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# Designing of Antiepileptic Ligands by Esterification and Acetylation of Dipeptides

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**Abstract** □ Glycylglycine, alanylalanine and alanylglycine were synthesized, their free carboxylic and amino groups were converted to methyl esters of N-acetylglycylglycine, N-acetylalanylglycine and N-acetylalanylalanine.

The synthesized compounds were evaluated for antiepileptic activity, plasmaprotein binding, $TD_{50}$ and potentiating effect of phenobarbitone sodium.

**Keywords** □ N-acetyl glycylglycine methyl ester, N-acetylalanylglycine methyl ester, N-acetyl-alanylalanine methyl ester, plasma protein binding, antiepileptic activity.

Most of the antiepileptic drugs, i.e. derivatives of barbiturates, hydantoins, contain uride structure. Kohn[1] *et al.* synthesized various selectively derivatives of α-acetamido-N-benzyl-α-phenyl acetamide and evaluated in the maximal electroshock seizure (MES) and horizontal screen (tox) tests in mice. It was also reported that replacement of the α-phenyl substituent on α-acetamido-N-benzyl-α-phenyl acetamide by a relatively small electron-rich, heteroaromatic moiety led to a greater improvement in the anticonvulsant potency of the drug candidate. Ken[2] proposed an anticonvulsant nucleus containing the same moiety. Takashi[3] extracted a seizure producing substance (K-substance) during seizure condition from dog's brain and explained that the substance contained $-COO^-$, $-CH_2NH_2$,-CHNH, $-CH_2OH$ pharmacodynamic moieties present in the anticonvulsant nucleus.

## EXPERIMENTAL METHODS

Sheehan's method[4] was adapted for the preparation of glycylglycine, alanylalanine and alanylglycine.

***Synthesis of dipeptides***

Glycylglycine was synthesized in the following steps.

***Preparation of phthalylglycine*** : Glycine (0.2 mol) and phthalic anhydride (0.2 mol) was heated in an oil bath at 180-185℃ for 15 min. The mixture was then cooled and recrystallized from ethanol (10%), yield, 90%, mp. 191-192℃.

***Preparation of phthalylglycylchloride*** : A suspension of phthalylglycine (0.1 mol) and phosphorous pentachloride (0.1 mol) in 200 m*l* benzene was heated at 60℃ for 2 hr with constant stirring. The reaction mixture was cooled, concentrated under reduced pressure and the residue was recrystallized from benzene and petroleum ether, yield, 75%, mp. 81-82 ℃.

***Preparation of phthalylglycylglycine*** : A solution of phthalylglycylchloride (0.02 mol) was added slowly with constant stirring to a suspension of glycine (0.02 mol) and magnesium oxide (0.03 mol) in 75 m*l* of water at 5℃. After stirring for an additional 10 min at room temperature, the mixture was acidified with hydrochloric acid to get a precipitate. The precipitate was filtered and recrystallized from 90% ethanol, yield, 80%, mp. 227-228℃.

***Preparation of glycylglycine from phthalylglycylglycine*** : A suspension of phthalylglycylglycine (0.01 mol) in

30 m*l* ethanol was heated under reflux with 1 M ethanolic hydrazine hydrate (10 m*l*) for 1 hr. The reaction mixture was evaporated, the dried residue was warmed to 50℃ for 10 min with 25 m*l* hydrochloric acid (2N) and allowed to cool to room temperature. The phthalyl hydrazide was filtered off and the product (glycylglycine hydrochloride) was obtained by concentrating the filtrate under reduced pressure. The product was dissolved in water to get the free dipeptide by means of Amberlite IR-4B acid adsorbing resin. Yield, 7%, mp. 215- 216℃. IR (KBr): 3230 $cm^{-1}$, 2700 $cm^{-1}$, 1630 $cm^{-1}$, 1540 $cm^{-1}$, 1450 $cm^{-1}$, 1250 $cm^{-1}$.

***Synthesis of alanylglycine***

The procedure as stated under the preparation of glycylglycine was followed. Phthalylalanyl chloride (0.02 mol) was made to react with glycine (0.02 mol) and magnesium oxide (0.03 mol) in 75 m*l* water, yield, 70%, mp. 210-211℃. IR (KBr): 3270 $cm^{-1}$, 2720 $cm^{-1}$, 1700 $cm^{-1}$, 1490 $cm^{-1}$, 1250 $cm^{-1}$.

***Synthesis of alanylalanine***

The method given under the synthesis of glycylglycine was followed. Phthalylalanylchloride (0.02 mol) was made to react with alanine (0.02 mol), yield, 74%, mp. 190-192℃. IR (KBr): 3240 $cm^{-1}$, 2720 $cm^{-1}$, 1710 $cm^{-1}$, 1510 $cm^{-1}$, 1460 $cm^{-1}$.

***General procedure for the synthesis of methyl ester of N-acetylated dipeptides***

Utilizing the procedure of Ronald[5], methyl ester hydrochloride of glycylglycine, alanylalanine and alanylglycine were synthesized. Dipeptide (0.05 mol) was added to a solution of thionyl chloride (0.05 mol in methanol) and the reaction mixture was then stirred under reflux for 4 hr. The solvent was removed under reduced pressure and the residue was triturated with several 20 m*l* portions of cold ether at 0℃. The crude product was recrystallized from hot methanol (25 m*l*) by slow addition of ether (150-200 m*l*) followed by cooling.

