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SAR Studies of 2-*o*-Substituted-benzoyl- and 2-Alkanoyl-cyclohexane-1,3-diones as Inhibitors of 4-Hydroxyphenylpyruvate Dioxygenase

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Abstract—Inhibition studies of 4-hydroxyphenylpyruvate dioxygenase (HPPD) with various synthesized 2-*o*-substituted-benzoyl- and 2-alkanoyl-cyclohexane-1,3-diones suggest that the presence of a strongly electronegative group at the *ortho* position and the conformation of the benzene ring moiety on the benzoylcyclohexane-1,3-dione inhibitors are crucial for potent HPPD inhibition.

4-Hydroxyphenylpyruvate dioxygenase (HPPD)[1] is an important enzyme involved in the biosynthesis of plastoquinones and tocopherols in plants, as well as in the catabolism of the aromatic acids phenylalanine and tyrosine in most organisms. It catalyzes oxidative decarboxylation, hydroxylation of the aromatic ring, and a 1,2-shift of a carboxymethyl group from 4-hydroxyphenylpyruvate **1** to homogentisate **2** in the presence of oxygen, as shown in Scheme 1.

While the detailed mechanism of the reaction catalyzed by HPPD remains unclear, increasing evidence[2] suggests that HPPD is the target site of certain bleaching herbicides that contain the 2-benzoylcyclohexane-1,3-dione moiety, referred to as triketones. Inhibition of HPPD activity by triketone herbicides decreases tocopherol and plastoquinone levels in plants, indirectly reducing phytoene desaturation and leading to development of bleaching symptoms. The structures of two representative triketones 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC)[3] and 2-(2-chloro-4-methane-sulfonylbenzoyl)-cyclohexane-1,3-dione (sulcotrione)[4] are shown in Figure 1. Both NTBC and sulcotrione were found to be competitive HPPD inhibitors with IC_{50} values of 40 and 45 nM, respectively.

In 1997, Lee and co-workers,[5] after screening a wide range of triketone analogues and isosteres against the HPPD enzyme, demonstrated that a 2-benzoylethen-1-ol substructure, depicted in Figure 2, is the minimum substructure required for potent in vitro inhibition of HPPD. Nevertheless, the function of the "X" substituent at the *ortho* position of the benzene ring moiety was not elucidated.

In this study, we synthesized a series of 2-*o*-substituted-benzoylcyclohexane-1,3-diones and tested their relative competence as inhibitors of 4-hydroxyphenylpyruvate dioxygenase in an effort to investigate the role played by the *ortho* substituent on triketone inhibitors in HPPD inhibition. The synthesis of *ortho* substituted 2-benzoylcyclohexane-1,3-diones is outlined in Scheme 2. Preparations of **5a**–**h** were accomplished by the cyanide catalyzed isomerization of enol esters **4a**–**h** derived from the reaction of the corresponding benzoyl chloride **3a**–**h** with cyclohexane-1,3-dione using triethylamine as a base in CH_2Cl_2, as described in the literature.[6] The 2-*o*-fluorobenzoylcyclohexane-1,3-dione was not prepared since the consequent elimination of hydrogen fluoride proceeds relatively smoothly under the reaction conditions to yield the nucleophilic intramolecular heterocyclized product exclusively.[7]

Inhibition of 4-hydroxy-phenylpyruvate dioxygenase by 2-*o*-substituted-benzoylcyclohexane-1,3-diones was studied. Varying concentrations of **5a**–**h** were incubated with HPPD purified from pig liver[8] in the presence of the natural substrate 4-hydroxyphenylpyruvate. The enzyme activity was monitored by the spectrophotometric enolborate method as described by Lindstedt.[9] The inhibition

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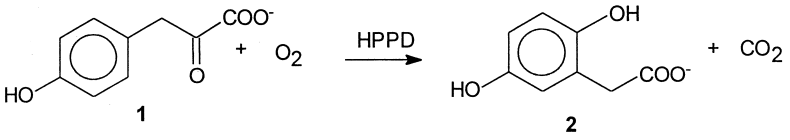


Scheme 1.

