o-Hydroxycinnamic derivatives as prospective anti-platelet candidates: in silico pharmacokinetic screening and evaluation of their binding sites on COX-1 and P2Y12 receptors

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o-Hydroxycinnamic derivatives as prospective anti-platelet candidates: *in silico* pharmacokinetic screening and evaluation of their binding sites on COX-1 and $P2Y_{12}$ receptors

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Abstract:
Background: The high prevalence of thrombotic abnormalities has become a major concern in the health sector. This is triggered by uncontrolled platelet aggregation, which causes complications and death. The problem becomes more complicated because of the undesirable side effects of the drugs currently in use, some of which have reportedly become resistant. This study aims to evaluate the potency of *o*-hydroxycinnamic acid derivatives (OCA1a–22a) and their pharmacokinetic properties and toxicity for them to be developed as new antiplatelet candidates.
Methods: *In silico* analysis of pharmacokinetics was carried out using pKCSM. Molecular docking of the compounds OCA 1a–22a was performed using the Molegro Virtual Docker. *In silico* evaluation of the potency of biological activity was done by measuring the bonding energy of each tested compound to the target receptor i.e. COX-1 and $P2Y_{12}$, as the Moldock score (MDS).
Results: pKCSM analyses showed that more than 90% of OCA 1a–22a are absorbed through the intestine and distributed in plasma. Most tested compounds are not hepatotoxic, and none is mutagenic. An evaluation of the COX-1 receptor showed that OCA 2a–22a have lower binding energy compared to aspirin, which is the COX-1 inhibitor used today. So, it can be predicted that OCA 2–22a have stronger activity. Interactions with $P2Y_{12}$ show lower MDS than aspirin, but slightly higher than ibuprofen, which is the standard ligand.
Conclusions: ADMET (absorption, distribution, metabolism, excretion, and toxicity) profile prediction shows that OCA 1a–22a have the potential to be developed as oral preparations. OCA 1a–22a have strong potential to interact with COX-1 and $P2Y_{12}$ receptors, so they are prospective anti-platelet candidates.

Keywords: anti-platelet, COX-1 inhibitor, Molegro Virtual Docker, $P2Y_{12}$ inhibitor, pKCSM
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Introduction

Research data have shown that thrombotic disorders are a major health problem [1], [2]. This is because hematological imbalance will cause the formation of pathological thrombi in veins, arteries, and cardiac chambers, leading to complications and death [3], [4].

To date, anti-platelet agents such as aspirin and $P2Y_{12}$ inhibitors are still the main choice for the management of vaso-occlusive diseases [5], [6]. Unfortunately, during the last decades, the clinical utility of these drugs have become limited because of some undesirable side effects such as thrombocytopenia, neutropenia, thrombotic thrombocytopenic purpura, and aplastic anemia [7], [8]. Furthermore, in the last decades, these platelet-aggregating agents are reported to have become resistant [9], [10], [11]. Therefore, continuing research to discover new prospective anti-platelet agents is inevitable.

Platelets are one of the most important components in hemostasis, which have δ-granules or dense bodies that contain platelet-activating factors such as adenosine diphosphate (ADP) [12]. The outside of the platelet is coated with receptors [13] including $P2Y_{12}$ [14]. During injury, platelets are activated, and secrete ADP from the dense granule. ADP then binds to $P2Y_{12}$ receptor, inhibits adenylate cyclase, and results in the amplification of

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stable platelet aggregation [11], [15], [16], [17]. Therefore, in this study, the $P2Y_{12}$ receptor is one of the targets in designing anti-platelet compounds.

Another considerable protein in platelet aggregation is cyclooxygenase-1 (COX-1) [18]. This enzyme is expressed by most tissues and controls basal prostaglandin levels [18]. Ligands that attenuate prostaglandin biosynthesis will reduce the pro-inflammatory response, including platelet aggregation [19], [20]. Once platelets are activated, the phospholipid membrane releases free arachidonic acid, which then is metabolized to prostaglandin (PG) G2 by the prostaglandin H synthase-1 enzyme, also referred as COX-1. PGG2 is later converted to PGH2 and thromboxane A2 (TxA2) [21]. TxA2 then binds TP receptors to activate the protein Gq and leads to a change in platelet shape, degranulation, and aggregation [22].

Both TxA2 and $P2Y_{12}$ pathways have considerable pro-aggregatory effect on platelets. The use of COX-1 inhibitors, for example, will only negate the TxA2 pathway but does not affect the $P2Y_{12}$ pathway [23]. For that reason, our research group intends to develop drug compounds that are able to inhibit both COX-1 and $P2Y_{12}$.

The interest to develop cinnamic acid (Figure 1) derivatives as anti-platelet agents stemmed from the extensive research on the anti-inflammatory effect of *Kaempheria galangal* and its cinnamic content [24], [25], [26]. Our research group also found that a derivative of ferulic acid (Figure 1), namely 3-methoxy-2-hydroxycinnamic acid, showed the ability to bind $P2Y_{12}$ receptor in a molecular docking study [27]. In line with this, the methyl ester derivative of ferulic acid was proven to prolong the bleeding time and clot formation through *in vivo* tests conducted on mice [28]. Some derivatives of *p*-hydroxycinnamic acid also showed anti-platelet activity in mice in an animal model [29].

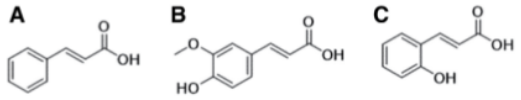

Figure 1: Chemical structure of (A) cinnamic acid, (B) ferulic acid, (C) *o*-hydroxycinnamic acid.A is the chemical structure of cinnamic acid which has aromatic groups, carboxylic acids and alkenes. B is the chemical structure of ferulic acid, a derivative of cinnamic acid with hydroxy and methoxy substituents in the benzene nucleus, which has interactions with P2Y12 receptors [28]. C is the chemical structure of o-hydroxycinnamic acid, which is investigated in this research.

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This study aims to examine the bonding of 22 derivatives of *o*-hydroxycinnamic acid (Figure 1) to both COX-1 and $P2Y_{12}$ receptors, by utilizing the Molegro Virtual Docker (MVD), to determine their prospective use as new anti-platelet candidates. This software will calculate the bond energy between each ligand and the target receptor, displayed as the Moldock score (MDS). Among all available methods, MVD was chosen because it has the highest accuracy [30], [31]. Furthermore, given the importance of the pharmacokinetic aspects in drug development [32], this study also aims to predict the physicochemical properties, pharmacokinetic profiles, and toxicity of these compounds by using the pKCSM web tool.

Materials and methods

Computer and software

We used an Intel(R) Core(TM) i7-4710MQ processor with CPU@2.50 GHz and RAM 8GB, with the software pKCSM and swissADME online tool. ChemDraw v. 15.0 of Perkin Elmer® was used along with MVD v. 5.5.

ADMET analysis

The structures of all *o*-hydroxycinnamic acid derivatives were translated into SMILES structures using the swissADME web tool. After that, each structure of the SMILES was analyzed using the pKCSM web tool. Among the physicochemical parameters calculated in this study were the nature of lipophilicity including water solubility and log P, molecular weight, torque, and the ability of molecules to donate and receive hydrogen. The absorption parameters included intestinal and skin absorption as well as Caco-2 permeability. Volume of distribution, excretion, and toxicity parameters were analyzed in one run analysis.

Molecular docking

Receptors

Both receptors were obtained from the Protein Data Bank (http://www.rcsb.org). For $P2Y_{12}$ receptors, the selected protein with the code PDB 4PXZ is $P2Y_{12}$ with 6AD_1201[A], (5-(6-amino-2-(methylthio)-9H-purine-9-yl)-3,4-dihydroxytetrahydro-furan-2-yl)-methyl diphosphate ligand complex [27]. COX-1 was downloaded from the Protein Data Bank (http://www. rcsb.org/-pdb) PDB ID:1EQG. Co-crystal of the reference ligand for the receptor was IBP_701[A] [33].

Method validation

To validate the docking process, reference ligands, namely 6AD_1201[A] for $P2Y_{12}$ and IBP_701[A] for COX-1, were re-docked to the appropriate binding site. The results were considered valid if the root-mean-square value (RMSD) was <2.0 Å [27].

Ligand preparation

Twenty-two *o*-hydroxycinnamic acid (OCA) derivatives, aspirin, and ibuprofen were drawn using Chem Draw professional 15.0. The three-dimensional structures were obtained by copying the structure to Chem3D v. 15.0. The preferred conformation was based on the minimum energy calculated by MMFF94. These results were then stored in the form of Sybil mol2.

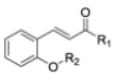
Docking study

Docking studies were conducted on the crystal structure of the enzyme COX-1 (PDB ID 1CQE) and $P2Y_{12}$ receptor (PDB ID 4PXZ) with 2.90 Å resolution. The crystal structure of each complex 1CQE-IBP_701[A] and 4PXZ-6AD_1201[A] was downloaded to its active site and determined for its binding sites. The 3D molecular structure of the test compounds was imported into the binding site and placed in a cavity according to each native ligand. The docking study was performed in a suitable cavity of the SE algorithm using MolDock with a maximum of 1500 iteratio The result of this molecular docking study was expressed as the MolDock score (MDS), which represented the interaction energy between ligand and receptor. The best docking result must meet the requirements of the lowest energy and the position of the molecule that is in the same bond with the native ligand, observed visually. Enzyme-ligand interaction was observed through hydrogen-bonding, steric (van der Waals), and electrostatic interactions performed to pose with the lowest MDS [29].

Results

Table 1 shows that the molecular weight of the 22 compounds ranges from 164.16 to 391.474. Log P value, which is a lipophilicity parameter, ranges from 1.4900 to 3.8705. A low bond rotation is calculated for OCA-1a, which is 2, while the highest one is for OCA-5a. The compounds with the least number of hydrogen donors are OCA-1a and OCA-15a, which is as small as 2, whereas the highest is OCA-22a, with 5. The most hydrogen-receiving candidate compound is OCA-22a, while OCA-17a does not have a hydrogen donor at all. The lowest water solubility is for OCA-1a and the highest is for OCA-21a.

