Development of Tyrosinase Inhibitors

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I, Sini Radhakrishnan, certify that the work in this thesis has not been previously submitted for a degree nor has it been submitted as part of requirements for a degree.

I also certify that the thesis has been written by myself. Any assistance that I have received in my research work and the preparation of the thesis itself has been acknowledged. Furthermore, I certify that all information sources and literature used are indicated in the thesis.

Sini Radhakrishnan

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ABSTRACT

The primary objective of this project was the drug discovery, synthesis and biological evaluation of a series of tyrosinase inhibitors. Chalcone skeleton was the chosen pharmacophore and a library of potent inhibitors of tyrosinase (polyphenol oxidase) were synthesized and their structure-activity (SAR) relationships were explored. Chalcone derivatives were synthesized by simple base catalyzed Claisen-Schmidt condensation of an aldehyde and an appropriate ketone in a polar solvent like methanol. The structures of the compounds synthesized were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and HRMS. Two compounds that are the reduction congeners of the pyridinyl azachalcones strongly inhibited the enzyme activity and were more potent than the positive control kojic acid. Two of the hydroxyazachalcones synthesized inhibited the diphenolase activity of tyrosinase and were identified as competitive reversible inhibitors and with their K_i values of 3.4 μM and 3.9 μM, respectively. This study showed that a more potent tyrosinase inhibitor can be obtained from methoxyazachalcones with a single step dealkylation reaction. A series of novel hydroxynaphthylchalcone compounds were synthesized and inhibited the diphenolase activity of tyrosinase in a dose dependent manner with much higher tyrosinase inhibitory activities than the positive control, kojic acid. The number and position of methoxy substituents on the aromatic rings appeared to be critical for cytotoxicity. Also placement of strongly electron-withdrawing groups such as NO_2 in ring B correlated with increase of cytotoxic activity. This study provides valuable information in utilizing methoxychalcone as a lead compound for the further design and development of potential tyrosinase inhibitors with antimelanogenic effects. Two pyridinyl methoxychalcone compounds exhibited higher tyrosinase inhibitory activities (IC_{50} values of 10.6 μM and 12.5μM, respectively) than the control kojic acid (IC_{50}: 22.83μM). Kinetic studies revealed them to act as competitive tyrosinase inhibitors. Both the compounds inhibited melanin production and tyrosinase activity in B16 cells. Docking results confirm that the active inhibitors strongly interact with mushroom tyrosinase residues. The current study portrays methoxychalcones with electrophilic character to be potent agents with antimelanogenic effects. A series of hydroxy substituted chalcone oxime derivatives have been synthesized and evaluated for their inhibitory activities on tyrosinase and melanogenesis in murine B16F10 melanoma cell lines. A library of 2′-acetylpyridinyl chalconeoxime compounds was also synthesized.

The study portrayed the significance of an oxime moiety on the chalcone framework that brought about better coordination with the copper metal centers at the active site of mushroom tyrosinase and also exhibited stronger hydrogen bonding interactions with the amino acid residues. From all the compounds synthesized, a novel 2′-acetylpyridinyl chalconeoxime compound exhibited the most potent tyrosinase inhibitory activity with an IC_{50} value that was found to be several times potent than the reference standard, kojic acid. The inhibition mechanism was competitive and was in complete agreement with the docking results. Furthermore, a solid-state based mechanochemical process was used to synthesize novel azachalcones and their oximes as tyrosinase inhibitors. Both the novel oxime compounds displayed competitive inhibition with their K_i values of 5.1μM and 2.5μM, respectively. This method minimizes waste disposal problems and provides a simple, efficient and benign method for the synthesis of novel tyrosinase inhibitors for use as skin whitening agents or as anti-browning food additives. The effects of novel 2,3-dihydro-1*H*-inden-1-one chalcone-like compounds for tyrosinase inhibition were studied. Hydroxy substituted 1-indanone chalcone-like compounds were found to be significantly more potent than kojic acid.

Assays were performed with L-DOPA as the substrate, using kojic acid, a well-known strong tyrosinase inhibitor as the positive control. The kinetic parameters and inhibition mechanisms of active tyrosinase inhibitors were illustrated with the help of Lineweaver-Burk plots and Dixon plots. Furthermore, the experimental results were integrated with simulation studies using Accelrys Discovery Studio 4.5 suite. Few of the active tyrosinase inhibitors were further evaluated for the *in vitro* cytotoxic activity on B16 F10 melanoma cell lines. The results suggested substituted chalcone derivatives to serve as an interesting candidate for the treatment of tyrosinase related disorders and as the lead compounds for the development of new and potent tyrosinase inhibitors.

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LIST OF PAPERS PUBLISHED

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LIST OF ABBREVIATIONS

DHI	5, 6-dihydroxyindole
DMSO	Dimethyl sulfoxide
DHICA	5, 6-dihydroxyindole-2-carboxylic acid
UV	Ultraviolet
DOPA	3, 4-dihydroxyphenylalanine
TOPA	2, 4, 5-trihydroxyphenylalanine
DHICA	5, 6-dihydroxyindole-2-carboxylic acid
TRP-1	tyrosinase related protein-1
PPO	polyphenol oxidase
HC	hemocyanins
GHB	glutaminyl-4-hydroxybenzene
NMR	Nuclear magnetic resonance
HRMS	High resolution mass spectroscopy
FTIR	Fourier transform infra-red
PDB	Protein data bank
SAR	Structure activity relationship
RT	Room temperature
TLC	Thin layer chromatography
SE	Standard error
Mp	Melting point
DNA	Deoxyribonucleic acid
ROS	Reactive oxygen species
MeOH	Methanol
MTT	3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide