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Synthesis, Spectroscopic Characterization and Biological Activity of *N*-1-Sulfonylcytosine Derivatives*

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Large scale preparation of *N*-1-sulfonylcytosine derivatives has been optimized. The best method was the condensation reaction of silylated cytosine (**1**) with *p*-toluenesulfonyl chloride in acetonitrile. Depending on the isolation procedure, 1-(*p*-toluenesulfonyl)cytosine **2** and 1-(*p*-toluenesulfonyl)cytosine hydrochloride **3** were isolated in 80 % and 75 % yields, respectively. The NMR evidence presented shows that **2** appears as a common keto-amino tautomer in DMSO-d_6 solution while its hydrochloride **3** forms exclusively the rare keto-imino tautomer. *N*-1-Sulfonylcytosine derivatives **2** and **3** were investigated for possible cytotoxic activity on human normal fibroblasts (WI38), human pancreatic adenocarcinoma cells (MIAPaCa2), poorly differentiated cells from lymph node metastases of colon carcinoma (SW-620), and human Burkitt lymphoma cells (Raji). MTT-cytotoxicity screens in human tissue culture cell lines showed that both investigated compounds demonstrated antiproliferative activity in different histological types of tumors. In comparison with 5-fluorouracil, some of *N*-1-sulfonylcytosine derivatives showed 10 times stronger activity, with respect IC_{50}. The inhibitory effect of the investigated derivatives on normal human cells was lower compared to their antitumor effects. In addition to antitumor effects, hematological findings following the parenteral administration of substances were also investigated.

Keywords
N-1-sulfonylcytosine derivatives
in vitro antiproliferative effect
antitumor activity
hematological findings

INTRODUCTION

From the aspect of biological activity, *N*-sulfonylpyrimidine derivatives are very interesting compounds because they have a combination of biologically active components: a sulfonylcyclourea fragment and a nucleic acid base. Sulfonylurea and its derivatives have been found to have multifold biological and pharmacological interest. Sulfonylureas are compounds with anticolesterolemic,[1] antihypoglycemic,[2] herbicidal[3] and antitumor[4] activity. Diarylsulfonylureas and diphenylsulfonylureas display very good antitumor features by inhibiting plasma membrane NADH oxidase displayed on the surface of (lung,

* Dedicated to Dr. Edward C. Kirby on the ocassion of his 70th birthday.

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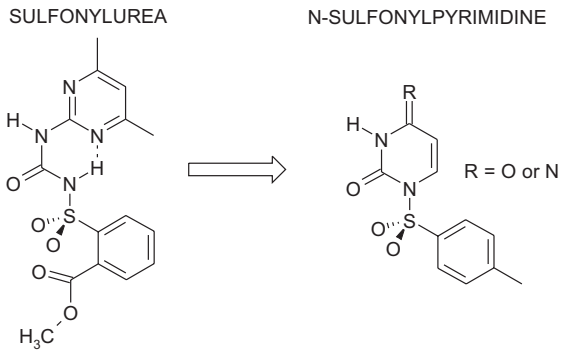


Figure 1. From sulfonylurea derivatives to novel *N*-sulfonylpyrimidine derivatives as a new type of sulfonylcycloureas.

breast, colon, pancreas and kidney) tumor cells but not on the surface of normal cells.[4–8] On the other hand, these compounds can be considered as modified nucleosides with potential biological activity.

In the last few years, we have been involved in the synthesis and biological evaluation of novel pyrimidine derivatives possessing a sulfonamide pharmacophore as potential antitumor agents (Figure 1). We have prepared sulfonylcyclourea derivatives by attaching the sulfonyl fragment onto N-1 of pyrimidine bases.[9–11]

The compounds showed potent growth inhibitory activity against human tumor cell lines *in vitro* at concentrations of 10^{-5}–10^{-8} M.[12] In comparison with 5-FU, some *N*-1-sulfonylpyrimidine derivatives showed 10 times stronger inhibitory effects while the effects on normal human cell lines were much lower. Our additional studies showed that *N*-1-sulfonylpyrimidine derivatives have a strong antiproliferative activity and ability to induce apoptosis in treated tumor cells.[12]

In the present work, we report on the large-scale synthesis and spectroscopic studies of *N*-1-sulfonylcytosine derivatives. The aim of the present study was to investigate the *in vitro* antitumor activity of *N*-1-sulfonylcytosine derivatives. In addition to the *in vitro* antitumor activity, we also investigated the effects of parenteral administration of sulfonylcyclourea derivatives (compared to 5-FU) on the peripheral blood hematological findings. 5-FU was used for comparison with *N*-1-sulfonylcytosine derivatives because it is a standard chemotherapeutic agent with pyrimidine structure.

