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SYNTHESIS OF CYTOTOXIC DERIVATIVES OF 2-OXO-1-AZETIDINYLACETAMIDE

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New derivatives of 2-oxo-1-azetidinylacetamide have been synthesized by the four-component condensation of β-amino acids with aldehydes and isonitriles. Study of their cytotoxic activity in vitro revealed a cytotoxic effect of individual compounds in relation to cancer cells of human fibrosarcoma, mouse hepatoma, and mouse neuroblastoma.

Keywords: N-cyclohexyl-α-(4-benzyloxycarbonyl-2-oxo-1-azetidinyl)-α-(aryl)acetamides, N-cyclohexyl-α-(2-oxo-4-phenyl-1-azetidinyl)-α-(aryl)acetamides, N-cyclohexyl-α-(3-benzyloxycarbonylamino-2-oxo-1-azetidinyl)-α-(4-cyanophenyl)acetamide, N-cyclohexyl-α-(3-*tert*-butoxycarbonylamino-2-oxo-1-azetidinyl)-α-(aryl)acetamides, N-tolylsulfonyl-α-(2-chloro-4-nitrophenyl)-α-(2-oxo-1-azetidinyl)-acetamide, cytotoxic activity.

In a continuation of investigations devoted to the synthesis of 1,3,4-trisubstituted β-lactams and to the analysis of the interconnection between their structure and cytotoxic properties [1-3], we have selected derivatives of 2-oxo-1-azetidinylacetamide as the next subject.

The Ugi one-step four-component condensation of β-amino acids with aldehydes and isonitriles [4-7] was used for their preparation. Its advantage in comparison with other methods of obtaining 2-oxo-1-azetidinylacetamide is the possibility of the simultaneous introduction of substituents directed into the 3C and 4C positions of the heterocycle, and also of replacing protons in the methylene and amide fragments of acetamide.

The synthesis of the new azetidinones **4a-j** with the aid of this condensation was effected by the interaction of structural analogs of β-amino acids **1a-e**, aldehydes **2a-e**, and isocyanates **3a,b** (Scheme 1).

The condensation was carried out in methanol at room temperature during 3-5 days. The end of the reaction was checked by TLC. Substances were isolated from the reaction mixtures by column chromatography. Their structures were demonstrated by ^{1}H NMR spectra, elemental analysis, and their homogeneity by HPLC.

The structural specificity of this reaction is characterized by the formation of a chiral center at the C-5 atom of the resulting compounds **4a-j.** Additional chiral centers are formed at the C-3 and C-4 atoms of **4a-i** due to the presence of substituents in the α- and β-positions of the initial amino acids **1b-e**. Theoretically this must lead to the preparation of the final product as a complex diastereoisomeric mixture. However, judging by HPLC data and ^{1}H NMR spectra the majority of the isolated products were equilibrium mixtures of only two isomers. In the case of azetidinone **4f** their ratio proved to be displaced in the direction of one representative (1:4), but azetidinones **4h** and **4j** proved to be diastereoisomeric products.

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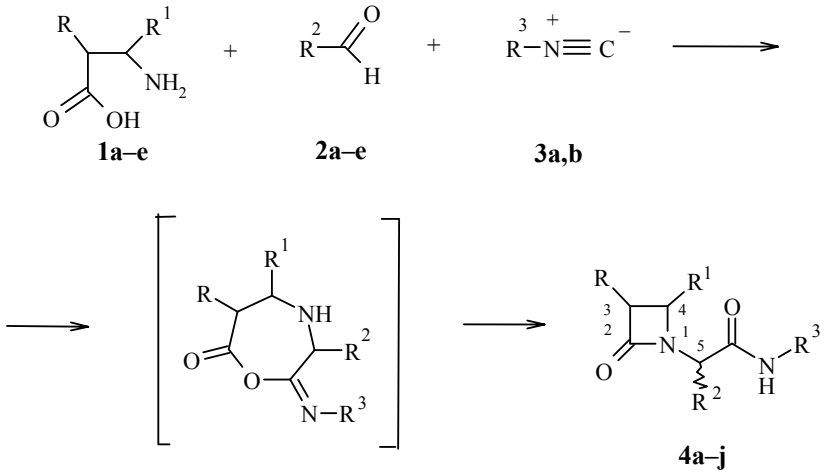
Scheme 1



