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Research Article

IN SILICO DOCKING STUDIES ON THE COMPONENTS OF *INONOTUS* SP., A MEDICINAL MUSHROOM AGAINST CYCLOOXYGENASE-2 ENZYME

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ABSTRACT

Objective: *Inonotus* spp., the mushroom is widely present and known for its medicinal property. This mushroom produces several bioactive compounds with various pharmaceutical uses. In the present study, about 8 biologically active compounds from *Inonotus* spp., were analyzed for its drug likeliness based on Lipinski's rule of five and inhibitor property against the cyclooxygenase (COX-2) enzyme, a protein responsible for inflammation.

Methods: The compounds which satisfy the Lipinski's rule namely inotilone, 4-(3,4-dihydroxyphenyl)butanone, and hispidin were subjected to docking experiments using AutoDock 4.2.

Results: Molecular docking studies revealed that inotilone, hispidin, and 4-(3,4-dihydroxyphenyl) butanone bind effectively to the active site region of COX-2 with a binding energy of −6.73, −7.78, and −5.63, respectively.

Conclusion: The in silico studies on compounds reported from *Inonotus* sp., were found to possess potential medicinal value with anti-inflammatory properties and this provides potential insight to develop new COX-2 inhibitors.

Keywords: *Inonotus* spp., Inflammation, Cyclooxygenase-2, AutoDoc.

INTRODUCTION

Fungi are organisms inhabited by all ecological niches of the earth and utilize various substrates as a consequence of their diverse biological and biochemical evolution [1]. The fungal kingdom consists of many species characterized by unique and unusual biosynthetic pathways [2]. Important secondary metabolites are produced by these fungi which are useful in agricultural, pharmaceutical, and industrial sectors [3]. *Inonotus* spp., is a black parasitic fungus belonging to the family of basidiomycetes with its recognized potential as a source of pharmaceuticals and its biotechnological utility [4]. Experimental studies on *Inonotus* spp., show that it produces a wide variety of secondary metabolites such as polyphenolic compounds, triterpenoids, steroids, trametenolic acids, ergosterol peroxide, melanins, etc., [5-7]. These compounds were shown to exhibit anti-tumor, antioxidant, hypoglycemi, and hepatoprotective activities [8]. Extraction of *Inonotus* spp., with ethanol and $CHCl_3$: MeOH produces inonotic acid, inotilone, (E)-4-(3,4-dihydroxyphenyl)but-3en-2-one, hispidin, iso-hispidin, etc., which were found to possess anti-inflammatory activity [9].

Cyclooxygenase (COX)-1 and COX-2 enzymes play an important role in the production of prostaglandins [10]. The COX-1 enzyme helps in protecting the stomach from acids and digestive chemicals, whereas COX-2 enzyme is responsible for inflammation by binding to arachidonic acid [11]. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibits COX-2 enzyme, but produces a wide range of side effects ranging from pain, nausea, indigestion, and lack of thrombotic activity [12]. The drug-likeness of compounds can be predicted by Lipinski's rule of five which refers to the similarity of compounds to oral drugs. The molecular docking process predicts ligand confirmation and orientation within their targeted binding site which holds great promise in the field of computer-based drug design [13]. *In silico*, docking studies on secondary metabolite from *Trichoderma* spp., against the skin cancer protein showed that the compounds heptadecanoic acid, 16 methyl, methyl ester were found to be better inhibitors of cancer protein than the standard drug dyclonine [14]. Diketopiperazine produced by the endophytic fungi *Penicillium* spp., were found to be potent inhibitors of cancer chaperone Hsp90 by *in silico* docking approaches [15].

In the present study, we have studied the drug-likeness of secondary metabolites from the fungus *Inonotus* spp., using Lipinski's rule of five and the binding mechanism of the compounds with COX-2 enzymes using molecular docking studies.

METHODS

Compounds from *Inonotus* spp.

