

Synthesis and Metabotropic Receptor Activity of the Novel Rigidified Glutamate Analogues (+)- and (-)-*trans*-Azetidine-2,4-dicarboxylic Acid and Their *N*-Methyl Derivatives

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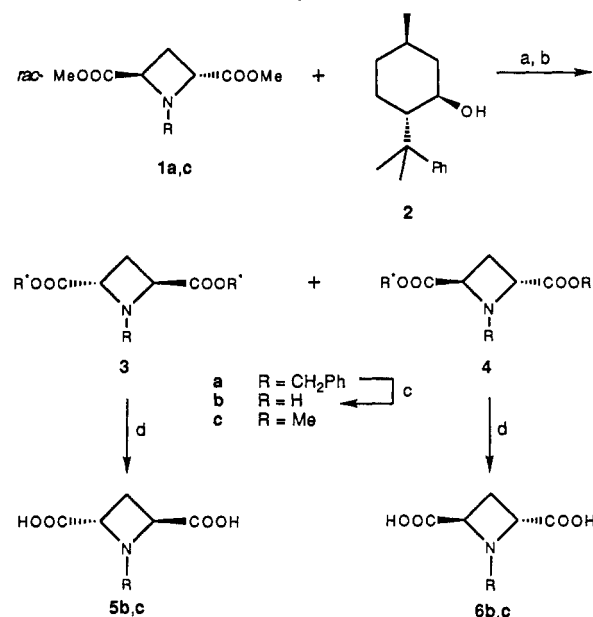
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The excitatory amino acid (EAA) receptor field constitutes currently one of the most intensively investigated areas of neuroscience. Much of the excitement in this area relates to the importance of structural modifications in glutamatergic synapses to processes underlying memory and learning.¹

Primary cultures of cerebellar granule cells express several subtypes of ionotropic and metabotropic glutamate receptors that have been extensively characterized in terms of associated signal transduction mechanisms.² Activation of the ionotropic *N*-methyl-D-aspartate (NMDA) receptors in these neurons increases the influx of extracellular Ca²⁺.³ Glutamate and NMDA, applied to primary cultures of cerebellar granule cells, cause a sustained increase of intracellular Ca²⁺ concentration followed by delayed cell death.⁴ This effect is mediated predominantly by the activation of NMDA-sensitive glutamate receptors. Cerebellar granule cells in culture also express metabotropic glutamate receptors (mGluRs) coupled to the hydrolysis of inositol phospholipids, which are activated by glutamate, quisqualate, and ibotenate.⁵ This response is also induced by the conformationally restricted analogue of glutamate, 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*t*-ACPD),⁶ the active enantiomer being (1*S*,3*R*)-ACPD.⁷ The relative efficacy of agonists, as well as their rank order of potency, show a large variation depending on the brain area and the age of animals, suggesting a heterogeneity of phosphatidylinositol (PI)-coupled mGluRs.⁸ While selective and potent ligands for the NMDA and AMPA receptors have been developed, ligands for the metabotropic glutamate receptors are few. Thus, it is essential to develop new agonists with greater selectivity for the different subtypes of mGluRs. In this regard it is worth noting that the 2-(carboxycyclopropyl)glycine (L-CCG-I) acts as a potent and selective agonist at mGluR2, a metabotropic receptor subtype linked to the inhibition of adenylate cyclase.⁹

We have shown previously that the *cis* isomer of azetidine-2,4-dicarboxylic acid (ADA) positively modulates the NMDA receptor at low (<50 μ M) concentrations and exhibits glutamate-like agonist activity at higher concentrations, whereas the *trans* isomer is inactive.¹⁰ Upon considering the structure of (1*S*,3*R*)-ACPD and carrying out overlay comparisons with the azetidines, we concluded that the *trans* diacid, in contrast to its *cis* isomer, might

Scheme I. Synthesis of the Optically Pure *trans*-Azetidine-2,4-dicarboxylic Acids^a



(R' = (-)-8-phenylmenthyl)

^a Reagents and conditions: (a) 2 (6 equiv) + 2 equiv (relative to 1) CH₃Li, THF, 0 °C, 25 min; add 1; 0 °C, 2 h; 72% (3/4a), 63% (3/4c); (b) silica gel chromatography, ethyl acetate/hexane (ratio 4:96, HPLC for 3/4a, ratio 1/9 for 3/4c); (c) 1 bar H₂, 20% Pd(OH)₂/C, ethyl acetate, room temp; 89% (3a), 98% (4a); (d) 4 equiv 5 M NaOH, THF/MeOH (1:1), room temp, 6–12 h, then add HCl (for 5,6b); Dowex 50W-X8 (acid form; used for 5,6c); 60% (5b), 72% (6b), 60% (5c), 55% (6c).

also show metabotropic receptor activity. For this reason, we prepared the optically pure *trans* diacids together with their *N*-methyl derivatives in order to test this hypothesis.

