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Original Scientific Article

Synthesis of Novel Aliphatic *N*-sulfonylamidino Thymine Derivatives by Cu(I)-catalyzed Three-component Coupling Reaction†

Luka Krstulović,[a] Hamit Ismaili,[b] Miroslav Bajić,[a] Aleksandar Višnjevac,[c] Ljubica Glavaš-Obrovac,[d,e] and Biserka Žinić[f,*]

[a]*Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, HR-10000 Zagreb, Croatia*
[b]*Faculty of Mathematics and Natural Sciences, University of Pristina, Agim Ramadani n.n. 10000, Pristina, Kosovo*
[c]*Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia*
[d]*Department of Clinical Chemistry and Biochemistry, School of Medicine, J. J. Strossmayer University of Osijek, Huttlerova 4, HR-31000 Osijek, Croatia*
[e]*Clinical Hospital Centre Osijek, Clinical Institute of Nuclear Medicine and Radiation Protection, Huttlerova 4, HR-31000 Osijek, Croatia*
[f]*Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia*

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Abstract. A series of new aliphatic *N*-sulfonylamidino thymine derivatives containing nucleobase, *N*-sulfonyl and amidine pharmacophores in the structure were synthesized by Cu(I)-catalyzed three-component coupling of 1-propargyl thymine, benzenesulfonyl azides and amines or ammonium salts. Preliminary *in vitro* antitumor screening (human cervix adenocarcinoma -HeLa and leukemia cells - Jurkat) revealed promising activities of *N*,*N*-diethyl- (**2**) and *N*-4-cyanobenzyl- (**6**) derivatives of 4-acetamidobenzenesulfonyl amidine. (doi: 10.5562/cca2198)

Keywords: alkynes, azides, copper, nucleobases, *N*-sulfonylamidine

INTRODUCTION

Search for new and more efficient anticancer drugs still presents one of major challenges of modern medicine and medicinal chemistry.[1,2] Due to the vast biological diversity of cancer, including more than 100 different types, there is a constant urge to discover of new molecules possessing more specific anticancer activity and lower toxicity.[3] Sulfonamides are known as extremely useful pharmaceutical compounds exhibiting a wide range of biological activities.[4–7] We have recently shown that *N*-sulfonylpyrimidine derivatives of type **I** (Figure 1) possess strong antitumor activity under *in vitro* and *in vivo* conditions.[8–11] In comparison with 5-FU (5-fluorouracil), some of the *N*-sulfonylpyrimidine derivatives showed up to 10 times stronger growth inhibitory effects on a number of tested tumor cells while the effects on normal human fibroblasts were much less pronounced.[12] These types of nucleic base sulfonamides were found to inhibit DNA, RNA and protein synthesis and induce apoptosis in human tumor cells.[12,13] *In vivo* experiments showed that some *N*-sulfonylcytosine derivatives exhibited strong antitumor activity against mouse mammary carcinoma.[9,14] To further explore the biological potential of this type of molecules, their structure was modified by combining them with another potent anticancer pharmacophore - that of amidine. The amidine group is present in many compounds capable of interacting with a wide range of biological targets, resulting in anti-degenerative, anticancer and antimicrobial activities.[15–18] Here, we report on the synthesis of a series of novel aliphatic thymine derivatives **II** (Figure 1) containing *N*-sulfonylamidino fragment attached to the

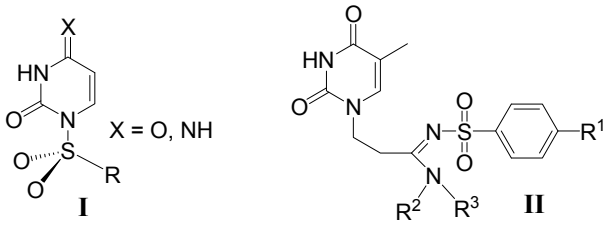

Figure 1. *N*-sulfonylpyrimidine **I** and *N*-sulfonylamidino thymine **II** derivatives.

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* Author to whom correspondence should be addressed. (E-mail: bzinic@irb.hr)

N1 position of thymine by ethylene spacer using the Cu(I)-catalyzed three-component coupling reaction of 1-propargyl thymine, selected benzenesulfonyl azides and amines or ammonium salts.

In this way, a series of potentially biologically active *N*-sulfonylamidino thymine derivatives **II**, representing a combination of pyrimidne nucleobase, *N*-sulfonyl group and amidine group pharmacophores, was prepared in moderate to good yields. Structures of *N*-sulfonylamidino thymines were approved by spectroscopic methods and X-ray crystallography. In preliminary *in vitro* screening, some of the prepared type **II** compounds showed promising anticancer activity against two selected human tumor cell lines. The results presented illustrate the versatility and potential of Cu(I) catalyzed three-component alkyne, sulfonyl azide, amine coupling for the preparation of *N*-sulfonylamidino nucleobase derivatives using propargylated nucleobase as the terminal alkyne component. The described synthetic approach also appears suitable for the preparation of libraries of *N*-sulfonylamidino nucleobase derivatives representing a new structural type of molecules with potential anticancer activity.

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}. 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. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on Bruker AV 300 and 600 MHz spectrometers 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.

5-Methyl-1-(prop-2-ynyl)pyrimidine-2,4(1*H*,3*H*)-dione (1-propargyl thymine)

To an acetonitrile suspension of thymine (2 g, 15.86 mmol), *N,O*-bis(trimethylsilyl)acetamide (BSA) (8.08 mL, 31.72 mmol) was added under argon and stirred at 80 °C for 30 min. The solution was cooled to room temperature and propargyl bromide (3.77 g, 31.72 mmol) was added. The reaction mixture was kept in dark for 14 days, acetonitrile was partly evaporated and then 5.0 mL of MeOH was added, inducing crystallization of 1-propargyl thymine (1.63 g, 63 %) m.p. = 152–154 °C (Ref. 34) m.p. = 157–158 °C); R_f = 0.77 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.32 (s, 1H, NH-3), 7.54 (s, 1H, H-6), 4.45 (d, 2H, J = 2.4 Hz, CH_2-1'), 3.35 (t, 1H, J = 2.5 Hz, CH-3'), 1.75 (s, 3H, CH_3-5); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 166.36 (s, C-4), 150.31 (s, C-2), 140.07 (d, C-6), 109.36 (s, C-5), 78.60 (s, C-2'), 75.55 (s, CH-3'), 36.26 (t, CH_2-1'), 11.84 (q, CH_3-5); IR (KBr) ν/cm^{-1}: 3406 (w), 3254 (s), 2126 (m), 1707 (s), 1691 (s), 1652 (s), 1473 (m), 1424 (s), 1356 (m), 1245 (m), 1136 (m), 933 (m).

