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Analogues designing for dephosphorylation of acetylcholinesterase enzyme

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Organophosphate (OP) causes phosphorylation of acetylcholinesterase enzyme which leads to accumulation of acetylcholine. This phosphorylation generally occurs due to exposure of nerve agents and intake of pesticides, *etc*. Various standard drugs specifically oxime derivatives (HI-6, Obidoxime, 2-PAM, *etc*.) are used as AChE enzyme reactivation agents. These standard drugs show least penetration to CNS. Taking them into consideration with the help of structure and ligand based screened compounds, various small molecules analogues targeting CNS have been designed. These analogues pass all the pharmacokinetic parameters structurally and have also shown better results than that of standards. Among various charged and uncharged analogues, **4g**, **4h** and **4j** have attained docking scores –13.11, –12.84 and –12.75Kcal/mol respectively which is better than that of the standard (HI-6) –12.13kcal/mol.

Keywords: Ligand based drug design, structure based drug design, LOPAC database, virtual screening, docking, analogue designing, pharmacokinetic parameters

Acetylcholinesterase enzyme is responsible for acetylcholine degradation which further terminates neural transmission[1]. AChE have mainly two sites i.e., active and peripheral. Active site includes residue (Ser203, His447, and Glu334) and peripheral sites (aromatic residues Tyr72, Tyr124, Trp286, and Tyr341 and acidic Asp74). These sites responsible for binding inhibitors, etc[2]. AChE enzyme is being blocked or phosphorylated sue to presence of phosphate group in organophosphate containing compounds (nerve agents, pesticides, etc)[1]. Due to phosphorylation toxicity may occur which leads to death further. Covalent bond formation occurs between hydroxyl group of Ser203 residue and phosphorus atom of organophosphate which leads to irreversible inhibition at Ser203 residue[3]. Various oxime derivatives are used for the above treatment. These derivatives have poor penetration in CNS. In this research paper with the help of ligand based drug design and structure based drug design analogues designing was planned. In ligand based drug design with the help of oxime derivatives pharmacophore was generated whereas in structure based drug design with the help of 2WHP enzyme pharmacophore was generated. Once pharmacophore were generated by both methods, then LOPAC database was screened and with the help of potential leads analogue designing was performed targeting CNS[4].

Methodology

With the help of structure and ligand based pharmacophore, screening of LOPAC database was done. Essential leads were taken into consideration and analogue designing was processed. For docking three dimensional target enzyme 2WHP (PDB ID: 2WHP) was downloaded from RCSB. It is crystal structure of acetylcholinesterase enzyme which is being phosphonylated by sarin and in complex with HI-6.

Design of Analogues

PharmacoPredicta flagship module of Inventus software which has ability to predict relevant pharmacokinetic and ADME properties of selected Hits/Lead molecules before proceeding ahead with cell line and animal studies[5,6]. This is particularly useful when physiology is not known. With the help of this module designed analogues targeting CNS were being run through it. Structure model was being carried with this module. Data has being analysed further.

Results and Discussion

Analogues Designing

The principle of analogues designing is analogues possessing only chemical similarities[7]. Total 104 analogues (charged and uncharged) were being

designed according to screened and potential leads which are obtained through structure based drug design and ligand based drug design including molecular factors which influence BBB transport, enhanced flux across the BBB (and thus improved efficacy). This could be attained by designing a reactivator with a short carbon linker (C1–C2) in the absence of additional functional groups, mono quaternary as bisquaternary AChE reactivators have a relatively low predicted permeability value (range 0.538–1.780) which indicates that these compounds may be poorly absorbed across the BBB and preferably with one or two oxime groups in the para position[8]. The nucleus of the analogues was based on the molecular factors mentioned above and the lipophilic moiety was being added though screened and potential leads. As synthesised charged reactivators (HI-6, 2-PAM, etc) show less reactivation and results to toxicity, to overcome with this, an attempt is being made by designing charged and uncharged analogues which can show optimal reactivation with least toxicity (Figure 1, Figure 2). As analogues were designed for CNS target, out of 104 analogues, 51 passed blood brain barrier. These analogues are presented below in Table I and studied further.

