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Synthesis and Anticonvulsant Evaluation of *N-Cbz*-α-amino-*N*-alkoxysuccinimides

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In previous studies for the development of new anticonvulsants, we found that *N-Cbz*-α-amino-*N*-alkylsuccinimides exhibited significant anticonvulsant activities in the Maximal electroshock seizure (MES) and Pentylenetetrazole induced seizure (PTZ) tests, and also their anticonvulsant activities were dependent on the *N*-alkyl substituents existent in their structures. Based on these estimations, *N-Cbz*-α-amino-*N*-hydroxysuccinimide and various *N-Cbz*-α-amino-*N*-alkoxysuccinimides were prepared in order to develop more active anticonvulsants and to examine the effects of *N*-hydoxy or *N*-alkoxy groups on their anticonvulsant activities. The (*R*)- or (*S*)-*N-Cbz*-α-amino-*N*-hydroxysuccinimide and *N-Cbz*-α-amino-*N*-alkoxysuccinimides were prepared from the corresponding (*R*)- or (*S*)-*N-Cbz*-aspartic acid through the known synthetic procedures. Their anticonvulsant activities in the MES and PTZ test were evaluated. All of these compounds except **3a** showed significant anticonvulsant activities against the PTZ test, but these compounds were not active in the MES test. The most active compound in the PTZ test was (*R*)-*N-Cbz*-α-amino-*N*-benzyloxysuccinimide (ED_{50}=62.5 mg/kg). In addition, the anticonvulsant activities of these compounds were dependent on their N-substited groups. The order of anticonvulsant activity against the PTZ test , as judged from the ED_{50} values for (*R*) series was *N*-benzyloxy > *N*-hydroxy > *N*-isopropoxy > *N*-methoxy > *N*-ethoxy; for the (*S*) series *N*-ethoxy > *N*-benzyloxy > *N*-methoxy > *N*-isopropoxy.

Key words: Anticonvulsant, Succinimide, MES test, PTZ test

INTRODUCTION

In our previous research for the development of new anticonvulsants of broad spectrum, *N*-Acyl-α-amino-*N*-alkylsuccinimide, such as **1** in Fig. 1, was found to show significant anticonvulsant activities in the Maximal electroshock seizure test (MES test) and the Pentylenetetrazole induced seizure test (PTZ test) (Park *et al*, 1996). Also the anticonvulsant activities were found to be dependent on the *N*-acyl groups and *N*-alkyl groups of their structure (Lee *et al*, 1997; Jung *et al.*, 1998).

Based on these results, *N-Cbz*-α-amino-*N*-hydroxysuccinimide and *N-Cbz*-α-amino-*N*-alkoxysuccinimide **2**, **3**, which were substituted with hydroxy or alkoxy groups instead of imide *N*-H, were prepared and their anticonvulsant activities were examined against the MES and PTZ tests in order to develop more active anticonvulsants and to define the effect of *N*-hydroxy or alkoxy groups on pharmacological activities.

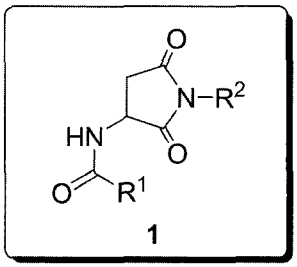

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Fig. 1. The *N*-acyl-α-amino-*N*-alkylsuccinimides

Here, we report the synthesis of (*R*)- and (*S*)-*N-Cbz*-α-amino-*N*-hydroxysuccinimide and various *N-Cbz*-α-amino-*N*-alkoxysuccinimides **2**, **3** and their *in vivo* anticonvulsant activities in the MES and PTZ tests.

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MATERIALS AND METHODS

Melting points were determined by the Electrothermal Melting point Apparatus and were uncorrected. IR spectra were taken in 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 δ 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 Development Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1989).

Synthesis

(*R*)- and (*S*)-*N*-*Cbz*-α-amino-*N*-alkoxysuccimides (**2**, **3**) were prepared from the corresponding (*R*)- or (S)-*N*-*Cbz*-aspartic acid through the known synthetic procedure as shown in Scheme 1.

The (*R*)- or (S)-*N*-*Cbz*-aspartic acid anhydride **5** was prepared in quantitative yield by treating the corresponding *N*-Cbz-aspartic acid **4** with acetic anhydride, and the treatment of hydroxylamine to *N*-*Cbz*-aspartic acid anhydride **5** gave to the *N*-*Cbz*-α-amino-*N*-hydroxysuccinimide (**2a**, **3a**). The *N*-*Cbz*-α-amino-*N*-alkoxy succinimides (**2b-e** and **3b-e**), could be obtained in moderate yields by alkylation of **2a** or **3a** with the corresponding alkyl halide and sodium hydride in dry *N,N*-dimethylformamide.

