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SAR Studies of 3-Cyclopropanecarbonyloxy-2-cyclohexen-1-one as Inhibitors of 4-Hydroxyphenylpyruvate Dioxygenase

Yung-Lung Lin, Chung-Shieh Wu, Shean-Woei Lin, Jian-Lin Huang, Yang-Sheng Sun and Ding-Yah Yang*

Department of Chemistry, Tunghai University, 181, Taichung-Kang Rd. Sec.3, Taichung, Taiwan 40704, ROC

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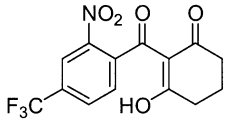
Abstract—Various 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one **1** derivatives have been synthesized and tested as inhibitors of 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) from pig liver. The inhibition results indicated that well-positioned dicarbonyl groups as well as the cyclopropyl group of **1** were essential for potent inhibition. Substitution at the 2-position of the ring system has a significant effect on inhibitor potency, while the 5-position can undergo substantial variations and retain inhibitor potency. In the compounds examined, 2-chloro substituted **12** is the best inhibitor of all with IC_{50} of 15 nM, the rest of the synthesized analogues were less potent inhibitors than the parent compound.

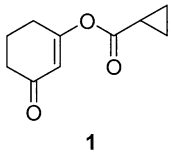
Introduction

4-Hydroxyphenylpyruvate dioxygenase (4-HPPD)[1] is an important enzyme involved in the catabolism of tyrosine and phenylalanine in most organisms which catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate. The biotransformations catalyzed by 4-HPPD require an oxidative decarboxylation of the 2-oxoacid side chain of the substrate with an accompanying hydroxylation of the aromatic ring and a 1,2-migration of the carboxymethyl group.[2] In the past 10 years, 4-HPPD has been the target site to develop effective drug therapy for the fatal hereditary disease tyrosinemia type I[3] which is characterized by a deficiency of fumarylacetoacetase activity that leads to the accumulation of hepatotoxic and nephrotoxic compounds like succinylacetone.[4] Inhibition of liver 4-HPPD activity will prevent the formation of homogentisate, thereby blocking tyrosine catabolism. Currently, 2-(2-(nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione[5] (NTBC), a potent 4-HPPD inhibitor with a triketone type structure, has been on the clinical trial as the first effective drug therapy to treat tyrosinemia type I patients.



NTBC

Recently, we reported[6] the discovery of a new potent, low molecular weight, non-triketone type 4-HPPD inhibitor 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one **1**. Inhibition studies indicated that compound **1** was a potent 4-HPPD inhibitor with $IC_{50} = 30$ nM, which is comparable to that of NTBC ($IC_{50} = 40$ nM). Here we report the structure–activity relationships of the parent compound **1** as inhibitors of 4-HPPD in an effort to provide insights into its mode of action at the molecular level.



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Results and Discussion

The molecules designed and synthesized for SAR are compounds **2**–**16**, shown in Figure 1, Scheme 1 and 3. Vinyl ether **2** was prepared by acid-catalyzed dehydration of cyclohexanedione and cyclopropanemethanol in benzene at 140 °C for 6 h. Ester **3** was synthesized in two steps by first converting cyclohexanone to the corresponding

*Corresponding author. Tel.: +886-4-2359-7613; fax: 886-4-2359-0426; e-mail: yang@mail.thu.edu.tw

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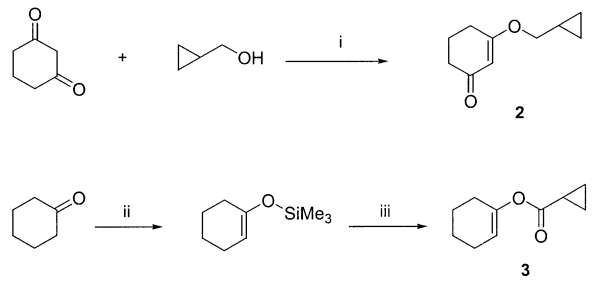
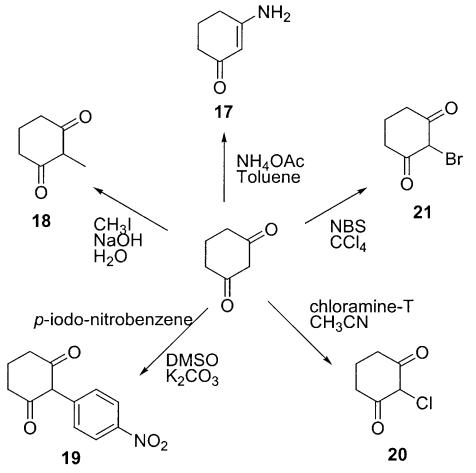


