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Synthesis, Molecular Docking and Molecular Dynamics Simulation Studies of Some Pyridazinone Derivatives as Lipase Inhibitors

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Research Article

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ABSTRACT

Human health and illness are dependent on lipases, which play a key role in maintaining cell integrity, storing fat for energy and serving as signaling molecules. In this study, 4 compounds that carry 6-phenylpyridazin-3(2*H*)-one main nucleus, which can be effective as lipase inhibitors, were synthesized and their structures were elucidated. The biological activity of synthesized compounds was evaluated via the porcine pancreatic lipase type II (PLL) inhibitor assay. Orlistat, a lipase inhibitor, was used as a positive control. Compound **8d** was found to be the most effective compound, with an IC_{50} value of 32.66±2.8265 (µg/mL). In addition, molecular docking and molecular dynamics simulations studies were carried out to examine the interactions of the compounds with the target in detail. The results obtained as a result of these in silico studies were found to be compatible with the lipase inhibition effects of the compounds. It was observed that the compounds may have potential lipase inhibitory effects as a result of the substitutions of the 3-(6-oxo-3-phenylpyridazin-1(6*H*)-yl)propanehydrazide structure.

Keywords: Pyridazinone, Molecular docking, Molecular dynamics simulations studies, Lipase inhibition.

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Introduction

Lipids play an important role in regulating the physicochemical properties and functions of cellular membranes, membrane repair, and cellular signaling. It is an efficient energy storage unit. In particular, they play a critical role in the metabolism of drug molecules. Changing the amounts of various lipid species by activating or deactivating their biosynthetic or degradation processes has been demonstrated to be either beneficial or harmful [1].

Lipases have an important role in both human health and disease. Lipases operate in dietary lipid digestion, transport, and processing. Lipases also hydrolyze a range of lipid substrates to control membrane integrity, lipid signaling, and the creation and dynamics of lipid rafts [2]. Most lipases are members of the serine hydrolase superfamily, which uses the nucleophilic active-site serine catalyze the hydrolysis of various lipid substrates. Because of the common biochemistry across this enzyme class, as well as particular chemical scaffolds that target serine hydrolases, various lipase inhibitors have been developed [3,4].

Pyridazinone derivatives have been suggested to offer such intriguing bioactivity as an antitumoral, antibacterial-antifungal, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, and anticancer impact [5-12]. Also, many of the pyridazinone derivatives have been synthesized by our research group as an inhibitor of cholinesterases which are from the serine hydrolase enzyme family [13-17]. It has been reported that compounds with heterocyclic ring containing nitrogen and oxygen atoms, such as oxadiazolone, benzo[1,3]oxazinon and benzoxazole, inhibit PLL. [18-20]. Cetilistat, which has a benzo[1,3]oxazin-4-one structure, inhibits pancreatic lipase, and is used in the clinic. In addition, it has been reported that compounds containing benzylidenehydrazide and thiosemicarbazide group (Figure 1) have significant lipase inhibition effects [21-23].

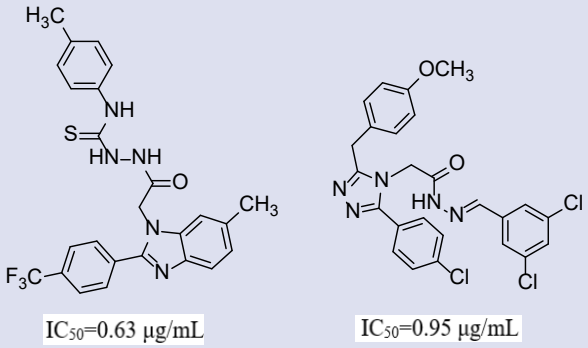


Figure 1. Pancreatic lipase inhibitors with IC_{50} value

In the light of this information, four pyridazinone derivative compounds (8a-d) were designed, synthesized and their lipase inhibitory properties were investigated with the MTT assay. Molecular docking simulations were employed to determine the compounds to interactions to

the targeted enzyme. By comparing the outcomes of in vitro enzyme inhibition and molecular docking investigations, the relationships between structure and activity was established [24].