The following compounds from *Inonotus* sp., are (1) Inotilone, (2) Hispidin, (3) 4-(3,4-dihydroxyphenyl) butanone, (4) Retinol (O-acetyl-all-*trans*-), (5) Henecoisane, (6) Hexatriacontane, (7) Docosane, (8) Hexadeconic acid were subjected to docking experiments [16]. The structures and the physiochemical properties of these compounds were taken from the PubChem database (www. ncbi. nlm.nih.gov/pubchem) which is shown in Table 1. Lipinski's rule of five parameters such as molecular

Table 1: Lipinski's properties of the compounds from *Inonotus* spp.,

Serial number	Compound	Molecular weight (<500 Da)	Log P (<5)	H-bond donor (5)	H-bond acceptor (<10)
1	Inotilone	218.21	1.8	2	4
2	Hispidin	246.22	1.7	3	5
3	4-(3,4-dihydroxyphenyl) butanone	180.2	−0.4	2	3
4	Retinol (O-acetyl-all-*trans*-)	328.5	6.3	0	2
5	Henecoisane	296.6	11	0	0
6	Hexatriacontane	506.9	19.1	0	0
7	Docosane	310.6	11.5	0	0
8	Hexadeconic acid	256.42	6.4	1	2

weight, log P, and number of hydrogen bond donors and number of hydrogen bond acceptors were taken from the PubChem database for the *Inonotus* spp., derived compounds.

COX-2 enzyme protein structure

The three-dimensional (3D) structure of the COX-2 enzyme was taken from the Protein Data Bank (PDB) database (www.rcsb.pdb) is given in Fig. 1.

The PDB acts as a repository for the 3D structural data of large biological macromolecules such as proteins and nucleic acids. The PDB ID of COX- 2 enzyme is 6COX, which is a complex of COX-2 enzyme with selective inhibitor compound SC-558. The active site region of the COX-2 enzyme is given in Fig. 2. The docking process was performed using AutoDock 4.2. The pdb coordinates of the protein and the ligand were submitted to AutoDock 4.2. The binding energy and the binding contacts of each ligand were obtained. Analysis of the docked complexes was done using Discovery Studio 3.1 visualizer.

Docking studies - AutoDock 4.2

Molecular docking studies were performed for the selected compounds with COX-2 enzyme by an automated docking tool, AutoDock 4.2. Which works by Lamarckian Genetic Algorithm. The precise interaction of bioactive agents or candidate molecules with their targets is important in the drug development process. AutoDock combines two methods to achieve these goals Rapid grid-based energy evaluation and efficient search of torsional freedom.

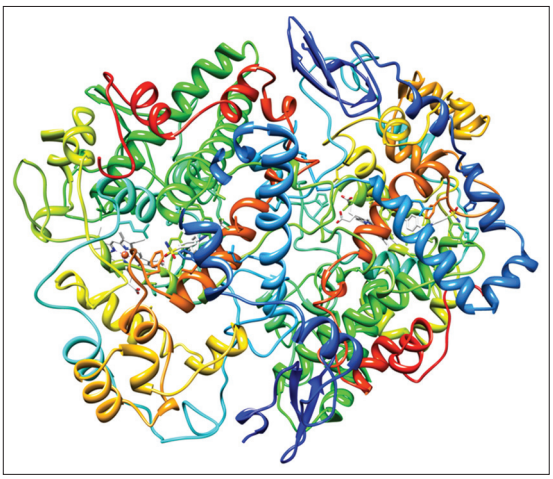

Fig. 1: Cyclooxygenase-2 (COX-2) (Prostaglandin Synthase-2) in complex with a COX-2 selective inhibitor

