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Discovery of a Potent, Non-Triketone Type Inhibitor of 4-Hydroxyphenylpyruvate Dioxygenase

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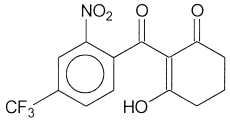
Abstract—3-Cyclopropanecarbonyloxy-2-cyclohexen-1-one has been found to be a new, potent, low molecular weight non-triketone type inhibitor of 4-hydroxyphenylpyruvate dioxygenase with IC_{50} value of 30 nM. Preliminary studies suggest that the two carbonyl groups present in the compound are crucial for the inhibition activity.

2-(2-Nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione[1] (NTBC), a triketone type compound, is the first effective drug therapy for the fatal hereditary disease tyrosinemia type I,[2] which is characterized by a deficiency in the activity of fumarylacetoacetase, leading to the accumulation of hepatotoxic and nephrotoxic compounds like succinylacetone.[3] Recent studies[4] have demonstrated that the primary site of action of NTBC is 4-hydroxyphenylpyruvate dioxygenase[5] (HPPD, EC 1.13.11.27), a non-heme Fe(II)-dependent enzyme involved in the catabolism[6] of tyrosine and phenylalanine in most organisms which catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate. Inhibition of liver HPPD by NTBC will prevent the formation of homogentisate, thereby blocking tyrosine catabolism. Here, we report the discovery of a new, potent, low molecular weight, non-triketone type HPPD inhibitor.



NTBC

Our recent discovery[7] that the herbicide sethoxydim which targets the enzyme acetyl-CoA carboxylase (ACCase) exhibited modest in vitro inhibition activity against 4-hydroxyphenylpyruvate dioxygenase suggested that the biological distinct HPPD and ACCase may share at least some similar binding pockets in the enzyme active site. Although more evidence is needed to confirm this hypothesis, other potent ACCase inhibitors may serve as a good starting point to search for non-triketone type potent HPPD inhibitors. In light of the information that various enol ethers of allyloximes[8] have been found to be more effective and selective herbicides than cyclohexanedione class herbicides, we synthesized a series of alkanoic acid 3-oxo-cyclohex-1-enyl esters **2a–g** as potential inhibitors of HPPD. Esters **2a–g** were easily prepared by a reaction coupling the various acyl chlorides **1a–g** with 1,3-cyclohexanedione in the presence of triethylamine in methylene chloride in an ice bath, as shown in Scheme 1.

The synthesized compounds were evaluated in vitro for inhibition activity against 4-hydroxyphenylpyruvate dioxygenase from pig liver[9] by the spectrophotometric enol-borate method.[10] The inhibition constants[11] for the reactions of these esters with HPPD are listed in Table 1. The most potent inhibitor tested was 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one **2d** with an IC_{50} of 30 nM, which is comparable to that of NTBC ($IC_{50} = 40$ nM). The fact that **2c** decreased inhibition activity up to 140-fold relative to **2d** suggests that the cyclopropyl group present in **2d** is important for potent HPPD inhibition. Similarly, either reducing the carbon number of the R group or increasing its bulkiness resulted in a decrease in potency, indicating that HPPD inhibition by this class of molecules appears to have higher steric and structural demands on the alkyl group.

In an effort to investigate the role played by the two carbonyl groups of **2d** in HPPD inhibition, we selectively removed each of the carbonyl groups present in **2d** and tested the competence of the resulting compounds as HPPD inhibitors as described above. The synthesis of analogues **4** and **6** is shown in Scheme 2. Compound **4**

