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Original article

Synthesis and bioevaluation of pyrazole-benzimidazolone hybrids as novel human 4-Hydroxyphenylpyruvate dioxygenase inhibitors

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ABSTRACT

4-Hydroxyphenylpyruvate dioxygenase (HPPD), an essential enzyme in tyrosine catabolism, is an important target for treating type I tyrosinemia. Inhibition of HPPD can effectively alleviate the symptoms of type I tyrosinemia. However, only one commercial HPPD inhibitor, 2-(2-nitro-4-trifluoromethylbenzoyl) cyclohexane-1,3-dione (NTBC), has been available for clinical use so far. In the present study, a series of novel pyrazole-benzimidazolone hybrids were designed, synthesized and evaluated as potent human HPPD inhibitors. Most of the new compounds displayed significant inhibitory activity against the recombinant human HPPD. Moreover, compound **9l** was identified as the most potent candidate with IC_{50} value of 0.021 μM against recombinant human HPPD, about 3-fold more potent than NTBC. Thus the pyrazole-benzimidazolone hybrid has great potential to be further developed for the treatment of type I tyrosinemia.

1. Introduction

4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD), catalyzing the conversion of 4-hydroxyphenylpyruvate (HPPA) to homogentisate (HGA), is an essential enzyme implicated in pigment synthesis and tyrosine catabolism in most organisms, and has great importance in drug discovery in both agricultural and therapeutic areas (Scheme 1) [1–3]. Inhibition of HPPD in plants leads to the deficiency in isoprenoid redox cofactors such as plastoquinone and tocopherol, finally causing growth inhibition and plant death. In the past thirty years, a series of inhibitors targeting HPPD have been discovered as potent herbicides, such as 2-(2-nitro-4-trifluoromethylbenzoyl) cyclohexane-1,3-dione (NTBC), sulcotrione and mesotrione et al. [4–8].

In human, abnormal metabolism in the tyrosine catabolism pathway gives rise to various diseases, including type I tyrosinemia. More specifically, the excessive HPPD indirectly causes the accumulation of succinylacetone which in turn induces severe liver and kidney dysfunction along with mutagenic changes and hepatocellular carcinoma, and even death [9]. At first, liver transplantation was the only effective treatment for type I tyrosinemia. Later it was found that the born defects in mammalian tyrosine catabolism could be treated effectively by HPPD inhibitors. Animal model and clinical test further indicated that NTBC, with a market name of orfadin, dramatically changed the disease course by improving liver and kidney functions and reducing risk of liver cancer [10–12]. Thereafter, NTBC was approved by the US FDA to clinically treat hereditary type I tyrosinemia [13,14]. So far, considerably less research has been focused on the discovery of new drugs targeting human HPPD. In addition, recent study by animal model indicated that some adverse effect occurred after NTBC treatment. Administration of NTBC was reported to produce corneal lesions in rats fed tyrosine in a low-protein diet [15–18]. Thus, it is of great interest to explore novel HPPD inhibitor as drug candidate for type I tyrosinemia treatment [19,20].

As heterocyclic aromatic compounds, benzimidazolones are important chemical scaffolds and widely applied in pesticide and pharmaceutical synthesis due to their extensive biological activities

Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; HGA, homogentisate; DKN, diketonitriles; CDI: 1, 1'-carbonyldiimidazole; IPTG, isopropyl β-D-1-thiogalactopyranoside; DMSO, dimethyl sulfoxide.

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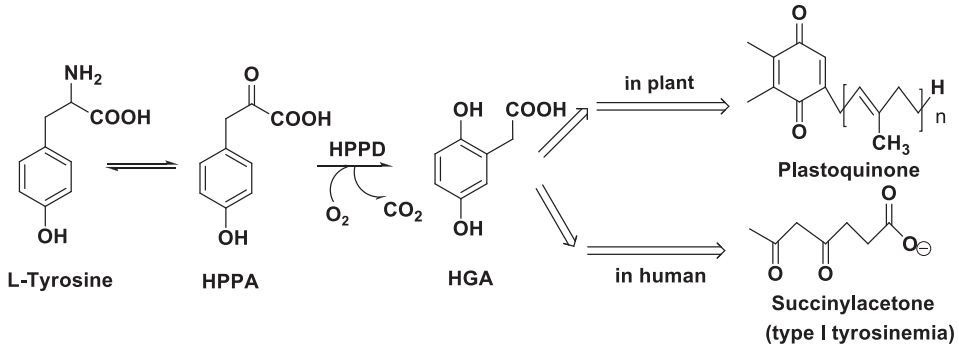

Scheme 1. The simple diagram for the catabolic pathways from tyrosine in plants and type I tyrosinemia patients.

[21–23]. Thus, the heterocyclic compounds incorporating the benzimidazole moiety have received considerable attention [24,25]. We envisioned that by applying scaffold hopping, hybrid (**1** in Fig. 1) of quinazolone with an identified pharmacophore of herbicides targeting HPPD (like pyrazole) would lead to novel HPPD inhibitors with improved potency. Therefore, we designed and synthesized a novel series of pyrazole-benzimidazolone hybrid, and the structure-activity relationship (SAR) was subsequently investigated in current study. As anticipated, more than ten new HPPD inhibitors (compounds **9** in Table 1) were identified with good potency at the nanomolar level in this study. Moreover, their activities were significantly improved compared to NTBC. Among them, compound **9l**, was identified as the most active human HPPD inhibitor, and its IC_{50} value is about 3-fold higher than that of NTBC.

