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An Efficient Synthesis and *In vitro* Cytostatic Activity of 5-Aminosulfonyl Uracil Derivatives

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THIS PAPER IS DEDICATED TO PROF. KATA MLINARIĆ-MAJERSKI ON THE OCCASION OF HER 70TH BIRTHDAY

Abstract: Efficient synthesis of 5-aminosulfonyl uracil derivatives **2**–**9** and results of their antiproliferative activity are provided. Sulfonylation of the amino group in 5-aminouracil **1** with selected arylsulfonyl chlorides occurs regioselectively when the reaction is carried out in pyridine at room temperature. Simple isolation of the products by recrystallization of the crude product mixture from aqueous methanol provides good to excellent yields. The prepared 5-aminosulfonyl uracil derivatives **2**–**9** were tested for the antiproliferative activity on a panel of seven tumor cell lines of different histological origin (HeLa, Caco-2, NCI-H358, Raji, HuT78, Jurkat, K562) and normal MDCK I cells. Derivatives **2**–**9** were found more efficient to lymphoma and leukemia cells compared to solid tumor and normal cells.

Keywords: 5-aminouracil, arylsulfonyl chlorides, 5-aminosulfonyl uracil derivatives, *in vitro*, anticancer activity.

INTRODUCTION

THE pyrimidine derivatives are known as essential constituents of nucleic acids and exhibit a variety of biological activities[1] notable among which are the anti-bacterial,[2–4] anticancer,[5–8] anti-inflammatory,[9,10] anti-tubercular,[11] analgesic,[12] and antiviral[13–15] activities. The pyrimidine base with modification at C5 position provides the enhanced biological activity of the nucleobase and nucleoside analogs.[16–18]

5-Fluorouracil[19,20] and the nucleoside floxuridine[21] are frequently used for the treatment of various cancers. Uramustine[22] is used orally in the treatment of leukemia and thymine derivative HEPT is considered as a non-nucleoside reverse transcriptase inhibitor in HIV-infection therapy (Figure 1).[23] 5-Bromouracil and 5-aminouracil could be incorporated into nucleic acids and could interfere with their transcription or translation processes. They also represent good starting material for the synthesis of the fluorescent uracil derivatives suitable for incorporation into PNA's or nucleic acids.[24–29]

Our group has a long-term experience in design, synthesis, and characterization of biologically active nucleoside derivatives.[30–33] Previously, we have synthesized *N*-sulfonylpyrimidine derivatives **I** (Figure 2) as a new type of sulfonylcycloureas.[34–37] These compounds showed strong antiproliferative activity on human tumor cell lines, in *in vitro*[38–41] and *in vivo*[42–45] conditions.

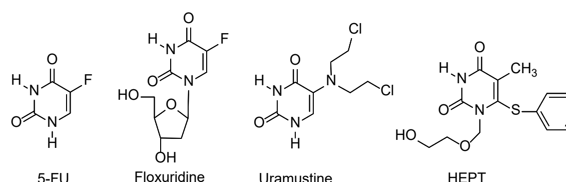

Figure 1. Structures of some biologically active C-5 substituted pyrimidine derivatives.

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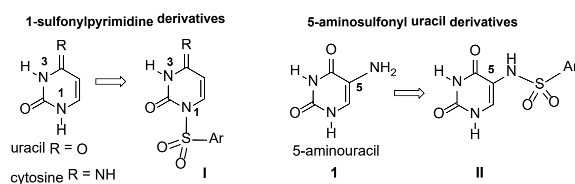


Figure 2. Structures of 1-sulfonylpyrimidines I and 5-aminosulfonyl uracil derivatives II.

The promising anticancer activity of 1-sulfonylpyrimidines **I** inspired the design and synthesis of novel series of 5-aminosulfonyl uracil derivatives of general structure **II** (Figure 2; compounds **2**–**9**, Scheme 1). Sulfonylation of 5-aminouracil **I** appears particularly interesting due to its enhanced hydrogen bonding ability by 5-amino hydrogens which enables the formation of quadruplex and triplex structures.[46,47] By 5-amino sulfonylation of **I** its hydrogen bonding potential is retained and could be even enhanced due to increased acidity of sufonylamide hydrogen.

For sulfonylation of **1**, the aromatic sulfonyl chlorides with different size and lipophilicity of aromatic groups with electron-donating or electron-withdrawing substituents on the aromatic system were selected. In this way, the molecules with variable electronic and lipophilic characteristics could be obtained, which may influence their chemical stability as well as physical and biological properties. Here we report the synthesis of 5-aminosulfonyl uracil derivatives **2**–**9** as well as their antiproliferative activity on normal MDCK I cells, and HeLa, Caco-2, NCI-H358, Raji, HuT78, Jurkat, and K562 tumor cell lines.

EXPERIMENTAL

General

Chemicals and solvents were obtained from Sigma–Aldrich Chemical Company (St. Louis, USA) and used without further purification. R_f values refer to analytical TLC performed using pre-coated silica gel 60 F254 plates purchased from Sigma–Aldrich (Steinheim, Germany) and developed in the solvent system indicated. Compounds were visualized by use of UV light (254 and 365 nm). Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV spectra were taken on a Philips PU8700 UV/VIS spectrophotometer (Philips Analytical, Cambridge, Great Britain). IR spectra were obtained in KBr pellets on a Perkin-Elmer 297 spectrophotometer (Perkin-Elmer, Waltham, MA, USA). NMR spectra were recorded on AV600 and AV300 MHz spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany), operating at 150.92 or 75.47 MHz for ^{13}C and 600.13 or 300.13 MHz for ^{1}H nuclei using DMSO-d_6 as the internal standard. Elemental analyses were performed by the Applied Laboratory Research Department at INA, d.d. Research and Development Sector, Central Analytical Laboratory.

