Molecular Docking of Natural Product-Derived Compounds: Estimation of Selectivity on Cyclo-oxygenase-2

นิพนธ์ต้นฉบับ

Original Article

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วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ 2554;6(2):79-85

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษากลไกระดับโมเลกุลในการจับกันและความเฉพาะเจาะจงระหว่างเอนไซม์ COX-1 และ COX-2 กับสารจากธรรมชาติ 12 ชนิดที่มีฤทธิ์เป็นสารต้านอักเสบโดยเทคนิค *in silico* **วิธีการศึกษา:** ใช้โปรแกรม AutoDock 4.2 ในการคำนวณค่าพลังงานอิสระที่เกิดจากอันตรกิริยาที่สารจับกับเป้าหมาย (ΔG_b) และค่าคงที่ของความสามารถในการยับยั้ง(K_i) ในการศึกษานี้ใช้ค่าคงที่ของความสามารถในการยับยั้งทำนายดัชนีความเฉพาะเจาะจงของสารแต่ละชนิด (อัตราส่วนระหว่าง COX-2 K_i และ COX-1 K_i) **ผลการศึกษา:** γ-Mangostin มีดัชนีความเฉพาะเจาะจงเท่ากับ 0.0269 เทียบเท่ากับ rofecoxib (0.0407) ดัชนีความเฉพาะเจาะจงของ gingerol, [8]-paradol, isorhapontigenin และ rutaecarpine อยู่ในช่วง 0.2 - 0.5 ซึ่งอาจจัดเป็น preferential COX-2 inhibitor **สรุป:** จากการศึกษากลไกการจับกันและความเฉพาะเจาะจงระหว่างเอนไซม์ COX-1 และ COX-2 กับสารจากธรรมชาติ 12 ชนิดที่มีฤทธิ์เป็นสารต้านอักเสบให้ข้อมูลอันตรกิริยาระหว่างเอนไซม์และตัวยับยั้งและดัชนีความเฉพาะเจาะจงที่น่าจะเป็นประโยชน์ในการออกแบบยาต้านอักเสบที่มีโครงสร้างแบบใหม่และมีความปลอดภัย

คำสำคัญ: docking, ตัวยับยั้ง COX-2 แบบเฉพาะเจาะจง, สารจากธรรมชาติ, AutoDock

Abstract

Objective: To investigate the binding modes and molecular selectivity on COX-1 and COX-2 enzymes of 12 natural product-derived compounds reported as anti-inflammatory agents using *in silico* prediction. **Method:** AutoDock 4.2 was employed to determine the free energy of binding (ΔG_b) and inhibition constants (K_i). The evaluated inhibition constant (K_i) from docking result was used to estimate the calculated selectivity index (ratio of COX-2 K_i to COX-1 K_i of each compound). **Results:** γ-Mangostin gained the lowest calculated selectivity index (0.0269) comparable to rofecoxib (0.0407). The calculated selectivity indices of gingerol, [8]-paradol, isorhapontigenin and rutaecarpine were in a range of 0.2-0.5 that could be defined as preferential COX-2 inhibitors. **Conclusion:** Binding modes and molecular selectivity of 12 natural product-derived compounds reported as anti-inflammatory agents were determined. This information from the inhibitor-enzyme interactions and calculated selectivity indices could be useful in designing NSAIDs with new scaffold and favorable safety profile.

Keywords: docking, COX-2 selective inhibitors, natural product-derived compounds, Autodock

