Arch. Pharm. Res. Vol.19, No.4, pp. 312-316, 1996

Synthesis and Anticonvulsant Evaluation of A Series of (R)- and (S)-*N*-Cbz-α-aminosuccinmide and their Structure Activity Relationship

Jaewon Lee[1], Jongwon Choi[2], and Minsoo Park*[2]

[1]Koshin Medical center, Amnam-Dong, Seo-Gu, Pusan 602-702, Korea, [2]College of Pharmacy, Kyung-sung University, 110-1, Daeyeon-Dong, Nam-Gu, Pusan 608-736, Korea

(Received April 10, 1996)

A series of *N*-Cbz-α-aminosucinimides (**1**), combining common moieties of various anticonvulsants such as N-CO-C-N and cyclic imide in a single molecule, were synthesized from the corresponding (R)- and (S)-*N*-Cbz-aspartic acid (**2**). And their *in vivo* anticonvulsant evaluations in MES and PTZ test were investigated. And also the rotorod test for neurotoxicity was investigated. All the tested compounds (**1**), except **1c** and **1f**, showed significant anticonvulsant activities in both MES and PTZ test. And the most active compound among them in MES test was (R)-*N*-Cbz-α-amino-*N*-methylsuccinimide (**1b**) (ED_{50}=52.5 mg/kg) and (S)-*N*-Cbz-aminosuccinimide((**1d**) was most active in PTZ test (ED_{50}=78.1 mg/kg). And the TD_{50} values of the tested compounds were above 117.5 mg/kg. These pharmacological data were comparable to those of currently available anticonvulsants. And also we found that the pharmacological effects were dependent on their *N*-substituted alkyl chains and their stereochemistry.

Key words : Anticonvulsant, Maximal Elecectric Shock Seizure (MES), Pentylenetetrazole induced Seizure (PTZ), *N*-Cbz-α-aminosuccinimide, Imide

INTRODUCTION

Epilepsy is a collective designation of seizure disorders that affect about 1% of population (Harvey and Champe, 1992). And currently marketed anticonvulsants do not often provide complete control of seizure, which consists of various form of seizures (Wilder, 1985). So no single agent is effective against the various forms and degrees of convulsive disorders. Furthermore, it was reported that about 20-40% of epileptic patients failed to experience significant seizure control with the currently available drugs (Harvey and Champe, 1992).

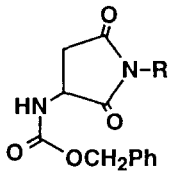
Therefore, the past dacade has witnessed a resurgence of interest in developement of new anticonvulsant drugs including modification of drugs, such as hydantoin (Sun *et al.*, 1994; Brouillette *et al.*, 1994; Gaoni *et al.*, 1994) and succinimide (Borenstein and Dukas, 1987; Kornet, 1984), GABA related compounds (Anderson *et al.*, 1993; N'Goka *et al.*, 1991), NMDA antagonists (Heckendorn *et al.*, 1993; Hays *et al.*, 1993) and derivatives of various amino acids (Kohn *et al.*, 1994; idem, 1993). But these compounds were reported to have limitations of narrow anticonvulsant spectrum.

Consquently, there is a need for the developement of antiepileptic substances having broader clinical spectrum and lower side effects.

In connection with the developement of new anticonvulsant, we examined the structural similarities of various anticonvulsant compounds, known to act by different mechanisms. From the above inspection, we found out the interesting facts that hydantoins and *N*-acylamino acid amides, showing anticonvulsant effect in maximal electric shock seizure (MES) test, had the N-CO-C-N and succinimides such as phensuximide, ethosuximide and methsuximide, showing anticonvulsant effect in pentylenetetrazole induced seizure (PTZ) test, had cyclic imides as common moiety in their structure. And also NMDA antagonists, showing anticonvulsant activity, had the structural similarity to the aspartic acid or glutamic acid in view of bioisoster.

