Articles

Inhibition of nNOS Activity in Rat Brain by Synthetic Kynurenines: Structure−Activity Dependence

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The overstimulation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors is involved in excitotoxicity, a process participating in neurodegeneration that characterizes some neurological disorders and acute cerebral insults. In looking for compounds with neuroprotective properties, a series of kynurenine derivatives were synthesized, and their effects on both the NMDA and nNOS activity in rat striatum were evaluated. Two compounds, **15a** (2-acetamido-4-(2-amino-5-methoxyphenyl)-4-oxobutyric acid) and **15c** (2-butyramido-4-(2-amino-5-methoxyphenyl)-4-oxobutyric acid), displayed more potent activities than the other synthetic compounds tested for the inhibition of NMDA excitability and nNOS activity. Two other compounds, **18a** (2-acetamido-4-(3-methoxyphenyl)-4-oxobutyric acid) and **18c** (2-butyramido-4-(3-methoxyphenyl)-4-oxobutyric acid), that have the same structure as **15a** and **15c**, except the amino group in R_1, showed different effects. Whereas compound **18a** showed lower electrophysiological potency than compounds **15a** and **15c** in the inhibition of the NMDA-dependent excitability, compound **18c** showed the opposite effect. Moreover, compounds **18a** and **18c** were unable to modify nNOS activity. The remaining kynurenines tested behave like compound **18a**. These results suggest that a structure-related activity of these synthetic kynurenines and a N-H bond in a specific direction is necessary for some kynurenine analogues to inhibit nNOS activity.

Introduction

The neuronal damage that occurs in chronic neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral esclerosis, and epilepsy, and the neuronal damage caused by acute insults such as ischemia/reperfusion and stroke cannot be prevented by currently available drugs. It is believed that an important part of the brain damage occurring in these situations results from the overactivation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, which leads to a Ca^{2+} influx into the cell and excitotoxicity. The accumulation of intracellular Ca^{2+} activates in turn a series of enzymes leading to the formation of reactive oxygen species (ROS), responsible for the neuronal damage.[1,2]

The kynurenine metabolic pathway of L-tryptophan in the brain turns out to be of great interest in neuroprotection because kynurenic and quinolinic acids, two intermediate metabolites of this pathway, modulate the NMDA receptor in a functionally opposite manner.[3−6] Kynurenic acid is a nonselective antagonist of the $glycine_B$ recognition site at the NMDA receptor and has neuroprotective properties, whereas quinolinic acid, a selective agonist of the NMDA binding site at the NMDA receptor, is a potent neurotoxin and has been involved in the pathogenesis of a variety of neurological disorders.[7−10]

Among the strategies aimed to reduce overstimulation of NMDA receptors, the current investigations address the development of selective therapeutic agents structurally related with kynurenines but with higher NMDA antagonistic properties than kynurenic acid.[11−15]

In this report, we describe a new class of synthetic compounds structurally related with L-kynurenine showing different effects on the NMDA-dependent neuronal excitability in rat striatum, a large subcortical nucleus involved in the regulation of motor activity. This nucleus was selected to test these compounds because it receives numerous glutamate projections from the motor cortex.[16,17] In electrophysiological experiments, we have previously shown that in rats, the excitatory response of the striatum to the sensorimotor cortex (SMCx) stimulation is mediated by the NMDA subtype of glutamatergic receptor.[18,19] Since the NMDA-induced excitotoxicity depends on an excess of nitric oxide (NO) produced by activation of the neuronal subtype of nitric oxide synthase (nNOS),[20] the effects of these compounds on the activity of striatal nNOS were also evaluated.[21,22]

The results of this study show that some synthetic kynurenines reduce the NMDA-dependent neuronal activity through the inhibition of the nNOS activity in rat striatal homogenate. The possibility that these

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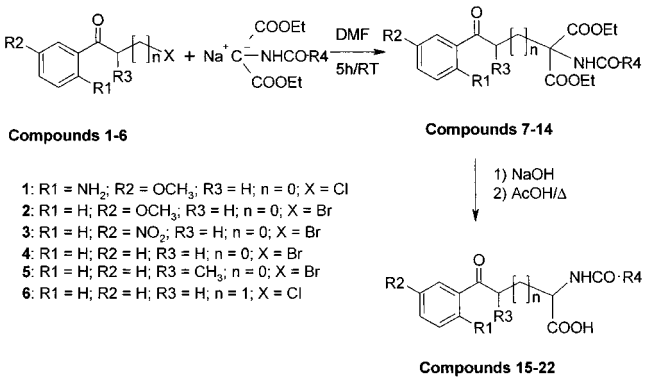

Figure 1. Schematic pathway followed for the synthesis of kynurenine derivatives.

Table 1. Structure of (A) 2-Substituted Diethyl Esters of the Amidomalonic Acid Derivatives and (B) α-Amido-*ω*-benzoyl-substituted Carboxylic Acid Derivatives

compd	R_1	R_2	R_3	R_4	*n*	solubility[c]
		(A) 2-Substituted Diethyl Esters of the Amidomalonic Acid Derivatives				
7a	NH_2	OCH_3	H	CH_3	0	S
7c	NH_2	OCH_3	H	C_3H_7	0	N
8a	$N(CH_3)_2$	OCH_3	H	CH_3	0	N
9a	$NHCH_2Ph$	OCH_3	H	CH_3	0	N
10a	H	OCH_3	H	CH_3	0	S
10b	H	OCH_3	H	C_2H_5	0	S
10c	H	OCH_3	H	C_3H_7	0	N
10d	H	OCH_3	H	C_6H_5	0	N
10e	H	OCH_3	H	C_6H_{11}	0	N
10f	H	OCH_3	H	C_3H_5[a]	0	N
10g	H	OCH_3	H	C_3H_5[b]	0	N
11a	H	NO_2	H	CH_3	0	N
11b	H	NO_2	H	C_2H_5	0	N
12a	H	H	H	CH_3	0	S
12b	H	H	H	C_2H_5	0	S
12c	H	H	H	C_3H_7	0	S
13c	H	H	CH_3	C_3H_7	0	N
14c	H	H	H	C_3H_7	1	N
		(B) α-Amido-*ω*-benzoyl-substituted Carboxylic Acid Derivatives				
15a	NH_2	OCH_3	H	CH_3	0	S
15c	NH_2	OCH_3	H	C_3H_7	0	S
16a	$N(CH_3)_2$	OCH_3	H	CH_3	0	S
17a	$NHCH_2Ph$	OCH_3	H	CH_3	0	S
18a	H	OCH_3	H	CH_3	0	S
18b	H	OCH_3	H	C_2H_5	0	S
18c	H	OCH_3	H	C_3H_7	0	S
18d	H	OCH_3	H	C_6H_5	0	S
18e	H	OCH_3	H	C_6H_{11}	0	S
18f	H	OCH_3	H	C_3H_5[a]	0	S
18g	H	OCH_3	H	C_3H_5[b]	0	S
19a	H	NO_2	H	CH_3	0	S
19b	H	NO_2	H	C_2H_5	0	S
20a	H	H	H	CH_3	0	S
20b	H	H	H	C_2H_5	0	S
20c	H	H	H	C_3H_7	0	S
21c	H	H	CH_3	C_3H_7	0	S
22c	H	H	H	C_3H_7	1	S

[a] 1-Propenyl. [b] Cyclopropyl. [c] S = soluble in 3% ethanol:water or in 3% methanol:water; N = not soluble in these solutions.

compounds may be used in the treatment of neurological disorders involving altered NMDA and/or nNOS responses is discussed.

Chemistry. Figure 1 represents the general pathway synthesis of the compounds included in Table 1A (2-substituted diethyl esters of the amidomalonic acid derivatives) and Table 1B (α-amido-*ω*-benzoyl-substituted carboxilic acid derivatives).

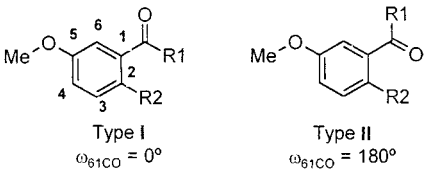

Figure 2. The two main conformational families found for the studied compounds. Because of the conjugation with benzene, the carbonyl group prefers to be coplanar (or almost coplanar) with the aromatic ring and can adopt two possible conformations: in one of them the oxygen atom points toward the methoxy group (type I), and in the other it is orientated in the opposite direction (type II).

The synthesis of compounds **7a** and **7c** was done starting from 4-methoxyaniline, which is converted under Houben-Hoesch conditions into 2-amino-5-methoxy-α-chloroacetophenone (**1**) by the procedure reported by Sugasawa.[23] The condensation of **1** with diethyl sodioacetamidomalonate in DMF at room temperature for 5 h afforded **7a** with a yield of 60%. When the reaction was carried out between **1** and diethyl sodiobutyramidomalonate, **7c** was obtained with a 50% yield.[24] Basic hydrolysis of **7a** and **7c** afforded **15a** and **15c**, respectively.[25] Similarly, starting from 3-methoxy-α-bromoacetophenone (**2**), 3-nitro-α-bromoacetophenone (**3**) (synthesized by bromination of 3-nitroacetophenone in dry ether),[26] α-bromoacetophenone (**4**), α-bromopropiophenone (**5**), and *β*-chloropropiophenone (**6**), compounds **10a**−**g**, **11a**−**b**, **12a**−**c**, **13c**, and **14c** were obtained and which were transformed to the corresponding acids **18a**−**g**, **19a**−**b**, **20a**−**c**, **21c**, and **22c**, respectively. Compounds **8a** and **9a** came from **7a** by dimethylation[27] and benzylation,[28] respectively, and produced **16a** (100%) and **17a** (70%) by basic hydrolysis.

The synthesis of diethyl 2-acylamidosubstituted malonates was carried out starting from diethyl aminomalonate hydrochloride and the adequate acyl chloride (propionyl chloride, butiryl chloride, benzoyl chloride, cyclohexanecarbonyl chloride, *trans*-crotonyl chloride, and cyclopropanecarbonyl chloride).[29]

Molecular Modeling and Conformational Analysis. Molecular modeling studies were performed using the Sybyl software version 6.5,[30] running on a Silicon Graphics workstation. Three-dimensional models of all compounds were built from a standard fragment library, and their geometries were subsequently optimized using the Tripos force field[31] including the electrostatic term calculated from Gasteiger and Huckel[32] charges ($\epsilon = 1$, distance dependent). The method of Powell[33] included in the Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.01 kcal/mol·Å^2.

For each compound, a conformational analysis was done by means of the random search procedure implemented in SYBYL (maximun hits = 10, RMS threshold = 0.2 Å, energy cutoff = +20 kcal/mol).

Two main conformational families could be identified depending on the orientation of the carbonyl group; in one of them (type **I**) the oxygen atom points toward the methoxy group, and in the other it is orientated in the opposite direction (type **II**) (Figure 2).

