BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, April 2015. Vol. **12**(1), 433-444

In Silico Drug Design on Aspirin for Cyclooxygenase I and II, Target for Reduce the Effects of Inflammatory

Ali Kazemi Babaheydari

[1]Department of Medicinal Plants, Researches Centre of Medicinal Plants & Ethno-veterinary, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

DOI: http://dx.doi.org/10.13005/bbra/1683

(Received: 10 February 2015; accepted: 22 March 2015)

Aspirin is part of a group of medications called nonsteroidal anti-inflammatory drugs (NSAIDs), but differs from most other NSAIDs in the mechanism of action. cyclooxygenases I (COX I) is mainly found in non-inflammatory cells such as cells of the gastric while the cyclooxygenases II (COX II) are found in inflammatory cells and white blood cells. COX I inhibition of coagulation disorders in the gastrointestinal adverse effects. In this paper, we simulated the protein GROMACS force filde, AutoDock (4.2) and Hex (6.1) try to locate the structural change in aspirin. We purpose making changes in conformation of aspirin that have a greater impact on COX II. These study the five new structure aspirin for further investigation. Dynamics analysis and molecular dynamics (MD) simulation were used to simulate protein-ligand complexes for observing the interactions and protein variations. The comparative results demonstrated three software that all the designed compounds have good binding energy when compared with the binding energies of standard structures such as for docking COX II with Aspirin-S1 (-5.59), Aspirin-S2 (-5.28), Aspirin-S3 (-3.26), Aspirin-S4 (-4.45) and Aspirin-S5 (-4.44). Among all the designed compounds, the compound COX II-Aspirin-S1 and S2 indicate more binding energy g hbond was used to analyze hydrogen bonds. The nonbonded interaction energies (Lennard-Jones (LJ) and Coulomb terms) were calculated using g energy.

Key words: AutoDock (4.2), cyclooxygenases I, cyclooxygenases II, Molecular dynamics, GROMACS force fild

Aspirin (acetylsalicylic acid, ASA), the most widely used nonsteroidal anti-inflammatory drug (NSAID) has been prescribed for over 100 years, because of its analgesic, antipyretic and anti-inflammatory properties (Mojtabavi *et al*., 2014).

Aspirin completely inhibited bis-oxygenation of arachidonate by prostaglandin endoperoxide PGH synthase-1; in contrast, aspirin-treated PGH synthase-2 metabolized arachidonate primarily to 16-hydroxyeicosatetraenoic acid (15-HETE) instead of PGH2. IDao values were determined for a panel of common NSAIDs by measuring instantaneous inhibition of cyclooxygenase activity using an oxygen electrode. Among common NSAIDs tested, indomethacin, sulindac sulfide, and piroxicam preferentially inhibited PGH synthase-1; ibuprofen, flurbiprofen, and meclofenamate inhibited both enzymes with comparable potencies; and 6-methoxy-2-naphthylacetic acid preferentially inhibited PGH synthase-2. These results demonstrate that the two PGH synthases are pharmacologically distinct and indicate that it may

* To whom all correspondence should be addressed.
Tel: +98381-3361001
E-mail: kazemiali@iaushk.ac.ir
kazemibali@gmail.com

be possible to develop isozyme-specific cyclooxygenase inhibitors useful both for anti-inflammatory therapy and for delineating between the biological roles of the PGH synthase isozymes (Meade *et al*., 1993).

Pain associated with inflammation involves prostaglandins synthesized from arachidonic acid (AA) through cyclooxygenase-2 (COX-2) pathways while thromboxane A (Meade *et al*., 1993) formed by platelets from AA via cyclooxygenase-1 (COX-1) mediates thrombosis (Rimon *et al*., 2010). COX-1 and COX-2 are both targets of nonselective nonsteroidal antiinflammatory drugs (nsNSAIDs) including aspirin whereas COX-2 activity is preferentially blocked by COX-2 inhibitors called coxibs (Meade *et al*., 1993). COXs are homodimers composed of identical subunits, but we have shown that only one subunit is active at a time during catalysis; moreover, many nsNSAIDS bind to a single subunit of a COX dimer to inhibit the COX activity of the entire dimer. Smith and *et al* based on our experimental studies show that thatcelecoxib and other coxibs bind tightly to a subunit of COX-1. Although celecoxib binding to one monomer of COX-1 does not affect the normal catalytic processing of AA by the second, partner subunit, celecoxib does interfere with the inhibition of COX-1 by aspirin in vitro. X-ray crystallographic results obtained with a celecoxib/COX-1 complex show how celecoxib can bind to one of the two available COX sites of the COX-1 dimer. COX-2 inhibitors such as celecoxib are widely used for pain relief. Because coxibs exhibit cardiovascular side effects, they are often prescribed in combination with low-dose aspirin to prevent thrombosis (Rimon *et al*., 2010).

ASA irreversibly inhibits cyclooxygenase enzyme (COX) or prostaglandin endoperoxide synthase (PGHS) by acetylating a serine residue at position 529 and places a bulky substituent on serine oxygen, which inhibits binding of arachidonic acid (Mojtabavi *et al*., 2014).

ASA also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, ASA is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots (Lewis *et al*., 1983). It has also been established that low doses of ASA may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue (Krumholz *et al*., 1995; Julian *et al*., 1996). ASA may be effective at preventing certain types of cancer, particularly colorectal cancer (Algra AM, Rothwell, 2010; Rothwell *et al*., 2012a; Rothwell *et al*., 2012b).

The widely used theoretical methods include MD method based on molecular mechanics (Huang *et al*., 2012) quantum mechanical method (Wang *et al*., 2012) and other high delity computational model based on rst principles (Ellabaan *et al*., 2012) The method used to study lipid bilayer systems usually is equilibrium MD method using atomistic (Heine *et al*., 2007) or coarse grain model (Yuan *et al*., 2009) This method depends largely on the simulation time and the initial conguration. The simulation time is limited to tens to hundreds of nanoseconds for most biological system, so equilibrium MD cannot be used to explore portions of the energy landscape separated by high barriers from the initial minimum (Hamelberg and McCammon, 2004). To overcome this defect of equilibrium MD, a number of approaches have been introduced: replica exchange, umbrella sampling, accelerated MD (aMD), and so on.

