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Pharmacoinformatics exploration of polyphenol oxidases leading to novel inhibitors by virtual screening and molecular dynamic simulation study

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ABSTRACT

Polyphenol oxidases (PPOs)/tyrosinases are metal-dependent enzymes and known as important targets for melanogenesis. Although considerable attempts have been conducted to control the melanin-associated diseases by using various inhibitors. However, the exploration of the best anti-melanin inhibitor without side effect still remains a challenge in drug discovery. In present study, protein structure prediction, ligand-based pharmacophore modeling, virtual screening, molecular docking and dynamic simulation study were used to screen the strong novel inhibitor to cure melanogenesis. The 3D structures of PPO1 and PPO2 were built through homology modeling, while the 3D crystal structures of PPO3 and PPO4 were retrieved from PDB. Pharmacophore modeling was performed using LigandScout 3.1 software and top five models were selected to screen the libraries (2601 of Aurora and 727, 842 of ZINC). Top 10 hit compounds (C1-10) were short-listed having strong binding affinities for PPO1-4. Drug and synthetic accessibility (SA) scores along with absorption, distribution, metabolism, excretion and toxicity (ADMET) assessment were employed to scrutinize the best lead hit. C4 (name) hit showed the best predicted SA score (5.75), ADMET properties and drug-likeness behavior among the short-listed compounds. Furthermore, docking simulations were performed to check the binding affinity of C1-C10 compounds against target proteins (PPOs). The binding affinity values of complex between C4 and PPOs were higher than those of other complexes (−11.70, −12.1, −9.90 and −11.20 kcal/mol with PPO1, PPO2, PPO3, or PPO4, respectively). From comparative docking energy and binding analyses, PPO2 may be considered as better target for melanogenesis than others. The potential binding modes of C4, C8 and C10 against PPO2 were explored using molecular dynamics simulations. The root mean square deviation and fluctuation (RMSD/RMSF) graphs results depict the significance of C4 over the other compounds. Overall, bioactivity and ligand efficiency profiles suggested that the proposed hit may be more effective inhibitors for melanogenesis.

1. Introduction

Polyphenol oxidases (PPOs), also known as tyrosinases, are membrane bound copper containing enzymes which regulate the melanin biosynthesis in melanocytes (Mayer, 2006). PPOs/tyrosinases are bifunctional enzymes which actively take part in two stepwise biochemical reactions for melanin synthesis in the presence of molecular oxygen (Ando et al., 2007; Nerya et al., 2003). Firstly, it catalyzes the hydroxylation of monophenols. Secondly, it aids in the conversion of *o*-diphenol to respective *o*-quinone which is directly involved in the synthesis of brown or black melanin (Kumar et al., 2010). The human skin color is directly controlled by the type of melanin and its distribution patterns around the keratinocytes. Melanin also controls the human hair and eye color (Parvez et al., 2006). Expression studies revealed that PPOs are highly expressed in melanocytes located at the epidermal

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junction region (Costin and Hearing, 2007; Hearing, 2011; Mort et al., 2015). In humans, melanin plays a significant role in skin protection against radiation. But the abnormal melanin accumulation causes pigmentation disorders such as melasma, freckles and senile lentigines (Slominski et al., 2004).

The PPOs/tyrosinases connection in the melanin production justifies their significance as target molecules to cure melanin-associated disorders. Recently, it has been observed that six tyrosinase isoforms are encoded by six genes located on two different chromosomes (PPO1 and 6 on chromosome 8; PPO2-5 on chromosome 12) of common edible mushroom (*Agaricus bisporus*) (Wichers et al., 2003; Wu et al., 2010).

To cure the melanin-associated diseases, large numbers of natural and synthetic potent tyrosinase inhibitors have been reported previously (Khan, 2012; Bao et al., 2010; Kim and Uyama, 2005; Solano et al., 2006; Chang, 2009). Moreover, standard tyrosinase inhibitors (arbutin, kojic acid and hydroquinones) were also used to cure hyperpigmentation by suppressing the dermal melanin production (Maeda and Fukuda, 1991; Jimbow et al., 1974; Zhu and Gao, 2008). They showed good therapeutic potential against melanin-associated disorders, but their toxicity and tumorigenicity risk were also observed at high-dose concentrations in humans (Cheng et al., 2006; Burdock et al., 2001).

