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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DEVELOPMENT OF NEW CYCLOPHILIN D RECEPTOR INHIBITORS FOR THE TREATMENT OF MULTIPLE SCLEROSIS

MS HASTALIĞININ TEDAVİSİ İÇİN YENİ SİKLOFİLİN D RESEPTÖR İNHİBİTÖRLERİNİN GELİŞTİRİLMESİ

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ABSTRACT

Objective: *In this study, it was aimed to carry out computational studies for the development of new molecules for the inhibition of the cyclophilin D (CypD) receptor, which causes the disability of mitochondrial function in multiple sclerosis (MS) disease.*

Material and Method: *Pharmacophore modeling study was applied via the PharmaGist Web server to the CypD inhibitors detected by the literature search. According to the best pharmacophore models from PharmaGist, 80 molecules were obtained from the ZINCPharmer database, and in silico ADME/Toxicology analysis was applied to these molecules. Then, molecular docking was performed with the ligands that gave the best results as a result of ADME/Tox analyses with the Autodock Vina program.*

Result and Discussion: *ZINC00390492 molecule shows the best binding affinity and binding profile with both CypD, Sphingosine-1-phosphate receptor 1. It has been demonstrated that this molecule may be a lead molecule for the treatment of MS.*

Keywords: *Cyclophilin D, in silico, molecular docking, multiple sclerosis, pharmacophore modeling*

ÖZ

Amaç: *Bu çalışmada, multipl skleroz (MS) hastalığında mitokondriyal fonksiyon bozukluğuna neden olan siklofilin D (CypD) reseptörünün inhibisyonu için yeni moleküllerin geliştirilmesine yönelik hesaplamalı çalışmaların yapılması amaçlanmıştır.*

Gereç ve Yöntem: *Literatür taraması ile tespit edilen CypD inhibitörlerine PharmaGist Web sunucusu üzerinden farmakofor modelleme çalışması uygulanmıştır. PharmaGist'in en iyi farmakofor modellerine göre*

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ZINCPharmer veri tabanından 80 molekül elde edilmiş ve bu moleküllere in silico ADME/Toksikoloji analizi uygulanmıştır. Daha sonra Autodock Vina programı ile ADME/Tox analizleri sonucunda en iyi sonucu veren ligandlar ile moleküler yerleştirme yapılmıştır.

Sonuç ve Tartışma: *ZINC00390492 molekülü hem CypD, hem de Sfingosin-1-fosfat reseptörü 1 ile en iyi bağlanma afinitesi ve bağlanma profilini göstermektedir. Bu molekülün, MS tedavisi için bir öncü molekül olabileceği gösterilmiştir.*

Anahtar Kelimeler: *Farmakofor modelleme, in silico, moleküler doking, multipl skleroz, siklofilin D*

INTRODUCTION

Multiple sclerosis (MS) is thought to be one of the main neurological diseases observed in young adults, as well as approximately 3 million people worldwide. In MS, which is a chronic and inflammatory disease that occurs in the central nervous system, the most important difference between a healthy neuron and MS neuron is demyelination. [1]. Clinical symptoms of MS include fatigue, motor dysfunction, nystagmus, loss of coordination or balance, acute paralysis, lethargy, speech and vision disorders, and cognitive impairment [2].

Mitochondrial damage known to be caused by reactive oxygen and nitrogen derivatives is the most common pathology in MS and causes chronic inflammation.[3]. Studies are carried out to elucidate the pathogenesis of MS using experimental autoimmune encephalomyelitis (EAE) animal models that mimic the characteristics of the disease.

Although it is known that demyelination in axons is of great importance in the development of physical disability, the underlying mechanism is not fully known. The relationship between mitochondrial dysfunction and axon demyelination has been examined in the literature, and it has been reported that a series of ionic imbalances lead to energy deficiencies that cause Ca^{2+} overload may be caused [4]. Ca^{2+} accumulation in mitochondria in healthy neurons stimulates oxidative metabolism. Ca^{2+} influx from the mitochondria is mediated by various transporters, and Ca^{2+} influx is directed by the activation of a protein complex called the permeability transition pore (PTP). Overloading of mitochondria with Ca^{2+} causes inappropriate activation of PTP, resulting in impaired mitochondrial functions and cell death.