The identification of two isoforms of COX has led to a reevaluation of the mechanism through which nonsteroidal anti-inflammatory drugs (NSAIDs) cause injury to the gastric mucosa. The observation that selective inhibition of cyclooxygenases II (COX II) spares gastric prostaglandin (PG) synthesis and is associated with a greatly reduced incidence of gastric erosions compared with what is observed with conventional NSAIDs (Masferrer *et al*., 1994; Chan *et al*., 1995).

Cyclooxygenases I (COX I) is built in many different cells to create prostaglandins used for basic housekeeping messages throughout the body. The second enzyme is built only in special cells and is used for signaling pain and inflammation. Unfortunately, ASA attacks both. Since that it is the suppression of gastric COX-1 by NSAIDs that is the key mechanism responsible for erosion formation, so that with COX I is targeted,

ASA can lead to unpleasant complications, such as stomach bleeding (Wallace *et al*., 2000).

Fortunately, specific compounds that block just COX II, leaving COX I to perform its essential jobs, are now becoming available. These new drugs are selective pain-killers and fever reducers, without the unpleasant side-effects. In this study, we actually build two different COX for different purposes.

METHODS

Data Sets

In this study, we used bioinformatics from the RCSB PDB, Drug Bank databases. Drug Bank is a unique Bioinformatics/ Cheminformatics fountain that adds detailed drug (i.e., chemical) data comprehensive drug target (i.e., protein).

The docking procedure MD simulation is designed for the automated search of every entry of the protein database for potential protein targets of molecule. For comparison, the dynamic model based on the MD simulations is provided we was used to Isomers and derivatives of aspirin on features pharmacophore models; see Figure 1.

The 3D structures of ligands (Figure 1) were at first built using ArgusLab 4.0.1 molecular builder and then optimized using the GAUSSIAN package 0.3 using B3LYP with 6-31g* basis set (Morris *et al*., 2009). All rotatable bonds were assigned for the ligands, and also partial charges and nonpolar hydrogens were calculated and merged using the above mentioned method for the receptor. The crystal structure of COX I and II was taken from PDB database using PDB ID COX I: 1CQE and ID COX II: 6COX, the missing atoms and loops were cleaned (Cosconati *et al*., 2010), and all residues were protonated under pH 7.4 conditions.

Molecular docking

Docking is the process by which two molecules fit together in three-dimensional space and molecular docking is a useful tool in structural molecular biology and computer-assisted drug design and bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found and brought to the clinical trials and eventually released to the marketplace. This method is widely used to predict the predominant binding mode(s) oligand with a protein. Besides, reliability in docking of ligand molecules to protein or other targets is an important challenge for molecular modeling. One applications of the docking technique includes the prediction of the binding mode of novel drugs. Docking permits the scientist to virtually screen a database of compounds and bode the strongest binders based on variant scoring functions. The docking analyses were carried by Hex (6.1) and AutoDock (4.2) docking software. Hex calculates protein-ligand docking, and it can superpose pairs of molecules using only witting of their 3D shapes. It avails Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the effect. It explores ways in which two molecules, such as compounds (Figure 1) and COX I and II fit together and dock to each other well. The collection of compounds and COX I and II was discovered via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations. The parameters applied for the docking process via Hex (6.1) docking were:

- Correlation type – Shape only
- FFT Mode – 3D fast life
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

AutoDock (4.2), a similar suite of programs involved genetic algorithm, was employed to gain an insight into the compounds bindings with COX I and II (Shukla MK, Leszcynski, 2006; Xue *et al*., 2010; Forli S, Olson, 2012). This program is designed to predict how small molecules, such as drug candidates, bind to a receptor. For the ligand conformational searches the Lamarckian genetic algorithm (LGA) was selected. 3D atomic coordinates of COX I and II were obtained from the Protein Data bank (Morris *et al*., 2009) and prepared for docking. All nonpolar hydrogens were merged and partial atomic charges were assigned using the Gasteiger-Marsili method. Different grid boxes with different grid points in size with a grid-point spacing of 0.375 Å were considered for docking. Each map was centered such that it covered the entire protein including all possible binding sites.

All the AutoDock docking runs were performed in Intel (R) Xeon (R) CPU 5150 @ 2.55 GHz, 2.5GB RAM in Apple system. AutoDock was compiled and run under Windows ubuntu 13.01 operating system. Autodock results were analyzed to study the interactions and the binding energy of the docked structure.

Docking Analysis.

The generated ligands were docked into the defined binding site on the COX I and II protein structure. Ligplot plus was used to analysis docking poses for H-bond and hydrophobic interactions.

MolecularDynamics Simulation

The molecular dynamic simulation was carried out by GROMACS 4.5.4 package (Pronk *et al*., 2013) to simulate the dynamic structure of COX I and II with docked compounds. We utilize Gromacs96 force field for the simulation system (Shukla and Leszcynski, 2006). The distance between the edge of box and protein was set to 1.2 nm. Each protein-ligand system was placed in cubic cell containing water molecular by water model SPC (Simple point charge). Nonbonded interactions include repulsion, dispersion, and Coulomb terms. The repulsion and dispersion terms involve Lennard-Jones interaction (Jones, 1924) and Buckingham potential (Buckingham, 1938); the cut-off distance of define van der Waals (VDW) residues was set to 1.4 nm. Long-range electrostatic forces were performed using the PME method (Darden *et al*., 1993; Essmann *et al*., 1995). The equation of Lennard-Jones interaction is as follows:

$$U(r) = 4å\left[\left(\frac{\delta}{\gamma}\right)^{12} - \left(\frac{\delta}{\gamma}\right)^{6}\right]$$

The Buckingham potential is defined as

$$\cup_{ij}(r_{ij}) = \sum \frac{Z_i Z_j}{4\pi\varepsilon_0 r_{ij}} + \sum A_l \, exp\frac{-r_{ij}}{pl} + \sum c_l r_{ij}^{-nl}$$

