

Scientific paper

Design, Synthesis and *In Vitro* Cytotoxic Activity of New 6,9-Disubstituted Purine Analogues

Asligul Kucukdumlu,¹ Meral Tuncbilek,^{1,*} Ebru Bilget Guven²
and Rengul Cetin Atalay³

¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey

² Department of Bioinformatics and Genetics, Faculty of Engineering and Natural Sciences, Kadir Has University, 34083, Cibali-Fatih, Istanbul, Turkey

³ Department of Bioinformatics, Graduate School of Informatics, Middle East Technical University, 06800 Ankara, Turkey

* Corresponding author: E-mail: tuncbile@pharmacy.ankara.edu.tr

Received: 04-12-2019

Abstract

A series of new 6,9-disubstituted purine analogs with 4-substituted piperazine at C-6 and 4-substituted benzyl at N-9 were designed and synthesized in four steps. All synthesized compounds (**7–26**) were screened initially for their *in vitro* anticancer activity on Huh7 liver, HCT116 colon and MCF7 breast carcinoma cell lines. Cytotoxic bioactivity studies revealed that all compounds screened, with compound **19** being the exception, were found to have promising cytotoxic activities at IC₅₀ range of 0.05–21.8 μM against cancer cells Huh7, HCT116 and MCF7. Among the prepared purine analogs, two of them (**12** and **22**) exhibited excellent cytotoxic activities, with IC₅₀ 0.08–0.13 μM, on Huh7 cells comparable to camptothecin (CPT) and better than cladribine, fludarabine and 5-FU. Afterwards, the evaluation of cytotoxicity of the most potent purine analogs was screened against further hepatocellular cancer (HCC) cell lines. The 6-(4-(4-trifluoromethylphenyl)piperazine (**12**) and 6-(4-(3,4-dichlorophenyl)piperazine analogs (**25**) displayed a significant IC₅₀ values (IC₅₀ < 0.1–0.13 μM) comparable to CPT and better cytotoxic bioactivity when compared with 5-FU, cladribine and fludarabine on HCC cells (Huh7 and HepG2).

Keywords: Purine; piperazine; benzyl; cytotoxic activity

1. Introduction

The purine nucleus is involved in the biological molecules that play a key role in the signaling pathways of all living organisms.¹ For this reason, the purine structure is an interesting organic moiety included in new drugs. Purine and purine nucleoside analogs exhibit a variety of biological activities. These analogs have been extensively studied as enzyme inhibitors,^{2–5} cytotoxic,^{6–10} antiviral,^{11–14} antihyperglycemic,¹⁵ immunostimulatory,¹⁶ antifungal, and antibacterial^{17–22} agents due to their potential activities, even though their antiviral and anticancer effects are more commonly known. 5-Fluorouracil (5-FU), a well known fluorinated nucleobase analogue, is highly preferred for the treatment of various cancers in clinics.²³ Subsequently, other pyrimidine analogs, such as cytarabine, gemcitabine, capecitabine, decitabine, and 5-fluoro deoxyuridine have been used in solid tumors and hematologic

malignancies.^{24–26} Purine nucleobase analogs such as 6-mercaptapurine and 6-thioguanine, are specifically used in pediatric acute lymphoblastic leukemia as an inhibitor of nucleic acid metabolism.^{27,28} In addition, many purine nucleoside analogs (fludarabine, cladribine, pentostatin, nelarabine, and clofarabine) are clinically administered in the treatment of solid and hematological malignancies.^{29–32}

A large number of investigations indicate that purine nucleobases and purine nucleosides target a series of pathways in chemotherapy-induced cell death mechanisms, such as apoptosis, necrosis, senescence, autophagy, and mitotic catastrophe.^{25,33–35} Also, various purine scaffolds and their analogs have been studied as potential anticancer agents that contain cyclin-dependent kinase and heat shock protein inhibition. Olomoucine,³⁶ roscovitine,³⁷ purvalanol A, B, amino-purvalanol³⁸ (Figure 1) have been synthesized and screened as cyclin-dependent kinase inhibitors. Especially, R-roscovitine is under investigation as

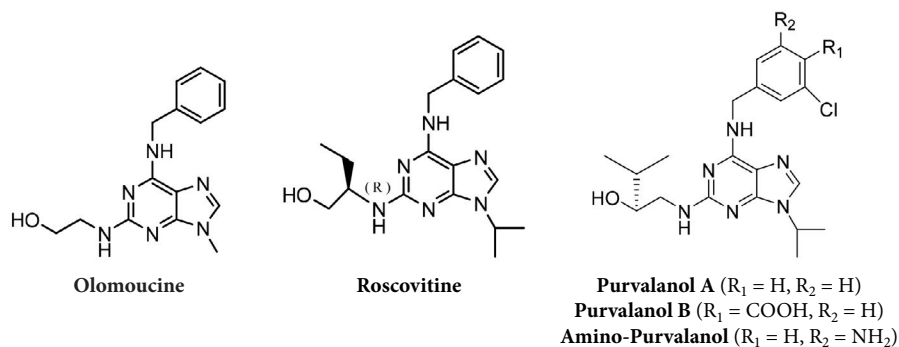


Figure 1. Structures of olomoucine, roscovitine, purvalanol A, B and amino-purvalanol

a chemotherapeutic agent against non-small cell lung cancer and other malignancies. Also, olomoucine with its 2,6,9-trisubstituted purine structure is another purine derivative with antiproliferative effects on cancer cell lines.

In addition to this, various heterocyclic analogs of purines, such as imidazo-pyrazines,³⁹ pyrazolo-pyridazines,⁴⁰ imidazo-pyridines,^{41,42} thieno-pyridines,⁴³ pyrrolo-pyrimidines,⁴⁴ pyrazolo-pyrimidines,^{45,46} thieno-pyrimidines⁴⁷ and triazolo-pyrimidines^{48,49} have been found to possess anticancer activities.

The heat shock protein 90 (Hsp90) has been an existing target in cancer since its inhibition may lead to the

breakdown of many cancer-associated proteins. Furthermore, inhibitors of Hsp90 kill cancer cells at lower concentrations than is required to harm normal human cells. In recent years, purine analogues, which are Hsp90 inhibitors, have entered clinical trials as drugs in the therapy of solid tumors and hematologic malignancies.⁵⁰

In our previous studies,^{51,52} we have reported important cytotoxic activities of 9-(cyclopentyl/ β -D-ribofuranosyl/*para*-toluenesulfonyl)-6-(4-substituted piperazino)purine analogs A, B, C (Figure 2). In this work, we report the synthesis of new analogs of purines A, B, C as 9-(4-substituted benzyl)purines and evaluate their cytotoxic activities against liver (Huh7), colon (HCT116) and breast (T47D) carcinoma cell lines. We further investigate the most active compounds (7–18, 20, 22–26) on a panel of liver cancer cells.

2. Experimental

2. 1. Chemistry

Melting points were recorded with a capillary melting point apparatus (Electrothermal 9100) and are uncorrected. NMR spectra were recorded on a VARIAN Mercury 400 FT-NMR spectrometer (400 MHz for ¹H, 100.6 MHz for ¹³C). TMS was used as internal standard for the ¹H NMR and ¹³C NMR spectra; values are given in δ (ppm) and *J* values are in Hz. Mass spectra were taken on Waters Micro-mass ZQ by using the ESI+ method. Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument and gave values within $\pm 0.4\%$ of the theoretical values. Column chromatography was accomplished on silica gel 60 (0.040–0.063 mm particle size). The chemical reagents used in the synthesis were purchased from Merck, Fluka, Sigma and Aldrich. 5-Amino-4,6-dichloropyrimidine (2) was synthesized according to the reported method.⁵³

2. 1. 1. General Procedure for the Synthesis of Compounds 3 and 4

5-Amino-4,6-dichloropyrimidine (2) (1 mmol) was dissolved in 5 mL of absolute EtOH, and then 4-substitut-

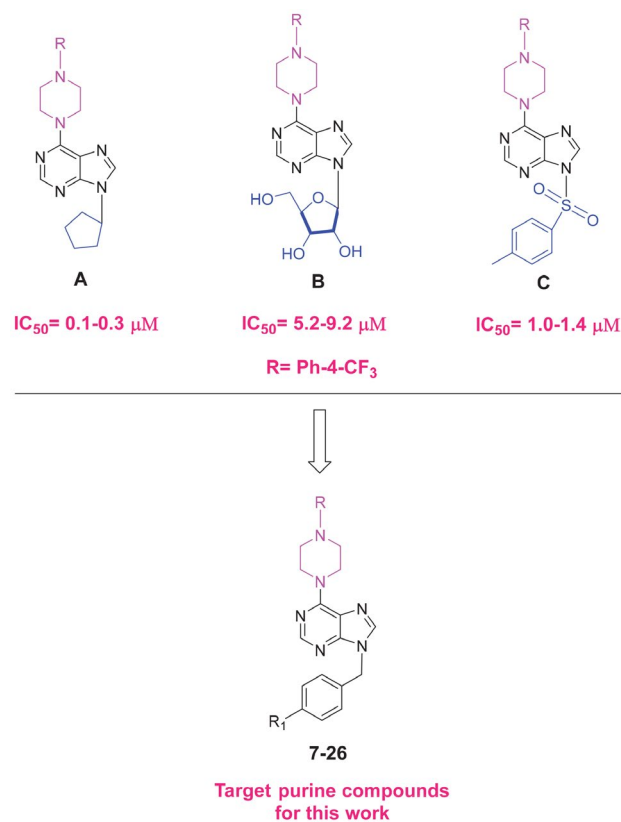


Figure 2. Structures of 9-(cyclopentyl/ β -D-ribofuranosyl/*para*-toluenesulfonyl)-6-(4-substituted piperazino)purine analogs A, B, C and target purine compounds 7–26.

ed benzylamines (2 mmol) and Et₃N (3 mmol) were added. The mixture was refluxed for 15 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (EtOAc-hexane, 1:4 to 1:2).