The main marker of mitochondrial damage is mitochondrial permeability transition (mPT). The permeability of the mitochondrial inner membrane increases and begins to pass all molecules less than 1500 Da non-selectively. cyclophilin D (CypD), which has a great effect on permeability by interacting with the mitochondrial inner membrane, is a peptidyl-prolyl isomerase found in the mitochondrial matrix [5]. CypD, which has an important role in the regulation of mitochondrial PTP, binds to PTP and facilitates permeability [6, 7]. Therefore, inhibition of CypD prevents mitochondrial and synaptic damage caused by $A\beta^{-}$ and oxidative stress and restores mitochondrial and cognitive functions.

Cyclophilins; It is a family of proteins found in mammals, plants, bacteria, and many other living

organisms. Cyclophilins are known to be involved in various processes such as protein folding [8], receptor signaling, and cellular response [9]. In addition, cyclophilins have become therapeutic targets because of their critical roles in inflammation [10], viral infections [11], vascular dysfunction [12], wound healing [13], and cancer progression [14].

There are 19 different isoforms of cyclophilin that are highly similar [15]. Among human cyclophilins, 5 isoforms are known [16, 17, 18]. One of these isoforms, CypD, is found in the mitochondrial matrix. CypD is involved in the modulation of mitochondrial permeability transition pores (mPTP) and affects mitochondrial function. CypD is thought to control PTP opening by sensitizing mPTP to calcium, inorganic phosphate, and reactive oxygen species. Opening of mPTP causes a rapid influx of cytosolic contents into the mitochondrial matrix thereby impairing mitochondrial respiration. CypD deficiency inhibits mPTP opening induced by excess Ca^{2+} and thus protects cells from oxidative stress-induced cell death in various disease models [19]. Blocking CypD did not alter responses to other PTP stimuli such as adenine nucleotides, membrane depolarization, ubiquinones, pH [20, 21].

One study developed experimental autoimmune encephalomyelitis (EAE) mice with MS lacking CypD; these mice appeared to recover partially, in contrast to the mice with CypD. Examining the spinal cords of both groups of mice revealed that the axons of the mice lacking CypD were preserved, despite a similar degree of inflammation. In addition, neurons of mice lacking CypD were found to be resistant to reactive oxygen and reactive nitrogen species. In addition, CypD-deficient brain mitochondria was found to sequester high Ca^{2+} levels [22]. Another study showed that the absence of CypD does not affect energy conservation and ATP synthesis [23].

CypD, a molecular target in mPTP, can be inhibited by cyclosporine A (CsA). However, toxicity due to long-term use of CsA prevented its use as MS treatment [24]. When molecules for inhibition of mPTP are screened in the literature, the molecule with the highest specificity is CypD (PPIase) inhibitor CsA. However, CsA lacks clinical significance due to its immunosuppressive effect by inhibiting calcinuria and its inability to cross the blood-brain barrier [16]. Antamanide (AA) and Sanglifehrin A (SfA) are produced for inhibition of mPTP, but they are insufficient to be drug molecules due to serious side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity [25, 26]. Other molecules with known CypD inhibitory properties have low solubility and high toxicity, as well as low blood-brain barrier and cell permeability. Considering all these reasons, CypD is an important target for drug development studies for the treatment of MS disease.

Sphingosine-1-phosphate receptor 1 (S1P1) is a G protein-coupled receptor. It is active in an important mechanism that leads to the emergence and progression of MS symptoms. This makes S1P1 an important target for drug development studies. Although there are preclinical and clinical studies on S1P1, no modulators with high selectivity have been found in the literature. Within the scope of the

study, which is the subject of this article, it was aimed to develop a multi-target drug by examining the effectiveness of molecules with cyclophilin D inhibitory properties on S1P1 using computational studies.

MATERIAL AND METHOD

Pharmacophore Modelling

Various molecules that inhibit cyclophilin D were determined by searching the literature. The molecules were loaded into the PharmaGist [27] Web tool, and pharmacophore analysis was performed. Conformational analysis was not implemented during the pharmacophore analysis process. The hypotheses generated by PharmaGist were uploaded to the ZINCPharmer [28] web tool to find new molecules with similar properties.

In Silico ADME/Toxicology Analysis

Absorption, Distribution, Metabolism, Excretion (ADME), and toxicology assessment were performed using SwissADME [29] for a total of 80 ligand molecules. In ADME-Toxicology evaluation, ligands were determined by Lipinski's rules, molecular weight, logP values, H-bond acceptor, donor numbers, CYP2D6 inhibition, and blood-brain barrier properties were investigated. It was determined that 3 of the ligand molecules that conflicted with Hypothesis 2 and 7 of the molecules that conflicted with Hypothesis 4 met the appropriate ADME-Toxicology conditions out of a total of 80 ligands.