Figure 3 shows, as an example, the energy of the different conformations found for compounds **15a**, **16a**,

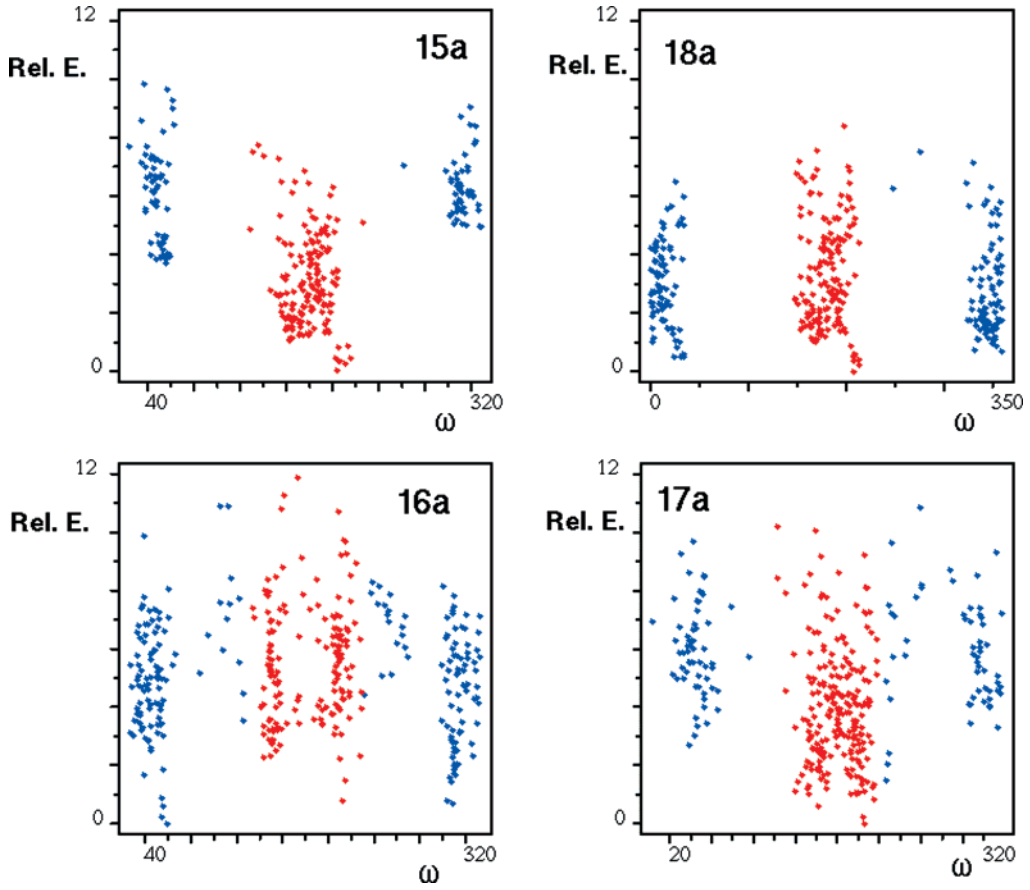

Figure 3. Representation of the relative energy (Rel. E., kcal/mol) versus ω_{61CO} (ω) for all the conformations found in compounds **15a**, **16a**, **17a**, and **18a**. Blue and red points represent type I and II conformational families, respectively.

17a, and **18a** as a function of the torsional angle $C_6-C_1-C{=}O$ (ω_{61CO}). Zones near 0° and 360° include conformations type I (blue points), and the zone near 180° corresponds to conformations type II (red points). When the 2-amine group is not present in the molecule (**18a**), the populations of both families are almost equal and both have similar energies. Nevertheless, in compound **15a**, the intramolecular hydrogen bond stabilizes the family II, and, consequently, the zone near 180° is more populated and includes the more stable conformers. These facts confirm the importance of this intramolecular hydrogen bond in the conformational behavior of these compounds. Compound **16a** shows a wide distribution of the conformations over all the values of ω_{61CO}, existing in more type I conformations, which are also the most stable ones. This is a consequence of the high volume of the dimethylamine group that prevents the conjugation with the aromatic ring of either the carbonyl or the amine groups. In compound **17a**, the 2-benzylamine substituent still allows the formation of the intramolecular hydrogen bond. The conformational behavior of this compound is similar to that of **15a**, showing a more populated region near 180° that includes more stable conformations. The presence of the benzyl group introduces high steric interactions in the molecule and allows the stabilization of some conformations with parallel benzene rings (π,π-stacking).

Figure 4 shows the most stable conformers of each one of these compounds belonging to both types (I and II) of conformations.

Results and Discussion

The effects of the kynurenine derivatives on the excitatory response of striatal neurones to SMCx stimulation were evaluated (Figure 5). Obviously, only soluble kynurenines were tested in our experimental protocols. Electrical stimulation was applied continuously to the deep layers of motor cortex, and recordings were made from the dorso-medial portion of the striatum. Most of the neurones in this region were quiescent unless excited by cortical stimulation.[34] Only those neurones showing an excitatory response to SMCx stimulation were selected for the study. When a striatal-responding neurone was identified, one kynurenine was microiontophoretically ejected onto this neurone to observe its effect on the excitatory response.

The kynurenine derivatives were classified into three groups according to their effects on the experimental paradigm described (Figure 5): (1) 21 compounds displaying similar attenuating effect on the striatal excitatory response (compounds **7a**, **10a**, **10b**, **12a**, **12b**, **12c**, **16a**, **17a**, **18a**, **18b**, **18d**, **18e**, **18f**, **18g**, **19a**, **19b**, **20a**, **20b**, **20c**, **21c**, and **22c**); (2) two compounds showing a stronger inhibitory effect than the former (compounds **15a** and **15c**); and (3) one compound displaying an excitatory effect (compound **18c**).

Taking compound **18a** as a prototype, we observed that there was no change in the inhibitory potency of these new compounds when replacing the methyl group at R_4 with ethyl (compound **18b**), phenyl (compound **18d**), cyclohexyl (compound **18e**), 1-propenyl (compound

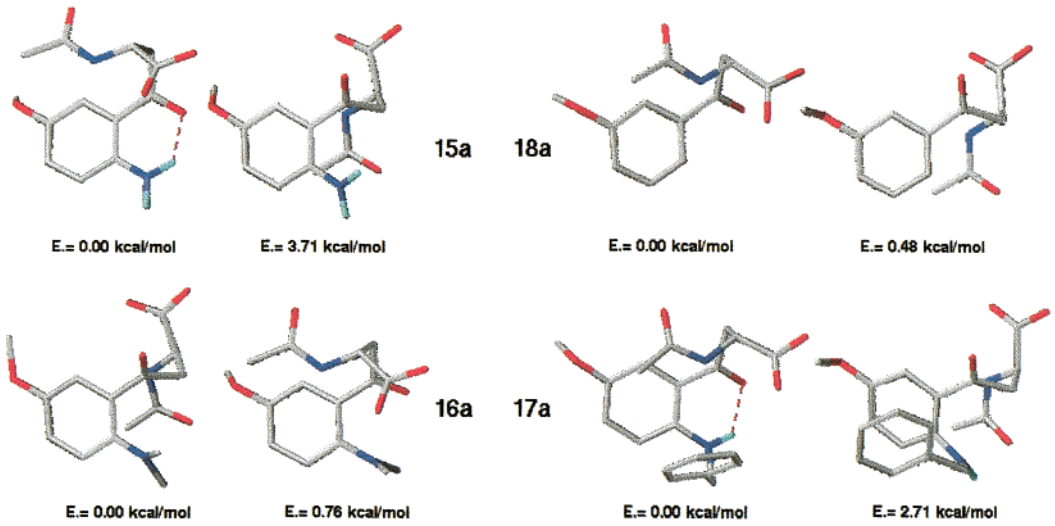

Figure 4. The two most stable conformers belonging to both types (I and II) of families for compounds **15a**, **16a**, **17a**, and **18a**. Relative energies (E) are calculated in relation to the most stable conformer. Intramolecular hydrogen bonds are clearly shown for type I conformations of **15a** and **17a**.

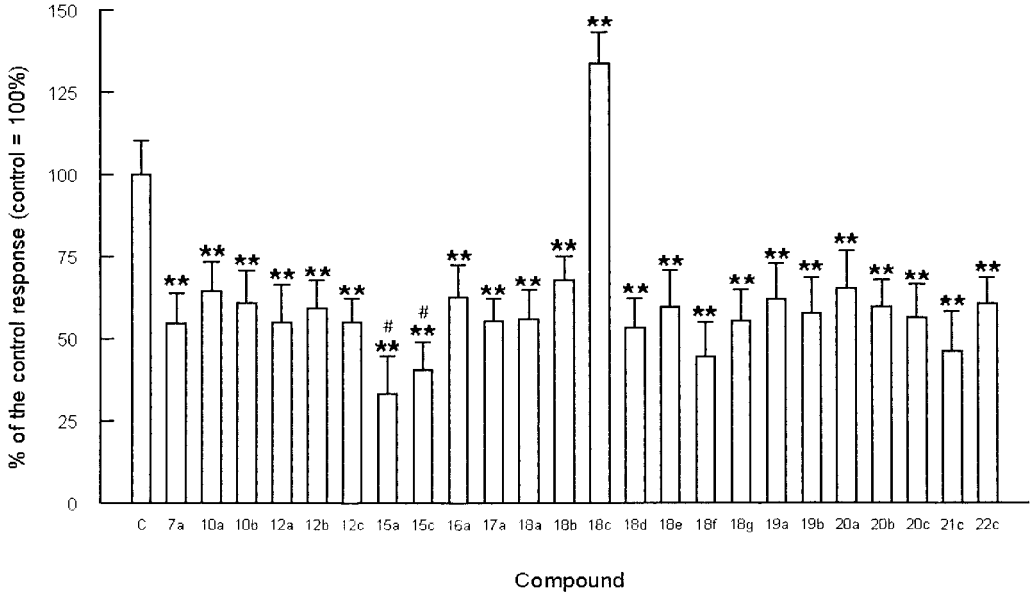

Figure 5. Effects of microiontophoresis of kynurenine derivatives (1 mM) on the excitatory response of striatal neurones to sensorimotor cortex (SMCx) stimulation. $^{**}P < 0.01$ vs control response (C); $^{\#}P < 0.05$ vs the other compounds.

18f), or cyclopropyl (compound **18g**) groups; removing the methoxy group at R_2 (compound **20a**) or its replacement with a nitro group (compound **19a**); replacing the methyl group at R_4 and the methoxy group at R_2 with an ethyl and nitro groups, respectively (compound **19b**); or replacing the methyl group at R_4 with an ethyl group and removing the methoxy group at R_2 (compound **20b**). The 2-substituted amidomalonic acid diethyl ester derivatives (compounds **7a**, **10a**, **10b**, **12a**, **12b**, and **12c**), with or without an amino group at R_1 position, also showed the same inhibitory effect as compound **18a**.

The presence of an amino group at R_1 in **18a** significantly increases the inhibitory potency on neuronal excitability (compound **15a**). However, when this amino group at R_1 in compound **15a** is blocked by either a dimethyl (compound **16a**) or a benzyl (compound **17a**) groups, the resultant compounds lost part of their inhibitory effect on neuronal excitability, showing similar potency as compound **18a**. Interestingly, the replacement of the methyl group at R_4 with *n*-propyl yields compound **18c**, with a stimulatory effect on neuronal excitability. It is also important that the presence of an amino group at R_1 in compound **18c** yields a compound **15c** with strong inhibitory effects on neuronal excitability. Besides, on removing the methoxy group at R_2 in **18c** (compound **20c**), the presence of a methyl group at R_3 (compound **21c**), or lengthening the side chain in one carbon atom (compound **22c**), a similar inhibitory effect was produced in compound **18a**.

