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Docking studies of some pyrazole containing compounds in the cyclooxygenase-2 active site

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Abstract

Whereas nonselective nonsteroidal anti-inflammatory drugs, such as aspirin, ibuprofen and diclofenac, inhibit both cyclooxygenase-1 and cyclooxigenase-2 enzymes, selective inhibitors target cyclooxygenase-2, which is overexpressed in inflammation, but also in cancer, atherosclerosis, Alzheimer's disease, and Parkinson`s disease. Potential cardiovascular and hepatic side effects of cyclooxygenase-2 inhibitors have limited their use. The development of selective and safe cyclooxygenase-2 inhibitors remains a high priority in drug discovery. Based on the structure of previously investigated newly synthesized β-hydroxy-β-arylpropanoic acids, two groups of compounds were designed: analogs in which one of the benzene rings was replaced by a pyrazole, while the carboxyl group was retained, and amides of β-hydroxy-β-arylpropanoic acids with pyrazole. The compounds were docked into the 3D structure of the catalytic site of the enzyme cyclooxygenase-2 using AutoDock Vina 1.2.0. and the obtained interactions were compared with the interactions of celecoxib, a selective inhibitor. The amides had lower binding energies than the designed acids, which makes them attractive target compounds for synthesis and further examination.

Key words: cyclooxygenase-2 inhibitors, molecular interactions, rational drug design, protein-ligand interactions, β-hydroxy-β-arylpropanoic acids

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Introduction

Inflammation is an intricate response mounted by the immune system to combat infections, injuries, and tissue damage. It involves a complex interplay of various immune cells, cytokines, and chemical mediators, aiming to restore tissue homeostasis (1). While acute inflammation is a crucial component of the body's defense mechanism, chronic or unresolved inflammation can contribute to the development and progression of numerous diseases, including diabetes (2), neurodegenerative diseases (3, 4), atherosclerosis (5), and cancer (6). Recently, the connection between inflammation and cancer has been extensively studied and there is evidence suggesting that selective NSAIDs could have benefical effects in the treatment of some cancers such as breast cancer (7, 8) and colon cancer (9, 10).

The two isoforms of enzyme cyclooxygenase (COX), COX-1 and COX-2, have emerged as key enzymes in the regulation of inflammatory processes. COX enzymes are responsible for converting arachidonic acid into prostaglandins, thromboxanes, and prostacyclins – collectively known as prostanoids. These prostanoids exert diverse effects on inflammation, vasodilation, platelet aggregation, and immune modulation (11).

COX-1 is constitutively expressed in many tissues and is involved in the maintenance of physiological functions such as gastric cytoprotection, renal blood flow regulation, and platelet aggregation. On the other hand, COX-2 was initially characterized as an inducible enzyme, expressed at low levels in healthy tissues but significantly upregulated in response to inflammatory stimuli. COX-2 plays a crucial role in the synthesis of prostanoids at the site of inflammation, amplifying the inflammatory response (11).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely utilized as a cornerstone in the management of inflammatory conditions. These drugs exert their therapeutic effects by inhibiting the activity of COX, thereby reducing the production of prostanoids. Traditional NSAIDs, which include drugs like aspirin, ibuprofen, and diclofenac, are nonselective and inhibit both COX-1 and COX-2, leading to a broad spectrum of anti-inflammatory and analgesic actions. However, the inhibition of COX-1 can also give rise to the adverse effects such as gastrointestinal ulcers and bleeding (12).

In recent years, selective COX-2 inhibitors have been developed with the aim of providing enhanced anti-inflammatory efficacy while minimizing the side effects associated with COX-1 inhibition. These selective inhibitors, exemplified by drugs like celecoxib, specifically target the inducible COX-2 isoform, offering a more focused approach to inflammation management (13). Because of the potential cardiovascular and hepatic side effects of current selective NSAIDs, there is still a need to develop new COX -2 inhibitors.

The advent of computational techniques such as molecular docking has revolutionized the field of drug discovery by enabling the prediction and analysis of protein-ligand interactions (14, 15). Computational docking of small molecules into the

active site of a protein has emerged as an essential tool for comprehending the molecular mechanisms of COX-2 inhibition and developing innovative therapeutics (16).