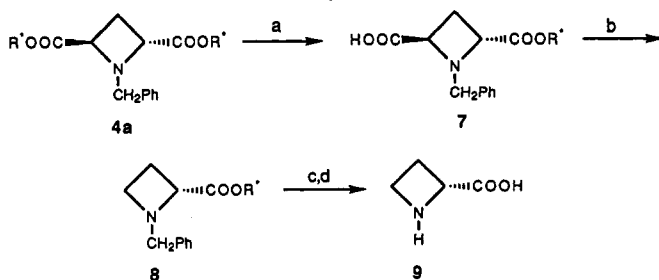
The synthesis of the racemic azetidine-2,4-dicarboxylic acid dimethyl esters 1a and 1b was carried out as described previously.¹⁰ The *N*-methyl derivative 1c was obtained from 1b by *N*-methylation (CH₃I, K₂CO₃, CH₃CN). Racemate resolution (Scheme I) was achieved by transesterification of the *N*-substituted dimethyl esters 1a,c with the lithium alkoxide derived from (-)-8-phenylmenthol¹¹ and chromatographic separation of the resulting diastereoisomers 3/4. No base-catalyzed *trans*-*cis* isomerization was observed under the conditions employed. Menthyl and 1-phenylethyl esters were similarly prepared in the *a* series but exhibited poorer separation. To obtain the amino acids unsubstituted at nitrogen, the *N*-benzyl groups were removed by hydrogenolysis. Both pairs of diastereoisomers 3/4b and 3/4c were then saponified with NaOH and purified by use of an ion-exchange resin to obtain the amino acids (5/6b were isolated as their hydrochlorides).¹² For the assignment of absolute configuration, compound 4a was degraded by the Barton decarboxylation method¹³ (Scheme II) to azetidine-2-carboxylic acid which showed the opposite sign of optical rotation from the commercially available¹⁴ *S* isomer. Accordingly, the stereochemistry of the (+)-isomer 6b is 2*R*,4*R*. The absolute configurations of the *N*-methyl diacids were established by the alternative synthesis of the precursor 3c from 3b by *N*-methylation (CH₃I, K₂CO₃, CH₃CN). All of the newly prepared compounds were characterized by IR and ¹H and ¹³C NMR together with mass spectral and/or elemental analysis.

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Scheme II. Assignment of Absolute Configuration of the *trans*-Azetidine-2,4-dicarboxylic Acids^a



(R' = (-)-8-phenylmenthyl)

^a Reagents and conditions: (a) 1 equiv 1 M NaOH, THF/MeOH 1:1, room temp.; 20 h; 36% + 50% of recovered 4a; (b) 1.15 equiv 1-hydroxypyridine-2-thione, 1.18 equiv DCC, CH₂Cl₂, room temp, dark, 1.5 h; filtration; 11 equiv *t*-BuSH, *hν* (incandescent lamp, 250 W), 30 min; 78%; (c) 1 bar H₂, 20% Pd(OH)₂/C, ethyl acetate, room temp; 90%; (d) 2 equiv 1 M NaOH, THF/MeOH 2:1, room temp, 11 h; 2 equiv 1 M HCl; Dowex 50W-X8 (H⁺ form), elution with aqueous NH₃; 79%.

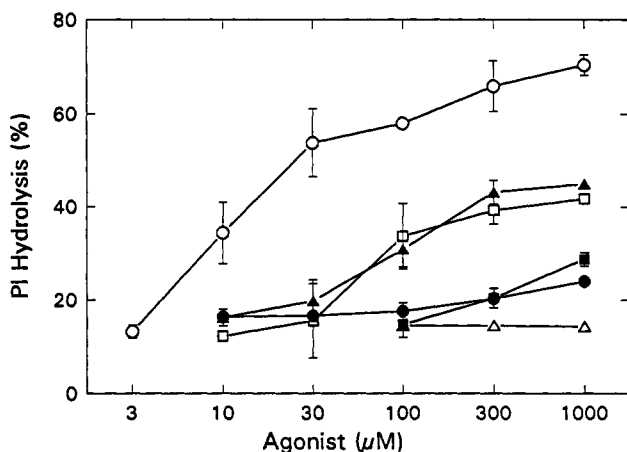


Figure 1. Effect of *trans*-ADA and analogues on PI hydrolysis in primary cultures of cerebellar granule cells. Cells were incubated for 20 min with the indicated concentrations of (±)-*t*-ACPD (○), (±)-ADA (5/6b) (□), (-)-ADA (5b) (△), (+)-ADA (6b) (■), (±)-*N*-methyl-ADA (5/6c) (●), and *trans*-2,5-PDA (△). PI hydrolysis is expressed as the percent of radioactivity present in the inositol phosphate fraction divided by the total incorporated radioactivity. Points are means from three to six experiments. Error bars represent SEM.

