ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA CCA

Croat. Chem. Acta **2017**, *90*(4), 625–636
Published online: April 9, 2018
DOI: 10.5562/cca3273

HKD 1926

Synthesis and *In vitro* Activity of *N*-sulfonylamidine-derived Pyrimidine Analogues

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RECEIVED: January 8, 2018 ✯ REVISED: March 12, 2018 ✯ ACCEPTED: March 26, 2018

THIS PAPER IS DEDICATED TO PROF. MLADEN ŽINIĆ ON THE OCCASION OF HIS 70TH BIRTHDAY

Abstract: Two novel series of *N*-sulfonylamidino pyrimidine derivatives were synthesized *via* Cu-catalyzed three-component reaction of propargylated nucleobases with different benzenesulfonyl azides and amines. In this way 4-acetamido, 4-methyl and 4-carboxybenzenesulfonyl amidine products **15**–**26** in the uracil series and 4-acetamidobenzenesulfonyl amidine derivatives **27**–**29** in the cytosine series were prepared in 34–69 % yields. Attempts to prepare *N*-sulfonylamidino cytosine derivatives in reaction with 4-methylbenzenesulfonyl azide were unsuccessful. The cytosine derivatives **32** and **33** were prepared from the *N*-sulfonylamidino uracil derivatives *via* the C4 triazole intermediates. The prepared *N*-sulfonylamidino pyrimidine derivatives **1**–**28** 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, K562, Jurkat) and on normal MDCK I cells. Most of the synthesized compounds showed antiproliferative activity on the tested cell lines.

Keywords: pyrimidines, *N*-sulfonylamidines, multi-component synthesis, copper(I), *in vitro*.

INTRODUCTION

In the last two decades, our group has been intensively involved in the design and synthesis of a new series of *N*-sulfonyl nucleobase derivatives that exhibit antitumor activity.[1–4] We have shown that *N*-1-sulfonylpyrimidine derivatives have strong antiproliferative activity on human tumor cell lines and an ability to induce apoptosis in the treated tumor cells.[5–10] *In vivo* experiments showed that some *N*-sulfonylcytosine derivatives had strong antitumor activity against mouse mammary carcinoma.[9,11–14]

Recently, we reported an efficient multicomponent synthesis of the new *N*-sulfonylamidino thymine derivatives **1**–**14** using Cu(I) catalyzed three component reactions of 1-propargyl thymine, selected benzenesulfonyl azides, and amines or ammonium salts (Table 1).[15] We have shown that this one-pot three component reaction appears to be advantageous for the preparation of variously substituted *N*-sulfonylamidino thymine derivatives in moderate to good yields and opens the way for the preparation of the libraries of other nucleobase *N*-sulfonylamidino derivatives as possible biologically active molecules.

As the next step in our research, we report here on the synthesis of novel *N*-sulfonylamidine derivatives in uracil and cytosine series and the results of *in vitro* activity of *N*-sulfonylamidine-derived pyrimidine analogues on the growth of different tumor cell lines.

EXPERIMENTAL

General

Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien Kieselgel 60 F_{254} and preparative thick-layer (2 mm) chromatography was done on Merck 60 F_{254} (Merck KGaA, Darmstadt, Germany). Purification for the removal of copper particles was carried out on short columns filled with neutral and activated Al_2O_3 (particle size 0.05–0.15 mm). Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV spectra [log ε / dm^3 mol^{-1} cm^{-1}, λ_{max} / nm] were taken on a Philips PU8700 UV/VIS spectrophotometer. IR spectra [ν_{max} / cm^{-1}] were recorded as KBr pellets on a Perkin-Elmer 297 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on Bruker AV300 and AV600 MHz spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany) using TMS or 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 Procedures for the Preparation of *N*-Sulfonyl Amidines (Table 1)

METHOD A

To a stirred mixture of alkyne (1 mmol), sulfonyl azide (1.2 mmol) and CuI (0.1 mmol) in dry THF (2 mL) amine nucleophile (1.2 mmol) was slowly added. The reaction mixture was stirred for 24 h at room temperature and diluted with a small amount of cold methanol. The product was collected by filtration and dissolved in hot MeOH. The crude amidine product was filtered through a short Al_2O_3 column, evaporated and the analytically pure product was obtained by recrystallization using methanol.

METHOD B

To a stirred mixture of alkyne (1 mmol), CuI (0.1 mmol) and amine / ammonium salt (1 mmol) in dry CH_2Cl_2 (2–5 mL) triethylamine (1.5 mmol) was slowly added and the color of the suspension turned to a light yellow. After that, sulfonyl azide (1 mmol) was added. The reaction mixture was stirred for 24 h at room temperature and diluted with a small amount of cold methanol. The product was collected by filtration and dissolved in hot MeOH. The crude amidine product was filtered through a short Al_2O_3 column, evaporated and the analytically pure product was obtained by recrystallization using methanol.

METHOD C

To a stirred mixture of alkyne (1 mmol), sulfonyl azide (1.2 mmol) and CuI (0.1 mmol) in THF (2 mL) cooled to 0 °C, amine nucleophile (2 mmol) was slowly added. The reaction mixture was stirred for 24 h (4 h in the case of compound **25**) at room temperature, dissolved in MeOH and filtered through a short Celite column. The filtrate was partially evaporated and the residue was filtered off. The crude product was dissolved in 5 % aq. $NaHCO_3$ and the water solution was washed with dichloromethane and ethyl acetate. The water phase was neutralized with 5 % CH_3COOH and partially evaporated. The product was collected by filtration and recrystallized from methanol.

N^1,*N*^{1}-DIISOPROPYL-*N*^{2}-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (15) METHOD A

White solid (69 %); R_f = 0.79 (CH_2Cl_2 / MeOH 9 : 1); m.p. = 229 °C; UV (MeOH): λ_{max} / nm: 264 (log ε / dm^{-3} mol^{-1}cm^{-1}: 4.6); IR (KBr) ν / cm^{-1}: 3310 (m), 3187 (m), 3046 (m), 2975 (m), 1714 (s), 1680 (s), 1552 (s), 1454 (s), 1399 (s), 1375 (s), 1257 (s), 1260 (s), 1136 (s), 1081 (s), 1054 (s); ^{1}H NMR (600 MHz, DMSO-d_6) δ / ppm: 11.32 (brs, 1H, NH-3'), 10.22 (s, 1H, NH-Ac), 7.72 (s, 4H, Ar), 7.45 (d,1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 5.63 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H-5'), 4.27 (m, 1H, CH-(CH$_3$)$_2$), 3.95 (t, 2H, $J_{3,2}$ = 7.4 Hz, CH$_2$-3), 3.65 (m, 1H, CH-(CH$_3$)$_2$), 3.20 (t, 2H, $J_{2,3}$ = 7.4 Hz, CH$_2$-2), 2.07 (s, 3H, CO-CH$_3$), 1.20 (pt, 12H, J = 6.5 Hz, CH-(CH$_3$)$_2$); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 168.8 (s, CO-CH$_3$), 163.7 (s, C-4'), 161.6 (s, C-1), 151.0 (s, C-2'), 145.0 (d, C-6'), 141.9 (s, Ar), 138.1 (s, Ar), 126.5 (d, Ar), 118.3 (d, Ar), 101.6 (d, C-5'), 50.2 (d, CH-(CH$_3$)$_2$), 47.3 (d, CH-(CH$_3$)$_2$), 45.3 (t, CH$_2$-3), 31.3 (t, CH$_2$-2), 24.0 (q, CO-CH$_3$), 20.1 (q, CH-(CH$_3$)$_2$), 19.6 (q, CH-(CH$_3$)$_2$). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{21}H_{29}N_5O_5S$ x 0.5H_2O (M_r = 472.56) are: C, 53.37; H, 6.40; N, 14.82; S, 6.78; found: C, 53.59; H, 6.47; N, 15.06; S, 6.54.

