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Research Paper

Targeting Serine Protease High-temperature RequirementFactorA1withSmallMoleculesLigandsasPotential Therapeutics for Alzheimer's Disease

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Abstract
Alzheimer'sdisease,theprimarycauseofdementia,israpidlybecomingoneofthemostexpensive,burdening,andlethaldiseasesofthecentury.Theprogressionofthisdiseaseinvolvestheaccumulationofbetaamyloidfragmentsformingamyloidplaqueswhichdisruptsbrainfunction.TheHTRA1proteinhasshownpromiseintheinhibitionofamyloidplaqueformationandADdevelopment,withstudiesofHTRA1exhibitingreducedaggregationofbetaamyloidplaquesanddegradedfibrillartauwhich contributesto Alzheimer's. Developinga smallmoleculeligandofHTRA1couldbeusefulin regulatingthe progressionof Alzheimer'sdisease.Computationalmethodsareusedasacosteffectivewayforaprimaryinvestigationofdeterminingpotentialligands.HTRA1wasscannedthroughFPocketWeb,CavityPlus,andPrankWebforpotentialligandbindingsites,whichwerefoundinpromisingquantities,warrantingfurtherinvestigationintomoleculesthatwouldinteractwiththesebindingsites.PocketQueryandZINCPharmerwithHTRA1andapeptide(PBDcode:2JOA)wereutilizedforapharmacophore basedvirtualscreeningtofind20hitcompoundsfittingfivepharmacophoremaps.Todeterminethecompoundwiththemost favorableinteractionwithHTRA1,theidentifiedcompoundsweresubjecttofurtherscreeningusingSwissDock.Thefivecompoundswiththelowest GibbsfreeenergyvaluesandmostfavorableinteractionswereinputtedintoSwissADME.Thiswas donetoanalyzedruglikenessproperties,whichshowedpromisingresultsforthreeout offivecompounds.

Keywords
Alzheimer'sdisease,Betaamyloidhypothesis,Drugdiscovery,liganddiscovery,HTRA1,Virtualscreening,Aging,Dementia

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I. Introduction

Alzheimer'sdiseaseisaneurodegenerativediseasethatisagrowingissueamongtoday'spopulation.Itresultsinthelossofmemory,language,problem-solvingandotherthinkingcapabilitiesandisconsideredthemaincauseofdementia,ageneraltermdescribingtheimpairmentofbrainfunction[1].About50millionpeoplehavedementiaworldwide,andthisnumberispredictedto triple,increasing to 152million globallyin 2050, asreported by the WorldAlzheimerReport2019[2].Alzheimer'sdiseaseismoreprevalentinindividuals overthe age of65,however,inrarecasesofearlyonsetAlzheimer'sitcanaffectindividualsunder65[3].Symptomsareknownto worsenovertime andeventually interferewithdaily tasks[3].While thediseasedoesnotdirectlycausedeath,itincreasesthevulnerabilityofanindividualtootherincidents[3].ThecauseofAlzheimer'sdiseaseiswidelybelievedtobearesultofacombinationofgenetic,lifestyleandenvironmentalfactorsthataffectthebrainovertime[4].PossibleriskfactorscontributingtoAlzheimer'sdiseaseincludeincreasingage,familyhistoryandgenetics, cardiovasculardiseases, andenvironmental influencesuch asair pollution[4].

ThedevelopmentofAlzheimer'susuallybeginswithdamagetotheregionofthebrainthatcontrolsmemory,includingtheentorhinalcortexandhippocampus[5].Thediseasethenprogressesinto thecerebral cortex, affecting many behaviorslike communication andreasoning[6].ADeventuallyreachesanddamagesotherareasofthebrain[6].Recentresearchonthecauseandpreventionof Alzheimer'shasbeenfocusedontheroleofamyloidplaquesandneurofibrillarytanglesthatarecommonlyfoundinthe

brainofAlzheimer's patients[7].Plaquesareabnormalclumpsandappeartohaveatoxiceffectonneuronsandtodisruptcommunicationbetweenbraincells[8].Theyaremadeofcellulardebrisandmisfoldedα-Synuclein(α-syn),aproliferateneuralproteinwhichassistsinregulationof synapticfunction,thatbecomesβ-sheetrichamyloidfibrils[9-11].Neurofibrillarytanglesareformedfromtauproteinsthatchangeshapeandorganizeintostructureswhichdisruptthebraintransportsystemandcausedamagetocells[8].Thepresenceofthe amyloid plaquesand neurofibrillarytanglesareconsidered themainindicators ofAD.

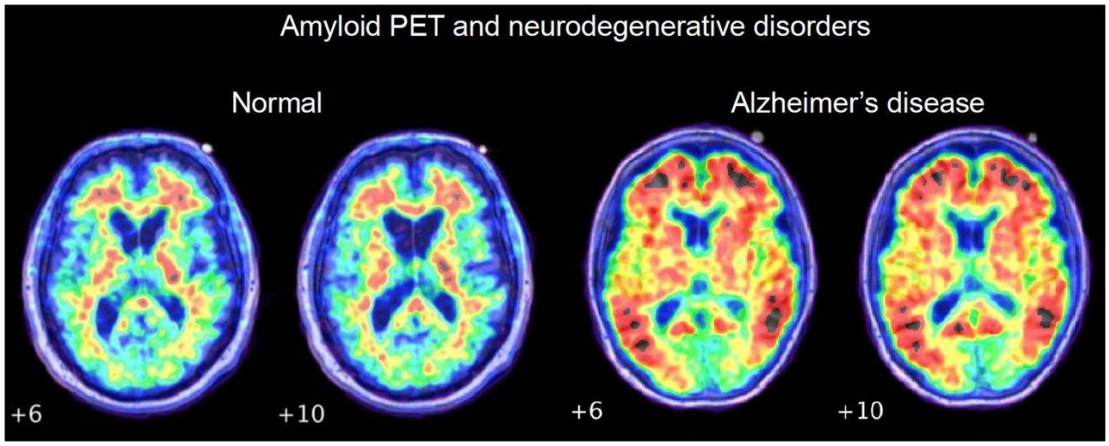


Fig.1ComparisonofHealthyBrainandabrainaffectedbyAlzheimer's. TheADbrainhassignificantlymore amyloidas a resultof the increasein amyloidplaques in thebrain [12].

