ApoE4-Targeted Small Molecules as Potential Therapies for Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory loss. The presence of the apolipoprotein E4 (ApoE4) allele is a significant genetic risk factor for AD, with ApoE4 influencing amyloid-beta aggregation, tau pathology, and neuroinflammation. This research focuses on finding suitable small molecules that inhibit ApoE4, preventing the protein from influence. In the beginning, one significant binding site on ApoE4 was identified. Pharmacophore models produced through ApoE4 x Leukocyte immunoglobulin-like receptor subfamily A member 6 (PocketQuery ID: 4MED9) interactions were then used to virtually screen for possible small molecule ApoE4 inhibitors. Furthermore, the molecules were then molecularly docked using SwissDock, which calculated the energy of each interaction. The top 5 candidates (ZINC09060047, ZINC40759037, ZINC09060047, ZINC09421183, ZINC40759203) were then screened with SwissADME to determine drug likeness, followed by a toxicity test via Protox. One small molecule was successfully identified and confirmed to inhibit ApoE4 in these tests. This result provides significant data that can be useful for future in vivo testing, where the effectiveness of the small molecule will be determined.

Keywords: Alzheimer's disease, neuroinflammation, Apolipoprotein E4, SwissDock, small molecule

Introduction

Alzheimer's Disease

Alzheimer's disease (AD) is the most common type of dementia (accounting for up to 80% of all diagnosis) and is classified as a slowly progressive neurodegenerative disease characterized by neuritic plaques and neurofibrillary tangles caused by the build-up of amyloid-beta peptides in certain regions of the brain. The neurotoxic potential of the amyloid-beta peptides is due to its favorability to aggregate into insoluble oligomers and protofibrils. These processes, along with diminishing clearance of amyloid-beta from the brain, leads to the accumulation of amyloid-betas extracellularly, aka senile plaques, leading to the activation of neurotoxic cascades that eventually end up causing neuronal dysfunction and cellular death [1].

At present, there are around 50 million AD patients worldwide and this number is projected to double every 5 years and will increase to reach 152 million by 2050. The burden of AD affects individuals, their families, and the economy, with estimated global costs of US $1 trillion annually. Currently, there is no direct cure for Alzheimer's disease, although there are available treatments that improve symptoms [2,3].

Amyloid Hypothesis

For decades, it was recognized that abnormal deposition of β-sheets in the central nervous system was strongly related with dementia, which led to the concept of the amyloid hypothesis. However, it was found that the amyloid plaques (AP) also deposit in normal healthy brains with aging, which raised the question of whether AP deposition was a defining factor in AD onset. Recently, alternative hypotheses were proposed for the non-inherited form of AD (NIAD), but at present, the amyloid hypothesis remains the most accepted pathological mechanism for inherited AD (IAD). The amyloid hypothesis suggests that the degradation of Aβ, derived from Aβ precursor protein (APP) by β- and γ-secretase, is decreased by age or pathological conditions, which leads to the accumulation of Aβ peptides (Aβ40 and Aβ42). Increasing the ratio of Aβ42/Aβ40 induces Aβ amyloid fibril formation, resulting in neurotoxicity and tau pathology induction, eventually leading to neuronal cell death and neurodegeneration. AD risk factors and mutations of several genes like APP, PSEN1, and PSEN2 were found to affect Aβ catabolism and anabolism, which rapidly cause an accumulation of Aβ and fast progression of neurodegeneration [4–6].