Preparation of Receptor and Ligands

The 3D structure (PDB ID: 2Z6W) of the CypD protein in complex with CsA was selected from the RCSB PDB Protein database [30]. The 3D receptor protein was isolated from *Homo sapiens* and *Tolypocladium inflatum*, expressed in the *Escherichia coli* BL21(DE3) expression system. The crystal structure of the protein was determined using the X-Ray Diffraction Crystallography method and has a resolution of 0.96 Å. The protein downloaded from the Protein Data Bank is in dimer form. Using the Discovery Studio Visualizer 2020 [31] program, the antagonist (CsA) in 3D structure was separated, water and other residual molecules were deleted, the antagonist was also recorded. Polar hydrogens and Gasteiger charges were added to the protein structure using the AutoDock Tools [32] package, the receptor protein was prepared in PDBQT format for AutoDock Vina.

The molecules were downloaded in sdf format via the link in the ZincPharmer database. Then H atoms were added to select 10 ligand molecules with the appropriate conditions using the Discovery Studio Visualizer 2020 program, and the ligands were corrected with the "clean geometry" option. Then ligands were edited with AutoDock Tools in PDBQT format and recorded.

Molecular Docking

Molecular docking studies were performed with ligands in Table 1 using the AutoDock Vina [33] program. A grid box was created with AutoDock Tools for the docking area. The x, y, z values of the grid box are 30, 46, 56 respectively; The x, y, z centers of the grid box were set to -13,812, -5.25, -30,799, respectively. Docking of 10 ligands with CyPD receptor protein was performed. The result files obtained as a result of molecular docking of 10 different ligand molecules with the receptor protein were analyzed in AutoDock Tools. In addition, molecular docking of these 10 ligands was performed with the sphingosine 1-phosphate receptor (PDB ID:3V2Y).

Table 1. Features of selected compounds for molecular docking

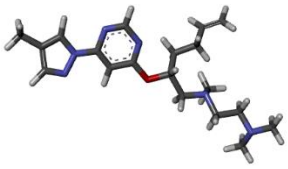
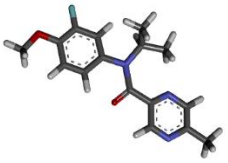
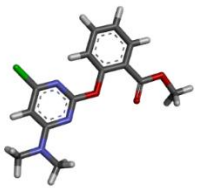
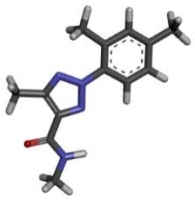
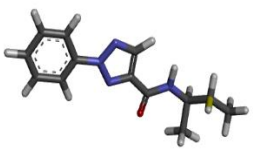
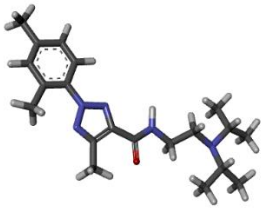
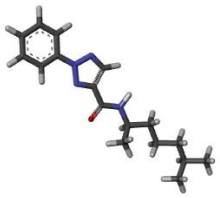
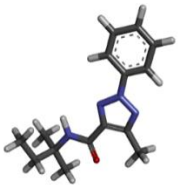
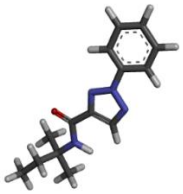
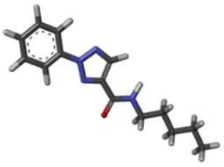
Compound	IUPAC Name	3D Structure
ZINC72474618	*N*,*N*,*N*'-trimethyl-*N*'-[2-[6-(4-methylpyrazol-1-yl)pyrimidin-4-yl]oxyhex-5-enyl]ethane-1,2-diamine	
ZINC60353659	*N*-(3-fluoro-4-methoxyphenyl)-5-methyl-*N*-propan-2-ylpyrazine-2-carboxamide	
ZINC01496377	Methyl 2-[4-chloro-6-(dimethylamino)pyrimidin-2-yl]oxybenzoate	
ZINC09603787	2-(2,4-dimethylphenyl)-*N*,5-dimethyltriazole-4-carboxamide	
ZINC32674515	*N*-butan-2-yl-2-phenyltriazole-4-carboxamide	
ZINC09950012	2-(2,4-dimethylphenyl)-*N*-[2-[di(propan-2-yl)amino]ethyl]-5-methyltriazole-4-carboxamide	

