DOI 10.1007/s11094-018-1708-6
Pharmaceutical Chemistry Journal, Vol. 51, No. 10, January, 2018 (Russian Original Vol. 51, No. 10, October, 2017)

SYNTHESIS AND BIOLOGICAL ACTIVITY OF (Z)-DIALKYLAMINOALKYLAMIDES OF N-BENZOYL-α,β-DEHYDROAMINO ACIDS AND THEIR IODOMETHYLATES

V. O. Topuzyan,[1] S. R. Tosunyan,[1,*] S. G. Chshmarityan,[1] and R. V. Paronikyan[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 51, No. 10, pp. 26 – 29, October, 2017.

Original article submitted November 28, 2014.

A series of *N*,*N*-(dialkylamino)alkylamides of several *N*-substituted α,β-dehydroamino acids and their quaternary ammonium salts were synthesized via the reaction of unsaturated 5(4*H*)-oxazolones with *N*,*N*-dialkyldiamines and characterized by physicochemical characteristics. Their reactions with human erythrocytic acetylcholinesterase (ACE) and plasmic butyrylcholinesterase (BuCE) were studied. The IC_{50} values [concentration at which the hydrolysis rate of cholinesterase was 50% inhibited by acetylthiocholine (0.1 mM)] of all synthesized compounds were determined. It was found that all synthesized compounds possessed anticholinesterase activity and were specific mainly for BuCE.

Keywords: α,β-dehydroamino acid derivatives, anticholinesterase properties, dialkylaminoalkylamide iodomethylates, ACE, BuCE.

Previously, we found that *N*-substituted α,β-dehydroamino-acid choline esters exhibited anticholinesterase activity [1 – 3]. Dialkylaminoalkylamides of several *N*-benzoyl-α,β-dehydroamino acids (**IX** – **XV**) were synthesized to reveal the chemical-structure—biological-activity relationship of this series of compounds. The last were prepared by reacting the corresponding unsaturated 5(4*H*)-oxazolones (**I** – **III**) with *N*,*N*-dialkylaminoalkylamines (**IV** – **VIII**) in ether at room temperature.

It is noteworthy that analogous amides of α,β-dehydrotryptophan were synthesized by the azlactone method using refluxing for 1-2 h in C_6H_6–MeCN [4]. According to the literature [5], 2-methyl-4-arylidene-5(4*H*)-oxazolones reacted with dialkylaminoalkylamines in $CHCl_3$ in 1 h.

Quaternization of synthesized amides **IX** – **XV** used the reaction with methyliodide in Me_2CO.

Yields of aminoamides **IX** – **XV** prepared in this manner varied in the range 91 – 99%; of their iodomethylates (**XVI** – **XXII**), in the range 61 – 98% (Table 1).

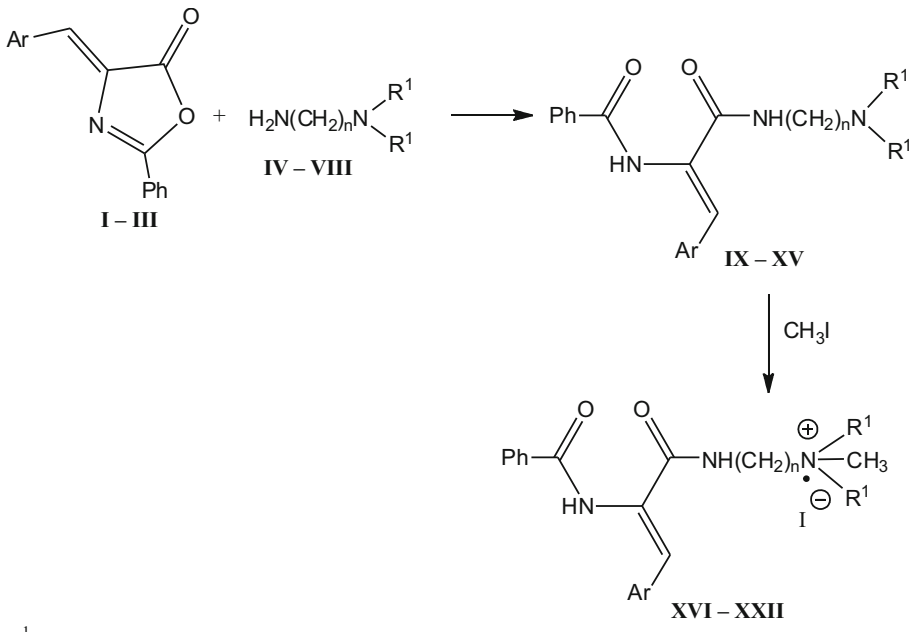
The structures of the synthesized compounds were confirmed by IR and PMR spectral data. IR spectra of **IX** – **XXII** had absorption maxima at 1624 – 1650 and 1644 – 1655 cm^{-1} that were characteristic of a double bond and amide carbonyl, respectively. The NH-amide stretching vibrations fell in the range 3190 – 3380 cm^{-1}. PMR spectra showed a singlet for the β-proton of the dehydroamino-acid residue at 7.05 – 7.24 ppm, which indicated that these compounds had the *Z*-configuration (Table 2).