Based on the above facts, we designed the following structure such as **1** in Fig .1, combining all the forementioned moieties in a single molecule, as new anticonvulsant of broader spectrum. Usually the stereoisomers exhibited different pharmacological ac-

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



1a: (R) R=H
b: (R) R=CH_3
c: (R) R=CH_2Ph
d: (S) R=H
e: (S) R=CH_3
f: (S) R=CH_2Ph

Fig. 1.

tivities, so we prepared the (R) and (S) compounds in order to investigate the pharmacological differences between their stereoisomers.

Herein we wish to report the synthesis of a series of *N*-Cbz-α-aminosuccinimides (**1**) and their *in vivo* anticonvulsant evaluations in MES and PTZ test.

METERIALS AND METHODS

Melting points were determined by Electrothermal 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}. ^1H 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.*, 1988).

Synthesis

The compounds (**3-5**) could be prepared from the corresponding (R)- or (S)-*N*-Cbz-aspartic acid in moderate yields by using known chemical reactions (Itho, 1969: Sandler *et al.*, 1972) as shown in Scheme 1. The synthetic procedures for the preparation of (R)- or (S)-*N*-Cbz-α-aminosuccinimide (**1a-f**) were as follows; the compound **3** could be prepared from *N*-Cbz-aspartic acid by treating paraformaldehyde (1.5 eq.) and catalytic amount of *p*-toluenesulfonic acid in quantitative yield and the treatment of **3** with excess amine in methanol gave **4** quantitatively. The compound **5** was obtained from **4** in 70-85% yields by treating with thionyl chloride in methanol and stirring at room temperature for 2-3 hrs. Then we prepared (R)- or (S)-*N*-Cbz-α-aminosuccinimides (**1a-f**), the final compounds, by refluxing of **5** with 0.5 equivalent of *p*-toluenesulfonic acid in toluene as follows.

(R)-*N*-Cbz-α-aminosuccinimide (1a)

(R)-*N*-Cbz-isoasparagine methyl ester (**5a**) (2.48 g, 0.01 mol) and *p*-touenesulfonic acid (954 mg, 0.005 mol) were suspended in toluene (248 mL) and this mixture was refluxed for 8hrs by using Dean-Stark apparatus. Then the reaction mixture was evaporated under reduced pressure and the residue was diluted with 300 mL of EtOAc. The EtOAc layer was washed with 5% $NaHCO_3$ (30 mL × 2), H_2O (30 mL × 2) and saturated saline (30 mL) successively and dried over anhydrous $MgSO_4$. And the filtrate was evaporated to give brown solid. This crude product was purified with silica gel column chromatography (230-400 mesh; EtOAc: hexane=2 : 1) to afford 1.73 g (70%) of white solid. mp: 103.4°C; IR(KBr)cm^{-1}: 3400, 3300, 1730, 1700, 1680; ^1H NMR (DMSO-d_6): δ 2.70-2.82 (1H, m), 2.95-3.07 (1H, m), 4.31-4.42 (1H, m), 5.09 (2H, s), 5.81-5.90 (1H, br), 7.31 (5H, s), 8.95-9.05 (1H, br)

By use of this procedure, the following compounds were prepared.

(R)-*N*-Cbz-α-amino-*N*-methylsuccinimide (1b)

Yield: 73%; mp: 106.6°C; IR(KBr)cm^{-1}: 3300, 1720, 1680; ^1H NMR(DMSO-d_6): δ 2.72-2.86 (1H, m), 3.03 (3H, s), 3.03-3.16 (1H, m), 4.26-4.38 (1H, m), 5.11 (2H, s), 5.40-5.60 (1H, br), 7.34 (5H, s).

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

Yield: 72%; mp: 140.5°C; IR(KBr)cm^{-1}: 3320, 1720, 1680; ^1H NMR(DMSO-d_6): δ 2.70-2.82 (1H, m), 3.03-3.16 (1H, m), 4.20-4.40 (1H, m), 4.68 (2H, s), 5.09 (2H, s), 5.35-5.45 (1H, br), 7.25-7.40 (10H, m).

(S)-*N*-Cbz-α-aminosuccinimide (1d)

Yield: 71%; mp: 107.4°C; IR and ^1H NMR spectra were identical to **1a**.

