Synthesis and SAR of 4-Aryl-2-hydroxy-4-oxobut-2-enoic Acids and Esters and 2-Amino-4-aryl-4-oxobut-2-enoic Acids and Esters: Potent Inhibitors of Kynurenine-3-hydroxylase as Potential Neuroprotective Agents

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The synthesis and structure−activity relationship of a series of 4-aryl-2-hydroxy-4-oxobut-2-enoic acids and esters and 2-amino-4-aryl-4-oxobut-2-enoic acids and esters as potent inhibitors of kynurenine-3-hydroxylase are described. These compounds are the most potent inhibitors of the kynurenine-3-hydroxylase enzyme so far disclosed. Additionally methyl 4-(3-chlorophenyl)-2-hydroxy-4-oxobut-2-enoate **(2d)**, 4-(3-chlorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3d)**, methyl 4-(3-fluorophenyl)-2-hydroxy-4-oxobut-2-enoate **(2f),** and 4-(3-fluorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3f)** prevent the increase in the interferon-γ-induced synthesis of quinolinic acid in primary cultures of cultured human peripheral blood monocyte-derived macrophages.

Introduction

Tryptophan is metabolized in vivo by the kynurenine pathway to yield kynurenic and quinolinic acids (Figure 1). In vivo, the former species is neuroprotective while the latter is neurotoxic.[1] Inhibitors of kynurenine-3-hydroxylase (KH) are particularly attractive since this enzyme can effectively control QUIN synthesis under conditions where the kynurenine pathway has been activated by cytokines.[2,3] Inhibition of KH has the ability to increase kynurenic acid (KynA) and decrease quinolinic acid (QUIN).

Consistent with this hypothesis, anticonvulsant activity was observed with the KH inhibitor (±)-*m*-nitrobenzoylalanine (Figure 2).[4] The anticonvulsant activity and KH inhibition were specifically associated with the *S*-(+) enantiomer. More recently, a series of benzenesulfonamide inhibitors of KH elevated KynA in brain dialysates.[5] However, the report did not demonstrate inhibition of QUIN formation by these compounds. We now report our synthesis and evaluation of 4-aryl-2-hydroxy-4-oxobut-2-enoic esters **2** and acids **3** and 2-amino-4-aryl-4-oxobut-2-enoic esters **4** and acids **5**, inhibitors of KH, and their inhibition of cellular QUIN synthesis in cultures of human macrophages.

Chemistry

The compounds described in Tables 1−4 were prepared as shown in Schemes 1 and 2.The methyl 4-aryl-2-hydroxy-4-oxobut-2-enoates **2** were prepared in modest to good yields by slowly adding the commercially available acetophenones **1** to a mixture of a freshly prepared solution of sodium methoxide in methanol and dimethyl oxalate cooled to 0 °C (Scheme 1). The corresponding acids **3** were obtained by acidic hydrolysis of the esters **2** using 6 N HCl at room temperature (Scheme 1). Proton NMR indicates that the acids **3** exist as a keto:enol mixture in a ratio of 1:9.

