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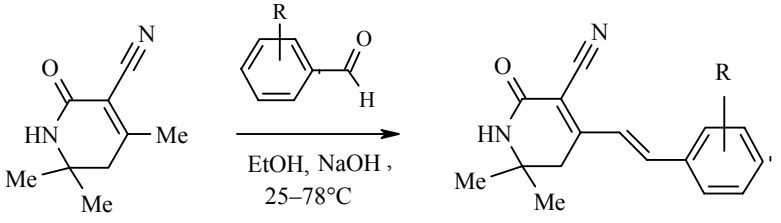
SYNTHESIS AND CYTOTOXICITY OF PHENYL-VINYL DERIVATIVES OF 4,6,6-TRIMETHYL-2-OXO-1,2,5,6-TETRAHYDROPYRIDINE-3-CARBONITRILE

E. Lukevics[1], D. Jansone[1]*, L. Leite[1], J. Popelis[1], G. Andreeva[1], I. Shestakova[1], I. Domracheva[1], V. Bridane[1], and I. Kanepe[1]

A series of phenylvinyl derivatives of 4,6,6-trimethyl-2-oxo-1,2,5,6-tetrahydropyridine-3-carbonitriles has been synthesized and their cytotoxic activity towards HT-1080 (human fibrosarcoma) and MG 22A (mouse hepatoma) tumor cells studied. It was found that the 2-nitro-, 2- and 3-chloro, 2-fluoro, and 2-bromophenyl derivatives showed high cytotoxic activity towards both cell lines. The toxicity towards NIH 3T3 normal mouse embryonic fibroblasts depends on the nature and position of the substituent in the phenyl ring. The greatest selectivity of cytotoxic effect was seen in the 2-bromophenyl derivative.

Keywords: 4,6,6-trimethyl-2-oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile, styryl derivatives, condensation, toxicity, cytotoxicity.

In the synthesis of novel antitumor compounds the method of introducing a δ-lactam [1-4] or cyanovinyl group [5-9] into the starting molecule has been successfully used. A δ-lactam ring is included in camptothecin series antitumor preparations (inhibitors of topoisomerase I [10] – irinotecan [10, 11], rubitecan [12], topotecan [10, 13], exatecan [14], and its metabolite 9-aminocamptothecin [15], silatecan [16], and also inhibitors of dihydrofolate reductase (pemetrexed [17]), thymidyl synthetase (raltitrexed [18]), and farnesyl transferase (tipifarnib [19]). A δ-lactam ring occurs in the structure of an immunoregulator [20] and the antiangiogenic agent roquinimex [21].



1 **2–26**

* To whom correspondence should be addressed, e-mail: jansone@osi.lv.

[1]Latvian Institute of Organic Synthesis, Riga LV-1006, Latvia.

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Several tyrosine kinase inhibitors contain a δ-lactam and an exocyclic β-cyanovinyl group in the same molecule [22].

We have prepared the δ-lactam – 4,6,6-trimethyl-2-oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile (**1**) on whose double bond a cyanovinyl group occurs in the lactam ring but this compound proved inert to the HT-1080 (human fibrosarcoma) and MG 22A (mouse hepatoma) tumor cells.

The introduction of a β-styryl group into the compound molecule often increases its antitumor activity [23] as occurs, for example, in the case of circumin [24], resveratrol [25], piceatannol [7], and the antitumor agent tamoxifen [26, 27]. Combrestatin A-4 (a natural *cis*-stilbene) and its analogs [28-32] are characterized by high cytotoxic activity *in vitro* relative to several types of human tumor cells. Hence we have carried out the condensation of lactam **1** with benzaldehyde to give the novel lactam – 6,6-dimethyl-2-oxo-4-styryl-1,2,5,6-tetrahydropyridine-3-carbonitrile (**2**) which contains all three of the discussed groups (δ-lactam, cyanovinyl, and β-styryl) in its molecule. This compound showed a marked cytotoxic effect on the studied tumor cells (LC_{50} 10 μg/ml).

