https://doi.org/10.31925/farmacia.2022.6.8 **ORIGINAL ARTICLE**

A COMPARISION OF BINDING AFFINITIES OF SOME DERIVATIVES OF ACETYLSALICYLIC ACID ON THE SURFACES OF COX1 AND COX2

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Abstract

The popularity of acetylsalicylic acid (aspirin-salicylates drugs class) used as a nonsteroidal antiinflamatory drug, declined after the market releases of paracetamol in 1956, and ibuprofen in 1969. Acetylsalicylic acid sales revitalized considerably in the last decades of the last century, and remain strong in the 21st century, because of its use, under lower concentrations, as a preventive treatment for cardiovascular diseases, like heart attacks and strokes, as an inhibitor of platelet aggregation and for the prevention of post-surgical thromboembolisms. However, it may be interesting to find and to try similar compounds in order to enlarge the spectrum of cyclooxygenase inhibitors used today in therapeutics. We used the Similar Compounds search type of the Chemical Structure Search of the PubChem Compound Database, to locate records that are similar to the chemical structure of acetylsalicylic acid, using pre-specified similarity thresholds. Using the threshold ≥ than 99% for the similar structures criteria, we found 14 compounds that meet this criterion (based on which chemical structures? Also salicylate?). In accordance with our calculations and molecular docking simulations, all these compounds have a better binding affinity to cyclooxygenase enzymes than acetylsalicylic acid, consequently they may be used as possible alternatives to this drug.

Rezumat

Popularitatea acidului acetilsalicilic (aspirină-clasa farmaco-terapeutică a salicilaților) folosită cu precădere drept antiinflamator non-steroidian, a scăzut după lansarea pe piață a paracetamolului în 1956 și a ibuprofenului în 1969. Vânzările de acid acetilsalicilic s-au revitalizat considerabil în ultimele decenii ale secolului trecut și rămân mari în secolul al XXI-lea, datorită utilizării sale pe scară largă, sub concentrații mult mai mici comparativ cu concetrațiile folosite pentru efectul antiinflamator, ca un tratament preventiv în boli cardiovasculare, ca atacul de cord și accidentul vascular cerebral, drept antiagregant plachetar și pentru prevenirea tromboembolismelor post chirurgicale. Cu toate acestea, poate fi utilă identificarea și încercarea unor compuși similari pentru a lărgi spectrul de inhibitori de ciclooxigenază folosiți astăzi în terapie. Am folosit algoritmul de căutare „compuși similari" al rutinei de căutare pe bază de structuri chimice a bazei de date PubChem, pentru identificarea unor molecule care sunt similare ca structură chimică acidului acetilsalicilic, utilizând praguri de similitudine predefinite (bazate pe ce structuri chimice de bază? Tot salicilați?). Pentru un prag ≥ 99%, pentru criteriul structuri similare, am găsit 14 compuși care au îndeplinit acest criteriu. Toți compușii au o afinitate de legare la ciclooxigenaze superioară acidului acetilsalicilic, deci ar reprezenta alternatve viabile la acestă moleculă.

Keywords: acetylsalicylic acid, substitutes, structural similarity, screening, docking

Introduction

Acetylsalicylic acid (2-acetyloxybenzoic acid, PubCHem Compound ID 2244, CAS 50-78-2) is an analgesic, antipyretic, anti-inflammatory and anti-rheumatic agent. In countries where Aspirin is a registered trademark owned by Bayer, the generic term is acetyl-salicylic acid (ASA).

Acetylsalicylic acid (an acetylated salicylate), is classified among the nonsteroidal anti-inflammatory drugs (NSAIDs). These agents reduce the signs and symptoms of inflammation and exhibit important pharmacologic activities, including analgesic, anti-pyretic, and antiplatelet properties. NSAIDs generally do not change the course of the disease process; they are used for symptomatic relief.

Acetylsalicylic acid's mode of action as an anti-inflammatory and antirheumatic agent may be due to inhibition of synthesis and release of prostaglandins, and produces analgesia by virtue of both a peripheral and central nervous system effect. Peripherally, acetylsalicylic acid acts by inhibiting the synthesis and release of prostaglandins. Acting centrally, it would appear to produce analgesia and antipyresis at a hypothalamic site in the brain, although the mode of action is not known [1].