For further study on the specificity of these compounds on the response of striatal neurons, a dose−response experiment was carried out (Figure 6). In this case, a striatal neuron was continuously stimulated throughout the experiment, and different doses (−25, −50, −100, and −150 nA) of representative compounds of each group were iontophoretized onto the neuron. The excitatory response decreased in a dose-dependent manner for the compounds **18a**, **15a**, and **15c**, whereas

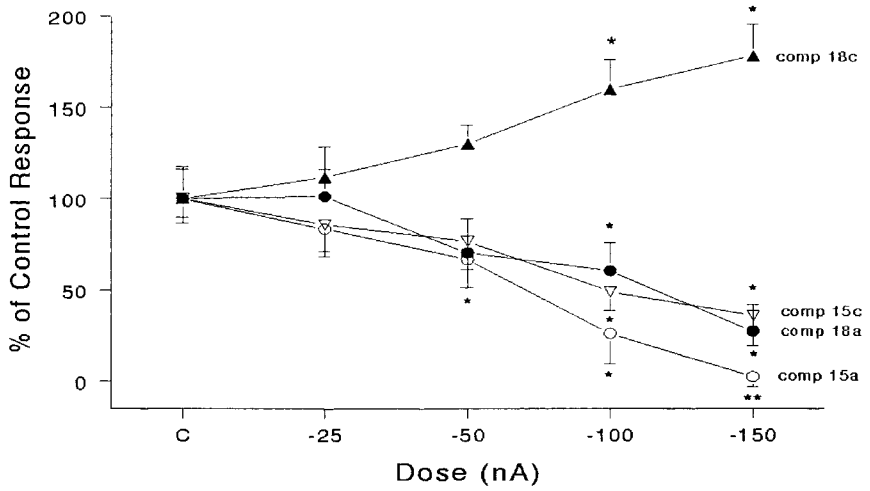

Figure 6. Dose-related effect of kynurenine derivatives iontophoretically applied to striatal neurones. Increasing the ejection current from −25 to −150 nA produces the striatal response to sensorimotor cortex (SMCx). Stimulation was attenuated when compounds **15a**, **15c**, and **18a** were iontophoresed. However, the application of compound **18c** increased the excitatory response. Data represent the percentage of change in firing rate with respect to control values (mean ± SEM of 10 neurones). *P < 0.05 and **P < 0.01 vs to control response.

Table 2. Dose-Dependent Effects of Six Kynurenine Derivatives on Rat Striatal nNOS Activity[a]

conc (M)	compd **18a**	compd **15a**	compd **18c**	compd **15c**	compd **16a**	compd **17a**
0	100.00 ± 7.71	100.00 ± 2.69	100.00 ± 0.56	100.00 ± 2.36	100.00 ± 0.84	100.00 ± 1.12
10^{-11}	92.65 ± 3.92	78.63 ± 5.97**	99.87 ± 7.22	85.80 ± 4.07*	100.18 ± 4.28	101.25 ± 6.52
10^{-9}	100.07 ± 10.10	73.38 ± 2.65**	100.56 ± 4.62	82.13 ± 2.03**	99.86 ± 4.43	100.53 ± 5.58
10^{-7}	87.24 ± 2.57	65.80 ± 4.53**	100.91 ± 3.54	77.27 ± 4.71**	99.63 ± 6.56	100.48 ± 5.27
10^{-5}	102.90 ± 8.62	64.00 ± 4.58**	99.74 ± 4.28	67.36 ± 6.06**	100.95 ± 5.22	98.16 ± 6.01
10^{-3}	95.72 ± 2.26	31.51 ± 9.92**	105.32 ± 5.10	54.95 ± 8.56**	98.99 ± 5.89	100.31 ± 4.62

[a] Data represent the mean ± SEM of the percentage of nNOS activity for each drug as compared with untreated samples. Each value is the mean of three experiments performed by triplicate in homogenates of four rat striata in each one. *P < 0.05 vs control; **P < 0.01 vs control.

iontophoresis of compound **18c** increased the excitatory response in a dose-dependent manner. A significant effect was found between −50 and −100 nA, and compound **15a** showed significant higher inhibition than the other compounds tested at doses between −100 and −150 nA.

The cortico-striatal pathway studied in our experimental paradigm was chosen because this is a glutamate-mediated circuit that mainly uses the NMDA receptor.[16,18,19,21,35] Therefore, we asked if the effects of the synthetic kynurenines described above involved NMDA receptors. To test this hypothesis, a striatal neurone showing excitatory response to SMCx stimulation was chosen for each compound. Once the neurone was found, the electrical stimulation was stopped and the neurone was silenced due to its lack of autoexcitation. Then, *N*-methyl-D-aspartate, the selective agonist for the NMDA subtype of glutamate receptor, was iontophoretized to induce the excitatory response in this neurone, and, during the NMDA ejection, a series of kynurenines were also iontophoretized. Figure 7 shows the effects of some representative kynurenines of each group. Compounds **15a** (Figure 7B) and **15c** (Figure 7D) display stronger inhibitory effects (longer duration of inhibition) than compound **18a** (Figure 7A). The results also confirm that when the amino group at R_1 of **15a** was blocked with a dimethyl or a benzyl group, the resulting compounds **16a** (Figure 7E) and **17a** (Figure 7F), respectively, behaved as **18a** (Figure 7A), thus losing part of their inhibitory effects. In contrast, **18c** (Figure 7C) exerts a proexcitatory effect. It is of interest to note that when these compounds were iontophoretized onto a silent neurone (in absence of NMDA ejection), there was no response (data not shown).

The results confirm that the assayed kynurenines act on the NMDA receptor in a structure-related manner. Two main possibilities should be kept in mind regarding their mechanism of action: (1) the kynurenines may bind to the NMDA receptor itself in a manner similar to kynurenic acid and some of its derivatives,[12,13,15] or (2) they can modulate nNOS activity, and thus, the intracellular production of NO, the second messenger of the NMDA receptor. However, since electrophysiological experiments do not report information regarding the mechanism of action, it is possible that the compounds tested may reduce quinolinic acid production or increase kynurenic acid production by inhibiting key enzymes of the kynurenine pathway.[24,27,36−38] This hypothesis is now being investigated in our laboratory.

Anyway, activation of the NMDA receptor increases Ca^{2+} influx into the cell, resulting in an increase of the nNOS activity by a Ca^{2+}-calmodulin (CaCaM)-dependent mechanism.[39] Consequently, intracellular NO concentration increases and diffuses out of the cell, increasing presynaptic glutamate output that further stimulates the NMDA receptor. Thus, the effect of **15a**, **15c**, **16a**, **17a**, **18a**, and **18c** on nNOS activity in rat striatal homogenates was assessed. Table 2 displays the results of this assay. It can be seen that 10 pM of **15a** or **15c** were able to significantly reduce nNOS activity to 78.6 and 85.8%, respectively. At the maximal concentration tested (1 mM), nNOS was reduced by **15a** and **15c** to

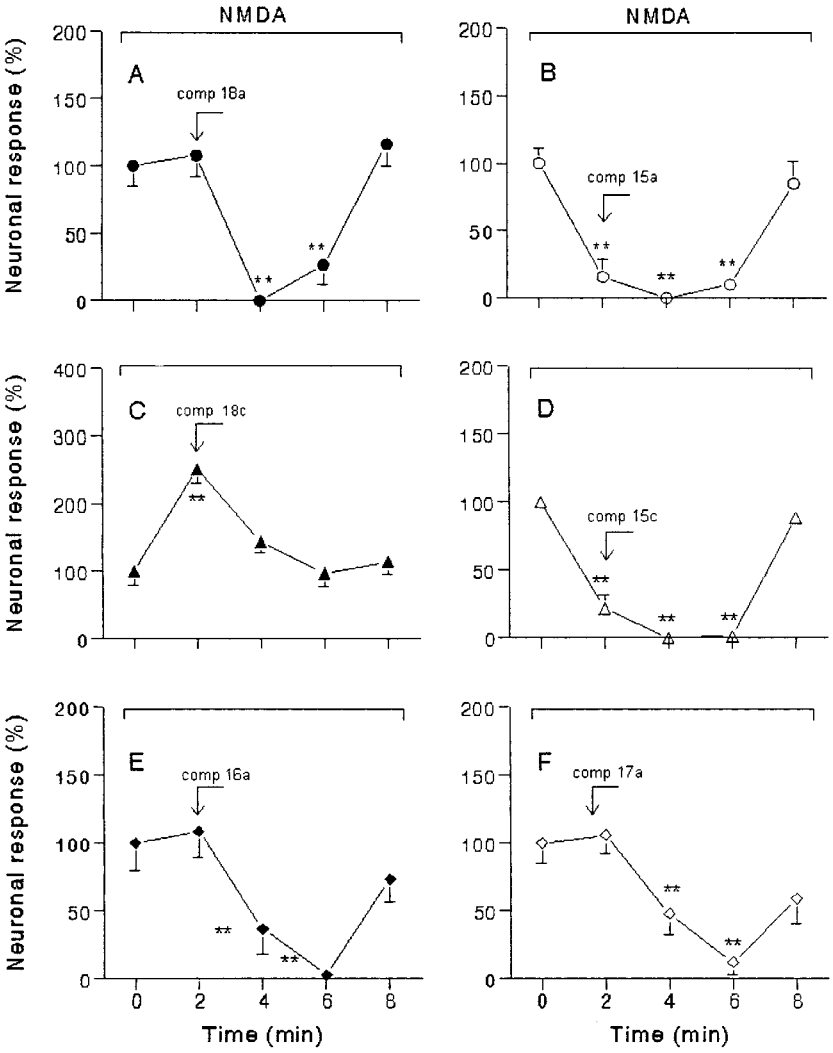

Figure 7. Time-dependent effects of the kinurenines derivatives on the NMDA excitation of striatal neurones. Iontophoretical application of NMDA and kynurenine derivatives are indicated by horizontal bars above histograms. The neurones are quiescent unless excited by NMDA ejection. In all the cells, NMDA ejection time was 600 s and produced excitation of the neurones. Compounds were co-iontophoresed with NMDA (time: 120−240 s) and produced inhibition except for compound **18c** that potentiated the NMDA-induced excitation. Ejection currents: NMDA, −30 nA; kynurenine derivatives, −100 nA. $^{**}P < 0.01$ vs time 0 (basal).

31.5 and 54.9%, respectively. These data suggest that **15a** is more potent than **15c** in the inhibition of striatal nNOS activity.

Interestingly, nNOS was unaffected by the other tested compounds at concentrations ranging from 10 pM to 1 mM (Table 2). Since compounds **15a** and **15c** share the same structure as **18a** and **18c** except for the presence of an amino group at R_1, these results suggested a relation between this chemical group and the biological activity of these compounds. This suggestion was further supported because nNOS activity was not affected by **16a** and **17a**, two compounds obtained from **15a** by blocking the amino group at R_1 with a dimethyl or a benzyl group, respectively. On the other hand, compound **18c**, which increases neuronal excitability, did not modify striatal nNOS activity. A conclusion that may be deduced from these results is the existence of three groups of kynurenines with different effects on the NMDA receptor: (a) those kynurenines inhibiting neuronal excitability and nNOS activity, such as **15a** and **15c**; (b) one kynurenine, **18c**, stimulating neuronal excitability but with no effect on nNOS activity; and (c) kynurenines with no effect on nNOS activity but maintaining inhibitory effects on neuronal excitability, including **18a**, **16a**, and **17a**. The electrophysiological and biochemical effects correlated very well. As can be seen in Table 3, kynurenines with stronger inhibitory effects on nNOS activity have smaller IC_{50} values for their electrophysiological effects than kynurenines with weak inhibitory effects on striatal nNOS.

Table 3. IC_{50} Values for Kynurenine Dervatives Obtained from Electrophysiological (nA) and nNOS Activity (M) Experiments

	IC_{50}	
compd	electrophysiological activity (nA)	nNOS activity (M)
18a	−111	
15a	−69	41×10^{-6}
18c	−83	
15c	−99	$>10^{-3}$
16a	−104	
17a	−106	

The conformational analysis of the kynurenines shows that conformations type **I** are favored by about 3 kcal/mol in compounds **15a** and **17a** and by 0.5 kcal/mol in

compound **18a**. Only in compound **16a** the most stable conformer belongs to type **II** conformational family. In general, the energy difference for all these conformers is small enough to allow molecules to change their conformation easily, explaining the similar inhibitory effects on the NMDA receptor. An exception is compound **18c**, whose proexcitatory effect cannot be explained at present, at least with respect to a clear different conformational behavior.

