Structural Features Resulting in Convulsive Activity of Carbapenem Compounds: Effect of C-2 Side Chain

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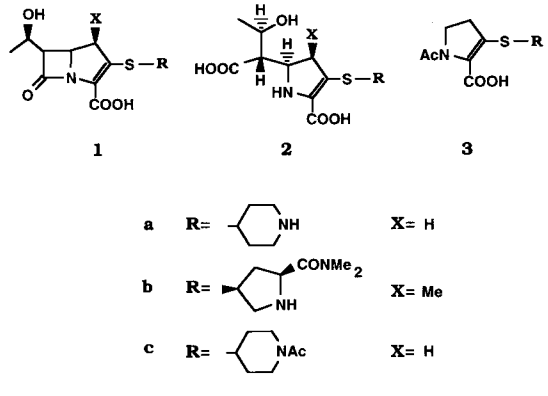
The neurotoxicity of meropenem was much lower than that of both imipenem and panipenem after intraventricular administration to mice. To clarify the major structural features responsible for the induction of convulsions by carbapenem antibiotics, the structure-activity relationship on convulsant activity was investigated in *N*-acetyl-2-pyrroline and cyclopentene derivatives which correspond to the 5-membered ring containing the C-2 side chain of carbapenem antibiotics. Among these derivatives, compounds with strong basicity in the side chain showed convulsant activity similar to that of the parent carbapenem compounds. In addition to the strength of the basicity of the amino group, the distance from the carboxyl to the amino group and steric crowding around the amino group also appeared to play an important role in the induction of convulsions. The results of gamma aminobutyric acid ($GABA_A$) receptor binding assays indicated that the induction of convulsions was caused predominantly by the inhibition of $GABA_A$-mediated inhibitory transmission. However, the *in vivo* convulsant activity of some of these compounds did not correlate with their *in vitro* inhibitory effect on $GABA_A$ receptor binding.

Induction of convulsions is a well known side effect of β-lactam antibiotics, such as penicillins and cephalosporins[1~3]. The carbapenem antibiotic, imipenem, administered together with the renal dehydropeptidase (DHP-I) inhibitor, cilastatin, has also been reported to induce convulsions clinically[4,5]. We have previously reported that the neurotoxic activity of meropenem, a 1-β-methylcarbapenem stable to DHP-I, was much lower than that of imipenem after intravenous and intraventricular injection to mice and rabbits[6,7]. The distribution of meropenem in the brain after intravenous administration to mice was similar to that of imipenem, suggesting that there was no difference in their blood brain barrier permeability. In another comparative study of imipenem/cilastatin and meropenem in mice, both imipenem alone and imipenem/cilastatin, but not meropenem, were found to cause a significant potentiation of metrazole-induced seizures[8].

We have recently studied the structure-activity relationships of 1-β-methylcarbapenem, carbapenem, and penem compounds with various C-2 side chains to determine their convulsant activity[9]. We found no significant difference between 1-β-methylcarbapenem and carbapenem in the induction of convulsions, and our results suggested that the presence of the basic amino group in the C-2 side chain, but not the carbapenem skeleton itself, was an important factor in inducing convulsions. Furthermore, the strength of basicity of the amino group was correlated with convulsant activity.

In the present study, carried out in an attempt to clarify the moiety responsible for convulsant activity, we investigated the relationship of structure to this convulsive activity. In this regard, we examined the degradation products of carbapenems (**2**), as well as *N*-acetyl-2-pyrrolines (**3**), which represent part of the carbapenem structure, and cyclopentene derivatives (**4**), which have intrinsic structures that might evoke convulsant activity. Since it has been reported that, when they accumulate in the central nervous system, imipenem and cephalosporins may induce convulsions through the inhibition of gamma-aminobutyric acid ($GABA_A$), receptor binding[10~12], we also examined the effects of these derivatives on $GABA_A$ receptor binding.

Fig. 1. Carbapenems and the products of β-lactam ring cleavage, and *N*-acetyl-2-pyrroline derivatives.



Chemistry

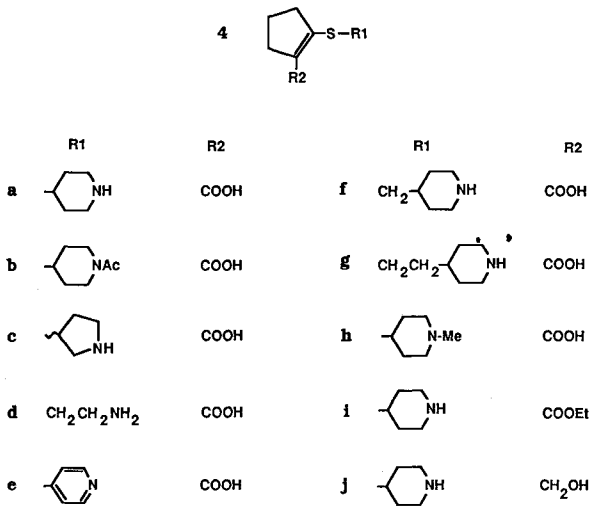
The degradation products of carbapenems (**2a** and **2c**) were prepared from the corresponding carbapenems as described for the preparation of **2b**[13].

The *N*-acetyl-2-pyrrolines (**3a**~**3c**) were synthesized by a procedure similar to that reported by TAKEMURA *et al.*[14] (Chart 1). The amino acid ester (**5**), prepared from Boc-β-alanine, was deprotected and then treated with *t*-butyl bromoacetate and triethylamine (Et_3N) in DMF to afford the corresponding amine (**6**) in 41% yield. After the acetylation of **6**, Dieckmann-type cyclization of **7**, using *t*-BuOK as a base in toluene, followed by treatment with diphenyl chlorophosphate in a mixture of toluene and DMF, afforded the enol phosphate (**8**) in 60% yield from **6**. Subsequently, the C-3 side chains were introduced into **8** by reaction with the corresponding thiolate anions to yield the protected 3-substituted *N*-acetylpyrrolines (**9**). Finally, the removal of the *t*-butyl and Boc groups in **9**, using trifluoroacetic acid followed by column chromatography purification on Diaion CHP-20P, provided the target compounds (**3**).