TABLE 1. Synthesis of 6,6-Dimethyl-2-oxo-4-styryl-1,2,5,6-tetrahydropyridine-3-carbonitriles **2-26**

Styryl-lactam	R	Molar ratio δ-lactam **1** : RC_6H_4CHO : NaOH	Reaction temperature, °C	Reaction time, h	Yield **2**–**26**, %
2	H	1 : 1.5 : 0.25	78	2	27
		1 : 1.5 : 0.25	78	4	27
3	2-F	1 : 1.5 : 0.25	78	6	38
4	2-Cl	1 : 1.5 : 0.25	25	2	75
		2 : 1: 0.05	25	2	100*
		1 : 1.5 : 0.25	78	2	29
5	3-Cl	1 : 1.5 : 0.25	78	2	28
6	4-Cl	1 : 1.5 : 0.25	78	2	45
7	2-Br	1 : 1.5 : 0.25	25	2	70
		1 : 1.5 : 0.25	78	2	64
8	4-Br	1 : 1.5 : 0.0625	65	1	36
9	2-I	1 : 1.5 : 0.25	78	2	90
10	2-OH	1 : 1.5 : 0.125	78	5	98.5
11	4-OH	1 : 1.5 : 0.25	78	6	84
12	2-OMe	1 : 1.5 : 0.25	78	2	17
13	4-OMe	1 : 1.5 : 0.125	78	3	55.5
14	$2\text{-}OCHF_2$	1 : 1.5 : 0.25	78	2	51
15	$2\text{-}NO_2$	1 : 1 : 0.0625	65	1	36
16	$3\text{-}NO_2$	1 : 1 : 0.0625	65	1	100
17	$4\text{-}NO_2$	1 : 1 : 0.0625	25	4.5	11
		1 : 1 : 0.0625	65	1	33
		1 : 1 : 0.0625	78	1	69
18	$4\text{-}NMe_2$	1 : 1 : 0.06	78	1	52
19	2-Me	1 : 1.5 : 0.25	78	3	55
20	$2\text{-}CF_3$	1 : 1.3 : 0.25	78	2	66
21	$2,4\text{-}Cl_2$	1 : 1 : 0.0625	65	1	88
		1 : 2 : 0.25	78	2	50
22	$2,6\text{-}Cl_2$	1 : 1.5 : 0.25	78	2	47
23	$3,4\text{-}(OH)_2$	1 : 1.5 : 0.25	78	4	0
		1 : 1.5 : 0.25	78	6	13.5
24	3-OMe, 4-OH	1 : 1.5 : 0.25	78	6	14
25	$3,4\text{-}(OMe)_2$	1 : 1.5 : 0.25	25	2	68.0
26	$3,4\text{-}OCH_2O$	1 : 1.5 : 0.25	25	2	76.5
		1 : 1.5 : 0.25	78	2	33

* Aldol condensation product **27**.

With the aim of increasing the cytotoxicity and the selectivity of cytotoxic activity of styryl cyanolactams we have synthesized a series of mono-(**3-20**) and disubstituted (**21-26**) phenyl derivatives (Tables 1 and 2) and studied their cytotoxicity on the two tumor cell lines HT-1080 and MG 22A as well as NIH 3T3 normal mouse fibroblasts which also served for evaluating the compound toxicity via the alternative method of determining LD_{50} [33].