The presence of the 2-amine groups in **15a** and **15c** restricts the flexibility of these compounds and allows the formation of an additional hydrogen bond between them and their potential biological target. These compounds are the only ones that inhibit nNOS activity and two hypothesis could be used to explain this biological property: (i) The restriction of the conformational flexibility due to the intramolecular hydrogen bond. The absence of the hydrogen bond in **18a** (R_1 = H) and **16a** [R_1 = $N(CH_3)_2$] would support this hypothesis. Nevertheless, **17a** (R_1 = $NHCH_2Ph$) is not active against nNOS although the intramolecular hydrogen bond is still formed in this compound restricting its conformational behavior. (ii) The second NH bond of the anilino-NH_2 group in compounds **15a** and **15c** may be a pharmacophore that can form an additional hydrogen bond with an active site residue on nNOS upon binding, and that compounds lacking that pharmacophore (**18a** and **18c**) or that have the nitrogen blocked by alkylation (**16a** and **17a**) are inactive. This second hypothesis is probably the correct one.

It is known that brain tryptophan metabolites are closely related to CNS activity. Tryptophan metabolism falls into two main pathways: (1) the kynurenine pathway, that yields a series of compounds with both excitatory and inhibitory effects on brain glutamate receptors,[3,4,24] and (2) the methoxyindole pathway, that produces melatonin, an indoleamine that inhibits brain NMDA receptors decreasing nNOS activity.[18,19,21,23,25] In normal conditions, an equilibrium between these two metabolic pathways yields primarily kynurenines during the day and methoxyindoles including melatonin during the night. An imbalance between these two metabolic pathways leads to an increase in CNS excitability and seizures.[40,41] In these situations, correction of the altered brain tryptophan metabolism may be a therapeutic approach.[42] Moreover, several attempts have been made to find melatonin-related compounds with neuroprotective properties suitable to be used in clinic.[43−45] Alternatively, in view of the remarkable effects of some synthetic kynurenines on both NMDA and nNOS, their use in status of excitotoxicity involving NMDA receptors activation may have clinical relevance.

Experimental Section

Melting points were determined in open capillaries using an Electrothermal-1A-6301 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using TMS as the internal standard, and peaks are reported in ppm (δ). The infrared spectra (IR) were recorded on a Perkin-Elmer 782 instrument connected to a 3600 data station, as a neat film over KBr or as KBr disks. High-resolution mass spectroscopy (HRMS) was carried out on a VG AutoSpec Q high-resolution mass spectrometer (Fisons Instruments). Elemental analyses were performed on a Eager 200 Custom Report and agreed with theoretical values within ± 0.4%. Flash-chromatography was carried out using silica gel 60, 230−400 mesh (Merck), and the solvent mixture reported within parentheses was used as eluent.

Preparation of 2-Substituted Amidomalonic Acid Diethyl Esters 7a, 7c, 10a−g, 11a−b, 12a−c, 13c, and 14c. General Method. Sodium hydride (25.32 mmol, from a 80% mineral oil suspension) was added to a magnetically stirred solution of diethyl amidomalonate (24.50 mmol) in anhidrous DMF (21 mL) at room temperature in a nitrogen atmosphere. Stirring was continued for 5 h, after which a solution of the appropriate haloacetophenone **1-6** (21.83 mmol) in anhidrous DMF (15 mL) was added dropwise over 20 min, and the resulting mixture was stirred continuously for 15 h.

The reaction mixture was then poured into water (250 mL) and, after acidification with 3 N HCl, extracted with ethyl acetate (5 × 100 mL). The combined organic phases were washed with brine (50 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was purified (chloroforme:acetone 10:1).

Diethyl 2-[2-(2-amino-5-methoxyphenyl)-2-oxoethyl]-acetamido malonate (7a): (60%) (eluted in ether:hexane 1:1); mp 99−102 °C; IR (KBr) 3473, 3385, 3344, 2993, 2845, 1770, 1662, 1644, 1597, 1552, 1517, 884, and 842 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, t, J = 7.1), 1.96 (3H, s), 3.76 (3H, s), 4.19 (2H, s), 4.24 (2H, q, J = 7.1), 4.25 (2H, q, J = 7.1), and 6.63−7.18 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 13.96, 23.05, 42.26, 55.33, 62.79, 64.05, 112.90, 118.50, 119.41, 124.53, 144.63, 151.10, 167.61, 169.47, and 198.25; MS (LSIMS) m/z 380.158462 (M^+), Calcd. mass for $C_{18}H_{24}N_2O_7$ 380.158351 (deviation −0.3 ppm).

Diethyl 2-[2-(2-amino-5-methoxyphenyl)-2-oxoethyl]-butyramido malonate (7c): (50%) (eluted in ether:hexane 1:3); IR (KBr, film) 3589, 3356, 2877, 2839, 1744, 1588, 908, 839, and 735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, t, J = 7.4), 1.22 (6H, t, J = 7.1), 1.58 (2H, m, J = 7.4), 2.14 (2H, t, J = 7.4), 3.76 (3H, s), 4.19 (2H, s), 4.24 (2H, q, J = 7.1), 4.25 (2H, q, J = 7.1), 6.58 (1H, d, J = 8.9), 6.96 (1H, dd, J = 2.8, 8.9), 7.07 (1H, s), and 7.15 (1H, d, J = 2.8); ^{13}C NMR ($CDCl_3$) δ 13.58, 13.96, 18.90, 38.08, 42.92, 56.10, 62.82, 64.03, 112.67, 116.95, 118.88, 124.79, 145.34, 150.24, 167.73, 172.31, and 198.21; MS (LSIMS) m/z 431.179181 ($M+Na)^+$, Calcd. mass for $C_{20}H_{28}N_2O_7Na$: 431.179421 (deviation 0.6 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]acetamido malonate (10a): (60%); mp 85−87 °C, IR (KBr) 3354, 3056, 2843, 1768, 1734, 1679, 1598, 1516, 871, 833, and 788 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (6H, t, J = 7.1), 1.96 (3H, s), 3.82 (3H, s), 4.23 (2H, s), 4.25 (2H, q, J = 7.1), 4.27 (2H, q, J = 7.1), and 7.12−7.56 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.97, 23.03, 42.26, 55.33, 62.79, 64.05, 111.99, 120.62, 121.09, 129.81, 137.40, 159.91, 167.34, 169.55, and 198.86; MS (LSIMS) m/z 388.137157 ($M+Na)^+$, Calcd. mass for $C_{18}H_{23}NO_7Na$: 388.137222 (deviation 0.2 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]propionamido malonate (10b): (64%); mp 70−73 °C; IR (KBr) 3362, 3056, 2842, 1767, 1733, 1673, 1598, 1514, 870, 833, and 786 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t, J = 7.6), 1.23 (6H, t, J = 7.1), 2.19 (2H, q, J = 7.6), 3.83 (3H, s), 4.23 (2H, s), 4.24 (2H, q, J = 7.1), 4.26 (2H, q, J = 7.1), and 7.07−7.56 (5H, m); ^{13}C NMR ($CDCl_3$) δ 9.47, 13.98, 29.27, 42.44, 55.52, 69.93, 63.98, 112.04, 120.61, 121.13, 129.81, 137.54, 159.9, 167.43, 173.20 and 196.94; MS (LSIMS) m/z 402.152653 ($M+Na)^+$, Calcd. mass for $C_{19}H_{25}NO_7Na$: 402.152872 (deviation 0.5 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]butyramido malonate (10c): (54%); mp 53−55 °C; IR (KBr) 3420, 3383, 2973, 2841, 2752, 1745, 1689, 1670, 1599, 1586, 859, 790, and 687 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, J = 7.4), 1.24 (6H, t, J = 7.1), 1.59 (2H, m, J = 7.4), 2.15 (2H, t, J = 7.4), 3.84 (3H, s), 4.24 (2H, s), 4.25 (2H, q, J = 7.1), 4.26 (2H, q, J = 7.1), and 7.10−7.57 (5H, m); ^{13}C NMR ($CDCl_3$): δ 13.58, 13.97, 18.90, 38.03, 42.45, 55.51, 62.92, 64.32, 111.97, 120.59, 121.11, 129.79, 137.48, 159.89, 167.41, 172.41, and 196.95; MS (LSIMS) m/z 416.168521 ($M+Na)^+$, Calcd. mass for $C_{20}H_{27}NO_7Na$: 416.168522 (deviation 0.0 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]benzamido malonate (10d): (62%); mp 82−83 °C; IR (KBr) 3436, 3062, 2844, 1743, 1681, 1664, 1601, 1507, 1484, 800, 718, and 689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (6H, t, J = 7.1), 3.81 (3H, s), 4.29 (2H, q, J = 7.1), 4.30 (2H, q, J = 7.1), 4.37 (2H, s), and 7.07−7.80 (10H, m); ^{13}C NMR ($CDCl_3$): δ 14.01, 42.55, 55.51, 63.06, 64.32, 112.01, 120.12, 121.12, 127.27, 128.65, 129.80, 132.06, 133.32, 137.45, 159.91, 166.49, 167.44, and 196.82; MS (LSIMS) m/z 450.152872 $(M+Na)^+$, Calcd. mass for $C_{23}H_{25}NO_7Na$: 450.152872 (deviation 0.0 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]cyclohexanecarboxamido malonate (10e): (58%); mp 71−74 °C; IR (KBr) 3368, 3082, 2672, 1766, 1740, 949, 804, and 786 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (6H, t, J = 7.1), 1.29 (6H, m), 1.67 (4H, m), 2.09 (1H, m), 3.82 (3H, s), 4.21 (2H, s), 4.24 (4H, q, J = 7.1), and 7.08−7.54 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.98, 25.52, 25.69, 29.24, 42.38, 44.73, 55.52, 62.87, 63.81, 111.98, 120.60 121.16, 129.78, 137.59, 159.89, 167.48, 175.36, and 197.03; MS (LSIMS) m/z 434.217885 $(M+1)^+$, Calcd. mass for $C_{23}H_{32}NO_7$: 434.217878 (deviation 0.0 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]-*trans*-crotonamido malonate (10f): (85%); mp 99−102 °C; IR (KBr) 3363, 3086, 1748, 1670, 1642, 977, 870, and 724 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1,22 (6H, t, J = 7.1), 1.80 (3H, dd, J = 1.6, 6.9), 3.81 (3H, s), 4.25 (4H, q, J = 7.1), 4.27 (2H, s), 5.83 (1H, dd, J = 6.9, 15.3,), 6.78 (1H, m, J = 1.6, 15.3), and 7.08−7.55 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.96, 17.75, 42.56, 55.49, 62.91, 64.04, 112.01, 120.55 121.08, 124.22, 129.75, 137.61, 141.29, 159.91, 164.97, 167.38, and 196.84; MS (LSIMS) m/z 414.152730 $(M+Na)^+$, Calcd. mass for $C_{20}H_{25}NO_7Na$: 414.152872 (deviation 0.3 ppm).

Diethyl 2-[2-(3-methoxyphenyl)-2-oxoethyl]cyclopropanecarboxamido malonate (10g): (65%); mp 94−96 °C; IR (KBr) 3376, 3081, 1748, 1689, 1599, 860, and 734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (2H, m), 0.85 (2H, m), 1.22 (6H, t, J = 7.1), 1.43 (1H, m), 3.83 (3H, s), 4.21 (2H, s), 4.23 (4H, q, J = 7.1), and 7.09−7.56 (5H, m); ^{13}C NMR ($CDCl_3$) δ 7.55, 13.93, 13.99, 14.46, 42.66, 55.53, 62.87, 64.13, 112.11, 120.56, 121.15, 129.79, 137.61, 159.94, 167.45, 172.89, and 196.98; MS (LSIMS) m/z 392.171729 $(M+1)^+$, Calcd. mass for $C_{20}H_{26}NO_7$: 392.170928 (deviation −0.2 ppm).

