Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1985–1990

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST ACTIVITY OF N^1-SUBSTITUTED ANALOGS OF 2*R*,4*R*-4-AMINOPYRROLIDINE-2,4-DICARBOXYLIC ACID

Matthew J. Valli,* Darryle D. Schoepp,[a] Rebecca A. Wright,[a] Bryan G. Johnson,[a] Ann E. Kingston,[a,b] Rosemarie Tomlinson,[a,b] and James A. Monn

Discovery Chemistry Research and [a]Neuroscience Discovery Research, Eli Lilly and Company, Indianapolis, Indiana, 46285, U.S.A.

[b]Lilly Research Centre, Ltd., Windelsham, Surrey GU20 6PH, U.K.

Received 22 April 1998; accepted 19 June 1998

Abstract: A series of N^1-substituted derivatives of (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC) has been prepared as constrained analogs of γ-substituted glutamic acids and examined for their effects at recombinant metabotropic glutamate receptor (mGluR) subtypes in vitro. Appropriate substitution of the N^1 position of 2*R*,4*R*-APDC resulted in the identification of a number of selective group II mGluR antagonists.

Introduction

L-Glutamic acid (L-Glu, Figure 1) is widely recognized as the primary excitatory neurotransmitter in the mammalian central nervous system (CNS).[1] L-Glu exerts its effects through activation of two classes of neuronal and glial cell surface receptors, the ion channel linked, or ionotropic glutamate receptors (iGluRs) and the G-protein-linked, or metabotropic glutamate receptors (mGluRs).[2–4] Molecular cloning of the metabotropic glutamate receptors has revealed eight individual receptor proteins ($mGluR_{1-8}$) that have been classified into three groups (I–III) based on primary amino acid sequence homology, agonist pharmacology and signal transduction mechanisms.[5–7] The group I mGluR subfamily consists of $mGluR_1$ and $mGluR_5$, while group II consists of $mGluR_2$ and $mGluR_3$, and group III of $mGluR_4$, $mGluR_6$, $mGluR_7$, and $mGluR_8$. A number of splice variants of these proteins have also been identified.

Our laboratory has been interested in the preparation of mGluR group selective and subtype selective agonists and antagonists that can be used as pharmacological tools for understanding the functioning of these receptors under physiological and pathophysiological conditions. To this end, we have previously reported the design, synthesis, and pharmacological identification of two potent and group II-selective mGlu receptor agonists, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (**1**, Figure 1)[8,9] and 1*S*,2*S*,5*R*,6*S*-2-aminobicyclo-[3.1.0]hexane-2,6-dicarboxylic acid (LY354740, **2**, Figure 1).[10,11]

0960-894X/98/$ - see front matter © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(98)00352-7

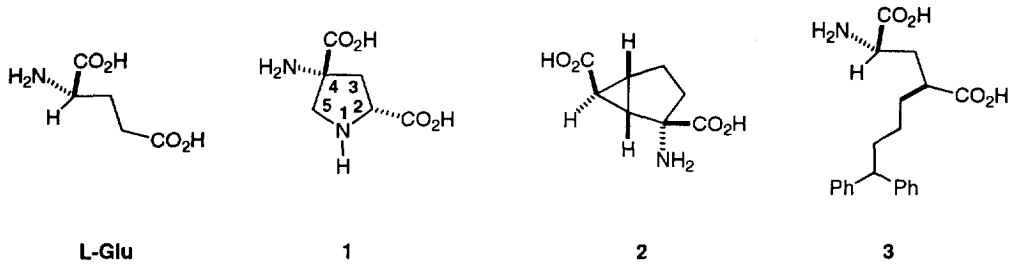
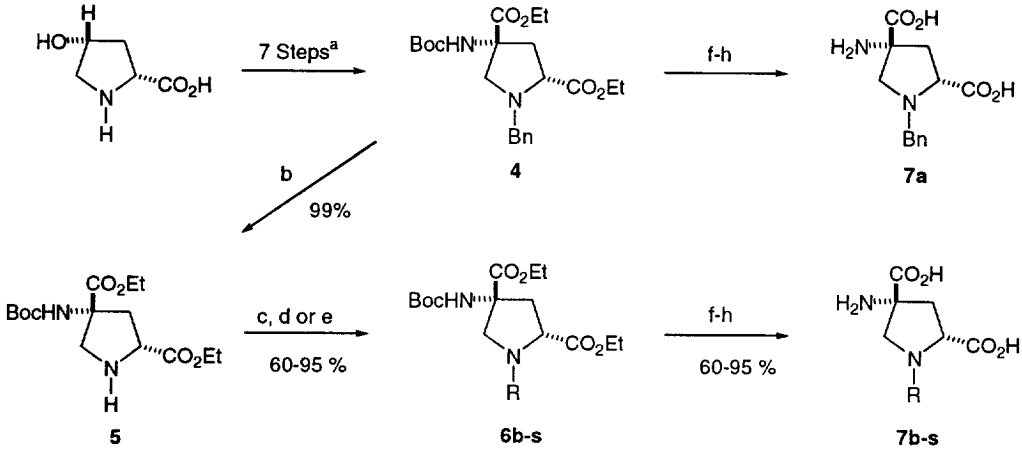