EXPERIMENTAL

Chemistry General

Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien Kieselgel 60 F254. Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV Spectra [λ_{max}/nm, log ε/dm^3 mol^{-1} cm^{-1}] were taken on a Philips PU8700 UV/VIS spectrophotometer. IR spectra [ν_{max}/cm^{-1}] were obtained for KBr pellets on a Perkin-Elmer 297 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C nucleus. The samples were dissolved in DMSO-d_6 and measured at 20 °C in 5 mm NMR tubes. Sample concentrations were 0.1 mol dm^{-3} for ^{1}H and 0.2 mol dm^{-3} for ^{13}C measurements. Chemical shifts (δ/ppm) were referred to TMS. Digital resolution was 0.3 Hz per point in ^{1}H- and 0.5 Hz per point in ^{13}C-NMR one-dimensional spectra. The applied techniques were standard ^{1}H and ^{13}C with broadband proton decoupling; ^{13}C gated decoupling, COSY, and HETCOR. For proton decoupling, the Waltz-16 modulation was used. COSY spectra were recorded in the magnitude mode with 1024 points in F2 dimension and 256 increments in F1 dimension, zero-filled to 1024 points. Increments were measured with 16 scans, 4500 Hz spectral width and a relaxation delay of 0.8 s. The corresponding digital resolution was 8.9 Hz per point and 17.6 Hz per point in F2 and F1 dimensions, respectively. HETCOR spectra were recorded with 2048 points in F2 dimension and 256 increments in F1 dimension, zero-filled to 512 points. Each increment was recorded by 96 scans with a relaxation delay of 1.0 s. Spectral widths were 19000 Hz in F2 and 4500 Hz in F1 dimensions, and the corresponding digital resolutions were 18.6 Hz per point and 17.6 Hz per point, respectively. All mass spectrometric investigations were performed using a Fourier transform mass spectrometer ThermoQuest FT/MS 2001 DD (Madison, WI, USA) equipped with a 3 T superconducting magnet. Following the electron impact (EI) ion formation at 70 eV, a time delay of 100 μs or 1 s was employed for relaxation of the initial cyclotron and axial motions by collision with background neutrals. The samples were introduced by direct insertion.

1-(p-Toluenesulfonyl)cytosine ***2***

A mixture of cytosine (**1**) (7 g, 63 mmol) and *N,O*-bis(trimethylsilyl)acetamide (BSA) (46.62 mL, 189 mmol) was heated under reflux in dry acetonitrile (140 mL) for 30 minutes. The solution was cooled to 0 °C and *p*-toluenesulfonyl chloride (14.42 g, 75.6 mmol) was added. After heating under reflux for 45 minutes, the solvent was evaporated. Methanol was added to the residue and the resulting solid was filtered off and recrystallized from hot methanol, yielding white crystals of the product **2**: 13.3 g (80 %); m.p. 216 °C; (Ref. 10: m.p. 216 °C).

Additional data for 1-(*p*-toluenesulfonyl)cytosine **2**: The phenyl-protons (Ts) assignments were confirmed by carbon-proton connectivity in the ^{1}H/^{13}C heteronuclear correlation spectra (HETCOR). Additionally, in the NOESY spectrum of **2**, the assignment of Ts-c protons was proved by their interaction with methyl-protons.

^{1}H-NMR (DMSO-d_6) δ/ppm: 8.14 (d, 1H, $J_{6,5}$ = 7.8 Hz, H-6), 7.95 (brs, 2H, NH_2), 7.87 (d, 2H, J = 8.1 Hz, Ts-b), 7.46 (d, 2H, J = 8.1 Hz, Ts-c), 5.98 (d, 1H, $J_{5,6}$ = 7.8 Hz, H-5), 2.42 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ/ppm: 166.27 (s, C-4), 151.22 (s, C-2), 145.61 (s, Ts-d), 139.73 (d,

C-6), 134.47 (s, Ts-a), 129.80 (d, Ts-c), 129.02 (d, Ts-b), 97.50 (d, C-5), 21.20 (q, CH_3); MS (EI) exact mass calcd. for $C_{11}H_{11}N_3O_3S$: *m/e* 265.051565 ($[M]^+$), found 265.040053.