Introduction

Prostaglandins (PGs) are involved in many important physiological functions including inflammation, pain, body temperature, as well as maintenance of gastric, renal and hematic systems.[1] The major enzymes responsible for the synthesis of PGs from their precursor, arachinodic acid, are cyclooxygenases (COXs) which have been identified into two isoforms, known as COX-1 and COX-2.[2,3] The constitutive COX-1 isoform is expressed in cells and normal tissues physiological functions (gastro-protection and keeping vascular and renal homeostasis). The inducible COX-2 is induced by mediators of inflammation in pathological conditions. Inhibition of both COX-1 and COX-2 enzymes with non-selective inhibitors lead to renal and gastro-intestinal side effects due to inhibition of COX-1.[4,5] Accordingly, the selective COX-2 inhibitors have been designed and developed based on the approach that they might have superior therapeutic action similar to non-selective inhibitors with reduced adverse effects.[6] The first selective COX-2 inhibitor, celecoxib[(7)], was launched in 1999 followed by rofecoxib[8], valdecoxib[9], parecoxib[10], etoricoxib[11] and lumiracoxib[12]. However, rofecoxib has been withdrawn from the market because its long-term treatment can increase the risk of serious thromboembolic events including myocardial infarction and valdecoxib has been withdrawn for serious cutaneous adverse reactions.[13,14] Recent reports demonstrated that selective COX-2 inhibitors may tip the balance between prothrombotic (TxA_2) and anti-prothrombotic prostraglandin (PGI_2) potentially increasing the possibility of a thrombotic cardiovascular events.[15,16] Currently, the extensive efforts to design new safer selective COX-2 inhibitors with different scaffold from the current ones are in the great deal of interest. The use of traditional medicine for

a relief from pain and inflammation has been reported for very long times. With their great structural diversity, these natural product-derived compounds are supposed to be devoid of severe adverse effects. With such promising advantages, their molecular selectivity on the target, COX-1 and COX-2 enzymes, need to be thoroughly scrutinized.

In this research, twelve natural product-derived compounds reported as anti-inflammatory agents were investigated for binding modes and selectivity of the molecules on COX-1 and COX-2 enzymes by means of docking studies. These natural compounds may serve as potential lead compounds to develop novel COX-2 selective inhibitors with new scaffold as safer anti-inflammatory drugs.

Materials and Methods

Preparation of Ligands

Twelve natural product-derived compounds (structures shown in Figure 1) having anti-inflammatory activities were selected from literatures.[17] The structures of all compounds were constructed and optimized by Sybyl 7.0 using the Tripos force field and Gasteiger Huckel charges. The Powell method was used for energy minimization with an energy convergence gradient of 0.001 kcal/mol. All possible flexible torsions of the ligand molecules were defined using AutoTor. The prepared ligands were used as input files for Autodock 4.2 in the next steps.

Preparation of receptors

The structures of COX-1 (1CQE) and COX-2 (1CX2) proteins were obtained from Protein DATA BANK [Research Collaboratory for Structural Bioinformatics (RCSB) (http://www.rcsb.org\pdb)]. All bound waters and ligands were eliminated from the proteins. The receptor models were prepared with AutoDock Tools adding hydrogens and Kollman charges. The auxiliary program, AutoGrid generated the grid maps. The affinity grid maps centered on and encompassing the active site were calculated for the relative ligand atom types with 0.375 À spaced box 40 x 40 x 40 À.

Docking simulations

The docking simulations were performed using Autodock version 4.2 software running on Itanium cluster.[18] The docking studies were carried out to evaluate the binding free energy of the inhibitors within the receptors. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. Default docking parameters were used with 100 independent docking runs for each ligand. The docking conformations of each ligand were clustered on the basis of root-mean-square deviation (RMSD) tolerance of 2.0 À and ranked on the basis of free energy of binding. The cluster with the lowest free energy of binding was visually analyzed using Discovery Studio Visualizer 2.5.