TABLE 2. Characteristics for the δ-Lactams **1-27**

Compound	Empirical formula	Found, % / Calculated, %			mp, °C
		C	H	N	
1	$C_9H_{12}N_2O$	65.81 / 65.83	7.36 / 7.37	17.09 / 17.06	196-199
2	$C_{16}H_{16}N_2O$	76.12 / 76.16	6.36 / 6.39	11.14 / 11.10	193-195
3	$C_{16}H_{15}FN_2O$	70.96 / 71.10	5.52 / 5.59	10.39 / 10.36	198-200
4	$C_{16}H_{15}ClN_2O$	66.62 / 67.02	5.15 / 5.27	9.71 / 9.77	228-230
5	$C_{16}H_{15}ClN_2O$	67.10 / 67.02	5.24 / 5.27	9.78 / 9.77	221-224 (decomp.)
6	$C_{16}H_{15}ClN_2O$	66.95 / 67.02	5.25 / 5.27	9.72 / 9.77	235-237 (decomp.)
7	$C_{16}H_{15}BrN_2O$	58.24 / 58.02	4.37 / 4.56	8.49 / 8.46	246-248
8	$C_{16}H_{15}BrN_2O$	58.00 / 58.02	4.53 / 4.56	8.39 / 8.46	253-255 (decomp.)
9	$C_{16}H_{15}IN_2O$	50.91 / 50.81	3.75 / 4.00	7.47 / 7.41	260-262
10	$C_{16}H_{16}N_2O_2$	71.48 / 71.62	6.02 / 6.01	10.56 / 10.44	255-260 (decomp.)
11	$C_{16}H_{16}N_2O_2$	71.33 / 71.62	6.03 / 6.01	10.43 / 10.44	288-291 (decomp.)
12	$C_{17}H_{18}N_2O_2$	72.36 / 72.32	6.39 / 6.43	9.85 / 9.92	230-232
13	$C_{17}H_{18}N_2O_2$	72.22 / 72.32	6.42 / 6.43	9.99 / 9.92	199-200
14	$C_{17}H_{16}F_2N_2O_2$	64.16 / 64.14	5.03 / 5.07	8.82 / 8.80	205-207
15	$C_{16}H_{15}N_3O_3$	64.47 / 64.64	5.03 / 5.09	14.16 / 14.13	246-248 (decomp.)
16	$C_{16}H_{15}N_3O_3$	64.59 / 64.64	5.03 / 5.09	14.12 / 14.13	299-302
17	$C_{16}H_{15}N_3O_3$	64.51 / 64.64	5.11 / 5.09	14.11 / 14.13	280-285 (decomp.)
18	$C_{18}H_{21}N_3O$	73.15 / 73.19	7.19 / 7.17	14.19 / 14.23	268-270 (decomp.)
19	$C_{17}H_{18}N_2O$	76.62 / 76.66	6.81 / 6.81	10.55 / 10.52	236-238
20	$C_{17}H_{15}F_3N_2O$	63.99 / 63.75	4.64 / 4.72	8.76 / 8.75	240-242 (decomp.)
21	$C_{16}H_{14}Cl_2N_2O$	59.96 / 59.83	4.35 / 4.39	8.70 / 8.72	283-286 (decomp.)
22	$C_{16}H_{14}Cl_2N_2O$	59.80 / 59.83	4.27 / 4.39	8.69 / 8.72	214-216
23	$C_{16}H_{16}N_2O_3 \times H_2O$	63.50 / 63.57	5.97 / 6.00	9.20 / 9.27	265-267 (decomp.)
24	$C_{17}H_{18}N_2O_3 \times 0.5\ C_2H_5OH \times H_2O$	64.29 / 63.70	6.64 / 6.83	8.22 / 8.25	213-215 (decomp.)
25	$C_{18}H_{20}N_2O_3$	69.03 / 69.21	6.40 / 6.45	8.91 / 8.97	205-207
26	$C_{17}H_{16}N_2O_3$	69.01 / 68.91	5.32 / 5.44	9.45 / 9.45	248-250
27	$C_{16}H_{17}ClN_2O_2$	63.05 / 63.05	5.53 / 5.55	9.18 / 9.19	222-225

