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In silico studies towards new BACE1 inhibitors

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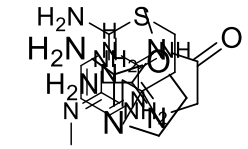
In silico studies towards new BACE1 inhibitors

Graphical Abstract

Design
Molecular hybridization



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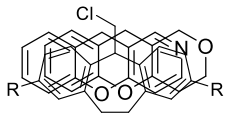
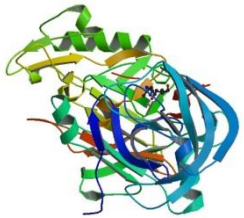
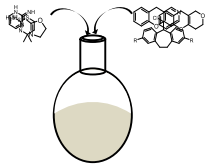


In silico studies
BACE1 catalytic pocket



- Binding energy
- Ligand efficiency
- Molecular visualization
- Drug likeness

Synthesis
Novel BACE1 inhibitors



- Design of suitable synthetic pathways
- Application of green chemistry principles

Abstract:

Beta-site APP-cleaving enzyme (BACE)1 is a type-1 membrane-anchored aspartyl protease playing an essential role in the release of Aβ peptides and Alzheimer's Disease (AD) progression. Hence, the development of potent BACE1 inhibitors represents a logical approach for AD therapy development and it have been widely explored by the pharmaceutical industry worldwide. Herein, we report the design of a virtual library of 300 compounds for *in silico* BACE1 inhibition assessment. These compounds were designed based on the hybridization of several hydrophobic fragments with aliphatic and aromatic amines, motifs identified in the literature by their ability to establish essential interactions with the amino acids present in the catalytic pocket of BACE1. Affinity for BACE1 was measure through the binding energy estimation of the ligand-protein complex. Additionally, the compounds designed were assessed through the Lipinski's rule of 5 and additional attributes crucial for central nervous system (CNS) drugs were also considered. The most promising compounds will be synthesized through suitable and green N-alkylation techniques and their biological activity will be assessed in *in vitro* studies.

Keywords: BACE1; ALZHEIMER'S DISEASE; IN SILICO STUDIES

Introduction

Figure from: https://saudeonline.pt/2017/03/03/descoberto-alvo-terapeutico-promissor-contra-progressao-de-doenca-de-alzheimer/

ALZHEIMER'S DISEASE (AD) – EPIDEMIOLOGY

- AD recognized as a global public health priority (WHO)
- Most prevalent cause of dementia -> dependence – disability – mortality
- Prevalence increasing up to 115.4 million cases in 2050
- Dramatic impact of AD in the health care systems

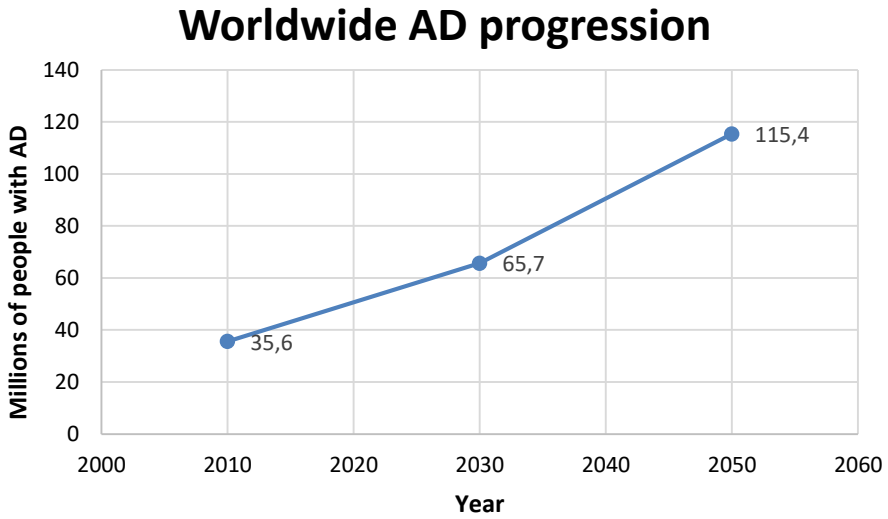


Figure 1. Worldwide AD progression. Adapted from [2]