The cyclopentene model structure fragments (**4a**~**4g**) were synthesized by the route outlined in Chart 2. Enol phosphate **10** was obtained from ethyl 2-oxocyclopentene-carboxylate with diphenyl chlorophosphate and sodium hydride in THF in 94% yield. Introduction of the C-2 side chains was performed with the corresponding thiols using sodium hydride as a base, to yield the protected 2-substituted cyclopentene esters (**11**). After the removal of the Boc protecting group of **11**, the ethyl ester of **12** was hydrolyzed to afford the desired compounds (**4a**~**4g**).

Reductive methylation of the ester (**4i**), followed by hydrolysis, gave the *N*-methyl derivative (**4h**). The corresponding hydroxymethyl compound (**4j**) was obtained by reduction of the ethyl ester (**11a**) with DIBAL, followed by deprotection. Each thiol was prepared according to established methods[15,16].

Fig. 2. *S*-Substituted cyclopentene derivatives.



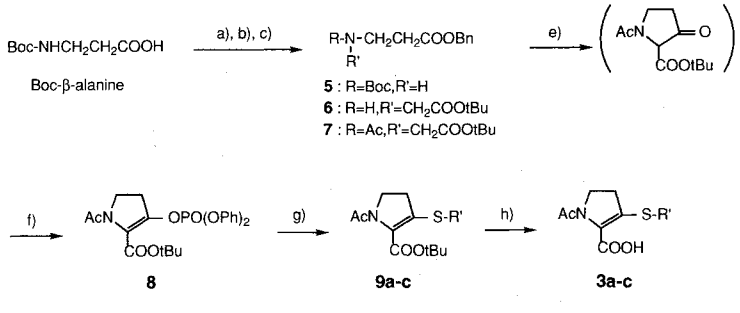
Results and Discussion

Convulsant Activity

The comparative convulsant activity of meropenem, imipenem, and panipenem, panipenem being a recently marketed carbapenem in Japan, after intraventricular injection in mice is shown in Table 1.

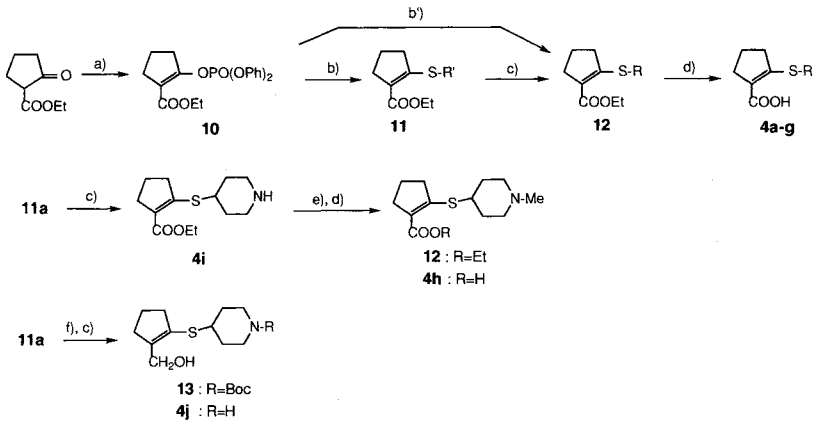
The doses of imipenem and panipenem that induce convulsions in 50% of mice (ED_{50}) are 11.3 and 28.7 μg/animal, respectively. These results are similar to those reported by SHIMADA *et al.*[12]. When imipenem and panipenem are co-administered with their companion drugs, *i.e.* cilastatin and betamipron, which are known to reduce the nephrotoxicity of the carbapenems[17,18], both combinations also show the same or slightly greater convulsant effects than the respective carbapenems administered alone. In contrast, meropenem shows no

Chart 1.



a) $BnBr,K_2CO_3$; b) 1:TFA; 2:$BrCH_2COOtBu,Et_3N$; c) $AcCl,Et_3N$; e) tBuOK; f) $(PhO)_2POCl$; g) R'-SH,NaH; h) TFA

Chart 2.



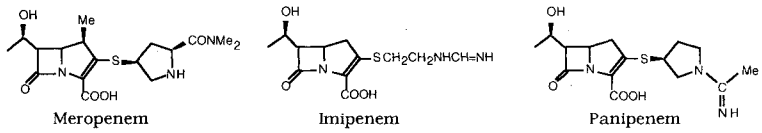
a) $(PhO)_2POCl$,NaH; b) R'-SH,NaH; b') R-SH,NaH; c) HCl in MeOH

d) NaOH; e) HCOOH,$NaBH_4$; f) DIBAL

Table 1. Convulsant activity of meropenem and other carbapenems after intraventricular injection in mice.

Compound	ED_{50} (μg/animal)		LD_{50}
	Clonic convulsion	Tonic convulsion	(μg/animal)
Meropenem	>300	>300	>300
Imipenem	11.3	16.6	16.6
Imipenem/cilastatin	8.9	13.4	13.4
Panipenem	28.7	40.8	40.8
Panipenem/betamipron	19.9	34.1	38.3

ED_{50} and LD_{50} were calculated by probit method.



convulsant effect, even at the highest dose examined, 300 μg/animal. The results of our previous structure-activity study suggested that the convulsant-inducing activity of imipenem and panipenem is due to their basic side chains at the C-2 position, whereas meropenem does not induce convulsions at comparable doses, due to the decreased basicity of its C-2 side chain.

