Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

IN SILICO ANALYSIS OF CYCLOOXYGENASE INHIBITORY ACTIVITY OF SOME NATURAL MOLECULES

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Received: 31 May 2013, Revised and Accepted: 23 Jun 2013

ABSTRACT

Objective: Cyclooxygenase-2 (COX-2) is inducible and is expressed only after an inflammatory stimulus. Molecular docking is used to predict and analyze interactions between protein receptors and ligands. In the present study efforts are made to identify novel natural compounds possessing anti-inflammatory activity using Autodock.

Methods: MGL tool was used for docking preparation and Autodock Vina was used for binding energy calculations. Virtual analysis of docking site was analyzed by PyRx and PyMol. The structure of cyclooxygenase-2 (4COX) was obtained from protein data bank and the structures of ligands were collected from Pubchem database.

Results: All the selected molecules except apigenin and naringin satisfied the Lipinski's rule of five. Naringin showed highest binding affinity (8.6 Kcal/mol) followed by rutin (8.3 Kcal/mol).

Conclusion: It is found that natural molecules can be potential anti-inflammatory agents.

Keywords: 4COX, Autodock, Anti-inflammatory, Naringin, rutin

INTRODUCTION

Cyclooxygenase (COX) is an endogenous enzyme involved in production of prostaglandins from arachidonic acid. Two common isoforms of this enzyme are COX-1 and COX-2. COX-1 is a constitutive enzyme whereas COX-2 is inducible and is expressed only after an inflammatory stimulus [1]. Rational drug design uses a variety of computational methods to identify novel compounds. One of those methods is molecular docking where interactions between protein receptors and ligands are predicted and analyzed [2]. In the present study efforts are made to identify novel natural compounds possessing anti-inflammatory activity using bioinformatics tools.

MATERIALS AND METHODS

Docking software

MGL tool was used for docking preparation and Autodock Vina was used for binding energy calculations. Autodock performs the docking of ligand to a set of grids (pre calculated by autogrid) describing the target protein. The energy grid was performed based on Lamarckian genetic algorithm [3]. Virtual analysis of docking site was analyzed by PyRx and PyMol.

Preparation of macromolecule

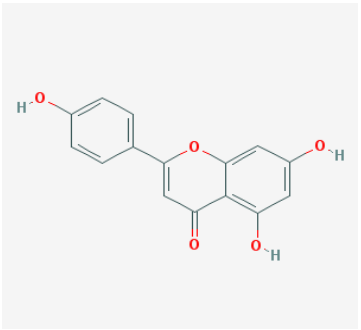
The 3D structure protein 4COX (cyclooxygenase-2) was obtained from protein data bank. All heteroatoms including water molecules and definitions for symmetry were excluded from the file using Discovery 3.5 software. Minimized structure was saved in PDBQT file format that contains a protein structure with hydrogen in all polar residues [4].

Ligand preparation

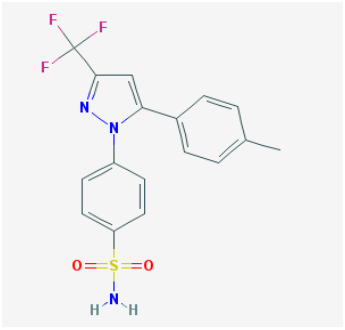
The 2D structures of few natural molecules and the standard drug celecoxib were searched against Pubchem database. Then these 2D structures were converted to 3D structures with the help of Open Babel using PyRx software. Ten of the natural molecules and the standard drug celecoxib and indomethacin (Figure-1) were minimized by computing gasteiger charges and saved in PDBQT file format [5].

Lipinski's Rule of Five

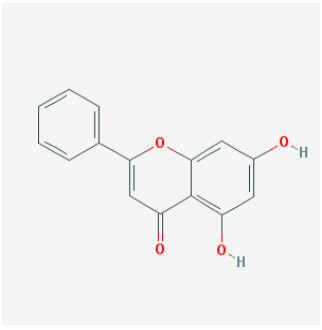
The molecules under study were checked to satisfy Lipinski's Rule of Five by using the data from Pubchem database [6,7].