As lipophilicity was the first of the descriptors to be identified as important for CNS penetration. However, ClogP correlates nicely with LogBBB with increasing lipophilicity increasing brain penetration. For several classes of CNS active substances, Hansch and Leo found that blood-brain barrier penetration is optimal when the LogP values are in the range of 1.5-2.7, with the mean value of 2.1 [9]. Considering the above range, out of 51 analogues, 15 analogues (5 charged and 10 uncharged) were found under Lipinski's rule (Table II).

Further these analogues were run for structure - based algorithms to predict pharmacokinetic properties. Although extremely valuable in early drug discovery because they require only structural features of the compound and not experimental data, these models usually correlate compound structures to a dataset for a

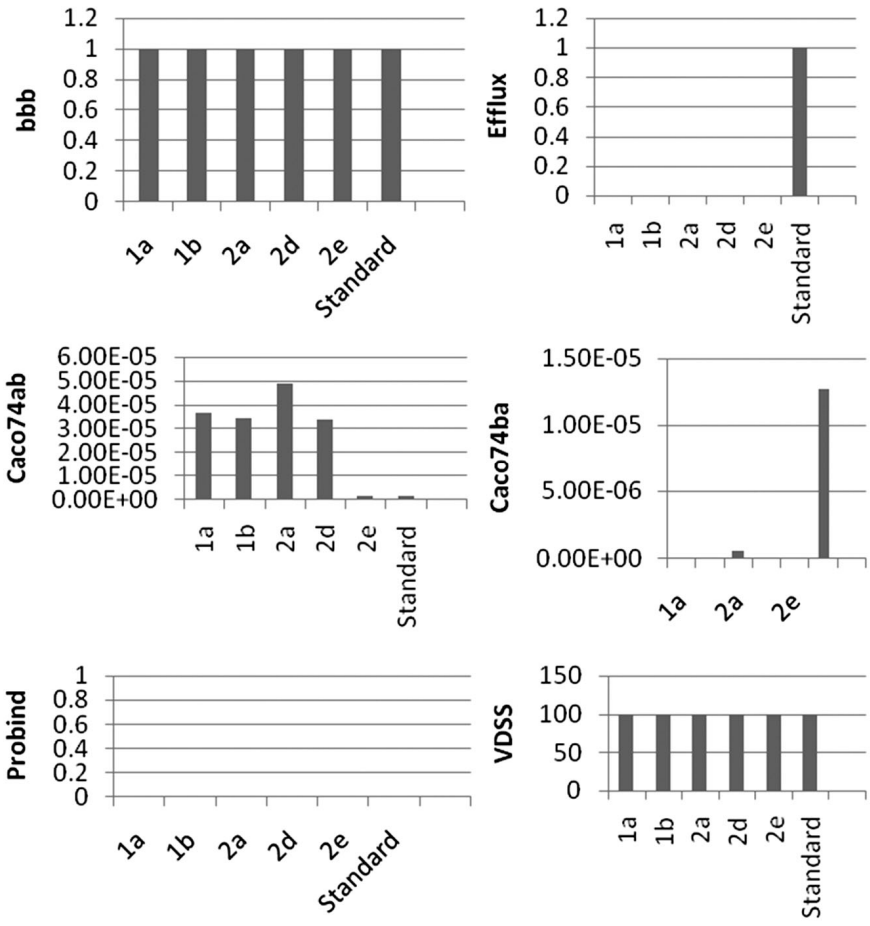


Figure 1 — Graphical Representation of Charged Analogues Data

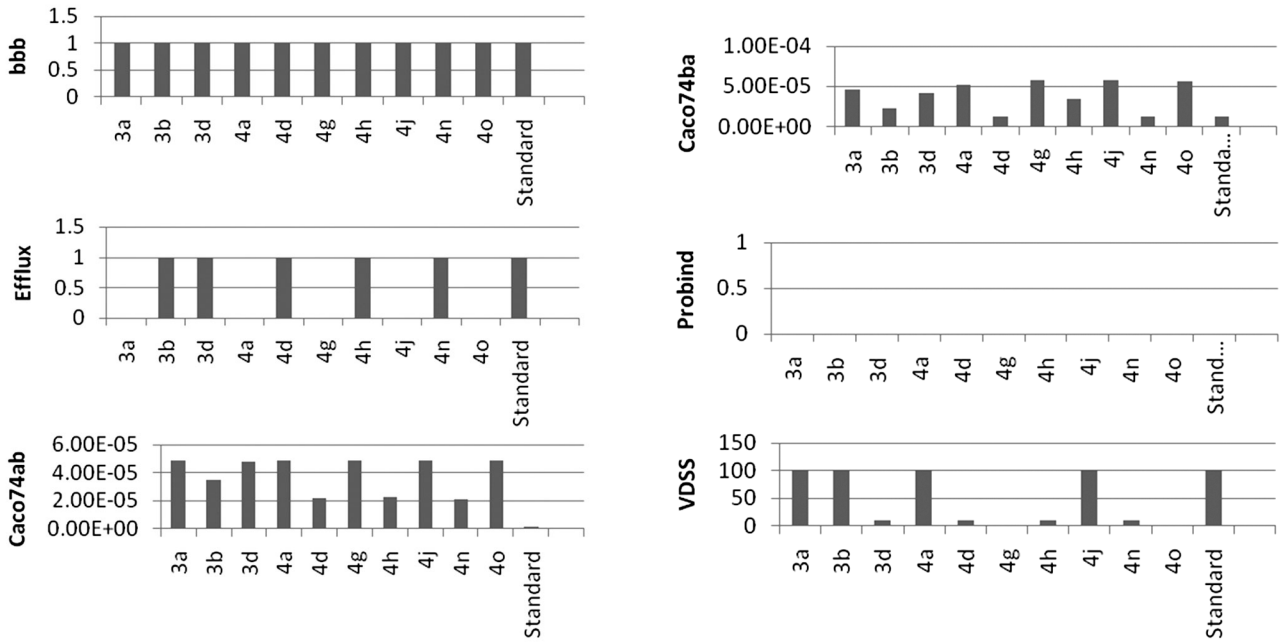


Figure 2 — Graphical Representation of Uncharged Analogues Data

Table I — Analogues structure along with lop P which passed BBB

Name	Structure	Log P
Ana 1a	O/N=C\C1=CC=[N+](CC2=CC=C(OC)C=C2)C=C1	2.65
Ana1b	O/N=C\C1=CC=[N+](CNC2CCCCC2)C=C1	2.76
