

Docking studies on butenolide derivatives as Cox-II inhibitors

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Abstract To supplement hits from a high through put screening, a docking study on butenolides derivatives was performed as COX-II inhibitors. The fourteen ligands were docked inside the ligand-binding domain (LBD) of protein data bank PDB ID: 3HS5 utilizing Maestro version 9. Out of 14 compounds, compounds I and XII were found to embed in the hydrophobic pocket by forming hydrogen bonds with the amino acids HOH902, THR212, and THR206. Nitrogen of the pyrrolone ring of compound XII formed a strong hydrogen bond with THR206 with the distance of 2.221 Å and had showed highest glide score (−9.3) and lowest energy −95.66 kJ/mol. Glide score of Diclofenac and Celecoxib was found to be −10.33 and −11.37, respectively. Some other docked compounds also showed good glide scores comparable to standard anti-inflammatory drug Diclofenac, were III, V, VI, VII, IX, and XIV. Docking results were further validated by calculation of conformational energy, which was higher in case of Diclofenac and Celecoxib, i.e., −33.57 and −43.7 kJ/mol, respectively, in comparison to hypothetically designed compounds. The compounds that had highest glide score, lowest conformational energy are generally considered

better and can be used for further drug designing and synthesize in laboratory. The most potent compound was XII having highest glide score and lowest conformational energy.

Keywords Butenolides · Anti-inflammatory · Docking studies · COX-II

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of rheumatoid arthritis and inflammatory diseases. However, long-term use of NSAIDs has been associated with gastrointestinal bleeding and nephrotoxicity (Omar *et al.*, 1996). Anti-inflammatory activity of non-steroidal anti-inflammatory drugs (NSAIDs) is mediated by inhibition of Cyclo-oxygenases, which results in decrease production of prostanoids, i.e. prostaglandins, prostacyclins, and thromboxane. This mechanism is believed to account for both therapeutic as well as adverse effects of NSAIDs. Two forms of COXs have been identified—COX-I and COX-II. While COX-I is expressed in most of body tissues, COX-II is present with low or undetectable levels in some tissues (Sandhu, 2003). The beneficial anti-inflammatory and analgesic activities are based on the inhibition of COX-II, but the gastro-intestinal toxicity and other side effects are due to the concurrent inhibition of COX-I (Gupta, 1999). Agent which inhibits COX-II, while sparing COX-I represents a new therapeutics goal for development of safer NSAIDs (Hawkey, 1999; Khannaa *et al.*, 1997; Boehm and Smietana, 1996; Li *et al.*, 1995; Manivannan *et al.*, 2004). Pharmacological inhibition of COX can provide relief from pain and inflammation (en.wikipedia.org/wiki/cyclooxygenase). Pharmacological inhibition of PGs

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not only play a central role in inflammation, but also regulate other critical physiological responses, i.e. blood clotting, ovulation initiation of labour pain, nerve growth, and development (Singh *et al.*, 2010) (Fig. 1).

Methods for predicting activity modes of organic molecules to protein receptors are widely used within drug discovery efforts. Ligand docking is typically achieved by generating a number of orientations (or poses) of a ligand within the active site, scoring of poses and identification of one or more poses that closely approximate the bioactive conformation. The bioactive conformations are determined by X-ray crystallography (Poulsen *et al.*, 2008). Docking algorithms are also used for identifying putative binders from virtual chemical databases and for estimating the

binding affinity of protein–ligand complexes (Prasanna *et al.*, 2009).

Butenolides, also known as furanones and pyrrolones, are reported to have anti-inflammatory, analgesic, antiviral, and many other medicinal properties (Husain *et al.*, 2005, Khan *et al.*, 2002). Using this background information and our current interest in the ligand–protein binding, we attempted *in-silico* study of various hypothetical butenolides as COX-II inhibitors, by computational docking method. The compounds which were found to have high glide score and low energy were synthesized and evaluated for anti-inflammatory and analgesic activities. However, only docking studies are presented in this research paper (Figs. 2, 3).