Small molecule Therapeutics for Alzheimer's disease

Due to the death of cholinergic neurons, AD is associated with cholinergic deficiency. By inhibiting acetylcholinesterase, these

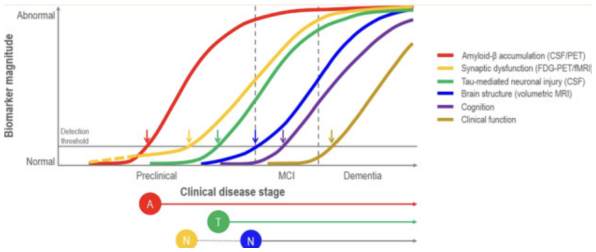


Fig. 1 Amyloid-B Hypothesis. Graph of Amyloid B (red) accumulation and the effects, such as Tau-mediated neuronal injury (green) and abnormal synaptic dysfunction (yellow)[7].

drugs restore acetylcholine levels, with the consequent symptomatic amelioration. There are three orally administered drugs in this group, namely rivastigmine, galantamine and donepezil. While donepezil and galantamine are rapid-action reversible acetylcholinesterase inhibitors, rivastigmine is a slow-action reversible inhibitor of both acetyl- and butyrylcholinesterase[8]. A transdermic formulation of rivastigmine is also available, which may enhance adherence to the treatment. Tacrine was also an acetylcholinesterase inhibitor used against AD, but due to hepatotoxicity issues it is no longer used in clinical practice[9].

ApoE4 in Alzheimer's

The ApoE protein is a glycoprotein that is highly expressed in liver, brain astrocytes, and some microglia and serves as a ligand for receptor-mediated endocytosis (e.g., cholesterol) for myelin production and normal brain function. The ApoE gene located on chromosome 19 has three subtypes: ApoE2, ApoE3, and ApoE4 due to changes in the coding sequence caused by single nucleotide polymorphisms (SNPs). The ApoE4 allele is a significant risk factor for AD compared with ApoE2 and ApoE3 alleles, which are associated with a lower risk and protective effect, respectively. ApoE4 plays an important role as Aβ plaque deposition in the elderly and leads to cerebral amyloid angiopathy (CAA), a known marker of AD. ApoE4 has also been shown to be associated with cerebral vascular damage, contributing to the pathogenesis of AD[10–12].

ApoE4-targeted therapeutics

The elementary principle of APoE4 immunotherapy is close to that applied in the immunotherapy of tau and Aβ[12], specifically to produce or introduce antibodies against these molecules in the periphery that can neutralize their target (this strategy presumes a toxic effect of ApoE4) after their penetration into the brain. Theoretically, the use of immunotherapy to APOE is encountered by the difficulty that the APOE level in the periphery is about 10-times greater than that in the brain[13], as a result, anti-APOE antibodies must be titrated out in the periphery prior to reaching the brain. Many studies demonstrated that the peripheral use of anti-mouse APOE antibodies in APP transgenic (TR) mice can suppress the amyloid deposition before the beginning of plaque and reduce its deposition after the formation of plaque[14,15]. Despite the mode of actions involving these pivotal effects of the anti-APOE monoclonal antibodies, these outcomes have an enormous significance and offer a fundamental idea about the reliability of anti-ApoE4 immunotherapy as a promising therapeutic strategy. Furthermore, this strategy has now been expanded to APOE3- and ApoE4-directed mice using an antibody that reacts particularly with ApoE4[16]. This reveals that repetitive intraperitoneal injection of these antibodies in mice leads to their aggregation in the brain and also in the generation of APOE/Immunoglobulin G complexes, especially in ApoE4 mice. Moreover, this was connected with the restoration of cognitive damages in ApoE4 mice as well as with the restoration of central synaptic and AD-associated pathological effects of ApoE4[17].

Results

DoGSiteScorer

DoGSiteScorer identified four potential binding sites on ApoE4 (Table 1 and Figure 2). Three facets of the binding sites are provided by DoGSiteScorer: volume, surface area, and drug score. The list of sites is ordered from largest to smallest area, with site P_0, having a volume of 359.87 $\AA^3$. P_0 also happens to have the most druggable binding site in ApoE4 with a drug score of 0.75.