2. 1. 1. 1. 5-Amino-6-chloro-4-[(4-trifluoromethylbenzyl)amino]pyrimidine (3)

Yield 120 mg (65%), m.p. 181–183 °C. ¹H NMR (CDCl₃) δ 3.40 (br s, 2H), 4.77 (d, 2H, *J* = 5.6 Hz), 5.24 (s, 1H), 7.46 (d, 2H, *J* = 8 Hz), 7.60 (d, 2H, *J* = 8 Hz), 8.11 (s, 1H). MS (ESI+) *m/z*: 303.34 (100%) (M+H), 305.35 (40%) (M+H+2). Anal. Calcd for C₁₂H₁₀ClF₃N₄: C, 47.62; H, 3.33; N, 18.51. Found: C, 47.63; H, 3.41; N, 18.45.

2. 1. 1. 2. 5-Amino-6-chloro-4-[(4-chlorobenzyl)amino]pyrimidine (4)

Yield 140 mg (88%), m.p. 192–194 °C. ¹H NMR (DMSO-*d*₆) δ 4.58 (d, 2H, *J* = 5.6 Hz), 5.07 (s, 2H), 7.28–7.40 (m, 5H), 7.70 (s, 1H). MS (ESI+) *m/z*: 269.24 (100%) (M⁺), 271.24 (55%) (M+2). Anal. Calcd for C₁₁H₁₀Cl₂N₄: C, 49.09; H, 3.75; N, 20.82. Found: C, 49.14; H, 3.66; N, 20.42.

2. 1. 2. General Procedure for the Synthesis of Compounds 5 and 6

A mixture of 5-amino-6-chloro-4-[(4-substituted-benzyl)amino]pyrimidines (**3**, **4**) (0.29 mmol), 2 mL triethyorthoformate and *para*-toluenesulfonic acid (0.03 mmol) was stirred at room temperature for 72 h. The residue was dissolved with CH₂Cl₂, washed with saturated NaHCO₃ and brine. The extract was dried over Na₂SO₄, the solvent was evaporated *in vacuo*, and the residue was purified by column chromatography (EtOAc-hexane, 1:4 to 1:2).

2. 1. 2. 1. 6-Chloro-9-(4-trifluoromethylbenzyl)-9H-purine (5)

Yield 50 mg (51%), m.p. 131 °C [Lit. 130–132 °C⁵⁴]. MS (ESI+) *m/z*: 313.34 (100%) (M+H), 315.36 (47%) (M+H+2).

2. 1. 2. 2. 6-Chloro-9-(4-chlorobenzyl)-9H-purine (6)

Yield 240 mg (87%), m.p. 133 °C [Lit. 130–133 °C⁵⁴]. MS (ESI+) *m/z*: 279.27 (100%) (M⁺), 281.27 (63%) (M+2).

2. 1. 3. General Procedure for the Synthesis of the Final Compounds 7–26

The appropriate 1-substituted piperazines (1 mmol) and Et₃N (3 mmol) were added to a solution of 6-chloro-purines (1mmol) (**5**, **6**) in 5 mL of absolute EtOH. The mixture was refluxed for 8–16 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography (EtOAc-hexane, 1:3 to 1:1).

2. 1. 3. 1. 6-[4-(2-Hydroxyethyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (7)

Yield 270 mg (65%), m.p. 141–143 °C. ¹H NMR (CDCl₃) δ 2.61 (t, 2H, *J* = 5.6 Hz), 2.66 (t, 4H, *J* = 5.2 Hz), 3.68 (t, 2H, *J* = 4.8 Hz), 4.34 (br s, 4H), 5.43 (s, 2H), 7.36 (d, 2H, *J* = 8 Hz), 7.59 (d, 2H, *J* = 8 Hz), 7.74 (s, 1H), 8.37 (s, 1H). ¹³C NMR (CDCl₃) δ 45.12 (CH₂ in piperazine), 46.44 (CH₂), 53.01 (CH₂ in piperazine), 57.77 (CH₂-N), 59.45 (CH₂-OH), 119.77, 122.46, 125.16 (C in phenyl), 125.97 (q, *J*_{CF} = 3.9 Hz), 127.75, 130.57 (q, *J*_{CF} = 32.9 Hz), 137.96 (C-5), 139.78 (C-8), 151.01 (C-6), 152.79 (C-2), 153.87 (C-4). MS (ESI+) *m/z*: 407.65 (100%) (M+H). Anal. Calcd for C₁₉H₂₁F₃N₆O·0.3CH₂Cl₂: C, 53.67; H, 5.04; N, 19.46. Found: C, 53.96; H, 5.08; N, 19.40.

2. 1. 3. 2. 6-[4-Cyclohexylpiperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (8)

Yield 360 mg (82%), m.p. 149–148 °C. ¹H NMR (CDCl₃) δ 1.12–1.89 (m, 10H), 2.33 (br s, 1H), 2.72 (t, 4H, *J* = 5.2 Hz), 4.32 (br s, 4H), 5.43 (s, 2H), 7.36 (d, 2H, *J* = 7.6 Hz), 7.59 (d, 2H, *J* = 8.4 Hz), 7.73 (s, 1H), 8.36 (s, 1H). ¹³C NMR (CDCl₃) δ 25.84, 26.25, 28.86 (CH₂ in cyclohexyl), 45.51 (CH₂ in piperazine), 46.41 (CH₂), 49.12 (CH₂ in piperazine), 63.71 (CH in cyclohexyl), 119.71, 122.46, 125.17 (C in phenyl), 125.95 (q, *J*_{CF} = 3.2 Hz), 127.72, 130.53 (q, *J*_{CF} = 32.9 Hz), 137.79 (C-5), 139.85 (C-8), 150.93 (C-6), 152.82 (C-2), 153.82 (C-4). MS (ESI+) *m/z*: 445.86 (100%) (M+H). Anal. Calcd for C₂₃H₂₇F₃N₆·0.05H₂O: C, 62.02; H, 6.13; N, 18.86. Found: C, 62.06; H, 6.13; N, 18.86.

2. 1. 3. 3. 6-[4-(Pyrimidine-2-yl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (9)

Yield 380 mg (86%), m.p. 171–173 °C. ¹H NMR (CDCl₃) δ 3.99 (t, 4H, *J* = 5.6 Hz), 4.41 (br s, 4H), 5.45 (s, 2H), 6.54 (t, 1H, *J* = 5.2 Hz), 7.37 (d, 2H, *J* = 8 Hz), 7.60 (d, 2H, *J* = 8 Hz), 7.77 (s, 1H), 8.35 (d, 2H, *J* = 4.4 Hz), 8.41 (s, 1H). ¹³C NMR (CDCl₃) δ 43.75 (CH₂ in piperazine), 44.99 (CH₂ in piperazine), 46.47 (CH₂), 110.24 (C-5 in pyrimidine), 119.89, 122.46, 125.16 (C in phenyl), 125.98 (q, *J*_{CF} = 3.9 Hz), 127.76, 130.57 (q, *J*_{CF} = 32.3 Hz), 138.07 (C-5), 139.78 (C-8), 151.06 (C-6), 152.80 (C-2), 154.04 (C-4), 157.77 (C-4,6 in pyrimidine), 161.68 (C-2 in pyrimidine). MS (ESI+) *m/z*: 441.8 (100%) (M+H). Anal. Calcd for C₂₁H₁₉F₃N₈: C, 57.27; H, 4.35; N, 25.44. Found: C, 57.36; H, 4.24; N, 25.42.

2. 1. 3. 4. 6-(4-Phenylpiperazine-1-yl)-9-(4-trifluoromethylbenzyl)-9H-purine (10)

Yield 430 mg (97%), m.p. 122 °C. ¹H NMR (CDCl₃) δ 3.33 (t, 4H, *J* = 5.2 Hz), 4.82 (br s, 4H), 5.44 (s, 2H), 6.90 (t, 1H, *J* = 7.6 Hz), 6.98 (d, 2H, *J* = 7.6 Hz), 7.29 (t, 2H, *J* = 7.6 Hz), 7.37 (d, 2H, *J* = 8 Hz), 7.59 (d, 2H, *J* = 7.6 Hz), 7.76 (s, 1H), 8.40 (s, 1H). ¹³C NMR (CDCl₃) δ 45.02 (CH₂ in piperazine), 46.47 (CH₂), 49.58 (CH₂ in piperazine), 116.51, 119.85, 120.30, 122.47, 125.17 (C in phenyl), 125.99 (q, *J*_{CF} = 3.8 Hz), 127.76, 129.22, 130.58 (q, *J*_{CF} =

32.8 Hz), 138.06 (C in phenyl), 139.79 (C-5), 151.06 (C-8), 151.20 (C-6), 152.83 (C-2), 153.90 (C-4). MS (ESI+) m/z : 439.64 (100%) (M+H). Anal. Calcd for $C_{23}H_{21}F_3N_6 \cdot 0.15H_2O$: C, 62.61. Found: C, 62.85; H, 4.68; N, 18.66.