Diethyl 2-[2-(3-nitrophenyl)-2-oxoethyl]acetamido malonate (11a): (52%); mp 114−116 °C; IR (KBr) 3280, 3001, 1743, 1702, 1646, 1615, 1533, 1301, 888, 814, and 735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (6H, t, J = 7.1), 1.98 (3H, s), 4.28 (4H, q, J = 7.1), 4.33 (2H, s), and 7.12−8.77 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.97, 23.01, 42.53, 63.19, 63.89, 123.10, 127.96, 130.13, 133.78, 137.26, 148.59, 166.98, 169.78, and 194.91; MS (LSIMS) m/z 403.111602 $(M+Na)^+$, Calcd. mass for $C_{17}H_{20}N_2O_8Na$: 403.111736 (deviation 0.3 ppm).

Diethyl 2-[2-(3-nitrophenyl)-2-oxoethyl]propionamido malonate (11b): (55%); mp 85−87 °C; IR (KBr) 3386, 3075, 2946, 1740, 1705, 1613, 1532, 889, 866, and 737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08 (3H, t, J = 7.6), 1.25 (6H, t, J = 7.1), 2.22 (2H, q, J = 7.6), 4.26 (4H, m, J = 7.1), 4.33 (2H, s), and 7.11−8.78 (5H, m); ^{13}C NMR ($CDCl_3$) δ 9.47, 14.01, 29.31, 42.58, 63.19, 63.90, 123.15, 127.95, 130.13, 133.81, 137.46, 148.70, 167.10, 173.50, and 195.02; MS (LSIMS) m/z 417.127289 $(M+Na)^+$, Calcd. mass for $C_{18}H_{22}N_2O_8Na$: 417.127386 (deviation 0.2 ppm).

Diethyl 2-(2-phenyl-2-oxoethyl)acetamido malonate (12a): (53%); mp 117−120 °C; IR (KBr) 3292, 3063, 1747, 1692, 1648, 1600, 1584, 1297, 1224, 1194, 765, 754, and 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (6H, t, J = 7.1), 1.96 (3H, s), 4.25 (4H, q, J = 7.1), 4.25 (2H, s), 7.12 (1H, s), 7.45 (2H, m), 7.57 (1H, m), and 7.95 (2H, m); ^{13}C NMR ($CDCl_3$) δ 13.96, 23.01, 42.34, 62.95, 64.04, 128.26, 128.76, 133.79, 136.11, 167.35, 169.53, and 196.98; MS (LSIMS) m/z 358.126680 $(M+Na)^+$, Calcd. mass for $C_{17}H_{21}NO_6Na$: 358.126657 (deviation −0.1 ppm).

Diethyl 2-(2-phenyl-2-oxoethyl)propionamido malonate (12b): (55%); mp 74−75 °C; IR (KBr) 3354, 3068, 2879, 1744, 1692, 1668, 1601, 1584, 913, 805, and 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (3H, t, J = 7.6), 1.22 (6H, t, J = 7.1), 2.18 (2H, q, J = 7.6), 4.22 (2H, q, J = 7.1), 4.24 (2H, s), 4.26 (2H, q, J = 7.1), and 7.08−7.94 (6H, m); ^{13}C NMR ($CDCl_3$) δ 9.45, 13.97, 29.26, 42.33, 62.90, 63.98, 128.26, 128.76, 133.74, 136.27, 167.43, 173.18, and 197.07; MS (LSIMS) m/z 372.142264 $(M+Na)^+$, Calcd. mass for $C_{18}H_{23}NO_6Na$: 372.142307 (deviation 0.1 ppm).

Diethyl 2-(2-phenyl-2-oxoethyl)butyramido malonate (12c): (71%); mp 62−64 °C; IR(KBr) 3491, 3300, 3058, 2876, 1748, 1695, 1658, 1599, 1583, 949, 822, and 765 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (3H, t, J = 7.4), 1.24 (6H, t, J = 7.1), 1.56 (2H, m, J = 7.4), 2.14 (2H, t, J = 7.4), 4.25 (2H, s), 4.26 (4H, m, J = 7.1), 7.10 (1H, s), and 7.40−7.96 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.56, 13.97, 18.93, 38.10, 42.36, 62.91, 64.01, 128.30, 128.77, 133.75, 136.31, 167.45, 172.42, and 197.12; MS (LSIMS) m/z 386.157885 $(M+Na)^+$, Calcd. mass for $C_{19}H_{25}NO_6Na$: 386.157958 (deviation 0.2 ppm).

Diethyl 2-(1-methyl-2-phenyl-2-oxoethyl)butyramido malonate (13c): (52%); IR (KBr, film) 3380, 3062, 2876, 1747, 1680, 1598, 1580, 1261, 1217, 708, and 688 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, J = 7.4), 1.09 (3H, t, J = 7.1), 1.23 (3H, t, J = 7.1), 1.38 (3H, d, J = 7.3), 1.60 (2H, m, J = 7.4), 2.17 (2H, t, J = 7.4), 4.13 (2H, q, J = 7.1), 4.25 (2H, q, J = 7.1), 4.85 (1H, q, J = 7.3), 6.83 (1H, s), 7.45 (2H, pt, J = 7.7), 7.56 (1H, pt, J = 7.7), and 7.94 (2H, d, J = 7.7); ^{13}C NMR ($CDCl_3$) δ 13.65, 13.78, 13.97, 14.70, 19.00, 38.19, 45.59, 62.49, 62.62, 67.01, 128.66, 128.78, 133.53, 135.78, 166.96, 167.97, 172.31, and 202.43; MS (LSIMS) m/z 378.190828 $(M+1)^+$, Calcd. mass for $C_{20}H_{28}NO_6$: 378.191663 (deviation 2.2 ppm).

Diethyl 2-(3-phenyl-3-oxopropyl)butyramido malonate (14c): (62%); mp 61−62 °C; IR (KBr) 3416, 3022, 2878, 1738, 1682, 1600, 1583, 1272, 1216, 757, 690, and 668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (3H, t, J = 7.4), 1.23 (6H, t, J = 7.1), 1.64 (2H, m, J = 7.4), 2.19 (2H, t, J = 7.4), 2.74 (2H, t, J = 7.4), 2.95 (2H, t, J = 7.4), 4.23 (4H, m, J = 7.1), 6.79 (1H, s), and 7.43−7.9 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.69, 13.99, 18.99, 27.90, 33.39, 38.16, 62.73, 65.54, 128.10, 128.67, 133.22, 136.54, 168.18, 172.27, and 198.74; MS (LSIMS) m/z 400.173680 $(M+Na)^+$, Calcd. mass for $C_{20}H_{27}NO_6Na$: 400.173608 (deviation −0.2 ppm).

Preparation of 2-Substituted Amidomalonic Acid Diethyl Esters 8a and 9a. 40% aq. formaldehyde (1.12 mL; 15 mmol) and sodium cyanoborohydride (0.3 g; 4.7 mmol) were added to a solution of **7a** (0.3 g; 0.79 mmol) in acetonitrile (6 mL). The pH was adjusted to 6 by addition of acetic acid, and the reaction mixture was stirred at room temperature for 8 h. After evaporation, the residue was taken up with water, basified with 2 N NaOH, and extracted with diethyl ether. The organic layer was washed with water, dried, and evaporated to dryness. The crude residue was purified by flash-chromatography (diethyl ether/hexane 2:1) to yield the acetamidomalonate **8a** as a yellow solid.

7a (0.93 g, 2.54 mmol) and benzyl bromide (0.121 mL, 1.016 mmol) were dissolved in 35 mL of DMF. Potassium carbonate (0.35 g) was then added, and the mixture was warmed overnight in an oil bath at 70 °C under nitrogen. The reaction mixture was brought to room temperature, and water (50 mL) was added. The product was taken up with ether (60 mL) and dried over Na_2SO_4. The crude product was collected and purified by flash column chromatography (silica gel). Elution with EtOAc/hexane (1:2) provided pure compound **9a** as a colorless solid and 0.38 g of **7a**.

Diethyl 2-[2-(2-(dimethylamino)-5-methoxyphenyl)-2-oxoethyl]acetamido malonate (8a): (67%); mp 200 °C (dec); IR (KBr) 3380, 2986, 2838, 1747, 1680, 1608, 1573, 1502, 858, and 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (6H, t, J = 7.1), 1.95 (3H, s), 2.65 (6H, s), 3.74 (3H, s), 4.24 (4H, q, J = 7.1), 4.35 (2H, s), and 6.85−7.07 (4H, m); ^{13}C NMR ($CDCl_3$) δ 13.96, 22.89, 45.16, 45.32, 55.62, 62.68, 64.10, 113.30, 118.35, 120.03, 134.19, 146.52, 154.65, 167.47, 169.12, and 202.70; MS (CI) m/z 409.197774 $(M+1)^+$, Calcd. mass for $C_{20}H_{29}N_2O_7$: 409.197477 (deviation −0.7 ppm).

Diethyl 2-[2-(2-benzylamino-5-methoxyphenyl)-2-oxoethyl]acetamido malonate (9a): (100%); mp 52−54 °C; IR (KBr) 3413, 2987, 2956, 1745, 1672, 1643, 1619, 1573, 1517,

859, 814, 755, and 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (6H, t, J= 7.1), 1.99 (3H, s), 3.77 (3H, s), 4.25 (2H, s), 4.27 (4H, q, J = 7.1), 4.43 (2H, d, J = 5.9), 6.58−7.36 (9H, m), and 8.87 (1H, t, J = 5.9); ^{13}C NMR ($CDCl_3$) δ 13.95, 23.05, 42.92, 47.01, 56.19, 62.82, 64.25, 113.85, 113.99, 116.54, 125.07, 126.90, 127.16, 128.68, 138.96, 146.56, 149.23, 167.68, 169.40, and 198.14; MS (LSIMS) m/z 493.194955 $(M+Na)^+$, Calcd. mass for $C_{25}H_{30}N_2O_7Na$: 493.195071 (deviation 0.2 ppm).

Preparation of α-Amido-ω-benzoyl Substituted Carboxylic Acid, 15a, 15c, 16a, 17a, 18a−g, 19a−b, 20a−c, 21c, and 22c. General Method. 2-Substituted amidomalonic acid diethyl esters **7a**, **7c**, **8a**, **9a**, **10a−g**, **11a−b**, **12a−c**, **13c**, or **14c** (2.74 mmol) were stirred with 10 mL of 1.00 N sodium hydroxide at room temperature until solution was complete (approximately 2 h). Glacial acetic acid (0.57 mL) was then added, and the solution was boiled for 20 min (evolution of carbon dioxide). The cooled solution was washed with water and rotaevaporated off several times to eliminate the acid. The residue was purified by flash chromatography ($CHCl_3$: methanol 7:3), generally yielding a quantitative amount of carboxylic acids.

2-Acetamido-4-(2-amino-5-methoxyphenyl)-4-oxobutyric acid (15a): (73%); mp 115−118 °C; IR (KBr) 3434, 3358, 3080, 2935, 2839, 1715, 1649, 1592, and 838 cm^{-1}; ^1H NMR (CD_3OD) δ 1.97 (3H, s), 3.44 (1H, dd, J = 5.4, 17.4), 3.52 (1H, dd, J = 5.6, 17.4), 3.72 (3H, s), 4.74 (1H, pt, J = 5.4, 5.6), 6.69 (1H, d, J = 9.1), 6.93 (1H, dd, J = 2.9, 9.1), and 7.19 (1H, d, J = 2.9); ^{13}C NMR (CD_3OD) δ 22.70, 42.25, 52.06, 55.41, 114.1, 118.23, 119.98, 125.05, 147.37, 151.22, 173.14, 176.75, and 200.74; MS (LSIMS) m/z 303.095659 $(M+Na)^+$, Calcd. mass for $C_{13}H_{16}N_2O_5Na$: 303.095692 (deviation 0.1 ppm); Anal. $C_{13}H_{16}N_2O_5$ (C, H, N).