General Procedure for the Condensation of 5-aminouracil 1 with sulfonyl chlorides

A suspension of 5-aminouracil **1** (1 mmol) in dry pyridine (7.5 mL) was cooled to 0 °C and the appropriate sulfonyl chloride (1 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the reaction was completed (1.5–20 h). The solvent was removed under reduced pressure and the crude product was recrystallized from methanol or aqueous methanol to afford the product.

N-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-methylbenzenesulfonamide (2)

Compound **2** was made in a different way than described in Ref. [48]. According to the general procedure, 5-aminouracil **1** (0.5 g, 3.93 mmol) and tosyl chloride (0.749 g, 3.93 mmol) in pyridine (29 mL) were reacted for 19 h. Recrystallization from aqueous methanol gave the analytically pure product **2** as white crystals: 1.08 g (98 %; lit.[48] 49 %); R_f = 0.55 (CH_2Cl_2/MeOH 9:1); m.p. >300 °C; UV (MeOH): λ_{max}/nm: 218 and 272; log ε/ dm^3 mol^{-1} cm^{-1}: 4.10 and 3.08; IR (KBr) ν_{max}/cm^{-1}: 3330 (s), 3125 (m), 3042 (m), 2921 (w), 1689 (s), 1647 (s), 1414 (s), 1358 (m), 1222 (s), 1169 (s), 1092 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.18 (s, 1H, NH-3), 10.88 (brs, 1H, NH-1), 9.24 (brs, 1H, NH-SO_2), 7.62 (d, J = 8.2 Hz, 2H, Ar), 7.32 (d, J = 8.2 Hz, 2H, Ar), 7.30 (brs, 1H, H-6), 2.35 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.0 (C-4), 150.3 (C-2), 142.9 (C_q, Ar), 138.6 (C-6), 137.5 (C_q, Ar), 129.3 (CH, Ar), 126.9 (CH, Ar), 110.2 (C-5), 21.0 (CH_3); (see Supporting Information Figures S1, S2). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{11}H_{11}N_3O_4S$ (M_r = 281.29) are: C, 46.97; H, 3.94; N, 14.94; S, 11.40; found: C, 47.02; H, 3.98; N, 15.00; S, 11.47.

N-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2,4,6-triisopropylbenzenesulfonamide (3)

According to the general procedure, 5-aminouracil **1** (0.5 g, 3.93 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (1.23 g, 3.93 mmol, 97 %) in pyridine (29 mL) were reacted for 12 h. Recrystallization from methanol gave the analytically pure product **3** as white crystals: 1.31 g (85 %); R_f = 0.32 (CH_2Cl_2/MeOH, 20:1); m.p. >300 °C (decom.); UV (MeOH): λ_{max}/nm: 206, 208, and 278; log ε/ dm^3 mol^{-1} cm^{-1}: 4.61, 4.61, and 3.89; IR (KBr) ν_{max}/cm^{-1}: 3318 (m), 3133 (m), 2958 (m), 1721 (s), 1654 (s), 1490 (w), 1425 (m), 1248 (m), 1144 (s), 902 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.17 (s, 1H, NH-3), 10.93 (d, 1H, J = 4.8 Hz, NH-1), 9.08 (s, 1H, NH-SO_2), 7.29 (d, 1H, J = 6.1 Hz, H-6), 7.16 (s, 2H, Ar), 4.00–3.87 (m,

2H, CH-Ar), 2.95–2.82 (m, 1H, CH-Ar), 1.20–1.14 (m, 18H, CH-CH$_3$); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.3 (C-4), 151.9 (C$_q$, Ar), 150.3 (C-2), 149.8 (C$_q$, Ar), 139.7 (C-6), 134.0 (C$_q$, Ar), 123.3 (CH, Ar), 109.2 (C-5), 33.1 (CH-CH$_3$), 29.5 (CH-CH$_3$), 24.5 (CH-CH$_3$), 23.3 (CH-CH$_3$); (see Supporting Information Figures S3, S4). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{19}H_{27}N_3O_4S$ (M_r = 393.50) are: C, 57.99; H, 6.92; N, 10.68; S, 8.15; found: C, 58.03; H, 6.98; N, 10.70; S, 8.17.

4-Acetamido-*N*-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-benzenesulfonamide (4)