Topology files and parameters of small compounds in protein-ligand complexes were generated for GROMACS simulation by SwissParam web server (Zoete *et al*., 2011). Bonds lengths were constrained by the linear constraint solver (LINCS) algorithm. Na+ and Cl" ion were randomly replaced with water molecular to neutralize the simulation systems, and the concentration was set as 0.145M in solvent system. The energy minimization was used to stabilize the solvent system by Steepest Descent algorithm with 5,000 steps, the follow by equilibration performed under position restraints to equilibrated water molecular in the protein for 1 ns under constant

Table 1. Results interaction COX I and II with ASA conformers by Hex (6.1)

Aspirin	ED	RD		EM		RM
COX1	-49.96	R1	63.9	-186.08	R1	8
		R2	48.8		R2	6
COX2	-37.63	R1	64.6	-164.90	R1	1.8
Aspirin-S1ED		RD		EM		RM
COX1	-58.00	R1	64.1	-188.32	R1	7.8
		R2	51.8			
COX2	-60.06	R1	64.9	-171.06	R1	7.8
		R2	51.8			
Aspirin-S2ED		RD		EM		RM
COX1	-65.3	R1	64.2	-192.3	R2	7.8
		R2	51.8			
COX2	-69.37	R1	65.1	-180.11	R2	6.8
		R2	51.8			
Aspirin-S3ED		RD		EM		RM
COX1	-57.35	R1	64.6	-187.53	R	7.8
		R2	51.8			
COX2	-63.94	R1	63.5	-169.71	R	5.8
		R2	48.8			
Aspirin-S4ED		RD		EM		RM
COX1	-59.11	R1	65.0	-189.74	R	7.8
		R2	51.8			
COX2	-68.05	R1	63.0	-168.32	R	6.8
		R2	48.8			
Aspirin-S5ED		RD		EM		RM
COX1	-55.99	R1	65.6	-188.64	R1	7.8
		R2	51.8			
COX2	-68.75	R1	64.0	-177.86	R1	6.8
		R2	48.8			

*ED: Energy Docking
RD: R Docking (Hex results for the docking of COX with aspirin (ASA) derivatives (position R1&R2))
EM: Energy Maching
RM: R Maching (Hex results for the maching of COX with aspirin (ASA) derivatives (position R1&R2))

temperature dynamics (NVT type) conditions. In final step, production running for 5000 ps under constant pressure and temperature dynamics (NPT type); all of the temperature simulation system was under 310K condition. MD conformations are sampled every 20 ps and all frames are analyzed under GROMACS 4.5.4.

RESULTS AND DISCUSSION

Hex Results

We used the Hex (6.1) score to select potent ASA compounds which have high affinity with COX I and II. The results of the docking score are listed in Table 1. Prediction of interaction

Table 2. Results interaction Cyclooxgenase I with ASA conformers by Auto dock.

parameters	Aspirin	S1	S2	S3	S4	S5
Cluster RMSD	0.00	0.00	0.00	0.00	0.00	0.00
Reference RMSD	200.62	200.81	200.815	187.93	191.77	219.59
Eb (K.Cal/mol)	-5.68	-5.05	-4.68	-3.12	-4.13	-3.97
Intermolecular energy	-6.58	-5.94	-5.94	-6.40	-6.22	-6.65
Internal energy	+0.23	-0.12	-0.12	+0.33	-1.30	-0.29
Unbound energy	0.21	-0.12	-0.12	+0.33	-1.3	-0.29
Ki	-68.17	+199.59	+199.59	5.18	932.75	1.24
Hydrogen Bond	Arg374	Arg374(A)	Lys532(B)	His446(A)	His207(B)	———————-
		Arg376(B)	Gln372(B)	Asp450(A)		
hydrophobic	Asn375	Asn375(A)	Ser126(B)	Lys453(A)	Leu294(B)	Lys211(A)
interaction	Phe149	Phe142(B)	Phe371(B)	Pro218(A)	Val291(B)	Gln289(A)
	Arg376	Gly225(A)	Asn122(B)	Glu454(A)	Lys211(B)	Glu290(A)
		Leu2249(A)	Gln370(B)		Thr212(B)	His207(A)
			Tyr373(A)			Thr212(A)
						Val291(A)

Table 3. Results interaction Cyclooxgenase II with ASA conformers by Auto dock.

Parameters	Aspirin	S1	S2	S3	S4	S5
Cluster RMSD	0.00	0.00	0.00	0.00	0.00	0.00
Reference RMSD	69.31	68.57	69.15	84.52	69.70	37.77
Eb (K.Cal/mol)	-5.53	-5.59	-5.28	-3.26	-4.45	-4.44
Intermolecular energy	-6.42	-6.49	-6.17	-6.54	-5.94	-7.13
Internal energy	-0.06	-0.16	+0.00	-1.18	-0.14	-0.78
Unbound energy	-0.06	-0.16	+0.00	-1.18	-0.14	-0.78
Ki	88.90	79.56	135.75	4.08	546.74	552.84
Hydrogen Bond	Lys532	Lys532	Lys532	Glu465(B)	Lys532	Cys41(A)
	Gln372	Gln372	Gln372	Asn39(B)	Gln372	Asn39(A)
hydrophobic interaction	Gln370	Phe371	Gln370	Leu152(B)	Ser121	Pro40(A)
	Ser121	Gln370	Ser121	Cys41(B)	Gln370	Glu465(A)
	Phe371	Tyr373	Tyr122	Cys36(B)	Phe371	Leu152(A)
			Phe371	Tyr130(B)		Gly45(A)
				Cys47(B)		Pro153
				Glu46(B)		Glu46(A)
				Gly45(B)		Cys47(A)
						Gly135(A)

energies between ligand and receptor has been a major challenge for molecular docking. Hex uses scoring algorithms to calculate these energy values of the docked complexes and stability of the docked complexes increases with decrease in energy value. While comparing the results obtained with all the six compounds ligand it is prominent that all of them show better stability when docked with COX I and II receptor.