Table 1: Prediction of physicochemical properties of *o*-hydroxycinnamic acid derivatives.



Code	R1	R2	MW	Log P	Bond rotation	HBA	HBD	PSA	Water solubility
OCA-1a	OH	H	164.16	1.4900	2	2	2	69.587	−1.792
OCA-2a	OH		206.197	1.7097	3	3	1	86.797	−2.624
OCA-3a	OH		220.224	2.0998	4	3	1	93.162	−2.9
OCA-4a	OH		234.251	2.4899	5	3	1	99.527	−3.189
OCA-5a	OH		248.278	2.8800	6	3	1	105.892	−3.488
OCA-6a	OH		268.268	3.0036	4	3	1	115.489	−4.18
OCA-7a	OH		282.295	3.31202	4	3	1	121.854	−4.316
OCA-8a	OH		298.294	3.0122	5	4	1	126.968	−4.384
OCA-9a	OH		302.713	3.657	4	3	1	125.793	−4.715
OCA-10a	OCH_3	H	178.187	1.5784	2	3	1	76.271	−1.835
OCA-11a	OC_2H_5	H	192.214	1.9685	3	3	1	82.636	−2.058
OCA-12a	OC_3H_7	H	206.241	2.3586	4	3	1	89.001	−2.426
OCA-13a	OC_4H_9	H	220.268	2.7487	5	3	1	95.366	−2.821
OCA-14a	OC_5H_{11}	H	234.295	3.1388	6	3	1	101.731	−3.236
OCA-15a	H	CH_3	178.187	1.793	3	2	1	76.271	−2.958
OCA-17a	CH_3	OCH_3	192.214	1.8814	3	3	0	82.955	−2.863
OCA-18a		CH_3	312.394	3.2216	4	3	2	134.357	−4.462
OCA-19a		CH_3	326.421	3.53002	4	3	2	140.722	−4.775
OCA-20a		CH_3	342.42	3.2302	5	4	2	145.836	−4.275
OCA-21a		CH_3	346.839	3.8750	4	3	2	144.66	−5.139
OCA-22a		CH_3	391.474	1.8690	5	5	3	156.458	−4.243

Table 2 shows the ADMET (absorption, distribution, metabolism, excretion, and toxicity) data of the test compounds, from which it can be seen clearly that there are no compounds that fall into the AMES toxicity category.

Table 2: ADMET properties of *o*-hydroxycinnamic acid derivatives.

Code	Absorption			Distribution			Metabolism		Excretion	Toxicity		
	Intestinal absorption	Skin permeability	Caco-2 permeability	VDss (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	Total clearance	AMES toxicity	Hepatotoxicity	LD_{50}
OCA-1a	92.105	−2.499	1.164	−0.553	−0.237	−2.404	No	No	0.794	No	No	2.069
OCA-2a	97.15	−2.664	−0.270	−1.168	−0.135	−2.486	No	No	0.876	No	No	2.657
OCA-3a	97.247	−2.658	1.054	−1.116	−0.182	−2.497	No	No	0.903	No	No	2.675
OCA-4a	96.872	−2.654	0.998	−1.065	−0.230	−2.502	No	No	0.920	No	No	2.683
OCA-5a	96.038	−2.653	0.960	−1.020	−0.258	−2.505	No	No	0.937	No	No	2.687
OCA-6a	94.55	−2.641	1.034	−0.918	−0.300	−2.25	No	Yes	0.849	No	No	2.37
OCA-7a	95.38	−2.577	1.002	−1.581	−0.126	−2.138	No	No	0.794	No	No	2.622
OCA-8a	95.854	−2.471	1.315	−1.694	-0.489	−2.413	No	No	0.769	No	No	2.688
OCA-9a	93.321	−2.639	1.049	−0.857	−0.33	−2.144	No	Yes	−0.087	No	No	2.591
OCA-10a	95.671	−2.701	1.209	−0.105	−0.026	−1.797	No	No	0.807	No	No	1.955
OCA-11a	95.252	−2.771	1.230	−0.033	−0.041	−1.896	No	No	0.836	No	No	2.028
OCA-12a	94.853	−2.848	1.256	0.031	−0.089	−1.841	No	No	0.847	No	No	2.017
OCA-13a	94.233	−2.908	1.279	0.091	−0.104	−1.777	No	No	0.864	No	No	1.991
OCA-14a	93.386	−2.942	1.302	0.145	−0.116	−1.695	No	No	0.882	No	No	1.975
OCA-15a	95.089	−2.283	1.247	−1.231	0.091	−2.085	No	No	0.845	No	No	2.248
OCA-17a	98.951	−2.481	1.046	−0.104	0.049	−2.017	No	No	0.898	No	No	1.995
OCA-18a	89.633	−2.817	1.397	−0.168	0.127	−2.014	No	Yes	−0.138	No	No	2.185
OCA-19a	90.169	−2.858	1.393	−0.087	0.096	−1.947	No	Yes	−0.196	No	Yes	2.262
OCA-20a	92.670	−2.915	1.347	−0.083	0.034	−2.215	No	Yes	−0.096	No	Yes	2.335
OCA-21a	88.711	−2.855	1.420	−0.139	0.083	−1.907	No	Yes	−0.270	No	No	2.33
OCA-22a	73.901	−3.053	0.193	−0.510	−0.838	−2.672	No	Yes	−0.388	No	Yes	1.913