N-acetylglycylglycine methyl ester hydrochloride, N-acetylalanine hydrochloride and N-acetylalanylglycine methyl ester hydrochloride were synthesized utilizing modified Baumann reaction[6]. Methyl ester of dipeptide (0.02 mol) was added slowly to a cold potassium carbonate solution (10%, 50 m*l*) and was stirred to get a clear solution. Acetyl chloride (0.02 mol) was added slowly to the above solution, with constant stirring, at 10℃. The separated compound was collected, dried, washed with sodium hydroxide solution (0.5%) and recrystallized from chloroform.

## EVALUATION

***Plasma protein binding***

The plasma protein binding of compounds was determined by equilibrium dialysis method, explained by Pippenger[7] using gout plasma and egg membrane. Results are shown in Table I.

**Table I Characteristics of methyl ester of acetylated dipeptides**

$$CH_3-\underset{\underset{O}{\|}}{C}-NHR$$

| S. No. | R | mp. (℃) | $TD_{50}$ (mg/kg) | % Plasma protein binding |
|---|---|---|---|---|
| 1. | $-\underset{\underset{CH_3}{\mid}}{CH}-CONH-\underset{\underset{CH_3}{\mid}}{CH}-COOCH_3$ | 190-192 | 105 | 50.9 |
| 2. | $-\underset{\underset{CH_3}{\mid}}{CH}-CONH-CH_2-COOCH_3$ | 188-191 | 152 | 61.2 |
| 3. | $-CH_2-CONH-CH_2-COOCH_3$ | 182-184 | 108 | 68.7 |

All compounds were analysed for C, H and N. The values were within ± 0.4% of the theoretical value. The IR spectra in KBr phase showed the presence of amide and ester linkage in all compounds. The mp. were determined in open capillaries and are uncorrected. The plasma protein binding was determined by equlibrium dialysis method.

**Table II Phamacological evaluation of methyl esters of N. acetylated dipeptides**

| Group | Treatment | Antiepileptic activity | | | | Synergistic activity | |
|---|---|---|---|---|---|---|---|
| | | TLF Mean time± SD in (sec.) | P.value | FEL Mean time± SD in (sec.) | P.value | Mean time± SD time in (h) | P. value |
| A | Control | 2.93± 0.0948 | | 14.60± 2.1954 | | 3.1933± 0.6679 | |
| B | AAME | 1.86± 0.4339* | 0.05 | 9.56± 1.8574* | 0.05 | 4.17± 0.9181$^{+}$ | 0.5 |
| C | AGME | 1.53± 1.3304$^{+}$ | 0.5 | 7.00± 2.3806* | 0.05 | 3.43± 0.9280$^{++}$ | 0.8 |
| D | GGME | 1.70± 0.3905* | 0.05 | 9.00± 1.8261* | 0.05 | 5.72± 0.8678$^{+}$ | 0.5 |

* The difference was found to be statistically significant ($p<0.05$) when compared with control. $^{+,++}$ The difference was found to be statistically insignificant when compared to control ($p>0.05$). TLF-Tonic limb flexation time, FEL-Full extension of limb time.
AAME-N. acetyl alanylalanine methyl ester.
AGME-N. acetyl alanylglycine methyl ester.
GGME-N. acetyl glycylglycine methyl ester.
Phenylhydantion and phenobarbitone sodium were administered intraperitoneally into control group of mice for antiepileptic and synergistic activity respectively.

***Toxic dose ($TD_{50}$)***

Karber's method[8] was used for determining the toxic dose of synthesized compounds on different sets of mice. Results are shown in Table I.

***Antiepileptic activity***

Maximal electroshock seizures (MES) were produced in mice by electroconvulsiometer through electrodes attached to the pinna according to the method of Swinyard *et al*[9]. The amount of current given to mice was 48 mA for 0.2 seconds. Tonic limb flexation time and full extension of limb time were observed. Results are shown in Table II.

***Synergistic activity***

Aqueous solution of phenobarbitone sodium 5 mg/ml, 10 mg/ml and 15 mg/ml were administered intraperitoneally to one set of mice, which served as control and the sleeping time was determined. Aqueous solution of synthesized compounds 5 mg/ml, 10 mg/ml and 15 mg/ml were administered similarly to different set of mice with same concentration of phenobarbitone sodium and the sleeping time, was determined. Resuluts are shown in Table II.

## RESULTS

All the synthesized compounds contained $CH_3$ CO, $COOCH_3$ and -CONH linkages. The compounds showed plasma protein binding between 50-69% and were found to possess antiepileptic activity.

## DISCUSSION

All the synthesized compounds showed low plasma protein binding and antiepileptic activity which may be due to structural modification of dipeptides. n the synthesized compounds, free carboxylic and amino groups were converted to methyl ester and acetamido groups, respectively, which are essential for antiepileptic activity. These conversions limited plasma protein binding. Hence a large amount of free drug was available in serum for producing higher pharmacological response. The conversion of carboxylic group to methyl ester increased the lipophilic properties which facilitate drug to cross the blood brain barrier to produce better dose response.

## CONCLUSION

Physical properties reflected the amino acid nature of compounds. The compounds were found to be active against epileptic seizures.

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