N^1,*N*^{1}-DIETHYL-*N*^{2}-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (16) METHOD A

White solid (57 %); m.p. = 229–231 °C; R_f = 0.58 (CH_2Cl_2 / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 264 (log ε / dm^{-3} mol^{-1} cm^{-1} : 4.1); IR (KBr) ν / cm^{-1}: 3311 (m), 3276 (m), 3190 (w), 3053 (m), 2975 (w), 1698 (s), 1680 (s), 1565 (s), 1532 (s), 1317(s), 1239 (s), 1130 (m), 1077 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.34 (brs, 1H, NH-3'), 10.24 (s, 1H, NH-Ac), 7.75 (d, 2H, J = 8.9 Hz, Ar), 7.70 (d, 2H, J = 8.9 Hz, Ar), 7.43 (d,1H, $J_{6',5'}$ = 7.9 Hz, H-6'), 5.62 (d, 1H, $J_{5',6'}$ = 7.9 Hz, H-5'), 3.97 (t, 2H, $J_{3,2}$ = 7.3 CH$_2$-3), 3.49 (q, 2H, J = 6.9 CH$_2$-CH$_3$), 3.38 (q, 2H, J = 6.9 CH$_2$-CH$_3$), 3.15 (t, 2H, $J_{2,3}$ = 7.3 Hz, CH$_2$-2), 2.07 (s, 3H, CO-CH$_3$), 1.16 (t, 3H, J = 7.8 Hz, CH$_2$-CH$_3$), 1.01 (t, 3H, J = 7.8 Hz, CH$_2$-CH$_3$), ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 168.9 (s, CO-CH$_3$), 163.7 (s, C-4'), 162.9 (s, C-1), 151.0 (s, C-2'), 144.9 (d, C-6'), 142.0 (s, Ar), 138.1 (s, Ar), 126.6 (d, Ar), 118.4 (d, Ar), 101.7 (d, C-5'), 45.4 (t, CH$_2$-3), 43.2 (t, CH$_2$-CH$_3$), 43.0 (t, CH$_2$-CH$_3$), 29.9 (t, CH$_2$-2), 24.1 (q,

CO-CH$_3$), 13.9 (q, CH$_2$-CH$_3$), 11.8 (q, CH$_2$-CH$_3$). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{19}H_{25}N_5O_5S$ (M_r = 435.49) are: C, 52.40; H, 5.78; N, 16.08; S, 7.36; found: C, 52.16; H, 5.76; N, 16.30; S, 7.07.

N^1-ISOPROPYL-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (17) METHOD A

White solid (50 %); m.p. = 146 °C; R_f = 0,74 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 262 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4,5); IR (KBr) ν / cm^{-1}: 3499 (m), 3062 (m), 2980 (m), 2928 (m), 1697 (s), 1673 (s), 1596 (m), 1562 (m), 1541 (m), 1366 (m), 1328 (m), 1229 (m), 1137 (m), 1088 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.24 (brs, 1H, NH-3'), 10.27 (s, 1H, NH-Ac), 8.68 (d, 1H, J = 7.1 Hz, NH-CH), 7.73 (d, 2H, J = 8.8 Hz, Ar), 7.71 (d, 2H, J = 8.8 Hz, Ar), 7.38 (d,1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 5.55 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H-5'), 4.02 (t, 2H, $J_{3,2}$ = 6.1 Hz, CH$_2$-3), 3.86 (m, 1H, CH-(CH$_3$)$_2$), 2.89 (t, 2H, $J_{2,3}$ = 6.1 Hz, CH$_2$-2), 2.07 (s, 3H, CO-CH$_3$), 1.01 (d, 6H, J = 6.5 Hz, CH-(CH$_3$)$_2$), ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 169.3 (s, CO-CH$_3$), 164.3 (s, C-4'), 163.6 (s, C-1), 151.2 (s, C-2'), 145.7 (d, C-6'), 142.0 (s, Ar), 138.6 (s, Ar), 127.1 (d, Ar), 118.8 (d, Ar), 101.5 (d, C-5'), 45.8 (t, CH$_2$-3), 43.2 (d, CH-(CH$_3$)$_2$), 33.3 (t, CH$_2$-2), 24.1 (q, CO-CH$_3$), 21.1 (q, CH-(CH$_3$)$_2$). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{18}H_{23}N_5O_5S \times 1.5H_2O$ (M_r = 448.49) are: C, 48.20; H, 5.84; N, 15.62; S, 7.15; found: C, 48.29; H, 5.48; N, 15.76; S, 7.13.

N^1-CYCLOPENTYL-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (18) METHOD A

White solid (48 %); m.p. = 147–149 °C; R_f = 0.43 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 263 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.5); IR (KBr) ν / cm^{-1} 3327 (s), 3012 (m), 2959 (m), 1696 (s), 1672 (s), 1251 (s), 1264 (s), 1255 (s), 1148 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.22 (brs, 1H, NH-3'), 10.24 (s, 1H, NH-Ac), 8.72 (brs, 1H, NH-cyclopentyl), 7.74 (d, 2H, J = 8.9 Hz, Ar), 7.70 (d, 2H, J = 8.9 Hz, Ar), 7.37 (d,1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 5.55 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H-5'), 3.99 (t, 2H, $J_{3,2}$ = 6.2 Hz, CH$_2$-3), 3.98 (m, 1H, CH-cyclopentyl), 2.91 (t, 2H, $J_{2,3}$ = 6.2 Hz, CH$_2$-2), 2.07 (s, 3H, CH$_3$-CO), 1.75 (m, 2H, CH$_2$-cyclopentyl), 1.56 (m, 2H, CH$_2$-cyclopentyl), 1.45 (m, 2H, CH$_2$-cyclopentyl), 1.38 (m, 2H, CH$_2$-cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 168.8 (s, CO-CH$_3$), 164.0 (s, C-4'), 163.8 (s, C-1), 150.7 (s, C-2'), 145.2 (d, C-6') 141.9 (s, Ar), 138.1 (s, Ar), 126.6 (d, Ar), 118.3 (d, Ar), 101.0 (d, C-5'), 52.9 (d, CH-cyclopentyl), 45.7 (t, CH$_2$-3), 33.1 (t, CH$_2$-2), 31.4 (t, CH$_2$-cyclopentyl), 24.1 (q, CO-CH$_3$), 23.5 (t, CH$_2$-cyclopentyl). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{20}H_{25}N_5O_5S$ x H_2O (M_r = 465.52) are: C, 51.60; H, 5.84; N, 15.04; S, 6.88; found: C, 51.53; H, 5.71; N, 15.08; S, 6.77.

N^1-(QUINOLIN-6-YL)-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (19) METHOD A

Pale yellow solid (49 %); m.p. = 221–223 °C; R_f = 0.43 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 262 (log ε / dm^{-3} mol^{-1} cm^{-1} : 4.4); IR (KBr) ν / cm^{-1}: 3317 (m), 3104 (m), 2970 (m), 2765 (m), 1695 (s), 1676 (s), 1553 (s), 1442 (s), 1276 (s), 1147 (s), 1089 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.26 (brs, 1H, NH-3'), 10.70 (brs, 1H, NH-quinolinyl), 10.30 (s, 1H, NH-Ac), 8.84–7.48 (m, 10H, Ar, quinolinyl), 7.54 (d, 1H, $J_{6',5'}$ = 7.7 Hz, H-6'), 5.56 (d, 1H, $J_{5',6'}$ = 7.7 Hz, H-5'), 4.20 (brt, 2H, CH$_2$-3), 3.21 (brt, 2H, CH$_2$-2), 2.08 (s, 3H, CO-CH$_3$); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 169.0 (s, CO-CH$_3$), 163.8 (s, C-4'), 163.1 (s, C-1), 151.0 (s, C-2'), 150.1 (d, Ar), 145.3 (s, Ar), 145.0 (d, C-6'), 142.5 (s, Ar), 137.1 (s, Ar), 135.7 (s, Ar), 135.6 (d, Ar), 129.3 (d, Ar), 127.7 (s, Ar) 127.0 (d, Ar), 125.2 (d, Ar), 122.0 (d, Ar), 119.1 (d, Ar), 118.5 (d, Ar), 101.3 (d, C-5') 45.9 (t, CH$_2$-3), 34.2 (t, CH$_2$-2), 24.1 (q, CO-CH$_3$). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{24}H_{22}N_6O_5S \times 1.5$ H_2O (M_r = 533.56) are: C, 54.03; H, 4.72; N, 15.75; S, 6.01; found: C, 53.86; H, 4.69; N, 15.70; S, 6.30.

N^1-(4-CYANOBENZYL)-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (20) METHOD B

White solid (34 %); m.p. = 230–234 °C; R_f = 0.47 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 237 and 264 (log ε / dm^{-3} mol^{-1} cm^{-1}: 3.7 and 3.7); IR (KBr) ν / cm^{-1}: 3310 (s), 3014 (m), 2973 (m), 2816 (m), 2221 (w), 1698 (s), 1666 (s), 1526 (s), 1268 (s), 1139 (s), 1092 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.26 (brs, 1H, NH-3'), 10.24 (s, 1H, NH-Ac), 9.35 (brs, 1H, NH-CH$_2$), 7.72 (d, 2H, J = 8.0 Hz, Ar), 7.63 (d, 2H, J = 8.7 Hz, Ar), 7.60 (d, 2H, J = 8.7 Hz, Ar), 7.38 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 7.36 (d, 2H, J = 8.0 Hz, Ar), 5.52 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H-5'), 4.37 (t, 2H, J = 5,4 Hz, NH-CH$_2$), 4.05 (t, 2H, $J_{3,2}$ = 6.0 Hz, CH$_2$-3), 3.01 (t, 2H, $J_{2,3}$ = 6.0 Hz, CH$_2$-2), 2.07 (s, 3H, CO-CH$_3$); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 168.8 (s, CO-CH$_3$), 165.0 (s, C-4'), 163.7 (s, C-1), 150.7 (s, CO-2'), 145.0 (d, C-6'), 143.1 (s, Ar), 142.0 (s, Ar), 137.6 (s, Ar), 132.1 (d, Ar), 128.5 (d, Ar), 126.6 (d, Ar), 118.7 (s, CN), 118.3 (d, Ar), 109.9 (s, Ar), 101.2 (d, C-5) 45.7 (t, NH-CH$_2$), 44.5 (t, CH$_2$-3), 33.0 (t, CH$_2$-2), 24.0 (q, CO-CH$_3$). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{23}H_{22}N_6O_5S$ (M_r = 494.52) are: C, 55.86; H, 4.48; N, 16.99; S, 6.48; found: C, 55.68; H, 4.38; N, 16.98; S, 6.31.