The anti-inflammatory, the analgesic and antipyretic activity are due to actions by both the acetyl and the salicylate fragments of the intact molecule as well as by the metabolite of active salicylate. Acetylsalicylic acid inhibits the activity of both types of cyclo-oxygenase (COX-1 and COX-2) to reduce the formation of precursors of prostaglandins and thromboxanes from arachidonic acid (AHA). Unlike other non-steroidal anti-inflammatory drugs (NSAIDS), such as diclofenac and ibuprofen, acetylsalicylic acid is an irreversible inhibitor.

Acetylsalicylic acid is used in the temporary relief of various forms of pain, inflammation associated with various conditions (rheumatoid arthritis, lupus erythematosus, osteoarthritis, and spondylitis), and is also used to reduce the risk of death and/or non-fatal myocardial infarction in patients with a previous infarction or unstable angina pectoris [1, 2].

It should not be taken by people who are allergic to ibuprofen or naproxen, or who have salicylate intolerance and caution should be exercised in those with asthma. Aspirin use has been shown to increase the risk of gastrointestinal bleeding. People with kidney disease, gout or hyperuricemia should not take acetylsalicylic acid because it inhibits the kidney's ability to excrete uric acid [3, 4]. It should not be given to children or adolescents to control cold or influenza symptoms, as this has been linked with Reye's syndrome [5, 6].

The popularity of this NSAID declined after the market releases of paracetamol (acetaminophen, N-(4-hydroxyphenyl)acetamide) in 1956 and ibuprofen (brufen, 2-(4-(2-methylpropyl)phenyl)propanoic acid) in 1969. In the 1960s and 1970s, John Vane and others discovered the basic mechanism of aspirin's effects [7], while clinical trials and other studies from the 1960s to the 1980s established aspirin's efficacy as an anticlotting agent that reduces the risk of clotting diseases.

However, other similar compounds must be found to replace or to complete the action of this active substance, or to enlarge the spectrum of prostaglandin-endoperoxide synthase inhibitors used today in therapeutics.

Materials and Methods

Hardware: Asus X401A PC, CPU Dual Core Intel 820, 1.7 GHz, 4 GB RAM.

Software: OS Windows 7 - 64 bit, Chem 3D Ultra 10.0, ChemDraw Pro 10.0, AutoDock Tools 1.5.6 Molecular Graphics Laboratory The Scripps Research Institute [8], AutoDock Vina by Sargis Dallakyan, The Scripps Research Institute [9], Open Babel 2.3.2. [10], PyRx 0.8, PubChem Compound Database, Firefox 28.0.

The Similar Compounds search type of the Chemical Structure Search of the PubChem Compound Database [11] allows locating records that are similar to a chemical structure query using pre-specified similarity thresholds. Similarity is measured using the Tanimoto equation and the PubChem dictionary-based binary fingerprint. This fingerprint consists of series of chemical substructure "keys". Each key denotes the presence or absence of a particular substructure in a molecule. The fingerprint does not consider variation in stereo chemical or isotopic information. Collectively, these binary keys provide a "fingerprint" of a particular chemical structure valence-bond form [11].

The degree of similarity is dictated by the Threshold parameter. A threshold of "100%" effectively acts as an "exact match" to the provided chemical structure query (ignoring stereo or isotopic information), while a threshold of "0%" would return all chemical structures in the PubChem Compound database. Various predefined thresholds between 99-60% are allowed [11].

Searching the databases (with over 30 million entries) is possible for a broad range of properties including chemical structure, name fragments, chemical formula, molecular weight, XLogP, and hydrogen bond donor and acceptor count. PubChem can be accessed for free through a web user interface.

AutoDock Vina [9] significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4 [8]. For its input and output, Vina uses the same PDBQT molecular structure file format used by AutoDock. PDBQT files can be generated (interactively or in batch mode) and viewed using MGLTools. Other files, such as the AutoDock and AutoGrid parameter files (GPF, DPF) and grid map files are not needed. As described in the software documentation, the docking calculation consists of a number of independent runs, starting from random conformations. Each of these runs consists of a number of sequential steps. Each step involves a random perturbation of the conformation followed by a local optimization (using the Broyden-Fletcher-Goldfarb-Shanno algorithm) and a selection in which the step is either accepted or not [8, 9]. Each local optimization involves many evaluations of the scoring function as well as its derivatives in the position-orientation-torsions coordinates. The number of evaluations in a local optimization is guided by convergence and other criteria. The number of steps in a run is determined heuristically, depending on the size and flexibility of the ligand and the flexible side chains. However, the number of runs is set by the exhaustiveness parameter. Since the individual runs are executed in parallel, where appropriate, exhaustiveness also limits the parallelism [8, 9]. Unlike in AutoDock 4, in AutoDock Vina, each run can produce several results: promising intermediate results are remembered. These are merged, refined, clustered and sorted automatically to produce the final result [12-16].