Ana1c	O/N=C\C1=CC=[N+](CC2=CC=C(Cl)C(Cl)=C2)C=C1	3.95
Ana1d	O/N=C\C1=CC=[N+](CCNC2CCCCC2)C=C1	3.08
Ana2a	O/N=C\C1=CC=[N+](CC2=CC=CC=C2)C=C1	2.64
Ana2b	O/N=C\C1=CC=[N+](CC2CCCCC2)C=C1	3.17
Ana2c	O/N=C\C1=CC=[N+](CC2=CC=C(Cl)C=C2)C=C1	3.29
Ana2d	O/N=C\C1=CC=[N+](C[C@H]2NC3=CC=CC=C3C2)C=C1	2.91
Ana2e	O/N=C\C1=CC=[N+](COC2=CC=CC=C2)C=C1	2.63
Ana2f	O/N=C\C1=CC=[N+](C[C@@H](C2=CC=CC=C2)C3CCCCC3)C=C1	4.96
Ana2g	O/N=C\C1=CC=[N+](CCC2CCCCC2)C=C1	3.56
Ana2h	O/N=C\C1=CC=[N+](CCC(C2)=NC3=C2C=CC=C3)C=C1	3.31
Ana3a	O/N=C\C1=CCN(CC2=CC=C(OC)C=C2)C=C1	2.87
Ana3b	O/N=C\C1=CCN(CNC2CCCCC2)C=C1	2.82
Ana3c	O/N=C\C1=CCN(CC(NC2CCCCC2)=S)C=C1	3.48
Ana3d	O/N=C\C1=CCN(CC2=CC=C(Cl)C(Cl)=C2)C=C1	4.16
Ana3e	O/N=C\C1=CCN(CC2=CC3=CC(Cl)=CC=C3N2)C=C1	3.84
Ana3f	O/N=C\C1=CCN(COC2=CC=CC3=CC=CC=C23)C=C1	3.85
Ana3g	O=C(C(C=C1)=CC=C1F)CN2CC=C(/C=N\O)C=C2	2.96
Ana3h	O/N=C\C1=CCN(CCC2=CC=C(OC)C=C2)C=C1	3.19
Ana3i	O/N=C\C1=CCN(CCNC2CCCCC2)C=C1	3.44
Ana3j	O/N=C\C1=CCN(CCC(NC2CCCCC2)=S)C=C1	3.87
Ana3k	O/N=C\C1=CCN(CCC2=CC=C(Cl)C(Cl)=C2)C=C1	4.49
Ana3l	O/N=C\C1=CCN(CCC2=CC3=CC(Cl)=CC=C3N2)C=C1	4.16
Ana3m	O/N=C\C1=CCN(CCOC2=CC=CC3=CC=CC=C23)C=C1	4.46
Ana3n	O/N=C\C1=CCN(CCC(C(C=C2)=CC=C2F)=O)C=C1	3.35
Ana4a	O/N=C\C1=CCN(CC2=CC=CC=C2)C=C1	2.86