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

(R)-*N*-*Cbz*-aspartic acid (10 g, 0.039 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*-aspartic acid anhydride as a white solid in quantitative yield. mp: 96.5°C: IR(KBr) cm^{-1}: 1610, 1700, 1755, 3100, 3300.

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

mp: 96.1°C; IR spectrum was identical to **5a**.

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

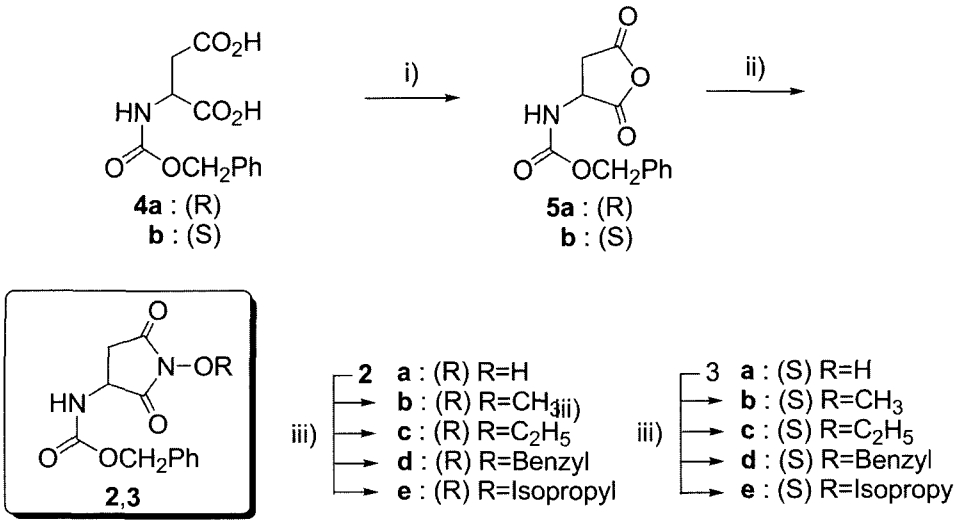
Hydroxylaminehydrochloride (833 mg, 0.012 mol) was dissolved with H_2O (2 mL), and Na_2CO_3 (636 mg) was added to this solution. Then, *N*-*Cbz*-aspartic acid anhydride (2.49 mg, 0.01 mol) was added slowly. The reaction mixture was refluxed for 2-3 h. And the reaction mixture was cooled in an ice bath to afford *N*-*Cbz*-α-amino-*N*-hydroxysuccinimide as a white solid. The crude *N*-*Cbz*-α-amino-*N*-hydroxysuccinimide was recrystallized with ethanol and water. 44%; mp: 134.7°C; IR(KBr) cm^{-1}: 1700, 1730, 3110, 3370: ^{1}H-NMR (DMSO-d_6): δ2.46-2.55 (1H, m), 2.92-3.05 (1H, m), 4.42-4.58 (1H, br), 5.02 (2H, s), 7.38 (5H, s), 10.82 (1H, br).

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

43%; mp: 132.3°C; IR and ^{1}H-NMR spectra were identical to **2a**.

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

To a suspension of NaH (160 mg, 4 mmol) in dry *N,N*-dimethylformamide (5 mL), (*R*)-*N*-*Cbz*-α-amino-*N*-hydroxy-succinimide **2a** (2 mmol) in *N,N*-dimethylformamide (5 mL) was added, and CH_3I (341 mg, 2.4 mmol) in *N,N*-



Scheme 1. The preparation of *N*-*Cbz*-α-amino-*N*-alkoxysuccinimides

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), saturated aqueous NaCl solution (10 mL×2), and dried over anhydrous magnesium sulfate successively. The filtrate was evaporated to give 330 mg of brown solid as a single spot in TLC (ethyl acetate: hexane=3:1). Then, the crude product was recrystallized with ethyl acetate and hexane. 59% : mp: 113.5°C; IR(KBr) cm^{-1}: 1710, 1740, 3350; ^{1}H-NMR (DMSO-d_6): δ2.60-2.75 (1H, m), 2.80-2.94 (1H, m), 3.17 (3H, s), 4.60-4.69 (1H, m), 5.07 (2H, s), 7.38 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-methoxysuccinimide (3b)
57%; mp: 110.3°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*-ethoxysuccinimide(2c)
43%; mp: 148.3°C; IR(KBr) cm^{-1}: 1690, 1710, 3300; ^{1}H-NMR (DMSO-d_6); δ1.28 (3H, t, J = 7.1 Hz), 2.60-2.77 (1H, m), 2.81-2.95 (1H, m), 3.97 (2H, q, J = 7.1 Hz), 4.51-4.65 (1H, m), 5.06(2H, s), 5.92-5.94 (1H, br), 7.36 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-ethoxysuccinimide (3c)
41%; mp: 144.5°C; IR and ^{1}H-NMR spectra were identical to **2c**.

