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Synthesis and Anticonvulsant Evaluations of *N-Cbz*-α-amino-*N*-alkoxyglutarimides

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In our previous studies for the development of new anticonvulsant of broad spectrum, we found that *N-cbz*-α-aminoglutarimides showed significant anticonvulsant activities of broad spectrum enough to be recommended for the new anticonvulsants and their anticonvulsant activities were dependent on their imide substituent groups. Based on these results, various *N-cbz*-α-amino-*N*-alkoxyglutarimides, where the imide N-H was substituted with the hydroxy and alkoxy group, were prepared and evaluated for their anticonvulsant activities using the Maximal electroshock seizure (MES) and Pentylenetetrazole induced seizure (PTZ) tests and also the rotorod test. A series of (*R*) or (*S*)-*N-cbz*-α-amino-*N*-alkoxyglutarimides could be prepared from the corresponding (*R*) or (*S*)-*N-cbz*-glutamic acid following the usual synthetic procedure. Among them, (*R*)-*N-cbz*-α-amino-*N*-hydroxyglutarimide (ED_{50}=86.25 mg/kg) was most active in the MES test. In the case of the PTZ test, (*R*)-*N-cbz*-α-amino-*N*-benzyloxyglutarimide (ED_{50} = 62.5 mg/kg) was most active. Among the tested compounds, **2a-c**, **3a**, and **3b** showed anticonvulsant activities in the MES and PTZ test. All of the tested compounds, except **2f** and **3f**, showed significant anticonvulsant activities in the MES or PTZ test. In addition, the neurotoxicities of these compounds were comparable to other anticonvulsant drugs.

Key words: Anticonvulsant, MES, PTZ, Rotorod test, Neurotoxicity, Glutarimide

INTRODUCTION

In connection with the development of new anticonvulsants of broad spectrum, we found that the *N*-acyl-α-aminoglutarimides such as **1** in Fig. 1 showed significant anticonvulsant activities of broad spectrum enough to be selected as lead compounds for the development of new anticonvulsants (Park *et al.*, 1966; Lee *et. al.*, 1996). Interestingly, their anticonvulsant activities were dependent on their substituents of the imide group or the α-amino group (Son *et al.*, 1998; Lee *et al.*, 1999).

These facts prompted us to prepare various derivatives of *N-cbz*-α-amino-*N*-hydroxyglutarimides **2** and **3** in Scheme 1, which were substituted with the hydoxy or alkoxy group instead of the imide N-H, and to evaluate their anticonvulsant activities in order to develop more active anticonvulsants and also define the effect of *N*-hydroxy or *N*-alkoxy groups on their anticonvulsant activities.

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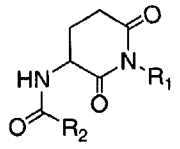

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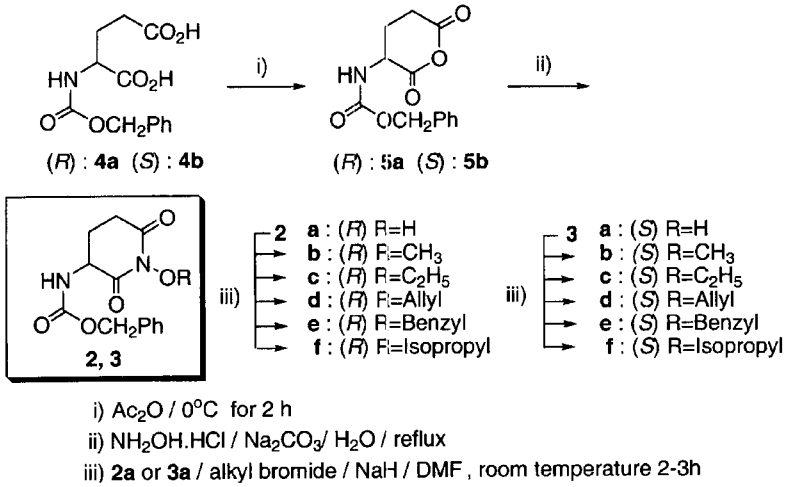
Fig. 1. *N*-Acly-α-amino-*N*-alkylglutarimides

Herein, we wish to report the synthesis of a series of *N-cbz*-α-amino-*N*-alkoxyglutarimides (**2**, **3**) and their *in vivo* anticonvulsant activities against the MES test, the PTZ test and also the rotorod test.

