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Silicon-Containing GABA Derivatives, Silagaba Compounds, as Orally Effective Agents for Treating Neuropathic Pain without Central-Nervous-System-Related Side Effects

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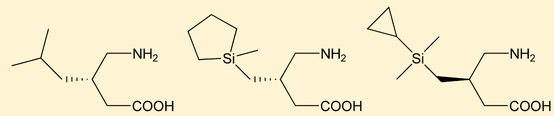
Supporting Information

ABSTRACT: Neuropathic pain is a chronic condition resulting from neuronal damage. Pregabalin, the (*S*)-isomer of 3-isobutyl-γ-aminobutyric acid (GABA), is widely used to treat neuropathic pain, despite the occurrence of central nervous system (CNS)-related side effects such as dizziness and somnolence. Here we describe the pharmacology of novel GABA derivatives containing silicon−carbon bonds, silagaba compounds. Silagaba131, 132, and 161 showed pregabalin-like analgesic activities in animal models of neuropathic pain, but in contrast to pregabalin they did not impair neuromuscular coordination in rotarod tests. Pharmacokinetic studies showed that brain exposure to silagaba compounds was lower than that to pregabalin. Surprisingly, despite their potent analgesic action in vivo, silagaba compounds showed only weak binding to α2-δ protein. These compounds may be useful to study mechanisms of neuropathic pain. Our results also indicate that silagaba132 and 161 are candidates for orally effective treatment of neuropathic pain without CNS-related side effects.



pregabalin silagaba132 silagaba161

KEYWORDS: *Silyl chemistry, neuropathic pain, pregabalin, allodynia, CNS side effect*

Neuropathic pain is a chronic condition resulting from damage or dysfunction in the peripheral and/or central nervous system. Treatment of neuropathic pain is still a challenge, because the pathophysiology is complex and the underlying mechanism remains poorly understood.[1] Chronic pain often responds unsatisfactorily to opioids and nonsteroidal anti-inflammatory drugs. However, adjuvant analgesics, including antidepressants and antiepileptics, are effective.[2] The *S*-isomer of 3-isobutyl-γ-aminobutyric acid (GABA), pregabalin [(*S*)-**1**], which was reported in the early 1990s as a novel antiepileptic, is widely used for treatment of diabetic peripheral neuropathy, postherpetic neuralgia, neuropathic pain following spinal cord injury, fibromyalgia, and also partial-onset seizures,[3−5] although dizziness and somnolence (sleepiness) are common side effects.[6] The higher incidence of these side effects in elderly patients, which may be due to age-related decrease of renal clearance, sometimes has a significant impact on quality of life. Thus, there is a significant unmet need for an orally effective analgesic without central nervous system (CNS)-mediated side effects to treat neuropathic pain.

The specific binding of pregabalin to the α_2-δ subunit of voltage-gated calcium channel, which is expressed at presynaptic terminals of neurons in the spinal cord and brain, is thought to be responsible for its analgesic and anticonvulsant actions.[5,7,8] Sites of dense α_2-δ expression in the brain include the insula and the cingular cortex, which are involved pain-encoding, partial epilepsy, vestibular sensation, and also sleep stages.[5,9] Excessive sedative effect of pregabalin in these areas may result in dizziness and somnolence. Previously, we reported the synthesis of (*R*)- and (*S*)-isomers of 4-amino-3-(trimethylsilyl)methylbutanoic acid, designated as silagaba121 [(*R*)-**2a**] and silagaba122 [(*S*)-**2a**], respectively, and we evaluated their analgesic efficacy in a spinal nerve ligation (SNL) model, the so-called Chung model, in rats.[10,11] In SNL rats, pregabalin showed CNS-mediated hypalgesic effects, as indicated by an increase of the normal pain threshold on the nonoperated side. Silagaba121 and 122 did not show such hypalgesic effects, and appear to be candidates for orally effective analgesics without CNS-mediated side effects. Here, we synthesized a series of silagaba derivatives and evaluated their analgesic effects. The results of rotarod tests and pharmacokinetic studies were consistent with the absence of CNS-mediated effects of these compounds.

■ RESULTS AND DISCUSSION

In order to prepare novel silagaba compounds with bulkier substituents than the trimethylsilyl group of silagaba121 [(*R*)-**2a**] and 122 [(*S*)-**2a**], we introduced a (1-methyl-1-silacyclopentan-1-yl)methyl substituent or a [cyclopropyl(dimethyl)silyl]methyl substituent at the 3-position of GABA. The *R*- and *S*-stereoisomers of each compound were separated by enzymatic optical resolution of a racemic synthetic intermediate, as previously reported in the case of silagaba121 and 122.[11] The resulting compounds were designated as silagaba131 [(*R*)-**2b**], 132 [(*S*)-**2b**], 161 [(*R*)-**2c**] and 162 [(*S*)-**2c**], as shown in Figure 1.

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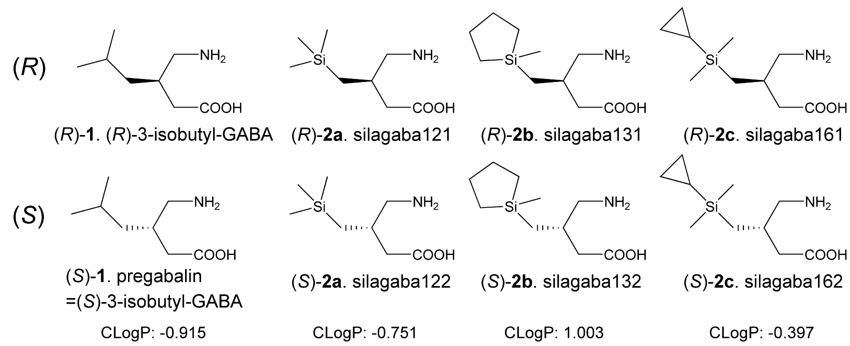

Figure 1. Structures of pregabalin and silagaba.