Compound **4** was prepared by modification of the described procedures.[49,50] According to the general procedure, 5-aminouracil **1** (0.5 g, 3.93 mmol) and 4-acetamidobenzenesulfonyl chloride (0.937 g, 3.93 mmol, 98 %) in pyridine (29 mL) were reacted for 20 h. Recrystallization from methanol gave the analytically pure product **4** as light brown crystals: 0.828 g (65 %): R_f = 0.22 (CH_2Cl_2/MeOH, 20:1); m.p. >290 °C (decom.), (lit.[50] m.p. >285–287 °C); UV (MeOH): λ_{max}/nm: 206 and 263; log ε/ dm^3 mol^{-1} cm^{-1}: 4.13 and 4.08; IR (KBr) ν_{max}/cm^{-1}: 3446 (w), 3321 (m), 3277 (m), 3035 (m), 2842 (m), 1735 (s), 1667 (s), 1586 (s), 1534 (s), 1494 (m), 1396 (m), 1313 (m), 1152 (s), 1011 (w), 846 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.17 (s, 1H, NH-3), 10.88 (brd, 1H, J = 5.0 Hz, NH-1), 10.28 (s, 1H, NH-C=O), 9.16 (s, 1H, NH-SO$_2$), 7.70 (d, J = 8.9 Hz, 2H, Ar), 7.65 (d, J = 9.0 Hz, 2H, Ar), 7.29 (d, 1H, J = 6.1 Hz, H-6), 2.07 (s, 3H, CH$_3$); ^{13}C NMR (DMSO-d_6) δ/ppm: 168.9 (O=C-CH$_3$) 161.0 (C-4), 150.3 (C-2), 143.0 (C$_q$, Ar), 138.5 (C-6), 133.8 (C$_q$, Ar), 128.0 (CH, Ar), 118.2 (CH, Ar), 110.1 (C-5), 24.1 (q, CH$_3$); (see Supporting Information Figures S5, S6). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{12}H_{12}N_4O_5S$ (M_r = 324.31)) are: C, 44.44; H, 3.73; N, 17.28; S, 9.89; found: C, 44.49; H, 3.74; N, 17.30; S, 9.91.

***N*-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-nitro-benzenesulfonamide (5)**

Compound **5** was prepared by modification of the described procedure.[50] According to the general procedure, 5-aminouracil **1** (0.5 g, 3.93 mmol) and 4-nitrobenzenesulfonyl chloride (0.898 g, 3.93 mmol, 97 %) in pyridine (29 mL) were reacted for 19 h. Recrystallization from aqueous methanol gave the analytically pure product **5** as a light brown solid: 0.761 g (62 %); R_f = 0.52 (CH_2Cl_2/MeOH, 20:1); m.p. >300 °C (decom.), (lit.[50] m.p. >300 °C); UV (MeOH): λ_{max}/nm: 202, 204, and 266 log ε/ dm^3 mol^{-1} cm^{-1}: 4.03, 4.06, and 3.99; IR (KBr) ν_{max}/cm^{-1}: 3338 (s), 3113 (s), 3038 (m), 2923 (m), 1689 (s), 1648 (s), 1541 (s), 1416 (s), 1352 (s), 1313 (m), 1221 (s), 1174 (s), 1090 (m), 925 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.21 (s, 1H, NH-3), 11.03 (brs, 1H, NH-1), 9.77 (brs, 1H, NH-SO$_2$), 8.36 (d, 2H, J = 8.8 Hz, Ar), 8.01 (d, 2H, J = 8.8 Hz, Ar), 7.48 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.1 (C-4), 150.4 (C-2), 149.6 (C$_q$, Ar), 146.1 (C$_q$, Ar), 140.5 (C-6), 128.5 (CH, Ar), 124.2 (CH, Ar), 109.2 (C-5); (see Supporting Information Figures S7, S8). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{10}H_8N_4O_6S$ (M_r = 312.26) are: C, 38.46; H, 2.58; N, 17.94; S, 10.27; found: C, 38.51; H, 2.59; N, 17.96; S, 10.30.

***N*-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-nitro-benzenesulfonamide (6)**

According to the general procedure, 5-aminouracil **1** (0.5 g, 3.93 mmol) and 2-nitrobenzenesulfonyl chloride (0.871 g, 3.93 mmol, 97 %) in pyridine (29 mL) were reacted for 20 h. Recrystallization from methanol gave the analytically pure product **6** as red crystals: 0.726 g (59 %); R_f = 0.22 (CH_2Cl_2/MeOH, 20:1); m.p. 280 °C (decom.); UV (MeOH): λ_{max}/nm: 268 and 326; log ε/ dm^3 mol^{-1} cm^{-1}: 3.99 and 2.92; IR (KBr) ν_{max}/cm^{-1}: 3313 (m), 3108 (m), 3027 (m), 2830 (m), 1715 (s), 1679 (s), 1542 (s), 1421 (s), 1385 (s), 1325 (m), 1168 (s), 910 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.23 (s, 1H, NH-3), 11.02 (brd, 1H, J = 5.4 Hz, NH-1), 9.70 (brs, 1H, NH-SO$_2$), 8.08–7.80 (m, 4H, Ph), 7.41 (d, 1H, J = 6.3 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.4 (C-4), 150.5 (C-2), 147.6 (C$_q$, Ar), 140.8 (C-6), 134.3 (CH, Ar), 132.6 (C$_q$, Ar), 132.2 (CH, Ar), 130.4 (CH, Ar), 123.8 (CH, Ar), 109.2 (C-5); (see Supporting Information Figures S9, S10). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{10}H_8N_4O_6S$ (M_r = 312.26) are: C, 38.46; H, 2.58; N, 17.94; S, 10.27; found: C, 38.49; H, 2.61; N, 17.99; S, 10.28.