Scheme 1. Synthesis of compounds **2** and **3**: (i) *p*-TsOH, benzene, reflux; (ii) Et_3N, trimethylsilyl chloride, DMF, 120 °C; (iii) potassium *t*-butoxide, cyclopropanecarbonyl chloride, THF, −20 °C.



Scheme 2. Preparation of compounds **17**–**21**.

trimethylsilyl enol ether,[7] followed by treatment with cyclopropanecarbonyl chloride in the presence of potassium *t*-butoxide in THF at −20 °C with 63% overall yield. Compounds **4**–**7** were easily prepared in one step by a standard coupling reaction of cyclopropanecarbonyl chloride in the presence of triethylamine as a base in methylene chloride with 5,5-dimethylcyclohexane-1,3-dione, cyclopentane-1,3-dione, resorcinol, and 4-hydroxy-coumarin, respectively. Preparation of compounds **8**–**13**, however, called for first preparing the appropriate cyclohexane-1,3-dione derivatives **17**–**21** shown in Scheme 2 according to the literature procedures,[8] followed by the same coupling reaction mentioned above. Base-catalyzed self-condensation of the commercially available diethyl acetonylsuccinate afforded 5-ethoxycarbonyl-cyclohexane-1,3-dione,[9] which upon treatment with cyclopropanecarbonyl chloride under basic conditions gave rise to compound **14** with 57% combined yield. The synthesis of compounds **14** and **15** was started by first introducing an aldehyde functional group onto 1,3,5-trimethoxybenzene via the Vilsmeier[10] reaction as illustrated in Scheme 3. The resulting aldehydes were then oxidized under Baeyer–Villiger[11] conditions to produce phenols **23a** and **23b**. After coupling with cyclopropanecarbonyl chloride as described above, the esters **24a** and **24b** were exposed to silver oxide and aqueous 6 N nitric acid in acetone to afford the final benzoquinones **15** and **16** with 48 and 35% overall yield, respectively.

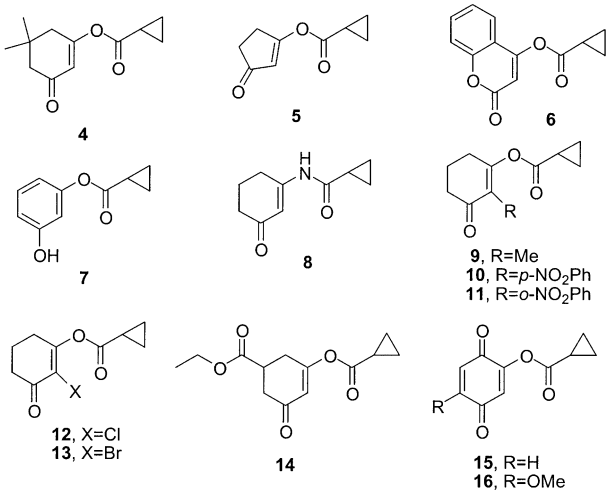


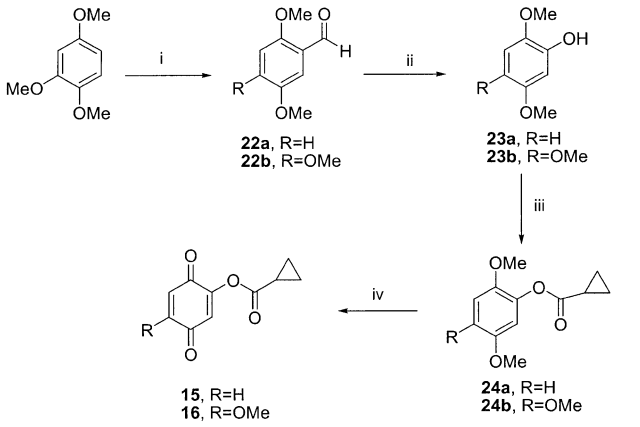
Figure 1. Various compounds synthesized for SAR studies.

