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Potential interaction of natural dietary bioactive compounds with COX-2

Wilson Maldonado-Rojas, Jesus Olivero-Verbel*

Environmental and Computational Chemistry Group, University of Cartagena, Cartagena, Colombia

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ABSTRACT

Bioactive natural products present in the diet play an important role in several biological processes, and many have been involved in the alleviation and control of inflammation-related diseases. These actions have been linked to both gene expression modulation of pro-inflammatory enzymes, such as cyclooxygenase 2 (COX-2), and to an action involving a direct inhibitory binding on this protein. In this study, several food-related compounds with known gene regulatory action on inflammation have been examined *in silico* as COX-2 ligands, utilizing AutoDock Vina, GOLD and Surflex-Dock (SYBYL) as docking protocols. Curcumin and all-*trans* retinoic acid presented the maximum absolute AutoDock Vina-derived binding affinities (9.3 kcal/mol), but genistein, apigenin, cyanidin, kaempferol, and docosahexaenoic acid, were close to this value. AutoDock Vina affinities and GOLD scores for several known COX-2 inhibitors significatively correlated with reported median inhibitory concentrations ($R^2 = 0.462$, $P < 0.001$ and $R^2 = 0.238$, $P = 0.029$, respectively), supporting the computational reliability of the predictions made by our docking simulations. Moreover, docking analysis insinuate the synergistic action of curcumin on celecoxib-induced inhibition of COX-2 may occur allosterically, as this natural compound docks to a place different from the inhibitor binding site. These results suggest that the anti-inflammatory properties of some food-derived molecules could be the result of their direct binding capabilities to COX-2, and this process can be modeled using protein–ligand docking methodologies.

1. Introduction

Foods have small amounts of bioactive compounds that act as extra nutritional constituents [1]. The diversity of these chemicals is large and some of the most representative include flavonoids, isothiocyanates, proanthocyanidins, terpenoids, carotenoids, anthocyanins, and omega-3 polyunsaturated fatty acids, among many others [2]. The presence of these natural bioactive molecules in fruits and foods has been considered relevant, not only due to their unique organoleptic properties, but also because of their beneficial effects on human health, as demonstrated in numerous studies [3,4]. A recent review paper by Pan et al. [2], detailed how natural bioactive compounds exert their anti-inflammatory activities by modulating gene expression of diverse inflammation-related genes. However, it is also well known that some anti-inflammatory molecules carry out their action by directly inhibiting inflammatory proteins such cyclooxygenase 2 (COX-2) [5]. This enzyme catalyzes the first step in the synthesis of prostaglandins, thromboxanes and other eicosanoids in several inflammatory processes [6].

Although several natural products have been shown to modulate COX-2 expression [7–9], it is not clear if those are able to directly interact with the gene product or its modulating transcription factors. Computational chemistry offers the possibility to explore these interactions through protein–ligand docking procedures. Docking methods are valuable tools for drug development, and most current approaches assume a rigid receptor structure to allow virtual screening of large numbers of possible ligands and putative binding sites on a receptor molecule [10]. Among those tools used for this purpose are AutoDock Vina, GOLD and Surflex-Dock (SYBYL) [11–13]. Docking strategies generate binding or affinity scores for different sites and poses on targets, and the protein 'hits' identified by using this method can serve as potential candidates for experimental validation [14,15].

In this study, docking methodologies were used to test the ability of 29 natural bioactive compounds, isolated from different food sources, to bind COX-2. In addition, ligands known to bind COX-2 were submitted to docking protocols to establish relationships between their biological activity and the predicted binding affinities.

2. Materials and methods

2.1. Protein and ligand structure preparation

Experimental coordinates of three COX-2 structures (PDB_codes: 1CX2, 1PXX and 1CVU) were obtained from

* Corresponding author at: Environmental and Computational Chemistry Group, Faculty of Pharmaceutical Sciences, University of Cartagena, Campus of Zaragocilla, Cartagena, Colombia. Tel.: +57 5 6698179/6698180; fax: +57 5 6698323.
E-mail addresses: jesusolivero@yahoo.com, joliverov@unicartagena.edu.co (J. Olivero-Verbel).

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Table 1
Examined natural products and their sources.

Compound	Dietary source	Reference
Apigenin	Celery	[20]
Tangeretin	Citrus peel	[21]
Silybinin	Milk thistle	[22]
Cyanidin	Cherries	[23]
Delphinidin	Dark fruits	[24]
Genistein	Soybean	[25]
Epicatechin and epigallocatechin-3-gallate	Green tea	[26]
Naringenin	Citrus peel	[27]
Quercetin and kaempferol	Broccoli	[28]
5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone	Citrus peel	[29]
Curcumin	Turmeric powder and curcuma	[30,31]
Resveratrol	Grape skins and red wine	[32]
[6]-Gingerol and [6]-shogaol	Ginger	[33]
Carnosol	Rosemary	[34]
Pterostilbene	Blueberries	[35]
Benzyl isothiocyanate and phenethyl isothiocyanate	Cabbage	[36]
Sulforaphane	Cabbage	[37]
Proanthocyanidins	Berries	[38]
All-*trans* retinoic acid	Carrot, peppers and broccoli	[39,40]
Menthone	Mentha	[41]
Lycopene and β-carotene	Tomato and carrot	[42,43]
Lutein	Spinach and eggs	[44]
Eicosapentaenoic acid and docosahexaenoic acid	Fish and fish oil	[45,46]

Protein Data Bank (PDB) [16] and prepared with SYBYL 8.1.1 package [17]. Anti-inflammatory natural products chosen to perform this study were those reported to modulate expression of genes related to inflammation [2]. All these chemicals are present in foods and vegetables (Table 1), and they have been proven to have good anti-inflammatory properties. Structures were drawn with SYBYL 8.1.1 package, exactly as presented by Pan et al. [2], and optimized using DFT at the B3LYP/6-31G level, and calculations were carried out with Gaussian 03 package program [18]. The resultant geometry was translated to Mol2 format with Open Babel [19]. To determine structural similarities between 1CX2, 1PXX and 1CVU, a molecular superposition was conducted using SYBYL 8.1.1 program.

