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Design and Synthesis of 3-Fluoro-2-oxo-3-phenylpropionic Acid Derivatives as Potent Inhibitors of 4-Hydroxyphenylpyruvate Dioxygenase from Pig Liver

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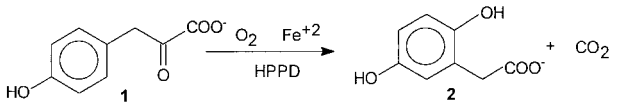
Abstract—Several rationally designed analogs of 3-fluoro-2-oxo-3-phenylpropionic acid were chemically synthesized, and the reactions of the hydrate form of these compounds with 4-hydroxyphenylpyruvate dioxygenase from pig liver as inhibitors were examined. Compounds **14a** and **14b** were found to be potent competitive inhibitors of the enzyme with K_i values of 10 and 22 μM, respectively.

Introduction

4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD)[1] is a key enzyme involved in the catabolism of tyrosine and phenylalanine in most organisms. It catalyzes the conversion of 4-hydroxyphenylpyruvate (HPP, **1**) and molecular oxygen to homogentisate **2** and carbon dioxide as shown below.



This enzyme has been isolated homogeneously from avian liver,[2] mammalian liver,[3] and a *pseudomonad* strain.[4] Most of these purified proteins have been shown to contain a non-heme iron as a cofactor essential for catalytic activity. Early studies of HPPD have established that one atom each from molecular oxygen ends up in carboxyl and hydroxyl groups of the product homogentisate and the produced carbon dioxide comes from the carboxylate of HPP.[5] Although kinetic studies of the enzymes have indicated the binding of substrates is ordered with oxygen as the second substrate,[6] none of the proposed enzyme-bound intermediates in the reaction have been observed or trapped. Thus, the sequential events catalyzed by HPPD leading to decarboxylation, hydroxylation and rearrangement of HPP remains unclear. Nevertheless, a couple of possible mechanisms have been proposed by Witkop[7] and Hamilton[8] as depicted in Schemes 1 and 2.

In Scheme 1, the metal ion–oxygen complex reacts with the carbanion of HPP to form a peroxide ion **3**, which then attacks the α-carbon of the keto acid yielding cyclic peroxide **4**. After decarboxylation, the carboxymethyl side chain of **5** migrates to the *ortho*-position to yield the product **2** by a mechanism similar to the NIH shift.[9]

In Scheme 2, the metal ion–oxygen complex reacts with the carbonyl group of the α-keto acid to form the cyclic peroxide **6**, followed by oxidative decarboxylation to yield the oxoiron (IV) species **7**. The proposed high-valent iron-oxo species then attacks the ring to give arene oxide-like intermediate **8**, followed by enzyme-catalyzed 1, 2-shift of the carboxymethyl group and final aromatization to afford homogentisate **2**.

According to the proposed mechanisms for HPPD in Schemes 1 and 2, both mechanisms share a common step of nucleophilic attack of activated oxygen onto the carbonyl group of the α-keto acid to generate the tetrahedral enzyme-substrate intermediate either **4** or **6**. We speculate the catalytic process will be blocked if the α-keto group of the natural substrate HPP change from keto form to hydrate form, since tetrahedral structure of α-carbon of HPP hydrate form is no longer feasible for nucleophilic attack by activated oxygen. Thus, the hydrate form of HPP may serve as a potential inhibitor for HPPD. The desired α-keto hydrated HPP can be prepared by simply introducing a fluorine atom at C-3