1. Maia MA, Sousa E. BACE-1 and γ -Secretase as Therapeutic Targets for Alzheimer ' s Disease. Pharmaceuticals. 2019;12(41). doi:10.3390/ph12010041
2. Lane CA, Hardy J, Schott JM. Alzheimer's disease. *Eur J Neurol*. 2018;25(1):59-70. doi:10.1111/ene.13439

Introduction

ALZHEIMER'S DISEASE (AD) – PATHOLOGY

- Main characteristics

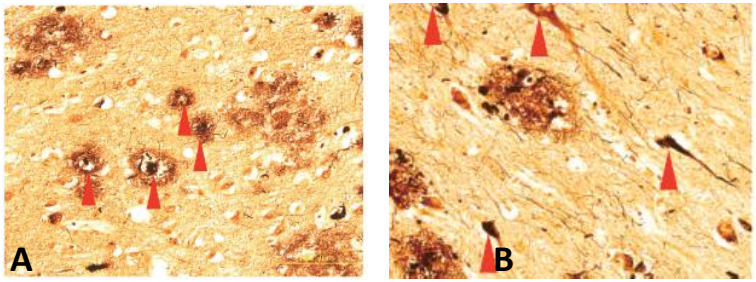


Figure 2. (**A**) Amyloid plaques and (**B**) Neurofibrillary tangles. Adapted from [4]

Amyloid plaques

- Neuropil threads
- Astrogliosis

Neurofibrillary tangles
(aggregates of hyperphosphorylated tau protein)

- Dystrophic neurites
- Microglial activation

Synaptic and neuronal loss (Neurodegeneration) ⇨ **Dementia**

- Brain changes with AD may begin 20 or more years before symptoms appear

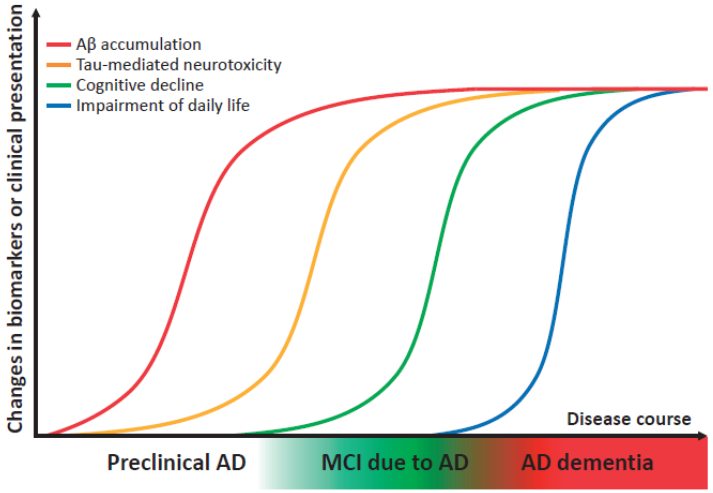


Figure 3. Hypothetical model of biomarker changes in AD. MCI: mild cognitive impairment. Adapted from [4].

1. Maia MA, Sousa E. BACE-1 and γ -Secretase as Therapeutic Targets for Alzheimer ' s Disease. Pharmaceuticals. 2019;12(41). doi:10.3390/ph12010041
4. Suzuki K, IWata A, IWatsubo T. The past , present , and future of disease-modifying therapies for Alzheimer 's disease. *Proc Japan Acad Ser B*. 2017;93(10):757-771.

Introduction

THE AMYLOID HYPOTHESIS OF AD

- Currently the prevalent theory of AD pathogenesis
- Accumulation of pathological forms of amyloid β (Aβ) as primary pathological process in AD