Table 1 (*continued*). Features of selected compounds for molecular docking

Compound	IUPAC Name	3D Structure
ZINC23228074	*N*-(6-methylheptan-2-yl)-2-phenyltriazole-4-carboxamide	
ZINC36719024	5-methyl-*N*-(2-methylbutan-2-yl)-2-phenyltriazole-4-carboxamide	
ZINC44514282	*N*-(2-methylbutan-2-yl)-2-phenyltriazole-4-carboxamide	
ZINC17322385	*N*-pentyl-2-phenyltriazole-4-carboxamide	

RESULT AND DISCUSSION

Pharmacophore Modelling

Based on the number of high scores and high feature matching (hydrophobicity, aromaticity, hydrogen bond acceptor, hydrogen bond donor, negative ionization) by pharmacophore analysis, 4 different hypotheses were selected (Table 2).

Table 2. Hypotheses and properties obtained as a result of pharmacophore modeling

Hypo	Score	Mols	Features	Aromatic	Hydrophobic	H-Donors	H-Acceptors	Negatives	Positives
1	33.07	3	7	2	0	1	4	0	0
2	31.18	14	4	2	0	0	2	0	0
3	31.75	4	6	2	0	1	3	0	0
4	31.75	4	6	2	0	1	3	0	0

The hypotheses and their features are given in Figure 1. In the figure, the hydrogen bond acceptor feature is shown in pink, the negative ionization feature is shown in gray, and the feature of having a ringed aromatic structure is shown in blue.

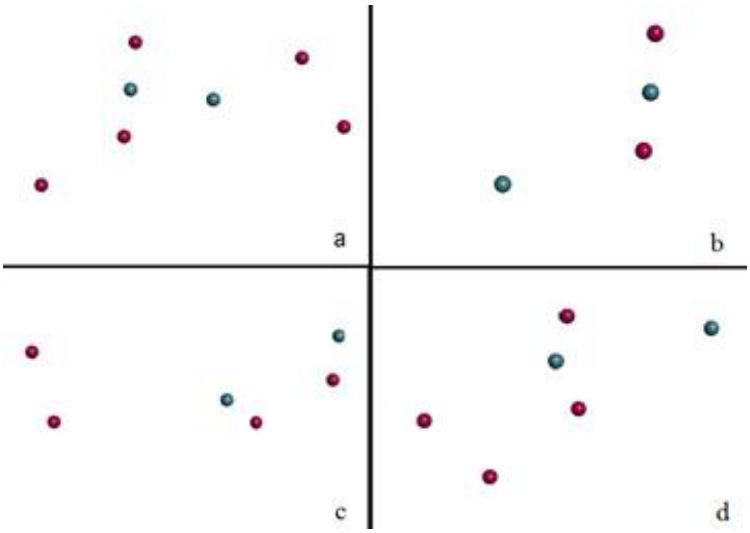


Figure 1. Features of Hypothesis 1 (a), Hypothesis 2 (b), Hypothesis 3 (c), and Hypothesis 4 (d)

The hypothesis were uploaded to the ZINCPharmer web tool to find new molecules with similar properties. Molecules were selected considering low RMSD value, low molecular weight, and a high number of rotatable bonds (Rbnds). 20 molecules for each of the 4 hypothesis were selected and recorded (Table 3).

***In Silico* ADME/Toxicology Analysis**

ZINC72474618, ZINC60353659, and ZINC01496377 molecules that conflict with Hypothesis 2 and ZINC09603787, ZINC32674515, ZINC09950012, ZINC23228074, ZINC36719024, ZINC44514282, and ZINC17322385 molecules that conflict with Hypothesis 4 was determined to meet the ADME-toxicity requirements.

For a molecule to provide drug properties, it must obey the Lipinski rules. According to Lipinski rules; the molecular weight should be less than 500 Da, the mLogP value should be less than 4.15, the number of hydrogen bond acceptors should be less than 10 and the number of hydrogen bond donors should be less than 5. It was determined that ZINC72474618, ZINC60353659, ZINC01496377, ZINC09603787, ZINC32674515, ZINC09950012, ZINC23228074, ZINC36719024, ZINC44514282 and ZINC17322385 molecules obey Lipinski conditions. TPSA value of less than 30 is a desirable feature. TPSA values of these molecules; 59.31 for ZINC72474618, 55.32 for ZINC60353659, 64.55 for ZINC01496377, 59.81 for ZINC09603787, ZINC32674515, ZINC23228074, ZINC36719024, ZINC4451422382, and ZINC445173223, 63.05 for ZINC09950012 (Table 4).