*1-(p-Toluenesulfonyl)cytosine Hydrochloride **3***

Method A: 1-(*p*-Toluenesulfonyl)cytosine **2** (3 g, 11.3 mmol) was dissolved in hot methanol (*ca* 400 mL) and treated with 3 % HCl/CH_3OH (20 mL). After storage at 4 °C, the main part of product **3** (1.91 g, 65 %) was crystallized from solution and filtered off.

Method B: A mixture of cytosine (**1**) (7.75 g, 69.8 mmol) and bis(trimethylsilyl)acetamide (BSA) (52 mL, 209 mmol) was heated under reflux in dry acetonitrile (160 mL) for 30 minutes. The solution was cooled to 0 °C and *p*-toluenesulfonyl chloride (16 g, 84 mmol) was added. After heating under reflux for 45 minutes, the solvent was evaporated. The residue was treated with 3 % HCl/CH_3OH (*ca* 100 mL) and the resulting white crystals of 1-(*p*-toluenesulfonyl)cytosine hydrochloride **3** were filtered off: 15.73 g (75 %); m.p. 175–178 °C; UV (MeOH): λ_{max}/nm 232,0 and 248,0, log ε/ dm^3 mol^{-1} cm^{-1} 3,97 and 4,00; IR (KBr) ν_{max}/cm^{-1}: 3350 (m), 3100 (s), 2700 (m, br), 2600 (vw), 1740 (s), 1668 (m), 1520 (m), 1485 (s), 1390 (w), 1375 (w), 1351 (w), 1300 (vw), 1255 (s), 1235 (vw), 1190 (w), 1184 (w), 1170 (s), 1136 (w), 1111 (w), 1080 (w), 1020 (w), 962 (vw), 865 (vw), 804 (w), 760 (w), 705 (w), 688 (m), 672 (m), 654 (m); ^{1}H-NMR (DMSO-d_6) δ/ppm: 10.40 (s, 1H, NH), 9.85 (brs, 1H, NH), 9.15 (s, 1H, NH), 8.30 (d, 1H, $J_{6,5}$ = 7.8 Hz, H-6), 7.89 (d, 2H, J = 8.3 Hz, Ts-b), 7.48 (d, 2H, J = 8.3 Hz, Ts-c), 6.28 (d, 1H, $J_{5,6}$ = 7.8 Hz, H-5), 2.40 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ/ppm: 160.91 (s, C-4), 146.93 (s, C-2), 145.28 (s, Ts-d), 141.49 (d, C-6), 132.40 (s, Ts-a), 129.82 (d, Ts-c), 129.49 (d, Ts-b), 97.07 (d, C-5), 21.39 (q, CH_3); *Anal*. Calcd. for $C_{11}H_{12}N_3O_3SCl$ (M_r = 301,76): C 43.78, H 4.01, N 13.93 %; found: C 43.81, H 3.93, N 13.85 %; MS (EI) exact mass calcd. for $C_{11}H_{12}N_3O_3SCl$: *m/e* 266,05939 ($[M\text{-Cl}]^+$), found 266,066003.

In vitro *Study:*

1. Cell lines

Human pancreatic adenocarcinoma cells (MIAPaCa2), poorly differentiated cells from lymph node metastases of colon carcinoma (SW-620), human Burkitt lymphoma cells (Raji) and normal human fibroblasts (WI38) were obtained from the Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia.

2. Chemicals

RPMI-1640 medium and fetal bovine serum (FBS) were obtained from Gibco BRL, Life Technologies (Paisley, UK), Dulbecco's Modified Eagle Medium (DMEM) with 10 % heat inactivated FBS and tripsine-EDTA were purchased from the Institute of Immunology Inc. (Zagreb, Croatia); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) and dimethylsulfoxyde (DMSO) were purchased from Merck (Darmstadt, Germany). Glutamine, penicillin, streptomycin, and all others chemicals were obtained from Sigma Chem. Co. (St. Louis, USA).

3. Cell Culture

Cells (MIAPaCa2, WI38, SW620) were grown as a monolayer in tissue culture flasks (250 mL) in DMEM supplemented with 10 % FBS, 2 mM glutamine, 100 U of penicillin and 0.1 mg streptomycin in CO_2 incubator (Shell Lab, Sheldon Manufacturing, USA). Leukemia cells (Raji) were grown in RPMI-1640 medium supplemented with 10 % FBS. The cultures were equilibrated with humidified 5 % CO_2 in air at 37 °C. Trypan blue dye exclusion method was used to assess cell viability.