4-Cyano-*N*-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-benzenesulfonamide (7)

According to the general procedure, 5-aminouracil **1** (0.250 g, 1.97 mmol) and 4-cyanobenzenesulfonyl chloride (0.410 g, 1.97 mmol, 97 %) in pyridine (15 mL) were reacted for 1.5 h. Recrystallization from methanol gave the analytically pure product **7** as orange crystals: 0.403 g (70 %); R_f = 0.44 (CH_2Cl_2/MeOH, 20:1); m.p. > 300 °C decom.; UV (MeOH): λ_{max}/nm: 202, 231, and 271; log ε/ dm^3 mol^{-1} cm^{-1}: 4.03, 3.95, and 3.76; IR (KBr) ν_{max}/cm^{-1}: 3341 (s), 3112 (s), 2815 (m), 2236 (w), 1782 (w), 1690 (s), 1517 (w), 1416 (s), 1362 (s), 1221 (s), 1172 (s), 1089 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.19 (s, 1H, NH-3), 10.99 (d, 1H, J = 4.8 Hz, NH-1), 9.66 (s, 1H, NH-SO$_2$), 8.02 (d, 2H, J = 8.4 Hz, Ar), 7.92 (d, 2H, J = 8.4 Hz, Ar), 7.43 (d, 1H, J = 6.2 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.1 (C-4), 150.4 (C-2), 144.6 (C$_q$, Ar), 140.4 (C-6), 133.0 (CH, Ar), 127.7 (CH, Ar), 117.8 (C$_q$, Ar), 114.9 (C≡N), 109.2 (C-5); (see Supporting Information Figures S11, S12). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{11}H_8N_4O_4S$ (M_r = 292.27) are: C, 45.20; H, 2.76; N, 19.17; S, 10.97; found C, 45.25; H, 2.80; N, 19.19; S, 11.01.

***N*-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)naphthalene-1-sulfonamide (8)**

According to the general procedure, 5-aminouracil **1** (0.5 g, 3.93 mmol) and 1-naphtalenesulfonyl chloride (0.918 g, 3.93 mmol, 97 %) in pyridine (29 mL) were reacted for 6 h.

Recrystallization from methanol gave the analytically pure product **8** as yellow crystals: 0.723 g (58 %); R_f = 0.75 (CH_2Cl_2/MeOH, 20:1); m.p. 294–296 °C; UV (MeOH): λ_{max}/nm: 203, 220, and 279 log ε/ dm^3 mol^{-1} cm^{-1}, 4.81, 4.86, and 4.28; IR (KBr) ν_{max}/cm^{-1}: 3182 (m), 2923 (m), 1711 (s), 1663 (s), 1430 (m), 1354 (m), 1226 (m), 1164 (s), 1134 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.09 (s, 1H, NH-3), 10.86 (d, 1H, J = 5 Hz, NH-1), 9.61 (s, 1H, NH-SO_2), 8.69 (d, 1H, J = 9.5 Hz, Ar), 8.21–8.04 (m, 3H, Ar), 7.71–7.56 (m, 3H, Ar), 7.23 (d, 1H, J = 6.1 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.1 (C-4), 150.3 (C-2), 138.7 (C-6), 135.7 (C_q, Ar), 134.1 (CH, Ar), 133.8 (C_q, Ar), 128.8 (CH, Ar), 127.9 (C_q, Ar), 127.6 (CH, Ar), 126.7 (CH, Ar), 125.1 (CH, Ar), 124.3 (CH, Ar), 110.0 (C-5); (see Supporting Information Figures S13, S14). Anal. Calcd for $C_{14}H_{11}N_3O_4S$ (M_r = 317.32) are: C, 52.99; H, 3.49; N, 13.24; S, 10.10; found: C, 53.04; H, 3.51; N, 13.28; S, 10.12.

(*E*)-4-((4-(Dimethylamino)phenyl)diazenyl)-*N*-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)benzenesulfonamide (9)
According to the general procedure, 5-aminouracil **1** (0.098 g, 0.77 mmol) and 4-*N,N*-dimethylaminoazobenzene-4'-sulfonyl chloride (0.256 g, 0.77 mmol, 97.5 %) in pyridine (6 mL) were reacted for 6 h. Recrystallization from methanol gave the analytically pure product **9** as a brown solid: 0.268 g (84 %); R_f = 0.71 ($CH_3CH_2CH_2OH/NH_3/H_2O$ 7:1:2); m.p. >300 °C; UV (MeOH): λ_{max}/nm: 204, 272, and 427; log ε/ dm^3 mol^{-1} cm^{-1}: 3.65, 3.38, and 3.62; IR (KBr) ν_{max}/cm^{-1}: 3320 (s), 3112 (s), 1691 (s), 1603 (s), 1519 (s), 1411 (s), 1361 (s), 1233 (s), 1145 (s), 1026 (s), 838 (s), 764 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.20 (s, 1H, NH-3), 10.96 (d, 1H, J = 5.9 Hz, NH-1), 9.43 (s, 1H, NH-SO_2), 7.88–7.73 (m, 6H, Ar), 7.38 (d, J = 6.2 Hz, 1H, H-6), 6.86 (d, J = 9.1 Hz, 2H, Ar), 3.08 (d, J = 5.2 Hz, 6H, NCH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 161.1 (C-4), 154.5 (C_q, Ar), 153.2 (C_q, Ar), 150.4 (C-2), 142.6 (C_q, Ar), 140.0 (C_q, Ar), 139.3 (C-6), 128.3 (CH, Ar), 125.6 (CH, Ar), 121.9 (CH, Ar), 111.8 (CH, Ar), 109.9 (C-5), 39.9 (N-CH_3); (see Supporting Information Figures S15, S16). *Anal.* Calcd. mass fractions of elements, w/%, for $C_{18}H_{18}N_6O_4S$ (M_r = 414,44) are: C, 52.17; H, 4.38; N, 20.28; S, 7.74; found: C, 52.21; H, 4.40; N, 20.31; S, 7.76.