AutoDock Results

It was noted that the reaction of ASA with nucleophilic reagents occurs via a direct nucleophilic attack on the phenolic ester carbonyl carbon atom (Scheme 1) instead of via an anhydride intermediate. The same mechanism appears to be straightforward for the ASA–COX reaction as well. Molecular modeling using the SCC-DFTB method in QM/MM dynamics supports this assumption (Toth *et al*., 2013). Derivatization of the carboxylate moiety in moderately selective COX-1 inhibitors, such as 5, 8, 11, 14-eicosatetraynoic acid (ETYA) and arylacetic and fenamic acid NSAIDs, exemplified by indomethacin and meclofenamic acid, respectively, generated potent and selective COX-2 inhibitors. In the indomethacin series, esters and primary and secondary amides are superior to tertiary amides as selective inhibitors. Only the amide derivatives of ETYA and meclofenamic acid inhibit COX-2; the esters are either inactive or nonselective. Inhibition kinetics reveal that indomethacin amides behave as slow, tight-binding inhibitors of COX-2 and that selectivity is a function of the time-dependent step. Site-directed mutagenesis of murine COX-2 indicates that the molecular basis for selectivity differs from the parent NSAIDs and from diarylheterocycles (Kalgutkar *et al*., 2000). COX I and II, the Macromolecule and the ligands (six compounds) were subjected to docking analysis using Autodock (4.2). Molecular docking simulations were conducted with this software suite. 20 docking runs were performed. Grid parameters were set as mentioned earlier and spacing between grid points was 0.375 Å. After the simulations were complete, the docked structures were analyzed and the interactions were seen. Hydrogen bond interactions and the binding distance between the donors and acceptors were measured for the best conformers. Distinct conformational clusters were formed at an RMSD-tolerance of 2.0 Å. Van der waals scaling factor was found to be 1.0 Å. To predict the appropriate interaction of six compounds with COX I and II, the ligand molecules were docked with the target protein using Autodock. The analysis command in the docking parameter file in this software causes AutoDock to

Table 4. Results of Total energy by Docking COX I with ASA and structures its.

COX I	Err. Est	Average	RMSD	Tot-Drift (kj/mol)
aspirin	-1.40158e+07	200	5033.17	-788.96
S1	-1.40157e+07	230	4837.53	-798.75
S2	-1.41862e+07	430	4993.47	-1597
S3	-1.42051e+07	170	5060.79	-1043
S4	-1.42006e+06	110	1590.08	-655.17
S5	-1.39774e+07	390	5209.04	-2787.99

Table 5. Results of Total energy by Docking COX II with ASA and structures its.

COX II	Err. Est	Average	RMSD	Tot-Drift(kj/mol)
aspirin	-1.6906e+06	230	1814.89	-1505.07
S1	-1.69064e+06	290	1868.79	-1793.06
S2	-1.6949e+06	260	1836.81	-1523.39
S3	-1.6976e+06	240	1839.13	-1496.06
S4	-1.67242e	200	1795.72	-1233.64
S5	-1.0436e	220	4277.91	-1646.87

perform a cluster analysis of the different docked conformations and find the minimum energy in each run. Every docking contains some useful knowledge which includes Binding Energy (Eb) which is the sum of the Intermolecular Energy, the Torsional Energy, and the Internal Energy which are reported in table 2 and 3. kI is the dissociation constant for a ligand with this Binding Energy, Cluster RMS is the root mean square difference in coordinates between this conformation and the cluster reference. In all of our cases it was found zero, because the selected conformations are the cluster reference. Reference RMS is the rms difference between this structure and the input structure.

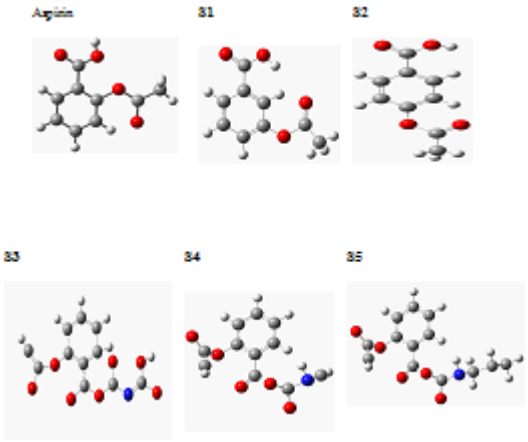

Fig. 1. Chemical scaffold of S1, S2, S3, S4 and S5.

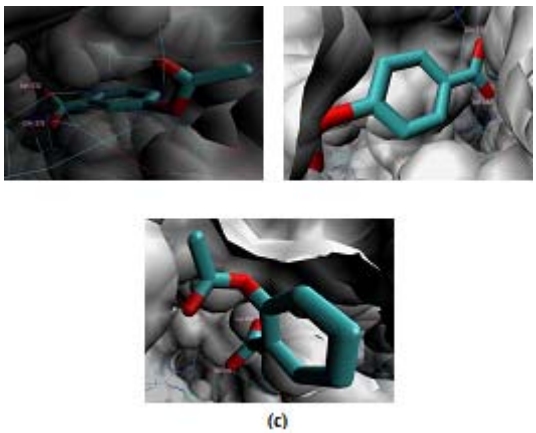

Fig. 3. Docking poses of top three candidates COX II: (a) S1, (b) S2, and (c) ASA. The small molecular and amino acids are colored in green and yellow, respectively.

The study and compare of all dockings indicates that ASA, S1 and S2 ligands with the receptor protein, COX I and COX II, gave the best docking results. According to in table 2 and 3.

Scaffold of the top six compounds is shown in Figure 1, and the docking poses of each ligand are displayed in Figure 2 and 3.

For H-bond analysis, compounds docked ligand formed with any residue of COX I and COX II that results are shown in Table 2 and 3. In the 2D diagram of docking poses by Ligplot for compounds with COX I and COX II (Figure 3 and 4).

