

# Generating a Homology Model of the Human M1 Muscarinic Receptor and the Design of Cognate Modulators at this Locus for the Management of Alzheimer's Disease

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## ABSTRACT

The link between Alzheimer's disease and the M1 muscarinic receptor subtypes makes the latter a viable target for modulating the pathogenesis involved in the development of the disease. The aim of this project was to create a novel drug to modulate an *in silico*-created homology model of the M1 receptor to manage Alzheimer's disease. The preliminary part of this study involved creation of a homology model of the M1 receptor. This was followed by analysis of the ligand-binding pocket and *in silico* design of novel molecules capable of modulating this proposed structure. SYBYL-X®, X-SCORE®, LigBuilder®, Visual Molecular Dynamics (VMD), Accelrys® Draw, Accelrys® Discovery Studio 3.5 and the Protein Data Bank were used to generate the results. A homology model for the M1 receptor was created. Analysis of the ligand binding pocket resulted in 12 varying conformers; that with optimal binding affinity was chosen to create a seed. This generated 200 molecules, classified into 12 chemical families, 124 of which were retained due to conformity to Lipinski's Rules. Highest & lowest-ranked molecules in each chemical family were structurally-analysed, which yielded chemical moieties responsible for optimal chemical binding to the proposed ligand binding pocket. The *de novo* molecules created and optimized present viable leads for high-throughput screening in subsequent drug-design studies, potentially leading to identification of novel M1 muscarinic receptor subtype modulators for the use in managing Alzheimer's disease.

## Introduction:

Alzheimer's disease (AD) is the most commonly diagnosed form of dementia, affecting more than 35 million patients globally <sup>1</sup>. The regions of the brain that are linked with specialised mental functionality - especially the neopallium, isocortex and hippocampus - are the most adversely affected by the distinctive progress of Alzheimer's disease.

This includes extracellular accumulation of  $\alpha$ -amyloid (from APP, amyloid precursor protein) in ageing plaques, intracellular arrangement of a neurofibrillary mesh containing an unusually phosphorylated form of a microtubule-associated protein, tau ( $\tau$ ), as well as a reduction in number of pyramidal neurons and neuronal synapses.

Drug therapy currently available on the market is symptomatic and does not treat the underlying cause of the disease. Patients still show signs of clinical and communicative deterioration irrespective of pharmacological therapy <sup>2</sup>.

The M1 mAChR subtype has long been known to be the most prevalent subtype found within the CNS and is associated with cognition in regions of the brain such as the striatum and hippocampus <sup>3</sup>. The M1 and M3 mAChRs have been linked with one hypothesised pathology of AD <sup>4</sup>. This correlation suggests that T-Proteins are released from cells as a reaction to neuronal disintegration, resulting in a

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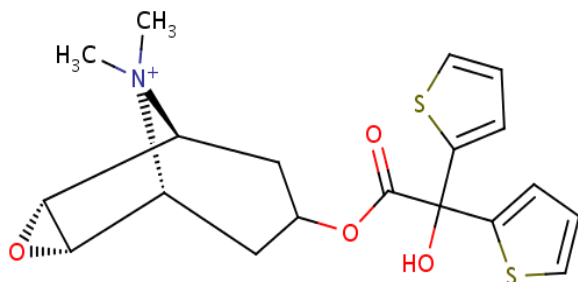
toxic effect on the surrounding cells through modulation of the M1 and M3 receptors. This prompts neuronal disintegration through a sustained increase in intracellular calcium levels<sup>5</sup>. This association followed the theory that “M1 receptors are associated with muscarinic antagonist-induced amnesia”<sup>6</sup>.

Continuation of this theory led to the proposal that an activated M1 mAChR enables bypassing of the causative amyloidogenic pathways, preventing production of A $\beta$  peptides, deterring disease progression<sup>7</sup>.

The strong relationship between the M1 receptors and cognitive deficit, neuronal inflammation and mitochondrial dysfunction was emphasised, resulting in a new locus for drug targeting in AD<sup>8</sup>. T-Phosphorylation was shown to be reduced when M1 stimulation was carried out, reducing one of the pathogeneses suggested for the disease<sup>9</sup>. Therefore, targeting the M1 muscarinic receptor is a viable therapeutic target for AD and other neurodegenerative disease<sup>10</sup>.