Cell Culturing and MTT Test[51]

5-Aminosulfonyl uracil derivatives **1**–**9** and 5-fluorouracil (5-FU) as the positive control, were selected for preliminary *in vitro* cytotoxicity testing against normal Madin-Darby canine kidney (MDCK I) cells, and seven tumor cell lines of different histological origin: cervix adenocarcinoma cells (HeLa), human epithelial colorectal adenocarcinoma cells (Caco-2), human caucasian bronchioalveolar carcinoma cells (NCI-H358), human Burkitt lymphoma cells (Raji), human T cell lymphoma cells (HuT78), human acute T cell leukemia cells (Jurkat, and chronic myelogenous leukemia cells (K562).

The NCI-H358, Raji, HuT78, Jurkat, and K562 cells were grown in RPMI 1640 medium (Gibco, EU) supplemented with 10 % heat-inactivated fetal bovine serume FBS (Gibco, EU), 2 × 10^{-3} mol dm^{-3} glutamine (Gibco, EU), 1 × 10^{-3} mol dm^{-3} sodium pyruvate (Gibco, EU), 1 × 10^{-2} mol dm^{-3} HEPES (Sigma-Aldrich, USA) and 100 U/0.1 mg penicillin/streptomycin. The MDCK I, HeLa and Caco-2 cells were grown in Dulbecco's Modified Eagle Medium DMEM (Gibco, EU), supplemented with 10 % FBS, 2 × 10^{-3} mol dm^{-3} glutamine and 100 U/0.1 mg penicillin/ streptomycin; in tissue culture flasks and grown as mono-layers. To detach them from the flask surface, cells were trypsinized using a 0.25 % trypsin/EDTA solution. Cells were cultured in a humidified atmosphere under the conditions of 37 °C / 5 % of CO_2 gas in a CO_2 incubator (Shell Lab, Sheldon Manufacturing, USA).

Tested compounds were dissolved in dimethyl sulfoxide as a 1 × 10^{-2} mol dm^{-3} stock solution. Working dilutions of derivatives **2**–**8** and 5FU were prepared in high pure water at a concentration range 10^{-4}–10^{-7} mol dm^{-3} and in the case of insoluble compound **9** at a concentration range 10^{-5}–10^{-7} mol dm^{-3}.

For the MTT test, the adherent cells, MDCK I, HeLa, and Caco-2, were seeded in 96 micro-well plates at concentration of 2×10^4 cells/cm^3 and allowed to attach overnight in the CO_2 incubator. After 72 hours of the exposure to tested compounds, medium was replaced with 5 mg/cm^3 MTT solution and the resulting formazane crystals were dissolved in DMSO.

Leukemia cells (1 × 10^5 cells/cm^3) were plated onto 96 micro-well plates and after 72 hours of incubation, 5 mg/cm^3 MTT solution was added to each well and incubated 4 hours in the CO_2 incubator. To each well, 10 % SDS with 0.01 mol dm^{-3} HCl was added to dissolve water-insoluble MTT-formazane crystals. The microplate reader (iMark, BIO RAD, Hercules, CA, USA) was used for measurement of the absorbance at 595 nm.

All experiments were performed three times in triplicates. The percentage of treated tumor cells growth inhibition was calculated relative to the growth of untreated (control) cells.

Selectivity index (SI) was calculated for each compound using formula: SI = IC_{50} for normal cell line MDCK I / IC_{50} for respective tumor cell line. Higher values of SI indicate greater antitumor specificity and the SI > 1.00 identifies compounds with efficacy against tumor cells greater than toxicity against normal cells.[52–54]

RESULTS AND DISCUSSION

Synthesis

Depending on the reaction conditions, alkylation of uracil with alkyl halides may result in *N*-1 or *N*-3, alkylated and *N*-1,*N*-3-dialkylated products. For example, in the reaction

of uracil with a high excess of methyl iodide, in the presence of alkali, 1,3-dimethyl uracil was formed,[55] while the alkylation with a small excess alkyl halide in the presence of a base in DMF yields *N*-1-alkylated products with a small amount of *N*-1,*N*-3-dialkylated compounds.[56–57]

On the other hand, 5-aminouracil **1** provides greater substitution possibilities due to the presence of a basic amino group at the C5 position of the ring. When potassium salt of 5-aminouracil is allowed to react with methyl iodide alkylation takes place in the N1 and N3 positions of the pyrimidine ring with formation of 1,3-dimethyl-5-amino-uracil.[48] Treatment of 5-aminouracil **1** with *p*-toluene-sulfonyl chloride in aq. sodium hydroxide gave 5-amino-tosyl uracil **2** in 49 % yield,[48] while 5-sulfanilamidouracil[49] was synthesized by reacting 5-aminouracil **1** and *N*-acetyl-sulfanilyl chloride in pyridine, followed by deprotection of *N*-acetyl group in aqueous sodium hydroxide (52 % yield). Pecorari *et al.*[50] showed that in the reaction of 4-acetamido and 4-nitrobenzenesulfonyl chloride with 5-aminouracil **1** in aq. sodium hydroxide, mono C5 aminosulfonyl products **4** and **5** are formed in a mixture with different amounts of disubstituted products (N^5,N^5-bis-sulfonyl), depending on pH.