4. Cell Proliferation Assay

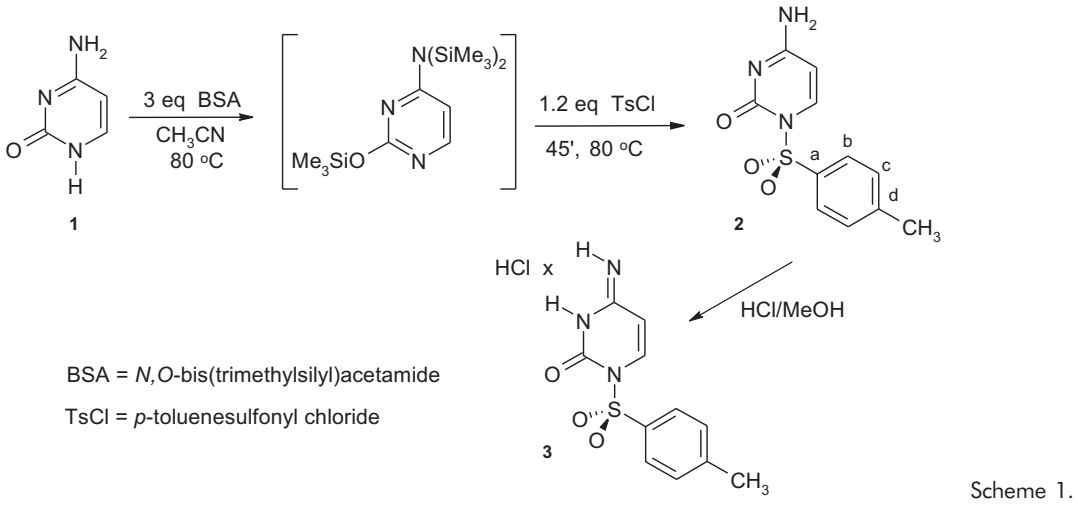
Cell growth was quantified by the MTT assay.[13] Cells (2×10^4 cells per mL) were seeded onto 96-microwell plates and allowed to attach overnight. After 24 hours, cells were treated by addition of various concentration substances and placed into a CO_2 incubator for 72 hours. Controls were grown under the same conditions without addition of the test substances. After 72 hours of incubation, the medium was removed and cells were washed 3 times with 100 μL Hank's balanced salt solution (HBSS) before adding 40 μL of MTT (5 mg/mL). DMSO (160 μL) was added to each well to dissolve water-insoluble MTT-formazane crystals. The plates were transferred to an Elisa plate reader (Stat fax 2100, Pharmacia Biotech, Uppsala, Sweden). Absorbance was measured at 570 nm. Experiments were performed in triplicates.

In vivo *Study:*

All experiments were performed on 13–15 week-old Wistar rats weighting 200–250 g, bred at the Zagreb University School of Medicine. Six animals were used in each group per experiment. Food and water were supplied *ad libitum*. Rats were divided into 7 groups. The first group was used as a negative control, and physiological saline was administered intraperitoneally. Groups 2 and 3 were treated with 7 mg/kg and 30 mg/kg of 1-(*p*-toluenesulfonyl)cytosine **2** (TsC), and groups 4 and 5 with 7 mg/kg and 30 mg/kg of 1-(*p*-toluenesulfonyl)cytosine hydrochloride **3** (TsC×HCl). Groups 6 and 7 were used as positive controls and were treated with 7 mg/kg and 30 mg/kg of 5-FU, respectively.

Blood samples were taken on days 15 and 30 after the treatment. The samples were collected and kept in tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. We analyzed peripheral blood values of red blood cells (RBC), white blood cells (WBC), thrombocytes, packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using the Serono Baker System 9120 (Allentown, Pennsylvania). Blood smears stained according to Romanovski were used to measure the differential WBC count. The investigation protocol complied with the European Community guidelines for the use of experimental animals.

Statistical analysis and data plotting were performed with the STATISTICA™ software (version 5.0). Differences between the effects of different drug doses were tested by means of ANOVA and Duncan's test.



Scheme 1.

