Original Article

Molecular Docking Study of Rosmarinic Acid and Its Analog Compounds on Sickle Cell Hemoglobin

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ABSTRACT

Introduction: Voxelotor, also known as GBT 440, binds to the alpha cleft in HbS tetramers and promotes the stability of the relaxed or oxygenated state of HbS. This process hinders the conformational change of the HbS tetramers into the deoxygenated state. Voxelotor prevents interactions between HbS tetramers in the deoxygenated state, ultimately inhibiting the polymerization of HbS tetramers and resulting in significant clinical improvements, particularly in raising hemoglobin levels in patients. In this study, we have explored the use of herbal compound models, such as rosmarinic acid and compounds with similar structures that exhibit high binding affinity to Voxelotor's hemoglobin binding site.

Materials and methods: The molecular model of hemoglobin (PDB: 5E83) was initially obtained from the RCSB PDB database. In addition, we collected 453 ligand models with structural similarity to rosmarinic acid from the PubChem database. To prepare these models for molecular docking, we utilized the Molegro Virtual Docker tool. Subsequently, we used the SwissADME web tool to predict the physicochemical properties and pharmacokinetics of these compounds.

Results: We investigated the affinity and binding site of 453 compounds similar to rosmarinic acid on the hemoglobin model (PDB: 5E83). Our focus was on the alpha cleft between two alpha chains of the hemoglobin model (PDB: 5E83). The results showed that most compounds had molecular weights above 500 daltons, and some exhibited acceptable hydrophobicity. Furthermore, their solubility in aqueous solutions was good. None of the compounds were able to cross the blood-brain barrier or have gastrointestinal absorption. However, they did have varying inhibitory effects on CYP2C9 cytochromes. The skin penetration rate was generally low.

Conclusion: Through our study, we identified three compounds (CID: 162739375, CID: 141386569, and CID: 24015539) with promising potential for further research. These compounds demonstrated high binding affinity to the hemoglobin model, favorable dissolution and digestive absorption rates, as well as suitable hydrophobicity, making them ideal candidates for continued laboratory investigation.

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Introduction

The effect of allosteric factors and natural mutations on the primary physiological function of hemoglobin (Hb) in oxygen binding and transport, as well as the Hb structures including tense (T state) and relaxed (R state) forms, and the dynamics of conformational change between them, have been studied. Allosteric factors can modulate the conformational change of Hb by binding to specific sites on Hb. Endogenous allosteric factors, such as 2,3-bisphosphoglycerate (2,3-BPG) and inositol hexaphosphate (IHP), bind to deoxygenated hemoglobin and stabilize the T-state form. Exogenous allosteric agents have been further investigated for their potential therapeutic applications. For example, aromatic aldehydes form adducts with the N-terminal nitrogen of αVal1 to increase hemoglobin's affinity for oxygen, making them potential anti-sickle agents [1].

Attempts to stabilize HbS with small molecules began with vanillin derivatives, but 5-(hydroxymethyl) furfural (5-HMF) was deemed unsuitable due to its poor affinity for HbS. Subsequent studies focused on the development, commercialization, and clinical use of new compounds such as Voxelotor (GBT 440), which binds to HbS tetramers in a 1:1 ratio. This compound has demonstrated clinical efficacy, with an increase in hemoglobin levels observed in 50% of patients who received a daily dose of 900 or 1500 mg [2].

While the effectiveness of R-state-stabilizing voxelotor has been confirmed in clinical trials to increase tissue oxygenation, previous studies have raised concerns about this approach. Some studies completely reject the idea of using R-state stabilizers in the treatment of sickle cell disease based on evidence that does not support current clinical practice. However, clinical trials on voxelotor have shown promising results in increasing hemoglobin levels and decreasing hemolysis, with no reported hypoxia-related side effects. Further research is needed to examine the effectiveness of this treatment while concerning the potential side effects of this drug on the central nervous system [3].

The recent discovery of PF-7059013 as a non-covalent modifier of hemoglobin that stabilizes the oxygenated state offers potential as a potent and effective treatment for sickle cell disease (SCD). In a study using a mouse model of SCD, treatment with PF-7059013 showed significant changes in markers of hemolytic anemia, including reduced red blood cell sickling under hypoxic conditions [4].

The research process has always focused on discovering compounds that have improved performance in treating sickle cell anemia. With this goal in mind, we are endeavoring to propose new patterns for designing more effective medicinal compounds using known herbal compounds [1-4].

The use of herbs containing bioactive components, such as rosmarinic acid (RA), a natural polyphenol found in herbs like Salvia rosmarinus L., has been found to intervene in carcinogenesis by inhibiting tumor cell proliferation, inducing apoptosis, suppressing metastasis, and reducing inflammation. In addition, RA exhibits potent antimicrobial, anti-inflammatory, antioxidant, and even antidepressant and anti-aging effects [5,6].

In our molecular docking studies, we observed a high binding affinity of rosmarinic acid to the space between the two chains of alpha globin (alpha cleft) in hemoglobin, which is the binding site of voxelotor on hemoglobin. Therefore, we conducted further investigations to determine the affinity of compounds with a similar structure to rosmarinic acid to the binding site of the Voxelotor drug on the alpha cleft of the hemoglobin molecular model [PDB: 5E83].

Materials and Methods

Hemoglobin Model Preparation

We obtained the molecular model of Hemoglobin [PDB: 5E83] from the RCSB PDB

database. This crystal structure of carbonmonoxyhemoglobin S (HbS) complexed with GBT440 (Voxelotor) was determined at a resolution of 1.80 Å [7].

Ligand Model Preparation

We obtained ligand models with structural similarity to rosmarinic acid (453 compounds) from the PubChem database. We used the Molegro Virtual Docker ligand preparation tool [8, 9].

Predicting Physicochemical Properties and Pharmacokinetics

The SwissADME web tool provides a user-friendly interface for predicting important parameters related to drug development, such as physicochemical properties and pharmacokinetics. This tool offers quick and reliable predictive models like the BOILED-Egg, iLOGP, and others. These models can be accessed without the need for logging in through the website http://www.swissadme.ch. We used the SwissADME web tool for our study.

Molecular Docking

Molecular docking is used to predict the binding affinity and orientation of small molecules to target proteins. Molegro Virtual Docker (MVD) is a powerful software that uses advanced computational methods for molecular docking. It employs a flexible docking algorithm that considers multiple conformations of both the ligand and receptor molecules, providing accurate results for both rigid and flexible docking scenarios. MVD also offers advanced features such as grid-based scoring functions, support for multiple docking engines, and extensive output analysis tools, which facilitate further investigation of docking results. Overall, MVD is a reliable and versatile tool for drug discovery and structure-based design applications. In our study, we utilized this software. MVD is a protein-ligand docking simulation program that offers four search algorithms and four native scoring functions, allowing for 32 docking protocols that take into account the presence or absence of water molecules [9].

Results and Discussion

In this study, we investigated the affinity and binding site of 453 compounds with structural similarities with rosmarinic acid. We performed the study using a docking grid box on the hemoglobin model [PDB: 5E83], focusing specifically on the alpha cleft between the two alpha chains. From the large amount of data, only 99 ligands showed higher binding affinity based on the molecular docking results for the alpha cleft in the hemoglobin tetramer. These results were then presented (see Table 1). In addition, the physicochemical properties (see Table 2) and pharmacological characteristics (see Table 3) of the 49 compounds with the highest binding affinity to the hemoglobin model [PDB: 5E83] are reported.

Docking Results

Among the 99 compounds with the highest binding energy for the grid box determined on hemoglobin, the lightest compound is CID: 46210733, which has an interaction (binding) affinity of -173.972 (kcal/mol), molecular weight = 401.344 (Dalton), and LE1 (MolDock Score / Heavy Atoms) = -5.00852 (kcal/mol). Among the 99 compounds, the highest LE1 = -5.6295 (kcal/mol) was for the compound CID number: 6450178. Likewise, the binding affinity of this compound for the binding site was -185.901 (kcal/mol) and its molecular weight was equal to 417.343 (Dalton).

