Identification of Novel Ligands for the Gabapentin Binding Site on the $\alpha_2\delta$ Subunit of a Calcium Channel and Their Evaluation as Anticonvulsant Agents

Justin S. Bryans,* Natasha Davies, Nicolas S. Gee, Visaka U. K. Dissanayake, Giles S. Ratcliffe, David C. Horwell, Clare O. Kneen, Andrew I. Morrell, Ryszard J. Oles, John C. O'Toole, Gemma M. Perkins, Lakhbir Singh, Nirmala Suman-Chauhan, and Jacqueline A. O'Neill

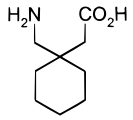
Parke Davis Neuroscience Research Centre, Cambridge University Forvie Site, Robinson Way, Cambridge CB2 2QB, U.K.

Received September 25, 1997

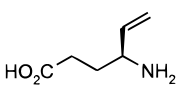
As part of a program to investigate the structure−activity relationships of Gabapentin (Neurontin), a number of alkylated analogues were synthesized and evaluated in vitro for binding to the Gabapentin binding site located on the $\alpha_2\delta$ subunit of a calcium channel. A number of other bridged and heterocyclic analogues are also reported along with their in vitro data. Two compounds showing higher affinity than Gabapentin were selected for evaluation in an animal model of epilepsy. One of these compounds, *cis*-(1*S*,3*R*)-(1-(aminomethyl)-3-methylcyclohexyl)acetic acid hydrochloride (**19**), was shown to be effective in this model with a profile similar to that of Gabapentin itself.

Introduction

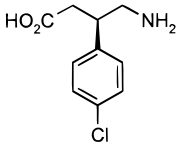
Gabapentin (Neurontin) (**1**) has been introduced as an anticonvulsant agent which is useful as add-on therapy in the treatment of epileptic seizures.[1] It has also recently been shown to be a potential treatment for neurogenic pain.[2−4] Gabapentin was originally designed as a lipophilic γ-aminobutyric acid (GABA) analogue,[5] similarly to other GABA analogues such as Vigabatrin (**2**) and Baclofen (**3**). However, unlike Vigabatrin (an irreversible GABA transaminase inhibitor)[6] and Baclofen (a $GABA_B$ agonist),[7] Gabapentin does not interact with any of the enzymes on the GABA metabolic pathway, nor does it interact directly with the $GABA_A$ or $GABA_B$ receptors.[8] However, it is able to efficiently cross the blood−brain barrier by an L-system amino acid transporter.[9] Gabapentin shows few, if any, toxic side effects at clinically relevant doses.[10] It does, however, possess a relatively short half-life, being excreted unchanged, possibly due to its very high water solubility and apparent lack of protein binding in vivo.[11]



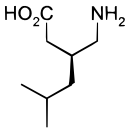
(1)



(2)



(3)



(4)

Recently, it has been shown that Gabapentin binds with high affinity to a novel binding site on the $\alpha_2\delta$ subunit of a calcium channel.[12−14] It is currently proposed that Gabapentin exerts its anticonvulsant and antinociceptive actions via interaction with this site. It is interesting to note that (*S*)-isobutylgaba (**4**), a novel anticonvulsant agent undergoing clinical trials, also binds to the Gabapentin binding site with slightly better affinity than Gabapentin itself−67 nM compared to 140 nM for Gabapentin. Moreover, neither Vigabatrin nor Baclofen show any significant binding to the Gabapentin binding site at 10 mM.

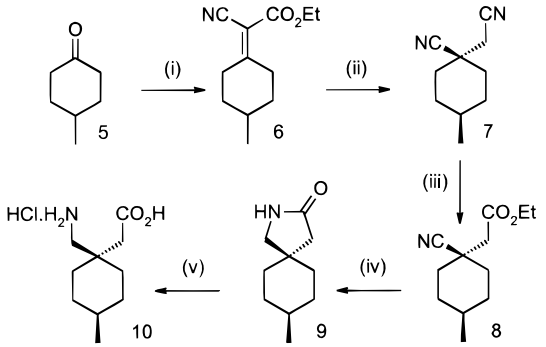
Results and Discussion

Gabapentin and (*S*)-isobutylgaba are both GABA analogues which bind to the Gabapentin binding site located on the $\alpha_2\delta$ subunit of a calcium channel. It has recently been reported that the likely binding conformation of Gabapentin is such that the aminomethyl moiety sits in an equatorial position relative to the cyclohexane ring.[15] It has also been reported that a number of neutral α-amino acids (for example (*S*)-leucine) also bind to the Gabapentin binding site,[13] but in all known cases the (*S*)-enantiomer of these amino acids is the eutomer. Thus it was proposed that the GABA portion of Gabapentin probably sits in a conformation which positions the acid and amine moieties such that they mimic an α-amino acid with the aminomethyl moiety existing in the equatorial position relative to the cyclohexane ring. If such a conformation of Gabapentin is modeled, together with a similar conformation of (*S*)-isobutylgaba, and the two are overlayed using the acid, amine, and β-carbon of the GABA backbone, it is clear that the isobutyl side chain of (*S*)-isobutylgaba overlaps with the cyclohexane ring of Gabapentin except that one of the terminal methyl groups extends from the (*pro-R*) 3_{eq}-position of Gabapentin.[16] This led to an investigation based on alkyl-substituted Gabapentin analogues.

For each of the monoalkylated Gabapentin analogues, either the cis or trans isomer may exist. These could be accessed individually using the routes exemplified for *cis*-(1-(aminomethyl)-4-methylcyclohexyl)acetic acid hydrochloride (**10**) and *trans*-(1-(aminomethyl)-4-methylcyclohexyl)acetic acid hydrochloride (**14**) in Schemes 1[17] and 2, respectively. In Scheme 1 the attack of

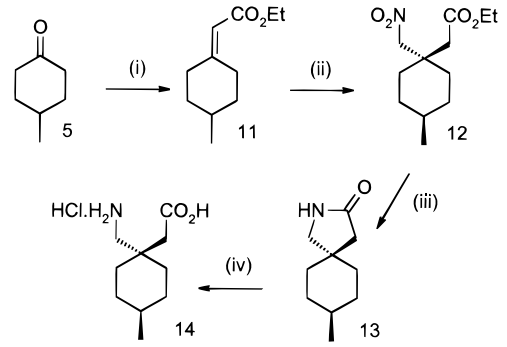
S0022-2623(97)00649-3 CCC: $15.00
Published on Web 04/29/1998

Scheme 1[a]



[a] (i) $NCCH_2CO_2Et$, NH_4OAc, PhMe; (ii) KCN, EtOH, H_2O; (iii) EtOH, HCl; (iv) H_2, Raney Ni, MeOH; (v) HCl.

Scheme 2[a]



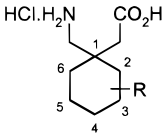
[a] (i) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (ii) $MeNO_2$, $Bu_4N^+F^-$, THF, 70 °C; (iii) Raney Ni, H_2, MeOH; (iv) HCl.

cyanide is reversible,[18] and so the reaction proceeds to minimize the 1,3-diaxial interactions between the axial protons on C-3 and C-5 and the axial group on C-1 by placing the cyanide moiety on C-1 in the axial position as opposed to the bulkier acetonitrile moiety. This gave rise to Gabapentin analogues where the aminomethyl ends up in the axial position.

Conversely, in Scheme 2, nitromethane attacks the α,β-unsaturated ester **11** from the least hindered equatorial direction giving rise to analogues where the aminomethyl lies in the equatorial position. The conformations of each of the products **10** and **14** were determined by NMR spectroscopy in DMSO-d_6.[15] The ^1H and ^{13}C chemical shifts of compounds **10** and **14** were assigned using $^1H-^1H$ double-quantum-filtered COSY and ^1H observed heteronuclear multiple quantum coherence (HMQC) for the ^{13}C resonances. The relative stereochemistries of the alkyl side chains to the aminomethyl and acetyl groups were determined by NOE difference. In both compounds the methyl moieties occupy equatorial positions, as expected, shown by the large coupling of the adjacent proton to nearby axial protons. From previous data[15] it was not expected that any of the analogues where the aminomethyl moiety existed in the axial conformation would have good affinity for the Gabapentin binding site on the $\alpha_2\delta$ subunit of a calcium channel, and indeed this was found to be the case. Thus this investigation centered mainly on compounds where the aminomethyl moiety adopted an equatorial conformation.