Table 3. Selected 20 molecules for each of the 4 hypothesis

Pharmacophore	RMSD	Molecular Weight	Rbnds	ZINC No	RMSD	Molecular Weight	Rbnds	ZINC No
Hypothesis 1	0.309	411	6	05260168	0.132	369	8	06818708
	0.137	381	8	06733121	0.124	383	9	06818722
	0.146	341	6	06733126	0.139	409	7	09027329
	0.137	397	10	06733127	0.147	327	5	09066439
	0.138	355	7	06733131	0.136	431	8	09599121
	0.136	369	8	06733132	0.136	446	9	09599123
	0.148	381	5	09166421	0.147	343	6	13552014
	0.128	417	7	06733134	0.145	448	7	17046011
	0.137	424	7	06733137	0.146	357	6	20151896
	0.138	341	6	06818697	0.131	356	5	01228678
Hypothesis 2	0.024	383	7	05333824	0.071	354	10	16000765
	0.073	389	8	07979410	0.361	308	8	01496377
	0.038	361	10	32675907	0.162	344	8	02488877
	0.460	359	15	72474618	0.118	391	7	04979909
	0.059	303	9	60353659	0.402	354	9	13207213
	0.059	311	9	58180599	0.385	377	7	31766509
	0.037	385	11	48401200	0.400	323	9	37868558
	0.055	350	9	34865485	0.038	335	8	15778627
	0.030	415	8	32682599	0.075	375	7	15317824
	0.049	337	10	30007860	0.038	317	7	08339390
Hypothesis 3	0.500	323	6	02157976	0.447	314	6	72229631
	0.279	480	9	02701874	0.336	388	7	08670100
	0.428	351	7	04906283	0.336	414	10	27756652
	0.428	409	11	04906311	0.332	297	6	32541243
	0.336	413	10	08584529	0.428	405	6	04906324
	0.382	346	5	69776578	0.279	442	11	34932032
	0.450	325	5	78619622	0.382	404	8	71281426
	0.279	474	9	15229604	0.382	374	6	71283848
	0.455	364	5	00801224	0.445	314	6	72229628
	0.336	370	7	27756603	0.382	374	6	71287695
Hypothesis 4	0.378	235	7	01686721	0.239	346	12	31820955
	0.248	319	10	04721581	0.415	371	13	32931677
	0.419	244	5	09603787	0.284	316	10	40162051
	0.278	244	5	32674515	0.417	272	7	36719024
	0.234	247	7	12406819	0.280	258	6	44514282
	0.418	359	13	09950012	0.221	402	8	45946126
	0.383	274	7	20729515	0.278	258	6	17322385
	0.239	318	10	20736887	0.241	319	10	20737156
	0.239	272	6	20745632	0.280	357	10	65010034
	0.279	300	9	23228074	0.285	394	11	71812077

Table 4. Hypotheses and properties obtained as a result of pharmacophore modeling

Compound (ZINC code)	Lipinski	TPSA	Consensus Log (Po/w)	Bioavailability Score	GI absorption	BBB permeant	P-gp substrate	CYP2D6 inhibitor	Log Kp (cm/s)
72474618	Yes	59.31	2.54	0.55	High	Yes	No	No	-6.51
60353659	Yes	55.32	2.60	0.55	High	Yes	No	No	-6.45
01496377	Yes	64.55	2.63	0.55	High	Yes	No	No	-5.81
09603787	Yes	59.81	2.08	0.55	High	Yes	No	No	-5.92
32674515	Yes	59.81	2.14	0.55	High	Yes	No	No	-5.77
09950012	Yes	63.05	3.42	0.55	High	Yes	No	No	-5.44
23228074	Yes	59.81	3.29	0.55	High	Yes	No	No	-4.92
36719024	Yes	59.81	2.74	0.55	High	Yes	No	No	-5.53
44514282	Yes	59.81	2.38	0.55	High	Yes	No	No	-5.73
17322385	Yes	59.81	2.53	0.55	High	Yes	No	No	-5.53