Fig. 1 Binding mode of compound I into COX-II

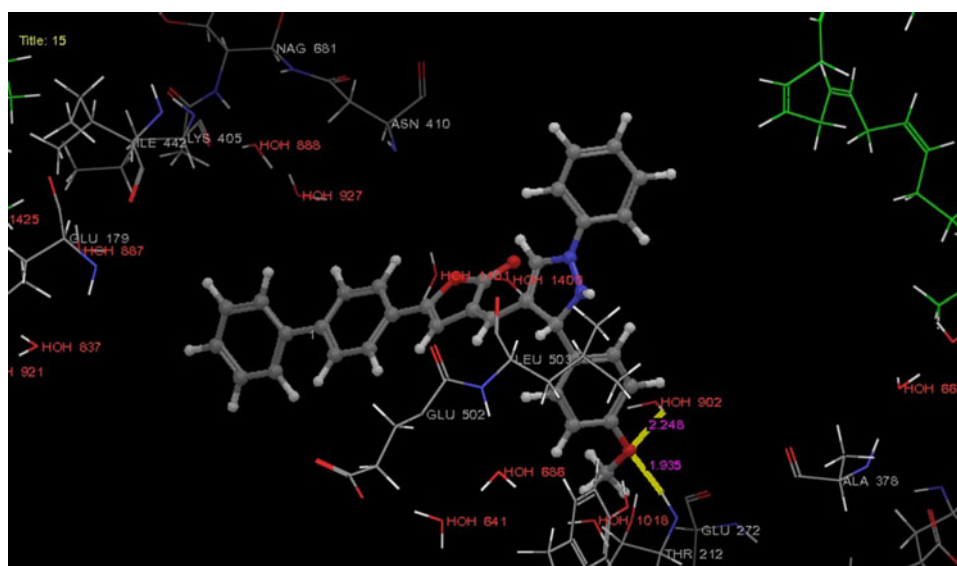


Fig. 2 Binding mode of compound II into COX-II

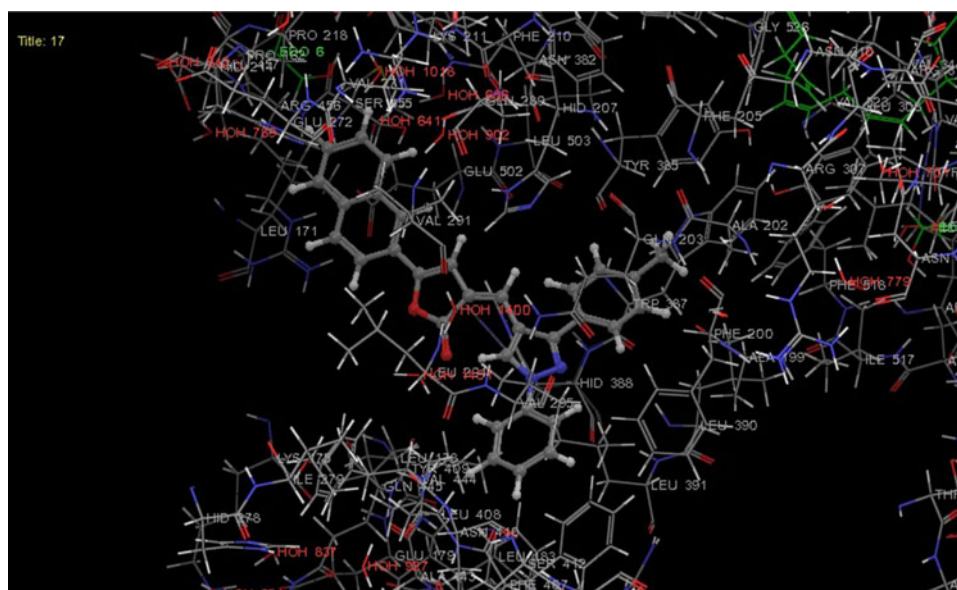
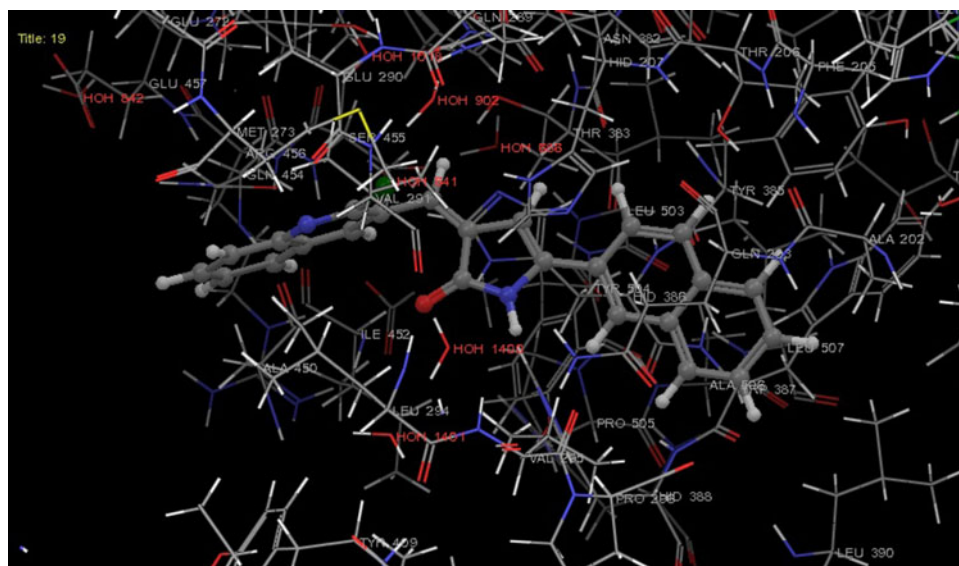