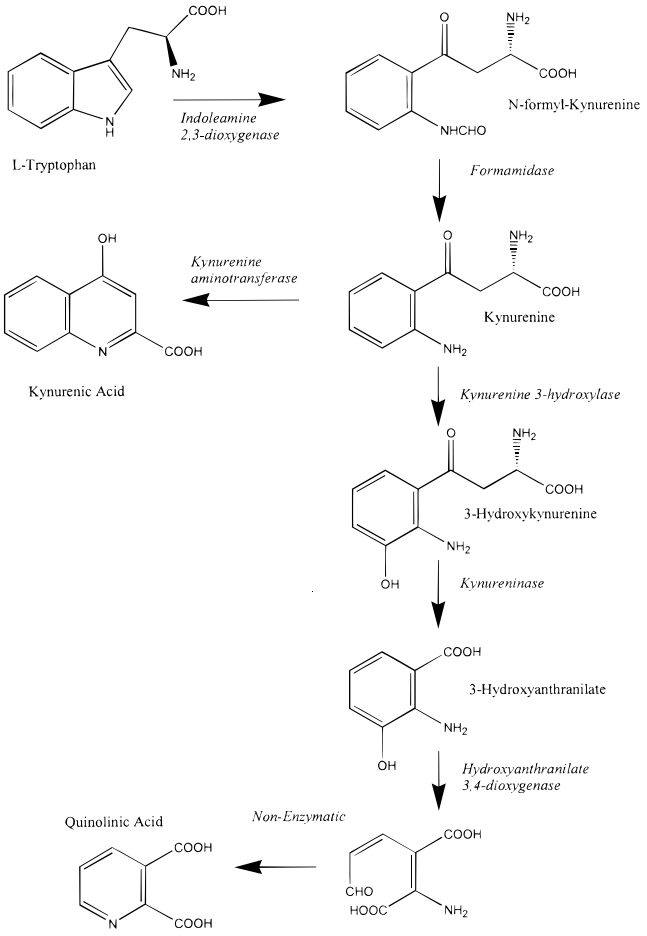

Figure 1. Overview of the kynurenine pathway.

The methyl 2-amino-4-aryl-4-oxobut-2-enoates **4** were synthesized by refluxing the hydroxy compounds **2** in

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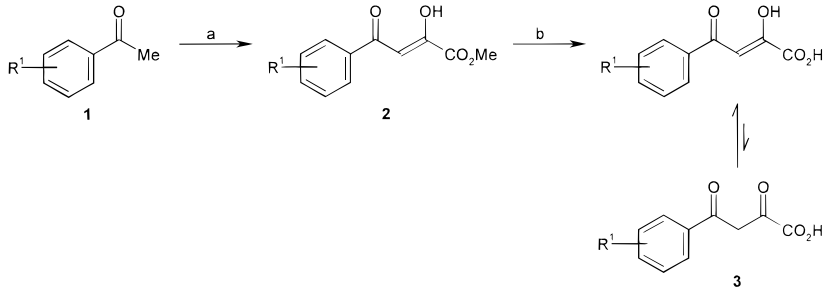
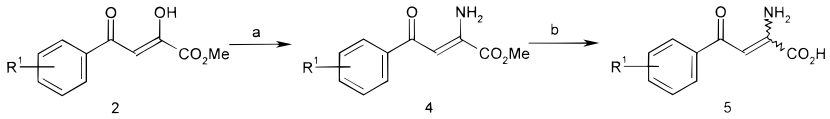
Scheme 1[a]

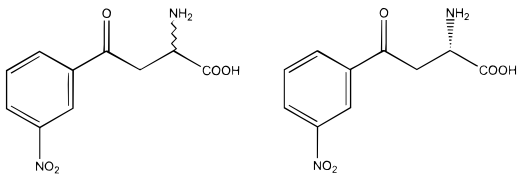


[a] Reagents: (a) NaOMe, MeOH, dimethyl oxalate, 0 °C−rt; (b) 6 N HCl, rt.

Scheme 2[a]



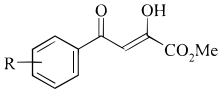
[a] Reagents: (a) NH_4OAc, benzene, CH_3CO_2H, reflux; (b) 0.5 N KOH, THF, 0 °C−rt.



(±)-*m*-Nicotinylbenzoylalanine S(+)-*m*-Nicotinylbenzoylalanine

Figure 2. (±)-*m*-Nitrobenzoylalanine and *(S)*-(+)-*m*-nitrobenzoylalanine.