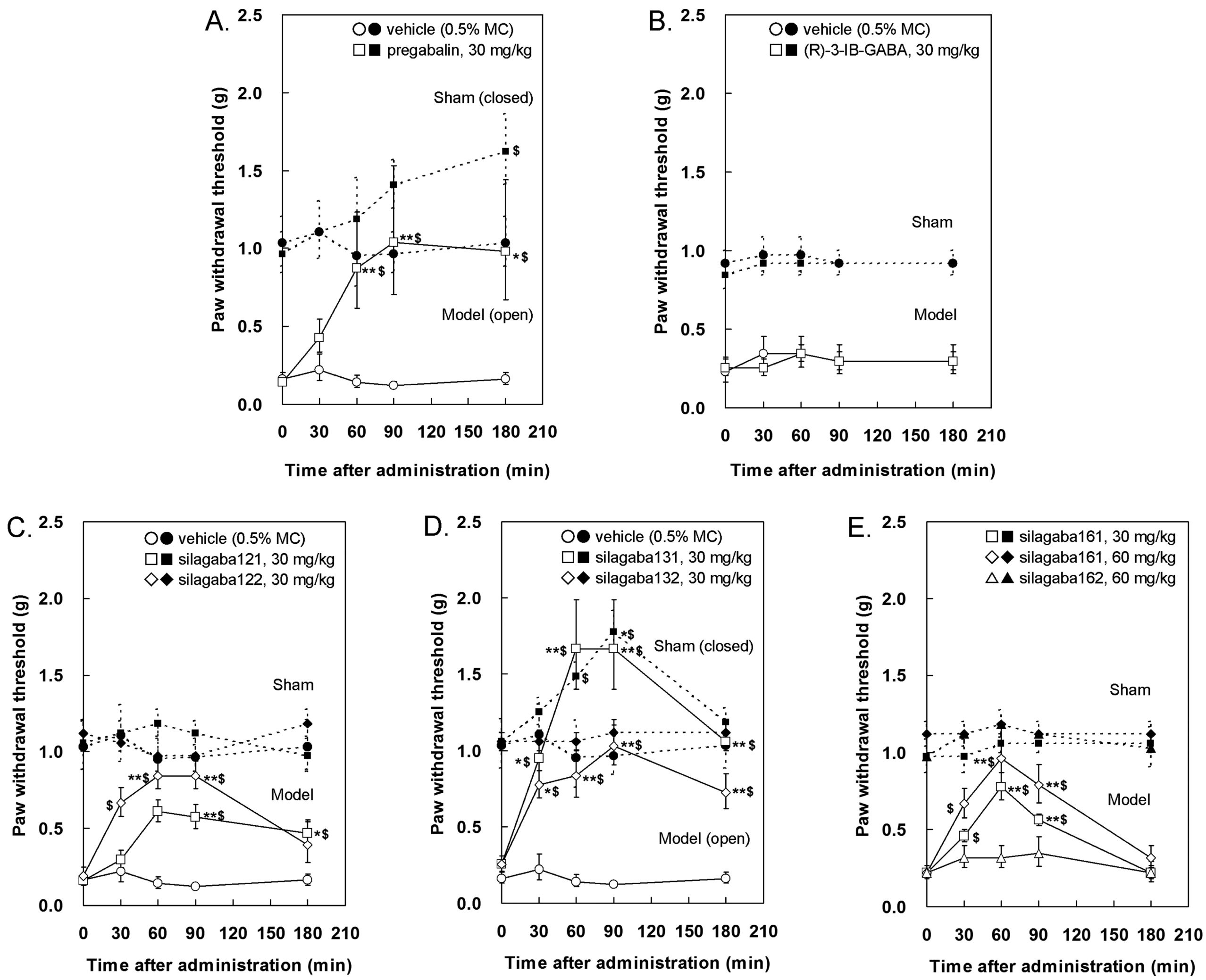

Figure 2. Analgesic activities of pregabalin [(*S*)-**1**], (*R*)-3-isobutyl-GABA [(*R*)-**1**], silagaba121 [(*R*)-**2a**], 122 [(*S*)-**2a**], 131 [(*R*)-**2b**], 132 [(*S*)-**2b**], 161 [(*R*)-**2c**], and 162 [(*S*)-**2c**] for alleviation of mechanical allodynia in SNL mice. Mechanical allodynia was induced by tight ligation of the right L5 and L6 spinal nerves in mice. Test compounds were administered to mice by oral gavage 28 days after surgery (35 days after surgery only for (*R*)-**1**). Paw withdrawal thresholds (pain thresholds) were measured in the right hind paws by stimulation with von Frey filaments at 30, 60, 90, and 180 min after administration. Data for SNL mice (model) and sham mice are shown by open and closed symbols, respectively. Vehicle (0.5% MC) control data are shown as circles. Symbols: (A) squares for 30 mg/kg pregabalin [(*S*)-**1**]; (B) squares for 30 mg/kg [(*R*)-**1**] (*R*-3-IB-GABA); (C) squares for 30 mg/kg (R)-**2a**, diamonds for 30 mg/kg (*S*)-**2a**, (D) squares for 30 mg/kg (*R*)-**2b**, diamonds for 30 mg/kg (*S*)-**2b**; (E) squares for 30 mg/kg (*R*)-**2c**, diamonds for 60 mg/kg (*R*)-**2c**, triangles for 60 mg/kg (*S*)-**2c**. Data are expressed as geometric mean ± SEM (n = 5 or 6, each group). Statistical analysis was done by using Excel with Analyze-it (Analyze-it Software, Ltd., UK). *,**: $p < 0.05$, $p < 0.01$, respectively, t test with Bonferroni correction (vs vehicle). $: $p < 0.05$, Dunnett's test (vs 0 min) following repeated measures ANOVA ($P < 0.05$) in each group.

SNL mice were used to evaluate the analgesic efficacies of pregabalin and these silagaba compounds. Mechanical allodynia, a symptom of neuropathic pain, was successfully induced in these mice, whose pain thresholds were assessed in terms of paw withdrawal responses to mechanical hind-paw stimulation with von Frey filaments. Each compound was orally administered at