Vina creates *_out.pdbqt files where it stores all docked poses and scores [9].

The predicted binding affinity of bound structures is given in kcal/mol. To compare the accuracy of the predictions of the experimental structure, AutoDock Vina uses a measure of distance between the experimental and predicted structures, RMSD, root-mean-square deviation.

As described in the software documentation, RMSD values are calculated relative to the best mode and using only movable heavy atoms. For scoring, AutoDock Vina uses a united-atom function, which involves only the heavy atoms [9].

Two variants of RMSD metrics are provided by the software, rmsd/lb (RMSD lower bound) and rmsd/ub (RMSD upper bound), differing in how the atoms are matched in the distance calculation: (i) *rmsd/ub* matches each atom in one conformation with itself in the other conformation, ignoring any symmetry; (ii) *rmsd'* matches each atom in one conformation with the closest atom of the same element type in the other conformation (rmsd' cannot be used directly, because it is not symmetric); (iii) *rmsd/lb* is defined as follows: $rmsd/lb(c_1, c_2) = max((rmsd'(c_1, c_2), rmsd'(c_2, c_1))$ [9].

Results and Discussion

Using the Chemical Structure Search of the PubChem Compound Database and a threshold ≥ than 99% for the similar structures criteria, we detected 14 compounds that meet this criterion. We calculated the binding affinities for the ligands (including acetyl-salicylic acid) to the surface of COX-1 and COX-2 enzymes. The structures of enzymes were retrieved from Protein Data Bank [17-19] in PDB format with Chem 3D's "*Online Find Structure from PDB id*" option. The water molecules, solvent molecules and other relics of the isolation and crystallization procedures were removed.

X-ray crystallography usually does not locate hydrogens; hence most PDB files do not include them. But hydrogens, particularly those that can form hydrogen bonds, are important in binding ligands, so hydrogens were added to backbone N, and to amine and hydroxyl side chains. Atoms were renumbered, and PDBQT files generated with AutoDock Tools 1.5.6 [8].

14 substances (with PubChem Compound ID = 298996, 94717, 22619484, 21481530, 95938, 94716, 3055063, 301846, 54224926, 301958, 19049630, 198203, 135269, 10176491) are better ligands for COX-1 and COX-2 than acetylsalicylic acid. These ligands are shown in Table I and below, together with acetylsalicylic acid (CID 2244). All substances accomplish the Lipinski Rule of Five [20], also known as the Pfizer's rule of five or simply the Rule of five (RO5), which is a rule of thumb to evaluate drug likeness or determine, if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans: (1) no more than five hydrogen bond donors; (2) no more than ten hydrogen bond acceptors; (3) a molecular mass under 500 daltons; (4) an octanol-water partition coefficient, LogP value under five.

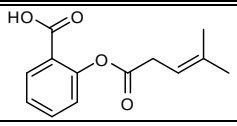
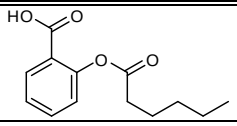
Most of these compounds are known in the literature as potential acetylsalicylic acid analogues [21], however, molecular docking investigations and molecular dynamics calculations have not been performed.

Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market. All 14 substances have 1 H-bond donor and 4 H-bond acceptors, and LogP values under 3.1.

Table I

Ligands with a similarity threshold ≥ 99%, and acetylsalicylic acid (CID 2244)