MATERIAL AND METHODS

Melting points were determined by the Electrothermal digital melting point apparatus and were uncorrected. IR spectra were taken in the KBr disk with JASCO FT/IR 200 and were reported in cm^{-1}. ^{1}H-NMR spectra were recorded in DMSO-d_6 on JNM-EX90A, and chemical shifts were reported as values in parts per million from TMS as an internal standard. The pharmacological tests were carried out according to the protocol of the Antiepileptic Drug



Scheme 1. Synthesis of *N-cbz*-α-amino-*N*-alkoxyglutarimides

Development Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1989).

Synthesis

(*R*) and (*S*)-*N-cbz*-α-amino-*N*-alkoxyglutarimides (**2**, **3**) were prepared from the corresponding (*R*) or (*S*)-*N-cbz*-glutamic acid following the usual synthetic procedure as shown in Scheme 1.

The *N-cbz*-glutamic acid anhydride **5** was prepared quantitatively by treating *N-cbz*-glutamic acid with acetic anhydride, and the treatment of hydroxylamine to *N-cbz*-glutamic anhydride **5** gave rise to the *N-cbz*-α-amino-*N*-hydroxyglutarimide (**2a** and **3a**). And *N-cbz*-α-amino-*N*-alkoxy glutarimides **2b-f** and **3b-f**, were obtained in moderate yields by alkylation of **2a** and **3a** with the corresponding alkyl halide and sodium hydride in dry *N,N*-dimethylformamide.

(*R*)-*N-Cbz*-glutamic acid anhydride (5a)

(*R*)-*N-Cbz*-glutamic acid (10 g, 0.037 mol) was dissolved with acetic anhydride (100 mL) and the reaction mixture was stirred at 0°C for 2 h. Then, the excess acetic anhydride was evaporated *in vacuo* and the residue was treated with diethyl ether to give *N-cbz*-glutamic acid anhydride as a white solid in quantitative yield. mp: 96.4°C: IR (KBr) cm^{-1}: 1700, 1755, 1820, 3300.

(*S*)-*N-Cbz*-glutamic acid anhydride (5b)

mp: 96.6°C; IR spectrum was identical to **5a**.

(*R*)-*N-Cbz*-α-amino-*N*-hydroxyglutarimide (2a)

Hydroxylaminehydrochloride (833 mg, 12 mmol) was dissolved with H_2O (2 mL) and Na_2CO_3 (636 mg, 6 mmol) was added to this solution. Then *N-cbz*-glutamic acid anhydride (2.63 g, 10 mmol) was added slowly. The reaction mixture was refluxed for 2-3 h, and then it was cooled in an ice bath to precipitate *N-cbz*-α-amino-*N*-hydroxyglutarimide as white solid. The crude *N-cbz*-α-amino-*N*-hydroxyglutarimide was recrystallized with ethanol and water. 44%; mp: 136.6°C; IR (KBr) cm^{-1}: 1700, 1755, 1820, 3300: ^{1}H-NMR (DMSO-d_6): δ 1.75-1.90 (2H, m), 2.40-2.80 (2H, m), 4.30-4.35 (1H, m), 5.07 (2H, s), 5.62-5.70 (1H, br), 7.35 (5H, s), 10.58-10.63 (1H, br).

(*S*)-*N-Cbz*-α-amino-*N*-hydroxyglutarimide (3a)

48%; mp: 132.3°C; IR and ^{1}H-NMR spectra were identical to **3a**.

(*R*)-*N-Cbz*-α-amino-*N*-methoxyglutarimide (2b)

To a suspension of NaH (160 mg, 4 mmol) in dry *N,N*-dimethylformamide (5 mL), (*R*)-*N-cbz*-α-amino-*N*-hydroxyglutarimide **2a** (556 mg, 2 mmol) in *N,N*-dimethylformamide (5 mL) was added. And CH_3I (341 mg, 2.4 mmol) in *N,N*-dimethyformamide (5 mL) was added successively. Then the reaction mixture was stirred for 4 h at room temperature. The excess *N,N*-dimethylformamide was evaporated *in vacuo*, and the residue was dissolved with 200 mL of ethyl acetate. The ethyl acetate solution was washed with H_2O (10 mL×2) and saturated aqueous NaCl solution (10 mL×2) and the solution was dried over anhydrous magnesium sulfate successively. The filtrate was evaporated to give 348 mg of brown solid as a single spot in TLC (ethy acetate : hexane = 3:1). Then the crude product was recrystallized with ethyl acetate and hexane. 63% : mp: 116.9°C; IR (KBr) cm^{-1}: 1690, 1710, 1770, 3350; ^{1}H-NMR (DMSO-d_6): δ 1.78-2.04 (2H, m), 2.33-2.80 (2H, m), 3.85 (3H, s), 4.34-4.49 (1H, m), 5.09 (2H, s), 5.63-5.80 (1H, br), 7.36 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-methoxyglutarimide (3b)