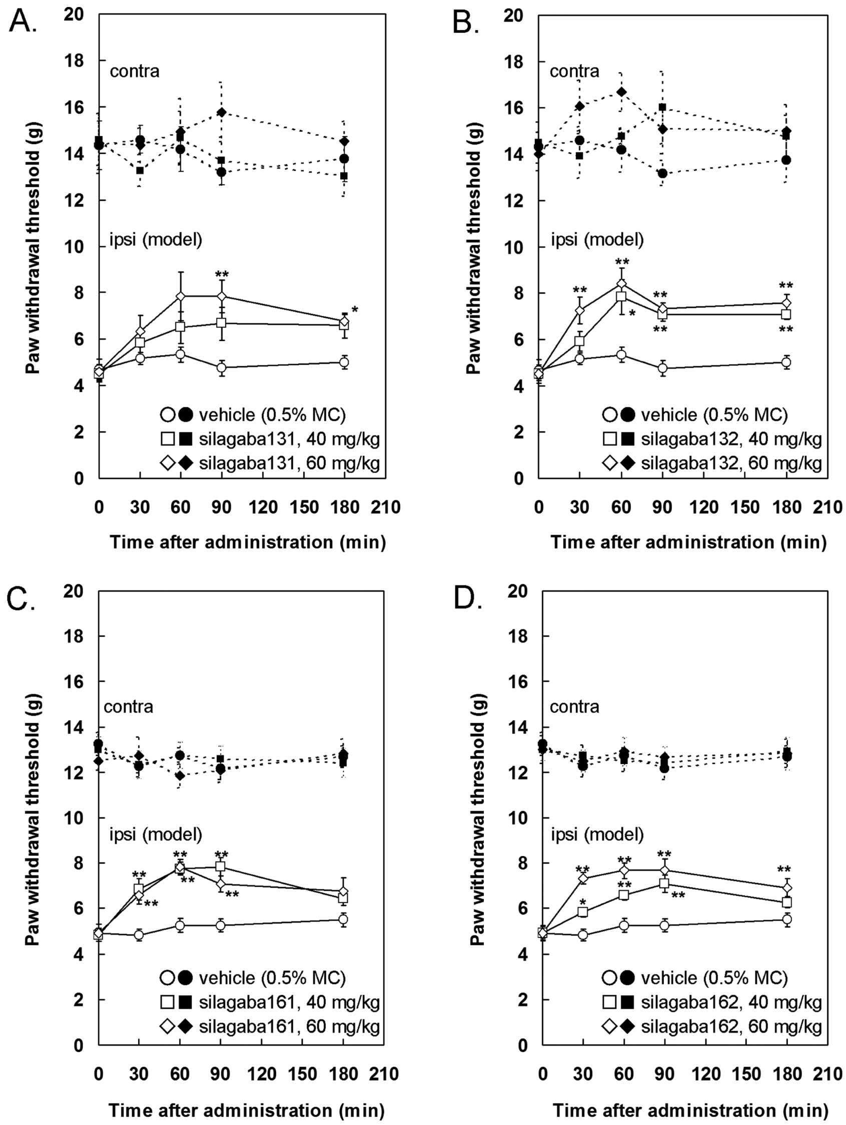

Figure 3. Analgesic activities of silagaba131 [(*R*)-**2b**], 132 [(*S*)-**2b**], 161 [(*R*)-**2c**], and 162 [(*S*)-**2c**] for alleviation of mechanical allodynia in SNL rats. Mechanical allodynia was induced by tight ligation of the left L5 and L6 spinal nerves in rats. Test compounds were administered to rats by oral gavage 7 days after surgery. Paw withdrawal thresholds were measured in both hind paws with an automatic dynamic plantar aesthesiometer at 30, 60, 90, and 180 min after administration. Data for the operated left paws (ipsilateral side: ipsi) and nonoperated right paws (contralateral side: contra) are shown by open and closed symbols, respectively. Vehicle (0.5% MC) control data are shown as circles. Symbols: (A) squares for 40 mg/kg (*R*)-**2b**, diamonds for 60 mg/kg (*R*)-**2b**, (B) squares for 40 mg/kg (*S*)-**2b**, diamonds for 60 mg/kg (*S*)-**2b**, (C) squares for 40 mg/kg (*R*)-**2c**, diamonds for 60 mg/kg (*R*)-**2c**, (D) squares for 40 mg/kg (*S*)-**2c**, diamonds for 60 mg/kg (*S*)-**2c** Data are expressed as mean ± SEM ($n = 6$, each group). Statistical analysis was done by using SAS9 software (SAS Institute Japan). *, **: $p < 0.05$, $p < 0.01$, respectively, in Dunnett's test (vs vehicle), for which data for 20 mg/kg (not significantly varied) were included.

30 mg/kg by gavage. As we previously found in SNL rats,[11] silagaba121 and 122 significantly increased the pain thresholds of SNL mice: that is, they are antiallodynic (Figure 2C). The (*S*)-isomer, silagaba122, was more effective than the (*R*)-isomer, silagaba121. Pregabalin showed significant antiallodynic activity in SNL mice, but also significantly increased the pain thresholds in sham mice at the late time point of 180 min (Figure 2A). This delayed hypalgesic effect is similar to that observed in paws on the contralateral, nonoperated side of SNL rats. In contrast, silagaba121 and 122 did not increase the pain thresholds of sham mice. These results suggest that silagaba121 and 122 are effective for neuropathic pain without unintended effects on normal nociception, unlike pregabalin. (*R*)-3-isobutyl-GABA [(*R*)-**1**], the stereoisomer of pregabalin, showed no analgesic activity in our SNL mice, as expected from previous reports showing that it lacks activity in animal models of epilepsy and thermal hyperalgesia when systemically administered (Figure 2B).[4,12]

Next, we tested the analgesic efficacy of the novel silagaba compounds 131, 132, 161, and 162 in SNL mice. As expected, they (but, except for 162) showed significant antiallodynic activities (Figure 2D,E). Silagaba132, the (*S*)-isomer of (1-methyl-1-silacyclopentan-1-yl)methyl-substituted silagaba, was as potent as pregabalin and silagaba122. Although the (*R*)-isomer, silagaba131, showed high potency in SNL mice, it also significantly increased the pain threshold in sham mice. The anti-allodynic activity of silagaba161, the (*R*)-isomer of [cyclopropyl-(dimethyl)silyl]methyl-substituted silagaba, was significant but less persistent as compared with the other compounds examined. Unexpectedly, the (*S*)-isomer, silagaba162, did not show antiallodynic activity even at 60 mg/kg in SNL mice.

Then, we evaluated the analgesic efficacy of silagaba compounds in SNL rats. We previously reported that pregabalin showed bilateral hypalgesic activity, increasing the pain thresholds on both sides of SNL rats at later time after administration, whereas silagaba121 and 122 increased the pain thresholds only on the ipsilateral operated side.[11] Silagaba131 and 132 showed similar antiallodynic efficacy in the ipsilateral paws (Figure 3A,B). In contrast to the results in SNL mice, however, silagaba131 did not increase the pain threshold in contralateral nonoperated paws, and therefore silagaba131 and 132 were not hypalgesic in SNL rats. The potencies of silagaba131 and 132 were moderately higher than those of silagaba121 and 122 (Supporting Information Figure S1). Silagaba 162, which was not effective in SNL mice, showed similar antiallodynic effects to the (*R*)-isomer silagaba161 at 60 mg/kg but weaker effects than silagaba 161 at 30 mg/kg in SNL rats (Figure 3C,D). Although the reason for these discrepancies between the effects in mice and rats is unclear, differences of genetic background, such as variability in segmental distributions to the sciatic nerve, may be a contributory factor.[13,14] The (*S*)-isomer silagaba132 and (*R*)-isomer silagaba161, which show consistent effects in both mice and rats, may therefore be the best choice in this series of silagaba compounds as candidate orally effective treatment for neuropathic pain without CNS-related side effects.