Table 1. Physical and Chemical Data of Methyl 4-Aryl-2-hydroxy-4-oxobut-2-enoates



compd	R	synth meth	mp (°C)	formula	anal.
2a	H	A	56−57	$C_{11}H_{10}O_4$	C, H, N
2b	2-Cl	A	77−79	$C_{11}H_9ClO_4 \cdot 0.1H_2O$	C, H, N
2c	2-NO_2	A	85−88	$C_{11}H_9NO_6$	C, H, N
2d	3-Cl	A	82−85	$C_{11}H_9ClO_4$	C, H, N
2e	3-Br	A	100−101	$C_{11}H_9BrO_4$	C, H, N
2f	3-F	A	77−79	$C_{11}H_9FO_4 \cdot 0.1H_2O$	C, H, N
2g	4-Cl	A	103−105	$C_{11}H_9ClO_4 \cdot 1.5H_2O$	C, H, N
2h	4-Br	A	105−106	$C_{11}H_9BrO_4$	C, H, N
2i	4-F	A	125	$C_{11}H_9FO_4$	C, H, N
2j	4-Me	A	76−78	$C_{12}H_{12}O_4$	C, H, N
2k	2,3-di-Cl	A	98−99	$C_{11}H_8Cl_2O_4$	C, H, N
2l	2,3-di-F	A	82−84	$C_{11}H_8F_2O_4$	C, H, N
2m	3,4-di-Cl	A	120−122	$C_{11}H_8Cl_2O_4$	C, H, N
2n	3,4-di-F	A	107−108	$C_{11}H_8F_2O_4$	C, H, N
2o	3-Cl,4-F	A	102−103	$C_{11}H_8ClFO_4$	C, H, N
2p	3-Me,4-Cl	A	87−89	$C_{12}H_{11}ClO_4$	C, H, N
2q	3-NO_2,4-Cl	A	137−139	$C_{11}H_8ClNO_6$	C, H, N

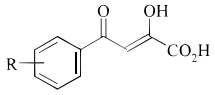
benzene with ammonium acetate and acetic acid under Dean and Stark conditions (Scheme 2). The acids **5** were obtained as a mixture of *E* and *Z* isomers by base hydrolysis of the ester **4** using 0.5 N KOH in THF (Scheme 2).

Representative examples of these structures are exemplified in the Experimental Section.

Results and Discussion

The initial targets at the outset of this work were the 2-amino-4-aryl-4-oxobut-2-enoic acids and esters **4** and **5** (Scheme 2) which were viewed as 'dehydrobenzoylalanine analogues' and thus as rational targets based on the structure of the endogenous ligand for KH, kynurenine (Figure 1). However during the synthesis of these

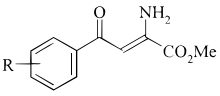
Table 2. Physical and Chemical Data of 4-Aryl-2-hydroxy-4-oxobut-2-enoic Acids



compd	R	synth meth	mp (°C)	formula	anal.
3a	H	B	148−150	$C_{10}H_8O_4 \cdot 0.25H_2O$	C, H, N
3b	2-Cl	B	138−139	$C_{10}H_7ClO_4 \cdot 0.2H_2O$	C, H, N
3c	2-NO_2	B	152−153	$C_{10}H_7NO_6 \cdot 0.15H_2O$	C, H, N
3d	3-Cl	B	154−155	$C_{10}H_7ClO_4 \cdot 0.2H_2O$	C, H, N
3e	3-Br	B	153−154	$C_{10}H_7BrO_4 \cdot 0.35H_2O$	C, H, N
3f	3-F	B	129−131[a]	$C_{10}H_7FO_4 \cdot 0.3H_2O$	C, H, N
3g	4-Cl	B	154−157	$C_{10}H_7ClO_4$	C, H, N
3k	2,3-di-Cl	B	153−154	$C_{10}H_6Cl_2O_4 \cdot 0.1H_2O$	C, H, N
3m	3,4-di-Cl	B	170−171[a]	$C_{10}H_6Cl_2O_4 \cdot 0.2H_2O$	C, H, N
3n	3,4-di-F	B	154−156	$C_{10}H_6F_2O_4 \cdot 0.1H_2O$	C, H, N
3o	3-Cl,4-F	B	163−164	$C_{10}H_6ClFO_4 \cdot 0.2H_2O$	C, H, N
3p	3-Me,4-Cl	B	149−151	$C_{11}H_9ClO_4 \cdot 0.35H_2O$	C, H, N
3q	3-NO_2,4-Cl	B	178−180	$C_{10}H_6ClNO_6$	C, H, N

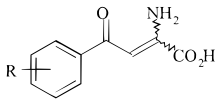
[a] Decomposed.

