RJC

RASĀYAN *J. Chem.*
Vol. 16 | No. 1 |227-232| January - March | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

COMPUTATIONAL STUDY OF *Acetylcholine esterase* INHIBITION BY AZAPHENOTHIAZINE ANALOGUES

K. Premavathi[1,✉], P. Valentina[2] and N. Ramalakshmi[3]

[1]Jaya College of Pharmacy, Thiruninravur, Chennai-602024, Tamil Nadu, India,
[Affiliated to The Tamil Nadu Dr. M.G.R Medical University]
[2]PERI College of Pharmacy, Mannivakkam, West Tambaram,
Chennai-600048, Tamil Nadu, India
[3]C.L. Baid Metha College of Pharmacy, Thorapakkam, Chennai- 600 097, Tamil Nadu, India
✉Corresponding Author: premavathi04@gmail.com

ABSTRACT

The present research is focused on new findings of azaphenothiazine analogs which is the promising lead moiety as the phenothiazine ring modified with azine ring as the basic nucleus reported from the literature survey that it has a wide spread of biological activity. The fifteen compounds were subjected to a molecular docking investigation using autodock pyrex, and it was discovered that ligand 4 with an ethyl group replaced gave the receptor a strong binding affinity of -8.6. Ligand 6having methyl group (showed the lowest binding affinity of -7.8. ligand 10 which is a substituted chlorine group gave good binding affinity and Ligand 13 showed significant binding affinity of -7.4 and -7.3 for the 2CMF active site of *Acetylcholine esterase* enzyme compared to imipramine (standard). The ADME property of selected moiety which is most important for drug development and discovery was done using SWISS adme and obeyed Lipinski rule 5 which plays an important role to predict physiochemical properties.

Keywords: Azaphenothiazine, 2CMF, Alzheimer's Disease, Drug likeness.

RASĀYAN*J. Chem.*, Vol. 16, No.1, 2023

INTRODUCTION

Alzheimer's is one of the types of dementia related to the loss of memory or cognition, serious enough that interferes with daily life and current therapies are unsuccessful to treat symptoms such as cognition.[1] Amyloid beta (A) plaque and neurofibrillary tangle buildup, decreased acetylcholine (Ach) levels, and oxidative stress is all beta-neuropathological characteristics of AD that are crucial to the loss of synaptic and neuronal function.[2,3] The main cause of dementia, in which brain cells die, is observed that causes a decrease in memory, language, and problem-solving skills.[4] The key feature of Alzheimer's disease pathogenesis is lead the formation of amyloid senile plaques.[5] The β-amyloid precursor protein (APP) is act as a proteolytic processing to produce Aβpeptides which are made up of 40 and 42 amino acids.[6,7] Acetylcholinesterase inhibitors and partial N-methyl D-aspartate antagonists are used to treat AD. According to various studies, the Ach level in the cholinergic system is declining and is closely associated with memory impairment in AD patients. The cortex, basal ganglia, and basal forebrain all contain the main neurotransmitter acetylcholine esterase.[8] The Ach neurotransmitter levels in the synaptic cleft are stabilizing by using acetylcholinesterase inhibitors. There are two binding sites involved in the AChE enzymes a catalytic ionic site (CAS) and a peripheral anionic site (PAS).[9,10] The acetylcholine esterase (AChE) enzyme plays an important role in the execution of synaptic transmission by rapid hydrolysis of the neurotransmitter acetylcholine (Ach), strength makes available symptomatic relief for early-stage AD patients. The ChEIs are also used discovered for the treatment of other dementias and other neurological indications.[11,12] Acetylcholinesterase and Butyrylcholinesterase are the two types of receptors found in the blood and neural synapses whereas *later* in the liver. Many drugs are available to target both receptors of Alzheimer's disease. The various inhibitory drugs are inactivating the AChE, the available drugs are Galantamine, Rivastigmine, and Donepezil[13], which acts as an acetylcholinesterase inhibitor. The reports of the azaphenothiazine analogs were mainly focused on butyl cholinesterase inhibitors for our research in the new findings of moiety for AD treatment[14], to develop the disrupted cortex region of the brain and to regularise the neurotransmitter.[15] Azaphenothiazine possesses various biological activities like