2.2. Protein–ligand docking calculations

The feasibility of natural compounds to be ligands for COX-2 structures was evaluated using molecular docking. This was performed utilizing three different programs that rely on several distinct scoring functions to evaluate the performance of the protein–ligand docking: AutoDock Vina, Surflex-Dock (SYBYL) and GOLD program.

AutoDock Vina combines some advantages of knowledge-based potentials and empirical scoring functions: it extracts empirical information from both the conformational preferences of the receptor–ligand complex and from experimental affinity measurements. Ligands are ranked based on an energy scoring function and, to speed up the score calculation, a grid-based protein–ligand interaction is used [11]. The docking site for the ligands on 1CX2, 1PXX and 1CVU was defined by establishing a cube at the geometrical center of the native ligand present in each one of the evaluated PDB structures, with the dimensions 24 × 24 × 24 Å, covering the ligand binding site with a grid point spacing of 0.375 Å. The coordinates *X*, *Y* and *Z* for 1CX2 from center grid boxes were 25.374, 21.657 and 17.292; for 1PXX 27.058, 24.431 and 15.437, and finally for 1CVU 25.277, 22.358 and 49.308, respectively. Ten runs were performed per each ligand, and for each run the best pose was saved. Finally, the average binding affinity for best poses was accepted as the binding affinity value for a particular complex.

GOLD utilizes a score function called fitness to rank different binding modes. It comprises four terms: the protein–ligand hydrogen-bond score, the protein–ligand van der Waals score, the contribution to the fitness due to intramolecular hydrogen bonds in the ligand and the contribution due to intramolecular strain in the ligand. It also has a mechanism for placing the ligand in the binding site using fitting points; and finally, it uses a search algorithm to explore possible binding modes [12]. The docking site was defined for each structure (1CX2, 1PXX and 1CVU) using the same coordinates *X*, *Y* and *Z* employed to localize the binding site with AutoDock Vina. A radius sphere of 10 Å was defined around the geometrical center of the native ligand for each evaluated protein. For each independent algorithm run, a maximum number of 125,000 operations were performed. Operator weights for crossover, mutation, and migration were set in mode auto, the maximum distance between hydrogen donors and fitting points was set to 3.0 Å, and non-bonded Van der Waals energies were cut-off at 6.0 Å.

The Surflex-Dock module of SYBYL is a molecular docking unit that performs flexible alignments. Its results are presented as both docking accuracy and screening utility [13]. The docking procedure was started with the protomol generation. The protomol was created using a ligand-based approach (native ligand for each COX-2 structure). Proto_threshold was set to 0.5 and proto_bloat was left at 0 as a default parameter. For each protein–ligand pair, twenty top ranked docked solutions were saved and the Surflex-Dock score presented as the mean for these values.

These docking platforms were also used to calculate docking scores for COX-2 inhibitors, SC558 and diclofenac, as well as for the natural substrate arachidonic acid. These molecules were also obtained from PDB. All protein–ligand docking calculations conducted on COX-2 proteins were performed using the inhibitor binding site on the crystal structure (PDB: 1CX2 and 1PXX) or the substrate binding site (PDB: 1CVU). These binding sites are the same in these COX-2 structures. In all cases, affinities were reported as the mean value obtained for 10 docking runs performed per ligand.

2.3. Identification of residues interacting with the natural bioactive compounds on COX-2 binding site

The identification of protein residues that interact with the natural bioactive compounds having the greatest affinities was carried out using LigandScout 3.0 [47]. This program creates simplified pharmacophores to detect the number and type of primary existing ligand–residue interactions on the protein active site.

2.4. Docking validation with biological data for COX-2 inhibitors

The 2D structures and the biological data of 21 COX-2 inhibitors were obtained from the PubChem chemical library [48] and literature [49,50]. Docking procedures were performed with three docking tools: AutoDock Vina, GOLD and Surflex-Dock [11–13], following the same protocols previously described for studied natural products. The biological data consisted of median inhibitory concentrations (IC_{50}), and the details of the testing protocols and materials are available on PubChem BioAssay [48]. The relationship between AutoDock Vina-calculated affinities of inhibitors on the three tested COX-2 (average values) and experimental activity data ($Log\,IC_{50}$) was performed by linear correlation [51], using Graph Instat Software (Version 3.06, 2003).

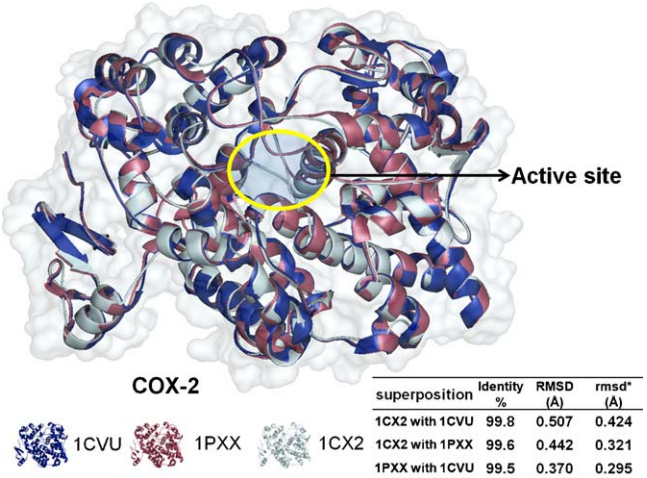
2.5. Theoretical approach to study the synergistic effect between curcumin and celecoxib on COX-2

It has been reported that curcumin acts synergistically with celecoxib in the inhibition of prostaglandin E2 synthesis by COX-2 [52,53]. In order to gain insight in this process, we performed docking simulations on the whole COX-2 (3LN1) structure with both compounds. Aiming to evaluate if the curcumin shares the same binding site as celecoxib, a series of 500 AutoDock Vina docking runs were performed using the following docking parameters. The docking procedure on the 3LN1 structure was performed by establishing a cube with the dimensions 60 × 84 × 72 Å covering the whole protein (Chain A), with a grid point spacing of 1.0 Å, using as center of the grid box the protein itself.