(S)-*N*-Cbz-α-amino-*N*-methylsuccinimide (1e)

Yield: 72%; mp: 106.6°C; IR and ^1H NMR spectra were identical to **1b**.

(S)-*N*-Cbz-α-amino-*N*-benzylsuccinimide (1f)

Yield: 70%; mp: 138.1°C; IR and ^1H NMR spectra were identical to **1c**.

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

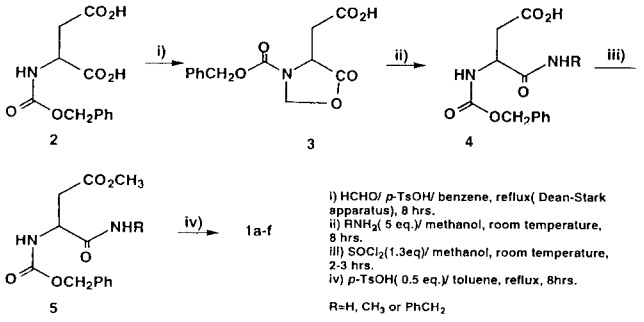
(ICR) and 30 min after administration. And also we determined the lowest dose that all the tested animals could be induced seizure at the stage of primary screening. Seizures were then artificially induced by either electric shock or pentylenetetrazole. The maximal electric shock seizure (MES) tests 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 Baseline, Itlay) as follows. The five animals were placed on a rod 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, The compounds (**1a-f**) could be prepared from the corresponding (R)- or (S)-*N*-Cbz-aspartic acid in moderate yields by using known chemical reactions. And the compounds (**1a-f**) were submitted to the following anticonvulsant tests of primary screening to select the compounds for the further pharmacological investigations, including the determination of ED_{50} and TD_{50} for neurotoxicity.

It was reported that the MES test was correlated to generalized tonic clonic seizure and the PTZ test to generalized absence seizure (Swinyard *et al.*, 1988). So these two kinds of seizure tests are very meaningful for the clinical prediction of anticonvulsant drug candidates. Therefore we investigated the anticonvulsant activities for those compounds (**1**) in MES test and PTZ test and neurotoxicity in rotorod test in mice according to the protocol of the Antiepileptic Drug Developement Program, National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1988). The results of primary anticonvulsant tests were summarized in Table I.

As seen in Table I, all the tested compounds (**1**), except **1c** and **1f**, showed significant anticonvulsant activities at the lower dose of 100 mg/kg in both MES and PTZ tests. And the anticonvulsant activities were revealed as dose dependent pattern. According to the protocol for the development of new anticonvulsant,



Scheme 1.

Table I. Anticonvulsant Activities of *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
			150	2/4(0/4)[d]	
1b	R	CH_3	25	4/4[e]	
			50	1/4	
			75	1.4[f]	4/4[f],3/4[g]
			100		0/4
1c	R	$PhCH_2$	50		4/4
			75		
			100	4/4	2/4
			150	4/4	1/4(0/4)[d]
1d	S	H	25	4/4	4/4
			50	3/4	3/4
			75	2/4	2/4
			100	2/4	1/4
			150	1/4(0/4)[d]	0/4
1e	S	CH_3	25	4/4	
			50	0/4	
			75	0/4	4/4
			100	0/4	3/4
1f			150		0/4
	S	$PhCH_2$	100	4/4	4/4
			150		4/4

[a]All compounds were dissolved in polyethyleneglycol and administerd i.p to ICR male mice. Dose is denoted in mg/kg
[b]The MES test: 50 mA, 60 Hz,ac, 0.2 sec., via corneal electrods, 30min post administration of 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 resuts were denoted as non-protected animals/ tested animals
[d]at dose of 200 mg/kg
[e]at dose of 30 mg/kg
[f]at dose of 70 mg/kg
[g]at dose of 80 mg/kg

Table II. The Selected Anticonvulsant Evaluations and Neurotoxicities

Compound	Config.	R	TD^{b}_{50} (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)
1d	S	H	160.8	103.0(1.6)	78.1(2.1)
1e	S	CH_3	117.5	61.2(1.9)	113.3(1.0)
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, 60 Hz, ac, 0.2 s. and PI is protective index (TD_{50}/ED_{50}).
[d]Sucutaneous pentylenetetrazole (80 mg/kg) induced seizure test.
[e]Data from Witak *et al.* (1972).
[f]not effective.

