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Research paper

Kinetic and in silico studies of novel hydroxy-based thymol analogues as inhibitors of mushroom tyrosinase

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ABSTRACT

The present studies reports the synthesis of hydoxylated thymol analogues **(4a–e)** and **(6a–c)** as mushroom tyrosinase inhibitors. The title compounds were obtained in good yield and characterized by FTIR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and X-ray crystallography in case of compound **(6a)**. The inhibitory effects on mushroom tyrosinase and DPPH were evaluated and it was observed that 2-[5-methyl-2-(propan-2-yl)phenoxy]-2-oxoethyl (2E)-3-(4-hydroxyphenyl)prop-2-enoate **(6b)** showed tyrosinase inhibitory activity (IC_{50} 15.20 μM) comparable to kojic acid (IC_{50} 16.69 μM) while 2-[5-methyl-2-(propan-2-yl)phenoxy]-2-oxoethyl 3,4-dihydroxybenzoate **(4d)** exhibited higher antioxidant potential (IC_{50} 11.30 μM) than standard ascorbic acid (IC_{50} 24.20 μM). The docking studies of synthesized thymol analogues was also performed against tyrosinase protein (PDBID 2ZMX) to compare the binding affinities with IC_{50} values. The predicted binding affinities are in good agreement with the IC_{50} values as compound **(6b)** showed highest binding affinity −7.1 kcal/mol. The kinetic mechanism analyzed by Lineweaver–Burk plots exhibited that compound **(4d)** and **(6b)** inhibit the enzyme by two different pathways displayed mixed-type inhibition. The inhibition constants *Ki* calculated from Dixon plots for compounds **(4d)** and **(6b)** are 34 μM and 25 μM respectively. It was also found from kinetic analysis that derivative **(6b)** formed reversible enzyme inhibitor complex. It is propose on the basis of our investigation that title compound **(6b)** may serve as lead structure for the design of more potent tyrosinase inhibitors.

1. Introduction

Tyrosinase (EC 1.14.18.1) as a binuclear copper-containing metalloenzyme catalyzes two distinct reactions of melanin biosynthesis. It catalyzes L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and further oxidation of (L-DOPA) to dopaquinone which then transformed through several reactions into brown to black melanin. The distribution patterns of melanin in the surrounding keratinocytes determine the color of human skin [1,2]. Kadekaro et al. and Petit et al. also reported that there are certain other factors such as UV exposure, α-melanocyte-stimulating hormone, melanocortin 1 receptor and agouti-related protein which involved in melanin biosynthesis [3,4]. Abnormal melanin production such as observed in melasma, freckles, lentigo senilis, and other forms of melanin hyperpigmentation can be a serious aesthetic problem often causing emotional disturbance [5–7]. Taking into account the key role of tyrosinase in melanin production, many tyrosinase inhibitors have found application in cosmetics and pharmaceutical products [8–10]. A large number of tyrosinase inhibitors have been reported but only a few are used because of their limitations with regards to cytotoxicity, selectivity and stability. Thus, it is in great need of developing new tyrosinase inhibitors without causing adverse reactions.

A number of cinnamic acid and benzoic acid analogues possessing hydroxy groups at position 3 and 4 of the phenyl ring have been reported as antioxidants and mushroom tyrosinase inhibitors [11,12]. Liu (2003) and Chen (2005) also reported that alkoxy benzoic acids and hydroxy benzoic acids showed potent mushroom tyrosinase inhibitory activity [13,14]. Thymol a naturally occurring monoterpene phenol which is the main constituent of thyme

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possesses tyrosinase inhibitory activity [15] along with other numerous pharmacological activities. As phenolic antioxidants, thymol protects food qualities and organisms from damage induced by oxidative stress. Thymol is used as meat preservatives or flavoring agent in the food industry. It exhibited anti-inflammatory effect in human neutrophils incubated [16] and also inhibits the formation of lethal products through reactive nitrogen species [17]. Thymol displays antimicrobial [18–20] and wound-healing activity [21] and is able to rise the levels of macrophage migration inhibitory factor (MIF) in central nervous system [22]. Thymol increased the in vitro fibroblast growth [23], effectively inhibited COX-1 [24] and prevented inducible lymphocyte proliferation [25].

Keeping in view the importance of these structural features we have synthesized the hydroxylated thymol analogues having benzoic acid and cinnamic acid moieties in order to discover their tyrosinase inhibitory potential to offer a source for the development of new effective tyrosinase inhibitors. The antioxidant activity was also carried out as most of the clinically used tyrosinase inhibitors kojic acid, arbutin, kaempferol, hydroquinone, etc. all possess antioxidant activity. In addition to evaluate the tyrosinase inhibitory potential and antioxidant activity of synthesized hydroxylated thymol derivatives molecular docking was carried out to predict the position of the synthesized compounds in the active site of the 3D structure of tyrosinase (PDB ID 2ZMX). The investigation of the binding interactions during docking analysis between ligand-protein functionalities is important to elucidate the possible molecular mechanism [26,27].

