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Pyrazolone–quinazolone hybrids: A novel class of human 4-hydroxyphenylpyruvate dioxygenase inhibitors

Yu-Ling Xu [a,†], Hong-Yan Lin [a,†], Run-Jie Cao [a], Ze-Zhong Ming [a], Wen-Chao Yang [a,*], Guang-Fu Yang [a,b,*]

[a] Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, PR China
[b] Collaborative Innovation Center of Chemical Science and Engineering, Tianjin 30071, PR China

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ABSTRACT

4-Hydroxyphenylpyruvate dioxygenase (HPPD), converting 4-hydroxyphenylpyruvate acid to homogentisate, is an important target for treating type I tyrosinemia and alkaptonuria due to its significant role in tyrosine catabolism. However, only one commercial drug, NTBC, also known as nitisinone, has been available for clinical use so far. Herein, we have elucidated the structure-based design of a series of pyrazolone–quinazolone hybrids that are novel potent human HPPD inhibitors through the successful integration of various techniques including computational simulations, organic synthesis, and biochemical characterization. Most of the new compounds displayed potent inhibitory activity against the recombinant human HPPD in nanomolar range. Compounds **3h** and **3u** were identified as the most potent candidates with K_i values of around 10 nM against human HPPD, about three-fold more potent than NTBC. Molecular modeling indicated that the interaction between the pyrazolone ring and ferrous ion, and the hydrophobic interaction of quinazolone with its surrounding residues, such as Phe347 and Phe364, contributed greatly to the high potency of these inhibitors. Therefore, compounds **3h** and **3u** could be potentially useful for the treatment of type I tyrosinemia and other diseases with defects in tyrosine degradation.

1. Introduction

The Fe(II)-dependent non-heme oxygenase, 4-hydroxyphenylpyruvate dioxygenase (HPPD; E.C. 1.13.11.27) with a subunit mass of 40–50 kDa has both agricultural and therapeutic significance due to its catalytic role in the transformation of 4-hydroxyphenylpyruvate acid (HPPA) to homogentisate (HGA).[1–4] This conversion in the presence of oxygen and ferrous ion is the second step of the catabolic pathway, with tyrosine as the starting material (Scheme 1). In plants, the HGA produced in this catabolic pathway is an important aromatic precursor for the biosynthesis of the plastoquinone and tocopherol, which are essential cofactors for the normal growth of plants.[5] Thus, in the past thirty years, a series of particular inhibitors targeting HPPD have been discovered as potent herbicides, due to their ability to block HPPA–HGA conversion, thereafter causing the intense bleaching and even death of weeds.

In the human, abnormal metabolism in the tyrosine catabolism pathway gives rise to various diseases, including type I tyrosinemia, type II tyrosinemia, type III tyrosinemia, hawkinsinuria, and alkaptonuria.[1,6–8] The most severe defect of tyrosine catabolism is type I tyrosinemia, attributed to the deficiency of fumarylacetoacetase. The metabolites from the previous two steps, fumarylacetoacetate and maleylacetoacetate, could be easily transformed to succinylacetone and induce the symptoms that cause type I tyrosinemia patients to suffer from liver dysfunction and renal proximal tubular failure and to incur a high rate of mortality. Type II tyrosinemia usually results in mild mental retardation at birth and corneal opacities due to the deficiency of tyrosine aminotransferase. Type III tyrosinemia caused by the deficiency of active HPPD is very similar to type II tyrosinemia in the clinical phenotype. Hawkinsinuria is believed to be the result of a gene mutation in HPPD resulting in the conversion of HPPA to an alternative product that is very reactive and forms covalent adducts with cellular thiols.[9] Deficiency of HGA dioxygenase incurs the accumulation of large quantities of quinone that readily form the so called ochronotic pigment, the structure of which remains unknown. Among the above diseases associated with tyrosine catabolism, it is believed that specific inhibition of HPPD could alleviate the symptoms of patients suffering from type I tyrosinemia and alkaptonuria.[10–13]

* Corresponding authors. Tel.: +86 27 67867800; fax: +86 27 67867141.
E-mail addresses: tomyang@mail.ccnu.edu.cn (W.-C. Yang), gfyang@mail.ccnu.edu.cn (G.-F. Yang).
† These two authors made equal contributions.

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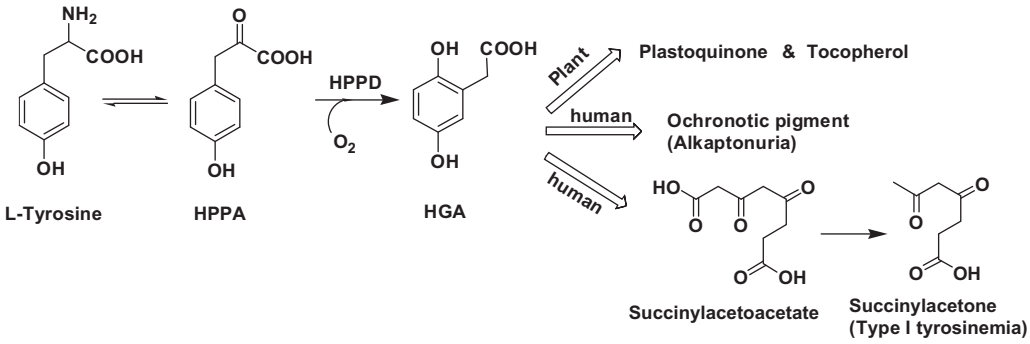

Scheme 1. The simple diagram for the catabolic pathways from tyrosine in plants, and alkaptonuria and type I tyrosinemia in human patients.