Figure 2 shows A: COX-1 co-crystal (PDB ID: 1EQG) ligand reference: IBP_701A. Docking study was performed in cavity 4 Vol 61.44. B: $P2Y_{12}$ co-crystal (PDB ID: 4PXZ) ligand reference: 6AD-1201. Docking study was conducted in cavity 2 Vol 74.52.

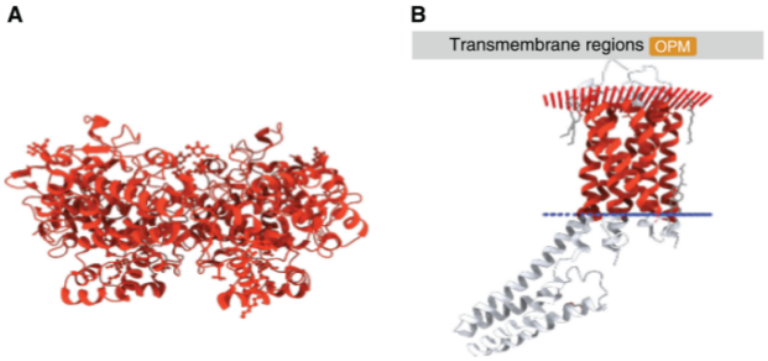


Figure 2: (A) COX-1 receptor with PDB ID: 1EQG [33]. (B) $P2Y_{12}$ receptor with PDB: ID 4PXZ [27]. A is a co-crystal structure of COX-1 enzymes and B is a co-crystal structure of $P2Y_{12}$ receptor with their reference ligand respectively, by which the binding site of the reference ligand and protein will be occupied by the test compounds.

Table A and Table B summarize the results of the docking study of all compounds OCA-1a–22a, aspirin, and ibuprofen against COX-1 enzyme. All binding energies are lower than that of aspirin, except OCA-1a.

Table A: Results of the docking of the test ligand (OCA-1a–12a) at the binding site of COX-1 receptor.

Ligand	OCA											
	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12a
MDS kCal/-mol	−95.893	−115.21	−116.11	−118.75	−119.58	−130.54	−132.37	−138.727	−136.41	−102.26	−109.46	−114.51
H-bond kCal/-mol	−3.7307	−2.5	−0.8395	−4.3413	−2.5429	−1.9859	−1.9859	−2.5	−2.5	−2.5348	−2.5	−1.9472
Amino acid residue	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe – – 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 200-Phe 202-Ala 203-Gln 206-Thr 210-Phe – 348-Tyr – 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe – 348-Tyr 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe – 348-Tyr 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe – – 382-Asn 385-Tyr 386-His 387-Trp 390-Leu	– 202-Ala 203-Gln 206-Thr 207-His 210-Phe – – – 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe – – 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 210-Phe 212-Thr – 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe 212-Thr – 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr – 210-Phe – – 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr – 210-Phe – – 382-Asn 385-Tyr 386-His 387-Trp 388-His	199-Ala 202-Ala 203-Gln 206-Thr 207-His 210-Phe – – 382-Asn 385-Tyr 386-His 387-Trp 388-His

	390-Leu	390-Leu	390-Leu	390-Leu	391-Met	390-Leu	390-Leu	390-Leu	390-Leu	–	–	–
	–	391-Met	391-Met	–	–	391-Met	391-Met	391-Met	–	–	–	391-Met

Table B: Results of the docking of the test ligand (OCA-13a–22a, ibuprofen, and aspirin) at the binding site of COX-1 receptor.