Adinarayana and *et al*, four selective COX-2 (Valdecoxib, Celecoxib, Rofecoxib and Etoricoxib) inhibitors chose for study to correlate the associated non-bonded interactions with receptor and the binding energy and of all the selective COX-2 inhibitors and Celecoxib analogs studied, that show one of analog relatively high dock score of about -172.267 kcal/mol while the score for Celecoxib is -140.018 kcal/mol (Adinarayana *et al*., 2012). The efficiency and selectivity properties of ASA are somewhat astonishing because no neighboring groups such as glutamate and histidine are present in the vicinity of Ser529 to increase its nucleophilicity and reactivity. Since, the reaction of other acetylating agents with COX-1 does not lead to acetylation of Ser529, it has been suggested that the cause of

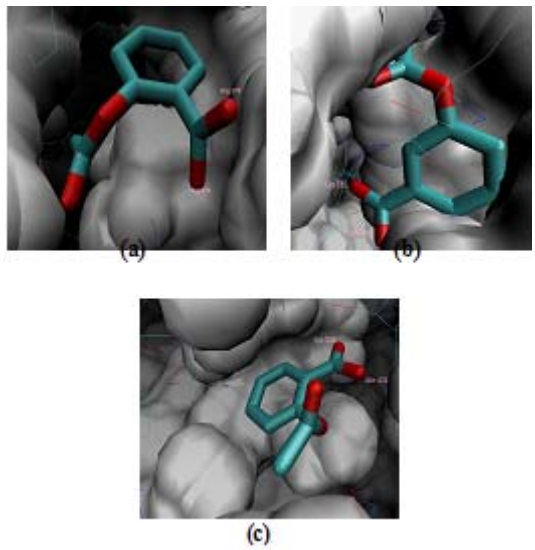

Fig. 2. Docking poses of top three candidates COX I: (a) S1, (b) S2, and (c) ASA. The small molecular and amino acids are colored in green and yellow, respectively.

this selective acetylation was due to salicylic acid moiety. The only residue with positive charge in the active site is Arg119, which is located 9.7 Å below the hydroxyl group of Ser529. Consequently, this resulted in a well-positioned situation to arrange the binding of salicylic acid moiety near Ser529 (Mojtabavi Naeini *et al*., 2014). Loll *et al*. illustrated that the hydroxyl group of Tyr384 and carbonyl oxygen of the bromoacetyl group in bromoacetylaspirin-COX-1complex were within H-bonding distance. This could suggest stabilizing the negative charge on the putative tetrahedrial intermediate during acetylation process, and therefore, increasing the reactivity of the acetyl

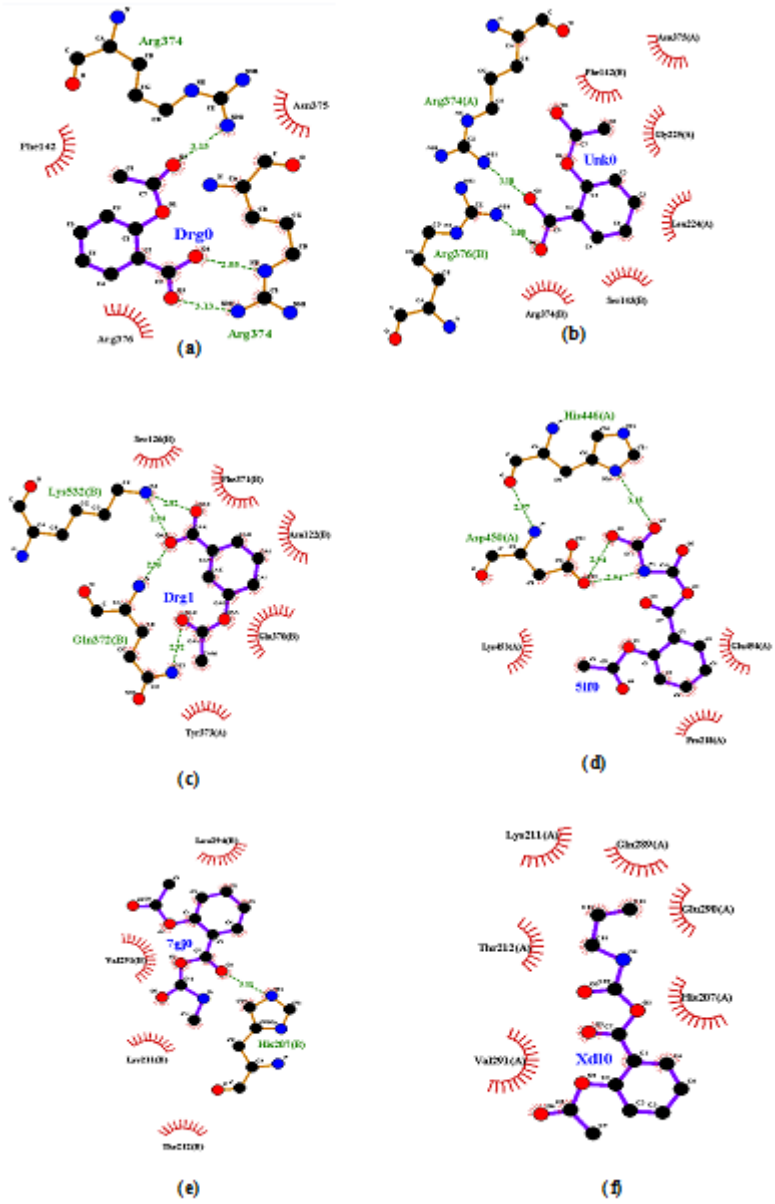

Fig. 4. A. Ligplot analyses results. 2D representation of ligand–protein interactions were analyzed between COX I with ASA: (a) ASA, (b) compound S1, (c) compound S2, (d) compound S3 (d) compound S4 (e), compound S5 (f). Hydrogen bond forming residues were shown in lines with hydrogen bonds shown as dotted lines and residues interacting by hydrophobic interactions were represented as lines in red.

group of ASA near the site of Ser529 would increase (Loll *et al.*, 1995). Experimental site-directedmutagenesis of Arg119 and Tyr384 (the reduced potency on R119Q, loss of function on R119A as well as loss of activity on Y384F mutants) suggested a critical role for these two residues (Hochgesang *et al*., 2000).