Research studies show that advances in the molecular pathogenesis of AD have led to new drug candidates with disease-modifying potential, which are currently within the testing phases of clinical trials<sup>11</sup>, while antineoplastic drugs administered to mice with AD symptoms show small but consistent signs of improvement<sup>12</sup>.

**Figure 1** Chemical Structure of Tiotropium Drawn in Accelrys® Draw version 4.1



Tiotropium is a long-acting anticholinergic agent indicated in the treatment of chronic obstructive pulmonary disorder (COPD)<sup>13</sup>. It is a potent muscarinic receptor antagonist through its high binding affinity and long dissociation time from M1 and M3 receptors, having an almost ten-fold increase in binding strength and potency when compared to ipratropium<sup>14</sup>.

Two significant properties of tiotropium make it a suitable candidate for a drug design study – it is kinetically-selective for the M1 mAChR and has a prolonged duration of action<sup>15</sup>. This implies that the structure-activity relationship (SAR) of the molecule is optimal to be studied and manipulated in order to achieve a novel drug with a favourable ligand binding profile.

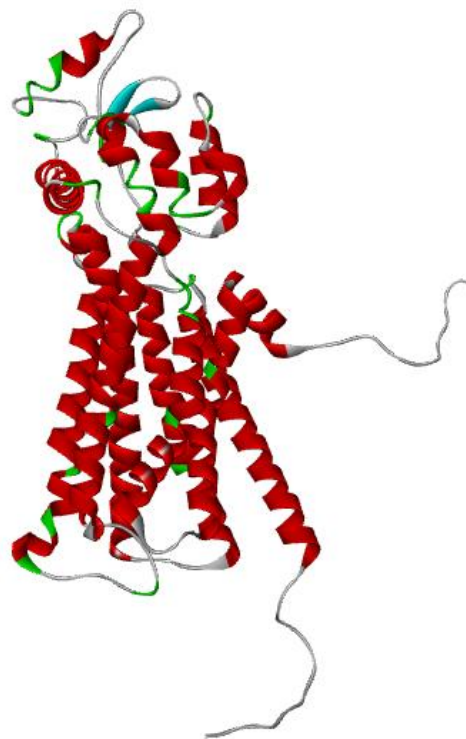
## Methodology:

### Homology Modelling :

X-ray crystallographic resolution of the human M1 mAChR has to date not been achieved. The generation of a robust homology model was considered as vital in the context of a receptor-based rational design study. Homology modelling was, in this case, carried out using UCSF Chimera,

following the premise that primary amino acid sequences result in similar three-dimensional configurations.

**Figure 2:** Homology Model of the M1 mAChR



The isoform sequences of the M1, M2 and M3 mAChR subtypes<sup>16</sup> were aligned in order to obtain a visual representation of the similarity proportions that existed within the receptor subtypes. The M3 receptor sequence was isolated as a suitable template for a homology model of the M1 receptor to be generated *in silico*. This was done through analysis of similar structures available on the Protein Data Bank, which revealed that the M3 receptor had the highest degree of amino acid homology.

### Identification of Ligand Binding Contacts:

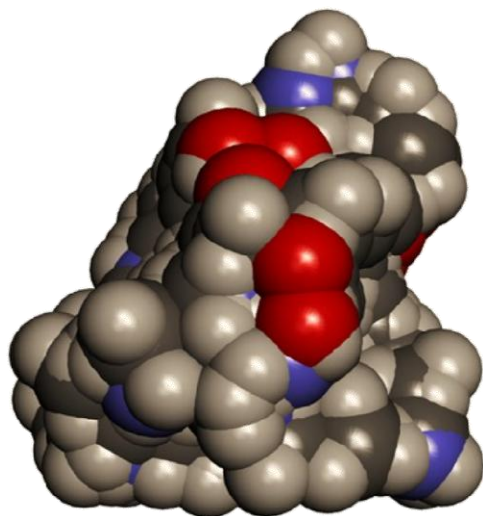
The amino acids within the ligand binding pocket (LBP) of the template generated were identified using UCSF Chimera. The same procedure was carried out for the template M3mAChR and the results compared. Differences within the ligand binding contacts of the two implied that the homology modelling process yielded a different structure than the original. Altered points within the LBP are loci which should be targeted in the context of a drug design study.