Fifteen of the studied ligands had an interaction affinity (the binding affinity between a ligand and the ligand binding site on hemoglobin) above 200 (Kcal/mol) for the binding site on the hemoglobin model (Figures 1, 2, and 3), of which only CID number: 134458289 compound has a weight below 500 daltons. This ligand has Ligand efficiency (LE1) = -5.43307 (Kcal/mol), which is relatively high. The compound CID: 10052949 binds to the Voxelotor special binding site between two alpha globin chains with an interaction affinity

of -217.488 (kcal/mol). For this compound, the total affinity of hydrogen bonds is equal to -10.8468 (kcal/mol). This compound with 39 heavy atoms and molecular weight of 537.448 dalton showed the strongest interaction affinity with the studied binding site on the alpha cleft.

The binding site of compound CID number: 10052949 includes Val1, Ala88, Arg92, Val93, Asp94, Pro95, Thr134, Thr137, Ser138, and Lys139 residues of alpha 1 globin chain, and includes residues Val1, Leu2, Val35, Met76, Pro77, Ala130, Ser131, Thr134, Val135, Asp136, and Lys137 from alpha 2 globin chain. It also includes residues Val34, Tyr35, Pro36, and Trp37 from the beta 2 globin chain of hemoglobin model.

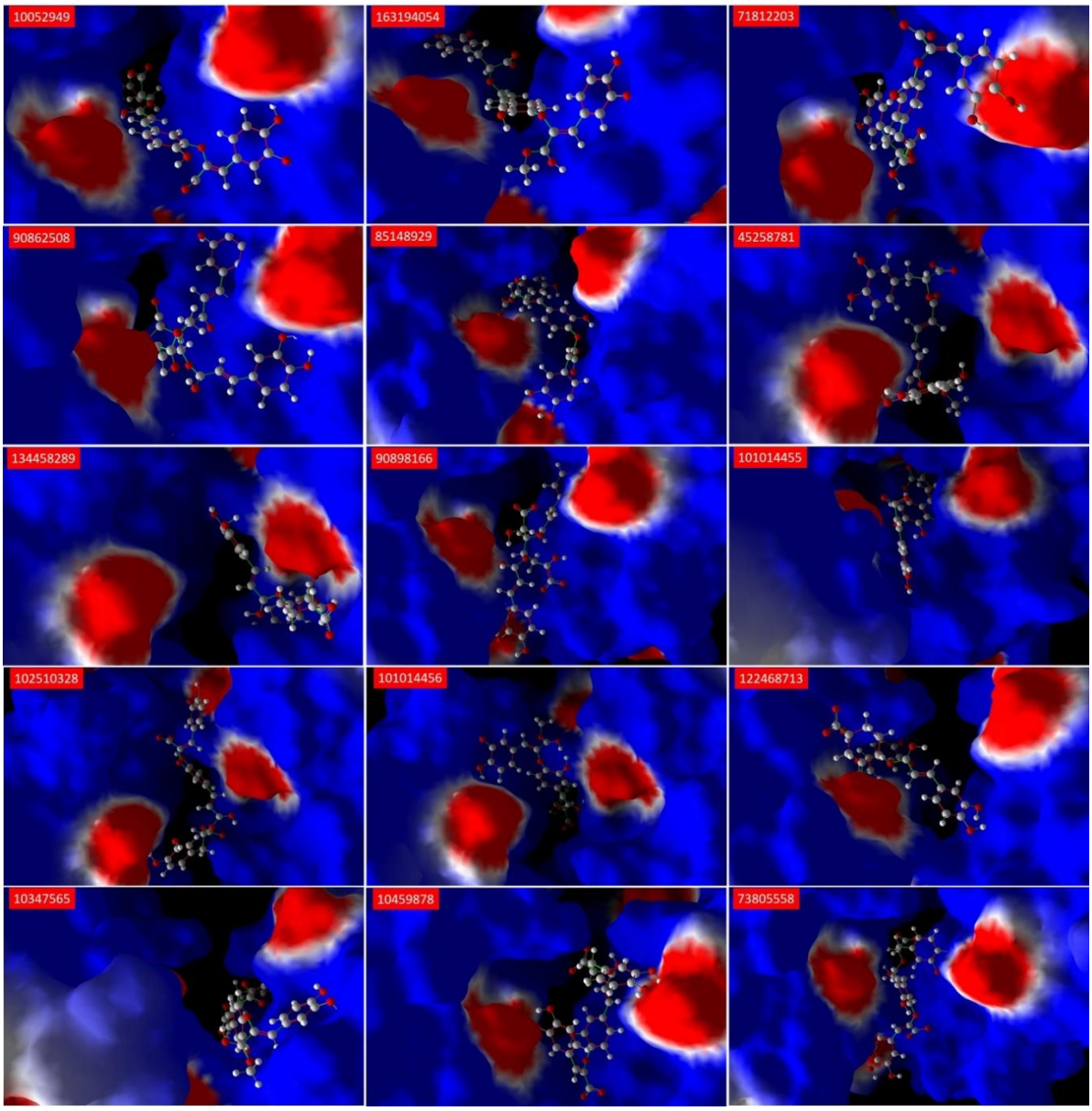

Figure 1 Ligand-hemoglobin binding sites for fifteen ligands with the maximum interaction affinity. The two alpha globin chains of hemoglobin are shown as the electrostatic surfaces

