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α-SUBSTITUTED QUISQUALIC ACID ANALOGS: NEW METABOTROPIC GLUTAMATE RECEPTOR GROUP II SELECTIVE ANTAGONISTS.

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Abstract: Syntheses of both the α-methyl and benzyl analogs of quisqualic acid are described. Testing of these compounds for their activity at excitatory amino acid receptors revealed a striking change in activity in comparison to quisqualic acid. This structural modification results in the loss of quisqualate's potent agonist action at both non-NMDA ionotropic glutamate receptors as well as at group I mGluRs, while allowing these analogs to acquire antagonist properties with relative selectivity for group II metabotropic glutamate receptors.

The ability to selectively regulate synaptic transmission in the central nervous system (CNS) has the potential to alleviate human disease states such as brain ischemia, cerebral trauma, epilepsy, and chronic neurodegenerative disorders like Alzheimer's disease.[1] The principal excitatory neurotransmitter in the mammalian CNS is L-glutamate which activates both ionotropic glutamate receptors (iGluR) and metabotropic glutamate receptors (mGluR). The overactivation of the iGluRs is believed to result in the excessive influx of extracellular calcium, leading to the overactivation of certain enzymes, and eventual nerve cell death, a process generally referred to as excitotoxicity. While considerable research effort has focussed on the identification of iGluR antagonists (and in particular NMDA receptor antagonists), the results have been somewhat disappointing. mGluRs constitute a more recently identified class of receptors and are subdivided into three main groups. The group I receptors include mGluR1 and mGluR5, and these subtypes are coupled to phosphoinositide hydrolysis and calcium mobilization. One of the group I subtypes is located presynaptically, and its activation enhances the release of glutamate. Both the group II receptors comprised of mGluR2 and mGluR3 and the group III receptors comprised of mGluR4, mGluR6, mGluR7, and mGluR8 are negatively coupled to adenylate cyclase and to voltage operated calcium channels.[2] Additionally, agonists of some of the group II and III receptors have been shown to reduce glutamate release. In general, ligands acting as antagonists of the group I receptors and agonists of the group II and III receptors reduce synaptic excitation and consequently, are expected to result in neuroprotection.[1] Due to the fact that the mGluRs may modulate synaptic transmission only under conditions of synaptic hyperactivity, their manipulation as drug targets may overcome the limitations seen with NMDA receptor based interventions.

Quisqualic acid is a natural product isolated from *Quisqualis fructus*, which is active at a variety of excitatory amino acid receptors including both AMPA and kainate receptors.[3] Additionally, this compound has been shown to be one of the most potent agonists of the group I mGluRs.[1] In light of the fact that two mGluR agonists L-AP4 and L-CCG-1 have been shown to become antagonists when methylated at their α-position (Figure 1),[4] we were interested to explore the effect of a similar modification on the activity of quisqualic acid. If this α-methylation effect is general, then α-methylquisqualic acid (MetQuis) may prove to be a good antagonist

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at one or more of these receptors subtypes. Herein we describe the synthesis of α-methylquisqualic acid together with its sterically bulkier α-benzyl derivative, and present the pharmacology for these new compounds.