To determine the portion of the structure of the carbapenems which plays an essential role in the induction of convulsions, we conducted a further study on convulsant activity with the cleaved products of the β-lactam ring (**2**) and with some of the structural moieties, including the C-2 side chain of carbapenem (**3**, **4**). In this study, we selected a 4'-piperidinylthio group as a model of an amino side chain exhibiting strong convulsant activity[9]. The product of cleavage of the β-lactam bond (**2a**) and the *N*-acetyl-2-pyrroline derivative containing a piperidinylthio side chain (**3a**) shows almost the same convulsant activity as the corresponding carbapenem compound (**1a**). On the other hand, none of compounds with the 3'-(5'-dimethylaminocarbonyl) pyrrolidinylthio group (**1b**, **2b**, and **3b**) exhibite convulsant activity. Similar to our previous findings with carbapenems, acetylation of the amino group, as in **1c** and **3c**, significantly blocks the induction of convulsions.

To clarify the contribution to convulsant activity of the N atom or the *N*-acetyl group in the pyrroline ring of **3a**, we synthesized a cyclopentene derivative containing a piperidinylthio group in its (**4a**) side chain. As shown in Table 3, **4a** induces convulsions at an incidence as high as that exhibited by **3a**. Consequently, the finding that the N atom and the *N*-acetyl group of the 5-membered ring is not involved with the induction of convulsions prompted us to study further structure-activity relationships, using cyclopentene derivatives as a simpler model of intrinsic structure for neurotoxic carbapenem compounds. Table 3 shows the convulsant activity of cyclopentene derivatives with various side

Table 2. Induction of convulsions after intraventricular injection in mice and effect on $GABA_A$ receptor binding.

Compound	ED_{50} (μg/animal)		LD_{50}	Inhibition of $GABA_A$ receptor binding (%)	
	Clonic convulsion	Tonic convulsion	(μg/animal)	0.1 mM	0.01mM
1a	2.0	2.6	2.8		
1b (meropenem)	>300	>300	>300		
1c	>300	>300	>300		
2a	2.9	2.9	3.1	65	18
2b	>300	>300	>300	<10	<10
3a	1.7	1.7	2.8	49	18
3b	>300	>300	>300	<10	<10
3c	>300	>300	>300	<10	<10

ED_{50} and LD_{50} were calculated by probit method.
Per cent of inhibition for 1nM [^{3}H]-mucimol binding to the $GABA_A$ receptor in the presence of the indicated concentration of the test compounds.

Table 3. Induction of convulsions after intraventricular injection in mice and effect on $GABA_A$ receptor binding.

Compound	ED_{50} (μg/animal)		LD_{50}	Inhibition of $GABA_A$ receptor binding (%)	
	Clonic convulsion	Tonic convulsion	(μg/animal)	0.1 mM	0.01mM
4a	0.6	1.9	2.4	67	23
4b	170	290	>300	15	<10
4c	0.9	1.7	2.3	70	26
4d	12	17	23	22	18
4e	>300	>300	300	16	16
4f	0.1	0.2	0.3	78	40
4g	13	20	20	81	28
4h	34	40	40	41	<10
4i	55	140	160	76	23
4j	1.0	2.2	3.8	82	29

ED_{50} and LD_{50} were calculated by probit method.
Per cent of inhibition for 1 nM [^{3}H]-mucimol binding to the $GABA_A$ receptor in the presence of the indicated concentration of the test compounds.

chains. *N*-Acetylation of the piperidinyl group (**4b**) reduce markedly convulsant activity. The pyrrolidinyl derivative (**4c**) exhibites high convulsant activity, comparable to that of the piperidinyl derivative (**4a**). The aminoethylthio derivative (**4d**) has one-tenth the convulsant activity of **4a**, and the less basic pyridylthio derivative (**4e**) has even less convulsant activity.

As described above, cyclopentene derivatives gave results very similar to those of carbapenem compounds. We therefore conclude that the central nerve system (CNS) toxicity of carbapenem compounds is related to the part of the structure only, including the C-2 side chain, and that the strength of basicity of the amino group is related to the convulsant activity. In addition, the introduction of one methylene spacer (CH_2) between the S atom and the piperidinyl group (**4f**) increases the convulsant activity, whereas the introduction of two methylene spacers (**4g**) reduce convulsant potency. *N*-Methylation of the piperidinyl group (**4h**) significantly blocks the induction of convulsions. These findings suggest that, in addition to basicity, the distance from the carboxyl to the amino group and steric crowding around the amino group plays an important role in the induction of convulsions.

Finally, ethylation of the carboxyl group (**4i**) significantly lowers the convulsant activity, while the hydroxymethyl derivative (**4j**) showed neurotoxicity comparable to that of **4a**. These results indicate that the presence of the carboxyl group is not necessary for the induction of convulsions, but that suitable hydrophilicity at this position could be a requirement.

Inhibition of $GABA_A$ Receptor Binding

To elucidate the possible mechanism responsible for the convulsions induced by these compounds, we carried out $GABA_A$ receptor binding assays in the presence of 0.1 mM and 0.01 mM of the compounds. Only **2a** and **3a**, which induced strong convulsions, have a significant inhibitory effect on $GABA_A$ receptor binding (Table 2). Cyclopentene derivatives, which induce convulsions at a high incidence, generally show strong inhibitory effects on $GABA_A$ receptor binding (Table 3). These results

suggested that these compounds, as well as imipenem and other β-lactam antibiotics, could induce convulsions through the inhibition of $GABA_A$-mediated inhibitory transmission. However, there are a few exceptions, in that, although there is a significant difference between **4d** and **4h** in their inhibitory effect on $GABA_A$ receptor binding (22% and 81%, respectively), the compounds show similar neurotoxicity. HIKIDA *et al.*[19] reported that imipenem also inhibits the benzodiazepine recognition site on the $GABA_A$ receptor complex and inhibits glycine receptor binding in rat synaptic membranes. These receptors are also known to participate in an inhibitory neurotransmission system. Therefore, it is possible that these compounds may affect the binding of receptors other than the $GABA_A$ agonist site.

