Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 9, pp. 1161-1166, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00185-6

STRUCTURE–ACTIVITY STUDIES OF ARYL-SPACED DECAHYDROISOQUINOLINE-3-CARBOXYLIC ACID AMPA RECEPTOR ANTAGONISTS

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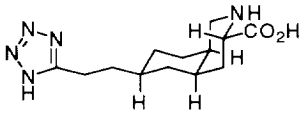
Abstract. We report the synthesis and structure activity studies of a series of decahydroisoquinoline AMPA antagonists where the distal acid is joined to the bicyclic ring nucleus with a spacer that contains an aromatic ring. These phenyl and thienyl substituted compounds are characterized as relatively potent AMPA antagoinsts.

Glutamic acid is the major excitatory neurotransmitter in the central nervous system, and it plays an important role in nervous system physiology. Ionotropic glutamate receptors (iGluRs) are multi-subunit-protein ligand operated ion channels, which modulate cell excitability through gating the flux of calcium and sodium ions into the cell.[1] There are three subclasses of iGluRs, named for the selective agonsits that activate each type: NMDA (for *N*-methyl-D-aspartic acid), AMPA (for 2-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic acid), and kainic acid.[2]

In a neuropathological state glutamic acid can cause cell death by a process known as excitotoxicity.[3] There is evidence to suggest that glutamic acid is involved in the neuropathology of cerebral ischemia,[4] spinal cord and head trauma,[5] as well as Alzheimer's disease;[6] and that AMPA antagonists may have therapeutic value in treating these conditions. AMPA anatagonists have also been shown to be anticonvulsants and may prove to be useful therapies for epilepsy.[7]



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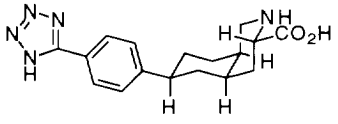

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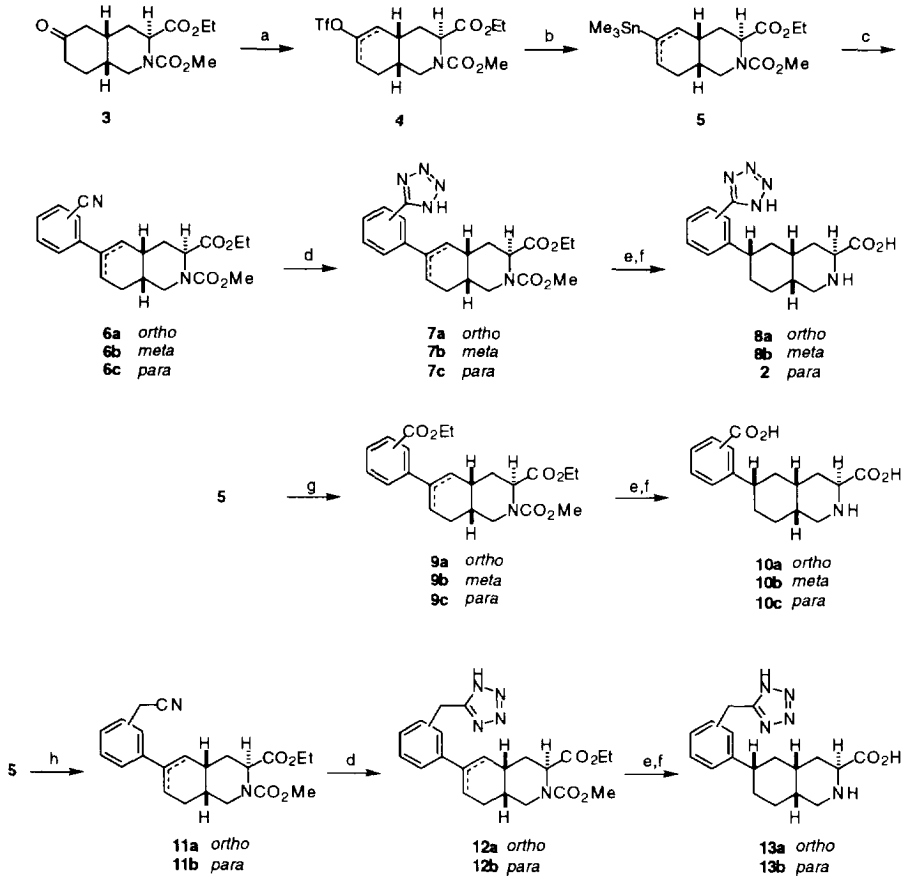
We recently disclosed that the 6-tetrazolylethyl substitituted decahydroisoquinoline-3-carboxylic acid **1** (LY293558) is a potent and selective AMPA receptor antagonist.[8] During the course of this structure activity study we identified a novel aryl-linked decahydroisoquinoline **2** (LY301199) with comparable affinity towards the AMPA receptor. This is consistent with earlier findings which showed that the length and nature of the tether between the distal acid and the bicyclic nucleus determines EAA receptor subclass selectivity.[8,12] Herein we report some of the interesting aspects of the structure–activity relationship that evolved from our discovery of **2**. We examined the effects of either phenyl or thienyl as the aromatic connector, and the relative orientation of acid and decahydroisoquinoline on that ring; we looked at addition of a methylene spacer adjacent to the acid or decahydroisoquinoline; and we looked at either carboxylic acid or teterazole as the distal acid.