General Procedures for the Preparation of *N*-Sulfonyl Amidines

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 mL), triethylamine (1.5 mmol) was slowly added and the colour of the suspension turned 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), amine nucleophile (2.4 mmol) was slowly added. The reaction mixture was stirred for 24 h (4 h in the case of compound **13**) 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 a water solution of $NaHCO_3$ (w = 5 %) 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-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl) propanamidine* ***(1)*** *Method A.* White solid (78 %); m.p. = 226 °C; R_f = 0,78 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 264 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4,7); ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm: 11.35 (brs, 1H, NH-3'), 10.24 (s, 1H, NH-Ac), 7.72 (s, 4H, Ph), 7.26 (s, 1H, H-6'), 4.33-4.26 (m, 1H, CH-(CH_3)$_2$), 3.91 (t, 2H, $J_{3,2}$ = 7.8 Hz, CH_2-3), 3.66 (m, 1H, CH-(CH_3)$_2$), 3.19 (t, 2H, $J_{2,3}$ = 7.4 Hz, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.77 (s, 3H, CH_3-5'), 1.19 (pt, 12H, J = 6.5 Hz, CH-(CH_3)$_2$); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.86 (s, CO-CH_3), 164.27 (s, C-4'), 161.66 (s, C-1), 150.93 (s, C-2'), 141.90 (s, Ph), 140.6 (d, C-6'), 138.11 (s, Ph), 126.51 (d, Ph), 118.32 (d, Ph), 109.11 (s, C-5'), 50.20 (d, CH-(CH_3)$_2$), 47.34 (d, CH-(CH_3)$_2$), 45.08 (t, CH_2-3), 31.21 (t, CH_2-2), 24.09 (q, CO-CH_3), 20.08 (q, CH-(CH_3)$_2$), 19.58 (q, CH-(CH_3)$_2$), 11.98 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3323 (m), 3182 (m), 3123 (m), 3006 (m), 2982 (m), 2838 (m), 1707 (s), 1685 (s), 1540 (s), 1375 (m), 1269 (m), 1085 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{22}H_{31}N_5O_5S$ × 0.5 H_2O (M_r = 486.58): C, 54.30; H, 6.63; N, 14.39; S, 6.59. Found: C, 54.14; H, 6.50; N, 14.43; S, 6.42.

N^1,N^1-*diethyl*-N^2-*(4-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4–dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-propanamidine* ***(2)*** *Method A.* White solid (64 %); m.p. = 230–232 °C; R_f = 0.55 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 264 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4,7); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.35 (brs, 1H, NH-3), 10.25 (s, 1H, NH-Ac), 7.75 (d, 2H, J = 8.9 Hz, Ph), 7.70 (d, 2H, J = 8.9 Hz, Ph), 7.24 (s, 1H, H-6'), 3.94 (t, 2H, $J_{3,2}$ = 7.2 Hz, CH_2-3), 3.48 (q, 2H, J = 6.6 Hz, CH_2-CH_3), 3.38 (q, 2H, J = 6.6 Hz, CH_2-CH_3), 3.13 (t, 2H, $J_{2',1'}$ = 7.3 Hz, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.76 (s, 3H, CH_3-5'), 1.15 (t, 3H, J = 7.1 Hz, CH_2-CH_3), 0.99 (t, 3H, J = 7.0 Hz, CH_2-CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.86 (s, CO-CH_3), 164.23 (s, C-4'), 162.89 (s, C-1), 150.91 (s, C-2'), 142.00 (s, Ph), 140.55 (d, C-6'), 138.15 (s, Ph), 126.58 (d, Ph), 118.39 (d, Ph), 109.14 (s, C-5'), 45.27 (t, CH_2-3), 43.09 (t, CH_2-CH_3), 42.96 (t, CH_2-CH_3), 29.75 (t, CH_2-2), 24.09 (q, CO-CH_3), 13.88 (q, CH_2-CH_3), 11.98 (q, CH_2-CH_3) 11.77 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3290 (m), 3124 (w), 3124 (w), 3035 (w), 2980 (w), 2840 (w), 1714 (m), 1663 (s), 1554 (s), 1255 (m), 1140 (m), 832 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{20}H_{27}N_5O_5S$ (M_r = 449.52): C, 53.44; H, 6.05; N, 15.58; S, 7.13. Found: C, 53.38; H, 5.65; N, 15.79; S, 6.96.

N^1-*isopropyl*-N^2-*(4-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-propanamidine* ***(3)*** *Method A.* White solid (54 %); m.p. = 216–217 °C; R_f = 0.50 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 263 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4.6); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.24 (brs, 1H, NH-3'), 10.28 (s, 1H, NH-Ac), 8.67 (brs, 1H, NH-CH), 7.73 (d, 2H, J = 9,3 Hz, Ph), 7.72 (d, 2H, J = 9.3 Hz, Ph), 7.19 (d, 1H, H-6'), 4.0 (t, 2H, $J_{3,2}$ = 6.3 Hz, CH_2-3), 3.87 (m, 1H, CH-(CH_3)$_2$), 2.88 (t, 2H, $J_{2,3}$ = 6.1 Hz, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.71 (s, 3H, CH_3-5'), 1.00 (d, 6H, J = 6.8 Hz, CH-(CH_3)$_2$); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.85 (s, CO-CH_3), 164.32 (s, C-4'), 163.62 (s, C-1), 150.69 (s, C-2'), 141.97 (s, Ph), 141.03 (d, C-6'), 138.15 (s, Ph), 126,65 (d, Ph), 118.80 (d, Ph), 108.33 (s, C-5'), 45.45 (t, CH_2-3), 43.14 (d, CH-(CH_3)$_2$), 33.22 (t, CH_2-2), 24.08 (q, CO-CH_3), 21.09 (q, CH-(CH_3)$_2$), 12.00 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3309 (m), 3124 (w), 3055 (w), 2983 (w), 1711 (s), 1675 (s), 1554 (s), 1384 (m), 1321 (m), 1250 (m), 1141 (m), 1090 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{19}H_{25}N_5O_5S$ × H_2O (M_r = 453.51): C, 50.32; H, 6.00; N, 15.44; S, 7.07. Found: C, 50.16; H, 5.61; N, 15.35; S, 6.94.

