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Acylcyclohexanedione Derivatives as Potential In Vivo Sequential Inhibitors of 4-Hydroxyphenylpyruvate Dioxygenase and GA_{20} 3β-Hydroxylase

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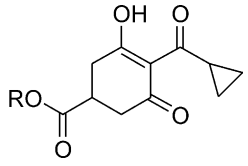
Abstract—Acylcyclohexanedione derivatives have been designed, synthesized, and evaluated for in vitro inhibition activity against the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD). The biological data demonstrated that **7** is a potent inhibitor of 4-HPPD with an IC_{50} value of 40 nM. After metabolism, compound **7** has the potential to become a potent inhibitor of a second enzyme, GA_{20} 3β-hydroxylase.

GA_{20} 3β-hydroxylase (EC 1.14.11.15)[1] is a non-heme, Fe(II) and α-keto acid-dependent dioxygenase involved in the later stages of gibberellins (GAs) biosynthetic pathway, a class of plant hormones.[2] It catalyzes the conversion of gibberellin 20 (GA_{20}) and 2-oxoglutarate to bioactive gibberellin 1 (GA_1) and succinate, as shown in Scheme 1.

The GAs play an essential role in many aspects of plant growth and development, such as seed germination, stem elongation and flower development. For example, gibberellin 1 (GA_1) is a tetracyclic diterpene carboxylic acid that induces stem elongation in maize and pea. Inhibition of GA_{20} 3β-hydroxylase activity will prevent the formation of the active hormone GA_1, thereby blocking GA biosynthesis and resulting in decreased cellular elongation and internode length. Therefore, a potent GA_{20} 3β-hydroxylase inhibitor could be a good candidate to serve as a plant growth regulator (PGR). In fact, trinexapac-ethyl[3] (**1**) is a commercially available PGR for turfgrass management which prevents the conversion of GA_{20} to GA_1. The molecule responsible for this inhibition has been identified as the corresponding acid **2**, and the mode of action is believed to be competition with the natural co-substrate, 2-oxoglutarate, at the active site of GA_{20} 3β-hydroxylase.[4]



1. R=Et
2. R=H

4-Hydroxyphenylpyruvate dioxygenase (4-HPPD, EC 1.13.11.27)[5] is a non-heme Fe(II)-dependent enzyme involved in the biosynthesis of plastoquinones and tocopherols in plants.[6] Unlike GA_{20} 3β-hydroxylase, 4-HPPD is an α-keto acid-dependent enzyme, where the α-keto acid is not a cofactor but part of the substrate. It catalyzes the conversion of 4-hydroxyphenylpyruvate (4-HPP) and molecular oxygen to homogentisate and carbon dioxide, as shown in Scheme 2.

Inhibition of 4-HPPD has recently become the focus of considerable research interest because potent 4-HPPD inhibitors could serve as a new class of bleaching herbicides for control of grass and broadleaf weeds.[7] Earlier, we reported the discovery of a potent, low molecular weight 4-HPPD inhibitor 3-cyclopropanecarbonyloxy-

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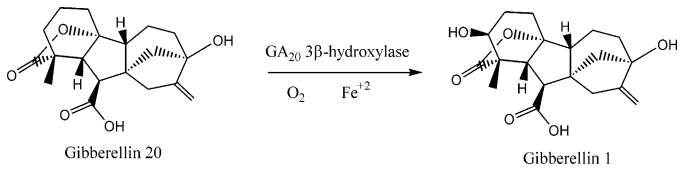
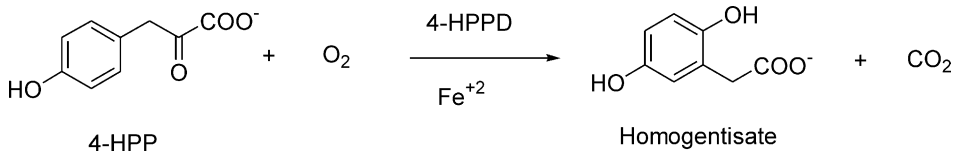
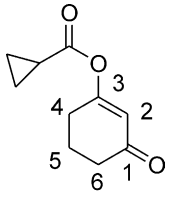


Scheme 1.



Scheme 2.

