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In silico molecular docking studies of isolated phytochemicals from the *Ledebouria* genes as COX-2 inhibitors

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Abstract
Ledebouria is a genus of weakly evergreen bulbs in the Hyacinthaceae family. The anti-inflammatory activities of the isolated phytochemicals 1-4 from the bulbs of the Southern African *Ledebouria socialis* and *Ledebouria ovatifolia* were evaluated against cyclooxygenase-1 and cyclooxygenase-2 isoenzymes. All the compounds exhibited significant activity against cyclooxygenase-2 at <10 µM. In this report, to study the possible interactions of isolates with the crystal structure of *human* cyclooxygenase-2 (PDB ID: 5F1A) from the molecular docking studies. Among all compounds, compound 2 exhibited higher COX-2 inhibitory activity (IC_{50} = 2.87±1.2 µM) was supported by highest docking score of 139.079 in molecular docking studies.

Keywords: cyclooxygenase, hyacinthaceae, *Ledebouria*, docking studies

Introduction
The non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used drugs for the treatment of pain and inflammation. Cyclooxygenase (COX) is responsible for the synthesis of prostaglandins in sensitive inflammatory conditions [1]. NSAIDs develop their mode of action by blocking the COX enzyme and thus the biosynthesis of PGs (Prostaglandins) [2]. cyclooxygenases are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain and increased body temperature (hyperpyrexia) [3]. NSAIDs block the COX enzymes and reduce prostaglandins throughout the body. As a consequence, ongoing inflammation, pain and fever are reduced. However, NSAIDs have a number of adverse effects, mainly because of their inhibition of the constitutive isoform of COX. COX-1 and COX-2 are two isomeric forms of COX enzyme. Non-selective inhibitors of cyclooxygenase decrease the inflammatory response but have also been linked to gastro-intestinal tract bleeding at high doses [4]. Selective COX-2 inhibitors decreased this effect [5], it has also been demonstrated in several studies that cyclooxygenase inhibitors can have cancer preventative effects [6, 7] especially when specific against COX-2 and the therapeutic anti-inflammatory action of NSAIDs is produced by the inhibition of COX-2 [8].
Nature has been a source of medicinal products with many useful drugs developed from plants. Plants are good sources of biologically active compounds and are used as traditional medicines. The phytochemical separation of plants has resulted in many bioactive compounds with different pharmacological activities in traditional medicine. Nowadays, more than 50% natural drugs are being used for medication, finding their origin in some way from plants. According to the survey of the World Health Organization (WHO), about 80% of the world population is using herbs and other traditional medicines for their primary healthcare. *Ledebouria* is a weak evergreen bulb of the Hyacinthaceae family. Phytochemical studies were carried out on several genera of *Ledebouria* [9, 10, 11]. The plant chemicals of *Ledebouria* are widely used in traditional medicine. Molecular docking is a method to predict the orientation of protein macromolecules when they bind to synthetic ligands, and a stable complex is formed at the atomic level [12]. The phytochemicals from the bulbs of the Southern African *Ledebouria socialis* and *Ledebouria ovatifolia* have previously been isolated and screened for cyclooxygenase-2 (COX-2) inhibitory activity [13]. By taking this, the isolated phytochemicals from plant origin of *Ledebouria*, our present study initiates a docking methodology to predicts the plausible modes of interaction of the potent phytochemicals from plant origin within the human COX-2 enzyme.

Materials and Methods

Protein preparation

The X-Ray crystal structure of a *human* cyclooxygenase-2 was retrieved from Protein Data Bank (PDB ID: 5F1A) with a resolution of 2.38 Å, R value of 0.174 and R free value of 0.218. In the first step, the protein preparation protocol of Discovery Studio (DS) was used to prepare the protein structure retrieved from the PDB. The water molecules and the hetero atom were removed before docking study. Hydrogen atoms were added to the protein structures corresponding to pH value of 7.4. Then the protocol performs protein structure refinement that corrects their bond orders, modeling missing loop regions, inserting missing atoms in incomplete residues, deleting alternate conformations, standardizing names of the atoms and protonating titratable residues. Finally, all-atom restrained energy minimization of the protein structure was carried out using CHARMm force field with steepest descent algorithm followed by conjugant gradient algorithm until the convergence gradient satisfied with a root mean square deviation (RMSD) tolerance of 0.01 Å. After energy minimisation, Using Define and Edit Binding Site tools in DS, the active site of the protein was selected based on the bound ligand benzamidine conformation and a active site sphere was defined with a radius of 10 Å respectively.