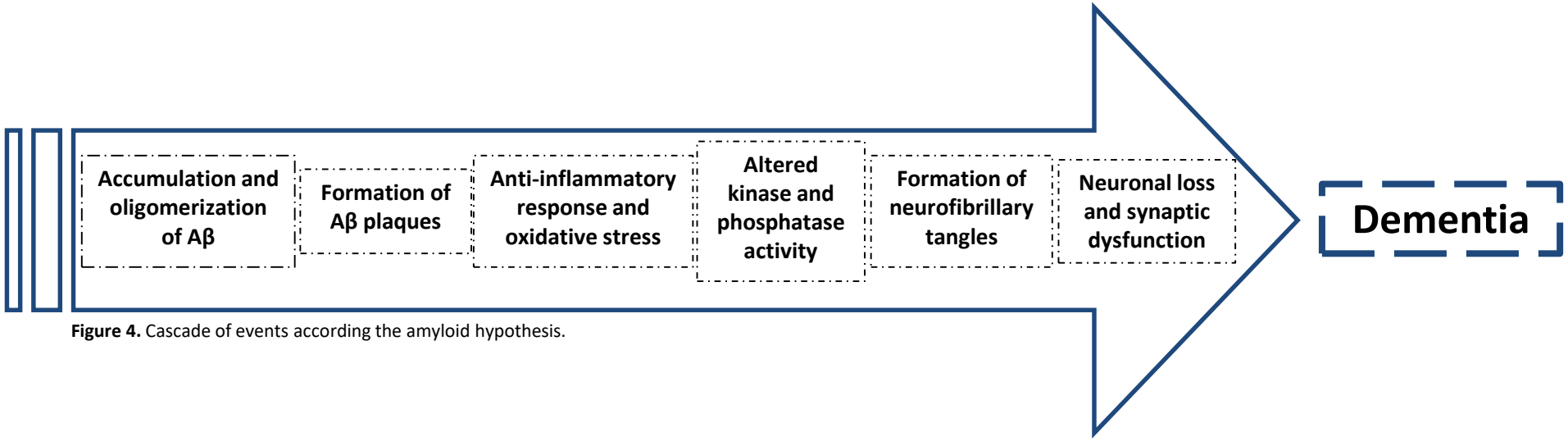


Figure 4. Cascade of events according the amyloid hypothesis.

1. Maia MA, Sousa E. BACE-1 and γ -Secretase as Therapeutic Targets for Alzheimer ' s Disease. Pharmaceuticals. 2019;12(41). doi:10.3390/ph12010041.

Introduction

BACE1 (beta-site APP-cleaving enzyme) is a type-1 membrane-anchored aspartyl protease responsible for the first step of the proteolysis of the amyloid precursor protein (APP)

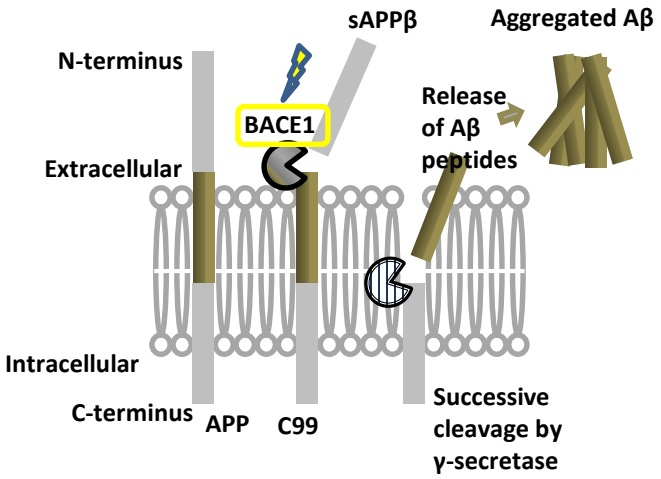


Figure 5. Scheme of the production of Aβ by the two step sequential cleavage of APP by BACE-1 and γ-secretase. Adapted from [4].

- BACE1 cleavages APP in the luminal surface of the plasma membrane and releases the soluble ectodomain of APP, leaving C99 (Aβ plus APP intracellular domain (AICD)) in the membrane to be subsequently cleavage by gamma-secretase (GS);
- APP mutations that increase the efficiency of β-cleavage and result in overproduction of Aβ peptides strongly influence the risk of AD;
- Mutation in APP gene (A673T) which results in a lifelong decrease in APP cleavage by BACE1 confers reduced clinical risk of AD

Inhibition of APP proteolysis by BACE1 as a rational strategy for clinical intervention

1. Maia MA, Sousa E. BACE-1 and γ -Secretase as Therapeutic Targets for Alzheimer ' s Disease. Pharmaceuticals. 2019;12(41). doi:10.3390/ph12010041

Introduction

112 AGENTS IN AD PIPELINE (2018)

29 Aβ related

13 immunotherapeutic agents

16 small molecules

5 BACE1 inhibitors (≈30%)

Table 1. Small molecules in the AD pipeline (2018) [5].

