

POSSIBLE SUBSTITUTES FOR PARACETAMOL: THE RESULTS OF A COMPREHENSIVE SCREENING BASED ON STRUCTURAL SIMILARITY AND DOCKING SIMULATION ON THE SURFACE OF ENZYMES

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Abstract

While generally safe for use at recommended doses, acute overdoses of paracetamol can cause potentially fatal liver damage. For this reason it may be interesting to find similar compounds in order to enlarge the spectrum of prostaglandin-endoperoxide synthase (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 paracetamol, using pre-specified similarity thresholds. Using the threshold $\geq 95\%$ for the similar structures criteria, we found 37 compounds that met these criteria. Many of these compounds have a better binding affinity to cyclooxygenase than paracetamol, consequently they may be used as possible substitutes of this drug.

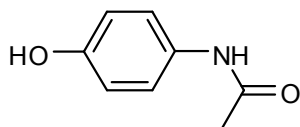
Rezumat

Considerat în general sigur în dozele recomandate, supradozele acute de paracetamol pot cauza leziuni ale ficatului potențial letale. Din această cauză poate fi interesantă găsirea unor compuși similari pentru lărgirea spectrului de inhibitori ai prostaglandin-endoperoxid sintazei (ciclooxygenazei), utilizați în terapeutică. Am folosit algoritmul de căutare “compuși similari” al rutinei de căutare pe bază de structuri chimice a bazei de date PubChem, pentru localizarea unor înregistrări care sunt similare ca structură chimică paracetamolului, utilizând praguri de similitudine predefinite. Pentru un prag $\geq 95\%$ pentru criteriul structuri similare, am găsit 37 de compuși care au îndeplinit acest criteriu. Mai mulți compuși dintre aceștia au o afinitate de legare la ciclooxygenaze superioară paracetamolului, deci ar putea înlocui cu succes acest medicament.

Keywords: paracetamol, structural similarity, screening, docking

Introduction

Paracetamol is a worldwide-used medicine for its analgesic and antipyretic actions. It has a spectrum of action similar to that of non-steroidal anti-inflammatory drugs (NSAIDs) and resembles particularly the cyclooxygenase-2 (COX-2) selective inhibitors.



CID 1983

Figure 1.

Acetaminophen, PubChem Compound ID 1983

Paracetamol is, on average, a weaker analgesic than NSAIDs or COX-2 selective inhibitors, but is often preferred because of its better tolerance. Despite the similarities to NSAIDs, the mode of action of paracetamol has been uncertain, but it is now generally accepted that it inhibits COX-1 and COX-2

through metabolism by the peroxidase function of these isoenzymes [1].

It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pains such as post-surgical pain and providing palliative care in advanced cancer patients [2].

In recommended doses and for a limited course of treatment, the side effects of paracetamol are mild to non-existent. While generally safe for use at recommended doses, acute overdoses of paracetamol can cause potentially fatal liver damage. According to the US Food and Drug Administration, “Acetaminophen can cause serious liver damage if more than directed is used” [3].

However, other similar compounds must be found to replace 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, 1.7GHz, 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 [4], AutoDock Vina by Sargis Dallakyan, The Scripps Research Institute [5], Open Babel 2.3.2. [6], PyRx 0.8, PubChem Compound Database, Firefox 28.0.

The Similar Compounds search type of the Chemical Structure Search of the PubChem Compound Database [7] 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 stereochemical or isotopic information. Collectively, these binary keys provide a "fingerprint" of a particular chemical structure valence-bond form.

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.

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 significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4. 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. 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. 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. 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 [8-13].

Vina creates *_out.pdbqt files where it stores all docked poses and scores [5].

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).

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.

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:

- *rmsd/ub* matches each atom in one conformation with itself in the other conformation, ignoring any symmetry;
- *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);
- *rmsd/lb* is defined as follows: $\text{rmsd/lb}(c_1, c_2) = \max((\text{rmsd}'(c_1, c_2), \text{rmsd}'(c_2, c_1)))$.