N^1-*cyclopentyl*-N^2-*(4-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-propanamidine* ***(4)*** *Method A.* White solid (58 %); m.p. = 155–157 °C; R_f = 0,77 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 264 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4.5); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.24 (brs, 1H, NH-3'), 10.24 (s, 1H, NH-Ac), 8.70 (d, 1H, J = 6.6 Hz, NH-cyclopentyl), 7.75 (d, 2H, J = 9.0 Hz, Ph), 7.69 (d, 2H, J = 9.0 Hz, Ph), 7.16 (s, 1H, H-6'), 3.98 (t, 2H, $J_{1',2'}$ = 5.8 Hz, CH_2-3), 3.98 (m, 1H, CH-cyclopentyl), 2.88 (t, 2H, $J_{2',1'}$ = 5.9 Hz, CH_2-2), 2.07 (s, 3H, CO-CH_3), 1.70 (s, 3H, CH_3-5'), 1.29-1.81 (m, 8H, CH_2-cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.83 (s, CO-CH_3), 164.30 (s, C-1), 164.08 (s, C-4'), 150.68 (s, C-2'), 141.94 (s, Ph), 140.93 (d, C-6'), 138.16 (s, Ph), 126.66 (d, Ph), 118.32 (d, Ph), 108.33 (s, C-5'), 52.87 (d, CH-cyclopentyl) 45.44 (t, CH_2-3), 33.11 (t, CH_2-2), 31.35 (t, CH_2-cyclopentyl), 24.09 (q, CO-CH_3), 23.49 (t, CH_2-cyclopentyl),12.00 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3327 (s), 3012 (m), 2959 (m), 2836 (w), 1696 (s), 1672 (s), 1568 (s), 1521 (s), 1316 (m), 1255 (s), 1148 (s), 1102 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{21}H_{27}N_5O_5S$ (M_r = 461.53): C, 54.65; H, 5.90; N, 15.17; S, 6.95. Found: C, 54.36; H, 5.82; N, 15.08; S, 6.66.

N^1-*(quinolin-6-yl)*-N^2-*(4-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-propanamidine* ***(5)*** *Method A.* Skin-colored solid (54 %); m.p. = 219–221 °C; R_f = 0.40 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 263 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4.5); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.26 (brs, 1H, NH-3'), 10.68 (brs, 1H, NH-quinolinyl), 10.29 (s, 1H, NH-Ac), 8.84 (d, 1H, J = 3.7 Hz, Ph), 8.11 (d, 2H, J = 13.6 Hz, Ph), 7.96 (d, 1H, J = 8.9 Hz, Ph) 7.85 (d, 2H, J = 8.8 Hz, Ph), 7.76 (d, 2H, J = 8.8 Hz, Ph), 7.70 (m, 1H,

Ph), 7.50 (m, 1H, Ph), 7.37 (s, 1H, H-6'), 4.17 (t, 2H, $J_{1',2'}$ = 5.6 Hz, CH_2-3), 3.20 (t, 2H, $J_{2',1'}$ = 5.5 Hz, CH_2-2), 2.08 (s, 3H, CO-CH_3), 1.68 (s, 3H, CH_3-5'); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.93 (s, CO-CH_3), 164.34 (s, C-4'), 163.19 (s, C-1), 150.92 (s, C-2'), 150.07 (d, Ph), 145.32 (s, Ph), 142.43 (s, Ph), 141.04 (d, C-6'), 137.08 (s, Ph), 135.61 (d, Ph), 129.27 (d, Ph), 127.68 (s, Ph) 126.95 (d, Ph), 125.22 (d, Ph), 121,97 (d, Ph), 119.13 (d, Ph), 118.45 (d, Ph), 108.64 (s, C-5') 45.62 (t, CH_2-3), 34.15 (t, CH_2-2), 24.12 (q, CO-CH_3), 11.98 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3486 (m), 3292 (m), 3066 (m), 2956 (m), 2770 (m), 1670 (s), 1546 (s), 1281 (s), 1149 (s), 1090 (s). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{25}H_{24}N_6O_5S \times 1.5\ H_2O$ (M_r = 547.58): C, 54.83; H, 4.97; N, 15.34; S, 5.85. Found: C, 55.21; H, 4.94; N, 15.18; S, 5.68.

N^1*-(4-cyanobenzyl)*-N^2*-(4-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine* ***(6)*** *Method B.* White solid (45 %); m.p. = 142–145 °C; R_f = 0.54 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 237 (log ε/dm^3 mol^{-1} cm^{-1}: 4.7); λ_{max}/nm: 264 (log ε/ dm^{-3} mol^{-1}cm^{-1}: 4,7) ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.25 (brs, 1H, NH-3'), 10.25 (s, 1H, NH-Ac), 9.34 (brs, 1H, N<u>H</u>-CH_2), 7.72 (d, 2H, J = 8.0 Hz, Ph), 7.66 (d, 2H, J = 8.7 Hz, Ph), 7.60 (d, 2H, J = 8.7 Hz, Ph) 7.36 (d, 2H, J = 8.0 Hz, Ph), 7.21 (s, 1H, H-6'), 4.37 (d, 2H, J = 5.4 Hz, NH-C<u>H</u>$_2$), 4.01 (t, 2H, $J_{2,3}$ = 6.0 Hz, CH_2-3), 3.00 (t, 2H, $J_{3,2}$ = 6.3 Hz, CH_2-2), 2.07 (s, 3H, CO-C<u>H</u>$_3$), 1.68 (s, 3H, CH_3-5'); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.92 (s, <u>C</u>O-CH_3), 165.18 (s, C-4'), 164.35 (s, C-1), 150.78 (s, C-2'), 143.24 (d, Ph), 142.11 (s, Ph), 140.90 (d, C-6'), 137.64 (s, Ph), 132.21 (s, Ph), 128.57 (d, Ph), 126.68 (d, Ph), 118.77 (s, CN), 118.33 (d, Ph), 109.89 (s, Ph), 108.67 (s, C-5'), 45.55 (t, NH-<u>C</u>H$_2$), 44.57 (t, CH_2-3), 33.04 (t, CH_2-2), 24.12 (q, CO-<u>C</u>H$_3$), 12.00 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3333 (s), 3110 (m), 3059 (m), 2922 (m), 2860 (m), 2230 (w), 1675 (s), 1562 (s), 1246 (m), 1145 (m), 1091 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{20}H_{24}N_6O_5S \times H_2O$ (M_r = 526.56): C, 54.74; H, 4.98; N, 15.95; S, 6.09. Found: C, 55.12; H, 4.71; N, 15.88; S, 5.95.

N-*(4-acetoamidobenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine* ***(7)*** *Method B.* White solid (56 %); m.p. = 219 °C; R_f = 0,47 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 264 (logε/dm^3 mol^{-1} cm^{-1}: 4.5); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.22 (s, 1H, NH-3'), 10.28 (s, 1H, N<u>H</u>-Ac), 8.67 (s, 1H, NH_2), 7.91 (s, 1H, NH_2), 7.77 (d, 2H, J = 8.8 Hz, Ph), 7.71 (d, 2H, J = 8,8 Hz, Ph), 7.18 (s, 1H, H-6'), 3.80 (t, 2H, $J_{3,2}$ = 6,1 Hz, CH_2-3), 2.57 (t, 2H, $J_{2,3}$ = 6.3 Hz, CH_2-2), 2.08 (s, 3H, CO-CH_3), 1.62 (s, 3H, CH_3-5'); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 168.89 (s, <u>C</u>O-CH_3), 165.89 (s, C-4'), 164.21 (s, C-1), 150.67 (s, C-2'), 142.55 (s, Ph), 141.59 (d, C-6'), 136.16 (s, Ph), 127.12 (d, Ph), 118.32 (d, Ph), 108.01 (s, C-5'), 44.92 (t, CH_2-3), 34.81 (t, CH_2-2), 24.10 (q, CO-<u>C</u>H$_3$), 11.89 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3366 (s), 3197 (s), 3096 (m), 3050 (m), 2960 (m), 1669 (s), 1573 (s), 1255 (s), 1128 (s), 1074 (s). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{16}H_{19}N_5O_5S$ (M_r = 393.42): C, 48.85; H, 4.87; N, 17.80. Found: C, 48.87; H, 4.90; N, 17.66.