TheAmyloidHypothesisisapossibleexplanationforthedevelopmentofAD. Itwas previouslydiscoveredthatthebeta-amyloidproteinisthecentralcomponentofextracellularamyloidplaquesinAD[13].Sincethen,theAmyloidHypothesis, believingthattheflawsintheprocessesgoverningproduction,accumulationordisposalofbeta-amyloid is the primarycauseofAlzheimer's,hasbecomealeadingtheoryofADpathogenesis[14].Overthepastdecades,targetingbeta-amyloidproteinhasbeenthefocus ofdevelopingADtreatment[14].

Furtherevidenceforthishypothesiscomesfromgeneticstudiesthathavesupportedthecausativeroleofbeta-amyloidaccumulationinADpathogenesis[14].MutationsinthegenesAPP,presenilin1(PSEN1)andPSEN2thatincrease beta-amyloidformationareshownto promoteplaqueformationandleadtoautosomaldominantearly-onsetfamilialAD(FAD)[15,16].Inaddition,Down syndrome(DS)patients haveanextracopy oftheAPP gene,whichleadstomostpatientsexhibitingtypicalADneuropathologywithsignificantlevelsofbeta-amyloidplaquesandtautanglesbyage40[17-19].Ontheotherhand,amutationintheAPPgeneinicelandicpopulationsreducesbeta-amyloidproduction,whichisshowntoreducetheriskofdementiainthepopulation[20].Bothgeneticandnon-geneticriskfactorsforlate-onsetAlzheimer's(SAD)havebeenfoundtoaffectbeta-amyloidproduction[14].Beta-amyloid'sinvolvementinADprogressionsupportsthemotivationofAlzheimerresearchtopursueanti-beta-amyloidtherapies.Whilefailureshaveoccurredinthetargetingofbeta-amyloid,recentdevelopmentson anti-beta-amyloidtherapieshave shownfurtherprogress [14].Recently,AducanumabandLecanemab,boththerapeuticstargetingAβaggregation,obtained

FDA-approval[21,22].Thesedrugshaveseenpositiveresultsinslowingtheprogressionof AD,displayingpromisethattargeting amyloidplaquescansuccessfullyresult inmodification ofdiseaseprogression[23].

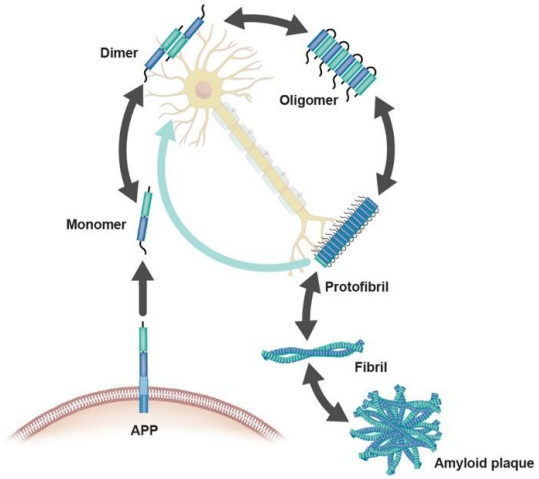


Fig.2Illustrationofaggregationinthebrainfrombeta-amyloidmonomerstoformanAmyloidplaque[24]. Themonomersareshowntobegeneratedfromthe APPgene,withan extracopyin DSpatients causinganincrease inAmyloid plaques[17-19].

HTRA1isapromisingtargetgeneforinhibitingamyloid plaqueformationand ADdevelopment.HTRA1,knownashigh-temperaturerequirementfactorA1(HTRA1),isaserineprotease,anenzymeresponsibleforbreakingdownotherproteins. TheproteinisATP-independentwithaPDZdomain[25].HTRA1anditsisoformsarefoundinbacteria,fungi, plants,andanimals andfunctioninthestressresponsebybindingfromitsPDZdomaintodamagedproteinstobreakthemdown[26,27].Theprot einisfoundthroughouttheextracellularmatrixand isfound inthecytoplasmandnucleusintracellularly[23,28].PDZdomainproteinsareknowntobindtosubstratescontainingβ-sheets,whichsuggestsitmaybindtoβ-sheetrichamyloidspeciespresentinAD[23].

Otherstudiessupportthisprediction,suggestingthatHTRA1maybeinvolvedinthereducedaggregationofbetaamyloidpl aques,and alterationsinHTRA1 activitycould impactthe buildupoftheseplaques[23,29].Amyloidandothermisfoldedproteinsareseentobehighly resistant

todegradationandcurrentlywithouttherapeuticstoremovemisfoldedproteins;however,HTRA1wasfoundtodegradeam yloidfibrilaggregates[23,30]DevelopingadrugtoregulateHTRA1expressionwouldbebeneficial inanattempt toinhibitAD development.Thegoal ofthisprojectistodeterminesmallmoleculeligandsofHTRA1whichcanserveasthestartingpointforfuturedevelopmento fADtreatmentintargetingamyloidplaques.