### Ligand Docking :

In order to dock tiotropium into the target M1 receptor LBP, bound conformations of the ligand were extracted from the M3 mAChR using SYBYL-X<sup>®</sup>. A Surflex-Dock preparation was carried out to generate multiple conformers of tiotropium which identified 18 different three-dimensional conformations that the ligand was allowed to adopt within the fixed target LBP.

A protomol was generated from this data, (Ref. Figure 3) which represented the target receptor's binding cavity to which future ligands would be aligned. Regions indicating hydrophobic and hydrophilic moieties in the LBP were used to manipulate the molecule *in silico* so as to further complement the binding process and lower the subsequent kinetic energy of the receptor-ligand complex.

**Figure 3:** Protomol of Tiotropium Conformations within M1 Muscarinic Receptor Binding Site

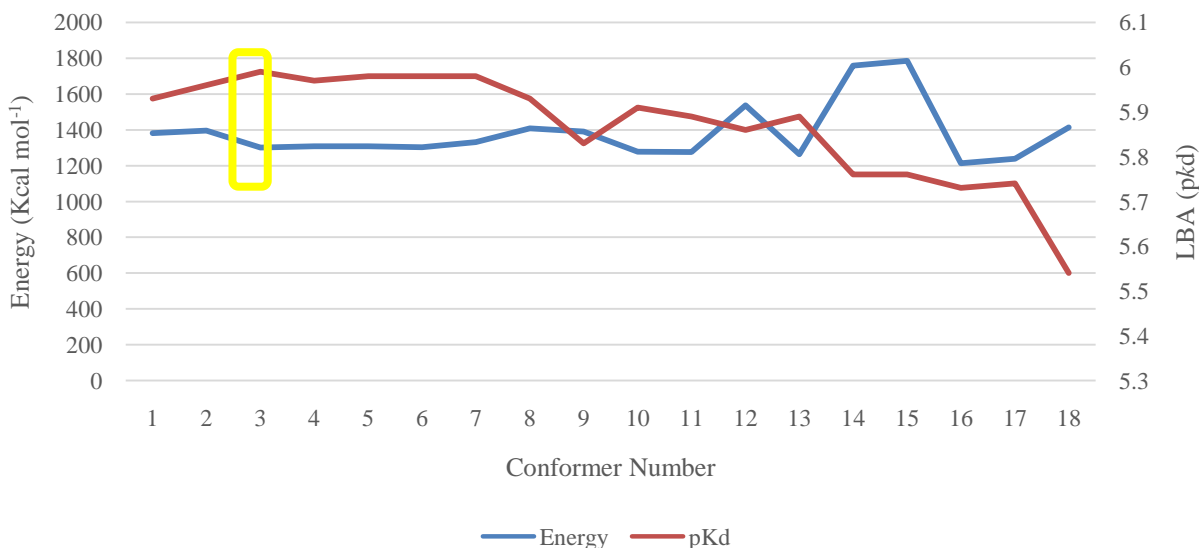


Energy ( $\text{Kcal mol}^{-1}$ ) and ligand-binding affinity (LBA; pKd) of each of the conformational isomers of tiotropium were plotted against conformer number. The conformer with the highest LBA value and the lowest energy values was chosen to be the most likely to yield a drug with the desirable characteristics. Drug 3 [ref. Graph 1] was selected, having a LBA pKd of 5.99 and an energy value of  $1300.3 \text{ kcal mol}^{-1}$ .

**Table 1:** LBA (pKd) of each tiotropium conformer to the M1 model generated in silico; arranged in order of descending LBA.