Ligand	OCA									Ibuprofen	Aspirin
	13a	14a	15a	17a	18a	19a	20a	21a	22a		
MDS kCal/-mol	−118.773	−121.945	−99.5915	−110.611	−137.417	−136.754	−137.127	−144.911	−117.789	−93.9403	−113.392
H-bond kCal/-mol	−2.5	−2.21449	−0.371838	−0.161469	−1.08633	−5	−3.2979	−0.806575	−2.07898	−2.95147	−2.5
Amino acid residue	–	–	–	–	–	–	–	–	–	113-Met	–
	–	–	–	–	–	–	–	116-Val	–	116-Val	–
	–	–	–	–	–	–	–	117-Leu	–	117-Leu	–
	–	–	–	–	–	–	–	120-Arg	–		–
	–	–	–	–	–	–	–	–	–	129-Arg	–
	–	–	–	–	–	–	–	–	148-Tyr	–	–
	–	–	–	–	–	–	–	–	198-Phe	–	–
	199-Ala	199-Ala	199-Ala	199-Ala	199-Ala	199-Ala	199-Ala	–	199-Ala	–	199-Ala
	–	–	–	–	–	–	–	–	200-Phe	–	–
	202-Ala	202-Ala	202-Ala	202-Ala	202-Ala	–	202-Ala	–	202-Ala	–	202-Ala
	–	203-Gln	–	203-Gln	203-Gln	203-Gln	203-Gln	–	203-Gln	–	203-Gln
	206-Thr	206-Thr	206-Thr	206-Thr	206-Thr	206-Thr	206-Thr	–	206-Thr	–	206-Thr
	207-His	–	207-His	207-His	207-His	207-His	–	–	207-His	–	207-His
	210-Phe	210-Phe	210-Phe	210-Phe	210-Phe	210-Phe	210-Phe	–	210-Phe	–	210-Phr
	–	–	211-Lys	–	–	–	211-Lys	–	211-Lys	–	–
	212-Thr	212-Thr	–	–	–	212-Thr	212-Thr	–	212-Thr	–	–
	–	–	–	–	–	–	–	–	–	345-Ile	–
	–	–	–	–	348-Tyr	348-Tyr	–	348-[illegible]r	–	–	–
	–	–	–	–	–	–	–	349-Val	–	349-Val	–
	–	–	–	–	–	–	–	352-Leu	–	352-Leu	–
	–	–	–	–	–	–	–	353-Ser	–	353-Ser	–
	–	–	–	–	–	–	–	355-Tyr	–	355-Tyr	–
	–	–	–	–	–	–	–	359-Leu	–	359-Leu	–

–	–	–	–	–	381-Phe	–	381-Phe	381-Phe	381-Phe	–
382-Asn	–	382-Asn	–	382-Asn	382-Asn	382-Asn	–	382-Asn	–	–
–	–	–	–	–	383-Gln	–	–	–	–	385-Tyr
–	–	–	–	–	–	–	384-Leu	–	384-Leu	386-His
385-Tyr	385-Tyr	385-Tyr	385-Tyr	385-Tyr	385-Tyr	385-Tyr	385-Tyr	385-Tyr	385-Tyr	387-Ttp
386-His	386-His	386-His	386-His	386-His	386-His	386-His	–	386-His	–	388-His
387-Trp	387-Trp	387-Trp	387-Trp	387-Trp	387-Trp	387-Trp	387-Trp	387-Trp	387-Trp	390-Leu
388-His	388-His	388-His	388-His	388-His	388-His	388-His	–	388-His	–	391-Met
390-Leu	390-Leu	–	390-Leu	390-Leu	–	390-Leu	–	390-Leu	–	–
–	–	391-Met	391-Met	391-Met	391-Met	–	–	391-Met	–	–
–	–	–	–	–	444-Ile	–	–	444-Ile	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	518-Phe	–	518-Phe	–
–	–	–	–	–	–	–	522-Met	–	–	–
–	–	–	–	–	–	–	523-Ile	–	523-Ile	–
–	–	–	–	–	–	–	526-Gly	–	526-Gly	–
–	–	–	–	–	–	–	527-Ala	–	527-Ala	–
–	–	–	–	–	–	–	530-Ser	–	530-Ser	–
–	–	–	–	–	–	–	531-Leu	–	531-Leu	–
–	–	–	–	–	–	–	–	–	552-Met	–
–	–	–	–	–	–	–	–	–	552-Met	–

Table C and Table D show the results of docking studies on $P2Y_{12}$ receptors. OCA-1A–22A are lower than aspirin but higher than ibuprofen.

Table C: Results of the docking of the test ligand (OCA-1a–12a) at the binding site of $P2Y_{12}$ receptor.

Ligand	OCA											
	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12a
MDS kCal/-mol	−88.092	−109.277	−114.255	−115.816	−121.981	−138.376	−141.739	−134.862	−143.635	−90.055	−99.3538	−101.611
H-bond kCal/-mol	−5	−4.45498	−3.85612	−3.00134	0	−4.13639	−4.49929	−3.10572	−2.65512	−4.22953	−5.84453	−2.5
Amino acid residue	–	–	–	–	97-Cys	97-Cys	–	–	97-Cys	–	–	–
	–	–	–	101-Ser	101-Ser	101-Ser	101-Ser	101-Ser	–	–	–	–
	102-Val	102-Val	–	102-Val	102-Val	–	102-Val	102-Val	102-Val	102-Val	102-Val	102-Val
	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr	–	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr
	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	–	106-Phe	106-Phe	106-Phe	–	106-Phe	106-Phe