48%; mp: 113.2°C; IR and ^{1}H-NMR spectra were identical

to **2b**.

The following compounds were prepared according to the procedure above.

(*R*)-*N-Cbz*-α-amino-*N*-ethoxyglutarimide (2c)
53%; mp: 122.4°C; IR (KBr) cm^{-1}: 1670, 1690, 1710, 3300; ^{1}H-NMR (DMSO-d_6): δ 1.28 (3H, t, J=7.1 Hz), 1.75-2.05 (2H, m), 2.35-2.84 (2H, m), 3.97-4.12 (2H, q, J=.7.1 Hz), 4.31-4.35 (1H, m), 5.06 (2H, s), 5.82-5.94 (1H, br), 7.30 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-ethoxyglutarimide (3c)
46%; mp: 126.5°C; IR and ^{1}H-NMR spectra were identical to **2c**.

(*R*)-*N-Cbz*-α-amino-*N*-benzyloxyglutarimide (2e)
67%; mp: 122.3°C; IR (KBr) cm^{-1}: 1650, 1695, 1720, 3300; ^{1}H-NMR (DMSO-d_6): δ 1.78-2.05 (2H, m), 2.34-2.85 (2H,m), 4.30-4.34 (1H, m), 5.08 (2H, s), 5.10 (2H, s), 5.83-5.94 (1H, br.) 7.33 (5H, s), 7.46 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-benzyloxyglutarimide (3e)
74%; mp: 127.6 °C; IR and ^{1}H-NMR spectra were identical to **2e**.

(*R*)-*N-Cbz*-α-amino-*N*-isopropoxyglutarimide (2f)
42%; mp: 122.3°C; IR (KBr) cm^{-1}: 1640, 1690, 1720, 3300; ^{1}H-NMR (DMSO-d_6): δ 1.13 (6H, d, J = 8.37 Hz), 1.74-2.08 (2H, m), 2.35-2.83 (2H, m), 4.06-4.10 (1H, m), 4.30-4.34 (1H, m) 5.08 (2H, s), 5.62-5.83 (1H, br), 7.32 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-isoproxyglutarimide (3f)
37%; mp: 121.5°C; IR and ^{1}H-NMR spectra were identical to **2f**.

Pharmacology

The anticonvulsant tests for *N-cbz*-α-amino-*N*-hydroxyglutarimides **2a**, **3a** and *N-cbz*-α-amino-*N*-alkoxyglutarimides **2b-f** and **3b-f** were carried out according to the protocol of the Antiepileptic Drug Development Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1989) as follows: All tested compounds were dissolved in polyethylene glycol 400 and administered ip to ICR male mice at dose of 25, 50, 75, and 100 mg/kg, and anticonvulsant tests were performed 30 min after the administration in groups of 4 mice. Also we determined the lowest dose that all of the tested animals could be induced with a seizure at the stage of preliminary screening. The seizure was artificially induced by either electric shock or pentylenetetrazole. The maximal electroshock seizure (MES) tests were elicited with a 60-cycle a.c. of 50 mA intensity delivered for 0.2 s *via* corneal electrods with ECT unit (UGO Baseline, Italy). A drop of 0.9% saline was instilled in the eye prior to application of electrods. Protection in this test was defined as the abolition of the hind limb tonic extension component of the seizure. The pentylenetetrazole induced seizure (PTZ) test entailed the subcutaneous administration of 80 mg/kg of pentylenetetrazole as a 0.5% solution in the posterior midline of the mice, and the animal was observed for 30 min. Protection was defined as the failure to observe even a threshold seizure (single episode of clonic spasms of at least 5 sec. duration). The ED_{50} was estimated from the dose-response data. The effects of the compounds on the forced and spontaneous motor activity were evaluated in mice by the rotorod test with the Rotorod treadmill for mice (UGO Baseline, Italy) as follows. The previously trained animals were placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm after the administration of the tested compounds. Normal mice can remain on a rod at this speed indefinitely. Neurological toxicity was defined as the failure of the animal to remain on the rod for 2 min. The median neurotoxic dose (TD_{50}) was estimated from the dose-response data.