N-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (21) METHOD B

White solid (47 %); m.p. = 215 °C; R_f = 0.35 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 263 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.5);

IR (KBr) ν / cm^{-1}: 3369 (s), 3204 (s), 3091 (m), 2955 (w), 2798 (w), 1707 (s), 1669 (s), 1597 (m), 1569 (s), 1528 (m), 1254 (s), 1129 (s), 1074 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.20 (s, 1H, NH-3'), 10.27 (s, 1H, NH-Ac), 8.68 (s, 1H, NH$_2$), 7.91 (s, 1H, NH$_2$), 7.79 (d, 2H, J = 8.8 Hz, Ar), 7.71 (d, 2H, J = 8.8 Hz, Ar), 7.16 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 5.28 (dd, 1H, $J_{5',6'}$ = 7.8 Hz, $J_{5',NH-3'}$ = 2.1 Hz, H-5'), 3.81 (t, 2H, $J_{3,2}$ = 6.4 Hz, CH$_2$-3), 2.57 (t, 2H, $J_{2,3}$ = 6.4 Hz, CH$_2$-2), 2.08 (s, 3H, CO-CH$_3$). ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 168.9 (s, CO-CH$_3$), 165.8 (s, C-4'), 163.6 (s, C-1), 150.7 (s, C-2'), 145.6 (d, C-6'), 142.6 (s, Ar) 136.1 (s, Ar), 127.2 (d, Ar), 118.4 (d, Ar), 100.4 (d, C-5'), 45.2 (t, CH$_2$-3), 34.8 (t, CH$_2$-2), 24.1 (q, CO-CH$_3$). *Anal.* Calcd. mass fractions of elements, w / %, for $C_{15}H_{17}N_5O_5S$ (M_r = 373.39) are: C, 47.49; H, 4.52; N, 18.46; S, 8.45; found: C, 47.19; H, 4.51; N, 18.09; S, 8.37.

N^1,N^1-DIISOPROPYL-N^2-(4-METHYLBENZENE-1-SULFONYL)- 3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (22) METHOD A

White solid (53 %); m.p. = 210–213 °C; R_f = 0.41 (CH$_2$Cl$_2$ / MeOH 20 : 1); UV (MeOH): λ_{max} / nm: 253 (log ε / dm^3 mol^{-1} cm^{-1}: 4.7); IR (KBr) ν / cm^{-1}: 3172 (m), 3109 (w), 3029 (m), 2976 (m), 2925 (m), 2829 (w), 1714 (s), 1679 (s), 1546 (s), 1454 (s), 1400 (m), 1377 (s), 1454 (s), 1377 (s), 1344 (s), 1261 (s), 1081 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.35 (s, 1H, NH-3'), 7.68 (d, 2H, J = 7.9 Hz, Ar), 7.45 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 7.35 (d, 2H, J = 7.9 Hz, Ar), 5.64 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H-5'), 4.27 (m, 1H, CH-(CH$_3$)$_2$), 3.96 (t, 2H, $J_{3,2}$ = 7.1 Hz, CH$_2$-3), 3.67 (m, 1H, CH-(CH$_3$)$_2$), 3.20 (t, 2H, $J_{2,3}$ = 7.1 Hz, CH$_2$-2), 2.37 (s, 3H, CH$_3$-Ts), 1.20 (brs, 12H, CH-(CH$_3$)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ / ppm : 163.7 (s, C-4'), 162.3 (s, C-1), 151.0 (s, C-2'), 144.9 (d, C-6'), 141.5 (s, Ar), 141.4 (s, Ar) 129.2 (d, Ar), 125.5 (d, Ar), 101.6 (d, C-5'), 50.3 (d, CH-(CH$_3$)$_2$), 47.3 (d, CH-(CH$_3$)$_2$), 45.3 (t, CH$_2$-3), 31.4 (t, CH$_2$-2), 20.9 (q, CH$_3$-Ts), 20.1 (q, CH-(CH$_3$)$_2$), 19.6 (q, CH-(CH$_3$)$_2$). *Anal.* Calcd. mass fractions of elements, w / %, for $C_{20}H_{28}N_4O_4S$ (M_r = 420.52) are: C, 57.12; H, 6.71; N, 13.32; S, 7.62; found: C, 56.92; H, 6.79; N, 13.08; S, 7.74.

N^1-CYCLOPENTYL-N^2-(4-METHYLBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (23) METHOD A

White solid (39 %); m.p. = 181–184 °C; R_f = 0.60 (CH$_2$Cl$_2$ / MeOH 20 : 1); UV (MeOH): λ_{max} / nm: 243 (log ε / dm^3 mol^{-1} cm^{-1}: 4.5); IR (KBr) ν / cm^{-1}: 3330 (s), 3041 (m), 2966 (m), 2869 (m), 2829 (w), 1697 (s), 1558 (s), 1267 (s), 1151 (s), 1093 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 11.24 (s, 1H, NH-3'), 8.74 (d, 1H, J = 6.5 Hz, NH-cyclopentyl), 7.70 (d, 2H, J = 7.8 Hz, Ar), 7.38 (d, 1H, $J_{6',5'}$ = 7.9 Hz, H-6'), 7.33 (d, 2H, J = 7.8 Hz, Ar), 5.55 (d, 1H, $J_{5',6'}$ = 7.9 Hz, H-5'), 4.0 (t, 2H, $J_{3,2}$ = 6.0 Hz, CH$_2$-3), 3.98 (m, 1H, CH-cyclopentyl), 2.91 (t, 2H, $J_{2,3}$ = 6.0 Hz, CH$_2$-2), 2.36 (s, 3H, CH$_3$-Ts), 1.74 (m, 2H, CH$_2$-cyclopentyl), 1.59–1.41 (m, 6H, CH$_2$-cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 164.2 (s, C-4'), 163.8 (s, C-1), 150.7 (s, C-2'), 145.2 (d, H-6'), 141.5 (s, Ar), 141.4 (s, Ar), 129.2 (d, Ar), 125.6 (d, Ar), 101.0 (d, C-5'), 52.9 (d, CH-cyclopentyl) 45.8 (t, CH$_2$-3), 33.2 (t, CH$_2$-2), 31.4 (t, CH$_2$-cyclopentyl), 23.5 (t, CH$_2$-cyclopentyl), 20.9 (q, CH$_3$-Ts). *Anal.* Calcd. mass fractions of elements, w / %, for $C_{19}H_{24}N_4O_4S$ (M_r = 404.48) are: C, 56.42; H, 5.98; N, 13.85; S, 7.93; found: C, 56.15; H, 5.80; N, 13.47; S, 7.96.

N^1,N^1-DIISOPROPYL-N^2-(4-CARBOXYBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (24) METHOD C

White solid (46 %); m.p. = 265 °C; R_f = 0.30 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 257 (log ε / dm^3 mol^{-1} cm^{-1}: 4.6); IR (KBr) ν / cm^{-1}: 3213 (w), 3132 (w), 3109 (w), 2975 (w), 2794 (w), 2611 (w), 2495 (w), 1546 (s), 1442 (m), 1375 (s), 1276 (s), 1120 (m), 1083 (s), 788 (m), 705 (m), 628 (m), 547 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 13.30 (brs, 1H, COOH), 11.34 (brs, 1H, NH-3'), 8.09 (d, 2H, J = 8.4 Hz, Ar), 7.90 (d, 2H, J = 8.4 Hz, Ar), 7.48 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 5.63 (dd, 1H, $J_{5',6'}$ = 7.8 Hz, $J_{5',NH-3'}$ = 2.1 Hz, H-5'), 4.28 (m, 1H, CH-(CH$_3$)$_2$), 3.97 (t, 2H, $J_{3,2}$ = 7.5 Hz, CH$_2$-3), 3.69 (m, 1H, CH-(CH$_3$)$_2$), 3.22 (t, 2H, $J_{2,3}$ = 7.5 Hz, CH$_2$-2), 1.19 (pt, 12H, J = 6.6 Hz, CH-(CH$_3$)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ / ppm: 166.3 (s, COOH), 163.7 (s, C-4'), 151.0 (s, C-2'), 147.5 (s, Ar), 145.0 (d, C-6'), 133.3 (s, Ar), 129.8 (d, Ar), 125.7 (d, Ar), 101.6 (d, C-5'), 50.5 (d, CH-(CH$_3$)$_2$), 47.5 (d, CH-CH$_3$), 45.3 (t, CH$_2$-3), 31.6 (t, CH$_2$-2), 20.0 (q, CH-(CH$_3$)$_2$), 19.5 (q, CH-(CH$_3$)$_2$). *Anal.* Calcd. mass fractions of elements, w / %, for $C_{20}H_{26}N_4O_6S \times 0.25H_2O$ (M_r = 455.01) are: C, 52.79; H, 5.87; N, 12.31; S, 7.05; found: C, 52.74; H, 6.19; N, 12.03; S, 7.12.