1a–e **2a–e** **3a,b** **4a–j**

1 a R = H; R^1 = H; **b** R = H, R^1 = CO_2CH_2Ph; **c** R = H, R^1 = Ph; **d** R = $Me_3COCONH$, R^1 = H; **e** R = $PhCH_2OCONH$, R^1 = H; **2 a** 4-ClC_6H_4; **b** R^2 = 4-NCC_6H_4; **c** R^2 = 3-$O_2NC_6H_4$; **d** R^2 = 1-naphthyl; **e** R^2= 2,4-$Cl(O_2N)C_6H_3$; **3 a** R^3 = C_6H_{11}, **b** R^3 = $SO_2C_6H_4Me$-4; **4 a-e, j** R = H; **f** R = $PhCH_2OCONH$; **g-i** R = BocNH; **4 a** R^1 = CO_2CH_2Ph, R^2 = 4-ClC_6H_4; **b** R^1 = CO_2CH_2Ph, R^2 = 4-NCC_6H_4, **c** R^1 = CO_2CH_2Ph, R^2 = 1-naphthyl; **d** R^1 = Ph, R^2 = 4-ClC_6H_4, **e** R^1 = Ph, R^2 = 4-NCC_6H_4; **f** R^1 = H, R^2 = 4-ClC_6H_4; **g** R^1 = H, R^2 = 4-NCC_6H_4; **h** R^1 = H, R^2 = 3-$O_2NC_6H_4$; **i** R^1 = H, R^2 = 1-naphthyl; **j** R^1 = H, R^2 = 2,4-$Cl(O_2N)C_6H_3$; **a-i** R^3 = C_6H_{11}; **j** R^3 = $SO_2C_6H_4Me$-4.

The biological portion of the investigations *in vitro* (Table 1) comprised the determination of the cytotoxic properties of the synthesized compounds in relation to cancer cell monolayers, and also their ability to initiate the biosynthesis of nitric oxide radical (TG_{100}), the high reactivity of which is an important component of the cytotoxic effect [8, 9].

TABLE 1. Biological Properties of Derivatives of 1,3,4-Trisubstituted 2-Azetidinones

Compound	Cytotoxic effect (μg/ml) and specific NO· generating ability in relation to tumor cells					
	HT-1080*			MG-22A*		
	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}
4a	0.9	0.6	150	55	51	100
4b	>100	>100	8	>100	>100	9
4c	44	45	250	60	59	250
4d	0.9	0.7	250	39	35	125
4e	39	33	13	>100	>100	6
4f	5.4	6.5	350	8.8	41	50
4g	47	58	200	81	83	15
4h	52	51	89	44	55	160
4i	8.3	8.3	500	9.6	24	550
4j	73	73	125	54.5	49	200

* TD_{50} is the concentration providing 50% cell death [staining with CV crystal violet, staining with MTT 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide]; TG_{100} is the specific NO· generating ability [9].

TABLE 2. Biological Properties of Derivatives of 1,3,4-Trisubstituted 2-Azetidinones

Compound	Cytotoxic effect (μg/ml) and specific NO· generating ability in relation to tumor cells					
	B 16			Neuro 2A		
	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}
4a	71	71	21	67	31	13
4d	44	52.3	44	32	43.4	250
4f	7.4	7.3	67	29	38	56
4i	29	32	300	18	32	250

The concentration of substances leading to the death of 50% cells (TD_{50}) was determined by standard methodology on four tumor cell lines: human fibrosarcoma HT-1080, mouse hepatoma MG-22A , mouse melanoma B 16, and mouse neuroblastoma Neuro 2A [9].

The synthesized compounds may be divided into two groups according to the biological effect displayed. Compounds **4b,c,e,g,h,j** belong to one and are characterized by the absence of a cytotoxic effect or a weak display in relation to cultures of HT-1080 and MG-22A.

The second group, consisting of azetidinones **4a,d,f,i,** displayed high cytotoxic activity in relation to human fibrosarcoma cells and more moderate activity in relation to mouse hepatoma cancer cells (Table 2). Additional testing showed good activity for compound **4f** in relation to mouse neuroblastoma.

In difference to previous investigations [1-3], a comparative analysis of the intensity of the intracellular generation of nitric oxide radicals and of the cytotoxic properties of the synthesized compounds has enabled an interconnection to be shown between these properties only for individual types of cancer cells.