Since **4a**, **4i** and **4j** shows strong inhibition of $GABA_A$ receptor binding, it would appear that the carboxyl group is not essential for the receptor binding interaction. As noted above, however, it is probable that a suitable hydrophilicity at this position is required for distribution to the site of action. SUZUKI *et al.*[20] demonstrated that, after intraventricular injection, imipenem is cleared from the CNS much less rapidly than benzylpenicillin, *via* an active organic anion transport system. Pharmacokinetic studies in the CNS are required to further elucidate the convulsant activity of these compounds.

It is uncertain whether the inhibitory action on $GABA_A$ receptor binding *in vitro* is correlated with CNS toxicity *in vivo*. To clarify this question, the concentration of these compounds in the hippocampus, a brain region recognized as being implicated in convulsions should be determined. If these compounds, like the β-lactam antibiotics, are distributed only in brain extracellular fluid and CSF (estimated as around 70 μl, equal to 20% of the total brain volume for the adult mouse[21]), and if rapid efflux from CSF does not occur, then the diffused concentration of **4a** would be between 9 and 30 μg/ml (concentration of solution of the compound inducing clonic convulsion in 50% of animals). We found here that **4a** inhibited $GABA_A$ receptor binding by 67% at a concentration of 0.1 mM (corresponding to approximately 20 μg/ml). Accordingly, this rough estimation suggests that the inhibitory effect on receptor binding *in vitro* could be closely related to the induction of seizures *in vivo*.

In conclusion, the neurotoxicity of carbapenem compounds is related to only part of the structure, including the C-2 side chain, and not to the carbapenem skeleton itself. In addition to the strength of basicity of the amino group, the distance from the carboxyl to the amino group and/or steric crowding around the amino group also plays an important role in the convulsant activity of the compounds. The induction of convulsions could be caused predominantly through the inhibition of $GABA_A$-mediated inhibitory transmission. Since there are some exceptions to the relation between convulsant activity and inhibitory effect on $GABA_A$ receptor binding, further molecular-biological and pharmacokinetic studies are necessary to elucidate the neurotoxic potential of carbapenem antibiotics. The findings presented here should be helpful in the development of novel potent carbapenems with low neurotoxicity.

Experimental

Melting points were determined on a Thomas-Hoover capillary melting points apparatus and were not corrected. Infrared (IR) spectra were measured on a Perkin Elmer 1600 infrared spectrometer. ^1H NMR spectra were recorded on a JEOL GX-270 (270 MHz) spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard (σ-values). Column chromatography was conducted with silica gel 60 (70~230 mesh, E. Merck). Preparative thin layer chromatography was done on silica gel 60 F_{254} TLC plates (E. Merck). The organic solution was dried over $MgSO_4$ before vacuum evaporation.

(5*S*)-5-[(1*S*,2*R*)-1-Carboxyl-2-hydroxypropyl]-3-(piperidin-4-yl-thio)-2-pyrrolinecarboxylic Acid (**2a**)

A solution of (5*R*,6*S*)-2-(piperidin-4-ylthio)-6-[(*R*)-1-hydroxyethyl]carbapen-2-em-3-carboxylic acid (150 mg, 0.48 mmol) in 1 N HCl (6 ml) was stirred at 0~5°C for 3 hours, and then neutralized with 1 N NaOH (6 ml). After lyophilization of the aqueous solution, the resulting residue was subjected to column chromatography on CHP-20P which was successively eluted with water. The fractions having UV absorption at 230 nm were combined and lyophilized to give **2a** (101 mg, 64%) as a white amorphous powder: IR (KBr) cm^{-1} 3393, 1712; ^1H NMR (D_2O) δ 1.29 (3H, d, J=5.9 Hz), 1.65~1.89 (3H, m), 2.10~2.40 (3H, m), 2.51~2.74 (2H, m), 2.96~3.17 (4H, m), 3.33~3.54 (3H, m).

Benzyl 3-*t*-Butoxycarbonylaminopropionate (**5**)

To a solution of Boc-β-alanine (9.45 g, 0.05 mol) and benzyl bromide (10.25 g, 0.06 mol) in acetone (150 ml) was added K_2CO_3 (13.8 g, 0.1 mol) and stirred under reflux for 5 hours. The reaction mixture was cooled and filtered. The filtrate was concentrated *in vacuo* and the residue was dissolved in EtOAc. This solution was washed with brine, dried and concentrated *in vacuo* to give an oily residue, which was purified by silica gel chromatography to give **5** (13.9 g, quantitative yield): IR (neat) cm^{-1} 3370, 1714; ^1H NMR ($CDCl_3$) δ 1.43 (9H,

s), 2.58 (2H, t, J=6.1 Hz), 3.41 (2H, q, J=6.1 Hz), 5.14 (2H, s), 7.35 (5H, s).