All compounds described herein were tested for their ability to inhibit the activity of 4-hydroxyphenylpyruvate dioxygenase using the spectrophotometric enol borate method described by Lindstedt and Rundgren.[12] Results of the inhibition reactions of these compounds with pig liver 4-HPPD are summarized in Table 1. The importance of the position of the two carbonyl groups of **1** was investigated by comparing analogues **2** and **3**. When the ring carbonyl group of **1** was removed to form **3**, the inhibition potency decreased 23-fold relative to **1** indicating that the ring carbonyl group is essential for activity. In addition, replacement of the cyclic α,β-unsaturated keto moiety of **1** with a phenol derivative **7** also resulted in significant losses in potency. In the case in which the ester carbonyl group of **1** was removed to form **2**, no enzyme inhibition was observed up to the concentration of 0.2 mM. This result suggests the carbonyl oxygen atom on the ester functionality is crucial for binding, presumably by chelating with ferric ion in the enzyme active site.[13] The role played by the substitution on the six-membered ring of **1** toward 4-HPPD inhibition was explored by investigation of the inhibition activity of compounds **4**–**16**. Contraction of the six-membered ring to the five-membered ring of **1** to **5**, fusion a benzene ring to the 4- and 5-position of the ring system to a 4-hydroxycoumarin derivative **6**, as well as the dimethyl and ethoxycarbonyl substitutions at 5-position of the ring system of **1** to compounds **4** and **14**

Table 1. Inhibition constants for reactions of **1**–**16** with 4-HPPD from pig liver by the enol borate method

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)
1	0.03 (7.52)[b]	**9**	5.6 (−0.75)
2	NA[c]	**10**	135 (−2.13)
3	0.7 (6.15)	**11**	20 (−1.30)
4	0.33 (6.48)	**12**	0.015 (7.82)
5	0.07 (7.15)	**13**	0.028 (7.55)
6	0.11 (6.96)	**14**	0.1 (7.00)
7	NA	**15**	25 (−1.40)
8	92 (−1.96)	**16**	30 (−1.48)

[a]Assay detail is described in Experimental. Values are the means of at least three independent determinations.
[b]pIC_{50} unit is given in parentheses.
[c]NA, not active; $IC_{50} > 200$ μM.



Scheme 3. Preparation of compounds **15** and **16:** (i) $POCl_3$, DMF; (ii) H_2O_2, H_2SO_4, MeOH; (iii) cyclopropanecarbonyl chloride, Et_3N, CH_2Cl_2; (iv) AgO, 6 N HNO_3, acetone.

all have moderate effects on inhibition potency, but modifications at the 2-position of the ring system gave more variable results. When the 2-substituents were a chlorine or bromine atom, the resulting **12** or **13** were as potent as **1**, whereas bulkier and less electronegative substituents like methyl (**9**) or aryl groups (**10** and **11**) led to a major reduction of activity. These results implied that both the steric and electrostatic effects of these substituents at the 2-position of the ring system might promote a conformational change to give a lower binding affinity for the 4-HPPD enzyme. Furthermore, the observation of 3-order of magnitude reduction of inhibition potency when the ester bond of **1** was replaced with the amide functionality might be attributed to the resulting conformational-constrained structure **8** that prevents tight binding with the enzyme active site.