Phase Clinical Trial(s)	Agent	Mechanism of Action	Sponsor
I	NGP 555	GSM	NeuroGenetic Pharmaceuticals
II	ID1201	Phosphatidylinositol 3-kinase/ Akt pathway activation	II Dong Pharmaceutical Co
II	Nilotinib	Tyrosine kinase inhibitor	Georgetown University
III	CNP520	(γ-secretase modulator)	Alzheimer's Association
III	ALZT-OP1a (cromolyn)+ ALZT-OP1b (ibuprofen)	**BACE1 inhibitor**	AZTherapies
III	Sodium Oligo-mannurarate (GV-971)	Increases amyloid clearance	Shanghai Green Valley
III	TTP488 (Azeliragon)	RAGE antagonist	vTv Therapeutics
II, III	JNJ-54861911	**BACE1 inhibitor**	Janssen
II,III	E2609 (Elenbecestat)	**BACE1 inhibitor**	Eisai, Biogen
II	LY3202626	**BACE1 inhibitor**	Eli Lilly
II	Atomoxetine	Adrenergic uptake inhibitor, SNRI	Emory University, NIA
II	AZD0530 (Saracatinib)	Kinase inhibitor	Yale University, ATRI,
II	CT1812	Sigma-2 receptor competitive inhibitor2)	Cognition Therapeutics
II	Posiphen	Selective inhibitor of APP production	QR Pharma, ADCS
II	Valacyclovir	Antiviral agent 4)	Umea University
III	AZD3293 (LY3314814)	**BACE1 inhibitor**	AstraZeneca, Eli Lilly

5. Cummings, J.; Lee, G.; Ritter, A.; Zhong, K. Alzheimer 's disease drug development pipeline: 2018. *Alzheimer's Dement. Transl. Res. Clin. Interv.* **2018**, *4*, 195–214.

Introduction

LIGAND INTERACTIONS WITH BACE1 CATALYTIC POCKET

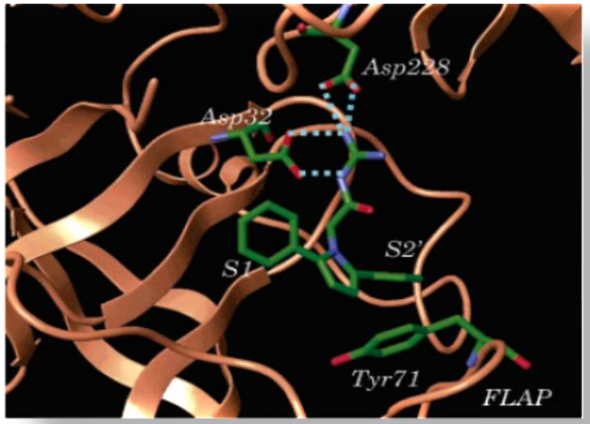
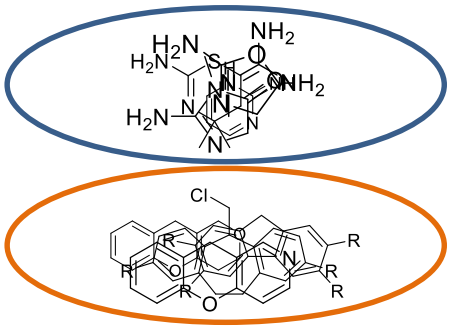


Figure 6. Crystal structure of BACE1 complexed with an acylguanidine-based inhibitor.Adapted from [6]

Hydrogen bond interaction with the catalytic aspartic acids residues Asp32 and Asp228



Hydrophobic interactions with Tyr71

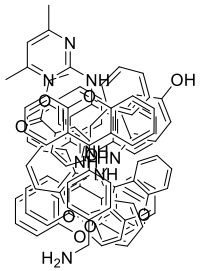
Drug design based on
Molecular hybridization

1. Maia MA, Sousa E. BACE-1 and γ -Secretase as Therapeutic Targets for Alzheimer ' s Disease. Pharmaceuticals. 2019;12(41). doi:10.3390/ph12010041
6. Cole DC, Manas ES, Stock JR, et al. Acylguanidines as small-molecule β-secretase inhibitors. J Med Chem. 2006;49(21):6158-6161. doi:10.1021/jm0607451