Table 3. Physical and Chemical Data of Methyl 2-Amino-4-aryl-4-oxobut-2-enoates



compd	R	synth meth	mp (°C)	formula	anal.
4a	H	C	33−34	$C_{11}H_{11}NO_3$	C, H, N
4b	2-Cl	C	126−128	$C_{11}H_{11}ClNO_3$	C, H, N
4d	3-Cl	C	63−64	$C_{11}H_{10}ClNO_3$	C, H, N
4e	3-Br	C	58−60	$C_{11}H_{10}BrNO_3$	C, H, N
4f	3-F	C	75−77	$C_{11}H_{10}FNO_3$	C, H, N
4g	4-Cl	C	117−119	$C_{11}H_{10}ClNO_3 \cdot 0.2H_2O$	C, H, N
4h	4-Br	C	121−123	$C_{11}H_{10}BrNO_3$	C, H, N
4i	4-F	C	80	$C_{11}H_{10}FNO_3$	C, H, N
4j	4-Me	C	96−97	$C_{12}H_{13}NO_3$	C, H, N
4k	2,3-di-Cl	C	80−81	$C_{11}H_9Cl_2NO_3$	C, H, N
4m	3,4-di-Cl	C	131−132	$C_{11}H_9Cl_2NO_3 \cdot 0.1H_2O$	C, H, N
4n	3,4-di-F	C	107−109	$C_{11}H_9F_2NO_3$	C, H, N
4o	3-Cl,4-F	C	120−123	$C_{11}H_9ClFNO_3$	C, H, N
4p	3-Me,4-Cl	C	116−117	$C_{12}H_{12}ClNO_3$	C, H, N

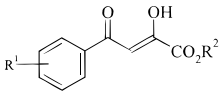
Table 4. Physical and Chemical Data of 2-Amino-4-aryl-4-oxobut-2-enoic Acids



compd	R	synth meth	mp (°C)	formula	anal.
5a	H	D	176−177	$C_{10}H_9NO_3$	C, H, N
5b	2-Cl	D	210−211[a]	$C_{10}H_8ClNO_3$	C, H, N
5d	3-Cl	D	187−188[a]	$C_{10}H_8ClNO_3$	C, H, N
5g	4-Cl	D	196−197	$C_{10}H_8ClNO_3 \cdot 0.3H_2O$	C, H, N
5i	4-F	D	180	$C_{10}H_8FNO_3 \cdot 0.25H_2O$	C, H, N
5k	2,3-di-Cl	D	220−221[a]	$C_{10}H_7Cl_2NO_3$	C, H, N
5m	3,4-di-Cl	D	210−212	$C_{11}H_7Cl_2NO_3 \cdot 0.1H_2O$	C, H, N
5n	3,4-di-F	D	193−195[a]	$C_{10}H_7F_2NO_3 \cdot 0.1H_2O$	C, H, N
5o	3-Cl,4-F	D	188−191	$C_{10}H_7ClFNO_3$	C, H, N

[a] Decomposed.

