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SYNTHESIS AND BIOLOGICAL PROPERTIES OF NEW DERIVATIVES OF 2-ARYLPYRROLIDINECARBONITRILES AND PYRROLIDINECARBOXAMIDES

S. P. Gasparyan,[1] M. V. Alexanyan,[1] G. K. Arutyunyan,[1] V. E. Oganesyan,[1] V. V. Martirosyan,[1] R. V. Paronikyan,[1] G. M. Stepanyan,[1] and A. O. Martirosyan[1]

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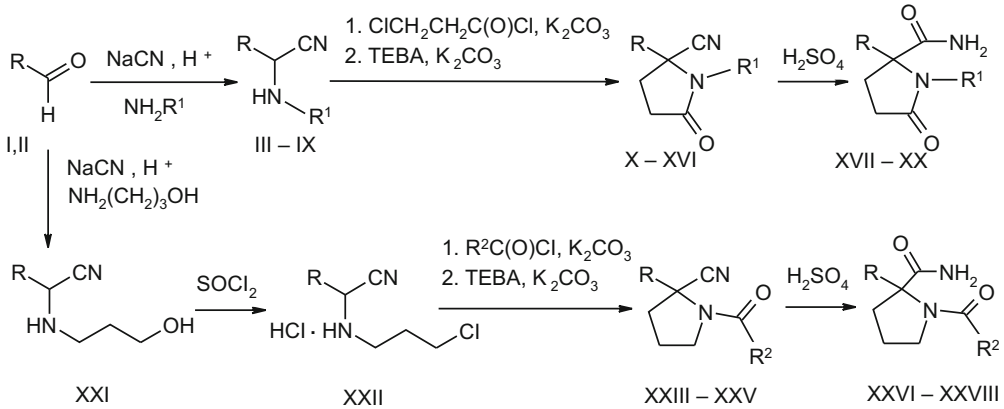
A series of new analogs of 2-arylpyrrolidinecarbonitriles were synthesized under phase-transfer catalysis conditions. New analogs of pyrrolidinecarboxamides were synthesized based on these carbonitriles. Biological evaluation showed that the synthesized derivatives of pyrrolidinecarbonitriles and pyrrolidinecarboxamides possessed moderate anticancer activity.

Key words: cyclic amino acids, proline, phase-transfer catalysis, intramolecular cyclization, acylation, pyrrolidine.

Cyclic analogs of á-amino acids, in particular proline, are interesting as starting materials for synthesizing new drugs. However, they are little studied because of the limited number of available synthetic pathways to them [1, 2]. A new method for synthesizing derivatives of 2-phenylproline that consisted of the synthesis of phenylglycine derivatives and intramolecular cyclization under phase-transfer catalysis conditions was developed by us earlier [3, 4].

Herein we report the synthesis and biological testing of new derivatives of 2-arylpyrrolidinecarboxylic acid that were prepared in a search for antitumor drugs among proline analogs. The starting materials were benzaldehyde and 3,4-dimethoxybenzaldehyde, the reaction of which with sodium cyanide and various aromatic amines gave the corresponding acetonitriles **III** – **IX**, acylation of which by

[1] Mndzhoyan Institute of Fine Organic Chemistry, Scientific and Technological Center of Organic and Pharmaceutical Chemistry, National Academy of Sciences of Armenia, Yerevan, 375014, Armenia.



I: R-C_6H_5; II: R=3,4-$(CH_3O)_2C_6H_3$; III, X, XVII: R=R^1=C_6H_5; IV, XI, XVIII: R=C_6H_5, R^1=$C_6H_5CH_2$; V, XII, XIX: R=C_6H_5, R^1=4-$CH_3C_6H_4$; VI, XIII: R=C_6H_5, R^1=2-$CH_3OC_6H_4$; VII, XIV, XX: R=C_6H_5, R^1=4-$CH_3OC_6H_4$; VIII, XV: R=3,4-$(CH_3O)_2C_6H_3$, R^1=4-$CH_3C_6H_4$; IX, XVI: R=3,4-$(CH_3O)_2C_6H_3$, R^1=3,5-$(CH_3)_2C_6H_3$; XXI – XXIII, XXVI: R=C_6H_5, R^2=2-BrC_6H_4; XXIV, XXVII: R=C_6H_5, R^2=4-BrC_6H_4; XXV, XXVIII: R=C_6H_5, R^2=4-CH_3O-3-NO_2-C_6H_3.