3. Results and discussion

3.1. Structural similarities of COX-2 structures

The superpositioning of the 3D COX-2 structures (PDB: 1CX2, 1CVU and 1PXX) as well as the RMSD values for each pair of them are presented in Fig. 1. As can be seemed, these three-dimensional structures of COX-2 have only minor differences (sequence identity > 99.5 and RMSD < 0.507 Å).



superposition	Identity %	RMSD (A)	rmsd* (A)
1CX2 with 1CVU	99.8	0.507	0.424
1CX2 with 1PXX	99.6	0.442	0.321
1PXX with 1CVU	99.5	0.370	0.295

Fig. 1. 3D-Superposition of COX-2 structures (1CVU, 1PXX and 1CX2), showing sequence identity and RMSD values. *RMSD for the binding site.

3.2. Docking calculations using AutoDock Vina, GOLD and SYBYL programs

The docking affinities of natural products for different COX-2, as calculated by three distinct docking programs are presented in Table 2. Results indicate that compared to the examined natural products, AutoDock Vina-calculated binding affinities for SC558, diclofenac (inhibitors) and arachidonic acid (substrate) were more consistent in terms of the magnitude of the expected predicted value, than the values generated for the scores calculated by GOLD and SYBYL. In the case of GOLD, the presence of the nitrogen seems to generate conflicting scores (negative values) for diclofenac, and high variability for binding scores obtained for the different COX-2 structures. SYBYL, on the other hand, also showed considerable variability for the scores obtained for the COX-2 structures. Therefore, successive calculations and discussions are referred solely to results provided by AutoDock Vina.

According to the AutoDock Vina-obtained affinity values (kcal/mol), several natural compounds are potential ligands for COX-2, with best scores obtained for PDB: 1CVU, including curcumin, all-*trans* retinoic acid (greatest docking scores, with identical mean absolute affinity value of 9.3 kcal/mol), as well as genistein, apigenin, cyanidin, kaempferol and docosahexaenoic acid.

3.3. Interaction between residues in COX-2 and natural products

The complex COX-2 (PDB: 1CVU) with curcumin and all-*trans* retinoic acid, as well as the interactions between residues in the protein binding site and these ligands are shown in Fig. 2. Both ligands fit into the same binding site (Fig. 2A). The most important residues on the 1CVU–curcumin complex (Fig. 2B) are Met113, Val116, Ile345, Val349, Leu359, Leu384, Trp387, Phe518, Ala527, Val523, and Ser530. Most interactions are hydrophobic and

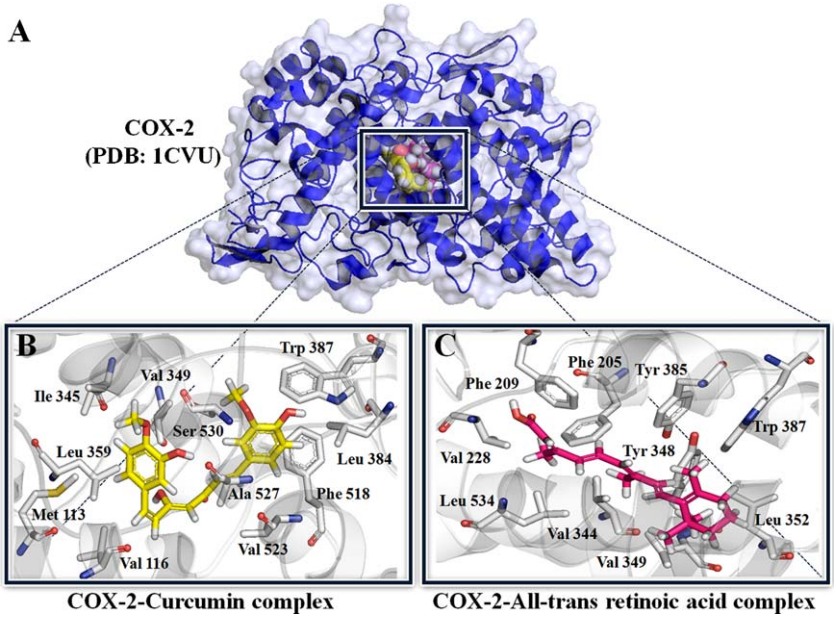


Fig. 2. 3D structure of COX-2(1CVU)-ligand complexes. (A) COX-2 bound to curcumin or all-trans retinoic acid (box). (B) Residues in the interaction COX-2-curcumin. (C) Residues in the interaction COX-2-all-*trans* retinoic acid.

Table 2
Docking results for natural bioactive compound on three COX-2 structures.