1. MaterialsandMethods

1.1. AnalysisofbindingsitesinHTRA1:

Inthefirstexperiment,threetoolswereusedtodetermineviableligandbindingsitesonHTRA1,includingCavityPlus(http: //www.pkumdl.cn:8000/cavityplus/index.php#/),FPocketWeb(https://durrantlab.pitt.edu/fpocketweb/),andPrankW eb(https://prankweb.cz/).ThePDBcode3TJNwasusedineachoftheseprograms.ThisPDBcodeisoftheunboundprotein HTRA1withoutadditionalmolecules.

1.1.1 CavityPlus

UsingCavityPlus(http://www.pkumdl.cn:8000/cavityplus/index.php#/)andinputtingthePDBcode3TJN,thenselectin gchainsA,B,andDandpressing“Submit”yieldstheresults.Thedefaultsettingsarekeptforotherpartsoftheprogram.Goog lesheetswereusedtogeneratethedatatablebasedon theresultsfromCavityPlus.

1.1.2 FPocketWeb

UsingFPocketWeb(https://durrantlab.pitt.edu/fpocketweb/)andinputtingthePDBcode3TJN,proceedtopress“Load”t hen“Start FPocketWeb” whichyieldstheresults.EnsurethatchainsA,B,andDareselectedonthefirstpagebeforepressing“Start FPocketWeb.” Thedefaultsettingsarekeptforotherpartsoftheprogram.Googlesheetswereusedtogeneratethedatatablebasedontheresu

ltsfrom FPocketWeb.

1.1.3 PrankWeb

UsingPrankWeb(https://prankweb.cz/)andinputtingthePDBcode3TJN,thenpressingsubmityieldstheresults.Thedefaultsettingsarekeptforotherpartsoftheprogram.Googlesheetswereusedtogenerate thedatatablebasedon theresultsfrom PrankWeb.

2.2 PharmacophoremapusingPocketQuery

PocketQuery(http://pocketquery.csb.pitt.edu/)wasusedtogeneratepharmacophoremapsofHTRA1.EnteringthePDBcode2JOAyieldedtheresultsinthedatatableandfigures.Fiveclusterswere selectedbased onif theclusters were chainB andhighest score.

2.3 MoleculeidentificationutilizingZINCPharmer

ThetopfiveclustersidentifiedbyPocketQuerywereexportedtoZINCPharmer(http://zincpharmer.csb.pitt.edu)toproducetheresults.Inclusterstwothrough five,someoftheduplicatepharmacophoreelementswereremovedtowhereeachclusterhadfourinteractionsremaining.Thiswastoensuretherewasareliablenumberofhitmoleculesbelow RMSD.4 toconductfurtherstudies.Fourmoleculesfromeachclusterwereselectedbasedonthelowest RMSDvaluebyclickingRMSDtwice.

2.4 MoleculardockingwithSwissDock

ThebindingabilityofthecompoundspreviouslyidentifiedbyZINCPharmertoHTRA1wasobservedusingSwissDock(http://old.swissdock.ch/docking).HTRA1(PBDcode:3TJN)wasuploadedasthetargetselection.ThetwentyZINCcodesfoundpreviouslybyZINCPharmerwereinputtedforligandselection.TwoofthetwentyZINCcodeswererepeated,resultinginSwissDockbeingrun18times.IftheZINCcodewas recognizedby SwissDock,the ligandwasselectedanddesignatedto"Dockselectedligand."IftheZINCcodewasnotrecognizedbySwissDock,afileofthecompoundwasobtainedthroughOPENBABEL(https://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html) byconvertingthe

2.5 DrugLikenesspredictionbySwissADME

DruglikenesspropertieswereanalyzedbySwissADME(http://www.swissadme.ch/).ASMILEcodewasenteredforeachofthetop5compoundsidentifiedbySwissDock.TheSMILEcodewasobtainedusingZINC12(https://zinc12.docking.org/)byentering ZINCcodeintothe searchbar.AftertheSMILEcodewasinputted,RUNwaspressedondefaultsettings.MolecularWeight(g/mol),Num.H-bondacceptors,Num.H-bonddonors,LogPo/w(iLOGP),Lipinski,Violations,WaterSolubilityLogS(ESOL)Class,GIabsorption,andBBBpermeantpropertieswererecorded inthe datatable.

2. ResultsandDiscussion

3.1 Analysisofpotentialligandbindingsites

Thefirstexperiment isfocused ondetermining bindingsites forsmall moleculesin proteins.Thisiscrucial as theavailability of suchbinding sites for smallmolecules will allowfor further studiestoidentifysmaller moleculesthatbind tothe protein.Fromthe threeprogramsused to determinebindingsites, overfiftypossible bindingsites werefound.

3.1.1 CavityPlus

Asafirst approachto findsuch bindingsites, CavityPlusis used,which isa geometricbasedmethodconsideringthestructure ofthesite todeterminea Cavityscorefor thesite[31]. Itthenutilizesthisscore toquantitatively determineasite's druggability,showing ifasite issuitable forbindingdrug-likemolecules [31].The resultsofCavityPlus predict23 possibleligandbindingsites(Fig.3),with fiveprospective bindingsites havingstrong andmedium druggabilityand drugscore.The bindingsite withthe highestdrug score(blue) issignificantly largerthan theotherswitha scoreof 2768,over two timesthe secondhighest drug score.