Ligand preparation

The isolated phytochemicals 5-acetyl-3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one (1), 3-(3,4-dihydroxybenzylidene)-7-hydroxy-5-methoxychroman-4-one (2), 1,3,6-trihydroxy-2-methoxy-9H-xanthen-9-one (3) and 3-(3,4-dihydroxybenzyl)-7-hydroxy-5-methoxychroman-4-one (4) were chosen in the present study (Figure 1). The chemical structures of the compounds are sketched using Chem Draw tool of PerkinElmer Chem Office Ultra14.0 software [14] and saved in mol2 format. These ligands were then subjected to prepare ligands protocol of DS. They were converted from 2D to 3D structures by including stereo chemical, ionization, tautomeric variations, as well as energy minimization and optimized for their geometry, desalted and corrected for their chiralities and missing hydrogen atoms. The bonds orders of these ligands were fixed and the charged groups were neutralized. The ionization and tautomeric states were generated between pH of 6.8 to 7.2. In the final stage of Ligand preparation, compounds were minimized using CHARMm force field until a root mean square deviation of 0.01 was achieved. Steepest descent algorithm was used for minimization, followed by conjugate gradient method. A single low energy confirmation per ligand was generated and the optimized ligands were used for docking analysis.

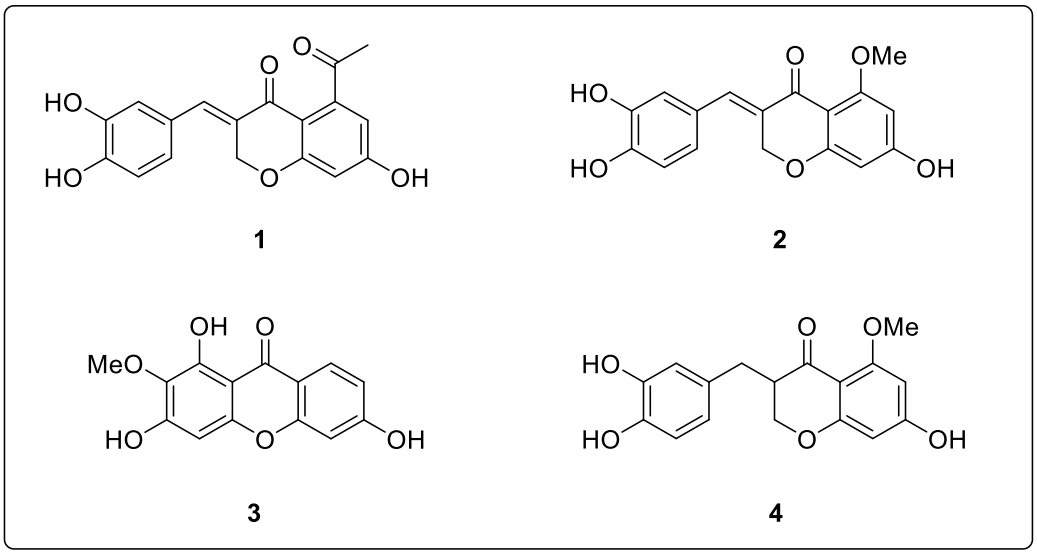

Fig 1: Structures of isolated phytochemicals used in the docking studies.