In addition to the experiments described above, we investigated the preferred solution conformation for Gabapentin itself. A ^1H NMR spectrum of Gabapentin

Table 1. $\alpha_2\delta$ Subunit Binding Site Affinities for 4- and 3-Substituted Gabapentin Analogues[14]



compd	R	stereochemistry	IC_{50} (nM)[a]
1	H		140 (11)
10	4-Me	*cis*	>10000
14	4-Me	*trans*	440 (35)
15	4-Et	*trans*	700 (18)
16	4-^{i}Pr	*trans*	7020 (510)
17	4-Ph	*trans*	>10000
18	4,4-$(Me)_2$		1312 (344)
19	3-Me	1*S*,3*R*	42 (6)
20	3-Me	1*R*,3*S*	73 (11)
21	3-Me	1*S*,3*S* and 1*R*,3*R*	>10000
22	3-Et	1*S*,3*R* and 1*R*,3*S*	280 (45)
23	3-^{i}Pr	1*S*,3*R* and 1*R*,3*S*	6270 (456)
24	3-^{n}Pr	1*S*,3*R* and 1*R*,3*S*	3300 (512)
25	3-Ph	1*S*,3*R* and 1*R*,3*S*	>10000
26	3,3-$(Me)_2$	±	880 (300)
27	3,5-$(Me)_2$	*cis*-(3*R*,5*S*)	140 (11)
28	3,5-$(Me)_2$	*trans*-(3*R*,5*S*)	>10000
29	3,3,5,5-$(Me)_4$		>10000

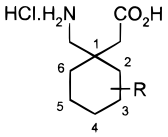
[a] IC_{50} is the concentration (nM) producing half-maximal inhibition of the specific binding of [^{3}H]Gabapentin to Gabapentin binding sites located on the $\alpha_2\delta$ subunit of a calcium channel. Values shown represent the geometric mean of at least three experiments with the standard error of the mean in parentheses.[14]

was recorded in deuteriomethanol. At ambient temperature this revealed a single compound, but on cooling the sample to −80 °C the spectrum showed two distinct conformations in a ratio of 2:1. These were assigned as the conformers with the aminomethyl moiety equatorial and axial. By comparison of the pair of methylene signals from the aminomethyl moiety and the analogous pair from the acetic acid moiety with the relative shifts of the methylene protons adjacent to the amine and carboxyl moieties in compounds **10** and **14**, it was possible to assign the peaks of the major conformer of Gabapentin to that with the aminomethyl in the equatorial position and the peaks from the minor conformer to that with the aminomethyl in the axial frame.

It can be seen from Table 1 that appending substituents to the 4-position of Gabapentin led to a reduction of binding affinity. Even *trans*-(1-(aminomethyl)-4-methylcyclohexyl)acetic acid hydrochloride (**14**) showed a 3-fold decrease in affinity, and larger groups reduced the binding still further. Evaluation of the 4,4-*gem*-dimethyl analogue **18** showed a significant decrease beyond the monomethylated analogue **14**. We investigated the preferred conformation of **18** in a manner similar to the methods used for Gabapentin, by recording low-temperature ^1H NMR spectra in deuteriomethanol. Analogously to the experiments with Gabapentin, it was determined that the preferred (2:1) solution conformation of **18** was that with the aminomethyl moiety in the equatorial frame. Thus it was deemed likely that the inactivity of **18** in the binding assay was due to steric factors around the 4-position and not to conformational biasing of the compound in favor of an inactive conformer.

Molecular modeling suggested that (*S*)-isobutylgaba could be overlayed with Gabapentin such that one of the terminal methyl groups of (*S*)-isobutylgaba over-

Table 2. Summary of $\alpha_2\delta$ Subunit Binding Site Affinities (nM) for 2-Substituted Gabapentin Analogues[14]



compd	R	stereochemistry	IC_{50} (nM)[a]
30	2-Me	1*S*,2*R* and 1*S*,2*S*	1300 (246)
31	2-OMe	1*S*,2*S* and 1*R*,2*R*	>10000
32	2-cC_6H_{11}	1*R*,2*R* and 1*S*,2*S*	>10000

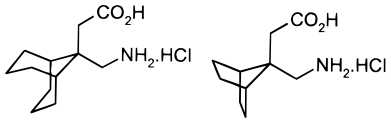
[a] See footnote to Table 1.

layed with the (*pro-R*) 3-position of Gabapentin.[16] Thus the 3-position of Gabapentin was also investigated (Table 1). The compounds were synthesized in the same manner as the 4-position analogues, which gave rise to the same degree of stereocontrol during the addition of cyanide or nitromethane. The conformations were elucidated in the same manner utilizing NMR techniques. It can be seen (Table 1) that appending a methyl group to the 3-position of Gabapentin to generate *cis*-(1*S*,3*R*)−(1-(aminomethyl)-3-methylcyclohexyl)acetic acid hydrochloride (**19**) gave a significant improvement in affinity (3-fold) for the Gabapentin binding site. The binding site shows a preference for this isomer over all the other possibilities, with its enantiomer, the (1*R*,3*S*)-isomer (**20**), having a reduced affinity of 73 nM compared to the (1*S*,3*R*)-isomer (**19**) which showed an affinity of 42 nM. Moreover, the (1*S*,3*S*)- and (1*R*,3*R*)-isomers have much lower affinities for the binding site (>10 μM) showed by the racemate (**21**). However, synthesis of the gem dimethylated 3,3-dimethylgabapentin (**26**) led to a large decrease in in vitro binding. A second methyl group was also introduced in the 5-position to produce the achiral analogue 1α,3α,5α-(1-(aminomethyl)-3,5-dimethylcyclohexyl)acetic acid hydrochloride (**27**) which showed comparable affinity to Gabapentin at 143 nM (its *trans*-isomer (**28**) was more than 100 times less active, as expected). The 3,3,5,5-tetramethyl analogue (**29**) was devoid of any in vitro activity at 10 μM.

As *cis*-(1*S*,3*R*)-(1-(aminomethyl)-3-methylcyclohexyl)-acetic acid hydrochloride (**19**) showed a significant improvement over its parent, the methyl group was extended to investigate larger groups. It can be seen from Table 1 that as the group increases in size beyond a methyl group, the affinity for the Gabapentin binding site on the $\alpha_2\delta$ subunit of a calcium channel decreases, with a large decrease progressing from ethyl (**22**) to isopropyl (**23**) or *n*-propyl (**24**).

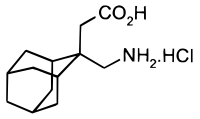
The 2-position of Gabapentin was also examined (Table 2). Synthesis was undertaken using the two routes outlined above (Schemes 1 and 2), but here much less cis/trans control was observed; in all the cases examined in this study a single pair of enantiomers was obtained by crystallization from an ethyl acetate/methanol mixture. These compounds were found to have the aminomethyl moiety in the equatorial conformation via use of analogous NMR techniques to those used for the 4- and 3-substituted analogues.[15] All the compounds examined existed in a conformation placing the 2-substituent in the equatorial position. In every case we found that the affinity for the Gabapentin binding site was significantly reduced when compared to that for Gabapentin itself.

