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# PHOSPHONOETHYLPHENYLALANINE DERIVATIVES AS NOVEL ANTAGONISTS OF NON-NMDA IONOTROPIC GLUTAMATE RECEPTORS

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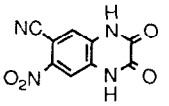
**Abstract**: Substituted phosphonoethylphenylalanines were evaluated for their ability to antagonize kainic acid (KA)- and AMPA-induced currents in *Xenopus* oocytes. Compounds which were substituted in the 5-position of the aromatic ring were found to selectively antagonize these currents. In addition, selected members of the series of compounds were demonstrated to possess anticonvulsant activity and to protect against KA-induced striatal toxicity *in vivo*. The effects of altering the position and identity of the aryl substituents were explored.

Excitatory amino acids (EAAs) mediate a substantial portion of the neurochemical synaptic activity occurring in the vertebrate central nervous system. Ionotropic EAA receptors are activated by glutamic and aspartic acids and are pharmacologically classified by their affinities for N-methyl-D-aspartatic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (AMPA) and kainic acid (KA).[1,2]

The development of selective NMDA antagonists has expanded the understanding of EAA neurotransmission, physiology and pathophysiology in the mammalian brain. Substantial preclinical evidence suggests that NMDA receptor antagonists may be useful as anxiolytics, anticonvulsants, antiemetics, antipsychotics and neuroprotectants.[3] Given the broad therapeutic potential of EAA antagonists, it is not surprising that efforts have been initiated to identify additional antagonist compounds. While substantial success has been achieved in identifying competitive and non-competitive antagonists of NMDA receptors,[4] there are few reports of potent and selective antagonists of KA- or AMPA-type EAA receptors. Identification of such antagonists is important, since in addition to possessing unique clinical utility, these agents are expected to share many of the potential therapeutic actions of antagonists of NMDA receptors. To date, the quinoxalinedione compounds (**1**) described by Honore et al.[5] (DNQX, CNQX, NBQX and related compounds) are the principle KA/AMPA antagonists being used in the study of EAA neurotransmission and its associated pathologies.

As part of a program to identify EAA receptor subtype-selective antagonists, we developed substituted phenylalanines of the type shown below (**2**) as potent and selective antagonists of KA- and AMPA-receptors. These compounds competitively antagonized KA- and AMPA-induced currents in *Xenopus* oocytes injected with rat brain mRNA (Table I) and inhibited the specific binding of [$^3$H]CNQX or [$^3$H]AMPA (data not shown). Selected members of this class of compounds were also found to possess anticonvulsant activity and to protect against KA-induced striatal toxicity *in vivo* Tables II and III). These compounds represent a novel class of non-NMDA antagonist which is structurally distinct from those previously reported.



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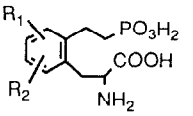

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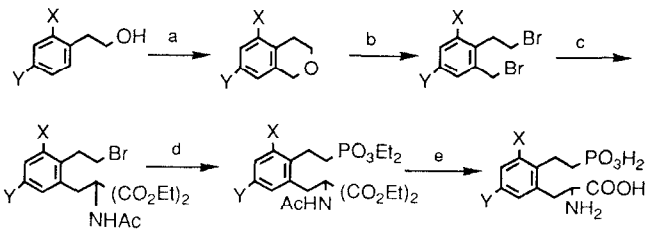
We began our investigations by preparing a series of monosubstituted 2-phosphonoethylphenylalanines. All four monomethyl substituted compounds were prepared by the routes shown below.

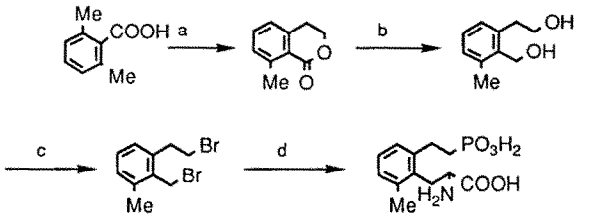
The 5-methyl compound, alone of the group, showed potent activity against KA-induced inward currents in oocytes (Table I; $K_I$ = 11 μM). The 3-, 4- and 6-methyl compounds were weakly active ($K_I$ > 100 μM) against both KA- and NMDA-induced currents (data not shown). Reduction of the aromatic ring resulted in a complete loss of activity for the 5-methyl compound (data not shown).