Key words: HPPD; enzyme inhibition; keto form; hydrate form.
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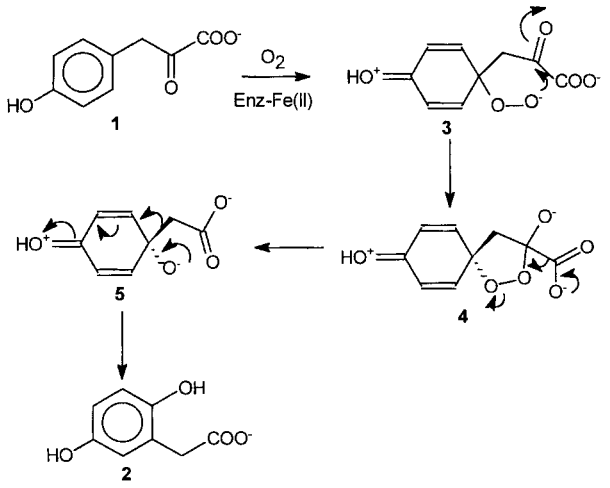
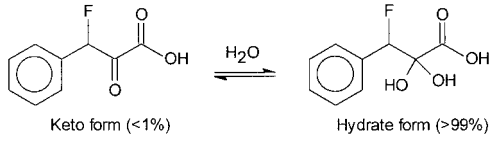
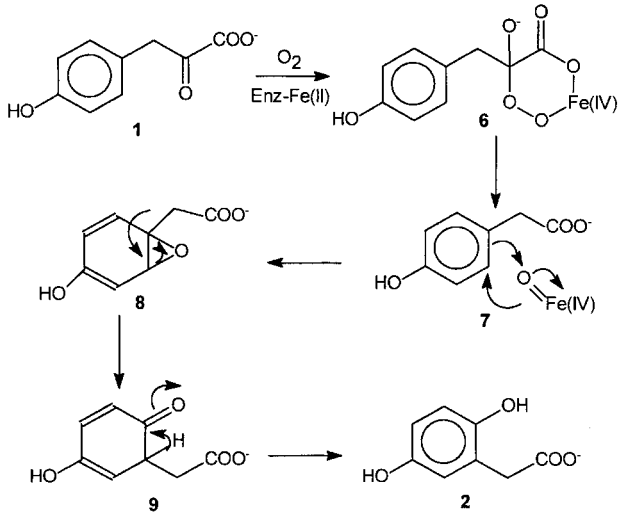


Scheme 1. Proposed HPPD mechanism by Witkop.

position of the natural substrate HPP. The substitution of a hydrogen atom by fluorine in organic molecules introduces few steric demands, but the high electronegativity of fluorine may induce large changes in reactivity. For example, the α-keto group of 3-fluoro-3-phenylpyruvic acid, flanked by an electron-withdrawing fluorine atom and a carboxylic acid group, is essentially hydrated in aqueous solution because of the adjacent fluorine atom.[10]



In this paper, we report studies of the reactions of a series of chemically synthesized fluorinated α-keto hydrated 3-fluoro-3-phenylpyruvic acids with 4-hydroxyphenylpyruvate dioxygenase from pig liver as our initial

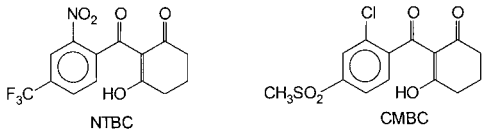


Scheme 2. Proposed HPPD mechanism by Hamilton.

effort to elucidate the pathway for this interesting enzymatic transformation and to develop strong inhibitors for this enzyme.

Results and Discussion

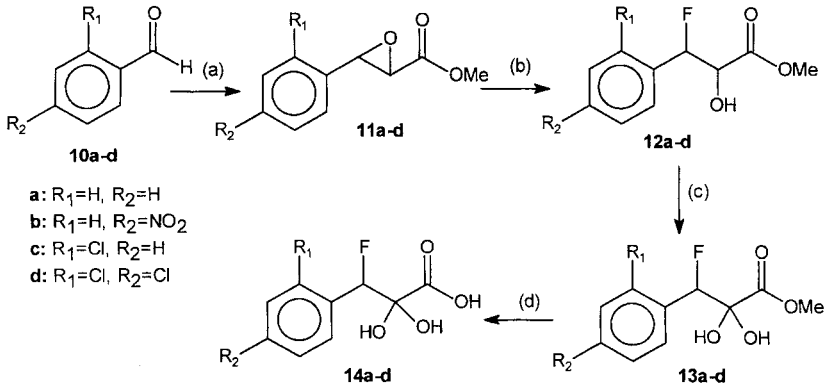
Since several well-known potent inhibitors of HPPD, such as 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione[11a] (NTBC, $IC_{50} = 40$ nM) and 2-(2-chloro-4-methanesulfonylbenzoyl)-cyclohexane-1,3-dione[11b] (CMBC, $K_i = 10$ nM), contain relatively large substituents at *ortho* and *para*-positions of the phenyl ring,[11c–d] it is conceivable that these bulky groups on the aromatic ring may play an important role in HPPD inactivation. Thus, the efficacy of single fluorinated substrate analogues as potential HPPD inhibitors may be enhanced by selectively introducing the bulky groups at *ortho* and/or *para*-position of aromatic ring to increase the number of favorable interactions between inhibitor and enzyme active site. In order to test this hypothesis, several *ortho* and/or *para*-substituted 3-fluoro-2-oxo-3-phenylpropionic acids have also been synthesized to investigate their effects on enzyme inactivation.