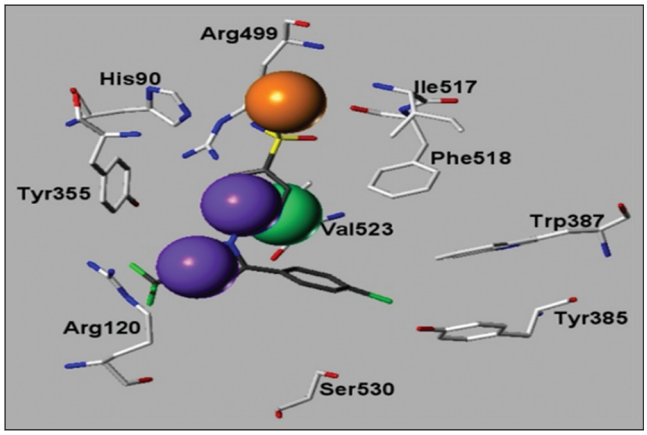

Fig. 2: Cyclooxygenase-2 (COX-2) enzyme in complex with selective inhibitor SC-558 showing the active site region of COX-2 enzyme

Discovery studio visualizer 3.1

Discovery studio visualizer developed by Accelrys is a free, molecular modeling environment, for both small molecule, and macromolecule applications. It generates 2D receptor-ligand interaction plots and analyzes the ligand binding patterns between a protein and its bound ligands.

RESULTS

In silico, studies on compounds from *Inonotus* spp., using AutoDock 4.2 showed the following results. Molecular weight, Log P, Number of H bond donor, and H bond acceptor are tabulated in Table 1. Compounds which obey Lipinski's rule of five are alone subjected to docking experiment. Of the 8 compounds studied, Inotilone, Hispidin, and 4-(3,4-dihydroxyphenyl) butanone satisfy Lipinski's rule of five for drug-likeness. The results are shown in Table 1. The other compounds (4-8 in table) which do not follow the Lipinski's properties were not considered for further docking studies. The structures of drug-likeness compounds from *Inonotus* spp., are shown in Fig. 3

The binding energy for each chosen compound with the COX-2 enzyme using AutoDock 4.2 is given in Table 2. Docking studies show that the ligands bind to the active site region of COX-2 enzyme with good binding energy in the same hydrophobic pocket. The docking models of the selected compounds (1) Inotilone, (2) Hispidin, (3) 4-(3, 4-dihydroxyphenyl) butanone in 3D view are shown in Figs. 4-6. The hydrogen contacts of the ligands are given in Table 3.

DISCUSSION

Cyclooxygenase enzyme plays a key role in the conversion of arachidonic acid to prostaglandins [17]. Prostaglandins regulate pathological processes such as inflammatory and cardiovascular responses [18]. COX-1, a constitutive enzyme is present in mammalian cells and COX-2, an inducible enzyme is found in inflammatory

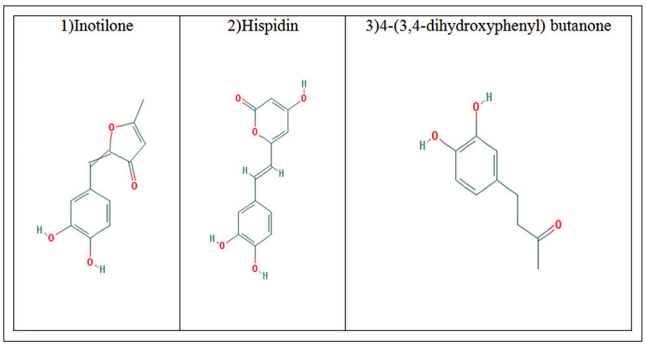

Fig. 3: The structures of compounds (1) Inotilone, (2) Hispidin, (3) 4-(3,4-dihydroxyphenyl) butanone obtained from PubChem database

Table 2: Dock scores of the compounds

Serial number	Compound	Dock score
1	Inotilone	−6.73
2	Hispidin	−7.78
3	4-(3,4-dihydroxyphenyl) butanone	−5.63

