Arch. Pharm. Res. Vol.20, No.1, pp. 53-57, 1997

The Effect of N-Substituted Alkyl Groups on The Anticonvulsant Activities of *N*-Cbz-α-amino-*N*-alkylsuccinimides

Jaewon Lee[1], Kichun Son[2], Kyungim Jung[2], Jongwon Choi[2] and Minsoo Park*[2]

[1]Koshin Medical Center, Amnam-Dong, Seo-Gu, Pusan, Korea 608-720 and [2]College of Pharmacy, Kyungsung University, 110-1, Daeyeon-Dong, Nam-Gu, Pusan 608-736, Korea

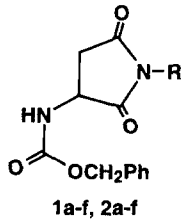
(Received August 7, 1996)

For the purpose of defining the effects of the *N*-substituted alkyl groups on the anticonvulsant activities of *N*-Cbz-α-aminosuccinimides, various (*R*)- and (*S*)-*N*-alkyl substituted *N*-Cbz-α-aminosuccinimides (**1** and **2**) were prepared from the corresponding (*R*)- and (*S*)-*N*-Cbz-aspartic acid by using known reaction and were evaluated the anticonvulsant activies in the MES and PTZ tests, including their neurotoxicities. The most active compound in the MES test was (*R*)-*N*-Cbz-α-amino-*N*-methylsuccinimide (**1b**) (ED_{50}=52.5 mg/kg, PI=3.2). And in case of the PTZ test, (*R*)-*N*-Cbz-α-amino-*N*-ethylsuccinimide (**1c**) was the most active compound (ED_{50}=32.5 mg/kg, PI=3.1). The order of anticonvulsant activities of these compounds against the MES test, as judged from the ED_{50} values for the *R* series (**1**), was *N*-methyl > *N*-isobutyl > non-substituted > *N*-ethyl, *N*-allyl > *N*-benzyl compound; for the *S* series (**2**) *N*-methyl > *N*-allyl > non-substituted > *N*-isobutyl > *N*-ethyl > *N*-benzyl compound. The anticonvulsant activities in the PTZ tests of these compounds exhibited somewhat different pattern ; for the *R* series (**1**) *N*-ethyl > *N*-methyl > *N*-isobutyl> non-substituted > *N*-allyl > *N*-benzyl compound in order of decreasing activity; for *S* series (**2**) *N*-ethyl > *N*-allyl, non-substituted > *N*-isobutyl > *N*-methyl > *N*-benzyl compound in order of decreasing activity.

Key words : Anticonvulsant, Maximal electric shock seizure(MES), Pentylenetetrazole induced seizure(PTZ), *N*-Cbz-α-aminosuccinimide, Imide, Structure-activity relationship

INTRODUCTION

In connection with the developement of new anticonvulsants of broader spectrum, the preceding papers (Lee *et al.*, 1996; Park *et al.*, 1996) reported that *N*-Cbz-α-amino-*N*-alkylsuccinimides, combining the common structures of currently available anticonvulsants such as N-CO-C-N and imide in a single molecule, exhibited significant anticonvulsant activities in both the maximal elecric shock seizure (MES) test and the pentylenetetrazole induced seizure (PTZ) test enough to be recommended as new anticonvulsants. The anticonvulsant activities of these compounds depended on the *N*-substituted alkyl chains. From the previous studies, it was conceivable that *N*-alkyl groups might play an important role for the anticonvulsant activities of this series of compounds.



1a-f, 2a-f

1a: (R), R= H
b: (R), R= CH_3
c: (R), R= C_2H_5
d: (R), R= allyl
e: (R), R= isobutyl
f : (R), R= benzyl

2a: (S), R= H
b: (S), R= CH_3
c: (S), R= C_2H_5
d: (S), R= allyl
e: (S), R= isobutyl
f : (S), R= benzyl

Fig. 1.

This estimates promted us to prepare the various *N*-alkyl substituted analogues of these compounds and investigate their anticonvulsant activities in order to clarify the effects of *N*-substituted alkyl groups on their anticonvulsant activities.

Herein we wish to report the synthesis and the anticonvulsant activities of the various *N*-alkyl substituted *N*-Cbz-α-aminosuccinimides (**1** and **2**) as shown in Fig. 1. And in this paper, we focused on the effects of *N*-substituted alkyl groups on the anticonvulsant activities of these compounds (**1** and **2**).