2-Butyramido-4-(2-amino-5-methoxyphenyl)-4-oxobutyric acid (15c): (70%); mp 82−85 °C; IR (KBr) 3446, 2966, 2840, 2354, 1715, 1645, 1591, 992, 838, and 675 cm^{-1}; ^1H NMR (CD_3OD) δ 0.90 (3H, t, J = 7.4), 1.60 (2H, m, J = 7.4), 2.18 (2H, t, J = 7.4), 3.42 (1H, dd, J = 4.5, 16.9), 3.52 (1H, dd, J = 6.5, 16.9), 3.75 (3H, s), 4.76 (1H, dd, J = 4.5, 6.5), 6.69 (1H, d, J = 9.0), 6.94 (1H, dd, J = 2.8, 9.0), and 7.23 (1H, d, J = 2.8); ^{13}C NMR (CD_3OD) δ 13.94, 20.21, 39.03, 42.46, 51.59, 56.42, 114.09, 118.43, 119.92, 124.93, 147.32, 151.27, 175.44, 176.98, and 200.54; MS (LSIMS) m/z 309.145071 $(M+1)^+$, Calcd. mass for $C_{15}H_{21}N_2O_5$: 309.145047 (deviation −0.1 ppm); Anal. $C_{15}H_{20}N_2O_5$ (C, H, N).

2-Acetamido-4-(5-methoxy-2-dimethylaminophenyl)-4-oxobutyric acid (16a): (100%); mp 64−65 °C; IR (KBr) 3324, 3062, 1740, 1706, 1690, 1672, 1500, 867, and 696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.99 (3H, s), 2.83 (6H, s), 3.47 (1H, m), 3.80 (3H, s), 3.84 (1H, m), 4.70 (1H, bd), 6.98−7.35 (4H, m), and 8.0 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 23.06, 43.70, 46.36, 50.26, 55.86, 113.26, 118.69, 120.65, 134.47, 142.91, 156.39, 170.69, 174.69, and 202.92; MS (LSIMS) m/z 331.126974 $(M+Na)^+$, Calcd. mass for $C_{15}H_{20}N_2O_5Na$ 331.126992 (deviation 0.1 ppm); Anal. $C_{15}H_{20}N_2O_5$ (C, H, N).

2-Acetamido-4-(2-benzylamino-5-methoxyphenyl)-4-oxobutyric acid (17a): (70%); mp 68−70 °C; IR (KBr) 3343, 3086, 2931, 2857, 1731, 1643, 1619, 1518, 813, and 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.99 (3H, s), 3.53 (1H, m), 3.73 (3H, s), 3.75 (1H, m), 4.39 (2H, s), 4.96 (1H, pt), and 6.57−7.32 (10H, m); ^{13}C NMR ($CDCl_3$) δ 22.98, 46.99, 56.06, 113.94, 114.10, 116.27, 124.95, 126.94, 127.17, 128.71, 138.86, 146.63, 149.09, 171.06, 176.40, and 199.20; MS (EI) m/z 370.152836 (M^+), Calcd. mass for $C_{20}H_{22}N_2O_5$ 370.152872 (deviation 0.1 ppm); Anal. $C_{20}H_{22}N_2O_5$ (C, H, N).

2-Acetamido-4-(3-methoxyphenyl)-4-oxobutyric acid (18a): (100%); mp 152−155 °C; IR (KBr) 3376, 3083, 2839, 1681, 1600, 1488, 869, 787, and 685 cm^{-1}; ^1H NMR (CD_3OD) δ 1.78 (3H, s), 3.22 (1H, dd, J = 7.0, 16.2), 3.36 (1H, dd, J = 5.4, 16.2), 3.79 (3H, s), 4.54 (1H, dd, J = 5.4, 7.0) and 7.14−7.85 (5H, m); ^{13}C NMR (CD_3OD) δ 22.66, 41.61, 50.59, 55.24, 112.47, 118.81, 120.51, 129.66, 138.29, 159.30, 168.73, 175.21, and 197.48; MS (LSIMS) m/z 310.066709 $(M-1+Na_2)^+$, Calcd. mass for $C_{13}H_{14}NO_5Na_2$: 310.066737 (deviation 0.1 ppm); Anal. $C_{13}H_{15}NO_5$ (C, H, N).

2-Propionamido-4-(3-methoxyphenyl)-4-oxobutyric acid (18b): (93%); mp 119−122 °C; IR (KBr) 3346, 3068, 2844, 1739, 1685, 1616, 1550, 1487, 895, 781, and 686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.02 (3H, t, J = 7.6), 2.17 (1H, q, J = 7.6), 2.20 (1H, q, J = 7.6), 3.52 (1H, dd, J = 4.1, 8.1), 3.65 (1H, dd, J = 4.3, 8.1), 3.76 (3H, s), 4.9 (1H, pt, J = 4.1, 4.3), 7.02−7.45 (5H, m), and 9.75 (1H, bs); ^{13}C NMR (CD_3OD) δ 9.60, 29.32, 40.58, 49.09, 55.43, 112.34, 120.26, 121.05, 129.75, 137.34, 159.79, 174.90, 176.03, and 198.45; MS (LSIMS) m/z 302.100335 $(M+Na)^+$, Calcd. mass for $C_{14}H_{17}NO_5Na$: 302.100443 (deviation 0.4 ppm); Anal. $C_{14}H_{17}NO_5$ (C, H, N).

2-Butyramido-4-(3-methoxyphenyl)-4-oxobutyric acid (18c): (70%); mp 100−102 °C; IR (KBr) 3323, 3063, 2842, 1750, 1697, 1602, 1556, 1486, 788, 775, and 682 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, J = 7.3), 1.62 (2H, m, J = 7.3), 2.20 (2H, t, J = 7.3), 3.58 (1H, dd, J = 3.7, 18.4), 3.77 (1H, dd, J = 3.6, 18.4), 3.83 (3H, s), 5.02 (1H, pt, J = 3.6, 3.7), 6.76−7.52 (5H, m), and 9.76 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 13.67, 19.06, 38.31, 40.62, 48.24, 55.51, 112.22, 120.62, 121.07, 129.85, 137.16, 159.92, 173.77, 175.22, and 198.11; MS (LSIMS) m/z 316.116052 $(M+Na)^+$, Calcd. mass for $C_{15}H_{19}NO_5Na$: 316.116093 (deviation 0.1 ppm); Anal. $C_{15}H_{19}NO_5$ (C, H, N).

2-Benzamido-4-(3-methoxyphenyl)-4-oxobutyric acid (18d): (100%); mp 166−168 °C; IR (KBr) 3325, 3017, 2963, 2840, 1706, 1681, 1640, 1582, 1535, 1487, 788, 717, and 685 cm^{-1}; ^1H NMR (CD_3OD) δ 3.69 (2H, m), 3.83 (3H, s), 5.14 (1H, m) and 7.15−7.83 (9H, m); ^{13}C NMR (CD_3OD) δ 41.20, 50.59, 55.89, 113.59, 120.73, 121.81, 128.40, 129.56, 130.89, 132.88, 135.21, 139.22, 161.43, 170.01, 175.10, and 198.81; MS (LSIMS) m/z 350.101513 $(M+Na)^+$, Calcd. mass for $C_{18}H_{17}NO_5Na$: 350.100443 (deviation −3.6 ppm); Anal. $C_{18}H_{17}NO_5$ (C, H, N).

2-Cyclohexanecarboxamido-4-(3-methoxyphenyl)-4-oxobutyric acid (18e): (70%); mp 121−124 °C; IR (KBr) 3445, 3022, 2485, 1699, 1689, 1552, 1488, 946, 883, 785, and 686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.28 (5H, m), 1.73 (5H, m), 2.17 (1H, m), 3.48 (2H, d, J = 5.6), 3.83 (3H, s), 4.67 (1H, t, J = 5.6), and 7.13−7.58 (4H, m); ^{13}C NMR (CD_3OD) δ 26.82, 30.28, 30.79, 46.35, 42.36, 52.12, 55.87, 113.60, 120.41, 121.86, 130.74, 139.71, 161.36, 177.50, 178.24, and 199.77; MS (LSIMS) m/z 334.164562 $(M+1)^+$, Calcd. mass for $C_{18}H_{24}NO_5$: 334.165448 (deviation 2.7 ppm), Anal. $C_{18}H_{23}NO_5$ (C, H, N).

2-*trans*-Crotonamido-4-(3-methoxyphenyl)-4-oxobutyric acid (18f): (85%); mp 164−166 °C; IR (KBr) 3360, 2948, 1727, 1689, 1667, 1612, 1536, 1486, 972, 868, 787, and 687 cm^{-1}; ^1H NMR (CD_3OD) δ 1.84 (3H, m, J = 1.6, 6.9), 3.58 (2H, d, J = 5.6), 3.64 (3H, s), 4.56 (1H, t, J = 5.6), 5.99 (1H, dd, J = 6.9, 15.3), 6.8 (1H, m, J = 1.6, 15.3), and 7.16−7.59 (4H, m); ^{13}C NMR (CD_3OD) δ 17.81, 41.50, 49.93, 55.90, 113.56, 120.70, 121.76, 130.87, 139.22, 161.46, 125.70, 141.57, 172.02, 176.50, and 198.61; MS (LSIMS) m/z 314.100419 $(M+Na)^+$, Calcd. mass for $C_{15}H_{17}NO_5Na$: 314.100443 (deviation 0.1 ppm); Anal. $C_{15}H_{17}NO_5$ (C, H, N).

2-Cyclopropanecarboxamido-4-(3-methoxyphenyl)-4-oxobutyric acid (18g): (80%); mp 121−122 °C; IR (KBr) 3377, 3935, 1737, 1689, 1629, 1547, 1487, 872, 788, and 688 cm^{-1}; ^1H NMR (CD_3OD) δ 0.69−0.88 (4H, m), 1.63 (1H, m), 3.55 (2H, d, J = 5.4), 3.83 (3H, s), 4.92 (1H, t, J = 5.4) and 6.70−7.59 (4H, m); ^{13}C NMR (CD_3OD) δ 7.53, 7.57, 14.67, 41.50, 49.86, 55.90, 113.55, 120.71, 121.77, 130.89, 139.17, 161.43, 174.68, 176.40, and 198.56; MS (LSIMS) m/z 314.100536 $(M+Na)^+$, Calcd. mass for $C_{15}H_{17}NO_5Na$: 314.100443 (deviation −0.3 ppm); Anal. $C_{15}H_{17}NO_5$ (C, H, N).

2-Acetamido-4-(3-nitrophenyl)-4-oxobutyric acid (19a): (86%); mp 70−74 °C; IR (KBr) 3395, 3092, 1696, 1659, 1565, 1532, 1222, 810, 736, and 672 cm^{-1}; ^1H NMR (CD_3OD) δ 1.90 (3H, s), 3.51 (1H, dd, J = 6.4, 17.0), 3.59 (1H, dd, J = 5.1, 17.0), 4.72 (1H, dd, J = 5.1, 6.4), 7.76 (1H, m), 8.41 (2H, m) and 8.75 (1H, m); ^{13}C NMR (CD_3OD) δ 22.74, 42.68, 52.56, 123.71, 128.21, 131.26, 135.09, 139.66, 149.88, 172.76, 180.70, and 198.09; MS (LSIMS) m/z 303.059235 $(M+Na)^+$, Calcd. mass for $C_{12}H_{12}N_2O_6Na$: 303.059306 (deviation 0.2 ppm); Anal. $C_{12}H_{12}N_2O_6$ (C, H, N).