RESULTS

Silylation of cytosine (**1**) was accomplished with *N,O*-bis(trimethylsilyl)acetamide (BSA) in acetonitrile at 80 °C. Condensation of silylated cytosine with *p*-toluenesulfonyl chloride in acetonitrile gave 1-(*p*-toluenesulfonyl)-cytosine **2** (TsC) in 80 % yield after recrystallization from hot methanol (Scheme 1). If after heating under reflux for 45 minutes and evaporation of the solvent the residue was treated with 3 % HCl/CH_3OH, the resulting white crystals of 1-(*p*-toluenesulfonyl)cytosine hydrochloride **3** (TsC×HCl) were obtained in 75 % yield. Direct treatment of a methanolic solution of TsC **2** with 3 % HCl/CH_3OH also gave product TsC×HCl **3** but in lower yield (65 %).

The *in vitro* cytotoxic effects of *N*-1-sulfonylcytosine derivatives **2** and **3** on the growth of human fibroblasts (WI38) and human tumor cells are summarized in Table I. 5-Fluoruracil was used as the reference compound. As shown in Table I, the investigated compounds inhibited the growth of different tumor cell lines with varying IC_{50} values (1×10^{-2} – 8×10^{-6} M). SW620 cells were the most sensitive to substances **2** and **3**. In comparison with 5-fluorouracil, *N*-1-sulfonylcytosine derivatives **2** and **3** showed 10 times stronger activity. Cytotoxicity of the tested compounds **2** and **3** on human fibroblasts was remarkably lower than their cytotoxicity on tumor cells.

TABLE I. Comparison of *in vitro* cytotoxic activity of 1-(*p*-toluenesulfonyl)cytosine (TsC), 1-(*p*-toluenesulfonyl)cytosine hydrochloride (TsC×HCl) and 5-FU on human normal fibroblasts (WI38) and different human tumor cell lines (SW620, MIAPaCa2, Raji)

	IC_{50} / mol dm^{-3}			
Compound	WI38	SW620	MIAPaCa2	Raji
TsC	3×10^{-2}	8×10^{-6}	7×10^{-4}	5×10^{-4}
TsC×HCl	1×10^{-2}	5×10^{-5}	5×10^{-4}	8×10^{-4}
5-FU	1×10^{-3}	3×10^{-5}	1×10^{-5}	1×10^{-4}

IC_{50} = Drug concentration that inhibited cell growth by 50 %. Exponentially growing cells were treated with substances for 72 hours. Cytotoxicity was analyzed with the MTT survival assay. Experiments were performed at least 3 times.

Three-day parenteral administration of TsC, TsC×HCl and 5-FU did not cause statistically significant changes in the peripheral blood thrombocytes, red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Laboratory findings were measured 15 and 30 days following the administration of substances. Significant deviations from the untreated control values were detected in the treated groups for the parameters of white blood cell count (WBC) and differential WBC (Table II). WBC values were significantly elevated on day 15 in the groups treated with 7 mg/kg and 30 mg/kg of TsC×HCl (Table III, Figure 2a). Segmented leukocytes were also significantly elevated on day 15 in the group treated with 7 mg/kg of TsC×HCl (Table IV). On day 30, we observed significantly elevated values of the WBC count in groups treated with 7 mg/kg and 30 mg/kg of 5-FU (Table V, Figure 2b).

TABLE II. ANOVA results of the white blood cell parameters in controls and different treatment groups

VARIABLE	SS Effect	MS Effect	SS Error	MS Error	F	*p*
WBC (15 days)	183.89*	30.65*	350.75*	10.96*	2.80*	0.026601*
SEGMENTED WBC (15 days)	2074.69*	345.78*	1797.70*	52.87*	6.54*	0.000117*
WBC (30 days)	263.57*	43.93*	123.81*	4.13*	10.64*	0.000002*

*$p < 0.05$

TABLE III. Duncan's multiple comparison test of the white blood cell count in controls and different treatment groups on day 15

GROUP	{1}M=9.0	{2}M=12.3	{3}M=11.7	{4}M=15.7	{5}M=15.1	{6}M=11.7	{7}M=11.1
{1}CONTROL		0.150955	0.221402	0.005292*	0.010539*	0.234349	0.322104
{2}TsC_7	0.150955		0.763133	0.118160	0.180324	0.754248	0.565991
{3}TsC_{30}	0.221402	0.763133		0.078768	0.130809	0.993515	0.754248
{4}TsC×HCl_7	0.005292*	0.118160	0.078768		0.746746	0.073601	0.045986*
{5}TsC×HCl_{30}	0.010539*	0.180324	0.130809	0.746746		0.120292	0.080232
{6}5-FU_7	0.234349	0.754248	0.993515	0.073601	0.120292		0.763133
{7}5-FU_{30}	0.322104	0.565991	0.754248	0.045986*	0.080232	0.763133	