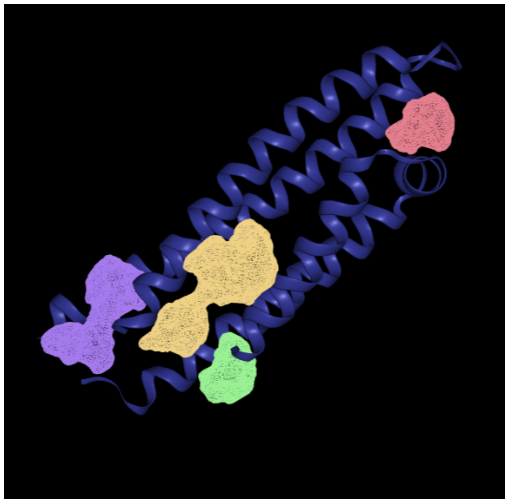


Fig. 2 Binding sites in ApoE4 as determined by DoGSiteScorer. Binding sites are represented as colored spheres (P_0 in yellow, P_1 in purple, P_2 in green, P_3 in red).

Table 1 The four binding sites on ApoE4 predicted by DoGSiteScorer.

Name	Volume ($Å^3$)	Surface ($Å^2$)	Drug Score
P_0	359.87	531.67	0.75
P_1	245.18	559.93	0.56
P_2	133.5	320.38	0.22
P_3	106.18	188.69	0.23

FT Site

One binding site is detected using FT site, represented in red in Figure 3.

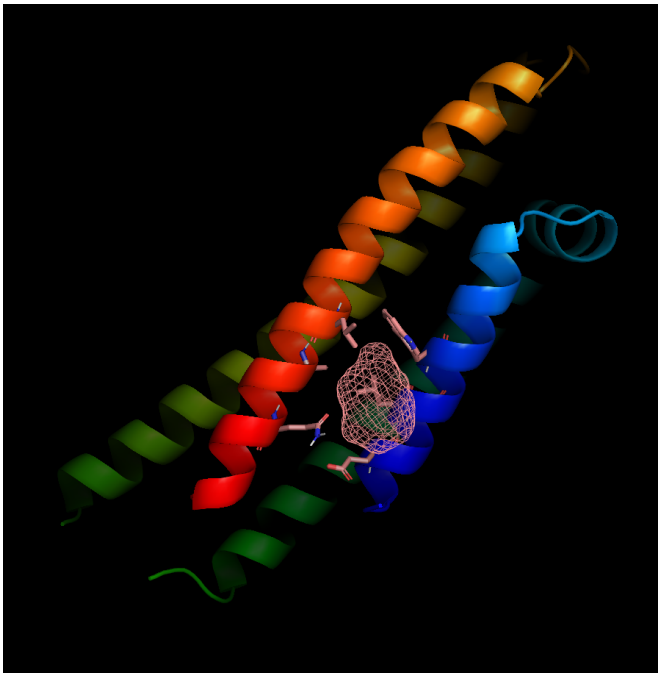


Fig. 3 FT site predicted binding site (in red) on ApoE4. Interacting residues are shown on the side next to the binding pocket.

PrankWeb

Using Prankweb as a machine learning tool to identify the binding sites in ApoE4, one binding site is predicted, with a pocket score of 1.00 and a probability score of 0.007, represented in red in Figure 4.

PocketQuery

The top 3 scoring results from PocketQuery are listed below. The higher the score is, the higher the residue affinity and matchability is to the binding sites of ApoE4.

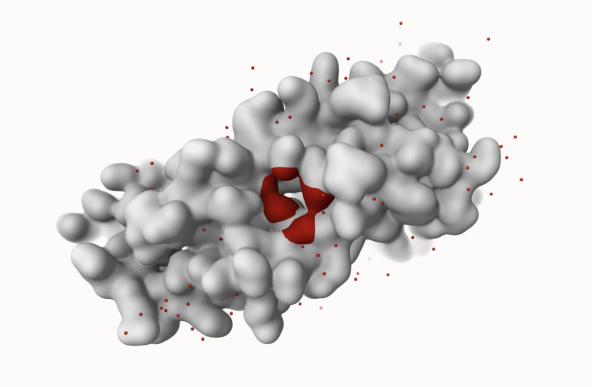


Fig. 4 PrankWeb predicted binding sites (represented in red). Identified through spatial and electrochemical favorability within the protein ApoE4 using machine learning.