3-chloropropionyl chloride and subsequent intramolecular cyclization under phase-transfer catalysis conditions afforded the target products **X** – **XVI**.

Benzaldehyde and 1-amino-3-hydroxypropane were reacted in the same manner to give the corresponding aminopropanol derivative **XXI**, which was converted to chloro derivative **XXII** by $SOCl_2$ [3]. Acylation of **XXII** by 2-bromo-, 4-bromo-, and 4-methoxy-3-nitrobenzoic acids also under phase-transfer catalysis conditions with intramolecular cyclization produced proline derivatives **XXIII** – **XXV**.

Amides **XVII** – **XX** and **XXVI** – **XXVIII** were synthesized for biological testing from the corresponding pyrrolidinecarbonitriles **X** – **XVI** and **XXIII** – **XXV** via reaction with cooling of the corresponding nitriles and conc. H_2SO_4.

EXPERIMENTAL CHEMICAL PART

The structures of the synthesized compounds were confirmed by PMR spectra recorded on a Mercury-300 instrument (Varian) and by elemental analyses. The course of reactions and purity of products were monitored using TLC on Silufol UV-254 plates and Me_2CO:nonane (1:1, a; 2:1, b).

General method for preparing substituted 2-arylacetonitriles (III – IX). A solution of aldehyde **I** or **II**

TABLE 1. Properties of Synthesized **III** – **XX** and **XXIII** – **XXVIII**

Compound	Yield, %	mp, °C	R_f	Empirical formula
III	90	83 – 85	0.50 (a)	$C_{14}H_{12}N_2$
IV	70	119 – 121	0.48 (a)	$C_{15}H_{14}N_2$
V	75	105 – 107	0.43 (a)	$C_{15}H_{14}N_2$
VI	80	71 – 72	0.51 (a)	$C_{15}H_{14}N_2O$
VII	85	73 – 75	0.41 (a)	$C_{15}H_{14}N_2O$
VIII	79	137 – 139	0.49 (b)	$C_{17}H_{18}N_2O_2$
IX	94	142 – 144	0.56 (b)	$C_{18}H_{20}N_2O_2$
X	97	138 – 139	0.46 (a)	$C_{17}H_{14}N_2O$
XI	98	116 – 118	0.48 (a)	$C_{18}H_{16}N_2O$
XII	98	155 – 157	0.51 (a)	$C_{18}H_{16}N_2O$
XIII	98	156 – 158	0.42 (a)	$C_{18}H_{16}N_2O_2$
XIV	95	134 – 136	0.45 (a)	$C_{18}H_{16}N_2O_2$
XV	92	158 – 160	0.50 (a)	$C_{20}H_{20}N_2O_3$
XVI	95	194 – 196	0.49 (b)	$C_{21}H_{22}N_2O_3$
XVII	87	92 – 96	0.49 (b)	$C_{17}H_{16}N_2O_2$
XVIII	70	182 – 184	0.50 (a)	$C_{18}H_{18}N_2O_2$
XIX	75	215 – 217	0.44 (b)	$C_{18}H_{18}N_2O_2$
XX	80	217 – 220	0.50 (b)	$C_{18}H_{18}N_2O_3$
XXIII	78	118 – 121	0.45 (b)	$C_{18}H_{15}BrN_2O$
XXIV	65	157 – 158	0.60 (b)	$C_{18}H_{15}BrN_2O$
XXV	88	197 – 199	0.58 (b)	$C_{19}H_{17}N_3O_4$
XXVI	65	188 – 189	0.44 (b)	$C_{18}H_{17}BrN_2O_2$
XXVII	90	219 – 220	0.48 (b)	$C_{18}H_{17}BrN_2O_2$
XXVIII	80	183 – 185	0.52 (b)	$C_{19}H_{19}N_3O_5$