Fig. 3 Binding mode of compound V into COX-II



Materials and methods

Docking is a computational method to determine possible binding modes of a ligand to the active site of a receptor. Docking studies has been performed with a set of hypothetical butenolide derivatives using Maestro 9.0 on COX-II (PDB ID 3HS5). The X-ray structures of which were accessed from the protein data bank (PDB). The basic structure of analogues is shown in Table 1. The COX-II (PDB ID 3HS5) X-ray structures were accessed from the protein data bank (PDB) (Fig. 4).

Ligand preparation

The molecules were built using Maestro 9.0 and converted to 3D structure from the 2D using Lig Prep version 5.5 (Maestro version 9). Lig Prep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with the 2D structures in SD or Maestro format. The resulting structures were saved in Maestro format. The simplest use of Lig Prep produces a single, low energy, 3D structure with correct chiralities for each successfully proposed input structure. While performing this step, chiralities were determined from 3D structure and original states of ionization were retained. Tautomers were generated using Macromodel method discarding current conformers (Fig. 5).

The conformational space was searched using the Monte Carlo (MCM) method as implemented in Macromodel version 9.6 (Halgren, 1999). Each search was continued until the global energy minima were carried out using the truncated Newton Conjugate Gradient (TNCG) and the

MMFFs force field as implemented in Macromodel. The conformational searches were done for aqueous solution using the generalized born/solvent accessible surface (GB/SA) continuum solvation (Hasel *et al.*, 1988; Still *et al.*, 1990) (Fig. 6).

Protein preparation

The COX-II (PDB ID 3HS5) X-ray structures were accessed from the protein data bank (PDB). The protein structure with polar hydrogen was prepared using the protein preparation wizard in Maestro (Maestro version, 2010). In this step, bond orders were assigned, all hydrogen's in the structure were added, and bonds to metals were deleted and adjust the formal charge on the metal and the neighboring atoms and deleting waters that were more than the 5 Å specific distance. With generated Het states options, prediction of ionization, and tautomeric states of the het group at pH 7 was achieved. In protein preparation, optimization of hydrogen bond network was carried out by reorienting hydroxyl group, water molecules, and amino acids. The final step in the preparation process was to refine the structure, with the help of restrained minimization. It was initiated in the imperfect minimization with the 0.3 Å RMSD for the minimization OPLS_2001 force field (Figs. 7, 8).

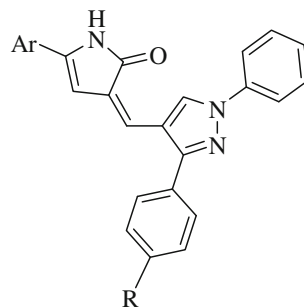
Docking

Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor were represented on a grid by several different sets of field that

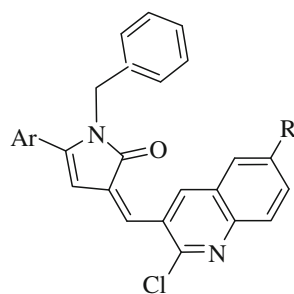
Table 1 Chemical structures of hypothetical compounds used for docking studies