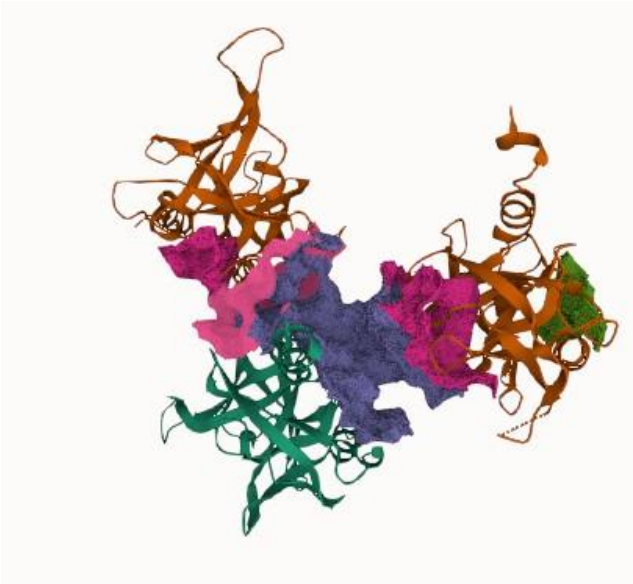


Fig.3GeneratedimageofHTRA1usingCavityPlusshowingselectbindingsites(PDBID:3TJN). Thefigures showfour Strongandone Mediumpredicted bindingsitesand arehighlightedingreen, magenta,pink,and blue.

Table 1.DrugScoreandDruggabilityoffirst10bindingsitesbyCavityPlus

# ↑↓	DrugScore↑↓	Druggability↑↓
1	2768	Strong
2	1011	Strong
3	1339	Strong
4	907	Strong
5	-842	Weak
6	153	Medium
7	-273	Weak
8	-874	Weak
9	-563	Weak
10	-1085	Weak

3.1.2 FPocketWeb

FPocketWeb isawebsitethatrunstheprogramfpocket3[32]. Itisoneofthefewprogramsthatconsideran energy-based approachto identify possibleligand binding siteswith energeticallyfavorableconditions[33]. Thisprogram resultedin 43possible bindingsites, with5 ligandbindingsitesover .1druggability.Out ofthesesites, pocketnumber43 hadthehighestdruggability,morethantwo timesthedruggabilityof thenexthighest druggability.

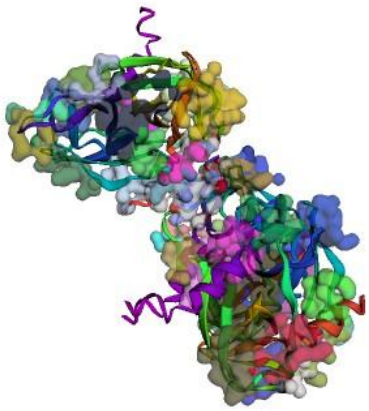


Fig.4Illustrationof43predictedbindingsitesbyFPocketWeb(PDBID:3TJN).

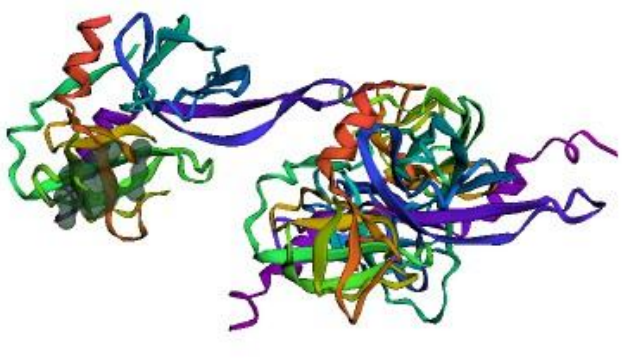


Fig.5Imageofpocket#29withthehighestdruggabilityscore.GeneratedbyFPocketWeb(PDBID:3TJN).

Table 2.Table oftop5bindingsitesonFPocketWebbasedondruggabilityover0.1

PocketNumber	Druggability	Score	Volume
29	0.582	-0.017	314.663
16	0.238	0.046	393.467
28	0.177	-0.015	481.56
6	0.114	0.134	321.631
2	0.107	0.182	690.074

3.1.3 PrankWeb

PrankWebwasusedtodeterminepotentialbindingsites.PrankWebistheonlineinterfacefortheprogramP2Rank,amachinelearning-basedmethodfordeterminingligandbindingsites[34].Itisshowntopredictnovelsitesduetoitstemplatefreemethod,allowingittodiscovernewpossibleprotein–ligandcomplexes[35].Outofthe5bindingsites,therearethreeprospectivebindingsiteswithscoresabovethree.Outofthesethree,thescoreofthefirstbindingsite(red)issignificantlyhigherthantheotherones,13.47 comparedto3.7. PrankWeb,FPocketWeb,andCavityPlusallyieldedpromisingresultswithabundantprospectivebindingsitesforfurtherinvestigationoftargetingHTRA1forinhibitingamyloid productionusingligands.

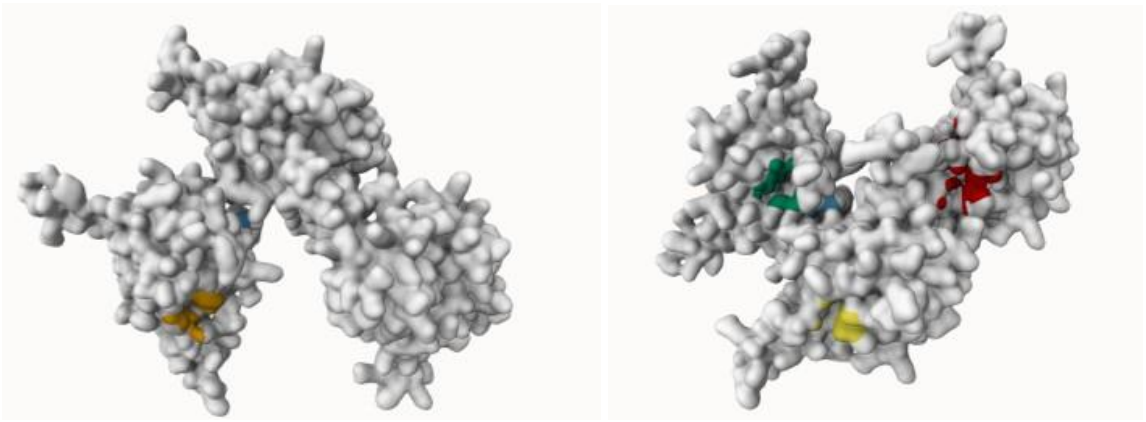


Fig.6PredictedbindingsitesofHTRA1byPrankWeb(PDBID:3TJN). Bindingsitesarehighlightedin green,red, yellow,dark yellow,and blue.