Benzyl 3-(*t*-Butoxycarbonylmethyl)aminopropionate (**6**)

A mixture of **5** (11.2 g, 0.04 mol) and trifluoroacetic acid (TFA) (13.7 g, 0.12 mol) was stirred at room temperature for 22 hours and then concentrated *in vacuo*. The oily residue was dissolved in toluene and evaporated *in vacuo* to give TFA salt of β-alanine benzylester (11.5 g), which was used for the next step without purification. A solution of the TFA salt (5.4 g, *ca.* 18 mmol) and Et_3N (2.02 g, 20 mmol) in THF (50 ml) was added to a suspension of NaH (60%, 0.96 g, 24 mmol) in THF (20 ml) with ice-water cooling and stirred for 10 minutes. *t*-Butyl bromoacetate (3.9 g, 20 mmol) was added dropwise at 0~5°C and then followed by stirring at the same temperature for 5 hours. The reaction mixture was diluted with EtOAc and washed with brine, dried and evaporated *in vacuo* to give an oily residue, which was purified by silica gel chromatography to give **6** (2.22 g, 41%) and benzyl 3-di(*t*-butoxycarbonylmethyl)aminopropionate (2.08 g, 28%) as a by-product: IR (neat) cm^{-1} 1733, 1157; ^1H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.84 (1H, br s), 2.56 (2H, t, J=6.6 Hz), 2.91 (2H, t, J=6.6 Hz), 3.30 (2H, s), 5.14 (2H, s), 7.35 (5H, s).

Benzyl 3-Acetyl-3-(*t*-butoxycarbonylmethyl)aminopropionate (**7**)

To a solution of **6** (500 mg, 1.71 mmol) and Et_3N (216 mg, 2.14 mmol) in CH_2Cl_2 (10 ml) was added acetyl chloride (160 mg, 2.04 mmol) dropwise with ice-water cooling. The mixture was stirred for 3 hours. The reaction mixture was washed successively with 0.5 N HCl, water, 5% $NaHCO_3$ and brine, dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **7** (562 mg, 98%): IR (neat) cm^{-1} 1740, 1657; ^1H NMR ($CDCl_3$) δ 1.45 (9H/2, s), 1.47 (9H/2, s), 1.98 (3H/2, s), 2.16 (3H/2, s), 2.67 (2H/2, t, J=6.9 Hz), 2.70 (2H/2, t, J=6.3 Hz), 3.63 (2H/2, t, J=6.3 Hz), 3.69 (2H/2, t, J=6.9 Hz), 3.97 (2H/2, s), 3.99 (2H/2, s), 5.11 (2H/2, s), 5.13 (2H/2, s), 7.35 (5H, s).

1-Acetyl-2-*t*-butoxycarbonyl-3-diphenoxyphosphoryloxy-2-pyrroline (**8**)

To a solution of **7** (1.0 g, 3.0 mmol) in toluene (30 ml) was added *t*-BuOK (405 mg, 3.6 mmol) with ice-water cooling and stirred for 1 hour. After further stirring for 1 hour at room temperature, DMF (10.5 ml) and then diphenylchlorophosphate (970 mg, 3.0 mmol) were added and stirred overnight. The reaction mixture was diluted with EtOAc and successively washed with 5% $NaHCO_3$ and brine. The organic phase was dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **8** (840 mg, 61%): IR (neat) cm^{-1} 1732, 1651; ^1H NMR ($CDCl_3$) δ 1.48 (9H, s), 2.05 (3H, s), 3.01 (2H, t, J=7.9 Hz), 3.97 (2H, t, J=7.9 Hz), 7.20~7.26 (6H), 7.35 (4H, d, J=7.8 Hz).

1-Acetyl-2-*t*-butoxycarbonyl-3-(1-*t*-butoxycarbonylpiperidin-4-ylthio)-2-pyrroline (**9a**)

A solution of 1-*t*-butoxycarbonyl-4-mercaptopiperidine (586 mg, 2.7 mmol) in CH_3CN (3 ml) was added dropwise to a suspension of **8** (1.15 g, 2.5 mmol) and NaH (60%, 0.12 g, 3 mmol) in CH_3CN (30 ml) with ice-water cooling and stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc, washed with brine 3 times, dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **9a** (830 mg, 78%): IR (neat) cm^{-1} 1733, 1694, 1668; ^1H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.57 (9H, s), 1.91 (2H, br d, J=13 Hz), 2.07 (3H, s), 2.64 (1H, dd, J=6.5, 13 Hz), 2.83 (2H, t, J=8.7 Hz), 2.98 (2H, m), 3.09 (1H, m), 3.92 (2H, br d, J=13 Hz), 3.97 (2H, t, J=8.7 Hz).

Compounds **9b** and **9c** were similarly prepared from **8** with the corresponding thioacetates according to the procedure described for **9a**.

1-Acetyl-2-*t*-butoxycarboxy-3-[(3*S*,5*S*)-1-*t*-butoxycarboxy-5-dimethylcarbamoylpyrrolidin-3-ylthio]-2-pyrroline (**9b**)

IR (neat) cm^{-1} 1709, 1660; ^1H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.57 (9H, s), 2.07 (3H, s), 2.62 (1H, m), 2.86 (2H, m), 2.96 (3H × 1/3, s), 2.98 (3H × 2/3, s), 3.05 (3H × 1/3, s), 3.10 (3H × 2/3, s), 3.43 (2H, m), 4.70 (1H, t, J=8.0 Hz).

1-Acetyl-2-*t*-butoxycarbonyl-3-(1-acetyl-piperidin-4-ylthio)-2-pyrroline (**9c**)

IR (neat) cm^{-1} 1732, 1652; ^1H NMR ($CDCl_3$) δ 1.57 (9H, s), 2.00 (2H, m), 2.08 (3H, s), 2.09 (3H, s), 2.83 (2H, t, J=8.6 Hz), 3.18 (2H, m), 3.97 (2H, t, J=8.6 Hz).

1-Acetyl-2-carboxy-3-(piperidin-4-ylthio)-2-pyrroline (**3a**)

A mixture of **9a** (626 mg, 1.47 mmol), TFA (1 ml) and CH_2Cl_2 (2 ml) was stirred at room temperature for 5.5 hours and then concentrated *in vacuo*. The oily residue was dissolved in 5% Na_2CO_3 (3 ml) and was purified by column chromatography on Diaion CHP-20P to give **3a** (304 mg, 76.5%) as a powder: IR (KBr) cm^{-1} 3503, 1634; ^1H NMR (D_2O) δ 1.85 (2H, m), 2.13 (3H, s), 2.84 (1H, m), 3.01~3.48, (5H, m), 4.03 (2H, m). UV λ_{max}: 278 nm.