Molecular docking studies

Molecular docking study was performed for title compounds using the *human* cyclooxygenase-2 enzyme as receptor with the Discovery Studio software [15]. To study the molecular interaction of compounds, crystallographic data of *human* Cyclooxygenase-2 (PDB: 5F1A) was retrieved from Protein Data Bank [16]. LibDock uses protein site features, referred to as hot spots, consisting of two types states (polar and apolar). The ligand poses are placed into the polar and apolar receptor interactions site. A polar hotspot is preferred by a polar ligand atom (e.g., a hydrogen bond donor or acceptor), and an apolar hotspot is preferred by an apolar atom (e.g., a carbon atom). The protocol allows the user to specify several modes for generating ligand conformations for docking. Scoring function of the LibDock [17] calculates the binding affinity score or docking score (LibDock score) of protein-ligand complex. Also the possible binding energies, possible hydrogen bonding and various interaction poses are calculated. The top ranked docked complexes of each compound are selected on the basis of LibDock Score. Binding poses with highest LibDock Score and lowest binding energy are preferred as the best pose and further binding interactions of the best pose for each compound are analyzed.

Results and Discussion

Cyclooxygenase activity

Isolated compounds 1, 2, 3 and 4 were screened for cyclooxygenase inhibitory activity by *in vitro* mode at 10 µM. All the compounds were also screened against COX-1 to measure their selectivity for the COX-2 enzyme. Compounds 3 and 4 were found to be non-specific inhibitors of both the COX-1 and COX-2 enzyme during these studies [13]. On the other hand, compound 2 was completely selective for COX-2 at a concentration of 10 µM, and had an IC_{50} for COX-2 of 2.87±1.2 µM. Compound 1 was partially selective, inhibiting COX-1 with an IC_{50} of 2.56±1.2 µM and COX-2 with an IC_{50} of 1.12±0.56 µM. However, the isolates show IC_{50} values

which are within the range of widely used and non-selective inhibitors such as paracetamol [18] has IC_{50} of 26 µM for COX-2. It can therefore be concluded that the isolates above could be therapeutically valuable and that compound **2** could be developed into a clinically relevant and COX-2 specific inhibitor.

In silico molecular docking studies

In vitro inhibition potential studies of isolated title compounds against cyclooxygenase-2 were supported by performing molecular docking studies. Molecular docking has huge applications in drug discovery and development. All the title compounds were docked against target enzyme and all of them show different interactions with different residues of the active site. X-ray crystal structure of *Human* Cyclooxygenase-2 (PDB: 5F1A) downloaded from PDB. Binding affinity evaluation and inhibitory potential of these compounds were measured through LibDock docking score and H-bond interactions. Of all the conformations generated for each compound, the compound with the highest LibDock score is taken for interaction analysis of the hydrogen bonding. The hydrogen bond interaction is significant for the bioactivity of compounds. The stability of the best docked pose of these compounds was evaluated by determining the hydrogen bonding interactions of the protein with compounds which revealed the critical amino acids involved in hydrogen bond formation. The high LibDock score of the ligand pose with least binding energy was taken into account for the prediction of the best ligand binding conformation. Apart from hydrogen bonding interactions, other non-bonded interactions like hydrophobic bonding were also observed. Structural model of *Human* COX-2 active site and binding pattern of title compounds have shown in Figure 2.