Table 3.Resultsofthe5bindingsitesfoundbyPrankWeb

Rank	Score	Probability
1	13.47	0.706
2	3.7	0.147
3	3.51	0.133
4	1.62	0.025
5	1.13	0.01

3.2 VirtualScreening

Thesecondexperimententaileddeterminingsuitablesmallmoleculesthatcouldbindto HTRA1.ThePDBcode2JOAwasusedanditconsistedofHTRA1 and a peptide. The binding sitesof the peptide to HTRA1 were observed using PocketQuery and through ZINCPharmer, 20small molecules were identified as possible ligands.

3.2.1 mapusingPocketQuery

PharmacophoremapsweregeneratedusingPocketqueryandthefivemapswiththehighestscorewereselectedforfurthera nalysis.Pocketqueryisanonlineinterfacethatobservesthepropertiesofprotein–proteininteraction(PPI)forthe purposeofdrugdiscovery[36].
Itidentifiespotentialbindingsitesthroughagivendruggabilityscore[36].Thedruggabilityscoreofthe

clusterrepresentsitsabilitytoform interactionswith asmall moleculeinhibitor [36].The scoreisona scaleof 0-1. A scoreof over 0.85indicating good druggabilityand a possiblebinding site ofHTRA1.Thetop5clusterswiththehighestscoresallhavescoresabove0.85,andthuswasfurtherinvestigatedusingZINC Pharmer.Visualillustrationsoftheclustersandtheirinteractionsareshownbelow (Fig.7).

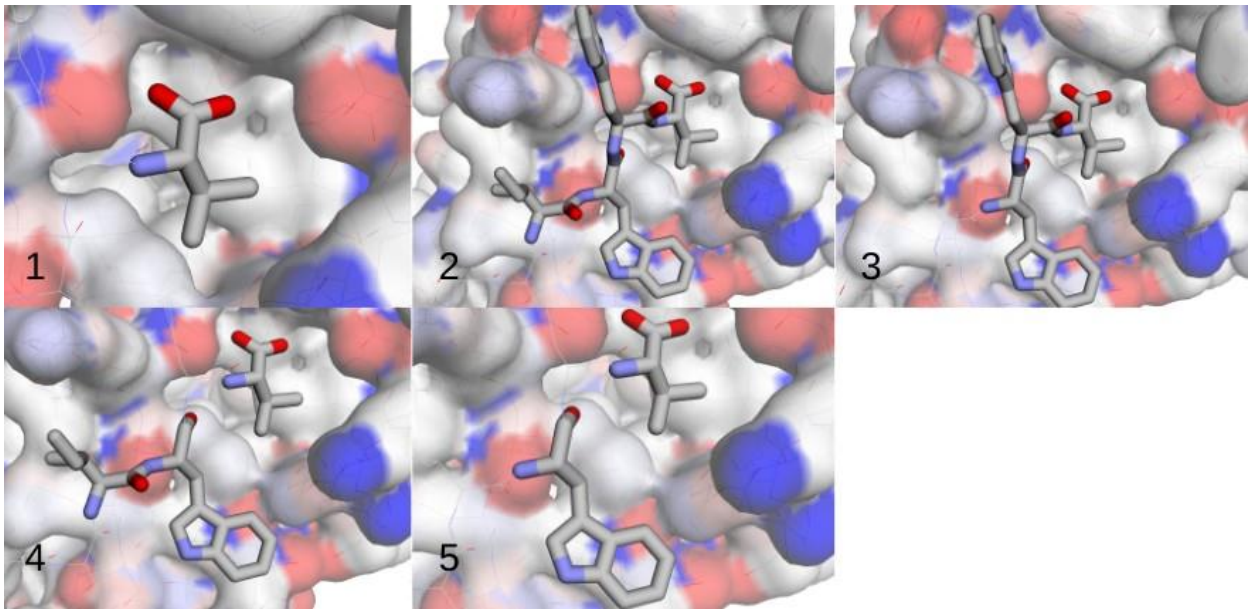


Fig.7PocketQuerygeneratedPharmacophoremapsofHTRA1boundtoapeptide(PDBID:2JOA)

Table4.Resultsofthetop5clustersrankedbyscore(PDBID:2JOA)

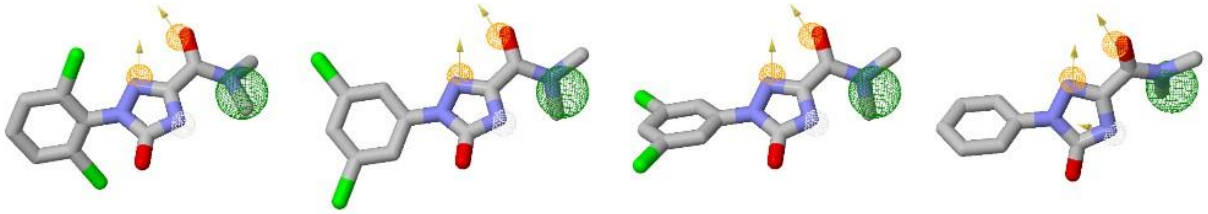
Cluster#	Score	Residue	Residue#	Size	Distance	Chain
1	0.898084	VAL	7	1	0	B
2	0.884624	ILE TRP TRP VAL	4 5 6 7	4	9.5778	B
3	0.872467	TRPTRPVAL	5 6 7	3	8.8039	B