the compounds, showing significant anticonvulsant activity at dose of 100 mg/kg in mice, were recomended as promising anticonvusants to submit to further pharmacological evaluations. From this estimate, we selected the *N*-Cbz-α-aminosuccinimides (**1a,b,d** and **e**) for the the determination of ED_{50} values and TD_{50} values in rotorod test to define the neurotoxicities, and the results were summarized in Table II.

As seen in Table II, the *N*-Cbz-α-aminosuccinimides showed high anticonvulsant activity in both MES and PTZ tests, and the ED_{50} values were comparable to those of other currently available anticonvulsants. And the most active compound in MES test was (R)-*N*-Cbz-α-amino-*N*-methylsuccinimide (**1b**) (ED_{50}=52.5 mg/kg) and the most active one in PTZ test was (S)-*N*-Cbz-α-aminosuccinimide (**1d**) (ED_{50}=82.5 mg/kg). Interestingly, we found that the pharmacological effects were dependant on their *N*-substituted alkyl chains. In MES test, the *N*-methylated compounds (**1b** or **1e**) were more active than non-alkylated one (**1a** or **1d**) of same configuration, but the benzylated compounds (**1c** and **1f**) had no anticonvulsant effect at the dose of 100 mg/kg. In PTZ test, the effects of *N*-substituted alkyl groups on anticonvulsant activities were found to be somewhat different as follows. Among the (R)-*N*-Cbz-α-aminosuccinimides (**1a,1b** and **1c**), *N*-methylated compound (**1b**) was more active than non-methyalted one (**1a**) and *N*-benzylated compound (**1c**) was not active. But in case of the (S) compounds (**1d,1e** and **1f**), non-alkylated one (**1d**) was more active than *N*-methylated one (**1e**). From the above results, we thought the *N*-substituted alkyl chain might play an important role for their anticonvulsant activities. And also the tested *N*-Cbz-aminosuccinimides exhibited pharmacological differences according to their stereochemistry. In case of the (R)- and (S)-*N*-Cbz-α-aminosuccinimide (**1a** and **1d**), the (S) configurated one (**1d**) showed more active anticonvulsant activity than (R) configurated one (**1a**) in both MES and PTZ tests. But the stereoisomeric pharmacological patterns of the (R) and (S)-*N*-Cbz-α-amino-*N*-methylsuccinimides (**1b** and **1e**) were inversed. So it was conceivable that the pharmacological mechanisms of *N*-Cbz-α-amino-*N*-methylsuccinimide (**1b** and **1e**) and *N*-Cbz-α-aminosuccinimide (**1a** and **1d**) might be different.

We examined the rotorod test for neurotoxicity and determined the TD_{50} values for these compounds (**1a, b,d** and **e**). The TD_{50} value of (R)-*N*-Cbz-α-amino-*N*-methylsuccinimide (**1b**), showing the most active anticonvulsant activity in MES test, was 166.7 mg/kg and the protective index (PI,TD_{50}/ED_{50}) was 3.2. And the TD_{50} value of (S)-*N*-Cbz-α-aminosuccinimide (**1d**), the most active one in PTZ test, was 160.8 mg/kg and the PI was 2.1. The TD_{50} values of other compounds were above 117.5 mg/kg.

In conclusion, the anticonvulsant activities of succinimides (**1**) in this study were approached to those of currently available drugs in both MES and PTZ tests. Especially, diphenylhydantoin, a typical anticonvulsant, was reported to be active in only MES test, so this compound was limited to the treatement of generalized tonic clonic seizure clinically. From the above fact, we thought that the succinimides (**1**) in this study were warrented to be promising anticonvulsant drug candidates of broader clinical spectrum.

Now we are continuing to synthesize their analogs and evaluate their anticonvulsant activities in order to define the structure activity relationship more precisely and develop more active anticonvulsant compounds.