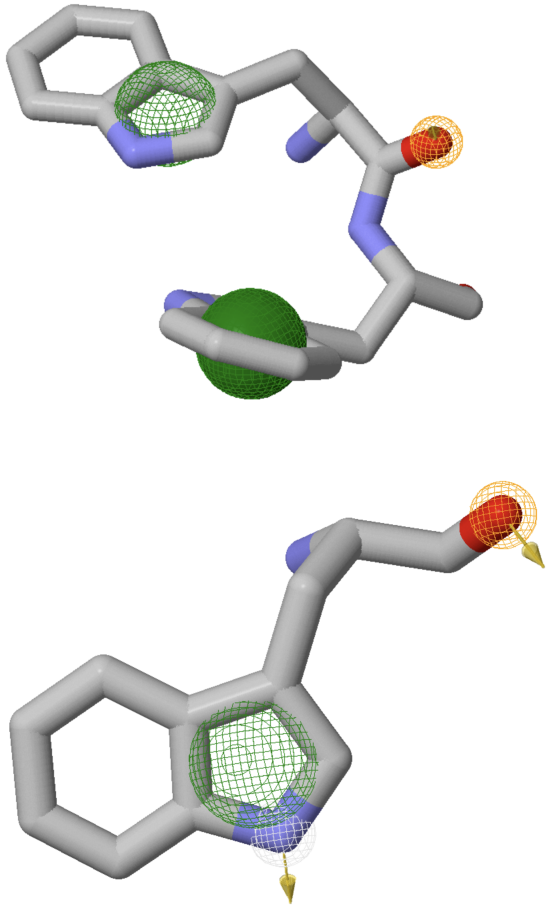


Fig. 5 Pharmacore maps produced by Pocketquery using the interaction of ApoE4 and a protein receptor (LilrB3), PDB: 8GRX. (A) consists of two tryptophans. (B) consists of only one.

SwissDock

In attempt to quantify the energetic interaction of the compounds with ApoE4 using SwissDock as a tool for molecular

docking, ZINC29492665 possessed the highest score (-7.1842 kcal/mol), followed by ZINC40759037 (-7.1842 kcal/mol), then ZINC09060047 (-6.9961 kcal/mol). With all three scores at around 7, these interactions prove to create strong bonds.

Table 2 Top 3 Swiss Param Scores from SwissDock. Scores were obtained through each compound's interaction with ApoE4.

Name	Swiss Param Score (kcal/mol)
ZINC29492665	-7.1842
ZINC40759037	-7.0182
ZINC09060047	-6.9961

SwissADME

While all 3 of the top results followed Lipinski's rule, the third compound, ZINC09060047 produced a Log P smaller than 1, proving it too hydrophilic as a candidate. The other two molecules (X and X) are promising candidates as they are in agreement with Lipinski's rule (Table 4).

Toxicity Report

Using Protox I was able to screen my top two compounds from SwissDock for their LD50, Toxicity classification, and areas of concerning toxicity. LD50, or lethal dose 50, is the amount of dosage needed for half of the test population to pass away. Both compounds were also classified as a 4 on a toxicity scale from 1-6, 1 meaning most toxic, 6 being least toxic.