We have previously investigated the anti-inflammatory activity of newly synthesized β-hydroxy-β-arylpropanoic acids with varying substituents on one of the benzene rings (17, 18). Based on the structure of examined acids, two groups of compounds were designed: 1) analogs in which one of the benzene rings was replaced with a pyrazole ring while keeping the carboxyl group, and 2) amides of β-hydroxy-β-arylpropanoic acids with pyrazole. In both cases, pyrazole was either unsubstituted or substituted with various groups. We docked the designed compounds into the 3D catalytic site of COX-2 in order to identify compounds with lower binding energies and reveal interactions they form with key amino acids.

Experimental

Autodock Vina 1.1.2. (19, 20), an open-source software, was used to predict binding modes of designed compounds into 3D catalytic site of isoform COX-2. It uses a modified form of the genetic algorithm based on the Lamarckian genetic algorithm for searching ligand conformations and predicting their binding affinity to a target protein. AutoDock Vina was developed as an improved version of the original AutoDock software, incorporating several advancements to enhance docking accuracy and computational efficiency. Autodock Vina performs the docking of ligand to a set of grids (precalculated by autogrid) describing the target protein. Each docking experiment consisted of 10 docking runs with 150 individuals and 500,000 energy evaluations. Other parameters were left at their default values. The search was conducted in a grid of 40 points per dimension and a step size of 0.375 centred on the binding site of enzyme. Prior to docking, water molecules and other nonessential residues were removed, and polar hydrogens were added using AutoDock Tools. Virtual analysis of the docking site was analyzed by PyMol (21).

The crystal structure of COX-2 (pdb code: 1cx2) was retrieved from Protein Data Bank and represents COX-2 in a complex with selective inhibitor SC-558. Since COX-2 is tetramer, all the calculations were performed using one chain.

Structures of all possible stereoisomer forms of ligands were generated using the ChemOffice v7.0 Ultra software package and have been MM2 optimized (22). Structures of acids were docked in their anionic forms.

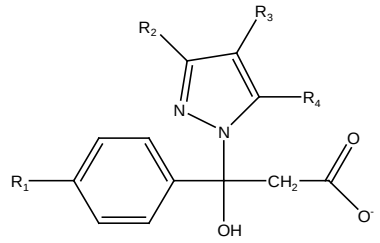
Structures were designed taking into account the availability of starting components so that a synthetic procedure could be carried out.

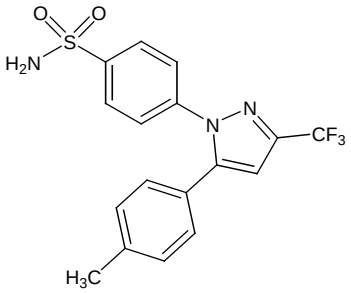
Results

Designed acids, celecoxib, as well as their binding energies, are presented in Table I. Since the disparities in the lowest binding energies of enantiomers were not significant, the results are displayed for S enantiomer.

Table I Structures of designed acids, celecoxib and their minimal binding energies

Tabela I Strukture dizajniranih kiselina, celekoksiba i njihove minimalne energije vezivanja

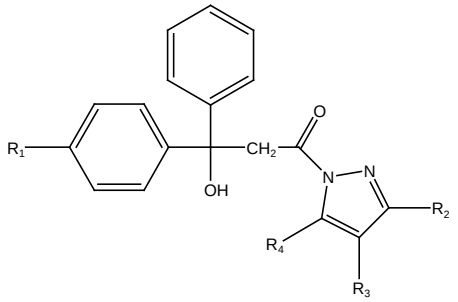


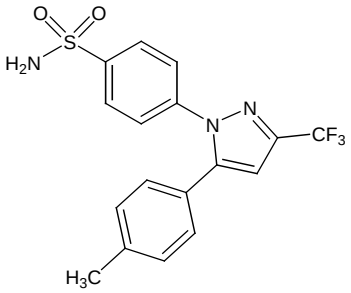
Compound	R_1	R_2	R_3	R_4	Binding energy (kcal/mol)
1	H	H	H	H	-7.4
2	Cl	H	H	H	-7.6
3	NO_2	H	H	H	-8.1
4	OCH_3	H	H	H	-7.3
5	F	H	H	H	-7.5
6	I	H	H	H	-7.7
7	CF_3	H	H	H	-8.3
8	H	H	H	NO_2	-7.4
9	H	CF_3	H	H	-7.8
10	H	H	I	H	-7.9
11	H	H	Br	H	-7.7
12	H	H	Cl	H	-8.2
13	H	H	NO_2	H	-7.4
14	H	H	CH_3	H	-7.6
15	H	CH_3	CH_3	H	-8.1
16	H	CF_3	H	CH_3	-7.3
Celecoxib					-9.7

Amides (Table II) have lower binding energies than acids. SC-558 was redocked into COX-2 and the root main sqare deviation (RMSD) was below 2Å, which proved that the docking settings were valid.