Finally, we evaluated the analgesic effects of silagaba132 and 161 in another peripheral nerve injury model of chronic pain, the partial sciatic nerve ligation (PSL) model (so-called Seltzer model) in rats (Figure 4).[15] The ligation procedure in the Seltzer model is less extensive and more peripheral than in the Chung model. As expected, pregabalin also showed significant analgesic effects in Seltzer rats. However, pregabalin (30 mg/kg) also showed a remarkable hypalgesic effect on the contralateral side at 180 min after administration. This seemed more evident in the Seltzer model than in the Chung model. The apparently greater sensitivity of the Seltzer model to delayed hypalgesic activity of pregabalin on the contralateral side may reflect the contrasting features of the two models.[16−18] Silagaba132 and silagaba161, orally administered at 30 mg/kg, showed almost equivalent antiallodynic effects to that of pregabalin at 60 min after administration on the ipsilateral side. Although the efficacy of pregabalin on the ipsilateral side peaked at 180 min and was sustained up to 300 min, being apparently superior to those of silagaba compounds, this may at least partly be explained by its bilateral hypalgesic effect mediated by the upper CNS.

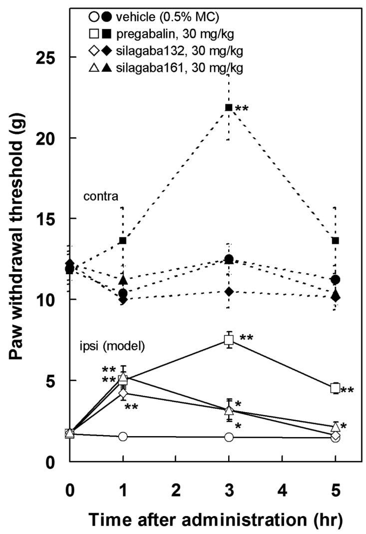

Figure 4. Analgesic activities of pregabalin [(*S*)-**1**], silagaba132 [(*S*)-**2b**], and 161 [(*R*)-**2c**] for alleviation of mechanical allodynia in PSL rats. Mechanical allodynia was induced by partial ligation of the left sciatic nerves in rats. Test compounds were administered by oral gavage at 30 mg/kg 14 days after surgery. Paw withdrawal thresholds were measured in both hind paws by stimulation with von Frey filaments at 1, 3, and 5 h after administration. Data for the left operated paws (ipsi) and right nonoperated paws (contra) are shown by open and closed symbols, respectively. Vehicle (0.5% MC) control data are shown as circles. Symbols: squares for pregabalin [(*S*)-**1**], diamonds for (*S*)-**2b**, triangles for (*R*)-**2c**. Data are expressed as mean ± SEM (n = 8, each group). Statistical analysis was done by using SAS9 software. *, **: $p < 0.05$, $p < 0.01$, respectively, in Student's t test (vs vehicle).

To further confirm the lack of CNS-mediated effects, we used the rotarod test, commonly used in CNS safety pharmacology, to assess the effect of the compounds on neuromuscular coordination in rats. In this test, pregabalin significantly and dose-dependently reduced the duration for which rats could maintain their balance on the rotating rods (Table 1).

Table 1. Results of Rotarod Tests of Pregabalin and Silagaba Compounds[a]

		duration (s)		
	dose (mg/kg)	1 h	2 h	3 h
vehicle		180 (0.0)	180 (0.0)	168.8 (11.2)
pregabalin [(*S*)-**1**]	10	180 (0.0)	160 (19.8)	125 (29.7)[##]
	30	180 (0.0)	93 (24.4)[##]	12 (3.7)[##]
	100	53 (11.5)[#]	13 (6.3)[##]	6 (2.1)[##]
(*R*)-**2a** (121)	300	180 (0.0)	180 (0.0)	180 (0.0)
(*S*)-**2a** (122)	100	180 (0.0)	180 (0.0)	180 (0.0)
	300	180 (0.0)	135 (28.8)	178 (2.2)
(*R*)-**2b** (131)	300	180 (0.0)	180 (0.0)	180 (0.0)
(*S*)-**2b** (132)	30	180 (0.0)	180 (0.0)	180 (0.0)
	100	180 (0.0)	180 (0.0)	180 (0.0)
	300	180 (0.0)	180 (0.0)	180 (0.0)
(*R*)-**2c** (161)	30	180 (0.0)	168 (12.2)	154 (25.8)
	100	180 (0.0)	180 (0.0)	180 (0.0)
	300	180 (0.0)	180 (0.0)	180 (0.0)

[a]Duration at each measured time point after administration is shown as mean (SEM). Statistical analysis was done by using SAS9 software. #, ##: $p < 0.05$, 0.01, respectively, in Dunnett's test (vs before test) following repeated measures ANOVA ($P < 0.05$) in each group.

The reduction of the duration following administration of pregabalin was greater at later times, suggesting delayed distribution of pregabalin to the brain. In rats administered 10 mg/kg of pregabalin, the duration was significantly shortened at 3 h after administration. Rats treated with silagaba121, 131, 132, and 161 showed unchanged duration on the rotating rod (180 s) at all the measured time points. At the dose of 300 mg/kg, silagaba122 reduced the duration at 2 h after administration slightly, but not significantly. Silagaba 161 at 30 mg/kg also slightly reduced the duration at 2 and 3 h after administration, but the change was not dose-dependent, suggesting that silagaba161 did not have a marked effect on neuromuscular coordination. In contrast to pregabalin, whose effective dose in the rotarod test is close to its analgesic dose in the SNL model, silagaba compounds showed no significant effects in the rotarod test at their analgesic dose in SNL model, suggesting that they would have a superior safety margin compared with pregabalin in pain treatment.

Table 2. Pharmacokinetic Parameters Obtained after Single Oral Dosing in Rats[a]

compd	dose (mg/kg)	C_{max} (μM)	T_{max} (h)	T1/2 (h)	AUC (μg·h/mL)	plasma, 1 h (μM)	brain, 1 h (μM)	$K_{p,brain}$	$C_{max,brain}$ (μM)
(*S*)-**1** (pregabalin)	30					109.3	12.4	0.114	
(*R*)-**2a** (121)	30	54.0	1.00	2.52	32 658	50.3	1.4	0.028	1.5
	100	102.1	1.00	2.49	69 670				2.9
(*S*)-**2a** (122)	30	125.4	0.83	2.19	91 673	98.8	5.1	0.052	6.5
	100	481.7	0.50	2.58	275 205				25.0
(*R*)-**2b** (131)	30	34.3	0.67	2.60	7390	32.1	2.6	0.082	2.8
	100	97.5	1.00	2.62	21 000				8.0
(*S*)-**2b** (132)	30	39.6	0.50	1.51	15 668	33.0	4.0	0.122	4.8
	100	111.8	0.67	2.23	53 737				13.6

[a]Three male SD rats at the age of 7 weeks (220−280 g) were orally administered with each dose of each compound in 0.5% MC solution by gavage. Serial plasma samples were collected at 0.25, 0.5, 1, 2, 4, 6, and 24 h after administration, and the plasma concentration of each compound was determined by LC-MS/MS. To measure the concentration in the brain, rats were euthanized 1 h after administration and the brain tissues were isolated and homogenized in phosphate-buffered saline with a weight-to-volume ratio of 1 to 5. The concentration of each compound in the homogenate was determined by LC-MS/MS. $K_{p,brain}$ values were calculated from the plasma and brain concentrations 1 h after administration (brain, 1 h/plasma, 1 h). $C_{max,brain}$ values are tentative values obtained by multiplying the C_{max} and $K_{p,brain}$ values.

To understand the effects of silagaba in vivo from the pharmacokinetic viewpoint, silagaba compounds were orally administered to rats and their concentrations in plasma and brain were quantified by LC-MS/MS. The obtained pharmacokinetic parameters of silagaba12x and 13x are summarized in Table 2. The pharmacokinetics (PK) of silagaba161, the most recently developed silagaba, has not yet been examined, but might be similar to those of other silagaba compounds, because the molecular mass of silagaba161 is the same as that of silagaba132 and the CLogP of silagaba161 lies between those of 12x and 13x. However, we cannot exclude the possibility that silagaba161 might have a distinct PK profile. For all the compounds tested, almost linear pharmacokinetics was observed at doses from 10 to 300 mg in rats (the plasma concentration—time plot of silagaba132 is shown in Figure 5). Oral absorption of silagaba compounds appears to be acceptable. The mean values of plasma half-life ($T_{1/2}$) ranged from 1.5 to 2.5 h, being shorter than that of pregabalin (around 6 h in dog and human).[19] The times of maximum drug concentration (T_{max}) ranged from 0.5 to 1.0 h, which is rather shorter than or similar to the T_{max} of pregabalin reported in healthy human volunteers (0.85−1.38 h).[19] Brain distribution of orally administered silagaba compounds and pregabalin at 30 mg/kg was evaluated at 1 h, which is close to the T_{max} values. The plasma concentration of silagaba122 at 1 h was as high as that of pregabalin, nearly 100 μM, but its concentration in the brain (5.1 μM) was less than half of that of pregabalin (12.4 μM). The plasma concentrations of silagaba131 and 132 at 1 h were about one-third of that of pregabalin, and their concentrations in the brain (2.6, 4.0 μM, respectively) was one-fifth and one-third of that of pregabalin, respectively. Calculated values for brain-to-plasma concentration ratio ($K_{p,\ brain}$) of silagaba compounds were lower than that of pregabalin, except for silagaba132, whose $K_{p,brain}$ was similar to that of pregabalin. The $K_{p,brain}$ value of each (*R*)-isomer seems lower than that of the corresponding (*S*)-isomer. Overall, the brain distribution of silagaba compounds is lower than that of pregabalin. Taking the longer plasma half-life of pregabalin into account, the brain concentration of pregabalin is expected to be high after 1 h and may increase further at time points later than 1 h. Therefore, brain exposure to pregabalin may become increasingly higher than exposure to silagaba compounds. Thus, the PK profiles of silagaba compounds could at least partially explain their lack of CNS-mediated effects in rats.

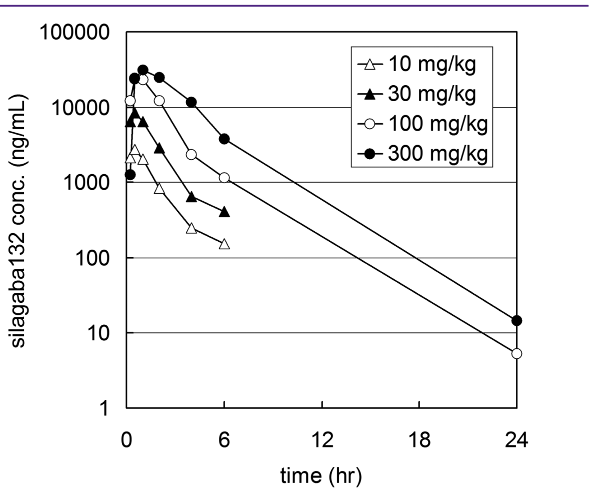

Figure 5. Plasma silagaba132 [(*S*)-**2b**] concentrations versus time in three rats after a single oral administration of 10, 30, 100, or 300 mg/kg.

The analgesic and anticonvulsant action of pregabalin is thought to be mediated by its specific binding to the α_2-δ subunit of voltage-gated calcium channel, which is expressed at presynaptic terminals of neurons in the brain and spinal cord.[5,7,8] The wide distribution of this protein may contribute to the diverse actions of pregabalin, including its CNS-mediated side effects. With regard to peripheral nerve injury models, increased protein level of α_2-δ-1 isoform in dorsal root ganglion (DRG) neurons on the ipsilateral side and its correlation with onset of allodynia have been reported.[20−22] Binding of pregabalin to α_2-δ-1 proteins in the affected DRG neurons could contribute to its unilateral early antiallodynic effect observed in our study. Thus, we evaluated the binding activities of silagaba compounds to the gabapentin-binding sites, presumably α_2-δ subunit proteins, in rat brain cortex by means of [^{3}H]gabapentin-binding assay (Figure 6; Table 3). In this assay, the IC_{50} value of pregabalin was 89 nM, in accordance with reported values.[8,12] The IC_{50} values of silagaba122 and 132 were 1.35 and 2.41 μM, respectively, which correspond to 6.6% and 3.7% relative binding affinity (RBA) versus pregabalin. The estimated RBA of the pregabalin stereoisomer, (*R*)-3-isobutyl-GABA, is 6.0 to 10.9%.[4,12,23] (*R*)-3-isobutyl-GABA lacked anticonvulsant and antiallodynic activities in previous studies and our study.[4,12] Therefore, the weak binding of silagaba compounds to α_2-δ protein in spite of their significant analgesic action in vivo is surprising, and may indicate