Compound no.	Ar	R
I		OCH ₃
II		H
III		CH ₃
IV		NO ₂
Compound no.	Ar	R
V		H
VI		Cl
VII		CH ₃
VIII		NO ₂
IX		NO ₂

Table 1 continued



Compound no.	Ar	R
X		NO ₂
XI		OCH ₃



Compound no.	Ar	R
XII		H
XIII		CH ₃

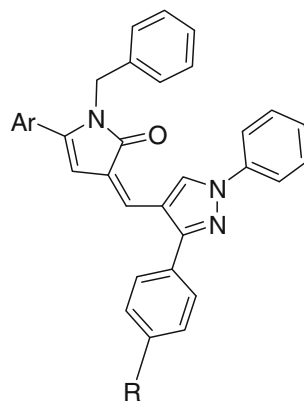


Table 1 continued

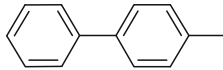
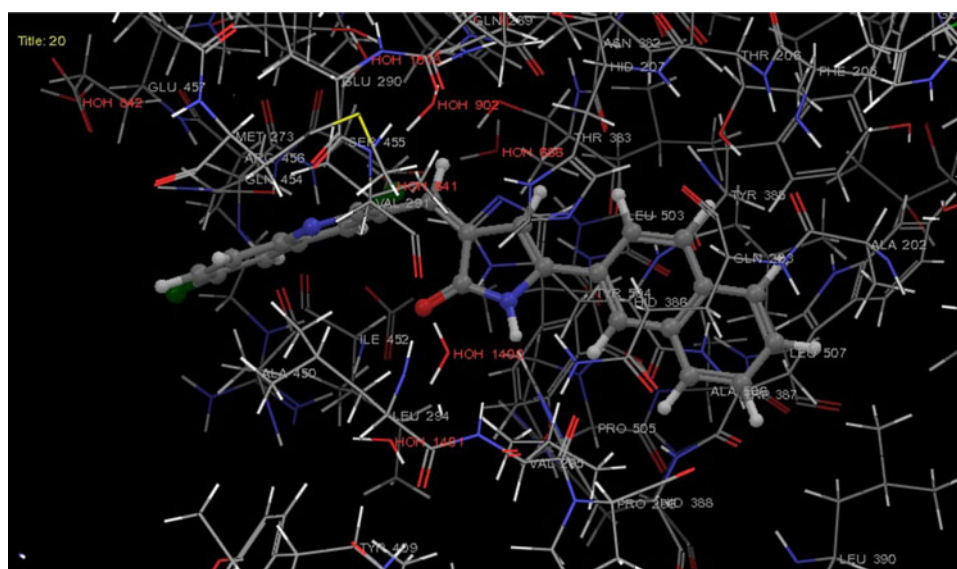
Compound no.	Ar	R
XIV		OCH ₃

Fig. 4 Binding mode of compound VI into COX-II

provide progressively more accurate scoring of the ligand poses. For receptors that adopted more than one conformation on binding, grids were prepared for each conformation, to ensure that possible activities were not missed. Ligand molecule was picked so it could be excluded from the grid generation with Vander waals radius scaling 1.00 and partial charge cutoff of 0.25. The compounds were docked using Glide with standard settings in standard precision (SP) mode. Grids were generated using Glide version 5.0 following the standard procedure recommended by Schrodinger. The docked poses discussed in it were not necessarily the highest scoring, but were selected as the highest scoring pose with a reasonable conformation and binding mode as judged by the modeler (Fig. 9).

Calculation of the conformational energy

The docked conformation was minimized with Macro Model using default restraining force constant of 100 kJ/mol. This allowed the docked conformations to relax (adjust) to the MMFFs force field. Without the relaxation the energy calculated by MMFFs would be meaningless. This relaxation did not change the conformation as RMS between the docked and relaxed structure are <0.1 Å.