Tiotropium Conformer	LBA (pKd)
Tiotropium in M3	6.46
Tiotropium in M1	6.14
3	5.99
5	5.98
6	5.98
7	5.98
4	5.97
2	5.96
1	5.93
8	5.93
10	5.91
11	5.89
13	5.89
12	5.86
9	5.83
14	5.76
15	5.76
17	5.74
16	5.73
18	5.54

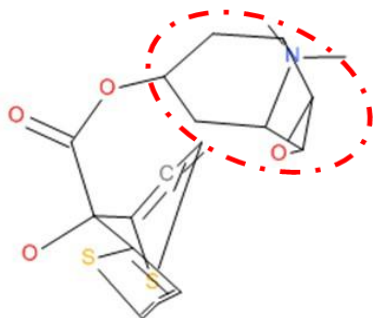
**Graph 1:** Energy & LBA vs Conformer No. of Tiotropium



## Seed Creation

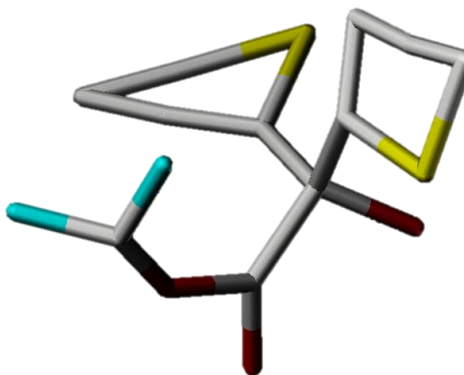
Structure-activity relationship (SAR) analysis of tiotropium identified *loci* within its structure considered essential to activity<sup>17</sup>. The “non-essential” parts of the molecule were considered redundant and replaceable by other efficient, high-affinity moieties.

**Figure 4** : Conformer 3 of Tiotropium



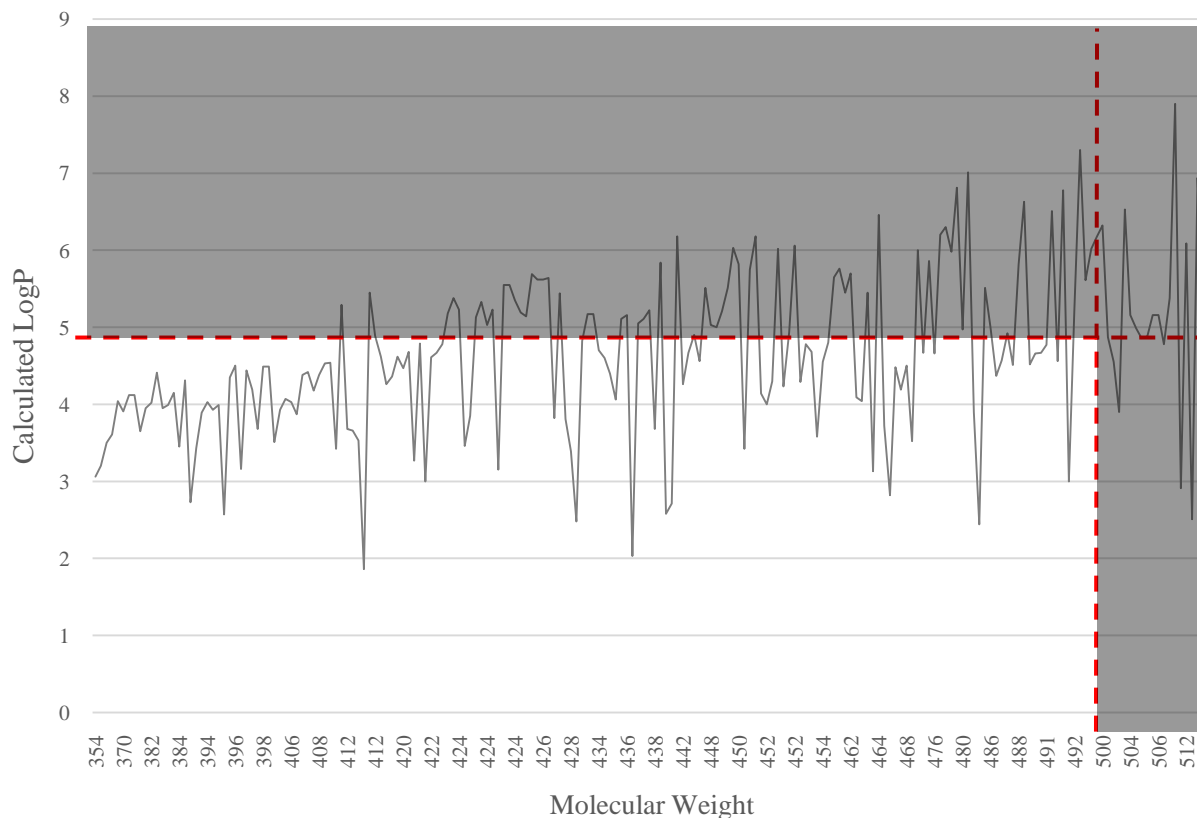
A seed was created using SYBYL-X® maintaining the essential regions of the tiotropium and choosing structural features of the molecule which could be modified to create seeds. Specifically, the high-torsion ring circled in Figure 4 was earmarked for elimination and substitution with lower-energy moieties in an attempt not only to increase affinity, but also molecular stability.