Discussion

Affecting over 55 million people worldwide, Alzheimer's is the most common type of dementia and is classified as a slowly progressive neurodegenerative disease. The ApoE gene, specifically the subtype ApoE4 allele, a key factor in Aβ deposition, has been proven to be associated with cerebral vascular damage, playing an important role in the pathogenesis of AD. This study uses computational tools, combining virtual screening, docking, and ADMET predictions to identify the most suitable small molecules for interaction with the crystal structure of the ApoE4 protein. Out of the 15 small molecules, five from three different pharmacophore maps, ZINC29492665 had the lowest G score, with a score of -7.1842 kcal/mol. ZINC29492665 also fulfilled lipinsky's rule and displayed low toxicity risks, however, because of its inability to permeate the blood brain barrier (BBB) it is hindered as a candidate. On the other hand, ZINC40759037 with a G of -7.0182 kcal/mol fulfills lipinski's rule, displays low toxicity risks and is able to permeate the BBB. Limited to predictions with computational models, these small molecules lack experimental data supporting thes inhibition of ApoE4. Furthermore, the identification of only a singular binding site on ApoE4 by Prankweb and FTsite also poses concerns as to the drug compatibility of the protein. Further experiments in vitro can be used to determine the drugs credibility, such as testing the compound on brain cells to check for a modified brain response. In vivo experiments can also be performed on Alzheimer's induced mice. ZINC40759037 provides promising potential as a drug to combat Alzheimer's disease while also serving as a starting point for future developments of small molecules in Alzheimer's disease

Methods

There are 4 main methods to identify binding sites in a protein: the geometric method, the energetic method, the machine learning method, and the template-based method. In this study, we utilized the first 3 to identify the binding sites on ApoE4.

DoGSiteScorer

DoGSiteScorer provides the functionality to detect potential binding pockets of a protein of interest. It analyzes the geometric properties of these pockets, like volume and surface area, and estimates the druggability of the protein by compiling these values into scores[18]. By inputting a PDB code into the protein plus query and pressing enter, the site will display a 3D structure of that protein. Then, by clicking on DoGSiteScorer in the settings list, the site will be prompted to run the program and produce the protein with possible binding sites colored in.

FTSite

FTSite uses the energetic method to identify potential binding pockets in a protein. By placing 16 probes on a dense grid with the protein, FTsite finds favorable binding positions using empirical free energy functions and by detecting where the probes cluster[19]. By inputting a PDB code into the FTsite job search and pressing "Find My Binding Site", the site will place you into a queue for your job to be run. After the job finishes, click "Finished" in the queue tab and the site will display your protein along with the binding pockets it found. By clicking "download pymol session" you are redirected to pymol and provided more information regarding the specific sites.

PrankWeb

PrankWeb is a template-free machine learning method based on the prediction of local chemical neighborhood ligandability centered on points placed on a solvent-accessible protein surface. Points with a high ligandability score are then clustered to form the resulting ligand binding sites[16] .By inputting a PDB code

Table 3. All 15 compounds interact with ApoE4. Generated using SwissDock. Sorted in order of largest to smallest (Top to bottom).