Over the last two decades, three main classes of potent HPPD inhibitors have been discovered as herbicides, including triketone, diketonitriles (DKN) and pyrazole.[14,15] Among them, the triketone herbicides are the most widely used and represent the greatest variety of commercialized HPPD inhibitors. The major pharmacophore of the triketone HPPD inhibitors is typically based around the 2-benzoyl or 2-heteroaroyl cyclohexane-1,3-dione and its similar derivatives. Considerably less research has been focused on the discovery of drugs targeting human HPPD. So far, only NTBC, also known as nitisinone (with a market name of orfadin), has been approved as the first effective drug to clinically treat hereditary type I tyrosinemia by the US Food and Drug Administration.[7,16–18] In fact, being a simple and classical representative triketone HPPD inhibitor, NTBC has also shown excellent herbicidal activity and has even been used as herbicide. Inspired by this case, we intended to design new inhibitors of human HPPD by taking advantage of the existing pharmacophore of herbicides targeting HPPD.

As a heterocyclic aromatic compound, quinazolone has been widely used in pesticide and pharmaceutical synthesis, and has displayed extensive biological activities, such as antihypertensive, antidiabetic, anti-inflammatory, anticonvulsive and antibacterial properties.[19–21] For example, by employing quinazolone as the major active subunit, raltitrexed (ZD1964) has been developed for effectively treating patients with advanced and previously untreated colorectal cancer.[22] Thus, 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 against human HPPD. In addition, structure-based drug design usually provides valuable hints for the rational discovery of enzyme inhibitors with high activity and good selectivity.[23–25] Encouraged by these ideas and by the identification of the structural differences and conformational changes of the key residues surrounding the enzyme active site, we judiciously designed and subsequently optimized the pyrazole–quinazolone hybrid on the basis of experimental and computational studies. As anticipated, dozens of novel HPPD inhibitors (compounds **2** and **3** in Scheme 2) were identified with good potency in the nanomolar range in this study, whose activities were significantly improved compared to NTBC. In addition, with the most active compound (**3h**) as the representative, the structural basis of inhibitory mechanism for the title compounds was determined through the use of collaborative methods, such as molecular modeling, spectral characterization, inhibitory kinetics and thermodynamics.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 2, an eight-step protocol was applied to prepare the target compounds (**2** and **3**). Commercially available 2,4-dimethyl aniline was chosen as the starting material. The acylation of 2,4-dimethyl aniline with acetic anhydride provided compound **4**, which was oxidized with $KMnO_4$ to generate the intermediate **5**. The selective esterification of **5** with methanol in the presence of concentrated H_2SO_4 afforded the intermediate **6**, with the concomitant deprotection of the amine group. To avoid side reactions in the next cyclization step, the amine group of **6** was re-protected. Then the cyclization of compound **7** with various substituted anilines was accomplished under the optimal conditions to give intermediate **8** with good yields (65–85%).[26] This raw material **8** was then treated with aqueous sodium hydroxide directly to afford carboxylic acids **9** without further purification. To convert **9** to **10**, we first tried a two-step one-pot strategy, acylating **9** to its corresponding acyl chloride form and then reacting with pyrazol-5-ol. However, none of reactions with various regular acylation reagents, such as $SOCl_2$, Cl(O=)CC(=O)Cl, $POCl_3$, PCl_5, worked for the production of **10**. We next followed a reported method, using the coupling reagent EDCI or DCC, produced **10** in moderate yields.[27] Finally, the desired products were obtained by the rearrangement 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, elemental analysis, and MS spectra. Their purities were also checked with HPLC analysis and are no less than 96%.

2.2. Hit to lead optimization

As mentioned in the introduction, we envisioned that hybrid (**1**) of pyrazole–quinazolone would afford novel HPPD inhibitors with improved potency (Fig. 1). Prior to the chemical synthesis, we performed molecular modeling to investigate the binding mode of hybrid (**1**) with the crystal structure of human HPPD (PDB entry: 3ISQ). Since no crystal structure of human HPPD coupled with inhibitor is available, the existing binding mode of *Arabidopsis thaliana* HPPD (PDB entry: 1SQD and 1TG5) coupled with inhibitors as reference. As shown in the middle portion of Figure 1, the modeling revealed that compound **1** did bind into the active center of HPPD because of the apparent chelation with the ferrous ion, while the quinazolone moiety occupied only a minor part of the hydrophobic cavity, which implied that introduction of a larger group could result in a better interaction. Bearing this consideration in mind, we then designed alternative lead compounds (**2**) which employed substituted phenyl groups on the amide nitrogen. The most common electron withdrawing and donating groups, such as halogen, methyl, trifluoromethyl, methoxy, and trifluoromethoxyl, were considered as the substituent groups. After chemical synthesis, the inhibitory activity of the new compounds was evaluated (Table 1).

As expected, all the compounds demonstrated potent inhibitory activity against human HPPD with inhibitory constants (K_i) at the submicromolar level or nanomolar level. The selectivity of new