The biological properties of these rigidified glutamate analogues were evaluated through a variety of functional experiments using primary cultures of cerebellar granule cells prepared as described previously. Phosphoinositide hydrolysis was measured using the procedure of Berridge *et al.*¹⁵ As is apparent from Figure 1, among the azetidines tested, the (-)-*trans* diacid 5b is active, producing a dose-dependent increase in PI hydrolysis, with the (+)-isomer being virtually inactive. The *N*-methyl derivatives were inactive. We further note that the newly identified metabotropic receptor antagonist (*R,S*)-4-carboxy-3-hydroxyphenylglycine was found capable of blocking the PI stimulatory effects of 5b (data not shown).¹⁶ For comparison purposes, we also tested racemic *trans*-pyrrolidine-2,5-dicarboxylic acid (2,5-PDA) and found this five-membered ring counterpart of the azetidine diacid to be inactive. The azetidines were also tested for their ability to antagonize ACPD-stimulated PI hydrolysis. None of these compounds exhibited antagonist activity (data not shown).

Perhaps most exciting is the finding that, in embryonic

kidney 293 cells transfected with mGluR1 cDNA, ACPD was found to stimulate phosphoinositide hydrolysis, while 5b was found to be inactive. In this experimental model, the effects of these compounds were examined both in the presence and in the absence of extracellular calcium, for mGluR1 has also been shown to stimulate phosphoinositide hydrolysis by allowing calcium influx. Since among the six cloned mGluRs only mGluR1 and mGluR5 are clearly linked to phosphoinositide hydrolysis and ACPD functions as an agonist or partial agonist at both,¹⁷ the present findings would suggest that 5b may act selectively on mGluR5 or some other mGluR subtype, and therefore prove useful in differentiating the function of various mGluR subtypes.

In summary, the present work reveals the (-)-(*S,S*)-isomer of the simple amino acid *trans*-azetidine-2,4-dicarboxylic acid to be an activator of the metabotropic receptor. This result is particularly intriguing in view of the fact that its *cis* isomer possesses NMDA modulatory activity. Since 5b is more rigid than (1*S*,3*R*)-ACPD (which possesses two envelope extremes¹⁸) and embodies a conformationally fixed amino group, it provides important information as to the precise nature of the glutamate conformation that is essential to recognition by the metabotropic receptor. More importantly, this compound and its analogues should prove useful in sorting out the subtle structural differences inherent in the G-protein linked metabotropic receptor subtypes.¹⁷

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Supplementary Material Available: Spectroscopic data for all new compounds reported herein and a figure showing the results of PI hydrolysis in 293 cells transfected with mGluR1 cDNA (5 pages). Ordering information is given on any current masthead page.

References

- (1) Kozikowski, A. P.; Barrionuevo, G., Eds. *Neurobiology of the NMDA Receptor, from Chemistry to the Clinic*; VCH Publishers, Inc.: New York, 1991.
- (2) Costa, E.; Fadda, E.; Kozikowski, A. P.; Nicoletti, F.; Wroblewski, J. T. Classification and allosteric modulation of excitatory amino acid signal transduction in brain slices and primary cultures of cerebellar neurons. *Neurobiology of Amino Acids, Peptides and Tropic Factors*; Ferrendelli, J. A., Collins, R. C., Johnson, E. M., Eds.; Kluwer Academic: Boston, 1988, pp 35-50.
- (3) (a) Wroblewski, J. T.; Fadda, E.; Mazzetta, J.; Lazarewicz, J. W.; Costa, E. Glycine and D-serine act as positive modulators of signal transduction at *N*-methyl-D-aspartate-sensitive glutamate receptors in cultured cerebellar granule cells. *Neuropharmacol.* 1989, 28, 447-452. (b) Wroblewski, J. T.; Nicoletti, F.; Costa, E. Different coupling of excitatory amino acid receptors with Ca²⁺ channels in primary cultures of cerebellar granule cells. *Neuropharmacol.* 1985, 24, 919-921.
- (4) Manev, H.; Favaron, M.; Guidotti, A.; Costa, E. Delayed increase of Ca²⁺ influx elicited by glutamate: role in neuronal death. *Mol. Pharmacol.* 1989, 36, 106-112.
- (5) (a) Nicoletti, F.; Wroblewski, J. T.; Novelli, A.; Alho, H.; Guidotti, A.; Costa, E. The activation of inositol phospholipid metabolism as a signal transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. *J. Neurosci.* 1986, 6, 1905-1911. (b) Rauli, R.; Danyasz, W.; Wroblewski, J. T. Pretreatment of cerebellar granule cells with concanavalin A potentiates quisqualate-stimulated phosphoinositide hydrolysis. *J. Neurochem.* 1991, 56, 2116-2124.
- (6) (a) Manzoni, O.; Fagni, L.; Pin, J. P.; Rassendren, F.; Poulat, F.; Sladeczek, F.; Bockaert, J. (*trans*)-1-Amino-cyclopentyl-1,3-dicarboxylate stimulates quisqualate phosphoinositide-coupled receptors but not ionotropic glutamate receptors in striatal neurons and *Xenopus* oocytes. *Mol. Pharmacol.* 1990, 38, 1-6. (b) Palmer, E.; Monaghan, D. T.; Cotman, C. W. *Trans*-ACPD, a selective agonist