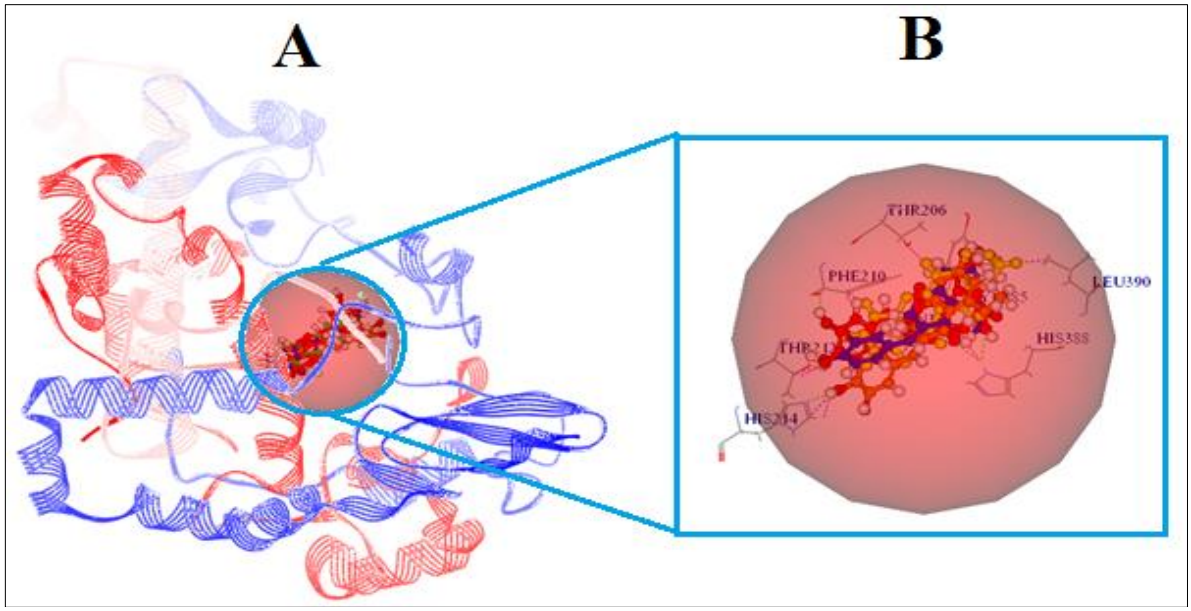

Fig 2: (A) Structural model of *Human* cyclooxygenase-2 (PDB: 5F1A) binding site (sphere); (B) Binding site and binding pattern of candidate compounds.

Docking results showed that LibDock program successfully docked compounds 1, 2, 3 and 4 into the binding site of *human* COX-2 with LibDock scores of 128.727, 139.079, 127.586 and 130.714 respectively. Table 1 shows the LibDock scores, interaction data and binding energies of the docked compounds. After scrutinizing all the results of docking and interaction analysis, interactions of the most active compound 2 (IC_{50} = 2.87±1.2 µM) was analyzed with the residues of the target protein. Receptor-ligand hydrogen bonds of compounds 1, 2, 3 and 4 with active site residues of *human* COX-2 have shown in Figure 3. The best docking score of for compound 2 was achieved against the human COX-2 receptor forming four hydrogen bonds with the ASN382 and THR212 amino acid residues of active site pocket of COX-2. The compound 1 has shown a LibDock score of 128.727 forming three hydrogen bonding interactions with the HIS388 and HIS214 amino acid residues. Similarly, the compounds 3 and 4 forming one hydrogen bond interactions each with the ASN382 and PHE210 amino acid residues of active site pocket of COX-2 respectively. Among all the compounds, compound 2 was considered as the best compound indicating high binding affinity and better hydrogen bond interactions with the *human* COX-2 receptor active site residues.

Table 1: Details of LibDock score and ligand interaction data revealed through molecular docking of title derivatives on *human* cyclooxygenase-2 (PDB: 5F1A).

Compound	LibDock score	Interacting atoms	Bond Distance (A^0)	No. of H-bonds
1	128.727	A:HIS388:HD1 - 1:O24	2.332000	3
		A:HIS388:HD1 - 1:O12	2.329000	
		1:H34 - A:HIS214:NE2	2.147000	
		1:H34 - A:HIS214:HE1	1.816000	
		1:H34 - A:HIS214:CE1	2.175000	
2	139.079	2:H34 - A:ASN382:OD1	2.473000	4
		A:ASN382:HD22 - 2:O21	2.058000	
		2:H33 - A:THR212:OG1	1.900000	

		A:THR212:HN - 2:O20	2.493000	
		A:THR212:CB - 2:H33	2.212000	
		A:ASN382:HD22 - 2:H34	1.518000	
		A:THR212:HB - 2:H33	1.747000	
3	127.586	A:ASN382:HD22 - 3:O16	2.018000	1
		3:H30 - A:LEU390:CD1	2.056000	
		3:H21 - A:THR206:OG1	1.618000	
		3:H22 - A:PHE210:CB	1.850000	
4	130.714	4:H36 - A:PHE210:O	2.018000	1
		A:HIS388:HD1 - 4:H25	1.783000	

Conclusion

In conclusion, *In vitro* inhibition potential studies of isolated title compounds 1, 2, 3 and 4 from the bulbs of *Ledebouria socialis* and *Ledebouria ovatifolia* against cyclooxygenase-2 were supported by adopting molecular docking studies. All the title compounds were docked against *Human* cyclooxygenase-2 (PDB: 5F1A). Among them, compound 2 was ranked highest docking score of 139.079 by involving four hydrogen bonds and also compound 2 exhibited higher COX-2 inhibitory activity IC_{50} = 2.87±1.2 µM was supported by molecular docking studies. The study of highlighted isolates as potential compounds to be further developed as novel COX-2 inhibitors for therapeutic applications.