TABLE 3. ^{1}H NMR Characteristics for the Compounds Synthesized

Compound	Chemical shifts, δ, ppm (*J*, Hz)*
1	2
1	1.31 (6H, s, 6-CH_3); 2.27 (3H, s, 4-CH_3); 2.46 (2H, s, H-5); 6.5 (1H, br. s, NH)
2	1.38 (6H, s, 6-CH_3); 2.74 (2H, s, H-5); 7.0 (1H, br. s, NH); 7.20 and 7.45 (2H, two d, *J* = 16.4, CH=CH); 7.41 (3H, m, H-3,4,5 Ph); 7.60 (2H, m, H-2,6 Ph)
3	1.38 (6H, s, 6-CH_3); 2.76 (2H, s, H-5); 6.35 (1H, br. s, NH); 7.39 and 7.50 (2H, two d, *J* = 16.1, CH=CH); 7.2-7.8 (4H, m, H Ph)
4	1.30 (6H, s, 6-CH_3); 2.68 (2H, s, H-5); 6.4 (1H, br. s, NH); 7.33 and 7.53 (2H, two d, *J* = 16.3, CH=CH); 7.1-7.3 (3H, m, H-3,5,6 Ph); 7.69 (1H, m, H-4 Ph)
5	1.37 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 6.13 (1H, br. s, NH); 7.11 and 7.51 (2H, two d, *J* = 16.0, CH=CH); 7.37 (1H, m, H-2 Ph); 7.47 (3H, m, H-4,5,6 Ph)
6	1.37 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 6.2 (1H, br. s, NH); 7.13 and 7.41 (2H, two d, *J* = 16.0, CH=CH); 7.39 and 7.52 (4H, A_2B_2 system, *J* = 8.4, H-3,5 Ph and H-2,6 Ph)
7	1.40 (6H, s, 6-CH_3); 2.77 (2H, s, H-5); 6.8 (1H, br. s, NH); 7.37 and 7.58 (2H, two d, *J* = 16.0, CH=CH); 7.2–7.4 (2H, m, H-4,5 Ph); 7.62 (1H, dd, *J* = 7.7 and *J* = 1.3, H-3 Ph); 7.77 (1H, dd, *J* = 7.8 and *J* = 1.5, H-6 Ph)
8	1.37 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 6.1 (1H, br. s, NH); 7.11 and 7.42 (2H, two d, *J* = 16.0, CH=CH); 7.44 and 7.55 (4H, A_2B_2 system, *J* = 8.8, H-3,5 Ph and H-2,6 Ph)
9	1.40 (6H, s, 6-CH_3); 2.78 (2H, s, H-5); 6.0 (1H, br. s, NH); 7.30 and 7.43 (2H, two d, *J* = 15.8, CH=CH); 7.09 (1H, dt, *J* = 8.0 and *J* = 1.6, H-4 Ph); 7.41 (1H, dt, *J* = 7.8 and *J* = 1.4, H-5 Ph); 7.72 (1H, dd, *J* = 7.8 and *J* = 1.6, H-3 Ph); 7.91 (1H, dd, *J* = 8.0 and *J* = 1.4, H-6 Ph)
10	1.24 (6H, s, 6-CH_3); 2.82 (2H, s, H-5); 6.8-7.6 (6H, m, H Ph, CH=CH); 8.1 (1H, br. s, OH); 10.5 (1H, br. s, NH);
11	1.34 (6H, s, 6-CH_3); 2.71 (2H, s, H-5); 6.88 and 7.46 (2H and 2H, A_2B_2 system, *J* = 8.6, H-3,5 Ph and H-2,6 Ph); 6.9 (1H, br. s, OH); 7.14 and 7.25 (2H, two d, *J* = 16.1, CH=CH); 7.4 (1H, br. s, NH)
12	1.37 (6H, s, 6-CH_3); 2.76 (2H, s, H-5); 3.92 (3H, s, OCH_3); 6.3 (1H, br. s, NH); 6.93 (1H, dd, *J* = 8.4 and *J* = 0.8, H-5 Ph); 7.00 (1H, dt, *J* = 7.4 and *J* = 0.8, H-4 Ph); 7.38 (1H, dd, *J* = 7.4 and *J* = 1.7, H-3 Ph); 7.52 and 7.59 (2H, two d, *J* = 16.4, CH=CH); 7.66 (1H, dd, *J* = 8.2 and *J* = 1.6, H-6 Ph)
13	1.36 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 3.86 (3H, s, OCH_3); 6.0 (1H, br. s, NH); 6.93 and 7.55 (2H and 2H, A_2B_2 system, *J* = 8.8, H-3,5 Ph and H-2,6 Ph); 7.14 and 7.33 (2H, two d, *J* =16.4, CH=CH)
14	1.39 (6H, s, 6-CH_3); 2,75 (2H, s, H-5); 6.3 (1H, br. s, NH); 6.58 (1H, t, J_{HF} = 73.2, $OCHF_2$); 7.16 (1H, dd, *J* = 7.8 and *J* = 0.9, H-3 Ph); 7.28 (1H, dt, *J* = 7.8 and *J* = 1.2, H-4 Ph); 7.43 (1H, dt, *J* = 7.8 and *J* = 1.7, H-5 Ph); 7.44 and 7.53 (2H, two d, *J* = 16.2, CH=CH); 7.81 (1H, dd, *J* = 7.8 and *J* = 1.7, H-6 Ph)
15	1.25 (6H, s, 6-CH_3); 2.82 (2H, s, H-5); 7.19 and 7.78 (2H, two d, *J* = 16.0, CH=CH); 7.69 (1H, dt, *J* = 8.4 and *J* = 1.4, H-5 Ph); 7.83 (1H, dt, *J* = 7.6 and *J* = 1.2, H-4 Ph); 7.94 (1H, dd, *J* = 8.0 and *J* = 1.4, H-6 Ph); 8.10 (1H, dd, *J* = 9.0 and *J* = 1.2, H-3 Ph); 8.3 (1H, br. s, NH)
16	1.25 (6H, s, 6-CH_3); 2.87 (2H, s, H-5); 7.39 and 7.74 (2H, two d, *J* =16.1, CH=CH); 7.9-8.4 (4H, m, H-4,5,6 Ph and NH); 8.49 (1H, m, H-2 Ph)

