ORIGINAL ARTICLE

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

LÁSZLÓ FERENCZ[1]*, DANIELA LUCIA MUNTEAN[2]

[1]*Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Str. Sighişoarei 1C, Tîrgu-Mureş – Corunca, 540485, Romania*
[2]*University of Medicine and Pharmacy Tîrgu-Mureş, Faculty of Pharmacy, Str. Gh. Marinescu, 38, Tîrgu-Mureş, 540139, Romania*

**corresponding author: lferencz@inbox.com*

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Abstract

Nimesulide is a controversial non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. For this reason it may be interesting to found 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 nimesulide, using pre-specified similarity thresholds. Using the threshold ≥ than 95% for the similar structures criteria, we found 14 compounds that met these criteria. Nine of these compounds have a better binding affinity to cyclooxygenase enzymes than nimesulide, consequently they may be used as possible substitutes to nimesulide.

Rezumat

Nimesulidul este un antiinflamator nestereoidian controversat cu proprietăţi analgezice şi antipiretice. Din această cauză poate fi interesantă găsirea unor compuşi similari pentru lărgirea spectrului de inhibitori ai prostaglandin-endoperoxid sintazei (ciclooxigenazei), utilizaţi în terapeutică. Am folosit algoritmul de căutare "compuşi 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 nimesulidului, utilizând praguri de similitudine predefinite. Pentru un prag ≥ 95% pentru criteriul structuri similare, am găsit 14 compuşi care au îndeplinit acest criteriu. Nouă compuşi au o afinitate de legare la ciclooxigenaze superioară nimesulidului, deci ar putea înlocui cu succes nimesulidul.

Keywords: nimesulide, structural similarity, screening, docking

Introduction

Nimesulide is a non-steroidal antiinflamatory drug (NSAID), reported to be a selective inhibitor of cyclooxygenase-2 (COX-2), with analgesic and antipyretic properties.

Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhea, but its use in European Union (EU) is limited due to reports of hepatotoxicity. Its mechanism of action is multifactorial and is characterized by a fast onset of action, giving a unique and broad action on inflammatory processes. Nimesulide was the first marketed NSAID which inhibited selectively COX-2, and belonged to a class of compounds (sulfonamides), that is unique among commercially available NSAIDs.

Nimesulide is different from other selective COX-2 inhibitors and classical non-steroidal anti-inflammatory drugs (NSAIDs). The anti-inflammatory effect mechanism of nimesulide (inhibition of inflammatory mediators) is similar to other classic NSAIDs, but Nimesulide exhibits a superior gastrointestinal safety as compared to other NSAIDS. It is known that nimesulide prevents NSAID-induced ulcers, while celecoxib and rofecoxib, which are more selective to COX-2, failed to prevent these ulcers. Nimesulide produces gastro-protective effects *via* a completely different mechanism. In addition, while selective COX-2 inhibitors increase the risk for cardio-vascular diseases, nimesulide does not exert significant cardiotoxicity. This data suggests that gastrointestinal side effects of classic NSAIDs cannot be related to the COX-1 inhibition alone and also suggest that nimesulide is an atypical NSAID, which is different from both non-selective and selective COX-2 inhibitors [1, 2].

The European Medicines Agency has completed a review of the safety and effectiveness of systemic medicines containing nimesulide (capsules, tablets, suppositories and powder or granules for oral

suspension). The Agency's Committee for Medicinal Products for Human Use (CHMP) concluded that the benefits of nimesulide used systemically continue to outweigh its risks but that its use should be restricted to the treatment of acute pain and primary dysmenorrhea. It issued a recommendation that it should no longer be used for the treatment of painful osteoarthritis [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], 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 was 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.

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, 9, 10, 11 and 12].

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 metrics are expressed as RMSD lower bound (rmsd/lb) and RMSD upper bound (rmsd/ub), differing in how the atoms are matched in the distance calculation. For scoring, AutoDock Vina uses a united-atom function, which involves only the heavy atoms.

RMSD values are calculated relative to the best mode and using only movable 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 ≥ than 95% for the similar structures criteria, we detected 14 compounds that met criteria. Substances are shown in Table I, together with nimesulide (**CID 4495**).