Compound	Protein name: cyclooxygenase-2 (COX-2)								
	1CX2			1PXX			1CVU		
	AV[a] affinity (kcal/mol)	G fitness	S total score	AV affinity (kcal/mol)	G fitness	S total score	AV affinity (kcal/mol)	G fitness	S total score
Curcumin	−8.4	51.41	7.60	−8.7	52.18	5.44	−9.3	52.62	7.03
Silibinin	−7.8	25.33	4.67	−3.6	44.59	0.79	−7.8	37.70	3.97
Apigenin	−8.4	47.99	5.24	−8.6	49.48	4.96	−8.9	48.68	6.08
Genistein	−8.4	43.42	4.49	−9.1	49.05	5.13	−8.8	48.03	5.15
Naringenin	−8.3	50.78	6.04	−8.4	49.66	5.89	−8.6	47.11	5.43
[6]-Shogahol	−8.0	54.43	9.10	−7.6	49.73	8.34	−7.8	53.17	7.02
[6]-Gingerol	−8.0	55.73	7.54	−7.6	46.76	8.24	−7.7	55.00	7.69
Docosahexaenoic acid	−7.7	63.84	9.10	−7.5	60.62	9.57	−8.8	64.05	10.73
Cyanidin	−7.6	46.71	5.25	−8.1	49.41	3.24	−8.9	51.37	5.70
Quercetin	−7.6	46.78	5.84	−8.1	49.55	4.57	−8.8	47.97	6.23
Resveratrol	−7.6	45.19	5.11	−8.0	47.15	6.49	−8.0	46.02	5.31
Eicosapentaenoic acid	−7.4	59.86	9.37	−7.5	58.10	8.98	−8.5	61.63	8.78
Tangeretin	−7.5	48.91	3.90	−7.7	65.16	6.82	−8.1	60.72	5.21
Epicatechin	−7.4	43.09	6.32	−8.5	47.23	5.19	−8.7	46.58	5.68
Kaempferol	−7.6	46.93	4.13	−7.9	48.48	3.85	−8.8	47.29	4.45
Delphinidin	−7.1	48.64	5.79	−8.1	49.77	3.84	−8.4	50.75	6.04
Pterostilbene	−6.9	49.10	6.94	−7.9	49.03	7.15	−8.2	48.09	6.79
All-*trans* retinoic acid	−7.2	36.84	5.08	−7.3	39.23	5.36	−9.3	46.28	6.90
Carnosol	−6.8	17.40	2.81	−5.6	44.06	4.33	−8.1	48.73	5.56
Menthone	−6.3	29.56	3.30	−6.6	30.11	4.15	−6.6	29.63	4.03
Benzylisothiocyanate	−5.9	39.73	2.83	−6.0	40.34	3.19	−6.1	39.02	2.95
Phenethylisothiocyanate	−6.1	44.28	4.42	−6.1	41.84	3.48	−6.5	38.86	3.29
Epigallocatechin-3-gallate	−7.2	52.43	4.88	−6.7	54.01	3.26	−8.2	59.86	6.43
β-carotene	−5.7	20.8	6.58	3.3	−103.52	5.60	−6.1	−21.11	6.66
Lycopene	−5.3	20.59	5.33	−5.6	21.64	3.20	−7.6	2.11	4.04
5-Hydroxy-3,6,7,8,3′,4-hexamethhoxyflavone	−6.1	48.27	8.33	−6.7	55.94	9.59	−7.9	49.00	8.06
Sulforaphane	−4.4	45.61	3.38	−4.7	42.94	4.04	−4.8	43.43	3.72
Lutein	−3.9	31.69	4.99	3.6	−89.77	4.56	−5.6	−65.32	2.77
Proanthocyanidin B2	−1.8	−44.04	2.70	3.8	−48.54	−1.26	−4.5	−39.79	1.15
SC558 (inhibitor)	−10.7	51.75	6.03	−10.0	45.21	5.59	−10.1	43.44	4.55
Diclofenac (inhibitor)	−8.0	−122.97	4.42	−8.6	−119.46	5.46	−8.8	−117.28	2.24
Arachidonic acid (substrate)	−8.0	59.70	8.64	−7.5	58.18	9.58	−7.8	66.83	10.81

[a] Docking scoring function values calculated for each protein: AV, AutoDock Vina; G, GOLD; S, Surflex-Dock (SYBYL).

aromatic in nature, except for Ser 530, which interacts with curcumin through a hydrogen bond. For the 1CVU-all-*trans* retinoic acid complex (Fig. 2C), relevant aminoacids are Phe205, Phe209, Val228, Val344, Tyr348, Val349, Leu352, Tyr385, Trp387, and Leu534, showing only hydrophobic interactions with the ligand. Most of these residues have also been reported for chemicals having strong interactions with COX-2 [54,55].

The most favorable conformation resulted from the docking of curcumin into the active site of COX-2 is similar to that experimentally found for the COX-2 substrate arachidonic acid (Fig. 3). Accordingly, it is plausible to suggest that curcumin may be exerting its action by acting as a competitive inhibitor of arachidonic acid during prostaglandin E_2 synthesis by COX-2.

Among many natural products with known anti-inflammatory properties, curcumin is one of the most commonly referenced [56–59]. It is a phenolic yellow pigment present in curry powder, which has been associated with beneficial effects on human health as a result of its consumption in food [2]. It has been shown that curcumin exhibits antioxidant, anti-inflammatory and pro-apoptotic activities. Other food-related phenolic compounds with anti-inflammatory properties have also been reported in grapes, peanuts, blueberries, cranberries and red wine [60].

All-*trans* retinoic acid is a terpenoid derived from the mevalonate and isopentenyl pyrophosphate pathway [61]. This compound has been used for the treatment or alleviation of inflammatory diseases [62].

Other molecules that docked into COX-2 were genistein, apigenin, cyanidin and kaempferol. These are flavonoids commonly present in foods that have been used for the treatment of many diseases, mainly due to their anti-allergic, antiviral, anti-inflammatory and vasodilatory properties [63–67]. Similarly, docosahexaenoic acid has been reported to possess systemic anti-inflammatory effects and cardiovascular protection [68].

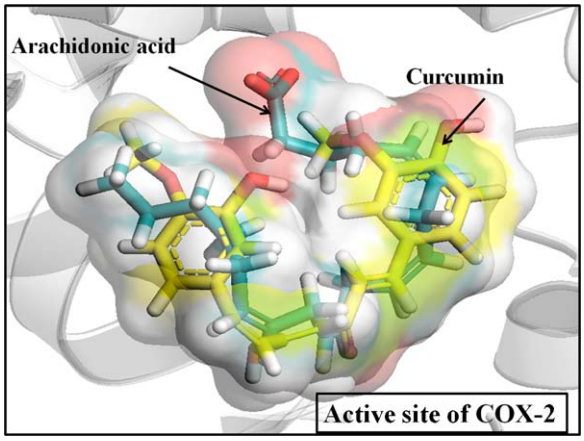


Fig. 3. Docking conformation of curcumin and arachidonic acid (experimental) on the active site of COX-2 (1CVU).

Although values obtained by docking analysis should be considered just as a theoretical approximation, this information could be useful to explore possible mechanisms by which these chemicals behave as anti-inflammatory compounds, in particular if those could directly bind proteins such as COX-2.