Table 3: Hydrogen contacts of the ligands

Serial number	Compounds	Hydrogen contacts
1	Inotilone	His 90, Leu 352, Ser 530
2	Hispidin	His 90, Leu 352, Ser 353, Trp 387, Gly 526.
3	4-(3,4-dihydroxyphenyl) butanone	His 90, Leu 352, Ser 353

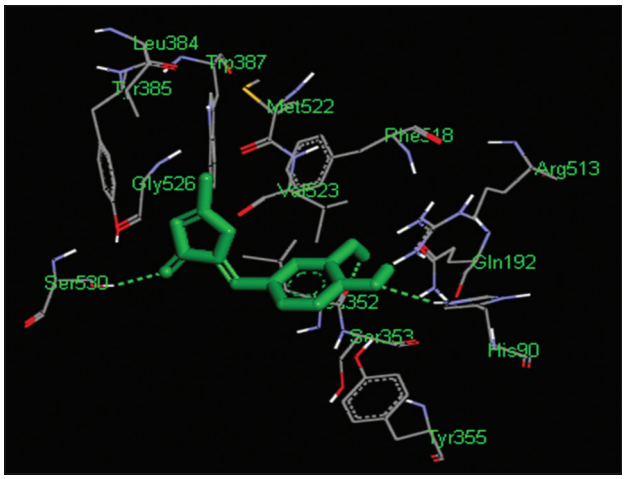

Fig. 4: Docking complex of COX-2 enzyme (PDB ID: 6COX) with Inotilone

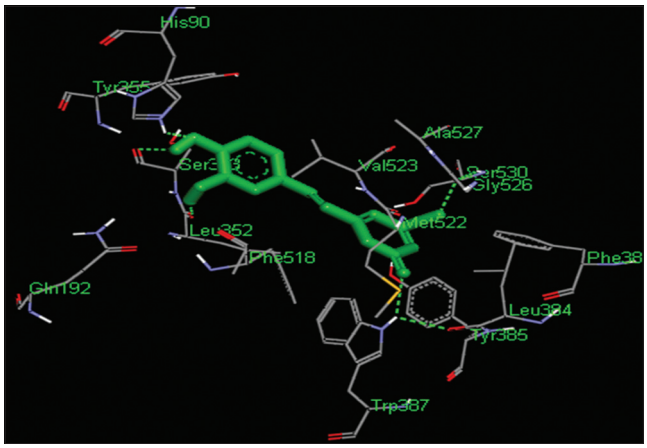

Fig. 5: Docking complex of COX-2 enzyme (PDB ID: 6COX) with Hispidin

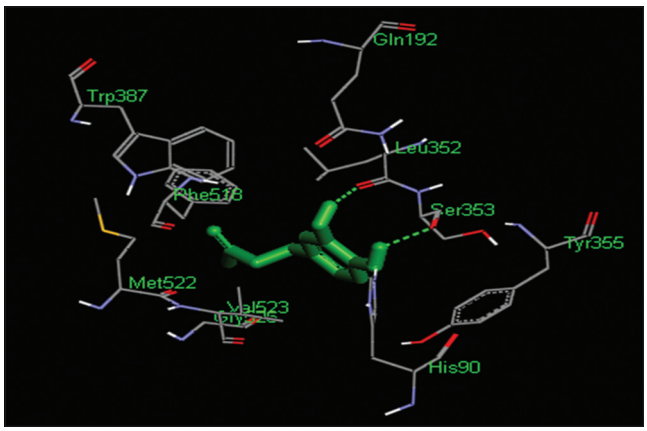

Fig. 6: Docking complex of COX-2 enzyme (PDB ID: 6 COX) with 4-(3,4-dihydroxyphenyl) butanone