*$p < 0.05$; M = mean value (× 10^9/L)

TABLE IV. Duncan's multiple comparison test of the segmented leukocyte count in controls and different treatment groups on day 15

GROUP	{1}M=13.3	{2}M=8.50	{3}M=10.8	{4}M=31.5	{5}M=14.6	{6}M=12.3	{7}M=13.0
{1}CONTROL		0.320607	0.598650	0.000266*	0.768019	0.827235	0.938148
{2}TsC_7	0.320607		0.587376	0.000037*	0.217936	0.403243	0.343931
{3}TsC_{30}	0.598650	0.587376		0.000093*	0.438563	0.726915	0.636146
{4}TsC×HCl_7	0.000266*	0.000037*	0.000093*		0.000460*	0.000197*	0.000263*
{5}TsC×HCl_{30}	0.768019	0.217936	0.438563	0.000460*		0.633205	0.726789
{6}5-FU_7	0.827235	0.403243	0.726915	0.000197*	0.633205		0.876602
{7}5-FU_{30}	0.938148	0.343931	0.636146	0.000263*	0.726789	0.876602	

*$p < 0.05$; M = mean value (× 10^9/L)

TABLE V. Duncan's multiple comparison test of the white blood cell count in controls and different treatment groups on day 30.

GROUP	{1}M=9.5	{2}M=9.9	{3}M=8.4	{4}M=10.5	{5}M=10.3	{6}M=14.3	{7}M=16.2
{1}CONTROL		0.745144	0.400370	0.472862	0.550123	0.001486*	0.000055*
{2}TsC_7	0.745144		0.274153	0.660423	0.754998	0.002910*	0.000097*
{3}TsC_{30}	0.400370	0.274153		0.149291	0.181703	0.000192*	0.000026*
{4}TsC×HCl_7	0.472862	0.660423	0.149291		0.875971	0.005819*	0.000210*
{5}TsC×HCl_{30}	0.550123	0.754998	0.181703	0.875971		0.005218*	0.000174*
{6}5-FU_7	0.001486*	0.002910*	0.000192*	0.005819*	0.005218*		0.151997
{7}5-FU_{30}	0.000055*	0.000097*	0.000026*	0.000210*	0.000174*	0.151997	

*$p < 0.05$; M = mean value (× 10^9/L)

DISCUSSION

Only a few reports on 1-sulfonyluracil and 1-sufonyl-5--fluorouracil could be found in the literature. Martirosyan *et al.*[14] isolated 1-(*p*-toluenesulfonyl)uracil as an unwanted product in the transformation of C-4 keto group of uracil, and Kaldrikyn *et al.*[15] examined the synthesis of 1-*p*-alkoxybenzenesulfonyl-5-bromouracil derivatives possessing antibacterial activity. According to Tada,[16] benzoyl and arenylsulfonyl-5-fluorouracil derivatives are more active and less toxic than 1-(2-tetrahydrofuryl)-uracil in the Leukemia L/1210 system. However, the synthesis and *in vitro* anticancer activity of 1-sulfonyl-cytosine derivatives was described for the first time in our patent.[11]

We have recently described the synthesis of several novel *N*-1-sulfonyl, and *N*-1,*NH*-4-disulfonylpyrimidine derivatives.[9–11] Two methods were applied to the synthesis of *N*-1-sulfonylpyrimidine derivatives: a) condensation of silylated pyrimidine bases with different sulfonyl chlorides in acetonitrile, and b) reaction of pyrimidine bases with sulfonyl chlorides in pyridine. In comparison, *N*-1 sulfonylation of silylated cytosine **1** in acetonitrile gave only one product, 1-(*p*-toluenesulfonyl)cytosine **2** in 80 % yield, while the reaction of **1** and *p*-toluenesulfonyl chloride in pyridine, besides the mono-sulfonylated product **2** (44 %), also gave the disulfonylated product 4-*N*,1-di(*p*-toluenesulfonyl)cytosine in 24 % yield.[10]

Here we report a large scale synthesis of TsC **2** and its hydrochloride TsC×HCl **3**. The best method was the