EXPERIMENTAL

The ^{1}H NMR spectra were taken on a Bruker WH 90/DS (90 MHz) spectrometer in $CDCl_3$ or DMSO-d_6, internal standard was TMS. Microanalytical data were determined with a Carlo Erba 1108 analyzer. A check on the progress of reactions was effected by TLC on Merck Kieselgel plates with UV visualization. The HPLC data were obtained on a Dupont Model 8800 instrument fitted with a UV detector (λ = 254 nm) and column (4.6 × 250 mm), packed with Symmetry C_{18} phase or Ultrasphere octyl in the system acetonitrile–water or acetonitrile–0.1 N pH 2.5 phosphate buffer (60:40), flow rate was 0.8-1.5 ml/min. Silica gel of type Merck Kieselgel (0.063-0.230 mm) was used for preparative column chromatography. Reagents and materials from Aldrich, Acros, Sigma, and SIA BAPEKS were used in experiments.

Synthesis of 2-Oxo-1-azetidinylacetamides 4a-j (General Procedure). Isonitrile **3a,b** (1.2 mmol) was added to a suspension of β-alanine **1a-e** (1 mmol) and aldehyde **2a-e** (1 mmol) in methanol (4 ml). The mixture was stirred at room temperature until the end of the reaction (3-5 days) according to TLC. The solvent was evaporated, and the residue was fractionated on a chromatographic column of silica gel in the system ethyl acetate–petroleum ether (1:2). Fractions containing the desired product were combined and evaporated.

(3*S,R*)-N-Cyclohexyl-α-(4-benzyloxycarbonyl-2-oxo-1-azetidinyl)-α-(4-chlorophenyl)acetamide (4a) was obtained by the condensation of *DL*-aspartic acid α-benzyl ester **1b**, 4-chlorobenzaldehyde **2a**, and cyclohexyl isonitrile **3a**. The desired product, isolated from the fraction of R_f 0.27 as an amorphous substance, was a mixture of isomers (1:1) of total content >98% according to HPLC. Yield 32%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.78-2.04 (10H, m, cyclohexyl); 2.80-3.48 (1H, m, H-3); 3.57-3.88 (1H, m,

cyclohexyl); 3.82 and 4.44 (1H, dd, $^3J_{trans}$ = 2, $^3J_{cis}$ = 5, H-4); 4.95 and 5.13 (2H, two s, CH_2); 5.24 and 5.31 (1H, two s, NCHCO); 6.08 (1H, d, *J* = 8, NH); 7.02-7.53 (10H, m, NH, C_6H_4, C_6H_5). Found, %: C 66.13; H 6.18; N 6.06. $C_{25}H_{27}ClN_2O_4$. Calculated, %: C 66.0; H 5.98; N 6.16.

(3*S,R*)-N-Cyclohexyl-α-(4-benzyloxycarbonyl-2-oxo-1-azetidinyl)-α-(4-cyanophenyl)acetamide (4b) was obtained by the condensation of compound **1b**, 4-cyanobenzaldehyde **2b**, and compound **3a**. The desired product, isolated from the fractions with R_f 0.32 as an amorphous substance, was a mixture of isomers (1:1) with total content >98% according to HPLC. Yield 37%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.73-2.08 (10H, m, cyclohexyl); 2.95 and 3.02 (1H, dd, ^2J = 16, $^3J_{trans}$ = 2, H-3); 3.33 (1H, dd, ^2J = 16, $^3J_{cis}$ = 5, H-3); 3.57-3.88 (1H, m, cyclohexyl); 3.91 and 4.46 (1H, dd, $^3J_{trans}$ = 2, $^3J_{cis}$ = 5, H-4); 5.02 and 5.15 (2H, two s, CH_2); 5.24 and 5.33 (1H, two s, NCHCO); 6.35 (1H, d, *J* = 8, NH); 7.15-7.66 (10H, m, NH, C_6H_4, C_6H_5). Found, %: C 69.70; H 6.13; N 9.28. $C_{26}H_{27}N_3O_4$. Calculated, %: C 70.10; H 6.11; N 9.43.

(3*S,R*)-N-Cyclohexyl-α-(4-benzyloxycarbonyl-2-oxo-1-azetidinyl)-α-(1-naphthyl)acetamide (4c) was obtained by the condensation of compound **1b**, 1-naphthaldehyde **2d**, and compound **3a**. The desired product, isolated from fractions with R_f 0.35 as an amorphous substance, was a mixture of isomers (45:55) with overall content >97% according to HPLC. Yield 12%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.95-2.07 (10H, m, cyclohexyl); 2.73-3.40 (1H, m, H-3); 3.55-3.93 (2H, m, cyclohexyl, H-3); 4.51 (1H, br. s, NCHCO); 5.07 and 5.49 (2H, two d, *J* = 6, CH_2); 6.28 (1H, d, *J* = 8, NH); 6.87-7.82 (13H, m, NH, $C_{10}H_7$, C_6H_5). Found, %: C 73.80; H 6.55; N 5.70. $C_{29}H_{30}N_2O_4$. Calculated, %: C 74.02; H 6.43; N 5.95.