Table I. Anticonvulsant activity of (*R*)-*N-cbz*-α-amino-*N*-alkoxy-glutarimides (**2**) in Mice

Compound	Config.	R	Dose[a]	MES[b]	PTZ[c]
2a	R	H	50	4/4	4/4
			75	3/4	3/4
			100	2/4	1/4
			125	0/4	0/4
2b	R	CH_3	75	4/4	4/4
			100	3/4	3/4
			125	2/4	2/4
			150	0/4	1/4(0/4)[d]
2c	R	C_2H_5	50	4/4	4/4
			75	3/4	3/4
			100	2/4	2/4
			125	0/4	1/4(0/4)[d]
2e	R	benzyl	25		4/4
			50		3/4
			75		1/4
			100	4/4	0/4
2f	R	isopropyl	100	4/4	4/4

[a]All compounds were dissolved in polyethyleneglycol400 and administered i.p. to ICR male mice. Dose was denoted in mg/kg. [b]The MES test : 50 mA, 60 Hz, ac, 0.2 sec., *via* corneal electrods, 30 min post administration of test compound. The results were denoted as non-protected animals/tested animals. [c]The PTZ test: Subcutaneous pentylenetetrazol (80 mg/kg) 30 min post administration of test compound. The results were denoted as non-protected animals/tested animals. [d]at a dose of 175 mg/kg.

RESULTS AND DISCUSSION

As seen in Scheme 1, all of the tested compounds except **2d** and **3d**, could be prepared from the corresponding (*R*)- or (*S*)-*N*-*cbz*-glutamic acid in moderate yields. The (*R*)- or (*S*)-*N*-*cbz*-α-amino-*N*-allyloxyglutarimide **2d** and **3d** could not be isolated owing to their instability. All of the tested compound gave the satisfactory spectral data. We investigated the anticonvulsant activities for these compounds in both the MES and PTZ test. The results of preliminary anticonvulsant activities were summarized in Table I and II.

As shown in Table I and II, the compound **2a-c**, and **3a**, **b** showed anticonvulsant activity against the MES test at a dose of 100 mg/kg. The other compounds such as **2d-f** and **3c-f**, did not show anticonvulsant activity in the MES test at a lower dose of 100 mg/kg. In the case of the PTZ test, all of the compounds except **2f** exhibited significant anticonvulsant activities at a lower dose of 100 mg/kg. According to the protocol for the development of a new anticonvulsant, the compounds, which showed an anticonvulsant activity at dose of 100 mg/kg, were recommended for further investigation of quantification. So we selected the compounds **2a-c**, **e**, **3a-c**, and **e** for the quantitative anticonvulsant evaluation, and the rotorod test for these compounds were carried out to evaluate the neurotoxicity. The results of quantitative anticonvulsant activities and rotorod tests were summarized in Table III.

As seen in Table III, (*S*)-*N*-*cbz*-α-amino-*N*-methoxyglutarimide **3b** (ED_{50} = 86.25 mg/kg) was most active among the tested compounds in the MES test. The anticonvulsant activity in the MES test of **3b** was 3.1 times more active than that of valproic acid as judged from ED_{50}. In the case of the PTZ test, **2e** was most active and this compound was 2.4 times more active than that of valproic acid. In addition, the compound **2a-c**, **3a,** and **3b** exhibited a broad spectrum of anticonvulsant activities. In the rotorod test for neurotoxicity, the TD_{50} values of these compounds were 100-118.7 mg/kg, and the protective indices (PI) were 0.86-1.5. The PI of **3b**, the most active compound in the MES test, was 1.23 and the PI of **2e**, the most active one in the PTZ test, was 1.6. The PI values of these

Table II. Anticonvulsant activity of (S)-*N*-*cbz*-α-amino-*N*-alkoxyglutarimides (**3**) in Mice