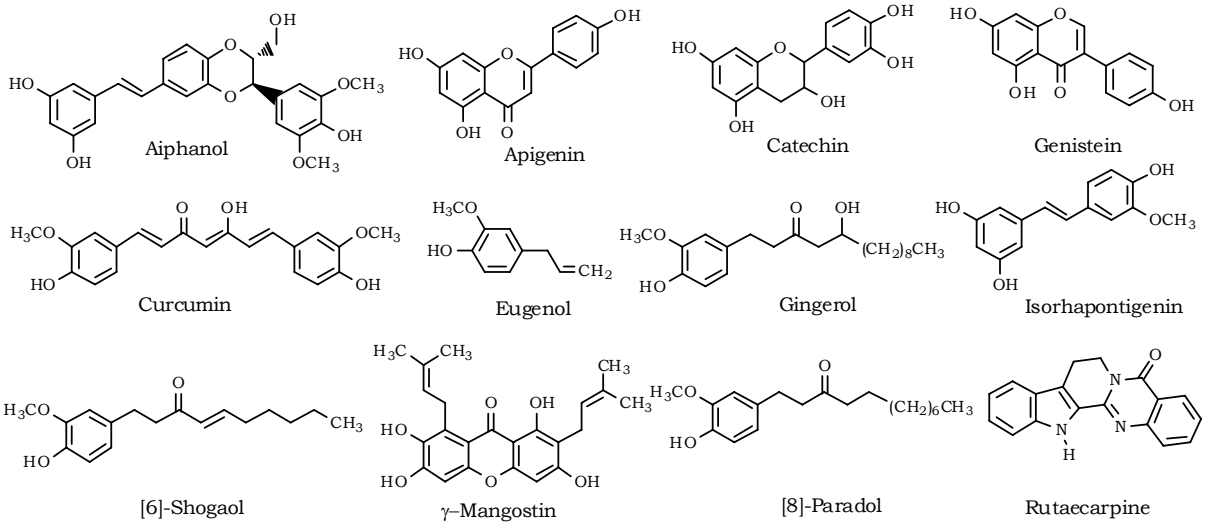

Figure 1 Structures of natural product-derived with anti-inflammatory activities.

The docking studies results were used to generate inhibitor thermodynamic properties, such as free energy of binding (ΔG_b) and inhibition constants (K_i). The inhibition constant was used to estimate the calculated selectivity index (ratio of COX-2 K_i to COX-1 K_i of each complex). The experimental selectivity index was calculated from the ratio of COX-2 IC_{50} to COX-1 IC_{50}. The correlation between calculated selectivity indices and experimental selectivity index was determined using Spearman rank correlation in order to demonstrate a method validation.[19,20] Molecular volume was calculated by Gaussian 03, Revision D.01.

Results and Discussions

The Autodock docking parameters were validated to ensure that the ligand orientation and the position obtained from the docking studies represent valid reasonable binding modes of inhibitors. The ligands, fluribufen and SC-58 in the conformation found in the crystal structure of 1CQE and 1CX2, respectively were extracted and docked back into the corresponding binding pocket. The results of docking simulation predicted the binding conformation of fluribufen for COX-1 enzyme and SC-58 for COX-2 enzyme with a root-mean-square deviation (RMSD) of 0.62 and 1.51 Å compared with conformations from X-ray crystallographic studies. These were within 2.0 Å RMSD, a value typically used in evaluating the success of docking algorithms, indicating our docking methods were valid for the given structures. The orientation of the SC-58 found within the crystal structure (only carbon atoms presented in green) and the predicted conformation by Autodock (colored by atom type) shown in Figure 2a. The orientation of SC-58 from docking simulation was reproduced except that the *p*-sulfonyl amino group was rotated 180° and formed hydrogen bonding with HIS90 and ARG513 as shown in Figure 2b.

To verify the ability of the docking method to differentiate selectivity of COX inhibitors, the docking calculations were also performed on three different classes of NSAIDs, including classical, preferential and selective NSAIDs. The classification of the three groups was defined based on their experimental selectivity indices (ratio of COX-2 IC_{50} to the COX-1 IC_{50}) which were more than 1, between 1 to 0.1 and less than 0.1, respectively. Eleven NSAIDs were docked to COX-1 and COX-2 crystal structures according to the above docking protocol. Table 1 shows the results of docking experiments, estimated binding free energy (ΔG_b), inhibition constants for each complex (K_i) and their corresponding experimental selectivity indices. The correlation between calculated selectivity indices and experimental selectivity index using Spearman correlation coefficient is significant ($r = 0.827$, *P*-value = 0.009,). These results demonstrate that this method was considerably robust and suitable for assessing the interaction and selectivity of the ligands and proteins.