TABLE 2. PMR Spectra of **III** – **XX** and **XXIII** – **XXVIII**

Compound	PMR spectra, DMSO-d_6, δ, ppm, *J*/Hz
III	5.70 (d, 1H, *J* = 9.3, CH), 6.44 (d, 1H, *J* = 9.3, NH), 6.68 – 7.64 (m, 10H, arom. H)
IV	3.96 and 4.15 (d, 2H, *J* = 12.8, NCH_2), 4.00 (br, 1H, NH), 5.83 (s, 1H, CH), 7.33 – 7.84 (m, 10H, arom. H)
V	($CDCl_3$), 2.30 (s, 3H, CH_3), 3.88 (br, 1H, NH), 5.41 (s, 1H, CH), 6.72 – 7.64 (m, 9H, arom. H)
VI	3.75 (s, 3H, OCH_3), 5.65 (d, 1H, *J* = 9.2, CH), 6.10 (d, 1H, *J* = 9.2, NH), 6.85 (s, 4H, C_6H_4), 7.40 – 7.70 (m, 5H, arom. H)
VII	3.71 (s, 3H, OC_3), 5.60 (d, 1H, *J* = 9.3, 1H, NH), 6.01 (d, 1H, *J* = 9.3, NH), 6.72 (s, 4H, C_6H_4), 7.33 – 7.60 (m, 5H, arom. H)
VIII	2.24 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.51 (d, 1H, *J* = 9.3, CH), 6.08 (d, 1H, *J* = 9.3, NH), 6.67 and 6.94 (m, 4H, C_6H_4), 6.89 (d, 1H, *J* = 8.2, H-5 C_6H_3), 7.10 (dd, 1H, ^2J = 8.2, ^2J = 2.1, H-6 C_6H_3) and 7.13 (d, 1H, *J* = 2.1, H-2 C_6H_3)
IX	($CDCl_3$), 2.23 (s, 6H, CH_3-Ar), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.51 (d, 1H, *J* = 9.3, CH), 6.07 (d, 1H, *J* = 9.3, NH), 6.34 [s, 1H, H-4 $C_6H_3(CH_3)_2$] and 6.38 [s, 2H, H-2, 6 $C_6H_3(CH_3)_2$], 6.90 [d, 1H, *J* = 8.1, H-5 $C_6H_3(OCH_3)_2$], 7.09 [dd, 1H, ^1J = 8.1, ^2J = 2.1, H-6 $C_6H_3(OCH_3)_2$] and 7.12 [d, 1H, *J* = 2.1, H-2 $C_6H_3(OCH_3)_2$]
X	2.54 – 3.00 (m, 4H, CH_2CH_2), 7.10 – 7.56 (m, 10H, arom. H)
XI	2.46 – 2.79 (m, 4H, CH_2CH_2), 3.97 and 4.54 (d, 2H, *J* = 14.9, NCH_2), 6.97 – 7.43 (m, 10H, arom. H)
XII	2.28 (s, 3H, CH_3), 2.59 (dt, 1H, ^1J = 11.9, ^2J = 8.9, CH_2CH_2) and 2.70 – 2.98 (m, 3H, CH_2CH_2), 7.04 (s, 4H, arom. H), 7.30 – 7.54 (m, 5H, arom. H)
XIII	2.66 – 3.01 (m, 4H, CH_2CH_2), 3.76 (s, 3H, OCH_3), 6.76 (t, 1H, *J* = 7.6, arom. H), 6.88 – 5.58 (m, 8H, arom. H)
XIV	2.64 – 2.97 (m, 4H, CH_2CH_2), 3.72 (s, 3H, OCH_3), 6.76 and 7.03 (m, 2H, $C_6H_4OCH_3$), 7.31 – 7.53 (m, 5H, arom. H)
XV	2.29 (s, 3H, CH_3), 2.61 – 2.86 (m, 4H, CH_2CH_2), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.80 (d, 1H, *J* = 8.4, H-5 C_6H_3), 6.98 (d, 1H, *J* = 2.3, H-2 C_6H_3), 7.03 (dd, 1H, ^1J = 8.4, ^2J = 2.3, H-6 C_6H_3), 7.06 (s, 4H, C_6H_4)
XVI	2.24 (s, 6H, CH_3-Ar) 2.55 – 2.87 (m, 4H, CH_2CH_2), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.77 – 6.81 [br, 3H, $C_6H_3(OCH_3)_2$], 6.80 [d, 1H, *J* = 8.3, H-5 $C_6H_3(OCH_3)_2$], 6.97 [d, 1H, *J* = 2.3, $C_6H_3(OCH_3)_2$] and 7.03 [dd, 1H, ^1J = 8.3, ^2J = 2.3, H-6 $C_6H_3(OCH_3)_2$]
XVII	2.28 – 2.84 (m, 4H, CH_2CH_2), 7.02 (m, 1H, H-4 C_6H_5), 7.10 – 7.30 (m, 9H, H-arom. and NH_2), 7.36 (m, 2H, arom. H)
XVIII	2.50 – 2.90 (m, 4H, CH_2CH_2), 4.30 (q, 2H, CH_3), 5.62 (s, 2H, NH_2), 7.0 – 7.54 (m, 10H, arom. H)
XIX	2.26 (s, 3H, CH_3), 2.32 – 2.81 (m, 4H, CH_2CH_2), 6.93 and 7.07 (m, 4H, arom. H), 7.12 (br, 2H, NH_2), 7.21 – 7.29 and 7.35 (m, 5H, arom. H)
XX	2.30 – 2.79 (m, 4H, CH_2CH_2), 3.71 (s, 3H, OCH_3), 6.66 and 7.05 (m, 4H, arom. H), 7.07 and 7.14 (br, 2H, NH_2), 7.20 – 7,35 (m, 5H, arom. H)
XXIII	2.02 – 2.42 and 2.86 (m, 4H, CH_2CH_2), 3.52 – 3.74 (m, 2H, NCH_2), 7.12 – 7.70 (m, 9H, arom. H)
XXIV	2.12 – 2.41 and 2.85 (m, 4H, CH_2CH_2), 3.86 (br, 1H) and 3.91 (m, 1H, NCH_2), 7.28 – 7.61 (m, 9H, arom. H)
XXV	2.12 – 2.38 and 2.75 (m, 4H, CH_2CH_2), 3.84 and 4.15 (m, 2H, NCH_2), 4.03 (s, 3H, OCH_3), 7.28 – 7.41 (m, 7H) and 8.19 (br. 1H, arom. N)
XXVI	1.70 – 2.04 (m, 3H), 2.90 – 3.10 (m, 1H) and 3.36 – 3.60 (m, 2H, CH_2CH_2), 7.12 (br, 1H, NH_2), 7.18 – 7.66 (m, 10H, NH_2 and arom. H)
XXVII	1.71 – 2.00 (m, 3H), 2.89 – 2.98 (m, 1H) and 3.63 and 3.75 (m, 2H, CH_2CH_2), 7.09 (br, 1H, NH_2), 7.18 – 7.33 (m, 5H), 7.40 (m, 1H) and 7.51 – 7.62 (m, 4H, NH_2 and arom. H)
XXVIII	1.90 – 3.40 (m, 6H, CH_2CH_2), 4.12 (s, 3H, OCH_3), 5.90 and 7.05 (ss, 2H, NH_2), 7.62 – 8.64 (m, 8H, arom. H)