(*Contd.*)

Table I — Analogues structure along with lop P which passed BBB

Name	Structure	Log P
Ana4b	O/N=C\C1=CCN(CC2CCCCC2)C=C1	3.52
Ana4c	O/N=C\C1=CCN(CN)C=C1	0.57
Ana4d	O/N=C\C1=CCN(CC2=CC=C(Cl)C=C2)C=C1	3.51
Ana4e	O/N=C\C1=CCN(CC(N)=S)C=C1	1.23
Ana4f	O/N=C\C1=CCN(CC(C2)CC3=C2C=CC=C3)C=C1	3.36
Ana4g	O/N=C\C1=CCN(C[C@H]2NC3=CC=CC=C3C2)C=C1	3.27
Ana4h	O/N=C\C1=CCN(CC2=CN=CCC2=O)C=C1	1.87
Ana4i	O/N=C\C1=CCN(C[C@@H](O)C2=CC=CC=C2)C=C1	2.68
Ana4j	O/N=C\C1=CCN(CC[C@@H](O)C2CCCCC2)C=C1	3.56
Ana4k	O/N=C\C1=CCN(COC2=CC=CC=C2)C=C1	2.69
Ana4l	O/N=C\C1=CCN(C[C@H](C2CCCCC2)C3=CC=CC=C3)C=C1	5.31
Ana4m	O/N=C\C1=CCN(CC2=CC=C(F)C=C2)C=C1	3
Ana4n	O/N=C\C1=CCN(CCC2=CC=CC=C2)C=C1	3.18
Ana4o	O/N=C\C1=CCN(CCC2CCCCC2)C=C1	3.91
Ana4p	O/N=C\C1=CCN(CCN)C=C1	1.19
Ana4q	O/N=C\C1=CCN(CCC2=CC=C(Cl)C=C2)C=C1	3.84
Ana4r	O/N=C\C1=CCN(CCC(N)=S)C=C1	1.62
Ana4s	O/N=C\C1=CCN(CCC2CC3=CC=CC=C3C2)C=C1	3.75
Ana4t	O/N=C\C1=CCN(CCC2=NC3=CC=CC=C3C2)C=C1	3.66
Ana4u	O/N=C\C1=CCN(CCC2=CN=CCC2=O)C=C1	2.26
Ana4v	O/N=C\C1=CCN(CCC[C@@H](O)C2=CC=CC=C2)C=C1	3.46
Ana4w	O/N=C\C1=CCN(CCOC2=CC=CC=C2)C=C1	3.31
Ana4x	O/N=C\C1=CCN(CCC[C@H](C2CCCCC2)C3=CC=CC=C3)C=C1	6.09
Ana4y	O/N=C\C1=CCN(CCC2=CC=C(F)C=C2)C=C1	3.32

Table II — Under Lipinski's rule (charged and uncharged analogues)

Analogues	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Molecular Weight	Log P
Charged Ana 1a	4	1	243.28	2.65
Ana 1b	4	2	234.32	2.76
Ana 2a	3	1	213.26	2.64
Ana 2d	4	2	233.81	2.91
Ana 2e	4	1	229.25	2.63
Uncharged Ana 3a	4	1	244.29	2.87
Ana 3b	4	2	235.33	2.82
Ana 3d	4	1	260.26	2.96
Ana 4a	3	1	214.26	2.86
Ana 4d	4	2	197.26	1.23
Ana 4g	5	1	231.25	1.87
Ana 4h	4	2	244.29	2.68
Ana 4j	4	1	230.26	2.69
Ana 4n	4	2	211.28	1.62
Ana 4o	5	1	245.26	2.26

particular pharmacokinetic endpoint, without regard for the underlying processes, i.e., physiology[10] (Table III).

