Scientific paper

Synthesis of Some Substituted 6-Phenyl Purine Analogues and Their Biological Evaluation as Cytotoxic Agents

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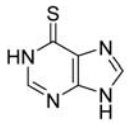
Abstract

A series of 6-(4-substituted phenyl)-9-(tetrahydropyran-2-yl)purines **3**–**9**, 6-(4-substituted phenyl)purines **10**–**16**, 9-((4-substituted phenyl)sulfonyl)-6-(4-substituted phenyl)purines **17**–**32** were prepared and screened initially for their *in vitro* anticancer activity against selected human cancer cells (liver Huh7, colon HCT116, breast MCF7). 6-(4-Phenoxyphenyl)purine analogues **9**, **16**, **30**–**32**, had potent cytotoxic activities. The most active purine derivatives **5–9**, **14**, **16**, **18**, **28**–**32** were further screened for their cytotoxic activity in hepatocellular cancer cells. 6-(4-Phenoxyphenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (**9**) had better cytotoxic activity (IC_{50} 5.4 μM) than the well-known nucleobase analogue 5-FU and known nucleoside drug fludarabine on Huh7 cells. The structure–activity relationship studies reported that the substitution at C-6 positions in purine nucleus with the 4-phenoxyphenyl group is responsible for the anti-cancer activity.

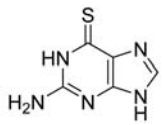
Keywords: Antitumor agents; Hepatocellular carcinoma; Heterocycles; Purine derivatives; Structure-activity relationships

1. Introduction

Cancer is a major human health problem and one of the principal reasons of death in both developing and industrialized countries. Purine and purine nucleoside analogues are important anti-cancer drugs used for the treatment of both hematological malignancies and solid tumors in chemotherapy. In 1953 and 1966, among the first anticancer drugs 6-mercaptopurine and 6-thioguanine (Fig. 1) were used as an inhibitor of nucleic acid metabolism in childhood acute lymphoblastic leukemia, respectively.[1–4]



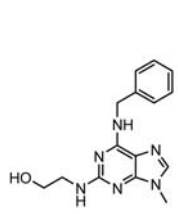
6-Mercaptopurine



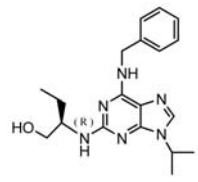
6-Thioguanine

Figure 1. Structures of 6-mercaptopurine and 6-thioguanine

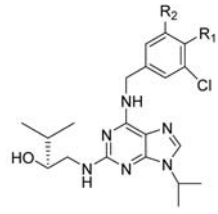
Potent purine-based cyclin-dependent kinase inhibitors olomoucine,[5] roscovitine,[6] purvalanol A, B, amino-purvalanol[7] (Fig. 2) and heterocyclic analogues of these compounds imidazo-pyrazines,[8] pyrazolo-pyrida-



Olomoucine



Roscovitine



Purvalanol A
(R_1= H, R_2= H)
Purvalanol B
(R_1= COOH, R_2= H)
Amino- Purvalanol
(R_1= H, R_2= NH_2)

Figure 2. Structures of olomoucine, roscovitine, purvalanol A, B and amino-purvalanol

zines,[9] imidazo-pyridines,[10,11] thieno-pyridines,[12] pyrrolo-pyrimidines,[13] pyrazolo-pyrimidines[14,15] and triazolo-pyrimidines[16,17] have been investigated as anticancer agents.

Furthermore, purine nucleosides such as fludarabine, cladribine, and pentostatine (Fig. 3) were approved in FDA for the therapy of hematologic disorders between 1991 and 1992.[18,19]

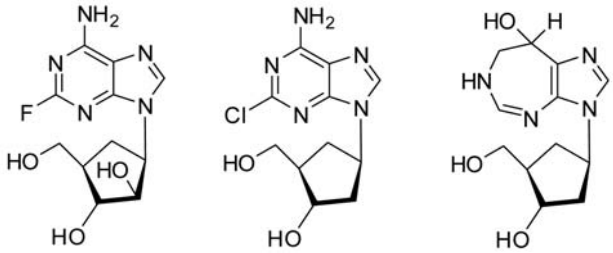


Figure 3. Structures of fludarabine, cladribine and pentostatine

Hepatocellular carcinoma (HCC) is one of the deadly cancers and affects most of the world population. It is also the fifth most common cancer in men and seventh in women, accounting for 7% of all cancer cases, and the third most common reason of cancer-connected death worldwide, with around 700,000 new cases each year.[20–21]