ACKNOWLEDGEMENT

This work was supported by grant of Grant of KOSEF(951-0710-065-2).

REFERENCES CITED

Andersen, K. E., Braestrup, C. B., Gronvald, F. C., Jorgensen, A. S., Nielsen, E. B., Sonnewald, U., Sorensen, P. O., Suzdak, P. D. and Knutsen, L. J. S., The Synthesis of Novel GABA Uptake Inhibitors. 1. Elucidation of the Structure-Activity Studies Leading to the Choice of (R)-1-[4,4-Bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid (Tiagabine) as an Anticonvulsant Drug Candidate, *J. Med. Chem.*, 36,1716-1725 (1993).

Bornstein, M. R. and Doukas, P. H., Anticonvulsant Activity of Indanylspirosuccinimide Mannich Bases, *J. Pharm. Sci.*, 76, 300-302 (1987).

Brouillette, W. J., Jestkov, V. P., Brown, M. L., Akhatar, M. S., DeLrey, T. M. and Brown, G. B., Bicycloc Hydantoins with a Bridgehead Nitrogen, Comparison of Anticonvulsant Activities with Binding to the Neuronal Voltage-Dependent Sodium Channel, *J. Med. Chem.*, 37, 3289-3293 (1994).

Harvey, R. A. and Champe, P.C., *Lippincott's illustrated Reviews: Pharmacology*, J.B. Lippincott company, 1992, Chap.15.

Hays, S. J., Rice, M. J., Ortwine, D. F., Johnson, G., Schwarz, R. D., Boyd, D. K., Coperando, L.F., Vartanian, M. G., and Boxer, P. A., Substituted 2-Benzothiazolamines as Sodium Flux Inhibitors: Quantitative Structure-Activity Relationships and Anticonvulsant Activity, *J. Pharm. Sci.*, 83, 1425-1432 (1994) and references cited therein.

Heckendorn, R., Allgeier, H., Baud, J., Gundenhauser, W. and Angst, C., Synthesis and Binding Properties of 2-Amino-5-phosphono-3-pentenoic acid Photoaffinty Ligands as Probes for the Glutamate Recognition Site of the NMDA Receptor. *J. Med. Chem.*, 36, 3721-3726 (1993).

Itoh, M., Peptide. I. Selective protection of α- or Side chain Carboxyl Groups of Aspartic and Glutamic Acid. A Facile Synthesis of β-Aspartyl and γ-Glutamyl Peptides. *Chem. Pharm. Bull.*, 17, 1679-1686 (1969).

Kohn, H., Sawhney, K. N.,Robertson, D. W. and Leander, J. D., Anticonvulsant Properties of N-substituted α,α-Diamino acid Derivatives. *J. Pharm. Sci.*, 83, 689-691 (1994).

Kohn, H., Sawhney, K. N., Bardel, P., Robertson, D. W. and Leander, J. D., Synthesis and Anticonvulsant Activities of α-Heterocyclic α-Acetamido-*N*-benzylacetamide Derivatives. *J. Med. Chem.*, 36, 3350-3360 (1993).

Kornet, M. J. Synthesis and Anticonvulsant Evaluation of *N*-Aminosuccinimides. *J. Pharm. Sci.*, 73, 405-406 (1984).

N'Goka, V., Schlewer, G., Linget, J. M., Chambon, J. P. and Wermuth, C. G., GABA uptake Inhibitors: Construction of a General Pharmacophore Model and Successful Prediction of a New Representative., *J. Med. Chem.*, 34, 2547-2557 (1991).

Sandler, S. R. and Karo, W., Organic Functional Group Preparation, Vol. III, Academic Press, N. Y., 1972, p. 253.

Sun, Z. Y., Kwon, C. H. and Wurpel, N. D., Srtucture-Activity Study of 5-Substituted 1-Carbobenzoxy-2-iminohydantoins as Potential Anticonvulsant Agents. *J. Med. Chem.*, 37, 2841-2845 (1994) and references cited therein.

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.

Wilder, B. J., *Seizure disorder-A Pharmcological Approach to Treatment*, Ravan Press, N.Y., 1985, pp. 23-27.

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).