Table 3
Calculated affinities (AutoDock Vina), binding scores values (GOLD and SYBYL) and median inhibitory concentrations [IC_{50}] for selected COX-2 inhibitors.

COX-2 inhibitor	COX-2 structure									Mean AV values	Mean G values	Mean S values	IC_{50} (μM)	Log IC_{50} (μM)
	PDB_code: 1CX2			PDB_code: 1PXX			PDB_code: 1CVU							
	AV affinity (kcal/mol)	G fitness	S total score	AV affinity (kcal/mol)	G fitness	S total score	AV affinity (kcal/mol)	G fitness	S total score					
Valdecoxib (AID: 162347)	−9.5 ± 0.0	65.86 ± 0.02	4.80 ± 0.00	−8.5 ± 0.0	61.78 ± 0.02	4.62 ± 0.00	−10.0 ± 0.0	62.32 ± 0.02	6.60 ± 0.00	−9.4 ± 0.1	63.32 ± 0.34	5.34 ± 0.00	0.005	−2.3
Celecoxib (AID: 270014)	−10.8 ± 0.0	68.42 ± 0.02	6.63 ± 0.00	−9.7 ± 0.0	65.53 ± 0.06	8.14 ± 0.00	−9.8 ± 0.1	65.42 ± 0.08	6.52 ± 0.00	−10.1 ± 0.1	66.46 ± 0.26	7.10 ± 0.00	0.0022	−2.7
Meloxicam (AID: 162326)	−7.4 ± 0.1	35.65 ± 0.62	5.96 ± 0.00	−7.0 ± 0.0	38.51 ± 0.15	3.90 ± 0.00	−7.6 ± 0.0	54.82 ± 0.03	4.14 ± 0.00	−7.3 ± 0.1	42.99 ± 1.58	4.67 ± 0.00	0.16	−0.8
Piroxicam (AID: 162326)	−8.3 ± 0.0	39.06 ± 0.04	3.54 ± 0.00	−8.0 ± 0.0	35.07 ± 0.24	3.80 ± 0.00	−8.5 ± 0.0	50.56 ± 0.16	4.55 ± 0.00	−8.3 ± 0.0	41.56 ± 1.22	3.96 ± 0.00	0.1	−1.0
Diclofenac (AID: 313125)	−8.0 ± 0.0	−122.97 ± 0.07	4.42 ± 0.00	−8.6 ± 0.0	−119.24 ± 0.24	5.46 ± 0.00	−8.8 ± 0.0	−117.28 ± 0.11	2.24 ± 0.00	−8.4 ± 0.1	−119.90 ± 0.11	4.04 ± 0.00	0.02	−1.7
Flosulide (AID: 162338)	−8.5 ± 0.0	67.40 ± 0.08	7.35 ± 0.00	−8.8 ± 0.0	63.56 ± 0.14	6.47 ± 0.00	−9.4 ± 0.0	62.38 ± 0.10	7.43 ± 0.00	−8.9 ± 0.1	64.45 ± 0.40	7.08 ± 0.00	0.021	−1.7
Tenidap (AID: 160880)	−8.4 ± 0.1	56.04 ± 0.15	6.47 ± 0.00	−8.3 ± 0.0	61.57 ± 0.08	4.20 ± 0.00	−9.1 ± 0.0	59.80 ± 0.10	5.56 ± 0.00	−8.6 ± 0.1	59.14 ± 0.43	5.41 ± 0.00	0.01	−2.0
Nimesulide (AID: 162655)	−7.6 ± 0.0	57.79 ± 0.10	6.77 ± 0.00	−7.6 ± 0.0	56.15 ± 0.13	5.28 ± 0.00	−8.2 ± 0.0	55.11 ± 0.24	4.92 ± 0.00	−7.8 ± 0.1	56.35 ± 0.22	5.66 ± 0.00	0.015	−1.8
Etodolac (AID: 52141)	−7.2 ± 0.1	49.14 ± 0.23	7.09 ± 0.00	−7.9 ± 0.0	45.72 ± 0.13	6.05 ± 0.00	−8.3 ± 0.0	51.35 ± 0.08	6.24 ± 0.00	−7.8 ± 0.1	48.74 ± 0.44	6.53 ± 0.00	0.025	−1.6
Rofecoxib (AID: 241308)	−9.8 ± 0.0	63.49 ± 0.04	6.60 ± 0.00	−8.8 ± 0.0	60.60 ± 0.03	7.47 ± 0.00	−9.8 ± 0.0	59.57 ± 0.19	6.37 ± 0.00	−9.3 ± 0.1	61.20 ± 0.31	6.81 ± 0.00	0.032	−1.5
Dup 697 (AID: 162346)	−9.9 ± 0.2	73.47 ± 0.04	6.18 ± 0.00	−9.5 ± 0.0	69.63 ± 0.09	5.88 ± 0.00	−9.4 ± 0.0	66.28 ± 0.15	5.27 ± 0.00	−9.6 ± 0.1	69.80 ± 0.55	5.78 ± 0.00	0.01	−2.0
L-745337 (AID: 162346)	−9.3 ± 0.0	62.69 ± 0.18	6.92 ± 0.00	−10.9 ± 0.0	63.73 ± 0.03	6.19 ± 0.00	−9.6 ± 0.0	64.33 ± 0.08	5.90 ± 0.00	−9.9 ± 0.1	63.58 ± 0.14	6.34 ± 0.00	0.02	−1.7
SC558 Filizola [49]	−10.7 ± 0.2	51.75 ± 0.25	6.03 ± 0.00	−10.0 ± 0.1	45.21 ± 0.36	5.59 ± 0.00	−10.1 ± 0.2	43.44 ± 0.26	4.55 ± 0.00	−10.3 ± 0.1	46.80 ± 0.68	5.39 ± 0.00	0.0093	−2.0
NS 398 (AID: 46852)	−7.6 ± 0.0	60.55 ± 0.07	6.23 ± 0.00	−7.7 ± 0.0	57.99 ± 0.05	5.18 ± 0.00	−8.5 ± 0.0	59.26 ± 0.06	7.39 ± 0.00	−7.9 ± 0.1	59.93 ± 0.10	6.27 ± 0.00	0.19	−0.7
SC-58125 (AID: 162346)	−10.2 ± 0.2	69.83 ± 0.10	6.72 ± 0.00	−9.6 ± 0.2	67.76 ± 0.08	6.40 ± 0.00	−9.7 ± 0.0	66.09 ± 0.08	6.58 ± 0.00	−9.8 ± 0.1	67.89 ± 0.29	6.57 ± 0.00	0.05	−1.3
CID: 10459826 (AID: 254745)	−7.0 ± 0.1	60.72 ± 0.64	6.28 ± 0.00	−7.3 ± 0.0	27.84 ± 1.31	7.27 ± 0.00	−10.6 ± 0.0	51.30 ± 0.41	9.24 ± 0.00	−8.3 ± 0.3	46.62 ± 2.61	7.60 ± 0.00	0.05	−1.3
CID: 10895294 (AID: 162484)	−9.4 ± 0.0	73.59 ± 0.03	7.25 ± 0.00	−7.6 ± 0.0	69.78 ± 0.08	7.55 ± 0.00	−9.3 ± 0.0	68.08 ± 0.19	6.49 ± 0.00	−8.8 ± 0.2	70.48 ± 0.43	7.10 ± 0.00	0.034	−1.5
CID: 9885354 (AID: 162507)	−10.6 ± 0.0	68.32 ± 0.03	7.49 ± 0.00	−9.4 ± 0.0	65.24 ± 0.06	8.29 ± 0.00	−9.3 ± 0.0	65.07 ± 0.12	6.23 ± 0.00	−9.8 ± 0.1	66.21 ± 0.28	7.32 ± 0.00	0.013	−1.9
2,3-Diarylcyclobutenone methylsulfone Dewitt [50]	−9.4 ± 0.0	66.20 ± 0.03	6.64 ± 0.00	−8.7 ± 0.0	64.91 ± 0.09	5.34 ± 0.00	−9.2 ± 0.0	64.09 ± 0.06	6.45 ± 0.00	−9.1 ± 0.1	64.09 ± 0.17	6.14 ± 0.00	0.003	−2.5
2,3-Diarylphenyl sulfonamide Dewitt [50]	−11.0 ± 0.0	66.32 ± 0.05	6.80 ± 0.00	−10.7 ± 0.0	65.70 ± 0.03	7.27 ± 0.00	−11.4 ± 0.0	63.38 ± 0.09	6.93 ± 0.00	−11.0 ± 0.1	65.10 ± 0.24	7.00 ± 0.00	0.002	−2.7
2,3-Diarylthiazolotriazole methylsulfone Dewitt [50]	−8.9 ± 0.0	66.62 ± 0.03	7.23 ± 0.00	−9.3 ± 0.0	72.88 ± 0.05	6.42 ± 0.00	−9.5 ± 0.0	67.86 ± 0.07	4.86 ± 0.00	−9.3 ± 0.0	69.12 ± 0.50	6.17 ± 0.00	0.01	−2.0