N^1*,N*^{1}*-diisopropyl-N*^{2}*-(4-methylbenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-propanamidine* ***(8)*** *Method A.* White solid (54 %); m.p. = 243–245 °C; R_f = 0.78 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 252 (log ε/dm^3 mol^{-1} cm^{-1}: 4.3); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.35 (brs, 1H, NH-3'), 7.68 (d, 2H, J = 8.0 Hz, Ph), 7.35 (d, 2H, J = 8.0 Hz, Ph), 7.26 (s, 1H, H-6'), 4.27 (m, 1H, C<u>H</u>-$(CH_3)_2$), 3.92 (t, 2H, J = 7.3 Hz, CH_2-3), 3.66 (m, 1H, C<u>H</u>-$(CH_3)_2$), 3.19 (t, 2H, J = 7.2 Hz, CH_2-2), 2.36 (s, 3H, CH_3-Ts), 1.76 (s, 3H, CH_3-5'), 1.19 (pt, 12H, J = 5.8 Hz, CH-(C<u>H</u>$_3$)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 164.27 (s, C-4'), 161.81 (s, C-1), 150.93 (s, C-2'), 141.5 (s, Ph), 141.4 (s, Ph), 140.59 (s, C-6'), 129.24 (d, Ph), 125.48 (d, Ph), 109.11 (s, C-5'), 50.24 (d, <u>C</u>H-$(CH_3)_2$), 47.35 (d, <u>C</u>H-$(CH_3)_2$), 45.08 (t, CH_2-3), 31.30 (t, CH_2-2), 20.91 (q, CH_3-Ts), 20.09 (q, CH-(<u>C</u>H$_3$)$_2$), 19.57 (q, CH-(<u>C</u>H$_3$)$_2$),11.99 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3157 (w), 3042 (w), 2979 (w), 2933 (w), 1686 (s), 1553 (s), 1463 (m), 1344 (m), 1266 (s), 1136 (m), 1085 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{21}H_{30}N_6O_5S$ (M_r = 434.55): C, 58.04; H, 6.96; N, 12.89. Found: C, 57.73; H, 6.64; N, 12.85.

N^1*-cyclopentyl-*N^2*-(4-methylbenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine* ***(9)*** *Method A.* White solid (45 %); m.p. = 216–219 °C ; R_f = 0.77 (CH_2Cl_2/MeOH 20:1); UV (MeOH): λ_{max}/nm: 212 and 241 (log ε/dm^3 mol^{-1} cm^{-1}: 3.9 and 3.9), ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.21 (s, 1H, NH-3'), 8.69 (d, 1H, J = 6.4 Hz, N<u>H</u>-cyclopentyl), 7.70 (d, 2H, J = 8.5 Hz, Ph), 7.33 (d, 2H, J = 8.0 Hz, Ph), 7.16 (s, 1H, H-6'), 3.99 (t, 2H, J = 6.4 Hz, CH_2-3), 3.99 (m, 1H, C<u>H</u>-cyclopentyl), 2.90 (t, 2H, J = 6.1 Hz, CH_2-2), 2.36 (s, 3H, C<u>H</u>$_3$-Ts), 1.74 (m, 2H, C<u>H</u>$_2$-cyclopentyl), 1.71 (s, 3H, CH_3-5'), 1.51-1.39 (m, 6H, C<u>H</u>$_2$-cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 164.28 (s, C-4'), 164.20 (s, C-1), 150.66 (s, C-2'), 141.53 (s, Ph), 141.46 (s, Ph), 140.93 (d, H-6'), 126.20 (d, Ph), 125.01 (d, Ph), 108.33 (s, C-5'), 52.89 (d, <u>C</u>H-cyclopentyl) 45.45 (t, CH_2-3), 33.13 (t, CH_2-2), 31.34 (t, <u>C</u>H$_2$-cyclopentyl), 23.49 (t, <u>C</u>H$_2$-cyclopentyl), 20.89 (q, <u>C</u>H$_3$-Ts), 12.00 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3323 (s), 3142 (w), 3021 (w), 2960 (w), 2833 (w), 1692 (s), 1674 (s), 1559 (s), 1429 (m), 1339 (m), 1262 (s), 1149 (s), 1090 (m), 1047 (m) 717 (m), 701 (m), 683 (m), 600 (w). *Anal. Calcd*. mass fractions of elements,

w/%, for $C_{20}H_{26}N_4O_4S$ (M_r = 418.51): C, 57.46; H, 6.26; N, 13.39; S, 7.66. Found: C, 57.22; H, 6.24; N, 13.45; S, 7.58.

N^1-*(4-cyanobenzyl)*-N^2-*(4-methylbenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-propanamidine (10) Method B.* White solid (30 %); m.p. = 180–183 °C; R_f = 0.45 (CH_2Cl_2/MeOH 20:1); UV (MeOH): λ_{max}/nm: 230 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4.3); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.24 (s, 1H, NH-3'), 9.36 (brs, 1H, N<u>H</u>-CH_2), 7.73 (d, 2H, J = 7.7 Hz, Ph), 7.56 (d, 2H, J = 7.7 Hz, Ph), 7.37 (d, 2H, J = 7.7 Hz, Ph), 7.28 (d, 2H, J = 7.7 Hz, Ph), 7.22 (s, 1H, H-6'), 4.37 (d, 2H, NH-C<u>H</u>$_2$), 4.02 (t, 2H, $J_{3,2}$ = 6.4 Hz, CH_2-3), 3.03 (t, 2H, $J_{3,2}$ = 6.4 Hz, CH_2-2), 2.36 (s, 3H, C<u>H</u>$_3$-Ts), 1.68 (s, 3H, CH_3-5'); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 165.25 (s, C-4'), 164.29 (s, C-1), 150.73 (s, C-2'), 143.24 (s, Ph), 141.69 (s, Ph), 140.95 (d, C-6), 140.82 (s, Ph), 132.19 (d, Ph), 129.13 (d, Ph), 128.48 (d, Ph), 125,57 (d, Ph), 118.76 (s, CN), 109.85 (s, Ph), 108.62 (s, C-5') 45.51 (t, NH-<u>C</u>H$_2$), 44.55 (t, CH_2-3), 33.01 (t, CH_2-2), 20.89 (q, <u>C</u>H$_3$-Ts), 11.99 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3326 (m), 3210 (w), 3097 (w), 2228 (w), 1692 (s), 1674 (s), 1651 (s), 1256 (m), 1139 (m), 1097 (m). *Anal. Calcd*. mass fractions of elements, *w*/%, for $C_{23}H_{23}N_5O_4S$ (M_r = 465.52): C, 59.34; H, 4.98; N, 15.04; S, 6.89. Found: C, 59.25; H, 5.14; N, 14.65; S, 6.65.