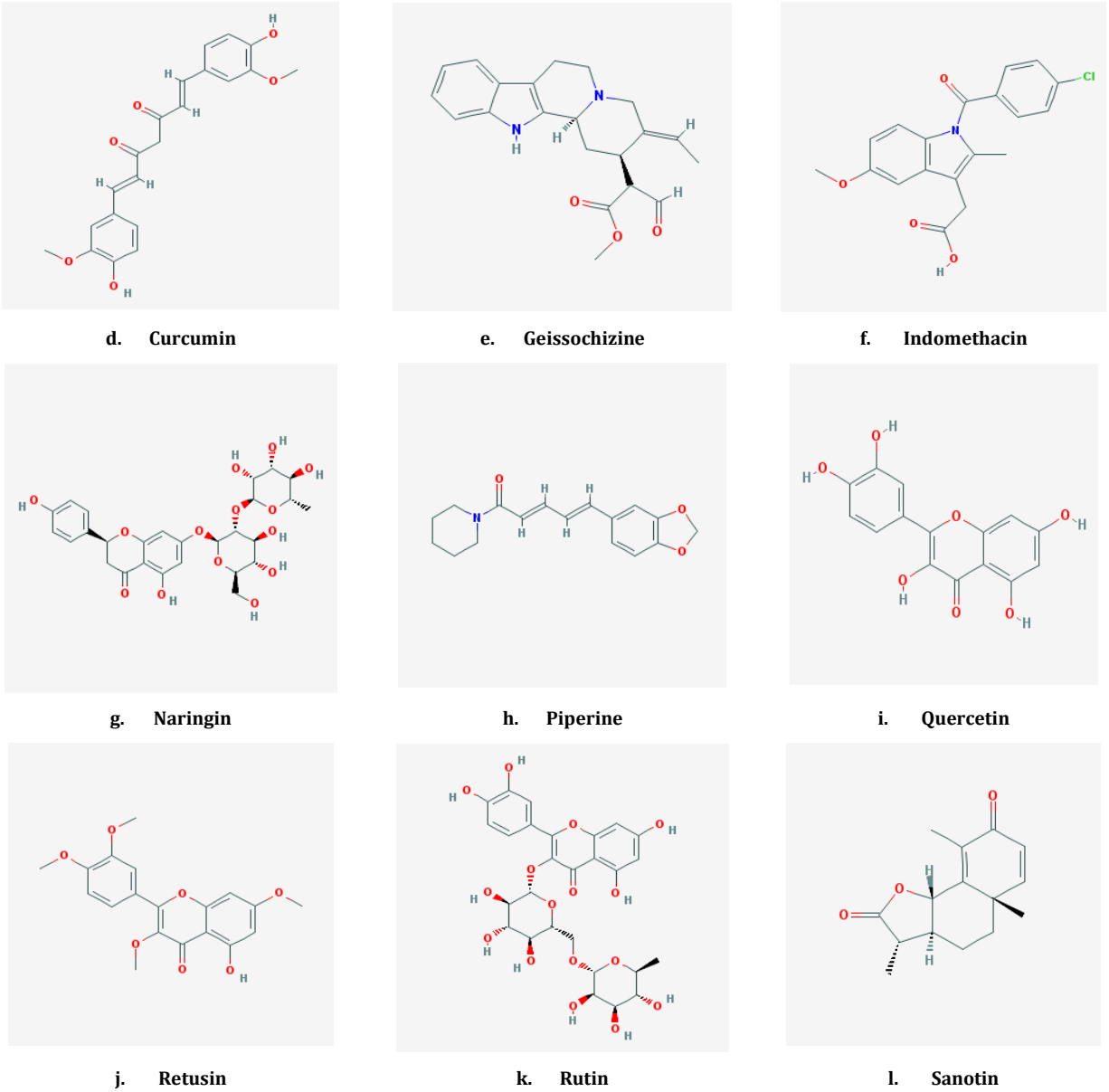
a. Apigenin



b. Celecoxib



c. Chrysin



d. Curcumin **e. Geissochizine** **f. Indomethacin**

g. Naringin **h. Piperine** **i. Quercetin**

j. Retusin **k. Rutin** **l. Sanotin**

Fig. 1: Structure of ligands

Structure validation

Native ligands present in the protein structure were removed. In order to check the confirmation root mean square deviation (RMSD) value was calculated between the original structure and the ligand deleted structure [8].