2. 1. 3. 5. 6-[4-(4-Methylphenyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (11)

Yield 390 mg (86%), m.p. 156 °C. 1H NMR ($CDCl_3$) δ 2.29 (s, 3H), 3.27 (t, 4H, $J = 5.2$ Hz), 4.48 (br s, 4H), 5.44 (s, 2H), 6.90 (d, 2H, $J = 8.4$ Hz), 7.10 (d, 2H, $J = 8$ Hz), 7.37 (d, 2H, $J = 8$ Hz), 7.60 (d, 2H, $J = 8$ Hz), 7.76 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.43 (CH_3), 45.12 (CH_2 in piperazine), 46.46 (CH_2), 50.18 (CH_2 in piperazine), 116.89, 119.84, 122.47, 125.18 (C in phenyl), 125.98 (q, $J_{CF} = 3.9$ Hz), 127.75, 129.74, 129.91, 130.58 (q, $J_{CF} = 32.2$ Hz), 138.02 (C in phenyl), 139.80 (C-5), 149.11 (C-8), 151.05 (C-6), 152.82 (C-2), 153.90 (C-4). MS (ESI+) m/z : 453.9 (100%) (M+H). Anal. Calcd for $C_{24}H_{23}F_3N_6$: C, 63.71; H, 5.12; N, 18.57. Found: C, 63.76; H, 5.10; N, 18.43.

2. 1. 3. 6. 6-[4-(4-Trifluoromethylphenyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (12)

Yield 60 mg (18%), m.p. 115–118 °C. 1H NMR ($CDCl_3$) δ 3.43 (t, 4H, $J = 5.2$ Hz), 4.48 (br s, 4H), 5.45 (s, 2H), 6.98 (d, 2H, $J = 8.4$ Hz), 7.38 (d, 2H, $J = 8.4$ Hz), 7.52 (d, 2H, $J = 8.4$ Hz), 7.60 (d, 2H, $J = 8$ Hz), 7.77 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 44.72 (CH_2 in piperazine), 46.51 (CH_2), 48.24 (CH_2 in piperazine), 114.85, 119.88, 120.92, 121.24, 123.28 (C in phenyl), 126.01 (q, $J_{CF} = 3.8$ Hz), 126.48 (q, $J_{CF} = 3.9$ Hz), 127.78, 130.47, 138.22 (C in phenyl), 139.71 (C-5), 151.10 (C-8), 152.81 (C-6), 153.15 (C-2), 153.83 (C-4). MS (ESI+) m/z : 507.51 (100%) (M+H). Anal. Calcd for $C_{24}H_{20}F_6N_6 \cdot 0.1H_2O \cdot 0.3CH_3COOC_2H_5$: C, 56.61; H, 4.26; N, 15.71. Found: C, 56.23; H, 3.93; N, 15.43.

2. 1. 3. 7. 6-[4-(4-Fluorophenyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (13)

Yield 240 mg (55%), m.p. 111–113 °C. 1H NMR ($CDCl_3$) δ 3.24 (t, 4H, $J = 5.2$ Hz), 4.48 (br s, 4H), 5.45 (s, 2H), 6.91–7.02 (m, 4H), 7.37 (d, 2H, $J = 8.4$ Hz), 7.60 (d, 2H, $J = 8$ Hz), 7.77 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 45.04 (CH_2 in piperazine), 46.48 (CH_2), 50.62 (CH_2 in piperazine), 115.65 (d, $J = 21.9$ Hz), 118.39 (d, $J = 7.7$ Hz), 119.85, 122.46, 125.16 (C in phenyl), 125.99 (q, $J_{CF} = 3.9$ Hz), 127.76, 130.60 (q, $J_{CF} = 32.8$ Hz), 138.08 (C in phenyl), 139.77 (C-5), 147.89 (C-8), 151.07 (C-6), 152.81 (C-2), 153.88 (C-4), 157.50 (d, $J = 239.3$). MS (ESI+) m/z : 457.57 (100%) (M+H). Anal. Calcd for $C_{23}H_{20}F_4N_6 \cdot 0.2H_2O$: C, 60.05; H, 4.47; N, 18.27. Found: C, 59.83; H, 4.30; N, 18.11.

2. 1. 3. 8. 6-[4-(2,4-Difluorophenyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (14)

Yield 420 mg (89%), m.p. 193 °C. 1H NMR ($CDCl_3$) δ 3.16 (t, 4H, $J = 5.2$ Hz), 4.49 (br s, 4H), 5.45 (s, 2H),

6.78–6.98 (m, 3H), 7.37 (d, 2H, $J = 8$ Hz), 7.60 (d, 2H, $J = 8$ Hz), 7.76 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 45.21 (CH_2 in piperazine), 46.48 (CH_2), 51.19 (CH_2 in piperazine), 104.84 (t, $J = 24.5$ Hz), 110.78 (dd, $J = 3.8$, $J = 21.9$), 119.81 (dd, $J = 3.9$ Hz, $J = 10.3$ Hz), 122.46, 125.16 (C in phenyl), 125.99 (q, $J_{CF} = 3.2$ Hz), 127.77, 130.59 (q, $J_{CF} = 32.2$ Hz), 136.49 (dd, $J = 3.8$, $J = 9$ Hz), (C in phenyl), 138.05 (C-5), 139.78 (C-8), 151.08 (C-6), 152.81 (C-2), 153.91 (C-4), 155.75 (dd, $J = 11.6$ Hz, $J = 237.3$), 158.22 (dd, $J = 7.7$ Hz, $J = 232.2$ Hz), (C in phenyl). MS (ESI+) m/z : 475.82 (100%) (M+H). Anal. Calcd for $C_{23}H_{19}F_5N_6$: C, 58.23; H, 4.04; N, 17.71. Found: C, 58.29; H, 4.17; N, 17.52.

2. 1. 3. 9. 6-[4-(3,4-Dichlorophenyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (15)

Yield 450 mg (88%), m.p. 136 °C. 1H NMR ($CDCl_3$) δ 3.30 (t, 4H, $J = 4.8$ Hz), 4.47 (br s, 4H), 5.45 (s, 2H), 6.79 (dd, 1H, $J = 9.2$ Hz, $J = 3.2$ Hz), 7.00 (d, 3H, $J = 2.8$ Hz), 7.30 (d, 1H, $J = 9.2$ Hz), 7.37 (d, 2H, $J = 8.4$ Hz), 7.60 (d, 2H, $J = 8$ Hz), 7.77 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 44.71 (CH_2 in piperazine), 46.50 (CH_2), 49.02 (CH_2 in piperazine), 115.69, 117.65, 119.88, 122.45, 122.80, 125.16, (C in phenyl), 126.01 (q, $J_{CF} = 3.8$ Hz), 127.78, 130.54, 130.62 (q, $J_{CF} = 32.9$ Hz), 132.90, 138.21 (C in phenyl), 139.71 (C-5), 150.55 (C-8), 151.11 (C-6), 152.80 (C-2), 153.81 (C-4). MS (ESI+) m/z : 507.7 (100%) (M⁺), 509.7 (63%) (M+2), 511.9 (5%) (M+4). Anal. Calcd for $C_{23}H_{19}Cl_2N_6$: C, 54.45; H, 3.77; N, 16.56. Found: C, 54.43; H, 3.70; N, 16.54.

2. 1. 3. 10. 6-[4-(Diphenylmethyl)piperazine-1-yl]-9-(4-trifluoromethylbenzyl)-9H-purine (16)

Yield 340 mg (65%), m.p. 179–182 °C. 1H NMR ($CDCl_3$) δ 2.53 (t, 4H, $J = 5.2$ Hz), 4.28 (s, 1H), 4.31 (br s, 4H), 5.40 (s, 2H), 7.17–7.36 (m, 8H), 7.44 (d, 4H, $J = 7.2$ Hz), 7.58 (d, 2H, $J = 8.4$ Hz), 7.67 (s, 1H), 8.33 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 42.82 (CH_2 in piperazine), 43.82 (CH_2), 49.45 (CH_2 in piperazine), 73.53 (CH), 117.19, 119.91, 122.61 (C in phenyl), 123.3 (q, $J_{CF} = 3.9$ Hz), 124.51, 125.17, 125.41, 125.96, 128.95 (q, $J_{CF} = 32.9$ Hz), 135.17 (C in phenyl), 137.28 (C-5), 139.64 (C-8), 148.35 (C-6), 150.24 (C-2), 151.32 (C-4). MS (ESI+) m/z : 529.68 (100%) (M+H). Anal. Calcd for $C_{30}H_{27}F_3N_6$: C, 68.17; H, 5.15; N, 15.90. Found: C, 67.90; H, 5.09; N, 15.71.

2. 1. 3. 11. 6-[4-(2-Hydroxyethyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (17)

Yield 290 mg (77%), m.p. 138–141 °C. 1H NMR ($CDCl_3$) δ 2.61 (t, 2H, $J = 5.6$ Hz), 2.66 (t, 4H, $J = 5.6$ Hz), 3.68 (t, 2H, $J = 5.2$ Hz), 3.77 (br s, 4H), 5.34 (s, 2H), 7.21 (d, 2H, $J = 8.4$ Hz), 7.31 (d, 2H, $J = 8.4$ Hz), 7.71 (s, 1H), 8.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 45.11 (CH_2 in piperazine), 46.34 (CH_2), 53.01 (CH_2 in piperazine), 57.76 (CH_2-N), 59.46 (CH_2-OH), 119.79, 128.98, 129.16, 134.26 (C in phenyl), 134.31 (C-5), 137.99 (C-8), 150.98 (C-6), 152.68 (C-2), 153.84 (C-4). MS (ESI+) m/z : 373.61 (100%)

(M+H), 375.62 (33%) (M+H+2). Anal. Calcd for $C_{18}H_{21}ClN_6O \cdot 0.4H_2O$: C, 56.88; H, 5.78; N, 22.11. Found: C, 56.65; H, 5.44; N, 21.88.