Chronic liver damage is due to viral diseases, chemical exposure, environmental toxins or autoimmune diseases that are the risk factors for HCC. These conditions cause an acquired tolerance to genotoxic stress, but finally result in a cancerous case that does not respond to the mechanism of cell death.[23]

The diagnosis of HCC patients is usually very poor and HCC tumors are resistant to chemotherapeutic agents. Lately, a multikinase inhibitor Sorafenib, was approved by the FDA and the EU for the treatment of hepatocellular carcinoma.[24] Sorafenib HCC Assessment Randomised Protocol (SHARP) indicated significantly improved overall survival and the time to progression by almost three months in cases with advanced HCC upon treatment with the antiangiogenic and antiproliferative agent Sorafenib.[25–27] Therefore, it is essential to discover new liver-cancer-specific drugs for hepatocellular carcinoma treatment.

As a result of our ongoing investigations of purine and purine nucleoside derivatives, which have displayed promising cytotoxic activity,[28,29] herein, we synthesized new series of substituted purines (**3**–**9**, **10**–**16**, **17**–**32**) and screened their anticancer activities on selected human cancer cells (liver Huh7, colon HCT116, breast MCF7); and the most potent purine derivatives (**5**–**9**, **14**, **16**, **18**, and **28**–**32**) were further tested on a panel of hepatocellular cancer cell.

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 for ^{1}H, 100.6 MHz for ^{13}C). TMS was used as internal standard for the ^{1}H and ^{13}C NMR spectra; values are given in δ (ppm) and J values are in Hz. Mass spectra were taken on Waters Micromass ZQ by using ESI+ ionization method. Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument and gave values within ±0.4% of the theoretical values. Column chromatography was accomplished on silica gel 60 (40–63 mm particle size). The chemical reagents used in synthesis were purchased from Merck, Fluka, Sigma and Aldrich.

2. 1. 1. 6-Chloro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (2)[30]

p-TSA (0.01 g) was added to a solution of 6-chloropurine (0.15 g, 1 mmol) in dry THF at reflux. After 3,4-dihydro-2*H*-pyran (0.098 g, 1.18 mmol) was added and the mixture refluxed for 15 h. After cooling to ambient temparature the reaction mixture was treated with 1 mL 25% NH_4OH and sitirred for 5 min. The solution was evaporated in vacuo and treated with 25 mL EtOAc, washed with brine and water. The organic phase was dried over Na_2SO_4, the solvent was evaporated in vacuo, and recrystallized from hexane petroleum ether to yield **2** (220 mg; 95%): mp 69–71 °C (67–69 °C[30]). ^{1}H NMR ($CDCl_3$) δ 1.64–1.88 (m, 3H, H-pyran), 2.02–2.21 (m, 3H, H-pyran), 3.80 (td, J_1 = 2.8 Hz, J_2 = 12 Hz, 1H, H-5'a in pyran), 4.20 (d, 1H, H-5'b in pyran), 5.80 (dd, J_1 = 10.8 Hz, J_2 = 2.4 Hz, 1H, H-1' in pyran), 8.35 (s, 1H, H-8 in purine), 8.76 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 239.70 (10%) (M+H).

2. 1. 2. General Procedure for the Synthesis of 6-(4-Substituted Phenyl)-9-(tetrahydropyran-2-yl)-9*H*-purines 3–9

6-Chloro-9-(tetrahydropyran-2-yl)-9*H*-purine (**2**) was dissolved in 5 mL toluene, then K_2CO_3 (1.5 mmol), 4-substituted phenylboronic acid (1.5 mmol) and $Pd(Ph_3)_4$ (0.05 mmol) were added. The mixture was refluxed for 12 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and purified by column chromatography (EtOAC–hexane, 1:3 to 1:6).

6-Phenyl-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (3)[31]

Yield 60 mg (55%), mp 189–191 °C. ^{1}H NMR ($CDCl_3$) δ 1.67–1.86 (m, 3H, H-pyran), 2.01–2.21 (m, 3H, H-pyran), 3.83 (td, J_1 = 11.6 Hz, J_2 = 2.8 Hz, 1H, H-5'a in pyran), 4.21 (d, 1H, H-5'b in pyran), 5.86 (dd, J_1

= 10 Hz, J_2 = 2.8 Hz, 1H, H-1' in pyran), 7.53 (t, J = 7.6 Hz, 2H, H-3,5 in phenyl), 7.60 (t, J = 7.6 Hz, 1H, H-4 in phenyl), 8.35 (s, 1H, H-8 in purine), 8.77 (d, J = 6.4 Hz, 2H, H-2,6 in phenyl), 9.03 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 197.52 (100%) (M+H–THP), 281.71 (77%) (M+H).

6-(4-Fluorophenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (4)[31]

Yield 190 mg (63%), mp 161–163 °C. ^{1}H NMR (DMSO-d_6) δ 1.57–1.65 (m, 2H, H-pyran), 1.70–1.82 (m, 1H, H-pyran), 1.97–2.03 (m, 2H, H-pyran), 2.29–2.40 (m, 1H, H-pyran), 3.72 (td, J_1 = 3.2 Hz, J_2 = 11.2 Hz, 1H, H-5'a in pyran), 4.03 (d, 1H, H-5'b in pyran), 5.81 (dd, J_1 = 2 Hz, J_2 = 10.8 Hz, 1H, H-1' in pyran), 7.43 (t, J = 8.8 Hz, 2H, H-3,5 in phenyl), 8.87–8.91 (m, 3H, H-2,6 in phenyl, H-8 in purine), 8.98 (s, 1H, H-2 in purine) (Ref. [31] 1.6–1.9 and 2.0–2.2 (2 × m, 6H, CH_2), 3.81 (dt, 1H, J_1 = 2.2 Hz, J_2 = 11.5, H-5'a), 4.20 (brd, 1H, J = 11.5 Hz, H-5'b), 5.84 (dd, 1H, J_1 = 10.3 Hz, J_2 = 2.3 Hz, H-1'), 7.22 (t, 2H, J = 8.7 Hz, H-o-Ar), 8.31 (s, 1H, H-8), 8.84 (dd, 2H, J_1 = 8.7 Hz, J_2 = 5.7 Hz, H-m-Ar), 8.99 (s, 1H, H-2)). MS (ESI+) *m/z*: 215.5 (100%) (M+H–THP), 299.7 (100%) (M+H).

6-(4-Chlorophenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (5)

Yield 240 mg (77%), mp 173–175 °C. ^{1}H NMR ($CDCl_3$) δ 1.65–1.86 (m, 3H, H-pyran), 2.02–2.18 (m, 3H, H-pyran), 3.80 (td, J_1 = 2.8 Hz, J_2 = 11.6 Hz, 1H, H-5'a in pyran), 4.19 (d, 1H, H-5'b in pyran), 5.83 (dd, J_1 = 2.8 Hz, J_2 = 10.8 Hz, 1H, H-1' in pyran), 7.51 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 8.32 (s, 1H, H-8 in purine), 8.76 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 8.99 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 231.5 (100%) (M+H–THP), 315.7 (100%) (M+H). Anal. Calcd for $C_{16}H_{15}ClN_4O \cdot 0.2EtOAc \cdot 0.2H_2O$: C, 60.05; H, 5.09; N, 16.67. Found C, 59.82; H, 4.69; N, 16.32.

6-(4-Bromophenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (6)

Yield 250 mg (70%), mp 160–162 °C. ^{1}H NMR ($CDCl_3$) δ 1.64–1.87 (m, 3H, H-pyran), 2.02–2.20 (m, 3H, H-pyran), 3.81 (td, J_1 = 2.8 Hz, J_2 = 11.2 Hz, 1H, H-5'a in pyran), 4.19 (d, 1H, H-5'b in pyran), 5.84 (dd, J_1 = 2.4 Hz, J_2 = 10 Hz, 1H, H-1' in pyran), 7.67 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 8.33 (s, 1H, H-8 in purine), 8.69 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 8.99 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 275.6 (100%) (M–THP), 359.7 (78%) (M). Anal. Calcd for $C_{16}H_{15}BrN_4O$: C, 53.50; H, 4.21; N, 15.60. Found C, 53.29; H, 4.30; N, 15.99.

6-(4-Trifluoromethylphenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (7)

Yield 250 mg (73%), mp 164–165 °C. ^{1}H NMR ($CDCl_3$) δ 1.67–1.88 (m, 3H, H-pyran), 2.05–2.21 (m, 3H, H-pyran), 3.82 (td, J_1 = 2.4 Hz, J_2 = 11.6 Hz, 1H, H-5'a in pyran), 4.21 (d, 1H, H-5'b in pyran), 5.86 (dd, J_1 = 2.8 Hz, J_2 = 10.8 Hz, 1H, H-1' in pyran), 7.80 (d, J = 8 Hz, 2H, H-2,6 in phenyl), 8.37 (s, 1H, H-8 in purine), 8.91 (d, J = 8 Hz, 2H, H-3,5 in phenyl), 9.05 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 265.6 (100%) (M+H–THP), 349.8 (75%) (M+H). Anal. Calcd for $C_{17}H_{15}F_3N_4O \cdot 0.04CH_2Cl_2 \cdot 0.15EtOAc$: C, 58.05; H, 4.49; N, 15.35. Found C, 58.44; H, 4.09; N, 14.95.

6-(4-*tert*-Butylphenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (8)

Yield 230 mg (67%), mp 161–162 °C. ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, CH_3), 1.66–1.84 (m, 3H, H-pyran), 2.04–2.20 (m, 3H, H-pyran), 3.81 (td, J_1 = 2.8 Hz, J_2 = 11.6 Hz, 1H, H-5'a in pyran), 4.20 (d, 1H, H-5'b in pyran), 5.84 (dd, J_1 = 2.8 Hz, J_2 = 10.4 Hz, 1H, H-1' in pyran), 7.58 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 8.31 (s, 1H, H-8 in purine), 8.67 (d, J = 8 Hz, 2H, H-2,6 in phenyl), 9.0 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 253.7 (100%) (M+H–THP), 337.8 (100%) (M+H). Anal. Calcd for $C_{20}H_{24}N_4O$: C, 71.40; H, 7.19; N, 16.65. Found C, 71.0; H, 7.34; N, 16.78.

6-(4-Phenoxyphenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (9)

Yield 220 mg (60%), mp 147–149 °C. ^{1}H NMR ($CDCl_3$) δ 1.64–1.85 (m, 3H, H-pyran), 2.02–2.18 (m, 3H, H-pyran), 3.80 (td, J_1 = 2.4 Hz, J_2 = 11.6 Hz, 1H, H-5'a in pyran), 4.19 (d, 1H, H-5'b in pyran), 5.83 (dd, J_1 = 2.8 Hz, J_2 = 10.4 Hz, 1H, H-1' in pyran), 7.08 (d, J = 8.8 Hz, 2H, H-2,6 in O-phenyl), 7.13–7.16 (m, 3H, H-3,5, H-4' in phenyl), 7.36 (t, J = 8 Hz, 2H, H-3,5 in O-phenyl), 8.30 (s, 1H, H-8 in purine), 8.78 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 8.98 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 289.7 (88%) (M+H–THP), 373.8 (100%) (M+H). Anal. Calcd for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04. Found C, 71.23; H, 5.44; N, 15.30.

2. 1. 3. General Procedure for the Synthesis of 6-(4-Substituted Phenyl)-9*H*-purines 10–16

A mixture of 6-(4-substituted phenyl)-9-(tetrahydropyran-2-yl)-9*H*-purines (1 mmol) **3–9**, Dowex 50 × 8 (H^+) (700 mg), MeOH (10 mL) and H_2O (1 mL) was refluxed. Then the reaction mixture was filtered and washed with saturated methanolic NH_3 and MeOH. The filtrate was evaporated in vacuo, and recrystallized from EtOAc –hexane.

6-Phenyl-9*H*-purine (10)[31]

Yield 160 mg (80%), mp 279–281 °C (280–282 °C[31]). ^{1}H NMR (DMSO-d_6) δ 7.28–7.40 (m, 3H, H-3,4,5 in phenyl), 7.76 (d, J = 6.4 Hz, 2H, H-2,6 in phenyl), 8.01 (s, 1H, H-8 in purine), 8.81 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 197.6 (100%) (M+H).

6-(4-Fluorophenyl)-9*H*-purine (11)[31]

Yield 190 mg (87%), mp 295–298 °C (299–302 °C[31]). ^{1}H NMR (DMSO-d_6) δ 7.41 (t, J = 8.8 Hz, 2H, H-3,5 in phenyl), 8.63 (s, 1H, H-8 in purine), 8.86–8.91 (m, 2H, H-2,6 in phenyl,), 8.92 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 215.6 (100%) (M+H).

6-(4-Chlorophenyl)-9*H*-purine (12)

Yield 190 mg (83%), mp 290–292 °C. ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 8.8 Hz, 2H, H-3,5 in phenyl), 8.68 (s, 1H, H-8 in purine), 8.87 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 8.97 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 231.4 (100%) (M+H). Anal. Calcd for $C_{11}H_7ClN_4$: C, 57.28; H, 3.06; N, 24.29. Found C, 57.08; H, 3.05; N, 24.32.

6-(4-Bromophenyl)-9*H*-purine (13)

Yield 150 mg (55%), mp 310–311 °C. ^{1}H NMR (DMSO-d_6) δ 7.82 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 8.69 (s, 1H, H-8 in purine), 8.80 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 8.97 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 275.6 (100%) (M), 277.7 (M+2) (90%). Anal. Calcd for $C_{11}H_7BrN_4 \cdot 1.0H_2O$: C, 45.07; H, 3.09; N, 19.11. Found C, 45.44; H, 2.97; N, 19.52.

6-(4-Trifluoromethylphenyl)-9*H*-purine (14)

Yield 210 mg (80%), mp 221–223 °C. ^{1}H NMR (DMSO-d_6) δ 7.66 (d, J = 8 Hz, 2H, H-2,6 in phenyl), 7.95 (d, J = 7.6 Hz, 2H, H-3,5 in phenyl), 8.33 (s, 2H, H-2,8 in purine). MS (ESI+) *m/z*: 265.6 (100%) (M+H). Anal. Calcd for $C_{12}H_7F_3N_4$: C, 54.54; H, 2.65; N, 21.21. Found C, 54.37; H, 2.46; N, 21.39.

6-(4-*tert*-Butylphenyl)-9*H*-purine (15)

Yield 220 mg (88%), mp 291–293 °C. ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, CH_3), 7.61 (d, J = 8.8 Hz, 2H, H-3,5 in phenyl), 8.34 (s, 1H, H-8 in purine), 8.71 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 9.10 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 253.7 (100%) (M+H). Anal. Calcd for $C_{15}H_{16}N_4 \cdot 0.1H_2O$: C, 70.89; H, 6.42; N, 22.04. Found C, 71.23; H, 6.65; N, 21.66.

6-(4-Phenoxyphenyl)-9*H*-purine (16)

Yield 260 mg (90%), mp 240–241 °C. ^{1}H NMR (DMSO-d_6) δ 7.14–7.25 (m, 5H, H-3,5 in phenyl, H-2,4,6 in O-phenyl), 7.46 (t, J = 7.6 Hz, 2H, H-3,5 in O-phenyl), 8.63 (s, 1H, H-8 in purine), 8.88 (d, J = 7.6 Hz, 2H, H-2,6 in phenyl), 8.92 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 289.7 (100%) (M+H). Anal. Calcd for $C_{17}H_{12}N_4O \cdot 0.22$EtOAc: C, 69.79; H, 4.50; N, 18.20. Found C, 70.08; H, 4.47; N, 17.81.

2. 1. 4. General Procedure for the Sulfonylation of 6-(4-Substituted Phenyl)-9*H*-purines (Preparation of Compounds 17–32)

A solution of (substituted phenyl)sulfonyl chloride (2 mmol) in 5 mL CH_2Cl_2 was slowly added to a solution of 6-(4-substituted phenyl)-9*H*-purines **10–16** (1 mmol) in 1 mL pyridine. The reaction mixture was stirred for 40–48 h in an ice bath. The reaction mixture was treated with 1N HCl (5 mL) and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, the solvent was evaporated in vacuo, and the residue was purified by column chromatography (hexane:CH_2Cl_2, 1:1).

9-(4-Fluorophenylsulfonyl)-6-phenyl-9*H*-purine (17)

Yield 71 mg (20%), mp 255–256 °C. ^{1}H NMR ($CDCl_3$) δ 7.29 (d, 2H, H-2,6 in phenyl), 7.55 (t, 3H, H-3,4,5 in phenyl), 8.39 (dd, J_1 = 4.8 Hz, J_2 = 8.8 Hz, 2H, H-2',6' in phenyl), 8.56 (s, 1H, H-8 in purine), 8.69 (dd, 2H, H-3',5' in phenyl), 9.09 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 355.7 (100%) (M+H). Anal. Calcd for $C_{17}H_{11}FN_4O_2S$: C, 57.62; H, 3.13; N, 15.81; S, 9.05. Found C, 57.96; H, 3.27; N, 15.59; S 9.09.

9-(4-Trifluoromethylphenylsulfonyl)-6-phenyl-9*H*-purine (18)

Yield 190 mg (48%), mp 222–224 °C. ^{1}H NMR ($CDCl_3$) δ 7.55 (t, 3H, H-3,4,5 in phenyl), 7.87 (d, 2H, J = 8.8 Hz, H-2,6 in phenyl), 8.50 (d, J = 8.4 Hz, 2H, H-2',6' in phenyl), 8.56 (s, 1H, H-8 in purine), 8.67–8.70 (m, 2H, H-3',5' in phenyl), 9.10 (s, 1H, H-2 in purine). ^{13}C NMR ($CDCl_3$) δ 127.02 (q) (CF_3), 129.04, 129.62, 130.13, 131.51, 132.02, 134.78, 137.0, 137.34 (C in phenyl), 140.32 (C-5), 141.06 (C-8), 151.21 (C-6), 154.33 (C-2), 156.73 (C-4). MS (ESI+) *m/z*: 405.7 (100%) (M+H). Anal. Calcd for $C_{18}H_{11}F_3N_4O_2S$: C, 53.46; H, 2.74; N, 13.86; S, 7.93. Found C, 53.69; H, 2.81; N, 13.59; S 7.97.

9-(4-*tert*-Butylphenylsulfonyl)-6-phenyl-9*H*-purine (19)

Yield 160 mg (40%), mp 236–237 °C. ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H, CH_3), 7.52–7.55 (m, 3H, H-3,4,5 in phenyl), 7.59 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 8.23 (d, J = 9.2 Hz, 2H, H-3',5' in phenyl), 8.56 (s, 1H, H-8 in purine), 8.67–8.69 (m, 2H, H-2',6' in phenyl), 9.11 (s, 1H, H-2 in purine). ^{13}C NMR ($CDCl_3$) δ 31.12 (CH_3), 35.74 (C in *tert*-butyl), 126.93, 128.76, 128.99, 130.07, 131.58, 131.79, 133.86, 135.01 (C in phenyl), 141.62 (C-5), 151.34 (C-8), 154.19 (C-6), 156.33 (C-2), 160.08 (C-4). MS (ESI+) *m/z*: 393.9 (100%) (M+H). Anal. Calcd for: $C_{21}H_{20}N_4O_2S$: C, 64.27; H, 5.14; N, 14.28; S, 8.17. Found C, 63.88; H, 5.19; N, 13.97; S 8.11.

9-(4-Fluorophenylsulfonyl)-6-(4-fluorophenyl)-9*H*-purine (20)

Yield 130 mg (35%), mp 265–267 °C. ^{1}H NMR ($CDCl_3$) δ 7.19–7.29 (m, 4H, H-3,5, H-3',5' in phenyl), 8.37 (dd, J_1 = 4.4 Hz, J_2 = 8.4 Hz, 2H, H-2',6' in phenyl), 8.54 (s, 1H, H-8 in purine), 8.77 (dd, J_1 = 5.6 Hz, J_2 = 8.4 Hz, 2H, H-2,6 in phenyl), 9.10 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 373.8 (100%) (M+H). Anal. Calcd for: $C_{17}H_{10}F_2N_4O_2S \cdot 0.5H_2O$: C, 53.54; H, 2.90;

N, 14.69; S, 8.40. Found C, 53.14; H, 2.70; N, 14.45; S 8.47.