**Figure 5**: Generated Seed



Molecular editing was carried out in SYBYL-X®. The editing process included the computational removal of the redundant, not crucial to binding moieties from the optimal tiotropium conformation and the assignment of H.*spc* hydrogens at *loci* which were consequently pre-designated as growing sites.

**Graph 2: Calculated LogP vs Molecular Weight**



## Novel Analogue Series Creation:

Novel molecular growth was carried out using LigBuilder v1.2. Through its Pocket module, the LBP of the M1 mAChR model was mapped, and a general pharmacophore proposed. The grow module of LigBuilder used this data in order to delineate the pharmacophoric space available for *in silico de novo* molecular growth. Finally, implementation of the process module resulted in generated data organisation. This meant that the molecular cohort generated by the grow module was organised into a molecular database that was segregated into structurally similar families, and ranked according to LBA (pKd). Other physicochemical data including LogP and molecular weight were included in the database.

## Analysis and Optimisation:

The generated molecular cohort was assessed for Lipinski rule compliance on the basis that this is the recognised gold standard for predicting oral bioavailability<sup>18</sup>. All 76 non-Lipinski rule compliant molecules were eliminated from the designed cohort [Ref. Graph 2].

Each molecule was structurally analysed in comparison with the rest of the members of the chemical family in order to understand the difference in binding affinities found. Molecules from each chemical family having the highest LBA were compared to those having lower LBA values, with a structure-activity relationship target in mind. Structural features in molecules with higher binding affinities which were not present in those with lower affinities imply a significance in the binding process to the ligand-binding site.

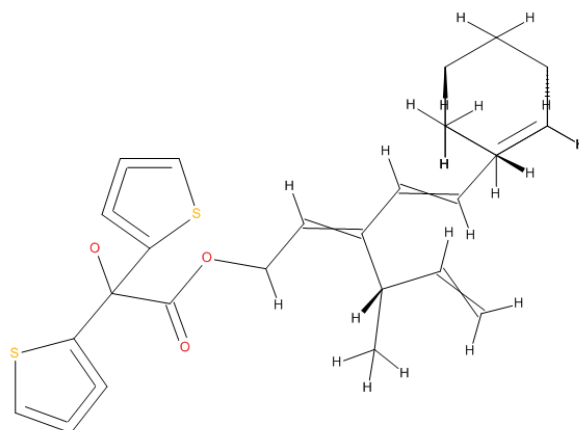
**Table 2:** LBA (pKd) of highest-ranking molecule of each chemical family generated in silico.

Chemical Family	Molecule Number	LBA (pKd)
1	3	6.9
2	21	6.17
3	73	6.66
4	84	6.81
5	127	7.07
6	140	6.53
7	152	7.51
8	162	6.44
10	182	6.41
11	189	6.83
12	18	6.05

Analysis of the molecule with the highest binding affinity with the highest-ranking molecule from the chemical family which yielded the greatest number of Lipinski rule-compliant molecules indicated structural moieties which may be exploited in order to formulate a structure with optimal binding properties. This molecule incorporated the structural backbone desirable which

yielded the greatest number of compliant molecules, with side-chain moieties necessary for high binding affinity.