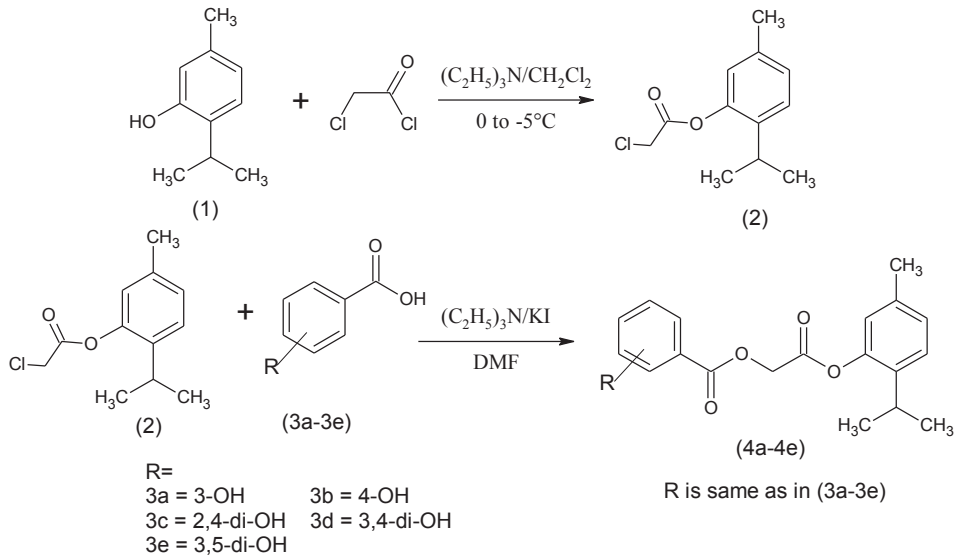
2. Results and discussion

2.1. Chemistry

The title compounds **(4a–e)** and **(6a–c)** were synthesized by following the already reported method [28] with some modification shown in Schemes 1 and 2. The thymol chlroacetyl derivative **(2)** was synthesized by esterification of phenolic –OH group of thymol with chloroacetyl chloride in the presence of $(C_2H_5)_3N$ and anhydrous methylene chloride as solvent. The presence of ester carbonyl stretching at 1723 cm^{-1} and disappearance of the –OH stretching in FTIR spectra confirmed the formation intermediate **(2)**. The final products **(4a–e)** and **(6a–c)** were prepared by simple nucleophilic substitution at intermediate **(2)** with hydroxy substituted benzoic acids **(3a–e)** and substituted and unsubstituted cinnamic acids **(5a–c)** respectively. All of the synthesized compounds have been characterized by FTIR, ^{1}HNMR, ^{13}CNMR and Mass spectroscopic data. Compound **(6a)** yielded single crystals, suitable for X-ray diffraction studies. The X-ray diffraction analysis also confirmed the formation of the desired product and this is an ambiguous evidence of the structure.

2.2. Bioassay for tyrosinase inhibitory activity

Hydroxylated thymol analogues have been designed to evaluate their inhibitory effects on mushroom tyrosinase activity. Kojic acid a competitive tyrosinase inhibitor was used as standard for comparison purpose. The hydroxylated tyrosinase inhibitors as described in preceding section are of special interest because of their high activity (IC_{50} < 10 μM). Novel thymol analogues **(4a–e)** and **(6a–c)** have been synthesized by incorporation of hydroxylated benzoic acids and cinnamic acids. The synthesis of mono and di-hydroxylated derivatives with different position of –OH at phenyl ring was carried out to explore the role of multiple hydroxyl groups in tyrosinase inhibition. It has been exposed from our bioassay results (Table 1) that the major determining factor of inhibitory activity is the position and not the number of the hydroxyl groups. Interestingly, compound **(4d)** bearing 3,4-dihydroxy substituted benzoic acid moiety showed higher activity (IC_{50} 45.0 μM) than **(4c)** and **(4e)** having IC_{50} 56.1 and 220.9 μM respectively. The derivatives **(4c)** and **(4e)** possess 2,4- and 3,5-dihydroxy substituted benzoic acid residues respectively. In case of compound **(4d)** two hydroxy groups are present on adjacent positions of phenyl ring; this impedes the molecule to interact well with enzyme. This structural feature can be well correlated with L-DOPA which is used as substrate for tyrosinase enzyme during bioassay. Thus compound **(4d)** because of close structural similarities with L-DOPA is more active among the dihydroxylated thymol analogues. Table 1 presented the IC_{50} values of the synthesized thymol analogues and it was observed that kojic acid is more active than all of the synthesized thymol analogues except compound **(6b)**. Compound **(6b)** showed excellent tyrosinase inhibitory activity with IC_{50} 15.2 μM and is so inhibitor as kojic acid (IC_{50} 16.69 μM). The synthesized thymol derivatives **(6a)**, **(6b)** and **(6c)**



Scheme 1. Synthesis of thymol analogues **(4a–e)**.