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# Synthesis and Anticonvulsant Activities of *N*-Cbz-α-aminoglutarimidooxy Carboxylate Derivatives

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Previous studies on the anticonvulsant activity of *N*-Cbz-α-aminoglutarimides have shown that the derivatives of *N*-Cbz-α-amino-*N*-alkoxy glutarimide have significant anticonvulsant activity. In addition, their anticonvulsant activities are dependent on the presence of *N*-alkoxy groups. Based on these results, a series of *N*-Cbz-α-amino-glutarimidooxy carboxylates derivatives (**3a-e**) were synthesized in moderate yield using a known synthetic procedure. Their anticonvulsant activities were evaluated using the maximal electroshock seizure (MES) test, the pentylene tetrazole induced seizure (PTZ) test, and the strychinine (Str) threshold test with the ultimate aim of developing more active anticonvulsants. None of the compounds (**3a-e**) tested showed anticonvulsant activity in the MES and PTZ test. However, all the compounds tested exhibited significant anticonvulsant activity in the Str. test. The most active compound in the Str. test was the methyl ester of *N*-Cbz-α-amino-glutarimidooxy acetic acid **3a** ($ED_{50}$ = 42.9 mg/kg).

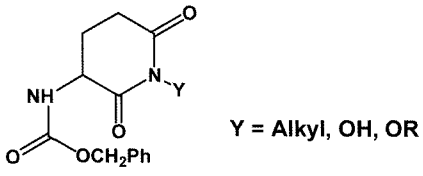
**Key words:** Anticonvulsant, MES test, PTZ test, Strychnine threshold test, Glutarimide. Glutarimidooxy carboxylate

## INTRODUCTION

Various *N*-Cbz-α-aminoglutarimides derivatives **1** (Fig. 1) have been reported to have significant anticonvulsant activity . (Son *et al.*, 1998; Lee *et al.*, 1999)

Their anticonvulsant activities were found to be dependent on the type of substituents on the imide moiety such as alkyl or alkoxy groups (Lee *et al.*, 1999; Kim *et al.*, 2004)

Recently, (Aminooxy) acetic acid (AOAA) and imidoxy esters were reported to show moderate anticonvulsant activity as GABA AT inhibitors (Edafiogho *et al.*, 1991).




**1**

**Fig. 1.** *N*-Cbz-α-aminoglutarimides

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From this, a series of *N*-Cbz-α-amino-glutarimidooxy carboxylate derivatives, such as compound **2** in Fig. 2, which contains AOAA, imidooxy ester and glutarimide structures in a single molecule, were prepared and their anticonvulsant activities were examined.

This paper reports the synthesis of *N*-Cbz-α-amino-glutarimidooxy carboxylates **3** and their *in vivo* anticonvulsant activities against the following tests: the maximal electroshock seizure (MES) test, the pentyl-enetetrazole induced seizure (PTZ) test, and the strychnine (Str.) threshold test.

## MATERIALS AND METHODS

The melting points were determined using an Electrothermal melting point apparatus and were uncorrected.

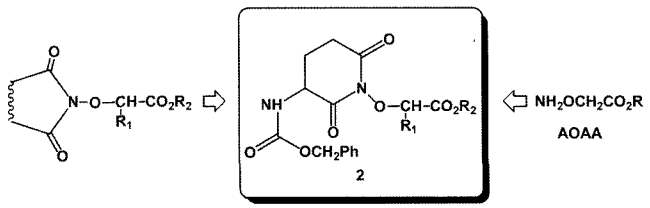



**Fig. 2.** *N*-Cbz-α-aminoglutarimidooxy carboxylate

The IR spectra were obtained using a JASCO FT/IR 200 and are reported in wave numbers ($cm^{-1}$). The $^1$H-NMR spectra were recorded in DMSO-$d_6$ on a JNM-EX 90A, and the chemical shifts were reported as $\delta$ values in parts per million from TMS as the 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

A series of *N*-Cbz-α-amino-glutarimidooxy carboxylate derivatives (**3**) were prepared from *N*-Cbz-glutamic acid the procedure shown in Scheme 1.