sites [19]. Thus, suppressing levels of COX-2 will be an effective method for inhibiting inflammation. Currently, NSAID used widely to control inflammation, and it has been estimated that 30-60% of NSAID users have gastrointestinal side effects and abdominal discomfort [20]. The current study focuses on the *in silico* investigation of natural compounds from the medicinal mushroom *Inonotus* spp., for anti-inflammatory property to avoid any undesirable side effects. The results of docking studies using AutoDock 4.2 shows that out of 8 compounds only three compounds satisfying Lipinski's properties namely inotilone, hispidin, and 4-(3,4-dihydroxyphenyl) butanone bind in the active site region of COX-2 enzyme with good binding energies of −6.73, −7.78, and −5.63, respectively. The strength and the catalytic activity of a binding complex are predicted by their hydrogen bonds between them [21]. The amino acid residues with which hydrogen bonds formed are His 90, Leu 352, Ser 353, Trp 387, and Gly 526. These residues are in the active site region of COX-2 enzyme. Hispidin binds in the catalytic site with more number of hydrogen bonds when compared to other compounds under study. This proves that it has better anti-inflammatory property. Hispidin analog from the mushroom, *Inonotus xeranticus* was proved to have anti-inflammatory property [22]. Inotilone prevents the inflammation-associated tumorigenesis using a classical two-stage mouse skin carcinogenesis model which proves its potential to be developed as an effective chemopreventive agent especially for the treatment of epithelial skin cancer [23]. 4-(3, 4-dihydroxyphenyl) butanone binds in the active site of COX-2 but with less hydrogen bonds and modification of its structure provides better inhibitor of COX-2. *Inonotus* spp., has many medicinal functions and the compounds extracted from them have clinical uses and a detailed study on this is needed. *In silico* analysis on these compounds helps us to understand the binding potential of compounds with the target enzyme. More number of *in silico* studies on secondary metabolites from plants against COX-2 enzyme has been done. The anti-inflammatory activity of secondary metabolites from *Cissus quadrangularis* were elucidated by molecular docking approaches [24]. The diverse pharmacological activities of fungal metabolites are less explored by *in silico* approaches. Molecular docking studies of marine-derived fungal secondary metabolites with Hsp90a cancer protein proves their ability to be used as drug leads for cancer treatment [25]. From this study, it was concluded that the active compounds from *Inonotus* spp., such as Inotilone, Hispidin, and 4-(3,4-dihydroxyphenyl) butanone were more potent COX-2 inhibitors through comparative analysis in this docking experiment which hold lots of promise to develop as COX-2 inhibitor.

To conclude with the compounds from the mushroom *Inonotus* spp. showed better binding features with the COX-2 enzyme. Thus, these compounds can be effectively used as drugs for treating inflammation which is predicted on the basis of docking scores. The insights gained in this work can be further used in experimental studies for designing anti-inflammatory drugs with novel targets and mechanism of action.

REFERENCES

1. Moore D, Robson GD, Trinci A. Biochemistry and developmental biology of fungi. In: 21st Century Guidebook to Fungi. Cambridge: Cambridge University Press; 2011. p. 237-9.
2. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism - from biochemistry to genomics. Nat Rev Microbiol 2005;3(12):937-47.
3. Cole R, Schweikert M. Handbook of Secondary Fungal Metabolites. Amsterdam: Elsevier; 2003. p. 1-3.
4. Kim YO, Park HW, Kim JH, Lee JY, Moon SH, Shin CS. Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of *Inonotus obliquus*. Life Sci 2006;79(1):72-80.
5. Rzymowska J. The effect of aqueous extracts from *Inonotus obliquus* on the mitotic index and enzyme activities. Boll Chim Farm 1998;137(1):13-5.
6. Song Y, Hui J, Kou W, Xin R, Jia F, Wang N, *et al.* Identification of *Inonotus obliquus* and analysis of antioxidation and antitumor activities of polysaccharides. Curr Microbiol 2008;57(5):454-62.
7. Kukulyanskaya TA, Kurchenko NV, Kurchenk VP, Babitskaya VG. Physicochemical properties of melanins produced by the sterile form of *Inonotus obliquus* ("chaga") in natural and cultivated fungus. Appl Biochem Microbiol 2002;38(1):58-61.
8. Mu H, Zhang A, Zhang W, Cui G, Wang S, Duan J. Antioxidative Properties of Crude Polysaccharides from *Inonotus obliquus*. Int J Mol Sci 2012;13(7):9194-206.
9. Wangun K, Vignie H. Isolation, Structure Elucidation and Evaluation of Anti-inflammatory and Anti-infectious Activities of Fungal Metabolites. Ph. D Dissertation, Council of Chemistry and Geo Science Faculty of the Friedrich-Schiller, University Jena; 2006.