2. 1. 3. 12. 6-[4-Cyclohexylpiperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (18)

Yield 380 mg (93%), m.p. 125 °C. 1H NMR ($CDCl_3$) δ 1.09–1.99 (m, 10H), 2.54 (br s, 1H), 2.86 (br s, 4H), 4.47 (br s, 4H), 5.31 (s, 2H), 7.19 (d, 2H, $J = 8.4$ Hz), 7.29 (d, 2H, $J = 8.4$ Hz), 7.70 (s, 1H), 8.35 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.57, 25.82, 28.11 (CH_2 in cyclohexyl), 44.31 (CH_2 in piperazine), 45.78 (CH_2), 48.86 (CH_2 in piperazine), 64.53 (CH in cyclohexyl), 119.80, 129.01, 129.16 (C in phenyl), 134.26 (C-5), 138.20 (C-8), 151.02 (C-6), 152.66 (C-2), 153.55 (C-4). MS (ESI+) m/z : 411.75 (100%) (M+H), 413.74 (30%) (M+H+2). Anal. Calcd for $C_{22}H_{27}ClN_6$: C, 64.30; H, 6.62; N, 20.45. Found: C, 64.28; H, 6.84; N, 20.12.

2. 1. 3. 13. 6-[4-(Pyrimidine-2-yl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (19)

Yield 280 mg (69%), m.p. 202 °C. 1H NMR ($CDCl_3$) δ 3.99 (t, 4H, $J = 5.6$ Hz), 4.40 (br s, 4H), 5.35 (s, 2H), 6.54 (t, 1H, $J = 4.4$ Hz), 7.21 (d, 2H, $J = 8.8$ Hz), 7.32 (d, 2H, $J = 8$ Hz), 7.74 (s, 1H), 8.34 (d, 2H, $J = 4.4$ Hz), 8.41 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 43.76 (CH_2 in piperazine), 44.98 (CH_2 in piperazine), 46.36 (CH_2), 110.22 (C-5 in pyrimidine), 119.91, 128.99, 129.17, 134.26 (C in phenyl), 134.30 (C-5), 138.12 (C-8), 151.02 (C-6), 152.69 (C-2), 154.01 (C-4), 157.76 (C-4,6 in pyrimidine), 161.67 (C-2 in pyrimidine). MS (ESI+) m/z : 407.77 (100%) (M+H), 409.84 (32%) (M+H+2). Anal. Calcd for $C_{20}H_{19}ClN_8 \cdot 0.43MeOH$: C, 58.34; H, 4.96; N, 27.54. Found: C, 58.73; H, 5.18; N, 26.27.

2. 1. 3. 14. 6-(4-Phenylpiperazine-1-yl)-9-(4-chlorobenzyl)-9H-purine (20)

Yield 120 mg (82%), m.p. 140–143 °C. 1H NMR ($CDCl_3$) δ 3.33 (t, 4H, $J = 5.2$ Hz), 4.48 (br s, 4H), 5.35 (s, 2H), 6.91 (t, 1H, $J = 7.6$ Hz), 6.98 (d, 2H, $J = 8$ Hz), 7.21 (d, 2H, $J = 8.8$ Hz), 7.25–7.34 (m, 4H), 7.73 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 45.04 (CH_2 in piperazine), 46.36 (CH_2), 49.58 (CH_2 in piperazine), 116.50, 119.87, 120.28, 128.99, 129.18, 129.21, 134.28, 134.30 (C in phenyl), 138.10 (C-5), 151.04 (C-8), 151.21 (C-6), 152.73 (C-2), 153.88 (C-4). MS (ESI+) m/z : 405.69 (100%) (M+H), 407.69 (47%) (M+H+2). Anal. Calcd for $C_{22}H_{21}ClN_6$: C, 65.26; H, 5.23; N, 20.76. Found: C, 65.24; H, 5.04; N, 20.70.

2. 1. 3. 15. 6-[4-(4-Methylphenyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (21)

Yield 310 mg (75%), m.p. 193 °C. 1H NMR ($CDCl_3$) δ 2.28 (s, 3H), 3.26 (t, 4H, $J = 4.8$ Hz), 4.47 (br s, 4H), 5.34 (s, 2H), 6.90 (d, 2H, $J = 8.4$ Hz), 7.10 (d, 2H, $J = 8$ Hz), 7.21 (d, 2H, $J = 8.8$ Hz), 7.31 (d, 2H, $J = 8$ Hz), 7.73 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.44 (CH_3), 45.03 (CH_2 in piperazine), 46.37 (CH_2), 50.23 (CH_2 in piperazine), 116.92, 119.85, 128.99, 129.18, 129.74, 129.97, 134.29 (C in

phenyl), 138.08 (C-5), 149.06 (C-8), 151.01 (C-6), 152.69 (C-2), 153.84 (C-4). MS (ESI+) m/z : 419.78 (100%) (M+H), 421.81 (45%) (M+H+2). Anal. Calcd for $C_{23}H_{23}ClN_6 \cdot 0.2H_2O$: C, 65.38; H, 5.58; N, 19.89. Found: C, 65.94; H, 5.53; N, 20.06.

2. 1. 3. 16. 6-[4-(4-Trifluoromethylphenyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (22)

Yield 330 mg (69%), m.p. 162 °C. 1H NMR ($CDCl_3$) δ 3.43 (t, 4H, $J = 4.8$ Hz), 4.48 (br s, 4H), 5.35 (s, 2H), 6.98 (d, 2H, $J = 8.4$ Hz), 7.22 (d, 2H, $J = 8.8$ Hz), 7.31 (d, 2H, $J = 8.4$ Hz), 7.51 (d, 2H, $J = 8.4$ Hz), 7.40 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 44.78 (CH_2 in piperazine), 46.43 (CH_2), 48.22 (CH_2 in piperazine), 114.84, 119.87, 120.89, 121.21, 125.97 (C in phenyl), 126.48 (q, $J_{CF} = 3.8$ Hz), 129.02, 129.21, 134.20 (C in phenyl), 138.31 (C-5), 151.01 (C-8), 152.55 (C-6), 153.13 (C-2), 153.67 (C-4). MS (ESI+) m/z : 473.54 (100%) (M+H), 475.52 (40%) (M+H+2). Anal. Calcd for $C_{23}H_{20}ClF_3N_6$: C, 58.42; H, 4.26; N, 17.77. Found: C, 58.55; H, 4.30; N, 17.60.

2. 1. 3. 17. 6-[4-(4-Fluorophenyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (23)

Yield 280 mg (67%), m.p. 186–188 °C. 1H NMR ($CDCl_3$) δ 3.23 (t, 4H, $J = 5.2$ Hz), 4.47 (br s, 4H), 5.35 (s, 2H), 6.91–7.02 (m, 4H), 7.21 (d, 2H, $J = 8.4$ Hz), 7.31 (d, 2H, $J = 8$ Hz), 7.73 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 40.31 (CH_2 in piperazine), 41.62 (CH_2), 45.87 (CH_2 in piperazine), 110.89 (d, $J = 21.9$ Hz), 113.64 (d, $J = 7.7$ Hz), 115.12, 124.24, 124.43, 129.52, 129.54 (C in phenyl), 133.38 (C-5), 143.13 (C-8), 146.28 (C-6), 147.93 (C-2), 149.08 (C-4), 152.75 (d, $J = 239.9$). MS (ESI+) m/z : 424 (100%) (M+H), 425.94 (35%) (M+H+2). Anal. Calcd for $C_{22}H_{20}ClFN_6$: C, 62.48; H, 4.77; N, 19.87. Found: C, 62.55; H, 4.57; N, 19.84.

2. 1. 3. 18. 6-[4-(2,4-Difluorophenyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (24)

Yield 410 mg (93%), m.p. 183–185 °C. 1H NMR ($CDCl_3$) δ 3.15 (t, 4H, $J = 4.8$ Hz), 4.48 (br s, 4H), 5.35 (s, 2H), 6.78–6.96 (m, 3H), 7.22 (d, 2H, $J = 8.4$ Hz), 7.32 (d, 2H, $J = 8$ Hz), 7.73 (s, 1H), 8.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 40.53 (CH_2 in piperazine), 41.63 (CH_2), 46.44 (CH_2 in piperazine), 100.08 (t, $J = 24.4$ Hz), 106.03 (dd, $J = 21.3$ Hz, $J = 3.9$ Hz), 115.05 (dd, $J = 9.1$ Hz, $J = 3.8$ Hz), 124.25, 124.43, 129.52, 131.71 (dd, $J = 9.1$ Hz, $J = 3.9$), (C in phenyl), 133.35 (C-5), 145.51 (C-8), 146.28 (C-6), 147.90 (C-2), 149.09 (C-4), 151.03 (dd, $J = 240.6$, $J = 11.6$ Hz), 153.46 (dd, $J = 231.6$, $J = 12.2$) (C in phenyl). MS (ESI+) m/z : 441.8 (100%) (M+H), 443.8 (37%) (M+H+2). Anal. Calcd for $C_{22}H_{19}ClF_2N_6$: C, 59.93; H, 4.34; N, 19.06. Found: C, 59.87; H, 4.24; N, 19.11.

2. 1. 3. 19. 6-[4-(3,4-Dichlorophenyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (25)

Yield 440 mg (92%), m.p. 198–200 °C. 1H NMR ($CDCl_3$) δ 3.30 (t, 4H, $J = 4.8$ Hz), 4.46 (br s, 4H), 5.35 (s,

2H), 6.79 (dd, 1H, $J = 2.8$ Hz, $J = 9.2$ Hz), 7.00 (d, 1H, $J = 2.8$ Hz), 7.22 (d, 2H, $J = 8.8$ Hz), 7.29–7.33 (m, 3H), 7.74 (s, 1H), 8.40 (s, 1H). ^{13}C NMR (CDCl_3) δ 44.75 (CH_2 in piperazine), 46.41 (CH_2), 49.01 (CH_2 in piperazine), 115.69, 117.64, 119.89, 122.79, 129.01, 129.20, 130.54, 132.90, 134.22, 134.32 (C in phenyl), 138.26 (C-5), 150.56 (C-8), 151.05 (C-6), 152.65 (C-2), 153.75 (C-4). MS (ESI+) m/z : 473.7 (100%) (M^+), 475.8 (90%) ($\text{M}+2$), 477.8 (35%) ($\text{M}+4$). Anal. Calcd for $\text{C}_{22}\text{H}_{19}\text{Cl}_3\text{N}_6$: C, 55.77; H, 4.04; N, 17.74. Found: C, 55.50; H, 4.08; N, 17.86.