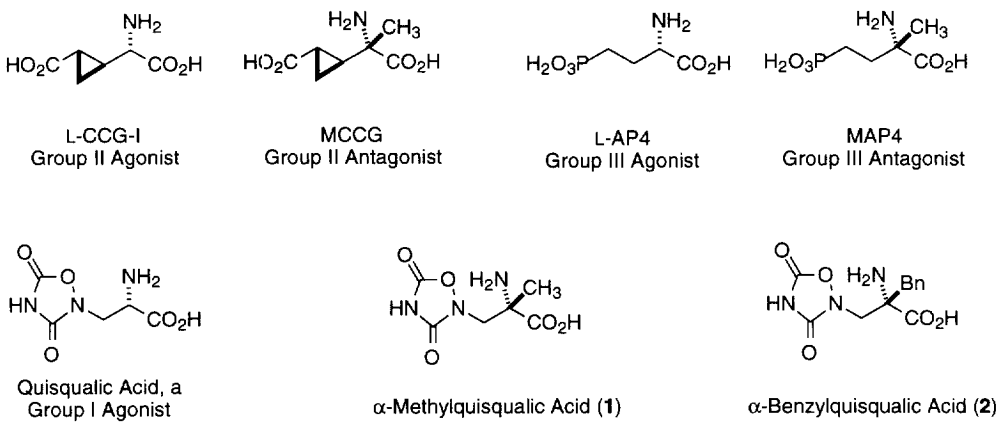


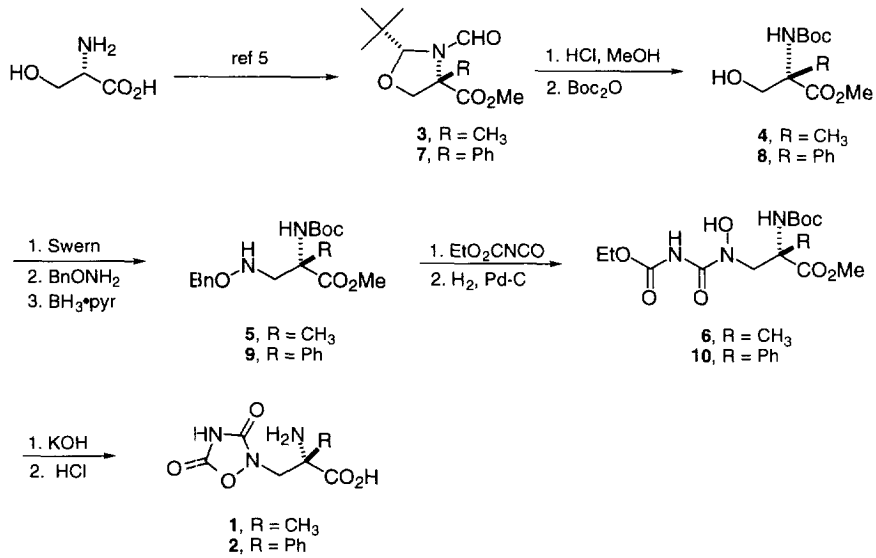
Figure 1. Structures of some previously known mGluR agonists and their α-methylated antagonist counterparts, including quisqualic acid and the two new derivatives **1** and **2** described in this article.

Chemical Synthesis Methods

The synthesis of α-methylquisqualic acid was undertaken as follows. Following a known procedure,[5] L-serine was transformed to oxazolidine **3** (Scheme 1). Acid catalyzed methanolysis followed by treatment with di-*tert*-butyl dicarbonate afforded the α-methylated L-methylserine derivative **4**. Following a procedure for the synthesis of quisqualic acid analogs reported by Venkatraman et al.,[6] the alcohol group of **4** was oxidized using Swern conditions, and the resulting aldehyde was reacted with *O*-benzylhydroxylamine hydrochloride to afford the corresponding oxime. Reduction of this oxime with sodium cyanoborohydride according to the literature precedent was tried next. Since this reaction was unsuccessful, the borane-pyridine complex was used instead to reduce the oxime to benzyloxyamine **5**. N-Acylation with ethoxycarbonyl isocyanate followed by palladium catalyzed hydrogenolysis afforded the penultimate intermediate **6**. Lastly, treatment with 2 equiv of KOH followed by HCl afforded α-methylquisqualic acid (**1**).[7]

α-Benzylquisqualic acid (**2**) was also synthesized starting from L-serine which was converted by way of the same chemistry to the oxazolidine **7**. Methanolysis followed by protection produced alcohol **8**. Oxidation and oxime formation went smoothly, but the borane reduction did not proceed to completion even after an extended reaction time. Therefore, the inseparable mixture of oxime and the benzyloxyamine **9** was alkylated with ethoxycarbonyl isocyanate, and the unreacted oxime was removed at this stage. Next, **9** was deprotected with Pd/C under an atmosphere of hydrogen to afford compound **10**. Hydrolysis of the methyl ester also proved to be problematic, and 18 equiv of base and an extended reaction time were required for complete hydrolysis. Treatment with 4 N HCl then produced the desired quisqualic acid derivative **2**.

Scheme 1. Synthesis of the quisqualic acid derivatives **1** and **2**.



Biological Methods and Results

Cell Cultures. Lines of Chinese hamster ovary (CHO) cells with stable expression of mGluR1a, mGluR2, mGluR5a and mGluR6 receptors were prepared using cDNAs for rat mGluRs kindly donated by Dr. S. Nakanishi.[8] The baby hamster kidney (BHK) cell line espressing mGluR4a was a generous gift from Zymogenetics Inc. (Seattle). All cell lines were cultured in 96-well plates and passaged every 6-7 days. Primary cultures of neurons were prepared from cerebella of 7-day old rats and cultured in 96-well plates. Cerebellar neurons were used for experiments after 8-9 days in culture.

Assay of Inositol Phosphate (IP) Accumulation. The activity of agonists at the phospholipase C-coupled receptors (mGluR1a and mGluR5a) was determined by measurement of their ability to increase the hydrolysis of membrane phosphoinositides. CHO cells expressing mGluR1a or mGluR5a cultured in 96-well plates were incubated overnight in glutamine-free culture medium supplemented with 0.75 μCi *myo*-[^{3}H]inositol (Amersham) to label the cell membrane phosphoinositides.[9] Incubations with agonists were carried out for 30 min at 37 °C in Locke's buffer (156 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 1 mM $MgCl_2$, 1.3 mM $CaCl_2$, 5.6 mM glucose and 20 mM Hepes, pH 7.4) containing 20 mM LiCl to block IP degradation. The reaction was terminated by aspiration of the medium, and the inositol phosphates were extracted for 10 min with 0.5 mL of 0.1 M HCl. The separation of [^{3}H]inositol phosphates was performed by anion exchange chromatography as described previously.