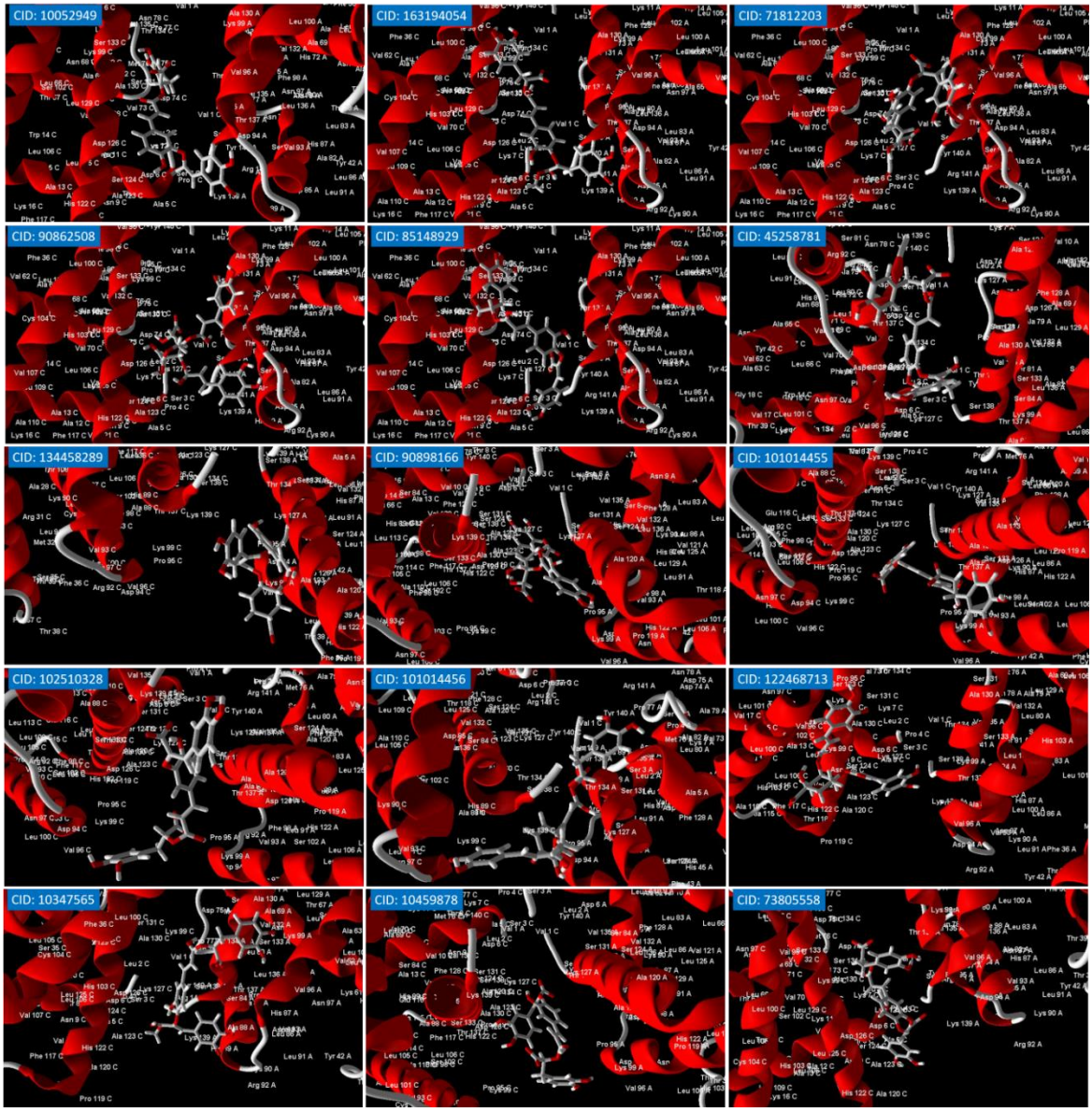

Figure 2 Ligand-hemoglobin binding sites for fifteen ligands with the maximum interaction affinity. The two alpha globin chains of hemoglobin are shown as secondary structures

Hydrogen bonds between the functional group on the backbone of the compound (CID number: 10052949) with the *N*-terminal nitrogen of Val1C, carbonyl oxygen in Ala88A, carbonyl oxygen in Val34D, carbonyl oxygen in carboxylic acid Ser131C, hydroxyl oxygen Ser131C, hydroxyl oxygen Thr134C, peptide bond nitrogen Lys139A, and the oxygen of the carbonyl in Lys137C are displayed in Figure 1.

The binding site of the compound CID number: 163194054 includes residues Ala88, Arg92, Val93, Asp94, Pro95, Thr134, Thr137, Ser138, and Lys139 on alpha-1 globin chain. It includes residues Pro95, Phe98, Lys99, Ala123, Asp126, Lys127, Ala130, Ser131, Ser133, Thr134, and Thr137 on alpha-2 globin chain. This ligand is the second high-affinity compound for the studied binding site. The binding site and hydrogen bonds of other compounds are also depicted in Figure 3.

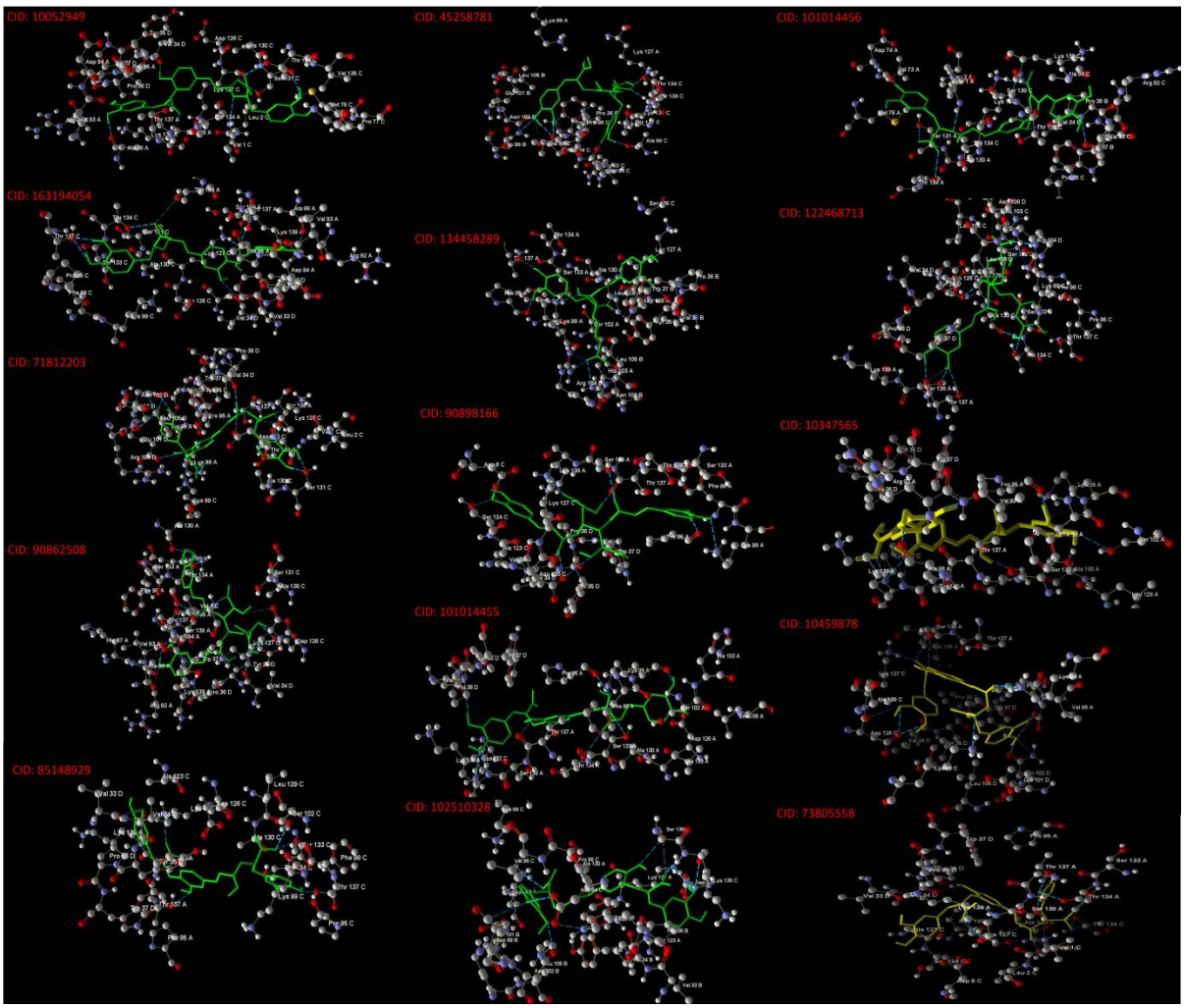

Figure 3 Ligand-hemoglobin binding sites for fifteen ligands with the maximum interaction affinity.Each picture displays the binding site of the ligands with the globin chains represented as a ball and stick model. The image also illustrates the presence of hydrogen bonds between the ligands and amino acids in the binding site

Physicochemical Properties Results

The compounds with CID numbers 163194054, 45258781, and 129905773 exhibited the highest level of hydrophobicity, with iLogP values above 2.8 and XLOGP3 values above 4.00. On the other hand, the compound with CID number 163188664 had the lowest hydrophobicity, with an iLogP value of 0.57. This compound demonstrated very high solubility in aqueous solutions. Most of the studied compounds are soluble or moderately soluble in aqueous solutions. The compounds with CID numbers 5281792, 24015539, 141386569, 46210733, 90862508, 90898166, 163188664, 5316647, 51399539, 54610010, 99719312, 154495931, 510, 129316856, 5281769, 13520496, 165387154, 91538896, 162950211, 14446, 92543361, 59977180, 163193404, and 77915995 exhibited the highest level of dissolution in aqueous solutions, with ESOL solubility (mg/ml) values above 0.1. All of these compounds demonstrated solubility in aqueous solutions. The compounds with CID numbers 90898166, 163188664, 10531154, 162926590, and 163193404 exhibited the highest total polar surface, with values above 280 (Å2) (Table 2).