EXPERIMENTAL CHEMICAL PART

IR spectra were recorded in mineral oil on a Nicolet Avatar 330 FT-IR spectrometer; PMR spectra, in DMSO-d_6 on a Varian Mercury-300 spectrometer. TLC used Silufol UV-254 plates, PrOH—H_2O (7:3) eluent, and I_2 detector. Unsaturated 5(4*H*)-oxazolones were prepared as before [6].

2-(Dialkylaminoalkyl)amides of *N*-benzoyl-α,β-dehydroamino acids (IX – XV). A solution of the appropriate

[1] A. L. Mnjoyan Institute of Fine Organic Chemistry, Scientific and Technological Center of Organic and Pharmaceutical Chemistry, National Academy of Sciences of Armenia, 26 Azatutyan Pr., 0014 Yerevan, 375014 Armenia.
[*] e-mail: syuzitos@mail.ru



X, **XVI**: Ar = C_6H_5, n = 2, $R^1 = C_2H_5$;
X, **XVII**: Ar = C_6H_5, n = 2, $N(R^1)_2$ = pyrrole;
XI, **XVIII**: Ar = C_6H_5, n = 2, $N(R^1)_2$ = morpholine;
XII, **XIX**: Ar = C_6H_4Br-4, n = 2, $R^1 = CH_3$;
XIII, **XX**: Ar = C_6H_4Br-4, n = 2, $R^1 = C_2H_5$;
XIV, **XXI**: Ar = C_6H_4Br-4, n = 3, $R^1 = CH_3$;
XV, **XXII**: Ar = $C_6H_4OCH_3$-4, n = 2, $R^1 = C_2H_5$

5(4*H*)-oxazolone (**I** – **III**, 0.004 mol) in Et_2O (50 mL) was treated with diamine (**IV** – **VIII**, 0.004 mol) and stirred at room temperature for 1 h. The resulting precipitate was filtered off and recrystallized from C_6H_6.

TABLE 1. Yields, Melting Points, and TLC Data for Dialkylaminoalkylamides of *N*-Substituted α,β-Dehydroamino Acids (**IX** – **XV**) and Their Iodomethylates (**XVI** – **XXII**)

Compound	Yield, %	mp, °C	R_f (PrOH–H_2O, 7:3)	Empirical formula
IX	95.8	150 – 152	0.53	$C_{22}H_{27}N_3O_2$
X	91.1	98 – 101	0.51	$C_{22}H_{25}N_3O_2$
XI	96.9	179 – 182	0.70	$C_{22}H_{25}N_3O_3$
XII	99.4	208 – 211	0.52	$C_{20}H_{22}BrN_3O_2$
XIII	99.9	148 – 150	0.50	$C_{22}H_{26}BrN_3O_2$
XIV	97.3	165 – 167	0.50	$C_{21}H_{24}BrN_3O_2$
XV	98.8	153 – 156	0.48	$C_{23}H_{29}N_3O_3$
XVI	98.0	190 – 192	0.83	$C_{23}H_{30}JN_3O_2$
XVII	67.0	116 – 120	0.74	$C_{23}H_{28}JN_3O_2$
XVIII	61.0	146 – 150	0.79	$C_{23}H_{28}JN_3O_3$
XIX	64.1	125 – 128	0.74	$C_{21}H_{25}BrJN_3O_2$
XX	87.9	186 – 190	0.83	$C_{23}H_{29}BrJN_3O_2$
XXI	97.5	197 – 200	0.79	$C_{22}H_{27}BrJN_3O_2$
XXII	73.2	128 – 133	0.74	$C_{24}H_{32}JN_3O_3$

