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Research paper

Chemical exploration of 4-(4-fluorobenzyl)piperidine fragment for the development of new tyrosinase inhibitors

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ABSTRACT

Tyrosinase is involved in the production of melanin through the hydroxylation of monophenols to *o*-diphenols. The role of this enzyme was extensively studied in order to identify new therapeutics preventing skin pigmentation and melanoma. In this work we initially identified the 3-(4-benzylpiperidin-1-yl)-1-(1*H*-indol-3-yl)propan-1-one (**1a**) as promising mushroom tyrosinase inhibitor (IC_{50} = 252 μM). Then, several chemical modifications were performed and new analogues related to compound **1a** were synthesized. Biochemical assays demonstrated that several obtained compounds proved to be effective inhibitors showing IC_{50} values lower both than "lead compound" **1a** and reference inhibitor kojic acid, as a well-known tyrosinase inhibitor. The inhibition kinetics analyzed by Lineweaver–Burk plots revealed that compounds **2 a-c** and **10b** act as non-competitive inhibitors while the most active inhibitor **2d** (IC_{50} = 7.56 μM) is a mixed-type inhibitor. Furthermore, experimental and computational structural studies were performed in order to clarify the binding mode of the derivative **2d**.

1. Introduction

Tyrosinase (monophenol monooxygenases, EC 1.14.18.1) is a multifunctional enzyme widely distributed in nature and containing two copper ions coordinated with histidine residues in the active site. Tyrosinase is the key enzyme in the melanin biosynthesis, the main pigment commonly observed in bacteria, fungi, plants, and animals. Melanin is also responsible for human skin color [1–3]. The biosynthetic process for melanin formation occurs in two distinct reactions [4–6]. In the first two steps tyrosinase catalyzes both the hydroxylation of monophenol L-tyrosine to *o*-diphenol 3,4-dihydroxyphenylalanine (L-DOPA) (monophenolase activity) and the further oxidation of L-DOPA to *o*-quinone (diphenolase activity). Then dopaquinone, a highly reactive compound determinant in the melanogenesis, rapidly and spontaneously evolves towards the formation of various melanin pigments.

Although the melanin production shields the human skin from UV radiation, inhibiting photocarcinogenesis and affecting the synthesis of the vitamin D3 [7], an excessive accumulation of epidermal pigmentation, such us senile lentigines, freckles, ephelide, melasma and other melanin hyperpigmentation, causes serious esthetic problems in human beings [8,9].

Interestingly, it was recently demonstrated that tyrosinase is not only involved in the melanin synthesis of peripheral tissues but also in the substantia nigra (SN) of mice and humans playing a relevant role in the brain neuromelanin formation [10]. In this case, an excessive production of dopaquinones, by oxidation of dopamine, results in neuronal damage and cell death, linking tyrosinase to Parkinson's and other neurodegenerative diseases [11–13]. Thus, it extensively was highlighted the relevance of tyrosinase and there is an active interest among researchers to identify enzymatic inhibitors useful in clinical therapeutic applications as well as in cosmetic industry [14–16]. To achieve this goal different chemical classes of compounds, occurring from several sources, such as flavonoids [17–20], stilbene derivatives [21–24], kojic acid [25–29],

Abbreviations: L-DOPA, *o*-Diphenol 3,4-dihydroxyphenylalanine; SN, Substantia nigra.

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tropolone [30] and novel synthetic compounds [31–38], have been investigated. Obviously, more efforts are still needed in this direction and, therefore, we recently focused our interest to this biological target.

In a previous paper [39] we have reported the serendipitous discovery of two indole derivatives (**CHI 1043** and **CHI 1164**, Fig. 1) able to inhibit the mushroom tyrosinase, displaying IC_{50} values of 224 and 372 μM, respectively.

Searching for further tyrosinase inhibitors from synthetic source, we herein describe the development of a novel series of indole derivatives in which several structural modifications were carried out. The main goal was to improve the inhibitory effects and expand our knowledge about chemical structural requirements controlling the interaction with the enzyme. Thus, the synthesized compounds were screened as mushroom tyrosinase inhibitors and their mechanism of action was explored. Moreover, experimental and computational analyses have been performed to highlight their interactions within the catalytic binding site.

2. Results and discussion

2.1. Design, synthesis and evaluation of tyrosinase inhibitory activity

In the first step of this research we selected several indole-compounds by means of a screening campaign throughout our CHIME 1.5 database, which consists of a collection of small molecules synthesized in our laboratory in the last decade. Mushroom tyrosinase has been employed for the estimation of inhibitory effects. The best effective compound was the 3-(4-benzylpiperidin-1-yl)-1-(1*H*-indol-3-yl)propan-1-one (**1a**) (Fig. 2) which proved to inhibit the diphenolase activity showing IC_{50} value of 255 μM.

Hence, we chose the active compound **1a** for generating the first series of indole analogues possessing the methoxy substituent at 5 and/or 6 position of the indole nucleus and fluorine atom on the benzyl ring (Fig. 2).

