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Allosteric Modulation of SULT2A1 by Celecoxib and Nimesulide: Computational Analyses

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Abstract

We used protein-ligand docking and minimization to identify celecoxib as an allosteric modulator of SULT2A1-catalyzed estradiol sulfonation. Subsequent to celecoxib docking and complex minimization, conformational changes in SULT2A1 allowed estradiol docking to an alternative binding region with predicted preference for 17 β -OH-E₂ sulfonation over 3-OH-E₂ sulfonation.

Keywords

Estradiol; sulfotransferase; allosteric; docking; metabolism; Autodock 4

INTRODUCTION

Cytosolic sulfotransferase enzymes are Phase II xenobiotic metabolizing enzymes that play a key role for the sulfonation of physically important steroid phenols and alcohols including estradiol (E₂), estrone, testosterone, dehydroepiandrosterone, pregnenolone, cortisol, cholesterol and bile acids. In addition, they sulfonate catechol and phenolic monoamine neurotransmitters and thyroid hormone phenols. Variation in the activity of sulfotransferases which metabolize the endogenous substrates may cause disruption in the normal regulation of the activity of these steroids, thyroid hormones, and neurotransmitters [1]. Sulfotransferase enzymes are also responsible for metabolism of many pharmacological agents—usually to inactive or easily excreted forms, but occasionally to reactive or genotoxic forms. In contrast to the expectation that tight regulation of sulfotransferase activity towards these endogenous substrates would be evolutionarily advantageous, a vast number of sulfotransferase inhibitors have been identified, and many of these inhibitors exhibit non-competitive kinetic behavior [2,3].

Of the 13 known human cytosolic sulfotransferases, 11 have been sub-classified into SULT1 and SULT2 families based on their amino acid sequence identity and enzymatic properties. SULT1 family mostly sulfonates phenols while SULT2 family mostly catalyzes sulfonation of alcohols, especially hydroxysteroids [4]. Thus, the common name for SULT1 family is “phenol sulfotransferase”, and the common name for the SULT2 family is “hydroxysteroid sulfotransferase”. The SULT1 family includes SULT1A1, SULT1A2, SULT1A3, SULT1A4, SULT1B1, SULT1C1, SULT1C2, SULT1C3, and SULT1E1. The SULT2 family includes SULT2A1, SULT2B1_v1, and SULT2B1_v2. Single members of other

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mammalian SULT families include mouse SULT3A1; mouse, human, and rat SULT4A1; and human SULT6B1 [5].

Under *in vitro* conditions in the absence of inhibitors, three SULTs are capable of sulfonating estradiol: SULT1E1, SULT1A1, and SULT2A1 [6–8]. The K_m of estradiol for SULT1E1 is very low (1–20 nM range); thus SULT1E1 sulfonates E_2 at so-called ‘physiological’ estradiol concentrations. The K_m ’s of estradiol for SULT1A1 and SULT2A1 are higher (11–31 μ M and 1.5–12.4 μ M, respectively) [9–11]; thus SULT1A1 and 2A1 sulfonate estradiol at so-called ‘supra-physiological’ estradiol concentrations. All three enzymes (SULT2A1, 1A1, and 1E1) are expressed in the liver. SULT2A1 is also found in the small intestine and, at high levels, in the adrenal gland [12,13]. SULT1E1 is also expressed in the intestine and, at lower levels, in the kidney. SULT1A1 is present at highest levels in the liver, with significant expression in small intestine and other tissues. Thus, in the liver SULT1A1 and/or 2A1 could contribute to sulfonation of μ M concentrations of estradiol.

SULT2A1 is a human cytosolic sulfotransferase that catalyzes sulfate conjugation of many hydroxysteroid substrates including androgen precursor dehydroepiandrosterone, androgens, androsterone, bile acids, pregnenolone, estrogens, alcohol precarcinogens, and other hormonal and xenobiotic substrates [14]. In contrast to the generalization that a sulfotransferase from the SULT2 family should sulfonate hydroxysteroids (17 β -OH of E_2), human cytosolic SULT2A1 catalyzes sulfonation of the phenolic 3-OH- E_2 position under typical conditions (Fig. (1)). However, in the presence of celecoxib (a selective COX-2 inhibitor) the major sulfonation product was found to switch from the 3-OH position to the 17 β -OH position [11]. This is noteworthy because E_2 -3-O-sulfonate is hydrolyzable to active estrogen by sulfatase, while E_2 -17 β -O-sulfonate is not hydrolyzed and is excreted. Thus, 3-OH sulfonation would allow regeneration of E_2 activity, while 17 β -OH sulfonation is expected to be a terminal reaction *in vivo*.

The goal of this work was to identify the allosteric binding site within SULT2A1 for celecoxib, and to evaluate the structural interactions underlying the mechanism of ‘switching’ of the sulfonation position from 3-OH- E_2 to 17 β -OH- E_2 . Elucidation of the structural features on which the ‘switching’ is based may lead to better predictions of drug-steroid interactions involving sulfotransferases.

EXPERIMENTAL DESIGN AND METHODS

Software and Structural Coordinates

X-ray crystal structural coordinates were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB, www.pdb.org) [15]. Estradiol, PAPS, celecoxib and nimesulide structural coordinates were from 1AQU, 1HY3, 1OQ5 and 1ZWP, respectively. The ligands were prepared by fixing double and aromatic bonds, and by calculating atom partial charges. Three structures were available for SULT2A1 (1J99, 1EFH, and 1OV4) [16–18]. The three structures were evaluated for their suitability for this study, and 1J99 was judged the most appropriate structure based on fewer missing residues, good B-factors, and better average resolution. The A-chain of 1J99 in the presence of crystal waters was used for all docking experiments.

Binding cavities were calculated using Accelrys Discovery Studio 2.0. Each protein-ligand complex was energy-minimized with the CHARMM (Chemistry at Harvard Macromolecular Mechanics) module of Accelrys Discovery Studio, and the minimized complexes were used in docking experiments. AutoDockTools 1.4.6 [19] was used to prepare all grid and docking parameter files for SULT2A1, estradiol, PAPS, celecoxib and

nimesulide according to the software supplier's instructions [20]. Docking analyses were conducted with AutoDock 4 [21–22] on a Windows workstation. Autodock 4 uses a stochastic grid-based approach and a hybrid global-local search algorithm (Lamarckian genetic algorithm) to search the phase space of the ligand-protein system.

Binding Site Computations

Potential sites for the allosteric binding of celecoxib to SULT2A1 were calculated using the binding site analysis feature of Discovery Studio 2.0. This feature calculates solvent-accessible cavities within the receptor structural coordinates. An opening site of 10 Å and grid size of 0.7 Å were used. For visualization of the binding cavity surface, the cavity grid points were typed as hydrogen, and the soft closed surface was rendered by Discovery Studio.

Molecular Docking and Minimization of Docked Complexes

For the docking, Autodock 4's hybrid global-local search method, Lamarckian genetic algorithm, was used with 2500000 evaluations, 27000 generations, and population size 300. Multiple (≥ 100) distinct runs were executed for each docking experiment, and the ≥ 100 resulting conformations were clustered within 2.0 Å atom RMSD. The default values of other parameters were selected. The identified binding cavities were used to define the dimensions and centers of binding site grid boxes used for the docking experiments. Grid point and center coordinates are shown in Table 1.

Because current docking software only docks one ligand at a time, the docking and minimization steps were conducted in sequence. First PAPS was inserted into 1J99 based on the superimposition of 1HY3 and 1J99 protein coordinates. This enzyme-cofactor complex was energy-minimized, named [SULT2A1/PAPS]_m, and used as the receptor for the separate docking of estradiol, celecoxib, and nimesulide (**I**, **II**, **III**).

To investigate the binding of estradiol in the absence of allosteric modulator, estradiol was docked (**I**) into [SULT2A1/PAPS]_m as described in Table 1.

To indicate the effect of celecoxib on the binding of estradiol to SULT2A1, the binding affinity of estradiol was examined in the presence of celecoxib. For this approach, celecoxib was docked (**II**) into the binding site of the minimized SULT2A1/PAPS complex prior to estradiol docking. Several (14) docked conformations of celecoxib were selected for further study, and the energy of each SULT2A1/PAPS/celecoxib complex (1–14) was minimized. Multiple celecoxib conformations were chosen so as to cover a wide selection of celecoxib binding modes. Lastly estradiol was docked into each minimized SULT2A1/PAPS/celecoxib complex (**IV**_{1–14}). As a result, the binding of estradiol to SULT2A1 was observed in the absence (**I**) and in the presence (**IV**_{1–14}) of multiple conformations of celecoxib.