MATERIALS AND METHODS

Melting points were determined by Electrothermal

Correspondence to: Minsoo Park, College of Pharmacy, Kyungsung University, 110-1, Daeyeon-Dong, Nam-Gu, Pusan, Korea 608-736

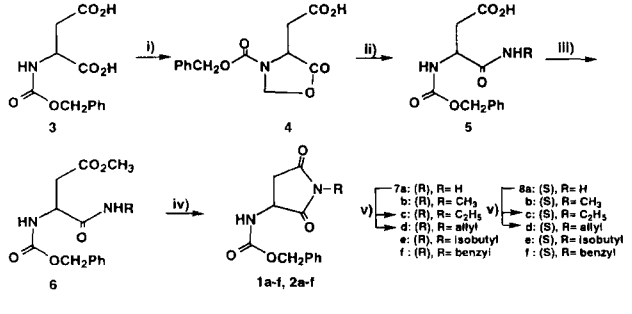
digital melting point apparatus and were uncorrected. IR spectra were taken in KBr disks with JASCO FT/IR 200 and were reported in cm^{-1}. ^{1}H-NMR spectra were recorded in DMSO-d_6 on JNM-EX90A and chemical shifts were reported as δ values in parts per million from TMS as an internal standard. All yields referred to chromatographically and spectroscopically homogeneous materials. The pharmacological tests were carried out according to the protocol of the Antiepileptic Drug Developement Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1989).

Synthesis

The synthesis of the compounds (**1a,b,f 2a, b, and f**) has been previously reported (Lee *et al.*, 1996). And also (*R*)- and (*S*)-*N*-Cbz-α-amino-*N*-isobutylsuccinimide (**1e** and **2e**) were prepared according to this synthetic procedure. *N*-Cbz-*N*-ethylsuccinimide (**1c** and **2c**) and *N*-Cbz-*N*-allylsuccinimide (**1d** and **2d**) were prepared by N-alkylation of the corresponding (*R*)-or (*S*)-*N*-Cbz-α-aminosuccinimide (**1a** or **2a**) in dry *N,N*-dimethylformamide with sodium hydride and corresponding alkyl bromide. The synthetic procedure was outlined in Scheme 1.

(*R*)-*N*-Cbz-α-amino-*N*-ethylsuccinimide (1c)

To a suspension of NaH (96 mg, 4 mmol) in dry *N,N*-dimethylformamide (5 mL), the solution of (*R*)-*N*-Cbz-α-aminosuccinimide (**1a**, 496 mg, 2 mmol) in *N,N*-dimethylformamide (5 mL) was added. And the mixture was stirred for 0.5 hrs in ice bath and followed by addtion of ethyl bromide (326 mg, 6 mmol). Then the reaction mixture was stirred at room temperature for 4-5 hrs. The reaction mixture was evaporated *in vacuo* and the residue was dissolved with EtOAc (250 mL). The EtOAc layer was washed with 5% $NaHCO_3$ (25 mL × 2), 5% HCl (25 mL × 2), H_2O (25 mL × 2) and saturated NaCl (25 mL × 2) successively and dried over anhydrous $MgSO_4$. The filtrate was evaporated to give brown solid. This crude product was purified with silicagel column chromatography (230-400 mesh, EtOAc: Hexane=2:1) to afford 419 mg of white solid (76%). mp: 72.5°C; IR (KBr) cm^{-1}: 3350, 1720, 1690; ^{1}H-NMR (DMSO-d_6): δ 1.18 (3H, t, *J*=6.4 Hz), 2.70-2.82 (1H, m), 3.02-3.16 (1H, m), 3.57 (2H, q, *J*= 6.4 Hz), 4.21-4.37 (1H, m), 5.10 (2H, s), 5.40-5.60 (1H, br), 7.34 (5H, s) The following compounds were prepared according to the above procedure.



Scheme 1. The preparatiion of *N*-Cbz-α-aminosucinimides (1 and 2).

(*S*)-*N*-Cbz-α-amino-N-ethylsuccinimide (2c)

75%; mp: 72.2°C; IR and ^{1}H-NMR spectra were identical to **1c**.