The known ketone **3**[9] was a pivotal intermediate for the synthesis of these aryl-spaced amino acids (Scheme 1).[10] To this end, enolization of **3** with lithium bis(trimethylsilyl)amide and quenching with *N*-phenyl triflamide afforded a inseperable regioisomeric mixture of enol triflates **4**. Regardless of the base or conditions used, we always obtained a regioisomeric mixture of enolate; however, the lack of regioselectivity in this process was not a concern because we reduced the olefin later in the synthesis. We first attempted to couple **4** with 4-bromobenzonitirle, using an in situ conversion to the aryl stannane with hexabutyldistannane and palladium tetrakis(triphenylphosphine). Results with this method were inconsistent, and yields were typically low. We found it more efficient to convert **4** to the corresponding vinyl stannane **5** (hexamethyldistannane and palladium tetrakis(triphenylphosphine)) which allowed us to utilize a variety of readily avialable aryl halides in the subsequent coupling reaction. This stannane was stable to purification by silica gel chromatography and the reaction was easily scaled-up to afford multigram quantities.

Scheme 1. Preparation of phenyl-spaced amino acids using palladium mediated coupling reactions.



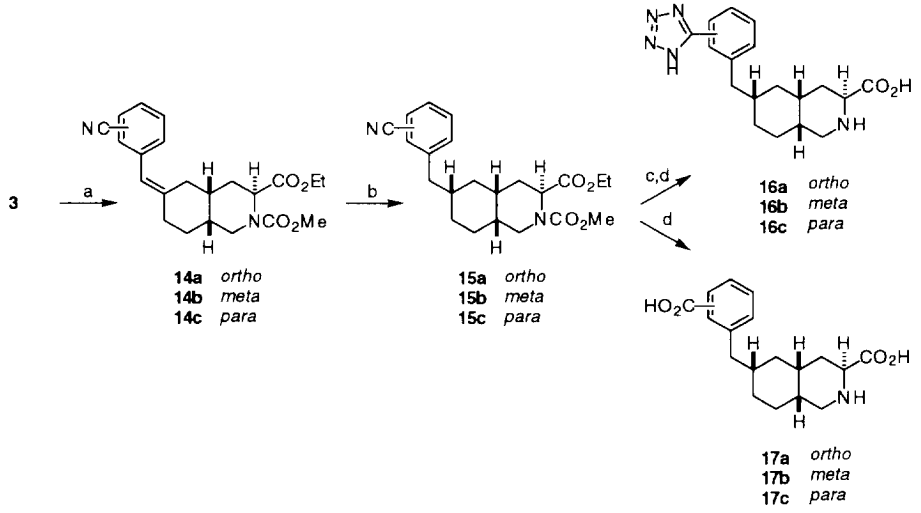
a. $LiN(TMS)_2$, THF, -78 °C; $PhN(OTf)_2$. b. $Me_3SnSnMe_3$, $Pd(Ph_3P)_4$, THF, LiCl, 66 °C. c. 2-, 3- or 4-bromobenzonitrile, $Pd(Ph_3P)_4$, xylene, 138 °C. d. *n*-Bu_3SnN_3, 80 °C. e. 60 psi H_2, 5% Pd/C, EtOH, 40 °C. f. 6 N HCl, reflux; Dowex 50-X8, 10% pyridine/water. g. Ethyl 2-, 3- or 4-bromobenzoate, $Pd(Ph_3P)_4$, xylene, 138 °C. h. 2- or 4-bromophenylacetonitrile, $Pd(Ph_3P)_4$, xylene, 138 °C.