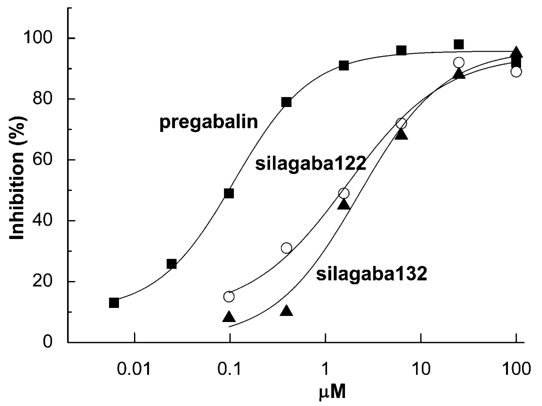

Figure 6. Dose-dependent inhibition of [^{3}H]gabapentin binding by pregabalin [(*S*)-**1**], silagaba122 [(*S*)-**2a**], and silagaba132 [(*S*)-**2b**]. Competitive binding assays for test compounds were performed at Eurofins Panlabs by using ^{3}H-labeled gabapentin (20 nM) and plasma membranes prepared from rat cerebral cortex, as reported.[23,30]

Table 3. Binding Characteristics of Pregabalin and Silagaba to Gabapentin-Binding Site[a]

	% inhibition (at μM)	IC_{50} (μM)	K_i (μM)	RBA (%)
pregabalin	79 (0.39 μM)	0.089	0.058	100
(*R*)-3-isobutyl-GABA	19 (1 μM)	0.95		9.4
silagaba121	78 (75 μM)			
silagaba122	31 (0.39 μM)	1.35	0.88	6.6
	72 (6.25 μM)			
silagaba132	10 (0.39 μM)	2.41	1.58	3.7
	68 (6.25 μM)			

[a]IC_{50} values were determined by nonlinear, least-squares regression analysis of the concentration-inhibition curves. The K_i values were calculated using the equation of Cheng and Prusoff, where the dissociation constants (K_d) and concentration of [^{3}H]gabapentin are 38 nM and 20 nM, respectively. Relative binding affinity (RBA %) for each test compound was calculated by dividing the IC_{50} value of pregabalin by the IC_{50} value of the test compound. The IC_{50} value of (*R*)-3-isobutyl-GABA was taken from a previous study and used for calculation of RBA of (*R*)-3-isobutyl-GABA.[12] We have no binding data for silagaba131, 161, and 162.

that α_2-δ protein is not the only target molecule of gabapentinoid compounds. However, silagaba121, 122, and 132 did not compete with binding of radio-labeled ligands to 75 other pain-related target sites, including GABA receptors, opioid receptors, and sodium channel site 2 (a target of local anesthetics) (Supporting Information Table S1). Nevertheless, it remains possible that silagaba has other target proteins that were not studied here. It remains an open question whether all of the diverse actions of pregabalin are mediated through their interaction with α_2-δ proteins.[5] At least it can be said that carbon−silicon substitution does not appear to cause nonspecific binding of silagaba compounds. On the other hand, it is noteworthy that Houghton reported comparable analgesic efficacy of (*R*)-3-isobutyl-GABA to pregabalin in an acute arthritis model when it was locally administered by microdialysis infusion into the dorsal horn.[24] Well differentiated local distribution and kinetics and a distinct mode of target binding (including to α_2-δ) in vivo could account for the analgesic activities of silagaba compounds. Further studies need to be done.

In conclusion, our series of newly synthesized GABA derivatives containing silicon−carbon bonds, silagaba compounds, showed significant analgesic effects with minimal CNS-related side effects. Their effects on the CNS were examined by means of the rotarod test, pharmacokinetic studies, receptor binding studies and animal pain models. Our findings in these studies are broadly consistent with the observed lack of pregabalin-type CNS-mediated effects, except for the hypalgesic effect of silagaba131 in SNL mice. In contrast, sleepiness and weakness were repeatedly observed in pregabalin-administered animals throughout our studies. Silagaba compounds are expected to be useful in studies of the mechanisms of neuropathic pain and these compounds are also candidates for improved treatment of patients with chronic pain. From the perspective of medicinal chemistry, it is an interesting question why the subtle changes of molecular size and shape resulting from silicon−carbon substitution, as compared to other types of substitution, can modify the strict preference for the isobutyl moiety and for *S*-stereochemistry at the 3-position of GABA for analgesic and anticonvulsant activities.[12,25−27] Their binding affinity for the gabapentin site (α_2-δ proteins) is too weak to account for their in vivo activities. The target molecule(s) responsible for the analgesic effect of silagaba compounds remains unclear, and at this stage we cannot exclude any possibilities. It is also unclear whether both enantiomers of silagaba work on the same molecule, though we have not yet found any critical difference between the enantiomers of silagaba in the tests to discriminate their target molecules. We believe the present findings warrant further studies of our silagaba compounds, especially silagaba132 and 161, to evaluate their potential application as orally effective analgesics without CNS-mediated side effects.

■ METHODS

General Synthetic Procedure. For preparation of each silagaba compound, ethyl cyanoacetate was alkylated with the corresponding (chloromethyl)trialkylsilane in the presence of potassium iodide and potassium carbonate. The obtained ethyl 2-cyano-3-alkylsilyl-propionate was condensed with ethyl bromoacetate in the presence of sodium hydride and then decarboxylated to obtain ethyl 4-(alkylsilyl)-3-cyanobutanoate. Optical resolution of the racemic cyanobutanoate ester was achieved by enzymatic hydrolysis with Novozyme 435 (Sigma-Aldrich) in 0.1 M phosphate buffer (pH 7.4)/dimethyl sulfoxide (5:1) to obtain optically active carboxylic acid (*R*-rich) and recovered ester (*S*-rich).[28] The enantiomeric ratios, estimated from the ^{1}H NMR spectra in the presence of a chiral shift reagent Chirabite-AR (Tokyo Chemical Industry Co., Japan),[29] were 5:1−9:1 when the extent of enzymatic hydrolysis reached about a half. To achieve high optical purity (at least 96% ee), the carboxylic acid was re-esterified, and the resulting *R*-rich ester as well as *S*-rich ester were both rehydrolyzed with Novozyme, if necessary. The resultant highly optically pure (*R*)-carboxylic acid or (*S*)-ester was hydrogenated with Raney Ni under a hydrogen atmosphere (0.45 MPa) at 25 °C in alkaline solvent (NaOH or KOH in MeOH/H_2O) for 2 days. The reaction mixture was neutralized with acetic acid and cooled. Precipitated silagaba powder was collected by filtration, washed with cooled water, and recrystallized from MeOH/iPrOH or MeOH/H_2O to obtain the optically pure silagaba compound (>99% ee). For details, see the Supporting Information.