All of these 10 ligand molecules can cross the blood-brain barrier. The consensus LogPo/w value represents lipophilic behavior, and the value is expected to be less than 5. Consensus LogPo/w value 2.54 for ZINC2474618, 2.60 for ZINC60353659, 2.63 for ZINC01496377, 2.08 for ZINC09603787, 2.14 for ZINC32674515, 3.42 for ZINC09950012, 3.29 for ZINC23228074, 2.74 for ZINC36719024, 2.38 for ZINC44514282, 2.53 for ZINC17322385 (Table 4). The bioavailability score must be higher than 0.1, all ten molecules have a bioavailability score of 0.55. Gastrointestinal absorption (GI) of all molecules is high. The P-glycoprotein substrate (P-gp) property indicates that the molecule can attach to proteins, and this leads to reduced absorption in the gastrointestinal tract; none of the ligands has P-gp property. Cytochrome P (CYP) enzymes affect liver functions, they should not be inhibited. ZINC72474618 and ZINC09603787 molecules inhibit one CYP enzyme, ZINC60353659, ZINC32674515, ZINC09950012, ZINC44514282 molecules inhibit two each, ZINC01496377, ZINC23228074, ZINC367190285, ZINC1732 molecules each. None of the molecules inhibits the CYP2D6 enzyme.

Molecular Docking

RMSD values and binding affinities obtained as a result of molecular docking with CyPD of ligands meeting ADME/toxicity conditions were examined. The initial binding mode of the CyPD receptor protein with its original ligand, CsA, was calculated as -7.1 kcal/mol. Initial binding modes of ligands; -6.9 for ZINC72474618, -7.6 for ZINC60353659, -7.2 for ZINC01496377, -7.6 for ZINC09603787, -7.0 for ZINC32674515, -7.4 for ZINC09950012, -8.0 for ZINC23228074, -7.0 for ZINC36719024, -7.2 for ZINC44514282, -7.4 kcal/mol for ZINC17322385 (Table 5). It is seen that the binding affinities of these ligands ZINC60353659, ZINC01496377, ZINC09603787, ZINC09950012, ZINC23228074, ZINC44514282, and ZINC17322385 molecules are better than the original antagonist, and the binding affinity of the ZINC23228074 molecule is the best score with –8.0 kcal/mol.

Considering the RMSD values of these ligands in the best 3 binding modes; ZINC09603787, ZINC36719024, ZINC23228074, and ZINC44514282 molecules seem to have RMSD values less than 2 in the majority. When the RMSD values and binding affinities obtained as a result of molecular docking of the S1P1 receptor protein with these 10 ligands are examined; The affinities of ZINC32674515, ZINC23228074, ZINC36719024, and ZINC44514282 molecules in the initial binding modes were calculated as -7.8 kcal/mol, the best score among 10 ligands. Looking at the RMSD values; ZINC32674515, ZINC23228074, ZINC44514282, and ZINC17322385 molecules seem to have RMSD values less than 2 in the majority. Considering these results, it can be said that the best affinity score and RMSD values were obtained as a result of molecular docking of ZINC23228074 and ZINC44514282 ligand molecules with both CyPD and S1P1 receptor proteins.

Molecular docking of CyPD receptor protein was performed with 4 ligands, which were molecularly docked with S1P1 receptor protein in our previous study [34]. When the binding affinities and RMSD values obtained as a result of this are examined; The affinity value of the ZINC00390492 molecule in the first binding mode was calculated as -8.0 kcal/mol, and low RMSD values were obtained as desired. As a result of molecular docking of ZINC00390492 with S1P1, the initial binding mode was calculated as -8.6 kcal/mol and appropriate RMSD values were obtained. Considering these results, it can be said that the ZINC00390492 molecule is an antagonist compatible with both S1P1 and CyPD receptor proteins.

Table 5. Hypothesis and properties obtained as a result of pharmacophore modeling

Compound	S1P1		CyPD	
	Mode	Affinity (kcal/mol)	Mode	Affinity (kcal/mol)
ZINC72474618	1	-6.9	1	-6.9
ZINC60353659	1	-7.3	1	-7.6
ZINC01496377	1	-7.5	1	-7.2
ZINC09603787	1	-7.2	1	-7.6
ZINC32674515	1	-7.8	1	-7.0
ZINC09950012	1	-7.3	1	-7.4
ZINC23228074	1	-7.8	1	-8.0
ZINC36719024	1	-7.8	1	-7.0
ZINC44514282	1	-7.8	1	-7.2
ZINC17322385	1	-7.7	1	-7.4
Inhibitor (CsA)			1	-7.1
ZINC00390492	1	-8.6	1	-8.0
ZINC67740009	1	-7.6	1	-7.7
ZINC19847253	1	-6.3	1	-7.0
ZINC19847241	1	-6.8	1	-6.9
Inhibitor (ML5)	1	-8.4		

When the interactions of ZINC32674515 with the receptor were examined in AutoDock Tools,

the ligand appears to interact with Gly75, Arg82, Asn102, Thr107, Gly109, and Gln111 amino acid residues in Van der Waals. When the interactions of the ZINC32674515 molecule with the receptor are examined in Discovery Studio Visualizer; It is seen that the ligand has alkyl interaction with Met51 and Ala101, *pi-alkyl* interaction with Trp121 and Lys125, *pi-cation* interaction with Arg55, conventional hydrogen bond interaction with His126, and *pi-pi stacked* interaction with Phe113 (Figure 2a).