Materials and Methods

Chemistry

Aldrich, Fluka, and Emanuel Merck supplied all of the compounds employed in this investigation. Scheme 1 entails the synthesis of 4-oxo-4-phenylbutanoic acid (3), 6-(p-tolyl)-4,5-dihydropyridazin-3(2H)-one (4), 6-(p-tolyl)pyridazin-3(2H)-one (5), ethyl 3-(6-oxo-3-(p-tolyl)pyridazin-1(6H)-yl In this study, **8b** and **8d** were synthesized for the first time. TLC on Merck Kieselgel F254 plates was used to track the progression of the reaction. Using the Electrothermal 9200 melting points device, the melting points were ascertained. The structures of these pyridazinone derivatives were confirmed by ^{1}H-NMR and ^{13}C-MNR spectra obtained with a Bruker avance 300 MHZ NMR spectrometer at İBTAM (İnonu University) and mass spectra obtained with an Agilent LC/MS instrument.

Synthesis of Compounds

Synthesis of 6-(p-tolyl)pyridazin-3(2H)-one (5)

Glyoxylic acid (2) (0.05 mol) and 4-methylacetophenone (1) (0.15 mol) were heated for 2 hours at 100°C. The reaction mixture was cooled to 40 °C at the conclusion of this interval, and then 20 mL water and 5 mL ammonium hydroxide solution (25%) were added to the reaction mixture until the medium pH reached 8. The reaction mixture was then extracted using dichloromethane. The separated aqueous layer was treated with hydrazine hydrate (0.05 mol), and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature when the reaction was completed. The precipitate that formed was filtered to yield chemicals [13,14].

Synthesis of ethyl 3-(6-oxo-3-(p-tolyl)pyridazin-1(6H)-yl)propanoate (6)

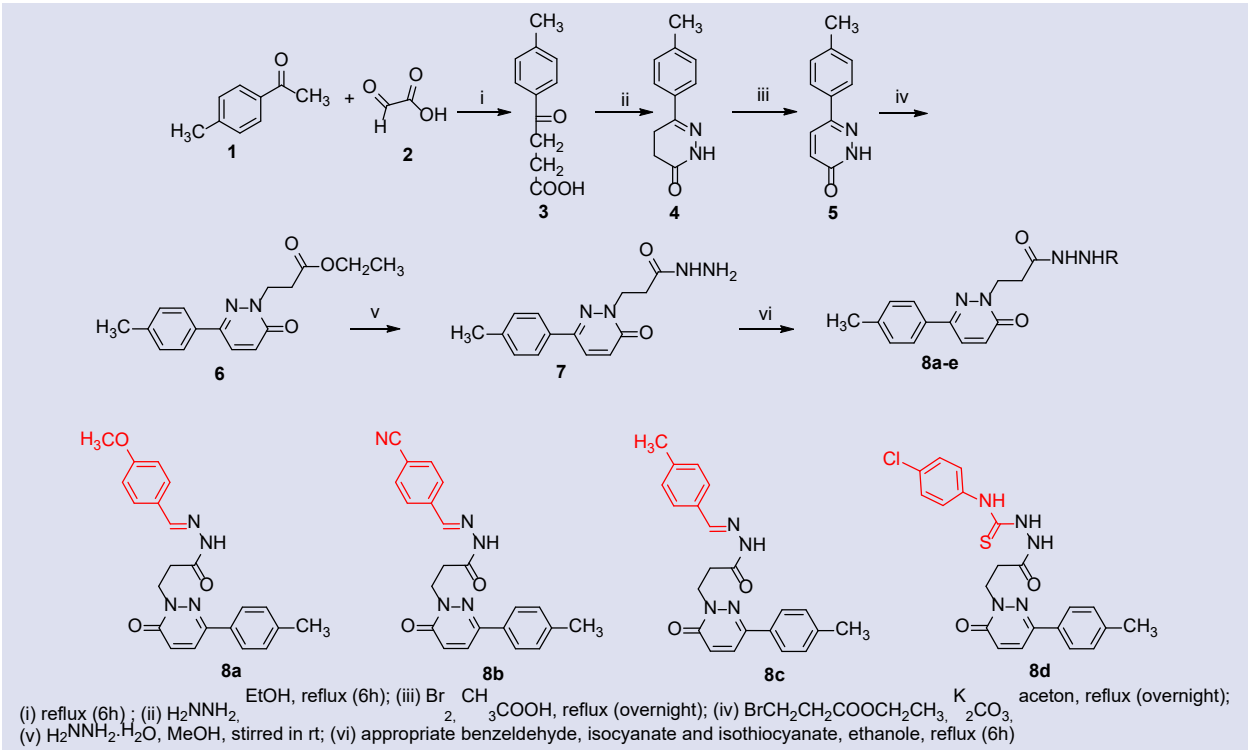
Overnight in acetone (40 mL), 0.01 mol 6-(p-tolyl)pyridazin-3(2H)-one (5), 0.02 mol (2.2252 mL) ethyl bromopropionate, and 0.02 mol (2.7636 g) potassium carbonate were refluxed. After cooling, the organic salts were filtered out, the solvent was removed, and the residue was refined by recrystallization with methanol to get the esters [15].