PubChem CID	IUPAC Name	Molecular Formula	MW [g/mol]	XLogP3-AA
298996	2-(4-methylpent-3-enoyloxy)benzoic acid	$C_{13}H_{14}O_4$	234.24786	2.8
94717	2-hexanoyloxybenzoic acid	$C_{13}H_{16}O_4$	236.26374	3.1
22619484	2-[(E)-but-2-enoyl]oxybenzoic acid	$C_{11}H_{10}O_4$	206.1947	2
21481530	2-(2,2-dimethyl-propanoyloxy)benzoic acid	$C_{12}H_{14}O_4$	222.23716	2.6
95938	2-propanoyloxybenzoic acid	$C_{10}H_{10}O_4$	194.184	1.7
94716	2-butanoyloxybenzoic acid	$C_{11}H_{12}O_4$	208.21058	1.7
3055063	2-heptanoyloxybenzoic acid	$C_{14}H_{18}O_4$	250.29032	3.6
301846	2-(5-methylhex-4-enoyloxy)benzoic acid	$C_{14}H_{16}O_4$	248.27444	3.1
54224926	2-(2-methylpropanoyloxy)benzoic acid	$C_{11}H_{12}O_4$	208.21058	2.2
301958	2-(3-methylbut-2-enoyloxy)benzoic acid	$C_{12}H_{12}O_4$	220.22128	2.6
19049630	2-but-3-enoyloxybenzoic acid	$C_{11}H_{10}O_4$	206.1947	1.9
198203	2-(2-methylprop-2-enoyloxy)benzoic acid	$C_{11}H_{10}O_4$	206.1947	2.2
135269	2-pentanoyloxybenzoic acid	$C_{12}H_{14}O_4$	222.23716	2.6
10176491	2-prop-2-enoyloxybenzoic acid	$C_{10}H_8O_4$	192.16812	1.8
2244	2-acetyloxybenzoic acid	$C_9H_8O_4$	180.15742	1.2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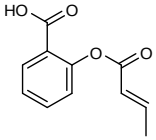	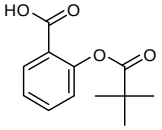
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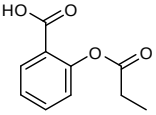	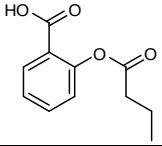
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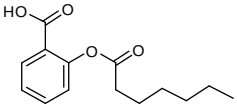
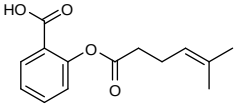
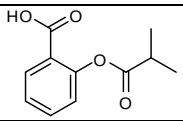
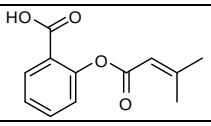
	
3055063	301846
	
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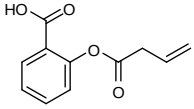	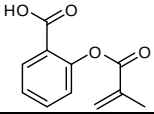
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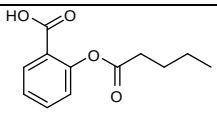	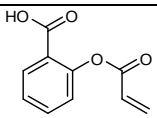
135269	10176491

In Table II and Table III are presented the calculated binding affinities in descending order for the ligands and the two enzymes (COX-1 and COX-2), only for rmsd/ub and rmsd/lb = 0.

When using a flexible docking engine, then minimizing the input conformation of the ligands can reduce problems that are known to occur in conformer generation inside the docking engine, that arise if the input 3D conformation is not relaxed into good bond lengths and angles. For small molecules a good choice is to use some of molecular mechanics to optimize the structure down to local energy minima, like UFF or mm^2. The assignment of Universal Force Field (UFF) atom types and the calculation of the molecular connectivity (identifying bonds, angular, torsional and inversion terms) has been performed using the routines available in the Open Babel package [9, 10]. OpenBabel GUI (2006) by Chris Morley converts chemical objects (currently molecules or reactions) from one file format to another. OpenBabel can be used for refining initial geometries with UFF molecular-mechanics optimizations, adding or removing hydrogens to PDB protein files.

Open Babel supports a number of force fields which can be used for energy evaluation as well as energy minimization. We used the following energy minimization parameters: Conjugate Gradients optimization algorithm, 200 total numbers of steps, stop if energy difference is less than 0.1 kcal/mol.

The virtual screening results show that the 14 compounds are strong inhibitors of COX-1, and COX-2 (Table II and Table III).

The RMSD cut-off of 2Å is usually used as criteria of the correct bound structure prediction [20]. Using the same cut-off value, the two metrics used for RMSD (summarized in Table II and Table III) indicate that all predictions for tested compounds are very accurate. Therefore, results indicate that 14 compounds are much better ligands of COX-1and COX-2 than acetylsalicylic acid (CID 2244), because they require lesser energy for binding. This suggests that these substances will successfully substitute acetylsalicylic acid.