Table 5. Inhibition of KH by 4-Aryl-2-hydroxy-4-oxobut-2-enoic Acids and Esters



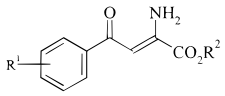
compd	R^1	R^2	IC_{50} (μM) or % inhibn at 10 μM[a]
2a	H	Me	23%
3a	H	H	0%
2b	2-Cl	Me	31%
3b	2-Cl	H	33%
2c	2-NO_2	Me	1%
3c	2-NO_2	H	24%
2d	3-Cl	Me	1.9
3d	3-Cl	H	0.32
2e	3-Br	Me	2.1
3e	3-Br	H	1.7
2f	3-F	Me	1.6
3f	3-F	H	0.58
2g	4-Cl	Me	38%
3g	4-Cl	H	3.0
2h	4-Br	Me	1.9
2i	4-F	Me	22%
2j	4-Me	Me	12%
2k	2,3-di-Cl	Me	14%
3k	2,3-di-Cl	H	33%
2l	2,3-di-F	Me	8.1
2m	3,4-di-Cl	Me	1.9
3m	3,4-di-Cl	H	1.4
2n	3,4-di-F	Me	3.3
3n	3,4-di-F	H	0.12
2o	3-Cl,4-F	Me	2.6
3o	3-Cl,4-F	H	0.27
2p	3-Me,4-Cl	Me	4.9
3p	3-Me,4-Cl	H	1.7
2q	3-NO_2,4-Cl	Me	11.2
3q	3-NO_2,4-Cl	H	2.0
(±)-*m*-nicotinylbenzoylalanine			8.0
(*S*)-(+)-*m*-nicotinylbenzoylalanine			4.0

[a] IC_{50} values are the mean values of at least 2 experiments performed in duplicate with a variation of <20%.

compounds, proton NMR showed that the intermediate methyl 4-aryl-2-hydroxy-4-oxobut-2-enoates **2** existed exclusively, and the corresponding acids **3** mainly, in their enol tautomeric form (Scheme 1). Thus the structural similarity of **2** and **3** to **4** and **5** made them equally attractive candidates.

As can be quickly seen in Tables 5 and 6, the parent unsubstituted compounds **2a**, **3a**, **4a,** and **5a** have little if any activity against KH at 10 μM. Introduction of a single substituent into the ring at the 2-position had little effect, but 3- or 4-substitution was much more promising.

Table 6. Inhibition of KH by 2-Amino-4-aryl-4-oxobut-2-enoic Acids and Esters



compd	R^1	R^2	IC_{50} (μM) or % inhibin at 10 μM[a]
4a	H	Me	3%
5a	H	H	17%
4b	2-Cl	Me	31%
5b	2-Cl	H	19%
4d	3-Cl	Me	7.9
5d	3-Cl	H	2.4
4e	3-Br	Me	0%
4f	3-F	Me	0%
4g	4-Cl	Me	13%
5g	4-Cl	H	33%
4h	4-Br	Me	0%
4i	4-F	Me	1%
5i	4-F	H	41%
4j	4-Me	Me	4%
4k	2,3-di-Cl	Me	33%
5k	2,3-di-Cl	H	32%
4m	3,4-di-Cl	Me	37%
5m	3,4-di-Cl	H	1.0
4n	3,4-di-F	Me	3.6
5n	3,4-di-F	H	1.5
4o	3-Cl,4-F	Me	4%
5o	3-Cl,4-F	H	0.82
4p	3-Me,4-Cl	Me	0%
(±)-*m*-nicotinylbenzoylalanine			4.0
(*S*)-(+)-*m*-nicotinylbenzoylalanine			8.0

[a] IC_{50} values are the mean values of at least 2 experiments performed in duplicate with a variation of <20%.

In the 4-aryl-2-hydroxy-4-oxobut-2-enoic acids and esters (Table 5), the introduction of a halogen at the 3-position gave the very potent 4-(3-chlorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3d)** ($IC_{50} = 0.32$ μM) and 4-(3-fluorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3f)** ($IC_{50} = 0.58$ μM). The corresponding esters of these two acids **(2d** and **2f)** had IC_{50} values of 1.9 and 1.6 μM, respectively. The possibility that this activity is due to partial hydrolysis to the active acid during the assay cannot be completely ruled out. Alternatively, the increased potency of **2d**, as compared with **2f**, may have been due to effects other than inhibition of KH (e.g. inhibition of IDO). Since IDO was not studied in the present series, IDO inhibition cannot be excluded. The corresponding 4-substituted acids were at least an order of magnitude less potent, e.g. 4-(4-chlorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3g):** $IC_{50} = 3.0$ μM.