2. 1. 3. 20. 6-[4-(Diphenylmethyl)piperazine-1-yl]-9-(4-chlorobenzyl)-9H-purine (26)

Yield 310 mg (62%), m.p. 145–147 °C. ^1H NMR (CDCl_3) δ 2.53 (m, 4H), 4.28 (s, 1H), 4.30 (br s, 4H), 5.28 (s, 2H), 7.14–7.22 (m, 4H), 7.25–7.31 (m, 6H), 7.44 (d, 4H, $J = 7.2$ Hz), 7.63 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (CDCl_3) δ 45.37 (CH_2 in piperazine), 46.28 (CH_2), 52.03 (CH_2 in piperazine), 76.10 (CH), 119.79, 127.06, 127.98, 128.52, 128.98, 129.14, 134.20, 134.36 (C in phenyl), 137.79 (C-5), 142.22 (C-8), 150.89 (C-6), 152.70 (C-2), 153.86 (C-4). MS (ESI+) m/z : 495.67 (100%) ($\text{M}+\text{H}$), 497.66 (39%) ($\text{M}+\text{H}+2$). Anal. Calcd for $\text{C}_{29}\text{H}_{27}\text{ClN}_6$: C, 70.36; H, 5.50; N, 16.98. Found: C, 70.17; H, 5.20; N, 16.92.

2. 2. Biological Evaluation

2. 2. 1. Cells and Culture

The human primary liver cancer cell lines (Huh7, HepG2, Mahlavu and FOCUS) were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen GIBCO) with 10% fetal bovine serum (FBS) (Invitrogen GIBCO), nonessential amino acids, and 1% penicillin (Biochrome). It was incubated at 37 °C with 5% CO_2 . DMSO (Sigma) was used as the solvent for the compounds. The concentration of DMSO was always less than 1% in the cell culture medium. The cytotoxic drugs (camptothecin (CPT), 5-fluorouracil (5-FU), fludarabine, and cladribine) used as positive controls were from Calbiochem.

2. 2. 2. Sulforhodamine B (SRB) Assay for Cytotoxicity Screening

Huh7, HCT116, MCF7, HepG2, Mahlavu, and FOCUS cells were inoculated (2000–10000 cells/well in 200 μL) in 96-well plates. The next day, the media was refreshed, and the compounds dissolved in DMSO were applied in concentrations between 1 and 40 μM in parallel with DMSO-only treated cells as negative controls. At the 72nd hour of treatment with compounds 7–26 and the other drugs, the cancer cells were fixed with 100 μL of 10% (w/v) trichloroacetic acid (TCA) and kept at +4 °C in dark for 1 h. TCA fixation was terminated by washing the wells with ddH_2O five times. Air-dried plates were stained with 0.4% sulforhodamine B (SRB) dissolved in 1% acetic acid solution for 10 min in the dark and at room tempera-

ture. The protein-bound and dried SRB dye was then solubilized with 10 mM Tris-Base pH 8. The absorbance values were obtained at 515 nm in a microplate reader. The data normalized against DMSO-only treated wells, which were used as controls in serial dilutions. In all experiments, a linear response was observed, with serial dilutions of the compounds and the drugs.

3. Results and Discussion

3. 1. Chemistry

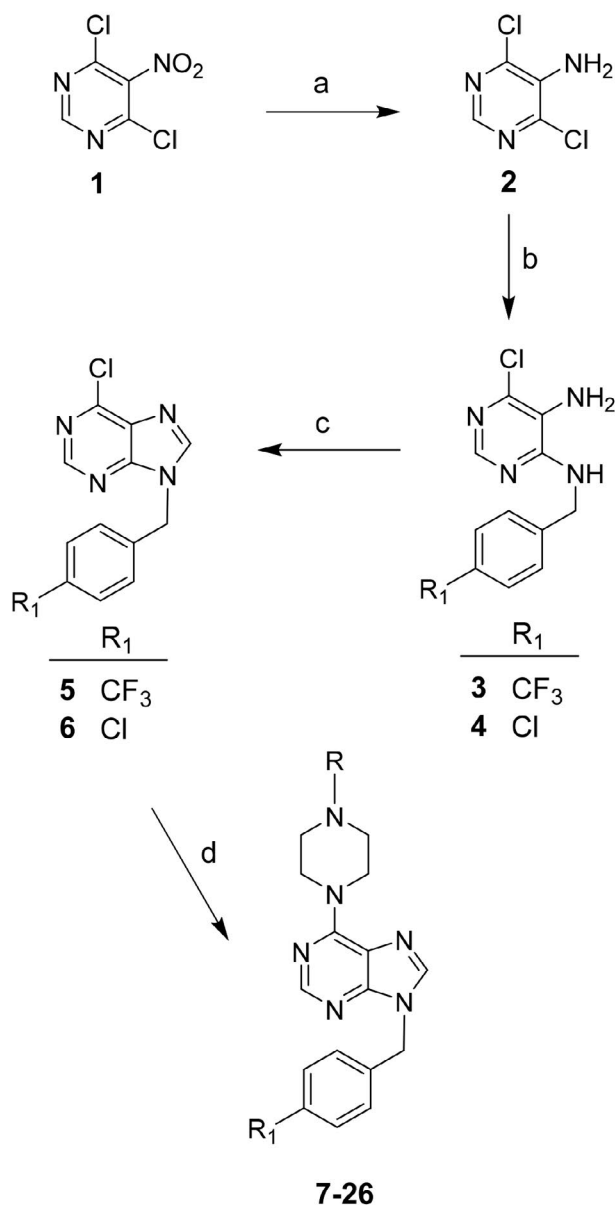
The 6-(4-substituted piperazine-1-yl)-9-(4-substituted benzyl)purine analogs were synthesized in four steps starting with commercially available 4,6-dichloro-5-nitropyrimidine (**1**) (Scheme 1). The dichloronitropyrimidine was reduced to the corresponding dichloroaminopyrimidine (**2**) with stannous chloride and ethanol.⁵³ Amination of 5-amino-4,6-dichloropyrimidine (**2**) with the appropriate benzylamines gave the 4-(4-substituted benzyl)pyrimidines (**3**, **4**). Condensation of compounds **3**, **4** with triethylorthoformate and *para*-toluenesulfonic acid afforded the intermediate 6-chloro-9-(4-substituted benzyl)purines **5**, **6**.⁵⁴ Purines substituted at C-6 (**7**–**26**) were synthesized by nucleophilic substitution of the chlorine of 9-substituted purines (**5**, **6**) with the appropriate 4-substituted piperazines in the presence of base.

3. 2. Biological Evaluation

The antitumor activities of newly synthesized purine analogues were first analyzed on three human cancer cell lines including Huh7 (liver), HCT116 (colon) and MCF7 (breast) cancer cells by using the sulforhodamine B (SRB) method. The IC_{50} values of the purine compounds were calculated in comparison with DNA topoisomerase inhibitor camptothecin (CPT) and the known cell growth inhibitors fludarabine, cladribine, 5-fluorouracil (5-FU). The data are summarized in Table 1.

All synthesized purine derivatives in this study, except for compound **19**, exhibited important cytotoxic activity against cancer cells Huh7, HCT116, MCF7 with IC_{50} from 0.05 to 21.7 μM .

As seen from the data in Table 1, all the 6-(trifluoromethylphenyl)piperazine purines, **12** and **22** exhibited excellent cytotoxic activities with IC_{50} 0.08–0.13 μM on Huh7 cells comparable to CPT and better than cladribine, fludarabine and 5-FU. In addition, compounds **15** and **25** bearing a 3,4-dichlorophenyl group at the piperazine of the purine, presented a higher cytotoxic activity than known nucleoside drugs cladribine, fludarabine and nucleobase drug 5-FU on Huh7 cells. For the 4-fluorophenyl substituted derivatives **13** and **23**, their best activity is observed for the 9-(4-chlorobenzyl) purine derivative **23** with IC_{50} value of 0.57 μM on Huh7. Cytotoxic activity differences were not observed in the unsubstituted phe-



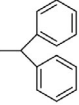


	R	R ₁		R	R ₁		R	R ₁
7		CF ₃	13		CF ₃	17		Cl
8		CF ₃	14		CF ₃	18		Cl
9		CF ₃	15		CF ₃	19		Cl
10		CF ₃	16		CF ₃	20		Cl
11		CF ₃				21		Cl
12		CF ₃				22		Cl
						23		Cl
						24		Cl
						25		Cl
						26		Cl

Scheme 1. (a) SnCl₂·2H₂O, EtOH; (b) 4-substituted benzylamine, EtOH, Et₃N; (c) HC(OEt)₃, pTSA; (d) 4-substituted piperazine, Et₃N, EtOH

Table 1. *In vitro* cytotoxicity of compounds 7–26 on different human cancer cell lines