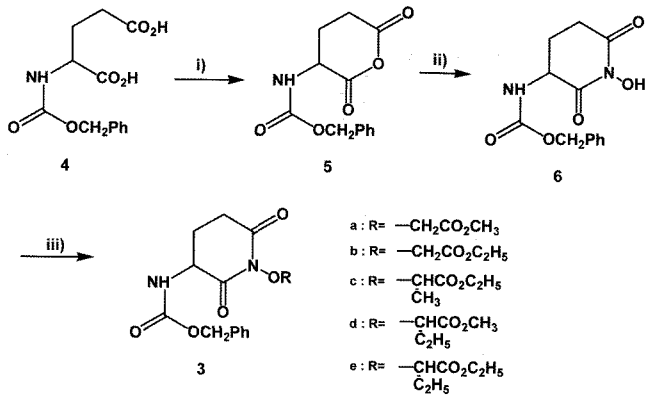
*N*-Cbz-glutamic acid anhydride **5** was prepared in quantitative yield by treating the corresponding *N*-Cbz-glutamic acid **4** with acetic anhydride. Compound 5 was treated with hydroxylamine to give *N*-Cbz-α-amino-*N*-hydroxy-glutarimide (**6**). The *N*-Cbz-α-amino-*N*-glutarimidooxy carboxylates (**3a-e**) could be obtained in moderate yields via the *O*-alkylation of compound **6** with the corresponding α-bromo carboxylic acid esters and sodium hydride in dry DMF.

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

(*S*)-*N*-Cbz-glutamic acid (10 g) was dissolved in acetic anhydride (100 mL), and the reaction mixture was stirred at 0 °C for 2 hrs. The excess acetic anhydride was then evaporated *in vacuo* and the residue was treated with diethyl ether to give *N*-Cbz-glutamic acid anhydride **5** as a white solid in quantitative yield. mp: 96.6 °C: IR (KBr) $cm^{-1}$: 1700, 1755, 1820, 3300

### (*S*)-*N*-Cbz-α-amino-*N*-hydroxyglutarimide (6)

Hydroxylamine hydrochloride (833 mg, 12 mmol) was dissolved in $H_2O$ (2 mL), which was followed by the addition of $Na_2CO_3$ (636 mg, 6 mmol). *N*-Cbz-glutamic acid anhydride **5** (2.63 g, 10 mmol) was then added slowly. The reaction mixture was heated under reflux for 2-3 hrs, and then cooled in an ice bath to precipitate the *N*-Cbz-α-amino-*N*-hydroxyglutarimide **6** as a white solid. The crude *N*-Cbz-α-amino-*N*-hydroxyglutarimide **6** was recrystallized with ethanol and water. 48%; mp: 132.3 °C; IR (KBr) $cm^{-1}$: 1700, 1755, 1820, 3300: $^1$H-NMR (90 MHz, 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).




4 5 6 3

i) $Ac_2O$ / room temp. ii) $NH_2OH$. HCl/ $Na_2CO_3$/ $H_2O$/ 80-90°C iii) R-Br/ NaH/ DMF, room temp. 4-5 hrs

**Scheme 1.** The Preparation of *N*-Cbz-aminoglutarimidooxy carboxylates

### (*S*)-Methyl-2-(3-Cbz-aminoglutaimidooxy)-acetate (3a)

A solution of (*S*)-*N*-Cbz-α-amino-*N*-hydroxyglutarimide **6** (556 mg, 2.0 mmol) in DMF (5 mL) was added to a suspension of NaH (96 mg, 2.4 mmol) in dry DMF (5 mL). Methyl bromoacetate (367 mg, 2.4 mmol) in DMF (5 mL) was then added slowely. The reaction mixture was then stirred for 4-5 hrs at room temperature. The excess DMF was evaporated *in vacuo*, and the residue was dissolved in 200 mL of ethyl acetate. The ethyl acetate solution was washed with $H_2O$ (10 mL x 2), and then with a saturated aqueous NaCl solution (10mL x 2), and dried over anhydrous magnesium sulfate. The crude product was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to give 528 mg of **3a** as viscous oil. 75%; IR (neat) $cm^{-1}$: 1250, 1750, 2900, 3000, 3350; $^1$H-NMR (90 MHz, DMSO-$d_6$): δ 1.90-2.10 (2H, m), 2.30-2.53 (2H, m), 3.72 (3H, s), 4.30-4.50 (1H, m), 4.55 (2H, s), 5.09 (2H,s), 5.80-5.90 (1H, br), 7.35 (5H, s.