Results and discussion

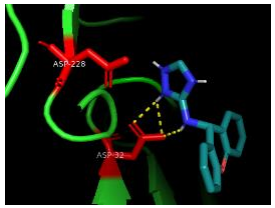
In silico studies

- Design of a virtual library of ≈300 molecular hybrids



- Docking against human BACE1

- Molecular visualization



Results and discussion

In silico studies

Design of a virtual library of ≈ 300 molecular hybrids

- Calculation of drug likeness properties

Lipinski's rule of five

- ✓ Molecular weight < 500
- ✓ logP < 5
- ✓ < 5 H-bond donors (sum of NH and OH)
- ✓ < 10 H-bond acceptors (sum of N and O)

Central Nervous System (CNS) drug rules [7]

- ✓ Molecular weight < 450
- ✓ logP < 5
- ✓ < 3 H-bond donors
- ✓ < 7 H-bond acceptors (sum of N and O)
- ✓ <8 rotatable bonds
- ✓ TPSA < 70 $Å^2$

7. Pajouhesh H, Lenz GR. Medicinal Chemical Properties of Successful Central Nervous System Drugs. *Neurotherapeutics*. 2005;2:541-553. doi:https://doi.org/10.1602/neurorx.2.4.541

Results and discussion

In silico studies

Docking against BACE1

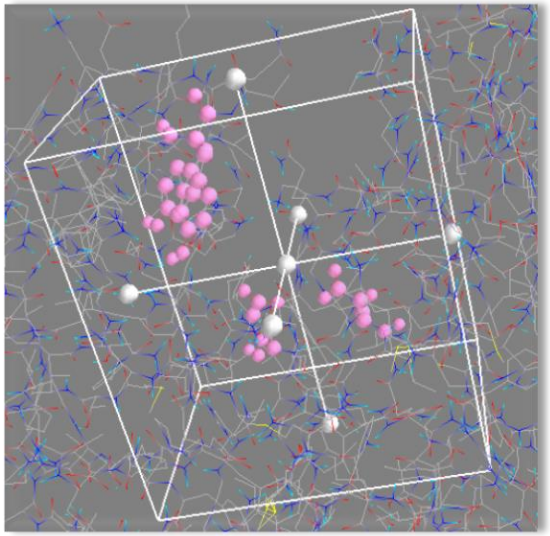


Figure 7. 3D scence visualization using PyRx, Ver 0.8.

- ❑ **Calculation of Binding Energy (kcal/mol)**
- ❑ **Calculation of Ligand Efficiency (Binding Energy/N^4)**

- BACE1 crystal structure available in Protein Data Bank (**PDB: 4RCF**)
- Docking performed considering the interactions with residues Asp32, Asp228 and Tyr71 from BACE1 catalytic pocket

4. N: Number of non-hydrogen atoms
Software tools: Chemdraw Professional ®, Ver. 16.0; Arguslab, Ver. 4.0.1; PyRx, Ver 0.8; Pymol, Ver. 2.3.0.

Results and discussion

In silico studies

Docking against BACE1

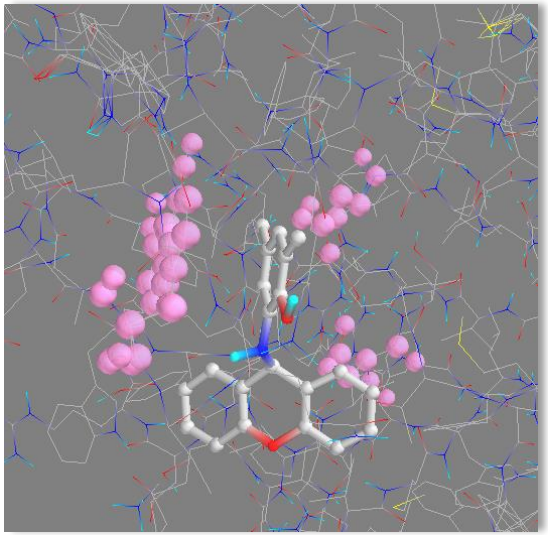


Figure 2. Predicting interaction between BACE1 and a ligand using PyRx, Ver 0.8.

Table 1. Binding energy estimation of the ligand-BACE1 complex and ligand efficiency values.