The preparation of **3b** and **3c** was carried out by a procedure similar to that described for **3a**.

1-Acetyl-2-carboxy-3-(5-dimethylcarbamoylpyrrolidin-3-ylthio)-2-pyrroline (**3b**)

IR (KBr) cm^{-1} 3448, 1654; ^1H NMR (D_2O)δ: 2.04 (2H, m), 2.14 (3H, s), 2.99 (3H, s), 3.05 (3H, s), 3.50 (1H, m), 3.79 (2H, m), 4.01 (2H, m), 4.10 (1H, m). UV λ_{max}: 277 nm.

1-Acetyl-2-carboxy-3-(1-acetylpiperidin-4-ylthio)-2-pyrroline sodium salt (**3c**)

IR (KBr) cm^{-1} 3448, 1624; ^1H NMR (D_2O) δ 1.53 (2H, m), 2.01 (2H, m), 2.11 (6H, s), 2.84 (2H, m), 3.08

(1H, m), 3.22, (2H, m), 3.64~4.22 (4H, m). UV λ_{max}: 280 nm.

Ethyl 2-(Diphenoxyphosphoryloxy)cyclopent-1-enecarboxylate (**10**)

To a solution of ethyl 2-oxocyclopentanecarboxylate (3.9 g, 25 mmol) in THF (30 ml) was added NaH (60%, 1.18 g, 29.5 mmol) and then diphenylchlorophosphate (7.32 g, 27.3 mmol) with ice-water cooling. The mixture was stirred at room temperature for 5 hours. The reaction mixture was diluted with benzene and successively washed with water, 5% $NaHCO_3$ and brine. The organic phase was dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **10** (9.12 g, 94%): IR (neat) cm^{-1} 1714, 1660; ^1H NMR ($CDCl_3$) δ 1.20 (3H, t, J=7.1 Hz), 1.92 (2H, m), 2.61 (2H, m), 2.79 (2H, m), 4.14 (2H, q, J=7.1 Hz), 7.29 (10H).

Ethyl 2-(1-*t*-Butoxycarbonylpiperidin-4-ylthio)cyclopent-1-enecarboxylate (**11a**)

To a solution of **10** (785 mg, 2.01 mmol) and 1-*t*-butoxycarbonyl-4-mercaptopiperidine (459 mg, 2.12 mmol) in CH_3CN (8 ml) was added NaH (60%, 88 mg, 2.2 mmol) with ice-water cooling. The mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with EtOAc, washed with water, dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **11a** (670 mg, 94%), which was recrystallized from EtOAc. mp 84~87°C: IR (neat) cm^{-1} 1694; ^1H NMR ($CDCl_3$) δ 1.29 (3H, t, J=7.3 Hz), 1.45 (9H, s), 1.96 (4H, m), 2.65 (2H, t, J=7.3 Hz), 2.83 (2H, t, J=7.3 Hz), 2.95 (2H, t, J=10.9 Hz), 3.23 (1H, m), 4.00 (2H, m), 4.20 (2H, q, J=7.3 Hz).

Compounds **11c**, **11d**, **11f**, **11g**, **12b** and **12e** were similarly prepared from **10** with the corresponding mercaptan according to the procedure described for **11a**.

Ethyl 2-(1-*t*-Butoxycarbonylpyrrolidin-3-ylthio)-cyclopent-1-enecarboxylate (**11c**)

IR (neat) cm^{-1} 1694, 1682; ^1H NMR ($CDCl_3$) δ 1.29 (3H, t, J=7.1 Hz), 1.46 (9H, s), 2.05 (2H, m), 2.29 (1H, m), 2.66 (2H, m), 2.82 (2H, m), 3.37 (2H, m), 3.69 (2H, m), 3.71 (2H, m), 4.20 (2H, q, J=7.1 Hz).

Ethyl 2-(*t*-Butoxycarbonylaminoethylthio)cyclopent-1-enecarboxylate (**11d**)

IR (neat) cm^{-1} 3370, 1694; ^1H NMR ($CDCl_3$) δ 1.32 (3H, t, J=7.1 Hz), 1.47 (9H, s), 1.98 (2H, m), 2.69 (2H, t, J=7.6 Hz), 2.84 (2H, t, J=7.6 Hz), 3.00 (2H, t, J=6.6 Hz), 3.38 (2H, m), 4.24 (2H, q, J=7.1 Hz), 4.98 (1H, br s).

Ethyl 2-(1-*t*-Butoxycarbonylpiperidin-4-ylmethylthio)cyclopent-1-enecarboxylate (**11f**)

IR (neat) cm^{-1} 1692; ^1H NMR ($CDCl_3$) δ 1.18 (2H, m), 1.29 (3H, t, J=7.3 Hz), 1.45 (9H, s), 1.64 (1H, m), 1.80~2.05 (4H, m), 2.30 (1H, m), 2.60~2.81 (7H, m), 4.11 (2H, m), 4.20 (2H, q, J=7.3 Hz).

Ethyl 2-[3-(1-*t*-Butoxycarbonylpiperidin-4-ylpropyl)-thio]cyclopent-1-enecarboxylate (**11g**)

IR (neat) cm^{-1} 1694; ^1H NMR ($CDCl_3$) δ 1.07 (2H, m), 1.29 (3H, t, J=7.0 Hz), 1.45 (9H, s), 1.63 (5H, m), 1.94 (2H, m), 2.23 (1H, m), 2.66 (5H, m), 2.81 (2H, q, J=7.0 Hz), 4.07 (2H, br d, J=11 Hz), 4.20 (2H, q, J=7.0 Hz).