Synthesis of 3-(6-oxo-3-(p-tolyl)pyridazin-1(6H)-yl)propanehydrazide (7)

Hydrazine hydrate (99%, 3 mL) was added to a 25 mL methanol solution containing 0.01 mol ethyl 3-(6-oxo-3-(p-tolyl)pyridazin-1(6H)-yl)propanoate (6) and stirred for 3 hours at room temperature. The resulting precipitate was filtered, washed with water, dried, and recrystallized from ethanol [16,17].

General procedure for the title compounds (8a-d)

In ethanol (15 mL), 0.01 mol of 3-(6-oxo-3-(p-tolyl)pyridazin-1(6H)-yl)propanehydrazide (7) and 0.01 mol of substituted benzaldehyde/substituted phenylisocyanate/substituted phenylisothiocyanate were mixed. refluxed for 6 hours; at the conclusion of the reaction The precipitate from methanol/water was filtered, dehydrated, and crystallized [16,17]. Compounds 8a and 8c were previously synthesized by our research team [15].



(i) reflux (6h) ; (ii) H_2NNH_2, EtOH, reflux (6h); (iii) Br_2CH_3COOH, reflux (overnight); (iv) $BrCH_2CH_2COOCH_2CH_3$, K_2CO_3, aceton, reflux (overnight);
(v) $H_2NNH_2.H_2O$, MeOH, stirred in rt; (vi) appropriate benzeldehyde, isocyanate and isothiocyanate, ethanole, reflux (6h)

Scheme 1. Synthesis of compounds 8a-d

(E/Z)-N'-(4-cyanobenzylidene)-3-(6-oxo-3-(p-tolyl) pyridazin-1(6H)-yl)propanehydrazide ***(8b);*** White powder, MP: 271-3 °C, Yield 81%, ^{1}H-NMR (DMSO-d6, 400 MHz) δ ppm; δ (ppm) 2.28 (3H; s; -CH_3, 33%), 2.33 (3H; s; -CH_3, 67%) 2.79 (2H; t; *J*=7.78 Hz; -N-CH_2- $C\underline{H_2}$-C=O, %33), 3.20 (2H; t; *J*=7.78 Hz; -N-CH_2- $C\underline{H_2}$-C=O, %67), 4.42 (2H; t; *J*=7.78 Hz; -N-$C\underline{H_2}$-CH_2-C=O), 6.98 (1H; s; -N=CH-, 67%), 7.04 (1H; s; -N=CH-,33%), 7.19-7.23 (2H, m, pyridazinone protons)7.68-8.18 (8H; m; phenyl protons), 11.67 (1H; s; -NH-N,67%). 11.77 (1H; s; -NH-N,33%). ^{13}C-NMR (DMSO-d6, 100 MHz) δ ppm; 172.89, 167.14, 159.30, 144.32, 142.64, 141.16, 135.97, 134.55, 133.74, 133.39, 131.33, 130.80, 130.68, 130.44, 129.96, 129.32, 127.99, 118.88, 112.45, 47.94, 33.31, 31.16. LC/MS (API): m/z calculated for $C_{22}H_{19}N_5O_2$: 385.15 found: 386.10.