Compound	R	R ₁	Cancer cell lines, IC ₅₀ (μM) ^a		
			Huh7	HCT116	MCF7
7		CF ₃	18.25 ± 0.55	19.14 ± 0.75	17.83 ± 0.56
8		CF ₃	7.86 ± 0.04	7.74 ± 0.08	6.51 ± 0.52
9		CF ₃	9.6 ± 0.6	11.3 ± 2.1	17.8 ± 0.2
10		CF ₃	0.86 ± 0.19	0.28 ± 0.003	0.16 ± 0.037
11		CF ₃	<1.0	<0.1	<1.0
12		CF ₃	0.13 ± 0.12	0.42 ± 0.08	0.4
13		CF ₃	1.54 ± 0.10	1.26 ± 0.13	1.12 ± 0.16
14		CF ₃	5.05 ± 0.52	5.51 ± 0.29	4.24 ± 1.17
15		CF ₃	0.6 ± 0.1	0.74 ± 0.05	<1.0
16		CF ₃	15.55 ± 1.09	12.97 ± 1.11	12.13 ± 0.38
17		Cl	21.8 ± 2.7	18.96 ± 0.06	21.8 ± 4.4
18		Cl	9.26 ± 0.59	8.87 ± 0.35	8.61 ± 0.26
19		Cl	38.0 ± 5.1	57.1 ± 8.8	100.9 ± 28.0
20		Cl	0.56 ± 0.12	0.26 ± 0.17	0.38 ± 0.07
21		Cl	<0.1	0.26 ± 0.35	0.48 ± 0.50
22		Cl	0.08 ± 0.06	0.04 ± 0.004	0.05
23		Cl	0.57 ± 0.12	0.14 ± 0.10	2.80 ± 3.22

24		Cl	9.38 ± 0.65	NI	NI
25		Cl	0.31 ± 0.10	13.0 ± 0.38	7.08 ± 3.03
26		Cl	20.1 ± 6.8	13.15 ± 0.06	14.02 ± 0.67
CPT			<0.1	0.034 ± 0.036	<0.1
5-FU			30.6 ± 1.8	4.1 ± 0.3	3.5 ± 0.7
Fludarabine			28.4 ± 19.2	8.0 ± 3.4	15.2 ± 0.1
Cladribine			0.9 ± 0.7	<0.1	2.4 ± 2.4

^a IC₅₀ values were calculated from the cell growth inhibition percentages obtained with 5 different concentrations (40, 20, 10, 5, and 2.5 μM) of each molecule incubated for 72 h. NI: no inhibition.

Table 2. IC₅₀ values of 7–18, 20, 22–26 against hepatocellular carcinoma (HCC) cell lines Huh7, HepG2, MAHLAVU, FOCUS.

Compound	HCC Cancer cell lines, IC ₅₀ (μM) ^a			
	Huh7	HepG2	Mahlavu	FOCUS
7	28.9 ± 4.0	25.2 ± 4	NI	NI
8	5.36 ± 0.2	6.4 ± 0.5	8.0 ± 0.2	6.4 ± 0.6
9	3.32 ± 1.3	4.3 ± 0.6	6.0 ± 0.1	7.2 ± 1.7
10	1.45 ± 0.2	1.4 ± 0.2	1.9 ± 0.4	1.5 ± 0.5
11	0.29 ± 0.4	<1.0	NI	0.5 ± 0.1
12	0.13 ± 0.1	0.1 ± 0.04	<0.1	<0.1
13	2.13 ± 0.1	1.6 ± 0.2	2.3 ± 0.3	1.9 ± 0.1
14	5.48 ± 0.2	3.8 ± 0.5	4.8 ± 0.2	5.1 ± 0.5
15	0.24 ± 0.1	<0.1	8.2 ± 1.4	1.4 ± 0.3
16	19.4 ± 1.7	32.3 ± 23.9	10.8 ± 0.4	11.6 ± 0.4
17	23.9 ± 0.5	69.4 ± 25.7	85.4 ± 26.9	83.3 ± 14.2
18	6.74 ± 0.3	7.3 ± 1.3	13.1 ± 1.2	9.1 ± 0.9
20	1.89 ± 0.1	1.5 ± 0.2	2.3 ± 0.1	0.2 ± 0.1
22	0.23 ± 0.1	<0.1	<0.1	<0.1
23	2.33 ± 0.2	1.8 ± 0.4	3.2 ± 0.2	4.5 ± 0.5
24	2.22 ± 0.4	1.4 ± 1.1	11.1 ± 1.9	5.1 ± 1.5
25	<0.1	<0.1	5.6 ± 0.1	1.2 ± 0.3
26	16.4 ± 1.7	11.7 ± 1.4	12.1 ± 0.6	1 ± 1
CPT	<0.1	<0.1	<0.1	<0.1
5-FU	30.6 ± 1.8	0.8 ± 0.26	10.0 ± 1.8	3.7 ± 0.5
Fludarabine	28.4 ± 19.2	17.0 ± 5.9	13.5 ± 4.9	13.7 ± 1.2
Cladribine	0.9 ± 0.7	0.4 ± 0.1	<0.1	<0.1

^a IC₅₀ values were calculated from the cell growth inhibition percentages obtained with 5 different concentrations (40, 20, 10, 5, and 2.5 μM) of each molecule incubated for 72 h. NI: No inhibition

nyl and 4-methyl phenyl group bearing purin derivatives (**10**, **11**, **20**, **21**) and these compounds had significantly higher bioactivity (IC₅₀ < 1.0 μM) compared to 5-FU, fludarabine, cladribine against Huh7 cell.

Within the tested purine analogs on HCT116 cell, compounds **11** and **22** showed superior cytotoxic activity (IC₅₀ < 0.1 and 0.04 μM, respectively) compared to 5-FU (IC₅₀ 4.1), fludarabine (IC₅₀ 8.0 μM), cladribine (IC₅₀ < 0.1

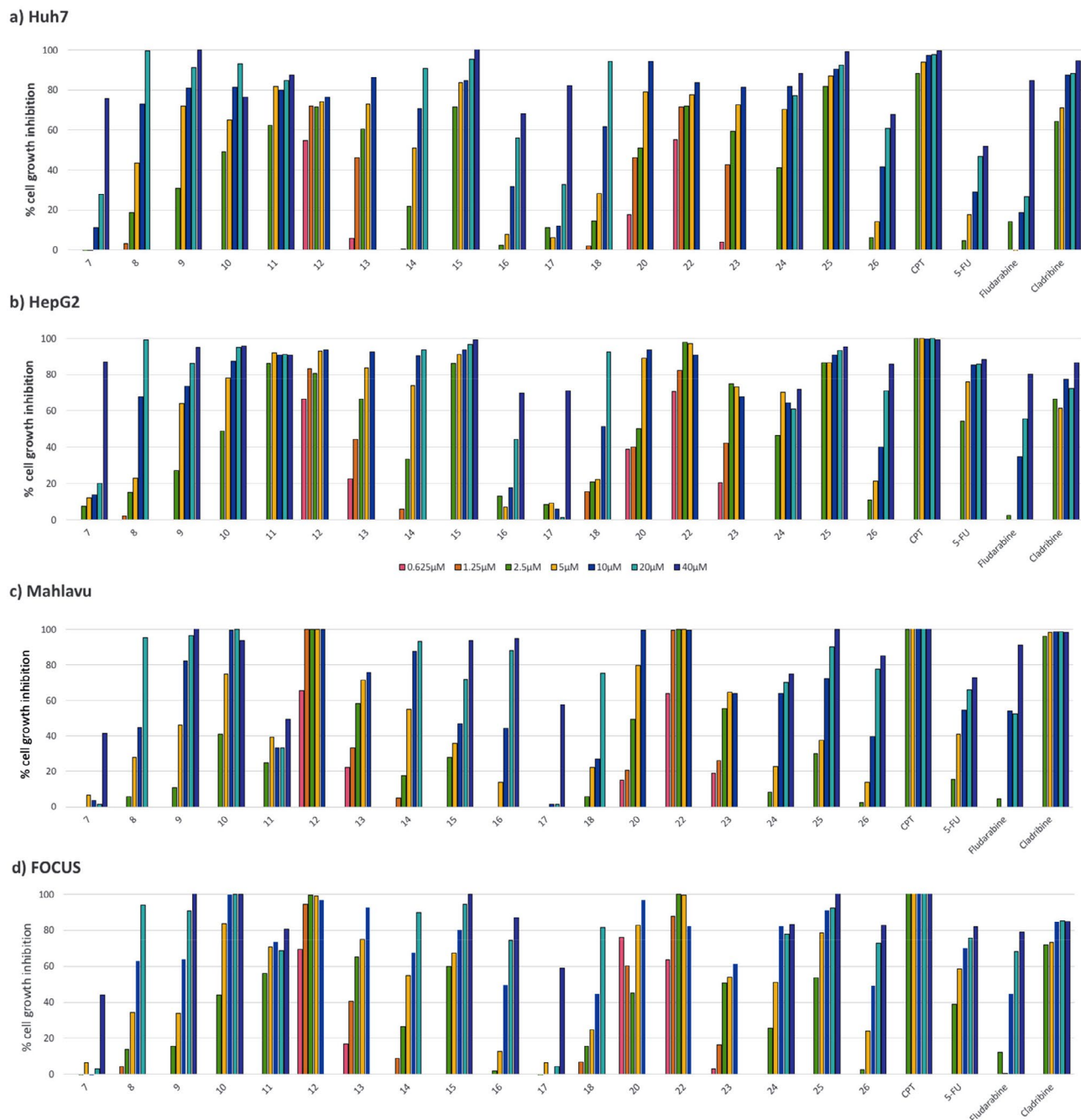


Figure 3. Percent cell death in the presence of most active compounds. Huh7, HepG2, Mahlavu and FOCUS cells were inoculated in 96-well plates. All molecules and their DMSO controls were administered to the cells in triplicate with five different concentrations: 40, 20, 10, 5, and 2.5 μM . After 72 h of incubation, SRB assays were generated, and the cell death percentages were calculated in comparison with DMSO-treated wells.

μM) and CPT (IC_{50} 0.034 μM). Furthermore, compounds **10**, **12**, **15**, **20**, **21**, **23** had a better cytotoxic activity (IC_{50} < 1.0 μM) than 5-FU and fludarabine against HCT116 cell line.