Table II

The calculated binding affinities greater than for acetylsalicylic acid, in descending order for Cyclooxygenase-1 (COX-1)

Enzyme-Ligand (ligand's energies with Babel, kcal/mol)	**Binding Affinity** [kcal/mol]	**rmsd/ub** [Å]	**rmsd/lb** [Å]
COX1_301846_uff_E = 195.59	-7.3	0	0
COX1_301958_uff_E = 196.55	-7	0	0
COX1_298996_uff_E = 155.58	-7	0	0
COX1_22619484_uff_E = 436.89	-6.8	0	0
COX1_21481530_uff_E = 152.88	-6.8	0	0
COX1_94717_uff_E = 142.53	-6.8	0	0
COX1_95938_uff_E = 132.22	-6.7	0	0
COX1_94716_uff_E = 137.52	-6.7	0	0
COX1_3055063_uff_E = 215.11	-6.6	0	0
COX1_54224926_uff_E = 141.55	-6.5	0	0
COX1_19049630_uff_E = 477.49	-6.4	0	0
COX1_198203_uff_E = 138.43	-6.4	0	0
COX1_135269_uff_E = 158.16	-6.4	0	0
COX1_10176491_uff_E = 128.76	-6.2	0	0
COX1_2244_uff_E = 419.53	-6.1	0	0

Table III

The calculated binding affinities greater than for acetylsalicylic acid, in descending order for Cyclooxygenase-2 (COX-2)

Enzyme-Ligand (ligand's energies with Babel, kcal/mol)	**Binding Affinity** [kcal/mol]	**rmsd/ub** [Å]	**rmsd/lb** [Å]
COX2_298996_uff_E = 155.58	-8	0	0
COX2_94717_uff_E = 142.53	-7.5	0	0
COX2_301958_uff_E = 196.55	-7.5	0	0
COX2_198203_uff_E = 138.43	-7.3	0	0
COX2_301846_uff_E = 195.59	-7.3	0	0
COX2_22619484_uff_E = 436.89	-7.3	0	0
COX2_54224926_uff_E = 141.55	-7.2	0	0
COX2_21481530_uff_E = 152.88	-6.9	0	0
COX2_95938_uff_E = 132.22	-6.8	0	0
COX2_10176491_uff_E = 128.76	-6.8	0	0
COX2_3055063_uff_E = 215.11	-6.6	0	0
COX2_94716_uff_E = 137.52	-6.4	0	0
COX2_135269_uff_E = 158.16	-6.3	0	0
COX2_19049630_uff_E = 477.49	-6.2	0	0
COX2_2244_uff_E = 419.53	-6.1	0	0

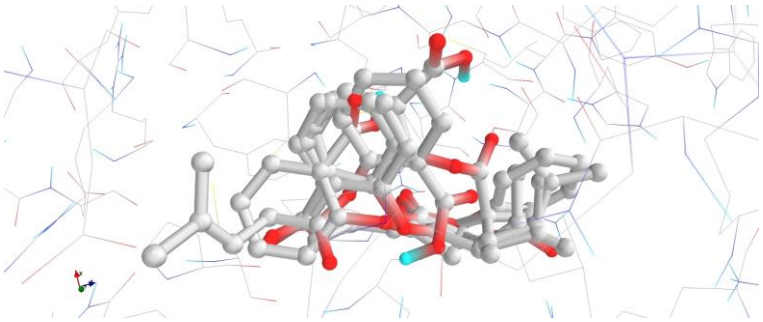

Figure 1.

Molecular docking of acetylsalicylic acid and other 6 ligands (CID 135269, 298996, 301846, 301958, 21481530, 22619484) in protein target (COX-1) for rmsd/ub and rmsd/lb = 0

For example, all calculated binding modes (scenarios) of CID 298996 have greater binding affinities than acetylsalicylic acid (Table IV).

We used the default docking parameters: 9 number of binding modes, and exhaustiveness (thoroughness of search): 8. Larger values increase the probability

of finding the global minimum, but also extend the computational time. Increasing the exhaustiveness value increases the time linearly and decreases the probability of not finding the minimum exponentially. Apart from exhaustiveness influenced by users, Vina has an internal heuristic algorithm to extend the search in accordance with an increasing number of atoms and rotatable bonds [23].

The article is a continuation of our investigations into NSAID class compounds (paracetamol and nimesulide) [24, 25].