Table I

The identified substances with a similarity threshold ≥ 95% and nimesulide (CID 4495)

PubChem CID	IUPAC Name	Molecular Formula	MW [g/mol]
10020363	N-[2-(2,4-difluorophenoxy)-4-nitrophenyl]methanesulfonamide	$C_{13}H_{10}F_2N_2O_5S$	344.29070
10902415	N-(4-nitroso-2-phenoxyphenyl)methanesulfonamide	$C_{13}H_{12}N_2O_4S$	292.31038
11493409	N-(2-hexoxy-4-nitrophenyl)methanesulfonamide	$C_{13}H_{20}N_2O_5S$	316.3733
11680642	N-(4-nitro-2-propoxyphenyl)methanesulfonamide	$C_{10}H_{14}N_2O_5S$	274.29356
11822507	N-[4-(hydroxyamino)-2-phenoxyphenyl]methanesulfonamide	$C_{13}H_{14}N_2O_4S$	294.32626
40489928	methylsulfonyl-(4-nitro-2-phenoxyphenyl)azanide	$C_{13}H_{11}N_2O_5S^-$	307.30184
4495	N-(4-nitro-2-phenoxyphenyl)methanesulfonamide	$C_{13}H_{12}N_2O_5S$	308.30978
4553	N-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide	$C_{13}H_{18}N_2O_5S$	314.35742
58701679	N-(4-nitro-2-phenoxyphenyl)ethanesulfonamide	$C_{14}H_{14}N_2O_5S$	322.33636
69899876	N-(3-cyclohexyloxy-4-nitrophenyl)methanesulfonamide	$C_{13}H_{18}N_2O_5S$	314.35742
71163623	4-nitro-2-phenoxy-1-(sulfinatoamino)benzene	$C_{12}H_9N_2O_5S^-$	293.27526
71364034	1,1,1-trifluoro-N-(4-nitro-2-phenoxyphenyl)methanesulfonamide	$C_{13}H_9F_3N_2O_5S$	362.28117
71365841	1-fluoro-N-(4-nitro-2-phenoxyphenyl)methanesulfonamide	$C_{13}H_{11}FN_2O_5S$	326.300243
71771973	N-methylsulfonyl-N-(4-nitro-2-phenoxyphenyl)methanesulfonamide	$C_{14}H_{14}N_2O_7S_2$	386.40016
9884264	N-[2-(4-methoxyphenoxy)-4-nitrophenyl]methanesulfonamide	$C_{14}H_{14}N_2O_6S$	338.33576

Further, we calculated the binding affinities of the 15 ligands (including nimesulide) to the surface of COX-1 and COX-2 enzymes. Nimesulide is a relatively COX-2 selective ligand, but linking of the other ligands to COX-1 enzyme may be interesting too.

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 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.

In Tables II and III are presented the calculated binding affinities in descending order for the ligands and the two enzymes. Rmsd/ub and rmsd/lb values are also shown.

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 local energy minima, like Universal Force Field (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 [5, 6]. OpenBabel can be used for refining initial geometries with UFF molecular-mechanics optimizations, adding or removing hydrogens to 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 RMSD cut-off of 2Å is usually used as criteria of the correct bound structure prediction [15]. Using the same cut-off value, the two metrics used for RMSD (summarized in Table II and III) indicate that 7 predictions for tested compounds are very accurate in case of COX-1 (71364034, 71771973, 9884264, 40489928, 71365841, 10902415, 11822507), and 4 predictions (10020363, 9884264, 71364034, 69899876) are very accurate for COX-2. Therefore, results indicate that **9 compounds are better ligands** of COX-1 and COX-2 **than nimesulide** (4495), because they require a smaller energy for binding.