Quaternization of tertiary aminoamides XVI – XXII. A solution of amide (**IX** – **XV**, 0.0006 mol) in Me_2CO (10 mL) was treated with methyliodide (0.13 g, 0.06 mL, 0.0009 mol) and left for 24 h at room temperature. The resulting precipitate was filtered off, dried in air, and recrystallized from Me_2CO or EtOH–Et_2O (1:1).

EXPERIMENTAL BIOLOGICAL PART

Acetylcholinesterase (ACE) from erythrocytes [7] and high-purity butyrylcholinesterase (BuCE) from human blood serum that was obtained as before [8] were used in the work.

ACE activity was measured by a modified method [9]. The reaction mixture (2.5 mL final volume) contained phosphate buffer (0.1 M, pH 7.6), 5,5′-dithio-*bis*-2-nitrobenzoic acid (0.4 mM), acetylthiocholine (ATC, 0.05 – 0.5 mM), and the required amount of enzyme. The reaction was carried out on a Specord UV-VIS spectrophotometer (Germany) in a thermostatted cell at 25°C. The initial reaction rate was determined from the slope of the tangent to the absorption curve of the product from ATC hydrolysis. The molar extinction coefficient of the Ellman reaction product $\varepsilon_{412-420}$ was taken as 14,150 $M^{-1} \cdot cm^{-1}$ [10]. BuCE activity was measured analogously.

Anticholinesterase activities, i.e., the IC_{50} values (compound concentration at which the cholinesterase hydrolysis rate of ATC was 50% inhibited), were determined with constant ATC substrate concentration (0.1 mM) and variable concentrations of the test compounds. IC_{50} values were determined from plots of the suppression of the ATC enzymatic

hydrolysis rate as a function of inhibitor concentration (Table 3).

Antibacterial activity of all compounds was studied using the literature method [11] with bacterial loading 20×10^6 microbes per mL of medium. The experiments used Gram-positive (*S. aureus* 1 and 209p) and Gram-negative bacteria (*S. flexneri* 6858 and *E. coli* 0-55). Solutions of the compounds and control drug were prepared in DMSO (1:20 dilution). Petri dishes with inoculations of the aforementioned microorganism strains were treated with the test compounds (0.1 mL each). Results were calculated from the diameter (*d*, mm) of the microbe growth inhibition zone at the application site of the compounds after test cultures were grown for 1 d in a thermostat at 37°C. The positive control under analogous conditions was furazolidone [12] taking into account the amount of active ingredient in the tablets (OAO Borosovskii Medicinal Preparation Plant, Belarus, Borisovo).

RESULTS AND DISCUSSION

Table 3 shows that the strongest ACE inhibitor of the tertiary aminoamides was **XIII** whereas 2-amide **XII** showed comparatively high anticholinesterase activity for BCE. Iodomethylates of amides **XIX** (for ACE) and **XX** (for BCE) of the quaternary ammonium salts had comparatively high inhibiting properties. The results led to the conclusion that quaternization of *N*-benzoyl-α,β-dehydroamino-acid aminoamides degraded the inhibiting properties for BuCE whereas the reverse situation was mainly observed for BCE. All tested compounds exhibited selectivity for BuCE. Iodome-

TABLE 2. PMR Spectra of Dialkylaminoalkylamides (**IX** – **XV**) and Their Quaternary Ammonium Salts (**XVI** – **XXI**)