Analysis of binding

The binding sites for docking were designed such that the entire ligand binding region was included within the GRID. Ligand binding region of the macromolecule was selected by using Autodock tools. Docking analysis of 4COX with the ligands was carried out using Autodock Vina. Throughout the docking study the macromolecule was kept as rigid and ligand molecules were flexible [9].

RESULTS

The structure of cyclooxygenase 2 (4COX) is given in Figure 2.

All the selected molecules except apigenin and naringin satisfied the Lipinski's rule of five. Celecoxib showed an binding affinity of 8.0 Kcal/mol with 4COX whereas Indomethacin showed less binding affinity of 7.0 Kcal/mol. Naringin showed highest binding affinity (8.6 Kcal/mol) followed by rutin (8.3 Kcal/mol). The details are given in Table 1.

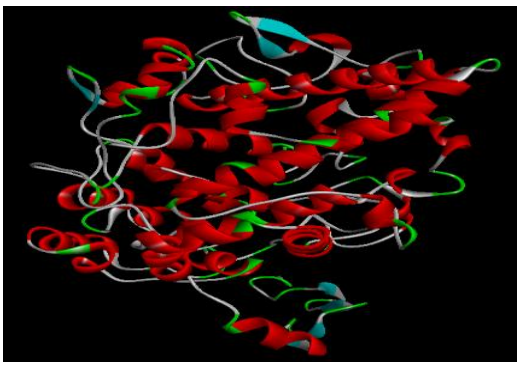


Fig. 2: Structure of cyclooxygenase (4COX)

Table 1: It shows the ligand parameters to satisfy Lipinski's Rule of Five and their binding affinity (Kcal/mol) with Cyclooxygenase (4COX)

Ligands	Molecular formula	Molecular weight	Log P	H-B Donor	H-B Acceptor	Remark	Binding affinity (Kcal/mol)
Santonin	$C_{15}H_{18}O_3$	246.30	2.3	0	3	P	-6.6
Naringin	$C_{27}H_{32}O_{14}$	580.53	0.5	8	16	F	-8.6
Piperine	$C_{17}H_{19}O_3$	285.33	1.5	5	7	P	-6.4
Curcumin	$C_{21}H_{20}O_6$	368.37	1.7	3	5	P	-6.4
Quercetin	$C_{15}H_{10}O_7$	302.23	2.2	1	4	P	-6.7
Apigenin	$C_{15}H_{10}O_5$	270.23	1.3	10	16	F	-6.6
Geissoschizine	$C_{21}H_{24}N_2O_3$	352.42	2.1	2	4	P	-7.2
Rutin	$C_{27}H_{30}O_6$	610.51	3.5	1	7	P	-8.3
Chrysin	$C_{15}H_{10}O_4$	254.23	3.5	0	3	P	-6.7
Retusin	$C_{19}H_{18}O_7$	358.34	3.2	2	6	P	-6.5
Celecoxib	$C_{17}H_{14}F_3N_3O_{25}$	381.37	3.4	1	7	P	-8.0
Indomethacin	$C_{19}H_{16}CINO_4$	357.78	4.3	1	4	P	-7.0

DISCUSSION

COX-2 is induced in inflammation. Celecoxib is a selective COX-2 inhibitor and Indomethacin is a nonselective COX inhibitor. Our finding is in agreement with this as the binding affinity of Celecoxib is more than Indomethacin [10].

This in-silico work makes use of Autodock Vina. Autodock has the ability to predict the interaction of small molecule with molecular targets with reasonable accuracy and speed [2].

Lipinski's rule of five is a rule of thumb to evaluate drug likeness. This rule is formulated by Christopher A. Lipinski in 1997. This rule describes molecular properties important for drug pharmacokinetics in human body. This rule says that an orally active drug has no more than one violation of following criteria i,e. has no more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, partition co-efficient log P less than 5. All the selected molecules passed Lipinski's rule of 5 except apigenin and naringin [6,7].

CONCLUSIONS

It is found that natural molecules can be potential anti-inflammatory agents. Naringin followed by rutin have the binding affinity comparable to that of Celecoxib for cyclooxygenase-2. However, further investigations and in vivo studies are needed for the development of natural COX-2 inhibitors for the treatment of inflammatory disorders.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Siksha O Anusandhan University for providing the facilities for this research work.

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