N^1-ISOPROPYL-N^2-(4-CARBOXYBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (25) METHOD C

White solid (37 %); m.p. = 186–188 °C; R_f = 0.22 (CH$_2$Cl$_2$ / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 247 (log ε / dm^3 mol^{-1} cm^{-1}: 4.3); IR (KBr) ν / cm^{-1}: 3271 (m), 3099 (m), 3051 (m), 2972 (m), 2935 (w), 2875 (w), 1670 (s), 1548 (s), 1465 (m), 1365 (s), 1269 (s), 1149 (s), 1089 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 13.30 (brs, 1H, COOH), 11.24 (brs, 1H, NH-3'), 8.83 (d, 1H, J = 7.2 Hz, NH-CH), 7.99 (d, 2H, J = 8.7 Hz, Ar), 7.79 (d, 2H, J = 8.5 Hz, Ar), 7.40 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 5.55 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H-5'), 4.04 (t, 2H, $J_{3,2}$ = 6.1 Hz, CH$_2$-3), 3.87 (m, 1H, CH-(CH$_3$)$_2$), 2.92 (t, 2H, $J_{2,3}$ = 6.1 Hz, CH$_2$-2), 1.01 (d, 6H, J = 6.6 Hz, CH-(CH$_3$)$_2$); ^{13}C-NMR (150 MHz, DMSO-d_6) δ / ppm: 163.3 (s, C-4'), 163.2 (s, C-1), 154.6 (s, C-2'), 150.2 (s, Ar) 144.7 (d, C-6'), 140.2 (s, Ar), 128.8 (d, Ar), 124.6 (d, Ar), 100.5 (d, C-5'), 45.2 (t, CH$_2$-3), 42.7 (d, CH-(CH$_3$)$_2$), 32.8 (t, CH$_2$-2), 20.6 (q, CH-(CH$_3$)$_2$). *Anal.* Calcd. mass fractions of elements, w / %, for $C_{17}H_{20}N_4O_6S \times 1.5H_2O$ (M_r = 435.45) are: C, 46.89; H, 5.32; N, 12.86; S, 7.36; found: C, 46.53; H, 5.20; N, 12.48; S, 7.19.

N^1-CYCLOPENTYL-N^2-(4-METHYLBENZENE-1-SULFONYL)-3-(2,4-DIOXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPANAMIDINE (26) METHOD C

White solid (42 %); m.p. = 190–193 °C; R_f = 0.30 (CH_2Cl_2 / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 247 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.1); IR (KBr) ν / cm^{-1}: 3255 (m), 3097 (m), 3037 (m), 2964 (m), 2869 (m), 1620 (s), 1552 (s), 1469 (m), 1423 (m), 1373 (s), 1282 (s), 1151 (s), 1087 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 13.48 (brs, 1H, COOH), 11.34 (s, 1H, NH-3'), 8.99 (d, 1H, J = 6.4 Hz, NH-cyclopentyl), 8.15 (d, 2H, J = 8.5 Hz, Ar), 8.00 (d, 1H, $J_{6',5'}$ = 7.9 Hz, H-6'), 7.47 (d, 2H, J = 8.0 Hz, Ar), 5.63 (d, 1H, $J_{5',6'}$ = 7.9 Hz, H-5'), 4.12 (t, 2H, $J_{3,2}$ = 6.4 Hz, CH_2-3), 4.10 (m, 1H, CH-cyclopentyl), 3.04 (t, 2H, $J_{2,3}$ = 6.4 Hz, CH_2-2), 1.76 (m, 2H, CH_2-cyclopentyl), 1.36 – 1.59 (m, 6H, CH_2-cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 164.2 (s, C-4'), 163.7 (s, C-1), 150.7 (s, CO-2'), 145.2 (d, H-6'), 144.4 (s, Ar), 142.6 (s, Ar), 129.1 (d, Ar), 124.8 (d, Ar), 101.0 (s, C-5'), 52.9 (d, CH-cyclopentyl) 45.7 (t, CH_2-3), 33.2 (t, CH_2-2), 31.4 (t, CH_2-cyclopentyl), 23.5 (t, CH_2-cyclopentyl). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{19}H_{22}N_4O_6S$ (M_r = 434.46) are: C, 52.52; H, 5.10; N, 12.89; found: C, 52.72; H, 5.14; N, 12.46.

N^1,N^1-DIISOPROPYL-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(4-AMINO-4-OXOPYRIMIDINE-2(1*H*)-YL)PROPANAMIDINE (27) METHOD A

White crystals (45 %); m.p. = 232–235 °C; R_f = (CH_2Cl_2 / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 265 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.4), IR (KBr) ν / cm^{-1}: 3586 (m), 3342 (s), 3115 (m), 2999 (m), 2974 (m), 1687 (s), 1663 (s), 1596 (m), 1552 (s), 1535 (s), 1361 (s), 1249 (s), 1132 (s), 1085 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 10.24 (s, 1H, NH-Ac), 7.73 (brs, 4H, Ar), 7.41 (d, 1H, $J_{6',5'}$ = 7.2 Hz, H-6'), 7.14 (brs, 2H, NH_2), 5.74 (d, 1H, $J_{5',6'}$ = 7.2 Hz, H-5'), 4.44 (m, 1H, CH-(CH_3)), 3.91 (t, 2H, $J_{3,2}$ = 7.2 Hz, CH_2-3), 3.66 (m, 1H, CH-(CH_3)$_2$), 3.14 (m, 2H, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.21 (d, 6H, J = 6.5 Hz, CH-(CH_3)$_2$), 1.18 (d, 6H, J = 6.5 Hz, CH-(CH_3)$_2$); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ / ppm: 168.8 (s, CO-CH_3), 166.2 (s, C-4'), 162.0 (s, C-1), 155.8 (s, C-2'), 145.3 (d, C-6'), 141.9 (s, Ar), 138.2 (s, Ar), 126.5 (d, Ar), 118.4 (d, Ar), 94.1 (d, C-5'), 50.2 (d, CH-(CH_3)$_2$), 47.3 (d, CH-(CH_3)$_2$), 46.6 (t, CH_2-3), 31.6 (t, CH_2-2), 24.1 (q, CO-CH_3), 20.1 (q, CH-(CH_3)$_2$), 19.6 (q, CH-(CH_3)$_2$); *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{21}H_{30}N_6O_4S \times H_2O$ (M_r = 462.56) are: C, 52.48; H, 6.71; N, 17.48; S, 6.67; found: C, 52.12; H, 6.39; N, 16.99; S, 6.56.

N^1,N^1-DIETHYL-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(4-AMINO-4-OXOPYRIMIDINE-2(1*H*)-YL)PROPANAMIDINE (28) METHOD A

White crystals (50 %); m.p. = 208–210 °C; R_f = 0.23 (CH_2Cl_2 / MeOH = 9 : 1); UV (MeOH): λ_{max} / nm: 264 (log ε / dm^{-3} mol^{-1} cm^{-1}: 3.8); IR (KBr) ν / cm^{-1}: 3554 (m), 3434 (m), 3344 (m), 3105 (m), 2977 (m), 2937 (m), 1701 (m), 1639 (s), 1560 (s), 1527 (s), 1498 (s), 1365 (s), 1311 (s), 1251 (s), 1135 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 10.24 (s, 1H, NH-Ac), 7.77 (d, 2H, J = 9.0 Hz, Ar), 7.71 (d, 2H, J = 9.0 Hz, Ar), 7.38 (d, 1H, $J_{6',5'}$ = 7.2 Hz, H-6'), 7.13 (d, 2H, J = 20.1 Hz, NH_2), 5.71 (d, 1H, $J_{5',6'}$ = 7.2 Hz, H-5'), 3.92 (t, 2H, $J_{3,2}$ = 7.6 Hz, CH_2-3), 3.55 (q, 2H, J = 6.8 Hz, CH_2-CH_3), 3.37 (q, 2H, J = 6.8 Hz, CH_2-CH_3), 3,10 (t, 2H, $J_{2,3}$ = 7.6 Hz, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.15 (t, 3H, J = 6.9 Hz, CH_2-CH_3), 1.00 (t, 3H, J = 6.9 Hz, CH_2-CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm : 169.3 (s, CO-CH_3), 166.6 (s, C-4'), 163.7 (s, C-1), 156.2 (s, C-2'), 145.8 (d, C-6'), 142.5 (s, Ar), 138.7 (s, Ar), 127.0 (d, Ar), 118.8 (d, Ar), 94.5 (s, C-5'), 47.2 (t, CH_2-3), 43.5 (t, CH_2-CH_3), 43.4 (t, CH_2-CH_3), 30.6 (t, CH_2-2), 24.6 (q, CO-CH_3), 14.4 (q, CH_2-CH_3), 12.3 (q, CH_2-CH_3). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{19}H_{26}N_6O_4S \times H_2O$ (M_r = 452.52) are: C, 50.42; H, 6.23; N, 18.57; S, 7.08; found: C, 50.75; H, 5.91; N, 18.21; S, 7.27.