Figure 1. Chemical structures of L-glutamic acid and structural derivatives that act selectively at group II metabotropic glutamate receptors

During the course of our studies related to these agonists, others in our laboratories demonstrated that incorporation of lipophilic, aromatic containing substituents at the γ-position of L-Glu (as in 2*S*,4*S*-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic acid, **3**, Figure 1)[12] afforded group II mGluR selective antagonists. We hypothesized that when interacting at group II mGluRs, the γ-substituent of **3** might occupy a region of space that could be accessed from the pyrrolidine scaffold of **1** by attachment of the appropriate substituent to the ring (N^1) nitrogen atom. If so, then N^1-substitution of **1** might convert this selective group II mGluR agonist into a series of selective group II mGluR antagonists.[13,14] In this account, we report the preparation of compounds of type **7** and the activity of these analogs at recombinant groups I, II, and III mGlu receptors in vitro.

Chemistry

The synthesis of compounds **7a–s** is depicted in Scheme 1. The requisite 2*R*,4*R*-N^4-(Boc-protected)-N^1-benzylpyrrolidine-2,4-dicarboxylate intermediate **4** was prepared in stereochemically controlled fashion from *cis*-4-hydroxy-D-proline in seven steps by our previously described procedure.[9] Hydrogenolysis of the N^1-benzyl functionality afforded **5**, a highly versatile intermediate for the preparation of a wide variety of N^1-substituted derivatives. Alkylations of the pyrrolidine ring (N^1) nitrogen atom with MeI, EtI, (substituted) benzyl chlorides, bromodiphenylmethane, or diphenylethyl iodide under standard conditions provided intermediates **6b–j** and **6l–p**, while reductive aminations employing diphenylacetaldehyde, cyclohexane carboxaldehyde, phenylacetaldehyde, or phenylpropionaldehyde afforded intermediates **6k** and **6q–s**. Both processes proceeded smoothly and in high overall yield. The final amino acid products (**7a–s**) were then obtained by sequential hydrolysis of the Boc and ester protecting groups followed by ion-exchange chromatography. The 2*S*,4*S* enantiomers (***ent*-7a** and ***ent*-7o**) were prepared in an identical fashion from the corresponding 2*S*,4*S*-N^4-(Boc-protected) pyrrolidine diester intermediate ***ent*-5**, itself obtained from L-*trans*-4-hydroxyproline.[9] All final products and synthetic intermediates exhibited satisfactory spectral and analytical (C, H, N) characteristics.



Cmpnd 6 or 7	R	Cmpnd 6 or 7	R
a	benzyl	j	diphenylmethyl
b	methyl	k	2,2-diphenylethyl
c	ethyl	l	3,3-diphenylpropyl
d	2-chlorobenzyl	m	1-naphthylmethyl
e	3-chlorobenzyl	n	2-naphthylmethyl
f	4-chlorobenzyl	o	2-phenylbenzyl
g	3,4-dichlorobenzyl	p	4-phenylbenzyl
h	2,6-dichlorobenzyl	q	cyclohexylmethyl
i	2,4-dichlorobenzyl	r	2-phenylethyl
		s	3-phenylpropyl

Scheme 1 Synthesis of N^1-substituted derivatives of 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylic acid (**1**).

Reagents and conditions. (a) see ref. 9; (b) H_2, 5% Pd-C, EtOH, rt; (c) RI, DMF, K_2CO_3, rt (examples b, c); (d) RBr, *i*Pr_2NEt, CH_2Cl_2, rt (examples j, n, o) or RCl, *i*PrNEt_2, Bu_4NI, CH_2Cl_2, rt (examples d–i, m, p) or RI (example l); (e) RCHO, H_2, 5% Pd-C, EtOH, rt (examples k, q–s); (f) HCl (g), Et_2O; (g) 1 N NaOH, THF, rt; (h) ion exchange chromatography.