4	0.867201	ILETRPVAL	4 5 7	3	9.5778	B
5	0.859819	TRPVAL	5 7	2	6.8737	B

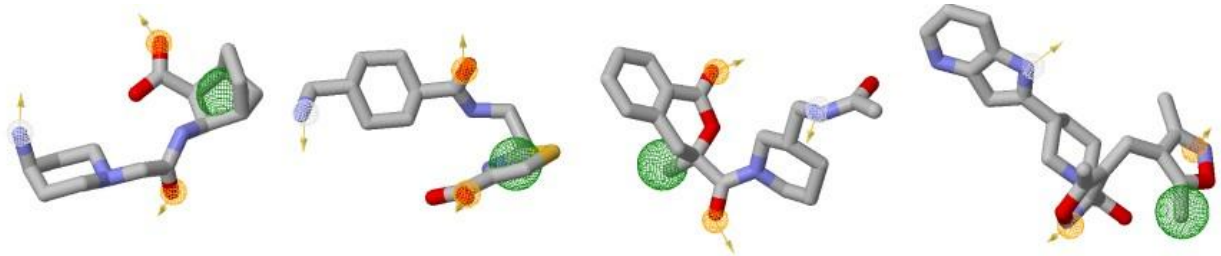
3.2.2 Moleculeidentification

ThetopclustersfromPocketQuerywereexportedtoZINCPharmer.ZINCPharmerisawebinterfacethatidentifiespurchasable compoundswhich align witha specific pharmacophoremap[37].ZINCPharmerquantifiesthealignmentbetweenthemoleculeandtheclusterthroughRootMeanSquareDeviationorRMSD.CompoundswithlowerRMSDhaveabettergeometricmatchtothepharmacophore[37].4compoundsfromeachclusterwerechosenforfurtherinvestigationbasedonRootMeanSquareDeviationorRMSDvalue.ZINCPharmerincludesdifferentconformerswhendeterminingpossibleligands,resultingintworepeatZINCcodesfor atotalof 18compounds.

Cluster1molecules



Cluster2molecules



Cluster3molecules

Cluster4molecules

Cluster5molecules

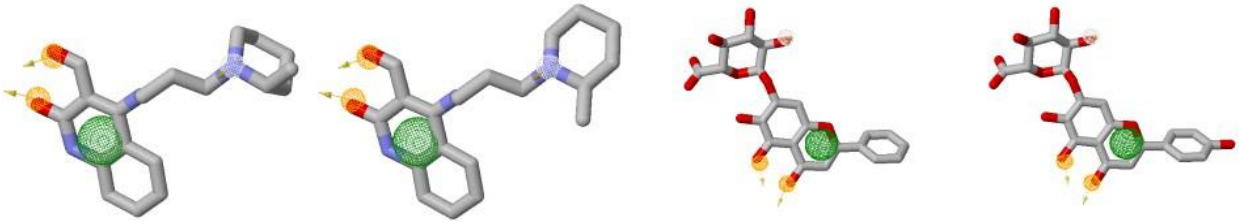


Fig.820smallmoleculesidentifiedby ZINCPHarmer. Thepharmacophoremapinteractionsarerepresentedby Green,hydrophobic,yellow, hydrogenacceptor,andwhite,hydrogendonor.

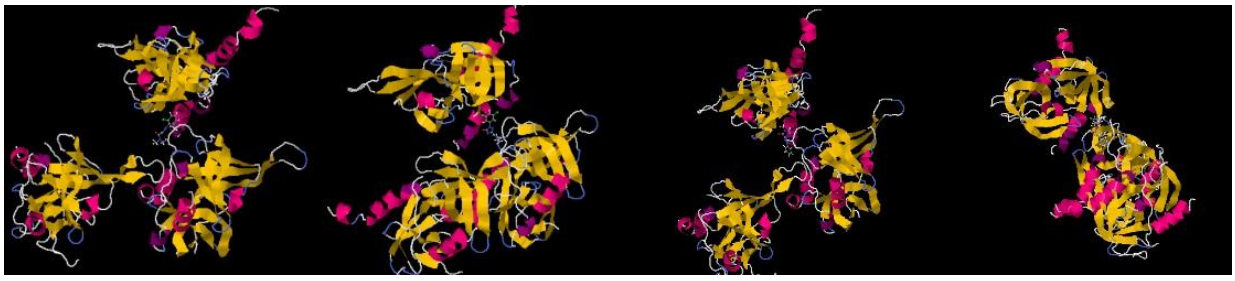
Table5.DetailsofthetopmoleculesofeachclusterbasedonRMSD

Cluster#	Name	RMSD	Mass	RBnds
1	ZINC36533601	0.353	301	5
	ZINC15444140	0.353	301	5
	ZINC15444140	0.353	301	5
	ZINC15444133	0.354	232	5
2	ZINC92715070	0.151	295	4
	ZINC93426091	0.215	311	6
	ZINC67363835	0.282	344	5
	ZINC19580940	0.282	419	8