TABLE 3 (continued)

1	2
17	1.25 (6H, s, 6-CH_3); 2.87 (2H, s, H-5); 7.40 and 7.69 (2H, two d, *J* = 16.0, CH=CH); 7.95 and 8.29 (2H and 2H, A_2B_2 system, *J* = 8.8, H-3,5 Ph and H-2,6 Ph); 8.27 (1H, s, NH)
18	1.34 (6H, s, 6-CH_3); 2.71 (2H, s, H-5); 3.05 (6H, s, $N(CH_3)_2$); 5.8 (1H, br. s, NH); 6.67 and 7.48 (2H and 2H, A_2B_2 system, *J* = 8.9, H-3,5 Ph and H-2,6 Ph); 7.11 and 7.23 (2H, two d, *J* = 16.0, CH=CH)
19	1.39 (6H, s, 6-CH_3); 2.45 (3H, s, CH_3); 2.75 (2H, s, H-5); 6.3 (1H, br. s, NH); 7.36 and 7.48 (2H, two d, *J* = 15.8, CH=CH); 7.25 (3H, m, H-3,4,5 Ph); 7.68 (1H, m, H-6 Ph)
20	1.40 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 6.4 (1H, br. s, NH); 7.40 and 7.55 (2H, two d, *J* = 16.0, CH=CH); 7.51 and 7.63 (2H, t, *J* = 6.9, H-4,5 Ph); 7.72 (2H, two br. d, H-3,6 Ph)
21	1.25 (6H, s, 6-CH_3); 2.86 (2H, s, H-5); 7.30 and 7.60 (2H, two d, *J* = 16.0, CH=CH); 7.54 (1H, dd, *J* = 8.6 and *J* = 2.0, H-5 Ph); 7.76 (1H, d, *J* = 2.0, H-3 Ph); 7.93 (1H, d, *J* = 8.6, H-6 Ph); 8.3 (1H, br. s, NH)
22	1.40 (6H, s, 6-CH_3); 2.78 (2H, s, H-5); 6.15 (1H, br. s, NH); 7.1-7.4 (3H, m, H-3,4,5 Ph); 7.26 and 7.64 (2H, two d, *J* = 16.4, CH=CH)
23	1.22 (6H, s, 6-CH_3); 2.80 (2H, s, H-5); 6.80 (1H, d, *J* = 8.0, H-5 Ph); 7.00 (1H, dd, *J* = 8.2 and *J* = 1.9, H-6 Ph); 7.00 and 7.40 (2H, two d, *J* = 15.7, CH=CH); 7.13 (1H, d, *J* = 1.9, H-2 Ph); 8.05 (1H, br. s, NH); 9.52 (2H, br. s, 3'- and 4'-OH)
24	1.36 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 3.96 (3H, s, OCH_3); 5.07 (1H, br. s, NH); 5.98 (1H, s, OH); 6.94 (1H, d, *J* = 7.0, H-5 Ph); 7.0–7.2 (2H, m, H-2,6 Ph); 7.13 and 7.28 (2H, two d, *J* = 16.3, CH=CH)
25	1.37 (6H, s, 6-CH_3); 2.73 (2H, s, H-5); 3.93 (6H, s, OCH_3); 6.4 (1H, br. s, NH); 6.89 (1H, d, *J* = 8.4, H-5 Ph); 7.10 (1H, d, *J* = 1.8, H-2 Ph); 7.15 (1H, dd, *J* = 8.5 and *J* = 1.8, H-6 Ph); 7.14 and 7.31 (2H, two d, *J* = 15.8, CH=CH)
26	1.23 (6H, s, 6-CH_3); 2.80 (2H, s, H-5); 6.11 (2H, s, OCH_2O); 7.01 (1H, dd, *J* = 8.2 and *J* = 1.7, H-6 Ph); 7.21 (1H, m, H-5 Ph); 7.30 (1H, br. s, H-2 Ph); 7.10 and 7.48 (2H, two d, *J* = 15.8, CH=CH); 8.11 (1H, s, NH)
27	1.19 (6H, s, 6-CH_3); 2.61 (2H, s, H-5); 5.20 (1H, m, CH); 5.84 (1H, d, OH); 7.3-7.5 (3H, m, Ph); 7.65 (1H, d, *J* = 7.8, H-6 Ph); 8.04 (1H, m, NH)