To investigate the 2- and 6-axial positions when the aminomethyl moiety is in the equatorial position, it was necessary to join the 2- and 6-axial substituents into a second ring, otherwise the structure would simply undergo a ring flip to place the two 2- and 6-position substituents in an equatorial position with concomitant placement of the aminomethyl group in the axial position, which would lead to poor affinity for the Gabapentin binding site.[15] The bridged compound **33** was derived from bicyclo[3.3.1]nonan-9-one in the usual manner. At the same time the bridged compounds derived from bicyclo[2.2.1]heptan-7-one[19] and adamantanone, **34** and **35**, respectively, were also investigated. It was noticed that hydrolysis of the lactam precursor (**36**) of the bicyclic compound **33** gave two compounds,

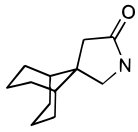


(33)

(34)



(35)



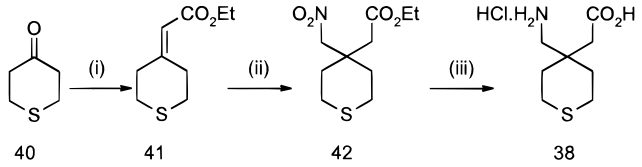
(36)

the desired amino acid hydrochloride **33** and unreacted lactam **36** in a 1:9 ratio. The lactam could be separated from the amino acid by trituration with ethyl acetate. Further hydrolysis of the recovered lactam (48 h) again provided a mixture of amino acid and lactam in a 1:9 ratio suggesting that there was an equilibrium under these conditions. To confirm this, a sample of the pure amino acid **33** was subjected to the same hydrolysis conditions again giving rise to amino acid and lactam in a ratio of 1:9. A similar scenario was discovered for the bicyclic compound **34** which gave a lactam:amino acid ratio of 1:1. However, in the case of the adamantane derivative **35**, no amino acid was detected, indicating that the equilibrium lay completely in favor of the lactam in this case. We repeated these experiments for Gabapentin and (*S*)-isobutylgaba and found that the equilibrium mixtures gave lactam:amino acid ratios of 1:9 and greater than 1:20, respectively. Thus it is clear that as the bulk of the lipophilic moiety increases, the equilibrium is displaced in favor of the lactam.

The two bridged compounds **33** and **34** were evaluated for their binding to the Gabapentin binding site located on the $\alpha_2\delta$ subunit of a calcium channel and were shown to have good affinity at 40 nM (±8 nM) and 44 nM (±9 nM), respectively, which compares to 140 nM for Gabapentin. This indicated that substitutions at the 2- and 6-axial positions in the preferred binding conformation of Gabapentin were well-tolerated.

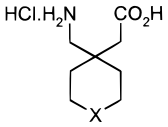
Heterocyclic Gabapentin analogues have also been examined with incorporation of oxygen (**37**), sulfur (**38**), or nitrogen (**39**) at the 4-position. For the tetrahydrothiopyran analogue **38**, the synthetic strategy had to be modified owing to incompatibility of the sulfur

Scheme 3[a]



[a] (i) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (ii) $MeNO_2$, tetramethylguanidine, reflux; (iii) $SnCl_2$, H_2O HCl.

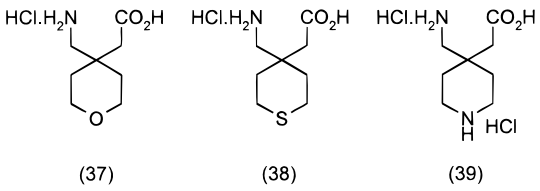
Table 3. Summary of $\alpha_2\delta$ Subunit Binding Site Affinities (nM) for Heterocyclic Gabapentin Analogues[14]



compd	X	IC_{50} (nM)[a]
37	O	2380 (537)
38	S	385 (95)
39	NH	>10000

[a] See footnote to Table 1.

moiety with the previously utilized catalytic hydrogenation. Thus a chemical reduction of the nitro group was used (Scheme 3). As can be seen from Table 3 the affinity for the Gabapentin binding site is in the order S > O > N. Thus as the heteroatom becomes more polar, the affinity for the binding site decreases. This implies that the 4-position of the Gabapentin molecule is sitting in a hydrophobic part of the $\alpha_2\delta$ subunit.



Pharmacological Evaluation. Two of the highest-affinity compounds, *cis*-(1*S*,3*R*)-(1-(aminomethyl)-3-methylcyclohexyl)acetic acid hydrochloride (**19**) and the bicyclic analogue **33**, were selected for in vivo evaluation in an animal model of epilepsy. The two compounds were examined to see if they were able to block semicarbazide (SEZ)-induced tonic seizures in mice. Groups of male TO mice (20−25 g) were injected subcutaneously with SEZ (750 mg/kg). The latency to the tonic extension of forepaws was noted. Any mice not convulsing within 2 h after SEZ were considered protected and given a maximum latency score of 120 min. Test compounds were administered intracerebroventricullarly (icv) or subcutaneously (sc) prior to the administration of SEZ. The time difference between administration of the test compound and SEZ is referred to as the pretreatment time. Gabapentin (Figure 1) when administered icv at doses of 1−100 μg/animal 0.5 h before SEZ produced a dose-dependent anticonvulsant effect with a minimum effective dose of 3 μg/animal. (1*S*,3*R*)-3-Methylgabapentin (**19**) was examined icv (Figure 2) at doses of 1−30 μg/animal and shown to have a minimum effective dose of 3 μg/animal as well. The bicyclic compound **33** was examined icv at doses of 3−100 μg/animal (Figure 3) and found to have a minimum effective dose of 30 μg/animal. The minimum effective dose values obtained compare to a minimum

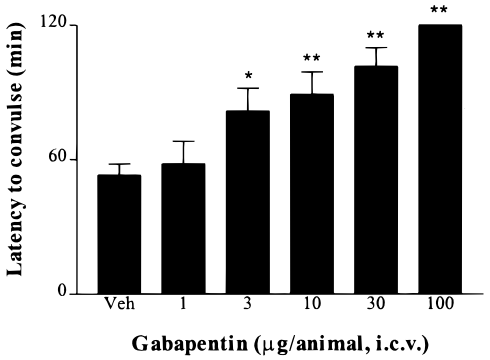


Figure 1. Effect of icv administration of doses of Gabapentin (1−100 μg/animal) on latency to seizure in a semicarbazide-induced seizure model of epilepsy in the mouse. (Each data point is the mean of at least seven experiments; *P < 0.05, **P < 0.01.)

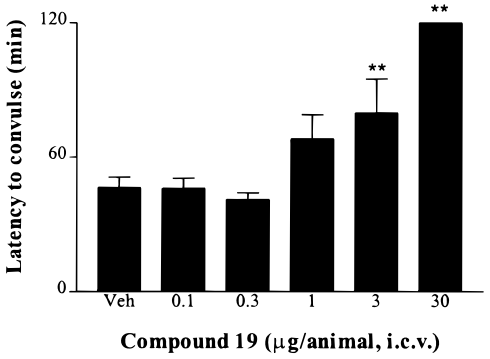


Figure 2. Effect of icv administration of doses of *cis*-(1*S*,3*R*)-(1-(aminomethyl)-3-methylcyclohexyl)acetic acid hydrochloride (**19**) (1−30 μg/animal) on latency to seizure in a semicarbazide-induced seizure model of epilepsy in the mouse. (Each data point is the mean of at least seven experiments; **P < 0.01.)

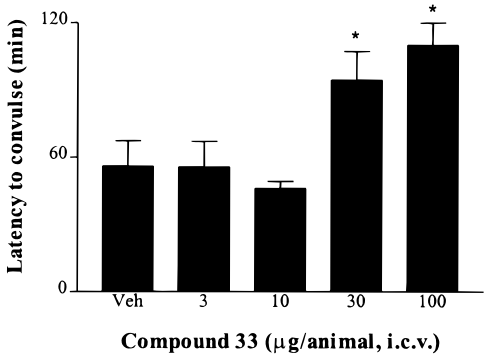


Figure 3. Effect of icv administration of doses of the gabapentin analogue **33** (3−100 μg/animal) on latency to seizure in a semicarbazide-induced seizure model of epilepsy in the mouse. (Each data point is the mean of at least seven experiments; *P < 0.05, **P < 0.01.)

effective dose for Vigabatrin of 30 μg when administered icv with a pretreatment time of 120 min. The minimum effective dose found for the bicyclic analogue **33** is somewhat worse than expected based on its binding affinity. This could be due to its propensity to cyclize to the spirolactam **36**, which does not bind to the Gabapentin binding site at any significant concentration.

Compound **19** was then evaluated alongside Gabapentin with both compounds being administered subcutaneously. Each compound was dosed at 30 mg/kg subcutaneously with pretreatment times of 30−240 min

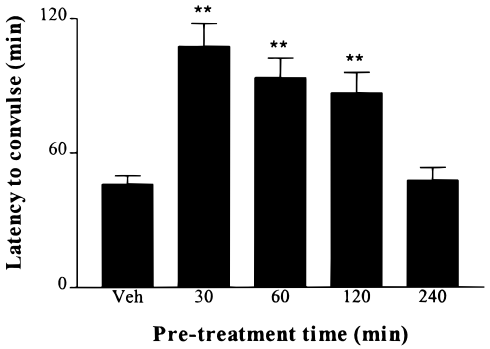


Figure 4. Effect of sc administration of Gabapentin (30 mg/kg) with different pretreatment times on the latency to seizure in a semicarbazide-induced seizure model of epilepsy in the mouse. (Each data point is the mean of at least 7 experiments; $^{**}P < 0.01$.)