http://doi.org/10.31788/RJC.2023.1616995

antimicrobial, antifungal, antibacterial agents, and antidepressant activity.[16-18] Cholinesterase inhibitor activity, inhibition of Aβ fibrils formation, and inhibition of aggregation of tau protein.[19] To the best of our knowledge there is no report on the anticholinesterase inhibitory activity of azaphenothiazine analogs. All these above facts focused our research in search of finding a new pharmacophore of a phenothiazine analog. In this paper, we describe the molecular docking studies of azaphenothiazine derivatives as acetylcholinesterase inhibitors.

EXPERIMENTAL

Preparation of Ligand and Protein Preparation

The compounds were drawn by using Marvin sketch and generated smiles notation (Table-1). The cholinergic system plays important role in memory[20] and maintenance of the neurotransmitter which is involved in thinking and learning was vital.[21]

Molecular Docking

The target protein details are collected from the Protein data bank PDB ID: 2CMF and used as templates for the study. A protein data bank was used to download the protein (https://www.rcsb.org/pdb)Docking was done using autodockpyrx to predict the interaction and selectivity for Acetylcholine esterase.

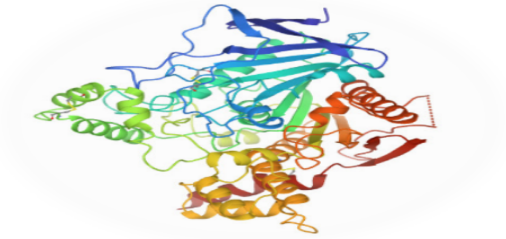


Fig-1: PDB ID:2CMF

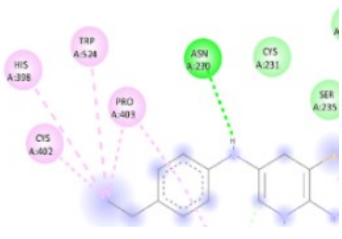
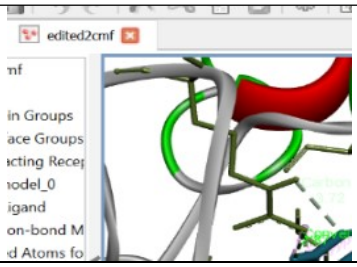
Protein-Ligand Docking

The receptor was docked against 2CMF to explore the protein-ligand interaction.[23] All the compounds were docked and the docking score was noted and the protein-ligand complex was analyzed and listed in the Table-2.

Residual Interaction with 2CMF

Ligand 4 shows binding affinity of azaphenothiazine is listed in Table-2 CYS A:231 and ethyl group to HISA: 406 and NH-group to ASN A: 230 and aromatic ring to ILE A: 296, LEU A:305, and pyridine group to ARG A: 289. The standard imipramine has a good binding affinity with the active residue azaphenothiazine with ASN A:525 and ethyl group to TRP A:524. Ligand 2 has shown good binding affinity given in the Table-2 aromatic ring with ILE A: 296, methoxy group to CYS A: 402, HIS A: 398, TRP A: 524, PRO A: 405, and NH group to ASN A: 233, and S group LEU A:3 05 and pyridine group to PRO A: 232. Ligand 3 shows the NH group to ASN A:230 and aromatic ring to ILE A: 290, LEU A: 305 PRO A: 403, and pyridine ring to ARS A:289.

The ADME properties were generated using Swiss ADME webserver[24] for 20 compounds in order to determine the physiochemical properties such as molecular weight, topological polar surface area (TPSA), number of H-bond acceptors, number of H-bond donors, and lipophilicity. The drug-likeness property and number of violations have been predicted by using molinspiration software.