Stability Analysis

Complexes of with docked COX I and II ligands were performed by MD simulation at 5000 ps, and COX I and II with no ligand (COX I & II protein) were regarded as the control for comparison. Each plot of the root mean square deviation (RMSD), mean square displacement (MSD). The COX I & II protein of COX II changed significantly from 4.5 to 7.5 nm after 5000 ps, indicating that COX II with ligand was more stable during MD simulation. The MSD analysis was used to calculate the migration distance among all simulation times. The MSD values for COX II

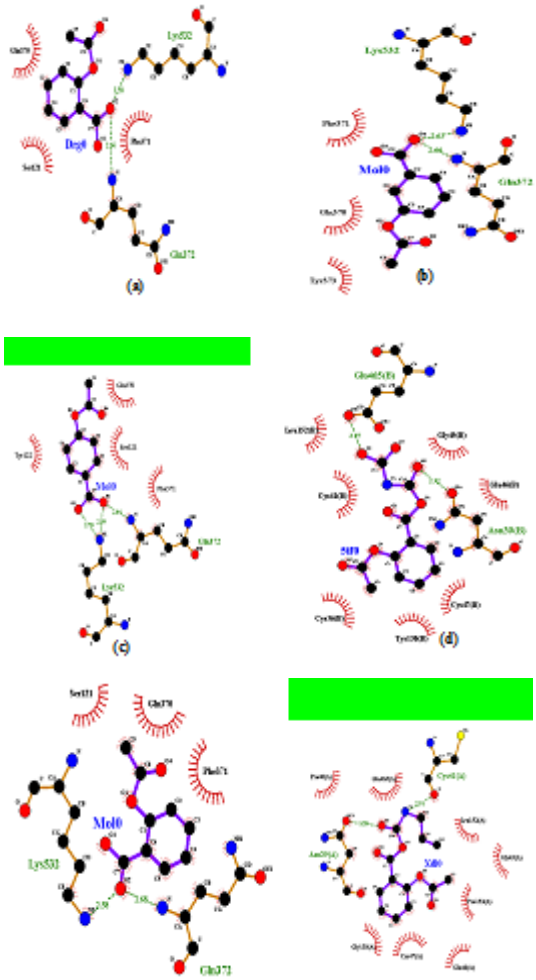

Fig. 4. Ligplot analyses results. 2D representation of ligand–protein interactions were analyzed between COX II with ASA: (a) ASA, (b) compound S1, (c) compound S2, (d) compound S3 (d) compound S4 (e), compound S5 (f). Hydrogen bond forming residues were shown in lines with hydrogen bonds shown as dotted lines and residues interacting by hydrophobic interactions were represented as lines in red

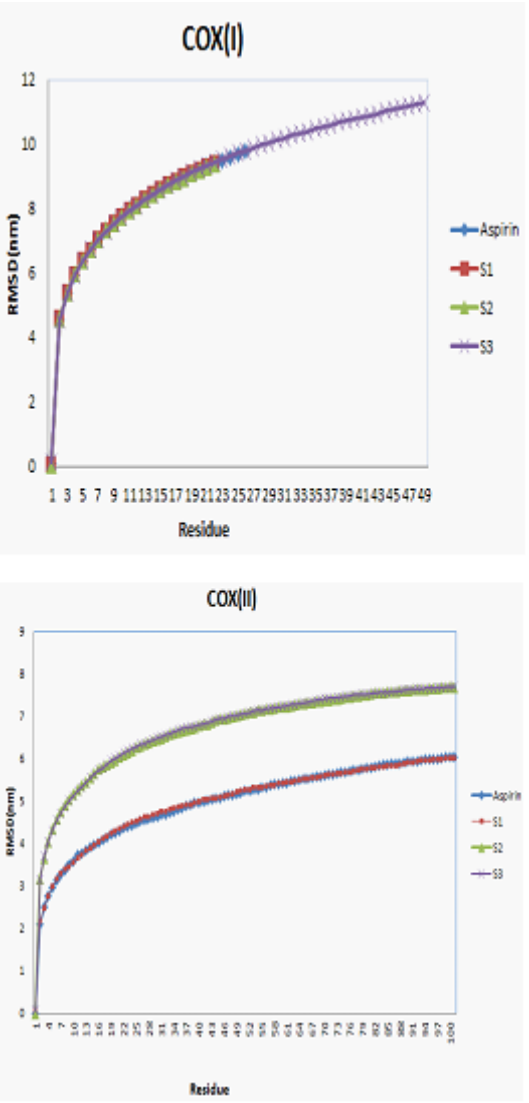

Fig 5. RMSD values of COX I and II with docked ligand with simulation times of 5000 ps; the no-ligand binding protein (d) was used as the control.

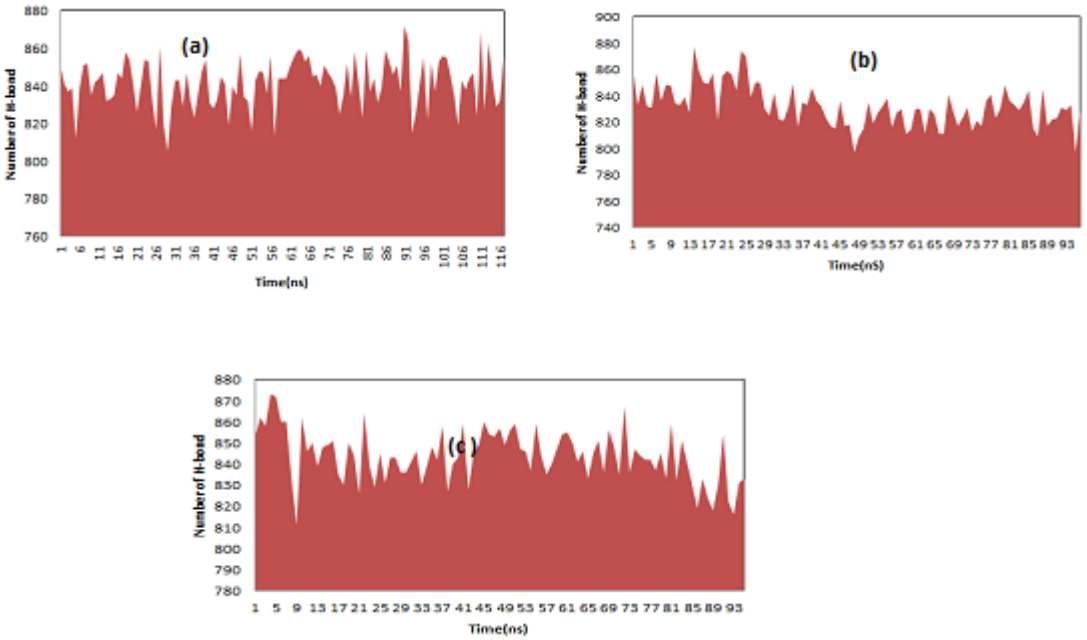

Fig 6. Hydrogen bonds formed between ASA (ASA and structures) and ASA (a), s1 (b), s2 (c) from accelerated simulations COX I.