Analogue **15** explored the effect of an additional oxo group at C-4 position when the six-membered ring was replaced by a benzoquinone moiety. In this case, inhibition potency of **15** decreased 833-fold ($IC_{50} = 25\ \mu M$) as compared to **1**, and 227-fold as compared to **6**. The results indicate that incorporation of a small but strongly electronegative atom like oxygen at 4-position of the ring system of **1** is highly detrimental to the inhibition activity, whereas incorporation of a large, planar, but less electronegative aromatic moiety like benzene at 4- and 5-positions of the ring (**6**) is not. We reasoned that the presence of a C-4 oxo group in the ring system might interfere with either of the two carbonyl groups in the molecule to chelate with the active site ferric ion and thus result in a weak affinity with the enzyme 4-HPPD, although more investigation is needed to confirm this assumption. Finally, further incorporation of a methoxy substituent on the 6-position of the benzoquinone moiety (**16**) resulted in little improvement on inhibition potency.

Conclusion

As a result of this investigation, we now have a general understanding of the SAR for this class of 4-hydroxyphenylpyruvate dioxygenase inhibitors, as depicted in Figure 2. The two carbonyl groups as well as the cyclopropyl group of 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one were required for potent 4-HPPD inhibition. Substitution at the 2-position of ring system has a significant effect on inhibitor potency, while the 5-position can undergo substantial variations and retain inhibitor potency. The ability to vary the 5-position of compound **1** should become important as this class of 4-HPPD inhibitors undergo further development, as it may be possible to vary the in vivo profile of these inhibitors without affecting inhibitor potency. The results of our studies will provide an alternate treatment for the fatal disease tyrosinemia type I.

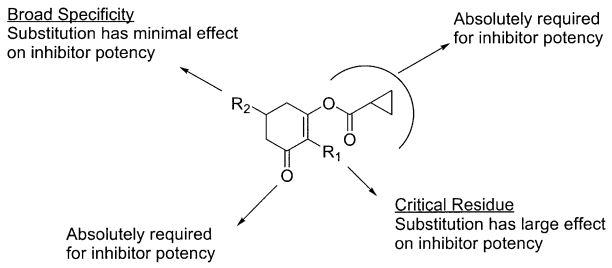


Figure 2. General SAR for 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one inhibitors.

Experimental

General

Melting points were determined on a Mel-Temp melting point apparatus in open capillaries and are uncorrected. Ultraviolet-visible spectroscopy was performed on a Hewlett-Packard 8453 spectrophotometer. High resolution FAB-MS was measured with a JEOL JMS-SX/SX 102A spectrometer. ^1H NMR spectra were recorded at 300 MHz on a Varian VXR300 spectrometer. Chemical shifts were reported in ppm on the δ scale relative to internal standard (tetramethylsilane, or appropriate solvent peaks) with coupling constants given in hertz. ^1H NMR multiplicity data were denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad). Flash chromatography was performed in columns of various diameters with Merck silica gel (230–400 mesh ASTM 9385 kieselgel 60H) by elution with the solvents reported. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60F-254 plates (0.2 mm) and developed with the solvents mentioned. All new compounds exhibited satisfactory spectroscopic and analytical data.

Enzyme purification and assay

4-HPPD was purified from pig liver by the method of Hamilton[14] with a specific activity of 0.1 μmol min^{-1} mg^{-1}. The protein concentration was determined by BCA method. Routine assay of the enzyme utilized the spectrophotometric enol borate method of Lindstedt and Rundgren.[12] The typical assay 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-hydroxyphenyl-

pyruvic 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), 0.01 mL phenylpyruvate tautomerase (10 U/mL, Sigma). The above solution was equilibrated for about 15 min (monitored at 308 nm), then 4-HPPD to be tested was added (0.05 mL solution). For calculation of dioxygenase activity the change in absorbance between the 8th and 10th min was used. The inhibition reaction of **1**–**16** with the enzyme 4-HPPD was evaluated by measuring the decrease in absorbance at 308 nm over a 15 min period following coadministration of varying concentration of the inhibitors and 4-HPPD. The IC_{50} values were determined by fitting the data to the equation $v_i = v_o/(1 + [\text{inhibitor}]/IC_{50})$, where v_i is the rate of absorbance change at a given inhibitor concentration and v_o the rate of absorbance change in the absence of inhibitor, and converted into pIC_{50} units for modeling SAR.