The following compounds were prepared using the above procedure.

### (*S*)-Ethyl-2-(3-Cbz-amino-glutarimidooxy)-acetate (3b)

67%; white solid; mp: 90.5 °C: IR (KBr) $cm^{-1}$: 1200, 1750, 2900, 3000, 3350; $^1$H-NMR (90 MHz, DMSO-$d_6$): δ 1.30 (3H, t, $J$ = 7.0 Hz), 1.80-2.10 (2H, m), 2.60-2.90 (2H, m), 4.20 (2H, q, $J$ = 7.0Hz), 4.30-4.50 (1H, m), 4.70 (2H, s), 5.10 (2H, s), 5.60-5.80 (1H, br), 7.30 (5H, s).

### (*S*)-Ethyl-2-(3-Cbz-amino-glutarimidooxy)-propinate (3c)

62%; viscous yellow oil; IR (neat) $cm^{-1}$: 1200, 1750, 2900, 3000, 3300; $^1$H-NMR (90 MHz, DMSO-$d_6$): δ 1.10 (3H, t, $J$ = 7.0Hz), 1.50 (3H, t, $J$ = 7.0Hz), 1.80-2.00 (2H, m), 2.60-2.90 (2H, m), 4.16 (2H, q, $J$ = 7.0Hz), 4.30-4.50 (1H, m), 4.50-4.60 (1H, m), 5.10 (2H, s), 5.70-5.90 (1H, br), 7.40 (5H, s).

### (*S*)-Methyl-2-(3-Cbz-amino-glutarimidoxy)-butyrate (3d)

70%; mp: 110.5°C; white solid; IR (KBr) $cm^{-1}$: 1250, 1750, 2900, 3000, 3350; $^1$H-NMR (90 MHz, DMSO-$d_6$): δ 1.00 (3H, t, $J$ = 7.0Hz), 1.90-2.10 (2H, m), 2.30-2.50 (2H, m), 2.60-2.90 (2H, m), 3.72 (3H, s), 4.20 (1H, q, $J$ = 7.0Hz),

4.30-4.50 (1H, m), 5.10 (2H, s), 5.50-5.70 (1H, br), 7.40 (5H, s).

**(*S*)-Ethyl-2-(3-Cbz-amino-glutarimidoxy)-butyrate (3e)**
71%; mp: 74.3°C; white solid; IR (KBr) $cm^{-1}$: 1200, 1750, 2900, 3000, 3350; $^1$H-NMR (90 MHz, DMSO-$d_6$): δ 1.00 (3H, t, *J* = 7.0Hz), 1.20 (3H, t, *J* = 7.0Hz), 1.90-2.10 (2H, m), 2.40-2.50 (2H, m), 2.60-2.90 (2H, m), 4.20 (2H, q, *J* = 7.0Hz), 4.30-4.50 (1H, m), 4.50-4.60 (1H, m), 5.10 (2H, s), 5.70-5.90 (1H, br), 7.40 (5H, s).

## Pharmacology

The anticonvulsant test for the *N*-Cbz-α-amino-*N*-glutarimidooxy carboxylates (**3a-e**) in the MES and the PTZ 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). All the compounds tested were dissolved in polyethylene glycol 400 and administered ip to ICR male mice at doses of 25, 50, 75, and 100 mg/kg. The anticonvulsant tests were performed in groups of four, 30 min after administering the test compound. In addition, the lowest dose that could induce a seizure in all the animals tested was determined during the preliminary screening stage. Seizure was then artificially induced by either electroshock or pentylenetetrazole. The MES test was elicited with a 60-cycle a.c. with an intensity of 50 mA delivered for 0.2 s via corneal electrodes with an ECT unit (UGO Baseline. Italy). One drop of a 0.9% saline solution was instilled in the eye before applying the 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. The animal was then 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 Sc. Str. seizure threshold test entailed injecting 1.20 mg/kg of strychnine as a 0.5% solution subcutaneously in the posterior midline of the mice. The animals were then observed for 30 min. Protection was defined as a failure to observe even a threshold seizure(single episode of clonic spasms of at least 5 sec. duration). The $ED_{50}$ as a quantitative anticonvulsant evaluation was estimated from the dose-response data.