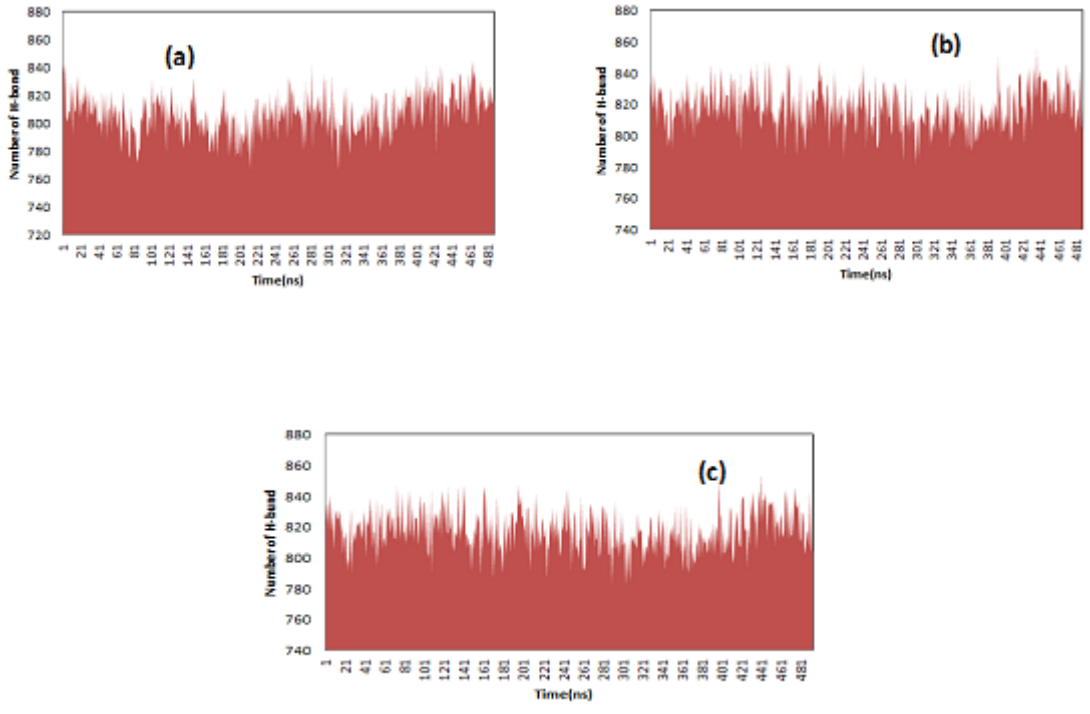

Fig 7. Hydrogen bonds formed between ASA (ASA and structures) and ASA (a), s1 (b), s2 (c) from accelerated simulations COX II.

increased from 19 to 100 ps, which displayed a similar movement distance to S3 at final simulation time. S2 had an increased RMSD value with a simulation time of 5000 ps. For total energy analysis significantly increased values were observed among all simulation times (Table 4 and 5).

Hydrogen bonds

As COX I and II have one hydrogen bond donor (OH) and one hydrogen bond acceptor (O), the probability to form hydrogen bonds is rare. Number of H-bonds formed between COX I and II and asprin, S1, S2 and S3 molecules during aMD simulation was calculated. Of all these three cases, the number of H-bonds fluctuates between 0 and 890 (Figure 6 and 7).

CONCLUSIONS

The Protein–Ligand interaction plays a main role in structural based drug charting. In the present work we have taken change structure ASA for effective further on COX II for novel drug design. The receptor (COX I and II) was docked with ASA, ASA-S1, ASA-S2, ASA-S3, ASA-S4 and ASA-S5 and the energy values obtained. After MD simulation, the top two isomers of aspirin remain as the same docking poses under dynamic conditions. Hence, we propose the aspirin isomers, as potential candidates for repression COX 2 is to reduce the side effects of aspirin that required to clinical research.

ACKNOWLEDGMENTS

This study was supported by Azad University, Shahrekord Branch, and the Young Research Club of Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