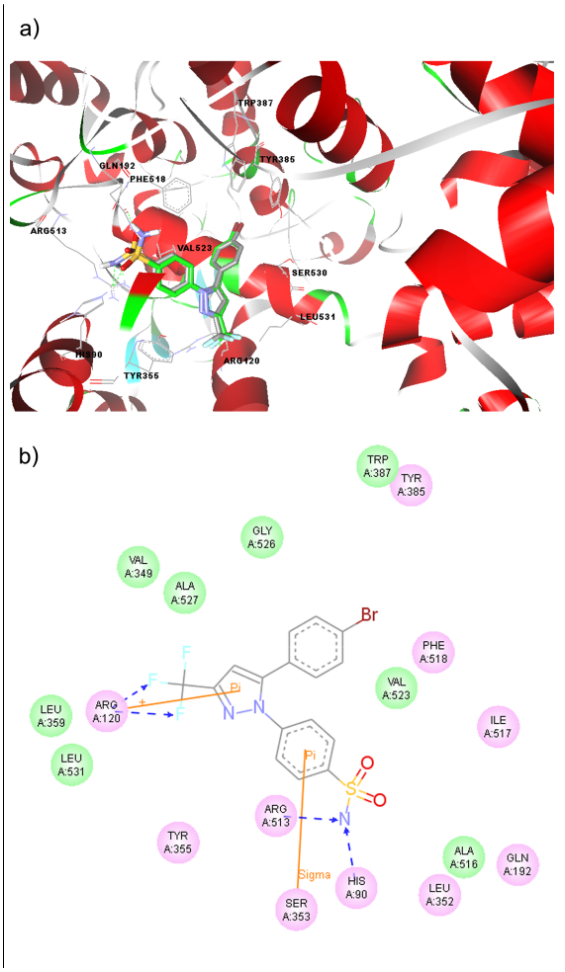

Figure 2 a) Orientation of the SC-58 found within the crystal structure (only carbon atoms presented in green) and the predicted conformation by Autodock (colored by atom type), b) The predicted binding mode of SC-58 in the binding site of COX-2; a) and b) made by Discovery Studio 3.0.

After being docked into the catalytic sites of COX-1 (1CQE) and COX-2 (1CX2) enzymes, 11 out of 12 natural product-derived compounds with anti-inflammatory activities (Figure 1) were found to be docked into the active sites of both enzymes; while aiphanol could not display any conformations to bind the active site of COX-1. The

experimental IC_{50} (some data are shown as %inhibitory activity), calculated molecular volume and the results of docking experiments with these inhibitors are summarized in Table 2. The estimated binding free energy (ΔG_b), calculated inhibition constants (K_i) as well as the calculated selectivity indices complex are shown.

γ-Mangostin, the tetraoxygenated diprenylated xanthone derivative isolated from pericarp of mangosteen fruit (*Garcinia mangostana*), exhibited a good COX-2 selectivity comparable to rofecoxib; while gingerol, isorhapotigenin, [8]-paradol and rutaecarpine were predicted as preferential COX-2 inhibitors. All five compounds play a crucial role in COX-2 selectivity by filling in the space called selectivity pocket as shown in Figure 3a-d. The docked model of γ-mangostin in the active site of COX-2 enzyme (Figure 3a) revealed 5 predicted hydrogen bonds, involving the hydrogen bonds interactions of hydroxyl group at C-1, C-3, C-6 and C-7 with LEU352, TYR355, ALA527 and SER530, respectively. Additional hydrogen bond interaction was observed between oxygen atom of C-1 hydroxyl group and ARG513. The prenylated moiety at position 8 was oriented toward a hydrophobic pocket comprised of TYR385, TRP387, PHE518 and LEU352. A few hydrophobic contacts have been

Table 1 Experimental selectivity indices, binding free energy (ΔG_b) and calculated K_i of selected NSAIDS.

NSAIDs	IC_{50} WBA*(μM)		Experimental selectivity indices	ΔG_b(Kcal/mol)		Calculated K_i (μM)		Calculated selectivity indices
	COX-1	COX-2		COX-1	COX-2	COX-1	COX-2	
Ibuprofen	7.6	7.2	0.9474	-6.17	-5.93	29.89	45.24	1.5135
Ketoprofen	0.047	2.9	61.7021	-7.52	-7.27	3.1	4.72	1.5226
Naproxen	9.3	28	3.0108	-6.80	-6.53	10.29	16.44	1.5977
Tolmetin	0.35	0.82	2.3429	-6.97	-6.43	7.73	19.37	2.5058
Etodolac	12	2.2	0.1833	-5.62	-7.41	75.95	3.68	0.0485
Meloxicam	5.7	2.1	0.3684	-6.43	-9.19	19.44	0.1843	0.0095
Nimesulide	10	1.9	0.1900	-7.05	-7.86	6.1	1.72	0.2820
Celecoxib	1.2	0.83	0.6917	-7.68	-8.48	2.34	0.613	0.2620
Rofecoxib	63	0.84	0.0133	-8.28	-10.18	0.8507	0.0346	0.0407
Valdecoxib	26.1	0.87	0.0333	-8.04	-9.93	1.27	0.0527	0.0415
Etoricoxib	116	1.1	0.0095	-7.45	-10.57	3.48	0.0117	0.0050

* IC_{50} was obtained from references 21-22.