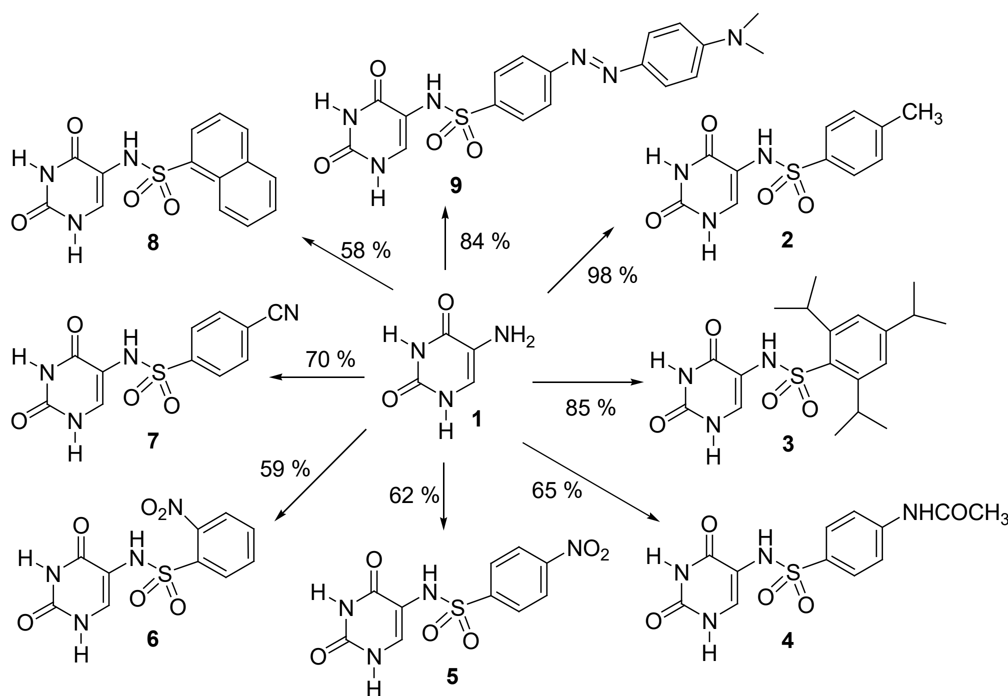
We have previously described two methods for the preparation of *N*-1-sulfonylpyrimidine derivatives of general formula **I** (Figure 2): a) condensation of silylated pyrimidine bases with different sulfonyl chlorides in acetonitrile; b) reaction of pyrimidine bases with sulfonyl chlorides in pyridine.[34,36] The above procedures worked well with aliphatic, aromatic, and heteroaromatic sulfonyl chlorides.

For the synthesis of the 5-aminosulfonyl uracil derivatives of general formula **II** (Figure 2), we used 5-aminouracil **1** and applied the method b) in pyridine for the regioselective introduction of the aromatic sulfonyl groups to the C5 amino group of uracil ring.

The substitution of the amino group in **1** proceeded well when equimolar amounts of 5-aminouracil **1** and *p*-toluenesulfonyl chloride were reacted in pyridine at room temperature. After simple isolation by recrystallization of the crude product mixture from aqueous methanol, 5-aminotosyl uracil **2** was isolated in 98 % yield (Scheme 1).

Employing the latter reaction conditions with commercially available arylsulfonyl chlorides, which have previously been associated with antitumor activity,[58–60] the respective 5-aminosulfonyl uracil derivatives **3**–**9** were obtained in good to excellent yields (Scheme 1).

The structures of the synthesized compounds were confirmed by elemental analysis and by IR and NMR data. The ^{1}H NMR spectra of the products **2**-**9** confirm the respective structures of 5-aminosulfonyl uracil derivatives. Singlets, in the NMR spectra of **2**-**9** appearing in the range of δ 11.09–11.23 ppm, are attributed to the uracil N3 protons. The uracil N1 protons appear as broad singlets (**2** and **5** at 10.88 and 11.03 ppm, respectively) or doublets in the range δ 10.86–11.02 ppm ($J_{1,6}$ 5–6 Hz), while the



Scheme 1. Synthesis of 5-aminosulfonyl uracil derivatives **2**–**9** in pyridine at room temperature (yields correspond to analytically pure products).

singlets of NH protons of C5 sulfonamido groups are shifted upfield and appear in the range δ 9.08–9.77 ppm. The multiplet signals within the δ 6.86–8.69 ppm region are assigned to the aromatic protons and H-6 protons of uracil moiety.

In vitro Antiproliferative Screening

5-Aminosulfonyl uracil derivatives **2**–**9** were tested on *in vitro* cytotoxicity against normal MDCK I cells, solid tumor (HeLa, Caco-2, NCI-H358), lymphoma (Raji, HuT78), and leukemia (Jurkat, K562) cell lines. 5-Fluorouracil (5-FU) was used as the positive control. All cells were treated by investigated compounds in the 10^{-4}–10^{-7} mol dm^{-3} range of concentrations (with exception of compound **9**, which was found insoluble at $c = 10^{-4}$ mol dm^{-3}) using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay method.