3-Cyclopropylmethoxy-2-cyclohexen-1-one (2). To a solution of 1,3-cyclohexanedione (166 mg, 1.48 mmol) in benzene (30 mL) was added cyclopropanemethanol (107 mg, 1.48 mmol) and catalytic amounts of *p*-toluenesulfonic acid. The resulting mixture was heated to 140 °C. The by-product water was removed by the Dean–Stark trap. The reaction was completed within 6 h, then quenched with water. The vinyl ether was extracted with ethyl acetate, dried in $MgSO_4$, and concentrated in vacuo to obtain the crude product, which was further purified by column chromatography to give a colorless liquid with a 90% yield. $R_f = 0.54$ (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 5.17 (s, 1H, 2-H), 3.54 (d, $J = 6.9$ Hz, 2H, OCH_2), 2.32–2.29 (m, 2H, 6-H), 2.23–2.20 (m, 2H, 4-H), 1.87–1.83 (m, 2H, 5-H), 1.10–1.02 (m, 1H, OCH_2CH), 0.52–0.47 (m, 2H, CH_2), 0.20–0.17 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_{14}O_2$ 166.0994, found 166.0989.

2-Cyclopropanecarbonyloxy-1-cyclohexene (3). A solution of potassium *t*-butoxide (560 mg, 5 mmol) in THF (5 mL) was added to a stirred solution of 1-cyclohexenyloxy-trimethylsilane[7] (850 mg, 5 mmol) in THF (10 mL) at −78 °C under nitrogen and the mixture was stirred at this temperature for 1 h. After this, the temperature was increased to −20 °C, then a solution of cyclopropanecarbonyl chloride (523 mg, 5 mmol) in THF (5 mL) was added to it. After 15 min, the mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O. The extract was dried and evaporated under reduced pressure and the crude product was purified by flash chromatography on silica gel to give a colorless liquid with a 70% yield. $R_f = 0.25$ (100% hexanes). ^{1}H NMR ($CDCl_3$) δ 6.59 (t, $J = 1.9$ Hz, 1H, 2-H), 2.57–2.49 (m, 4H, 3-H, 6-H), 2.09–2.03 (m, 2H, 4-H), 1.80–1.75 (m, 1H, CH), 1.65–1.50 (m, 2H, 5-H), 1.14–1.11 (m, 2H, CH_2), 1.00–0.97 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_{14}O_2$ 166.0994, found 166.0985.

General procedure for preparation of compounds 4–16, 24a, and 24b

The cyclopropanecarbonyl chloride (523 mg, 5 mmol) was added to a solution of 5,5-dimethylcyclohexane-1,3-dione (700 mg, 5 mmol) with triethylamine (1.4 mL, 10 mmol) in dry methylene chloride (30 mL). The mixture was stirred at room temperature for 1 h, then washed with water, diluted hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water. The organic phase was dried with $MgSO_4$ and evaporated to dryness in vacuo to give the crude enol ester, which was further purified by flash chromatography to give the desired product.

3-Cyclopropanecarbonyloxy-5,5-dimethyl-2-cyclohexen-1-one (4). This compound was obtained as a colorless liquid with a 87% yield. $R_f = 0.30$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 5.91 (s, 1H, 2-H), 2.42 (s, 2H, 6-H), 2.27 (s, 2H, 4-H), 1.75–1.68 (m, 1H, CH), 1.13–1.10 (m, 2H, CH_2), 1,11 (s, 6H, 5-Me), 1.04–1.00 (m, 2H, CH_2). HRMS: calcd for $C_{12}H_{16}O_3$ 208.1100, found 208.1092.

3-Cyclopropanecarbonyloxy-2-cyclopenten-1-one (5)

This compound was obtained as a colorless liquid with a 70% yield. $R_f = 0.17$ (50% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 6.20 (s, 1H, 2-H), 2.79–2.75 (m, 2H, 5-H), 2.47–2.43 (m, 2H, 4-H), 1.81–1.76 (m, 1H, CH), 1.19–1.16 (m, 2H, CH_2), 1.11–1.06 (m, 2H, CH_2). HRMS: calcd for $C_9H_{10}O_3$ 166.0630, found 166.0635.