Assay of cAMP formation. The activity of agonists at receptors coupled negatively to adenylyl cyclase (mGluR2, mGluR4a, and mGluR6) was determined by measurements of their ability to decrease the forskolin-induced elevation of cyclic AMP formation.[9] Cells cultured on 96-well plates were preincubated 10 min at 37 °C in Locke's medium containing 300 μM isobutylmethylxanthine to inhibit the activity of phosphodiesterases which degrade cAMP. Forskolin (5 μM) was then added without or with the mGluR agonists, and the

incubation was continued for 10 min. After the incubation the medium was rapidly aspirated. The cAMP was extracted for 10 min with 0.1 M HCl, and measured by radioimmunoassay using a magnetic Amerlex RIA kit.

Determination of Calcium Influx. The activity of the compounds at ionotropic glutamate receptors was determined by measurements of $^{45}Ca^{2+}$ influx in cerebellar neurons. Cells cultured in 96-well plates were incubated for 15 min at room temperature in Locke's buffer containing 1 μCi/mL of $^{45}CaCl_2$ and the indicated additions. Agonist activity at all ionotropic glutamate receptors was measured in Mg^{2+}-free medium containing 1 μM glycine to allow for activity at NMDA receptors and 10 μM cyclothiazide to inhibit densensitization of AMPA receptors. Antagonist activity at NMDA receptors was measured in a Mg^{2+}-free medium in the presence of 100 μM NMDA, 1 μM glycine, and 10 μM NBQX (to inhibit non-NMDA receptors). Antagonism of kainate action was tested in the presence of 1 mM Mg^{2+}, 50 μM kainate, and 1 μM MK-801 (to inhibit NMDA receptors), while the effects on AMPA action were measured in the presence of 1 mM Mg^{2+}, 30 μM AMPA, 10 μM cyclothiazide, and 1 μM MK-801. Incubations were terminated by 3 washes with ice-cold buffer, and the cells were dissolved in 0.5 M NaOH for detemination of accumulated radioactivity and protein content.

The results obtained from these biological assays are provided in Figure 2 and Tables 1 and 2.

Table 1. Antagonist effects of the quisqualate analogs on group I and group II mGluRs.

mGluR Subtype	MetQuis EC_{50} (μM)	BnQuis EC_{50} (μM)
mGluR1a	~500	~300
mGluR5a	~1000	~300
mGluR4a	>1000	>1000
mGluR6	>1000	>1000

Table 2. Effects of the quisqualate analogs on ionotropic glutamate receptors in cerebellar neurons.[a]

Compound	Concentration	% of NMDA effect	% of KA effect	% of AMPA effect
MetQuis	100 μM	105 ± 5	71 ± 6	98 ± 8
MetQuis	1 mM	30 ± 3	75 ± 6	89 ± 5
BnQuis	100 μM	84 ± 6	97 ± 10	113 ± 5
BnQuis	1 mM	52 ± 3	105 ± 7	103 ± 3

[a]Activity at ionotropic glutamate receptors was measured as $^{45}Ca^{2+}$ influx in cerebellar granule cells as described above. All values represent means ± S.E.M. from six measurements performed on two separate preparations of neuronal cultures. All antagonist effects are expressed as per cent of net $^{45}Ca^{2+}$ influx induced by the agonist alone.

Discussion

Quisqualic acid is a very potent agonist of phospholipase C-coupled metabotropic glutamate receptors (mGluR1 and mGluR5). However, it fails to activate mGluR2, and it acts as a weak agonist of mGluR6.[2] As is apparent from the data disclosed herein, the introduction of a methyl or benzyl group into the α position of quisqualic acid has a dramatic effect upon mGluR activity. When these compounds were tested as agonists in CHO cell lines stably expressing particular mGluR subtypes, wherein receptor activation was determined by measurements of phosphoinositide hydrolysis for group I mGluRs and of forskolin-induced cAMP accumulation for group II and III mGluRs, neither compound was found to show agonist properties (data not shown). In contrast, both compounds showed antagonistic properties with a similar degree of selectivity at the