- Caco-2 permeability (A→B or apical to basolateral), P eff at *p*H 7.4 (cm/s)
- Caco-2 permeability (B→A or basolateral to apical) at *p*H 7.4 (cm/s)
- Efflux at *p*H 7.4 (0 if ≤ 5.3, 1 if > 5.3)
- Blood Brain Barrier permeability (0 if no penetration, 1 if penetration).
- Human absorption, FDp (%) results are classified as:
- Low (0%-33% absorbed)
- Medium (34%-66% absorbed)
- High (67%-100% absorbed)
- Protein Binding (0 if ≤ 85%, 1 if > 85%)
- Volume of Distribution at Steady State, VDSS(lit.)
- Prediction Confidence (high, medium, low)[5].

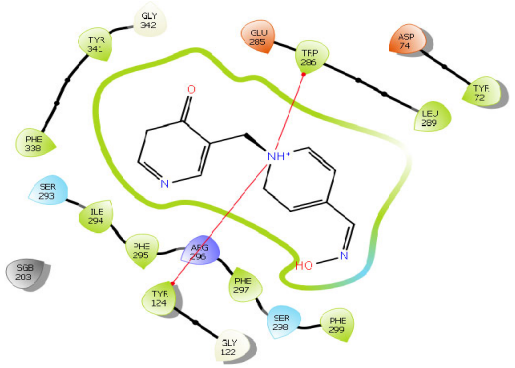
Further refinement of these analogues was done by docking. Among these best analogues were obtained

Table III — Structural ADME parameters for charged and uncharged analogues

Analogues	caco74ab	caco74ba	efflux	bbb	fdp	probind	Vdss
1a	3.65E-05	9.70E-08	0	1	High	0	100
1b	3.47E-05	5.05E-08	0	1	High	0	100
2a	4.90E-05	5.29E-07	0	1	High	0	100
2d	3.40E-05	6.12E-08	0	1	High	0	100
2e	1.42E-06	6.17E-08	0	1	High	0	100
3a	4.90E-05	4.60E-05	0	1	High	0	100
3b	3.47E-05	2.35E-05	1	1	High	0	100
3d	4.78E-05	4.22E-05	1	1	High	0	10
4a	4.90E-05	5.22E-05	0	1	High	0	100
4d	2.15E-05	1.36E-05	1	1	High	0	10
4g	4.90E-05	5.73E-05	0	1	High	0	1
4h	2.27E-05	3.41E-05	1	1	High	0	10
4j	4.90E-05	5.73E-05	0	1	High	0	100
4n	2.14E-05	1.37E-05	1	1	High	0	10
4o	4.90E-05	5.66E-05	0	1	High	0	1
Standard	1.87E-06	1.27E-05	1	1	High	0	100

Table IV — Docking of uncharged (4g, 4h, 4j) and charged (2e, 1b, 2d) analogues Standard (HI-6) docking score= -12.130KCAL/MOL

Analogues	Glide XP score (Kcal/mol) Residues and 2D images
Ana 4g	**Docking Score:** -13.11 **Pi cation –** TYR124, TRP286. **Hydrophobic Bonds-** TYR341, PHE338, ILE294, PHE295, TYR124, PHE297, PHE299, TRP286, LEU289, TYR72