For the 2-amino-4-aryl-4-oxobut-2-enoic acids and esters (Table 6), 3- or 4-halogenated analogues gave relatively inactive compounds with the exception of 2-amino-4-(3-chlorophenyl)-4-oxobut-2-enoic acid **(5d)** ($IC_{50} = 2.4$ μM) and methyl 2-amino-4-(3-chlorophenyl)-4-oxobut-2-enoate **(4d)** ($IC_{50} = 7.9$ μM).

The introduction of a second substituent into the phenyl ring showed that 3,4-dihalogenated analogues were preferred in both the 2-hydroxy series (Table 5) and the 2-amino series (Table 6). In the case of the 2-hydroxy series, 4-(3,4-difluorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3n)** ($IC_{50} = 0.12$ μM) and 4-(3-

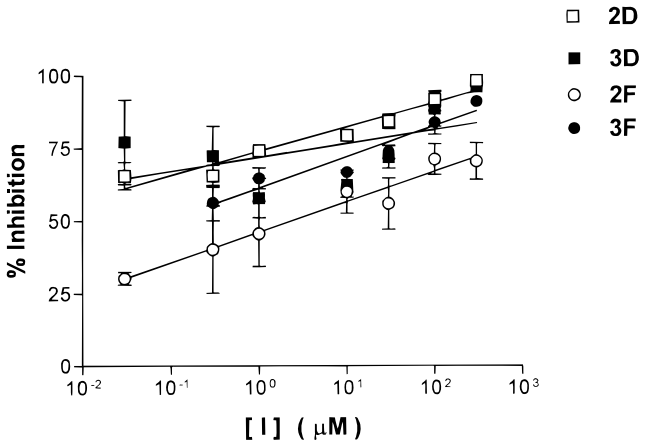


Figure 3. Inhibition of QUIN formation in human macrophages.

chloro-4-fluorophenyl)-2-hydroxy-4-oxobut-2-enoic acid **(3o)** (IC_{50} = 0.27 μM) are the most potent inhibitors of KH reported so far. In the 2-amino series, 3,4-dihalogenated analogues were considerably more potent than the corresponding monohalogenated analogues. The most potent of this type is the 3,4-chloro compound **5m** (IC_{50} = 1.0 μM) and the 3,4-difluoro compound **5o** (IC_{50} = 0.82 μM).

The ability of selected compounds to inhibit QUIN production was performed using macrophage cultures stimulated with interferon-γ as a model for QUIN formation in inflammatory disease. Normally QUIN levels are very low, but following activation of the type-2 interferon receptor, significant quantities of QUIN are produced. The present study measured QUIN synthesis (and its inhibition) directly from the conversion of [^{2}H]-tryptophan to [^{2}H]QUIN by intact cells. The data in Figure 3 reveal unexpected differences between the 3-chlorophenyl and 3-fluorophenyl matched ester/acid pairs **2d**,**3d** and **2f,3f,** respectively. The most potent inhibitor, on intact cells, was **2d** (IC_{50} = 6 nM). The corresponding acid **3d** was 4-fold less potent (24 nM). In contrast, the acid **3f** inhibited cellular QUIN production with an IC_{50} = 40 nM, while its ester **2f** was ~150-fold less potent (5600 nM). Such differences would not be expected if the esters were simply prodrugs for the acids and most likely reveal differences in binding to the substrate site of KH. Additionally, **2d** might be expected to have improved brain penetration as the ester compared to the corresponding acid **3d**. The high potency of **2d** may reflect the fact that hydrolysis of the ester may occur intracellularly and the active acid **3d** is retained within the cell. The lipophilicity and potency of **2d** make it an ideal candidate for neuroinflammatory diseases in which QUIN is pathogenic.