The procedure for the synthesis of 3-fluoro-2-oxo-3-phenylpropionic acid derivatives[12] is outlined in Scheme 3. According to this strategy, epoxy esters **11a–d** were obtained by Darzens[13] glycidic ester condensation of commercially available *ortho* and/or *para*-substituted benzaldehydes **10a–d** with methyl chloroacetate in the presence of sodium methoxide. Ring opening of **11a–d** with a hydrogen fluoride-pyridine solution produced the β-fluoro-α-hydroxy esters **12a–d**, which were oxidized to the corresponding hydrate form of β-fluoro-α-keto esters **13a–d** by Jones reagent and then hydrolyzed, under basic conditions, to give the target hydrate form of 3-fluoro-α-keto acids **14a–d**.

The extent of hydration of 3-fluoro-2-oxo-3-phenylpropionic acid derivatives in aqueous solution were determined by ^{1}H NMR analysis. 3-Fluoro-*p*-nitrophenylpyruvate **14b**, for example, was found to be in both keto and hydrate forms when dissolved in acetone-d_6 with hydrate/keto ratio of 2.6/1, based on the integration of β-H in ^{1}H NMR spectrum shown in Figure 1. When dissolved in deuterium oxide, however, **14b** was found exclusively in hydrate form with the complete disappearance of the keto form β-H signal at 6.83 ppm.

With these potential HPPD inhibitors at hand, incubation experiments were then conducted to determine kinetic and inhibition parameters as well as the type of inhibition of **14a–d** by the spectrophotometric enol borate assay method.[14] The time-course of the reaction of pig liver HPPD in the absence and presence of increasing amount of the hydrate form of 3-fluoro-3-



Scheme 3. Synthesis of compounds **14a–d**. (a) $ClCH_2CO_2Me$, NaOMe, MeOH; (b) HF/pyridine, CH_2Cl_2; (c) Jones reagent, acetone; (d) H_2O, *i*-PrOH, $NaHCO_3$.

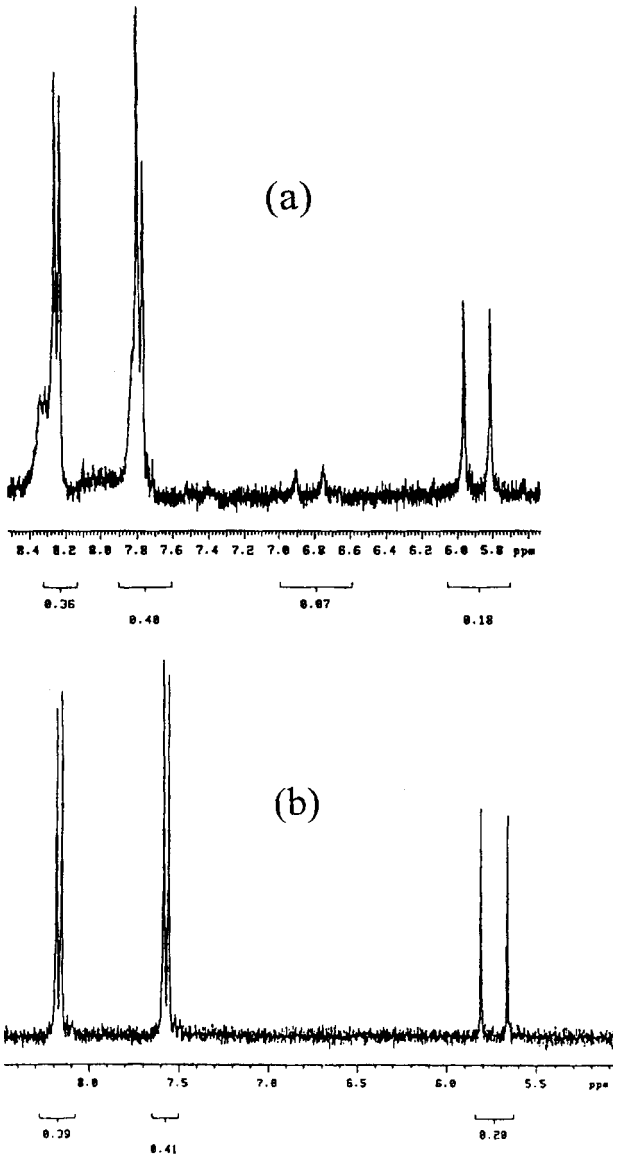


Figure 1. ^1H NMR spectra (300 MHz) of **14b** in (a) acetone-d_6 (b) D_2O. The more upfield doublet at 5.88 ppm in (a) was assigned to β-H of **14b** with $J_{H-F} = 44.7$ Hz and the small doublet at 6.83 ppm was assigned to β-H of keto form of **14b** with $J_{H-F} = 47.1$ Hz.

phenylpyurvate is shown in Figure 2. The activity of HPPD was obviously inhibited by **14a**. Almost complete inhibition was observed at a concentration of 100 μM.