Biochemical Evaluation

Analogs **7a–s**, ***ent*-7a**, and ***ent*-7o** were examined for their ability to influence the activity of recombinant human mGluRs expressed in RGT cells.[10,15,16] For group I mGlu receptors ($mGluR_{1a}$ and $mGluR_{5a}$), compounds were tested both for their ability to stimulate polyphosphoinositide (PI) hydrolysis (agonist activity) and to reverse PI hydrolysis (antagonist activity) induced by quisqualic acid as previously described.[15] For group II and group III mGlu receptors ($mGluR_2$, $mGluR_3$, $mGluR_4$ and $mGluR_8$), compounds were tested both for their ability to inhibit forskolin-stimulated cyclic adenosine 3',5'-mono phosphate (c-AMP) production (agonist activity) and to reverse 1*S*,3*R*-ACPD-inhibited, forskolin-stimulated c-AMP production (antagonist activity).[10,16] As none of the N^1-substituted analogs that were evaluated demonstrated agonist effects (up to a concentration of 300 μM) at any of the mGlu receptor subtypes examined, only

antagonist data are presented (Table 1). In addition to the compounds from the present study, two other structurally diverse mGluR antagonists, γ-substituted glutamate derivative **3** and (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG, Tocris)[15,17] are included in Table 1 for comparative purposes.

Table 1 Antagonist activity of N^1-substituted derivatives of **1** at recombinant human mGlu receptor subtypes expressed in RGT cells.

	IC_{50} (μM)					
No.	**$mGluR_{1a}$**[a]	**$mGluR_{5a}$**[b]	**$mGluR_2$**[c]	**$mGluR_3$**[d]	**$mGluR_4$**[e]	**$mGluR_8$**[f]
7a	> 300	> 300	121 ± 25	38%[g]	> 300	> 300
7b	> 300	> 300	> 300	NT	> 300	> 300
7c	> 300	> 300	> 300	NT	> 300	> 300
7d	> 300	> 300	75 ± 17.6	> 300	> 300	> 300
7e	> 300	> 300	> 300	> 300	> 300	> 300
7f	> 300	> 300	> 300	> 300	NT	NT
7g	> 300	> 300	99 ± 26	NT	NT	NT
7h	238.5 ± 31.5	> 300	42.0 ± 11.3	40.3 ± 4.7	> 300	> 300
7i	> 300	> 300	> 300	NT	NT	NT
7j	> 300	> 300	> 300	> 300	NT	NT
7k	> 300	> 300	37.3 ± 5.7	63.6 ± 14.5	> 300	> 300
7l	> 300	> 300	> 300	NT	> 300	NT
7m	> 300	> 300	20.0 ± 2.4	8.58 ± 1.64	> 300	> 300
7n	133[h]	> 300	50.0 ± 7.4	> 300	> 300	> 300
7o	> 300	> 300	36.0 ± 6.9	16.1 ± 0.52	> 300	> 300
7p	> 300	> 300	> 300	> 300	NT	NT
7q	> 300	> 300	46.2 ± 4.4	> 300	NT	NT
7r	> 300	> 300	61 ± 11.6	166 ± 20.7	> 300	NT
7s	> 300	> 300	> 300	NT	NT	NT
***ent*-7a**	> 300	> 300	> 300	> 300	> 300	NT
***ent*-7o**	> 300	> 300	> 300	> 300	> 300	NT
3	> 300	> 300	50 ± 19	30 ± 10	> 300	> 300
(+) MCPG	57 ± 20	195 ± 21	339 ± 83	NT	> 1000	> 1000

[a]Quisqualic acid (0.3 μM) employed as the agonist challenge.[15] [b]Quisqualic acid (0.2 μM) employed as the agonist challenge.[15] [c]1*S*,3*R*-ACPD (3 μM) employed as the agonist challenge.[16] [d]1*S*,3*R*-ACPD (30 μM) employed as the agonist challenge.[16] [e]L-AP4 (3 μM) employed as the agonist challenge.[16] [f]L-AP4 (0.3 μM) employed as the agonist challenge.[16] [g] % reversal of ACPD at 100 μM. [h]n = 2. NT: not tested.