Anti-Allodynia Tests. Two neuropathic pain models, the SNL model (Chung model) and PSL model (Seltzer model), were used to evaluate the analgesic activity of each compound, as described previously. In SNL mice, the right L5 and L6 spinal nerves of male ICR mice (Japan SLC, Inc., Hamamatsu, Japan) at 5 weeks of age were tightly ligated under anesthesia and the animals were used for tests 28 days after surgery. The sham mice were subjected to similar procedures except for the spinal nerve ligation. Each compound was suspended in 0.5% methylcellulose (MC) and administered by oral gavage. The hind paw withdrawal responses to a series of calibrated von Frey filaments were measured to quantify the pain threshold of mice. For SNL rats, the left L5 and L6 spinal nerves of male Sprague–Dawley (SD) rats at 6 weeks of age (Charles River Japan Inc., Yokohama, Japan) were tightly ligated under anesthesia. The degree of mechanical allodynia was automatically measured by using a dynamic plantar aesthesiometer at day 7 after surgery. Paw withdrawal thresholds were measured in both hind paws at 30, 60, 90, and 180 min after administration. For the PSL model, the left sciatic nerves of male SD rats at 5 weeks of age were partially (1/2–1/3) ligated. The degree of mechanical allodynia was manually measured by using von Frey filaments at day 14 after surgery.

Rotarod Test. On the day before tests, male Wistar rats at the age of 7 weeks (Japan SLC, Inc., Hamamatsu, Japan) were trained three times to walk on the rotating rod (10 rpm) for more than 180 s. Next day, 6 rats for each group were orally administered test compounds in 0.5% MC by gavage (10, 30, and 100 mg/kg for pregabalin, 30, 100, 300 mg/kg for silagaba compounds). Before and at 1, 2, and 3 h after administration, the time that each rat could stay on the rotating rods (10 rpm) up to 180 s was measured three times, and the maximum time was taken as the observed duration for each rat.

Pharmacokinetics Studies. Three male SD rats at the age of 7 weeks (220–280 g) were orally administered with each dose of each compound in 0.5% MC solution by gavage. Serial plasma samples were collected at 0.25, 0.5, 1, 2, 4, 6, and 24 h after administration and the plasma concentration of each compound was determined by LC-MS/MS. To measure the concentration in the brain, rats were euthanized 1 h after administration and the brain tissues were isolated and homogenized in phosphate-buffered saline with a weight-to-volume ratio of 1 to 5. The concentration of each compound in the homogenate was determined by LC-MS/MS.

Animal Ethics. The animals were maintained under appropriate conditions and allowed to access to food and water ad libitum. Animal experiments were performed according to the guidelines of the Science Council of Japan and also with the approval of the local animal ethics committee of Hoshi University, Mitsubishi Chemical Medience Corporation (Kumamoto, Japan), Hamamatsu Pharma Research, Inc. or ITSUU laboratory.

In Vitro Binding Study. Radioligand binding assays including gabapentin-binding site assay in the rat brain cortex (catalog no. 230000) were conducted at Eurofins Panlabs, Inc. (Taipei, Taiwan). For primary assay, silagaba121, 122, and 132 were tested in each assay at 75, 250, and 70 μM, respectively. These concentrations were chosen taking into account the calculated C_{max} after oral administration at 60 mg/kg. For secondary assay, IC_{50} and K_i values for the gabapentin-binding site were evaluated. Radioligand binding asssays for 75 other pain-related target sites were also performed at Eurofins Panlabs.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details and characterization of each compound. This material is available free of charge via the Internet at http://pubs.acs.org.

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H.F. and H.M. are responsible for pharmacology and chemistry in this study, respectively.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ANOVA, analysis of variance; AUC, area under the curve; ClogP, calculated partition coefficient; C_{max}, maximum concentration; CNS, central nervous system; DRG, dorsal root ganglion; GABA, γ-aminobutyric acid; IC_{50}, concentration resulting in 50% inhibition; K_d, dissociation constant; K_i, dissociation constant of inhibitor; $K_{p,\ brain}$, brain-to-plasma concentration ratio; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MC, methyl cellulose; PK, pharmacokinetics; PSL, partial sciatic nerve ligation; SEM, standard error of the mean; SNL, spinal nerve ligation; $T_{1/2}$, half-time; T_{max}, time of maximum drug concentration

■ REFERENCES

(1) Baron, R., Binder, A., and Wasner, G. (2010) Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol, 9*, 807–819.

(2) Dworkin, R. H., O'Connor, A. B., Audette, J., Baron, R., Gourlay, G. K., Haanpaa, M. L., Kent, J. L., Krane, E. J., Lebel, A. A., Levy, R. M., Mackey, S. C., Mayer, J., Miaskowski, C., Raja, S. N., Rice, A. S., Schmader, K. E., Stacey, B., Stanos, S., Treede, R. D., Turk, D. C., Walco, G. A., and Wells, C. D. (2010) Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin. Proc. 85*, S3–14.

(3) Silverman, R. B., Andruszkiewicz, R., Nanavati, S. M., Taylor, C. P., and Vartanian, M. G. (1991) 3-Alkyl-4-aminobutyric acids: the first class of anticonvulsant agents that activates L-glutamic acid decarboxylase. *J. Med. Chem. 34*, 2295–2298.

(4) Taylor, C. P., Vartanian, M. G., Yuen, P. W., Bigge, C., Suman-Chauhan, N., and Hill, D. R. (1993) Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to in vitro binding at a novel site labeled by tritiated gabapentin. *Epilepsy Res. 14*, 11–15.

(5) Stahl, S. M., Porreca, F., Taylor, C. P., Cheung, R., Thorpe, A. J., and Clair, A. (2013) The diverse therapeutic actions of pregabalin: is a single mechanism responsible for several pharmacological activities? *Trends Pharmacol. Sci. 34*, 332–339.

(6) Zaccara, G., Gangemi, P., Perucca, P., and Specchio, L. (2011) The adverse event profile of pregabalin: a systematic review and meta-analysis of randomized controlled trials. *Epilepsia 52*, 826–836.