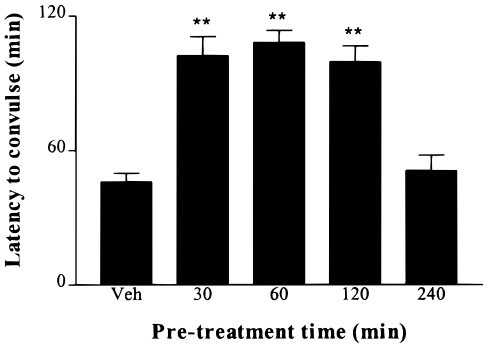


Figure 5. Effect of sc administration of *cis*-(1*S*,3*R*)-(1-(aminomethyl)-3-methylcyclohexyl)acetic acid hydrochloride (**19**) (30 mg/kg) with different pretreatment times on the latency to seizure in a semicarbazide-induced seizure model of epilepsy in the mouse. (Each data point is the mean of at least seven experiments; $^{**}P < 0.01$.)

(Figures 4 and 5). As can be seen, compound **19** gave a profile which was not significantly different from that of Gabapentin based on efficacy and duration of action.

Conclusion

The structure−activity relationships of a series of Gabapentin analogues have been examined, and from this it has been possible to identify a number of compounds having greater affinity for the Gabapentin binding site on the $\alpha_2\delta$ subunit of a calcium channel than Gabapentin itself. Two of these have been examined in an animal model of epilepsy using icv administration, and from this *cis*-(1*S*,3*R*)-(1-(aminomethyl)-3-methylcyclohexyl)acetic acid hydrochloride (**19**) has been identified as a potent novel anticonvulsant agent possessing a similar profile to that of Gabapentin in the animal model utilized. Compound **19** was further examined alongside Gabapentin utilizing sc administration which showed no significant difference between the two compounds.

Experimental Section

Melting points were determined with a Mettler FP80 or a Reichert Thermovar hot-stage apparatus. Proton NMR spectra were recorded on a Varian Unity +400 spectrometer; chemical shifts were recorded in ppm downfield from tetramethylsilane. IR spectra were recorded on a Perkin-Elmer System 2000 Fourier transform spectrophotometer. Mass spectra were recorded with a Finnigan MAT TSQ70 or Fisons VG Trio-2A instrument. Elemental analyses are within ±0.4% of theoretical values and were determined by Medac Ltd., Uxbridge, U.K. Normal phase silica gel used for chromatography was Merck no. 9385 (230−400 mesh), and reverse-phase silica gel used was Lichroprep RP-18 (230−400 mesh); both were supplied by E. Merck, AG, Darmstadt, Germany. Anhydrous solvents were purchased in Sureseal bottles from Fluka Chemicals Ltd., Glossop, U.K. All in vivo experiments were conducted in accordance with the U.K. Home Office Animals (Scientific Procedures) Act 1986.

General Route A (Scheme 1). Cyano(4-methylcyclohexylidene)acetic Acid Ethyl Ester (6). 4-Methylcyclohexanone (**5**) (15.30 mL, 125 mmol), ethyl cyanoacetate (13.20 mL, 124 mmol), ammonium acetate (0.96 g, 12.4 mmol), and glacial acetic acid (1.40 mL, 24.4 mmol) were dissolved in toluene (150 mL) and heated to reflux with azeotropic removal of water by a Dean−Stark trap for 24 h. The mixture was cooled and washed with H_2O (3 × 50 mL). The H_2O washes were combined and extracted with toluene (2 × 30 mL). The toluene extracts were combined with the original organic layer and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude oil was purified by Kugelrohr distillation to give 21.05 g (82%) of **6** as an oil: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 0.95 (3H, d, J = 6.8 Hz, −CHCH_3), 1.20−1.31 (2H, m, CH_2-CHCH_2), 1.35 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.80−1.90 (1H, m, CH_2CH(Me)CH_2), 1.90−2.10 (2H, m, CH_2CHCH_2), 2.15, 2.34, 3.02, 3.84 (each 1H, m, CH_2C(CN)(CH_2CO_2Et)CH_2), 4.27 (2H, q, J = 7.2 Hz, CH_2CH_3); IR (film) 2927, 2225, 1728 cm^{-1}; MS (CI) *m/e* 236, 209, 208 ([MH]$^+$, 100%). Anal. ($C_{12}H_{17}NO_2$) C, H, N.

***cis*-1-(Cyanomethyl)-4-methylcyclohexanecarbonitrile (7).** A solution of **6** (6.21 g, 30 mmol) in 95% ethanol (60 mL) was added to a solution of NaCN (1.47 g, 30 mmol) in H_2O (6 mL) and 95% ethanol (100 mL) and the resulting mixture heated to reflux. After 22 h the mixture was cooled and filtered. The solvent was removed in vacuo, and the crude oil was purified by flash chromatography (silica, heptane/ethyl acetate, 3:1) to give 3.208 g (66%) of **7** as a pale-yellow oil which solidified on standing: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 0.98 (3H, d, J = 5.6 Hz, CHCH_3), 1.30−1.40 (3H, m, CH_2CH(Me)CH_2), 1.50 (2H, m, CH_2CH(Me)CH_2), 1.73−1.92 (2H, m, CH_2C(CN)-(CH_2CN)CH_2), 2.10 (2H, m, CH_2C(CN)(CH_2CN)CH_2), 2.68 (2H, s, CH_2CN); IR (film) 2859, 2252, 2238, 1170 cm^{-1}; MS (CI) *m/e* 95, 136, 163 (100%, MH$^+$), 164, 182. Anal. ($C_{10}H_{14}N_2$) C, H, N.

***cis*-(1-Cyano-4-methylcyclohexyl)acetic Acid Ethyl Ester (8).** The bisnitrile **7** (2.00 g, 12.4 mmol) was dissolved in absolute ethanol (30 mL) and added to dry toluene (30 mL). The solution was chilled in an ice bath while saturating the solution with gaseous HCl. The flask was stoppered and the solution left to stand at room temperature for 24 h. The solvent was removed in vacuo, and the residue was triturated with diethyl ether (50 mL) to obtain a white solid which was collected by filtration and dried under vacuum. No purification was attempted, and the solid was used in next step. The crude product was dissolved in ice-cold H_2O (40 mL) and the pH adjusted with 1 N HCl to pH 1.5. The solution was stirred at room temperature for 20 h. The solution was extracted with ethyl acetate (3 × 30 mL), the organic extracts were washed with H_2O (30 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 1.94 g (75%) of **8** as a low-melting solid: ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 0.92−1.01 (3H, d, J = 7 Hz, CHCH_3), 1.27−1.31 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.37 (5H, m, CH_2CH(Me)CH_2), 1.70−1.73 (2H, m, 2 of CH_2C(CN)(CH_2-CO_2Et)CH_2), 2.10−2.13 (2H, m, 2 of CH_2C(CN)(CH_2CO_2Et)-CH_2), 2.54 (2H, s, CH_2CO_2Et), 4.21 (2H, q, J = 7.2 Hz, CH_2CH_3); IR (CH_2Cl_2) 2926, 2856, 2235, 1735 cm^{-1}; MS (CI) *m/e* 210 ([MH]$^+$, 12%), 182 ([MH − C_2H_5]$^+$, 100%), 164, 122. Anal. ($C_{12}H_{19}NO_2$) C, H, N.

***cis*-8-Methyl-2-azaspiro[4.5]decan-3-one (9).** The ester **8** (606 mg, 2.9 mmol) was dissolved in NH_3/EtOH (7%, 40 mL) and shaken over Raney nickel (catalytic) under an atmosphere of hydrogen gas (46 psi) at 30 °C. After 24 the catalyst was removed by filtration through Celite. The filtrate was collected and the solvent removed in vacuo to give 460 mg (95%) of **9** as

a white solid: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 0.86 (3H, d, J = 6 Hz, CH_3CH), 0.93−1.06 (2H, m, 2 of CH_2CHCH_2), 1.27−1.30 (3H, m, 2 of CH_2CHCH_2 and CH$_3$CH), 1.51 (2H, m, 2 of CH_2CCH_2), 1.62 (2H, m, 2 of CH_2CCH_2), 1.92 (2H, s, CH_2CO), 3.02 (2H, s, CH_2NH), 7.43 (1H, br s, NH); IR (CH_2Cl_2) 3189, 1679 cm^{-1}; MS (CI) *m/e* 168 ([MH]$^+$, 100%). Anal. ($C_{10}H_{17}$NO) C, H, N.