Results and Discussion

Using the Chemical Structure Search of the PubChem Compound Database and a threshold $\geq 95\%$ for the similar structures criteria, we detected 37 compounds that met these criteria. We have calculated the binding affinities for the ligands (including paracetamol) to the surface of COX-1 and COX-2 enzymes.

32 substances (CID = 21039831, 54373747, 28739598, 58156, 6435661, 12541316, 70185934, 13565130, 62679285, 190616, 837933, 21304453, 56990904, 62679286, 64794047, 70503032, 72907, 836029, 64795168, 29520, 836551, 10979586, 23424464, 28739570, 64780675, 64793306, 1725710, 64781061, 4301564, 57880597, 66874, 64782388) are better ligands for COX-1 than paracetamol, 28 compounds (CID = 21039831,

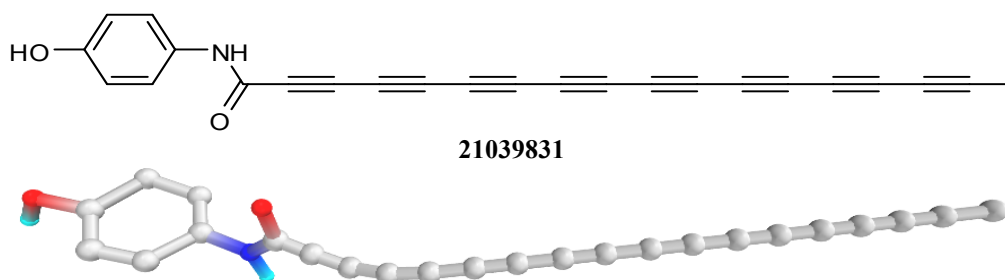
54373747, 58156, 64780675, 70185934, 23424464, 72907, 21304453, 836029, 6435661, 10979586, 12541316, 13565130, 70503032, 29520, 28739598, 62679286, 64781061, 1725710, 3014069,

21931829, 66874, 190616, 62679285, 64782388, 74325, 28739570, 70458847) are better ligands for COX-2. The best 7 ligands are shown in Table I, together with paracetamol (CID 1983).