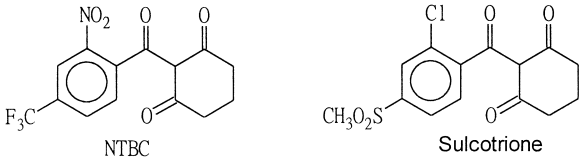


Figure 1.

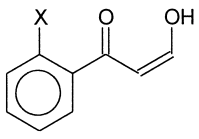


Figure 2.

constants for the reactions of **5a**–**h** with HPPD are listed in Table 1.

The inhibition results indicated that the presence of a strongly electronegative group like chloro, trifluoromethyl, or nitro at the *ortho* position of the benzene ring of triketones enhances the inhibition potency. A maximum 68-fold increase in potency was observed when a nitro group replaced the *ortho* hydrogen atom. When the *ortho* hydrogen was replaced with a methyl or methoxy group, however, no significant increase in inhibition potency was detected. It is assumed that the electrostatic interaction between the relatively electronegative atom (group) at the *ortho* position and the nearby enzyme active site residue induces a rotation of the benzene ring, with the resulting triketone conformation mimicking the structure of the enzyme–substrate complex to tightly bind with the enzyme. Thus, the active conformation adopted by the benzene ring moiety of the triketone

Table 1. Inhibition constants for reactions of **5a**–**h** with HPPD from pig liver by the enol-borate method

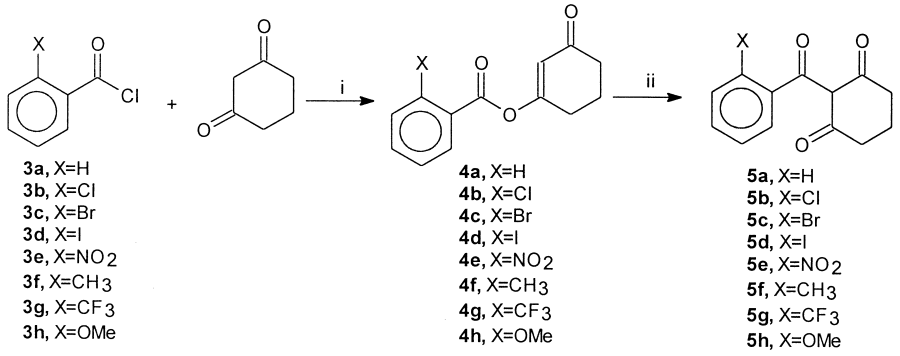
Compound	X	IC_{50} (μM)[a]	Compound	X	IC_{50} (μM)[a]
NTBC	—	0.04	**5e**	NO_2	0.16
5a	H	11.20	**5f**	CH_3	3.75
5b	Cl	0.50	**5g**	CF_3	0.25
5c	Br	0.56	**5h**	OMe	11.70
5d	I	0.76			

[a]Mean of two determinations.

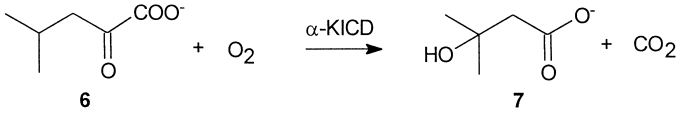
inhibitors might be crucial for potent HPPD inhibition, although more evidence is needed to support this assumption.

In 1995, Crouch and co-workers[10] demonstrated that 4-hydroxyphenylpyruvate dioxygenase also displays α-ketoisocaproate dioxygenase (α-KICD) activity. α-KICD[11] is also an internal keto-acid dependent dioxygenase responsible for the oxidative decarboxylation and hydroxylation of α-ketoisocaproate (**6**) to β-hydroxyisovalerate (**7**), as shown in Scheme 3. Further studies[12] have indicated that HPPD and α-KICD are actually the same enzyme, that is, both 4-hydroxyphenylpyruvate (**1**) and α-ketoisocaproate (**6**) serve as natural substrates for HPPD.