Results and discussion

Most important features of docking are the logical interaction of the ligand with the putative-binding site of the enzyme. The molecules built using Maestro 9.0 were converted to 3D structure using Lig Prep version 5.5 (Maestro version 9). Using Lig Prep, a single, low energy, 3D structure with correct chiralities for each successfully proposed input structure was designed. Tautomers were generated using Macro Model method, discarding current conformers, and conformational space. Each search was continued until the global energy minima were carried out. The protein structure with polar hydrogen was prepared and optimization of hydrogen bond network was carried out. The favorable interactions between one or more ligand molecules and a receptor molecule was carried out by preparing grids where ever required, to ensure that possible activities were not missed. The process of evaluating a particular pose was achieved by counting the no. of favorable interactions that might be hydrogen, hydrophobic or electrostatic bonding. Out of fourteen compounds included in this study, two compounds (I and XII) were found to have best results which showed hydrogen bond interaction with amino acids of COX-II protein. The compounds (XIV, IX, VII, VI, V, and III) did not show any hydrogen bond

Fig. 5 Binding mode of compound VII into COX-II

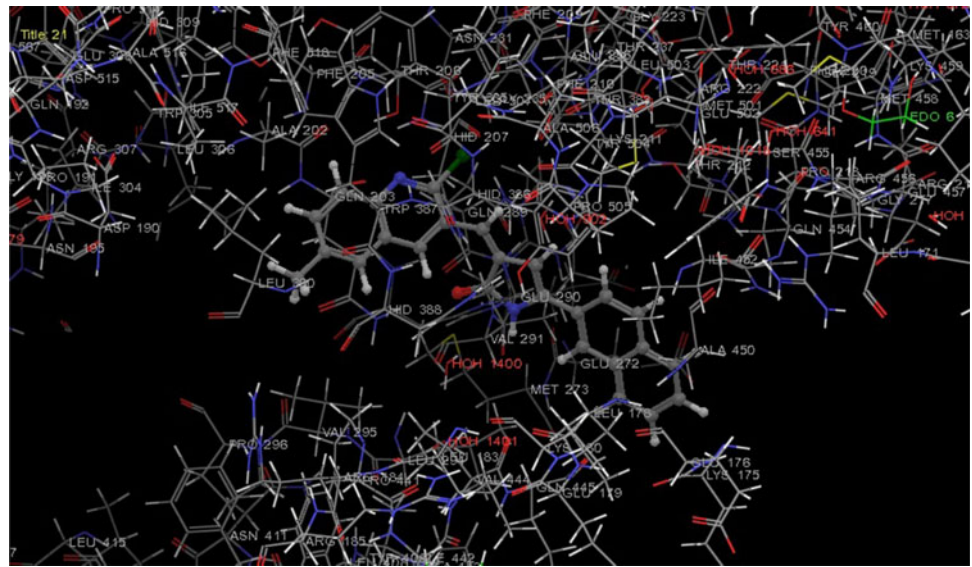


Fig. 6 Binding mode of compound IX into COX-II

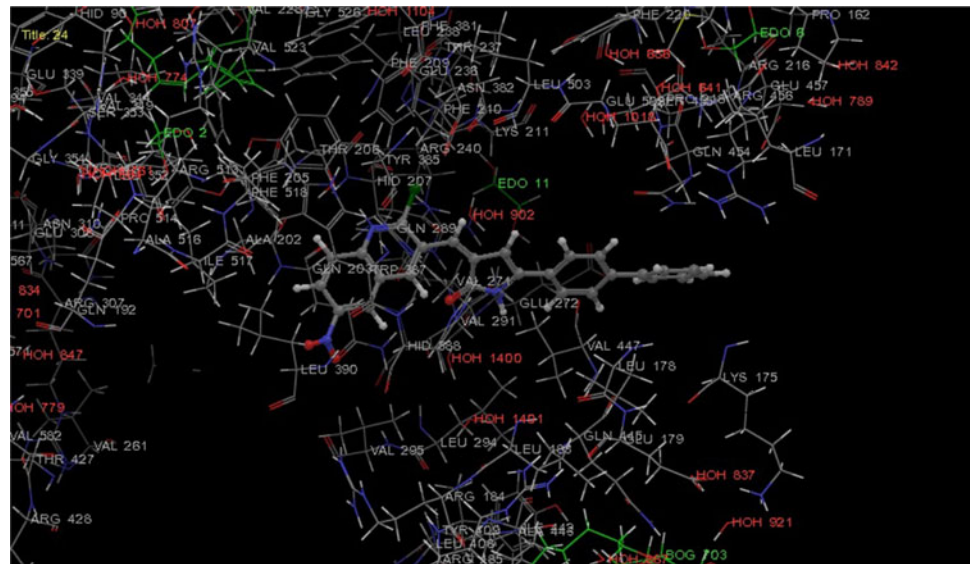


Fig. 7 Binding mode of compound XII into COX-II

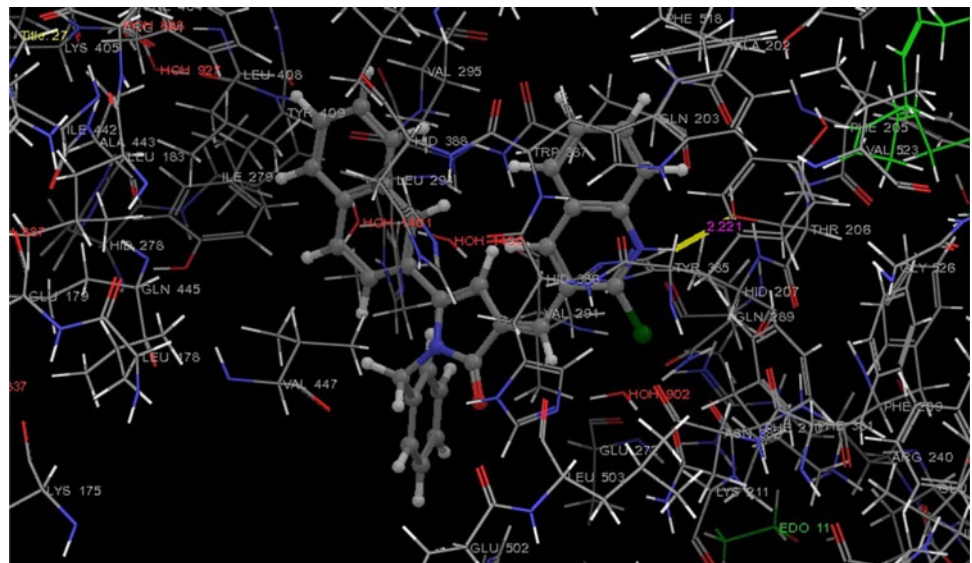
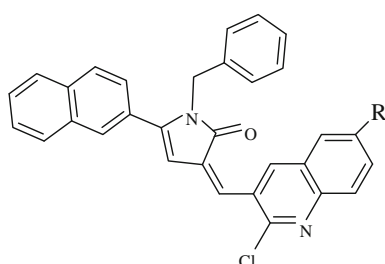
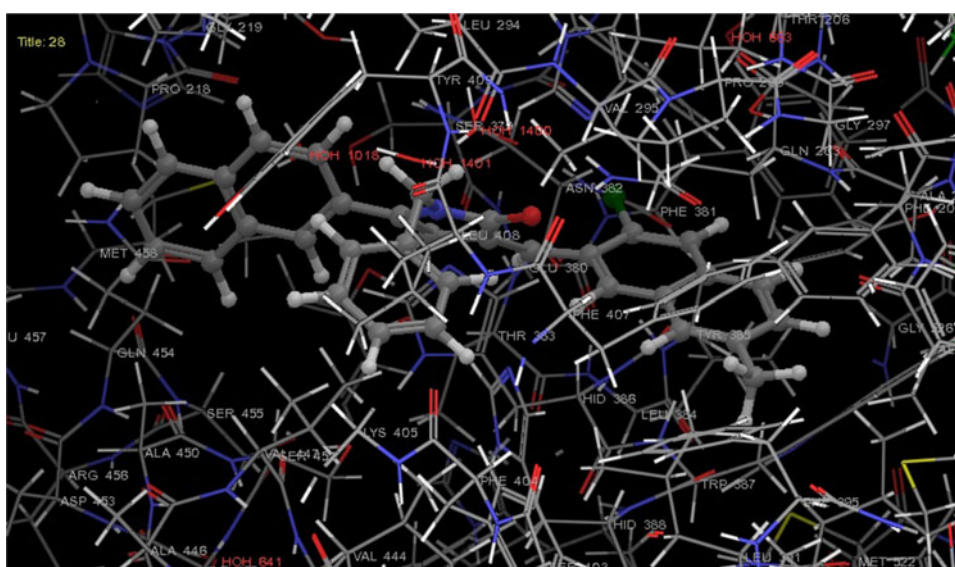
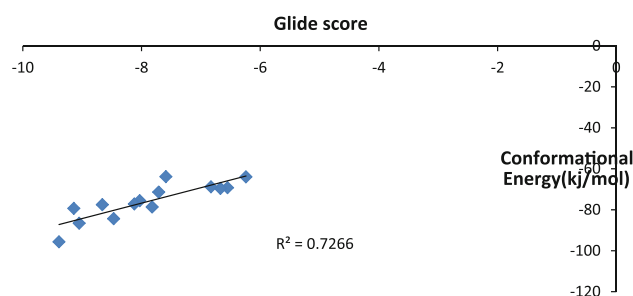