N-*(4-methylbenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine **(11)** Method B.* White solid (39 %); m.p. = 205–207 °C; R_f = 0.33 (CH_2Cl_2/MeOH 20:1); UV (MeOH): λ_{max}/nm: 236 and 268 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 3.4 and 3.7); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.20 (s, 1H, NH-3'), 8.68 (brs, 1H, NH_2), 7.94 (s, 1H, NH_2), 7.72 (d, 2H, J = 8.5 Hz, Ph), 7.33 (d, 2H, J = 8.1 Hz, Ph), 7.18 (d, 1H, H-6'), 3.73 (t, 2H, J = 6.6 Hz, CH_2-3), 2.60 (t, 2H, J = 6.4 Hz, CH_2-2), 2.36 (s, 3H, C<u>H</u>$_3$-Ts), 1.61 (s, 3H, CH_3-5'); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 166.01 (s, C-4'), 164.19 (s, C-1), 150.67 (s, C-2'), 142.29 (s, Ph), 141.5 (d, C-6), 139.59 (s, Ph), 129.27 (d, Ph), 126.02 (d, Ph), 108.0 (s, C-5'), 44.82 (t, CH_2-3), 34.80 (t, CH_2-2) 20.95 (q, <u>C</u>H$_3$-Ts), 11.88 (q, CH_3-5') ; IR (KBr) ν/cm^{-1}: 3403 (m), 3314 (m), 3217 (m), 2992 (m), 2832 (m), 1697 (s), 1657 (s), 1553 (m), 1463 (m), 1279 (s), 1144 (s), 1086 (m). *Anal. Calcd*. mass fractions of elements, *w*/%, for $C_{15}H_{18}N_4O_4S$ (M_r = 350.38): C, 51.41; H, 5.17; N, 15.99 S, 9.15. Found: C, 51.72; H, 5.15; N, 16.26; S, 9.28.

N^1,N^1-*diisopropyl*-N^2-*(4-carboxybenzenev-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine **(12)** Method C.* White solid (43 %); m.p. = 246–249 °C; R_f = 0.42 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 222 and 258 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4.5 and 4.6); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 13.35 (brs, 1H, COOH), 11.34 (s, 1H, NH-3'), 8.09 (d, 2H, J = 8.6 Hz, Ph), 7.91 (d, 2H, J = 8.5 Hz, Ph), 7.28 (s, 1H, H-6'), 4.30 (m, 1H, C<u>H</u>-$(CH_3)_2$), 3.94 (t, 2H, $J_{3,2}$ = 6.9 Hz, CH_2-3), 3.69 (m, 1H, C<u>H</u>-$(CH_3)_2$), 3.22 (t, 2H, $J_{2,3}$ = 7.4 Hz, CH_2-2), 1.76 (s, 3H, CH_3-5'), 1.19 (pt, 12H, J = 5.6 Hz, CH-(C<u>H</u>$_3$)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 166.33 (s, COOH) 164.26 (s, C-4'), 162.10 (s, C-1), 150.94 (s, C-2'), 147.52 (s, Ph), 140.63 (d, C-6'), 133.29 (s, Ph), 129.86 (d, Ph), 125.75 (d, Ph), 109.11 (s, C-5'), 50.46 (d, <u>C</u>H-$(CH_3)_2$), 47.48 (d, <u>C</u>H-$(CH_3)_2$), 45.09 (t, CH_2-3), 31.53 (t, CH_2-2), 19.99 (q, CH-(<u>C</u>H$_3$)$_2$), 19.53 (q, CH-(<u>C</u>H$_3$)$_2$), 11.97 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3097 (w), 3049 (w), 3014 (w), 2974 (w), 2933 (w), 1718 (s), 1689 (s), 1542 (s), 1452 (m), 1365 (m), 1269 (s), 1083 (m). *Anal. Calcd*. mass fractions of elements, *w*/%, for $C_{21}H_{28}N_4O_6S$ x 0.25 H_2O (M_r = 469.04): C, 53.26; H, 6.17; N, 11.83; S, 6.77. Found: C, 53.50; H, 6.49; N, 11.56; S, 6.95.

N^1-*isopropyl*-N^2-*(4-carboxybenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine **(13)** Method C.* White solid (43 %); m.p. = 245–248 °C; R_f = 0.22 (CH_2Cl_2/MeOH 9:1); UV (MeOH): λ_{max}/nm: 245 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4.4), ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 11.22 (brs, 1H, NH-3'), 8.81 (d, 1H, J = 7.4 Hz, N<u>H</u>-CH), 8.01 (d, 2H, J = 8.4 Hz, Ph), 7.82 (d, 2H, J = 8.1 Hz, Ph), 7.21 (d,1H, J = 1.2 Hz, H-6), 4.00 (t, 2H, $J_{3,2}$ = 6.3 Hz, CH_2-3), 3.88 (brs, 1H, C<u>H</u>-$(CH_3)_2$), 2.91 (t, 2H, $J_{2,3}$ = 6.3 Hz, CH_2-2), 1,71 (s, 3H, CH_3-5'), 1.01 (d, 12H, J = 6.6 Hz, CH-(C<u>H</u>$_3$)$_2$); ^{13}C-NMR (150 MHz, DMSO-d_6) δ/ppm: 167.07 (s, COOH), 164.25 (s, C-4'), 163.86 (s, C-1), 150.65 (s, C-2'), 145.72 (s, Ph), 140.95 (d, C-6'), 129.44 (d, Ph), 125.18 (d, Ph), 108.33 (s, C-5'), 45.41 (t, CH_2-3), 43.23 (d, <u>C</u>H-$(CH_3)_2$), 33.29 (t, CH_2-2), 21.01 (q, CH-(<u>C</u>H$_3$)$_2$), 11.91 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3317 (m), 3060 (w), 2975 (w), 2927 (w), 2821 (w), 1697 (s), 1556 (s), 1467 (m), 1334 (m), 1253 (m), 1147 (m), 1126 (m), 1093 (m), 1039 (m). *Anal. Calcd*. mass fractions of elements, *w*/%, for $C_{18}H_{22}N_4O_6S \times H_2O$ (M_r = 440.47): C, 49.08; H, 5.49; N, 12.71; S, 7.27. Found: C, 48.74; H, 5.26; N, 12.24; S, 7.46.