To indicate the effect of nimesulide on the binding of estradiol to SULT2A1, the binding affinity of estradiol was examined in the presence of nimesulide. For this approach, nimesulide was docked (**III**) into the binding site of minimized SULT2A1/PAPS complex prior to estradiol docking. Several (7) docked conformations of nimesulide were selected for further study and the energy of each SULT2A1/PAPS/nimesulide complex (1–7) was minimized. Lastly estradiol was docked into each minimized SULT2A1/PAPS/nimesulide complex (**V**_{1–7}). As a result, the binding of estradiol to SULT2A1 was observed in the absence (**I**) and in the presence (**V**_{1–7}) of multiple conformations of nimesulide.

The docking results were quantitatively analyzed by evaluation of calculated parameters: binding free energy (ΔG_b , kcal/mol), inhibition constant (K_i , μM), and root mean square deviation (RMSD, Å) from a reference structure. The binding free energy is estimated by

Autodock 4 as the energy resulting from the ligand moving from unbound to bound state. Specifically, Autodock 4 considers the free energy change due to van der Waals forces, hydrogen bonding, electrostatics, desolvation, and torsional free energy. The scoring function is based on the AMBER forcefield and has been shown to be accurate within 2.5 kcal/mol [21]. The K_i is calculated by Autodock 4 from the binding free energy change (ΔG_b).

We also analyzed specific binding interactions of the docked ligands with the receptor. First, we monitored the distance between the acceptor oxygen (3-OH or 17 β -OH) and the PAPS sulfur for each docking instance. Second, we monitored the hydrogen-bond distance between the conserved catalytic histidine (His99 in SULT2A1) and the acceptor oxygen. These atoms must be within bonding distance for the catalysis to occur [23]. His99 is thought to trigger catalysis by deprotonating the substrate hydroxyl, making the oxygen more nucleophilic and better able to initiate a covalent bond with the electrophilic sulfur of the donor PAPS.

RESULTS AND CONCLUSIONS

SULT2A1 Crystal Structure Evaluation

Three X-ray crystal structures were available for SULT2A1 in the PDB [16–18], but only 1J99 was used for the docking studies based on the initial criteria that 1J99 had the fewest missing residues when compared to the crystal structures of 1EFH and 1OV4. Further comparison of 1J99, 1EFH, and 1OV4 confirmed the appropriateness of 1J99 for this work. Superimposition of the three structures showed that the only significant folding difference ($\Delta 5$ –10 Å, C α atoms) amongst the three structures was in the region of residues 232–252. This region made up a portion of the PAPS cavity, as previously noted by Allali-Hassani et al [5] and Lu et al [24]. Specifically, we found that Ser251 of 1J99 clashed with the 3'-phosphate of superimposed PAP. We additionally found that this region made up a portion of the substrate binding cavity, and that Ser232–Ser235 of 1EFH clashed with the superimposed crystal substrates of 1J99 and 1OV4 (data not shown). Because of this lack of ability to accommodate substrate structures, we discarded 1EFH from further consideration. We next addressed the question of whether 1J99 could accommodate PAPS in a catalytic conformation. We found that, indeed, PAPS could dock to 1J99 with appropriate binding energy (-7.56 kcal/mol, K_i 2.9 μ M) simply by rotating the 3'-phosphate to a less hindered position, while maintaining the critical catalytic positioning of the 5'-phosphosulfate (data not shown).

Computational Prediction of Alternative SULT2A1 Allosteric Modulators

We chose to model celecoxib as a modulator based on the published biochemical data with estradiol and SULT2A1 [11]. We chose to model nimesulide as a potential modulator based on our published biochemical data on inhibitors of SULT1A1 [2]. Nimesulide was chosen after comparison of celecoxib with the 2D and 3D structures of several SULT1A1 non-competitive inhibitors. As shown in Fig. (2), we found that one of those inhibitors, nimesulide, has striking 2D and 3D structural similarity to celecoxib, including overlay of the sulfonamide functional group and major ring angles. Because of these structural similarities, we predicted that nimesulide may bind to SULT2A1 in an allosteric manner and modulate estradiol metabolism similar to celecoxib.

Binding Site Analysis and Prediction of Allosteric Binding Site

The substrate and cofactor binding cavity of SULT2A1 maintains the general shape of all crystallized cytosolic sulfotransferases: a 'dumbbell' shape of two cavities connected by a narrower channel (RS King, unpublished research) (Fig. (3)). It is especially noteworthy that

the cavity region available for substrate binding in SULT2A1 is large enough for two molecules (of size similar to known substrates and inhibitors) to bind simultaneously (Fig. (3)). This finding that celecoxib can bind into the cavity near the substrate binding site, without necessity to compete with substrate binding, is one key evidence that this, indeed, may be the allosteric binding site for celecoxib.