When the interactions of ZINC23228074 with the receptor are examined in AutoDock Tools, the ligand appears to hydrogen bond with Asn102 and Van der Waals interaction with amino acid residues Arg55, Phe60, Met61, Gly74, Gly75, Gln83, Ala101, Ala103, Phe113, Leu122, and His126. When the interactions of the ZINC23228074 molecule with the receptor are examined in the Discovery Studio Visualizer program, the ligand has *pi-donor* hydrogen bond interaction with Gln111, conventional hydrogen bond interaction with Asn102, *pi-alkyl* interaction with Ala101, alkyl interaction with Ala101, Phe113, and His126 (Figure 2b).

When the interactions of ZINC36719024 with the receptor were examined in AutoDock Tools, the ligand appears to interact with Gly75, Lys76, Ser81, Arg82, Gly109, and Gln111 amino acid residues in Van der Waals. When the interactions of the ZINC36719024 molecule with the receptor are examined in Discovery Studio Visualizer; It is seen that *pi-sulfur* interaction with Met61, conventional hydrogen bond interaction with Gln63, *pi-alkyl* interaction with Ala101, *pi-pi cluster* interaction with Phe113, and unfavorable donor-donor interaction with Arg55 (Figure 2c).

When the interactions of ZINC44514282 with the receptor were examined in Autodock Tools, the ligand generates hydrogen bonds with Thr73 and Van der Waals interaction with amino acid residues Asn69, Gly75, Lys76, Ala101, and Gln111. When the interactions of the ZINC44514282 molecule with the receptor are examined in the Discovery Studio Visualizer program, Met61, Ala101, and Trp121 residues interact with *pi-alkyl*, Arg55 with *pi-cation* interaction, and His126 interact with conventional hydrogen bonds (Figure 2d).

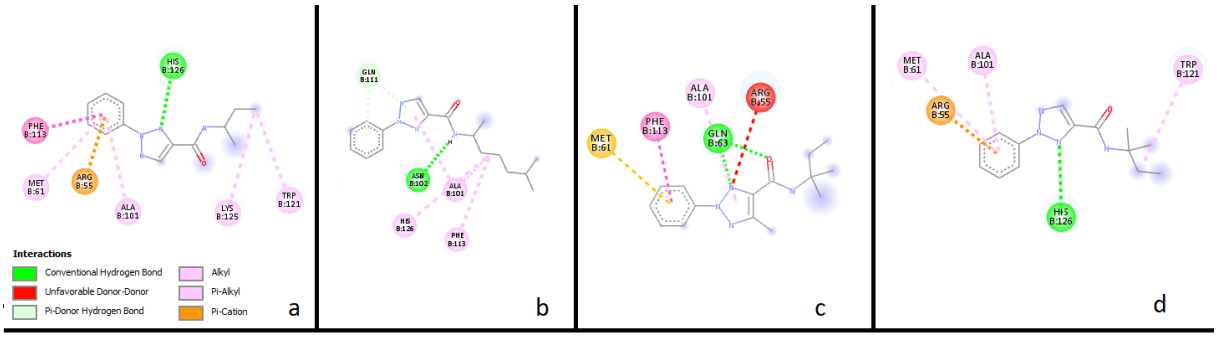


Figure 2. Binding profiles of the ligands with best binding affinity values (a) ZINC32674515, (b) ZINC23228074, (c) ZINC36719024, and (d) ZINC44514282

When the interactions of ZINC00390492 with CypD were examined in AutoDock Tools, the

ligand makes hydrogen bonds with Gly109 and Ser110 amino acid residues, and Van der Waals interaction with Gly74, Gly75, Arg82, Gly109, Ser110, and Gln111 amino acid residues. When the interactions of ZINC00390492 molecule with CypD are examined in Discovery Studio Visualizer; It is observed that Pro105 and Lys125 interact with *pi-alkyl*, with Asn102 and Lys125 halogen interaction, His126 with *pi-sigma* interaction and *pi-pi T-shaped* interaction, with Lys125 *pi-cation* interaction, and with Arg55 conventional hydrogen bond interaction (Figure 3).