Table 1 Molecular docking results

IUPAC Name	Ligand (CID number)	Interaction (kcal/mol)	MolDock Score (kcal/mol)	Rerank Score (kcal/mol)	HBond (kcal/mol)	LE1 (MolDock Score/ Heavy Atoms)	MW	Heavy Atoms	Torsions
2-hydroxy-6-[[2-(2-propan-2-ylpyrazol-3-yl)pyridin-3-yl]methoxy]benzaldehyde	71602803	-129.755	-107.506	-83.5389	-2.52355	-4.1330025	338.38	25	6
(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid	5281792	-144.831	-132.652	-103.745	-10.8458	-5.10201	360.315	26	7
(2R)-2-[(E)-3-[3-[(Z)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-4-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	10052949	-217.488	-195.366	-87.6047	-10.8468	-5.0094	537.448	39	11
(2S)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[4-[(Z)-1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yl]oxy-3-hydroxyphenyl]prop-2-enoyl]oxypropanoic acid	163194054	-216.231	-197.659	-80.1194	-12.3389	-4.94146	553.491	40	12
(2S)-2-[(E)-3-[4-[(Z)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-3-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	71812203	-214.34	-188.981	-133.997	-19.4825	-4.84566	537.448	39	11
3,4-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-2-hydroxy-7-methoxy-6,7-dioxoheptanoic acid	90862508	-212.088	-194.376	-81.1	-18.045	-4.8594	563.484	40	15
2-[3-[4-[1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-3-hydroxyphenyl]prop-2-enoyloxy]-3-(3,4-dihydroxyphenyl)propanoic acid	85148929	-212.084	-173.417	-128.383	-11.8435	-4.44659	537.448	39	11

(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[3-[(Z)-1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yl]oxy-4-hydroxyphenyl]prop-2-enoyl]oxypropanoic acid	45258781	-211.809	-176.968	-59.808	-15.2367	-4.4242	553.491	40	12	
(3R,4R)-3,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]cyclohexane-1-carboxylic acid	134458289	-208.487	-190.157	-138.849	-11.0894	-5.43307	485.46	35		
3,4-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-2-hydroxy-6-oxoheptanedioic acid	90898166	-206.962	-198.193	-125.261	-15.9118	-5.08188	547.442	39	13	
(2R)-2-[(E)-3-[4-[(Z)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-3-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	101014455	-205.61	-165.666	-88.9291	-16.4435	-4.24784	537.448	39	11	
(2R)-2-[(E)-3-[3-[(E)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-4-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	102510328	-205.599	-168.332	-117.427	-12.3373	-4.31621	537.448	39	11	
(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[4-[(Z)-1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yl]oxy-3-hydroxyphenyl]prop-2-enoyl]oxypropanoic acid	101014456	-204.496	-183.978	-56.8383	-10.7748	-4.59945	553.491	40	12	
(1R,3R,4S)-3,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1-hydroxycyclohexane-1-carboxylic acid	122468713	-203.747	-182.755	-134.597	-15.5307	-5.07654	501.459	36	9	
3-(3,4-dihydroxyphenyl)-2-[(E)-3-[4-[(Z)-1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yl]oxy-3-hydroxyphenyl]prop-2-enoyl]oxypropanoic acid	10347565	-202.999	-175.749	-46.5043	-13.8366	-4.39373	553.491	40	12	
2-[(E)-3-[4-[(Z)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-3-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	10459878	-202.049	-184.963	-119.197	-19.9935	-4.74263	537.448	39	11	
2-[3-[3-[1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-4-hydroxyphenyl]prop-2-enoyloxy]-3-(3,4-dihydroxyphenyl)propanoic acid	73805558	-200.742	-170.883	-110.566	-14.0992	-4.38161	537.448	39	11	
methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[3-[(Z)-1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yl]oxy-4-hydroxyphenyl]prop-2-enoyl]oxypropanoate	129905773	-199.935	-181.746	-135.774	-3.84234	-4.43284	569.533	41	13	

(2R)-2-[(E)-3-[4-[(E)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-3-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	162890634	-198.949	-176.423	-115.431	-9.55363	-4.52368	537.448	39	11	
(2R,3R,4S,5S)-2,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxyhexanedioic acid	163188664	-198.891	-194.584	-110.658	-17.4639	-5.12064	534.423	38	3	
1,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	5316647	-197.357	-162.228	-99.9024	-10.7556	-4.38453	517.459	37	9	
1-(1-hydroxypropan-2-yloxy)propan-2-yl (3S,5S)-3,5-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1-hydroxycyclohexane-1-carboxylate	137475239	-196.581	-177.519	-117.222	-12.9465	-4.03452	619.634	44	15	
(1R,3R,4S,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylate	51399539	-195.454	-176.117	-99.166	-15.795	-4.75991	517.459	37	9	
4-[(Z)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3,5-dihydroxycyclohexane-1-carboxylic acid	54610010	-195.114	-157.956	-97.689	-16.1237	-4.26908	517.459	37	9	
(1S,3R,4S,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	99719312	-194.802	-162.228	-113.665	-17.3875	-4.38455	517.459	37	9	
(1R,3S,4R,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	154495938	-194.801	-163.015	-116.469	-15.7144	-4.4058	517.459	37	9	
(1R,3R,5S,7S)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-7-[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxymethyl]-6,8-dioxabicyclo[3.2.1]octane-5-carboxylic acid	139298976	-194.625	-163.049	-123.799	-7.45472	-4.29076	529.469	38	10	
(1R,2R,3S,4R)-6-[(E)-3-[(1R)-1-carboxy-2-(3,4-dihydroxyphenyl)ethoxy]-3-oxoprop-1-enyl]-3-(3,4-dihydroxyphenyl)cyclohex-5-ene-1,2,4-tricarboxylic acid	10531154	-194.594	-174.41	-125.172	-13.4572	-4.2539	569.447	41	11	
4-[3-(3,4-dihydroxyphenyl)propanoyloxy]-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,5-dihydroxycyclohexane-1-carboxylic acid	70697815	-193.781	-184.816	-132.801	-16.4128	-4.99502	519.475	37	10	

2-[(E)-3-[3-[(Z)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenoxy]-4-hydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	21582559	-193.535	-170.563	-39.172	-9.99261	-4.37341	537.448	39	11
2-[(3S)-5-[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxymethyl]-3-hydroxy-2-oxooxolan-3-yl]ethyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	68103309	-192.68	-174.746	-102.278	-14.1529	-4.85405	503.475	36	1
(3S,5S)-3,5-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1-hydroxycyclohexane-1-carboxylic acid	138530142	-192.219	-174.866	-121.632	-10.861	-4.85739	501.459	36	9
(2R)-3-(3,4-dihydroxyphenyl)-2-[3-[2-[2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyloxy]propanoic acid	125990	-191.632	-179.703	-128.366	-12.2957	-4.99174	494.447	36	9
(2S,3S,4S,5R)-2,5-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-3,4-dihydroxyhexanedioic acid	162926590	-190.972	-165.989	-122.438	-17.8192	-4.36813	534.423	38	13
(1R)-1,3-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	129316856	-190.079	-165.889	-15.9925	-17.4178	-4.4835	517.459	37	9
(1R,3R,4S,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	5281769	-189.366	-163.246	-124.123	-9.91727	-4.41205	517.459	37	9
4,5-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-2,6-dihydroxy-2-methyloctanoic acid	143079495	-189.351	-180.585	-116.694	-5.41521	-4.63039	547.528	39	14
(1S,3S)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]cyclohexane-1-carboxylic acid	142555193	-189.093	-163.511	-114.097	-11.3555	-4.67175	485.46	35	9
(1R,3S,4S,5S)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	13520496	-189.076	-156.579	-122.835	-14.4673	-4.23186	517.459	37	9
(3R,4S,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	165387154	-188.82	-167.059	-122.008	-9.09982	-4.51511	517.459	37	9