N^1-CYCLOPENTYL-N^2-(4-ACETAMIDOBENZENE-1-SULFONYL)-3-(4-AMINO-2-OXOPYRIMIDINE-2(1*H*)-YL)PROPANAMIDINE (29) METHOD A

White crystals (62 %); m.p. = 160–162 °C; R_f = 0.52 (CH_2Cl_2 / MeOH / EtOAc = 3 : 1 : 1); UV (MeOH): λ_{max} / nm: 263 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.5); IR (KBr) ν / cm^{-1}: 3325 (m), 3097 (m), 1653 (s), 1593 (s), 1537 (s), 1493 (s), 1445 (m), 1400 (m), 1389 (m), 1371 (m), 1317 (m), 1261 (m), 1140 (s), 1086 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 10.28 (s, 1H, NH-Ac), 8.74 (d, 1H, J = 6.5 Hz, NH-cyclopentyl), 7.73 (q, 4H, J = 8.9 Hz, Ar), 7.36 (d, 1H, $J_{6',5'}$ = 7.2 Hz, H-6'), 7.03 (d, 2H, J = 26.9 Hz, NH_2), 5.65 (d, 1H, $J_{5',6'}$ = 7.2 Hz, H-5'), 3.99 (t, 2H, $J_{3,2}$ = 6.3 Hz, CH_2-3), 3.17 (s, 1H, CH-cyclopentyl), 2.94 (t, 2H, $J_{2,3}$ = 6.3 Hz, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.75 (m, 2H, CH_2-cyclopentyl), 1.45 (m, 6H, CH_2-cyclopentyl); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ / ppm: 168.9 (s, CO-CH_3), 166.0 (s, C-4'), 164.3 (s, C-1), 155.6 (s, C-2'), 145.5 (d, C-6'), 141.9 (s, Ar), 138.2 (s, Ar), 126.6 (d, Ar), 118.4 (d, Ar), 93.6 (d, C-5'), 52.9 (d, CH-cyclopentyl), 46.6 (t, CH_2-3), 33.5 (t, CH_2-2), 31.4 (t, CH_2-cyclopentyl), 24.1 (q, CO-CH_3), 23.5 (t, CH_2-cyclopentyl). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{20}H_{26}N_6O_4S$ (M_r = 446.52) are: C, 53.8; H, 5.87; N, 18.82; S, 7.18; found: C, 50.75; H, 5.94; N, 18.65; S, 7.35.

N^1,N^1-DIISOPROPYL-N^2-(4-METYLBENZENESULFONYL)-3-(4-(3-NITRO-1*H*-1,2,4-TRIAZOLE-1-YL)-2-OXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPIONAMIDINE (30)

To a stirred mixture of uracil derivative **15** (105.1 mg, 0.25 mmol) in pyridine (1 mL) under argon, 1-(mesitylsulfonyl)-3-nitro-1*H*-1,2,4-triazole) (MSNT) (265 mg, 1.14 mmol) and diphenylphosphate (30 mg, 0.12 mmol) were added. After stirring the reaction mixture for 48 hours at room temperature, the pyridine was evaporated and methanol (10 mL) was added. The product **30** precipitates from methanol as white crystals: 112 mg (86 %); R_f = 0.76 (CH_2Cl_2 /

MeOH = 20 : 1); ^{1}H NMR (600 MHz, DMSO-d_6) δ / ppm: 9.74 (s, 1H, CH-triazole), 8.42 (d, 1H, $J_{6',5'}$ = 7.0 Hz, H-6'), 7.67 (d, 2H, J = 8.0 Hz, Ar), 7.33 (d, 2H, J = 8.0 Hz, Ar), 7.08 (d, 1H, $J_{5',6'}$ = 7.0 Hz, H-5'), 4.37 (m, 1H, CH-(CH_3)), 4.24 (t, 2H, $J_{3,2}$ = 7.5 Hz, CH_2-3), 3.70 (brs, 1H, CH-(CH_3)$_2$), 3.34 (t, 2H, $J_{2,3}$= 7.5 Hz, CH_2-2), 2.36 (s, 3H, CH_3-Ts), 1.24 (dd, 12H, J = 6.4 Hz, J = 3.3 Hz, 2×CH-(CH_3)$_2$); ^{13}C-NMR (150 MHz, DMSO-d_6) δ / ppm: 163.1 (s, C-4'), 161.5 (s, C-1), 158.2 (s, triazole), 154.3 (d, C-6'), 154.1 (s, C-2'), 145.9 (s, triazole), 141.5 (s, Ar), 141.3 (s, Ar), 129.2 (d, Ar), 125.5 (d, Ar), 94.2 (d, C-5'), 50.3 (d, CH-(CH_3)$_2$), 48.2 (t, CH_2-3), 47.4 (d, CH-(CH_3)$_2$), 31.1 (t, CH_2-2), 20.9 (q, CH_3-Ts), 20.1 (q, CH-(CH_3)$_2$), 19.6 (q, CH-(CH_3)$_2$).

N^1-CYCLOPENTYL-*N*^{2}-(4-METYLBENZENESULFONYL)-3-(4-(3-NITRO-1*H*-1,2,4-TRIAZOLE-1-YL)-2-OXO-3,4-DIHYDROPYRIMIDINE-1(2*H*)-YL)PROPIONAMIDINE (31)

To a stirred mixture of uracil derivative **18** (100 mg, 0.25 mmol) in pyridine (1 mL) under argon, 1-(mesitylsulfonyl)-3-nitro-1*H*-1,2,4-triazole) (MSNT) (265 mg, 1.14 mmol) and diphenylphosphate (30 mg, 0.12 mmol) were added. After stirring the reaction mixture for 48 hours at room temperature, the pyridine was evaporated and methanol (10 mL) was added. Product precipitates from methanol as white crystals 67 mg (56 %): R_f = 0.52 (CH_2Cl_2 / MeOH 20 : 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 9.75 (s, 1H, CH-triazole), 8.76 (d, 1H, J = 6.9 Hz, NH-cyclopentyl), 8.34 (d, 1H, $J_{6',5'}$ = 7.0 Hz, H-6'), 7.70 (d, 2H, J = 7.0 Hz, Ar), 7.32 (d, 2H, J = 7.4 Hz, Ar), 7.01 (d, 1H, $J_{5',6'}$ = 7.0 Hz, H-5'), 4.31 (t, 2H, $J_{3,2}$ = 6.0 Hz, CH_2-3), 4.00 (m, 1H, CH-cyclopentyl), 3.06 (t, 2H, $J_{2,3}$= 6.0 Hz, CH_2-2), 2.35 (s, 3H, CH_3-Ts), 1.76 (m, 2H, CH_2-cyclopentyl), 1.54 (m, 2H, CH_2-cyclopentyl), 1.45 (m, 2H, CH_2-cyclopentyl), 1.38 (m, 2H, CH_2-cyclopentyl); ^{13}C-NMR (150 MHz, DMSO-d_6) δ / ppm: 163.9 (s, C-4'), 163.2 (s, C-1), 158.0 (s, triazole), 154.4 (d, C-6'), 153.8 (s, C-2'), 145.8 (s, triazole), 141.5 (s, Ar), 141.4 (s, Ar), 129.2 (d, Ar), 125.6 (d, Ar), 93.5 (d, C-5'), 52.9 (d, CH-cyclopentyl) 48.9 (t, CH_2-3), 32.5 (t, CH_2-2), 31.6 (t, CH_2-cyclopentyl), 23.5 (t, CH_2-cyclopentyl), 20.9 (q, CH_3-Ts).