Compounds	2D View	3D View
Compound 10		

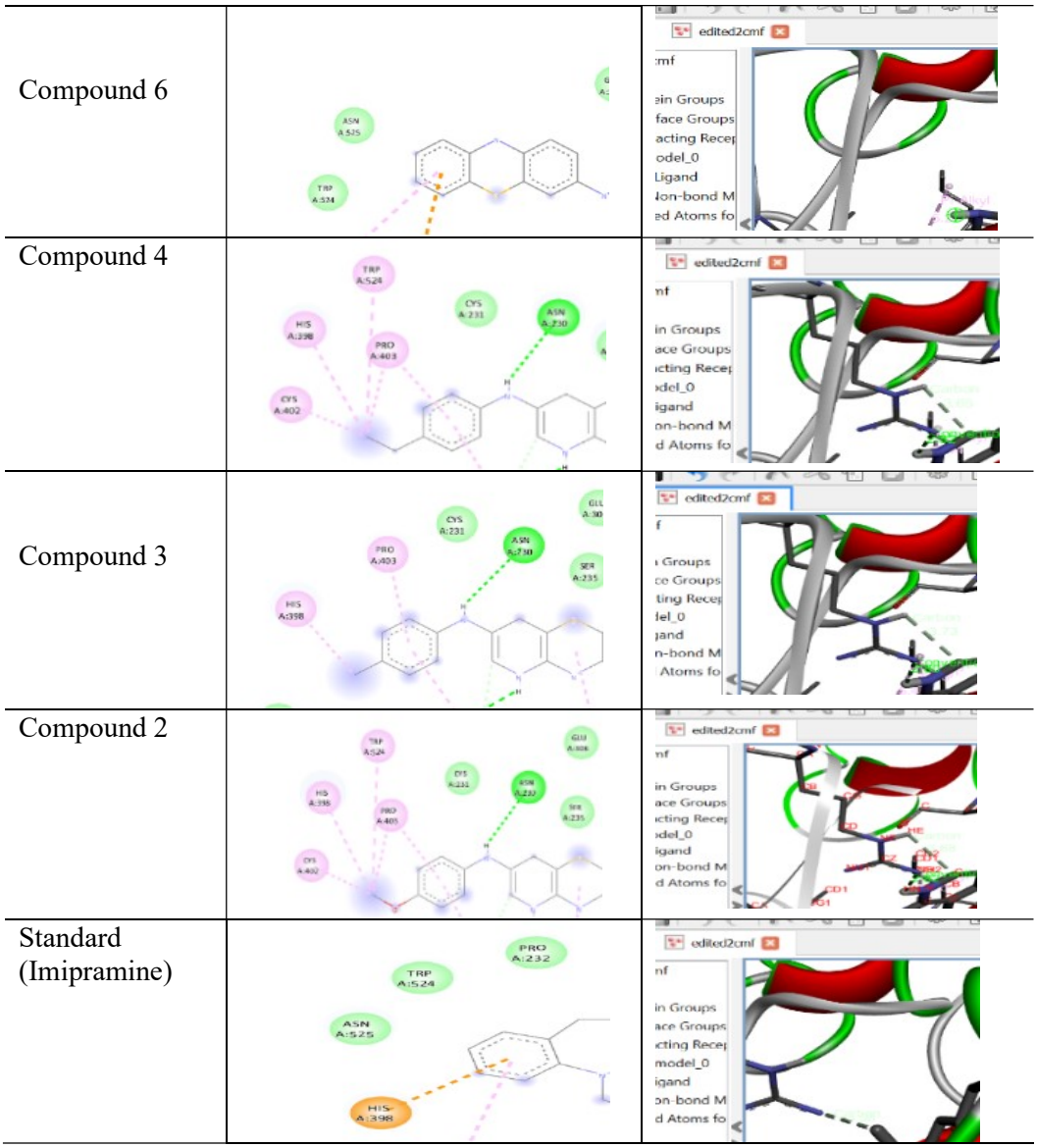


Fig.-2: 2D and 3D Representation of Compound 10, Compound 6, Compound 4, Compound 3, Compound 2 and Standard (Imipramine) Within the Active Site of Acetyl Cholinesterase *In-Silico* Physiochemical Properties

ADME Properties

ADME properties play a major role in drug development mainly in the success rate of drug candidates. The prediction of ADME properties of chemical compounds helps to detect the number of compounds to be synthesized. The ADME properties of the designed ligands are predicted using swiss ADME online software.[25] The smile notation of the designed compounds was entered at http://swissadme.ch website and studies were run to generate the ADME parameters (Table-2).