***cis*-(1-(Aminomethyl)-4-methylcyclohexyl)acetic Acid Hydrochloride (10).** The lactam (418 mg, 2.5 mmol) was heated to reflux in 5 N HCl (10 mL) for 5 h. The cooled solution was diluted with H_2O (10 mL) and washed with dichloromethane (2 × 15 mL). The aqueous layer was collected and the water removed in vacuo to give 551 mg (92%) of **10** as a white solid: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 0.88 (3H, d, J = 6 Hz, CH_3CH), 1.02−1.12 (2H, m, 2 of CH_2CHCH_2), 1.25−1.32 (3H, m, 2 of CH_2CHCH_2 and CH$_3$CH), 1.43−1.47 (4H, m, CH_2CCH_2), 2.33 (2H, s, CH_2CO$_2$H), 2.99 (2H, s, CH_2NH$_3^+$), 8.03 (3H, br s, NH$_3^+$), 12.33 (1H, br s, CO$_2$*H*); IR (MeOH) 3393, 2925, 2862, 1714, 1613 cm^{-1}; MS (CI) *m/e* 186 ([MH − HCl]$^+$,-100%), 168, 109. Anal. ($C_{10}H_{19}NO_2$·HCl) C, H, N.

General Route B (Scheme 2). *trans*-(4-Methyl-1-(nitromethyl)cyclohexyl)acetic Acid Ethyl Ester (12). Sodium hydride (60% dispersion in oil, 0.98 g, 24.45 mmol) was suspended in dry tetrahydrofuran (50 mL) and cooled to 0 °C in an ice bath. Triethylphosphonoacetate (5.12 mL, 25.67 mmol) was added dropwise over 5 min. After the addition was complete the mixture was stirred at 0 °C for an additional 15 min. 4-Methylcyclohexanone (3 mL, 24.45 mmol) was then added and the mixture allowed to warm to room temperature. After 1.5 h the mixture was partitioned between 2 N HCl (50 mL) and ethyl acetate (150 mL). The organic phase was separated, washed with brine (3 × 50 mL), and dried ($MgSO_4$) and the solvent removed in vacuo to give 5.05 g of a clear oil which was used without purification. The crude α,β-unsaturated ester (**11**) (2.94 g) was dissolved in tetrahydrofuran (20 mL) and stirred at 70 °C with nitromethane (1.75 mL, 32.3 mmol) and tetrabutylammonium fluoride (1 M in tetrahydrofuran, 24 mL, 24.0 mmol). After 18 h the mixture was cooled to room temperature, diluted with ethyl acetate (40 mL), and washed with 2 N HCl (20 mL) and then brine (2 × 30 mL). The organic phase was collected and dried ($MgSO_4$) and the solvent removed in vacuo. The residue was purified by flash chromatography (silica, ethyl acetate/heptane, 1:9) to give 2.74 g (70%) of **12** as a clear oil: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 1.27 (3H, d, J = 6 Hz, CHCH_3), 1.14 (2H, m, 2 of CH_2CHCH_2), 1.22−1.43 (6H, m, CH$_2$CH_3, CHCH$_3$, and 2 of CH_2CHCH_2), 1.60 and 1.75 (2H each, m, CH_2CCH_2), 2.59 (2H, s, CH_2CO$_2$Et), 4.15 (2H, q, J = 6 Hz, CH_2CH$_3$), 4.60 (2H, s, CH_2NO$_2$); IR (film) 1549, 1732 cm^{-1}; MS (APCI) *m/e* 244 ([MH]$^+$, 10%), 198 (100%). Anal. ($C_{12}H_{21}NO_4$) C, H, N.

***trans*-8-Methyl-2-azaspiro[4.5]decan-3-one (13).** The nitro ester **12** (2.70 g, 11.1 mmol) was dissolved in methanol (50 mL) and shaken over Raney nickel (catalytic) under an atmosphere of hydrogen gas (40 psi) at 35 °C. After 18 h the catalyst was removed by filtration through Celite and the solvent removed in vacuo. The residue was purified by flash chromatography (silica, ethyl acetate/heptane, 1:1) to give 0.72 g (39%) of **13** as a white solid: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 0.91 (3H, d, J = 6 Hz, CHCH_3), 1.01 (2H, m, 2 of CH_2CHCH_2), 1.36 (3H, m, CHCH$_3$ and 2 of CH_2CHCH_2), 1.58−1.79 (4H, m, CH_2CCH_2), 2.20 (2H, s, CH_2CO), 3.09 (2H, s, CH_2N), 5.75 (1H, br s, NH); IR (film) 1668, 1693, 3219 cm^{-1}; MS (APCI) *m/e* 168 ([MH]$^+$, 100%). Anal. ($C_{10}H_{17}$NO) C, H, N.

***trans*-(1-(Aminomethyl)-4-methylcyclohexyl)acetic Acid Hydrochloride (14).** The lactam (715 mg, 4.0 mmol) was heated to reflux in a mixture of 6 N HCl (15 mL) and 1,4-dioxane (5 mL). After 4 h the solvent was removed in vacuo and the solid residue recrystallized from a methanol/ethyl acetate/heptane mixture to give 664 mg (70%) of a white solid: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.88 (3H, d, J = 6 Hz, CH_3CH), 1.10 (2H, m, 2 of CH_2CHCH_2), 1.22 (3H, m, 2 of CH_2-CHCH_2 and CH$_3$CH), 1.48−1.53 (4H, m, CH_2CCH_2), 2.43 (2H, s, CH_2CO$_2$H), 2.85 (2H, s, CH_2NH$_3^+$), 7.92 (3H, br s, NH$_3^+$), 12.39 (1H, br s, CO$_2$*H*); MS (APCI) *m/e* 186 ([MH − HCl]$^+$, 100%). Anal. ($C_{10}H_{19}NO_2$·HCl) C, H, N.

***trans*-(1-(Aminomethyl)-4-ethylcyclohexyl)acetic Acid Hydrochloride (15).** Synthesized via route B in 16% overall yield from 4-ethylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.85 (3H, t, J = 6 Hz, CH_3), 1.06 (3H, m, CHEt and 2 of CH_2CHCH_2), 1.21 (4H, m, CH_2CH$_3$ and 2 of CH_2CHCH_2), 1.54 (4H, m, CH_2CCH_2), 2.44 (2H, s, CH_2CO$_2$H), 2.85 (2H, s, CH_2N), 8.03 (3H, br s, NH$_3^+$), 12.37 (1H, br s, CO$_2$*H*); MS (APCI) *m/e* 200 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl), C, H, N.

***trans*-(1-(Aminomethyl)-4-isopropylcyclohexyl)acetic Acid Hydrochloride (16).** Synthesized via route B in 13% overall yield from 4-isopropylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.85 (6H, d, J = 6 Hz, CH(CH_3)$_2$), 0.90−1.28 (5H, m, CH_2CHCH_2), 1.35−1.63 (5H, m, CH(CH$_3$)$_2$ and CH_2CCH_2), 2.43 (2H, s, CH_2CO$_2$H), 2.84 (2H, s, CH_2N), 8.00 (3H, br s, NH$_3^+$), 12.28 (1H, br s, CO$_2$*H*); MS (APCI) *m/e* 214 ([MH − HCl]$^+$, 100%). Anal. ($C_{12}H_{23}NO_2$·HCl), C, H, N.

***trans*-(1-(Aminomethyl)-4-phenylcyclohexyl)acetic Acid Hydrochloride (17).** Synthesized via route B in 12% overall yield from 4-phenylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 1.20 (2H, m, 2 of CH_2CHCH_2), 1.67 (6H, m, CH_2CCH_2 and 2 of CH_2CHCH_2), 2.43 (1H, CHPh), 2.60 (2H, CH_2CO$_2$H), 2.91 (2H, m, CH_2N), 7.16−7.33 (5H, m, Ph), 8.02 (3H, br s, NH$_3^+$), 12.44 (1H, br s, CO$_2$*H*); MS (APCI) *m/e* 248 ([MH − HCl]$^+$, 100%). Anal. ($C_{15}H_{21}NO_2$·HCl), C, H, N.