The inhibition constants for the reactions of **14a–d** with HPPD are listed in Table 1. The Lineweaver–Burk plots indicated that **14a** and **14b** were competitive inhibitors of HPPD with respect to HPP, whereas **14c** and **14d** were noncompetitive inhibitors. Compounds **14a** and **14b** were found to be potent inhibitors of the enzyme with K_i values of 10 and 22 μM, respectively. Apparently, the substituent on *para*-position of the phenyl ring is not essential for binding, since the introduction of *p*-nitro group resulted in little change in potency. The failures of **14c** and **14d** as competitive inhibitors of HPPD are consistent with the results reported by Pascal,[15] who has shown that *o*-chloro and *o*-methyl-HPP were noncompetitive inhibitors of HPPD. Perhaps the relatively bulky substituent at *ortho*-position of phenyl moiety distorts the molecular geometry which is required for binding. Furthermore, the fact that higher steric demands on the *ortho*-position of HPP ring substituents as the substrate for HPPD suggest that **14a** and **14b** may share different inhibition mechanism of action with NTBC and CMBC, since latter compounds required a bulky *ortho* subsutituent like nitro or chloro group for potent inhibition activity.[16]

Conclusion

A series of analogues of the hydrate form of 3-fluoro-2-oxo-3-phenylpropionic acid were rationally designed and synthesized to mimic the tetrahedral structure of enzyme–substrate complex, and the reactions of these compounds with 4-hydroxyphenylpyruvate dioxygenase purified from pig liver as inhibitors were examined. Compounds **14a** and **14b** were found to be potent competitive inhibitors of the enzyme HPPD, whereas compound **14c** and **14d** were noncompetitive inhibitors. The substrate analogues for potent HPPD inhibition appear to have higher steric demands on *ortho*-position than *para*-position of the phenyl moiety. The results of our studies will provide useful information for designing inhibitors for treatment of the fatal disease tyrosinemia

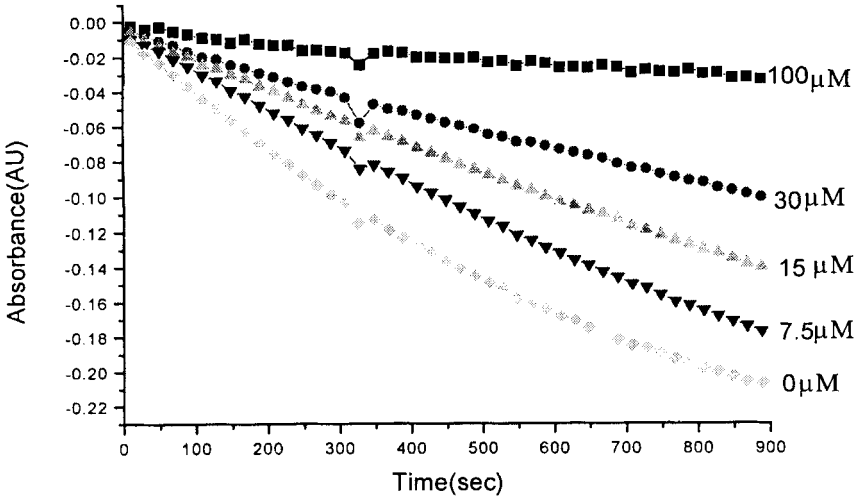


Figure 2. Time-course of the reaction of HPPD and 4-hydroxyphenylpyruvate (35 μM) with varying concentrations of **14a** (100, 30, 15, 7.5, and 0 μM). The protein concentration in the assay was 1 mg/mL.

type I[11a] and developing new class of bleaching herbicides[17] for selected plants as well.

Experimental

General

Melting points were determined on a Mel-Temp melting point apparatus in open capillaries and are uncorrected. High resolution FAB-MS was measured with a JEOL JMS-SX/SX 102A spectrometer. Ultraviolet-visible spectroscopy was performed on Hewlett–Packard 8453 spectrophotometer. ^1H and ^{13}C 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 and ^{13}C 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 60G-254 plates (25 mm) and developed with the solvents mentioned. Solvents, unless otherwise specified, were reagent grade and distilled once prior to use.