RESULTS AND DISCUSSION

The calculated free binding energy after molecular docking is given in Table-2. Compound 10 showed good binding affinity-8.9 and showed bonding interaction to ASN A: 230, ARG A: 289, and PRO A: 232 and alky bond to PRO S: 403, LEU A: 305, and ILE A: 296. Compound 4 gave a strong binding affinity of -8.6 with the receptors and ethyl group attached with hydrogen bonding to ASN A: 230, ARG A: 289, ASN A: 235, and SER A: 235 PRO A: 232, and methoxy group attached with (hydrogen bonding to ASN A: 230, ARG A: 289, PRO A: 232, and HIS A: 398. Compound 6 showed a binding energy of -7.8. Compounds 4 and 3 showed significant binding affinity -8.6 and -8.3 for 2CMF compared to imipramine

(standard). The ADME properties of the designed ligands are predicted using Swiss ADME online software and reveal the physicochemical parameters to predict the drug-likeness property. The results mentioned in the Table-3 indicate that the values obtained showed as per the limits mentioned i.e., Clog P (5.3 -8.3), TPSA (27.8-59.1), and iLog P (3.0-3.8) which is very useful for predicting the bioavailability of the drug.

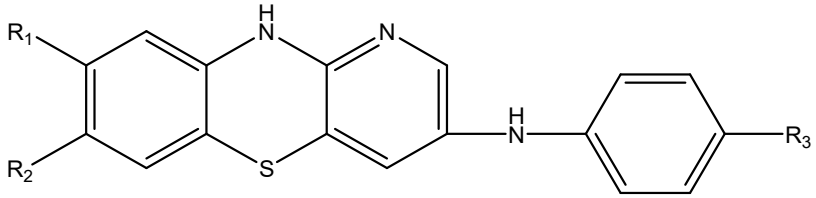


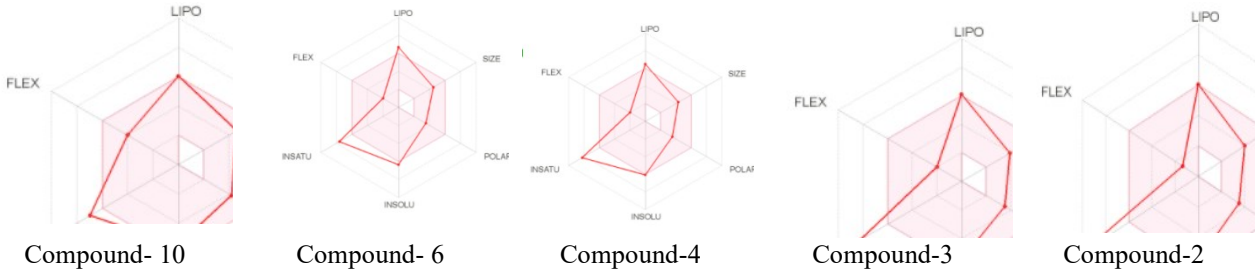
Table-1: Derivatives of 1-Azaphenothiazine

S.NO	COMPOUNDS	R1	R2	R3
01	Comp-01	Cl	CH_2CH_3	CH_3
02	Comp-02	H	H	OCH_3
03	Comp-03	H	H	Cl
04	Comp-04	H	H	CH_2CH_3
05	Comp-05	CH_3	CH_3	CH_3
06	Comp-06	H	H	CH_3
07	Comp-07	H	H	$CH_2CH_2CH_2CH_3$
08	Comp-08	OCH_2CH_3	OCH_2CH_3	H
09	Comp-09	CH_2CH_3	CH2CH3	H
10	Comp-10	Cl	H	CH2CH3
11	Comp-11	H	H	CH2CH2CH3
12	Comp-12	H	Cl	$CH_2CH_2CH_3$
13	Comp-13	CH_3	CH2CH3	CH3
14	Comp-14	CH_3	CH_3	$CH_2CH_2CH_3$
15	Comp-15	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	CH_2CH_3
16	Comp-16	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$
17	Comp-17	CH3	CH3	Cl
18	Comp-18	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	Cl
19	Comp-19	CH_2CH_3	CH_3	CH_2CH_3
20	Comp-20	CH_3	CH_2CH_3	CH_3