N^1-*cyclopentyl*-N^2-*(4-carboxybenzene-1-sulfonyl)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propanamidine **(14)** Method C.* White solid (45 %); m.p. = 234-237 °C; R_f = 0.37 (CH_2Cl_2/MeOH 20:1); UV (MeOH): λ_{max}/nm: 218 and 247 ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}: 4,6 and 4,6); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 13.32 (brs, 1H, COOH), 11.24 (s, 1H, NH-3'), 8.88 (d, 1H, J = 6.6 Hz, N<u>H</u>- cyclopentyl), 8.08 (d, 2H, J = 8.5 Hz, Ph), 7.93 (d, 2H, J = 8.5 Hz, Ph), 7.20 (pd, 1H, J = 1.2 Hz, H-6'), 4.01 (t, 2H, $J_{3,2}$ = 6.0 Hz, CH_2-3), 4.01 (m, 1H, C<u>H</u>- cyclopentyl), 2.93 (t, 2H, $J_{2,3}$ = 6.1 Hz, CH_2-2), 1.76 (m, 2H, C<u>H</u>$_2$- cyclopentyl), 1.71 (s, 3H, C<u>H</u>$_3$-5'), 1.58-1.33 (m, 6H, C<u>H</u>$_2$- cyclopentyl); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm: 166.35 (s, COOH), 164.57 (s,

C-4'), 164.31 (s, C-1), 150.71 (s, C-2'), 147.66 (s, Ph), 140.97 (d, H-6'), 133.41 (s, Ph) 129.83 (d, Ph), 125.88 (d, Ph), 108.39 (s, C-5'), 53.06 (d, CH- cyclopentyl) 45.49 (t, CH_2-3), 33.34 (t, CH_2-2), 31.35 (t, CH_2-cyclopentyl), 23.53 (t, CH_2- cyclopentyl), 12.00 (q, CH_3-5'); IR (KBr) ν/cm^{-1}: 3326 (m), 3139 (w), 3045 (w), 2958 (w), 2869 (w), 2810 (w), 1698 (s), 1678 (s), 1552 (s), 1284 (m), 1147 (m), 1087 (m). *Anal. Calcd*. mass fractions of elements, w/%, for $C_{20}H_{24}N_4O_6S \times 0{,}25\ H_2O$ (M_r = 453.00): C, 53.03; H, 5.45; N, 12.36; S, 7.08. Found: C, 53.00; H, 5.63; N, 11.96; S, 7.02.

X-ray Structures

Crystal data, data collection and refinement parameters are summarized in Table 1. Colourless prisms suitable for data collection were obtained by slow evaporation of a 1:1 mixture of methanol and dichloromethane at 4 °C. Data collection was performed on a prism 0.3 × 0.2 × 0.2 mm, on an Oxford Diffraction Xcalibur Nova R diffractometer with a microfocusing Cu tube (λ = 1.54179 Å). Data reduction and cell refinement were carried out using the CRYSALIS PRO software.[19] Intensities were measured at room temperature since crystals do not show any sign of decay. The structure was solved using direct methods with SHELXS86[20] and refined using full matrix least-squares refinement based on F2, with SHELX97.[21] Molecular illustrations were prepared with ORTEP-3,[22] and MERCURY[23] included into the WinGX package.[24] All non-solvent non-hydrogen atoms were refined anisotropically. A water molecules oxygen atom with a population of 0.25 was refined isotropically. All hydrogen atoms were included in their geometrically calculated positions and refined according to the riding model.

Cell culturing and MTT test[25]

N-sulfonylamidino thymine derivatives **2** and **6** were selected for preliminary *in vitro* testing on cytotoxicity using normal Madine-Darby canine kidney (MDCKI) cells, human cervix adenocarcinoma (HeLa), and human T-cell leukemia (Jurkat) cell lines. HeLa and MDCKI cells were grown in DMEM medium (Gibco, EU) while Jurkat cells were grown in RPMI 1640 medium (Gibco, EU). Both media were supplemented with 10 % heat-inactivated fetal bovine serum-FBS (Gibco, EU), 2×10^{-3} M glutamine (Gibco, EU), 1×10^{-3} M sodium pyruvate (Gibco, EU), 1×10^{-2} M HEPES (Sigma-Aldrich, USA) and 100 U/0.1 mg antibiotic/antimycotic (Gibco, EU). Cells were grown on 37 °C, with 5 % CO_2 gas in humidified CO_2 incubator (ShelLab, Sheldon Mfg. Inc., USA). Trypan blue dye exclusion method was used to assess cell viability. Tested compounds were dissolved in dimethyl sulfoxide as a 1×10^{-2} M stock solution. Working dilutions were prepared in high pure water at a concentration range $10^{-3}-10^{-6}$ mol dm^{-3}.

Table 1. Crystallographic data

Structure	**1**
Moiety formula	$C_{22}H_{31}N_5O_5S$, 0.25 (H_2O)
Formula weight / g mol^{-1}	481.59
Space group	*C2/c*
a / Å	50.8502 (19)
b / Å	5.9521 (1)
c / Å	52.055 (2)
β / °	161.773 (1)
V / Å^3	4928.0 (3)
Z	8
ρ_{calc} / g cm^{-1}	1.298
μ(CuK_α) / mm^{-1}	1.531
Absorption correction	multiscan
F(000)	2048
θ_{max} / °	62.07
No. refl. measured	14320
No. refl. unique	3827
No. refl. observed [$I > 2\sigma(I)$]	3318
R_{int}	0.0317
R_σ	0.0194
Parameters	290
R_1 [$I > 2\sigma(I)$]	0.0590
wR_2, all	0.1898
S	1.056
ρ_{max}, ρ_{min} / e Å^{-3}	0.60, –0.29

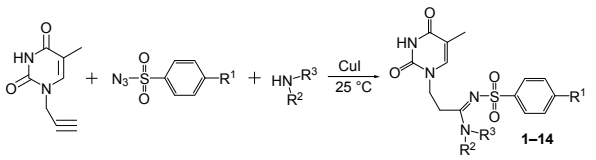
For the MTT test, cells were seeded on 96 micro well flat bottom plates (Greiner, Austria) at 2×10^4 cells/mL. After 72 hours of incubation with the tested compounds MTT (Merck, Germany) was added. DMSO (Merck, Germany) was used to dissolve the formed MTT-formazane crystals. Absorbency was measured at 570 nm on Stat fax 2100 plate reader (Awareness Technology Inc. USA). All experiments were performed three times in triplicates. The IC_{50} value, defined as the concentration of compound (1×10^{-6} M) achieving 50 % of cell growth inhibition, was calculated and used to compare cytotoxicity among the compounds.

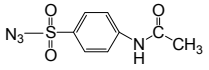
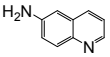
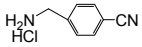
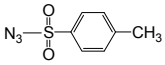
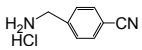
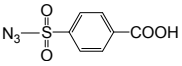
RESULTS AND DISCUSSION

Synthesis.