4-Cyclopropanecarbonyloxy-coumarin (6). This compound was obtained as a brown liquid with a 75% yield. $R_f = 0.39$ (50% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.66 (d, $J = 7.8$ Hz, 1H, Ar H), 7.59 (t, $J = 8.2$ Hz, 1H, Ar H), 7.38–7.28 (m, 2H, Ar H′s), 6.51 (s, 1H, 3-H), 1.99–1.59 (m, 1H, CH), 1.29–1.19 (m, 2H, CH_2), 1.18–1.15 (m, 2H, CH_2). HRMS: calcd for $C_{13}H_{10}O_4$ 230.0579, found 230.0570.

3-Cyclopropanecarbonyloxy-phenol (7). This compound was obtained as a light blue liquid with a 92% yield. $R_f = 0.44$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.18 (t, $J = 8.4$ Hz, 1H, Ar H), 6.68–6.62 (m, 2H, Ar H′s), 6.57 (s, 1H, Ar H), 5.90 (bs, 1H, OH), 1.85–1.80 (m, 1H, CH), 1.17–1.14 (m, 2H, CH_2), 1.05–1.00 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_{10}O_3$ 178.0630, found 178.0636.

***N*-(3-Oxo-cyclohex-1-enyl)-cyclopropanecarboxamide (8).** This compound was obtained from 3-amino-2-cyclohexen-1-one[15] **17** as a brown solid with a 65% yield. Mp 177–178 °C. $R_f = 0.40$ (100% EtOAc). ^{1}H NMR ($CDCl_3$) δ 7.18 (bs, 1H, NH), 6.48 (s, 1H, 2-H), 2.62 (t, $J = 6.0$ Hz, 2H, 6-H), 2.38 (t, $J = 6.3$ Hz, 2H, 4-H), 2.21–2.10 (m, 2H, 5-H), 1.49–1.41 (m, 2H, CH), 1.10–1.07 (m, 2H, CH_2), 0.91–0.87 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_{13}NO_2$ 179.0947, found 179.0957.

3-Cyclopropanecarbonyloxy-2-methyl-2-cyclohexen-1-one (9). This compound was obtained from 2-methylcyclohexane-1,3-dione[16] **18** as a colorless liquid with a 87% yield. $R_f = 0.36$ (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 2.53–2.51 (m, 2H, 6-H), 2.46–2.42 (m, 2H, 4-H), 2.04–1.99 (m, 2H, 5-H), 1.79–1.75 (m, 1H, CH), 1.67 (s, 3H, 2-CH_3), 1.34–1.11 (m, 2H, CH_2), 1.03–1.00

(m, 2H, CH_2). HRMS: calcd for $C_{11}H_{14}O_3$ 194.0943, found 194.0940.

2-*p*-Nitrophenyl-3-cyclopropanecarbonyloxy-2-cyclohexen-1-one (10). This compound was obtained from 2-*p*-nitrophenyl-cyclohexane-1,3-dione[17] **19** as a yellow solid with a 75% yield. Mp 92–93 °C. R_f = 0.33 (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 8.21 (d, J = 9.0 Hz, 2H, Ar H′s), 7.31 (d, J = 9.0 Hz, 2H, Ar H′s), 2.75 (t, J = 6.0 Hz, 2H, 6-H), 2.62 (t, J = 6.0 Hz, 2H, 4-H), 2.18 (quintet, J = 6.0 Hz, 2H, 5-H), 1.55–1.49 (m, 1H, CH), 0.90–0.85 (m, 4H, CH_2CH_2). HRMS: calcd for $C_{16}H_{15}NO_5$ 301.0951, found 301.0946.