Table II

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

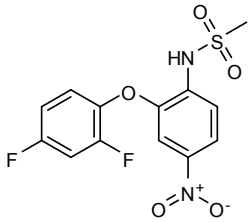
Enzyme-Ligand (ligand's energies with Babel, kcal/mol)	**Binding Affinity** [kcal/mol]	**rmsd/ub** [Å]	**rmsd/lb** [Å]
COX1_71364034_uff_E=729.52	**-8.9**	**0**	**0**
COX1_71364034_uff_E=729.52	-8.9	5.627	3.618
COX1_10020363_uff_E=717.74	-8.8	0	0
COX1_71364034_uff_E=729.52	-8.8	6.54	4.017
COX1_71771973_uff_E=1249.52	**-8.6**	**0**	**0**
COX1_9884264_uff_E=729.21	**-8.6**	**0**	**0**
COX1_71364034_uff_E=729.52	-8.5	9.832	7.52
COX1_71364034_uff_E=729.52	-8.5	11.877	9.105
COX1_71364034_uff_E=729.52	-8.5	5.479	3.512
COX1_71771973_uff_E=1249.52	-8.5	26.766	24.904
COX1_71771973_uff_E=1249.52	-8.5	26.905	25.149
COX1_10020363_uff_E=717.74	-8.4	49.274	47.526
COX1_40489928_uff_E=686.56	**-8.4**	**0**	**0**
COX1_40489928_uff_E=686.56	-8.4	6.153	2.924
COX1_71365841_uff_E=721.11	**-8.4**	**0**	**0**
COX1_71365841_uff_E=721.11	-8.4	9.387	6.84
COX1_10020363_uff_E=717.74	-8.3	49.062	47.206
COX1_10020363_uff_E=717.74	-8.3	3.327	2.66
COX1_40489928_uff_E=686.56	-8.3	11.074	8.37
COX1_71364034_uff_E=729.52	-8.3	13.191	10.23
COX1_71364034_uff_E=729.52	-8.3	11.028	8.725
COX1_71365841_uff_E=721.11	-8.3	8.874	7.141
COX1_9884264_uff_E=729.21	-8.3	48.916	46.889
COX1_10020363_uff_E=717.74	-8.2	6.471	3.237
COX1_10020363_uff_E=717.74	-8.2	47.894	46.133
COX1_71364034_uff_E=729.52	-8.2	9.6	5.341
COX1_71365841_uff_E=721.11	-8.2	8.869	6.743
COX1_71771973_uff_E=1249.52	-8.2	17.235	15.61
COX1_9884264_uff_E=729.21	-8.2	49.667	47.225
COX1_10902415_uff_E=719.40	**-8.1**	**0**	**0**
COX1_11822507_uff_E=715.58	**-8.1**	**0**	**0**
COX1_9884264_uff_E=729.21	-8.1	19.923	17.616
COX1_9884264_uff_E=729.21	-8.1	4.643	3.383
COX1_9884264_uff_E=729.21	-8.1	48.342	46.788
COX1_10020363_uff_E=717.74	-8	4.558	3.239
COX1_10902415_uff_E=719.40	-8	5.664	2.509
COX1_11822507_uff_E=715.58	-8	48.441	46.521
COX1_4495_uff_E=726.56	**-8**	**0**	**0**

Table III

The calculated binding affinities greater than for nimesulide, 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_10020363_uff_E=717.74	-8.9	6.735	4.247
COX2_10020363_uff_E=717.74	**-8.9**	**0**	**0**
COX2_10020363_uff_E=717.74	-8.8	27.241	24.927
COX2_10020363_uff_E=717.74	-8.8	34.578	31.98
COX2_9884264_uff_E=729.21	**-8.6**	**0**	**0**
COX2_71364034_uff_E=729.52	-8.6	6.05	3.247
COX2_71364034_uff_E=729.52	-8.6	34.496	33.242
COX2_71364034_uff_E=729.52	**-8.6**	**0**	**0**
COX2_71364034_uff_E=729.52	-8.5	34.864	33.455
COX2_71364034_uff_E=729.52	-8.4	20.365	17.335
COX2_10020363_uff_E=717.74	-8.4	34.445	31.683
COX2_71364034_uff_E=729.52	-8.3	5.588	2.806
COX2_71364034_uff_E=729.52	-8.3	34.965	33.105
COX2_71364034_uff_E=729.52	-8.3	18.933	16.92
COX2_69899876_uff_E=742.87	**-8.3**	**0**	**0**
COX2_4495_uff_E=726.56	**-8.3**	**0**	**0**

