SYNTHESIS AND ANTICONVULSANT ACTIVITY OF NEW N-BENZOYL DERIVATIVES OF ALIPHATIC ACIDS AND THEIR SALTS

S. A. Kazaryan,[1] K. P. Grigoryan,[1] R. G. Paronikyan,[1] A. S. Agaronyan,[1] A. I. Gevondyan,[1] and V. M. Samvelyan[1]

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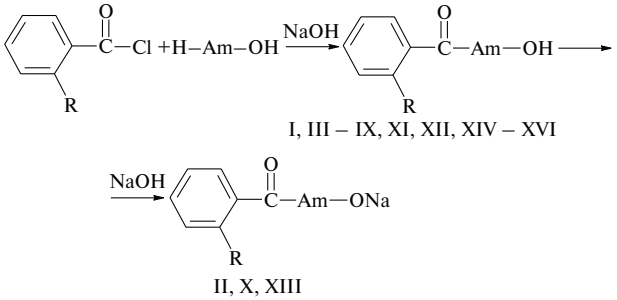
In the past years, there was a certain increase in the clinical use of preparations based on neuroactive amino acids such as glycine, β-alanine, and γ-aminobutyric acid (GABA) [1 – 6]. In connection with this, it was of interest to search for new biologically active substances in the class of neuroactive amino acid derivatives. We have synthesized and characterized with respect to anticonvulsant activity a series of N-(*ortho*-R-benzoyl) derivatives of glycine, β-alanine, ε-aminocaproic acid, GABA, and some salts of these acids (I – XVI) (Table 1).

Amino groups in the amino acids were benzoylated under the Schotten – Baumann reaction conditions [7]. Some physicochemical characteristics and proton chemical shifts in the ^{1}H NMR spectra of the synthesized N-substituted amino acids and their salts (I – XVI) are listed in Tables 1 and 2. The syntheses were conducted by the following scheme:



The initial amino acids H–Am–OH and substituents R are indicated in Table 1.

The proposed structures were confirmed by the data of elemental analyses and the results of IR and ^{1}H NMR spectroscopic measurements. The IR spectra of N-(*o*-R-benzoyl)amino acids display bands in the region of characteristic absorption of the carboxy carbonyl (1620 cm^{-1}), amide NH (1540, 3350 cm^{-1}), and aromatic CH (1600 cm^{-1}) groups. The parameters of the ^{1}H NMR spectra are presented in Table 2.

EXPERIMENTAL CHEMICAL PART

Purity of the synthesized compounds was checked by TLC on Silufol UV-254 plates. The TLC runs with benzoylated amino acids (compounds I, III – IX, XI, XII, XIV – XVI)

TABLE 1. Yields and Physicochemical Characteristics of N-Substituted Amino Acids and Their Sodium Salts (I – XVI)

Compound	R	Initial amino acid (H–Am–OH)	Yield, %	M.p., °C	R_f	Empirical formula
I	Cl	GABA	88.2	92 – 93	0.81	$C_{11}H_{12}ClNO_3$
II	Cl	GABA	70.0	80 – 82	0.74	$C_{11}H_{11}ClNO_3Na$
III	Cl	Glycine	69.0	171 – 172	0.84	$C_9H_8ClNO_3$
IV	Cl	β-Alanine	87.8	88 – 89	0.82	$C_{10}H_{10}ClNO_3$
V	Cl	ε-Amino-caproic	74.0	58 – 59	0.76	$C_{13}H_{16}ClNO_3$
VI	CH_3O	GABA	98.0	74 – 75	0.84	$C_{12}H_{15}NO_4$
VII	C_2H_5O	GABA	89.0	71 – 72	0.74	$C_{13}H_{17}NO_4$
VIII	C_3H_7O	GABA	91.3	78 – 79	0.79	$C_{14}H_{19}NO_4$
IX	C_4H_9O	GABA	59.6	62 – 63	0.69	$C_{15}H_{21}NO_4$
X	C_4H_9O	GABA	55.8	66 – 67	0.69	$C_{15}H_{20}NO_4Na$
XI	$C_5H_{11}O$	GABA	82.7	61 – 62	0.79	$C_{16}H_{23}NO_4$
XII	*iso*-$C_5H_{11}O$	GABA	62.0	75 – 76	0.85	$C_{16}H_{23}NO_4$
XIII*	$C_5H_{11}O$	GABA	68.8	86 – 87	0.66	$C_{16}H_{22}NO_4Na$
XIV	CH_3O	β-Alanine	67.7	79 – 80	0.82	$C_{11}H_{13}NO_4$
XV	$C_5H_{11}O$	β-Alanine	66.8	88 – 89	0.80	$C_{15}H_{21}NO_4$
XVI	*iso*-$C_5H_{11}O$	β-Alanine	83.6	73 – 74	0.73	$C_{15}H_{21}NO_4$

* Na salt.

[1] Mndzhoyan Institute of Fine Organic Chemistry, National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia.

were performed in a propanol – water (7 : 3) system, while sodium salts (compounds II, X, XIII) were eluted in a butanol – acetic acid – water (8 : 1 : 2) system. The spots were revealed by exposure to iodine vapor. The IR absorption spectra were recorded on an UR-20 (Germany) spectrophotometer using samples prepared as suspensions in Vaseline oil. The data of elemental analyses agree with the results of analytical calculations according to empirical formulas (Table 1).

General method for the synthesis of N-(*o*-chlorobenzoyl)- and N-(*o*-alkoxybenzoyl)amino acids. A solution of 0.1 mole of amino acid in 70 ml of 1 N aqueous sodium hydroxide solution was cooled on ice to 5°C. To this solution was gradually (over 30 min) and simultaneously (in approximately equal portions) added with intensive stirring a 1 N aqueous sodium hydroxide solution and 0.1 mole of *o*-alkoxybenzoic or *o*-chlorobenzoic acid chloroanhydride, with the acidity controlled at pH 7.5 – 8.5. Then the reaction mixture was stirred with cooling for 6 – 7 h and extracted with diethyl ether (3 × 30 ml). The aqueous layer was cooled on ice and acidified with 5 N hydrochloric acid to pH 3 – 4. The precipitate was separated by filtration, washed with water, and recrystallized from ethanol – water (1 : 1) mixture.

N-Aroylamino acid sodium salts (II, X – XIII). To a solution of 0.1 mole of aroylamino acid I, IX, or XI in 20 ml

TABLE 2. ^{1}H NMR Spectra of N-Substituted Amino Acids and Their Sodium Salts (I – XVI)