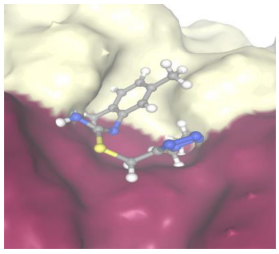
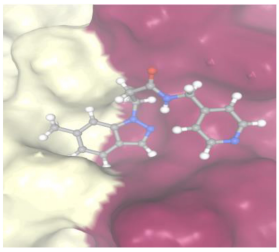
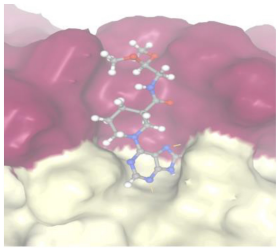
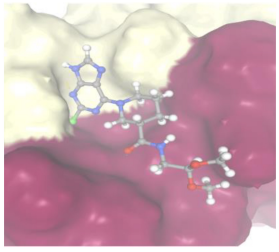
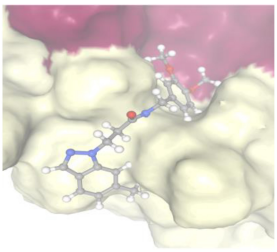
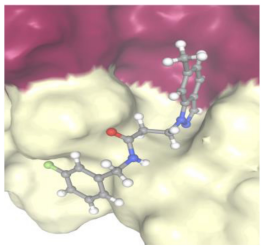
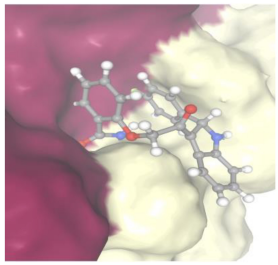
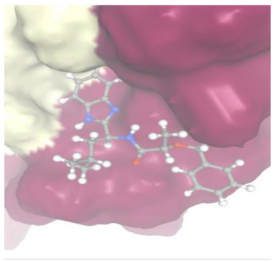
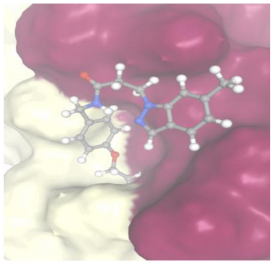
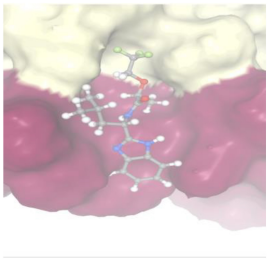
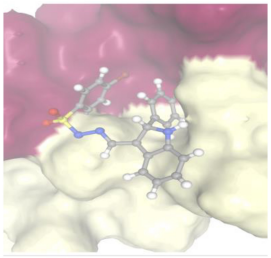
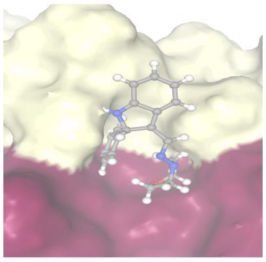
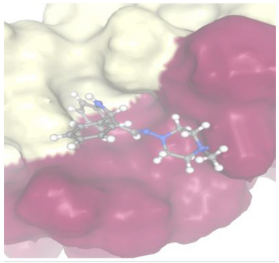
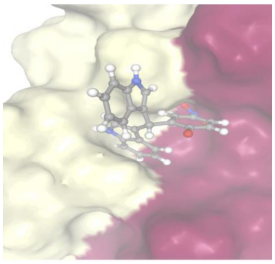
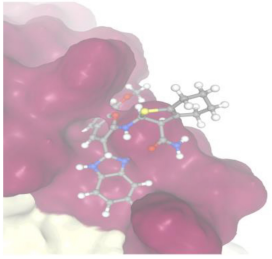
ZINC29492665	ZINC40759037	ZINC09060047
		
ZINC09421183	ZINC40759203	ZINC40759197
		
ZINC39934258	ZINC48109742	ZINC40759211
		
ZINC42853662	ZINC39792766	ZINC40010813
		
ZINC40071450	ZINC04954103	ZINC63406114
		

Table 3 SwissADME reports for ZINC29492665 and ZINC40759037, respectively. Both compounds pass Lipinski's rule. Since ZINC29492665 is not BBB permeant, further modifications will be needed unlike ZINC40759037.

Molecule	Canonical SMILES	Formula	MW	#Heavy atoms	#Aromatic heavy atoms
ZINC29492665	CCCn1nnnc1CS/C(=N/c1cc(C)ccc1C)/N	C14H20N6S	304.41	21	11
ZINC40759037	O=C(CCn1ncc2c1cc(C)cc2)NCc1ccncc1	C17H18N4O	294.35	22	15
Molecule	**Fraction Csp3**	**#Rotatable bonds**	**#H-bond acceptors**	**#H-bond donors**	**Consensus Log P**
ZINC29492665	0.43	6	4	1	2.49
ZINC40759037	0.24	6	3	1	2
Molecule	**GI absorption**	**BBB permeant**	**Lipinski #violations**	**Bioavailability Score**	**Synthetic Accessibility**
ZINC29492665	High	No	0	0.55	3.28
ZINC40759037	High	Yes	0	0.55	2.25