Table 1. Kinetic constants for reactions of **14a–d** with HPPD from pig liver

Compound	R_1	R_2	type[a]	K_i (μM)[b]
14a	H	H	C	10 ± 2
14b	H	NO_2	C	22 ± 2
14c	Cl	H	NC	250 ± 20
14d	Cl	Cl	NC	380 ± 25

[a] C = competitive inhibitor; NC = noncompetitive inhibitor.
[b] Assay detail is described in Experimental.

Enzyme purification and assay

Phenylpyruvate tautomerase was purified from pig liver by the method of Knox.[18] HPPD was purified from pig liver by the method of Hamilton[19] with the specific activity of 0.1 μmol min^{-1} mg^{-1}. The protein concentration was determined by BCA method.[20] Routine assay of the enzyme utilized the spectrophotometric enol borate method of Lindstedt and Rundgren.[14] 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 HPP (1.8 mM, in 0.2 M Na_3PO_4 buffer), 30 mL dichlorophenolindophenol (reduced form, prepared by mixing 1 mL of 3.3 mM sodium dichloro-phenolindophenol in H_2O and 0.16 M glutathione in 0.2 M sodium phosphate buffer), 0.01 mL phenylpyruvate tautomerase (10 U/mL). The above solution was equilibrated for about 15 min (monitored at 308 nm), then 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 interaction of 3-fluoro-2-oxo-3-phenylpropionic acid derivatives and the enzyme HPPD was evaluated by measuring the decrease in absorbance at 308 nm over a 15 min period following coadministration of varying concentration of the substrate and inhibitors.

General procedure for preparation of epoxy esters **11a–d**. A solution of benzaldehyde (2.12 g, 10.0 mmol) and methyl chloroacetate (2.0 g, 20.0 mmol) was added a solution of sodium methoxide in anhydrous MeOH (2 g of sodium dissolved in 40 mL of MeOH), with the temperature kept at −10 °C. After the addition was completed, the solution was stirred at 0 °C for 2 h and then 3 h at room temperature. The mixture was then poured into 0.1 N HCl cold water and the crude product was extracted with ethyl acetate, dried with $MgSO_4$, concentrated in vacuo to give the yellow solid or liquid which can be further recrystallized in ethanol or purified by flash chromatography to give the desired product.

Methyl 3-phenylglycidate (11a). This compound was obtained as a white solid in 52% yield, mp 84–85 °C (lit.[12] 84 °C).

Methyl *trans*-3-(4-nitrophenyl)glycidate (11b). This compound was obtained as a white solid in a 65% yield, mp 139–140 °C (lit.[21] 138–140 °C).

Methyl *trans*-3-(2-chlorophenyl)glycidate (11c).[22] This compound was obtained as a yellow liquid in a 68% yield.

Methyl *trans*-3-(2,4-dichlorophenyl)glycidate (11d). This compound was obtained as a colorless liquid with a 62% yield. R_f = 0.66 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.38–7.16 (3H; m; Ar H's), 4.38 (1H; d, J = 2.1; H_β), 3.85 (3H; s; OMe), 3.37 (1H; d, J = 2.1; H_α). ^{13}C NMR ($CDCl_3$) δ 167.8 (COOMe), 134.9, 133.9, 131.7, 129.1, 127.4, 126.8 (Ar C's), 55.7, 54.8 (Ca, Cb), 52.6 (OMe). HRMS: calcd for $C_{10}H_8Cl_2O_3$ 245.9851, found 245.9847.

General procedure for preparation of α-hydroxy esters **12a–d**. To a solution of epoxy ester **11** (392.0 mg, 2.2 mmol) in dry methylene chloride (10 mL) in a plastic beaker was added dropwise a solution of HF/pyridine (70%, 0.2 mL) under −20 °C. After the reaction is completed within 5 min, the mixture was added to ice-cold water, extracted with methylene chloride, dried with $MgSO_4$, concentrated in vacuo, and purified by column chromatography to give the pure product.

Methyl 3-fluoro-2-hydroxy-3-phenylpropionate (12a).[12] This compound was obtained as a light yellow liquid in a 90% yield.