N^1,*N*^{1}-DIISOPROPYL-*N*^{2}-(4-METYLBENZENESULFONYL)-3-(4-AMINO-2-OXOPYRIMIDIN-2(1*H*)-YL)PROPANAMIDINE (32)

To a suspension of compound **30** (77.8 mg, 0.15 mmol) in dioxane (3 mL) ammonium hydroxide (6 mL) was added. After stirring the reaction mixture for 24 hours at room temperature the solvent was evaporated under reduced pressure. The residue was purified by preparative chromatography (CH_2Cl_2/MeOH 9:1) to give product **32** as a white powder: 62 mg (98 %): m.p. = 220–222 °C; R_f = 0.47 (CH_2Cl_2 / MeOH = 9 : 1); UV (MeOH): λ_{max} / nm: 249 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.3); IR (KBr) ν / cm^{-1}: 3358 (m), 3099 (m), 2972 (m), 1664 (s), 1645 (s), 1630 (s), 1545 (s), 1522 (s), 1501 (s), 1447 (s), 1391 (s), 1371 (s), 1362 (s), 1252 (s), 1132 (s), 1078 (s); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 7.70 (d, 2H, J = 8.1 Hz, Ar), 7.41 (d, 1H, $J_{6',5'}$ = 7.2 Hz, H-6'), 7.36 (d, 2H, J = 8.2 Hz, Ar), 7.13 (d, 2H, J = 9.0 Hz, NH_2), 5.73 (d, 1H, $J_{5',6'}$ = 7.2 Hz, H-5'), 4.44 (m, 1H, CH-(CH_3)$_2$), 3.91 (m, 2H, CH_2-3), 3.67 (brs, 1H, CH-(CH_3)$_2$), 3.15 (m, 2H, CH_2-2), 2.37 (s, 3H, CH_3-Ts), 1.20 (dd, 12H, J = 10.7 Hz, J = 6.6 Hz, CH-(CH_3)$_2$); ^{13}C-NMR (150 MHz, DMSO-d_6) δ / ppm: 166.2 (s, C-4'), 162.1 (s, C-1), 155.8 (s, C-2'), 145.3 (d, C-6'), 141.5 (s, Ar), 141.4 (s, Ar), 129.3 (d, Ar), 125.5 (d, Ar), 94.1 (d, C-5'), 50.2 (d, CH-(CH_3)$_2$), 47.3 (d, CH-(CH_3)$_2$), 46.6 (t, CH_2-3), 31.8 (t, CH_2-2), 20.9 (q, CH_3-Ts), 20.1 (q, CH-(CH_3)$_2$), 19.6 (q, CH-(CH_3)$_2$). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{20}H_{29}N_5O_3S$ (M_r = 419.54) are: C, 57.26; H, 6.97; N, 16.69; S, 7.18; found: C, 57.40; H, 6.80; N, 16.65; S, 7.22.

N^1-CYCLOPENTYL-*N*^{2}-(4-METYLBENZENESULFONYL)-3-(4-AMINO-4-OXOPYRIMIDIN-2(1*H*)-YL)PROPANAMIDINE (33)

To a suspension of compound **31** (50 mg, 0.1 mmol) in dioxane (2 mL) ammonium hydroxide (4 mL) was added. After stirring the reaction for 24 hours at room temperature the solvent was evaporated under reduced pressure. The residue was purified by preparative chromatography (CH_2Cl_2/MeOH 9:1) to give product **33** as a white powder: 27 mg (66 %); m.p. = 122 °C; R_f = 0.60 (CH_2Cl_2 / MeOH 9 : 1); UV (MeOH): λ_{max} / nm: 214 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4,2), λ_{max} / nm: 238 (log ε / dm^{-3} mol^{-1} cm^{-1}: 4.2); IR (KBr) ν / cm^{-1}: 3433 (s), 2923 (m), 2852 (m), 1714 (s), 1652 (s), 1558 (s), 1496 (m) 1363 (s), 1267 (w), 1222 (w), 1143 (m); ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm: 8.84 (brs, 1H, NH-cyclopentyl), 7.70 (d, 2H, J = 8.2 Hz, Ar), 7.39 (d, 1H, $J_{6',5'}$ = 7.2 Hz, H-6'), 7.32 (d, 2H, J = 8.2 Hz, Ar), 7.06 (d, 2H, J = 7.07 Hz, NH_2), 5.66 (d, 1H, $J_{5',6'}$ = 7.2 Hz, H-5'), 4.00 (t, 2H, $J_{3,2}$ = 6.2 Hz, CH_2-3), 3.97 (m, 1H, CH-cyclopentyl), 2.94 (t, 2H, $J_{2,3}$ = 6.2 Hz, CH_2-2), 2.35 (s, 3H, CH_3-Ts), 1.60 (m, 8H, CH_2-cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ / ppm: 166.0 (s, C-4'), 164.3 (s, C-1), 155.6 (s, C-2'), 145.4 (d, C-6'), 141.5 (s, Ar), 141.5 (s, Ar), 129.2 (d, Ar), 125.6 (d, Ar), 93.6 (d, C-5'), 52.9 (d, CH-cyclopentyl) 45.6 (t, CH_2-3), 33.5 (t, CH_2-2), 31.4 (t, CH_2-cyclopentyl), 23.5 (t, CH_2-cyclopentyl), 20.9 (q, CH_3-Ts). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_{19}H_{25}N_5O_3S$ (M_r = 403.5) are: C, 56.56; H, 6.25; N, 17.36; S, 7.95; found: C, 56.40; H, 6.40; N, 17.22; S, 7.53.

Cell Culturing and MTT Test[16]

N-sulfonylamidino pyrimidine derivatives **1**–**28** were selected for preliminary *in vitro* cytotoxicity testing against normal Madine Darby canine kidney (MDCKI) 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), chronic myelogenous leukemia cells (K562) and human acute T cell leukemia cells (Jurkat).

The NCI-H358, Raji, HuT78, K562 and Jurkat cells were grown in RPMI 1640 medium (Gibco, EU) supplemented with 10 % heat-inactivated fetal bovine serum 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 MDCKI, 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 monolayers. 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 were prepared in high pure water at a concentration range 10^{-4}–10^{-7} mol dm^{-3}.

For the MTT test, the adherent cells, MDCK1, 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 GI_{50} value, defined as the concentration of compound achieving 50 % of cell growth inhibition was calculated and used to compare cytotoxicity among the compounds. Calculation of GI_{50} value curves and QC analysis is performed by using the Excel tools and GraphPadPrism software (La Jolla, CA), v. 5.03. Briefly, individual concentration effect curves are generated by plotting the logarithm of the concentration of tested compounds (X) *vs.* corresponding percent inhibition values (Y) using least squares fit. The best fit GI_{50} values are calculated using Log (inhibitor) versus normalized response - Variable slope equation, where *Y* = 100 / (1 + 10^((log GI50-X) * HillSlope)). QC criteria parameters (Z0, S:B, R2, HillSlope) were checked for every GI_{50} curve.

RESULTS AND DISCUSSION

Synthesis

Cu(I)-catalyzed 1,3-dipolar cycloadditions of azides with terminal alkynes (CuAAC) afford a 1,4-disubstituted 1,2,3-triazole. This powerful, widely used reaction[17,18] is the most representative example of click chemistry. Employment of electron-deficient phosphoryl or sulfonyl azides led to a path change in the copper-catalyzed reaction with 1-alkynes.[19] Proposed key intermediate ketenimine, which is generated *in situ* upon ring-opening of the corresponding copper-triazole intermediate, undergoes addition reactions with various nucleophiles such as amines, alcohols, water or heterocycle compounds to give amidines, imidates, amides and other coupled compounds.[20] These three component reactions allow access to biologically interesting compounds that are typically otherwise prepared by multistep functional group transformations.

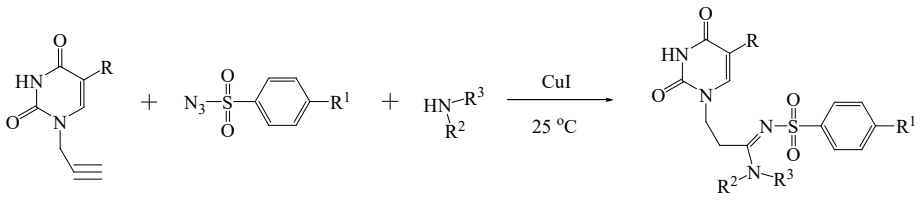
The synthesis of target *N*-sulfonylamidino uracils **15**–**26** was started by the preparation of 1-propargyl uracil.[21,22] First, the uracil was silylated with *N,O*-bis(trimethylsilyl)acetamide (BSA) in acetonitrile and then the silylated intermediate was treated with propargyl bromide, giving the *N*-1 alkylated product in 64 % yield.

The copper-catalyzed reactions of 1-propargyl uracil with three different commercially available sulfonyl azides (4-acetamidobenzenesulfonyl, 4-methylbenzenesulfonyl and 4-carboxybenzenesulfonyl) and amines or amine salts were performed in THF or dichloromethane at room temperature affording *N*-sulfonylamidino uracils **15**–**26** in the 34–69 % yields (Table 1). All reactions included 20 % molar excess of sulfonyl azide and amine, except the reaction with 4-carboxybenzenesulfonyl azide (entry 12–14), where a 100 % molar excess of amine was required. In the case of amine/ammonium salts (entry 10 and 11), additional triethylamine base in 50 % molar excess was used.[15]

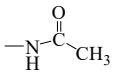
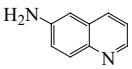
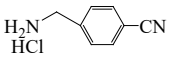
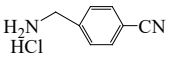
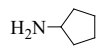
Table 1 provides a comparison between the previously reported[15] *N*-sulfonylamidino thymine derivatives **1**–**14** and newly synthesized compounds from the uracil series **15**–**26**. Compared to the propargyl thymine, in all reactions, a slightly lower reactivity of propargyl uracil is apparent, with the exception of compound **24** (entry 12, Table 1). Among used azides, reaction with 4-acetamidobenzenesulfonyl azide afforded products with highest yields.