2-cyclohexen-1-one **3** ($IC_{50} = 30$ nM).[8] Further SAR studies suggested that a C-2 substitution on **3** has a large effect on inhibition potency, while a C-5 substitution has minimal effect.[9]



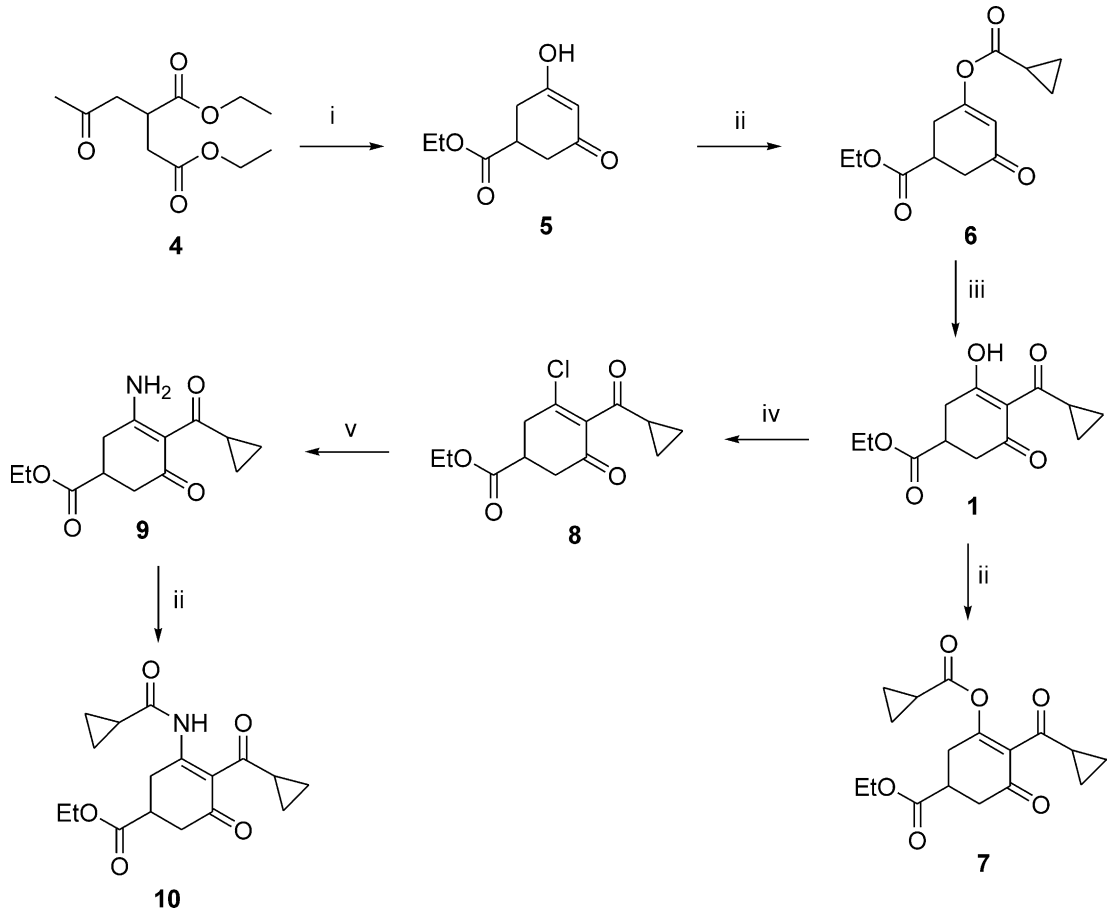
3

In this paper, we report our attempt to design and synthesize a potent inhibitor for 4-HPPD as a potential herbicide which would block the biosynthesis of plastoquinones and tocopherols in plants, and when metabolized in vivo could become a potent GA_{20} 3β-hydroxylase inhibitor and act as a plant growth regulator by interfering with gibberellin biosynthesis. The molecules designed and synthesized as potential sequential inhibitors are compounds **7** and **10**, as depicted in Scheme 3. The approach began with base-catalyzed cyclization of keto ester **4** via intramolecular Dieckmann condensation to afford enol **5**,[10] which upon treatment with cyclopropanecarbonyl chloride under basic conditions gave rise to enol ester **6**. Preparation of **1** was accomplished by the cyanide-catalyzed isomerization[11] of enol ester **6** using triethylamine as a base in methylene chloride. Further esterification of **1** with cyclopropanecarbonyl chloride under basic conditions yielded 2,3,5-trisubstituted cyclohexane-1,3-dione derivative **7**, quantitatively. When treated with oxalyl chloride at room temperature, enol **1** was converted to the corresponding chloride **8**.[12] Amination of **8** with liquid ammonia in methylene chloride at room temperature gave the expected enamine **9**.[13] Final *N*-acylation of **9** with cyclopropanecarbonyl chloride as described previously afforded amide **10**.