Table IV

Binding affinities, rmsd/ub and rmsd/lb values for 298996 on the surface of COX-2

Enzyme-Ligand	**Binding Affinity** [kcal/mol]	**rmsd/ub** [Å]	**rmsd/lb** [Å]
COX2_298996_uff_E=155.58	-8	0	0
COX2_298996_uff_E=155.58	-8	6.133	3.11
COX2_298996_uff_E=155.58	-7.7	44.88	43.181
COX2_298996_uff_E=155.58	-7.6	6.215	4.197
COX2_298996_uff_E=155.58	-7.4	45.977	43.85
COX2_298996_uff_E=155.58	-7.4	2.993	1.305
COX2_298996_uff_E=155.58	-7.2	46.297	43.652
COX2_298996_uff_E=155.58	-7.1	30.924	29.535
COX2_298996_uff_E=155.58	-7	32.545	31.01

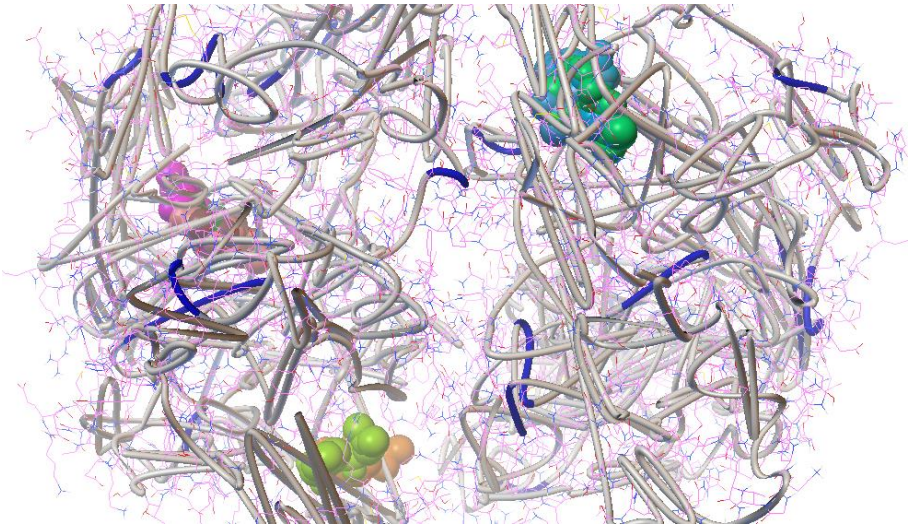

Figure 2.

Binding scenarios of CID 298996 on the surface of COX-2

Protein is represented with lines and beaded ribbons

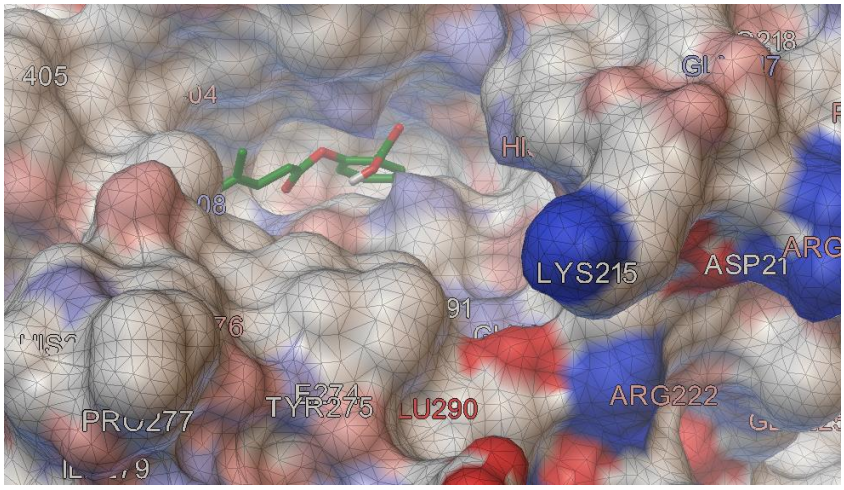

Figure 3.

Ligand CID 298996 (sticks) on the binding site of COX-2. Enzyme's surface represented with Gouraud-shaded polygons is coloured by polarity

Conclusions

Compounds with PubChem ID: 298996, 94717, 22619484, 21481530, 95938, 94716, 3055063, 301846, 54224926, 301958, 19049630, 198203, 135269 and 10176491 have better binding affinity to cyclo-oxygenase enzymes (COX-1 and COX-2) than acetyl-salicylic acid, they present the correct bound structure prediction, so they seem to be good substitutes for acetylsalicylic acid. Further investigations are needed to establish their pharmacodynamic properties and toxicity.

Conflict of interest

The authors declare no conflict of interest.

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