(1-(Aminomethyl)-4,4-dimethylcyclohexyl)acetic Acid Hydrochloride (18). Synthesized via route A in 23% overall yield from 4,4-dimethylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.89 (6H, s, 2 CH_3), 1.24 (4H, m, 2 CH_2), 1.40 (4H, m, 2 CH_2), 2.39 (2H, s, CH_2), 2.92 (2H, s, CH_2), 7.94 (3H, br s, NH$_3^+$), 12.36 (1H, br s, CO$_2$*H*); IR (film) 3409, 2946, 1713, 1594, 1498, 1408, 1234, 1218, 1186 cm^{-1}; MS (APCI) *m/e* 200 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{22}NO_2$Cl) C, H, N.

***cis*-(1*S*,3*R*)-(1-(Aminomethyl)-3-methylcyclohexyl)acetic Acid Hydrochloride (19).** Synthesized via route B in 33% overall yield from (*R*)-3-methylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.74−0.91 (5H, m, CH_2CH(CH_3)CH$_2$), 1.02−1.18 (1H, m, CHCH$_3$), 1.38−1.65 (6H, m, CH_2CH_2CCH_2), 2.46 (2H, s, CH_2CO$_2$H), 2.84 (2H, s, CH_2NH$_3^+$), 7.97 (3H, br s, NH$_3^+$), 12.37 (1H, br s, CO$_2$*H*); IR (KBr disk) 1187, 1214, 1400, 1515, 1710, 2922, 3370 cm^{-1}; MS (APCI) *m/e* 186 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl) C, H, N.

***cis*-(1*R*,3*S*)-(1-(Aminomethyl)-3-methylcyclohexyl)acetic Acid Hydrochloride (20).** (±)−(**20**) was synthesized via route B in 30% overall yield from 3-methylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.74−0.91 (5H, m, CH_2CH(CH_3)-CH$_2$), 1.02−1.18 (1H, m, CHCH$_3$), 1.38−1.65 (6H, m, CH_2CH_2-CCH_2), 2.46 (2H, s, CH_2CO$_2$H), 2.84 (2H, s, CH_2NH$_3^+$), 7.97 (3H, br s, NH$_3^+$), 12.37 (1H, br s, CO$_2$*H*); IR (KBr disk) 1187, 1214, 1400, 1515, 1710, 2922, 3370 cm^{-1}; MS (APCI) *m/e* 186 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl) C, H, N. Pure **20** was separated from **19** by HPLC using a Chirobiotic T (macrocyclic glycopeptide antibiotic) column, 250 × 4.6 mm, supplied by BAS Technicol, eluting with EtOH/H_2O (95:5 isocratic). Compound **20** had a retention time of 25−29 min, and compound **19** had a retention time of 30.5−36 min.

(±)-*trans*-(1-(Aminomethyl)-3-methylcyclohexyl)acetic Acid Hydrochloride (21). Synthesized via route A in 13% overall yield from 3-methylcyclohexanone: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 0.69−0.79 (1H, m, 1 of CH_2CH(CH$_3$)CH$_2$), 0.82 (3H, d, J = 6 Hz, CHCH_3), 0.87−0.90 (1H, m, CH_2CH-(CH$_3$)CH$_2$), 1.12−1.20 (1H, m, CHCH$_3$), 1.34−1.50 and 1.60−1.63 (each 3H, m, CH_2CH_2CCH_2), 2.30 (2H, s, CH_2CO$_2$H), 3.01 (2H, s, CH_2NH$_3^+$), 7.93 (3H, br s, NH$_3^+$), 12.04 (1H, br s, CO$_2$*H*); IR (MeOH) 2924, 2353, 1708, 1599, 1523, 1454, 1216 cm^{-1}; MS (CI) *m/e* 186 ([MH − HCl]$^+$, 13%), 168 (100%), 109. Anal. ($C_{10}H_{19}NO_2$·1.1HCl) C, H, N. Sample shown to be a homogeneous peak by HPLC utilizing a C_{18} prodigy ODS3 column eluting with a gradient of 40% MeCN/60% H_2O to 100% MeCN and also, using the same column, eluting with a gradient of 30% MeOH/70% H_2O to 100% MeOH.

(±)-*cis*-(1-(Aminomethyl)-3-ethylcyclohexyl)acetic Acid Hydrochloride (22). Synthesized via route B in 15% overall

yield from 3-ethylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.75 (2H, m, CH_2), 0.84 (3H, t, J = 7 Hz, CH_3), 1.15 (3H, m), 1.38 (2H, m), 1.54 (3H, m), 1.69 (1H, m), 2.45 (2H, s, CH_2), 2.84 (2H, s, CH_2), 8.02 (3H, br s, NH_3^+), 12.36 (1H, br s, CO_2H); IR (film) 3400, 2926, 1710, 1607, 1505, 1457, 1403, 1205 cm^{-1}; MS (APCI) *m/e* 200 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl) C, H, N.

(±)-*cis*-(1-(Aminomethyl)-3-isopropylcyclohexyl)acetic Acid Hydrochloride (23). Synthesized via route B in 17% overall yield from 3-isopropylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.83 (6H, d, J = 6 Hz, 2 CH_3), 0.86 (1H, m), 1.15 (1H, m), 1.20−1.45 (3H, m), 1.49−1.66 (4H, m), 2.45 (2H, s, CH_2), 2.85 (2H, s, CH_2), 7.98 (3H, br s, NH_3^+), 12.36 (1H, br s, CO_2H); IR (film) 3402, 2930, 2871, 1710, 1608, 1505, 1460, 1403, 1286, 1207 cm^{-1}; MS (APCI) *m/e* 214 ([MH − HCl]$^+$, 100%). Anal. ($C_{12}H_{23}NO_2$·HCl) C, H, N.

(±)-*cis*-(1-(Aminomethyl)-3-*n*-propylcyclohexyl)acetic Acid Hydrochloride (24). Synthesized via route B in 19% overall yield from 3-*n*-propylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.65−0.90 (2H, m), 0.84 (3H, t, J = 7 Hz, CH_3), 1.11 (3H, m), 1.26 (2H, m), 1.35−1.69 (6H, m), 2.46 (2H, s, CH_2), 2.83 (2H, s, CH_2), 8.01 (3H, br s, NH_3^+), 12.36 (1H, br s, CO_2H); IR (film) 3393, 2926, 2870, 1711, 1607, 1507, 1458, 1403, 1286, 1259, 1206 cm^{-1}; MS (APCI) *m/e* 214 ([MH − HCl]$^+$, 100%). Anal. ($C_{12}H_{23}NO_2$·HCl) C, H, N.

(±)-*cis*-(1-(Aminomethyl)-3-phenylcyclohexyl)acetic Acid Hydrochloride (25). Synthesized via route B in 30% overall yield from 3-phenylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 1.22−1.85 (8H, m, 4 CH_2 ring), 2.60 (2H, s, CH_2-CO_2H), 2.78 (1H, m, C*H*Ph), 2.90 (2H, s, CH_2N), 7.11−7.35 (5H, m, Ar), 7.96 (3H, br s, NH_3^+), 12.45 (1H, br s, CO_2H); IR (KBr disk) 1406, 1497, 1592, 1715, 2927 cm^{-1}; MS (ES$^+$) *m/e* 248 ([MH − HCl]$^+$, 100%). Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N.

(1-(Aminomethyl)-3,3-dimethylcyclohexyl)acetic Acid Hydrochloride (26). Synthesized via route A in 21% overall yield from 3,3-dimethylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.90 (3H, s, Me), 0.92 (3H, s, Me), 1.15−1.49 (8H, m, 4 CH_2), 2.45 (2H, s, CH_2CO_2H), 2.90 (2H, br q, J = 13.5 Hz, CH_2NH$_3$), 7.96 (3H, br s, NH_3), 12.36 (1H, br s, CO_2H); IR (film) 2930, 1728, 1272, 1123 cm^{-1}; MS (APCI) *m/e* 200 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl) C, H, N.