Scheme 1 summarizes the synthetic pathway employed to obtain the title compounds **1 b-d** and **2 a-d** prepared according to the previously reported method for the synthesis of "lead structure" **1a** [40].

As depicted in Scheme 1 the appropriate indole **3 a-d** was converted into the corresponding 3-acetyl derivative **4 a-d** under Vilsmeier–Haack conditions, using phosphoryl chloride and an excess of *N*,*N*-dimethylacetamide. In the next step, compounds **1 a-d** and **2 a-d** were prepared by a simple Mannich reaction between the 3-acetylindole intermediates **4 a-d**, the suitable amine derivatives and paraformaldehyde.

Inhibitory effects of indole derivatives **1 b-d** and **2 a-d** on mushroom tyrosinase activity have been evaluated and the results of the biochemical assays are summarized in Table 1. Kojic acid and 3-(4-benzylpiperidin-1-yl)-1-(1*H*-indol-3-yl)propan-1-one (**1a**) were used as reference compounds for comparative purpose.

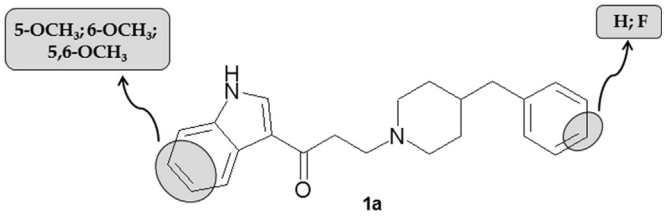

Fig. 2. The "lead compound" 3-(4-benzylpiperidin-1-yl)-1-(1*H*-indol-3-yl)propan-1-one (**1a**) retrieved from CHIME 1.5 database and the designed structural modifications.

The obtained data revealed that the first series of indole compounds **1 b-d** and **2 a-d** were effective inhibitors both of monophenolase and diphenolase activity, thus displaying IC_{50} values lower than compound **1a**. Notably derivatives **2 a-d** exhibited interesting inhibitory effect resulting more potent than kojic acid (see Table 1).

On the basis of data displayed in Table 1, the following structure-activity relationships might be drawn for this series of indole derivatives based on **1a** as lead structure.

By comparison of inhibitory effects of **1a** with the analogues **1 b-d** we can observe that the introduction of a methoxy substituent at 5- and/or 6-position of benzene fused ring generally improves the inhibitory potency toward monophenolase activity.

In addition, as depicted in Table 1 the 3-(4-benzylpiperidin-1-yl)-1-(5-methoxy-1*H*-indol-3-yl)propan-1-one (**1b**) was more potent that prototype **1a** against the two steps of melanin biosynthesis. Particularly, it was seven-fold more active than lead compound **1a** against the monophenolase activity (IC_{50} = 25.78 μM *vs* IC_{50} = 175 μM).

Furthermore, the presence of a fluorine atom at *para*-position of the benzyl moiety induces a significant improvement of activity so that the indoles **2 a-d** were up to 35-fold more potent than analogs **1 a-d** against diphenolase activity. Particularly, the most potent analogue was the 1-(5,6-dimethoxy-1*H*-indol-3-yl)-3-(4-(4-fluorobenzyl)piperidin-1-yl)propan-1-one (**2d**), in which the 5,6-dimethoxy substitution is combined with the introduction of a fluorine atom.

These data inspired us the synthesis of a further series of potential inhibitors maintaining the 3-(4-(4-fluorobenzyl)piperidin-1-yl)propan-1-one tail linked to different aromatic fragments. Pursuing the objective to explore the impact on inhibitory effects of the removal of indole nucleus of the most active compound **2d**, we planned the insertion of benzymidazole or (hetero)arylamino chemical portions. Thus, the designed *N*-substituted 3-(4-(4-fluorobenzyl)piperidin-1-yl)propanamides **7** and **10 a-c** have been synthesized as depicted in Scheme 2.

The synthesis of the 1-(1*H*-benzo[*d*]imidazol-1-yl)-3-(4-(4-fluorobenzyl)piperidin-1-yl)propan-1-one **7** (Scheme 2-A) has been carried out in two steps starting from benzimidazole **5**. By reaction with 3-chloropropionyl chloride we prepared the

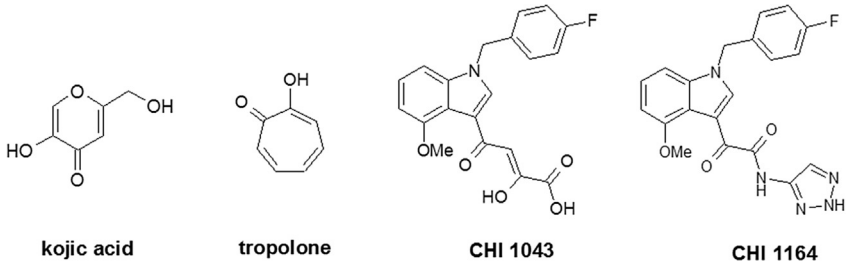

Fig. 1. Chemical structures of kojic acid, tropolone, **CHI 1043** and **CHI 1164**.