Ligand	Binding energy (kcal/mol)	Ligand efficiency (bind. energy/ N)
255	-10.2	-0.49
139	-9.1	-0.46
1	-9.1	-0.43
37	-8.4	-0.47
50	-8.7	-0.44

Software tools: Chemdraw Professional ®, Ver. 16.0; Arguslab, Ver. 4.0.1; PyRx, Ver 0.8; Pymol, Ver. 2.3.0.

Results and discussion

In silico studies

Molecular visualization

- Visualization of the ligand-receptor binding
- Interactions between BACE1 residues and ligands

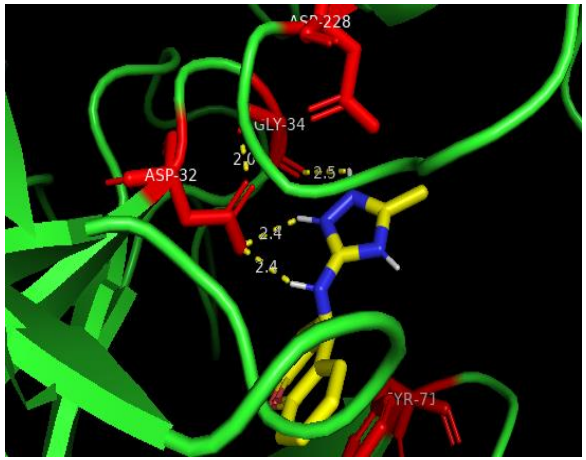


Figure 2. Predicted interaction between ligand 255 and BACE1 catalytic pocket. 3D image acquired using the visualization tool PyMol, Ver. 2.3.0.

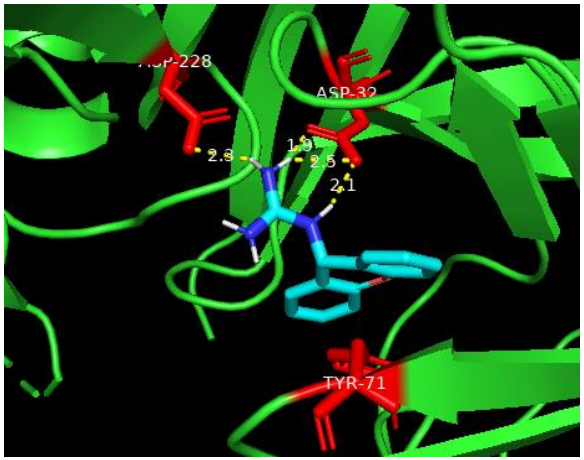


Figure 2. Predicted interaction between ligand 37 and BACE1 catalytic pocket. 3D image acquired using the visualization tool PyMol, Ver. 2.3.0.

Software tools: Chemdraw Professional ®, Ver. 16.0; Arguslab, Ver. 4.0.1; PyRx, Ver 0.8; Pymol, Ver. 2.3.0.

Results and discussion

Good scores for Binding Energy and Ligand Efficiency

Docking against BACE1

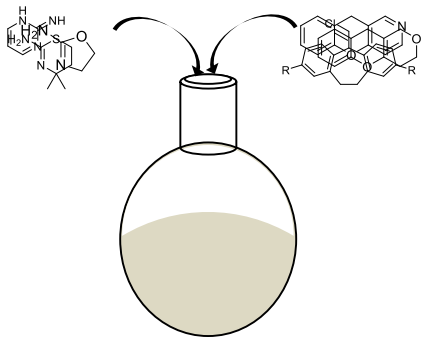
Drug likeness evaluation

Compliance with rules for CNS drugs

Assessment of suitable synthetic pathways

Synthesis

Ongoing work



Conclusions

- *In silico* results revealed promising results for BACE1 inhibition activity for the designed ligands;
- Preliminary results for the *N*-alkylation of the tricyclic moieties showed that a suitable synthetic pathway was found to synthesize the desire ligands;
- Future work will focus on *in vitro* studies and further structural modifications for hit to lead optimization.

Acknowledgments

This work was developed under the Strategic Funding UID/Multi/04423/2019 and Project No. POCI-01-0145-FEDER-028736, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds. Miguel Maia acknowledges his FCT grant (SFRH/BD/146211/2019).

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