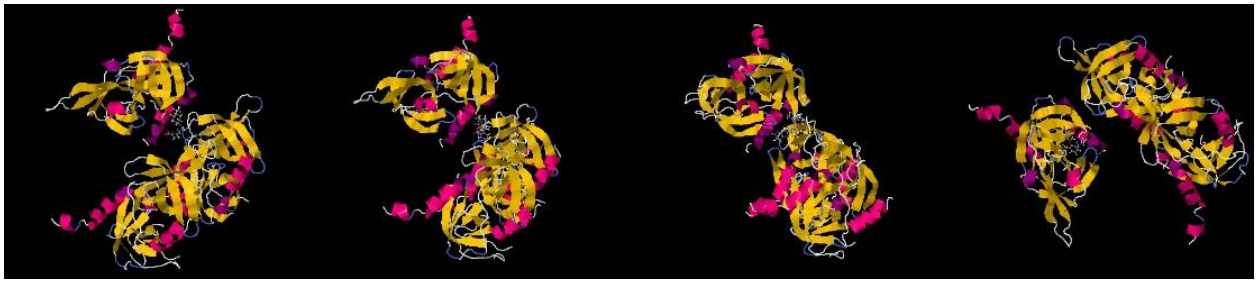
3	ZINC94679710	0.293	288	10
	ZINC85893874	0.304	750	15
	ZINC85444486	0.328	333	12
	ZINC81188012	0.331	319	11
4	ZINC94879818	0.147	255	8
	ZINC27928090	0.152	331	8
	ZINC27928791	0.155	303	6
	ZINC75487197	0.158	276	7
5	ZINC06681836	0.289	328	7
	ZINC06681836	0.289	328	7
	ZINC34114798	0.297	445	9
	ZINC21992914	0.297	461	10

3.3 MolecularDocking

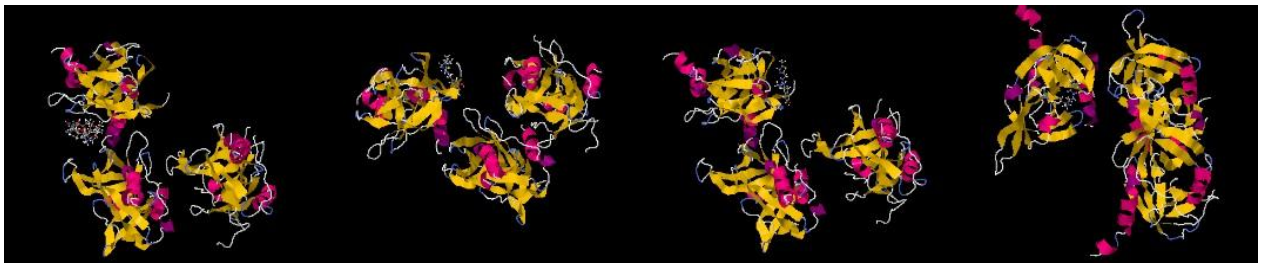
Moleculardockingwasusedtodeterminethefavorabilityoftheidentifiedcompoundsfrom PocketQueryasligandstoHTRA1.ThiswasdonethroughSwissDock,awebserverthat simulatesproteinliganddockingbasedontheprogramEADockDSS[38]. The results showed thattherewere multiple locations/pockets where the ligand could bind. There were also multiple conformationsthattheligandcouldbepresented.Thelocationandconformationcouldimpact thedeltagvalueoftheinteraction.ThelowestdeltaGvalueconsideringthelocationand conformationfromeachsimulatedprotein-ligandinteractionwasrecordedinthetable.A lower ormorenegativedeltaGvaluecorrelatestoamorefavorableproteinligandinteractionwhich means that the ligand has a higher binding ability to the protein.



ZINC36533601 ZINC15444140 ZINC15444133 ZINC92715070



ZINC93426091 ZINC67363835 ZINC19580940 ZINC94679710



ZINC85893874 ZINC85444486 ZINC81188012 ZINC94879818

ZINC27928090 ZINC27928791 ZINC75487197 ZINC06681836

ZINC34114798ZINC21992914

Fig.9ModelsofinteractionsbetweencompoundsidentifiedbyPocketQueryandbindingsiteofHTRA1usingSwiss Dock (PDBID:3TJN)

Table6.ΔGvaluesofinteractionsbetweencompoundsidentifiedbyPocketQueryandbindingsiteofHTRA1using SwissDockcomparingbindingfavorability(PDB ID:3TJN)

Molecule	Numberof bindingsites	EstimatedΔG(kcal/mol)
ZINC36533601	50	-9.64
ZINC15444140	37	-8.58
ZINC15444133	48	-9.06
ZINC92715070	43	-9.37
ZINC93426091	49	-10.77
ZINC67363835	48	-7.95
ZINC19580940	37	-8.16
ZINC94679710	35	-8.12
ZINC85893874	31	-8.74
ZINC85444486	42	-7.98
ZINC81188012	36	-8.39
ZINC94879818	40	-7.94

ZINC27928090	47	-9.68
ZINC27928791	40	-8.07
ZINC75487197	42	-8.62
ZINC06681836	47	-8.47
ZINC34114798	39	-10.62
ZINC21992914	32	-11.13

Outofthe18hitmolecules,thetop5moleculeswiththelowestdeltagvalueandthehighest bindingabilityhadestimatedΔG(kcal/mol)of-11.13,-10.77,-10.62,-9.68,-9.64.Thenumberof possible binding sites was shown to have little impact on the estimated ΔG value.