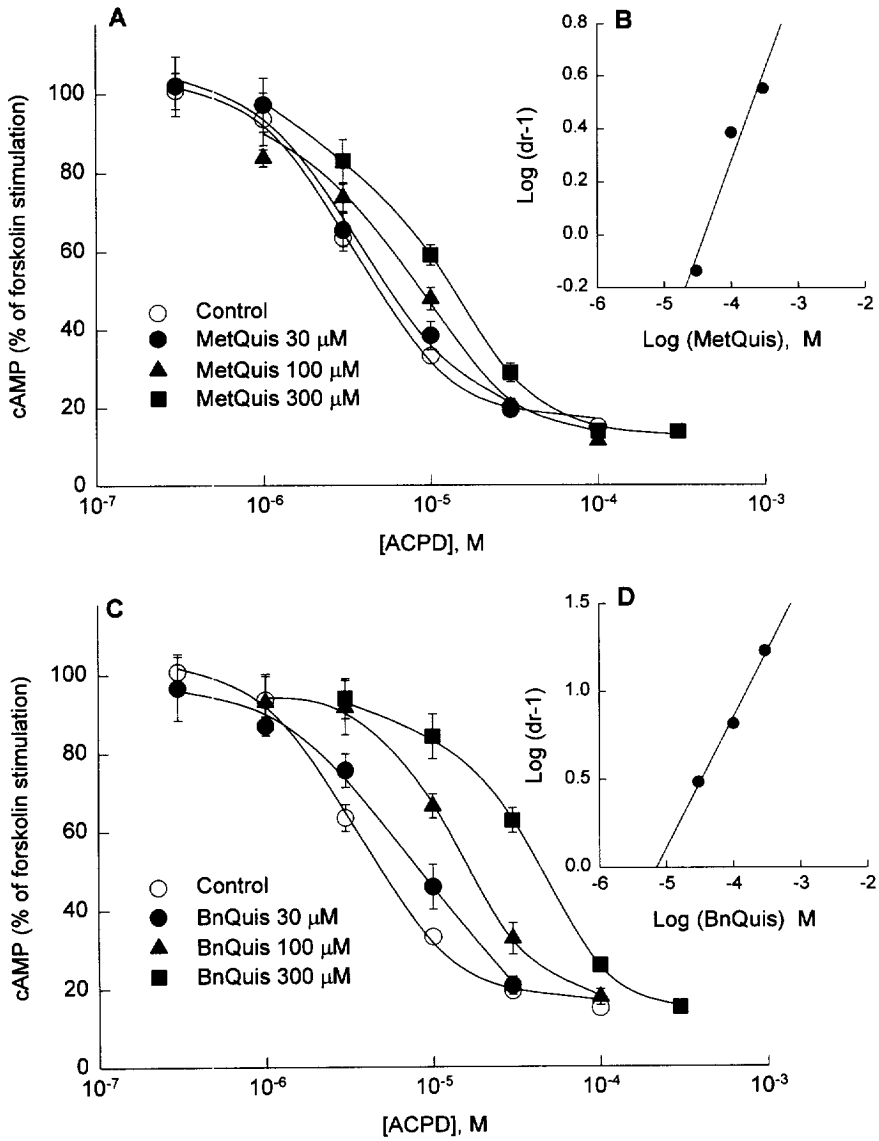


Figure 2. Inhibitory action of MetQuis and BnQuis on agonist-induced activation of mGluR2 receptors expressed in CHO cells. Receptor activity was determined by measurements of the ability of agonist to inhibit forskolin-induced cAMP accumulation. Inhibitory effects of MetQuis (A) and BnQuis (C) are shown as the right-shift of the ACPD dose-response curve. Insets show Schild's regressions which yield the following values: $pA_2 = 4.39$, $K_B = 40$ μM for MetQuis (B) and $pA_2 = 5.15$, $K_B = 7.1$ μM for BnQuis. Data points represent the mean ± S.E.M from three experiments performed in duplicate.

individual mGluR subtypes (Table 1 and Figure 2). α-Methylquisqualate (MetQuis) was a competitive antagonist of group II mGluRs as shown by its ability to inhibit the action of increasing concentrations of ACPD. The calculated K_B for MetQuis was 40 μM. It was less potent as an antagonist of mGluR5a and mGluR1a, and it had no activity at mGluR4a and mGluR6. Similarly, α-benzylquisqualate (BnQuis) antagonized mGluR2 receptors with an improved potency (K_B = 7.1 μM) over that of MetQuis, while it had little activity at group I receptors and no activity at mGluR4a and mGluR6. Both compounds were also tested for activity at ionotropic glutamate receptors and were found to partially antagonize NMDA receptors at high concentrations (1 mM) as shown in Table 2. Neither of the quisqualate analogs showed any activity at AMPA or kainate (KA) receptors.

In summary, one may conclude from this work that the introduction of either a methyl or benzyl group into the α-position of the parent quisqualate structure results in a remarkable change in its biological properties. This structural change leads to the loss of its potent agonist action at both non-NMDA ionotropic glutamate receptors as well as at group I mGluRs, while allowing it to acquire antagonist properties with relative selectivity for group II metabotropic glutamate receptors.[10]

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