

## Full Length Research Paper

# Design and optimisation of novel Huperzine A analogues capable of modulating the acetylcholinesterase receptor for the management of Alzheimer's disease

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Received 6 October, 2015; Accepted 4 April, 2016

This is a *de novo* drug design study that aimed to create novel structures based on the alkaloid Huperzine A, capable of inhibiting the acetylcholinesterase (AChE) enzyme ligand binding pocket (AChE\_LBP) for the management of Alzheimer's disease. The X-ray crystallographic model of the Torpedo Californica AChE complexed to Huperzine A was identified from the Protein Data Bank (PDB ID 1VOT). Molecular visualisation and modelling was carried out using SYBYL<sup>®</sup> 1.2, *in silico* predicted ligand binding affinity (LBA) was quantified using XSCORE\_V1.3 and *de novo* drug design was carried out using LIGBUILDER<sup>®</sup>V1.2. Two seed structures were constructed in SYBYL<sup>®</sup> 1.2 according to a methodology that took into account the relationship between molecular structure and biological activity as described in the literature. Based on SAR data derived from Huperzine A, the points considered to be critical for binding were retained in each seed and planted into the AChE\_LBP with growth being allowed according to defined parameters of LIGBUILDER<sup>®</sup>V1.2. The implication of this study consequently is that novel structures compliant to Lipinski's Rule of 5 may be promoted to second level drug design which could lead to identification of novel AChE inhibitors with better potency and a low side effect profile.

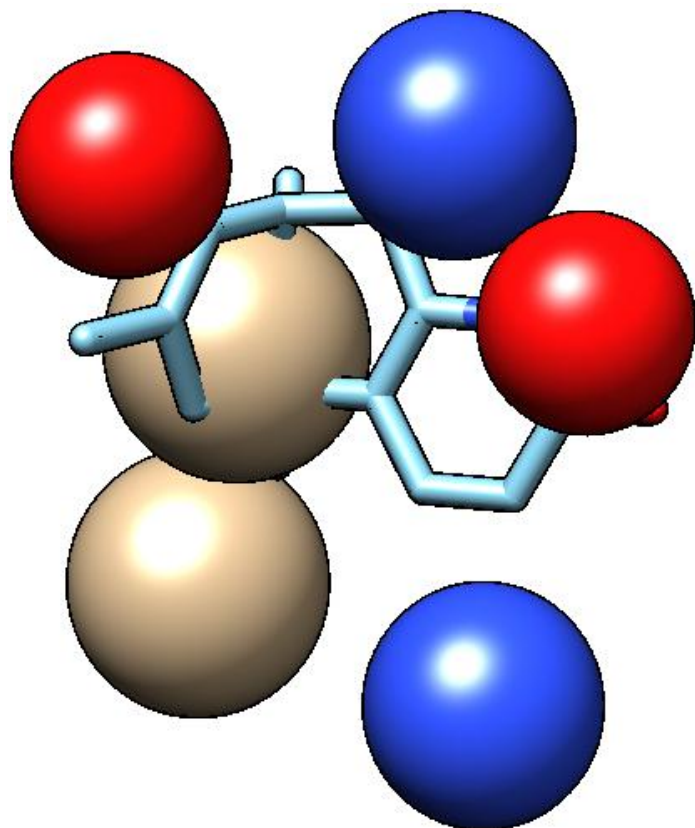
**Key words:** *de novo* drug design, Huperzine A, acetylcholinesterase, Alzheimer's disease, Lipinski's Rule of 5.

## INTRODUCTION

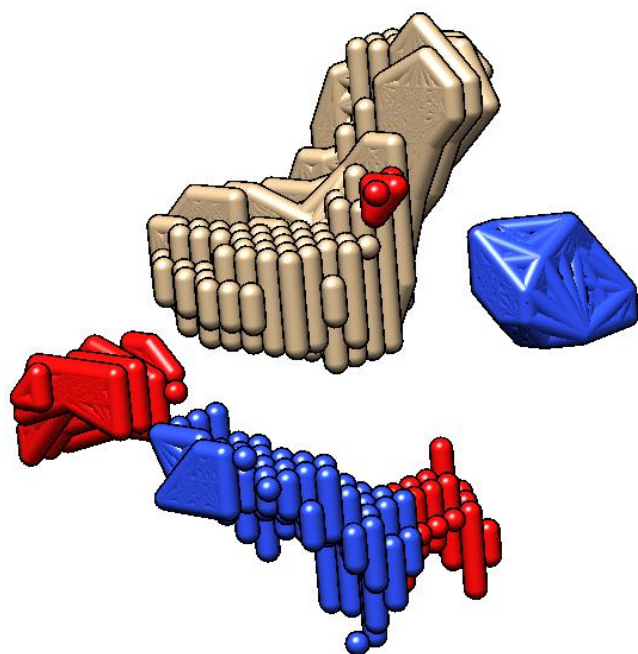
Alzheimer's disease is the most common cause of dementia (Akhondzadeh and Abbasi, 2006) characterised by the build-up in the brain of protein rich

plaques and leading to decreased cerebral nerve cell connectivity culminating in the death of nerve cells and loss of brain tissue (Wang et al., 2006a). It is also

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**Figure 1.** Huperzine A onto its pharmacophore shown in beads rendered in Chimera® v.1.7.