4,5-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-6-hydroxy-7-methoxy-2,7-dioxoheptanoic acid	91538896	-188.767	-175.383	-125.607	-7.01683	-4.38459	563.484	40	14	
3-(3,4-dihydroxyphenyl)-2-[2-[2-(3,4-dihydroxyphenyl)-4,5-dihydroxyinden-1-ylidene]acetyl]oxypropanoic acid	53776105	-188.536	-166.356	-95.6934	-12.0561	-4.62101	492.431	36	7	
(1S,3S,4R,5S)-1,3-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	162950211	-188.513	-164.024	-110.976	-12.7312	-4.43308	517.459	37	9	
1,4-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	14446	-188.402	-154.816	-65.1264	-9.52687	-4.1842	517.459	37	9	
(3R,5S)-1,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	92543361	-188.183	-157.214	-113.132	-16.6579	-4.24902	517.459	37	9	
(2R)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxy-3-(3-hydroxyphenyl)propanoic acid	153530089	-188.141	-155.816	-56.9232	-16.4088	-4.45189	478.447	35	9	
(1S,4S)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	59977180	-187.457	-150.816	-104.79	-12.443	-4.0761	517.459	37	9	
(2R,3S,4R,5S)-2,5-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,4-dihydroxyhexanedioic acid	163193404	-187.373	-179.178	-117.106	-14.2048	-4.71521	534.423	38	13	
4-[3-(3,4-dihydroxyphenyl)propanoyloxy]-3-[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-1,5-dihydroxycyclohexane-1-carboxylic acid	77915995	-186.851	-175.194	-122.244	-18.4995	-4.73498	519.475	37	10	
2-[(1R)-2-[carboxy-(3,4-dihydroxyphenyl)methoxy]-1-(3,4-dihydroxyphenyl)-2-oxoethoxy]-2-(3,4-dihydroxyphenyl)acetic acid	91051656	-186.523	-170.215	-54.1159	-13.0338	-4.60041	515.4	37	10	
3-(3,4-dihydroxyphenyl)-2-[3-[2-[2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyloxy]propanoic acid	502233	-186.291	-159.765	-122.921	-19.7331	-4.4379	494.447	36	9	

(1S,3R,4R)-3-[3-(3,4-dihydroxyphenyl)propanoyloxy]-4-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1-hydroxycyclohexane-1-carboxylic acid	117647846	-186.249	-164.194	-108.989	-13.4219	-4.56095	503.475	36	10
octyl 3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoate	101513776	-185.992	-176.801	-123.117	-10.6635	-5.20003	474.543	34	15
(3R,5R)-1,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	12358846	-185.918	-165.241	-122.978	-16.4191	-4.46598	517.459	37	9
(2R,3S)-2-[(3,4-dihydroxyphenyl)methyl]-2-hydroxy-3-[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]oxybutanedioic acid	6450178	-185.901	-168.885	-121.397	-9.88208	-5.6295	417.343	30	9
(1S,3S,5S)-1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-hydroxy-5-[(2E,4E)-6-hydroxy-4-methylhepta-2,4,6-trienoyl]oxycyclohexane-1-carboxylic acid	138535292	-185.59	-145.733	-103.357	-11.6454	-4.28627	476.473	34	10
(3R,5R)-4-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoxy]-1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3,5-dihydroxycyclohexane-1-carboxylic acid	155215195	-185.329	-151.981	-52.8782	-10.2779	-4.22169	502.467	36	9
(1R,4R,5R)-4,5-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1-hydroxycyclohex-2-ene-1-carboxylic acid	46230344	-185.296	-164.168	-116.137	-15.5228	-4.56022	499.444	36	9
methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxypropanoate	24862412	-185.087	-162.643	-92.9353	-15.2975	-4.39575	510.489	37	10
2-[(E)-3-[2-[(E)-1-carboxy-2-(3,4-dihydroxycyclohexa-1,3-dien-1-yl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxy-3-(3,4-dihydroxyphenyl)propanoic acid	129208109	-184.872	-174.603	-111.06	-20.1948	-4.47701	539.464	39	10
(1S,3S,4R,5S)-3-[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-4,5-dihydroxycyclohexane-1-carboxylic acid	134688153	-184.799	-162.356	-112.082	-18.3745	-4.388	517.459	37	9
(2S,4R,5R)-2,5-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4-hydroxy-2-methylhexanoic acid	130252131	-184.505	-152.062	-119.338	-13.7566	-4.22394	503.475	36	12

(1S,3R,4R,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	6474640	-184.076	-165.62	-97.5846	-13.5726	-4.47621	517.459	37	9
(1S,3S,5S)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-5-hydroxycyclohexane-1-carboxylic acid	138530157	-183.981	-149.344	-63.4149	-13.8143	-4.14846	501.459	36	9
(3R,4S,5R)-3-[3-(3,4-dihydroxyphenyl)propanoyloxy]-1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-4,5-dihydroxycyclohexane-1-carboxylic acid	167221902	-183.287	-165.367	-113.594	-11.7242	-4.46939	519.475	37	10
(1S,3R,4R)-1,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3-hydroxycyclohexane-1-carboxylic acid	137550958	-182.859	-151.952	-90.4835	-2.80394	-4.22088	501.459	36	9
(4R,5R)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	126755519	-182.814	-159.249	-110.499	-14.1914	-4.30404	517.459	37	9
(2S,3R)-2-[(3,4-dihydroxyphenyl)methyl]-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-2-hydroxybutanedioic acid	162905688	-182.49	-156.342	-83.3132	-12.8408	-5.04329	433.342	31	9
(3S,5S)-1,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	5316279	-182.441	-153.952	-77.7484	-7.61632	-4.16086	517.459	37	9
(2S)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid	9913656	-182.189	-145.169	-91.1375	-14.6706	-4.03248	494.447	36	9
3-(3,4-dihydroxyphenyl)-2-[(E)-3-[4-[(E)-1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yl]oxy-3-hydroxyphenyl]prop-2-enoyl]oxypropanoic acid	14580455	-181.84	-156.286	-108.888	-8.70533	-3.90715	553.491	40	12
(3R)-1,4-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	68326315	-181.838	-156.705	-95.8584	-14.8962	-4.23526	517.459	37	9
(1S,3R,4R,5R)-1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3,4-dihydroxy-5-[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylic acid	71719612	-180.951	-147.623	-92.3471	-11.4025	-4.10065	501.459	36	9

1,3-bis[[(Z)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	44306801	-180.737	-145.582	-94.1876	-10.9761	-3.93464	517.459	37	9
(1R,3S,5S)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-5-hydroxycyclohexane-1-carboxylic acid	138530158	-180.341	-152.037	-109.747	-14.667	-4.22324	501.459	36	9
(3R,5R)-1,4-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	121232733	-180.283	-162.744	-118.783	-10.6418	-4.39848	517.459	37	9
(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid	5281793	-180.253	-160.559	-97.6996	-14.1702	-4.45998	494.447	36	9
3-(3,4-dihydroxyphenyl)-2-[(E)-3-[2-[(E)-3-(3,4-dihydroxyphenyl)but-2-en-2-yl]-3,4-dihydroxyphenyl]-2-methylbut-2-enoyl]oxypropanoic acid	54381522	-179.316	-171.004	-92.5597	-11.2374	-4.27511	550.553	40	9
(1R,3R,4S,5R)-1,3-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	122685	-179.224	-148.107	-79.2488	-11.1722	-4.00288	517.459	37	9
(1R,3R,4S,5R)-1-[3-(3,4-dihydroxyphenyl)propanoyloxy]-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-4,5-dihydroxycyclohexane-1-carboxylic acid	138533022	-178.831	-159.017	-110.907	-14.9921	-4.29775	519.475	37	10
3-(3,4-dihydroxyphenyl)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid	6124301	-178.598	-153.289	-98.264	-19.3161	-4.25803	494.447	36	9
(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-4,5-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid	45272145	-178.523	-166.246	-115.439	-11.1028	-4.61794	494.447	36	9
(1S,4R)-1,3-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	54502128	-178.417	-166.827	-106.274	-9.23549	-4.50882	517.459	37	9
(1S,3R,4R,5R)-1-[3-(3,4-dihydroxyphenyl)propanoyloxy]-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-4,5-dihydroxycyclohexane-1-carboxylic acid	155204007	-177.996	-157.678	-89.2044	-15.7796	-4.26157	519.475	37	10