REFERENCES

1. Adinarayana KPS, Ashoka Reddy P, Ajay Babu P. Structural Studies on Docking Selective COX-2 Inhibitors. *J Bioinformatics Res.* 2012; **1** (1): 21-26.
2. **Algra AM, Rothwell PM. E**ffects of regular aspirin on long-term cancer incidence and metastasis: A systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol*. 2012; **13** (5): 518–527.
3. **Buckingham** RA. The classical equation of state of gaseous helium, neon and argon, Proceedings of the Royal Society of London Series A. *Math Phys*. 1938; **168** (993): 264–283.
4. Chan CC, Boyce S, Brideau C, Ford-Hutchinson AW, Gordon R, Guay D, Hill RG, Li CS, Mancini J, Penneton M, *et al*. Pharmacology of a selective cyclooxygenase-2 inhibitor, L-745,337: a novel nonsteroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and nonhuman primate stomach. *J Pharmacol Exp Ther*. 1995; **274**: 1531–1537.
5. Cosconati S, Forli S, Perryman AL, Harris R, Goodsell DS, Olson AJ. Virtual Screening with AutoDock: Theory and Practice. *Expert Opin Drug Discov*. 2010; **5**(6): 597-607. doi:10.1517/17460441.2010.484460.
6. Darden T, York D, Pedersen L. Particle mesh Ewald: an NÅ"log(N) method for Ewald sums in large systems, *J Chem Phys*. 1993; **98** (12): 10089–10092.
7. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle meshes Ewald method. *J Chem Phys*. 1995; **103** (19): 8577–8593.
8. Ellabaan MMH, Ong YS, Nguyen QC, Kuo JL. Evolutionary discovery of transition states in water clusters. *J Theor Comput Chem*. 2012; **11**: 965.
9. Forli S, Olson AJ. A force field with discrete displacement waters and desolvations entropy for hydrate ligand docking. *Med Chem*. 2012; **55**: 623-638.
10. Hamelberg D, McCammon JA. Accelerated molecular dynamics: A promising and e±- cient simulation method for biomolecules. *J Chem Phys*. 2004; **120**:11919-11929.
11. Heine DR, Rammohan AR, Balakrshnan J. Atomistic simulation of the interaction between lipid bilayers and substrates. *Mol Simulat*. 2007; **33**:391.
12. Hochgesang GP, Rowlinson SW, Marnett LJ. Tyrosine-385 is critical for acetylation of cyclooxygenase-2 by aspirin. *J Am Chem Soc*. 2000; **122**: 6514–6515.
13. Huang DW, Li XH, Xiu ZL. Molecular modeling of the interactions between histone deacetylase 8 and inhibitors. *J Theor Comput Chem*. 2012; **11**: 907.
14. Jones JE. On the determination of molecular fields. II. From the equation of state of a gas. *Proc R Soc B*. 1924; **106** (738): 463–477.
15. Julian DG, Chamberlain DA, Pocock SJ. A comparison of aspirin and anticoagulation following thrombolysis for myocardial infarction (the AFTER study): a multicentre unblinded randomised clinical trial. *BMJ*. 1996; **313** (70):

1429–1431.

16. Kalgutkar, AS, Crews BC, Rowlinson SW, Marnett AB, Kozak KR, Remmel RP, Marnett LJ. Biochemically based design of cyclooxygenase-2 (COX-2) inhibitors: Facile conversion of nonsteroidal antiinflammatory drugs to potent and highly selective COX-2 inhibitors. *Proc Natl Acad Sci USA*. 2000; **97**(2): 925–930.
17. Krumholz HM, Radford MJ, Ellerbeck EF, Hennen J, Meehan TP, Petrillo M, Wang Y, Kresowik TF, Jencks SF. Aspirin in the treatment of acute myocardial infarction in elderly Medicare beneficiaries. Patterns of use and outcomes. *Circulation*. 1995; **92** (10): 2841–2847.
18. Lewis HD, Davis JW, Archibald DG, Steinke WE, Smitherman TC, Doherty Je JE, Schnaper HW, Lewinter MM, Linares E, Pouget JM, Sabharwal SC, Chesler E, Demots H. Protective Effects of Aspirin against Acute Myocardial Infarction and Death in Men with Unstable Angina. *N Engl J Med*. 1983; **309** (7): 396–403.
19. Loll PJ, Picot D, Garavito RM. The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. *Nat Struct Mol Biol*. 1995L **2**: 637–643.
20. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. *Proc Natl Acad Sci USA*. 1994; **91**: 3228–3232.
21. Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J Biol Chem*. 1993; **268** (9): 6610-6614.
22. Mojtabavi NM, Mesrian TH, Emamzadeh R, Vallian S. A computational prospect to aspirin side effects: Aspirin and COX-1interaction analysis based on non synonymous SNPs. *Comput Biol Chem.*. 2014; **51**: 57-62.
23. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 2009; **30**: 2785-2791.
24. Pronk S, Páll S, Schulz R, Larsson P, Bjelkmar P, Apostolov R, Shirts MR, Smith JC, Kasson PM, van der Spoel D, Hess B, Lindahl E. GROMACS 4.5: a highthroughput and highly parallel open source molecular simulation toolkit, *Bioinformatics*. 2013; **29** (7): 845–854.
25. Rimon G, Sidhu RS, Lauver DA, Lee JY, Sharma NP, Yuan C, Frieler RA, Trievel RC, Lucchesi BR, Smith WL. Coxibs interfere with the action of aspirin by binding tightly to one monomer of cyclooxygenase-1. *Proc Natl Acad Sci USA*. 2010; **107** (1): 28-33.
26. Rothwell PM, Price JF, Fowkes FGR, Zanchetti A, Roncaglioni MC., Tognoni G, Lee R, (...), Meade TW. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. *The Lancet*. 2012a; **379** (9826): 1602- 1612.
27. Rothwell PM, Wilson M, Price JF, Belch JFF, Meade TW, Mehta Z, Effect of daily aspirin on risk of cancer metastasis: A study of incident cancers during randomised controlled trials. *The Lancet*. 2012b; **379** (9826): 1591-1601.
28. Shukla MK, Leszcynski J. *Chem Phys Lett*. 2006; **48**: 317.
29. Toth L, Muszbek L, Komaromi I. Mechanism of the irreversible inhibition of human cyclooxygenase-1by aspirin as predicted by QM/MM calculations. *J Mol Graphics Modell*. 2013; **40**: 99–109.
30. Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology*. 2000; **119** (3):706-714.
31. Wang CH, Zhang RQ, Lin ZJ. A comparative study on intermolecular hydrogen bond interactions in molecular dimmers using di®erent levels of computational method. *J Theor Comput Chem*. 2012; **11**: 1237.
32. Xue B, Dunbrack RL, Williams RW, Dunker AK, Uversky VN. PONDR-FIT: a meta-predictor of intrinsically disordered amino acids. *Biochim Biophys Acta*. 2010; **1804** (4): 996–1010.
33. Yuan H, Jameson CJ, Murad S. Exploring gas permeability of lipid membranes using coarse-grained molecular dynamics. *Mol Simulat*. 2009; **35**: 953.
34. Zoete V, Cuendet MA, Grosdidier A, Michielin O. SwissParam: a fast force field generation tool for small organic molecules. *J Comp Chem*. 2011; **32** (11): 2359–2368.