**Figure 2.** Huperzine A key interaction sites rendered in Chimera® v.1.7 based on the coordinates of PDB ID 1VOT (Raves et al., 1997).

associated with decreased acetylcholine (ACh) levels, due to the fact that this is broken down at a higher than average rate by the acetylcholinesterase (AChE). This also contributes to incomplete transmission of nerve impulses (Picoulin, 2002).

The naturally occurring alkaloid Huperzine A has been shown to be a potent inhibitor of the transport of choline through cholinesterase inhibition consequently resulting in an increase in ACh. AChE inhibition also results in a slower rate of breakdown of acetylcholine and in nerve impulses of strength and duration consequently enhancing cerebral performance (Zangara, 2003).

In this study, an analysis of the binding modality of Huperzine A within the AChE Ligand Binding Pocket and the use of the Huperzine A scaffold to generate analog series of lead molecules for further optimisation using a static algorithm were reported.

## METHODOLOGY

### Molecular modelling

X-ray crystallographic deposition 1VOT describing the bound coordinates of the Torpedo Californica AChE bound to the nootropic alkaloid Huperzine A was identified from the Protein Data Bank and used as a template for this study (Raves et al., 1997).

Molecular modelling was carried out in Sybyl®-X, Ligand Binding Affinity (LBA) was quantified in X-Score v1.2, and *de novo* ligand generation was carried out using LigBuilder v2.0. VMD® v.1.9 (Humphrey et al., 1996) and Chimera® v.1.7 were used for image generation.

Protein Data Bank crystallographic deposition 1VOT was then read into Sybyl®-X and all water molecules at a distance  $\geq 5\text{\AA}$  from the Ligand Binding Pocket were removed. The bound ligand, Huperzine A was extracted from the AChE Ligand Binding Pocket. The now *apo*-AChE and the extracted ligand were saved in PDB and *mol2* format, respectively.

### Quantifying the binding affinity (pKd) of huperzine A for the AChE

The *apo*-AChE (saved in *pdb* format) and the extracted ligand (saved in *mol2* format) were used as input files for X-Score® v1.2, which quantified, based on atomic interactions, the affinity (pKd) of the bioactive conformer of Huperzine A for its co-crystallised Ligand Binding Pocket (Wang et al., 1998). This procedure was considered as vital in the establishment of a baseline affinity against which that of the *de novo* generated Huperzine A analogs could be compared.

### AChE ligand binding pocket mapping

The POCKET module of LIGBUILDER® v2.0 (Wang et al., 2000) was used to generate a pharmacophore and a 3-Dimensional map of the AChE Ligand Binding Pocket as circumscribed around the bioactive conformation of Huperzine A. Both the general pharmacophoric structure and the 3-Dimensional map of the AChE Ligand Binding Pocket were colour coded by atom type, with hydrogen bond donors and acceptors being coloured blue and red, respectively and with hydrophobic sites being coloured cyan. These are described in Figures 1 and 2, respectively.

The output file in each case was in *pdb* format and could be

**Table 1.** The 2-dimensional and 3-dimensional structure of Huperzine A as rendered in Symyx<sup>®</sup> Draw 4.0 and VMD<sup>®</sup> v1.9, along with its predicted *in silico* ligand binding affinity to its cognate receptor 1VOT, as calculated in X-Score<sup>®</sup> v1.3.

2-Dimensional Structure	3-Dimensional Structure	Predicted <i>in silico</i> LBA within cognate receptor
<b>Huperzine A within 1VOT</b>		
		HPScore $-\log(K_d) = 6.41$ HMScore $-\log(K_d) = 6.41$ HSScore $-\log(K_d) = 6.54$ Predicted average $-\log(K_d) = 6.45$ Predicted binding energy = $-8.80$ kcal/mol

visualised in VMD<sup>®</sup> v.1.9 (Humphrey et al., 1996) and Chimera<sup>®</sup> v.1.7. This process was important because it established the pharmacophoric space available for *de novo* ligand construction, as well as the general pharmacophoric structure to which all the *de novo* generated ligands must necessarily comply for efficient binding.

#### Seed structure construction

A seed structure is essentially a molecular scaffold that is capable of sustaining molecular growth at user directed pre-designated growing sites. For this study, two seed structures were constructed in Sybyl<sup>®</sup>-X according to a methodology that took into account the relationship between molecular structure and biological activity as described in the literature. Seed A was created by removing the methyl group at the 15-position and the carbon group at the 14-position thereby opening the last ring of Huperzine A.

Seed B was created by removing the fused cyclic rings and retaining solely the aromatic ring bearing the carbonyl group and two methyl groups at the 6 and 12 positions. The carbonyl group was retained in both seeds because the pyridone oxygen forms a strong hydrogen bond with a protein residue of the ligand binding pocket. In Seed A, atoms 8 and 13 were designated as growing sites (*H.spc*) whereas in Seed B, atoms 6 and 12 were designated as growing sites (*H.spc*).

#### *de Novo* ligand design

The modelled seed structures were successively docked into the AChE Ligand Binding Pocket 3-Dimensional map, with which molecular growth was sustained according to the generic algorithm embedded in the GROW module of LigBuilder<sup>®</sup>V2.0.