In our present work, *in-silico* drug screening approaches were applied for the identification of novel tyrosinase inhibitors through Pharmacophore modeling, virtual screening, molecular docking and dynamic simulation. Four target receptors PPO1-4 were selected to screen the best tyrosinase potent inhibitors. The PPO1 and PPO2 crystal structures are absent in protein Data Bank (PDB). Therefore, homology modeling based approach was used to build their three dimensional (3D) structures using MODELLER 9v8 tool. The crystal structures of PPO3 and PPO4 were retrieved from PDB database (2Y9X and 4OUA, respectively). All the PPOs structures were further validated on the basis of stereo-chemical properties and rotamers/outliers positioning by using different online servers. The architecture and statistical percentage values of helices, beta sheets, coils and turns in PPOs were determined by using online tool VADAR 1.8. The 16 potent tyrosinase inhibitors were selected from data mining (Ley and Bertram, 2001; Criton and Le Mellay-Hamon, 2008; Devkota et al., 2007; Loizzo et al., 2012; Lee et al., 2005; Song et al., 2007; Shin et al., 1998; Ohguchi et al., 2003; Yokozawa and Kim, 2007; Likhitwitayawuid et al., 2006; Schmaus et al., 2006; Oozeki et al., 2008) and were employed for pharmacophore modeling and virtual screening using LigandScout 3.1. Top ten screening hits were selected for further chemo-informatic, docking and MD studies. Multiple computational approaches were used to predict their drug-likeness scores and to validate Lipinski Rule of 5. Moreover, their Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties along with tumorigenic/mutational effects were also calculated by online tools. In addition, docking and MD simulation techniques were utilized to assess the most active compound on the basis of binding energy values, RMSD, RMSF, radius of gyration and SASA analyses. Therefore, potent and novel candidate inhibitors to cure the melanin associated diseases were selected through comparative ligand-based approaches.

2. Methodology

2.1. Dataset

Four structures of mushroom (*A. bisporus*) PPOs were utilized to explore the novel inhibitor against melanogenesis. The 3D crystal structures of PPO1 and PPO2 (tyrosinases) are not reported in Protein Data Bank database (http://www.rcsb.org). A homology modeling approach was employed to predict 3D structures of PPO1 and PPO2. The amino acid sequences in FASTA format of PPO1 (568 AA, ID: Q00024) and PPO2 (556 AA, ID: O42713) were retrieved from UniProt Knowledge database (http://www.uniprot.org/). It has been observed that the selection of the correct template is most significant parameter to predict the good protein model (Sadowski and Jones, 2007). The template PPO4 structure (PDBID # 4OUA) was selected for prediction of PPO1 and PPO2 3D structures on the basis of sequence identities (33% and 63%, respectively). By using this template structure, 3D structures of mushroom tyrosinase PPO1 and PPO2 were modeled by using MODELLER 9v8 tool (Eswar et al., 2008). The two copper ions were adjusted in each predicted structure using Java based PDB Editor tool (Lee and Kim, 2009). Two crystal structures PPO3 and PPO4 were retrieved from PDB database with PDB IDs (2Y9W and 4OUA, respectively). Furthermore, the reliability and validity of PPOs structures were confirmed by using various evaluation tools like ERRAT (Colovos and Yeates, 1993), SAVES (Eisenberg et al., 1997) and MolProbity (Chen et al., 2010). The poor rotamers and outliers were removed to obtain optimized and reliable structures. Avogardo (Hanwell et al., 2012) and UCSF Chimera 1.10.1 (Pettersen et al., 2006) were utilized for energy minimization and visualization of PPOs structures, respectively. Structural superimposition was performed by UCSF Chimera 1.10.1. The statistical percentage values of helices, beta-sheets, coils and turns of PPOs were calculated by using online tool VADAR 1.8 (Willard et al., 2003).

2.2. Pharmacophore model generation

To design the ligand-based pharmacophore models, 16 potent inhibitors were selected on the basis of inhibition constant (IC_{50}) values from literature mining (Ley and Bertram, 2001; Criton and Le Mellay-Hamon, 2008; Devkota et al., 2007; Loizzo et al., 2012; Lee et al., 2005; Song et al., 2007; Shin et al., 1998; Ohguchi et al., 2003; Yokozawa and Kim, 2007; Likhitwitayawuid et al., 2006; Schmaus et al., 2006; Oozeki et al., 2008) as mentioned in supplementary Table S4. Our literature reviews justified that all 16 compounds have good therapeutic potential against melanogenesis. Molecular dockings studies were performed on these selected compounds against PPO1-4 separately by AutoDock VINA with default parameters (Dallakyan and Olson, 2015). The best docked complexes were screened for pharmacophore generation on the basis of good binding energy values. LigandScout 3.1 (Wolber and Langer, 2005) was employed to generate the pharmacophore models from training set. The pharmacophoric sites such as hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), aromatic ring, hydrophobic sites and positive and negative ionizable groups were prudently characterized. Merge feature model generation and atom overlap scoring function of LigandScout 3.1 were applied to incorporate the features of selected compounds. The top five models were selected for further compound screening/evaluation.

2.3. Virtual screening

The five best-fitting models exhibiting good score values were selected for virtual screening. The 3D pharmacophore models were screened against a total of 730,443 compounds (2601 of Aurora, http://www.aurorafinechemicals.com and 727, 842 of ZINC libraries, Irwin and Shoichet, 2005) to identify novel drug targets. Furthermore, novel screened compounds were utilized for docking analysis on the basis of best fit values relative to pharmacophore models.

2.4. Chemo-informatics and ADMET properties of screened compounds

The best ten novel screening hits were selected for chemo-informatics and ADMET properties. Multiple online servers like