Click chemistry developed by Meldal[26] and Sharpless[27] involving the Cu(I)-catalyzed 1,3-dipolar azide alkyne cycloaddition into 1,4-disubstituted 1,2,3-triazoles has found extensive application in the construction of complex molecules of interest in materials,[28] biological[29] and medicinal[30] chemistry. The scope and potential of

Table 2. Three-component coupling reactions of 1-propargyl thymine with various aromatic sulfonyl azides, amines and ammonium salts.



Product	Azide	Amine	Yield / %[a]
1			78[b]
2			64[b]
3			54[b]
4			58[b]
5			54[b]
6			45[c]
7		NH_4Cl	56[c]
8			54[b]
9			45[b]
10			30[c]
11		NH_4Cl	39[c]
12			43[d]
13			43[d]
14			45[d]

[a] Yields of analytically pure products.
[b] 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.
[c] 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.
[d] Method C: alkyne (1 mmol), sulfonyl azide (1.2 mmol), amine (2.4 mmol), CuI (0.1 mmol) in THF (2.0 mL) at 25 °C for 24 h.

the latter reaction have been extended by a recent discovery of Chang and co-workers.[31,32] They have found that by reacting alkynes with electron deficient azides such as acyl, sulfonyl and phosphoryl azides and primary or secondary amines, amidines are formed in excellent yields under mild reaction conditions. The reaction is of wide scope and enables an easy one-pot access to amidines, which could be otherwise conventionally prepared by functional group transformation from amides, thioamides, nitriles, isonitriles or oximes.[33] The three component reaction is shown to proceed *via* the ketenimine intermediate, generated in situ by the Cu(I)-catalyzed coupling of 1-alkynes and sulfonyl azides upon the release of N_2.[34]

The latter reaction has been selected as the most promising approach to the series *N*-sulfonylamidino thymine derivatives **II** (Figure 1), starting from easily accessible 1-propargyl thymine as the alkyne component, commercial 4-acetamidobenzenesulfonyl, 4-methylbenzenesulfonyl and 4-carboxybenzenesulfonyl azides and various primary and secondary amines. The starting 1-propargyl thymine was obtained by a slight modification of the Woodman method.[35] For this, thymine was activated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in acetonitrile and treated with propargyl bromide, giving exclusively the *N*-1 alkylated product in 63 % yield. Cu(I)-catalyzed three-component coupling reactions of 1-propargyl thymine with three different commercially available benzenesulfonyl azides and primary and secondary amines were conducted at room temperature in THF. In this way, *N*-alkyl- (**3**, **4**, **5**, **9**, **13**, **14**) and *N,N*-dialkyl-(**1**, **2**, **8**, **12**) sulfonylamidine derivatives were prepared in the 43–78 % yields (Table 2). In the couplings, 20 % molar excess of the sulfonyl azide and amine component was used. In the reactions with 4-carboxybenzenesulfonyl azide possessing a free carboxylic group addition of 140 % molar excess of amine was used. Benzenesulfonyl amidine products **12**, **13** and **14** possessing a free carboxylic group at *para* position were obtained in 43–45 % yield. Reactions of 1-propargyl thymine and 4-acetamidobenzenesulfonyl azide or 4-methylbenzenesulfonyl azide (tosyl azide) with secondary amines (Table 2, **1**, **2** and **8**) gave somewhat better yields compared to those with primary amines (Table 2, **3**, **4** and **9**) and aromatic amine (Table 2, **5**), presumably due to higher nucleophilicity of the former.[34b]

In all reactions with 4-acetamidobenzenesulfonyl azide, the presence of a small amount of 4-acetamidobenzenesulfonyl amide by-product was observed. It is reported in the literature that sulfonamide may be formed from the corresponding sulfonyl azide by its decomposition or during the transfer of diazo group to carbon or nitrogen; it can be also obtained from the azide under reductive conditions in the presence of copper powder in aqueous media.[36–38]

We have found that the amount of 4-acetamidobenzenesulfonyl amide by-product increases with increasing reaction temperature, which indicates that its formation could be mostly a consequence of thermal decomposition of the starting azide.

Ammonium salts were found to be a convenient substitute for the amine component in the Cu-catalyzed

three-component reactions.[39] Using ammonium chloride and 4-(aminomethyl)benzonitrile hydrochloride *N*-unalkylated (**7**, **11**) and *N*-monobenzylated (**6**, **10**) sulfonylamidines, respectively, were prepared (Table 2). The reactions were performed in dichloromethane at room temperature in the presence of excess triethylamine. Although it has been reported[39] that the use of ammonium hydroxide instead of ammonium chloride gave better yields of respective amidines, this was not the case in our reaction examples. By using ammonium hydroxide, the expected *N*-unalkylated sulfonylamidine **7** formed only in 30 % yield after heating the reaction mixture to 50 °C. The attempted preparation of *N*-unalkylated sulfonylamidine in the reaction with 4-carboxybenzenesulfonyl azide and ammonium chloride was unsuccessful even in refluxing dichloromethane.

Molecular and Crystal Structure.

Compound **1** crystallizes in the monoclinic centrosymmetric space group *C2*/*c* with one molecule in the asymmetric unit and a water molecule with the occupancy factor of 0.25. The X-ray structure discloses the *E*-form of the generated amidine C10=N9 double bond (torsion angle C18-C10-N9-S1, 2.9(3)°, see Figure 2). Bond lengths N9–C10 and C10–N11 of the amidine group (see Table 3) are compared with analogous bond lengths of the set of 78 related structures (138 structural fragments) extracted from the current version of the Cambridge Structural Database.[40] The R-N=C(R)-N(R_2) fragment was given as input, where R stands for any non-hydrogen atom. Structures with *R* factor exceeding 5 %, as well as metal or ion containing structures were excluded. The average value of the N=C bond length (analogue of N9=C10 in **1**) is 1.307 (2) Å, while 1.364(2) Å is the average value of the N-C bond (analogue of N11-C10 in **1**). Comparison of these values with the corresponding values for N9=C10 and C10-N11, listed in Table 3, strongly suggests that in the case of **1**, the double bond character in the generated amidine

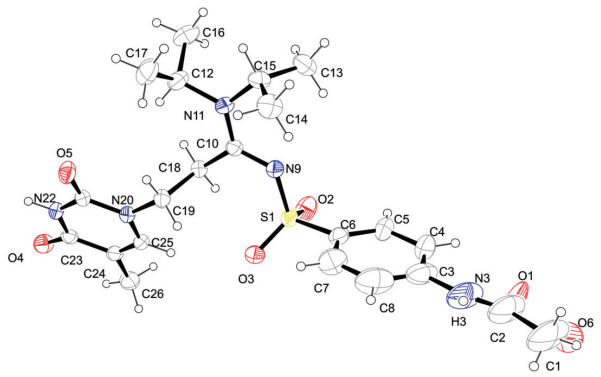


Figure 2. ORTEP drawing of compound **1** with atom numbering. Displacement parameters are scaled to 50 % probability value.

Table 3. Selected bond lengths in the structure of **1**.