Reaction of stannane **5** with commercially available *o*-, *m*- or *p*-bromobenzonitrile in the presence of catalytic palladium tetrakis(triphenylphosphine) afforded **6a–c**, all as mixtures of olefin regioisomers (Scheme 1).[10] Attempts to hydrogenate the double bond prior to tetrazole formation were thwarted by competing nitrile reduction. The nitriles **6a–c** were therefore first converted to the tetrazoles by treatment with azido tri-*n*-butylstannane neat at 80 °C for 3 days followed by hydrogenation at 60 psi for 6 hr to afford, after chromatography, the single diastereoisomers **7a–c**. Subsequent hydrolysis of **7a–c** then afforded the desired amino acids **8a**, **8b**, and **2**. The (–)- and (+)-isomers of **2** were also prepared by the same methodology as for racemic **2** (Scheme 1), starting with the known (–)- and (+)-isomers of ketone **3**.[11]

The coupling reaction with vinyl stannane **5** worked equally well with ethyl *o*-, *m*- or *p*-bromobenzoate to yield **9a–9c**, each as a mixture of olefin regioisomer (Scheme 1).[10] Hydrogenation and exhaustive hydrolysis afforded amino acids **10a–c**. The stereoselectivity of the hydrogenation is attributed to the preference of the carbethoxy to adopt an axial conformation in order to reduce $A_{1,3}$ strain. This conformation allows for selective beta face reduction of the olefin. Stereochemical assignments were confirmed by ^{1}H NMR and were consistent with previously published analogs in this series.[8,9,11]

The Stille coupling of 2-bromophenylacetonitrile and 4-bromophenylacetonitrile with **5** afforded **11a** and **11b**, respectively, but in lower yields than the more activated bromobezonitriles or carboxylates (Scheme 1).[10] **11a** and **11b** were easily converted to the tetrazoles **12a** and **12b**, and then hydrogenated and exhaustively hydrolyzed to yield amino acids **13a** and **13b**.

Scheme 2. Preparation of phenyl-spaced amino acids using Horner-Emmons chemistry.

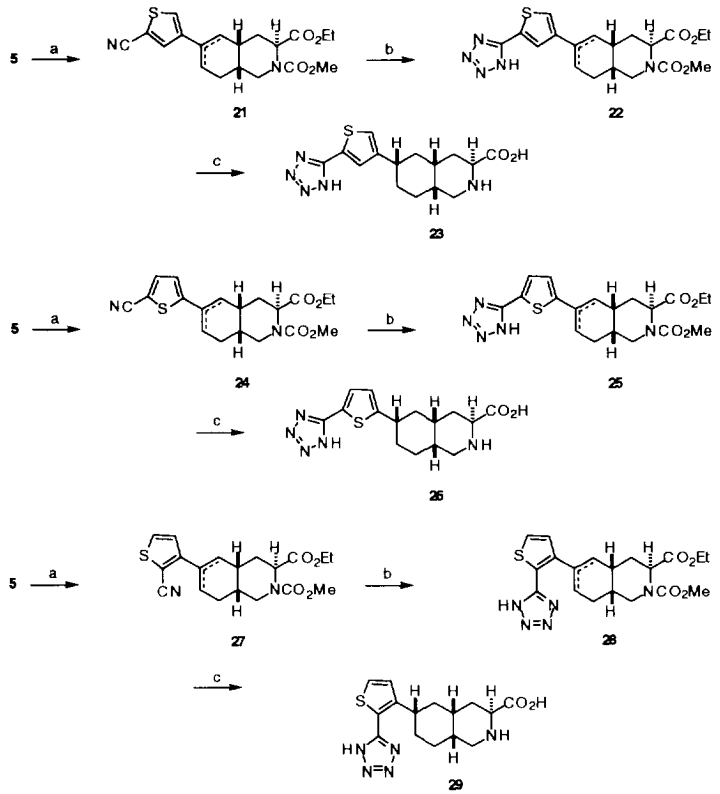


a. 2-, 3- or 4-(diethylphosphonomethyl)benzonitrile, $NaN(TMS)_2$, THF, room temperature. b. 15 psi H_2, 5% Pd/C,EtOH, room temperature. c. n-Bu_3SnN_3, 80 °C d. 6 N HCl, reflux; Dowex 50-X8, 10% pyridine/water.