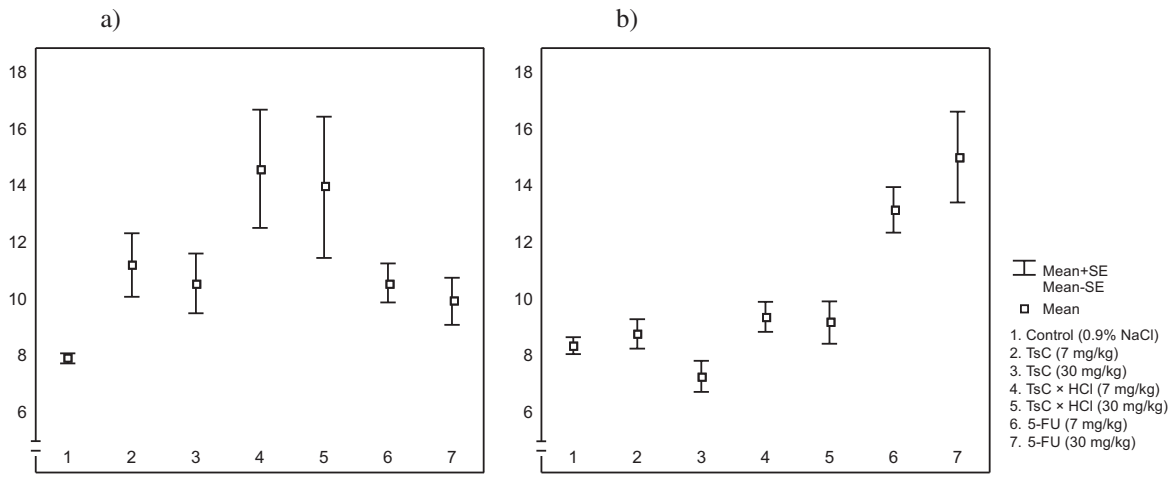


Figure 2. White blood cell count (× 10^9/L) in controls and different treatment groups on days 15 (a) and 30 (b).

condensation reaction of silylated cytosine (**1**) with TsCl in acetonitrile. Depending on the isolation procedure, 1--(*p*-toluenesulfonyl)cytosine **2** and 1-(*p*-toluenesulfonyl)-cytosine hydrochloride **3** were isolated in 80 % and 75 % yields, respectively (Scheme 1).

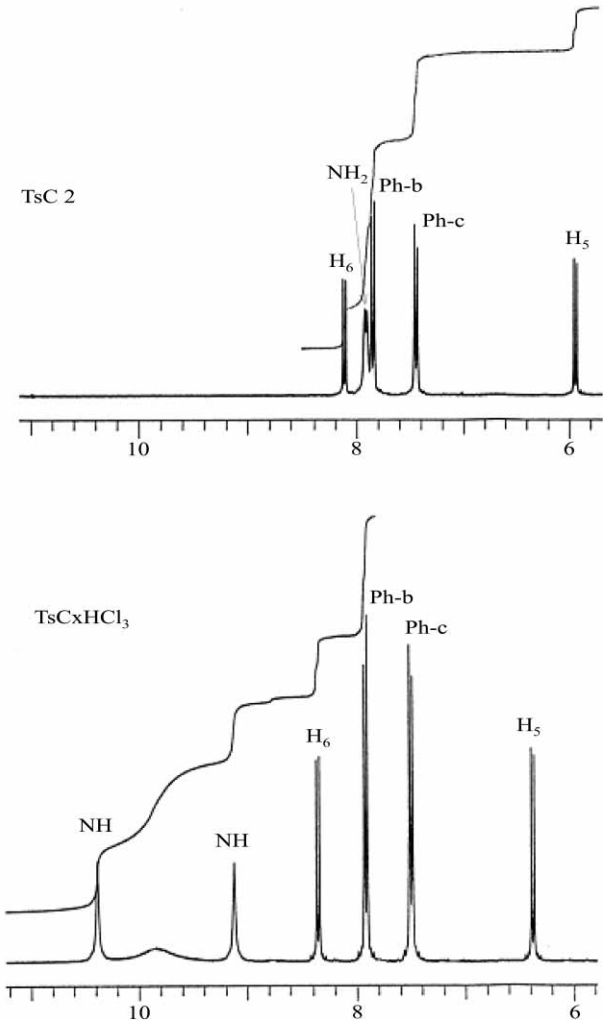


Figure 3. ^{1}H-NMR Spectra (DMSO-d_6) of *N*-1-sulfonylcytosine derivatives **2** (TsC) and **3** (TsC×HCl).