Table-2: ADME, Drug-Likeness Parameters, and Free Binding Energy of Synthesized Compounds

S. No	Compounds	Gi Absorption	Bbb Permiant	P-Gp Substrate	Mw[a]	C Logp	Nhbd	Nhba	Nrb	Tpsa	Binding Affinity
1	Comp-1	High	No	Yes	353.88	6.82	3	2	3	40.71	-7.7
2	Comp-2	High	No	Yes	321.40	5.30	4	2	3	49.90	-8.4
3	Comp-3	High	No	Yes	325.82	5.93	3	2	2	40.71	-8.3
4	Comp-4	High	No	Yes	319.43	6.16	3	2	3	40.71	-8.6
5	Comp-5	High	No	Yes	333.46	6.51	3	2	2	40.71	-7.7
6	Comp-6	High	No	Yes	318.44	5.69	2	2	3	27.82	-7.8
7	Comp-7	High	No	Yes	318.44	5.69	2	2	3	27.82	-7.6
8	Comp-8	High	No	Yes	379.49	5.62	5	2	6	59.18	-7.0
9	Comp-9	High	No	Yes	347.49	6.98	3	2	4	40.71	-6.3
10	Comp-10	High	No	Yes	353.88	6.82	3	2	3	40.71	-8.9
11	Comp-11	High	No	Yes	333.46	6.55	3	2	4	40.71	-7.4
12	Comp-12	High	No	Yes	367.90	7.21	3	2	4	40.71	-7.4
13	Comp-13	High	No	Yes	347.49	6.96	3	2	3	40.71	-7.3
14	Comp-14	High	No	Yes	361.51	7.36	3	2	4	40.71	-7.7

15	Comp-15	High	No	Yes	375.54	7.90	3	2	5	40.71	-6.9
16	Comp-16	High	No	Yes	389.57	8.25	3	2	6	40.71	-5.6
17	Comp-17	High	No	Yes	353.88	6.73	3	2	3	40.71	-8.0
18	Comp-18	Low	No	Yes	409.99	8.37	3	2	6	40.71	-6.9
19	Comp-19	High	No	Yes	361.51	7.43	3	2	4	40.71	-6.2
20	Comp-20	High	No	Yes	347.49	6.96	3	2	3	40.71	-6.9
21	Standard (Imipramine)	High	Yes	No	280.41	4.16	2	0	4	6.48	-6.2



Compound- 10 Compound- 6 Compound-4 Compound-3 Compound-2

Fig.-3: Bioavailability Radar Plots Depict the Drug Likeness Property of Azaphenothiazine Analogue

CONCLUSION

The twenty analogs of azaphenothiazine were prepared and docking is carried out. The *in-silico* studies of the compound showed that compound 10 showed good binding energy of -8.9 Kcal/mol compared with standard imipramine -6.2 Kcal/mol. The substituted electron withdrawing group chlorine group showed good binding affinity. The binding poses of these compounds (10, 6, 4, 2, and 3) in the binding cavities of the drug receptors are shown in Fig.-2. The concluded that compound 10 showed more binding affinity with ligands 10, 6, 4, 3, and 2 when compared with standard imipramine which shows that these compounds inhibit the Acetylcholinesterase receptors. The ADME properties performed by Swiss ADME were useful to predict the bioavailability of the lead azaphenothiazine derivatives and all the compounds satisfy the rule and are within the limit. The research is under progress for synthesis and *in-vivo* and *in-vitro* of these compounds for further study.

ACKNOWLEDGEMENTS

The authors thank the management of Jaya College of Pharmacy, Thiruninravur, Chennai- Tamil Nadu, and The Tamil Nādu Dr. M.G.R Medical University, Tamil Nadu, India to provide the necessary support to carry out this research.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

K. Premavathi https://orcid.org/0000-0001-5492-2885
P.Valentina https://orcid.org/0000-0001-8535-7196
N. Ramalakshmi https://orcid.org/0000-0002-6915-2235

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