Table 2 Estimated binding free energy (ΔG_b), calculate inhibition constants (K_i) and calculated selectivity indices of natural product-derived compounds.

Compounds	IC_{50} (μM)		Mol. Vol.* (cm^3/mol)	ΔG_b(Kcal/mol)		Calculated K_i (μM)		Calculated selectivity indices
	COX-1	COX-2		COX-1	COX-2	COX-1	COX-2	
γ-Mangostin	0.8	2	304.04	-6.38	-8.52	20.96	0.5657	0.0269
Gingerol	-	3.7	307.49	-5.71	-6.53	65.69	16.42	0.2499
Isohapotigenin	1.5	6.2	191.41	-6.63	-7.19	13.72	5.36	0.3906
[8]-paradol	-	3.4	269.55	-6.13	-6.68	32.32	12.76	0.3948
Rutaecarpine	8.7	0.28	223.41	-8.36	-8.78	0.7469	0.3657	0.4896
Catechin	80		178.44	-6.51	-6.70	16.97	12.30	0.7248
Apigenin	65%‡		147.84	-7.11	-7.09	6.14	6.34	1.0336
[6]-Shogaol	-	2.1	243.41	-6.58	-6.49	14.90	17.39	1.1671
Genistein	80	-	193.36	-6.83	-6.61	9.93	14.20	1.4300
Eugenol	97%‡	-	157.75	-5.01	-4.69	213.84	362.06	1.6931
Curcumin	-	0.5	290.02	-5.08	-4.68	188.17	369.77	1.9651
Aiphanol	1.9	9.9	270.36	N/A	-4.50	N/A	1070	-

* Mol. Vol. = Molecular volume.

‡ % Inhibition at the concentration of 1,000 μM.

N/A = unable to dock to the binding site.

observed between prenylated moiety at position 2 and ALA516, GLN192 and VAL523.

Through our docking studies, gingerol, oleoresin principles of ginger (*Zingiber officinale*), showed three hydrogen bonds to COX-2 enzyme (Figure 3b). Two hydrogen interactions were formed via oxygen atom and hydrogen atom of phenolic hydroxyl group with ILE517 and GLN192, respectively. The oxygen atom of *m*-methoxyl group interacted by means of hydrogen bond with HIS90. The alkyl side chain was embedded in the hydrophobic pocket, making lipophilic contact with side chain of TRY387, TYP385, MET522, VAL523, GLY526 and ALA527. Another oleoresin from ginger, [8]-paradol, was predicted as selective COX-2 inhibitor with calculated selectivity index of 0.3948. Its conformation in the active site of COX-2 enzyme was similar to gingerol in figure 3b.

The interaction observed for rutaecarpine, a major indoloquinazoline alkaloid isolated from Rutaceous plants such as *Evodia rutaecarpa* and *Evaodia officinalis*, was the hydrogen bond between the carbonyl oxygen and TYR385 as shown in Figure 3c. The quinazoline ring of the rutaecarpine was oriented toward the apex of COX-2 active site and bound to TRP387, LEU384, GLY526, ALA527, VAL523, and LUE531 via van der Waals contacts, while the indole ring was close to VAL349, ARG120 and TYR355.

Figure 3d shows the docked pose of isorhapentigenin bound in the active site of COX-2 that revealed six predicted hydrogen bonds with five amino acids, SER530, TYR385, MET522, HIS90 and SER353. One of the phenyl rings interacted with a hydrophobic pocket consisting of PHE381, TYR385, LEU384 and ALA527. Another phenyl ring accommodated in selectivity pocket surrounded by amino acids PHE518, ILE517, ARG513, HIS90, SER353, TYR355, THR94 and GLN192.