^{1}H-NMR spectra (all measured in DMSO-d_6) of *N*--1-sulfonylated cytosine **2** differ from that of its hydrochloride **3**, with respect to chemical shifts and multiplicity (Figure 3). The most striking difference refers to N-protons. In the spectrum of **2**, two amino protons at N-4, readily exchangeable for deuterium by addition of D_2O, appear as a broadened singlet at δ 7.95 ppm.[10]

This is in accord with the existence of the keto-amino tautomer, known to be the most stable form of unsubstituted cytosine. In the ^{1}H-NMR spectrum of hydrochloride **3**, two slightly broadened one-proton singlets were observed at δ 10.40 and 9.15 ppm (exchangeable with D_2O). The large chemical shift difference between these protons, $\Delta\delta$ of 1.25 ppm, strongly suggests the formation of the keto-imino tautomer. Inspection of the COSY spectrum showed no cross-peaks between two non-equivalent N-protons, completely ruling out the geminal spin-spin interaction. Therefore, the observed signals for N-protons are not due to the chemically non-equivalent geminal N-protons but to protons at two different N-atoms in **3**.

Large scale preparation of *N*-1-sulfonylcytosine derivatives **2** and **3** has been optimized. ^{1}H-NMR spectrum of hydrochloride **3** taken in DMSO-d_6 revealed that in contrast to 1-(*p*-toluensulfonyl)cytosine **2**, which in the same solvent forms the keto-amino tautomer, exclusively forms the keto-imino tautomer.

It is well known that 5-fluorouracil (5-FU) has antitumor activity, but the therapeutic index of 5-FU is low.[17] Consequently, there is much interest in finding molecules that cause less cytotoxicity. Novel compounds, *N*-1-sulfonylcytosine derivatives **2** and **3**, demonstrated antiproliferative activity up to 10 times stronger compared to 5-fluorouracil, with respect to IC_{50}. At the same time, we found them to be cell-specific because their cytotoxic effects on normal fibroblasts were weak. The given

results show that both of the investigated substances represent a promising class of compounds that could be of interest in cancer chemotherapy.

In vivo effects of TsC, TsC×HCl, and 5-FU show that on days 15 and 30, following 3 dose treatments with either 7 mg/kg or 30 mg/kg, the substances caused no significant influence on the peripheral blood thrombocyte, red blood cell and hemoglobin values. Significant deviations from the untreated control values were detected for the peripheral white blood cell count of the animals treated with 7 mg/kg and 30 mg/kg of TsC×HCl (on day 15) and 5-FU (on day 30 of the trial). The data indicate that different dynamic patterns of influence on the peripheral white blood cells exist between TsC×HCl and 5--FU. Tables III–V and Figure 2 show that TsC×HCl could be less toxic for the hematopoietic system than 5-FU, *e.g.,* due to faster recovery of white blood cells. Further investigations are needed to evaluate suppressive effects of the *N*-1-sulfonylcytosine derivatives on the blood white cell proliferation and/or maturation.

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SAŽETAK

Sinteza, spektroskopska karakterizacija i biološka aktivnost *N*-1-sulfonilcitozinskih derivata

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Optimizirana je priprava većih količina *N*-1-sulfonilcitozinskih derivata, kondenzacijom silililiranoga citozina i *p*-toluensulfonil klorida u acetonitrilu. Ovisno o načinu izolacije dobiveni su 1-(*p*-toluensulfonil)citozin **2** (80 %) i 1-(*p*-toluensulfonil)citozin hidroklorid **3** (75 %). NMR eksperimenti pokazuju isključivo nastajanje keto-imino tautomera **3** u DMSO-d_6 otopini, dok se **2** pojavljuje u uobičajenome keto-amino obliku. Ispitivani su potencijalni citotoksični učinci *N*-1-sulfonilcitozinskih derivata **2** i **3** na fibroblastima čovjeka (WI38), stanicama adenokarcinoma gušterače (MIAPaCa2), slabo diferenciranim metastazama adenokarcinoma debelog crijeva (SW-620) i stanicama Burkitt-ovog limfoma (Raji). Rezultati dobiveni MTT-testom pokazuju da ispitivani spojevi djeluju antiproliferativno na različite histološke tipove tumora. U usporedbi s 5-fluorouracilom, *N*-1-sulfonilcitozinski derivati pokazuju 10 puta jaču inhibiciju rasta izloženih tumorskih stanica. Inhibicijski učinci ispitivanih spojeva na normalne stanice značajno su slabiji u odnosu na protutumorske učinke. Osim antitumorskoga učinka, ispitivani su i hematološki parametri poslije parenteralne primjene ispitivanih tvari.