1α,3α,5α-(1-(Aminomethyl)-3,5-dimethylcyclohexyl)-acetic Acid Hydrochloride (27). Synthesized via route B in 11% overall yield from *cis*-3,5-dimethylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.47 (1H, m, 1 of CHCH_2CH), 0.77−0.91 (8H, m, 2-H$_{ax}$, 6-H$_{ax}$, and 2 CH_3), 1.46−1.63 (5H, m, 2-H$_{eq}$, 6-H$_{eq}$, 2 C*H*CH$_3$, and 4-H$_{eq}$), 2.45 (2H, s, CH_2CO_2H), 2.84 (2H, s, CH_2NH$_3^+$), 8.00 (3H, br s, NH$_3^+$), 12.37 (1H, br s, CO_2H); MS (APCI) *m/e* 200 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl), C, H, N.

1α,3β,5β-(1-(Aminomethyl)-3,5-dimethylcyclohexyl)-acetic Acid Hydrochloride (28). Synthesized via route A in 10% overall yield from *cis*-3,5-dimethylcyclohexanone: ^{1}H NMR (400 MHz) (DMSO-d_6) δ 0.43 (1H, m, 1 of CHCH_2CH), 0.75−0.90 (8H, m, 2-H$_{ax}$, 6-H$_{ax}$, and 2 CH_3), 1.47−1.64 (5H, m, 2-H$_{eq}$, 6-H$_{eq}$, 2 C*H*CH$_3$, and 4-H$_{eq}$), 2.31 (2H, s, CH_2CO_2H), 3.00 (2H, s, CH_2NH$_3^+$), 7.94 (3H, br s, NH$_3^+$), 12.32 (1H, br s, CO_2H); MS (APCI) *m/e* 200 ([MH − HCl]$^+$, 100%). Anal. ($C_{11}H_{21}NO_2$·HCl) C, H, N.

(1-(Aminomethyl)-3,3,5,5-tetramethylcyclohexyl)acetic Acid Hydrochloride (29). Synthesized via route A in 6% overall yield from 3,3,5,5-tetramethylcyclohexanone: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 0.94 (6H, s, 2 CH_3), 1.01 (6H, s, 2 CH_3), 1.20 (4H, m, CH_2), 1.40 (2H, d, CH_2), 2.53 (2H, s, CH_2), 2.93 (2H, br s, CH_2), 7.85 (3H, br s, NH_3^+), 12.38 (1H, br s, CO_2H); IR (film) 3429, 2954, 1714 cm^{-1}; MS (APCI) *m/e* 228 ([MH − HCl]$^+$, 100%). Anal. ($C_{13}H_{24}NO_2$·HCl), C, H, N.

(±)-(1-(Aminomethyl)-2-methylcyclohexyl)acetic Acid Hydrochloride (30). Synthesized via route A in 15% overall yield from 2-methylcyclohexanone: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 0.78 (3H, d, J = 6 Hz, CH_3), 1.18−1.65 (9H, m, 4 CH_2, C*H*CH$_3$), 2.22 (1H, J = 15 Hz, 1 of CH_2CO_2H), 2.49 (1H, J = 15 Hz, 1 of CH_2CO_2H), 2.91 (1H, d, J = 14 Hz, 1 of CH_2NH$_3^+$), 3.15 (1H, d, J = 14 Hz, 1 of CH_2NH$_3^+$), 7.90 (3H, br s, NH_3^+), 12.36 (1H, br s, CO_2H); MS (APCI) *m/e* 186 ([MH − HCl]$^+$, 90%). Anal. ($C_{11}H_{21}NO_2$·HCl) C, H, N.

(±)-(1-(Aminomethyl)-2-methoxycyclohexyl)acetic Acid Hydrochloride (31). Synthesized via route A in 12% overall yield from 2-methoxycyclohexanone: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 1.17−1.90 (8H, m, 4 CH_2), 2.46 (2H, s, CH_2CO_2H), 2.98 (2H, m, CH_2NH$_3^+$), 3.09 (1H, m, C*H*OMe), 3.24 (3H, s, OCH_3), 7.90 (3H, br s, NH_3^+), 12.25 (1H, br s, CO_2H); MS (APCI) *m/e* 202 ([MH − HCl]$^+$, 85%). Anal. ($C_{10}H_{19}NO_3$·HCl), C, H, N.

(±)-(1-(Aminomethyl)-2-cyclohexylcyclohexyl)acetic Acid Hydrochloride(32). Synthesized via route A in 13% overall yield from 2-cyclohexylcyclohexanone: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 0.87−1.75 (2H, m, 9 CH_2 and 2 C*H*), 2.47 (2H, s, CH_2CO_2H), 2.97 (2H, ABq, J = 13.5 and 13 Hz, CH_2NH$_3^+$), 7.96 (3H, br s, NH_3^+), 12.38 (1H, br s, CO_2H); MS (CI$^+$) *m/e* 254 ([MH − HCl]$^+$, 7%), 236 (100%). Anal. ($C_{15}H_{27}NO_2$·HCl) C, H, N.

(9-(Aminomethyl)bicyclo[3.3.1]non-9-yl)acetic Acid Hydrochloride (33). Synthesized via route A in 6% overall yield from bicyclo[3.3.1]nonan-9-one: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 1.24−1.66 (8H, m), 1.74−2.16 (6H, m), 2.63 (2H, s, CH_2CO_2H), 3.22 (2H, s, CH_2NH$_3^+$), 7.90 (3H, br s, NH$_3^+$), 12.43 (1H, br s, CO_2H); IR (MeOH) 3419, 3172, 2934, 1717, 1614 cm^{-1}; MS (CI) *m/e* 194 (100%, MH$^+$ − H_2O). Anal. ($C_{12}H_{21}$-NO_2·1.8HCl) C, H, N. Sample shown to be a homogeneous peak by HPLC utilizing a C_{18} prodigy ODS3 column eluting with a gradient of 40% MeCN/60% H_2O to 100% MeCN and also, using the same column, eluting with a gradient of 30% MeOH/70% H_2O to 100% MeOH.

(7-(Aminomethyl)bicyclo[2.2.1]hept-7-yl)acetic Acid Hydrochloride (34). Synthesized via route A in 7% overall yield from bicyclo[2.2.1]heptanan-7-one: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 1.24 (4H, m, 4 C*H*), 1.73 (4H, m, 4 C*H*), 2.00 (2H, s, 2 C*H*), 2.50 (2H, s, CH_2), 3.31 (2H, s, CH_2), 7.78 (3H, br s, NH_3^+), 12.36 (1H, br s, CO_2H); IR (film) 3439, 2957, 2887, 1703, 1675, 1610 cm^{-1}; MS (APCI) *m/e* 184 ([MH − HCl]$^+$, 90%). Anal. ($C_{10}H_{18}NO_2Cl$) C, H, N.

Spiro[pyrrolidine-3,2′-tricyclo[3.3.1.1^{3,7}]decane]-5-one (36). Synthesized via route A in 19% overall yield from adamantan-2-one: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 1.60−1.87 (14H, m, 4 C*H* and 5 CH$_2$), 2.37 (2H, s, CH_2), 3.35 (2H, s, CH_2), 5.67 (1H, br s, N*H*); IR (film) 3402, 3236, 1674, 1487, 1452 cm^{-1}; MS (ES$^+$) *m/e*: 206 ([MH]$^+$, 100%). Anal. ($C_{13}H_{19}NO$) C, H, N.

(4-(Aminomethyl)tetrahydropyran-4-yl)acetic Acid Hydrochloride (37). Synthesized via route A in 3% overall yield from tetrahydro-4H-pyran-4-one: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 1.40−1.60 (4H, m, 2 CH_2CH$_2$-O-), 2.53 (2H, s, CH_2-CO_2H), 3.02 (2H, s, CH_2−NH$_3^+$), 3.50−3.70 (4H, m, CH_2−O−CH_2), 8.02 (3H, br s, NH_3^+), 12.45 (1H, br s, CO_2H); IR (film) 1026, 1514, 1611, 1712, 2936 cm^{-1}; MS (ES$^+$) *m/e*: 174 ([(M − HCl)H]$^+$, 95%). Anal. ($C_8H_{15}NO_3$·HCl) C, H, N.