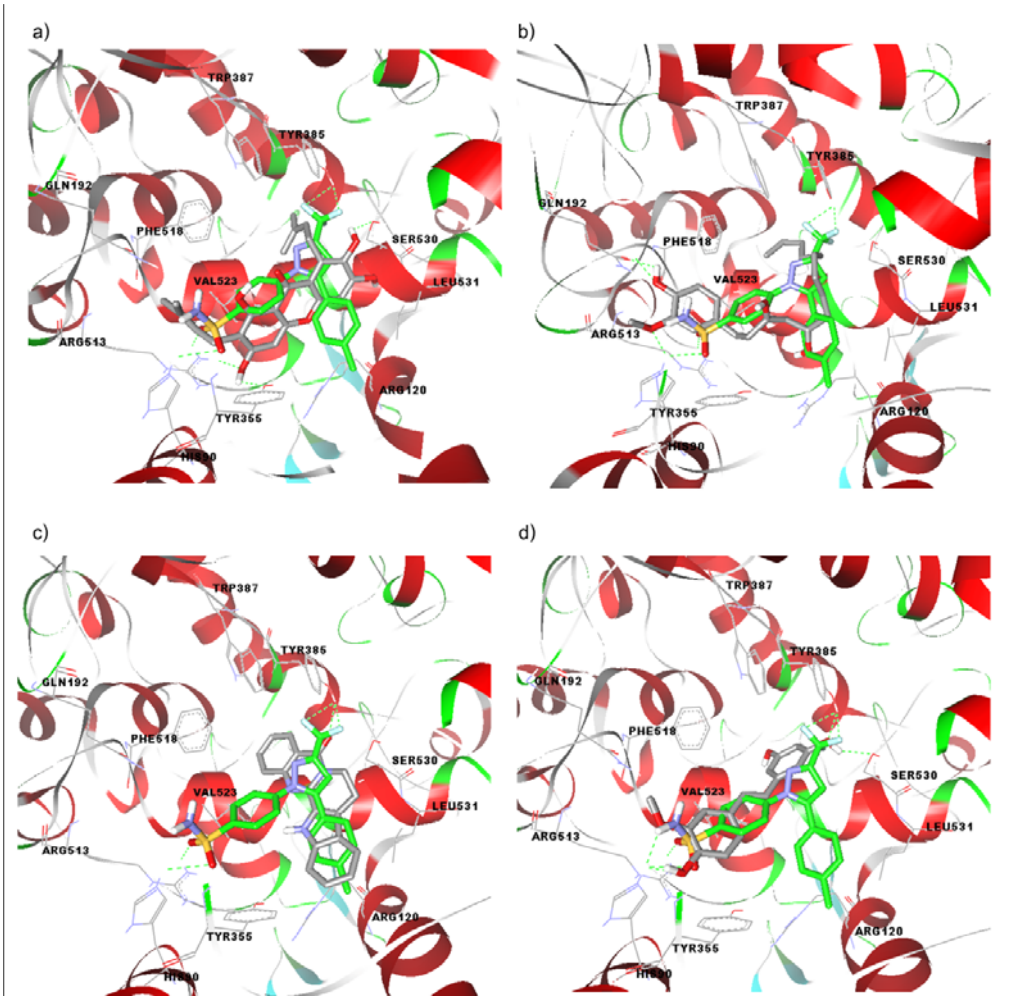

Figure 3 The predicted binding conformations of a) γ-mangostin, b) gingerol, c) rutaecarpine and d) isorhapentigenin superimpose with celecoxib (only carbon atom presented in green in the active site of COX-2 (*see also pdf file on the Journal website*). Hydrogen bonds are shown as green dashed lines.

The docking studies of γ-mangostin, gingerol, [8]-paradol, isorhapentigenin, and rutaecarpine with COX-1 enzyme were investigated through molecular simulation. As seen in Figure 4, all five compounds were placed in the hydrophobic pocket and pointed toward the mouth of the COX-1 channel. They were shifted from the selectivity pocket due to the steric clash with the bulky amino acid ILE523. γ-Mangostin, isorhapentigenin, [8]-paradol, and gingerol formed 5, 4, 2 and 2 hydrogen bonds with COX-1, respectively. Rutaecarpine had no hydrogen bond interaction with COX-1 enzyme. The hydrogen bonds formed by these compounds with COX-1 were weaker than those with respective COX-2 complexes. It could suggest that γ-mangostin, gingerol, isorhapontigenin, [8]-paradol and rutaecarpine provided more stable conformations in COX-2 rather than COX-1.