Experimental Section

Biological Assays. KH was isolated from rat liver as the P2 mitochondrial pellet, resuspended in 0.32 M sucrose, and used as the enzyme source. The assay of KH was based on the release of ^{3}HOH from 3-[^{3}H]-L-kynurenine[6] and was performed at 37 °C under conditions of linearity with time and enzyme protein at K_m concentrations of substrate at 5 concentrations of inhibitor. K_i values were calculated from secondary plots of K_m/[I] or estimated using the Chang−Prussoff equation where

$$K_i = IC_{50}/(1 + [S]/K_m)$$

Human peripheral blood monocytes were isolated using Ficoll gradients and differentiated into macrophages by exposure for 1 week to granulocyte−macrophage colony-stimulating factor[7] before being exposed to the human interferon-γ (100 U/mL). The compounds were added immediately after the IFN-γ as was [^{2}H]tryptophan and the samples were incubated for an additional 24 h at which time aliquots of the media were analyzed by gas chromatography/mass spectrometry for [^{2}H]-QUIN.[7,8]

Chemistry. Melting points were taken on either an Electrothermal or Gallenkamp digital melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Brucker AC200 spectrometer; chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. Elemental analyses were obtained using a Carlo Erba 1106 elemental analyzer. Anhydrous solvents were used directly as purchased from Aldrich Chemical Co. Ltd., Gillingham, England.

Method A. Methyl 2-Hydroxy-4-oxo-4-phenylbut-2-enoate (2a). To a freshly prepared solution of sodium methoxide made by dissolving sodium (1.42 g, 61.7 mmol) in dry methanol (30 mL) under a nitrogen atmosphere was added dimethyl oxalate (7.16 g, 60.6 mmol) at room temperature. This solution was cooled to 0 °C and acetophenone (7.20 g, 7.07 mL, 60.6 mmol) added dropwise. The reaction was allowed to warm to room temperature overnight and diluted with diethyl ether (50 mL) and the resulting solid filtered off. This solid was stirred in water (200 mL) for 0.5 h and the solution filtered and made to pH 4 using acetic acid. After cooling to 0 °C for 1 h the resulting white solid was filtered off, washed with water, and dried in a vacuum desiccator over P_2O_5 to yield the desired ester **2a** (5.13 g, 38%): mp 56−57 °C; NMR (DMSO-d_6) δ 3.88 (3H, s), 7.10 (1H, s), 7.55−7.77 (3H, m), 8.05−8.12 (2H, m).

Methyl 4-(3,4-Difluorophenyl)-2-hydroxy-4-oxobut-2-enoate (2n). Method as for **2a** except using 3,4-difluoroacetophenone to yield the desired ester **2n** (8.22 g, 56%): mp 107−108 °C; NMR ($CDCl_3$) δ 3.97 (3H, s), 7.00 (1H, s), 7.23−7.37 (1H, m), 7.73−7.91 (2H, m).

Method B. 2-Hydroxy-4-oxo-4-phenylbut-2-enoic Acid (3a). Methyl 2-hydroxy-4-oxo-4-phenylbut-2-enoate **(2a)** (200 mg, 0.970 mmol) was stirred in 6 N HCl (20 mL) for 4 h at room temperature. After this time the mixture was basified with 10 N NaOH and washed with dichloromethane (20 mL). The aqueous layer was filtered, cooled on ice, and acidified with 6 N HCl. The resulting solid was filtered off, washed with water, and dried in a vacuum desiccator over P_2O_5 to yield the desired acid **3a** (100 mg, 54%): mp 148−150 °C; NMR (DMSO-d_6) δ 4.55 (0.1H, s, keto form), 7.09 (0.9H, s, enol form), 7.52−7.77 (3H, m), 7.94−7.99 (0.2H, m, keto form), 8.02−8.11 (1.8H, m, enol form).