N-(4-chlorophenyl)-2-(3-(6-oxo-3-(p-tolyl)pyridazine-1(6H)-yl)propanoyl)hydrazine-1-carbothioamide ***(8d);*** White powder, MP: 228-30 °C, Yield 84%, ^{1}H-NMR (DMSO-d6, 400 MHz) δ ppm; 2.35 (3H; s; -CH_3), 2.72 (2H; t; *J*=7.88 Hz; -N-CH_2- $C\underline{H_2}$-C=O), 4.36 (2H; t; *J*=7.88 Hz; -N-$C\underline{H_2}$-CH_2-C=O), 7.00- 8.08 (10H; m; phenyl and pyridazinone protons), 8.36 (1H;s;O=C-N$\underline{H}$-NH-), 9.06 (1H;s; -NH-N$\underline{H}$-C=S-NH), 9.90 (1H; s; -NH-NH-C=S-N$\underline{H}$), ^{13}C-NMR (DMSO-d6, 100 MHz) δ ppm; 181.34, 168.27, 159.41, 149.88, 143.18, 138.58, 134.67, 132.87, 131.10, 130.72, 130.20, 130.08, 129.39, 128.41, 128.13, 128.01, 47.94, 32.61, 24.98. LC/MS (API): m/z calculated for $C_{21}H_{20}ClN_5O_2S$: 441.10 found: 442.10.

Biological Activity

Lipase Inhibitory Effect Assay

p-nitrophenyl butyrate was employed as the substrate for the porcine pancreatic lipase type II (PLL) inhibitory assay in the improved method [25]. Compounds (8a-8d) and orlistat (as a positive control) were produced at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. The following equation was used to compute the proportion of PLL inhibitory effect:

PLL Inhibition (%) = [[(A-B)-(C-D)]/(A-B)]*100

A: absorbance in the presence of the substrate (control group),

B: absorbance in the absence of the substrate and compound (blank),

C: absorbance in the presence of the substrate and compound (experimental group),

D: absorbance in the presence of a compound (blank of C).

The IC_{50} values of pyridazinone derivatives are given in Table 1.

Molecular Docking Studies

Molecular docking was conducted utilizing Maestro 11.8. Using 2D Sketcher, the structures of the ligands were created. The ligands were minimized using the Schrodinger utility MacroModel. 3D structures of ligands were constructed utilizing Maestro11.8 software LigPrep software and with OPLS 2005 force field, then were optimized with conjugated gradient method. Lipase complex (triacylglycerol lipase/colipase complex) 1ETH pdb encoded protein downloaded from www.rcsb.org [26-28].

Schrödinger's modules, Protein Preparation Wizard Prime, Impact, Epik, Propka, and Prime were used for removing ligands and solvent molecules in protein, adding hydrogens and assigning charges. After the target region of the proteins was determined, the grid box was created with the grid generation panel. It has been reported in the literature that the target site of the protein is the catalytic triad of Ser153, Asp177 and His264 [29]. Grid map was created in this region. Then prepared ligands were docked in this grid map 50 times in SP mode with Glide (Maestro 11.8) [30,31].

Molecular Dynamics Simulation Studies

The molecular dynamics (MD) simulations of the compounds were carried out using the Desmond software of the Maestro 12.8 (Schrodinger, NewYork) program. Protein ligand complexes were placed in a 10 Å thick cubic box. TIP3P water model was used in the simulation box. The system was neutralized using Na^+ and Cl^- ions. The concentration of the system was adjusted with 0.15M NaCl solution. Desmod's default relaxation protocol has been applied to the simulation system. These results suggest that compound 8d may interact with the protein in a similar way to orlistat.

ADMET predictions

Before the calculations, the structures of the compounds were drawn with 2D Sketcher and prepared with LigPrep. Some ADME properties (molecular weights, logP, volume, QPP Caco, Qplog Khsa, Percent Human Oral Absorption, Rule of Five) of the compounds were calculated with QikProp (Maestro) software. The estimated mutagenic, tumorogenic and irritant properties of the compounds were calculated with the DataWarrior 4.07.02 program [32].

Results and Discussion

Chemistry

The synthesis of the compounds was synthesized with a yield between 70% and 87% according to the literature are shown in Scheme 1. Compounds 8b and 8d were synthesized for the first time. Compounds 8a and 8c are registered in the literature. The molecular structures of these compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, and LC/MS. Compounds have *E/Z* isomers due to the C=N groups in their structures. Therefore, the peaks of the isomers may be seen at different ppm values and at different percentages. The signals of the protons in the CH_3, -N-$C\underline{H_2}$-CH_2-C=O, -N-$C\underline{H_2}$-CH_2-C=O, -N=CH, and, -NH-N groups of compounds 8b were 67% and 33%, in the *E* and *Z* isomers.