We proceeded to investigate the effects of altering the functional group at the key 5-position. The corresponding 5-halo (fluoro, chloro, bromo and iodo), 5-methoxy, 5-trifluoromethyl and higher alkyl analogues were all prepared and evaluated in the oocyte assay. The results for these compounds are summarized in Table I. In the cases where the corresponding phenethyl alcohols were available, the compounds were prepared as shown in Scheme I. In the case of the 5-trifluoromethyl, 4-methyl and 6-methyl analogues, alternative routes were required; these are shown in Schemes II, III and IV. The preparation of the 7-iodo compound was done via the 7-iodoisochroman, and this was prepared from 7-bromoisochroman by lithium-halogen exchange followed by iodine quench. All compounds were fully characterized by IR, $^1$H NMR, and elemental analysis.



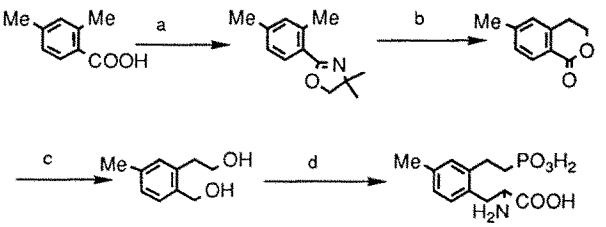
a i HCl, $(CH_2O)_x$, ii, $AlCl_3$ b: HBr/HOAc, 80°C c Diethyl acetamidomalonate, phase transfer conditions d $P(OEt)_3$, reflux e: 10N HCl, reflux

**SCHEME I. SYNTHESIS OF SUBSTITUTED 2-PHOSPHONOETHYLPHENYLALANINES**



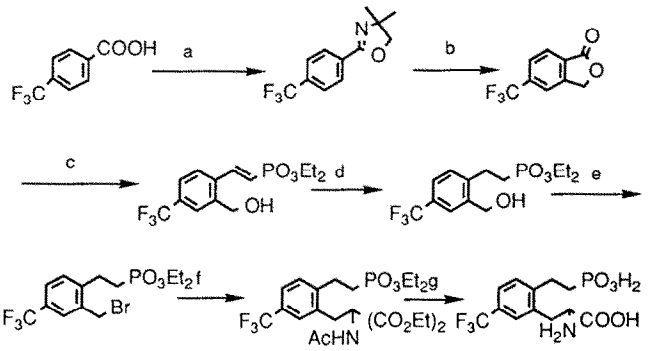
a. i, nBuLi, -78°C, then $(CH_2O)_x$, -78°C to r.t.; ii, $H_3O^+$, reflux. b: LAH/ether. c: $Ph_3PBr_2$, $CH_2Cl_2$, r t d. Remaining steps as in Scheme I.

**SCHEME II. SYNTHESIS OF 6-METHYL ANALOGUE**



a i, $SOCl_2$; ii, 2-amino-2-methyl-1-propanol; iii, $SOCl_2$ b i, nBuLi, -78°C; ii, $(CH_2O)_x$; iii, $H_3O^+$, reflux c. LAH/ether d Remaining steps as in Scheme II.

**SCHEME III: SYNTHESIS OF 4-METHYL ANALOGUE**



a. i, $SOCl_2$; ii, 2-amino-2-methyl-1-propanol, iii, $SOCl_2$ b. nBuLi, -78°C; ii, $(CH_2O)_x$, -78°C to r.t.; iii, $H_3O^+$, reflux c: i, DIBAL, -78°C, toluene; ii, sodio tetraethylmethylenebisphosphonate d: $H_2$, 5% Pd/C, 50psi e: $PPh_3Br_2$, $CH_2Cl_2$ f Diethyl acetamidomalonate, phase transfer conditions g: 10N HCl.

**SCHEME IV. SYNTHESIS OF 5-TRIFLUOROMETHYL ANALOGUE**

The data in Table I indicate that the 5-position substituent is critical for the activity at non-NMDA receptors; compounds which lacked a substituent at this position were inactive or were NMDA antagonists. Reduction of the aromatic ring to the cyclohexane compound completely abolished activity. A reduction in activity was noted when the alkyl group at the 5-position became quite bulky, the 5-tert-butyl ($K_i$ = 29 μM), 5-phenyl ($K_i$ = 76 μM) and 5-cyclohexyl ($K_i$ = 139 μM) compounds being successively less potent.