(3*S,R*)-N-Cyclohexyl-α-(4-chlorophenyl)-α-(2-oxo-4-phenyl-1-azetidinyl)acetamide (4d) was obtained by the condensation of *DL*-3-amino-3-phenylpropionic acid **1c**, 4-chlorobenzaldehyde **2a**, and compound **3a**. The desired product, isolated from fractions with R_f 0.45 as an amorphous substance, was a mixture of isomers (1:1) with total content >99% according to HPLC. Yield 42%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.73-2.04 (10H, m, cyclohexyl); 2.93 and 3.02 (1H, dd, ^2J = 15, $^3J_{trans}$ = 2, H-3); 3.42 and 3.48 (1H, dd, ^2J = 15, $^3J_{cis}$ = 5, H-3); 3.68-3.95 (1H, m, cyclohexyl); 4.55 and 4.84 (1H, dd, $^3J_{trans}$ = 2, $^3J_{cis}$ = 5, H-4); 4.91 and 4.93 (1H, two s, NCHCO); 6.46, 6.84 (1H, two d, *J* = 8, NH); 7.06-7.46 (9H, m, C_6H_4, C_6H_5). Found, %: C 69.19; H 6.38; N 7.02. $C_{23}H_{25}ClN_2O_2 \cdot 0.1H_2O$. Calculated, %: C 69.19; H 6.38; N 6.92.

(3*S,R*)-N-Cyclohexyl-α-(4-cyanophenyl)-α-(2-oxo-4-phenyl-1-azetidinyl)acetamide (4e) was obtained by condensing compound **1c**, 4-cyanobenzaldehyde **2b**, and compound **3a**. The desired product, isolated from fractions of R_f 0.53 as an amorphous substance, was a mixture of isomers (1:1) of total content >98% according to HPLC. Yield 38%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.73-2.00 (10H, m, cyclohexyl); 2.91 and 3.00 (1H, dd, ^2J = 15, $^3J_{trans}$ = 2, H-3); 3.40 and 3.46 (1H, dd, ^2J = 15, $^3J_{cis}$ = 5, H-3); 3.48-3.88 (1H, m, cyclohexyl); 4.55 and 4.77 (1H, dd, $^3J_{trans}$ = 2, $^3J_{cis}$ = 5, H-4); 4.89 and 4.93 (1H, two s, NCHCO); 6.64 and 6.91 (1H, two d, *J* = 8, NH); 7.30 and 7.58 (4H, two d, *J* = 9, C_6H_4); 7.02-7.50 (5H, m, C_6H_5). Found, %: C 73.57; H 6.56; N 10.60. $C_{24}H_{25}N_3O_2$. 0.25 H_2O. Calculated, %: C 73.39; H 6.50; N 10.72.

(4*S,R*)-N-Cyclohexyl-α-(3-benzyloxycarbonylamino-2-oxo-1-azetidinyl)-α-(4-chlorophenyl)-acetamide (4f) was obtained by condensing compound **1e**, 4-chlorobenzaldehyde **2a**, and compound **3a**. The desired product was isolated from fractions with R_f 0.50 as a crystalline substance of mp 188-189°C. According to HPLC it was a mixture of isomers (1:4) of total content >99%. Yield 35%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.90-2.01 (10H, m, cyclohexyl); 3.27 (1H, m, cyclohexyl); 3.37-3.88 (1H, m, H-4); 4.13-4.68 (1H, m, H-3); 5.03 (2H, s, CH_2); 5.06 and 5.09 (1H, two s, NCHCO); 5.26 and 5.42 (1H, two d, *J* = 8, NH); 6.41 (1H, m, OCONH); 7.01-7.57 (9H, m, C_6H_4, C_6H_5). Found, %: C 63.88; H 6.00; N 8.88. $C_{25}H_{28}ClN_3O_4$. Calculated, %: C 63.89; H 6.01; N 8.94.