109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	152-Met	109-Tyr	109-Tyr	109-Tyr
152-Met	152-Met	152-Met	152-Met	152-Met	–	–	–	–	–	–	152-Met
155-Leu	155-Leu	–	–	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu
156-Ser	156-Ser	156-Ser	156-Ser	156-Ser	156-Ser	159-Asn	159-Asn	159-Asn	156-Ser	156-Ser	156-Ser
159-Asn	159-Asn	159-Asn	159-Asn	159-Asn	–	–	–	–	159-Asn	159-Asn	159-Asn
–	–	–	–	175-Cys	175-Cys	175-Cys	175-Cys	175-Cys	–	–	–
–	–	179-Lys	–	179-Lys	179-Lys	–	–	179-Lys	–	179-Lys	–
–	–	–	186-Trp	–	–	–	–	–	–	–	–
–	–	187-His	187-His	187-His	187-His	187-His	187-His	187-His	187-His	187-His	–
190-Val	190-Val	–	190-Val	190-Val	190-Val	190-Val	190-Val	–	190-Val	190-Val	190-Val
191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn
194-Cys	194-Cys	–	194-Cys	194-Cys	194-Cys	194-Cys	194-Cys	194-Cys	194-Cys	194-Cys	194-Cys
–	–	–	–	–	–	–	–	–	–	–	195-Gln
252-Phe	252-Phe	252-Phe	252-Phe	252-Phe	252-Phe	252-Phe	252-Phe	252-Phe	–	–	252-Phe
–	–	–	–	–	–	–	–	–	–	–	253-His
256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg

Table D: Results of the docking of the test ligand (OCA-13a–22a, ibuprofen, and aspirin) at the binding site of $P2Y_{12}$ receptor.

Ligand	OCA									Ibuprofen	Aspirin
	13a	14a	15a	17a	18a	19a	20a	21a	22a		
MDS kCal/-mol	−104.067	−107.711	−90.295	−94.6112	−94.9611	−132.141	−138.165	−144.937	−136.335	−147.891	−84.6987
H bond kCal/-mol	−3.34754	−4.83363	−2.83019	−3.18851	−0.59485	−2.20782	−2.11142	−2.39911	−3.21049	−3.14894	−2.5
Amino acid residue	–	–	–	–	–	–	93-Arg	–	–	–	–
	–	–	–	–	–	–	–	–	–	96-Val	–
	–	–	–	–	–	97-Cys	97-Cys	97-Cys	97-Cys	–	97-Cys
	101-Ser	101-Ser	–	–	–	–	–	101-Ser	101-Ser	101-Ser	101-Ser
	102-Val	102-Val	102-Val	102-Val	102-Val	102-Val	102-Val	102-Val	102-Val	102-Val	–
	105-Tyr	105-Tyr	–	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr	105-Tyr
	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe	106-Phe
	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr	109-Tyr
	152-Met	152-Met	–	152-Met	–	152-Met	–	152-Met	152-Met	–	152-Met
	–	–	–	–	–	–	–	–	–	153-Phe	–

155-Leu	–	–	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu	155-Leu
156-Ser	156-Ser	156-Ser	156-Ser	156-Ser	156-Ser	159-Asn	156-Ser	156-Ser	156-Ser	156-Ser
159-Asn	159-Asn	159-Asn	159-Asn	159-Asn	159-Asn	–	159-Asn	159-Asn	159-Asn	159-Asn
–	–	–	–	–	163-Thr	–	–	–	–	–
175-Cys	–	–	–	–	–	–	175-Cys	–	175-Cys	–
–	–	–	–	–	–	–	176-Ser	–	–	–
179-Lys	179-Lys	–	–	–	179-Lys	179-Lys	179-Lys	179-Lys	179-Lys	179-Lys
187-His	–	187-His	–	–	187-His	187-His	187-His	187-His	187-His	187-His
190-Val	190-Val	190-Val	190-Val	–	190-Val	190-Val	190-Val	190-Val	190-Val	190-Val
191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn	191-Asn
194-Cys	194-Cys	194-Cys	194-Cys	194-Cys	194-Cys	–	194-Cys	–	194-Cys	194-Cys
–	–	–	–	195-Gln	–	–	–	–	–	–
–	–	252-Phe	252-Phe	252-Phe	–	–	–	–	–	–
–	–	–	–	253-His	–	–	–	–	–	–
256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	256-Arg	–
–	–	–	–	–	–	–	259-Tyr	263-Gln	259-Tyr	–
–	–	–	–	–	263-Gln	263-Gln	263-Gln	–	263-Gln	–
–	–	–	–	–	–	–	–	277-Phe	–	–
–	–	–	–	–	280-Lys	280-Lys	280-Lys	280-Lys	280-Lys	–
–	–	–	–	–	–	–	–	–	281-Glu	–

Figure 3 shows the interaction between ligands and amino acids at COX-1 receptors. It can be observed that aspirin (A) and OCA-1a (C) have similar amino acid residues. The addition of aromatic constituents to OCA-9a (D) adds to the amino acid bond. In contrast to interactions with COX-1, in Figure 4, the $P2Y_{12}$ receptor amino acid residues that interact with *o*-hydroxycinnamic derivatives differ from those of aspirin.