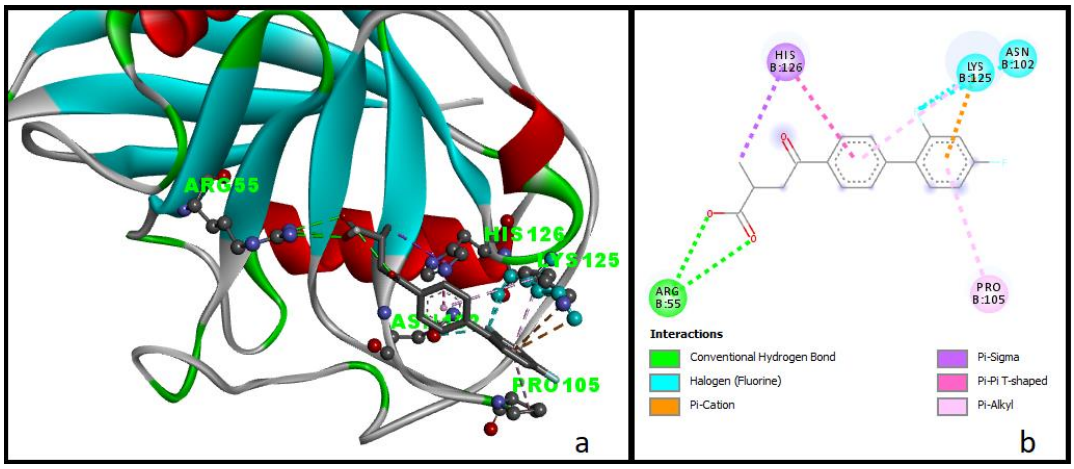


Figure 3. Binding profiles of the ZINC00390492 a) 3D structure view b)2D structure view

The superposition of ZINC00390492 with hypothesis 2 is given in Figure 4. Accordingly, it is seen that the molecule overlaps with the 1 ring aromatic feature, as well as the 2 hydrogen bond acceptor feature (green squares).

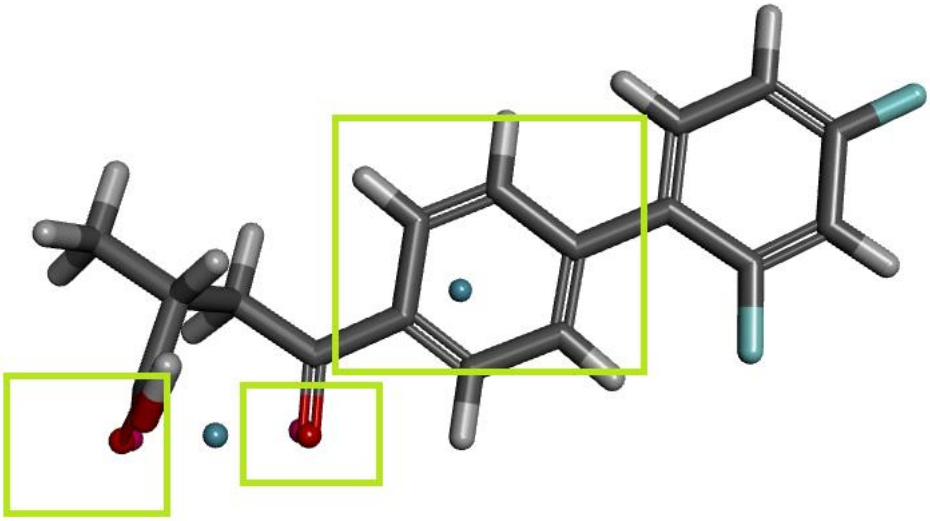


Figure 4. Superposition of ZINC00390492 and hypothesis 2. Features can be seen inside the light green squares.

In conclusion, the ZINC00390492 molecule shows the best binding affinity and binding profile with both CypD, S1P1. It has been demonstrated that this molecule may be a lead molecule for the treatment of MS disease.

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AUTHOR CONTRIBUTIONS

Concept: *G.Y.O.*; Design: *G.Y.O.;* Control: *G.Y.O.;* Sources: *G.Y.O., B.H.;* Materials: *G.Y.O., B.H.;* Data Collection and/or processing: *B.H.;* Analysis and/or interpretation: *G.Y.O., B.H.;* Literature review: *B.H.;* Manuscript writing: *G.Y.O., B.H.;* Critical review: *G.Y.O.;* Other: *G.Y.O.*

CONFLICT OF INTEREST

The authors declare that there is no apparent financial or personal conflict of interest affecting the work of this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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