(4*S,R*)-N-Cyclohexyl-α-(3-*tert*-butoxycarbonylamino-2-oxo-1-azetidinyl)-α-(4-cyanophenyl)-acetamide (4g) was obtained by condensing *D*-2-*tert*-butoxycarbonylamino-3-aminopropionic acid (**1d**) and compounds **2b** and **3a**. The desired product was isolated from fractions with R_f 0.45 as a crystalline substance of mp 118-120°C. According to HPLC it was a mixture of isomers (56:44) of total content >97%. Yield 42%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.04-2.09 (10H, m, cyclohexyl); 1.44 (9H, s, C_4H_9); 3.11 (2H,

two d, J = 5, H-4); 3.44-4.00 (2H, m, H-4, cyclohexyl); 4.13-4.60 (1H, m, H-3); 5.00 and 5.69 (1H, two s, NCHCO); 5.07-5.38 (1H, m, NH); 7.22-7.71 (4H, m, C_6H_4); 7.87 (1H, m, OCONH). Found, %: C 64.98; H 7.43; N 13.38. $C_{23}H_{30}N_4O_4$. Calculated, %: C 64.77; H 7.09; N 13.14.

N-Cyclohexyl-α-(3-*tert*-butoxycarbonylamino-2-oxo-1-azetidinyl)-α-(3-nitrophenyl)acetamide (4h) was obtained by condensing compound **1d**, 3-nitrobenzaldehyde **2c**, and compound **3a**. The desired product, isolated from fractions with R_f 0.40 as an amorphous substance, was one of the stereoisomers according to HPLC. Yield 16%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 1.06-2.04 (10H, m, cyclohexyl); 1.49 (9H, s, C_4H_9); 3.11 (1H, dd, ^2J = 5, $^3J_{cis}$ = 5, H-4); 3.67 (1H, dd, ^2J = 5, $^3J_{trans}$ = 2, H-4); 3.75-4.08 (1H, m, cyclohexyl); 4.08-4.35 (1H, m, H-3); 5.05 (1H, d, J = 7, NH); 5.62 (1H, s, NCHCO); 7.42-7.67 (2H, m, C_6H_5); 7.87 (1H, d, J = 7, OCONH); 8.11-8.29 (2H, m, C_6H_5). Found, %: C 59.40; H 7.12; N 12.00. $C_{22}H_{30}N_4O_6 \cdot 0.1$EtOAc. Calculated, %: C 59.09; H 6.82; N 12.31.

(4*S*,*R*)-N-Cyclohexyl-α-(3-*tert*-butoxycarbonylamino-2-oxo-1-azetidinyl)-α-(1-naphthyl)acetamide (4i) was obtained by condensing compound **1d**, 1-naphthaldehyde **2d**, and compound **3a**. The desired product, isolated from fractions of R_f 0.35 as an amorphous substance, was a mixture of isomers (56:44) of total content >97% according to HPLC. Yield 42%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 1.11-2.04 (10H, m, cyclohexyl); 1.38 and 1.49 (9H, two s, C_4H_9); 3.07 (1H, dd, ^2J = 5, $^3J_{cis}$ = 5, H-4); 3.60 (1H, dd, ^2J = 5, $^3J_{trans}$ = 2, H-4); 3.70-4.04 (2H, m, H-4, cyclohexyl); 4.04-4.33 and 4.58-4.82 (1H, m, m, 3-H); 4.93-5.24 (1H, m, NH); 5.33 and 5.67 (1H, two s, NCHCO); 6.28-6.60 (1H, m, OCONH); 7.26-7.33 (7H, m, $C_{10}H_7$). Found, %: C 68.53; H 7.52; N 8.93. $C_{26}H_{33}N_3O_4$. 0.25 H_2O. Calculated, %: C 68.45; H 7.41; N 9.21.

N-Tolylsulfonyl-α-(2-chloro-4-nitrophenyl)-α-(2-oxo-1-azetidinyl)acetamide (4j) was obtained by condensing 3-aminopropionic acid **1a**, 2-chloro-4-nitrobenzaldehyde **2e**, and tolylsulfonyl isonitrile **3b**. The desired product, isolated from fractions of R_f 0.36 as an amorphous substance, was one of the stereoisomers according to HPLC. Yield 6%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 2.40 (3H, s, CH_3); 2.80-3.04 (2H, m, H_2-4); 3.17 (1H, dd, ^2J = 6, $3J_{trans}$ = 3, H-3); 3.46 (1H, dd, ^2J = 6, $^3J_{cis}$ = 6, H-3); 5.37 (2H, d, J = 7, CH_2SO_2); 5.62 (1H, s, NCHCO); 7.17-7.84 (7H, m, C_6H_3, C_6H_4); 8.26 (1H, two d, J = 7, NH). Found, %: C 50.72; H 4.18; N 9.36. $C_{19}H_{18}ClN_3O_6S$. Calculated, %: C 50.50; H 4.01; N 9.30.

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