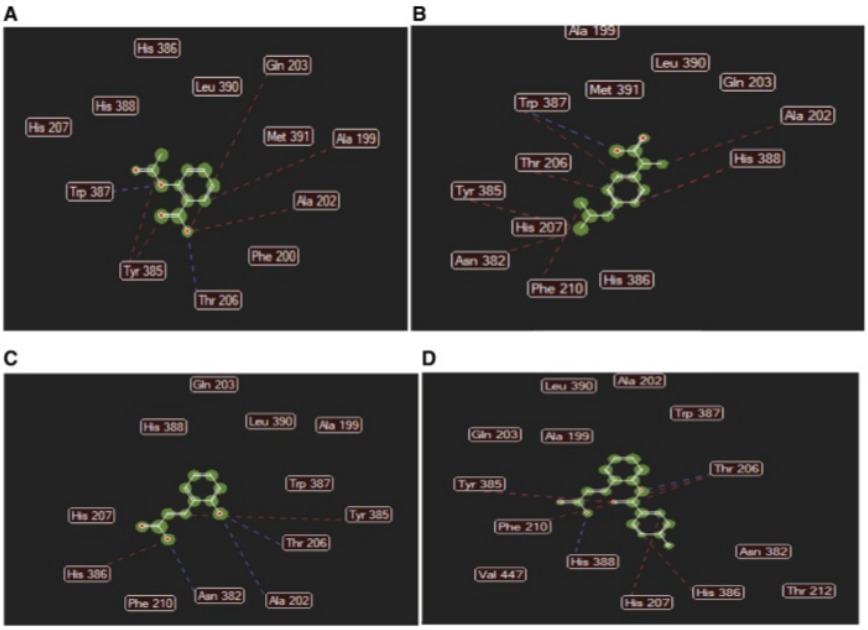


Figure 3: Map of the interaction between *o*-hydroxycinnamic acid derivatives and COX-1 receptor: (A) aspirin, (B) ibuprofen, (C) OCA-1a, (D) OCA-9a. Examples of interactions between functional groups of the test compounds with amino acid residues of the COX-1 enzyme, the blue line shows the hydrogen bonding interaction, and the red line shows the hydrophobic interaction.

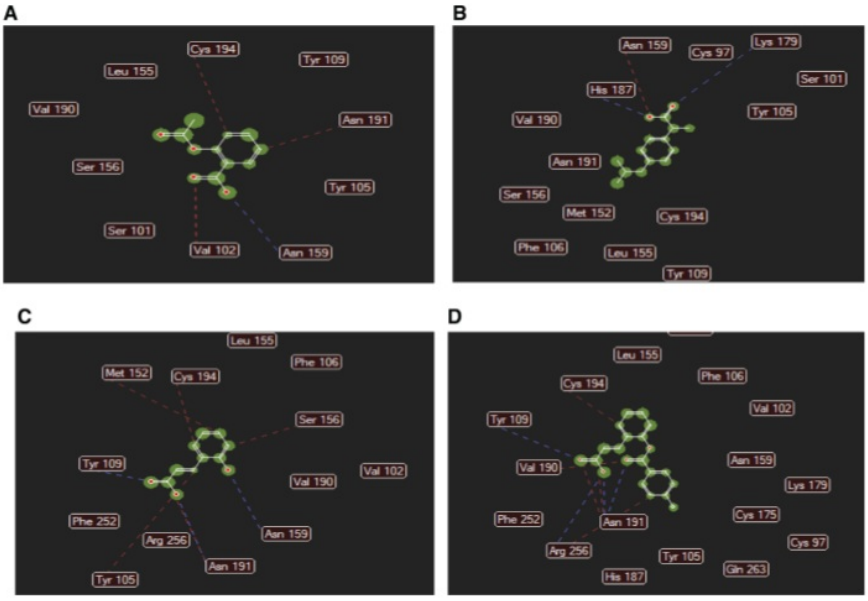


Figure 4: Map of interaction between *o*-hydroxycinnamic acid derivatives and COX-1 receptor: (A) aspirin, (B) ibuprofen, (C) OCA-1a, (D) OCA-9a. Examples of interactions between functional groups of the test compounds with amino acid residues of the $P2Y_{12}$ receptor, the blue line shows the hydrogen bonding interaction, and the red line shows the hydrophobic interaction.

Discussion

The failure of a drug candidate in clinical trial is commonly due to its poor ADMET profile [34], [35]. The success and efficacy of a drug compound are determined by its pharmacokinetic properties. Anti-platelets for the prevention of coronary heart disease recurrence are generally given orally [36] for the comfort and convenience of patients [37]. Consequently, the design of the drug compound must be such that it is well absorbed in the digestive tract.

Based on an in-depth evaluation of the molecular properties with the results of Phase II clinical trials, Lipinski et al. formulated five physical and chemical characteristics of drugs that can predict the absorption and permeability of drug candidates, known as the Lipinski rule of 5. Excluding the possibility of whether a drug compound can bind peptides in the intestinal membrane depends on the following criteria: molecular weight less than 500, log P not greater than 5, number of hydrogen bond donors (HBD) less than 5, and number of hydrogen bond acceptors (HBA) more than 10 [38], [39].

The results in Table 1 show that all compounds OCA1–22 meet the Lipinski rule. OCA-22 has the largest MW, HBA, and HBD, but they are still below 500, 10, and 5, respectively. Navia and Chaturvedi found that the molecular size of a drug is inversely proportional to its absorption ability [40]. Molecules with excessive HBD impair phospholipid membranes [41], while those with HBAs exceeding 10 will hinder absorption [37].