Compound	
IX	0.95 (t, 6H, J 7.1 Hz, NEt_2), 2.48 (q, 4H , J 7.1 Hz, NEt_2), 2.52 (t, 2H, J 6.8 Hz, NCH_2), 3.25 (dt, 2H, J_1 6.8 Hz, J_2 5.2 Hz, $NH\underline{CH_2}$), 7.21 (s, 1H, =CH), 7.23 – 7.34 (μ, 3H, Ar), 7.42 – 7.56 (μ, 6H, C_6H_5 and NH), 7.98 – 8.04 (μ, 2H, Ar), 9.73 (br.s, 1H, NH).
X	1.62 – 1.69 (μ, 4H , $NCH_2(\underline{CH_2})_2$), 2.43 – 2.52 (μ, 4H , $N(\underline{CH_2})_2$), 2.57 (t, 2H, J 6.7 Hz, NCH_2), 3.31 (dt, 2H, J_1 6.7 Hz, J_2 5.2 Hz, $NH\underline{CH_2}$), 7.19 (s, 1H, =CH), 7.21 – 7.34 (μ, 3H, Ar), 7.42 – 7.56 (μ, 5H, C_6H_5), 7.62 (br.t, 1H, J 5.2 Hz, $\underline{NH}CH_2$), 7.98 – 8.03 (μ, 2H, Ar), 9.73 (br.s, 1H, NH).
XI	2.37 – 2.42 (μ, 4H, $N(CH_2)_2$), 2.46 (t, 2H, J 6.6 Hz, NCH_2), 3.32 (dt, 2H, J_1 6.6 Hz, J_2 5.2 Hz, $NH\underline{CH_2}$), 3.44 – 3.47 (μ, 4H, $O(\underline{CH_2})_2$), 7.20 (s, 1H, =CH), 7.22 – 7.34 (μ, 3H, Ar), 7.44 – 7.55 (μ, 5H, Ar), 7.58 (br.t, 1H, J 5.2 Hz, $\underline{NH}CH_2$), 8.01 – 8.05 (μ, 2H, Ar), 9.77 (br.s, 1H, NH).
XII	2.20 (s, 6H, NMe_2), 2.39 (t, 2H, J 6.7 Hz, $N\underline{CH_2}CH_2NH$), 3.28 (td, 2H, J_1 6.7 Hz, J_2 5.7 Hz, $\underline{CH_2}NH$), 7.11 (s, 1H, =CH), 7.42 – 7.55 (μ, 3H, C_6H_5), 7.45 (s, 4H, C_6H_4Br), 7.70 (br.t, 1H, J 5.7 Hz, NH), 7.96 – 8.00 (μ, 2H, C_6H_5), 9.72 (br.s, 1H, NH).
XIII	0.95 (t, 6H, J 7.1 Hz, NEt_2), 2.48 (q, 4H, J 7.1 Hz, NEt_2), 2.52 (t, 2H, J 6.8 Hz, NCH_2), 3.24 (td, 2H, J_1 6.8 Hz, J_2 5.1 Hz, $NH\underline{CH_2}$), 7.16 (s, 1H, =CH), 7.42 – 7.55 (μ, 3H, C_6H_5), 7.45 (br.s, 4H, C_6H_4Br), 7.60 (br.t, 1H, J 5.1 Hz, NH), 7.97 – 8.02 (μ, 2H, C_6H_5), 9.73 (br.s, 1H, NH).
XIV	1.64 (2H, q, J 6.6 Hz, $NCH_2\underline{CH_2}$), 2.04 (s, 6H, NMe_2), 2.29 (t, 2H, J 6.6 Hz, NCH_2), 3.27 (td, 2H, J_1 6.6 Hz, J_2 5.3 Hz, $NH\underline{CH_2}$), 7.16 (s, 1H, =CH), 7.41 – 7.56 (μ, 3H, C_6H_5), 7.44 (s, 4H, C_6H_4Br), 7.99 – 8.04 (μ, 2H, C_6H_5), 8.10 (br.t, 1H, J 5.3 Hz, $\underline{NH}CH_2$), 9.68 (br.s, 1H, NH).