Table7.Top 5 moleculeswithmostfavorableinteractions

Molecule	Numberof bindingsites	EstimatedΔG(kcal/mol)
ZINC21992914	32	-11.13
ZINC93426091	49	-10.77
ZINC34114798	39	-10.62
ZINC27928090	47	-9.68
ZINC36533601	50	-9.64

3.4 SwissADMEDrugLikeness

FiveselectedligandsbasedonSwissDockwereinputtedintoSwissADMEtodeterminetheir theoreticaldruglikenesspropertiesforthegoalofdecidingwhetherthey would be viable small moleculedrugs.SwissADME(http://www.swissadme.ch/)isawebsitethat takes a SMILE codeordrawnchemicalstructureandpredictsitsphysicochemicalproperties,pharmacokinetics, drug-likenessandmedicinalchemistryfriendliness,estimating the success rate of the molecule asadrug[39].TheresultsofSwissADMEareshowninTable8.Threecompounds, ZINC93426091,ZINC27928090,andZINC36533601,wereshowntofollowLipinski'srule.Whendrugsaretakenorall y,thedrughastoremaininthebodyinsufficientconcentrationtobindtothetargetandachievethedesiredeffect.Thedrugha storemaininthebodythrough

theprocessesofabsorption,distribution,metabolism,andexcretion.Lipinski'srulehelpstopredictthis,requiringthatthe numberofhydrogen-bondacceptors≤10,numberof hydrogen-bonddonors≤5,molecularweight≤500g/mol,andacalculatedLogP (cLogP) ≤ 5 forsufficient absorption of the drug [40].Interestingly, ZINC21992914 with the lowest change inGibbsfreeenergydidnotfollowLipinski'srule.WaterSolubilityandGIabsorptionisalsoanimportantfactorinpredicti ngdruglikeness,withthemoleculebeingpreferablyatleast moderatelysolubleinwaterandhighGIabsorption.Thefivecompoundsallmeetthe requirementforwatersolubility.ZINC27928090andZINC36533601seempromising,fulfillingthequalificationsforap otentialdruginLipinski,watersolubility, and GI absorption. In addition,bothmoleculesarebloodbrainbarrier(BBB)permeant,whichisestimatedtoappearinonly 2%ofsmallmoleculedrugs[41].ThisiscrucialforpotentialHTRA1ligandsastheprotein resideswithintheBBB.Whilemoleculescanbemodifiedtoincrease BBBpermeability,thiswould shorten the drug synthesis process, making these molecules more advantageous.

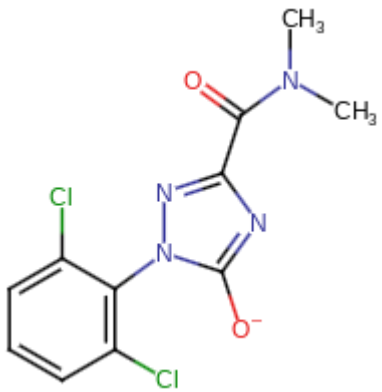


Fig.10Chemicalstructureofoneofthetopcompoundsfordruglikeness,ZINC36533601.

Table8.SwissADMEgeneratedruglikenesspropertiesofthetop5ligandswiththelowestΔGvalue

ZINCID	Molecula Weight(g/ mol)	Num. H-bondacceptor s	Num.H-bonddonors	LogPo/w(iLOGP)	Lipinski	Violati ns	WaterSolubilityo LogS(ESOL) Class	GI absorpto n	BBB permean t
ZINC2199	461.35	12	6	1.98	No	2	Soluble	Low	No

2914									
ZINC93426091	311.4	4	2	1.67	Yes	0	VerySoluble	Low	No
ZINC34114798	445.35	11	5	1.86	No	1	Soluble	Low	No
ZINC27928090	331.25	2	2	2.6	Yes	0	Soluble	High	Yes
ZINC36533601	300.12	4	0	2.25	Yes	0	Soluble	High	Yes

4. Conclusion

Alzheimer'sdiseaseisoneofthemostrapidlyexpandingdiseases,withestimatespredictingthat the affected population will triple to about 152 million people globally with dementia in 2050,withAlzheimer'scontributingto60–70%ofcases. The major indicators of AD are the formation ofamyloidplaques and neurofibrillary tangles, which inhibit brain functions. Preventing the build upofamyloidfragmentsandformationofamyloidplaquesto inhibit AD development has been thefocusoftheAmyloidHypothesisandADdrugdevelopmentinrecentyears.TheHTRA1 proteinhasshownpromiseinachievingthisgoal,withstudiesshowingitscorrelationof reducedamyloidaggregationanditsabilityto degrade fibrillartar, which is also associated withAD.HTRA1wasfirstscreenedforpotentialbindingsitesusingPrankWeb,FPocketWeb,andCavityPlus,with5from PrankWeb,43fromFPocketWeb,and23fromCavityPlus,withmorethanonebindingsitefromeachprogramshowingpromisingdruggabilityresults.Warrantingfurtherstudytoidentifypossibleligands,thesecondscreeninginvolvedPocketQuery conductingapharmacophorebasedvirtualscreening analyzing the druggability of binding sites ofHTRA1toaprotein(PBDcode:2JOA).5pharmacophoresmapswithascoreofover.85 weretheninputtedintoZINCPharmergenerating20compounds.SwissDockthendetermined thegibbsfreeenergyoftheidentifiedcompoundsbindingtoHTRA1,withZINC21992914, ZINC93426091,ZINC34114798,ZINC27928090,ZINC36533601havingthelowestgibbsfreeenergyvaluesandmost favorableinteractions.Thesecompoundswerethenscreenedby SwissADME,findingthatZINC93426091,ZINC27928090,ZINC36533601followLipinski'sruleandshowpromisingdruglikenessproperties.Interestingly,ZINC21992914withthemostfavorable interaction did not follow Lipinski's rule.

Limitationstothisstudyarethatresultsaretheoreticallycalculatedusingcomputerprograms andhavenotbeenverifiedphysically.Inaddition,theRMSDvaluesforcompoundsinthefirstclusterwerehigherthanrecommendedbeingover 0.35. There has also been a limited quantity ofcompoundstakenfromZINCPharmerwhiletherewereremoremoleculesthatmettheabove 1.35 mark due to the limited number of people working on this project. This project can be taken furtherbyphysicalscreeningofthe final compounds, testing if they successfully bind to HTRA1 inarealsetting.Further objectives if physical screening is successful could be testing the small molecule drugs in animal models.

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