Table 2 shows the ADMET data of all the tested compounds. The compounds OCA1a–22a have good intestinal absorption, which is in line with the physicochemical properties of the tested compounds that meet the Lipinski rule. All this indicates that all the test compounds are suitable for oral administration. A compound is considered to have a high Caco-2 permeability if it has Papp > 8 × 10^6 cm/s. In the case of the pkCSM predictive model, high Caco-2 permeability translates into predicted log Papp values >0.90 cm/s [42].

Table 2 shows that only OCA-2a and OCA 22-a have Caco-2 permeability values below 0.9, which means that the majority of tested compounds have very high permeability [44]. However, some experts believe that Caco-2 permeability applies only to drugs that are absorbed through passive diffusion. For drugs with active transport, it might give different results [44].

The skin permeability value was calculated from the logarithmic penetrating ability of 211 molecules to human skin *in vitro*. The result is considered low if log Kp exceeds −2.5 [43]. In Table 2, most of the test compounds have values lower than −2.5, which shows that these compounds have the potential to be developed as transdermal drugs.

The volume of distribution (Vd) illustrates how much blood plasma is needed so that drug compounds have the same levels. The higher the Vd value, the more the volume of the compound in the tissue. In pKCSM, Vd is considered low if log L/kg is below −0.15. Table 2 shows that most of the test compounds have values below −0.15. This means that there is less drug in in the tissues and more in the blood, which is what is expected for drug compounds that target platelets [42].

Toxicity data show that most of the test compounds do not cause hepatotoxicity. AMES toxicity predicts whether the test compound is carcinogenic, and our results indicate that none of the test compounds is predicted to cause genetic mutations [42]. LD50 estimates the threshold for chemicals to cause toxicity in humans. The result is considered low if it is less than or equal to 0.477 [43]. Table 2 shows that the LD50 values of all the test compounds are above 0.477, meaning that the values are high, so they are safe for use in humans.

Consistent with the good pharmacokinetic properties, the interaction of the test compounds with COX-1 and $P2Y_{12}$ receptors also gives promising results. MVD optimizes the pose of each ligand structure using an algorithm [45]. This software can also identify the binding sites, called cavity or active sites, which are pockets where the ligands are predicted to bind to the receptors. $P2Y_{12}$ and COX-1 receptors in this study were found to be in cavity 4 and cavity 2, respectively. The cavities were chosen because the native ligands were inside.

All ligands OCA1a–22a and the standard ligands ibuprofen and aspirin which were prepared were docked on the selected cavity. The MVD program uses a score to estimate the binding energy in a ligand-protein complex. To improve docking accuracy, an MDS is given, which can identify the most promising docking solution from the docking algorithm [31], [45]. The results of the *in silico* tests are in the form of bond energy values or MDS. MDS is a differential evolution algorithm in the Molegro Virtual Docker program. MDS or E_{score} is the sum of E_{inter} (the ligand-receptor interaction energy) and E_{intra} (the ligand internal energy) [46], 47]. The energy needed by ligands to bind to receptors is called the bonding energy. The smaller the energy, the more stable the bonds, so it can be estimated that the activity will be stronger [27].

Table A shows that the MDS of the test compounds are lower than that of aspirin, both on the COX-1 receptor and $P2Y_{12}$. The MDS difference between OCA-1a and aspirin on COX-1 is 18 kCal/mol, whereas in $P2Y_{12}$ the receptor it is 5 kCal/mol. Among them, the compounds OCA-6a, 7a, 8a, and 9a, 18a, 19a, 20a, and 21 show promising results because the MDS is very low in both receptors.

Based on the data in Table A and Table B, it is seen that the interaction of the test compounds, i.e. *o*-hydroxycinnamic acid derivatives, against COX-1 is with the amino acids 199-Ala, 202-Ala, 203-Gln, 206-Thr,

207-His, 210-Phe, 382-Asn, 385-Tyr, 386-His, 387-Trp, 388-His, and 390-Leu. This is similar to aspirin except that there is an additional 391-Met. Likewise, the interaction with $P2Y_{12}$ involves 101-Ser, 102-Val, 105-Tyr, 109-Tyr, 155-Leu, 156-Ser, 159-Asn, 190-Val, 191-Asn, and 194-Cys, the same as for aspirin. The tested compounds also have additional interactions with 106-Phe and 256-Arg.

Conclusions

pKCSM analysis showed that all compounds OCA-1a–22a have good absorption profiles and can be used as oral preparations. Their distribution and toxicity are also good enough for them to be developed as anti-platelet agents. Docking analysis showed that the interaction of amino acids that bind to Cox-1 and $P2Y_{12}$ receptors is not much different from that of aspirin. However, MDS for derivatives with aromatic substituents in OCA-6a, 7a, 8a, 9a, OCA-18a, 19a, 20a, and 21a have very low interaction energy compared to aspirin and ibuprofen, both on COX-1 and $P2Y_{12}$. Therefore, it can be predicted that these seven compounds have strong potential to inhibit both receptors.

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