XV	0.93 (t, 6H, J 7.1 Hz, NEt_2), 2.46 (q, 4H , J 7.1 Hz, NEt_2), 2.48 – 2.54 (μ, 2H, NCH_2), 3.20 – 3.28 (μ, 2H, $NH\underline{CH_2}$), 3.78 (s, 3H, OCH_3), 6.84 and 7.49 (2H each, both m, C_6H_4O), 7.22 (s, 1H, =CH), 7.41 – 7.55 (μ, 3H, C_6H_5 and NH), 8.01 – 8.05 (μ, 2H, C_6H_5), 9.66 (br.s, 1H, NH).
XVI	1.35 (t, 6H, J 7.1 Hz, $CH_2\underline{Me}$), 3.10 (s, 3H, NMe), 3.44 – 3.54 (μ, 6H, NCH_2), 3.62 – 3.69 (μ, 2H, NCH_2), 7.23 (s, 1H, =CH), 7.24 – 7.36 (μ, 3H, Ar), 7.44 – 7.59 (μ, 5H, Ar), 8.01 – 8.07 (μ, 2H, Ar), 8.34 (t, 1H, J 5.7 Hz, $\underline{NH}CH_2$), 9.92 (br.s, 1H, NH).
XVII	12.15 – 2.24 (μ, 4H, $2CH_2$-pyrrole), 3.18 (s, 3H, NMe), 3.60 – 3.74 (μ, 4H, $2CH_2$-pyrrole), 7.24 (s, 1H, =CH), 7.26 – 7.37 (μ, 3H, Ph), 7.43 – 7.60 (μ, 5H, Ph), 8.00 – 8.06 (μ, 2H, Ph), 8.42 (t, 1H, J 5.4 Hz, $\underline{NH}CH_2$), 9.95 (s, 1H, NH).
XVIII	3.35 (s, 3H, NMe), 3.55 – 3.68 (μ, 4H, $2CH_2$,-morpholine), 3.71 – 3.81 (μ, 2H, CH_2, NCH_2), 3.89 – 4.05 (μ, 4H, $2CH_2$-morpholine), 7.23 (s, 1H, =CH), 7.25 – 7.37 (μ, 3H, Ar), 7.45 – 7.60 (μ, 5H, Ar), 8.01 – 8.06 (μ, 2H, Ar), 8.42 (t, 1H, J 5.0 Hz, $\underline{NH}CH_2$), 9.97 (br.s, 1H, NH).
XIX	3.24 (s, 9H, NMe_3), 3.59 – 3.73 (μ, 4H, NCH_2), 7.20 (s, 1H, =CH), 7.44 – 7.58 (μ, 7H, Ar), 7.99 – 8.04 (μ, 2H, Ar), 8.42 (t, 1H, J 5.5 Hz, $\underline{NH}CH_2$), 9.93 (br.s, 1H, NH).
XX	1.35 (t, 6H, J 7.1 Hz, NEt_2), 3.11 (s, 3H, NCH_3), 3.43 – 3.55 (μ, 6H, CH_2NEt_2), 3.61 – 3.69 (μ, 2H, $NH\underline{CH_2}$), 7.20 (s, 1H, =CH), 7.44 – 7.57 (μ, 7H, C_6H_5 and C_6H_4), 8.00 – 8.05 (μ, 2H, C_6H_4), 8.38 (t, 1H, J 5.7 Hz, $\underline{NH}CH_2$), 9.92 (s, 1H, NH).
XXI	1.96 – 2.07 (μ, 2H, $NCH_2\underline{CH_2}$), 3.21 (s, 9H, NMe_3), 3.31 – 3.38 (μ, 2H, NCH_2), 3.48 – 3.55 (μ, 2H, NCH_2), 7.05 (s, 1H, =CH), 7.45 – 7.58 (μ, 7H, C_6H_5 and C_6H_4), 8.01 – 8.06 (μ, 2H, C_6H_4), 8.25 (t, 1H, J 6.0 Hz, $\underline{NH}CH_2$), 10.00 (br.s, 1H, NH).