Docking and Minimization

In the absence of celecoxib, 84% of the total E₂ conformations oriented with the 3-OH in position for sulfonation, while 16% of the total oriented with the 17β-OH in position for sulfonation (Fig. (3A) and Table 2A). This result is consistent with the fact that SULT2A1 normally sulfonates E₂ at the 3-OH position, with some sulfonation at the 17β-OH position [11]. The calculated binding energy for estradiol docked to the SULT2A1/PAPS complex was -7.64 kcal/mol ($K_i = 2.5 \mu\text{M}$). This result is similar to the published K_m (1.5–12.4 μM) of estradiol for SULT2A1 [11]. Comparison of the two docked estradiol binding modes with the reference (overlaid E₂ from 1HY3), showed that docked estradiol occupied the same region in the cavity as the reference, and we denote this region 1.

When celecoxib was docked (IV₁₋₁₄) prior to estradiol, we found that celecoxib also occupied region 1 which would require displacement of estradiol. However, subsequent docking of estradiol to the complex minimized in the presence of celecoxib showed that conformational changes allowed estradiol to dock with sufficient affinity to an alternative region in the cavity. We denote this region 2. Within region 2 estradiol preferred to dock with the 17β-OH in position for sulfonation or in a non-catalytic position (with both -OH's distant from donor PAPS) (Table 2B)). Specifically, of the seven celecoxib conformers (celecoxib₁₋₇) with most negative binding energy (-6.00 – -5.70 kcal/mol, 40–60 μM K_i), two allowed E₂ to dock only with the 17β-OH in position for catalysis, one allowed E₂ to dock with either the 3-OH or the 17β-OH in position for catalysis, and the remaining four allowed E₂ to dock only in non-catalytic positions (Table 2B). Celecoxib₁, the conformer with the most negative binding energy, showed a calculated K_i of 40 μM with minimized SULT2A1/PAPS, which is similar to the celecoxib concentration causing maximal 17β-OH sulfonation of E₂ with liver cytosol [11]. Thus, the docking experiments showed that celecoxib can induce a conformational change which stimulates catalysis at the 17β-OH position (Fig. 3B). The docking data in the presence of celecoxib predicts that the 3-OH and 17β-OH oriented binding modes in region 1 would be inhibited by celecoxib in a competitive manner, whereas conformational changes in the protein in the presence of celecoxib cause stimulation of the 17β-OH binding mode in region 2.

Similarly, when nimesulide was docked (V₁₋₇) prior to estradiol, we found that nimesulide also occupied region 1 which would require displacement of estradiol. Minimization of the complex in the presence of nimesulide again induced conformational changes in the SULT2A1 protein structure which allowed estradiol to dock to region 2 of the cavity (Table 2C), Fig. (3C)). Specifically, of the seven nimesulide conformers (nimesulide₁₋₇) with most negative binding energy (-3.50 – -3.04 kcal/mol, 2.7 – 5.9 mM K_i), one allowed E₂ to dock only with the 17β-OH in position for catalysis ($K_i = 12 \mu\text{M}$), one allowed E₂ to dock only with the 3-OH in position for catalysis ($K_i = 0.76 \mu\text{M}$), and the remaining five allowed E₂ to dock only in non-catalytic positions (Table 2C). This docking data predicts that the 3-OH and 17-OH oriented estradiol binding modes in region 1 would be competitively inhibited by mM nimesulide, but this inhibition may be masked by allosteric activation of 3-OH and 17β-OH binding modes in region 2.

Estradiol-SULT2A1 binding interactions and interatomic distances were used to determine whether estradiol was bound in catalytic (at 3-OH or 17β-OH) or non-catalytic position. As shown in Fig. (4A) in the absence of modulator, estradiol preferred to dock with the 3-OH

within hydrogen-bonding distance of the basic histidine 99 nitrogen (3.3 Å) with and the 3-oxygen within bonding distance of the sulfur of PAPS (3.7 Å). Additional binding interactions included a hydrogen bond between the 17β-OH of E₂ with the carbonyl backbone of lysine 138, and hydrophobic interactions between E₂ carbons and proline 14, threonine 15, methionine 16, phenylalanine 133, tryptophan 134, methionine 137, lysine 138, tyrosine 231, and tyrosine 238 of SULT2A1 (Fig. (4A)). These results are consistent with substrate binding regions identified previously for SULT2A1 [24], SULT1E1 [23], and for other sulfotransferases (R. King, unpublished).