Biological Activity

Orlistat, an inhibitor of pancreatic and other lipases, was utilized to investigate the PLL inhibition of produced drugs. By inhibiting at a concentration of 32.66±2.8265 µg/mL, the chemical 8d was shown to be the most potent. Compounds 8a and 8b have IC_{50} values of 92.70±3.2231 µg/mL and 93.15±4.2592 µg/mL, respectively, which are indicative of a modest lipase inhibitory action. At a dosage of 52.06±3.7526 µg/mL, the compound 8c inhibited the lipase enzyme.

Table 1. PLL inhibition of synthesized compounds 8a-d

Compounds	PLL Inhibition* (IC_{50} (µg/mL) ± SD**)
8a	92.70±3.2231
8b	93.15±4.2592
8c	52.06±3.7526
8d	32.66±2.8265
Orlistat	13.49±1.2262

*Porcine pancreatic lipase **Standard deviation

Molecular Docking Studies

Molecular docking studies were carried out to examine the interactions of the compounds and orlistat with the active site of the target protein. Docking results and activity results were found to be compatible. While the docking scores of the synthesized compounds are between -5.042 and -6.202 kcal/mol, the docking score of orlistat is -6.692 kcal/mol. The docking score of compound 8d (IC_{50}:32.66±2.8265) with the best PLL inhibition was calculated as -6.202 kcal/mol. The docking scores are given in Table 2.

Table 2. Docking scores of compounds 8a-d and orlistat against target protein

Compounds	Docking Scores (kcal/mol)
8a	-5.182
8b	-5.042
8c	-5.819
8d	-6.202
Orlistat	-6.692

As a result of docking studies, the interactions of ligands with residues in the active region of the protein were also investigated (Figure 2).