Compound	Proton chemical shift δ, ppm (solvent)
I	10.0 (s, 1H, COOH); 7.80 (t, J_{H-H} 6.1 Hz), (1H, NH); 7.45 (s, 4H, C_6H_4); 3.45 (q, J_{H-H} 6.2 Hz), (2H, N–CH_2); 2.45 (t, J_{H-H} 6.2 Hz), (2H, CH_2–CO); 1.96 (m, 2H, CH_2): (acetone).
II	8.65 (t, J_{H-H} 6.1 Hz), (1H, NH); 7.40 (s, 4H, C_6H_4); 3.10 (q, J_{H-H} 6.1 Hz), (2H, N–CH_2); 1.8 (m, 4H, $(CH_2)_2$): (DMSO).
III	8.60 (t, J_{H-H} 6.1 Hz), (1H, NH); 7.42 (s, 4H, C_6H_4); 3.90 (d, J_{H-H} 6.1 Hz), (2H, CH_2): (DMSO).
IV	8.50 (t, J_{H-H} 6.1 Hz), (1H, NH); 7.50 (s, 4H, C_6H_4); 3.50 (q, J_{H-H} 6.2 Hz), (2H, N–CH_2); 2.55 (t, J_{H-H} 6.2 Hz), (2H, CH_2 – CO): (DMSO).
V	7.40 (s, 4H, C_6H_4); 6.96 (t, J_{H-H} 6.1 Hz), (1H, NH); 3.38 (q, J_{H-H} 6.1 Hz), (2H, N–CH_2); 2.30 (t, J_{H-H} 6.1 Hz), (2H, CH_2–CO); 1.50 (m, 6H, $(CH_2)_3$): ($CDCl_3$).
VI	11.8 (ms, 1H, COOH); 7.98 (t, J_{H-H} 5.5 Hz), (1H, NH); 7.87 (dd, J_{H-H} 7.8 Hz, J_{H-H} 1.9 Hz), (1H, H – Ar); 7.40 (m, J_{H-H} 8.75 Hz, 6.9 Hz, 1.9 Hz), (1H, H–Ar); 7.03 (d, J_{H-H} 7.8 Hz), (1H, H–Ar); 7.00 (t, J_{H-H} 7.8 Hz), (1H, H–Ar); 3.96 (s, 3H, OCH_3); 3.37 (q, J_{H-H} 6.5 Hz), (2H, NCH_2); 2.29 (t, J_{H-H} 7.2 Hz), (2H, CH_2–CO); 1.83 (qu, J_{H-H} 7.0 Hz), (2H, CH_2): (DMSO).
VII	10.5 (s, 1H, COOH); 8.30 (t, J_{H-H} 6.0 Hz), (1H, NH); 8.22 (dd, J_{H-H} 2.0 Hz, J_{H-H} 7.9 Hz), (1H, H–Ar); 7.45 (td, J_{H-H} 2.0 Hz, J_{H-H} 7.9 Hz), (1H, H–Ar); 7.20 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.15 (t, J_{H-H} 7.9 Hz), (1H, H–Ar); 4.20 (q, J_{H-H} 6.2 Hz), (2H, OCH_2); 3.60 (q, J_{H-H} 6.3 Hz), (2H, NCH_2); 2.55 (t, J_{H-H} 6.3 Hz), (2H, CH_2–CO); 2.05 (q, J_{H-H} 6.2 Hz), (2H, CH_2); 1.55 (t, J_{H-H} 7.2 Hz), (3H, CH_3): ($CDCl_3$).
VIII	7.92 (dd, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.38 (td, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.00 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.98 (t, J_{H-H} 7.9 Hz), (1H, H–Ar); 4.00 (t, J_{H-H} 6.3 Hz), (2H, OCH_2); 3.45 (t, J_{H-H} 6.3 Hz), (2H, NCH_2); 2.41 (t, J_{H-H} 6.3 Hz), (2H, CH_2–CO); 1.90 (m, 4H, $2CH_2$); 1.02 (t, J_{H-H} 7.2 Hz), (3H, CH_3): (CD_3OD).
IX	8.27 (t, J_{H-H} 6.05 Hz), (1H, NH); 7.86 (dd, J_{H-H} 2.08 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.39 (d, J_{H-H} 2.0 Hz, J_{H-H} 8.04 Hz), (1H, H–Ar); 7.05 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.95 (t, J_{H-H} 8.0 Hz), (1H, H–Ar); 4.08 (t, J_{H-H} 6.12 Hz), (2H, OCH_2); 3.32 (q, J_{H-H} 6.35 Hz), (2H, NCH_2); 1.8 (m, 8H, $2(CH_2)_2$); 0.98 (t, J_{H-H} 7.2 Hz), (3H, CH_3): (DMSO).
X	8.25 (t, J_{H-H} 6.0 Hz), (1H, NH); 7.90 (dd, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.42 (td, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.10 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.05 (t, J_{H-H} 8.0 Hz), (1H, H–Ar); 4.08 (t, J_{H-H} 6.2 Hz), (2H, OCH_2); 3.30 (q, J_{H-H} 6.3 Hz), (2H, NCH_2); 1.80 (m, 8H, $2(CH_2)_2$); 0.98 (t, J_{H-H} 7.2 Hz), (3H, CH_3): (DMSO).
XI	10.4 (s, 1H, COOH); 8.2 (t, J_{H-H} 6.0 Hz), (1H, NH); 8.0 (dd, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.38 (td, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.05 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.97 (t, J_{H-H} 7.9 Hz), (1H, H–Ar); 4.15 (t, J_{H-H} 6.2 Hz), (2H, OCH_2); 3.45 (q, J_{H-H} 6.3 Hz), (2H, NCH_2); 2.40 (t, J_{H-H} 6.3 Hz), (2H, CH_2–CO); 1.80 (m, 8H, $(CH_2)_3$, CH_2); 0.95 (t, J_{H-H} 7.2 Hz), (3H, CH_3): (acetone).
XII	12.0 (s, 1H, COOH); 8.2 (t, J_{H-H} 6.0 Hz), (1H, NH); 7.95 (dd, J_{H-H} 2.0 Hz, J_{H-H} 7.8 Hz), (1H, H–Ar); 7.00 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.98 (t, J_{H-H} 7.8 Hz), (1H, H–Ar); 4.15 (t, J_{H-H} 6.3 Hz), (2H, O – CH_2); 3.55 (q, J_{H-H} 6.3 Hz), (2H, NCH_2); 2.48 (t, J_{H-H} 6.3 Hz), (2H, CH_2–CO); l.82 (m, 3H, CH_2 – CH); 1.00 (d, J_{H-H} 6.3 Hz), (6H, CH_3): (DMSO).
XIII	10.42 (s, 1H, COOH); 8.26 (t, J_{H-H} 6.0 Hz), (1H, NH); 7.98 (dd, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.4 (td, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.1 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.99 (t, J_{H-H} 7.98 Hz), (1H, H-Ar); 4.2 (t, J_{H-H} 6.2 Hz), (2H, OCH_2); 3.5 (q, J_{H-H} 6.32 Hz), (2H, NCH_2); 2.42 (t, J_{H-H} 6.3 Hz), (2H, CH_2–CO); 1.82 (m, 8H, $(CH_2)_3$–CH_2); 0.98 (t, J_{H-H} 7.22 Hz), (3H, CH_3): (DMSO).
XIV	12.02 (ms, 1H, COOH); 7.90 (t, J_{H-H} 5.45 Hz), (1H, NH); 7.78 (dd, J_{H-H} 7.85 Hz, J_{H-H} 2.01 Hz), (1H, H–Ar); 7.45 (m, J_{H-H} 8.70 Hz, J_{H-H} 7.01 Hz, J_{H-H} 1.94 Hz), (1H, H–Ar); 7.03 (d, J_{H-H} 7.1 Hz), (1H, H–Ar); 7.04 (t, J_{H-H} 7.8 Hz), (1H, H–Ar); 3.94 (s, 3H, OCH_3); 3.34 (q, J_{H-H} 6.5 Hz), (2H, N–CH_2); 2.32 (t, J_{H-H} 7.19 Hz), (2H, CH_2–CO): (DMSO).
XV	7.90 (dd, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 7.38 (td, J_{H-H} 2.0 Hz, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.95 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.90 (t, J_{H-H} 8.0 Hz), (1H, H–Ar); 4.00 (t, J_{H-H} 6.2 Hz), (2H, OCH_2); 3.55 (t, J_{H-H} 6.3 Hz), (2H, NCH_2); 2.50 (t, J_{H-H} 6.2 Hz), (2H, CH_2CO); l.60 (m, 6H$(CH_2)_3$); 0.9 (t, J_{H-H} 7.2 Hz), (3H, CH_3): (CD_3OD).
XVI	12.0 (s, 1H, COOH); 8.2 (t, J_{H-H} 6.0 Hz), (1H, NH); 7.95 (dd, J_{H-H} 2.0 Hz, J_{H-H} 7.8 Hz), (1H, H–Ar); 7.37 (td, J_{H-H} 2.0 Hz, J_{H-H} 7.8 Hz), (1H, H–Ar); 7.00 (d, J_{H-H} 8.0 Hz), (1H, H–Ar); 6.98 (t, J_{H-H} 7.8 Hz), (1H, H–Ar); 4.15 (t, J_{H-H} 6.3 Hz), (2H, O–CH_2); 3.55 (q, J_{H-H} 6.3 Hz), (2H, N–CH_2); 2.48 (t, J_{H-H} 6.3 Hz), (2H, CH_2–CO); 1.82 (m, 3H, CH_2–CH); 1.00 (d, J_{H-H} 6.3 Hz), (6H, CH_3): [DMSO].