Purine **22**, one of the most cytotoxic molecules, displayed a significant IC_{50} value of 0.05 μM comparable to CPT (IC_{50} < 0.1 μM) on MCF7. Compound **22** also displayed better cytotoxic bioactivities on MCF7 cells with respect to 5-FU (IC_{50} 3.5 μM) and known nucleoside drugs

cladribine (IC_{50} 2.4 μM) and fludarabine (IC_{50} 15.2 μM), on MCF7 cells. In addition, the cytotoxic activity against MCF7 cell line of purines 7–26 was evaluated.

Significant bioactivity was also observed for compounds **10** (IC_{50} 0.16 μM), **12** (IC_{50} 0.4 μM), **20** (IC_{50} 0.38 μM), **21** (IC_{50} 0.48 μM), **11**, **15** (IC_{50} < 1.0 μM) on MCF7 cells.

We then screened the anticancer activity of the most potent purine analogs against further hepatocellular cancer

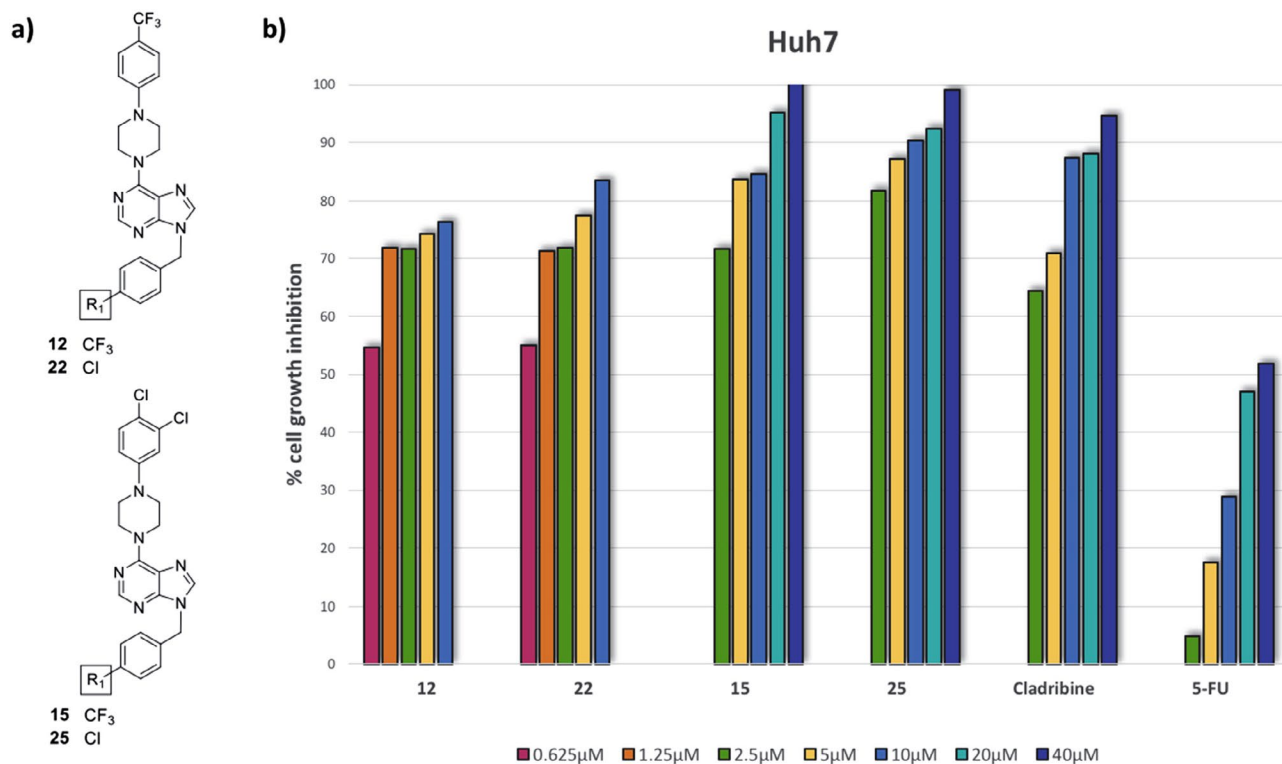


Figure 4. a) Chemical structures of the most active purine analogs **12**, **22**, **15** and **25** b) Percent cell death in the presence of most active compounds (**12**, **22**, **15** and **25**). Huh7 cells were inoculated in 96-well plates. All molecules and their DMSO controls were administered to the cells in triplicate with corresponding different concentrations: 0.625, 1.25, 2.5, 5, 10, 20, and 40 μM. After 72 h of incubation, SRB assays were generated, and the cell death percentages were calculated in comparison with DMSO-treated wells.

(HCC) cells lines (Table 2, Figure 3). We observed that the most important cell growth inhibition in the presence of 6-(4-(4-trifluoromethylphenyl)piperazine)-9-(4-trifluoromethylbenzyl)purine derivative **12** and its 9-(4-chlorobenzyl) analogue **22**, with IC₅₀ values of < 0.1–0.23 μM against all the HCC cell lines. Compounds **12** and **22** also showed comparable cytotoxic effects with CPT and cladribine on these cell lines. Furthermore, **12** and **22** showed a better biological activity than the standard anticancer agents 5-FU and fludarabine in HCC cell lines (Table 2). The 6-(4-(2,4-dichlorophenyl)piperazine) analogs **15**, **25** were also very active (IC₅₀ < 0.1–0.24 μM) against Huh7 and HepG2 cell lines.

4. Conclusion

We designed and synthesized twenty novel purine analogs **7–26** bearing substituted piperazine at the C-6, substituted benzyl group at the N-9, by the multistep reactions, starting from 4,6-dichloro-5-nitropyrimidine. The cytotoxic activities of the compounds were evaluated first in human liver (Huh7), breast (MCF7), colon (HCT116) and then in hepatocellular carcinoma cells (HCC): Huh7, HepG2, Mahlavu and FOCUS. Our results demonstrated that the 6-(trifluoromethylphenyl)piperazine analogs **12**, **22** with IC₅₀ values less than 0.5 μM were promising molecules as cytotoxic agents on Huh7, MCF7 and HCT116

cancer cells. In order to investigate the use of potential cytotoxic agents on HCC, the bioactivity of the purine analogs was also tested in a panel of liver cancer cells. Molecules **12** and **22**, that were synthesized as putative cytotoxic compounds, displayed the best anticancer bioactivities (IC₅₀ < 0.1–0.23 μM) against HCC cell lines (Figure 4). These results indicate that these compounds can be considered as promising lead molecules for the development of potential anticancer agents.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey-TUBITAK (TBAG-109T987), the KANSIL-2016H121540 (Ministry of Development, Turkey).

5. References

1. M. E. Welsch, S. A. Snyder, B. R. Stockwell, *Curr. Opin. Chem. Biol.* **2010**, *14*, 347–361. DOI:10.1016/j.cbpa.2010.02.018
2. I. Moriguchi, Y. Kanada, *Chem. Pharm. Bull.* **1977**, *25*, 926–935. DOI:10.1248/cpb.25.926
3. A. Brathe, G. Andresen, L. L. Gundersen, K. E. Malterud, F. Rise, *Bioorg. Med. Chem.* **2002**, *10*, 1581–1586. DOI:10.1016/S0968-0896(01)00427-8

