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GluK1 antagonists from 6-(carboxy)phenyl decahydroisoquinoline derivatives. SAR and evaluation of a prodrug strategy for oral efficacy in pain models

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ABSTRACT

The synthesis and structure–activity relationship of decahydroisoquinoline derivatives with various benzoic acid substitutions as GluK1 antagonists are described. Potent and selective antagonists were selected for a tailored prodrug approach in order to facilitate the evaluation of the new compounds in pain models after oral administration. Several diester prodrugs allowed for acceptable amino acid exposure and moderate efficacy in vivo.

Kainate receptors form ligand-gated ion channels which are activated by the primary excitatory neurotransmitter, glutamate.[1] Receptors containing the GluK1 subunit (previously known as GluR5)[2] have been implicated in pain transmission.[3] Excessive stimulation of these receptors can lead to abnormal pain processing, including allodynia and hyperalgesia, and therefore GluK1 receptor antagonists may be useful as analgesic agents.[4] Clinical studies by Sang et al.[5] have reported that iv administration of the non-selective kainate antagonist decahydroisoquinoline amino acid **1** (LY293558, Chart 1),[6] significantly reduced pain intensity and unpleasantness. Simmons et al. showed that **2** (LY382884, Chart 1),[7] a selective GluK1 receptor antagonist, was active in a rat model of persistent pain via intraperitoneal (ip) administration and in a model of peripheral neuropathy in monkeys after administration into the spinal cord.[8] Filla et al. demonstrated oral activity in animal models of migraine pain with the diethyl ester prodrug of a selective GluK1 receptor antagonist.[9]

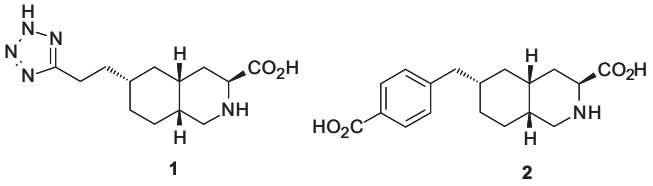

Chart 1. GluK1 receptor antagonists with anagelsic efficacy in vivo.

We sought to examine modifications of **2** (LY382884) in an attempt to discover potent and selective GluK1 antagonists with activity in preclinical models of persistent pain.[10]

Affinity in GluK1 and in a representative of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) family of ionotropic receptors (GluA2) were evaluated in ligand binding screens.[11] Potent and selective GluK1 antagonists were then transformed into diethyl ester prodrugs and evaluated in vivo for oral bioavailability and activity in established models of persistent pain.[12]

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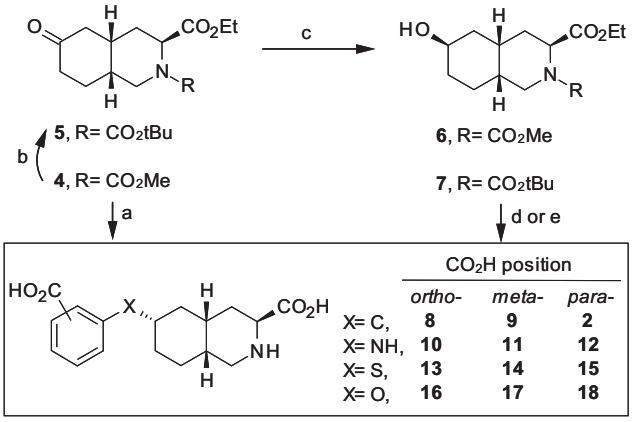
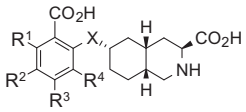


Scheme 1. Reagents and conditions: (a) (X = NH) (i) **4**, $ArNH_2$, $NaBH(OAc)_3$, rt (20–90%), (ii) 6 N HCl, 100 °C (50–100%); (b) (i) TMSI, rt, (ii) Boc_2O, Et_3N, rt (92%, two steps); (c) $CeCl_3$, $NaBH_4$, EtOH, −78 °C to rt (60%); (d) (X = S) (i) **6**, MsCl, Et_3N, rt (quant.), (ii) ArSH, K_2CO_3, acetone (40–70%), (iii) 6 N HCl, 100 °C (70–90%); (e) (X = O) (i) **7**, ArOH, DEAD, Ph_3P (60–75%), (ii) LiOH (80–100%), (iii) HCl, EtOAc, rt (90–100%). Ar: ethyl benzoate.

We first evaluated several heteroatom replacements of the linker atom between the decahydroisoquinoline nucleus and the aromatic acid domain, and prepared all three possible regio-isomeric benzoic acids for each linker atom. Compounds **8**–**18** were prepared from the known decahydroisoquinoline ketone **4** (Scheme 1).[13] The carbon-linked derivatives (**2**, **8**, **9**) were previously obtained via Wittig–Horner reaction and hydrogenation, followed by hydrolysis.[7] The nitrogen-linked compounds (**10**–**12**) required reductive aminations between appropriate anilines and the ketone **4** followed by acidic hydrolysis. Two key intermediates for the preparation of sulfur- and oxygen-linked derivatives were the known (6R)-alcohols **6** and **7**.[14] Successful reduction of ketones **4** or **5** was achieved with sodium borohydride in the presence of an equimolar quantity of cerium trichloride heptahydrate at low temperature. Alcohol **6** was previously obtained as the minor C-6 epimer by reduction with sodium borohydride in the absence of cerium.[14a] The nucleophilic substitution of the mesylate from alcohol **6** with thiophenols and the deprotection by acid hydrolysis allowed for the preparation of the sulfur-linked derivatives **13**–**15**. The oxygen-linked compounds (**16**–**18**) were synthesized by Mitsunobu reaction between appropriate phenols and alcohol **7** followed by mild ester hydrolysis and amine deprotection.[15]

Table 2
GluK1 in vitro affinity of phenyl-substituted acidic amino acids



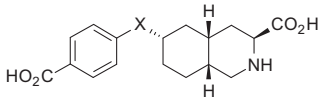
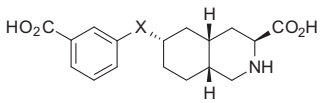
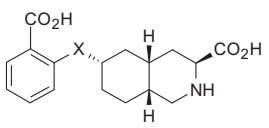
Compd[a]	X	R^1	R^2	R^3	R^4	GluK1 K_i (μM)[b]	GluA2 K_i (μM)[b]
19	O	F	H	H	H	9.7	93
20	O	H	Cl	H	H	1.5	19
21	S	H	Cl	H	H	3.7	42
22*	NH	H	Cl	H	H	3.6	3.3
23	O	H	Me	H	H	9.9	107
24	O	H	OMe	H	H	19	87
25	O	H	Ph	H	H	11	172
26	O	H	H	Cl	H	2.1	151
27	S	H	H	Cl	H	1.3	22
28	O	H	H	Me	H	5.9	89
29	O	H	H	OMe	H	6.2	111
30	O	H	H	Ph	H	0.5	8.8
31	O	H	H	H	Cl	316	1470
32	O	H	H	H	Me	228	818

[a] All compounds as hydrochloride salts except those with *.
[b] Values are means of at least two experiments.

The data in Table 1 summarizes the in vitro binding affinities for GluK1 and GluA2 of the reference compound **2** and of the amino acids **8**–**18**. The replacement of the carbon linker in **2** (LY382884) by a heteroatom, as in **12**, **15** or **18**, reduced the affinity for the GluK1. No improvement in the in vitro profile was observed with *meta*-benzoic acids derivatives (**9**, **11**, **14**, and **17**), regardless of the linker atom; on the contrary, affinity for GluA2 was significantly increased and compound **14** was more potent in GluA2 than in GluK1. Surprisingly, while **8** was the least potent GluK1 antagonist in the carbon-linked series, *ortho*-benzoic acids linked by a heteroatom showed an increased binding for GluK1 while retaining a moderate selectivity against GluA2.

Next, we evaluated derivatives of these potent *ortho*-benzoic acids (**10**, **13**, **16**, Table 1) with substitution in the phenyl ring as a straightforward entry to a broader set of GluK1 antagonists. The application of the same synthetic strategy from Scheme 1 with substituted anthranilates, thiosalicylates[16] and salicylates allowed for the preparation of compounds **19**-**32** (Table 2).[17]

Table 1
GluK1 and GluA2 in vitro affinity of amino acids derivatives

General structure	Compd[a]	X	GluK1 Ki[b]	GluA2 Ki[b]
	**2**	C	3.6	553
	12*	NH	528	1420
	15*	S	19	418
	18	O	267	913
	**9**	C	16	149
	11*	NH	8	238
	14*	S	46	9
	17	O	6	44
	**8**	C	67	29600
	10*	NH	0.9	77
	13*	S	0.8	15
	16	O	2.3	70

[a] All compounds as hydrochloride salts except those with *.
[b] Values (μM) are means of at least two experiments.

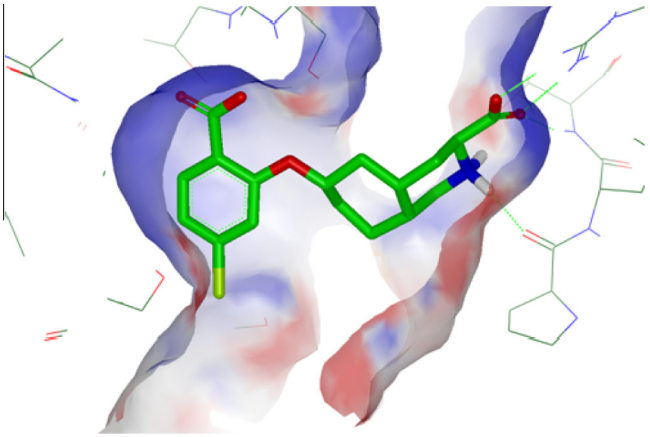


Figure 1. Docked model of binding of antagonist **26** based on the X-ray structure 2F35 (Glide 5.6 SP docking). Figure made using VIDA.[19]

Table 3
Rat PK data of GluK1 antagonist amino acids[a]

Compd[b]	$AUC_{0\ to\ \infty}$ (ng h/ml)	C_{max} (ng/ml)	T_{max} (h)	%F
2[c]	1506	539	1	62.5
13	7531	2981	0.5	38.8
16	24743	9205	0.5	75.3
26	9193	6449	0.5	32.9
30	7226	2695	1	22.2

[a] Pharmacokinetics (PK) parameters of amino acids in Fischer rats after 30 mg/kg oral dose. Three rats per time point were used. $AUC_{0\ to\ \infty}$: area under the amino acid plasma concentration curve. C_{max}: maximum observed amino acid plasma concentration. T_{max}: time at C_{max}. %F: the bioavailability of the amino acid following its oral administration as diethyl ester relative to the amino acid administered by the intravenous (iv) route.
[b] Amino acid dosed p.o. as diethyl ester hydrochloride salt.
[c] Data from a 10 mg/kg oral dose.