2-Propionamido-4-(3-nitrophenyl)-2-oxobutyric acid (19b): (75%); mp 218−220 °C; IR (KBr) 3394, 3086, 1695, 1614, 1534, 1220, 879, 812, and 736 cm^{-1}; ^{1}H-MNR (CD_3OD) δ 1.08 (3H, t, J = 7.6), 2.23 (2H, q, J = 7.6), 3.55 (1H, dd, J = 6.4, 17.4), 3.65 (1H, dd, J = 5.1, 17.4), 4.80 (1H, pt, J = 5.1, 6.4), 7.75 (1H, m), 8.38 (2H, m), and 8.70 (1H, s); ^{13}C NMR (CD_3OD) δ 10.16, 30.04, 42.03, 51.71, 123.70, 128.35, 131.32, 135.01, 139.45, 149.88, 176.00, 176.80, and 197.70; MS (LSIMS) m/z 317.0743 $(M+Na)^+$, Calcd. mass for $C_{13}H_{14}N_2O_6Na$: 317.074956 (deviation 2.0 ppm); Anal. $C_{13}H_{14}N_2O_6$ (C, H, N).

2-Acetamido-4-phenyl-4-oxobutyric acid (20a): (100%); mp 149−152 °C; IR (KBr) 3305, 3067, 1690, 1662, 1640, 1582, 1448, 1306, 758, and 689 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.93 (3H, s), 3.52 (2H, d, J = 5.6), 4.70 (1H, t, J = 5.6) and 7.46−8.00 (5H, m); ^{13}C NMR (CD_3OD) δ 24.20, 42.38, 52.44, 129.26, 129.69, 134.35, 138.25, 172.63, 178.37, and 200.30; MS (LSIMS) m/z 258.074193 $(M+Na)^+$, Calcd. mass for $C_{12}H_{13}NO_4Na$: 258.074228 (deviation 0.1 ppm); Anal. $C_{12}H_{13}NO_4$ (C, H, N).

2-Propionamido-4-phenyl-4-oxobutyric acid (20b): (100%); mp 119−121 °C; IR (KBr) 3335, 3179, 2987, 2879, 1714, 1633, 1573, 896, 823, and 751 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.22 (3H, t, J = 7.5), 2.51 (2H, q, J = 7.5), 3.47 (1H, dd, J = 5.5, 18.2), 3.83 (1H, dd, J = 3.6, 18.2), 4.45 (1H, dd, J = 3.6, 5.5), and 7.43−7.88 (5H, m); ^{13}C NMR (CD_3OD) δ 10.16, 30.14, 42.24, 52.08, 129.23, 129.70, 134.28, 138.34, 176.21, 177.47, and 199.96; MS (LSIMS) m/z 250.107933 $(M+1)^+$, Calcd. for $C_{13}H_{16}NO_4$: 250.107933 (deviation 0.0 ppm); Anal. $C_{13}H_{15}NO_4$ (C, H, N).

2-Butyramido-4-phenyl-4-oxobutyric acid (20c): (100%); mp 150−153 °C; IR (KBr) 3371, 3063, 2483, 1679, 1643, 1573, 970, 832, and 685 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.88 (3H, t, J = 7.4), 1.58 (2H, m, J = 7.4), 2.16 (2H, t, J = 7.4), 3.51 (2H, d, J = 5.7), 4.73 (1H, t, J = 5.7), and 7.47−7.98 (5H, m); ^{13}C NMR (CD_3OD) δ 13.95, 20.18, 39.05, 42.27, 52.14, 129.23, 129.69, 134.27, 138.33, 175.33, 177.50, and 199.94; MS (LSIMS) m/z 264.123153 $(M+1)^+$, Calcd. mass for $C_{14}H_{18}NO_4$: 264.123583 (deviation 1.6 ppm); Anal. $C_{14}H_{17}NO_4$ (C, H, N).

2-Butyramido-3-methyl-4-phenyl-4-oxobutyric acid (21c): (diastereom. mixt. A and B, relation 1.7:1) (94%); mp 162−165 °C; IR (KBr) 3444, 3354, 3070, 2878, 1680, 1598, 1449, 1384, 705, and 663 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.92 (3H, t, J = 7.3), 1.13 (3H, d, J = 7.0, isomer A), 1.18 (3H, d, J = 7.0, isomer B), 1.60 (2H, m, J = 7.3), 2.20 (2H, t, J = 7.3), 4.17 (1H, m), 4.70 (1H, d, J = 5.6, isomer B), 4.80 (1H, d, J = 6.4, isomer A), and 7.46−8.09 (5H, m); ^{13}C NMR (CD_3OD) δ 13.12, 14.05, 14.07, 20.28, 20.35, 38.96, 39.06, 43.90, 44.03, 57.86, 129.32, 129.64, 129.72, 129.79, 134.27, 137.87, 175.85, 176.11, 180.38, 203.97, and 204.82; MS (LSIMS) m/z 278.138218 $(M+1)^+$, Calcd. mass for $C_{15}H_{20}NO_4$: 278.139233 (deviation 3.6 ppm); Anal. $C_{15}H_{19}NO_4$ (C, H, N).

2-Butyramido-5-phenyl-5-oxopentanoic acid (22c): (70%); mp 136−138 °C; IR (KBr) 3300, 3051, 2876, 1727, 1669, 1598, 1547, 1449, 747, and 689 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.93 (3H, t, J = 7.4), 1.62 (2H, m, J = 7.4), 2.06 (1H, ddd, J = 8.2, 8.3, 14.1), 2.20 (2H, t, J = 7.4), 2.27 (1H, ddd, J = 5.2, 8.3, 14.1), 3.11 (2H, m, J = 8.3), 4.41 (1H, dd, J = 5.2, 8.2), 7.48 (2H, pt, J = 7.4), 7.59 (1H, pt, J = 7.4), 7.97 (2H, d, J = 7.4); ^{13}C NMR (CD_3OD) δ 14.00, 20.26, 27.95, 35.95, 38.97, 54.45, 129.11, 129.72, 134.29, 138.21, 175.79, 177.07 and 201.60; MS (LSIMS) m/z 278.138792 $(M+1)^+$, Calcd. mass for $C_{15}H_{20}NO_4$: 278.139233 (deviation 1.6 ppm); Anal. $C_{15}H_{19}NO_4$ (C, H, N).

Electrophysiological Methods. Male Wistar rats weighing 250−325 g, housed under a 12:12 h light:dark cycle and with free access to food and water were used. On the day of the experiment, the rats were anesthetized with urethane (1.2 g/kg of body weight, ip) and mounted in a stereotaxic apparatus, with the incisor bar 4 mm below the ear bars. Body temperature was maintained at 36.5−37.5 °C with a rectal probe and an electrically regulated heating pad. Recording electrodes were lowered through burr holes in the skull to the striatum, using the coordinates +1.3 to −0.7 mm to bregma, 1−2.5 mm lateral to the sagittal sinus, and 3−7 mm below the dura. Stimulating electrodes were placed in the SMCx at the coordinates 1.5 mm anterior to bregma, 1.5 mm lateral to the sagittal sinus, and 1.5 mm below the dura. Glass microelectrodes filled with 0.5 M sodium acetate and 2% pontamine sky blue dye, with a tip of 1 mm diameter and an impedance of 6−9 MΩ at 1 kHz, were used for extracellular recordings from single units in the striatum. Electrical stimulation was applied to the SMCx, using two monopolar stainless steel electrodes (Rhodes Medical NE-200X, 1 mm separation). During the recordings and microiontophoretic experiments, single monophasic square wave pulses (0.21 ms duration, 100−200 nA intensity, and 0.5 Hz frequency) were generated by a digital stimulator coupled to a stimulus isolation unit. Signals recorded from the microelectrode were digitalized and sampled by a computer that records spike frequencies and interspike intervals with a resolution of 1 ms. In microiontophoretic experiments, 5-barrel glass micropipets were used, assembled to the recording micropipet. Retention and ejection currents were +3 and −100 nA, respectively. The procedure has been previously published.[18,35] Only striatal neurones with an excitatory response to SMCx stimulation were selected to study the effects of kynurenine derivatives microiontop horesis.

All the compounds assayed were microiontophoretically ejected for 2 min (1 mM, 0.3% ethanol/water or 0.3% methanol/water, pH 6.0, −100 nA), and the response of the neurone was recorded. Following this period of ejection, and in the absence of kynurenine ejection, the response of the neurone was recorded for 10 min. Only those neurones that at the end of the experiment recovered to basal excitatory activity, i.e., the activity before kynurenine ejection, were used in the study. Controls were made regularly, passing a current of the same intensity and polarity through the solvent. To study the subtype of glutamatergic receptor involved in the actions of kynurenine derivatives, iontophoresis of NMDA (−30 nA; 0.05 M; pH: 8) was also used in some experiments.

Striatal nNOS Activity Determination. L-Arginine, L-citruline, *N*'-(2-hydroxymethyl)piperazine-*N*'-(2-hydroxypropanesulfonic acid) (HEPES), DL-ditriothreitol (DTT), leupeptin, aprotinin, pepstatin, phenilmethylsulfonylfluoride (PMSF), hypoxantine-9-β-D-ribofuranosid (inosine), ethylene-glycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA), bovine serum albumin (BSA), Dowex-50 W (50 × 8−200), FAD, NADPH, 5,6,7,8-tetrahydro-L-biopterin dihydrocloride (H_4-biopterin), and melatonin were obtained from Sigma Química (Spain). L-[^{3}H]-Arginine (58 Ci/mmol) was obtained from Amersham (Amersham, Bucks, UK). Tris (hydroxymethyl)-aminomethane (Tris-HCl) and calcium chloride were obtained from Merck (Spain).

The rats were killed by cervical dislocation, and the striata were quickly collected and immediately used to measure NOS activity. Upon removal, the tissues were cooled in ice-cold homogenizing buffer (25 mM Tris, 0.5 mM DTT, 10 μg/mL leupeptin, 10 μg/mL pepstatine, 10 μg/mL aprotinine, 1 mM PMSF, pH 7.6). Two striata were placed in 1.25 mL of the same buffer and homogenized in a Polytron (10 s × 6). The crude homogenate was centrifuged for 5 min at 1000*g*, and aliquots of the supernatant were either stored at −20 °C for total protein determination[46] or used immediately to measure NOS activity. The nNOS activity was measured by the Bredt and Snyder[47] method, monitoring the conversion of L-[^{3}H]-arginine to l-[^{3}H]-citruline. The final incubation volume was 100 μL and consisted of 10 μL crude homogenate added to a buffer to give a final concentration of 25 mM Tris, 1 mM DTT, 30 μM H_4-biopterin, 10 μM FAD, 0.5 mM inosine, 0.5 mg/mL BSA, 0.1 mM $CaCl_2$, 10 μM L-arginine, and 50 nM L-[^{3}H]-arginine, at pH 7.6. Increasing concentrations of melatonin (up to 1 mM) or of the other four compounds were added to the incubation medium. The reaction was started by the addition of 10 μL of NADPH (0.75 mM final) and continued for 30 min at 37 °C. Control incubations were performed by the omission of NADPH. The reaction was halted by the addition of 400 μL of cold 0.1 M HEPES, 10 mM EGTA, and 0.175 mg/mL L-citruline, pH 5.5. The reaction mixture was decanted into a 2-mL column packed with Dowex-50 W ion-exchange resin (Na^+ form) and

eluted with 1.2 mL of water. L-[^{3}H]-Citruline was quantified by liquid scintillation spectroscopy. The retention of L-[^{3}H]-arginine in this process was greater than 98%. Specific enzyme activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. The nNOS activity was expressed as picomoles of L-[^{3}H]-citruline produced (mg of protein)$^{-1}$ min^{-1}.

Statistical Analysis. Statistical analysis of data was performed by using the Newman-Keuls multiple range test. The ANOVA II (two-way) test, followed by the Dunnett's test, was used to decide the existence of significant differences in the total responsiveness, and $P < 0.05$ was considered as significant in the direction (increase or decrease) of the responses among neurones treated with kynurenine derivatives.