4. A. Brathe, L. L. Gundersen, K. E. Malterud, F. Rise, *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 159–166. DOI:10.1002/ardp.200400951
5. S. F. Laufer, D. M. Domeyer, T. R. Scior, W. Albrecht, D. R. J. Hauser, *J. Med. Chem.* **2005**, *48*, 710–722. DOI:10.1021/jm0408767
6. J. A. Montgomery, K. Hewson, *J. Med. Chem.* **1968**, *11*, 48–52. DOI:10.1021/jm00307a010
7. G. Andersen, L. L. Gundersen, J. Nissen-Meyer, F. Rise, B. Spilberg, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 567–569. DOI:10.1016/S0960-894X(01)00803-4
8. J. F. Wang, L. R. Zhang, Z. J. Yang, L. H. Zhang, *Bioorg. Med. Chem.* **2004**, *12*, 1425–1429. DOI:10.1016/j.bmc.2004.01.005
9. M. Hocek, P. Naus, R. Pohl, I. Votruba, P. A. Furman, P. M. Tharnish, M. J. Otto, *J. Med. Chem.* **2005**, *48*, 5869–5873. DOI:10.1021/jm050335x
10. A. Kucukdumlu, M. Tuncbilek, E. B. Guven, R. C. Atalay, *Acta Chim. Slov.* **2017**, *64*, 621–632. DOI:10.17344/acsi.2017.3419
11. R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, R. K. Robins, *Science.* **1972**, *177*, 705–706. DOI:10.1126/science.177.4050.705
12. E. De Clercq, *Biochem. Pharmacol.* **1987**, *36*, 2567–2575. DOI:10.1016/0006-2952(87)90533-8
13. E. De Clercq, *Nucleosides Nucleotides.* **1998**, *17*, 625–634. DOI:10.1080/07328319808005205
14. E. De Clercq, *Antiviral Res.* **2005**, *67*, 56–75. DOI:10.1016/j.antiviral.2005.05.001
15. B. Y. Kim, J. B. Ahn, H. W. Lee, S. K. Kang, J. H. Lee, J. S. Shin, S. K. Ahn, C. I. Hong, S. S. Yoon, *Eur. J. Med. Chem.* **2004**, *39*, 433–447. DOI:10.1016/j.ejmech.2004.03.001
16. G. Jin, C. C. N. Wu, R. I. Tawatao, M. Chan, D. A. Carson, H. B. Cottam, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4559–4563. DOI:10.1016/j.bmcl.2006.06.017
17. E. M. Peterson, J. Brownell, R. Vince, *J. Med. Chem.* **1992**, *35*, 3991–4000. DOI:10.1021/jm00100a003
18. A. Gangjee, A. Vasudevan, S. F. Queener, *J. Med. Chem.* **1997**, *40*, 3032–3039. DOI:10.1021/jm970271t
19. G. Gumina, C. K. Chu, *Org. Lett.* **2002**, *4*, 1147–1149. DOI:10.1021/ol025562x
20. F. Bordon-Pallier, N. Jullian, P. Ferrari, A. M. Girard, M. T. Bocquel, J. Biton, N. Bouquin, J. L. Haesslein, *Biochim. Biophys. Acta.* **2004**, *1697*, 211–223. DOI:10.1016/j.bbapap.2003.11.025
21. P. R. Patel, C. Ramalingam, Y. T. Park, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6610–6614. DOI:10.1016/j.bmcl.2007.09.060
22. M. Tuncbilek, Z. Ates-Alagoz, N. Altanlar, A. Karayel, S. Ozbey, *Bioorg. Med. Chem.* **2009**, *17*, 1693–1700. DOI:10.1016/j.bmc.2008.12.050
23. J. G. Kuhn, *Ann Pharmacother.* **2001**, *35*, 217–227. DOI:10.1345/aph.10096
24. C. M. Galmarini, J. R. Mackey, C. Dumontet, *Lancet Oncol.* **2002**, *3*, 415–424. DOI:10.1016/S1470-2045(02)00788-X
25. D. Sampath, V. A. Rao, W. Plunkett, *Oncogene.* **2003**, *22*, 9063–9074. DOI:10.1038/sj.onc.1207229
26. C. M. Galmarini, F. Popowycz, B. Joseph, *Curr. Med. Chem.* **2008**, *15*, 1072–1082. DOI:10.2174/092986708784221449
27. G. Escherich, S. Richards, L. J. Stork, A. J. Vora, *Leukemia.* **2011**, *25*, 95–959. DOI:10.1038/leu.2011.37
28. P. N. Munshi, M. Lubin, J. R. Bertino, *Oncologist.* **2014**, *19*, 760–765. DOI:10.1634/theoncologist.2014-0178
29. S. A. Johnson, W. Thomas, *Hematol. Oncol.* **2000**, *18*, 141–153. DOI:10.1002/1099-1069(200012)18:4<141::AID-HON666>3.0.CO;2-#
30. S. A. Johnson, *Expert Opin. Pharmacother.* **2001**, *2*, 929–943.
31. W. B. Parker, J. A. 3rd Secrist, W. R. Waud, *Curr. Opin. Invest. Drugs.* **2004**, *5*, 592–596.
32. E. Lech-Maranda, A. Korycka, T. Robak, *Mini-Rev Med Chem.* **2006**, *6*, 575–581. DOI:10.2174/138955706776876212
33. M. S. Ricci, W. X. Zong, *Oncologist.* **2006**, *11*, 342–357. DOI:10.1634/theoncologist.11-4-342
34. M. Collado, M. Serrano, *Nature Rev. Cancer.* **2006**, *6*, 472–476. DOI:10.1038/nrc1884
35. C. Nardella, J. G. Clohessy, A. Alimonti, P. P. Pandolfi, *Nature Rev. Cancer.* **2011**, *11*, 503–511. DOI:10.1038/nrc3057
36. J. L. Haesslein, N. Jullian, *Curr. Topics Med. Chem.* **2002**, *2*, 1037–1050. DOI:10.2174/1568026023393291
37. W. F. De Azevedo, S. Leclerc, L. Meijer, L. Havlicek, M. Strnad, S. H. Kim, *Eur. J. Biochem.* **1997**, *243*, 518–526. DOI:10.1111/j.1432-1033.1997.0518a.x
38. Y. T. Chang, N. S. Gray, G. R. Rosania, D. P. Sutherlin, S. Kwon, T. C. Norman, R. Sarohia, M. Leost, L. Meijer, P. G. Schultz, *Chem. and Biol.* **1999**, *6*, 361–375. DOI:10.1016/S1074-5521(99)80048-9
39. K. Zurbonsen, A. Michel, P. A. Bonnet, L. Gannoun-Zaki, M. N. Mathieu, C. Chevillard, *Eur. J. Pharmacol.* **1997**, *320*, 215–221. DOI:10.1016/S0014-2999(96)00890-4
40. M. F. Brana, M. Cacho, M. L. Garcia, E. P. Mayoral, B. Lopez, B. De Pascual-Teresa, A. Ramos, N. Acero, F. Llinares, D. Munoz-Mingarro, O. Lozach, L. Meijer, *J. Med. Chem.* **2005**, *48*, 6843–6854. DOI:10.1021/jm058013g
41. C. Jaramillo, J. E. Diego, C. Hamdouchi, E. Collins, H. Keyser, C. Sanchez-Martinez, M. Prado, B. Norman, H. B. Brooks, S. A. Watkins, C. D. Spencer, J. A. Dempsey, B. D. Anderson, R. M. Campbell, T. Leggett, B. Patel, R. M. Schultz, J. Espinosa, M. Vieth, F. M. Zhang D. E. Timm, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6095–6099. DOI:10.1016/j.bmcl.2004.09.053
42. R. M. Mohareb, A. A. Mohamed, A. E. M. Abdallah, *Acta Chim. Slov.* **2016**, *63*, 227–240. DOI:10.17344/acsi.2015.1668
43. R. M. Mohareb, N. Y. M. Abdo, F. O. Al-Farouk, *Acta Chim. Slov.* **2017**, *64*, 117–128. DOI:10.17344/acsi.2016.2920
44. A. Gaagjee, X. Lin, R. L. Kisliuk, J. J. McGuire, *J. Med. Chem.* **2005**, *48*, 7215–7222.
45. S. Schenone, O. Bruno, A. Ranise, F. Bondavalli, C. Brullo, P. Fossa, L. Mosti, G. Menozzi, F. Carraro, A. Naldini, C. Bernini, F. Manetti, M. Botta, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2511–2517.
46. J. A. Markwalder, M. R. Arnone, P. A. Benfield, M. Biosdir, M. Boisclair, C. R. Burton, C. H. Chang, S. S. Cox, P. M. Czerniak, C. L. Dean, D. Doleniak, R. Grafstrom, B. A. Harrison, R. F. Kaltenbach, D. A. Nugiel, K. A. Rossi, S. R. Sherk, L. M. Sisk, P. Stouten, G. L. Trainor, P. Worland, S. P. Seitz, *J. Med. Chem.* **2004**, *47*, 5894–5911. DOI:10.1021/jm020455u

47. E. H. El-Sayed¹, A. A. Fadda, *Acta Chim. Slov.* **2018**, *65*, 853–864. DOI:10.17344/acsi.2018.4506
48. L. Havlicek, K. Fuksova, V. Krystof, M. Orsag, B. Vojtesek, M. Strnad, *Bioorg. Med. Chem.* **2005**, *13*, 5399–5407. DOI:10.1016/j.bmc.2005.06.007
49. S. Botros, O. M. Khalil, M. M. Kamel, Y. S. El-Dash, *Acta Chim. Slov.* **2017**, *64*, 102–116. DOI:10.17344/acsi.2016.2901
50. M. A. Biamonte, R. Van de Water, J. W. Arndt, R. H. Scannevin, D. Perret, W. C. Lee, *J. Med. Chem.* **2010**, *53*, 3–17. DOI:10.1021/jm9004708
51. M. Tuncbilek, E. Bilget Guven, T. Onder, R. Cetin Atalay, *J. Med. Chem.* **2012**, *55*, 3058–3065. DOI:10.1021/jm3001532
52. Z. Demir, E. B. Guven, S. Ozbey, C. Kazak, R. C. Atalay, M. Tuncbilek, *Eur. J. Med. Chem.* **2015**, *89*, 701–720. DOI:10.1016/j.ejmech.2014.10.080
53. D. L. Romero, C. E. Masse, S. Robinson, J. R. Greenwood, G. Harriman, *PCT Int Appl WO 2015/048281 Al.* **2015**, 1–168
54. A. K. Bakkestuen, L. L. Gundersen, B. T. Utenova, *J. Med. Chem.* **2005**, *48*, 2710–2723. DOI:10.1021/jm0408924

Povzetek

Načrtovali in izvedli smo sintezo serije novih 6,9-disubstituiranih purinskih analogov, ki na položaju C-6 vsebujejo 4-substituiran piperazin, na položaju N-9 pa 4-substituiran benzilni fragment. Vse pripravljene spojine (**7–26**) smo *in vitro* testirali za morebitno protirakavo aktivnost na Huh7 celicah jeter, HCT116 celicah debelega črevesa in MCF7 pljučnih celicah rakavih celičnih linij. Študije citotoksične bioaktivnosti so pokazale, da so vse spojine, z izjemo **19**, obetavno citotoksične z IC₅₀ vrednostmi med 0.05–21.8 μM proti Huh7, HCT116 in MCF7 celičnim linijam. Med vsemi pripravljenimi purinskimi analogi sta dva (**12** in **22**) izkazala posebej odlično citotoksično aktivnost in sicer IC₅₀ 0.08–0.13 μM na Huh7 celicah, kar je primerljivo s kamptotecinom (CPT) in boljše od kladribina, fludarabina in 5-FU. Nato smo raziskali še citotoksičnost najbolj aktivnih purinskih analogov na hepatocelične (HCC) rakave celice ter ugotovili, da spojini 6-(4-(4-trifluorometilfenil)piperazin (**12**) in 6-(4-(3,4-diklorofenil)piperazin (**25**) izkazujeta zelo obetavne IC₅₀ vrednosti (IC₅₀ <0.1–0.13 μM), ki so primerljive z vrednostmi za CPT in boljše kot je citotoksična bioaktivnost 5-FU, kladribina in fludarabina na HCC celice (Huh7 in HepG2).



Except when otherwise noted, articles in this journal are published under the terms and conditions of the Creative Commons Attribution 4.0 International License