Compounds **2**–**9** showed modest to negligible effects on normal (MDCK I) and solid tumor (HeLa, Caco-2, and NCI-H358) cells growth (Supporting Information Figures S18-S20, Table S1). For example, at the highest applied concentration (10^{-4} mol dm^{-3}) 2,4,6-triisopropyl-benzenesulfonamide **3**, 4-nitrobenzenesulfonamide **5**, and 2-nitrobenzenesulfonamide **6** derivatives showed low (10–20 %) growth inhibition activities against MDCK I cell lines (Figure 3; Supporting Information Figure S17). Similarly, at 10^{-4} mol dm^{-3}, compounds **2**–**4** and compound **9** at $c = 10^{-5}$–10^{-6} mol dm^{-3}, caused the reduction (10–20 %) of HeLa cells growth (Supporting Information Figure S18); compounds **2** and **9** inhibited growth of CaCo-2 cells (Supporting Information Figure S19), while compounds **2**–**4**, **7**, and **9** inhibited growth of NCI-H358 cells (Supporting Information Figure S20). The lower concentrations of the tested compounds **2**–**8** ($c = 10^{-5}$–10^{-7} mol dm^{-3}) had no significant influence on the growth of normal and solid tumor cells.

However, 5-aminosulfonyl uracil derivatives **2**–**9** were more efficient to lymphoma and leukemia cells compared to normal and solid tumor (HeLa, Caco-2, NCI-H358) cell lines. Compounds **2**–**8** showed statistically significant influence at the highest concentrations ($c = 10^{-4}$ mol dm^{-3}) on the lymphoma (Raji, HuT78) and leukemia (Jurkat, K562) cells (Supporting Information Figures S21–S24).

The strongest cytotoxic effect was found for 4-acet-amidobenzenesulfonamide **4** (71.4 % of growth inhibition). Also, strong inhibition effects were observed for tosyl-sulfonamide **2** (64.8 %) and naphthalene-1-sulfonamide **8** (56.4 %). Other tested compounds showed only modest cytotoxic activity on Raji cells (less than 50 % of inhibition). At the lower range of concentrations (10^{-5}–10^{-7} mol dm^{-3}), tested compounds exhibited insignificant effects on Raji cell lines growth (Supporting Information Figure S21).

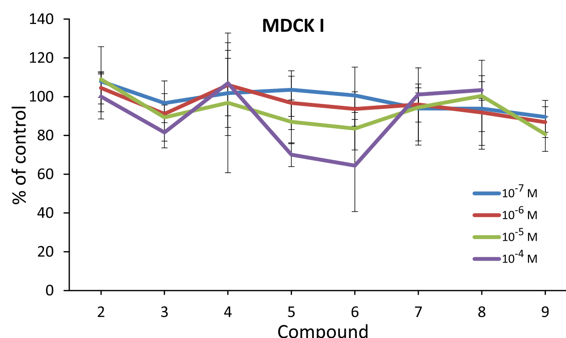


Figure 3. Cytotoxic effects of 5-aminosulfonyl uracil derivatives **2**–**9** on normal Madin-Darby canine kidney (MDCK I) cell lines growth after 72 h of incubation in the final concentration range (■ 10^{-4}, ■ 10^{-5}, ■ 10^{-6} and ■ 10^{-7} mol dm^{-3}). Cytotoxicity was analyzed using the MTT survival assay. Data are presented as the mean value ± SD of three independent experiments done in triplicates.

In comparison to effects on Raji cells, tested compounds demonstrated similar results on HuT78 cells (Supporting Information Figure S22). Compounds **2**–**8** applied at 10^{-4} mol dm^{-3} showed strong activity on HuT78 cells growth. The most potent effect showed 2-nitro benzenesulfonamide **6**, with 57 % of cell inhibition. Our results indicate that only dabsyl-sulfonamide **9** applied at concentration 10^{-5} mol dm^{-3} caused inhibition of 21 % of human T cell lymphoma. Other compounds applied at 10^{5}–10^{-7} mol dm^{-3} concentration ranges have no or only weak inhibition effect on the tested cell lines.

Applied at the highest concentration range, compounds **2**–**6**, and **8** inhibited more than 50 % of the growth of Jurkat cells (Figure 4, Supporting Information Figure S23). Also, 2,4,6-triisopropylbenzenesulfonamide **3** applied at $c = 10^{-5}$ mol dm^{-3}, caused a 20 % reduction of Jurkat cell growth. Interestingly, most of the tested compounds applied at lower concentration range (10^{-6} and 10^{-7} mol dm^{-3}) showed even a slight increase of Jurkat cell growth.

The most interesting results were obtained for the K562 cell lines (Figure 5; Supporting Information Figure S24). Compounds **2**, **3**, **7**, **8**, and **9** showed significant growth inhibitory effects in all applied range of con-centrations. The most pronounced cytotoxic effect was observed for 2,4,6-triisopropylbenzenesulfonamide **3** at 10^{-4} mol dm^{-3} (39.2 %). Also, at lower concentrations, significant inhibitory effects was observed for **3** (65.6 %, $c = 10^{5}$ mol dm^{-3}) and dabsyl-sulfonamide **9** (63–74 %, $c = 10^{5}$–10^{-7} mol dm^{-3}). 4-Nitrobenzenesulfonamide **5** and 2-nitrobenzenesulfonamide **6** failed to show any K562 cytotoxicity at the applied concentration range of 10^{-5}–10^{-7} mol dm^{-3}.

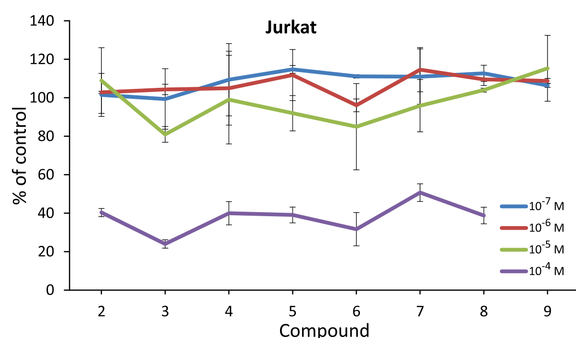


Figure 4. Cytotoxic effects of 5-aminosulfonyl uracil derivatives **2**–**9** on Jurkat cells growth after 72 h of incubation in the final concentration range (■ 10^{-4}, ■ 10^{-5}, ■ 10^{-6} and ■ 10^{-7} mol dm^{-3}). Cytotoxicity was analyzed using the MTT survival assay. Data are presented as the mean value ± SD of three independent experiments done in triplicates.

