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Structure–activity relationships of α-amino acid ligands for the $\alpha_2\delta$ subunit of voltage-gated calcium channels

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Abstract—A series of α-amino acids were identified as ligands which compete with gabapentin for binding to the $\alpha_2\delta$ subunit of voltage-dependent Ca^{2+} channels. Potent analogs were identified. Their activity in an in vivo pain assay is described.

Agents that modulate voltage-gated calcium channels are of interest for their ability to relieve chronic pain.[1] Two marketed drugs that act via direct or indirect modulation of these channels are the N-type calcium channel blocker ziconitide (Prialt®), an intrathecally administered treatment for chronic pain, and the anticonvulsant agent gabapentin (Neurontin®) (1), used in the treatment of neuropathic pain.[2] It has been proposed that gabapentin and related compounds containing a γ-aminobutyric acid backbone produce their analgesic effect through interaction with a site on the $\alpha_2\delta$ subunit of a voltage-dependent Ca^{2+} channel.[3] In addition, non-polar α-amino acids such as L-leucine have been reported to bind to the site with affinities comparable to that of gabapentin.[4] We sought to develop compounds with efficacy in rat in vivo pain models from amino acid analogs that bind to the reported gabapentin binding site.

Initially, amino acid analogs in the Abbott repository compound collection were screened for their ability to displace [^{3}H]L-leucine from $\alpha_2\delta$ subunit-containing calcium channels in murine brain membrane preparations.[5] Binding affinities of selected compounds are shown in Tables 1–3. The results of the screening confirmed that a range of neutral amino acids bind with affinities comparable to that of gabapentin. In addition, no amino acids with polar side chains were identified as hits in the screen. Although N- and C-terminally modified amino acid analogs which bind to N-type Ca^{2+} channels have been reported,[1c] all of the compounds that displaced [^{3}H]leucine binding in our screen contained unmodified amino and acid groups.

As shown in Table 1, amino acids with small cyclic side chains maintained binding potency comparable to that of gabapentin. The binding was sensitive to changes in substitution on the non-polar side chain and stereochemistry at the α carbon; in cases where both the *R* and *S* isomers of the amino acid were tested, only the *S* isomer was potent (e.g., phenylglycines **3** and **4** and cyclohexylalanines **5** and **6**). Compounds displaying a phenyl or cyclohexyl group at the end of a non-polar linker were active. Extending the linker to 4 atoms produced the most potent compound, benzylhomocysteine **12**. We chose to explore analogs of phenylglycine **3** and benzylhomocysteine **12** in an effort to further improve potency in the binding assay.

Syntheses of analogs of phenylglycine have been described in the literature,[6] and several are commercially available. For example, compound **16** (Table 2) was synthesized in 2 steps from *m*-chlorobenzaldehyde.[6b] Reaction with sodium cyanide gave the 5-arylhydantoin, which was cleaved under basic conditions to give the amino acid (47%, 2 steps). Other compounds shown in Table 1 were obtained from commercial sources.

Keywords: Voltage-gated calcium channel; $\alpha_2\delta$; Gabapentin; Amino acids.
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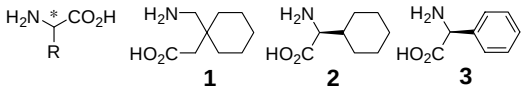
Table 1. Binding to the $\alpha_2\delta$ subunit by neutral amino acids: extending the non-polar side chain

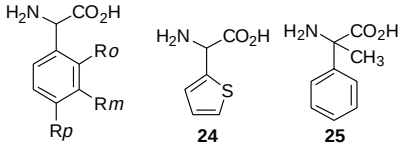


1 **2** **3**

Compound	Side chain	α-Stereo-chem	$\alpha_2\delta$ binding[a] K_i (μM)	pK_i (SEM)
1	NA	NA	0.12	−6.92 (0.09)
2	Cy[b]	S	0.060	−7.22 (0.08)
3	Ph[c]	S	0.18	−6.74 (0.21)
4	Ph	R	30	−4.52 (0.09)
5	CH_2Cy	S	0.090	−7.05 (0.03)
6	CH_2Cy	R	>100	
7	CH_2Ph	S	0.98	−6.01 (0.00)
8	$(CH_2)_2Ph$	*rac*	5.4	−5.27 (0.06)
9	$(CH_2)_3Cy$	*rac*	0.060	−7.23 (0.11)
10	CH_2SCH_2Ph	S	0.11	−6.96 (0.08)
11	$(CH_2)_2SCH_2CH_3$	S	0.10	−6.98 (0.07)
12	$(CH_2)_2SCH_2Ph$	S	0.028	−7.55 (0.18)

NA, not applicable.
[a] Displacement of ^{3}H-labeled Leu from murine brain.[5] Data reported are means of three determinations.
[b] Cyclohexyl.
[c] Phenyl.

Table 2. Analogs of phenylglycine **3**



24 **25**

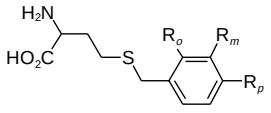
Compound	R_o	R_m	R_p	$\alpha_2\delta$ binding[a] K_i (μM)	pK_i (SEM)
3[b]	H	H	H	0.18	−6.74 (0.21)
13[b]	CH_3	H	H	0.89	−6.05 (0.03)
14	CF_3	H	H	>100	
15	F	H	H	0.26	−6.59 (0.16)
16	H	Cl	H	0.054	−7.27 (0.05)
17	H	Br	H	0.039	−7.41 (0.08)
18[b]	H	H	CH_3	4.9	−5.31 (0.07)
19	H	H	CF_3	65	−4.19 (0.05)
20	H	H	Cl	20	−5.73 (0.08)
21	F	F	H	0.096	−7.02 (0.03)
22	F	H	F	3.5	−5.46 (0.03)
23	H	Cl	F	0.31	−6.51 (0.07)
24	NA	NA	NA	0.21	−6.68 (0.19)
25	NA	NA	NA	0.17	−6.78 (0.07)

NA, not applicable.
[a] Displacement of [^{3}H]Leu from murine brain.[5] Data reported are means of three determinations.
[b] *S*-Configuration.

Benzylhomocysteine analogs were generated by standard methods as shown in Scheme 1. A Boc-protected homocysteine analog was reacted with various benzylating agents. The carbamate was cleaved by treatment with trifluoroacetic acid to give the desired amino acid.

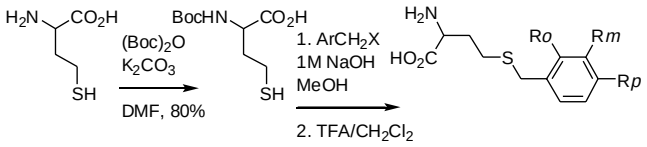
As shown in Table 2, substitution in the *ortho* position of phenylglycine led to a reduction in potency. The *o*-methyl group reduced affinity 5-fold (**13**), while the

Table 3. Substitutions on benzylhomocysteine



Compound	R_o	R_m	R_p	$\alpha_2\delta$ binding[a] K_i (nM)	pK_i (SEM)
12[b]	H	H	H	28	−7.55 (0.18)
26	Br	H	H	20	−7.69 (0.20)
27	H	Br	H	31	−7.51 (0.15)
28	H	H	Br	4.5	−8.34 (0.12)
29	H	Cl	H	26	−7.58 (0.14)
30	H	H	Cl	4.3	−8.37 (0.18)
31	H	F	H	19	−7.72 (0.14)
32	CH_3	H	H	7.6	−8.12 (0.03)
33	OCH_3	H	H	1500	−5.84 (0.09)
34	H	OCH_3	H	510	−6.29 (0.22)
35	CF_3	H	H	31	−7.51 (0.05)
36	H	CF_3	H	120	−6.93 (0.11)
37	H	OCF_3	H	320	−6.50 (0.03)
38	H	H	*t*Bu	79	−7.10 (0.22)
39	H	H	Ph[c]	260	−6.59 (0.10)
40	NO_2	H	H	1300	−5.90 (0.39)
41	H	NO_2	H	39	−7.40 (0.07)
42	CN	H	H	760	−6.12 (0.06)
43	H	CN	H	840	−6.08 (0.26)
44	H	CH_3	CH_3	66	−7.18 (0.11)
45	H	3,5-di-CH_3		450	−6.35 (0.11)
46	2,3-di-Cl		H	5.3	−8.27 (0.14)
47	Cl	Cl	Cl	18	−7.76 (0.14)
48	2,5-di-Cl		H	21	−7.68 (0.07)
49	H	Cl	Cl	3.7	−8.44 (0.26)
50	H	3,5-di-Cl		19	−7.73 (0.22)
51	CF_3	H	CF_3	100	−7.00 (0.13)

[a] Displacement of [^{3}H]Leu from murine brain.[5] Data reported are means of three determinations.
[b] *S*-Configuration.
[c] Phenyl.



Scheme 1. Preparation of benzylhomocysteine analogs.

more electron-withdrawing trifluoromethyl group abolished binding (**14**). Similarly, *para* substituents reduced binding, with a CF_3 group causing the greatest drop (**19**). In contrast, *meta*-substitution with a halogen was beneficial. The compound with the highest affinity was *m*-chloro analog **16**, more than 3-fold more potent than the parent compound **3** and 2-fold more potent than gabapentin (**1**). The intolerance of the SAR for *ortho* or *para* substitution may reflect steric constraints in the protein binding site. However, since substituents with different electronic properties have substantially different affinities, electron density in the phenyl ring or changes in the pK_a of the amino acid moiety could also contribute.[7] Replacement of phenyl with thiophene (**24**) or methylation of the α carbon (**25**) was tolerated, but were not beneficial changes to the parent.

The aromatic group of compound **12** proved to be more tolerant to substitution than compound **3**, and several analogs with increased potency were identified (Table 3). In general, non-polar substituents were allowed, but polar substituents were not well tolerated in any position. Appropriate substitution at the *para* or *ortho* position improved potency vs. the parent compound **12** (e.g., **28**, **30**, **32**, and **46**), while *meta* substitution showed no advantage. Halogen substitution was beneficial, and the *p*-Cl (**30**) and *p*-Br (**28**) analogs were 6-fold more potent than **12**. Di-substituted compounds, such as 2,3-dichloro compound **46**, maintained activity but did not further improve it. The most potent analogs in this series were approximately 30-fold more potent than gabapentin.

Five of the highest affinity compounds were selected for in vivo evaluation in a rat model of pain (Table 4). Compounds were tested for their ability to relieve persistent inflammatory pain induced by complete Freund's adjuvant (rat CFA model).[8] The compounds were dosed orally. In our hands, gabapentin (**1**) had an ED_{50} value of 125 μmol/kg in the assay. Compounds **12** and **3** were fully efficacious at similar doses, and compound **5** was effective with an ED_{50} value 2-fold higher. Compound **16** was partially efficacious (64% of the maximum efficacy observed in the model) at the highest dose tested, 300 μmol/kg, and compound **28** was inactive at this dose. The potency of the compounds in the binding assay did not correlate well with their efficacy in the CFA pain assay: the most potent and efficacious compounds in vivo, **1**, **3**, and **12**, had rather high binding constants (30–180 nM). In contrast, the potent binder **28** (5 nM) was inactive. Compound **16**, which is structurally similar to **3** and binds to the gabapentin binding site more potently, was less potent and efficacious in vivo. Since gabapentin is believed to gain access to the brain by an active transport mechanism,[11] structural changes could result in poor brain penetration. However, several compounds achieve brain levels similar to that of gabapentin, so poor access to the CNS target may not explain the discrepancy.

Table 4. In vivo potency of selected compounds

Compound	$\alpha_2\delta$ binding[a] K_i (nM)	CFA in vivo assay, ED_{50} (po)[a] (μmol/kg)	Concd in brain at 0.75 h[b] (μg/g (±SEM))
1	120	125	3.41 (0.28)
3	180	100	6.30 (0.55)
5	90	200	ND
16	54	64%[c] at 300	1.76 (0.06)
12	28	120	0.43 (0.03)
28	5	>300	ND

ND, not determined.
[a] See Ref. 8 for assay protocol.
[b] Mean brain concentrations of compounds after a 100 μmol/kg oral dose in rat.
[c] Of maximum efficacy.

Although the functional consequences of $\alpha_2\delta$ binding which lead to in vivo efficacy have not been fully established, ligands for $\alpha_2\delta$ continue to be pursued as an approach to finding new therapies for pain.[9] 3-Substituted γ-aminobutyric acid analogs have been reported with binding characteristics and in vivo efficacy similar to those of gabapentin.[2b,10] In the present study, changing the ligand backbone from a γ- to an α-amino acid did not preclude the identification of highly potent ligands, and preliminary in vivo studies identified compounds with analgesic activity. However, the difference in orientation between γ- and α-amino acids may translate into compounds that bind but have different functional activity. Since the mechanism of action of ligands like gabapentin is not fully understood, the two series of ligands may also interact differently with other targets in the CNS that were outside the scope of these studies. Compounds that modulate calcium channels via binding to $\alpha_2\delta$ may be useful in treating neuropathic pain. Thus, having achieved efficacy comparable to that of gabapentin **1** in the CFA model of hyperalgesia shown above, several of the active compounds were advanced to further pharmacological and in vivo studies, to be described elsewhere.

Conclusion. Several α-amino acids were identified as ligands which compete with gabapentin for binding to the $\alpha_2\delta$ subunit of voltage-dependent Ca^{2+} channels. A survey of variations on the side chains of phenylglycine **3** and benzylhomocysteine **12** revealed a stringent SAR which was sensitive to stereochemistry at the α carbon and substitution patterns on the rings. Two series of potent ligands were identified. Several compounds were determined to have analgesic efficacy in an in vivo inflammatory pain model.

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