Ethyl 2-(1-Acetylpiperidin-4-ylthio)cyclopent-1-enecarboxylate (**12b**)

IR (KBr) cm^{-1} 1689, 1644; ^1H NMR($CDCl_3$) δ 1.28 (3H, t, J=7.1 Hz), 1.62 (2H, m), 1.98 (4H, m), 2.09 (3H, s), 2.65 (2H, t, J=7.3 Hz), 2.83 (2H, t, J=7.3 Hz), 2.99 (1H, t, J=11.5 Hz), 3.1~3.4 (2H, m), 3.74 (1H, m), 4.19 (2H, q, J=7.1 Hz).

Ethyl 2-(Pyridin-4-ylthio)cyclopent-1-enecarboxylate (**12e**)

mp 62~66°C; IR (neat) cm^{-1} 1694; ^1H NMR ($CDCl_3$) δ 1.33 (3H, t, J=7.2 Hz), 1.89 (2H, quint, J=7.4 Hz), 2.42 (2H, t, J=7.4 Hz), 2.72 (2H, t, J=7.4 Hz), 4.21 (2H, q, J=7.2 Hz), 7.41 (2H, d, J=4.3 Hz), 8.57 (2H, d, J=4.3 Hz).

Ethyl 2-(Piperidin-4-ylthio)cyclopent-1-enecarboxylate hydrochloric acid salt (**4i**)

A solution of **11a** (1.14 g, 3.21 mmol) in MeOH (9 ml) was added dropwise to methanolic HCl, prepared from the addition of acetyl chloride (4.5 ml) to MeOH (24 ml) with ice-water cooling. The mixture was stirred at room temperature for 4 hours. The reaction mixture was concentrated *in vacuo* to give a crystalline residue which was recrystalized from EtOAc-MeOH (5:1) to give **4i** (790 mg, 85%). mp 192~194°C: IR (KBr) cm^{-1} 1678; ^1H NMR ($CDCl_3$) δ 1.29 (3H, t, J=7.1 Hz), 1.82 (2H, m), 1.99 (2H, quint, J=7.6 Hz), 2.23 (2H, m), 2.66 (2H, t, J=7.6 Hz), 2.91 (2H, t, J=7.6 Hz), 3.13 (2H, m), 3.3~3.5 (3H, m), 4.18 (2H, q, J=7.1 Hz).

Ethyl 2-(1-Methylpiperidin-4-ylthio)cyclopent-1-enecarboxylate (**12**)

To a solution of **4i** (495 mg, 1.7 mmol) and $NaBH_4$ (520 mg, 13.6 mmol) in THF (15 ml) was added formic acid (5 ml) with ice-water cooling and stirred at room temperature for 15 hours. With ice-water cooling water (30 ml) and 50% NaOH were added to the reaction mixture until it was strongly basic. The mixture was extracted with CH_2Cl_2, washed with brine, dried and evaporated *in vacuo* to give **12** (790 mg, 85%): IR (neat) cm^{-1} 1696; ^1H NMR ($CDCl_3$) δ 1.27 (3H, t, J=7.0 Hz), 1.65~1.81 (2H), 1.88~2.12 (4H), 2.25 (3H, s), 2.63 (2H, t, J=7.0 Hz), 2.75~2.85 (4H), 3.07 (1H, m), 4.18 (2H, q, J=7.0 Hz).

2-(1-*t*-Butoxycarbonylpiperidin-4-ylthio)-1-hydroxymethylcyclopentene (**13**)

To a solution of **11a** (970 mg, 2.73 mmol) in THF (25 ml) was added 0.93 M DIBAL-hexane solution

(13 ml, 12.1 mmol) with ice-water cooling. The mixture was stirred for 15 minutes and then for 30 minutes at room temperature. The reaction mixture was quenched with aq. NH_4Cl (50 ml), diluted with EtOAc and filtered on Celite. The organic phase was separated, dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **13** (583 mg, 68%). mp 102~103.5°C: IR (KBr) cm^{-1} 3424, 1674; ^1H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.68~1.97 (4H, m), 2.52 (4H, m), 2.94 (2H, dt, J=3.0, 12 Hz), 3.04 (1H, m), 3.93 (2H, br. d, J=13 Hz), 4.32 (2H, s); *Anal.* Calcd for $C_{16}H_{27}NO_3S$: C 61.31, H 8.68, N 4.47, S 10.23. Found: C 60.93, H 8.67, N 4.54, S 10.39.

2-(Piperidin-4-ylthio)cyclopent-1-enecarboxylic Acid (**4a**)

To a solution of **4i** (288 mg, 0.99 mmol) in MeOH (3 ml) and THF (1 ml) was added 1 N NaOH (3.0 ml) and stirred at 50°C for 4 hours. The reaction mixture was diluted with water and washed with EtOAc. The aqueous layer was acidified with 1 N HCl and extracted with EtOAc. The extracts were washed with brine, dried and evaporated *in vacuo*. The residue was dissolved in water (3 ml) and was purified by column chromatography on Diaion CHP-20P to give **4a** (125 mg, 55%) as a powder: IR (KBr) cm^{-1} 3447, 1617, 1395; ^1H NMR (D_2O) δ 1.71 (2H, m), 1.85 (2H, quint, J=7.5 Hz), 2.15 (2H, dd, J=3.0, 11.0 Hz), 2.50 (2H, t, J=7.5 Hz), 2.71 (2H, t, J=7.5 Hz), 3.04 (2H, dt, J=3.0, 10.5 Hz), 3.3~3.5 (3H, m); UV λ_{max} 271 nm.