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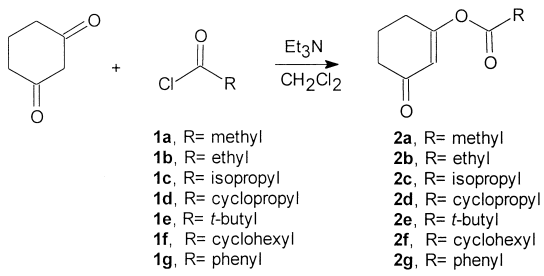
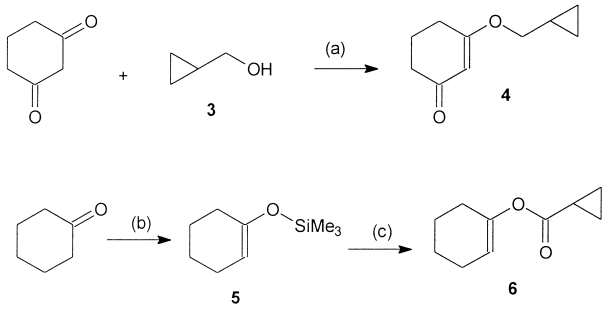


Scheme 1. Preparation of compounds **2a**–**g**.

Table 1. Inhibition constants for reactions of **2a**–**g** with HPPD from pig liver by the enol borate assay method

Compound	R	IC_{50} (μM)[a]	Compound	R	IC_{50} (μM)[a]
2a	CH_3	3.62	**2e**	$C(CH_3)_3$	79.60
2b	C_2H_5	0.11	**2f**	$CH(CH_2)_5$	3.70
2c	$CH(CH_3)_2$	4.16	**2g**	C_6H_5	1.58
2d	$CH(CH_2)_2$	0.03			

[a]Mean of two determinations.



Scheme 2. Synthesis of compounds **4** and **6**: (a) *p*-TsOH, benzene, reflux; (b) Et_3N, trimethylsilyl chloride, DMF, 120 °C; (c) potassium *t*-butoxide, cyclopropane carbonyl chloride, THF, −20 °C.

was prepared in one step by acid catalyzed dehydration of cyclohexanedione and cyclopropanemethanol **3**. Compound **6** was synthesized in two steps by first converting the cyclohexanone to the corresponding trimethylsilyl enol ether **5**,[12] followed by treatment with cyclopropane carbonyl chloride in the presence of potassium *t*-butoxide in THF.[13]

The inhibition results showed that analogue **6** was a much less potent inhibitor than **2d** with IC_{50} of 0.7 μM and analogue **4** was not a HPPD inhibitor. When the ring carbonyl group of **2d** was removed to form **6**, the inhibition potency decreased 23-fold relative to **2d** indicating that this carbonyl group is essential for binding. When the ester carbonyl group of **2d** was removed to form **4**, no enzyme inhibition was observed up to the concentration of 0.2 mM. This result implies the carbonyl oxygen atom on the ester functionality is crucial for inhibition, presumably by chelating with ferrous ion in the enzyme active site.[14]

In summary, we have discovered a new, potent, low molecular weight, non-triketone type HPPD inhibitor. This study revealed that 3-cyclopropane-carbonyloxy-2-cyclohexen-1-one has potent HPPD inhibition activity, comparable to that of NTBC. The preliminary SAR studies demonstrated that the well-positioned dicarbonyl groups of **2d** are essential for this potent inhibition. Thus, 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one or its derivatives has the potential to serve as a new therapeutic agent to treat the fatal human disease tyrosinemia type I. Further SAR studies are currently underway in our laboratory.

Acknowledgements

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11. The reaction mixtures contained 0.85 mL potassium phosphate/borate buffer (prepared by adjusting the pH of 0.42 M H_3BO_3 to 6.2 with a 0.17 M Na_3PO_4 solution), 0.06 mL 4-hydroxyphenylpyruvic acid (1.8 mM, in 0.2 M Na_3PO_4 buffer), 0.03 mL dichlorophenolindophenol (reduced form, prepared by mixing 1 mL of 3.3 mM sodium dichlorophenolindophenol in H_2O and 0.16 M glutathione in 0.2 M sodium phosphate buffer), and 0.01 mL phenylpyruvate tautomerase (10U/mL, Sigma). The above solution was equilibrated for 15 min, the inhibition reaction of **2a**–**g**, **4** and **6** with the enzyme HPPD was evaluated by measuring the decrease in absorbance at 308 nm over a 15 min period following coadministration of varying concentrations of the inhibitors and HPPD.
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