(*R*)-*N-Cbz*-α-amino-*N*-benzyloxysuccnimide (2d)
63%; mp: 114.7°C; IR(KBr) cm^{-1}: 1650, 1695, 1720, 3300; ^{1}H-NMR (DMSO-d_6): δ2.42-2.54 (1H, m), 2.83-2.99 (1H, m), 4.50-4.62 (1H, m), 5.06 (2H, s), 5.10 (2H, s), 5.91-5.94 (1H, br.), 7.33 (5H, s),7.35 (5H, s).

(*S*)-*N-Cbz*-α-amino-*N*-benzyloxysuccinimide (3d)
61%; mp: 112.3°C; IR and ^{1}H-NMR spectra were identical to **2d**.

(*R*)-*N-Cbz*-α-amino-*N*-isopropoxysuccinimide (2e)
32%; mp: 138.5°C; IR(KBr) cm^{-1}: 1630, 1695, 1720, 3310; ^{1}H-NMR (DMSO-d_6): δ1.16 (6H, d, J = 8.20 Hz), 2.40-2.55 (1H, m), 2.82-2.98 (1H, m), 4.03-4.11 (1H, m), 4.47-4.62 (1H, m), 5.09 (2H, s), 5.88-5.93 (1H, br), 7.32 (5H, s)

(*S*)-*N-Cbz*-α-amino-*N*-isoproxysuccinimide (3e)
35%; mp: 139.4°C; IR and ^{1}H-NMR spectra were identical to **2e**.

Pharmacology

The anticonvulsant test for *N-Cbz*-α-amino-*N*-hydroxyglutarimides **2a**, **3a** and *N-Cbz*-α-amino-*N*-alkoxyglutarimides **2b-e** and **3b-e** in the maximal electric shock seizure (MES) test and the pentylenetetrazole induced seizure (PTZ) test 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 were administered ip to ICR male mice at doses of 25, 50, 75, and 100 mg/kg. Thirty minwere after the administration, anticonvulsant tests were performed in groups of four. Also, the lowest doses that could induced seizure in all the tested animals were determined at the stage of preliminary screening. Seizure was then artificially induced by either electroshock or pentylenetetrazole. The MES test was elicited with a 60-cycle a.c. of 50 mA intensity delivered for 0.2 s *via* corneal electrodes with ECT unit (UGO Basline, Italy). A drop of 0.9% saline was instilled in the eye prior to the application of electrodes. Protection in this test was defined as the abolition of the hind limb tonic extension component of seizure. The PTZ test entailed the subcutaneous administration of 80 mg/kg of pentylenetetrazole as a 0.5% solution in the posterior midline of the mice. Then, 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} as quantitative anticonvulsant evaluations were estimated from the dose-response data.

RESULTS AND DISCUSSION

As shown in Scheme 1, all the tested compounds could be prepared from the corresponding (*R*)- or (*S*)-*N-Cbz*-aspartic acid in moderate yields. The spectral data of the tested compounds were satisfactory. And we investigated anticonvulsant activities for these compounds in both the MES and PTZ tests. The results of preliminary anticonvulsant activities were summarized in Table I and II.

As shown in Table I and II, the tested compounds did not show any anticonvulsant activity against the MES test at a dose of 100 mg/ kg. However, in the case of the PTZ test, all the compounds except **5a** exhibited a significant anticonvulsant activities at a dose of 100 mg/kg. According to these results, the tested compounds **2a-e**, **3b-e** could exhibit anticonvulsant activities only against the PTZ test. According to the protocol for the development of new anticonvulsants, the compounds, showing the anticonvulsant activity at a dose of 100 mg/kg, were recommended for further investigation of quantification. Thus the compounds **2a-e** and **3b-e** were selected for the quantitative anticonvulsant evaluation in the PTZ test. The results of the quantitative anticonvulsant activities in the PTZ test were summarized in Table III.

Table I. Anticonvulsant activities of (*R*)-*N*-*Cbz*-α-amino-*N*-alkoxy-succinimides (**2**) in mice

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

[a] All compounds were dissolved in polyethyleneglycol400 and were 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 electrodes, 30 min post administration of the test compound. And 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. And the results were denoted as non-protected animals/ tested animals. [g] at a dose of 125 mg/kg. [h] at a dose of 150 mg/kg. [i] at a dose of 175 mg/kg.