(10 mmol) in EtOH (20 mL) was stirred at room temperature, treated with a solution of NaCN (0.5 g, 10 mmol) in H_2O (10 mL), stirred for 10 min, treated with HOAc (0.6 g, 10 mmol), stirred for another 10 min, diluted with a solution of the corresponding amine (10 mmol) in EtOH (10 mL), stirred for 2 h, diluted with cold H_2O (10 mL), and left overnight. The resulting precipitate was filtered off, washed with H_2O, dried, and recrystallized from EtOH (Tables 1 and 2).

General method for preparing 2-aryl-2-pyrrolidinecarbonitriles (X – XVI, XXIII – XXV). A mixture of the corresponding 2-arylacetonitrile **III** – **IX** or **XXII** (10 mmol) [3] in 1,2-dichloroethane (20 mL) and anhydrous K_2CO_3 (1.4 g, 10 mmol) at 10 – 15°C was treated dropwise with 3-chloropropionyl chloride or substituted benzoic acid (10 mmol), stirred at room temperature for 30 min and at 40 – 45°C for 2 h, cooled, treated with 1,2-dichloroethane (20 mL), washed several times with H_2O, and dried over $CaCl_2$. The solvent was distilled off. The residue was treated with anhydrous K_2CO_3 (1.4 g, 10 mmol), triethylbenzylammonium chloride (0.1 g, 5 mmol), and CH_3CN (20 mL), stirred at 45 – 50°C for 4 h, and filtered. The filtrate was evaporated. The residue was dissolved in $CHCl_3$, washed with H_2O, and dried over $CaCl_2$. The solvent was distilled off. The residue was recrystallized from EtOH (Tables 1 and 2).