(Tetrahydrothiopyran-4-ylidene)acetic Acid Ethyl Ester (41). A solution of tetrahydrothiopyran-4-one (2.50 g, 21.6 mmol) and (carbethoxymethylene)triphenylphosphorane (9.0 g, 25.9 mmol) was heated to reflux in toluene (30 mL) for 18 h. The mixture was cooled to room temperature and evaporated to dryness in vacuo. The residue was purified by flash chromatography (silica, ether/hexane, 1:1) to give 3.77 g (94%) of **42** as an oil: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 1.28 (3H, t, J = 7.2 Hz, CH_3), 2.50−2.55 (2H, m, CH_2CH$_2$S), 2.74−2.80 (4H, m, CH_2−S−CH_2), 3.18−3.21 (2H, m, CH_2CH$_2$S), 4.15 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 5.67 (1H, s, vinylic); IR (film) 1649, 1713, 2908, 2981 cm^{-1}; MS (CI) *m/e* 187 ([M + H]$^+$, 15%). Anal. ($C_9H_{14}O_2S$) C, H, N.

(4-(Nitromethyl)tetrahydrothiopyran-4-yl)acetic Acid Ethyl Ester (42). The unsaturated ethyl ester **42** (1.00 g, 5.3 mmol) was heated to reflux under nitrogen in nitromethane (50 mL) with tetramethylguanidine (0.5 mL) for 10 h. The mixture was cooled to room temperature, diluted with ethyl acetate (100 mL), and washed with 1 N HCl (3 × 50 mL). The organic solution was separated and dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by flash chromatography (silica, ethyl acetate/heptane, 1:1) to give

0.41 g (31%) of **43** as a colorless oil: ^{1}H NMR ($CDCl_3$) (400 MHz) δ 1.28 (3H, t, J = 7.2 Hz, CH_3), 1.85−2.00 (4H, m, 2 CH_2CH_2S), 2.54 (2H, s, CH_2CO_2Et), 2.60−2.75 (4H, m, CH_2−S−CH_2), 4.17 (2H, q, J = 7.2 Hz, CH_2CH_3), 4.72 (2H, s, CH_2-NO_2); IR (film) 1374, 1458, 1549, 1728 cm^{-1}; MS (EI) *m*/*e* 247 ([M]$^+$, 100%). Anal. ($C_{10}H_{17}NO_4S$), C, H, N.

(4-(Aminomethyl)tetrahydrothiopyran-4-yl)acetic Acid Hydrochloride (38). The nitro ester **43** (0.40 g, 1.62 mmol) was dissolved in concentrated hydrochloric acid (20 mL) with tin(II) chloride (1.50 g). The mixture was heated to 100 °C for 2 h. The mixture was then evaporated to dryness in vacuo. The residue was purified by reverse-phase chromatography to give 0.10 g (26%) of **39** as colorless crystals: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 1.65−1.80 (4H, m, 2 CH_2CH_2−S), 2.44 (2H, s, CH_2CO_2H), 2.54−2.67 (4H, m, CH_2−S−CH_2), 2.95 (2H, s, $CH_2NH_3^+$), 7.99 (3H, br s, NH_3^+), 12.42 (1H, br s, CO_2H); IR (film) 1525, 1582, 1712, 2959, 3382 cm^{-1}; MS (ES$^-$) *m*/*e* 188 ([(M − HCl) − H]$^-$, 100%). Anal. ($C_8H_{15}NO_2S$·HCl) C, H, N.

(4-(Aminomethyl)piperidin-4-yl)acetic Acid Dihydrochloride (39). Synthesized via route A in 3% overall yield from *N*-(*tert*-butoxycarbonyl)-4-piperidone: ^{1}H NMR (DMSO-d_6) (400 MHz) δ 1.70 (4H, m, CH_2CCH_2), 2.14 (2H, s, CH_2-CO_2H), 2.9−3.2 (6H, m, $CH_2NH_3^+$ and $CH_2NH_2^+CH_2$), 8.15 (3H, br s, NH_3^+), 8.81 (2H, br s, NH_2^+), 12. 14 (br s, CO_2H); MS (ES$^+$) *m*/*e* 173 ([(MH − 2HCl)]$^+$, 80%). Anal. ($C_8H_{16}N_2O_2$·2HCl) C, H, N.

References

(1) Taylor, C. P. The Role of Gabapentin. In *New Trends in Epilepsy Management*; Chadwick, D., Ed.; Royal Society of Medicine Services Ltd.: London, 1993; pp 13−40.

(2) Singh, L.; Field, M. J.; Ferris, P.; Hunter, J. C.; Oles, R. J.; Williams, R. G.; Woodruff, G. N. The antiepileptic agent Gabapentin (Neurontin) possesses anxiolytic-like and antinociceptive actions that are reversed by D-serine. *Psycopharmacol. Berl.* **1996**, *127* (1), 1−9.

(3) Rosner, H.; Rubin, L.; Kestenbaum, A. Gabapentin adjunctive therapy in neuropathic pain states. *Clin. J. Pain* **1996**, *12* (1), 56−8.

(4) Zapp, J. J. Postpoliomyelitis pain treated with Gabapentin. *Am. Fam. Physician* **1996**, *53* (8), 2442, 2445.

(5) Satzinger, G. Antiepileptics from gamma-aminobutyric acid. *Arzneimittelforschung* **1994**, *44*, 261−6.

(6) Nanavati, S. M.; Silverman, R. B. Design of potential anticonvulsant agents: mechanistic classification of GABA aminotransferase inactivators. *J. Med. Chem.* **1989**, *32*, 2413−21.

(7) Bowery, N. G.; Pratt, G. D. $GABA_B$ receptors as targets for drug action. *Arzneimittelforschung* **1992**, *42* (2A), 215−23.

(8) Taylor, C. P. Emerging perspectives on the mechanism of action of Gabapentin. *Neurology* **1994**, *44*, S10−16.

(9) Thurlow, R. J.; Brown, J. P.; Gee, N. S.; Hill, D. R.; Woodruff, G. N. [^{3}H]Gabapentin may label a system-L-like neutral amino acid carrier in brain. *Eur. J. Pharmacol.* **1993**, *247*, 341−55.

(10) McLean, M. J. Gabapentin. *Epilepsia* **1995**, *36*, S73−86.

(11) Beydoun, A.; Uthman, B. M.; Sackellares, J. C. Gabapentin: pharmacokinetics, efficacy, and safety. *Clin. Neuropharmacol.* **1995**, *18* (6), 469−81.

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

(13) Hill, D. R.; Suman-Chauhan, N.; Woodruff, G. N. Localization of [^{3}H]Gabapentin to a novel site in rat brain: autoradiographic studies. *Eur. J. Pharmacol.* **1993**, *244*, 303−9.

(14) Gee, N. S.; Brown, J. P.; Dissanayake, V. U. K.; Offord, J.; Thurlow, R.; Woodruff, G. N. The novel anticonvulsant drug, Gabapentin (Neurontin), binds to the $\alpha_2\delta$ subunit of a calcium channel. *J. Biol. Chem.* **1996**, *271* (10), 5768−76.

(15) Bryans, J. S.; Davies, N.; Gee, N. S.; Horwell, D. C.; Kneen, C. O.; Morrell, A. I.; O'Neill, J. A.; Ratcliffe, G. S. Investigation into the preferred conformation of gabapentin for interaction with its binding site on the $\alpha_2\delta$ subunit of a calcium channel. *BioOrg. Med. Chem. Lett.* **1997**, *7* (19), 2481−4.

(16) Bryans, J. S.; Horwell, D. C.; Ratcliffe, G. S. Unpublished results.

(17) Griffiths, G.; Mettler, H.; Mills, L. S.; Previdoli, F. Novel syntheses of Gabapentin via addition of hydrocyanic acid to cyclohexylidenemalonate or cyano(cyclohexylidene)acetate. *Helv. Chim. Acta* **1991**, *74*, 309−14.

(18) Nagata, W.; Yoshioka, M. Hydrocyanation of conjugated carbonyl compounds. *Org. React.* **1977**, *25*, 255−476.

(19) Gassman, P. G.; Marshall, J. L. Bicyclo[2,2,1]hepten-7-one. *Organic Syntheses*; Wiley: New York, 1973; Coll. Vol. 5, pp 91, 424.

JM970649N