Reaction of **3** with the sodium salt of either 2-, 3-, or 4-(diethylphosphonomethyl)benzonitriles afforded the unsaturated (E/Z mixture) nitriles **14a–c**, respectively (Scheme 2).[10] The exocyclic olefins were carefully hydrogenated at 1 atm for 16 hrs to afford **15a–c**. Coversion to the tetrazole as above followed by exhaustive

hydrolysis gave the amino acids **16a–c**, whereas exhaustive hydrolysis of the nitriles yielded the carboxy-substituted amino acids **17a–c**.

Scheme 3. Synthesis of thiophene-linked amino acids.



a. 4-bromothiophene-2-carbonitrile (**18**) or 5-bromothiophene-2-carbonitrile (**19**) or 3-bromothiophene-2-carbonitrile (**20**), $Pd(Ph_3P)_4$, xylene, 138 °C. b. n-Bu_3SnN_3, 80 °C. c. 60 psi H_2, 5% Pd/C,EtOH, 40 °C; 6 N HCl, reflux; Dowex 50-X8, 10% pyridine/water.

We prepared thiophene-linked compounds in the same manner as the corresponding phenyl-linked compounds (Scheme 3).[10] Condensation of 4-bromothiophene-2-carboxaldehyde, 5-bromothiophene-2-carboxaldehyde or 3-bromothiophene-2-carboxaldehyde with hydroxylamine followed by dehydration with phenylphosphonic dichloride afforded 4-bromothiophene-2-carbonitirle (**18**), 5-bromothiophene-2-carbonitirle (**19**), or 3-bromothiophene-2-carbonitirle (**20**), respectively.[12] Stille coupling of **18**, **19**, or **20** with **5** gave **21**, **24**, or **27**, respectively; then tetrazole formation gave **22**, **25**, or **28**, respectively, followed by hydrogenation and hydrolysis to yield amino acids **23**, **26**, or **29**.

We evaluated these novel amino acids in binding assays in rat forebrain membranes (using the selective radioligands [^{3}H]AMPA,[13] [^{3}H]CGS19755[14] and [^{3}H]kainic acid[15]) to determine for affinity at AMPA, NMDA, and kainic acid receptors (Table; data not shown for kainic acid receptor binding). Selected compounds were then evalutated for functional antagonist activity by assessing their abitily to inhibit depolariztions induced by 40 uM AMPA, 40 uM NMDA and 10 uM kainic acid in a rat cortical slice preparation[16] (Table; data for kainic acid not shown). Also included for comparison is data for (±)- and (−)-**2**.

For amino acids with only the aromatic spacer between the bicyclic ring system and the distal acid, we found that para-substitution on the aromatic ring was the preferred orientation for AMPA antagonist activity. Compounds **2** and **10c** were more potent than the *ortho*- and *meta*-substituted compounds **8a**, **8b**, **10a**, and **10b**, although the *meta*-carboxy-substituted compound did show some affinity for the AMPA receptor. The tetrazole-substituted compound **2** was more potent than the carboxy-substituted compound **10c**. The affinity of **2** for AMPA receptors was 30-fold more potent than for NMDA receptors, making **2** more selective than **1**. Amino acid **2** was functionally active as an AMPA antagonist in the cortical slice preparation, although less potent than **1**, with an IC_{50} = 14.9 ± 2.0 uM. When **2** was resolved into its constituent isomers, AMPA receptor affinity and functional AMPA antagonist activity was found to reside in the (–)-3*S*,4a*R*,6*S*,8a*R*-isomer. The affinity of (–)-**2** was about 2.5-fold higher than (±)-**2** for the AMPA receptor, however, (–)-**2** was no more potent as a fucntional AMPA antagonist in the rat cortical slice preparation.

Inserting one methylene either between the tetrazole and the aromatic ring (as in **13a** and **13b**) or between the aromatic ring and the bicyclic ring system (as in **16a–c** and **17a–c**) significantly decreased affinity at AMPA receptors, with weak AMPA receptor affinity found only for the *ortho*-substituted amino acid **16a**. This compound was the only one of those prepared in this study that showed any appreciable affinity for kainic acid receptors, with an IC_{50} = 17.6 uM. **16a** had about threefold higher affinity for kainic acid than AMPA receptors. We also found that **16b** functionally antagonized depolarizations induced by 10 uM kainic acid in the rat cortical slice preparation, with an IC_{50} = 14.5 ± 2.7 uM.

The phenyl ring of **2** could be replaced with a thiophene ring. The 2,4- and 2,5-disubstituted amino acids **23** and **26**, respectively, were equipotent in terms of affinity to **2**, while the 2,3-disubstituted compound **29** was inactive at AMPA receptors. **23** and **26**, however, were less active in the cortical slice preparation.