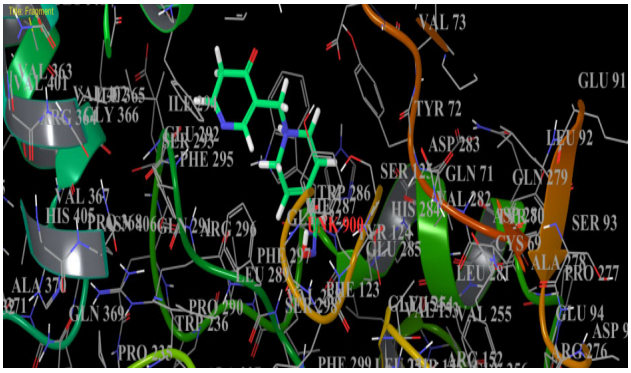


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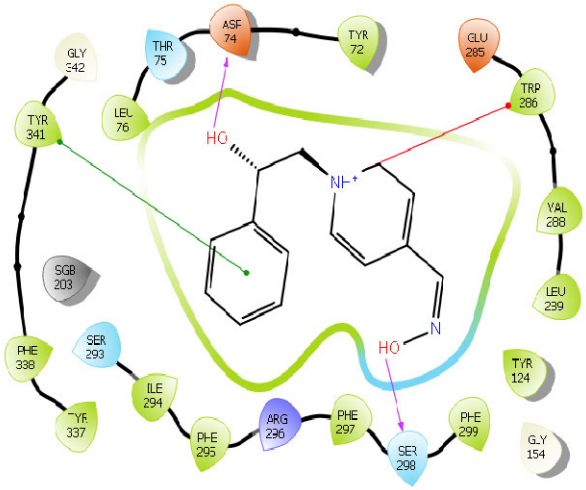
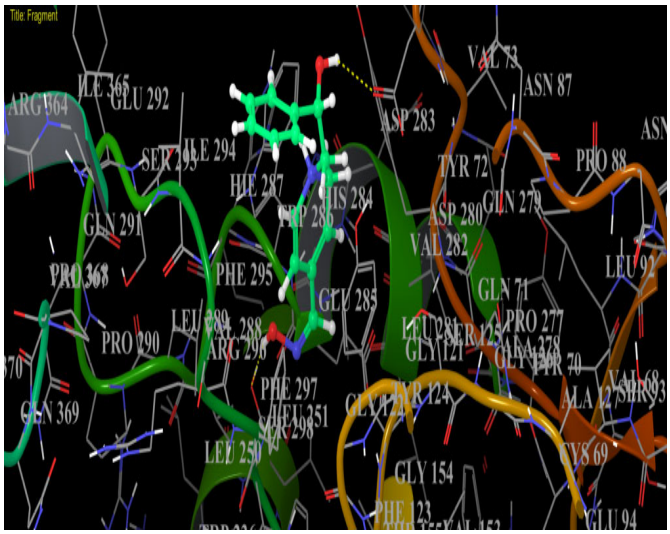
Table IV — Docking of uncharged (4g, 4h, 4j) and charged (2e, 1b, 2d) analogues Standard (HI-6) docking score= -12.130KCAL/MOL

Analogues	Glide XP score (Kcal/mol) Residues and 2D images
Ana 4h	**Docking Score:** -12.84 **Pi cation –** TRP286. **Pi-Pi stacking-** TYR341. **Salt Bridge-** TRP286. **Hydrogen Bond-** ASP74, SER298. **Hydrophobic Bonds-** TYR72, LEU76, TYR341, TRP286, VAL288, LEU289, TYR124, PHE299, PHE297, PHE295, ILE294, TYR337, PHE338.

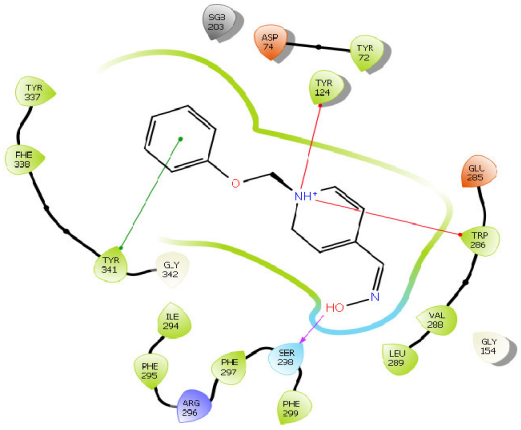
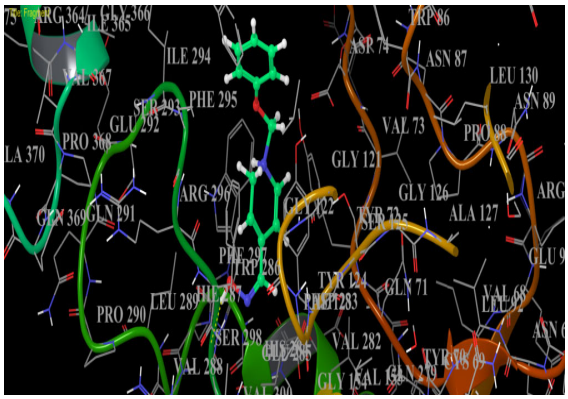
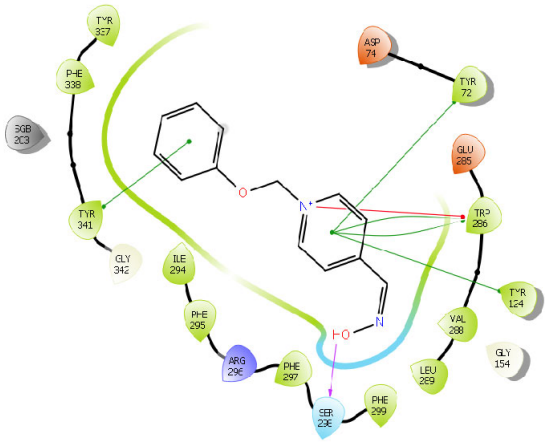