1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	6124212	-177.378	-166.138	-107.699	-9.40796	-4.49023	517.459	37	9
(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[6-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-2,3-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid	166778821	-177.317	-155.775	-104.855	-12.0935	-4.32707	494.447	36	9
(2S,4R,7R)-4-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-7-[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxymethyl]-2-hydroxyoxepane-2-carboxylic acid	126761059	-177.224	-150.22	-109.078	-10.7249	-3.95316	531.485	38	10
3-[4,5-dihydroxy-2-[(4-hydroxyphenyl)methyl]phenyl]-2-[3-(3,4-dihydroxyphenyl)prop-2-enoxy]propanoic acid	123482470	-176.985	-158.916	-112.948	-8.86259	-4.81563	451.445	33	9
3-(3,4-dihydroxyphenyl)-2-[1-[(3,4-dihydroxyphenyl)methylidene]-6,7-dihydroxyindene-2-carbonyl]oxypropanoic acid	53980234	-176.55	-150.873	-92.2113	-11.4591	-4.19092	492.431	36	7
1,4-bis[[(Z)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,5-dihydroxycyclohexane-1-carboxylic acid	44306981	-176.214	-138.306	-96.5701	-20.7367	-3.738	517.459	37	9
octyl 2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-(3-hydroxy-4-methylphenyl)propanoate	160629575	-175.939	-151.658	-120.285	-5.06545	-4.46053	472.571	34	15
(1S,3R,4R,5R)-1,3-bis[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-4,5-dihydroxycyclohexane-1-carboxylic acid	465405	-175.807	-153.99	-108.553	-15.7853	-4.1619	517.459	37	9
(2S)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-3,4-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid	29927682	-175.656	-164.618	-111.203	-7.35514	-4.57271	494.447	36	9
(1R,3R,4S)-1,3-bis[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4-hydroxycyclohexane-1-carboxylic acid	143968865	-175.563	-145.803	-93.2038	-15.9062	-4.05009	501.459	36	9
3-[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]-2-hydroxy-2-[(4-hydroxyphenyl)methyl]butanedioic acid	73082224	-175.533	-142.735	-99.8474	-11.3437	-4.75784	417.343	30	9

[(E,2R,3R,4S,5R)-9-(3,4-dihydroxyphenyl)-2,3,4,5,6-pentahydroxy-7-oxonon-8-enyl] (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	141330505	-175.214	-168.2	-94.6151	-14.128	-4.67223	508.472	36	12
2,3,4,5,6-pentahydroxyhexyl (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylate	146549680	-174.673	-175.468	-110.105	-20.3102	-4.87412	520.481	36	12
3-(3-hydroxypropoxy)propyl (1S,3S)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1-hydroxycyclohexane-1-carboxylate	137475029	-174.632	-160.321	-127.976	-11.2439	-5.17164	440.484	31	13
(2S)-3-(3,4-dihydroxyphenyl)-2-[(3S)-3-(3,4-dihydroxyphenyl)-5,6-dihydroxy-3,4-dihydronaphthalene-2-carbonyl]oxypropanoic acid	102232478	-174.073	-161.277	-113.573	-12.4592	-4.47991	494.447	36	7
(2R)-3-(3,4-dihydroxyphenyl)-2-[(3R)-3-(3,4-dihydroxyphenyl)-5,6-dihydroxy-3,4-dihydronaphthalene-2-carbonyl]oxypropanoic acid	102232477	-174.013	-166.815	-110.134	-14.1308	-4.63375	494.447	36	7
(2R,3S)-2-benzyl-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-2-hydroxybutanedioic acid	46210733	-173.972	-145.247	-89.7405	-10.6652	-5.00852	401.344	29	9
phenacyl 3,4-dihydroxybenzoate	24015539	-134.355	-118.589	-90.9547	-8.50036	-5.92943	274.269	20	5
methyl 1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylate	141386569	-147.417	-120.536	-96.5492	-8.31145	-5.24071	322.353	23	6
(2-phenylacetyl) (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	162739375	-138.53	-117.349	-95.7386	-8.41702	-5.33406	300.306	22	6

Table 2 Physicochemical properties of studied ligands

Ligand	MW	Heavy atoms	Rotatable bonds	H-bond acceptors	H-bond donors	MR	TPSA (Å2)	iLOGP	XLOGP3	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Class
71602803	337.37	25	17	6	5	94.72	77.24	2.68	2.69	-3.73	0.06	Soluble
5281792	360.31	26	7	8	5	91.4	144.52	1.48	2.36	-3.44	0.13	Soluble
10052949	538.46	39	11	12	7	136.1	211.28	1.33	3.7	-5.12	0.00404	Moderately soluble
163194054	552.48	40	12	12	6	140.42	200.28	2.82	4.03	-5.35	0.00249	Moderately soluble
71812203	538.46	39	11	12	7	136.1	211.28	1.11	3.7	-5.12	0.00404	Moderately soluble
90862508	560.46	40	15	14	6	134.1	234.42	1.95	1.54	-3.52	0.17	Soluble
85148929	538.46	39	11	12	7	136.1	211.28	1.11	3.7	-5.12	0.00404	Moderately soluble

45258781	134458289	90898166	101014455	102510328	101014456	122468713	10347565	10459878
552.48	484.45	546.43	538.46	538.46	552.48	500.45	552.48	538.46
40	35	39	39	39	40	36	40	39
12	9	14	11	11	12	9	12	11
12	10	14	12	12	12	11	12	12
6	5	7	7	7	6	6	6	7
140.42	124.53	129.78	136.1	136.1	140.42	125.73	140.42	136.1
200.28	170.82	245.42	211.28	211.28	200.28	191.05	200.28	211.28
2.83	2.15	1.15	1.66	1.48	2.14	1.85	2.59	1.11
4.03	3.26	1.21	3.7	3.7	4.03	2.5	4.03	3.7
-5.35	-4.56	-3.29	-5.12	-5.12	-5.35	-4.17	-5.35	-5.12
0.00249	0.0134	0.278	0.00404	0.00404	0.00249	0.0338	0.00249	0.00404
Moderately soluble	Moderately soluble	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble

73805558	129905773	162890634	163188664	5316647	137475239	51399539	54610010	99719312
538.46	566.51	538.46	534.42	516.45	616.61	515.44	516.45	516.45
39	41	39	38	37	44	37	37	37
11	13	11	13	9	15	9	9	9
12	12	12	14	12	13	12	12	12
7	5	7	8	7	6	6	7	7
136.1	144.74	136.1	125.94	126.9	156.34	124.95	126.9	126.9
211.28	189.28	211.28	248.58	211.28	209.51	214.11	211.28	211.28
1.46	3.81	1.53	0.57	1.91	2.05	1.65	1.84	1.79
3.7	4.35	3.7	0.78	1.52	2.86	1.52	1.52	1.52
-5.12	-5.56	-5.12	-3.02	-3.65	-4.68	-3.64	-3.65	-3.65
0.00404	0.00156	0.00404	0.51	0.117	0.013	0.118	0.117	0.117
Moderately soluble	Moderately soluble	Moderately soluble	Soluble	Soluble	Moderately soluble	Soluble	Soluble	Soluble

73805558	129905773	162890634	163188664	5316647	137475239	51399539	54610010	99719312
538.46	566.51	538.46	534.42	516.45	616.61	515.44	516.45	516.45
39	41	39	38	37	44	37	37	37
11	13	11	13	9	15	9	9	9
12	12	12	14	12	13	12	12	12
7	5	7	8	7	6	6	7	7
136.1	144.74	136.1	125.94	126.9	156.34	124.95	126.9	126.9
211.28	189.28	211.28	248.58	211.28	209.51	214.11	211.28	211.28
1.46	3.81	1.53	0.57	1.91	2.05	1.65	1.84	1.79
3.7	4.35	3.7	0.78	1.52	2.86	1.52	1.52	1.52
-5.12	-5.56	-5.12	-3.02	-3.65	-4.68	-3.64	-3.65	-3.65
0.00404	0.00156	0.00404	0.51	0.117	0.013	0.118	0.117	0.117
Moderately soluble	Moderately soluble	Moderately soluble	Soluble	Soluble	Moderately soluble	Soluble	Soluble	Soluble