We used the default docking parameters: 9 number of binding modes, and exhaustiveness (thoroughness of search): 8. Almost all binding scenarios to COX-2 of **10020363** (N-[2-(2,4-difluorophenoxy)-4-nitrophenyl]-methanesulfonamide) and **71364034** (1,1,1-trifluoro-N-(4-nitro-2-phenoxy-phenyl)methanesulfonamide) present higher binding affinity, than nimesulide. This suggests that these substances will successfully substitute nimesulide (Figures 1, 2):



CID 10020363

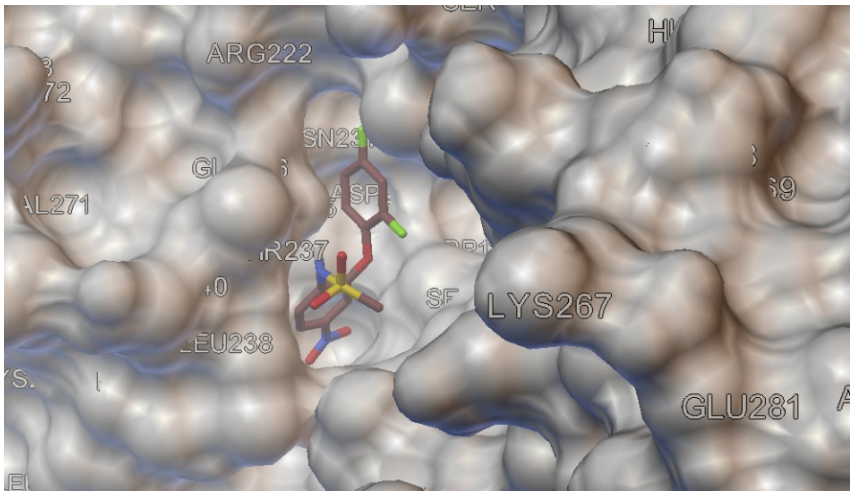
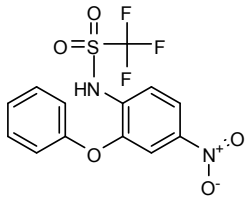


Figure 1.
An instance of binding of CID 10020363 to the COX-2's surface



CID 71364034

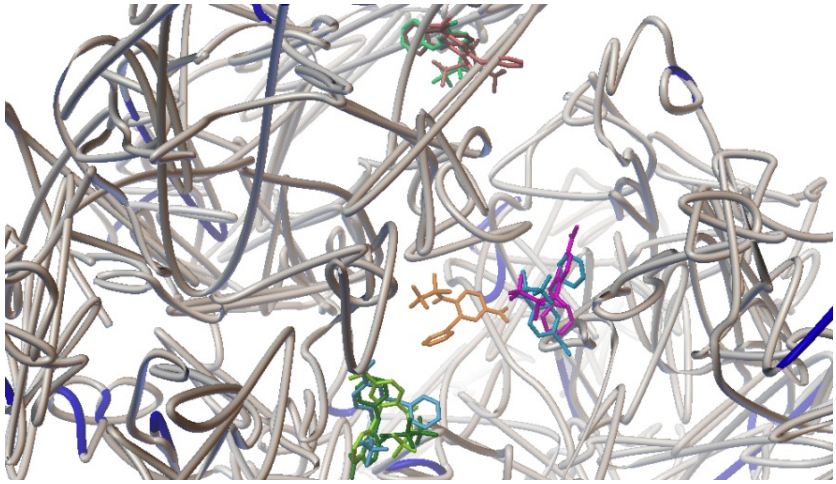
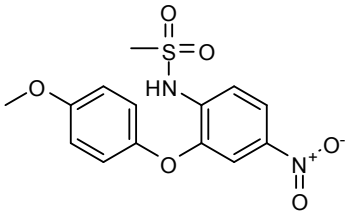


Figure 2.
Nine binding scenarios of CID 71364034 to COX-2 (represented with beaded ribbons)

The substance CID **9884264** N-[2-(4-methoxy-phenoxy)-4-nitrophenyl]methanesulfonamide (Figure 3) doesn't contain fluorine, but a methoxy group:



CID 9884264

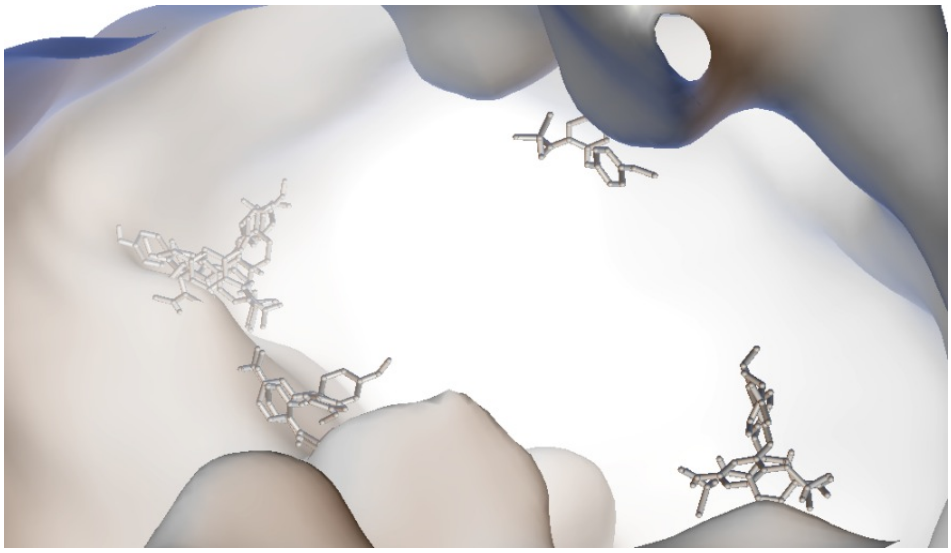
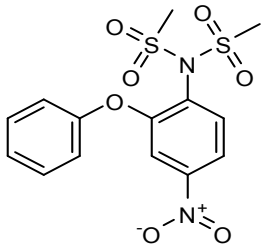


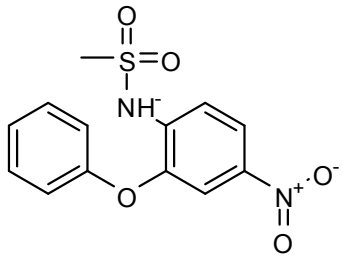
Figure 3.
Coarse molecular surface of the enzyme COX-2 with 9 CID 9884264 molecules

Substance **71771973** (N-methylsulfonyl-N-(4-nitro-2-phenoxy-phenyl)methanesulfonamide) binds well with COX-1:

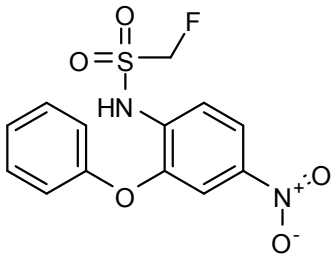


CID 71771973

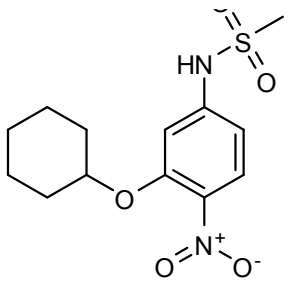
Other possible "candidates" are:



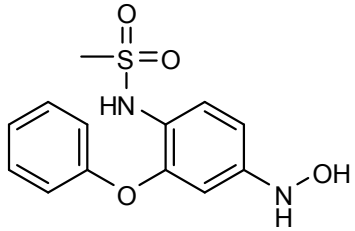
40489928



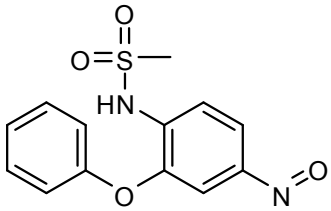
71365841



69899876



11822507



10902415

Conclusions

Compounds with PubChem ID: 71364034, 71771973, 9884264, 40489928, 71365841, 10902415, 11822507, 10020363 and 69899876 have better binding affinity to cyclooxygenase enzymes (COX-1 and COX-2) than nimesulide, they present the correct bound structure prediction, so they seem to be good substitutes for nimesulide. Further investigations are needed to establish their pharmacodynamics properties and toxicity.

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