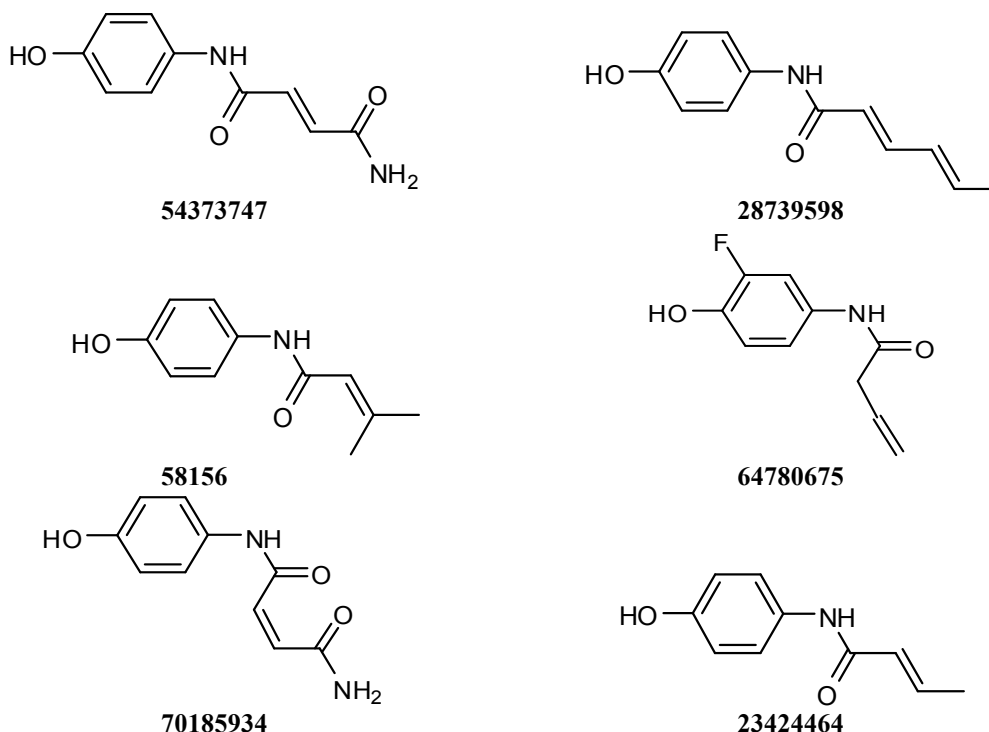
Table I

The best 7 ligands with a similarity threshold $\geq 95\%$ and paracetamol (CID 1983)

PubChem CID	IUPAC Name	Molecular Formula	MW [g/mol]
21039831	N-(4-hydroxyphenyl)octadeca-2,4,6,8,10,12,14,16-octaynamide	C ₂₄ H ₉ NO ₂	343.33376
54373747	N'-(4-hydroxyphenyl)but-2-enediamide	C ₁₀ H ₁₀ N ₂ O ₃	206.198
28739598	(2E,4E)-N-(4-hydroxyphenyl)hexa-2,4-dienamide	C ₁₂ H ₁₃ NO ₂	203.23712
58156	N-(4-hydroxyphenyl)-3-methylbut-2-enamide	C ₁₁ H ₁₃ NO ₂	191.22642
64780675	N-(3-fluoro-4-hydroxyphenyl)but-3-enamide	C ₁₀ H ₁₀ FNO ₂	195.190303
70185934	(Z)-N'-(4-hydroxyphenyl)but-2-enediamide	C ₁₀ H ₁₀ N ₂ O ₃	206.198
23424464	(E)-N-(4-hydroxyphenyl)but-2-enamide	C ₁₀ H ₁₁ NO ₂	177.19984
1983 (Paracetamol)	N-(4-hydroxyphenyl)acetamide	C ₈ H ₉ NO ₂	151.16256

**Figure 2.**

The N-(4-hydroxyphenyl)octadeca-2,4,6,8,10,12,14,16-octaynamide, CID 21039831 (Open Babel UFF molecular energy minimization)

**Figure 3.**

The best ligands structures (N'-(4-hydroxyphenyl)but-2-enediamide; (2E,4E)-N-(4-hydroxyphenyl)hexa-2,4-dienamide; N-(4-hydroxyphenyl)-3-methylbut-2-enamide; N-(3-fluoro-4-hydroxyphenyl)but-3-enamide; (Z)-N'-(4-hydroxyphenyl)but-2-enediamide; (E)-N-(4-hydroxyphenyl)but-2-enamide)

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.

The structures of enzymes were retrieved from Protein Data Bank [13,14,15] in PDB format with Chem 3D's "Online Find Structure from PDB id" option. The water molecules, other small molecules, like solvent molecules and other relics of the isolation and crystallization procedures were removed.

X-ray crystallography usually does not locate hydrogens; hence most Protein Data Bank (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.