Interestingly, we found in this SAR that some of the compounds showed modest affinity for NMDA receptors. For example, the *ortho*-tetrazole-substituted amino acid **8a**, the *meta*-tetrazole-substituted amino acid **16b** and the 2,3-disubstituted thiophene **29** had low micromolar affinity at the NMDA receptor. Weak functional NMDA antagonist activity was observed for **29**.

In this publication, we have shown that potent AMPA receptor affinity and antagonist activity can be maintained in a series of decahydroisoquinoline amino acids when the alkylene group that connects a distal acidic group, such as tetrazole, to the bicyclic ring system is replaced with an aromatic ring such as phenyl or thiophene. The para disposition of functionality on the aromatic ring is optimal for activity. Amino acid **2** is the most rigid AMPA antagonist that we have prepared in this series. The location of the tetrazole ring relative to the proximal carboxylate and amine can be known with some precision. In evaluating possible conformers of **1** that might represent the bioactive conformation, it was difficult to distinguish if a fully extended or more folded conformation was appropriate. Molecular modeling studies support the hypothesis that in the bioactive conformation of acidic amino acid NMDA antagonists, the distal and proximal acids are arrayed in such a way that it's possible for them to interact with a similar point on the receptor protein (folded conformation).[17] Based on the potent affinity of **2**, and its well defined extended conformation, one may conclude that a fully extended conformation of **1** (as shown above) may represent the bioactive conformation for acidic amino acid AMPA antagonists. Also consistent with the folded conformation being preferred for NMDA antagonist activity is the observation that the *ortho*-substituted amino acids **8a** and **29** show no affinity for AMPA receptors, but do show weak affinity for NMDA receptors.

Table. In Vitro and In Vivo Data for Aryl-Spaced Amino Acids

Compound[a]	IC_{50} (μM) for Inhibition of Rat Brain Membrane Radioligand Binding: AMPA Receptors [^{3}H]AMPA[b]	IC_{50} (μM) for Inhibition of Rat Brain Membrane Radioligand Binding: NMDA Receptors [^{3}H]CGS19755[c]	Rat Cortical Slice Preparation: IC_{50} (μM) Versus Agonist-Induced Depolarizations: AMPA[d]	Rat Cortical Slice Preparation: IC_{50} (μM) Versus Agonist-Induced Depolarizations: NMDA[d]
(±)-**1**[e]	4.8 ± 1.2	26.4 ± 2.0	6.0 ± 1.0	61 ± 3
(−)-**1**[e]	1.4 ± 0.1	12.1 ± 2.0	1.8 ± 0.2	---
8a	>100	6.4[f]	NT[g]	NT
8b	66.9[h]	>100	NT	NT
(±)-**2**	3.2 ± 0.5	98 ± 23	14.9 ± 2.0	>100
(−)-**2**	1.3 ± 0.4	>100	13.4 ± 1.8	>100
(+)-**2**	55.4[h]	>100	58.7 ± 17.4	>100
10a	>100	>100	NT	NT
10b	22.0[h]	70% @ 100[i]	15.5 ± 2.4	85.8 ± 24.9
10c	6.1[h]	>100	15.0 ± 2.0	>100
13a	>100	>100	NT	NT
13b	>100	>100	NT	NT
16a	46.8[h]	>100	NT	NT
16b	>100	14.0 ± 0.9	>100	>100
16c	>100	>100	NT	NT
17a	>100	>100	NT	NT
17b	>100	77 % @ 100[i]	>100	>100
17c	>100	>100	NT	NT
23	4.3[h]	>100	27.3 ± 2.4	75.4 ± 7.0
26	4.3[h]	20.1[h]	43.3 ± 8.6	>100
29	>100	11.0 ± 0.5	77.4 ± 16.7	55.2 ± 14.9

[a]All compounds are racemic, except for (−)-**2**, which is the 3*S*,4a*R*,6*S*,8a*R*-isomer and (+)-**2**, which is the 3*R*,4a*S*,6*R*,8a*S* -isomer. [b]Unless otherwise indicated, IC_{50}s were the average of three determinations. See 13. [c]Unless otherwise indicated, IC_{50}s were the average of three determinations. See 14. [d]See 16. [e]Data from reference 8. [f]Average of two determinations. [g]NT = not tested. [h]One determination. [i]Percent inhibition of receptor binding at 100 micromolar.

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(*Received in USA* 3 *February* 1997; *accepted* 28 *March* 1997)