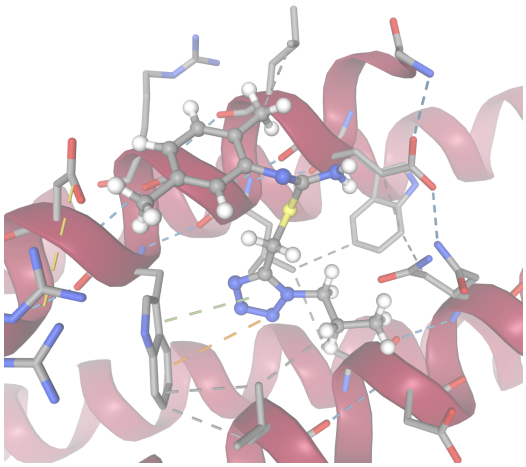


Fig. 6 SwissDock results with ZINC29492665 displaying molecular interactions with ApoE4

Table 4 LD50 and Toxicity class of top 2 compounds from SwissDock as promising ApoE4-targeted drug candidates.

Compound	LD50 (mg/kg)	Toxicity Class
ZINC29492665	500	4
ZINC40759037	580	4

into the PrankWeb query and pressing submit, PrankWeb runs the job and identifies available binding pockets inside your protein. It also provides information of the amino acids that the binding site consists of. If more than one site is found, each site is also ranked on the right hand side from most bindable to least, with a calculated score.

Pocketquery & ZincPharmer

PocketQuery (http://pocketquery.csb.pitt.edu) is a web interface for exploring the properties of protein-protein interaction (PPI) interfaces with a focus on the discovery of promising starting points for small-molecule design. PocketQuery rapidly focuses attention on the key interacting residues of an interaction using

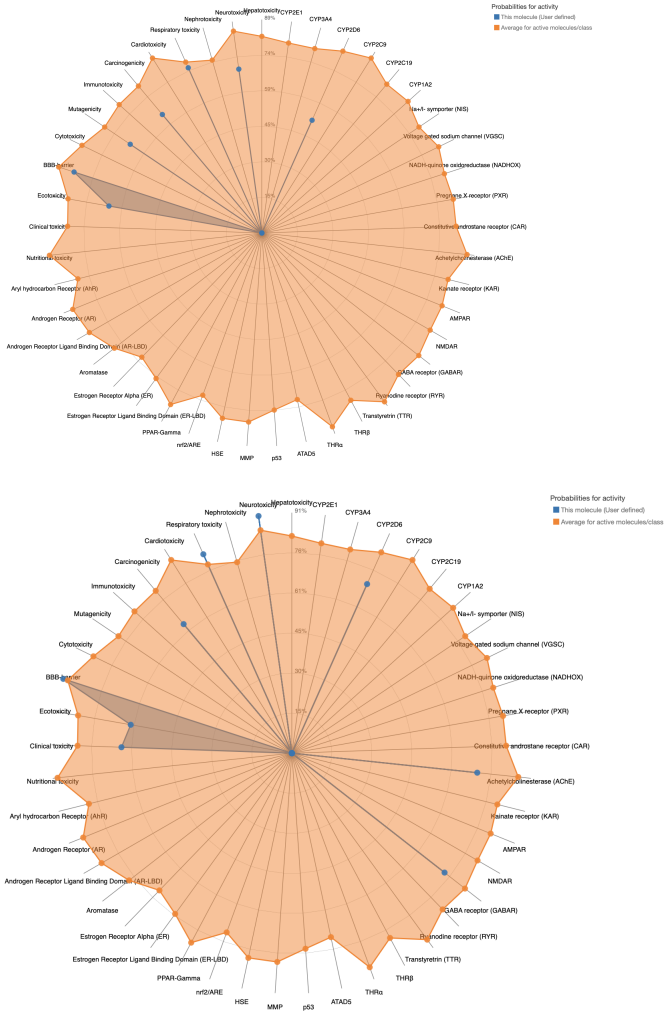


Fig. 7 ZINC29492665's (top) and ZINC40759037's (bottom) toxicity in different areas of the body compared to the average toxicity of FDA approved drugs.

a 'druggability' score that provides an estimate of how likely the chemical mimicry of a cluster of interface residues would result