(*R*)-N-Cbz-α-amino-*N*-allylsuccinimide (1d)

68%; mp: 75.0°C; IR (KBr) cm^{-1}: 3300, 1710, 1690; ^{1}H-NMR (DMSO-d_6): δ 2.70-2.88 (1H, m), 3.04-3.17 (1H, m), 4.15-4.23 (2H, m), 4.29-4.37 (1H, m), 5.10 (2H, s), 5.10-5.22 (2H, m), 5.40-5.60 (1H, br), 5.71-5. 80 (1H, m), 7.34 (5H, s).

(*S*)-*N*-Cbz-α-amino-*N*-allylsuccinimide (2d)

69%; mp: 79.0-82.2°C; IR and ^{1}H-NMR spectra were identical to **1d**.

The following compounds were prepared according to the previously reported synthetic methods (Lee *et al.*, 1996).

(*R*)-*N*-Cbz-α-amino-*N*-isobutylsuccinimide (1e)

67% (step iv), mp: 108.1°C; IR (KBr) cm^{-1}: 3300, 1700, 1680; ^{1}H-NMR (DMSO-d_6): δ 0.91 (6H, d, *J*= 6.6 Hz), 2.04-2.16 (1H, m), 2.78-2.90 (1H, m), 3.00-3.17 (1H, m), 3.40 (2H, d, *J*=7.4 Hz), 4.30-4.40 (1H, m), 5.11 (2H, s), 5.30-5.40 (1H, br), 7.25 (5H, s)

(*S*)-*N*-Cbz-α-amino-*N*-isobutylsuccinimide (2e)

65% (step iv), mp: 106.8°C; IR and ^{1}H NMR spectra were identical to **1e**.

Pharmacology

The anticonvulsant test for those compounds (**1**) in maximal electric shock seizure (MES) test, pentylenetetrazole induced seizure (PTZ) test, and neurotoxicity in rotorod test in mice were carried out according to the protocol of the Antiepileptic Drug Developement Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1988) as follows. All the tested compounds were dissolved in polyethylene glycol 400 and administered ip at dose of 25, 50, 75, 100 and 150 mg/kg and anticonvulsant tests were performed in groups of 4 mice

(ICR) and 30 min after administration. And also we determined the lowest dose that all the tested animals could be induced seizure at the stage of primary screening. Seizures were then artificially induced by either electric shock or pentylenetetrazole. The maximal electric shock seizure (MES) tests were elicited with a 60-cycle a.c. of 50 mA intensity delivered for 0.2 s. *via* corneal electrod with ECT unit (UGO Basline, Itlay) . 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 hind limb tonic extension component of seizure. The pentylenetetrazole seizure (PTZ) test entailed the administration of 80 mg/kg of pentylenetetrazole as a 0.5% solution subcutaneously in the posterior midline of 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). And the ED_{50} values were 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 Rotorod Treadmill for mice (UGO Basline, Itlay) as follows. The five animals were placed on an 1 inch diameter knurled plastic rod rotating at 6 rpm after the administration of the compounds. Normal mice could remain on a rod at this speed indifinitely. Neurological toxicity was defined as the failure of the animal to remain on the rod for 2 min. And the median neurotoxic dose (TD_{50}) was estimated from the dose-response data.

RESULTS AND DISCUSSION

As seen in Scheme 1, all the tested compounds (**1a-f** and **2a-f**) could be prepared from the corresponding (*R*)- or (*S*)-*N*-Cbz-asparic acid in moderate yields. And all products gave satisfactory spectral data. And we investigated the anticonvulsant activity for these compounds (**1** and **2**) in both the MES test and the PTZ test according to the protocol of the Antiepileptic Drug Development Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1989). The results of preliminary anticonvulsant activities were summarized in Table I and Table II.

As seen in Table I and II, all the tested compounds, except **1f** and **2f**, showed significant anticonvulsant activities in both the MES and PTZ test. And the anticonvulsant activities were exhibited as dose-dependent patterns. According to the prototocol for the development of new anticonvulsant, the compounds, showing significant anticonvulsant activities at dose of 100 mg/kg, were recommended as promising anticonvulsant to submit to further investigation. So we tried to investigate the further anticonvulsant evaluation to define the median effective dose (ED_{50}) and the median neurotoxic dose (TD_{50}). And the results were summarized in Table III.