The PROCESS module of LigBuilder<sup>®</sup>V2.0 was used to organise the *de novo* generated structured into families based on pharmacophoric similarity, and ranked in order of Ligand Binding

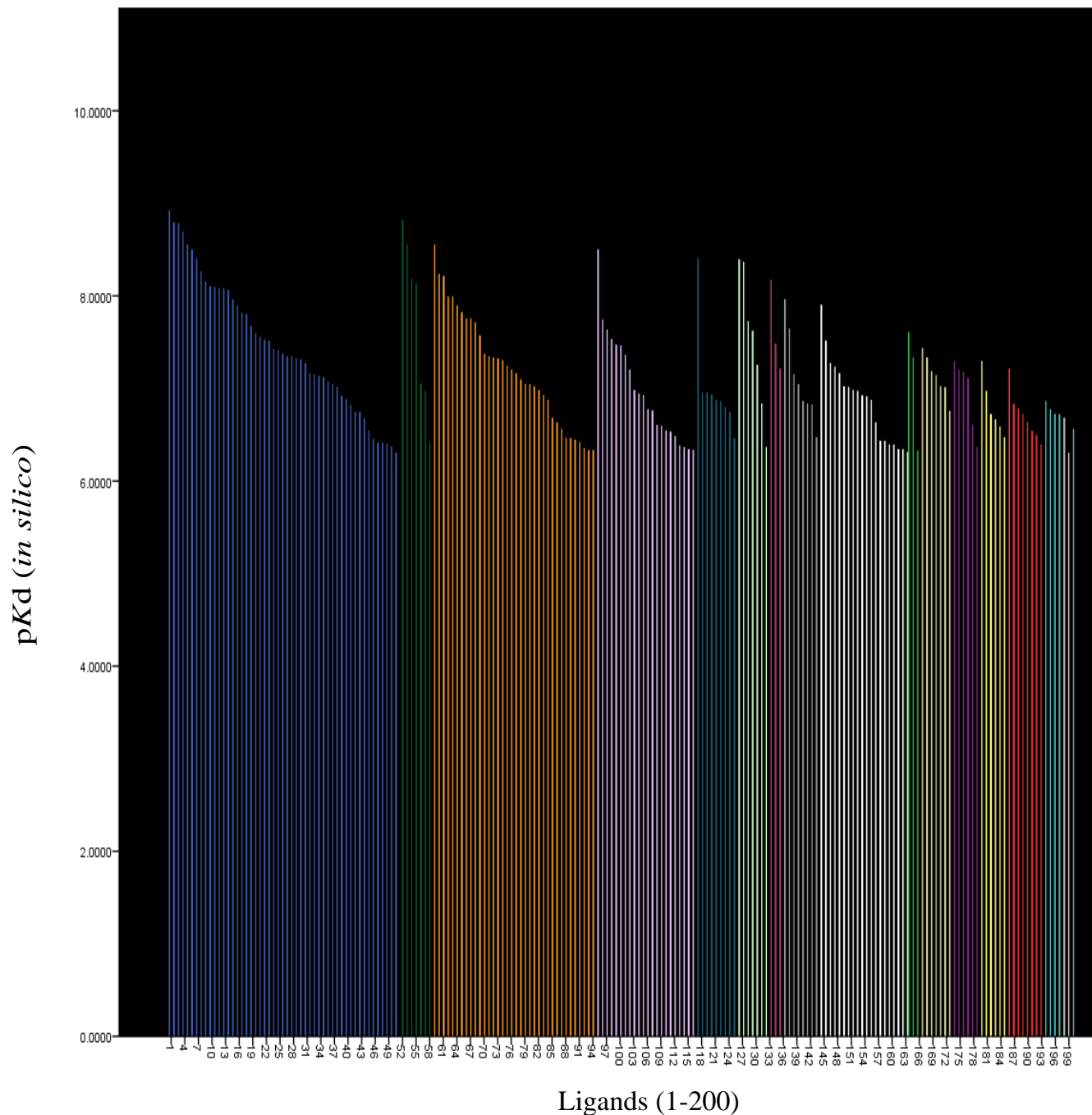
Affinity (pKd).

## RESULTS

The *in silico* LBA (pKd) of Huperzine A to its cognate receptor was predicted to be 6.45 as shown in Table 1. The algorithm embedded in the PROCESS module of LIGBUILDER<sup>®</sup>V2.0. This process resulted in 200 and 600 molecules to be generated from Seeds A and B, respectively. These *de novo* structures were divided into a number of families whose *in silico* affinity (pKd), molecular weight (Daltons/Da) and logP are displayed in Figures 3 to 8, respectively. Each molecular structure was assessed for Lipinski rule of 5 (predictors of *in vivo* bioavailability) rule compliance (Lipinski et al., 1997). The pKd for the *de novo* molecules ranged from 6.30 to 10, while molecular weight ranged from 300 to 526 and the LogP ranged from 3 to 5.99. A summary of all the values in each seed may be seen in Table 2.

## DISCUSSION

All 15 molecules chosen from Seed A and all 45 molecules chosen from Seed B were Lipinski rule compliant. In an ideal scenario, the molecules with the highest LBA (pKd) would also have the lowest LBE (kcal mol<sup>-1</sup>). The best molecule from the top 15 molecules chosen from Seed A is molecule number 6 having a pKd of 8.82 and a LBE of 126.999 kcal mol<sup>-1</sup>. Moreover, the



**Figure 3.** A graph showing the pKd (*in silico*) for the 200 *de novo* designed ligands from Seed A; the 17 different colours imply the 17 family series.