Table 4
In vivo effect of diethyl esters of GluK1 antagonists amino acids[a]

Compd[b]	Carr. Therm. Hyperalg. MED[c] (mg/kg)	Formalin% Reversal @ 30 mg/kg p.o	Rotorod MED[c] (mg/kg)
2	10 ($N = 6$)	13.4 ± 11.6 ($N = 9$)	>30 ($N = 8$)
13	10 ($N = 6$)	49.4 ± 4.6 ($N = 8$)[d]	>100 ($N = 8$)
16	10 ($N = 6$)	21.8 ± 13.6 ($N = 9$)	>30 ($N = 8$)
26	>10 ($N = 6$)	14.9 ± 8.0 ($N = 8$)	>30 ($N = 8$)

[a] The total number of animals used for in vivo characterization was 255, including vehicle controls. Vehicle controls for carrageenan, $N = 6$, formalin, $N = 8$–12, rotorod, $N = 8$. Experimental methods based on Ref. 14b.
[b] Amino acid dosed p. o. as diethyl ester hydrochloride salt.
[c] Several doses were evaluated. MED = minimal effective dose.
[d] Result obtained with 100 mg/kg p. o.

A small group like fluorine *ortho*- to the acid group (**19**) produced a modest weakening of GluK1 binding. Substitution at R^4, *ortho*- to the linker atom X, (**31**, **32**) was deleterious to the affinity for GluK1, which may indicate conformational preferences around the linker, disturbed by crowded environments. The substitutions at R^2 or R^3 were well tolerated when the linker atom was sulfur or oxygen, while a large reduction of the selectivity against GluA2 was noticed with the amino as linker (**22**). The most important position appears to be R^3 where a variety of substitution was allowed and offered the possibility of obtaining a set of potent and selective antagonists, such as **26**–**30**.

A model based on the public crystal structure of an antagonist (UBP302; pdb 2F35) bound to the ligand binding domain of GluK1 allowed rationalization of some of these observations (Fig. 1).[18] R^1 and R^2 were close to the protein surface and R^4 was close to the decahydroisoquinoline ring, limiting substitution in these cases. R^3 (Cl in Fig. 1) pointed to a large pocket in agreement with SAR results.

A selection of the above antagonists (Tables 1 and 2) was evaluated in vivo and compared with the lead compound **2**. While these amino acids showed poor pharmacokinetic properties (data not shown), we achieved acceptable oral bioavailability of the amino acids in rats with the use of the corresponding diethyl ester derivatives (Table 3).[9,20] Focusing on the AUC and C_{max} parameters in Table 3, the diethyl ester prodrug of **16** allowed for significantly higher plasma levels of the corresponding amino acid than was the case for diethyl ester prodrugs of our original compound **2** or the sulfur-linked **13**. Thus, the presence of oxygen as linker showed better pharmacokinetic parameters than other atoms. Phenyl ethers substituted at R^3 (**26** and **30**) still showed improved plasma levels of amino acid over **2**, though these substitutions reduced the bioavailability of the parent amino acid.

Diethyl ester prodrugs were chosen for evaluation in two animal models of pain via the oral route (Table 4). The diethyl ester prodrug of compound **2** showed moderate efficacy in the carrageenan-induced thermal hyperalgesia model, with a minimal effective dose of 10 mg/kg. It also showed weak reversal of the pain caused by formalin, without sedative or ataxic effects as measured by the rotorod test, at a single oral dose of 30 mg/kg.[8a]

The prodrugs of the new set of antagonists showed similar activity to the diethyl ester of **2** in both pain models despite modest improvements in affinity and/or exposure. Though the prodrug of compound **13** produced a significant larger reversal of pain in the formalin model than the prodrug of compound **2**, this occurred with a higher dose of 100 mg/kg. Efficacy in vivo of these new compounds may be limited by poor blood-brain barrier penetration of the parent amino acids. Indeed, experiments with compound **2** and related GluK1 antagonists, showed that higher in vivo efficacy was observed after central administration, and furthermore, this was in the same rank order as the compounds' in vitro potency.[21]

In conclusion, we have discovered a new family of GluK1 antagonists with a particular linker-acid group relationship. The phenyl substitution of these benzoic acid derivatives suggested the minimum requirements to maintain acceptable affinity for GluK1 and may be useful to develop other GluK1 antagonists. In addition, we have demonstrated that prodrug esters allow for a satisfactory rat oral bioavailability of the amino acids and for oral evaluation in pain models.

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