## RESULTS AND DISCUSSION

Previous studies on the anticonvulsant evaluations of various (*R*) and (*S*) glutarimides showed that there was no significant pharmacological difference between the steroisomers (Lee *et al.*, 1999; Kim *et al.*, 2004). Therefore, the compounds tested in this paper were synthesized from (*S*)-*N*-Cbz-glutamic acid only.

As shown in Scheme 1, all the compounds (**3a-e**) tested were prepared from the corresponding (*S*)-*N*-Cbz-glutamic acid in moderate yield. The spectral data of the tested compounds were satisfactory. The anticonvulsant activities for these compounds were investigated in both the MES and PTZ tests. Table I summarizes the primary anticonvulsant activity of these compounds.

As shown in Table I, none of the compounds tested (**3a-e**) showed any anticonvulsant activity against the MES test and PTZ test at a dose of 100 mg/ kg. The strychnine threshold test was then used to examine the anti-convulsant activity of these compounds. The strychnine threshold test is related to the glycine receptor, which is a part of convulsive receptor complex. (Thomson, 1989) Table II shows the primary anticonvulsant activities against the strychnine threshold test .

As shown in Table II, all the tested compounds **3a-e** showed significant anticonvulsant activity. In the case of compounds **3a** and **3b,** all the tested animals were protected at a dose of 75 mg/kg. According to the protocol for the development of new anticonvulsants, compounds showing anticonvulsant activity at a dose of 100 mg/kg

**Table I.** The Primary Anticonvulsant Activities of the *N*-Cbz-α-amino-glutarimidooxy carboxylates (3) in the MES and PTZ test

| Compound | R | Dose[a] | MES[b] | PTZ[c] |
|---|---|---|---|---|
| 3a | —$CH_2CO_2CH_3$ | 50 | 4/4 | 4/4 |
| | | 75 | 4/4 | 4/4 |
| | | 100 | 4/4 | 4/4 |
| 3b | —$CH_2CO_2C_2H_5$ | 50 | 4/4 | 4/4 |
| | | 75 | 4/4 | 4/4 |
| | | 100 | 4/4 | 4/4 |
| 3c | —$CHCO_2C_2H_5$ <br> $CH_3$ | 50 | 4/4 | 4/4 |
| | | 75 | 4/4 | 4/4 |
| | | 100 | 4/4 | 4/4 |
| 3d | —$CHCO_2CH_3$ <br> $C_2H_5$ | 50 | 4/4 | 4/4 |
| | | 75 | 4/4 | 4/4 |
| | | 100 | 4/4 | 4/4 |
| 3e | —$CHCO_2C_2H_5$ <br> $C_2H_5$ | 50 | 4/4 | 4/4 |
| | | 75 | 4/4 | 4/4 |
| | | 100 | 4/4 | 4/4 |

[a]All the compounds were dissolved in polyethyleneglycol 400 and administered i.p. to ICR male mice. The dose is reported as mg/kg [b]The MES test : 50mA, 60Hz, ac, 0.2 sec., via corneal electrodes, 30min after administering the test compound. The results are reported as the non-protected animals/tested animals. [c]The PTZ test: Subcutaneous pentylenetetrazol (80 mg/kg) 30min after administering the test compound. The results are reported as the non-protected animals/tested animals.