Table I. Anticonvulsant Activity of (*R*)-*N*-Cbz-α-aminosuccinimides (1) in Mice

Compounds	Config.	R	Dose[a]	MES[b]	PTZ[c]
1a	*R*	H	25		
			50	4/4	4/4
			75	3/4	3/4
			100	2/4(0/4)[d]	2/4 (0/4)[e]
1b	*R*	CH_3	25	4/4	
			50	2/4	4/4
			75	1/4[f]	3/4[f]
			100	0/4[g]	0/4[g]
1c	*R*	C_2H_5	25		2/4(4/4)[h]
			50		1/4
			75		0/4
			100	4/4(0/4)[d]	
1d	*R*	allyl	25		4/4
			50	4/4	3/4
			75	3/4	2/4(0/4)[e]
			100	2/4(0/4)[e]	
1e	*R*	isobutyl	25		4/4
			50		3/4
			75	4/4	1/4(0/4)[e]
			100	2/4(0/4)[e]	
1f		benzyl	25		4/4
			50		3/4
			75		2/4(0/4)[e]
			100	4/4	

[a]All compounds were dissolved in polyethyleneglycol 400 and administerd 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. And the resuts 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 resuts were denoted as non-protected animals/ tested animals. [d]at dose of 200 mg/kg. [e]at dose of 150 mg/kg. [f]at dose of 80 mg/kg. [g]at dose of 90 mg/kg. [h]at dose of 5 mg/kg.

As seen in Table III, the anticonvulsant activities of these compounds were comparable to the currently marketed antiepileptic drugs. The most active compound in the MES test was (*R*)-*N*-Cbz-α-amino-*N*-methylsuccinimide (**1b**) (ED_{50}=52.5 mg/kg, PI=3.2). As judged from the ED_{50} values, the anticonvulsant activity of **1b** was 5-fold more active than that of valproic acid, known as anticonvulsant of broad spectrum. And in case of the PTZ test, (*R*)-*N*-Cbz-α-amino-*N*-ethylsuccinimide (**1c**) was the most active one (ED_{50}=32.5 mg/kg, PI=3.1). The anticonvulsant activity of **1c** was 4.6-fold more active than that of valproic acid. The TD_{50} values and the protective indices (PI) of *N*-Cbz-α-aminosuccinimides (**1** and **2**) were comparable to those of other anticonvulsant drugs. Interestingly, the anticonvulsant activities were dependent on the *N*-substituted alkyl groups. The order of anticonvulsant activities against MES test of these compounds, as

Table II. Anticonvulsant activity of (*S*)-*N*-Cbz-α-aminosuccinimides (2) in mice

Compounds	Config.	R	Dose[a]	MES[b]	PTZ[c]
2a	*S*	H	25	4/4	4/4
			50	3/4	3/4
			75	2/4	2/4
			100	2/4(0/4)[d]	1/4(0/4)[e]
2b	*S*	CH_3	25	4/4[f]	
			50	2/4	
			75	2/4	4/4
			100	0/4[g]	3/4(0/4)[e]
2c	*S*	C_2H_5	25		3/4(4/4)[h]
			50		3/4
			75	1/4	2/4
			100	0/4	1/4(0/4)[e]
2d	*S*	Allyl	25		4/4
			50	4/4	3/4
			75	2/4	2/4
			100	1/4(0/4)[e]	1/4(0/4)[e]
2e	*S*	isobutyl	25		4/4
			50	4/4	2/4
			75	3/4	2/4
			100	2/4(0/4)[e]	2/4(0/4)[e]
2f	*S*	benzyl	25		
			50		4/4
			75		3/4
			100	4/4(4/4)[d]	2/4(0/4)[d]

[a]All compounds were dissolved in polyethyleneglycol400 and administerd 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. And the resuts 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 resuts were denoted as non-protected animals/tested animals. [d]at dose of 200 mg/kg. [e]at dose of 150 mg/kg. [f]at dose of 30 mg/kg. [g]at dose of 80 mg/kg. [h]at dose of 15 mg/kg.

judged from the ED_{50} values for the *R* series (**1**), was *N*-methyl > *N*-isobutyl > non-substituted > *N*-ethyl, *N*-allyl > *N*-benzyl compound; for the *S* series (**2**) *N*-methyl > *N*-allyl > non-substituted > *N*-isobutyl > *N*-ethyl > *N*-benzyl compound. The anticonvulsant activities in the PTZ tests of these compounds exhibited somewhat different pattern ; for the *R* series (**1**) *N*-ethyl > *N*-methyl > *N*-isobutyl > non-substituted > *N*-allyl > *N*-benzyl compound in order of decreasing activity; for *S* series (**2**) *N*-ethyl > *N*-allyl, non-substituted > *N*-isobutyl > *N*-methyl > *N*-benzyl compound. Based on the above results, it was conceivable that the *N*-substituted alkyl group might play an important role on the anticonvulsant activity of these compounds. In our previous papers about the anticonvulsant activities of *N*-Cbz-α-amino-glutarimides (Park *et al.*, 1996), it was reported that the (*S*) isomer was more active isomer. However, such stereoisomeric pharmacological patterns were not exhibited clearly in the series of *N*-Cbz-α-aminosuccinimide in this study.