Ana 4j	**Docking Score:** -12.75 **Pi cation –** TRP286, TYR124. **Pi-Pi stacking-** TYR341. **Hydrogen Bond-** SER298 **Hydrophobic Bonds-** TYR72, TYR124, TYR337, PHE338, TYR341, ILE294, PHE295, PHE297, PHE299, LEU289, VAL288, TRP286.

(*Contd.*)

Table IV — Docking of uncharged (4g, 4h, 4j) and charged (2e, 1b, 2d) analogues Standard (HI-6) docking score= -12.130KCAL/MOL

Analogues	Glide XP score (Kcal/mol) Residues and 2D images
	 
Ana 2e	**Docking Score:** -12.30 **Pi cation –** TRP286. **Pi-Pi stacking-** TYR72, TRP286, TRP286, TYR124, TYR341. **Hydrogen Bond-** SER298. **Hydrophobic Bonds-** TYR72, TYR124, TYR337, PHE338, TYR341, ILE294, PHE295, PHE297, PHE299, LEU289, VAL288, TRP286. 

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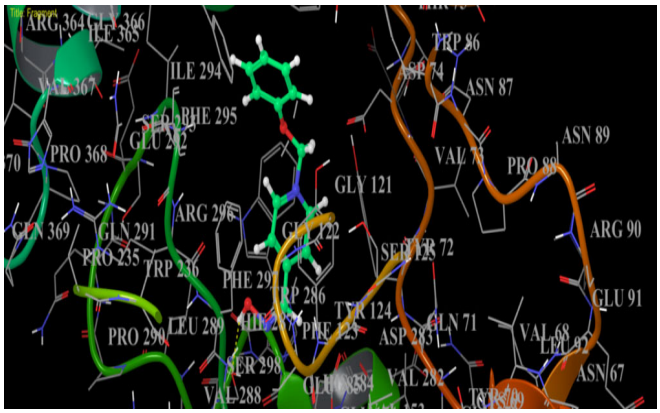
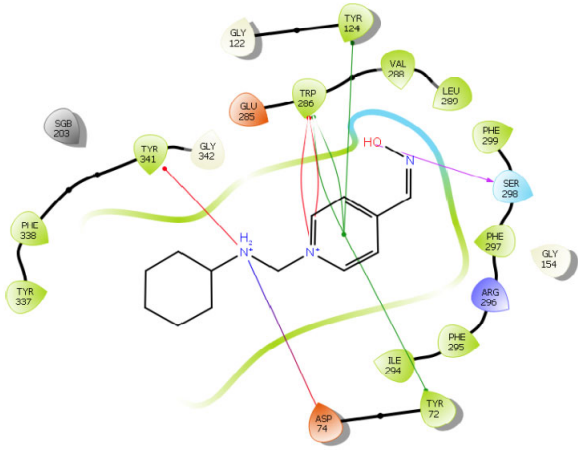
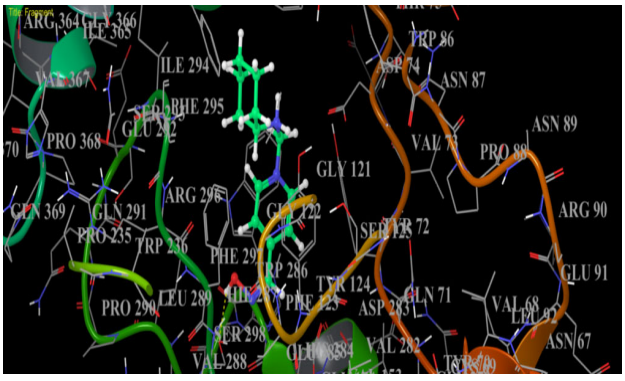
Analogues	Glide XP score (Kcal/mol) Residues and 2D images
	
Ana 1b	**Docking Score:** -12.03 **Pi cation –** TYR341, TRP286, TRP286. **Pi-Pi stacking-** TRP286, TRP286, TYR124, TYR72. **Salt Bridge-** ASP74. **Hydrogen Bond-** SER298. **Hydrophobic Bonds-** TYR72, TYR124, TYR337, PHE338, TYR341, ILE294, PHE295, PHE297, PHE299, LEU289, VAL288, TRP286.  