TABLE 3. Anticholinesterase Properties of **IX** – **XXII**

Compound	IC_{50}, $mM \cdot 10^{-3}$ (ACE) (A)	IC_{50}, $mM \cdot 10^{-5}$ (BuCE) (B)	A/B
IX	480 ± 22	62 ± 2.9	774
X	769 ± 11.5	104 ± 7.1	739
XI	830 ± 38	5000 ± 28.8	17
XII	300 ± 45	8.6 ± 0.48	3488
XIII	200 ± 15	33.4 ± 1.3	599
XIV	434 ± 10	82 ± 4.6	529
XV	500 ± 25	99 ± 3.8	505
XVI	1110 ± 261	78 ± 6.1	1423
XVII	710 ± 24	38 ± 2.1	1868
XVIII	1430 ± 89	72 ± 1.9	1986
XIX	180 ± 5	18 ± 0.14	10000
XX	470 ± 12	11 ± 0.9	4273
XXI	620 ± 18.5	19 ± 1.4	3263
XXII	590 ± 16.2	13.9 ± 1.7	4245

thylate **XIX** had the highest selectivity for BuCE (10,000 times). This selectivity is denoted in Table 3 by the ratio A/B, where A and B are the IC_{50} values of a given compound for ACE and BuCE, respectively. It is noteworthy that a hexahydrofuro[3,2-*b*]furan derivative showed selectivity for BuCE (51,000 times) in previous work [13].

Studies of the antibacterial properties of synthesized **IX** – **XXII** for Gram-positive *S. aureus* 1 and 209p and Gram-negative *S. flexneri* 6858 and *E. coli* 0-55 found that **XII** and **XIII** possessed high antibacterial activity. The other compounds had moderate activity (d = 10 – 19 mm) that was inferior to that of the control drug furazolidone (d = 24 – 25 mm).

Thus, compounds that inhibited selectively the activities of two types of cholinesterase and had antibacterial activity were prepared from *N*,*N*-(dialkylamino)alkylamides of several *N*-benzoyl-α,β-dehydroamino acids. This indicated that further searches in these directions were promising.

REFERENCES

1. A. A. Grigoryan, A. A. Ambartsumyan, M. V. Mkrtchyan, et al., *Khim.-farm. Zh.*, **40**(3), 18 – 23 (2006); *Pharm. Chem. J.*, **40**(3), 135 – 140 (2006).
2. H. A. Grigoryan, A. A. Hambardzumyan, M. V. Mkrtchyan, et al., *NAS RA Electron. J. Nat. Sci.*, **1**(6), 14 – 20 (2006).
3. H. A. Grigoryan, A. A. Hambardzumyan, M. V. Mkrtchyan, et al., *Chem.-Biol. Interact.*, **171**(1), 108 – 116 (2008).
4. A. A. Spasov, D. S. Yakovlev, and L. F. Suzdalev, *Khim.-farm. Zh.*, **46**(10), 14 – 17 (2012); *Pharm. Chem. J.*, **46**(10), 584 – 590 (2012).
5. A. Kawasaki, K. Meakawa, K. Kubo, et al., *Tetrahedron*, **60**(42), 9517 – 9524 (2004).
6. V. O. Topuzyan and S. R. Tosunyan, *Khim. Zh. Arm.*, **65**(3), 369 – 373 (2012).
7. G. Fairbanks, T. L. Stec, and D. F. Wallach, *Biochemistry*, **10**(13), 2606 – 2617 (1971).
8. B. N. La Du, C. F. Bartels, C. P. Nogueria, et al., *Clin. Biochem.*, **23**, 423 – 431 (1990).
9. G. L. Ellman, K. D. Coutney, V. Andres, Jr., et al., *Biochem. Pharmacol.*, **7**, 88 – 95 (1961).
10. P. W. Riddles, R. L. Blakeley, and B. Zerner, *Anal. Biochem.*, **94**(1), 75 – 81 (1979).
11. G. N. Pershin, *Methods of Experimental Chemotherapy* [in Russian], Meditsina, Moscow (1971), pp. 507 – 522.
12. M. D. Mashkovskii, *Drugs* [in Russian], Novaya Volna, Moscow (2010), p. 851.
13. C. G. Caloran, G. P. Dillon, J. M. Gaynor, et al., *J. Med. Chem.*, **51**(20), 6400 – 6409 (2008).