2-*o*-Nitrophenyl-3-cyclopropanecarbonyloxy-2-cyclohexen-1-one (11). This compound was obtained from 2-*o*-nitrophenyl-cyclohexane-1,3-dione[17] as a yellow solid with a 85% yield. Mp 82–83 °C. R_f = 0.42 (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 8.11 (dd, J = 8.1, 1.5 Hz, 1H, Ar H), 7.61 (dt, J = 7.5, 1.5 Hz, 1H, Ar H), 7.50 (dt, J = 8.4, 1.8 Hz, 1H, Ar H), 7.20 (dd, J = 7.5, 1.8 Hz, 1H, Ar H), 2.88–2.58 (m, 4H, 4-H, 6-H), 2.24–2.13 (m, 2H, 5-H), 1.53–1.45 (m, 1H, CH), 0.89–0.75 (m, 4H, CH_2CH_2). HRMS: calcd for $C_{16}H_{15}NO_5$ 301.0951, found 301.0935.

2-Chloro-3-cyclopropanecarbonyloxy-2-cyclohexen-1-one (12). This compound was obtained from 2-chlorocyclohexane-1,3-dione[18] **20** as a yellow liquid with a 75% yield. R_f = 0.23 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 2.71–2.62 (m, 2H, 6-H), 2.60–2.58 (m, 2H, 4-H), 2.11–2.03 (m, 2H, 5-H), 1.84–1.79 (m, 1H, CH), 1.26–1.18 (m, 2H, CH_2), 1.16–1.03 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_{11}ClO_3$ 214.0397, found 214.0403.

2-Bromo-3-cyclopropanecarbonyloxy-2-cyclohexen-1-one (13). This compound was obtained from 2-bromocyclohexane-1,3-dione[19] **21** as a yellow liquid with a 50% yield. R_f = 0.26 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 2.71–2.61 (m, 4H, 4-H, 6-H), 2.12–2.04 (m, 2H, 5-H), 1.84–1.76 (m, 1H, CH), 1.23–1.20 (m, 2H, CH_2), 1.09–1.05 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_{11}BrO_3$ 257.9891, found 257.9886.

3-Cyclopropanecarbonyloxy-5-ethoxycarbonyl-2-cyclohexen-1-one (14). This compound was obtained from 5-ethoxycarbonyl-cyclohexane-1,3-dione[9] as a yellow liquid with a 67% yield. R_f = 0.35 (35% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 5.96 (s, 1H, 2-H), 4.18 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 3.15–3.05 (m, 1H, 5-H), 2.93–2.55 (m, 4H, 4-H, 6-H), 1.77–1.71 (m, 1H, CH), 1.27 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.14–1.12 (m, 2H, CH_2), 1.06–1.01 (m, 2H, CH_2). HRMS: calcd for $C_{13}H_{16}O_5$ 252.0998, found 252.1002.

2, 4, 5-Trimethoxybenzaldehyde (22b). To a solution of 1, 2, 4-trimethoxybenzene (336 mg, 2.00 mmol) in DMF (10 mL) was added dropwise phosphorus oxychloride (307 mg, 2.00 mmol) at room temperature. The resulting mixture was stirred at that temperature for 1 h. After completion of the reaction, monitored by TLC, 100 mL of water and 10% NaOH solution (20 mL) was added to the solution. The precipitate was then filtered and the product was purified by column chromatography to give a gray solid with a yield of 81%. Mp 114–115 °C. R_f = 0.48 (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 10.32 (s, 1H, CHO), 7.33 (s, 1H, Ar H), 6.50 (s, 1H, Ar H), 3.98 (s, 3H, OMe), 3.93 (s, 3H, OMe), 3.88 (s, 3H, OMe). HRMS: calcd for $C_{10}H_{12}O_4$ 196.0736, found 196.0740.