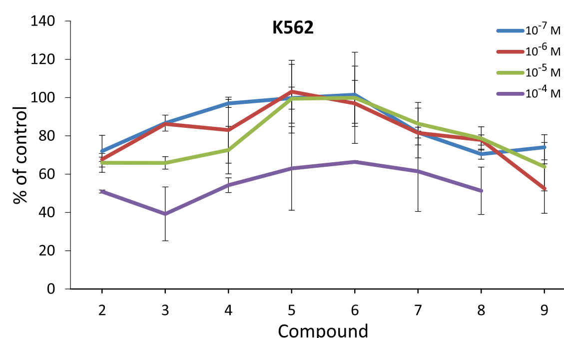


Figure 5. Cytotoxic effects of 5-aminosulfonyl uracil derivatives **2**–**9** on K562 cells growth after 72 h of incubation in the final concentration range (■ 10^{-4}, ■ 10^{-5}, ■ 10^{-6} and ■ 10^{-7} mol dm^{-3}). Cytotoxicity was analyzed using the MTT survival assay. Data are presented as the mean value ± SD of three independent experiments done in triplicates.

Table 1. Inhibitory effects of 5-aminosulfonyl uracil derivatives **2**–**9** and 5-FU on the growth of leukemia, lymphoma, and normal cells.

	IC$_{50}$ (1×10^{-6} mol dm^{-3})								
	Normal cells	Leukemia and lymphoma cells							
Comp.	MDCK I	Raji	SI	HuT78	SI	K562	SI	Jurkat	SI
2	>100	70.4 ± 0.6	**1.4**	>100		>100		80.6 ± 4.2	**1.2**
3	>100	>100		>100		78.4 ± 28.2	**1.3**	47.9 ± 4.3	**2.1**
4	>100	57.2 ± 13.6	**1.7**	>100		>100		79.9 ± 12.1	**1.3**
5	>100	>100		>100		>100		78.1 ± 8.2	**1.3**
6	>100	>100		86.0 ± 1.4	**1.2**	>100		63.3 ± 17.4	**1.6**
7	>100	>100		>100		>100		>100	
8	>100	87.1 ± 4.5	**1.1**	>100		>100		77.5 ± 8.7	**1.3**
9	>100	>100		>100		>100		>100	
5FU	55.0± 8.7	>100		>100		9.8±0.5	**5.6**	76.3± 11.4	**0.7**

IC$_{50}$: the concentration that causes 50 % growth inhibition. Data represent the mean IC$_{50}$ (1×10^{-6} mol dm^{-3}) value of three independent experiments ± SD.
SI = IC$_{50}$ for normal cell line MDCK I / IC$_{50}$ for respective tumor cell line.

IC$_{50}$ and SI values were also calculated for 5-aminosulfonyl uracil derivatives **2**–**9** for all the cell lines and compared to those calculated for 5-FU, the common chemotherapy drug (Supporting Information Table S1). IC$_{50}$ values calculated for solid tumor (HeLa, Caco-2, NCI-H358) cell lines along with the normal cell line, MDCK I, were more than100×10^{-6} mol dm^{-3}. Although compounds **2**–**8** showed a statistically significant influence at the highest applied concentrations (c = 10^{-4} mol dm^{-3}) on lymphoma (Raji, HuT78) and leukemia (Jurkat, K562) cells (Supporting Information Figures S21–S24) calculated selectivity index (SI) indicates that the new compounds generally lack anticancer specificities (Table 1). An exception is Jurkat cells where greater efficacy against tumor cells compared to normal cells is observed. The SI value calculated for 5-FU for Jurkat cell lines was low (0.7), indicating the superiority of compounds **2**-**6** and **8** (SI 1.2–2.1) compared to 5-FU.

CONCLUSIONS

A small library of 5-aminosulfonyl uracil derivatives **2**–**9** with different aromatic substituents at C5-NH-SO$_2$- of uracil moiety was efficiently synthesized (yields 58–98 %). The products were prepared using equimolar amounts of 5-aminouracil **1** and selected aromatic sulfonyl chloride in pyridine at room temperature.

The prepared compounds were tested for the antiproliferative activity on normal MDCK I cells and tumor HeLa, Caco-2, NCI-H358, Raji, HuT78, Jurkat, K562 cell lines. All of the newly synthesized compounds **2**–**9** showed only modest growth inhibition activity on normal (MDCK I) and solid tumor (HeLa, Caco-2, NCI-H358) cells. However, they were found more active on lymphoma (Raji, HuT78) and leukemia (Jurkat, K562) cells, showing statistically significant growth inhibition at the highest concentrations

(20–60 % growth inhibition at c =10^{-4} mol dm^{-3}). It should be noted that due to efficient synthetic protocol the sulfonyl group substituents of 5-aminosulfonyl uracil derivatives **II** could be easily varied providing the opportunity for diverse structural variations which may result with significant improvement of biological properties.

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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3567.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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