Method C. Methyl 2-Amino-4-(3,4-difluorophenyl)-4-oxobut-2-enoate (4n). To methyl 4-(3,4-difluorophenyl)-2-hydroxy-4-oxobut-2-enoate **(2n)** (4.55 g, 18.8 mmol) and ammonium acetate (1.70 g, 22 mmol) in benzene (50 mL) was added glacial acetic acid (1.70 mL, 29.7 mmol) and the mixture refluxed under Dean and Stark conditions for 12 h. After cooling to room temperature the mixture was washed with saturated sodium hydrogen carbonate solution (100 mL) and the organic layer dried (MgSO4) and concentrated in vacuo. The resulting solid was recrystallized from dichloromethane and hexane to yield the desired product **4n** as orange crystals (3.325 g, 74%): mp 107−109 °C; NMR ($CDCl_3$) δ 3.96 (3H, s), 6.10 (2H, br s), 6.55 (1H, s), 7.16−7.30 (1H, m), 7.67−7.86 (2H, m).

Method D. 2-Amino-4-(3,4-difluorophenyl)-4-oxobut-2-enoic Acid (5n). Methyl 2-amino-4-(3,4-difluorophenyl)-4-oxobut-2-enoate **(4n)** (500 mg, 2.07 mmol) was dissolved in anhydrous THF (10 mL) under an atmosphere of nitrogen and cooled to 0 °C. To this was added a solution of 0.5 N KOH (4.67 mL, 2.34 mmol) dropwise and the mixture allowed to stir at ambient temperature overnight. The reaction mixture was concentrated in vacuo and the residue partitioned between water and ethyl acetate. The aqueous layer was cooled on ice

and acidified with 2 N HCl. After chilling at 4 °C for 2 h the resulting precipitate was filtered off, washed with water, and dried in a vacuum desiccator over P_2O_5 to yield the desired acid **5n.** Recrystallization from acetone/hexane gave yellow crystals (294 mg, 62%): mp 193−195 °C; NMR (DMSO-d_6) δ 6.40 (0.8H, s, isomer 1), 7.08 (0.2H, s, isomer 2), 7.44−8.21 (5H, m).

References

(1) Reinhard, J. F., Jr.; Erickson, J. B.; Flanagan, E. M. Quinolinic acid in neurological disease: opportunities for novel drug discovery. *Adv. Pharmacol.* **1994**, *30*, 85−127.
(2) Heyes, M. P.; Chen, C. Y.; Major, E. O.; Saito, K. Different kynurenine pathway enzymes limit quinolinic acid formation by various human cell types. *Biochem. J.* **1997**, *326*, 351−6.
(3) Saito, K.; Heyes, M. P. Kynurenine pathway enzymes in brain. Properties of enzymes and regulation of quinolinic acid synthesis. *Adv. Exp. Med. Biol.* **1996**, *398*, 485−92.
(4) Carpenedo, R.; Chiarugi, A.; Russi, P.; et al. Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. *Neuroscience* **1994**, *61*, 237−43.
(5) Rover, S.; Cesura, A. M.; Huguenin, P.; Kettler, R.; Szente, A. Synthesis and biochemical evaluation of N-(4-phenylthiazol-2-yl)benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase. *J. Med. Chem.* **1997**, *40*, 4378−85.
(6) Erickson, J. B.; Flanagan, E. M.; Russo, S. M.; Reinhard, J. F., Jr. A radiometric assay for kynurenine 3-hydroxylase based on the release of ^3H_2O during the hydroxylation of L-[3,5-3H]-kynurenine. *Anal. Biochem.* **1992**, *205*, 257−62.
(7) Nottet, H. S.; Flanagan, E. M.; Flanagan, C. R.; et al. The regulation of quinolinic acid in human immunodeficiency virus-infected monocytes. *J. Neurovirol.* **1996**, *2*, 111−7.
(8) Espey, M. G.; Chernyshev, O. N.; Reinhard, J. F., Jr.; Namboodiri, M. A.; Colton, C. A. Activated human microglia produce the excitotoxin quinolinic acid. *Neuroreport* **1997**, *8*, 431−4.

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