As expected reactions of 1-propargyl uracil with 4-acetamidobenzenesulfonyl azide or 4-methylbenzenesulfonyl azide (tosyl azide) and with secondary amines (Table 1, compounds **15**, **16** and **22**) gave better yields compared to those with primary amines (Table 1,

Table 1. Three-component coupling reactions of 1-propargyl thymine and 1-propargyl uracil with various aromatic sulfonyl azides, amines, and ammonium salts.



1-14, R = CH_3 **15-26**, R = H

Entry	Azide-R^1	Amine	Product yield / % [Ref. 15],*		Product yield / %(*)	
1			**1**	78[a]	**15**	69[a]
2			**2**	64[a]	**16**	57[a]
3			**3**	54[a]	**17**	50[a]
4			**4**	58[a]	**18**	48[a]
5			**5**	54[a]	**19**	49[a]
6			**6**	45[b]	**20**	34[b]
7		NH_4Cl	**7**	56[b]	**21**	47[b]
8	—CH_3		**8**	54[a]	**22**	53[a]
9			**9**	45[a]	**23**	39[a]
10			**10**	30[b]	–	–
11		NH_4Cl	**11**	39[b]	–	–
12	—COOH		**12**	43[c]	**24**	46[c]
13			**13**	43[c]	**25**	37[c]
14			**14**	45[c]	**26**	42[c]

(*) Yields of analytically pure products.

(a) Method A: alkyne (1 mmol), sulfonyl azide (1.2 mmol), amine (1.2 mmol), CuI (0.1 mmol) in THF (2.0 mL) at 25 °C for 24 h.

(b) Method B: alkyne (1 mmol), sulfonyl azide (1 mmol), amine/ammonium salt (1 mmol), CuI (0.1 mmol) triethylamine (1.5 mmol) in CH_2Cl_2 (2–5 mL) at 25 °C for 24 h.

(c) Method C: alkyne (1 mmol), sulfonyl azide (1.2 mmol), amine (2 mmol), CuI (0.1 mmol) in THF (2.0 mL) at 25 °C for 24 h.

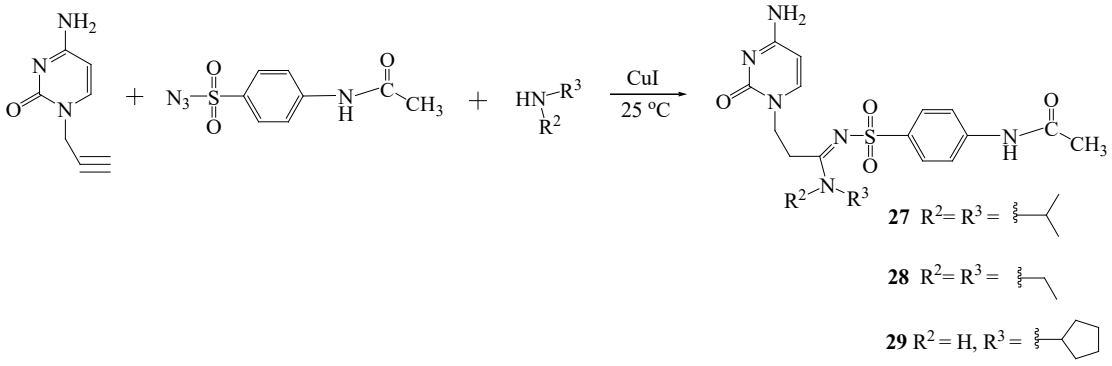
(–) unreacted 1-propargyl uracil was isolated.

compounds **17, 18** and **23**) and aromatic amine (Table 1, compound **19**). The reactions with 4-carboxybenzenesulfonyl azide afforded products **24–26** in lower yields (Table 1, entry 12–14). A similar trend has been noticed for thymine series. A significant difference between the reactivity of propargyl uracil and propargyl thymine was observed since the preparations of the uracil analogs of compounds **10** and **11**, failed. Although identical conditions were used, with the same reagents, there was no sign that the reaction occurred even at elevated temperatures, and the unreacted 1-propargyl uracil was isolated from the reaction mixture (> 90 %).

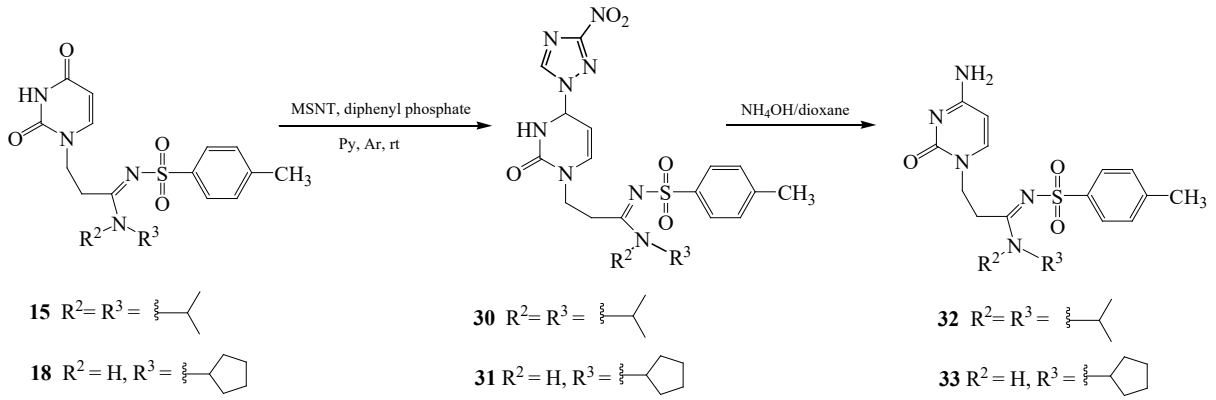
In the next step we decided to examine the conditions for the three-component coupling reactions with 1-propargyl cytosine, which was synthesized by the known condensation method of N^4-acetylcytosine with propargyl bromide.[23] The cytosine amino group was acetylated with acetic anhydride in pyridine[24] and obtained N^4-acetylcytosine was activated with K_2CO_3 in DMF and condensed with propargyl bromide. In the reaction mixture N1 and O2 regioisomers were obtained in the 96:4 ratio. N1 isomer was isolated by recrystallization from water and the acetyl group was readily removed by methanolic ammonia to give the desired *N*-1-propargyl cytosine.

Next, using the same conditions for Cu-catalyzed three-component reaction (Method A), 1-propargyl cytosine was reacted with 4-acetamidobenzenesulfonyl azide and secondary or primary amines giving desired products **27–29** in 45–62 % yields (Scheme 1). Cu-catalyzed three-component reactions of 1-propargyl cytosine and ammonium chloride (Method B) or 4-methylbenzenesulfonyl azide (Method A) failed to give any *N*-sulfonylamidine product and the starting material, 1-propargyl cytosine, was completely recovered.

Then we decided to solve this problem by known transformations of uracil into cytosine derivatives.[25–28] In these methods, the C4 carbonyl group of the uracil derivative is converted to a leaving group (chlorine, 1,2,4-triazole, *etc.*) which undergoes nucleophilic substitution with ammonia providing the corresponding cytosine derivative.



Scheme 1. Three-component coupling reactions of 1-propargyl cytosine [Method A: alkyne (1 mmol), sulfonyl azide (1.2 mmol), amine (1.2 mmol), CuI (0.1 mmol) in THF (2.0 mL) at 25 °C for 24 h].



Scheme 2. Synthesis of *N*-sulfonylamidino cytosine **32** and **33** by transformations of uracil derivatives.

The most successful method was with 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT).[27] The *N*-sulfonyl-amidino uracil derivatives **15** and **18** were treated with MSNT in the presence of diphenyl phosphate giving pyrimidine intermediates **30** and **31** in 86 % and 56 % yield, respectively. The latter compounds in the reaction with ammonium hydroxide in dioxane afforded desired *N*-sulfonylamidino cytosine derivatives **32** and **33** in 98 % and 66 % yield, respectively (Scheme 2).

In vitro Antiproliferative Screening

We have shown before that *N*-1-sulfonylpyrimidine derivatives have strong and selective antiproliferative activity on different human tumor cell lines *in vitro* and on xenograft model *in vivo*.[5–10] Amidines are important units in chemistry for the synthesis of heterocycles[29,30] and widely used in bioactive chemicals and drug molecular design.[31–33] We assumed the improved antiproliferative capacity of novel hybrid compounds could be obtained if

Table 2. Inhibitory effects of *N*-sulfonylamidine pyrimidine derivatives on the growth of normal and human tumor cells.