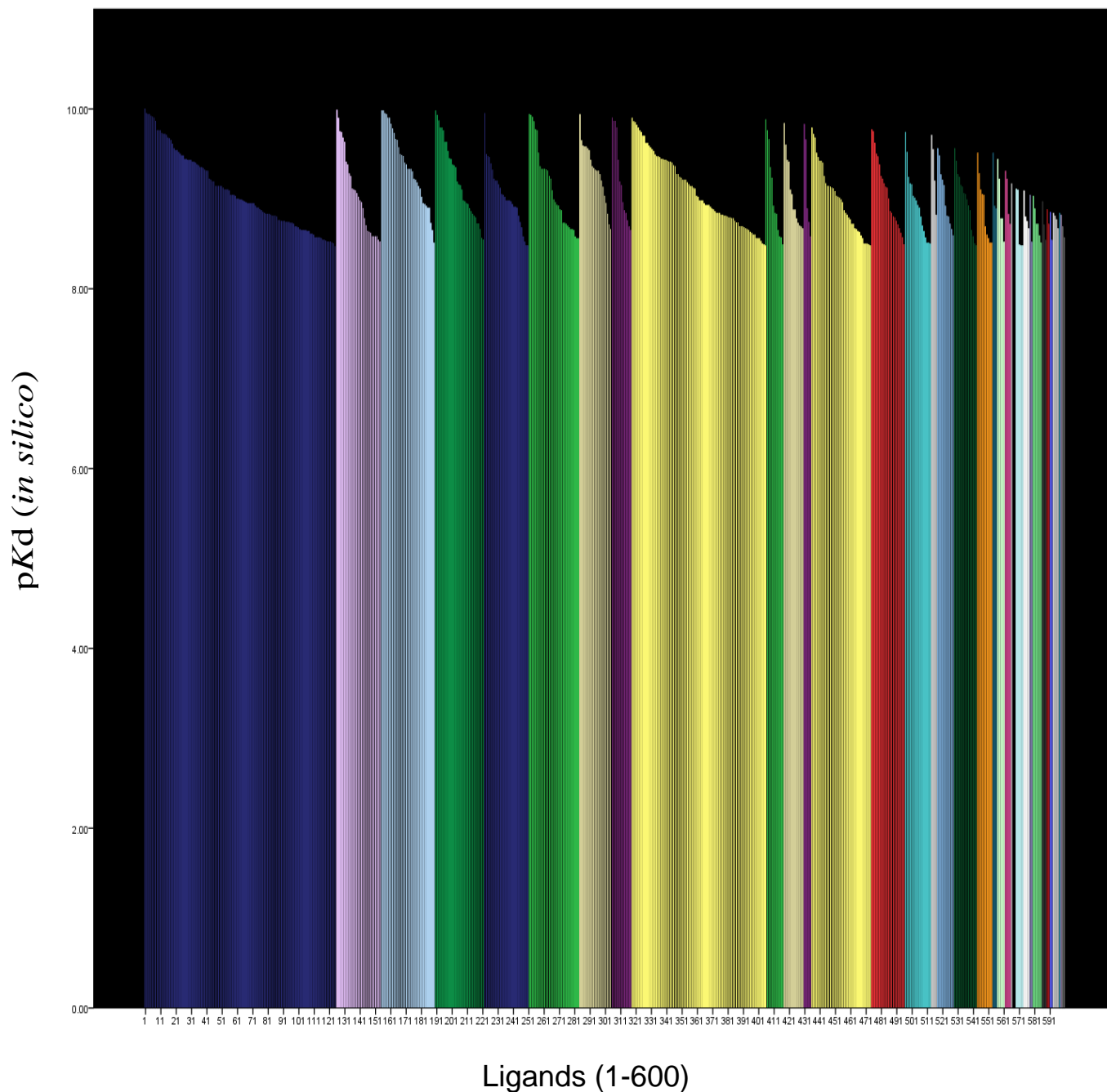
best molecule from the top 45 molecules chosen from Seed B is molecule number 3 having a pKd of 9.37 and a LBE of 45.796 kcal mol<sup>-1</sup>. The binding poses of molecules 6 and 4 are superimposed onto the bound co-ordinates of Huperzine A as shown in Figures 9 and 10.

From this *de novo* study, the following relationships between structure and activity have been identified:

1) Ligands having two aromatic rings connected by an

aliphatic chain have a high LBA (pKd) and a low LBE (kcal mol<sup>-1</sup>). This was evident from molecules generated from Seed B.

2) Molecules with an aromatic ring directly attached to a pyridine ring bearing a negatively charged oxygen atom have been observed to have a lower LBA (pKd) and a higher LBE (kcal mol<sup>-1</sup>). This was apparent from ligands generated from Seed A.



**Figure 4.** A graph showing the  $pK_d$  (*in silico*) for the 600 *de novo* designed ligands from Seed B; the 35 different colours imply the 35 family series.

(3) Ligands with lateral branching ending in an acidic group (specifically the carboxyl group) from the pyridine bearing the carbonyl group have a higher LBA ( $pK_d$ ) than those which did not have an acidic group.