Table II

The calculated binding affinities greater than for paracetamol, 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_21039831_uff_E=7278.98	-8.3	0	0
COX1_54373747_uff_E=135.65	-7.4	0	0
COX1_28739598_uff_E=434.80	-7.1	0	0
COX1_58156_uff_E=155.62	-6.9	0	0
COX1_6435661_uff_E=148.61	-6.8	0	0
COX1_12541316_uff_E=136.97	-6.8	0	0
COX1_70185934_uff_E=182.81	-6.8	0	0
COX1_13565130_uff_E=176.63	-6.7	0	0
COX1_62679285_uff_E=469.54	-6.7	0	0
COX1_190616_uff_E=420.09	-6.6	0	0
COX1_837933_uff_E=171.92	-6.6	0	0
COX1_21304453_uff_E=171.92	-6.6	0	0
COX1_56990904_uff_E=469.84	-6.6	0	0
COX1_62679286_uff_E=128.27	-6.6	0	0
COX1_64794047_uff_E=292.45	-6.6	0	0
COX1_70503032_uff_E=211.21	-6.6	0	0
COX1_72907_uff_E=774.64	-6.5	0	0
COX1_836029_uff_E=197.19	-6.5	0	0
COX1_64795168_uff_E=125.31	-6.5	0	0
COX1_29520_uff_E=465.06	-6.4	0	0
COX1_836551_uff_E=145.19	-6.4	0	0
COX1_10979586_uff_E=469.84	-6.4	0	0
COX1_23424464_uff_E=121.50	-6.4	0	0
COX1_28739570_uff_E=151.06	-6.4	0	0
COX1_64780675_uff_E=471.47	-6.4	0	0
COX1_64793306_uff_E=130.47	-6.4	0	0
COX1_1725710_uff_E=132.49	-6.3	0	0
COX1_64781061_uff_E=122.21	-6.3	0	0
COX1_4301564_uff_E=413.18	-6.2	0	0
COX1_57880597_uff_E=111.05	-6.2	0	0
COX1_66874_uff_E=122.15	-6.1	0	0
COX1_64782388_uff_E=123.73	-6.1	0	0
COX1_1983_uff_E=414.99	-6	0	0

When using a flexible docking engine, then minimising 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 a local minimal energy, like Universal Force Field (UFF) or mm².

The assignment of Universal Force Field (UFF) atom types and the calculation of the molecular connectivity (identifying bonds, angular, torsional and inversion terms) have been performed using the routines available in the Open Babel package [5, 6].

OpenBabel can be used for refining initial geometries with UFF molecular-mechanics optimizations, adding or removing hydrogens to Protein Data Bank (PDB) protein files, and many other utility tasks that often arise in molecular modeling projects.

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 number of steps, stop if energy difference is less than 0.1 kcal/mol.

The virtual screening results show that 32 compounds are strong inhibitors of COX-1, and 28 compounds for COX-2. (Table II and III).

Table III

The calculated binding affinities greater than for paracetamol, 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_21039831_uff_E=7278.98	-8	0	0
COX2_54373747_uff_E=135.65	-7.4	0	0
COX2_58156_uff_E=155.62	-7.3	0	0
COX2_64780675_uff_E=471.47	-7.2	0	0
COX2_70185934_uff_E=182.81	-7.2	0	0
COX2_23424464_uff_E=121.50	-7.1	0	0
COX2_72907_uff_E=774.64	-7	0	0
COX2_21304453_uff_E=171.92	-7	0	0
COX2_836029_uff_E=197.19	-6.9	0	0
COX2_6435661_uff_E=148.61	-6.9	0	0
COX2_10979586_uff_E=469.84	-6.9	0	0
COX2_12541316_uff_E=136.97	-6.9	0	0
COX2_13565130_uff_E=176.63	-6.9	0	0
COX2_70503032_uff_E=211.21	-6.9	0	0
COX2_29520_uff_E=465.06	-6.8	0	0
COX2_28739598_uff_E=434.80	-6.8	0	0
COX2_62679286_uff_E=128.27	-6.8	0	0
COX2_64781061_uff_E=122.21	-6.8	0	0
COX2_1725710_uff_E=132.49	-6.7	0	0
COX2_3014069_uff_E=417.03	-6.7	0	0
COX2_21931829_uff_E=462.37	-6.7	0	0
COX2_66874_uff_E=122.15	-6.6	0	0
COX2_190616_uff_E=420.09	-6.6	0	0
COX2_62679285_uff_E=469.54	-6.6	0	0
COX2_64782388_uff_E=123.73	-6.6	0	0
COX2_74325_uff_E=120.63	-6.5	0	0
COX2_28739570_uff_E=151.06	-6.5	0	0
COX2_70458847_uff_E=425.01	-6.5	0	0
COX2_1983_uff_E=414.99	-6.4	0	0