- of the phosphoinositide-coupled excitatory amino acid receptor. *Eur. J. Pharmacol.* **1989**, *166*, 585-587.
- (7) Irving, A. J.; Schofield, J. G.; Watkins, J. C.; Sunter, D. C.; Collingridge, G. L. 1S,3R-ACPD stimulates and L-AP3 blocks Ca^{2+} mobilization in rat cerebellar neurons. *Eur. J. Pharmacol.* **1990**, *186*, 363-365.
 - (8) Schoepp, D.; Bockaert, J.; Sladeczek, F. Pharmacological and functional characteristics of metabotropic excitatory amino acid receptors. *Trends Pharmacol. Sci.* **1990**, *11*, 508-515.
 - (9) Hayashi, Y.; Tanabe, Y.; Aramori, I.; Masu, M.; Shimamoto, K.; Ohfun, Y.; Nakanishi, S. Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.* **1992**, *107*, 539-543.
 - (10) Kozikowski, A. P.; Tückmantel, W.; Reynolds, I. J.; Wroblewski, J. T. Synthesis and bioactivity of a new class of rigid glutamate analogues: modulators of the N-methyl-D-aspartate receptor. *J. Med. Chem.* **1990**, *33*, 1561-1571.
 - (11) The required excess of this expensive chiral auxiliary is readily recovered by chromatography and distillation.
 - (12) Selected physical data follow. **5/6b-HCl**: ^1H NMR (D_2O) δ 4.83 (t, 2 H, $J = 8.5$ Hz), 2.88 (t, 2 H, $J = 8.5$ Hz). **5b-HCl**: $[\alpha]_{\text{D}} -180^\circ$ (c 2.7 g/L, H_2O). **6b-HCl**: $[\alpha]_{\text{D}} +173^\circ$ (c 1.65 g/L, H_2O). **5c**: mp 216-217 $^\circ\text{C}$; ^1H NMR (D_2O) δ 4.60 (t, 2 H, $J = 8$ Hz) 2.81 (s, 3 H), 2.69 (t, 2H, $J = 8$ Hz). Anal. Calcd for $\text{C}_8\text{H}_9\text{NO}_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.01; H, 5.61; N, 8.66. $[\alpha]_{\text{D}} -122^\circ$ (c 4.0 g/L, H_2O). **6c**: ^1H NMR (D_2O) δ 4.78 (t, 2 H, $J = 8.5$ Hz), 2.81 (s, 3 H), 2.76 (t, 2 H, $J = 8.5$ Hz); ^{13}C NMR (D_2O) δ 173.2, 66.8, 39.6, 27.5; $[\alpha]_{\text{D}} +127^\circ$ (c 4.0 g/L, H_2O).
 - (13) (a) Barton, D. H. R.; Hervé, Y.; Potier, P.; Thierry, J. Reductive radical decarboxylation of amino-acids and peptides. *J. Chem. Soc., Chem. Commun.* **1984**, 1298; (b) Barton, D. H. R.; Crich, D.; Motherwell, W. B. New and improved methods for radical decarboxylation of acids. *ibid.* **1983**, 939.
 - (14) Aldrich Chemical Co.
 - (15) Berridge, M. J.; Downes, C. P.; Hanley, M. R. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **1982**, *206*, 587-597.
 - (16) Jones, P. L. St. J.; Porter, R. H. P.; Birse, E. F.; Pook, P. C.-K.; Sunter, D. C.; Udvarhelyi, P. M.; Wharton, B.; Roberts, P. J.; Watkins, J. C. Characterization of a new excitatory amino acid (EAA) receptor type activated by (1S,3R)-ACPD and selectively blocked by (S)-4C3H-PG. *Br. J. Pharmacol.* **1992**, *106*, 47P.
 - (17) Schoepp, D. D.; Conn, P. J. Metabotropic glutamate receptors in brain function and pathology. *Trends Pharmacol. Sci.* **1993**, *14*, 13-20.
 - (18) Bridges, R.; Chamberlin, R. Conformationally constrained acidic amino acids as probes of glutamate receptors and transporters. *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven Press: New York, 1993.