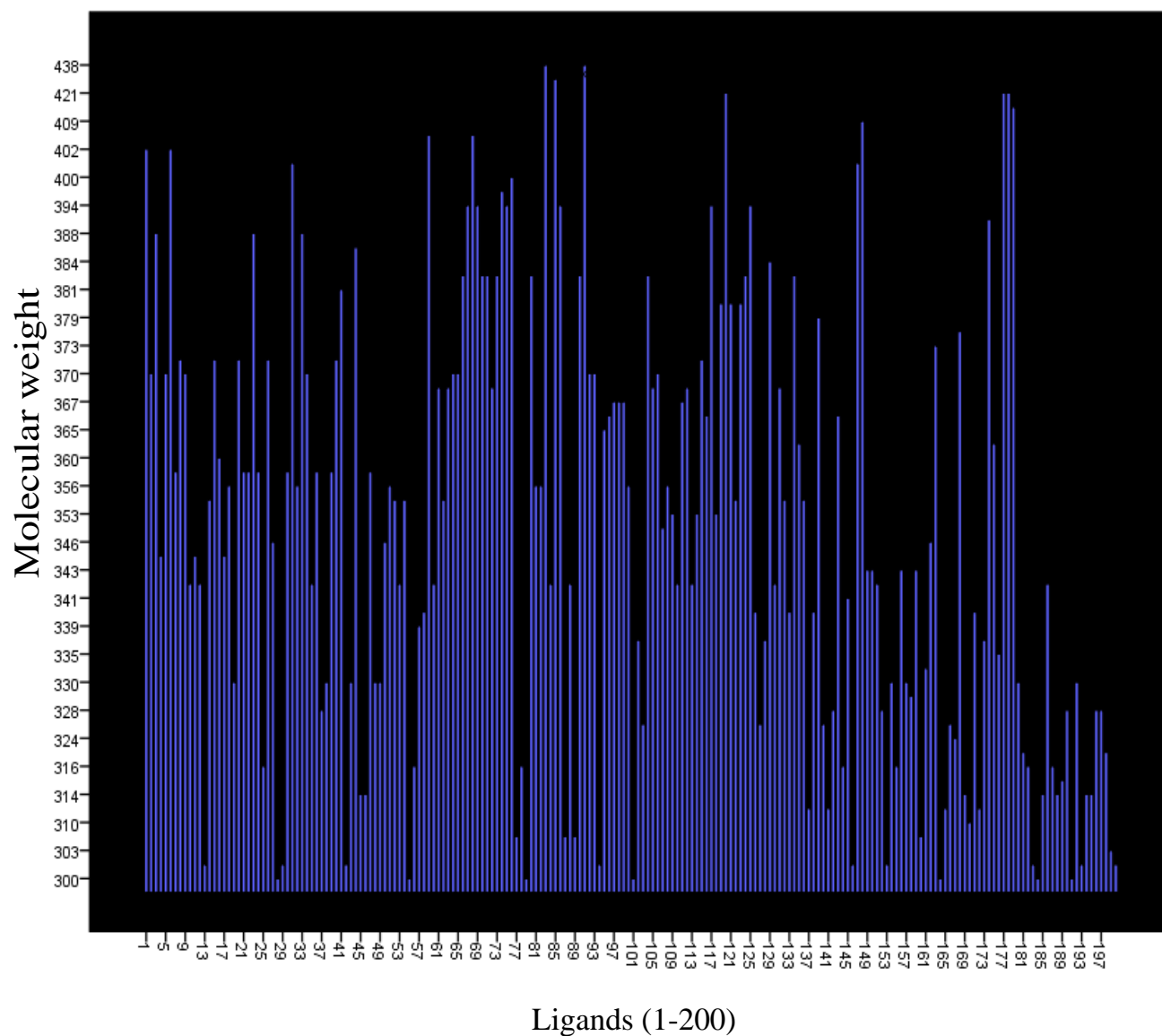
(4) Molecules having an aromatic ring linked to a pyridine bearing a negatively charged oxygen group exhibited a lower LBA ( $pK_d$ ) than molecules not having a negatively charged oxygen group connected to the pyridine ring.

(5) Ligands having an aromatic ring bearing a carboxylate anion had a higher LBA ( $pK_d$ ) than those without the

carboxylate anion.

The implication of this study is that all the novel molecules sampled from both seeds which are also compliant to Lipinski's Rule of 5, are candidates for subsequent iterative rounds of rational drug design and *in vitro* validation.