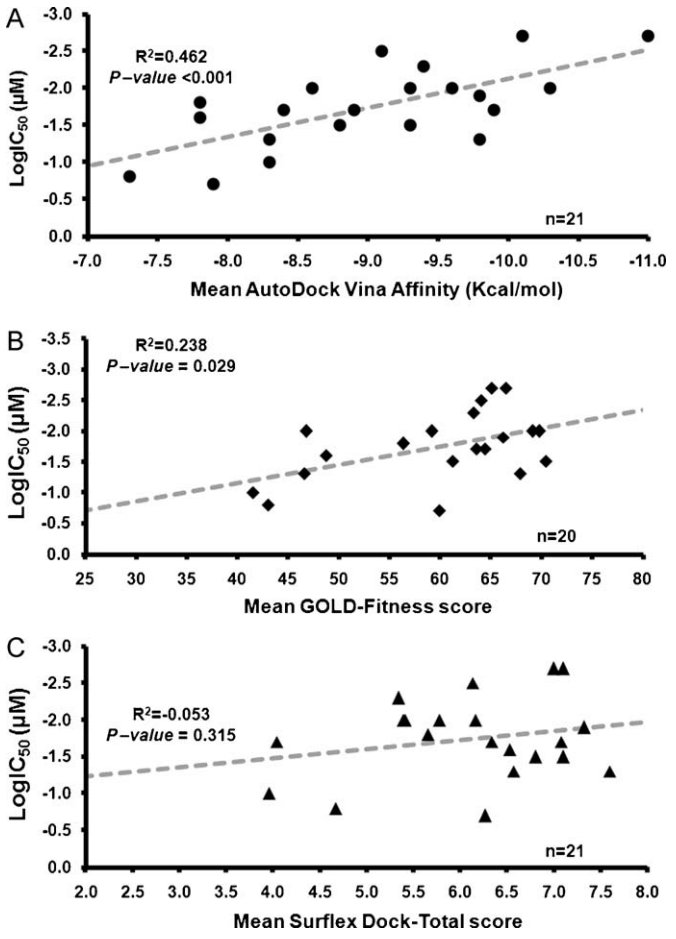


Fig. 4. Correlation between docking theoretical data for inhibitors on COX-2 structures (1CX2, 1PXX and 1CVU) and their half maximal inhibitory concentration ($Log\,IC_{50}$). (A) AutoDock Vina, (B) GOLD and (C) Surflex-Dock. The regression line is shown for illustrative purposes. The GOLD score value for diclofenac (-119.90 ± 0.11) was not included in the analysis.