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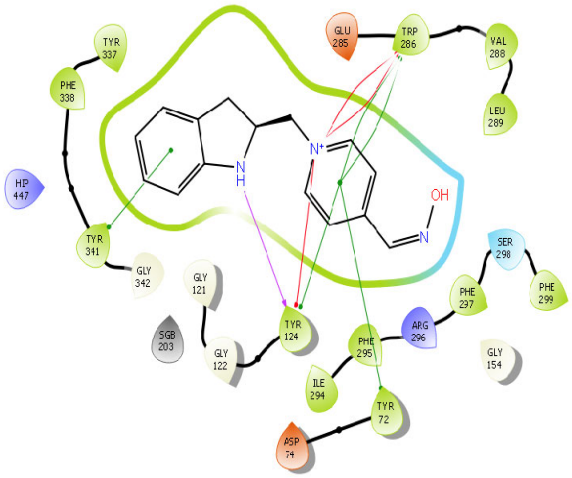
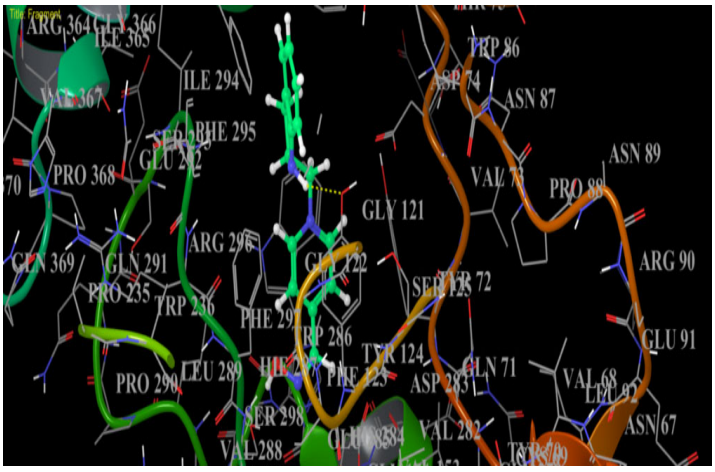
Table IV — Docking of uncharged (4g, 4h, 4j) and charged (2e, 1b, 2d) analogues Standard (HI-6) docking score= -12.130KCAL/MOL

Analogues	Glide XP score (Kcal/mol) Residues and 2D images
Ana 2d	**Docking Score:** -12.01 **Pi cation –** TRP286, TRP286, TYR124. **Pi-Pi stacking-** TRP286, TRP286, TYR124, TYR72, TYR341. **Hydrogen Bond-** TYR124. **Hydrophobic Bonds-** TYR72, TYR124, TYR337, PHE338, TYR341, ILE294, PHE295, PHE297, PHE299, LEU289, VAL288, TRP286.





by docking scores and interacting residues which are being compared with standard. Top three charged and uncharged analogues were being screened out and considered as potential leads as their docking scores are comparable to standard (HI-6,Table IV).

Conclusion

In this study, through ligand based drug design a pharmacophore model was generated with a dataset of 79 compounds as acetylcholinesterase reactivators in order to analyze the essential structural features which are required for binding. Later 3D-QSAR model was developed with the help of pharmacophore-based alignment. The model explains how the three dimensional arrangements of various substituent may affect the biological activity of the AChE reactivators[4]. With the use of structure based drug design most active site of acetylcholinesterase enzyme was being detected which is required for binding, as active site residues are responsible for

biological activity. Screening was done through structure and ligand based pharmacophore and screened leads were further taken for ADME screening. Various leads were considered as potential leads as they passed CNS parameters. Further refinement was done by performing docking studies as binding scores and residues were comparable with that of standard (HI-6). With this, for analyzing better potency these leads can be further taken for wet lab testing. With the help of potential leads, analogues designing were also performed. Structural ADME parameters, docking scores, residue information of potent analogues were comparable with that of standard (HI-6).

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Conflict of interests

The authors declare no conflict of interests.

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