A steady increase in potency in the halogen series was noted in going from 5-fluoro ($K_i$ = 68 μM) to 5-iodo ($K_i$ = 3.6 μM). The trifluoromethyl substituted compound was likewise quite active ($K_i$ = 10 μM), whereas the methoxy-substituted analogue was much less potent ($K_i$ = 67 μM). It may be that electron withdrawing substituents are preferred, but additional SAR will have to be developed to verify this conclusion. Disubstitution in the 3- and 5-positions appears to be well tolerated.

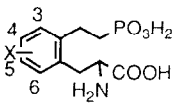



TABLE I

POTENCIES OF SUBSTITUTED 2-PHOSPHONOETHYLPHENYLALANINES TO INHIBIT KA- and AMPA-INDUCED CURRENTS IN RAT BRAIN mRNA-INJECTED *XENOPUS* OOCYTES[6]

| Compound | $K_i$ (μM) | |
|---|---|---|
| X = | KA | AMPA |
| 3-methyl | >100 | nt |
| 4-methyl | >100 | nt |
| 5-methyl | 11 | 13 |
| 6-methyl | >100 | nt |
| 3-phenyl | 83 | nt |
| 5-ethyl | 17 | 50 |
| 5-tBu | 29 | nt |
| 5-phenyl | 76 | nt |
| 5-cyclohexyl | 139 | nt |
| 5-fluoro | 68 | 167 |
| 5-chloro | 15 | 48 |
| 5-bromo | 10 | 61 |
| 5-iodo | 3.6 | 16 |
| 5-methoxy | 67 | nt |
| 5-trifluoromethyl | 10 | nt |
| 3,5-dimethyl | 12 | 39 |

(nt=not tested)

The 5-methyl and 3,5-dimethyl compounds were potent inhibitors of pentylenetetrazol-induced seizures when administered intracerebroventricularly to male CF-1 mice, being comparable in potency to the competitive NMDA antagonist CPP. However, these compounds were only weakly active when administered systemically. The 5-methyl compound protected against kainate-induced neuronal damage in rat striatum, as assessed by preventing reduction in choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD) activity (Table III). As shown in the Table, coadministration of the 5-methyl derivative (300 nmol) with KA (7.5 nmol) directly into the striatum nearly completely reversed the reduction in ChAT and GAD avtivity observed with KA alone.

TABLE II

POTENCY IN INHIBITING PENTYLENETETRAZOL-INDUCED SEIZURES IN MICE[7]

| Compound | $ED_{50}$ | |
|---|---|---|
| | i.c.v. (nmoles) | i.p. (mg/kg) |
| 5-methyl-2-phosphonoethyl-phenylalanine | 0 58 | 124 |
| 3,5-dimethyl-2-phosphonoethyl-phenylalanine | 0.30 | 111 |
| CPP | 0.15 | 7.3 |

TABLE III

PROTECTION AGAINST KAINIC ACID-INDUCED STRIATAL TOXICITY IN RATS[7]

| Example | % Activity of Contralateral Untreated Striatum | |
|---|---|---|
| | ChAT | GAD |
| KA | 32 ± 6 | 45 ± 9 |
| KA + 5-methyl | 98 ± 6 | 88 ± 17 |

In summary, we have developed a novel class of antagonists for non-NMDA ionotropic EAA receptors which is structurally distinct from previous KA/AMPA antagonists. Several of these compounds antagonize both KA- and AMPA-induced currents in *Xenopus* oocytes, suggesting that they will block EAA neurotransmission at KA/AMPA receptors in vivo. That two of the compounds inhibit seizures elicited by pentylenetetrazol can be taken as evidence for the potential utility of these agents as anticonvulsants. Also, the ability of the 5-methyl derivative to prevent KA-induced neuronal damage *in vivo* (Table III) and *in vitro* (data not shown) is indicative of neuroprotective properties. Further exploration and optimization of the activity of these compounds is underway in our laboratories and results will be disclosed in due course.

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