The preparation of **4b~4h** was carried out by a procedure similar to that described for the preparation of **4a** from **11a**.

Sodium 2-(1-Acetylpiperidin-4-ylthio)cyclopent-1-enecarboxylate (**4b**)

IR (KBr) cm^{-1} 1676, 1613, 1573; ^1H NMR (D_2O) δ 1.56 (2H, m), 1.93 (2H, m), 2.05 (2H, m), 2.14 (3H, s), 2.58 (2H, t, J=7.4 Hz), 2.78 (2H, t, J=7.4 Hz), 3.04 (1H, t, J=11 Hz), 3.33 (1H, t, J=11 Hz), 3.45 (1H, m), 3.90 (1H, d, J=14 Hz), 4.19 (1H, d, J=14 Hz); UV λ_{max} 286 nm.

2-(Pyrrolidin-3-ylthio)cyclopent-1-enecarboxylic Acid (**4c**)

IR (KBr) cm^{-1} 3461, 1674; ^1H NMR (D_2O) δ 2.02 (2H, t, J=7.6 Hz), 2.07 (1H, m), 2.55 (1H, m), 2.66 (2H, t, J=7.6 Hz), 2.89 (2H, m), 3.31~3.53 (3H, m), 3.79 (1H, dd, J=7.0, 13.0 Hz), 4.15 (1H, m); UV λ_{max} 278 nm.

2-(Aminoethylthio)cyclopent-1-enecarboxylic Acid (**4d**)

IR (KBr) cm^{-1} 3484, 1676; ^1H NMR (D_2O) δ 2.00 (2H, m), 2.67 (2H, t, J=7.6 Hz), 2.87 (2H, t, J=7.6 Hz), 3.24 (4H, m); UV λ_{max} 275 nm.

2-(Pyridin-4-ylthio)cyclopent-1-enecarboxylic Acid (**4e**)

IR (KBr) cm^{-1} 3522, 1672; ^1H NMR (D_2O) δ 1.99 (2H, m), 2.54 (2H, t, J=7.6 Hz), 2.74 (2H, t, J=7.6 Hz), 7.45 (2H, d, J=6.3 Hz), 8.42 (2H, d, J=6.3 Hz); UV λ_{max} 302 nm.

2-(Piperidin-4-ylmethylthio)cyclopent-1-enecarboxylic Acid (**4f**)

IR (KBr) cm^{-1} 3419, 1669; ^1H NMR (D_2O) δ 0.93 (2H, m), 1.31 (1H, m), 1.36 (2H, t, J=7.4 Hz), 1.54 (2H, br d, J=13.8 Hz), 2.03 (2H, t, J=7.4 Hz), 2.22 (2H, t, J=7.6 Hz), 2.29 (2H, d, J=6.6 Hz), 2.42 (2H, dt, J=2.6, 10.2 Hz), 2.88 (2H, d, J=12.9 Hz).

2-[3-(Piperidin-4-ylpropyl)thio]cyclopent-1-enecarboxylic Acid (**4g**)

IR (KBr) cm^{-1} 3357, 1669; ^1H NMR (D_2O) δ 1.38 (4H, m), 1.62 (3H, m), 1.94 (4H, m), 2.54 (2H, t, J=7.4 Hz), 2.72 (2H, t, J=7.6 Hz), 2.80 (2H, t, J=7.3 Hz), 2.96 (2H, dt, J=2.3, 12.9 Hz), 3.39 (2H, br d, J=12.5 Hz).

2-(1-Methylpiperidin-4-ylthio)cyclopent-1-enecarboxylic Acid (**4h**)

IR (KBr) cm^{-1} 3385, 1581; ^1H NMR (D_2O) δ 1.81 (2H, m), 1.93 (2H, quint, J=7.6 Hz), 2.23 (2H, m), 2.59 (2H, t, J=7.6 Hz), 2.76 (2H, t, J=7.6 Hz), 2.86 (3H, s), 3.13 (2H, m), 3.46 (3H, m); UV λ_{max} 254 nm.

2-(Piperidin-4-ylthio)-1-hydroxymethylcyclopentene Hydrochloric Acid (**4j**)

A solution of **13** (290 mg, 0.92 mmol) in MeOH (9 ml) was added dropwise to methanolic HCl, prepared from the addition of acetyl chloride (2.25 ml) to MeOH (12 ml) with ice-water cooling. The mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated to give an oily residue, which was dissolved in EtOAc. Evaporation *in vacuo* gave **4j** (164 mg, 84%). ^1H NMR ($CDCl_3$-CD_3OD 9:1) δ 1.77~1.87 (4H, m), 2.18~2.26 (4H, m), 3.09 (2H, m), 3.37~3.41 (3H, m), 3.84 (2H, s); UV λ_{max} 254 nm.

Testing of Convulsant Activity

The intraventricular administration of the compounds to mice was carried out as follows[7]. Groups of ten ICR mice weighing about 25 g were used. Each dose (0.3~300 μg/head) of the compounds dissolved in 20 μl of saline was injected into the left lateral ventricle of mice using a micro-syringe. Incidence of clonic and tonic convulsion and mortality were observed for 60 minutes after the administration. ED_{50} or LD_{50} values were estimated using the method of probit analysis.

$GABA_A$ Receptor Binding Assay

$GABA_A$ receptor binding assay was carried out by Panlabs, Inc. (Bothel, Washington, U.S.A.). Ten mg of synaptic membrane prepared by standard techniques was

incubated with 1 nM of [^{3}H]muscimol 10 minutes at 0°C. Non-specific binding was estimated in the presence of 100 nM muscimol. Membranes were filtered and washed, and the filters were counted to determine [^{3}H]muscimol specifically bound. All specific binding values were obtained in the presence of 0.1 mM and 0.01 mM of test compound.

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