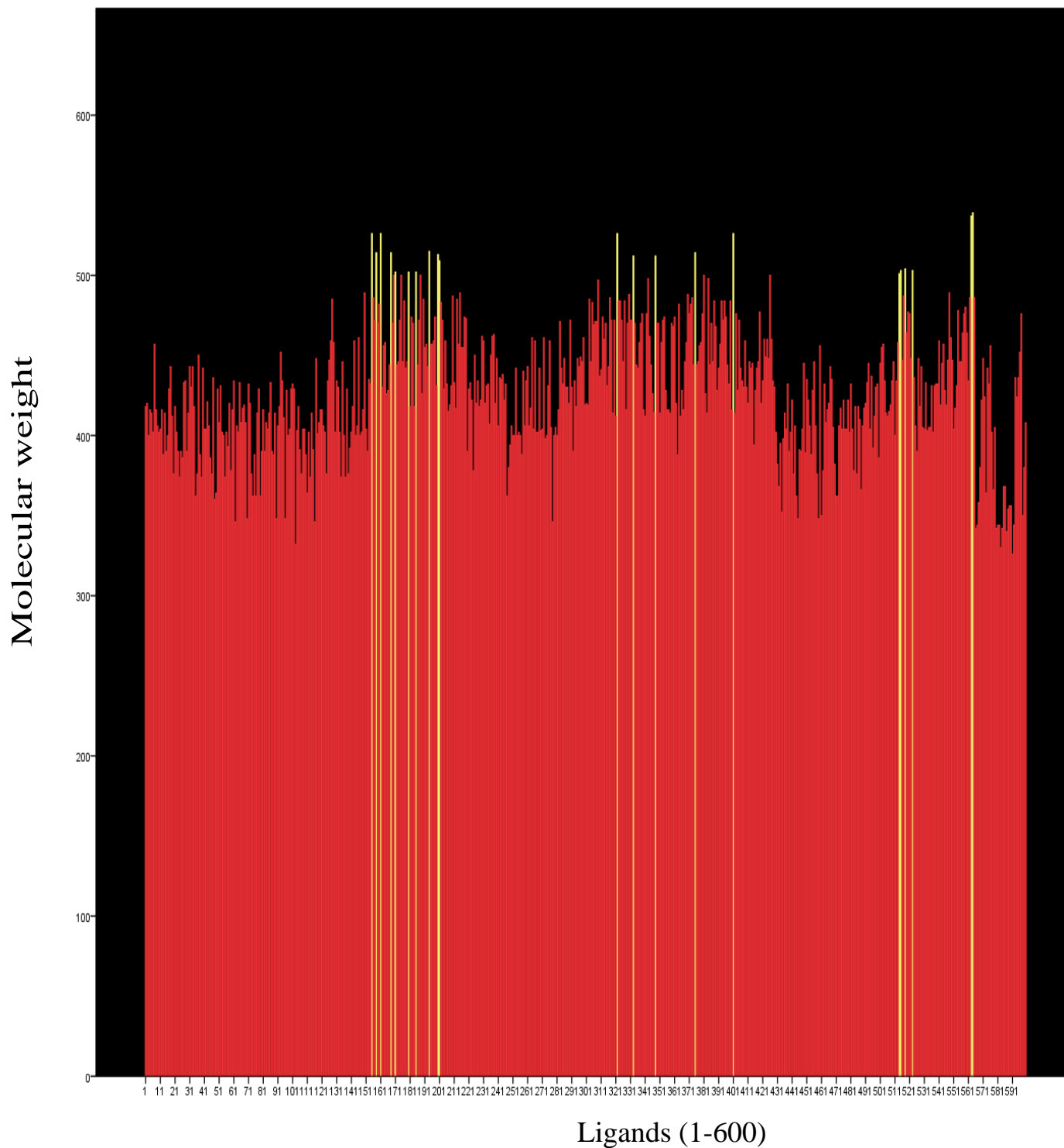
The best two ligands identified from this study may be considered as viable leads for further optimisation studies. Comparative molecular dynamics studies could shed further light on the way that these ligands interact



**Figure 5.** A graph showing the molecular weight for the 200 *de novo* designed ligands from Seed A. All ligands have a molecular weight of less than 500 and therefore are Lipinski rule of 5 compliant with respect to molecular weight only.