Comp.	GI_{50} (x 10^{-6} mol dm^{-3})[(a)]							
	Normal cells	Solid tumor cells			Leukemia and lymphoma cells			
	MDCK I	HeLa	Caco2	NCI-H358	Raji	K562	HuT78	Jurkat
1	> 100	> 100	> 100	> 100	> 100	> 100	> 100	54.1 ± 20.0
2	8.3 ± 1.8	8.9 ±1.8	8.4 ± 1.2	17.0 ± 4.8	13.1 ± 3.3	16.1 ± 3.4	> 100	37.2 ± 4.2
3	> 100	> 100	> 100	> 100	> 100	94.5 ± 6.4	> 100	76.7 ± 9.8
4	> 100	> 100	> 100	> 100	> 100	> 100	85.4 ± 4.1	79.9 ± 7.6
5	82.8 ± 17.8	> 100	> 100	> 100	> 100	> 100	> 100	44.8 ± 0.1
6	11.0 ± 3.4	9.8 ± 1.7	11.0 ± 4.6	16.4 ± 3.8	13.9 ± 3.5	15.7 ± 2.8	> 100	39.2 ± 2.5
7	> 100	> 100	> 100	> 100	> 100	> 100	> 100	61.9 ± 40.1
8	10.4 ± 4.6	8.6 ± 0.5	12.2 ± 2.9	15.4 ± 1.3	13.6 ± 4.0	15.7 ± 2.8	> 100	38.2 ± 3.2
9	9.0 ± 0.7	9.1 ± 2.0	10.8 ± 4.8	15.7 ± 3.6	12.8 ± 3.8	14.8 ± 0.6	> 100	38.0 ± 2.8
10	8.0 ± 0.4	7.9 ± 0.9	9.6 ± 0.3	17.3 ± 5.7	12.6 ± 2.7	13.8 ± 3.2	> 100	37.6 ± 2.1
11	> 100	52.9 ± 8.2	> 100	15.8 ± 3.1	69.9 ± 4.1	79.4 ± 26.0	> 100	38.3 ± 3.8
12	9.8 ± 5.6	7.7 ±1.3	11.3 ± 7.2	> 100	72.1 ± 0	2.4 ± 0	7.1 ± 0	4.1 ± 0
13	> 100	> 100	> 100	> 100	> 100	> 100	> 100	69.3 ± 0
14	> 100	> 100	> 100	> 100	89.7 ± 0	62.1 ± 0	21.9 ± 0	27.8 ± 0
15	> 100	> 100	> 100	> 100	85.4 ± 0	84.6 ± 4.1	> 100	57.5 ± 2.6
16	9.0 ± 1.8	8.1 ± 1.9	13.2 ± 0.7	15.4 ± 2.7	19.1 ± 10.8	15.3 ± 3.4	> 100	36.5 ± 1.7
17	> 100	> 100	> 100	> 100	75.3 ± 3.3	71.4 ± 37.0	79.9 ± 2.5	67.6 ± 23.1
18	> 100	> 100	> 100	> 100	> 100	> 100	> 100	69.5 ± 8.5
19	92.7 ± 5.6	> 100	> 100	> 100	77.0 ± 0	> 100	> 100	57.7 ± 13.9
20	9.2 ± 1.2	9.0 ± 2.3	12.5 ± 4.2	16.0 ± 3.8	14.4 ± 4.2	15.7 ± 2.8	> 100	38.3 ± 0.8
21	> 100	> 100	> 100	> 100	> 100	> 100	> 100	91.0 ± 3.3
22	8.3 ± 1.0	11.3 ± 4.7	9.3 ± 0.8	17.9 ± 6.3	13.1 ± 3.3	15.3 ± 3.4	> 100	37.2 ± 4.2
23	8.1 ± 0.1	11.0 ± 7.8	13.6 ± 0.8	18.0 ± 6.0	13.4 ± 2.9	15.6 ± 0.7	> 100	37.2 ± 1.8
24	> 100	> 100	> 100	> 100	> 100	48.7 ± 0	24.9 ± 0	38.2 ± 0
25	> 100	> 100	> 100	> 100	> 100	> 100	> 100	79.4 ± 8.8
26	> 100	> 100	> 100	> 100	82.5 ± 21.8	54.1 ± 0	16.0 ± 0	68.4 ± 25.2
27	11.3 ± 4.8	11.0 ± 4.2	18.2 ± 1.8	17.9 ± 6.0	15.6 ± 5.1	15.4 ± 2.1	> 100	37.9 ± 2.1
28	7.4 ±0.8	10.7 ± 4.1	16.6 ± 3.7	17.3 ± 6.8	12.3 ± 3.1	14.9 ± 3.9	> 100	36.3 ± 2.6
5-FU	55.1±3.3	8.2±1.9	5.9±0.7	8.0± 1.1	> 100	9.8±0.5	> 100	76.3± 11.4

[(a)] GI_{50} – Compound concentration that inhibited cell growth by 50 %. Exponentially growing cells were treated with compounds during 72-h period. Cytotoxicity was analyzed using MTT survival assay.

the hybrids contain an amidine unit, a sulfonyl group, and a pyrimidine nucleobase in the same structure.

In this study, *N*-sulfonylamidino pyrimidine derivatives **1**–**28** were selected for preliminary *in vitro* cytotoxicity testing against normal MDCKI cells, carcinoma cells (HeLa, Caco-2, NCI-H358), two lymphoma cells (Raji, HuT78), leukemia cells (K562, Jurkat) and in a parallel with 5-fluorouracil (5-FU) as a standard antitumor drug (Table 2). All cells were treated by investigated compounds in the range of concentration 10^{-7}–10^{-4} mol dm^{-3}.

As indicated in Table 2, two large groups of prepared *N*-sulfonylamidine-derived pyrimidine analogues showed great variation in the antiproliferative effect on tumor cell lines, depending on the cell line and structure of the tested compounds.

The first group of *N*-sulfonylamidino derivatives of thymine (**2**, **6**, **8**, **9**, **10**), uracil (**16**, **20**, **22**, **23**) and cytosine (**27**, **28**), which share the same or similar chemical characteristics and functional groups, shows strong antiproliferative effects on all treated cell lines. The GI_{50} ranged from 8 to 41 × 10^{-6} mol dm^{-3} for MDCK I, HeLa, Caco-2, NCI-H358, Raji, K562 and Jurkat cells, with exception of HuT78 cells where antiproliferative activity of tested compounds in applied highest concentration of 1 × 10^{-4} mol dm^{-3} was not observed.

The most prominent difference in the antitumor activity between thymine and uracil series was obtained between compounds **12** and **24**, having the same N1 substituent. The GI_{50} of normal MDCKI, tumor Caco-2, HeLa, HuT78, K562, and Jurkat cells induced by *N*-sulfonylamidino thymine **12** was between 2.4 × 10^{-6} and 11.3 × 10^{-6} mol dm^{-3}, whereas, as in the first group of tested compounds, antiproliferative activity on HuT78 cells in applied highest concentration of 10^{-4} mol dm^{-3} was not noticed. On the other hand, *N*-sulfonylamidino uracil **24** exhibits antiproliferative activity against lymphoma (HuT78 GI_{50} = 24.9 × 10^{-6} mol dm^{-3}) and leukemia (K562: GI_{50} = 48.7 × 10^{-6} mol dm^{-3}; Jurkat GI_{50} = 38.2 × 10^{-6} mol dm^{-3}) cell lines, while on normal (MDCK I) and tumor (HeLa, Caco2, NCI-H358, Raji) cell lines cytotoxic effects were not observed.

Compared to the first group of compounds, the second group of *N*-sulfonylamidino thymine (**1**, **3**–**5**, **7**, **13**) and uracil (**15**, **17**–**19**, **21**, **25**) derivatives, carrying the same N1 substituent, were mainly deprived of any inhibitory activities against the normal, solid tumor cell lines, leukemia and K562 lymphoma cell lines. Except compound **5** (GI_{50} = 44.8 × 10^{-6} mol dm^{-3}), all of them showed very weak antiproliferative capacity on the Jurkat cells (GI_{50} ~ 60–100 × 10^{-6} mol dm^{-3}).

In addition, *N*-sulfonylamidino thymine **14** displayed good cytostatic activities on Raji and Jurkat cell lines, with GI_{50} values 21.9 × 10^{-6} mol dm^{-3} and 27.8 × 10^{-6} mol dm^{-3}, respectively, while its structural analogue with uracil (**26**) shows strong antiproliferative effect on Raji cells (GI_{50} = 16.0 × 10^{-6} mol dm^{-3}).

CONCLUSIONS

In conclusion, the series of new aliphatic *N*-sulfonylamidino pyrimidine derivatives incorporating nucleobase, *N*-sulfonyl and amidine pharmacophores in the structure were synthesized by Cu(I)-catalyzed three-component coupling of 1-propargyl nucleobase, benzenesulfonyl azides and amines. New *N*-sulfonylamidino pyrimidine derivatives possess good inhibitory potential against tested tumor cells. These results stimulate further studies directed to investigate their mechanisms of action.

Acknowledgment. The authors gratefully acknowledge financial support from the Ministry of Science, Education and Sport of Croatia (Grants No098-0982914-2935, 219-0982914-2176), Croatian Science Foundation (Grant HRZZ-1477), J. J. Strossmayer University of Osijek supporting grant to Lj. Glavas-Obrovac (IZIP-2016-129) and VIF2016-MEFOS-25.

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