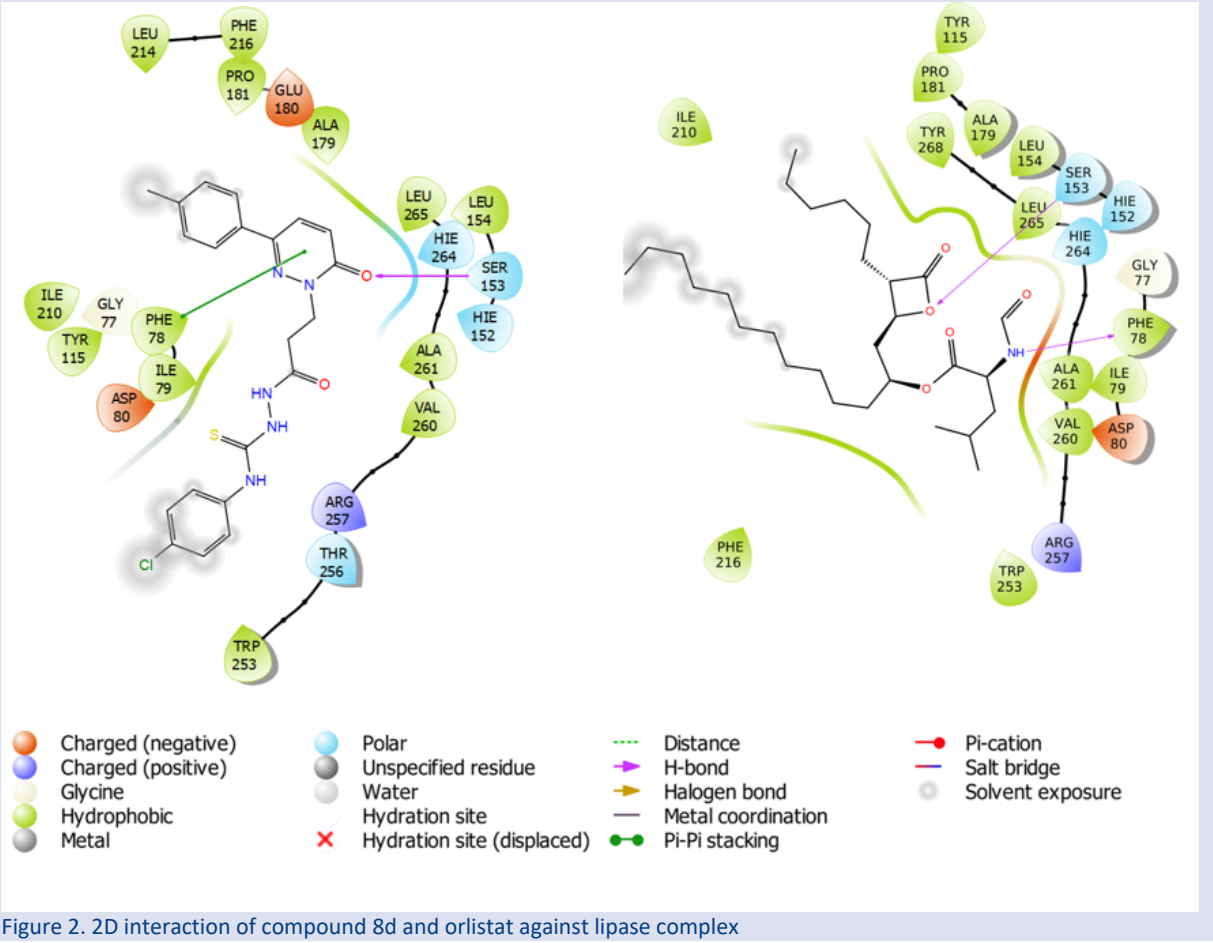


Figure 2. 2D interaction of compound 8d and orlistat against lipase complex

Orlistat made hydrogen bonds with SER153 and PHE78 in the active site of the protein. In addition, hydrophobic interaction with TYR115, PRO181, ALA179, TYR268, LEU154, LEU265, ALA261, VAL260, TRP 253 and PHE 216, polar interaction with SER153, HIS152, HIS264, charged (-) interaction with ASP80 and charged (+) interaction with

ARG257. It was observed that compound 8d, which has the best activity among the synthesized compounds, interacts similarly with orlistat and hydrogen bonds with SER153 and pi-pi stacking PHE 78. When we examined its 3D placement, we found that compound 8d and orlistat with the active site of the 1ETH pdb-encoded protein (Figure 3).

It was observed that compounds 8a and 8b with the lowest activity did not make hydrogen bonds with SER153, which is among the most important residues for activity. According to the molecular docking results, it is thought that the strong interaction with SER153 located in the active region of the protein is related to both the docking score and the activity.

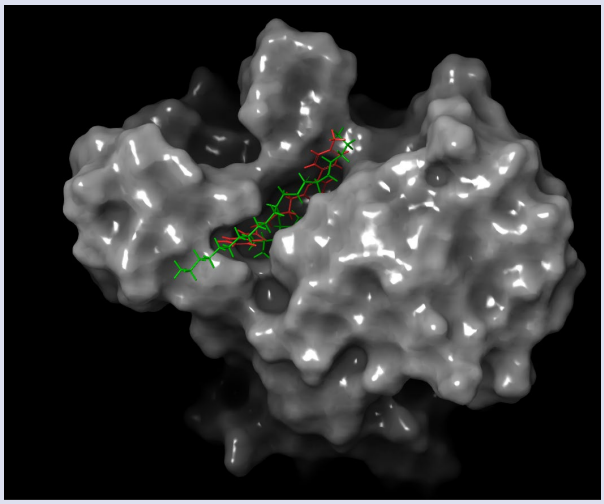


Figure 3. 3D placement of orlistat (green) and compound **8d** (red) with the active site of the 1ETH pdb-encoded protein

Molecular Dynamics Simulation Studies

Also, MD trajectory analysis of orlistat and compound 8d which showed the best lipase inhibition activity, with target protein for 50 ns was performed (Figure 4). Changes of the order of 1-3 Å are perfectly acceptable for small, globular proteins [33]. According to the analysis results, it is seen that the root mean square deviations (RMSD) values of the alpha carbons (Cα, blue line, left Y axis) of the enzyme (1ETH) vary between roughly 1.2 – 2.4 Å. Compound 8d has a RMSD value between 3.2 – 6.4 Å, orlistat has a RMSD value of 2.4 – 6.4 Å for 50 ns. These results are similar to compound 8d in orlistat (Figure 4). When the plot of compound 8d is examined, it is seen that RMSD value (red line, right Y axis) increased from 3.2 Å to 5.6 Å in about 2 ns and reached 6.4 Å in the following time. In this 48 ns period, the range of RMSD value under 1 Å.