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Supporting Information Available: ^{1}H and ^{13}C NMR assigments of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Herrling, P. L. Clinical implications of NMDA receptors. In *The NMDA Receptor*; Colingridge, G. L., Watkins, J. C., Eds.; Oxford University Press: Oxford, 1994; pp 376−394.
(2) Meldrum, B.; Garthwaite, J. Excitatory amino acid neurotoxicity and neurodegenerative diseases. *Trends Pharmacol. Sci*. **1990**, *11*, 379−387.
(3) Schwarcz, R. Metabolism and function of brain kynurenines. *Biochem. Soc. Trans*. **1993**, *21*, 77−82.
(4) Swartz, K. J.; During, M. J.; Freese, A.; Beal, M. F. Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. *J. Neurosci.* **1990**, *10*, 2965−2973.
(5) Stone, T. W.; Perkins, M. N. Quinolinic acid: a potent endogenous excitant at amino acid receptors in rat CNS. *Eur. J. Pharmacol*. **1981**, *72*, 411−412.
(6) Meldrum, B. S.; Chapman, A. G. Competitive NMDA antagonist as drugs. In *The NMDA Receptor*; Colingridge, G. L., Watkins, J. C., Eds.; Oxford University Press: Oxford, 1994; pp 376−394.
(7) Schwarcz, R.; Foster, A. C.; French, E. D.; Whetsell, W. O.; Köhler, C. Excitotoxic models for neurodegenerative disorders. *Life Sci*. **1984**, *35*, 19−32.
(8) Beal, M. F.; Kowall, N. W.; Ellison, D. W.; Mazurek, M. F.; Swartz, K. J.; Martin, J. B. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* **1986**, *321*, 168−171.
(9) Saito, K.; Nowak, T. S., Jr.; Markey, S. P.; Heyes, M. P. Mechanism of delayed increases in kynurenine pathway metabolism in damaged brain regions following transient cerebral ischemia. *J. Neurochem*. **1993**, *60*, 180−182.
(10) Heyes, M. P.; Saito, K.; Lackner, A.; Wiley, A.; Achim, C. L.; Markey, S. P. Sources of the neurotoxin quinolinic acid in the brain of HIV-1 infected patients and retrovirus-infected macaques. *FASEB J*. **1988**, *12*, 881−896.
(11) Moroni, F. Tryptophan metabolism and brain function: focus on kynurenine and other indole metabolites. *Eur. J. Pharmacol*. **1999**, *375*, 87−100.
(12) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestly, T.; Tridgett, R.; Iversen, L. L.; Woodruff, G. N. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. U.S.A*. **1988**, *85*, 6547−6550.
(13) Carling, R. W.; Leeson, P. D.; Moseley, A. M.; Baker, R.; Foster, A. C.; Grimwood, S.; Kemp, J. A.; Marshall, G. R. 2-Carboxytetrahydroquinolines. Conformational and stereochemical requirements for antagonism of the glycine site on the NMDA receptor. *J. Med. Chem*. **1992**, *35*, 1942−1953.
(14) Wu, H. Q.; Salituro, F. G.; Schwarcz, R. Enzyme-catalyzed production of the neuroprotective NMDA receptor antagonist 7-chlorokynurenic acid in the rat brain in vivo. *Eur. J. Pharmacol*. **1997**, *319*, 13−20.
(15) Wu, H. Q.; Lee, S. C.; Schwarcz, R. Systemic administration of 4-chlorokynurenine prevents quinolinate neurotoxicity in the rat hippocampus. *Eur. J. Pharmacol*. **2000**, *390*, 267−274.
(16) Alexander, G. E.; DeLong, M. R.; Strick, P. L. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* **1986**, *9*, 357−381.
(17) Calabresi, P.; Centonze, D.; Gubellini, P.; Marfia, G. A.; Pisani, A.; Sancesario, G.; Bernardi, G. Synaptic transmission in the striatum: from plasticity to neurodegeneration. *Prog. Neurobiol*. **2000**, *61*, 231−265.
(18) Escames, G.; Acuña-Castroviejo, D.; Vives-Montero, F. Melatonin-dopamine interaction in the striatal projection area of sensorimotor cortex in the rat. *NeuroReport* **1996**, *7*, 597−600.
(19) León, J.; Vives, F.; Gómez, I.; Camacho, E.; Gallo, M. A.; Espinosa, A.; Escames, G.; Acuña-Castroviejo, D. Modulation of rat striatal glutamatergic response in search for new neuroprotective agents: evaluation of melatonin and some kynurenine derivatives. *Brain Res. Bull*. **1998**, *45*, 525−530.
(20) Garthwaite, J. Glutamate, nitric oxide and cell-signaling in the nervous system. *Trends Neurosci*. **1991**, *14*, 60−67.
(21) León, J.; Vives, F.; Crespo, E.; Camacho, E.; Espinosa, A.; Gallo, M. A.; Escames, G.; Acuña-Castroviejo, D. Modification of nitric oxide synthase activity and neuronal response in rat striatum by melatonin and kynurenine derivatives. *J. Neuroendocrinol*. **1998**, *10*, 297−302.
(22) León, J.; Macías, M.; Escames, G.; Camacho, E.; Khaldy, H.; Martín, M.; Espinosa, A.; Gallo, M. A.; Acuña-Castroviejo, D. Structure-related inhibition of calmodulin-dependent nNOS activity by melatonin and synthetic kynurenines. *Mol. Pharmacol*. **2000**, *58*, 967−975.
(23) Sugasawa, T.; Adachi, M.; Sasakura, K.; Kitagawa, A. Amidohaloborane in organic synthesis. Simple synthesis of indoles and 1-acyl-3-indolinones using specific ortho-α-chloroacetylation of anilines. *J. Org. Chem*. **1979**, *44*, 573−586.
(24) Pellicciari, R.; Natalini, B.; Costantino, G.; Mahmoud, M. R.; Mattoli, L.; Sadeghpour, B. M. Modulation of the kynurenine pathway in search for new neuroprotective agents. Synthesis and preliminary evaluation of (*m*-nitrobenzoyl)alanine, a potent inhibitor of kynurenine-3-hydroxylase. *J. Med. Chem*. **1994**, *37*, 647−655.
(25) McKennis, H., Jr.; Turnbull, B.; Bowman, E. R.; Tamaki, E. The synthesis of Hydroxycotinine and studies on its structure. *J. Org. Chem*. **1963**, *28*, 383−387.
(26) Gaudry, M.; Marquet, A. Enolisation des cetones dissymmetriques-III. Acces facile aux bromomethylcetones par bromation en presence de methanol. *Tetrahedron* **1970**, *26*, 5611−5615.
(27) Varasi, M.; Della Torre, A.; Heidempergher, F.; Pevarello, P.; Speciale, C.; Guidetti, P.; Wells, D. R.; Schwarcz, R. Derivatives of kynurenine as inhibitors of rat brain kynurenine aminotransferase. *Eur. J. Med. Chem*. **1996**, *31*, 11−21.
(28) Dutta, A. K.; Xu, C.; Reith, M. E. A. Structure−activity peridine analogues: Synthesis and biological evaluation at the dopamine and serotonin transporter sites. *J. Med. Chem*. **1996**, *39*, 749−756.
(29) White, E. Deamination of amines. 2-Phenylethyl benzoate via the nitrosamide decomposition. *Org. Synth*. **1973**, *Coll. Vol 5*, 336−339.
(30) SYBYL 6.5. Tripos Associates, Inc., St. Louis, MO.
(31) Clark, M.; Cramer, R. D. III; Van Opdenbosch, N. Validation of the General Purpose Tripos 5.2 Force Field. *J. Comput. Chem*. **1989**, *10*, 982−1012.
(32) Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219−3228. (b) Marsili, M.; Gasteiger, J. *Croat. Chem. Acta* **1980**, *53*, 601−614. (c) Gasteiger, J.; Marsili, M. *Organ. Magn. Reson.* **1981**, *15*, 353−360.
(33) Powell, M. J. D. Restart Procedure for Conjugate Gradient Method. *Math. Prog*. **1977**, *12*, 241−254.
(34) Defrance, R.; Mocaer, E. The role of melatoninergic agonists in the clinic. *Eur. J. Med. Chem*. **1995**, *30*, S232−S241.
(35) Escames, G.; Macías, M.; León, J.; García, J. J.; Khaldy, H.; Martín, M.; Vives, F.; Acuña-Castroviejo, D. Calcium-dependent effects of melatonin inhibition of glutamatergic response in rat striatum. *J. Neuroendocrinol.* **2001**, *13*, 459−466.
(36) Cady, S. G.; Sono, M. 1-Methyl-DL-tryptophan, -(3-benzo-furanyl)DL-alanine (the oxygen analogue of tryptophan) and -[3-benzo-(b)thionyl]-DL-alanine (the sulfur analogue of tryptophan) are competitive inhibitors for indoleamine 2,3-dioxigenase. *Arch. Biochem. Biophys*. **1991**, *291*, 326−332.
(37) Dua, R. K.; Taylor, E. W.; Philips, R. S. S-aryl-L-cysteine S,S-dioxide: design, synthesis and evaluation of a new class of inhibitors of kynureninase. *J. Am. Chem. Soc*. **1993**, *115*, 1264−1270.
(38) Roever, S.; Cesura, A. M.; Huguenin, P.; Kettler, R.; Szente, A. Synthesis and biochemical evaluation of *N*-(4-phenylthiazol-yl)-benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase. *J. Med. Chem*. **1997**, *40*, 4378−4385.
(39) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev*. **1991**, *43*, 109−134.
(40) Muñoz Hoyos, M.; Molina Carballo, A.; Rodríguez Cabezas, T.; Uberos Fernandez, J.; Ruiz-Cosano, J. A.; Acuña-Castroviejo, D.

Relationships Between Methoxyindole and Kynurenine Pathway Metabolites in Plasma and Urine in Children Suffering from Febrile and Epileptic Seizures. *Clin. Endocrinol.* **1997**, *47*, 667−677.

(41) Muñoz-Hoyos, A.; Molina-Carballo, A.; Macías, M.; Rodriguez-Cabezas, T.; Martin-Medina, E.; Narbona-López, E.; Valenzuela-Ruiz, A.; Acuña-Castroviejo, D. Comparison between tryptophan methoxyindole and kynurenine metabolic pathway in normal and preterm neonates and in neonates with acute fetal distress. *Eur. J. Endocrinol.* **1998**, *139*, 89−95.

(42) Molina-Carballo, A.; Muñoz-Hoyos, A.; Reiter, R. J.; Sanchez-Forte, M.; Moreno-Madrid, F.; Rufo-Campos, M.; Molina-Font, J. A.; Acuña-Castroviejo, D. Utility of high doses of melatonin as adjuntive anticonvulsant therapy in a child with severe myoclonic epilepsy. Two years of experience. *J. Pineal Res.* **1997**, *23*, 97−105.

(43) Acuña-Castroviejo, D.; Escames, G.; Macías, M.; Muñoz-Hoyos, A.; Molina-Carballo, A.; Arauzo, M.; Montes, R.; Vives, F. Cell protective role of melatonin in the brain. *J. Pineal Res.* **1995**, *19*, 57−63.

(44) Depreux, P.; Lesieur, D.; Mansour, H. A.; Morgan, P.; Howell, H. E.; Renard, P.; Caignard, D. H.; Pfeiffer, B.; Delagrange, P.; Guardiola, B.; Yous, S.; Demarque, A.; Adam, G.; Andrieux, J. Synthesis and structure−activity relationships of novel naphthalenic and bioisosteric related amidic derivatives as melatonin receptor ligands. *J. Med. Chem.* **1994**, *37*, 231−239.

(45) Defrance, R.; Mocaer, E. The role of melatoninergic agonists in the clinic. *Eur. J. Med. Chem.***1995**, *30*s, 232s−241s.

(46) Lowry, O. H.; Rosebroigh N. J.; Farr, A. S.; Randall, R. J. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−267.

(47) Bredt, S. S.; Snyder, S. H. Isolation of nitric oxide synthase, a calmodulin requiring enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 682−685.

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