Bond	Distance / Å
S1–C6	1.750 (2)
S1–O2	1.427 (3)
S1–O3	1.439 (2)
S1–N9	1.610 (2)
N9–C10	1.316 (3)
C10–N11	1.335 (3)
N20–C21	1.362 (3)
C21–N22	1.370 (3)
N22–C23	1.382 (3)
C23–C24	1.447 (4)
C24–C25	1.338 (4)
C25–N20	1.384 (3)
C19–N20	1.461 (3)

is delocalized over two C–N bonds. Terminal acetamido moiety reveals a strong static disorder, resulting in unusually large displacement parameters of C1, C2, and O1 atoms, as well as somewhat unreliable geometry in this part of the molecule (See Figure 2). During refinement, the phenyl ring C3-C4-C5-C6-C7-C8 was constrained to the idealized six-membered ring geometry. Nevertheless, all non-solvent non-hydrogen atoms were refined anisotropically. Thymine rings in both molecules have the usual and expected geometries.

Crystal packing of **1** is guided by the centrosymmetrical hydrogen-bonded dimers formed via two neighboring thymine moieties. They use their N22 and O5 atoms as proton donors and acceptors, respectively. Two hydrogen bonds, N22-H22···O5*i* (1-*x*,2-*y*,1-*z*) and its centrosymmetric counterpart form an H-bonded ring of the graph-set notation R_2^2 (8).[41] These dimers are further interconnected via water bridges, where O6 plays the role of a pillar in the hydrogen bonded bridge between N3 and O1 from the neighboring dimer. In this way, a dimer of dimers is formed as a distinct building element of the crystal packing of **1** (Figure 3).

***In vitro* Cytotoxicity Screening**

N-sulfonylamidino thymine derivatives **2** and **6** were selected for preliminary *in vitro* cytotoxicity testing against normal Madine Darby canine kidney (MDCKI) cells, and two tumor cell lines of different histological origin, human cervix adenocarcinoma (HeLa) and T cell leukemia cells (Jurkat).[25] As shown in Figure 4. both compounds have a similar pattern of cytotoxic capacity. Their inhibition potential differs in dependence on the dose applied as well as on the cell line treated. Compound **2** showed 60 % growth inhibition of HeLa cells applied at 10^{-5} and 10^{-6} M concentrations and 50 % inhibition of Jurkat cells at the 10^{-5} M concentration. Compound **6** inhibited the growth of HeLa cells by 60 %

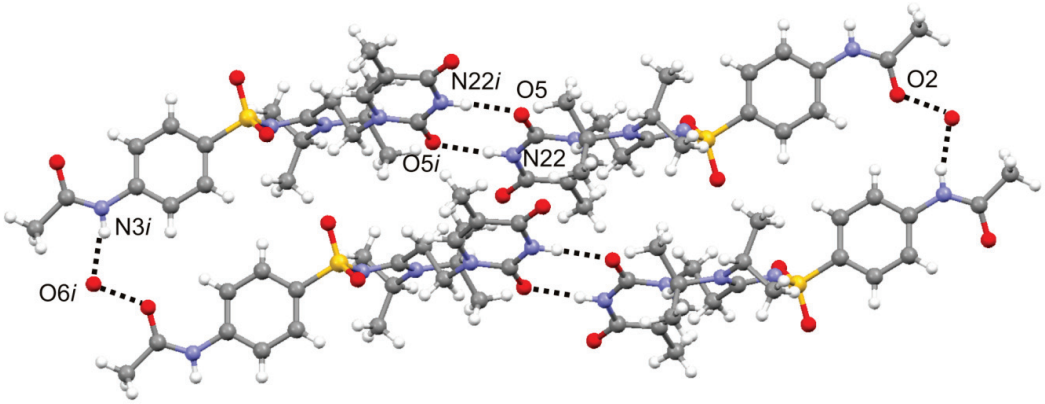


Figure 3. Hydrogen bonding in the structure of **1**.

at 10^{-5} and 10^{-6} M concentrations. In comparison with HeLa cells, Jurkat cells were less sensitive to the 10^{-5} M compound **6** and inhibition was near 40 %. The growth inhibitory effects on normal MDCKI cells were much smaller (Figure 4). Detailed cytotoxicity *in vitro* screening of the complete series of compounds is under way.

CONCLUSION

We report an efficient one-pot synthesis of a small library of novel *N*-sulfonylamidino thymine derivatives by Cu(I) catalyzed three component reaction of 1-propargyl thymine, selected benzenesulfonyl azides and primary amines, secondary amines and ammonium salts. The prepared compounds represent a combination of three important pharmacophores, thymine nucleobase, *N*-sufonyl group and amidine group. We show that this one-pot three component reaction appears to be favourable for the preparation of variously substituted *N*-sulfonylamidino thymine derivatives in moderate to good yields and opens the way for preparation of libraries of other nucleobase *N*-sulfonylamidino derivatives as potential biologically active molecules. Preliminary anticancer *in vitro* screening against human solid tumor (HeLa) and leukemia (Jurkat) cells showed that some of the prepared *N*-sulfonylamidino thymine derivatives of type **II** possess promising anticancer activity. Extensive studies on further evaluation of the biological potential of these new structures are under way.

Supplementary Materials. – CCDC 900214 contains the supplementary crystallographic data. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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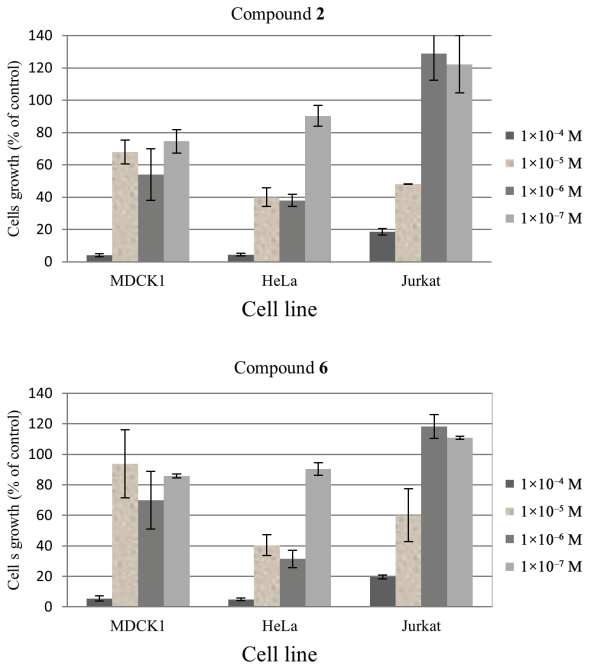

Figure 4. Cytotoxic effects of derivatives **2** and **6** on tumor and normal cell line growth after 72 h of incubation in the final concentration range (10^{-4}–10^{-7} M). Cytotoxicity was analyzed using the MTT survival assay. Data are presented as the mean value ± SD of three independent experiments done in triplicate.

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