Examining the plot of orlistat, the RMSD value ranges from 2.4 to 4.8 Å up to 35 ns. In this 35 ns period, the range of RMSD is less than 3 Å. However, after the 35th ns, the RMSD value increased up to 6.4 Å and decrease again from the 42nd ns.

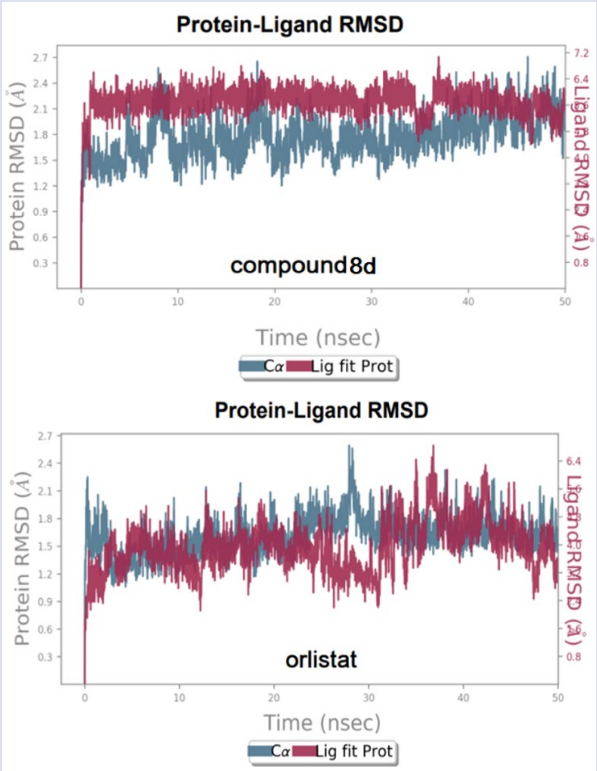


Figure 4. The Root Mean Square Deviations (RMSD) plots. RMSDs for compound 8d and orlistat.

ADMET Predictions

In addition to interacting with target macromolecules, it is very important for drugs to reach their target sites in order to have an effect. Many synthesized molecules cannot be used as drugs due to their unsuitable physicochemical properties. Although the compounds show high activity, they may have significant toxic effects, limiting their use. Therefore, it is important to determine the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of drug candidate compounds. In our study, various ADME and toxicological parameters of the synthesized compounds were calculated (Table 3). The logP values of the compounds range from 3.84 to 4.96. The volume values of the compounds were found close to each other. Caco permeability values and serum albumin binding values of the compounds were found to be suitable. The compounds have very high oral absorption values, which are important for the use of drugs. None of the compounds showed predicted mutagenic, teratogenic, and irritant effects. All compounds comply with Lipinski's rule of five.

Table 3. Some predicted ADME and toxicological, properties of compounds.

Comp.	Mol MW	logP	Volume	QPP Caco	QPlog Khsa	P. H. O.A.	Rule of five	Mutagenic	Teratogenic	Irritant
8a	390.44	4.79	1300.37	642.75	0.77	100.00	0	none	none	none
8b	385.42	3.84	1238.10	301.95	0.47	93.84	0	none	none	none
8c	374.44	4.97	1281.20	625.93	0.92	100.00	0	none	none	none
8d	441.93	4.96	1378.13	349.10	0.78	100.00	0	none	none	none
orlistat	495.74	6.56	1919.70	338.82	0.97	100.00	1	none	none	none

Conclusions

Orlistat was used as a test for the PLL inhibition of produced drugs. By inhibiting at 32.66±2.8265 µg/mL, compound 8d was revealed to be the most effective compound. Molecular modeling and MD studies were performed to examine the interaction of compounds 8a-d and orlistat with the target protein. The compounds were docked successfully, and the resulting activities were in sync. The most active chemical 8d has been demonstrated to interact with orlistat in a similar way. In this study, it was observed that the compounds obtained as a result of the substitutions of the 3-(6-oxo-3-phenylpyridazin-1(6*H*)-yl) propanehydrazide structure may have potential lipase inhibitory effects. It is planned to develop new, more effective and targeted (lipase complex) compounds by using the data obtained in further studies. The suitable ADMET properties of Compound 8d support its potential as a drug.

Conflicts of interest

The author declare that they have no conflict of interests

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