Table II Structures of designed amides, celecoxib and their minimal binding energies

Tabela II Strukture dizajniranih amida, celekoksiba i njihove minimalne energije vezivanja



17	H	H	H	H	-9.1
18	NO_2	H	H	H	-9.6
19	CF_3	H	H	H	-10.1
20	Cl	H	H	H	-9.4
21	CH_3	H	H	H	-9.5
22	H	CF_3	H	CH_3	-9.3
23	H	CF_3	H	H	-10.2
24	H	H	CH_3	CH_3	-9.2
25	NO_2	CF_3	H	H	-9.7
26	CF_3	CF_3	H	H	-10.2
27	CH_3	CF_3	H	H	-10.1
28	Cl	CF_3	H	H	-9.4
Celecoxib					-9.7

Discussion

COX-1 (consisting of 576 amino acids) and COX-2 (consisting of 587 amino acids) are isoforms of the COX enzyme, which belongs to the membrane proteins. These isoforms have 60% sequence similarity (23). The active sites can be observed in three regions: P1, P2, and P3. In the P1 region, the key amino acids responsible for interacting with the inhibitor are Arg120 and Tyr355. Nonselective NSAIDs that have a carboxyl group in the structure form the most important ionic interaction with the gvanidino group of Arg120 (24). It was previously thought that a compound need not have a carboxyl

group in its structure to be selective, but the discovery of lumiracoxib, a highly selective NSAID containing a carboxyl group, refuted this claim (25, 26).

P2 represents a hydrophobic channel with a size of 25 Å, which is lined by several amino acids, including Leu352, Phe518, Val523, and terminates with Tyr385 and Tyr387. This region branches into a side pocket called P3, which is only available in the COX-2 isoform for interactions with selective inhibitors. This is because the COX-2 isoform contains Val523, which is less bulky (than Ile523 in the COX-1 isoform) and allows the entry of inhibitors into the P3 region. The key amino acids in this region are His90, Gln192 and Arg513. Selective COX-2 inhibitors have a structure that can maintain interactions in the P3 region (25).

β-Hydroxy-β-arylalkanoic acids were prevously synthesized and tested for anti-inflammatory activity and potential selective COX-2 inhibition (17, 18). Based on their structure, with an aim to improve COX-2 selectivity, two groups of compounds were designed: acids in which one of the benzene rings was replaced with pyrazole (compounds **1**-**16**) and amides of selected acids with pyrazole (**17**-**28**).

All tested compounds had interactions in the P3 region, but amides had lower binding energies. Compound **25** had the same binding energy as celecoxib (-9.7 kcal/mol), while compounds **19**, **23**, **26** and **27** had an even lower one.

Compounds **1**-**7** are acids with carboxyl group, differing only in the substituent present at one of benzene rings. Amongst them, compounds **3** (containing nitro group) and **7** (containing trifluoromethyl group) have the lowest binding energies, -8.3 and -8.7 kcal/mol, respectively. However, these compounds occupy the active site differently. Carboxylic group in compound **3** maintains an ionic interaction with Arg120 (2.2 Å) and a hydrogen bond with Tyr355 (2.3 Å) (Figure 1), while compound **7** does not maintain such interactions. The orientation of compound **7** is more similar to that of celecoxib, since the polar carboxyl group interacts with the His90 (Figure 2).