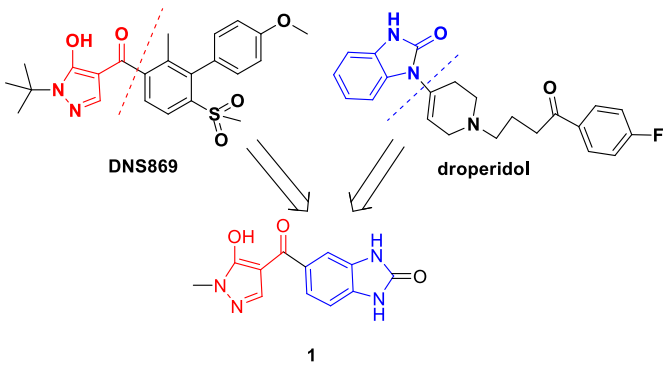

Fig. 1. Design strategy of the pyrazole-benzimidazolone hybrids as novel human HPPD inhibitors.

2. Results and discussion

2.1. Chemistry

The preparation of the target compounds (**9** and **10**) was illustrated in Scheme 2. The synthesis followed an eight-step synthetic route starting from the commercially available 4-chloro-3-nitro benzoic acid. The esterification of 4-chloro-3-nitro benzoic acid afforded the methyl benzoate in a yield of 83%, which was treated with diverse alkylamine or aromatic amine to generate the intermediate **3** ranging in yields from 60% to 90 %. The subsequent reduction of the nitro group in the molecule **3** was achieved by using palladium hydroxide in methanol to produce phenylenediamine **4**, which was subjected to the ring closure reaction without further purification by treating with 1,1'-carbonyldiimidazole (CDI) to give benzimidazolone derivatives **5**. Then, the alkylation reactions of intermediates **5** with alkyl iodide afforded the corresponding **6** in excellent yields (80%–95%). The raw material **6** was treated with lithium hydroxide to afford carboxylic acids **7**. The transformation from **7** to **8**, was furnished by a two-step one-pot strategy that the carboxylic acid **7** was first converted to the corresponding acyl chloride by treating with PCl_5 and the acyl chloride was treated with pyrazol-5-ol to afford compounds **8**. With the key intermediates **8** in hand, the desired products **9** and **10** were prepared though the rearrangement of the intermediates **8** in the presence of acetone cyanohydrin and triethylamine in anhydrous CH_3CN. The structures of all the target compounds were characterized by ^1H NMR, ^{13}C NMR and high resolution mass spectrum.

2.2. Lead compound design and SAR study

Although plenty of potent HPPD inhibitors have been commercialized, only NTBC, a triketone HPPD inhibitor, was approved for type I tyrosinemia therapy. Pyrazoles derivatives, covering almost 40% of commercial HPPD inhibitors which were applied as herbicides, may also be a potential candidate to be developed for medication, since pyrazole can tightly bind HPPD through chelation with the ferrous ion. For example, pyrasulfotole and topramezone, as two typical herbicides, are well-known HPPD inhibitors bearing pyrazole moiety. Another compound, DNS869 (Fig. 1) [26], has been reported as a potent inhibitor of both plant and mammalian HPPDs. As a heterocyclic aromatic compound, benzimidazolone has been widely used in pharmaceutical synthesis, and displays extensive biological activities, such as antihypertensive, antidiabetic, anti-inflammatory, anticonvulsive properties. For example, droperidol as a typical bnzimidzolone was developed and used as neuroleptic pharmaceutical (Fig. 1) [27]. Thus, we envisioned that hybrid of pyrazole-benzimidazolone would afford novel HPPD inhibitors with improved potency (Fig. 1).

The bioactivity of these newly synthesized compounds against human HPPD were evaluated (Table 1), using NTBC as the positive control. As expected, all compounds showed significant inhibitory activity against human HPPD. More than ten compounds have IC_{50} values in the nanomolar range. As indicated in Table 1, compound **9l**, displayed the highest activity with IC_{50} of about 0.021 μM, about 3-fold higher than of the commercial control NTBC (IC_{50} = 0.07 μM).

For the sake of clarity, all the compounds were divided into two groups according to the substitution on the pyrazole ring. As seen in Table 1, the inhibitory activities of the series of compounds **9** (R^1 is H) are generally about 10-fold more potent than that of the series of compounds **10** (R^1 is CH_3). More specifically, compound **9a**, **9f**, **9l** and **9q** exhibited excellent inhibition potency with respective IC_{50} values of 0.059 μM, 0.022 μM, 0.021 μM and 0.040 μM, which are about 17-fold, 44-fold, 45-fold and 18-fold higher than the corresponding compounds with methyl substitution at 3-position of pyrazole (**10a**, **10f**, **10k** and **10n**), respectively. This interesting