* ^{1}H NMR spectra taken in $CDCl_3$ (compounds **1-9, 12-14, 18-20, 22, 24, 25**), DMSO-d_6 (compounds **10, 15-17, 21, 23, 26, 27**) or in a 5: 1 mixture of DMSO-d_6 and $CDCl_3$ (compound **11**).

Condensation of lactam **1** with benzaldehydes was carried out in ethanol in the presence of a catalytic amount of NaOH (molar ratio of δ-lactam–aldehyde–NaOH 1:(1-2):(0.0625-0.25), respectively) at a temperature of 25-78ºC (Table 1). In all cases the main reaction product was the crotonic condensation product. According to ^{1}H NMR spectroscopy the exocyclic double bond has a *trans* configuration (Table 2), the spectroscopic signals for both protons forming an AB type spin system with a spin coupling typical of *trans*-related protons (15.7-16.4 Hz).

When carrying out the reaction of δ-lactam **1** with 2-chlorobenzaldehyde at room temperature and low content of catalyst in the reaction mixture (molar ratio of lactam–chlorobenzaldehyde–NaOH 2:1:0.05) the aldol condensation product 4-[2-(2-chlorophenyl)-2-hydroxyethyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile (**27**) is formed in quantitative yield. Increasing the amount of NaOH leads to dehydration of the aldol to give the lactam **4** in 75% yield. Increasing the temperature to 78ºC led to a more than twofold reduction in the product yield (Table 1).

The results of the biological studies (Table 4) show that the cytotoxicity of lactams **2-27** depends markedly on the nature and position of the substituent in the benzene ring and also on the number of them.

The cytotoxicity of the 2-substituted phenyl derivatives towards the HT-1080 and MG 22A tumor cells decreases in the substituent order NO_2 > Cl > F, Br > MeO > OH > CHF_2O > CF_3, Me > Me_2N. The 2-nitro derivative **15** shows the highest cytotoxicity towards both cell lines (LC_{50} 0.8-3 μg/ml). The 2- and 3-chloro derivatives (compounds **4** and **5** respectively) also show high cytotoxicity (LC_{50} 2-4 μg/ml). The compound with a 2-methoxy group **12** was somewhat less active (LC_{50} 6-10 μg/ml). All of these compounds show a much higher cytotoxicity towards tumor cells than the unsubstituted phenyl derivative **2** (LC_{50} 10 μg/ml).

The lowest toxicity (LD_{50} 2252 mg/kg) with the greatest selectivity in cytotoxicity action amongst this group was found in the 2-bromo derivative **7**. The toxicity of the 2-halo-substituted derivatives decreased in the order F > Cl > Br > I (compounds **3, 4, 7** and **9**).

The aldol condensation product **27** shows low cytotoxic activity. Morphological changes in cells are not implicated and the compound can be classified as non toxic.