3.4. Relationship between biological activity of COX-2 inhibitors and protein–ligand docking data

In order to determine if affinity values calculated by AutoDock Vina, as well as the scores calculated by GOLD and Surflex-Dock, could be utilized as an indication of the likeliness of a compound to behave as a COX-2 inhibitor, a group of 21 active compounds with confirmed inhibition activity, reported in PubChem BioAssay database [48], were docked to COX-2 (PDB: 1CX2, 1PXX and 1CVU). The PubChem chemical structure identifier (CID), biological activity (IC_{50}), AutoDock Vina affinity values, GOLD and Surflex-Dock scores for these compounds, and the biological activity ($Log\,IC_{50}$) are shown in Table 3. The relationships between biological activity and docking data are presented in Fig. 4. Results suggest that for all examined docking tools, COX-2 activity follows a linear relationship only with binding affinity (AutoDock Vina) and the docking scores from GOLD, being highly significant with the first one. Although the magnitude of the correlation was moderate ($R^2 = 0.462$, $P < 0.001$), this value is similar to that obtained for other docking studies [69].

Moreover, data showed that ligands with absolute affinities greater than 10 kcal/mol have a better chance of interaction with COX-2. For instance, celecoxib, SC558, and 2,3-diarylphenyl sulfonamide have absolute affinity values greater than 10 kcal/mol and low IC_{50}s. However, molecules with absolute affinities values around 9 kcal/mol have also a good probability of acting as COX-2 inhibitors. This is reassured when biological data is revised for our food-derived COX-2 inhibitors that presented best affinity values. Median inhibitory concentrations (IC_{50}) tested in different cell lines for curcumin (range 2–15 μM) [70–75], all-*trans* retinoic acid (20.5 μM) [76], genistein (range: <15–200 μM) [77–79], apigenin (range: 8.04–50 μM) [77,80], cyanidin (range: 40–90 μM) [81,82], kaempferol (range: <15–50 μM) [77,80], docosahexaenoic acid (range: 9.8–30 μM) [83,84], naringenin (7.9 ± 1.9 μM) [85], [6]-shogahol (2.1 μM) [86], resveratrol (range: 3.06 μM) [87], eicosapentaenoic acid (7.1 μM) [83] are supporting evidence that these compounds can modulate COX-2 activity not only at mRNA but also at the protein level.

3.5. Docking curcumin and celecoxib on COX-2

It is known that some of the chemicals studied here can modulate COX-2 activity not only by competitive inhibition, but also by allosteric binding [52,53]. It has been shown that curcumin produces a synergistic effect with celecoxib, a highly selective COX-2 inhibitor, almost abolishing all enzyme activity [52,53]. In order to determine if this additive process occurs due to curcumin (both the keto and the enol forms) binding on a site different from that used by celecoxib, a series of 500 AutoDock Vina docking runs were performed on the protein isolated from the complex COX-2-celecoxib (PDB: 3LN1), and the results are presented in Fig. 5. Celecoxib docks onto COX-2 (PDB: 3LN1) on two different sites (Fig. 5A). As expected, the most favorable was the active site of COX-2 (binding frequency, bf, 96.8%) (Fig. 5B), as found in the crystal structure of the celecoxib:COX-2 complex (PDB: 3LN1). An additional site (bf, 3.2%) was detected by the docking simulations, but it is less energetically favorable (−8.8 kcal/mol vs. −11.2 kcal/mol). On the other hand, in addition to the active site (celecoxib site), curcumin in the keto form prefers two additional (allosteric) sites on COX-2 (Fig. 5C) with binding frequencies of 38.6% and 38.2% (Fig. 5D). A different trend is observed for the enol form of curcumin. This form does not dock on the active site at all when the whole protein (3LN1) is used as docking surface (Fig. 5E), and it docks mainly to site 2 (bf, 94.12%), and in a minor grade to site 3 (bf, 5.88%) (Fig. 5F); however these interactions are less favorable than those detected for the keto form.

It is important to keep in mind that results from docking the keto and enol forms of curcumin on the whole protein surface (3LN1) are different from those acquired when the enol form is docked directly into the active site of 1CX2, 1PXX and 1CVU. In these last cases, the absolute binding affinities were greater by approximately 1–2 kcal/mol. The docking of the keto form of curcumin onto the active site of COX-2 generates not only different affinity values depending on the site, but also distinct spatial orientations. These last changes could require additional docking energy and this could be a reason explaining why this curcumin form prefers the other binding sites, where the docking implies less inner molecular consumption.

Docking runs ($n = 100$) for celecoxib and the two curcumin forms, performed using the three docking tools examined in this work, on the three binding sites predicted for curcumin (keto form, PubChem) using AutoDock Vina are shown in Table 4. Results showed that AutoDock Vina, GOLD and Surflex-Dock predicted that celecoxib prefers only the known inhibitor binding site (Site 1). In the case of curcumin, all three docking tools suggested that both forms of this natural product can at some point interact with any of the three binding sites. However, there are minor changes in the preferences based on the curcumin form and the docking tool used. Taken together, these results suggest that independent from the tautomeric state of curcumin, it has the ability to interact with COX-2 on a binding site different from celecoxib.

This *in silico* evaluation of curcumin binding on COX-2 offers a plausible explanation for the synergism observed for celecoxib and

Table 4
Binding affinity (AutoDock Vina) and binding score values (GOLD and Surflex-Dock) for curcumin (keto and enol forms) and celecoxib (inhibitor) on different predicted binding sites (1, 2, and 3) on COX-2.

Compound	Site 1			Site 2			Site 3		
	AV (kcal/mol)	G fitness	S total score	AV (kcal/mol)	G fitness	S total score	AV (kcal/mol)	G fitness	S total score
Celecoxib	−11.9 ± 0.1	68.54 ± 0.02	9.50 ± 0.00	−9.0 ± 0.0	61.04 ± 0.13	6.05 ± 0.00	−7.2 ± 0.0	60.87 ± 0.14	4.53 ± 0.00
Curcumin (keto)	−8.4 ± 0.0	56.51 ± 0.26	6.71 ± 0.00	−8.8 ± 0.0	52.58 ± 0.12	7.24 ± 0.00	−8.0 ± 0.0	52.64 ± 0.14	8.75 ± 0.00
Curcumin (enol)	−8.6 ± 0.2	48.39 ± 0.29	7.40 ± 0.00	−8.7 ± 0.0	51.97 ± 0.12	9.58 ± 0.00	−8.3 ± 0.0	51.52 ± 0.12	7.40 ± 0.00

AV, AutoDock Vina; G, GOLD; S, Surflex-Dock (SYBYL).