Methyl 3-fluoro-2-hydroxy-(4-nitrophenyl)propionate (12b). This compound was obtained as a yellow solid in a 60% yield. mp 86–89 °C. R_f = 0.39 (25% EtOAc/hexanes). ^{1}H NMR (*erythro* + *threo*) ($CDCl_3$) δ 8.27 (2H; d, J = 8.4; Ar H's), 8.24 (2H; d, J = 9.0; Ar H's), 7.58 (2H; d, J = 9.0; Ar H's), 7.51 (2H; d, J = 9.0; Ar H's), 5.91 (1H; dd, J = 45.0, 1.8; H_β), 5.84 (1H; dd, J = 45.3, 3.6; H_β), 4.69 (1H; d, J_{F-H} = 16.8; H_α), 4.48 (1H; dd, J = 28.2, 4.2, H_α), 3.91 (3H, s; OMe), 3.79 (3H; s; OMe), 3.21 (1H; d, J = 6.3; OH), 3.13 (1H; d, J = 6.6; OH). ^{13}C NMR (*erythro* + *threo*) ($CDCl_3$) δ 171.2, 171.1 (*C*OOMe), 148.2, 148.1 (Ar C-4's), 142.6 (d, J = 20.7; Ar C-1's), 127.2–126.8 (Ar C-2, Ar C-6's), 123.8–123.4 (Ar C-3, Ar C-5's), 92.8 (d, J = 183.2; C_β), 92.1 (d, J = 183.1; C_β), 73.3 (d, J = 22.6; C_α), 73.0 (d, J = 20.9; C_α), 53.3 (OMe), 52.9 (OMe). HRMS: calcd for $C_{10}H_{10}FNO_5$ 243.0543, found 243.0531.

Methyl 3-fluoro-2-hydroxy-(2-chlorophenyl)propionate (12c). This compound was obtained as a yellow liquid in a 78% yield. R_f = 0.45 (25% EtOAc/hexanes) ^{1}H NMR (*erythro* + *threo*) ($CDCl_3$) δ 7.62–7.31 (8H; m; Ar H's), 6.18 (1H; dd, J = 44.4, 1.2; H_β), 6.08 (1H; ddd, J = 45.3, 3.0, 0.6; H_β), 4.71 (1H; ddd, J = 18.0, 8.1, 3.0; H_α), 4.59 (1H; ddd, J = 30.1, 7.2, 1.2; H_α), 3.92 (3H; s; OMe), 3.68 (3H, s; OMe), 3.31 (1H; d, J = 8.1; OH), 3.24 (1H; d, J = 7.2; OH). ^{13}C NMR (*erythro* + *threo*) ($CDCl_3$) δ 171.5, 171.3 (COOMe), 133.5–126.7 (Ar C's), 91.4 (d, J = 180.9; C_β), 90.4 (d, J = 179.8; C_β), 72.4 (d, J = 25.3; C_α), 70.9 (d, J = 22.6; C_α), 53.1 (OMe), 52.4 (OMe). HRMS: calcd for $C_{10}H_{10}ClFO_3$ 232.0303, found 232.0312.

Methyl 3-fluoro-2-hydroxy-(2,4-dichlorophenyl)propionate (12d). This compound was obtained as a yellow liquid in a 68% yield. R_f = 0.53 (25% EtOAc/hexanes). ^{1}H NMR (*erythro* + *threo*) ($CDCl_3$) δ 7.52–7.29 (6H; m; Ar H's), 6.16 (1H; dd, J = 45.5, 1.5; H_β), 6.05 (1H; dd, J = 44.7, 3.0; H_β), 4.68 (1H; dd, J = 18.2, 8.1; H_α), 4.56 (1H; dd, J = 30.0, 6.9; H_α), 3.92 (3H; s; OMe), 3.71(3H; s; OMe), 3.31 (1H; d, J = 8.1; OH), 3.23 (1H; d, J = 7.2; OH). ^{13}C NMR (*erythro* + *threo*) ($CDCl_3$) δ 171.7, 171.5 (*C*OOMe), 135.3–126.9 (Ar C's), 91.0 (d, J = 181.4; C_β), 90.0 (d, J = 180.4; C_β), 72.4 (d, J = 22.0; C_α), 71.0 (d, J = 22.6; C_α), 53.1 (OMe), 52.5 (OMe). HRMS: calcd for $C_{10}H_9Cl_2FO_3$ 265.9914, found 265.9925.

General procedure for preparation of α-keto esters **13a–d**. To a solution of α-hydroxy ester **12** (500 mg, 2.53 mmol) in acetone (15 mL) was added Jones reagent dropwise under room temperature until the reaction was completed. The excess of Jones reagent was destroyed by adding isopropanol into the reaction mixture. After concentration by rotatory evaporation, the residue was added to water and followed by routine extraction and purification procedure to give the pure compound.

Methyl 3-fluoro-3-phenylpyruvate (13a).[12] This compound was obtained as a colorless liquid in a 55% yield.