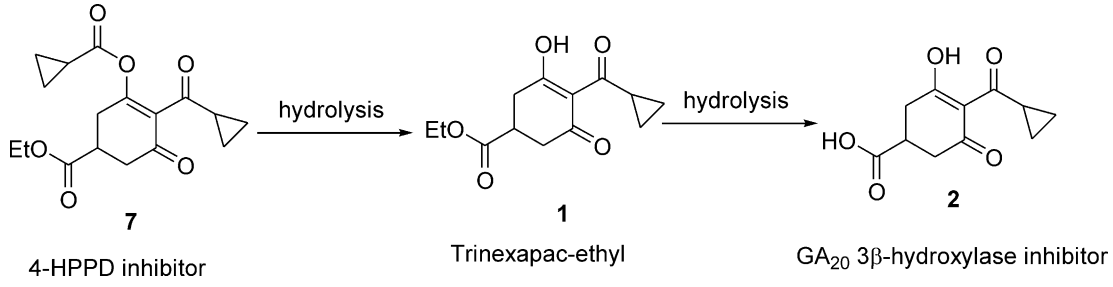
The synthesized compounds were evaluated in vitro for inhibition activity against 4-HPPD purified from pig liver[14] by the spectrophotometric enol-borate method.[15] The IC_{50} values for compounds **7** and **10** are 0.04 and 10 μM, respectively.[16] This result shows that compound **10** is 250-fold less potent than compound **7**. Apparently, the presence of an amide functionality at the C-3 position of **10** is highly detrimental to the inhibition potency. This observation is consistent with our previous SAR studies[9] and might be attributed to the resulting conformational-constrained structure that prevents tight binding with the enzyme active site. Having established that compound **7** is an effective in vitro 4-HPPD inhibitor, we turned our attention to investigating the possible metabolic fate of compound **7** in vivo. When compound **7** was dissolved in D_2O at room temperature for 24 h, more than 50% of **7** was hydrolyzed back to compound **1**, presumably due to the intrinsic electrostatic repulsion between the 2-acyl oxygen atom and the two 1,3-diketone oxygens.[16] Further basic hydrolysis of the ester functional group at the C-5 position gave the active GA_{20} 3β-hydroxylase inhibitor **2**. Thus, it is reasonable to assume that compound **7** will be nonenzymatically hydrolyzed or enzymatically degradated in vivo to give **1** and subsequently into its corresponding acid **2**, as indicated in Scheme 4.

Although further investigations of compound **7** as a sequential inhibitor for 4-HPPD and GA_{20} 3β-hydroxylase in vivo are needed, the results presented here demonstrate that compound **7** is a strong 4-HPPD inhibitor and it provides a good example of how to develop biologically active molecules with multi-functional purposes. Conceptually, successive inhibition of two enzymes in plants would offer the advantage of using a lower amount of the active ingredients for the same effect, as compared to selective inhibition of a single enzyme. Furthermore, it is less likely that the treated plants would develop resistance to the applied herbicides or PGRs.

In summary, we have designed and synthesized a potent 4-hydroxyphenylpyruvate dioxygenase inhibitor **7** with IC_{50} of 40 nM by functionalizing the 2-, 3-, and 5-positions of cyclohexane-1,3-dione. After metabolism, we expect compound **7** will have the potential to serve as a potent plant growth regulator by inhibiting the activity of a second enzyme, GA_{20} 3β-hydroxylase.



Scheme 3. Preparation of compounds **7** and **10**: (i) EtONa, EtOH; (ii) cyclopropanecarbonyl chloride, Et_3N, CH_2Cl_2; (iii) KCN, Et_3N, CH_2Cl_2; (iv) $(CO)_2Cl_2$, CH_2Cl_2; (v) NH_3, CH_2Cl_2.



Scheme 4.

Acknowledgements

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