10. Lipsky LP, Abramson SB, Crofford L, Dubois RN, Simon LS, van de Putte LB. The classification of cyclooxygenase inhibitors. J Rheumatol 1998;25(12):2298-303.
11. Chajjed SS, Hiwani PB, Bastikar VA, Upasani CD, Udavant PB, Dhake AS, *et al*. Structure based design and in-silico molecular docking analysis of some novel benzamidazoles. Int J Chem Tech Res 2010;2:1135-140.
12. Sudha KN, Shakira M, Prasanthi P, Sarika N, Kumar ChN, Babu PA. Virtual screening for novel COX-2 inhibitors using the ZINC database. Bioinformation 2008;2(8):325-9.
13. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: Methods and applications. Nat Rev Drug Discov 2004;3(11):935-49.
14. Kandasamy S, Sahu SK, Kandasamy K. *In Silico* studies on fungal metabolite against skin cancer protein (4, 5-Diarylisoxazole HSP90 Chaperone). ISRN Dermatol 2012;2012:626214.
15. Sharma R, Vijaya Kumar BS. *In silico* interaction studies on inhibitory action of endophytic fungal diketopiperazine and its related compounds on heat-shock protein 90 (hsp90). Asian J Biomed Pharm Sci 2014;4(28):25-9.
16. Mazurkiewicz W. Analysis of aqueous extract of *Inonotus Obliquus*. Pol Pharm Soc-Drug Res 2006;63(6):497-501.
17. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, *et al*. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 1996;384(6610):644-8.
18. Smith CJ, Morrow JD, Roberts LJ 2nd, Marnett LJ. Differentiation of monocytoid THP-1 cells with phorbol ester induces expression of prostaglandin endoperoxide synthase-1 (COX-1). Biochem Biophys Res Commun 1993;192(2):787-93.
19. Howe LR, Subbaramaiah K, Brown AM, Dannenberg AJ. Cyclooxygenase-2: A target for the prevention and treatment of breast cancer. Endocr Relat Cancer 2001;8(2):97-114.
20. Al Mofleh IA, Al Rashed RS. Nonsteroidal, antiinflammatory drug-induced gastrointestinal injuries and related adverse reactions: Epidemiology, pathogenesis and management. Saudi J Gastroenterol 2007;13(3):107-13.
21. Crabtree RH. A new type of hydrogen bond. J Sci 1998;282:2000-1.
22. Lee YG, Lee WM, Kim JY, Lee JY, Lee IK, Yun BS, *et al.* Src kinase-targeted anti-inflammatory activity of davallialactone from *Inonotus xeranticus* in lipopolysaccharide-activated RAW264.7 cells. Br J Pharmacol 2008;154(4):852-63.
23. Kuo YC. Effects of Inotilone on Inflammation and Inflammation Associated Tumorigenesis, Ph. D Dissertation, Graduate School-New Brunswick Rutgers, The State University of New Jersey, New Jersey; 2010.
24. Meenambiga SS, Rajagopal K. *In silico* studies on plant derived components of *Cissus quadrangularis* against COX-2 enzyme. Int J Pharm Pharm Sci 2014;6(8):483-7.
25. Virupakshaiah DB. Docking of Secondary metabolites derived from marine fungi with Hsp 90a protein in cancer treatment. J Adv Bioinform Appl Res 2014;5(2):92-6.