of a 50% aqueous ethanol solution was gradually added a 5% aqueous sodium hydroxide solution, with the acidity controlled at pH 8 – 9, and the mixture was allowed to stand overnight. Then the solvent was evaporated to minimum volume and the residue cooled to 0 – 5°C and diluted with 110 ml of acetone. The precipitate was separated by filtration and dried. Finally, the product was reprecipitated with diethyl ether from a solution in ethanol. The physicochemical data are presented in Table 1.

EXPERIMENTAL PHARMACOLOGICAL PART

The anticonvulsant properties of the synthesized compounds were studied on 250 male and female mice weighing 18 – 24 g. The activity was measured by the degree of protection from the maximum electroshock induced tonic extension or by the ability of preventing clonic convulsions induced by corazole (90 mg/kg, s.c.). The central *m*- and *n*-cholinolytic effects were evaluated by the antagonism with arecoline tremor (15 mg/kg, s.c.) and nicotine convulsions (8 mg/kg, i.p.) [8]. Reference was made to the well-known antiepileptic drug ethosuccimide (zarontin) [9].

The compounds to be tested were intraperitoneally injected as suspensions in carboxymethylcellulose solutions in a single dose of 200 mg/kg (i.p.). The drug injections were 45 min before inducing model convulsions or applying electric irritation. The active compounds were studied in a range of doses (25, 50, 100, 150, 250, and 300 mg/kg) and the effective doses (ED_{50}) producing an anticonvulsant effect in half of the tested animals were determined by the conventional Litchfield – Wilcoxon method [10].

Among the studied substances, a pronounced anticorazole effect was observed for N-(*o*-alkoxybenzoyl)-GABA and related sodium salts (compounds IX – XIII, Table 3). In particular, the derivative with amyloxybenzoyl radical (XI) was superior to ethosuccimide (zarontin).

None of the tested compounds offered protection against the maximum electroshock extension or exhibited central *m*- and *n*-cholinolytic effects.

TABLE 3. Anticonvulsant Activity of Some N-Substituted Amino Acids and Their Sodium Salts

Compound	R	Initial amino acid	ED_{50}, mg/kg
I	Cl	GABA	–
II	Cl	GABA	–
III	Cl	Glycine	–
IV	Cl	β-Alanine	–
V	Cl	ε-Aminocaproic	–
VI	CH_3O	GABA	–
VII	C_2H_5O	“	–
VIII	C_3H_7O	“	–
IX	C_4H_9O	“	145
X	C_4H_9O	“	210
XI	$C_5H_{11}O$	“	96
XII	*iso*-$C_5H_{11}O$	“	–
XIII*	$C_5H_{11}O$	“	120
Ethosuccimide		Zarontin	155

* Na salt.

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