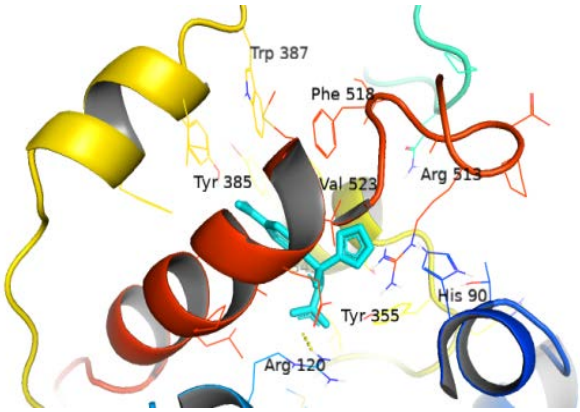


Figure 1. Position of the most favorable conformation of compound *3* (tyrquoise) in the active site of COX-2

Slika 1. Položaj najpovoljnije konformacije jedinjenja *3* (tirkizno) u aktivnom mestu COX-2

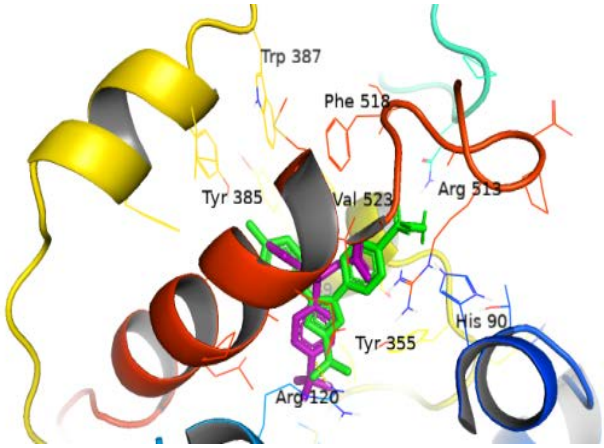


Figure 2. Position of the most favorable conformation of compound *7* (purple) and celecoxib (green) in the active site of COX-2

Slika 2. Položaj najpovoljnije konformacije jedinjenja *7* (ljubičasto) i celekoksiba (zeleno) u aktivnom mestu COX-2

Compounds **8-16** are acids designed to have different substituents on the pyrazole ring, and all compounds had the same orientation in the active site of COX-2, so the substituent on the pyrazole ring did not affect the orientation. Compounds **12** (containing iodo group) and **15** (containing two methyl groups in positions 2 and 3) had the lowest binding energies.

Compounds **17-28** are amides of β-hydroxy-β-arylalkanoic acids with pyrazole and they differ in the substituents present in the para position on one of the benzene rings or on the pyrazole ring in different positions. Amides which have the lowest binding energies (**19**, **23**, **25**, **26** and **27**) have the same orientation in the active site (Figure 3).

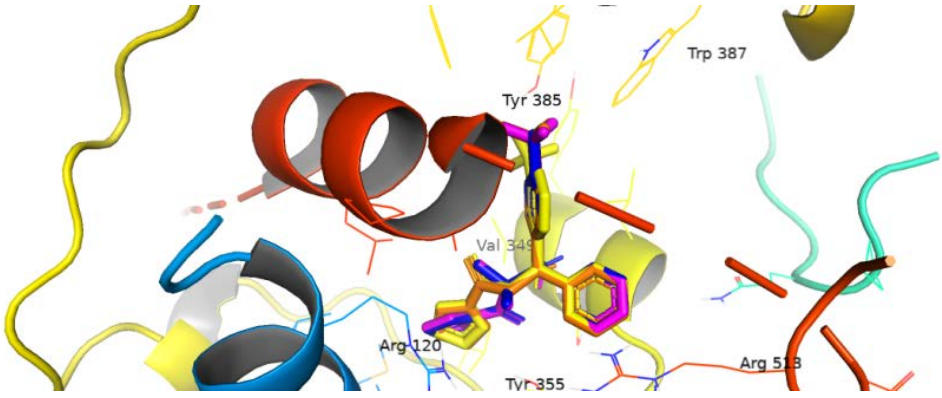


Figure 3. Position of the most favorable conformations of compounds *23* (yellow), *19* (orange), *26* (magenta), and *27* (blue) in the active site of COX-2

Slika 3. Položaj najpovoljnije konformacije jedinjenja *23* (žuta), *19* (narandžasta), *26* (purpurna) i *27* (plava) u aktivnom mestu COX-2

The pyrazole ring occupies the P1 region, the same as is the case with celecoxib maintaining hydrophilic interactions with Tyr355. The benzene ring without substituents is forced into the P3 region, where it interacts with His90. The substituted benzene ring occupies the P2 region and interacts with Tyr385. Hydroxyl group form close interaction with Val349. Compound **26** (the lowest binding energy -10.2 kcal/mol) has one trifluoromethyl group on benzene and the other on pyrazole. The pyrazole ring is embeded in the P1 region, maintaining hydrophylic interactions with Tyr355, while the trifluoromethyl group on pyrazole maintains a hydrogen bond with Arg120. The substituted benzene ring is embeded in the P2 region and the present trifluoromethyl group maintains hydrophylic interactions with Tyr385. The unsubstituted ring is forced into the P3 region. The orientation of amides is very similar to the orientation of celecoxib, as can be seen in Figure 4, where the position of compound **26** along with celecoxib is shown.