**Table II.** The Primary Anticonvulsant Activity of the *N*-Cbz-a-amino-glutarimidooxy carboxylates (3) against the Str. Threshold test

| Compound | R | Dose[a] | Str[b] |
|---|---|---|---|
| 3a | —$CH_2CO_2CH_3$ | 25 | 3/4 |
| | | 50 | 2/4 |
| | | 75 | 0/4 |
| | | 100 | 0/4 |
| 3b | —$CH_2CO_2C_2H_5$ | 25 | 4/4 |
| | | 50 | 2/4 |
| | | 75 | 0/4 |
| | | 100 | 0/4 |
| 3c | —$CHCO_2C_2H_5$ <br> $CH_3$ | 25 | 3/4 |
| | | 50 | 3/4 |
| | | 75 | 3/4 |
| | | 100 | 0/4 |
| 3d | —$CHCO_2CH_3$ <br> $C_2H_5$ | 25 | 4/4 |
| | | 50 | 4/4 |
| | | 75 | 4/4 |
| | | 100 | 1/4 |
| 3e | —$CHCO_2C_2H_5$ <br> $C_2H_5$ | 25 | 4/4 |
| | | 50 | 3/4 |
| | | 75 | 1/4 |
| | | 100 | 0/4 |

[a]All compounds were dissolved in polyethyleneglycol400 and administered i.p to ICR male mice. The dose is reported as mg/kg [b]The Str. threshold test: Subcutaneous strychnine (1.20 mg/kg) 30min after administering the test compound. The results are reported as non-protected animals/tested animals.

are recommended for further investigation of quantification. Hence, all the compounds were selected for the quantitative anticonvulsant evaluation using the Str. threshold test. Table III summarizes the results of the quantitative anti-convulsant activities in the Str. test.

As shown in Table III, the (*S*)-methyl 1-(3-Cbz-amino-glutarimidooxy) acetate **3a** was most active among the compounds tested using the Str. threshold test ($ED_{50}$ = 42.9 mg/kg). In addition the $ED_{50}$ values of compounds **3b**, **c**, and **e** were 52.5-96.8 mg/kg, which are comparable to other anticonvulsant drug candidates. As shown in table III, the glutarimidooxy acetate (**3a**, **3b**) were more active than the propionate (**3c**) and butyrate analogues (**3d**, **3e**). Moreover, based on the anticonvulsant activities of these compounds, the esters of glutarimidoxy carboxylic acid such as compound **3** can be recommended as a noble type of anticonvulsants. As mentioned above, the anticonvulsant activity against the Str. threshold test is related to the protection of the glycine site. Therefore, it is

**Table III.** The Anticonvulsant Activity of the *N*-Cbz-α-amino-glutarimidooxy carboxylates (3) against the Strychnine test

| Compound | R | $ED_{50}$[a,b] |
|---|---|---|
| 3a | —$CH_2CO_2CH_3$ | 42.9 |
| 3b | —$CH_2CO_2C_2H_5$ | 52.5 |
| 3c | —$CHCO_2C_2H_5$ <br> $CH_3$ | 70.3 |
| 3d | —$CHCO_2CH_3$ <br> $C_2H_5$ | 159.3 |
| 3e | —$CHCO_2C_2H_5$ <br> $C_2H_5$ | 96.8 |

[a]All the compounds were dissolved in polyethyleneglycol 400 and administered i.p. to ICR male mice. The $ED_{50}$ is reported as mg/kg [b]The Str test: Subcutaneous strychnine (1.20 mg/kg) 30min after administering the test compound.

conceivable that the glutarimidooxy ester can protect the glycine site.

## CONCLUSIONS

As A series of glutarimidooxy carboxylate, containing AOAA, glutarimidooxy and α-aminoglutarimide groups in a single molecule, were prepared from *N*-Cbz-glutamic acid as the starting material. Their anticonvulsant activities were examined using the MES, PTZ test and Str. threshold test. None of the compounds tested showed any anticonvulsant activity in the MES and PTZ test. However, all the compounds tested exhibited anticonvulsant activity against the Str. threshold test with an $ED_{50}$ comparable to that of other anticonvulsant drug candidates. The Str. threshold test is related to the glycine site of the convulsive receptor complex. Therefore, it is believed that the glutarimidooxy carboxylate protects the glycine site of the convulsive receptor complex. Overall, these glutarimidooxy carboxylates are recommended for further examination as anticonvulsant drug candidates with a novel structure.

## ACKNOWLEDGEMENTS

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