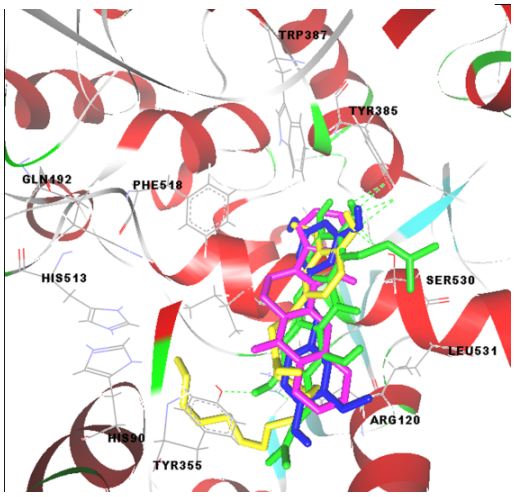

Figure 4 The predicted binding conformations of γ-mangostin (green), gingerol (yellow), c) rutaecarpine (purple) and d) isorhapentigenin (blue) in the active site of COX-1. (*see also pdf file on the Journal website*) Hydrogen bonds are shown as green dashed lines.

The volume of the COX-2 active site is larger than that of COX-1 which was a result of an additional hydrophilic side pocket in COX-2 enzyme. This side pocket is defined by HIS90, the less bulky VAL523 in COX-2 (isoleucine in COX-1) and contained ARG513 (Histidine in COX-1) at the base of the pocket.[23] With the docking simulations showing the same tendency, it was indicated that compounds with molecular volume higher than 200 cm^3/mol like γ-mangostin, gingerol, [8]-paradol and rutaecarpine gained lower K_i and were more suitable in COX-2 than COX-1. The compounds with molecular volume smaller than 200 cm^3/mol like apigenin, catechin, eugenol and genistein showed higher K_i in COX-2 than COX-1. Curcumin and [6]-shogaol were the compounds with high molecular volume but were predicted as classical COX-2 inhibitors. These compounds could not adapt their conformations to fit in COX-2 enzyme probably due to the less flexibility of the olefinic double bonds in their structures. Apigenin could not bind to the active site of COX-1. However, with its calculated K_i for COX-2 in a range of micromolar, it could not be classified as selective COX-2 inhibitors either. Isorhapontigenin was predicted as preferential COX-2 inhibitor which was not corresponding to its molecular volume (191.41 cm^3/mol), because of a large number of hydrogen bonds formed with the receptors.

Some calculated inhibition constants (K_i) did not correlate with the experimental results. This inconsistency could occur because the experimental K_is were obtained from the different literature sources with variable testing methods and sources of the enzyme. Another factor was the computational process. For example, during the calculation, Autodock did not consider the explicit water molecules. However, the predicted K_i was at least correct in the order of ranking and magnitude.

Conclusion

Molecular docking studies of twelve natural product-derived compounds with anti-inflammatory activities were carried out on COX-1 and COX-2 enzymes. AutoDock 4.2 estimated the binding free energy (ΔGb) and inhibition constant (K_i) of each complex. The calculated selectivity index of each compound was calculated from ratio of estimated COX-2 K_i to estimated COX-1 K_i. γ-Mangostin was predicted as selective COX-2 inhibitor with calculated selectivity index of 0.0269 comparable to rofecoxib. Gingerol, isorhapontigenin, [8]-paradol, and rutaecarpine were defined as preferential COX-2 inhibitors with the calculated selectivity indeices of 0.2499, 0.3906. 0.3948, and 0.4896. This study suggests that number of hydrogen bonding, molecular volume, orientation, flexibility, and functional groups which can form hydrogen bond are involved in COX-2 selectivity. These results could be useful in designing new derivatives

from these compounds as potent and selective COX-2 inhibitors with reduced toxicities.

Acknowledgement

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