When celecoxib was docked prior to estradiol, it consistently docked into the general space otherwise occupied by estradiol, as did nimesulide (Fig. (3, 4)). Subsequently, estradiol found an alternative binding position (in region 2), of equal affinity and within the extended cavity, which oriented the 17β-OH within catalytic distance of His 99 and the PAPS sulfur. In the presence of celecoxib₁ for example, the His 99 basic nitrogen was 3.1 Å from the 17β-OH and the PAPS sulfur was 3.0 Å from the 17β-oxygen. In the presence of nimesulide₆, the His 99 basic nitrogen was 3.5 Å from the 17β-OH and the PAPS sulfur was 3.2 Å from the 17β-oxygen. In the presence of other conformers (celecoxib₃₋₇, nimesulide_{1-3,5,7}), estradiol located to the same alternative binding region, but in such an orientation that catalysis could not occur. These results are consistent with the observed allosteric modulation of SULT2A1 estradiol sulfonation by celecoxib, and are consistent with our predicted allosteric modulation of SULT2A1 by high concentrations of nimesulide.

DISCUSSION

Within the large substrate binding cavity, we found two distinct binding regions. We denote these region 1 and region 2. It is noteworthy that only region 1 was available for estradiol binding prior to modulator docking. Region 2 became available for estradiol binding only after a conformational change induced by celecoxib (or nimesulide) docking & minimization. Within region 1 estradiol has two binding modes. One mode in the 3-OH position, and one mode in the 17-OH position (Table 2A). The binding constants (K_i 's) calculated by Autodock 4 for binding in the 3-OH and 17-OH mode (2.5 μM and 3.1 μM, respectively) are similar to the biochemical K_m 's published by Wang & James (1.5 μM and 3 μM, respectively [11]).

The results shown for celecoxib docking to SULT2A1/PAPS complex are representative of multiple docking runs under a variety of docking conditions. In all instances, celecoxib preferred to dock to the portion of the binding pocket normally filled by estradiol. This result is consistent with, but a modification our hypothesized interaction of celecoxib with SULT2A1. We had predicted that celecoxib would bind in the extended substrate binding pocket near enough to the estradiol to reverse the orientation of estradiol, yet not displace the estradiol. Yet, as shown in Fig. (3B) and (4B), celecoxib could instead force estradiol to bind in an alternate catalytic binding mode in the extended region of the binding cavity. We find it noteworthy that the binding affinity of estradiol for SULT2A1 was minimally affected by the presence of celecoxib conformers 1–3 (Table 2) even though estradiol was forced into the alternative binding mode. Because celecoxib displaced estradiol from region 1, our docking data implies that both 3-OSO₃-E₂ and 17-OSO₃-E₂ formation should be competitively inhibited by celecoxib. However, celecoxib binding also induced the conformational change allowing estradiol to bind to region 2 in catalytically active mode. Thus, celecoxib has the overall effect of an allosteric modulator of estradiol sulfonation by SULT2A1.

The results shown for nimesulide docking to SULT2A1/PAPS complex are also representative of multiple docking runs under a variety of docking conditions. Nimesulide

also preferred to dock to the portion of the binding pocket normally filled by estradiol, while estradiol found an alternative binding mode. However, the binding affinity of nimesulide to SULT2A1/PAPS complex was rather poor (K_i , 2–6 mM), and not likely to be clinically relevant under typical doses of nimesulide [2].

This research approach was designed to be transferrable to additional members of the sulfotransferase superfamily. Defining the allosteric binding site and interactions for sulfotransferase enzymes is significant because many cytosolic sulfotransferases are affected by allosteric (non-competitive) inhibitors and these sulfotransferases modulate the activity of several steroid phenols and steroid alcohols. Identification of the allosteric binding site and understanding of the underlying mechanism may be important for prediction of drug-drug and drug-steroid interactions involving sulfotransferases.

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LIST OF ABBREVIATIONS

Å	Angstrom
CEL	Celecoxib
E ₂	17β-estradiol
NIM	Nimesulide
PAP	3'-Phosphoadenosine-5'-phosphate
PAPS	3'-Phosphoadenosine-5'-phosphosulfate
RMSD	Root mean square deviation
SULT2A1	Human sulfotransferase member 2A1

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Fig. 1.
Regioselectivity of SULT2A1 catalyzed 17β-estradiol sulfonation.

Fig. 2.

(A) 2D- and 3D-Spatial similarity of celecoxib (purple) and nimesulide (green).