Results and Discussion

As part of our ongoing efforts to prepare structurally novel subtype selective agonists and antagonists for metabotropic glutamate receptors, we have designed and synthesized N^1-substituted derivatives of the selective group II mGluR agonist **1**. These amino acids may also be viewed as ring constrained analogs of γ-substituted glutamic acids (e.g., **3**), a novel class of group II mGluR antagonists. N^1-substitution of **1** with a methyl (**7b**) or ethyl (**7c**) group resulted in derivatives devoid of mGluR activity, while bulkier cyclohexylmethyl substitution (**7q**) gave rise to selective antagonist effects at $mGluR_2$ (IC_{50} = 46 μM). Similarly, N^1-benzyl substitution as in **7a** yielded a weak $mGluR_2$ antagonist (IC_{50} = 121 μM)[14] with limited antagonist activity at $mGluR_3$ (38% reversal of ACPD at 100 μM). Extension of the alkyl chain length between the pyrrolidine and phenyl ring systems present in **7a** afforded the 2-phenylethyl derivative **7r**, a compound possessing mixed antagonist activity in $mGluR_2$ and $mGluR_3$ expressing cells ($mGluR_2$ IC_{50} = 61 μM; $mGluR_3$ IC_{50} = 166 μM), while further extension to the 3-phenylpropyl analog **7s** resulted in a complete loss of activity (up to 300 μM) at all mGlu receptor subtypes examined. Addition of a phenyl ring at the chain terminus of **7a**, **7r** and **7s** resulted in a modest improvement in antagonist activity for the 2,2-diphenylethyl analog **7k** ($mGluR_2$ IC_{50} = 37 μM; $mGluR_3$ IC_{50} = 64 μM) compared to its monoaromatic counterpart **7r**, but the other diphenylalkyl derivatives, **7j** and **7l**, were inactive at the highest concentration tested (300 μM). Substitution of the phenyl ring in **7a** with chlorine atoms resulted in compounds **7d–i**. For $mGluR_2$ antagonist activity, chloro-substitution was found to be tolerated only for *ortho*-substituted analogs **7d** ($mGluR_2$ IC_{50} = 75 μM) and **7h** ($mGluR_2$ IC_{50} = 42 μM; $mGluR_3$ IC_{50} = 40 μM). Chloro-substitution at the *meta*- and/or *para*-positions (or phenyl-substitution at the *para*-position) of **7a** resulted in the total loss of group II mGluR activity, suggesting a region of steric intolerance about these positions of the aromatic ring when interacting with the ligand recognition site of $mGlu_2$ and $mGlu_3$ receptor subtypes. Interestingly, **7h** was also found to exhibit weak, yet significant antagonist activity at $mGluR_{1a}$ (IC_{50} = 239 μM), differentiating it from the majority of analogs within this series. To further probe the available steric bulk tolerance adjacent to the *ortho*-position of **7a**, biphenyl derivative **7o** was prepared. As with the *o*-chloro derivative **7d**, **7o** exhibited potent antagonist effects at $mGlu_2$ receptors (IC_{50} = 36 μM), but also reversed ACPD-mediated agonist effects in the $mGluR_3$ expressing cell line at similar concentrations (IC_{50} = 16 μM). The antagonist effect of **7o** was shown to be stereospecific, as ***ent*-7o** was inactive up to the highest concentration tested (300 μM). In support of an hypothesis that a region of steric tolerance exists adjacent to the *ortho*-position of **7a** at the ligand recognition site of the group II mGluRs (perhaps especially for $mGluR_3$), 1-naphthyl derivative **7m** displayed good antagonist activity at both $mGluR_2$ and $mGluR_3$ (IC_{50} = 20 μM and 9 μM, respectively), while isomeric 2-naphthyl derivative **7n** was found to elicit $mGluR_2$ antagonist effects (IC_{50} = 50 μM) but was inactive up to 300 μM at $mGluR_3$. The observed antagonist activity of **7n** at $mGluR_{1a}$ (IC_{50} = 133 μM) was unanticipated based on the preponderance of SAR data from this largely group II mGluR-active series, and may reflect a highly specific recognition element at the ligand binding domain of $mGluR_{1a}$ that prefers this particular substituent type. In conclusion, we have found that appropriate substitution at the N^1-position of the selective group II mGluR agonist **1** affords selective group II mGluR antagonists possessing micromolar potencies at recombinant $mGlu_2$/$mGlu_3$ receptor subtypes. Members from this series (e.g., **7h**, **7m**, and **7o**) appear to possess similar receptor subtype selectivities and potencies as that observed for γ-substituted glutamate **3**, and may offer advantages to the widely utilized phenylglycine antagonist (+)-MCPG when studying group II mGluR function in vitro or in vivo.