**Table 2.** Summary of important parameters of all the *de novo* molecules generated within LigBuilder® v2.0.

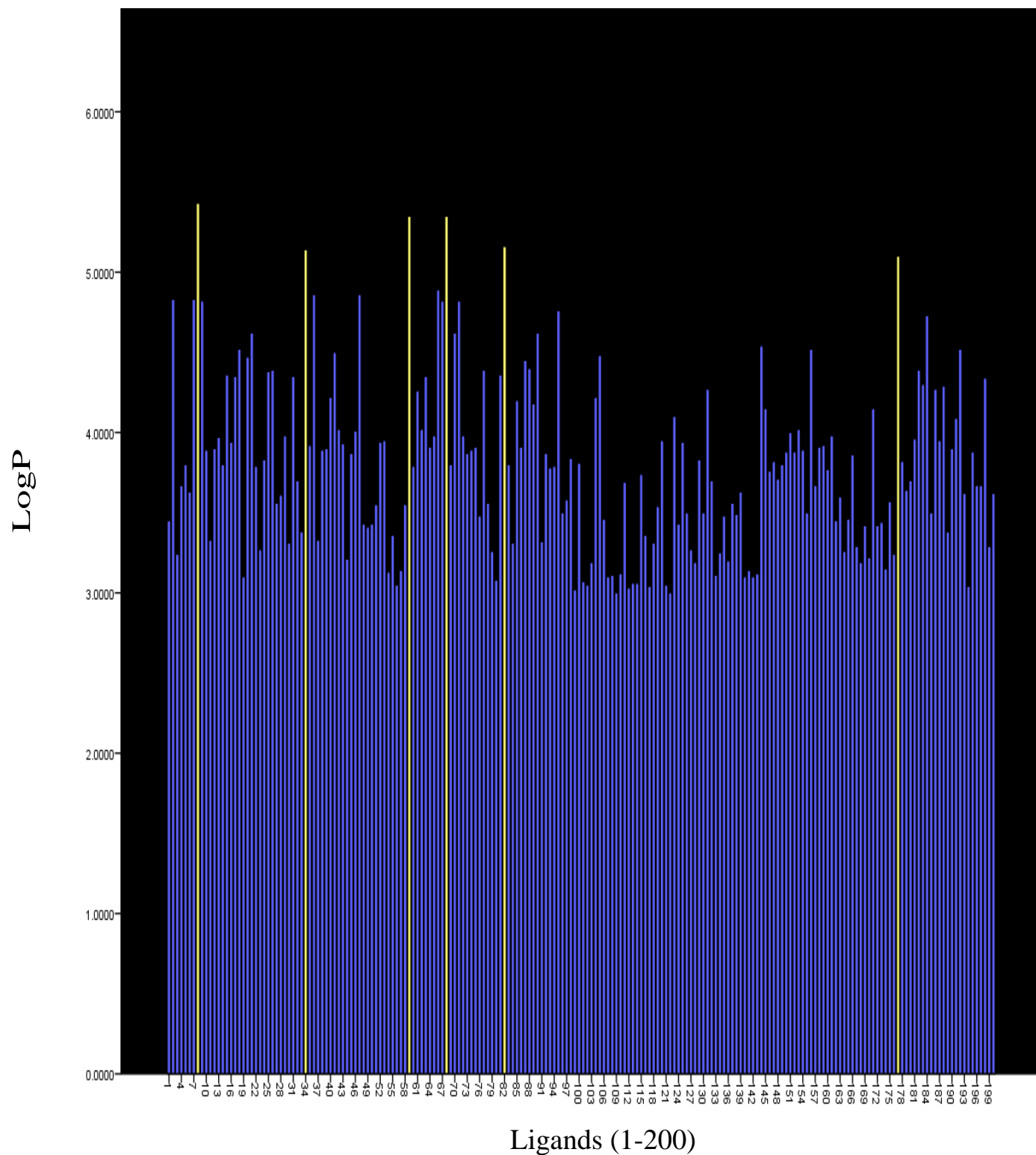
AChE_LBP	Seed A	Seed B
Total number of molecules	200	600
No. of families	17	35
Maximum number of molecules per family	51	125
Minimum number of molecules per family	1	1
Maximum pKd	8.92	10
Minimum pKd	6.30	8.47
Maximum molecular weight	438	526
Minimum molecular weight	300	326
Maximum LogP	5.43	5.99
Minimum LogP	3	3.01



**Figure 6.** A graph showing the molecular weight for the 600 *de novo* designed ligands from Seed B. The red colour indicates a molecular weight of less than 500 and therefore these ligands are Lipinski rule of 5 compliant with respect to molecular weight only. The yellow colour indicates a molecular weight exceeding 500 and thus such ligands are not Lipinski rule of 5 compliant with respect to molecular weight only.

with the AChE Ligand Binding Pocket relative to Huperzine A, with Principal Component Analysis indicating the most significant ligand driven

conformational changes which could be exploited in *in silico* attempts to identify novel entities with higher LBAs(pKd) and lower LBEs ( $\text{kcal mol}^{-1}$ ). This could lead