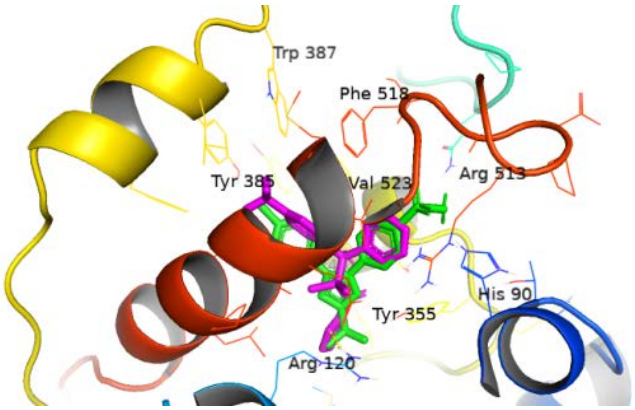


Figure 4. Position of the most favorable conformations of compounds *26* (magenta) and celecoxib (green) in the active site of COX-2

Slika 4. Položaj najpovoljnije konformacije jedinjenja *26* (purpurna) i celekoksiba (zelena) u aktivnom mestu COX-2

Conclusion

Docking experiments were performed using designed acids and amides in the active site of the COX-2 isoform to identify compounds that exhibit strong interactions with the key amino acids. These compounds were designed based on the structure of previously synthesized β-hydroxy-β-arylalkanoic acids, which were studied for their anti-inflammatory activity. The performed docking experiments showed that both the designed acids and amides can be effectively accommodated within the 3D catalytic site of COX-2. Some of the acids formed interactions with Arg120 and Tyr355, as commonly observed with nonselective COX-2 inhibitors, but compound 7 formed interactions more similar to selective inhibitors. Amides had lower binding energies than acids and formed interactions more reminiscent to those of celecoxib, a selective COX-2 inhibitor.

Compounds with one trifluoromethyl group on the benzene ring and another on the pyrazole ring were particularly well stabilized in the active site. The designed amides are target compounds for further synthesis and testing.

Acknowledgment

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Doking studije nekih jedinjenja sa pirazolom u aktivnom mestu ciklooksigenaze-2

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Kratak sadržaj

Neselektivni nesteroidni antiinflamatorni lekovi poput aspirina, ibuprofena i diklofenaka inhibiraju enzime ciklooksigenazu-1 i ciklooksigenazu-2, a selektivni inhibitori ciljaju ciklooksigenazu-2 koja je prekomerno izražena u inflamaciji, ali takođe i kod kancera, ateroskleroze, Parkinsonove i Alchajmerove bolesti. Potencijalni kardiovaskularni i hepatički neželjeni efekti selektivnih inhibitora ciklooksigenaze-2 su ograničili njihovu primenu. Razvoj selektivnih i bezbednih inhibitora ciklooksigenaze-2 ostaje veoma prioritetna oblast u otkrivanju lekova. Na osnovu strukture prethodno istraživanih novosintetisanih β-hidroksi-β-arilpropanskih kiselina dizajnirane su dve grupe jedinjenja: analozi u kojima je jedan od benzenovih prstenova zamenjen pirazolom, uz zadržavanje karboksilne grupe, i amidi β-hidroksi-β-arilpropanskih kiselina sa pirazolom. Program AutoDock Vina 1.2.0 je korišćen za dokovanje dizajniranih jedinjenja u 3D strukturu katalitičkog mesta enzima ciklooksigenaze-2, a ostvarene interakcije su upoređene sa interakcijama koje ostvaruje selektivni inhibitor celekoksib. Amidi su imali nižu energiju vezivanja od kiselina, što ih čini dobrim kandidatima za sintezu.

Ključne reči: ciklooksigenaza-2, molekularne interakcije, racionalno dizajniranje lekova, protein-ligand interakcije, β-hidroksi-β-arilpropanske kiseline