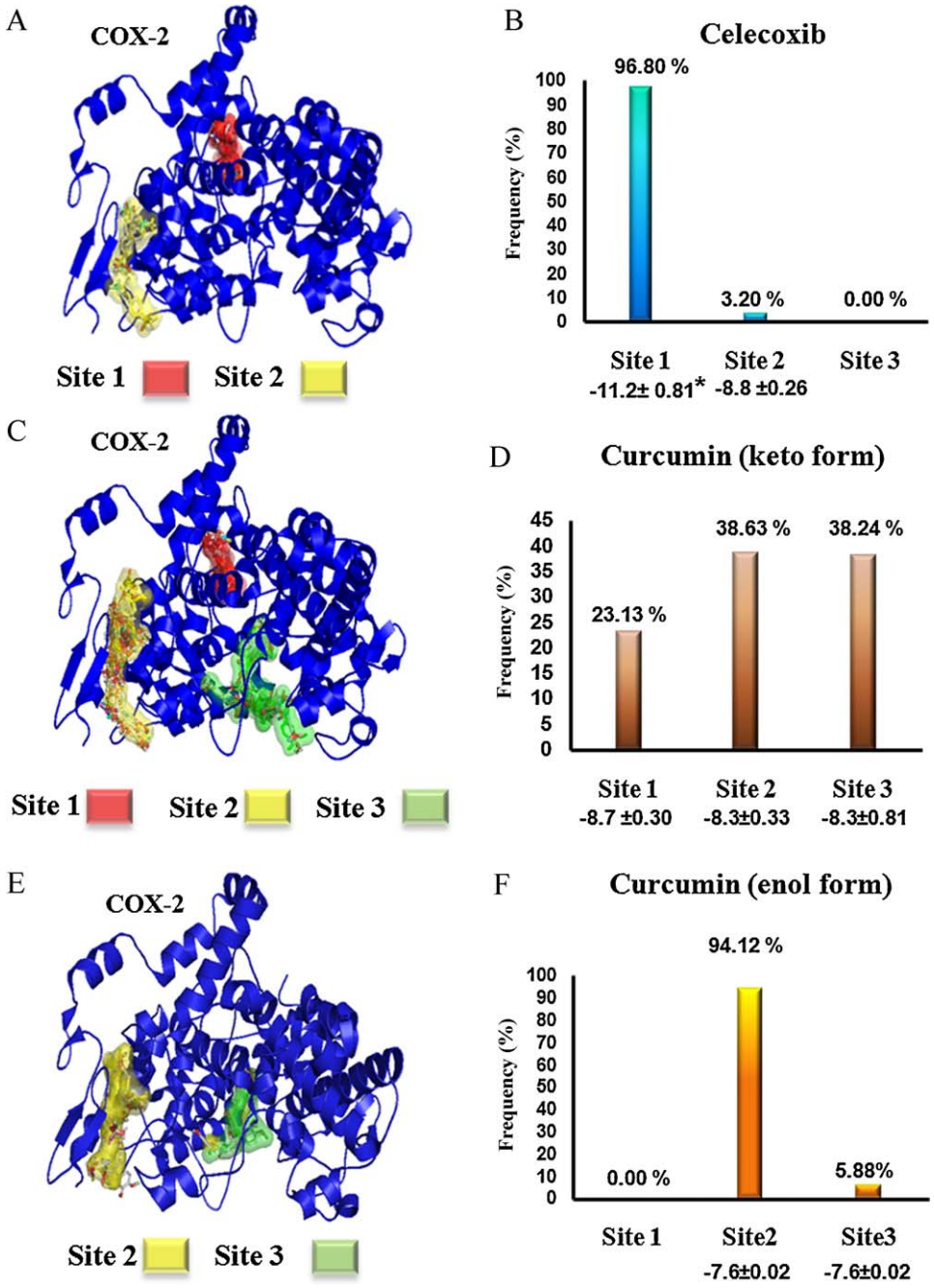


Fig. 5. Celecoxib (A) and curcumin (keto, C; enol E) binding sites on COX-2, and ligand binding site preferences for each one of them (B, D, and F, respectively). *The affinity values (mean ± standard deviation, $n = 500$) in kcal/mol obtained for each protein–ligand complex are shown in below site.

curcumin to inhibit the action of the enzyme. It also showed that the size of the used docking grid can have profound differences in the results. However, it was clear that for both keto and enol forms, a binding site different from the active site is preferred by curcumin, although this process is less energetically favorable.

Although the mechanisms involved in the anti-inflammatory action of chemicals present in edible plants may comprise distinct pathways, some of the compounds examined here are known for their actions on the regulation of transcription factors such as nuclear factor-kappa B (NFκβ) [88,89], signal transducers and activation of transcription-1 (STAT-1) [90], peroxisome proliferator-activated receptor gamma (PPARγ) [91], NF-E2-related factor-2 (Nrf2) [92], and also in the inhibition of mitogen-activated protein kinase (MAPK) (ERK, JNK, and p38) phosphorylation [93], among many other targets. These mechanisms may indeed alter the expression of COX-2. However, as shown here, it may be equally important to consider their direct action at the protein level, in order to have a better knowledge of their pharmacological benefits. In addition, it is clear that computational chemistry is a powerful tool that speeds up and lowers the cost of those approaches leading to find therapeutic agents to promote human health.

4. Conclusion

In silico docking calculations performed with AutoDock Vina showed that binding affinities obtained for some natural compounds on COX-2, such as curcumin and all-*trans* retinoic acid, are of similar magnitude than those generated for known inhibitors of this protein. Affinities from AutoDock Vina and scores given by the docking software GOLD showed significant correlations with experimental data for COX-2 inhibition. Docking studies performed with curcumin and celecoxib, this last a synthetic inhibitor of COX-2, suggest that curcumin may be able to bind this protein both competitively and allosterically. Therefore, natural products present in the diet are important not only as transcriptional regulators of COX-2, but also they may modulate its enzyme activity to control inflammatory processes.

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