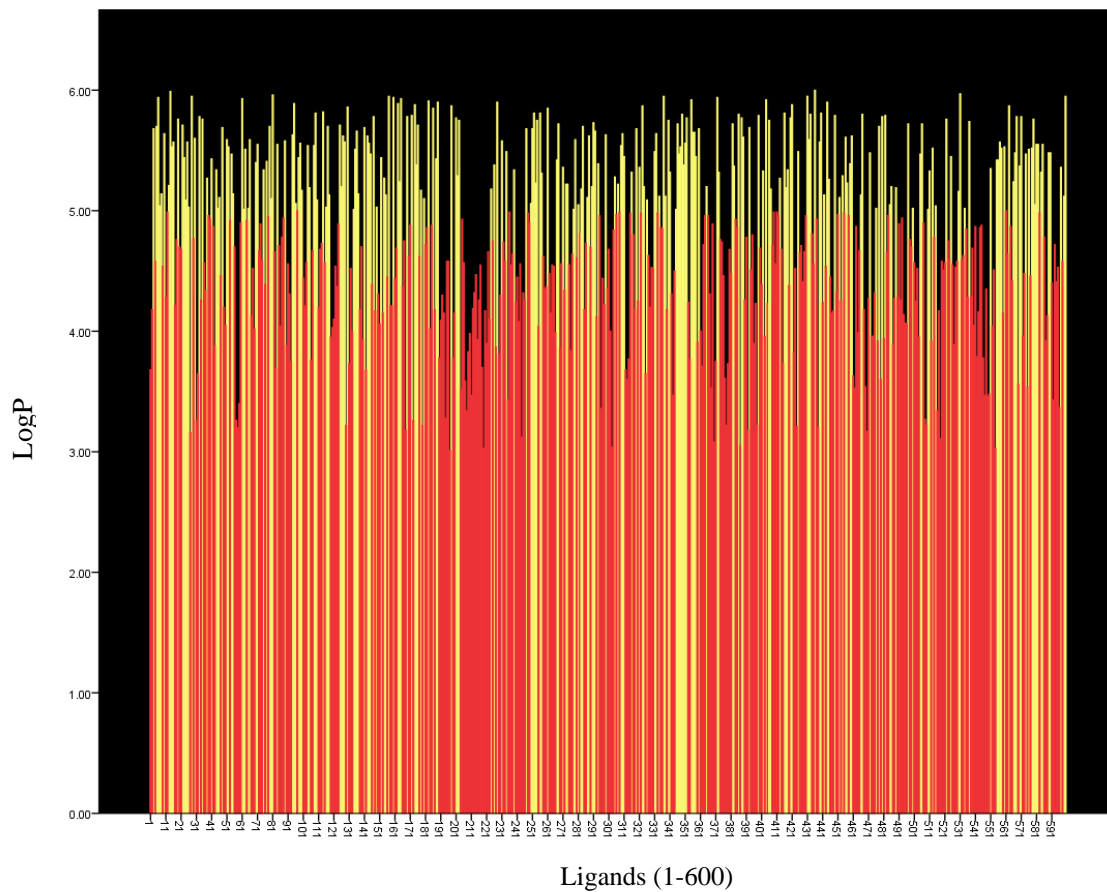


**Figure 7.** A graph showing the value of LogP for the 200 *de novo* ligands generated from Seed A. The yellow colour indicates a value of more than 5 and therefore such molecules are non-compliant with the Lipinski Rule of 5 with respect to LogP only.

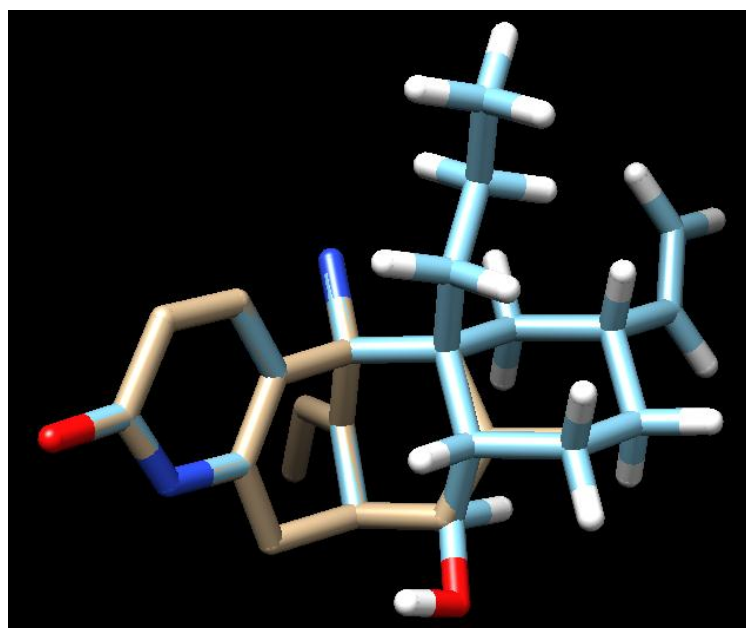
to the identification of innovative AChE inhibitors with better potency and a low side effect profile. AChE

inhibitors will continue to be developed, because this class of drugs has shown promise in symptomatic

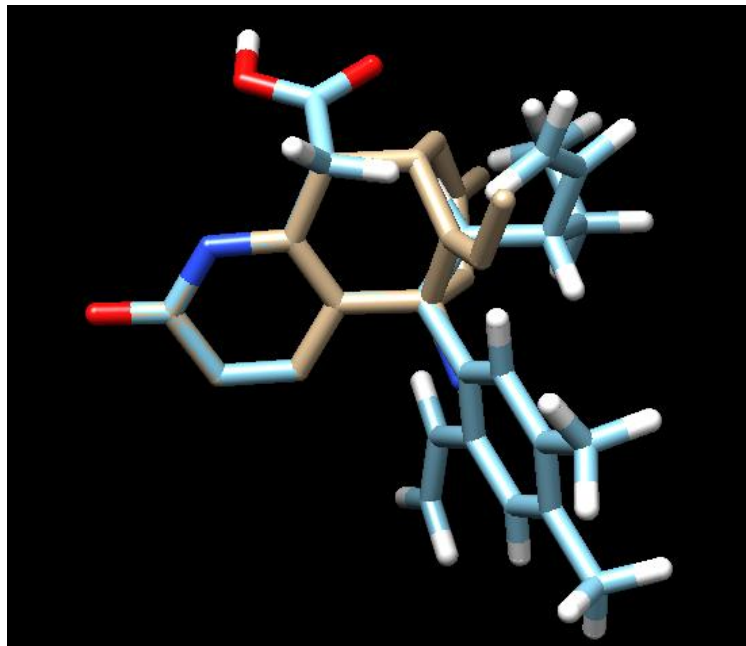




**Figure 8.** A graph showing the value of LogP for the 600 *de novo* ligands generated from Seed B. The yellow colour indicates a value of more than 5 and therefore such molecules are non-compliant with the Lipinski Rule of 5 with respect to LogP only.



**Figure 9.** Superimposition of Molecule 6 (shown in light blue and white) onto Huperzine A (shown according to molecular type).



**Figure 10.** Superimposition of Molecule 4 (shown in light blue and white) onto Huperzine A (shown according to molecular type).

therapy (Mehta et al., 2011).

### Conflict of interest

The authors have not declared any conflict of interest

### ACKNOWLEDGEMENT

"This submission is sponsored by APL Swift Services (Malta) Limited, a subsidiary of Aurobindo Pharma Limited."

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