Methyl 3-fluoro-*p*-nitrophenylpyruvate (13b). This compound was obtained as a yellow solid in a 40% yield. mp 106–108 °C. R_f = 0.48 (50% EtOAc/hexanes). ^{1}H NMR (*keto* form) ($CDCl_3$) δ 8.29 (2H; d, J = 8.4; Ar H's), 7.70 (2H; d, J = 8.7; ArH's), 6.54 (1H; d, J_{F-H} = 46.8; H_β), 3.88 (3H; s; OMe). ^{1}H NMR (*hydrate* form) ($CDCl_3$) δ 8.27 (2H; d, J = 8.4; Ar H's), 7.68 (2H; d, J = 8.7; Ar H's), 5.78 (1H; d, J_{F-H} = 44.7; H_β), 4.51 (1H; bs; OH), 3.95 (3H; s; OMe), 3.19 (1H; bs; OH). ^{13}C NMR (*hydrate* form) (Acetone-d_6) δ 171.5 (*C*OOMe), 149.2 (Ar C-4's), 142.9 (d, J = 20.2; Ar C-1's), 130.1 (d, J = 7.7; Ar C-2, Ar C-6's), 123.4 (Ar C-3, Ar C-5's), 94.3 (d, J = 26.3; C_α), 94.0 (d, J = 179.3; C_β), 53.2 (OMe). HRMS: calcd for $C_{10}H_{10}FNO_6$ 259.0492, found 259.0485.

Methyl 3-fluoro-*o*-chlorophenylpyruvate (13c). This compound was obtained as a yellow liquid in a 56% yield. R_f = 0.58 (50% EtOAc/hexanes). ^{1}H NMR (*keto* form) ($CDCl_3$) δ 7.51–7.36 (4H; m; Ar H's), 6.82 (1H; d, J = 46.2; H_β), 3.83 (3H; s; OMe). ^{1}H NMR (*hydrate* form) δ 7.51–7.36 (4H; m; Ar H's), 6.22 (1H; d, J = 44.3; H_β), 3.92 (3H; s; OMe). ^{13}C NMR (*keto* + *hydrate* form) ($CDCl_3$) δ 186.9 (d, J = 24.8; *keto* form C_α), 171.2 (*hydrate* form *C*OOMe), 160.0 (d, J = 2.2, *keto* form *C*OOMe), 134.3–127.5 (m; *keto* + *hydrate* form Ar C's), 93.5 (d, J = 29.1; *hydrate* form C_α), 89.9 (d, J = 187.0; *keto* form C_β), 88.9 (d, J = 186.4; *hydrate* form C_β), 53.9 (*hydrate* form OMe), 53.2 (*keto* form OMe). HRMS: calcd for $C_{10}H_{10}ClFO_4$ 248.0252, found 248.0257.

Methyl 3-fluoro-3-(2,4-dichlorophenyl)pyruvate (13d). This compound was obtained as a colorless liquid in a 64% yield. $R_f = 0.32$ (25% EtOAc/hexanes). ^{1}H NMR (*keto* form) ($CDCl_3$) δ 7.65–7.26 (3H; m; Ar H's), 6.76 (1H; d, $J = 45.9$; H_β), 3.87 (3H; s; OMe). ^{1}H NMR (*hydrate* form) δ 7.51–7.36 (4H; m; Ar H's), 6.18 (1H; d, $J = 43.8$; H_β), 4.49 (1H; s; OH), 3.96 (3H; s; OMe), 3.12 (1H; s; OH). ^{13}C NMR (*keto* + *hydrate* form) ($CDCl_3$) δ 188.1 (d, $J = 22.1$; *keto* form C_α), 173.2 (*hydrate* form *C*OOMe), 160.3 (*keto* form *C*OOMe), 134.1–127.2 (m; *keto* + *hydrate* form Ar C's), 93.5 (d, $J = 29.2$; *hydrate* form C_α), 89.2 (d, $J = 185.1$; *keto* form C_β), 88.6 (d, $J = 183.4$; *hydrate* form C_β), 53.6 (*hydrate* form OMe), 53.4 (*keto* form OMe). HRMS: calcd for $C_{10}H_9Cl_2FO_4$ 281.9863, found 281.9869.

General procedure for preparation of α-keto acids **14a–d**. To a solution of α-keto ester **13** (300 mg, 1.5 mmol) in 10 mL of H_2O/*i*-PrOH (1/1) was added solid sodium bicarbonate until it was saturated under room temperature. After the hydrolysis was completed within 1 h, aqueous HCl solution (0.1 N) was added to adjust pH to about 3. The product was then extracted with ethyl acetate, dried with $MgSO_4$, concentrated in vacuo, and purified by flash chromatography to give the final target α-keto acid **14** with low yield.