In light of these findings and subsequent comparisons of the molecular structures of both HPPD enzyme substrates and triketone type inhibitors, we speculate that 2-isopropylcarbonylcyclohexane-1,3-dione (**8c**), illustrated in Figure 3, is a potential HPPD inhibitor. Further structure–activity relationship studies of various 2-alkanoyl-cyclohexane-1,3-diones may not only discover new potent HPPD inhibitors but also shed light on the mode



Scheme 2. (i) Et_3N, CH_2Cl_2; (ii) Et_3N, KCN, CH_2Cl_2.



Scheme 3.

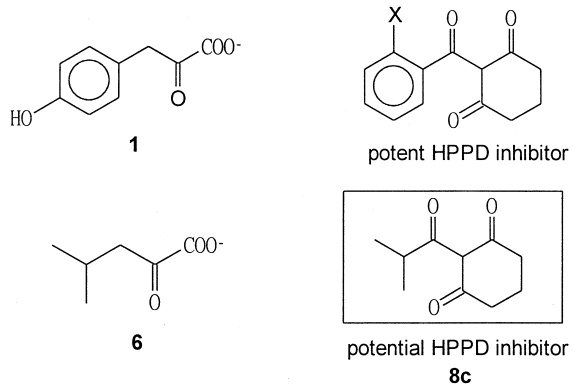


Figure 3.

of action of the benzene ring moiety of the existing 2-benzoylcyclohexane-1,3-dione inhibitors.

Several 2-alkanoylcyclohexane-1,3-diones (**8a–e**), shown in Figure 4, were then prepared as described earlier. Attempts to synthesize 2-*t*-butylcarbonylcyclohexane-1,3-dione from the corresponding enol ester by either cyanid-catalyzed isomerization or aluminum chloride-catalyzed Fries rearrangement were unsuccessful, presumably due to low reactivity of the bulky substrate. Similar findings have been reported in the literature.[13]

Subsequently, the synthesized compounds **8a–d** were evaluated in vitro for inhibition activity against HPPD, and the biological data are listed in Table 2. The results show that 2-alkanoylcyclohexane-1,3-diones were generally less potent HPPD inhibitors than 2-benzoylcyclohexane-1,3-diones. For instance, compound **8c** was almost 9-fold less potent than the corresponding compound **5a**. Furthermore, when the isopropyl group on **8c** was replaced by the cyclopropyl group, the inhibition potency of the resulting **8d** was up to 15 times higher. The major difference between the isopropyl group of **8c** and cyclopropyl group of **8d** when bound to the enzyme active site is their conformation. This result supports our assumption that the conformation of the benzene ring moiety plays an important role in potent HPPD inhibition. If both an *ortho* substituent and the benzene ring conformation of 2-benzoylcyclohexane-1,3-dione inhibitors are essential for tight binding, perhaps the mode of action of triketones in HPPD inactivation is more complex than simply acting as analogues to the substrate 4-hydroxyphenylpyruvate as previously surmised.[14] Further studies are needed to elucidate this point.

In summary, inhibition studies of 4-hydroxyphenylpyruvate dioxygenase with various synthesized 2-*o*-substituted-benzoyl and 2-alkanoyl-cyclohexane-1,3-diones suggest that the presence of a strongly electro-

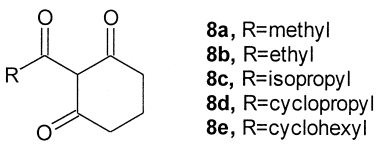


Figure 4.

Table 2. Inhibition constants for reactions of **8a-e** with HPPD from pig liver by the enol-borate method

Compound	R	IC_{50} (μM)[a]
8a	CH_3	11.2
8b	CH_2CH_3	17.8
8c	$CH(CH_3)_2$	93.3
8d	$CH(CH_2)_2$	6.0
8e	$CH(CH_2)_5$	364.5

[a]Mean of two determinations.

negative group at the *ortho* position and the conformation of the benzene ring moiety of the 2-benzoylcyclohexane-1,3-diones are both crucial for potent HPPD inhibition. The information provided in this study may allow us to better define the specific binding characteristics for inhibitors of the enzyme HPPD and may further reveal mechanistic details of this intriguing enzyme. Further characterization of the mechanism of action of the triketone-type HPPD inhibitors at a molecular level is being actively pursued in our laboratory.

Acknowledgements

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