General method for preparing 2-aryl-2-pyrrolidinecarboxamides (XVII – XX and XXVI – XXVIII). The corresponding 2-aryl-2-pyrrolidinecarbonitrile **X** – **XII**, **XIV**, or **XXIII** – **XXV** (10 mmol) was dissolved in conc. H_2SO_4 (10 mL) at 0 – 5°C, left at room temperature for 3 h, and slowly poured into a beaker with ice. The resulting crystals were filtered off, washed with dilute $NaHCO_3$ solution and H_2O, and recrystallized from EtOH (Tables 1 and 2).

EXPERIMENTAL BIOLOGICAL PART

Antibacterial activity of the synthesized compounds was studied using Gram-positive *Staphylococcus aureus* 209P, 1 and Gram-negative *Shigella dysenteriae Flexneri*-6858 and *Escherichia coli* 0 – 55 and the agar-diffusion method with bacterial load 20×10^6 microbes per millimeter of medium [5]. Compounds were studied at 1:20 concentrations. Results were calculated from the diameter (d, mm) of the microorganism growth inhibition zone at the application site of the compounds after growth for 1 d at 37°C (thermostatted).

Toxicity and antitumor activity of **XV** – **XX** and **XXV** – **XXVIII** were tested by the usual methods [6, 7] on 210 white laboratory mice (20 – 22 g) of both sexes.

Acute toxicity was studied in white mice with a single i.p. injection. The absolute lethality (LD_{100}) and maximum tolerated dose (MTD) were determined for each compound.

Antitumor activity was studied in mice with grafted sarcoma 37 and Ehrlich ascites carcinoma (EAC) tumors. Because of their poor solubility, the compounds were administered to the animals as suspensions in carboxymethylcellulose solution (0.5%) daily by i.p. injection for six days at doses from 1/10 to 1/15 of LD_{100}. The criteria for a therapeutic effect were the percent tumor growth inhibition (TGI, %) for sarcoma 37 and the increase of average lifespan (ALS, %) for EAC.

TABLE 3. Toxicity and Antitumor Activity of **XV** – **XX** and **XXV** – **XXVIII**

Compound	Acute toxicity, mg/kg		Sarcoma-37			EAC	
	LD_{100}	MTD	Dose, mg/kg	TGI, %	P	MLS, %	P
XV	2500	1250	200	45	< 0.05	39	= 0.05
XVI	2500	1250	200	40	= 0.05	35	= 0.05
XVII	2500	1200	250	59	< 0.05	57	< 0.05
XVIII	2200	1050	150	40	= 0.05	38	= 0.05
XIX	2500	1200	250	53	< 0.05	52	< 0.05
XX	2500	1200	250	50	= 0.05	45	= 0.05
XXV	2500	1250	200	39	= 0.05	0	-
XXVI	2200	1050	150	35	= 0.05	0	-
XXVII	2200	1050	150	32	= 0.05	0	-
XXVIII	2200	1000	150	43	= 0.05	0	-

Results were processed statistically using the Student—Fisher method.

It was found during the acute toxicity study that **XV** – **XX** and **XXV** – **XXVIII** had comparatively low toxicity (LD_{100} = 2,200 – 2,500 mg/kg).

The chemotherapy tests showed that **XVII**, **XIX**, and **XX** possessed moderate antitumor activity (Table 3). The other compounds had a weak suppressive effect on the TGI of sarcoma 37 and only a few of them, on EAC.

Thus, changing the nitrile of 5-oxopyrrolidines **XV** – **XX** to carboxamide increased the antitumor activity. The toxicity and antitumor activity of substituted 1-benzoylpyrrolidine derivatives **XXV** – **XXVIII** were inferior to those of aforementioned 5-oxopyrrolidine derivatives **XV** – **XX**.

The study of the antibacterial properties of **X** – **XX** and **XXV** – **XXVIII** showed that they did not possess antibacterial activity.

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