As seen in Table III, (*R*)-*N*-*Cbz*-α-amino-*N*-benzyloxy-succinimide **2d** (ED_{50} = 62.5 mg/kg) was most active among the tested compounds in the PTZ test. Judging from ED_{50} values, the anticonvulsant activity of **2d** in the PTZ test of was 2.3 times more active than that of valproic acid, one of the widely used anticonvulsant drug in clinical practice. Also, anticonvulsant activities against the PTZ test of other compounds were comparable to those of other anticonvulsant drugs.

Interestingly, the anticonvulsant activities of these compounds were dependent on the *N*-substituted hydroxy or alkoxy groups in these compounds as follows. The order of anticonvulsant activity against the PTZ test that was as judged from the ED_{50} values for (*R*) series was *N*-benzyloxy > *N*-hydroxy > *N*-isopropoxy > *N*-methoxy > *N*-ethoxy; for the (*S*) series *N*-ethoxy > *N*-benzyloxy > *N*-methoxy > *N*-isopropoxy. From these results, it was con-

Table II. Anticonvulsant activities of (*S*)-*N*-*Cbz*-α-amino-*N*-alkoxy-succinimides (**3**) in mice

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

[a] All compounds were dissolved in polyethyleneglycol400 and were 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 electrodes, 30min post administration of the test compound. And the results were denoted as non-protected animals/tested animals. [c] The PTZ test: Subcutaneous pentylene tetrazol (80 mg/kg) 30 min post administration of test compound. And the results were denoted as non-protected animals/ tested animals. [g] at a dose of 125 mg/kg. [h] at a dose of 150 mg/kg. [i] at a dose of 175 mg/kg. [j] at a dose of 200 mg/kg.

Table III. The selected anticonvulsant evaluations of *N*-*Cbz*-α-amino-*N*-alkoxysuccinimides (**2** and **3**) in mice

Compound	Config.	R	ED_{50}(mg/kg)[a]	
			MES(PI)[b]	PTZ[c]
2a	R	H	–	81.3
2b	R	CH_3	–	112.5
2c	R	C_2H_5	–	131.3
2d	R	benzyl	–	62.5
2e	R	isopropyl	–	87.5
3a	S	H	–	–
3b	S	CH_3	–	125
3c	S	C_2H_5	–	100
3d	S	benzyl	–	106.3
3e	S	isopropyl	–	143.8
Diphenylhydantoin[d]			9.5(6.9)	[f]
Phenobarbital[d]			21.8(3.1)	13.1(5.3)
Ethosuximide[d]			f	130.4(3.4)
Methosuximide[d]			42.6(3.1)	34.5(3.7)
Valproic aid[d]			271.1(1.6)	148.6(2.9)
Trimethadione[d]			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 the administration of test compound. [b] maximal electric shock seizure test : 50 mA, 60 Hz, ac, 0.2 sec. [c] Subcutaneous pentylenetetrazole (80 mg/kg) induced seizure test. [d] Witak., *et al.* 1972. [f] not effective.

ceivable that the *N*-substituted group might play an important role for their anticonvulsant activities.

CONCLUSION

In connection with the development of new anticonvulsants of the *N*-Acyl-α-aminosuccinimides, *N-Cbz*-α-amino-*N*-hydroxysuccinimide, and various *N-Cbz*-α-amino-*N*-alkoxysuccinimides, which were substituted with hydroxy or various alkoxy groups instead of imide *N*-H in the *N*-Cbz-α-aminosuccinimides, were prepared and their anticonvulsant activities were examined in the MES and PTZ test in order to develop more active anticonvulsants and to define the effects of the *N*-substituted group on their anticonvulsant activities.

All the tested compounds could be prepared from the corresponding (*R*)- or (*S*)-*N-Cbz*-aspartic acid in moderate yields. The tested compounds did not show any anticonvulsant activity against the MES test at the dose of 100 mg/kg. However, in the PTZ test, all the compounds except **5a** exhibited significant anticonvulsant activities. Among them, (*R*)-*N-Cbz*-α-amino-*N*-benzyloxysuccinimide **2d** (ED_{50} = 62.5 mg/kg) was most active in the PTZ test. Also, the anticonvulsant activities of other compounds against the PTZ test were comparable to those of other anticonvulsant drugs. In addition, the pharmacological activities of this series of compounds were dependent on their *N*-substituted hydroxy or alkoxy groups. According to these results, it was conceivable that the *N*-substituted group might play an important role for their anticonvulsant activities of the *N-Cbz*-α-aminosuccinimides.

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