162926590	129316856	5281769	143079495	142555193	13520496	165387154	91538896
534.42	516.45	516.45	546.52	484.45	516.45	516.45	560.46
38	37	37	39	35	37	37	40
13	9	9	14	9	9	9	15
14	12	12	12	10	12	12	14
8	7	7	7	5	7	7	6
125.94	126.9	126.9	138.62	124.57	126.9	126.9	134.1
248.58	211.28	211.28	211.28	170.82	211.28	211.28	234.42
0.75	2.08	1.34	2.59	1.87	1.83	1.34	2.29
0.78	1.52	1.52	2.65	3.47	1.52	1.52	1.54
-3.02	-3.65	-3.65	-4.2	-4.69	-3.65	-3.65	-3.52
0.51	0.117	0.117	0.0344	0.0099	0.117	0.117	0.17
Soluble	Soluble	Soluble	Moderately soluble	Moderately soluble	Soluble	Soluble	Soluble

53776105	162950211	14446	92543361	153530089	59977180	163193404	77915995
492.43	516.45	516.45	516.45	478.45	516.45	534.42	518.47
36	37	37	37	35	37	38	37
7	9	9	9	9	9	13	10
10	12	12	12	9	12	14	12
7	7	7	7	6	7	8	7
128.7	126.9	126.9	126.9	128.79	126.9	125.94	126.58
184.98	211.28	211.28	211.28	164.75	211.28	248.58	211.28
1.78	1.3	1.91	1.91	1.99	1.43	1.21	1.84
2.95	1.52	1.52	1.52	4.28	1.52	0.78	1.33
-4.66	-3.65	-3.65	-3.65	-5.29	-3.65	-3.02	-3.47
0.0108	0.117	0.117	0.117	0.00246	0.117	0.51	0.175
Moderately soluble	Soluble	Soluble	Soluble	Moderately soluble	Soluble	Soluble	Soluble

91051656	516.41	37	10	13	8	122.47	231.51	0.74	2.14	-4.09	0.042	Moderately soluble
502233	494.45	36	9	10	7	130.81	184.98	1.62	3.92	-5.15	0.00349	Moderately soluble
46210733	402.35	29	12	9	9	99.94	161.59	1.13	1.84	-3.21	0.25	Soluble
141386569	320.34	23	6	6	6	84.34	93.06	2.77	3.01	-3.52	0.1	Soluble
162739375	298.29	22	12	6	5	80.97	83.83	1.97	2.94	-3.55	0.08	Soluble
24015539	272.25	20	12	5	5	71.48	83.83	1.64	2.47	-3.2	0.17	Soluble

Pharmaceutical Properties Results

Except for Voxelotor, none of the studied compounds have the ability to cross the blood-brain barrier. Except for CID numbers 141386569, 162739375, 24015539, and Voxelotor, all ligands have low gastrointestinal absorption. Additionally, none of the compounds have an inhibitory effect on CYP1A2, CYP2C19, CYP2D6, or CYP3A4 cytochromes. However, a large number of these compounds do have an inhibitory effect on CYP2C9, including compounds 10052949, 163194054, 71812203, 85148929, 45258781, 101014455, 102510328, 101014456, 10347565, 10459878, 73805558, 12990573, 162890634, 21582559, 125990, 53776105, 153530089, and 502233. Among all of our

studied compounds, the compound with CID number 162739375 has the maximum skin permeability rate of -6.03 log Kp (cm/s). On the other hand, the compounds 163188664, 10531154, 162926590, and 163193404 have the lowest skin absorption log Kp (cm/s) with a value approximately -9.0 log Kp (cm/s).

Overall, the skin permeability rate for all of the studied compounds is low.

The curcumin-affected hemolysate samples lead to a transition to the deoxygenated state in the hemoglobin of healthy individuals and patients. Although this study was performed on blood samples from thalassemia patients, due to curcumin's antioxidant activity, it can be expected to have the same effect on blood samples from patients with sickle cell anemia. This study contradicted previous findings, which suggested that curcumin might stabilize hemoglobin in its oxygenated state [12-15].

Table 3 Pharmacokinetic properties of studied ligands

Ligand	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
71602803	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-6.45
5281792	Low	No	No	No	No	No	No	No	-6.82
46210733	Low	No	No	No	No	No	No	No	-7.45
24015539	High	No	No	No	No	No	No	No	-6.21
141386569	High	No	No	Yes	No	No	No	No	-6.12
162739375	High	No	No	No	No	No	No	No	-6.03
10052949	Low	No	No	No	No	Yes	No	No	-6.96
163194054	Low	No	No	No	No	Yes	No	No	-6.81
71812203	Low	No	No	No	No	Yes	No	No	-6.96
90862508	Low	No	Yes	No	No	No	No	No	-8.63
85148929	Low	No	No	No	No	Yes	No	No	-6.96
45258781	Low	No	No	No	No	Yes	No	No	-6.81
134458289	Low	No	Yes	No	No	No	No	No	-6.94
90898166	Low	No	Yes	No	No	No	No	No	-8.77
101014455	Low	No	No	No	No	Yes	No	No	-6.96
102510328	Low	No	No	No	No	Yes	No	No	-6.96
101014456	Low	No	No	No	No	Yes	No	No	-6.81
122468713	Low	No	Yes	No	No	No	No	No	-7.58
10347565	Low	No	No	No	No	Yes	No	No	-6.81
10459878	Low	No	No	No	No	Yes	No	No	-6.96
73805558	Low	No	No	No	No	Yes	No	No	-6.96
129905773	Low	No	No	No	No	Yes	No	No	-6.67
162890634	Low	No	No	No	No	Yes	No	No	-6.96
163188664	Low	No	Yes	No	No	No	No	No	-9.01
5316647	Low	No	Yes	No	No	No	No	No	-8.37
137475239	Low	No	Yes	No	No	No	No	No	-8.03
51399539	Low	No	Yes	No	No	No	No	No	-8.37
54610010	Low	No	Yes	No	No	No	No	No	-8.37
99719312	Low	No	Yes	No	No	No	No	No	-8.37
154495938	Low	No	Yes	No	No	No	No	No	-8.37

139298976	Low	No	Yes	No	No	No	No	No	-7.77
10531154	Low	No	No	No	No	No	No	No	-8.99
70697815	Low	No	Yes	No	No	No	No	No	-8.52
21582559	Low	No	No	No	No	Yes	No	No	-6.96
68103309	Low	No	Yes	No	No	No	No	No	-7.65
138530142	Low	No	Yes	No	No	No	No	No	-7.58
125990	Low	No	No	No	No	Yes	No	No	-6.53
162926590	Low	No	Yes	No	No	No	No	No	-9.01
129316856	Low	No	Yes	No	No	No	No	No	-8.37
5281769	Low	No	Yes	No	No	No	No	No	-8.37
143079495	Low	No	Yes	No	No	No	No	No	-7.75
142555193	Low	No	Yes	No	No	No	No	No	-6.79
13520496	Low	No	Yes	No	No	No	No	No	-8.37
165387154	Low	No	Yes	No	No	No	No	No	-8.37
91538896	Low	No	Yes	No	No	No	No	No	-8.63
53776105	Low	No	No	No	No	Yes	No	No	-7.21
162950211	Low	No	Yes	No	No	No	No	No	-8.37
14446	Low	No	Yes	No	No	No	No	No	-8.37
92543361	Low	No	Yes	No	No	No	No	No	-8.37
153530089	Low	No	No	No	No	Yes	No	No	-6.18
59977180	Low	No	Yes	No	No	No	No	No	-8.37
163193404	Low	No	Yes	No	No	No	No	No	-9.01
77915995	Low	No	Yes	No	No	No	No	No	-8.52
91051656	Low	No	Yes	No	No	No	No	No	-7.93