2,5-Dimethoxyphenol (23a). To a solution of commercially available 2,5-dimethoxybenzaldehyde (196 mg, 1.00 mmol) was added dropwise 30% hydrogen peroxide (0.23 mL, 2.00 mmol) and sulfuric acid (0.05 mL). The resulting mixture was then stirred at room temperature for 2 h. After completion of the reaction, water was added to the mixture and the solution was neutralized to pH 7. The product was extracted with ethyl acetate twice. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The resulting syrup was purified by column chromatography to give a yellow liquid with a yield of 65%. R_f = 0.45 (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 6.78 (d, J = 8.7 Hz, 1H, Ar H), 6.56 (d, J = 3.0 Hz, 1H, Ar H), 6.38 (dd, J = 8.7, 3.0 Hz, 1H, Ar H), 5.67 (bs, 1H, OH), 3.84 (s, 3H, OMe), 3.75 (s, 3H, OMe). HRMS: calcd for $C_8H_{10}O_3$ 154.0630, found 154.0634.

2, 4, 5-Trimethoxyphenol (23b). This compound was obtained as a white solid with a yield of 69% following the procedure of **23a**. Mp 60–61 °C. R_f = 0.40 (50% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 6.61 (s, 1H, Ar H), 6.58 (s, 1H, Ar H), 5.28 (bs, 1H, OH), 3.85 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.82 (s, 3H, OMe). HRMS: calcd for $C_9H_{12}O_4$ 184.0736, found 184.0739.

1-Cyclopropanecarbonyloxy-2,5-dimethoxybenzene (24a). This compound was obtained as a yellow liquid with a yield of 92%. R_f = 0.60 (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 6.89 (d, J = 8.7 Hz, 1H, Ar H), 6.72 (dd, J = 8.7, 3.0 Hz, 1H, Ar H), 6.65 (d, J = 3.0 Hz, 1H, Ar H), 3.38 (s, 3H, OMe), 3.75 (s, 3H, OMe), 1.91–1.83 (m, 1H, CH), 1.21–1.16 (m, 2H, CH_2), 1.05–0.98 (m, 2H, CH_2). HRMS: calcd for $C_{12}H_{14}O_4$ 222.0892, found 222.0880.

1-Cyclopropanecarbonyloxy-2,4,5-trimethoxybenzene (24b). This compound was obtained as a colorless liquid with a yield of 75%. R_f = 0.47 (50% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 6.64 (s, 1H, Ar H), 6.60 (s, 1H, Ar H), 3.87 (s, 3H, OMe), 3.85 (s, 3H, OMe), 3.82 (s, 3H, OMe), 1.92–1.84 (m, 1H, CH), 1.19–1.03 (m, 2H, CH_2), 1.02–0.99 (m, 2H, CH_2). HRMS: calcd for $C_{13}H_{16}O_5$ 252.0998, found 252.0993.

2-Cyclopropanecarbonyloxy-1,4-benzoquinone (15). To a solution of **24a** (127 mg, 0.64 mmol) in acetone was added silver (II) oxide (372 mg, 3.00 mmol), and 6 N nitric acid (1 mL) dropwise at room temperature. The reaction was finished within 1 h. After acetone was removed under reduced pressure, the residue was dissolved in water. The aqueous layer was neutralized with sodium bicarbonate and extracted with ether. The combined organic extracts were dried over $MgSO_4$, filtered, concentrated, and the residue was purified by column

chromatography to give a yellow solid with a yield of 80%. Mp 173–175 °C. $R_f = 0.55$ (40% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.26 (s, 1H, Ar H), 6.87 (s, 1H, Ar H), 6.70 (s, 1H, Ar H), 1.91–1.84 (m, 1H, CH), 1.27–1.20 (m, 2H, CH_2), 1.15–1.09 (m, 2H, CH_2). HRMS: calcd for $C_{10}H_8O_4$ 192.0422, found 192.0428.

2-Cyclopropanecarbonyloxy-5-methoxy-1,4-benzoquinone (16). This compound was obtained as a yellow solid with a yield of 83% following the procedure of **15**. Mp 85–87 °C. $R_f = 0.43$ (50% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 6.54 (s, 1H, Ar H), 5.95 (s, 1H, Ar H), 3.86 (s, 3H, OMe), 1.90–1.84 (m, 1H, CH), 1.26–1.20 (m, 2H, CH_2), 1.13–1.08 (m, 2H, CH_2). HRMS: calcd for $C_{11}H_{10}O_5$ 222.0528, found 222.0533.

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