Sulfonamide functional group on both celecoxib and nimesulide is colored by element

(oxygen, red; nitrogen, blue; sulfur, yellow). **(B)** Image A rotated 90 degrees about x-axis.

(C) Image A rotated 90 degrees about y-axis. Images prepared using Accelrys Discovery Studio.

Fig. 3.

Docked conformations of E₂ into SULT2A1 in (A) absence of modulator, (B) presence of celecoxib₁, and (C) presence of nimesulide₆. In the presence of celecoxib₁ or nimesulide₆, estradiol preferred to dock with the 17-OH in position for catalysis rather than the 3-OH. Estradiol is shown in blue color, celecoxib and nimesulide are shown in purple color, and PAPS is shown in red color. Binding cavity is depicted as transparent surface. Images prepared using Accelrys Discovery Studio 2.0.

Fig. 4. Binding interactions amongst SULT2A1 and estradiol in the (A) absence of modulator, (B) presence of celecoxib, and (C) presence of nimesulide. Estradiol is shown in blue color with red oxygen and white hydrogen, PAPS and other ligands are colored by element. SULT2A1 residues interacting with estradiol are displayed in wire form with text labels. Images produced with Discovery Studio 2.0; interactions calculated using Ligand Explorer.

Table 1

Grid Points and Center Coordinates for Each Docking Experiment

	Macromolecule	Docked Ligand	Grid Points x/y/z	Center Coordinates x/y/z
I	[SULT2A1/PAPS] _m	estradiol	41/41/41	-14.870/-49.838/17.943
II	[SULT2A1/PAPS] _m	celecoxib	51/51/51	-14.870/-51.931/15.211
III	[SULT2A1/PAPS] _m	nimesulide	51/51/51	-14.870/-51.931/16.801
IV_{I-14}	[SULT2A1/PAPS/celecoxib _{I-14}] _m	estradiol	61/61/61	-15.114/-51.648/17.468
V_{I-7}	[SULT2A1/PAPS/nimesulide _{I-7}] _m	estradiol	51/51/51	-14.870/-51.931/16.801

m indicates minimized complex.

Table 2A

Summary of Estradiol Docking in the Absence of Allosteric Modulators

Docking	E ₂ Affinity ^a kcal/mol	E ₂ K _i μ M	E ₂ binding orientation
I	-7.64 -7.45	2.5 3.1	84% 3-OH 16% 17-OH

^a Gibbs free energy of binding, ΔG_b .

Table 2B

Summary of Estradiol Docking in the Presence of Celecoxib

Docking	Modulator	CEL affinity ^a kcal/mol	CEL K _i μM	E ₂ affinity ^a kcal/mol	E ₂ K _i μM	E ₂ binding orientation
IV ₁	Celecoxib ₁	-6.00	40	-6.49	18	100% 17β-OH
IV ₂	Celecoxib ₂	-5.93	45	-6.80 -6.71	12 10	59% 17β-OH 41% 3-OH
IV ₃	Celecoxib ₃	-5.91	46	-6.90	9	100% nc ^b
IV ₄	Celecoxib ₄	-5.82	54	-5.61	79	100% nc
IV ₅	Celecoxib ₅	-5.79	57	-5.03	207	100% nc
IV ₆	Celecoxib ₆	-5.78	58	-5.24 -4.72	144 350	49% 17β-OH 51% nc
IV ₇	Celecoxib ₇	-5.70	60	-5.22	150	100% nc

^a Gibbs free energy of binding, ΔG_b;^b non-catalytic binding orientations.

Table 2C

Summary of Estradiol Docking in the Presence of Nimesulide

Docking	Modulator	NIM affinity ^a kcal/mol	NIM K _i mM	E ₂ affinity ^a kcal/mol	E ₂ K _i μM	E ₂ binding orientation
V ₁	Nimesulide ₁	-3.50	2.7	-6.33	23	100% nc ^b
V ₂	Nimesulide ₂	-3.38	3.3	-6.13	32	100% nc
V ₃	Nimesulide ₃	-3.24	4.3	-7.03	7	100% nc
V ₄	Nimesulide ₄	-3.19	4.6	-8.35	0.76	99% 3-OH
V ₅	Nimesulide ₅	-3.17	4.8	-4.89	240	100% nc
V ₆	Nimesulide ₆	-3.14	5.0	-6.71	12	100% 17β-OH
V ₇	Nimesulide ₇	-3.04	5.9	-4.91	250	100% nc

^a Gibbs free energy of binding, ΔG_b;

^b non-catalytic binding orientations.