(7) Field, M. J., Cox, P. J., Stott, E., Melrose, H., Offord, J., Su, T. Z., Bramwell, S., Corradini, L., England, S., Winks, J., Kinloch, R. A., Hendrich, J., Dolphin, A. C., Webb, T., and Williams, D. (2006) Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc. Natl. Acad. Sci. U.S.A. 103*, 17537–17542.

(8) Li, Z., Taylor, C. P., Weber, M., Piechan, J., Prior, F., Bian, F., Cui, M., Hoffman, D., and Donevan, S. (2011) Pregabalin is a potent and selective ligand for alpha(2)delta-1 and alpha(2)delta-2 calcium channel subunits. *Eur. J. Pharmacol. 667*, 80–90.

(9) Lopez, C. (2013) A neuroscientific account of how vestibular disorders impair bodily self-consciousness. *Front. Integr. Neurosci. 7*, 91.

(10) Kim, S. H., and Chung, J. M. (1992) An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain 50*, 355–363.

(11) Muratake, H., Ito, A., Toda, T., Suzuki, H., Fukasawa, H., Tsuda, M., Inoue, K., Sugiyama, K., and Shudo, K. (2012) (*R*)- and (*S*)-4-Amino-3-(trimethylsilyl)methylbutanoic acids ameliorate neuropathic pain without central nervous system-related side effects. *Bioorg. Med. Chem. Lett. 22*, 7602–7604.

(12) Belliotti, T. R., Capiris, T., Ekhato, I. V., Kinsora, J. J., Field, M. J., Heffner, T. G., Meltzer, L. T., Schwarz, J. B., Taylor, C. P., Thorpe, A. J., Vartanian, M. G., Wise, L. D., Zhi-Su, T., Weber, M. L., and Wustrow, D. J. (2005) Structure-activity relationships of pregabalin and analogues that target the alpha(2)-delta protein. *J. Med. Chem. 48*, 2294–2307.

(13) Mogil, J. S. (1999) The genetic mediation of individual differences in sensitivity to pain and its inhibition. *Proc. Natl. Acad. Sci. U.S.A. 96*, 7744–7751.

(14) Rigaud, M., Gemes, G., Barabas, M. E., Chernoff, D. I., Abram, S. E., Stucky, C. L., and Hogan, Q. H. (2008) Species and strain differences in rodent sciatic nerve anatomy: implications for studies of neuropathic pain. *Pain 136*, 188−201.

(15) Seltzer, Z., Dubner, R., and Shir, Y. (1990) A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain 43*, 205−218.

(16) Kim, K. J., Yoon, Y. W., and Chung, J. M. (1997) Comparison of three rodent neuropathic pain models. *Exp. Brain Res. 113*, 200−206.

(17) Lee, B. H., Yoon, Y. W., Chung, K., and Chung, J. M. (1998) Comparison of sympathetic sprouting in sensory ganglia in three animal models of neuropathic pain. *Exp. Brain Res. 120*, 432−438.

(18) Wang, L. X., and Wang, Z. J. (2003) Animal and cellular models of chronic pain. *Adv. Drug Delivery Rev. 55*, 949−965.

(19) Bockbrader, H. N., Radulovic, L. L., Posvar, E. L., Strand, J. C., Alvey, C. W., Busch, J. A., Randinitis, E. J., Corrigan, B. W., Haig, G. M., Boyd, R. A., and Wesche, D. L. (2010) Clinical pharmacokinetics of pregabalin in healthy volunteers. *J. Clin. Pharmacol. 50*, 941−950.

(20) Li, C. Y., Song, Y. H., Higuera, E. S., and Luo, Z. D. (2004) Spinal dorsal horn calcium channel alpha2delta-1 subunit upregulation contributes to peripheral nerve injury-induced tactile allodynia. *J. Neurosci. 24*, 8494−8499.

(21) Bauer, C. S., Nieto-Rostro, M., Rahman, W., Tran-Van-Minh, A., Ferron, L., Douglas, L., Kadurin, I., Sri Ranjan, Y., Fernandez-Alacid, L., Millar, N. S., Dickenson, A. H., Lujan, R., and Dolphin, A. C. (2009) The increased trafficking of the calcium channel subunit alpha2delta-1 to presynaptic terminals in neuropathic pain is inhibited by the alpha2delta ligand pregabalin. *J. Neurosci. 29*, 4076−4088.

(22) Bauer, C. S., Rahman, W., Tran-van-Minh, A., Lujan, R., Dickenson, A. H., and Dolphin, A. C. (2010) The anti-allodynic alpha(2)delta ligand pregabalin inhibits the trafficking of the calcium channel alpha(2)delta-1 subunit to presynaptic terminals in vivo. *Biochem. Soc. Trans. 38*, 525−528.

(23) Gee, N. S., Brown, J. P., Dissanayake, V. U., Offord, J., Thurlow, R., and Woodruff, G. N. (1996) The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J. Biol. Chem. 271*, 5768−5776.

(24) Houghton, A. K., Lu, Y., and Westlund, K. N. (1998) *S*-(+)-3-isobutylgaba and its stereoisomer reduces the amount of inflammation and hyperalgesia in an acute arthritis model in the rat. *J. Pharmacol. Exp. Ther. 285*, 533−538.

(25) Taylor, C. P., Vartanian, M. G., Andruszkiewicz, R., and Silverman, R. B. (1992) 3-alkyl GABA and 3-alkylglutamic acid analogues: two new classes of anticonvulsant agents. *Epilepsy Res. 11*, 103−110.

(26) Bryans, J. S., and Wustrow, D. J. (1999) 3-Substituted GABA analogs with central nervous system activity: a review. *Med. Res. Rev. 19*, 149−177.

(27) Mills, J. S., and Showell, G. A. (2004) Exploitation of silicon medicinal chemistry in drug discovery. *Expert Opin. Invest. Drugs 13*, 1149−1157.

(28) Felluga, F., Pitacco, G., Valentin, E., and Venneri, C. D. (2008) A facile chemoenzymatic approach to chiral non-racemic β-alkyl-γ-amino acids and 2-alkylsuccinic acids. A concise synthesis of (*S*)-(+)-Pregabalin. *Tetrahedron: Asymmetry 19*, 945−955.

(29) Ema, T., Tanida, D., and Sakai, T. (2006) Versatile and practical chiral shift reagent with hydrogen-bond donor/acceptor sites in a macrocyclic cavity. *Org. Lett. 8*, 3773−3775.

(30) Suman-Chauhan, N., Webdale, L., Hill, D. R., and Woodruff, G. N. (1993) Characterisation of [^{3}H]gabapentin binding to a novel site in rat brain: homogenate binding studies. *Eur. J. Pharmacol. 244*, 293−301.