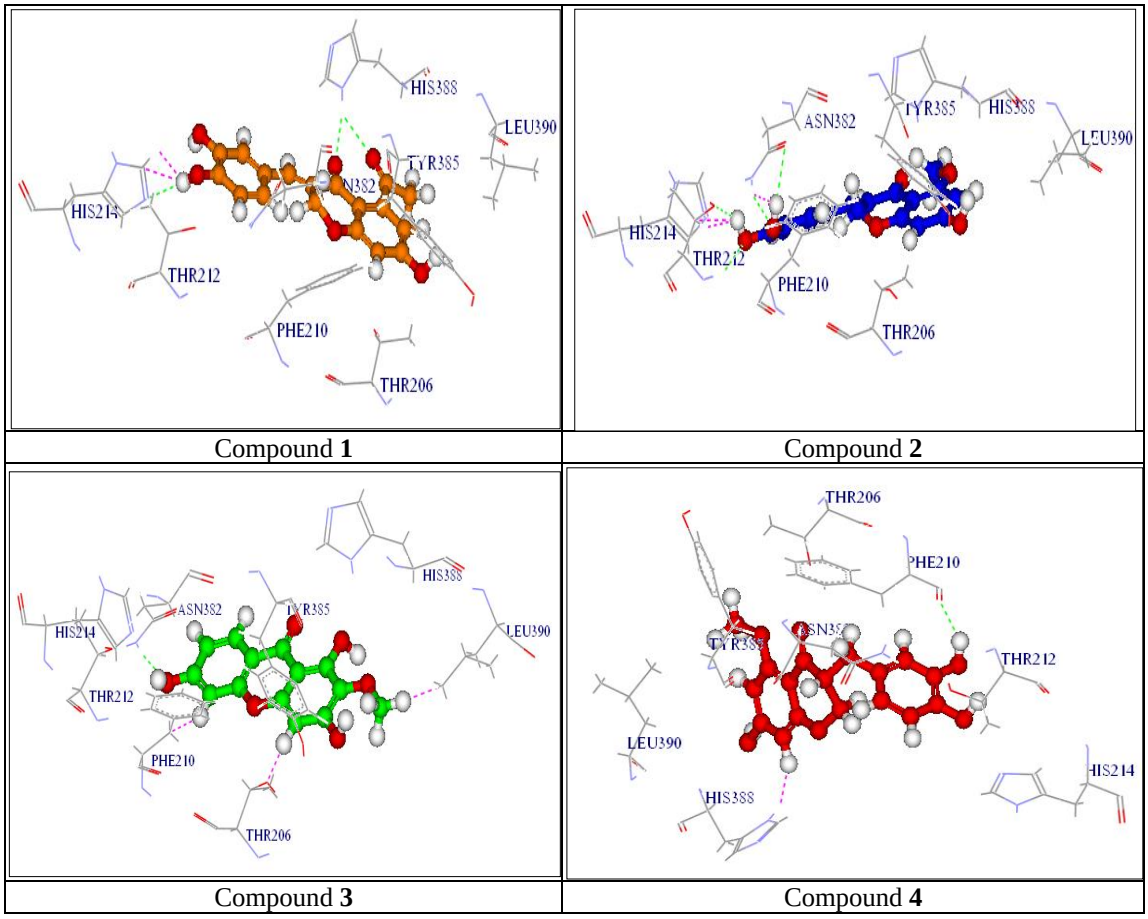

Fig 3: Receptor-ligand hydrogen bonds (green colour) and bumps (pink colour) of compounds 1, 2, 3 and 4 with active site residues of *human* cyclooxygenase-2 (PDB: 5F1A).

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