The RMSD cutoff of 2Å is usually used as criteria of the correct bound structure prediction [16]. Using the same cutoff value, the two metrics used for RMSD (summarized in Table II and III) indicate that all predictions for tested compounds are very accurate, but in case of COX-1 three predictions (CID 21039831, 54373747, 28739598), and in case of COX-2 six predictions (CID 21039831, 54373747, 58156, 64780675, 70185934,

23424464) have a binding affinity greater than 7 kcal/mol. Therefore, results indicate that 7 compounds with binding affinity greater than 7 kcal/mol, are much better ligands of COX-1 and COX-2 than paracetamol (CID 1983), because they require lesser energy for binding. This suggest, that these substances will successfully substitute paracetamol (Figure 4 and 5).

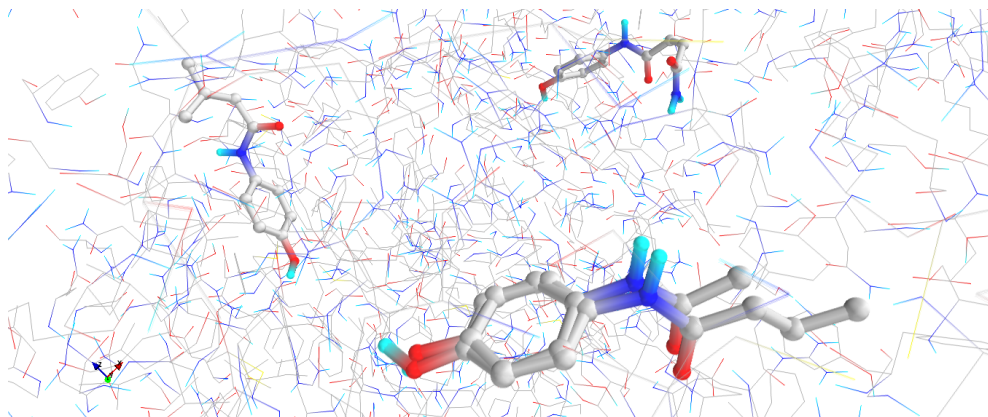


Figure 4.

Molecular docking and position of paracetamol and 3 “top” ligands in protein target (COX-2)

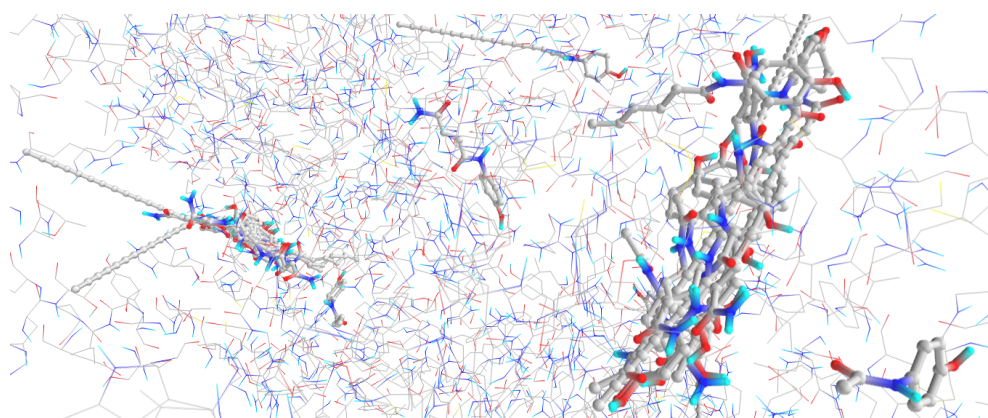


Figure 5.

All (36) binding scenarios of CID 21039831, 54373747, 28739598, paracetamol and COX-1

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 [17].

Conclusions

Compounds with PubChem ID: 21039831, 54373747, 28739598, 58156, 64780675, 70185934 and 23424464 have much better binding affinity to

cyclooxygenase enzymes (especially to COX-2) than paracetamol, they present the correct bound structure prediction, so they seem to be good substitutes for paracetamol. Further investigations are needed to establish their pharmacodynamic properties and toxicity.

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