Table III. The selected anticonvulsant evaluation of *N*-Cbz-α-aminosucinimides (1 and 2) in mice

Compound	Config.	R	TD_{50}[b] (mg/kg)	ED_{50} (mg/kg)[a]	
				MES (PI)[c]	PTZ(PI)[d]
1a	*R*	H	178.0	125.0(1.4)	110.0(1.6)
1b	*R*	CH_3	166.7	52.5(3.2)	82.5(2.0)
1c	*R*	C_2H_5	100.0	150.0(0.7)	32.5(3.1)
1d	*R*	allyl	98.8	150.0(1.0)	112.5(0.9)
1e	*R*	isobutyl	88.1	106.9(0.8)	91.3(1.0)
2a	*S*	H	160.8	103.0(1.6)	78.1(2.1)
2b	*S*	CH_3	117.5	62.2(1.9)	113.3(1.0)
2c	*S*	C_2H_5	94.3	119.4(0.8)	73.8(1.3)
2d	*S*	allyl	81.3	86.9(0.9)	78.1(1.0)
2e	*S*	isobutyl	63.1	106.9(0.6)	81.9(0.8)
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)
Methsoximide[e]			130.1	42.6(3.1)	34.5(3.7)
Valproic acid[e]			425.8	271.7(1.6)	148.6(2.9)
Trimethadione[e]			1070.0	704.2(1.5)	250.5(4.3)

[a]All compounds were administered ip 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, 60Hz, 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.

CONCLUSIONS

In conclusion, various (*R*)- and (*S*)- *N*-alkyl substituted *N*-Cbz-α-aminosuccinimides (**1** and **2**) were prepared in order to define the effects of the *N*-substituted alkyl groups on the anticonvulsant activities of *N*-Cbz-α-aminosuccinimides (**1** and **2**) and the anticonvulsant activites were evaluated in the MES and PTZ tests, including their neurotoxicities. The anticonvulsant activities and neurotoxicities of these compounds (**1** and **2**) were comparable to those of the currently marketed antiepileptic drugs. Interestingly, the anticonvulsant activities were depended on the *N*-substituted alkyl groups as shown in Table III. Based on the above results, *N*-methylated compound (**1b** or **2b**) showed more active anticonvulsant activity against the MES test than any other *N*-alkylated compounds. In the case of PTZ test, the *N*-ethylated compound (**1c** or **2c**) was more active one than any other *N*-alkylated compounds.

ACKNOWLEDGEMENTS

This work was supported by a grant of KOSEF (951-0710-2) and Research Foundation of Kyungsung University.

REFERENCES CITED

Lee, J., Choi, J., and Park, M., Synthesis and an-

ticonvulsant evaluation of a series of (*R*)- and (*S*)-*N*-Cbz-α-aminosuccinimide and their structure activity relathionship. *Arch. Pharm. Res.*, 19, 312-316 (1996).

Park, M., Lee, J., and Choi., J., Synthesis and anticonvulsant evaluation of a series of (*R*)- and (*S*)-*N*-Cbz-α-aminoglutarimide and succinimide. *Bioorg. Med. Chem. Lett.*, 6, 1298-1302 (1996).

Swinyard, E. A., Woodhead, J. H., White, H. S. and Frankline, M. R., *General Principles, Experimental Section, Quantification and Evaluation of Anticonvulsants in Antiepileptic Drugs*, 3rd Ed: In Levy, R., *et al.*, (Eds.), Ravan Press, N. Y., 1989, p. 88.

Witak, D. T., Seth, S. K., Baizman, E. R., Wiebel, S. L. and Wolf, H. H., Para-substituted *N*-acetyl-L(*S*)-and-D(*R*)-α-amino-*N*-phenylsuccinimides and -glutarimides. Substituent effects on stereoselective anticonvulsant activity. *J. Med. Chem.*, 15, 1117-1123 (1972).