Acknowledgment

The authors acknowledge the technical assistance of Mr. Jack Campbell in performing catalytic hydrogenations that provided reductive amination products **6k** and **6q–s**.

References and Notes

1. Collinridge, G. L.; Lester, R. A. *Pharmacol. Rev.* **1989**, *40*, 143.
2. Nakanishi, S. *Science* **1992**, *258*, 597.
3. Nakanishi, S.; Masu, M. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 319.
4. Hollmann M.; Heinemann S. *Annu. Rev. Neurosci.* **1994**, *17*, 31.
5. Schoepp, D. D.; Conn, P. J. *Trends in Pharmacol. Sci.* **1993**, *14*, 13.
6. Pin, J.-P.; Duvoisin, R. *Neuropharmacol.* **1995**, *34*, 1.
7. Conn, P. J.; Pin, J.-P. *Ann. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
8. Schoepp, D. D.; Johnson, B. G.; Salhoff, C. R.; Valli, M. J.; Desai, M. A.; Burnett, J. P.; Mayne, N. G.; Monn, J. A. *Neuropharmacol.* **1995**, *34*, 843.
9. Monn, J. A.; Valli, M. J.; Johnson, B. G.; Salhoff, C. R.; Wright, R. A.; Howe, T.; Bond, A.; Lodge, D.; Griffey, K; Tizzano, J. P.; Schoepp, D. D. *J. Med. Chem.*, **1996**, *39*, 2990.
10. Schoepp, D. D.; Johnson, B. G.; Wright, R. A.; Salhoff, C. R.; Mayne, N. G.; Wu, S.; Cockerham, S. L.; Burnett, J. P.; Belagaje, R.; Bleakman, D.; Monn, J. A. *Neuropharmacol.* **1997**, *36*, 1.
11. Monn, J. A.; Valli, M. J.; Massey, S. M.; Wright, R. A.; Salhoff, C. R.; Johnson, B. G.; Howe, T.; Alt, C. A.; Rhodes, G. A.; Robey, R. L.; Griffey, K. R.; Tizzano, J. P.; Kallman, M. J.; Helton, D. R.; Schoepp, D. D. *J. Med. Chem.* **1997**, *40*, 528.
12. Wermuth, C. G.; Mann, A.; Schoenfelder, A.; Wright, R. A.; Johnson, B. G.; Burnett, J. P.; Mayne, N. G.; Schoepp, D. D. *J. Med. Chem.* **1996**, *39*, 814.
13. Aspects of this work have been previously presented: Valli, M. J., Campbell, J. B., Johnson, B. G., Salhoff, C. R., Wright, R. A., Griffey, K. I., Tizzano, J. P., Bond, A., Lodge, D., Schoepp, D. D. and Monn, J. A. 112th Meeting of the American Chemical Society, August 25-29, 1996, Orlando, FL. Abstract MEDI 0077.
14. 2*R*,4*R*-1-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate has recently been reported to possess agonist activity at rat $mGlu_6$ receptors (EC_{50} = 20 μM) and antagonist activity at $mGlu_2$ (IC_{50} = 200 μM) and $mGlu_5$ (IC_{50} = 600 μM) receptors, with no agonist or antagonist activity observed at $mGlu_1$ receptors up to 1000 μM: Tuckmantel, W.; Kozikowski, A. P.; Wang, S.; Pshenichkin, S.; Wroblewski, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 601.
15. Kingston, A. E.; Burnett, J. P.; Mayne, N. G.; Lodge, D. *Neuropharmacology* **1995**, *34*, 887-894.
16. Wu, S.; Wright, R. A.; Rockey, P. K.; Burgett, S. G.; Arnold, J. S.; Rosteck, P. R. Jr.; Johnson, B. G.; Schoepp, D. D.; Belagaje, R. M. *Mol. Brain Res.* **1998**, *53*, 85.
17. Hayashi, Y.; Sekiyama, N.; Nakanishi, S.; Jane, D. E.; Sunter, D. C.; Birse, E. F.; Udvarhelyi, P. M.; Watkins, J. C. *J. Neurosci.* **1994**, *14*, 3370.