Fig. 8 Binding mode of compound XIII into COX-II**Fig. 9** Structure of compound XII

interaction with pocket but good glide score between -9.14 and -8.03 and reasonable conformational energy. The compounds III and XIV did not showed hydrogen bonding but glide score and conformational energy comparable to I and XII, it may be due to some other type of bond interaction, other than hydrogen. From these studies it was observed that binding pocket within the COX-II structure are formed mainly by three amino acid residue HOH902, THR212, and THR206, as shown by most potent compounds I and XII. The results of docking studies by Schrodinger provided very useful information as given in Table 2.

The docking studies revealed useful information with respect to interaction of butenolide derivatives with COX-II receptor. Oxygen of the methoxyl group of compound I showed one hydrogen bond interaction to hydroxy group of amino acid residue HOH902 and same group also formed another hydrogen bond with residue THR212. They are highlighted in yellow colour. H-bond with the HOH902 had a distance of 2.248 and with THR212 had a distance of 1.935. The compound I had Glide score of -6.55 .

The nitrogen atom of the quinoline ring of compound XII, formed a strong hydrogen bond with THR206, and showed

**Fig. 10** Plot of glide score of the docked poses against the conformational energy

the distance of 2.221. The compound XII had highest glide score (-9.3) and lowest energy, i.e. -95.66 kJ/mol.

Glide score of standard drug Diclofenac was found to be 10.33, whereas the glide scores of selected compounds III, V, VI, VII, IX, XII, and XIV were found as -9.14 , -8.66 , -8.03 , -8.12 , -8.47 , 9.39 , and 9.03 , respectively. These glide scores data indicated that interaction of selected compounds was comparable to standard drug Diclofenac. Moreover, from the literature we found that Glide score of Celecoxib (Bhandari *et al.*, 2009) was found to be -11.37 , which was also comparable to hypothetical compounds. The results of glide score were further validated by calculation of conformational energy of Diclofenac (-33.57 kJ/mol) and Celecoxib (-43.7 kJ/mol), which was higher than the hypothetical compounds used in the study. The compounds that had highest glide score and lowest conformational energy, can be used for further drug designing and also provides a way to synthesize new potent compounds in laboratory. The most potent compound of our study was XII, which was having highest glide score and lowest conformational energy.

Table 2 Ligand–receptor interaction data of butenolides using Schrodinger software

Compound no.	Interaction with H-bond (Å)	No. of H-bond	Glide score	Conformational energy (kJ/mol)
I	HOH902(2.248),THR212(1.935)	2	−6.55	−69.25
II	–	–	−7.59	−63.87
III	–	–	−9.14	−79.32
IV	–	–	−6.67	−69.59
V	–	–	−8.66	−77.52
VI	–	–	−8.03	−75.59
VII	–	–	−8.12	−77.18
VIII	–	–	−7.82	−78.63
IX	–	–	−8.47	−84.38
X	–	–	−6.24	−63.95
XI	–	–	−6.83	−68.82
XII	THR206(2.221)	1	−9.39	−95.66
XIII	–	–	−7.71	−71.41
XIV	–	–	−9.05	−86.57
Standard (Diclofenac)	–	–	−10.33	−33.57

Bold values indicate most potent and highest glide score

From the graph shown in Fig. 10, we found that there was a good correlation between glide score and conformational energy. As the conformational energy decreases, glide score increases in the same passion. The highest is glide score, lowest is conformational energy and compound is more stable in binding pocket.

Conclusion

Docking studies have helped us to know about the binding modes of the furanones to elicit their COX-II inhibitory activity. These investigations were found to be very useful during the synthesis of selected compounds, which were more potent and selective COX-II inhibitors. Moreover it was also proved from above discussion that geometry of receptor plays important role in defining drug action.

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