TABLE 4. Cytotoxicity of Lactams **1-27***

Compound	LC_{50}, μg/ml							LD_{50}, mg/kg
	HT 1080			MG 22A			3T3	
	CV	MTT	NO	CV	MTT	NO	NR	
1	*[2]	*[2]	7	*[2]	*[2]	7	*[2]	>2000
2	10	10	300	10	10	120	100	706
3	4	6	120	5	3	300	31	432
4	3	3	200	2	2	133	316	1233
5	4	4	140	2	2	71	32	454
6	21	12	33	10	10	83	100	774
7	5	5	22	5	2	17	1000	2252
8	*[2]	14	6	*[2]	100	6	1174	2418
9	*[2]	10	15	100	12	17	*[2]	>2000
10	30	18	200	15	9	150	37	483
11	70	21	11	88	50	7	*[2]	>2000
12	10	6	15	7	10	23	477	1496
13	*[2]	*[2]	8	*[2]	100	9	574	1609
14	40	10	450	10	30	15	*[2]	>2000
15	3	1.8	200	1.5	0.8	100	31	476
16	*[2]	100	5	92	73	9	1000	2111
17	*[2]	17	11	103	10	18	206	1070
18	*[2]	71	10	*[2]	>100	10	*[2]	>2000
19	100	90	8	100	100	5	*[2]	>2000
20	100	75	12	100	100	10	5	221
21	20	10	20	4	2	80	1000	2252
22	12	10	23	6	6	18	*[2]	>2000
23	20	10	250	10	20	100	202	1094
24	36	28	39	47	30	36	670	1760
25	20	10	250	10	20	100	202	1013
26	*[2]	100	6	*[2]	*[2]	5	*[2]	>2000
27	86	100	24	100	100	15	496	1585

* LC_{50} is the concentration of lactams **1-27** causing the death of 50% of the cells; CV = crystal violet (action on cell membranes); MTT = 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (effect on the activity of cell mitochondrial enzymes); NR = neutral red; NO = extent of generation of NO determined and calculated as in method [34].

*[2] Cytotoxic effect absent.

In the series of methoxy (compounds **12** and **13**), chloro (compounds **4** and **6**), hydroxy (compounds **10** and **11**), and nitro derivatives (compounds **15** and **17**) the 4-isomers are less toxic than the corresponding 2-isomers. The 4-isomers are also inferior to their 2-isomers in their cytotoxic activity towards tumor cells.

The introduction of a second chlorine atom into position 4 of the 2-chloro derivative (compound **21**) or position 6 (compound **22**) leads to a modest lowering of cytotoxicity towards MG 22A cells but towards HT-1080 cells this lowering is somewhat greater. At the same time, their toxicity towards normal fibroblasts is markedly decreased (316 to 1174 μg/ml).

The introduction of a second substituent (hydroxy or methoxy) into position 3 of the low activity 4-substituted hydroxy (compound **11**) or methoxy derivative (compound **13**) increases their cytotoxicity (compounds **23-25**) but also increases their toxicity towards normal cells.

It was interesting to find that a series of compounds having high (**15, 3-5**) or modest (**2, 10, 23, 25**) cytotoxicity towards tumor cells substantially induced the formation of nitric oxide in them (Table 4).

Hence we have shown a marked effect of the nature of the substituent and of its position in the phenyl ring on the antitumor activity and toxicity of styryl cyanolactams. The greatest selectivity of cytotoxic activity is seen in the 2-bromo derivative **7**.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Mercury-400 (400 MHz) instrument using TMS as internal standard. HPLC Analysis was performed under reversed phase chromatographic conditions and carried out on a Varian ProStar chromatograph consisting of a ProStar 240 gradient pump, ProStar 330 diode array detector, and ProStar 240 autosampler. The Alltech column (4.6×150 mm) was filled with Apollo C18 sorbent and the mobile phase was acetonitrile-0.1% aqueous phosphoric acid in (pH 2.3). The gradient was linear from 40 to 100% over 15 min and then isocratic for 5 min with 100% acetonitrile. The mobile phase flow rate was 1 ml/min. Using HPLC it was shown that the purity of the compounds exceeded 99%.

The starting δ-lactam **1** was prepared using the method we developed [35] through condensation of 4-hydroxy-4-methyl-2-pentanone with cyanoacetic ester in the presence of ammonium acetate.

Condensation of 4,6,6-Trimethyl-2oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile with Benzaldehydes (General Method). A mixture of δ-lactam **1**, the benzaldehyde, and NaOH in ethanol was stirred and held at room temperature or heated to reflux and refluxed for 1-6 h. The precipitated reaction product was filtered, washed on the filter with a small amount of ethanol, and then recrystallized from ethanol.

Cytotoxicity of Compounds 2-27 (Table 3) *in vitro* with respect to HT-1080 monolayer tumor cells (human fibrosarcoma), MG 22A (mouse hepatoma), and normal NIH 3T3 cells (mouse embryonic fibroblasts) was determined in 96 well plates using the dyes CV, MTT, and NR following the method reported in [36]. The median acute toxicity (LD_{50}) was calculated using method [33] from the data obtained for the 3T3 cell culture.

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