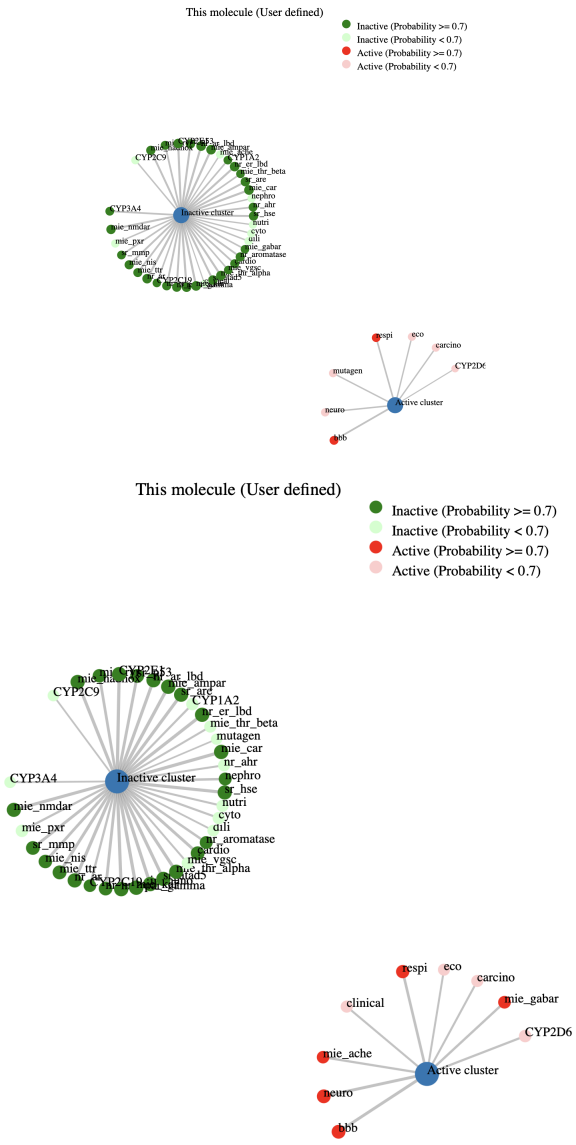


Fig. 8 ZINC29492665's (up) and ZINC40759037's (down) active areas of toxicity concern (bottom right cluster in red) and inactive areas of toxicity concern (top left in green).

in a small-molecule inhibitor of an interaction[20]. Zinc Pharmer is then used in consequence to the produced pharmacore map in order to identify small molecules that fit these specific sites.

By inputting the PDB code for the interaction between two proteins, Pocketquery produces results of key points within the protein in which binding may be available and lists them in a table with scores.

Molecular Docking

Swissdock (http://www.swissdock.ch/docking) is an online platform that simulates the docking for small molecules against target proteins. Swissdock uses theoretical methods to predict and calculate the energy of interaction between small molecules and proteins. It uses the EADock DSS engine to operate[21]. The docking data, the target protein structures, and the ligands are presented[21]. Submit the target protein in the "Target selection" by searching it with the protein's URL and PDB code or uploading a mol2 standard protein file. Submit the "Ligand selection" by searching the ZINC AC or uploading a mol2 standard ligand file. Then, enter the project's name and user email to receive the notification when the result comes out. Press "Start Docking" to begin the molecular docking.

Swiss ADME

Swiss ADME http://www.swissadme.ch is an online platform, where molecules are estimated for ADME, physicochemistry, drug-likeness, pharmacokinetics and medicinal chemistry friendliness properties[22]. By pasting a smile code or drawing the compound of interest and pressing submit, a result screen will pop up, displaying calculations made by ADME.

Toxicity Report

ProTox is a web server that incorporates molecular similarity, pharmacophores, fragment propensities and machine-learning models to predict various toxicity results; such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes pathways (Tox21) and toxicity targets. The predictive models are built on data from both in vitro assays and in vivo cases[23]. By pasting a smile code into the search box and pressing submit, ProTox will run its algorithm and produce a table of results.

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