Discussion

In our study, most of the compounds that had a high binding affinity to the alpha cleft of the hemoglobin model also had a high molecular weight and were heavier than the Voxelotor drug. Compared to Voxelotor's molecular weight of 337.37 (Dalton), rosmarinic acid's molecular weight is only slightly higher at 360 (Dalton). However, it has a higher binding affinity (-144.831 kcal/mol) than Voxelotor. While Voxelotor has high digestive absorption, rosmarinic acid has low digestive absorption. Voxelotor is highly soluble in aqueous liquids, which increases its dissolution and transfer in the blood. It also has high hydrophobicity, allowing it to pass through cell membranes and facilitating increased digestive absorption and passage through the red blood cell membrane (iLOGP=2.68). In addition, the sum of its polar surfaces is much less than other compounds. On the other hand, rosmarinic acid has a much lower hydrophobicity (iLOGP=1.48), but it has high solubility in aqueous liquids and more polar surfaces. These results indicate that Voxelotor is a more suitable compound in terms of physicochemical properties compared to rosmarinic acid [10-11,13-14].

Among all the compounds with a binding affinity near or above Voxelotor, the best ligand efficiency (LE=-5.92943 kcal/mol) was calculated for phenacyl 3,4-dihydroxybenzoate with CID number: 24015539. It has a binding affinity of -134.355 kcal/mol, a total hydrogen bond energy of -8.50036 kcal/mol, 20 heavy atoms, and a molecular weight of 274.269 (Dalton). This compound has higher binding affinity and lower molecular weight (Figure 4). It has high gastrointestinal absorption but cannot cross the blood-brain barrier. It does not have an inhibitory effect on all studied types of Cyt-p450. This compound has good digestive absorption, although its hydrophobicity (iLOGP=1.64) is not very suitable. However, the sum of its polar levels is close to Voxelotor [10, 11, 15-17].

The compound (2-phenylacetyl) (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate with CID number: 162739375, with a molecular weight of 298.29 daltons and 22 heavy atoms, has good dissolution in aqueous liquids but lacks good hydrophobicity (iLOGP=1.97). The compound methyl 1-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylate with CID number: 141386569, with a molecular weight of 320.34 daltons and 23 heavy atoms, has high hydrophobicity (iLOGP=2.77) and a polar surface of 93.06 Angstroms. It exhibits good dissolution in physiological fluids and good digestive absorption. This ligand may be a good option for laboratory studies. The binding affinity indices, as well as the location of interaction between the ligand and hemoglobin, are not the only effective indices for producing drug compounds with properties similar to Voxelotor. If the physicochemical and pharmacological characteristics of Voxelotor allow for its presence in red blood cells in patients with sickle cell disease [1-3], and then compounds with CID numbers 141386569, 162739375, and 24015539 are considered to have potential for laboratory investigations.

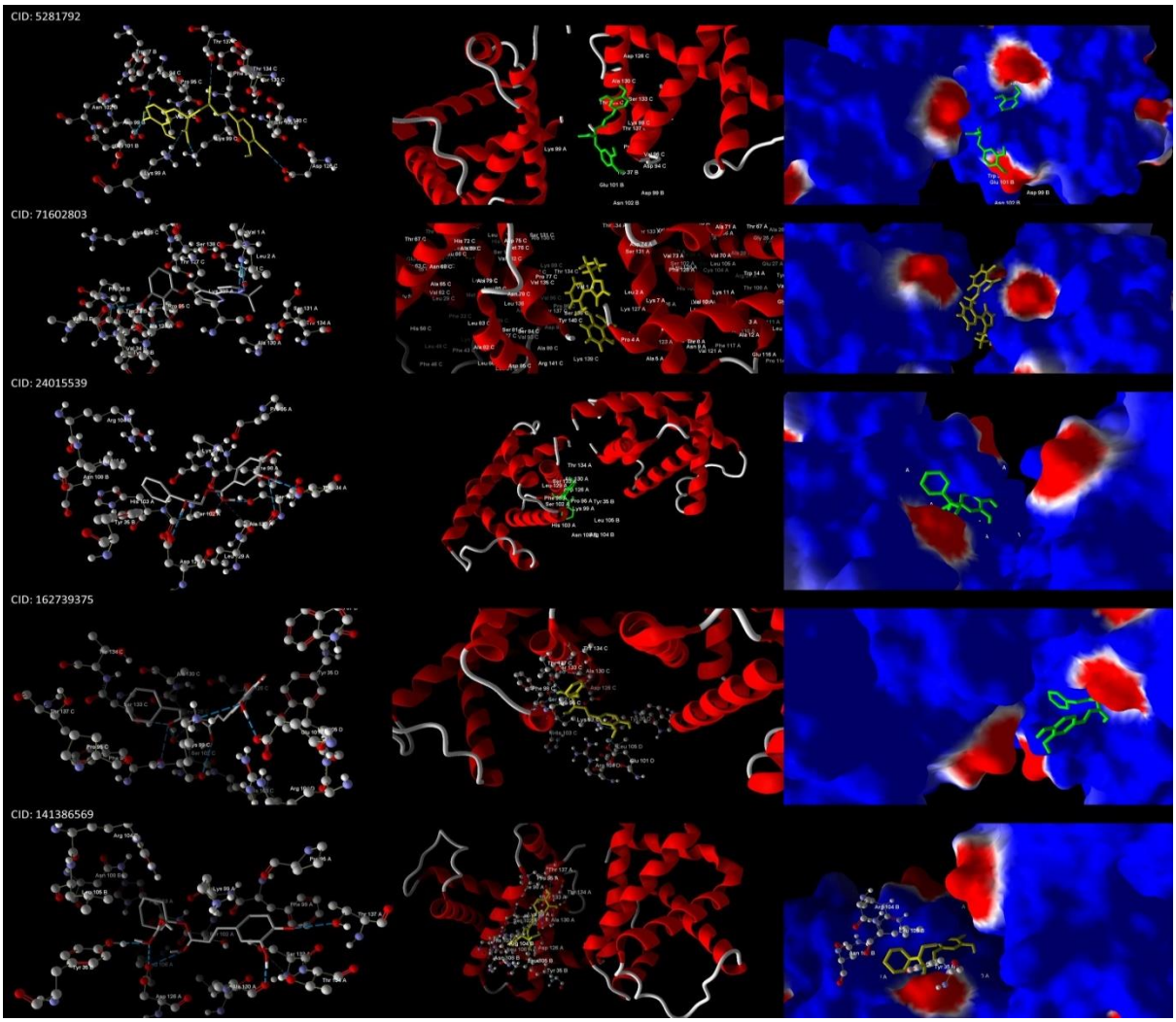

Figure 4 Binding sites of ligands with CID numbers 71602803, 5281792, 46210733, 24015539, 141386569, and 162739375 are depicted on the hemoglobin model. The left column displays the binding site of the ligands with the globin chains represented as a ball and stick model. The image also illustrates the presence of hydrogen bonds between the ligands and amino acids in the binding site. In the central column, the two alpha chains of hemoglobin are shown as secondary structures. The right column displays the electrostatic surfaces of the two alpha chains when in contact with the ligands.

Conclusion

In our study comparing studied ligands with Voxelotor, a drug used to treat sickle cell anemia, we found that while some compounds had a higher binding affinity to the hemoglobin model, their physicochemical properties were not as favorable as Voxelotor. However, three compounds with potential for further research: (2-phenylacetyl) (*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoate (CID: 162739375), methyl 1-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxycyclohexane-1-carboxylate (CID: 141386569), and phenacyl 3,4-dihydroxybenzoate (CID: 24015539) showed potential in terms of dissolution, digestive absorption, and hydrophobicity, making them suitable for further laboratory investigations.

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Conflict of Interest

The authors report no conflict of interest for the current study**.**

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