Compound	Config.	R	Dose[a]	MES[b]	PTZ[c]
3a	R	H	25		3/4(4/4)[e]
			50	4/4	2/4
			75	3/4	2/4
			100	2/4(0/4)[d]	1/4(0/4)[d]
3b	R	CH_3	25		4/4
			50	4/4	3/4
			75	3/4	3/4
			100	1/4(0/4)[d]	0/4
3c	R	C_2H_5	25		4/4
			50		3/4
			75		3/4
			100	4/4	0/4
3e	R	benzyl	25		4/4
			50		3/4
			75		2/4
			100	4/4	0/4
3f	R	isopropyl	25		
			50		4/4
			75		3/4
			100	4/4	2/4(0/4)[f]

[a]All compounds were dissolved in polyethyleneglycol400 and administered i.p. to ICR male mice. Dose was denoted in mg/kg. [b]The MES test : 50 mA, 60 Hz, ac, 0.2 sec., *via* corneal electrods, 30 min post administration of test compound. The results were denoted as non-protected animals/tested animals. [c]The PTZ test: Subcutaneous pentylenetetrazol (80 mg/kg) 30 min post administration of test compound. The results were denoted as non-protected animals/tested animals. [d]at a dose of 125 mg/kg. [e]at dose of 12.5 mg/kg. [f] at a dose of 150 mg/kg.

Table III. The Selected anticonvulsant evaluation of *N*-*cbz*-α-amino-*N*-alkoxyglutarimides (**2** and **3**) in Mice

Compound	Config.	R	TD_{50}[b] (mg/kg)	ED_{50}(mg/kg)[a]	
				MES(PI)[c]	PTZ(PI)[d]
2a	R	H	100	93.75(1.07)	87.5(1.14)
2b	R	CH_3	118.7	118.75(1.0)	137.5(0.86)
2c	R	C_2H_5		118.75	125.0
2e	R	benzyl	100	–	62.5(1.6)
2f	R	isopropyl		f	f
3a	S	H	100	93.75(1.07)	84.06(1.19)
3b	S	CH_3	106.5	86.25(1.23)	75(1.42)
3c	S	C_2H_5	112.5	–	75(1.5)
3e	S	benzyl	–	–	68.75
3f	S	isopropyl		–	100
Diphenylhydantoin[e]			65.4	9.5(6.9)	f
Phenobarbital[e]			69.0	21.8(3.1)	13.1(5.3)
Ethosuximide[e]			440.8	f	130.4(3.4)
Methosuximide[e]			130.1	42.6(3.1)	34.5(3.7)
Valproic aid[e]			425.8	271.1(1.6)	148.6(2.9)
Trimethadione[e]			1070.0	704.2(1.5)	250.5(4.3)

[a]All compounds were administered i.p. to ICR male mice, and all anticonvulsant tests were performed in groups of 4 mice 30 min after test compound administration. [b]Rotorod test for neurotoxicity in groups of 5 mice. [c]maximal electric shock seizure test : 50 mA, 60 Hz, ac, 0.2 s. and PI is protective index (TD_{50}/ED_{50}). [d]Subcutaneous pentylenetetrazole (80 mg/kg) induced seizure test. [e] Witak. *et al.*, 1972. [f]not effective.

compounds were comparable to those of valproic acid. Estimated from these results, **2a-c**, **3a**, and **3b** can be recommended for new anticonvulsant drugs.

CONCLUSION

In conclusion, *N-cbz*-α-amino-*N*-hydroxyglutarimide **2a**, **3a** and *N-cbz*-α-amino-*N*-alkoxyglutarimide **2b-f** and **3b-f** were prepared and evaluated for the anticonvulsant activities against the MES and PTZ test, which included the rotorod test, in order to develop a new anticonvulsant of broad spectrum. The most active compound among the tested compounds was **3b** in the MES test. In the case of the PTZ test, **2e** was the most active one. As judged from the ED_{50} value, **3b** was 3.1 times more active than valproic acid in the MES test, and in the case of the PTZ test, **2e** was 2.4 times more active than valproic acid, a currently marked antiepileptic drug. And also, the neurotoxicities of these compounds were comparable to those of other anticonvulsant drugs. Especially **2a-c**, **3a**, and **3b** showed a broad spectrum of anticonvulsant activities. Based on these estimates, *N-cbz*-α-amino-*N*-alkoxyglutarimide are anticonvulsant compounds adequate enough to be recommended for new anticonvulsant drug candidates.

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