**Figure 6:** Proposed Molecule



## Discussion:

This study is valuable in creating a homology model of the human M1 muscarinic receptor, delineating a proposed 3D ligand binding pocket within which novel molecular growth could be sustained. The tiotropium scaffold proved viable in generating novel *in silico* high-affinity structures capable of modulating the M1 receptor. Bioassays are recommended as a subsequent step to further validate the hypotheses made throughout this study.

## Conclusion:

*De novo* molecules created and optimized present viable leads for high-throughput screening in subsequent drug-design studies, leading to identification of novel M1 muscarinic receptor subtype modulators for the use in managing Alzheimer's disease.

## Reference:

1. Batsch NL, Mittelman MS (2012). World Alzheimer Report 2012: Overcoming the Stigma of Dementia. London: Alzheimer's Disease International.
2. Azzopardi LM, Editor (2010). Lecture Notes in Pharmacy Practice. United Kingdom: Pharmaceutical Press.
3. Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J Neurosci.* 11(10):3218–3226.
4. Avila J (2011). Muscarinic Receptors and Alzheimer's Disease. Future Medicine Ltd. Neurodegenerative Disease Management. 1(4):267-269.
5. Mieltska-Porowska A, Wasik U, Goras M, Filipek A & Niewiadomska G (2014). Tau Protein Modifications and Interactions: Their Role in Function and Dysfunction. *International Journal of Molecular Sciences.* 15:4671–4713.
6. Kihara T, Shimohama S (2004). Alzheimer's Disease and Acetylcholine Receptors. *Acta Neurobiologiae Experimentalis.* 64:99-105.
7. Shirey, J.K. et al. (2009) A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. *J. Neurosci.* 29:14271–14286

8. Fisher A, Medeiros R, Barner N, Natan N, Brandeis R, Elkon H, Nahum V, Grigoryan G, Segal M, LaFerla F (2014). M1 Muscarinic Agonists and a Multipotent Activator of Sigma1/M1 Muscarinic Receptors: Future Therapeutics of Alzheimer's Disease. *Alzheimer's Disease & Dementia*. 10(4):123.
9. Tarr, J.C. et al. (2012) Targeting selective activation of M1 for the treatment of Alzheimer's disease: further chemical optimization and pharmacological characterization of the M1 positive allosteric modulator ML169. *ACS Chem. Neurosci.* 3, 884–895
10. Jiang S, Li Y, Zhang C, Zhao Y, Bu G, Xu H *et al* (2014). M1 Muscarinic Acetylcholine Receptor in Alzheimer's Disease. *Neuroscience Bulletin*. 30(2):295-307.
11. Ballard C, Gauthier S, Corbett A, Brayne C, Arsaland D, Jones E (2011). Alzheimer's Disease. *The Lancet*. 377(9770):1019-31.
12. Sanders L (2012). Cancer Drug Shows Promise as Treatment for Alzheimer's. *Science News*.
13. Barnes PJ, Belvisi MG, Mak JCW, Haddad EB, O'Connor B (1995). Tiotropium Bromide, a Novel Long Acting Muscarinic Antagonist for the Treatment of Obstructive Airways Disease. *Life Sciences Journal*. 56:853–859.
14. Disse B, Speck GA, Rominger KL, *et al* (1999). Tiotropium (Spiriva): Mechanistical considerations and clinical profile in obstructive lung disease. *Life Sciences*. 64:457–464.
15. Heredia J (2009). Tiotropium Bromide: An Update. *The Open Respiratory Medicine Journal*. 3:43-52.
16. Higgins D, Thompson J, Gibson T, Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research: Oxford Journals*. 22:4673-4680.
17. Kolluru S (2012). Cholinergic Antagonists. *Health Science Center, Irma Lerma Rangel College of Pharmacy*.
18. Lipinski CA (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies* 1.4:337–341.