3-Fluoro-3-phenylpyruvic acid (14a). This compound was obtained as a white solid in a 45% yield, mp 70–71 °C. $R_f = 0.35$ (20% MeOH/EtOAc). ^{1}H NMR (*keto* form) (Acetone-d_6) δ 7.50–7.33 (5H; m; Ar H's), 6.64 (1H; d, $J_{F-H} = 47.1$; H_β). ^{1}H NMR (*hydrate* form) (Acetone-d_6) δd 7.50–7.33 (5H; m; Ar H's), 5.69 (1H; d, $J_{F-H} = 44.7$; H_β). ^{13}C NMR (*keto* + *hydrate* form) (Acetone-d_6) δ 188.3 (d, $J = 24.5$; *keto* form C_α), 171.8 (*hydrate* form *C*OOH), 160.5 (*keto* form *C*OOH), 135.1–127.3 (m; *keto* + *hydrate* form Ar C's), 96.3 (d, $J = 27.2$; *hydrate* form C_α), 94.0 (d, $J = 177.0$; *keto* form C_β), 93.2 (d, $J = 184.2$; *hydrate* form C_βb). HRMS: calcd for $C_9H_9FO_4$ 200.0485, found 200.0512.

3-Fluoro-*p*-nitrophenylpyruvic acid (14b). This compound was obtained as a white solid in a 25% yield. mp 88 °C dec. $R_f = 0.38$ (20% MeOH/EtOAc). ^{1}H NMR (*hydrate* form) (D_2O) δ 8.17 (2H; d, $J = 8.4$; Ar H's), 7.57 (2H; d, $J = 8.7$; Ar H's), 5.74 (1H; d, $J_{F-H} = 44.7$; H_β). ^{13}C NMR (D_2O) δ 172.9 (*C*OOH), 148.0 (Ar C-4's), 141.1 (d, $J = 20.4$; Ar C-1's), 128.3, (d, $J = 7.7$; Ar C-2, Ar C-6's), 123.2 (Ar C-3, Ar C-5's), 93.6 (d, $J = 180.4$, C_β), 93.3 (d, $J = 27.6$; C_α). HRMS: calcd for $C_9H_8FNO_6$ 245.0335, found 245.0330.

3-Fluoro-*o*-chlorophenylpyruvic acid (14c). This compound was obtained as a white solid in a 37% yield. mp 72–73 °C. $R_f = 0.43$ (20% MeOH/EtOAc). ^{1}H NMR (*keto* form) (Acetone-d_6) δ 7.62–7.42 (4H; m; Ar H's), 6.94 (1H; d, $J = 46.5$; H_β). ^{1}H NMR (*hydrate* form) (Acetone-d_6) δ 7.62–7.42 (4H; m; Ar H's), 6.28 (1H; d, $J = 45.0$; H_β). ^{13}C NMR (*keto* + *hydrate* form) (Acetone-d_6) δ 189.2 (d, $J = 24.2$; *keto* form C_α), 172.2 (*hydrate* form *C*OOH), 161.8 (*keto* form *C*OOH), 135.1–127.3 (m; *keto* + *hydrate* form Ar C's), 94.3 (d, $J = 28.5$; hydrate form C_α), 91.4 (d, $J = 183.6$; *keto* form C_β), 90.2 (d, $J = 184.7$; *hydrate* form C_β). HRMS: calcd for $C_9H_8ClFO_4$ 234.0095, found 234.0089.

3-Fluoro-3-(2,4-dichlorophenyl)pyruvic acid (14d). This compound was obtained as a white solid in a 42% yield. mp 79–81 °C. $R_f = 0.46$ (20% MeOH/EtOAc). ^{1}H NMR (*keto* form) (Acetone-d_6) δ 7.82–7.41 (3H; m; Ar H's), 6.93 (1H; d, $J = 45.9$; H_β). ^{1}H NMR (*hydrate* form) (Acetone-d_6) δ 7.82–7.41 (3H; m; Ar H's), 6.24 (1H; d, $J = 44.1$; H_β). ^{13}C NMR (*keto* + *hydrate* form) (Acetone-d_6) δ 188.5 (d, $J = 22.7$; *keto* form C_α), 171.8 (*hydrate* form *C*OOH), 160.6 (*keto* form *C*OOH), 133.1–127.6 (m; *keto* + *hydrate* form Ar C's), 93.8 (d, $J = 27.6$; *hydrate* form C_α), 90.9 (d, $J = 184.8$; *keto* form C_β), 99.8 (d, $J = 181.9$; *hydrate* form C_β). HRMS: calcd for $C_9H_7Cl_2FO_4$ 267.9706, found 267.9715.

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