9-(4-Trifluoromethylphenylsulfonyl)-6-(4-fluorophenyl)-9*H*-purine (21)

Yield 140 mg (32%), mp 214–216 °C. ^{1}H NMR ($CDCl_3$) δ 7.22 (t, J = 8.8 Hz, 2H, H-3,5 in phenyl), 7.87 (d, J = 8.8 Hz, 2H, H-2',6' in phenyl), 8.49 (d, J = 8.4 Hz, 2H, H-3',5' in phenyl), 8.55 (s, 1H, H-8 in purine), 8.77 (dd, J_1 = 5.6 Hz, J_2 = 9.2 Hz, 2H, H-2,6 in phenyl), 9.07 (s, 1H, H-2 in purine). ^{13}C NMR ($CDCl_3$) δ 115.88, 116.09, 125.84 (C in phenyl), 126.82 (q) (CF_3), 129.42, 130.81, 131.0, 132.22, 132.31 (C in phenyl), 140.25 (C-5), 140.87 (C-8), 151.0 (C-6), 154.08 (C-2), 155.25 (C-4). MS (ESI+) *m/z*: 423.8 (100%) (M+H). Anal. Calcd for: $C_{18}H_{10}F_4N_4O_2S \cdot 0.4CH_2Cl_2$: C, 48.42; H, 2.38; N, 12.27; S, 7.02. Found C, 48.21; H, 2.48; N, 12.00; S 7.26.

9-(4-*tert*-Butylphenylsulfonyl)-6-(4-fluorophenyl)-9*H*-purine (22)

Yield 80 mg (20%), mp 226–227 °C. ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H, CH_3), 7.21 (t, J = 8.8 Hz, 2H, H-3,5 in phenyl), 7.59 (d, J = 8.8 Hz, 2H, H-3',5' in phenyl), 8.23 (d, J = 8.4 Hz, 2H, H-2',6' in phenyl), 8.55 (s, 1H, H-8 in purine), 8.77 (dd, J_1 = 5.6 Hz, J_2 = 9.2 Hz, 2H, H-2,6 in phenyl), 9.09 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 411.9 (100%) (M+H). Anal. Calcd for: $C_{21}H_{19}FN_4O_2S$: C, 61.45; H, 4.67; N, 13.65; S, 7.81. Found C, 61.83; H, 4.78; N, 13.25; S 8.02.

9-(4-Fluorophenylsulfonyl)-6-(4-chlorophenyl)-9*H*-purine (23)

Yield 190 mg (49%), mp 237–239 °C. ^{1}H NMR ($CDCl_3$) δ 7.51 (d, J = 8.4 Hz, 2H, H-3',5' in phenyl), 7.87 (d, J = 8.8 Hz, 2H, H-3,5 in phenyl), 8.49 (d, J = 8.8 Hz, 2H, H-2',6' in phenyl), 8.70 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 9.11 (s, 1H, H-8 in purine), 9.19 (s, 1H, H-2 in purine). ^{13}C NMR (DMSO-d_6) δ 126.75, 129.08, 129.40, 131.21, 133.02, 136.84, 138.21 (C in phenyl), 140.10 (C-5), 140.94 (C-8), 151.07 (C-6), 154.04 (C-2), 155.10 (C-4). MS (ESI+) *m/z*: 231.6 (100%) [M+H–(4-F-Ph-SO_2)]. Anal. Calcd for: $C_{17}H_{10}ClFN_4O_2S \cdot 0.4\ CH_2Cl_2$: C, 49.43; H, 2.57; N, 13.25; S, 7.58. Found C, 49.11; H, 2.46; N, 12.85; S 7.38.

9-(4-Fluorophenylsulfonyl)-6-(4-bromophenyl)-9*H*-purine (24)

Yield 110 mg (25%), mp 243–245 °C. ^{1}H NMR ($CDCl_3$) δ 7.21 (t, J = 8.8 Hz, 2H, H-3',5' in phenyl), 7.61 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 8.32 (dd, J_1 = 5.2 Hz, J_2 = 7.2 Hz, 2H, H-2',6' in phenyl), 8.50 (s, 1H, H-8 in purine), 8.56 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 9.01 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 433.7 (100%) (M), 435.8 (M+2) (60%). Anal. Calcd for: $C_{17}H_{10}BrFN_4O_2S$: C, 47.13; H, 2.33; N, 12.93; S, 7.40. Found C, 47.39; H, 2.27; N, 12.89; S 7.62.

9-(4-Fluorophenylsulfonyl)-6-(4-trifluoromethylphenyl)-9*H*-purine (25)

Yield 110 mg (26%), mp 240–242 °C. ^{1}H NMR ($CDCl_3$) δ 7.28 (t, J = 8.8 Hz, 2H, H-3',5' in phenyl), 7.79 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 8.39 (dd, J_1 = 5.2 Hz, J_2 = 9.2 Hz, 2H, H-2',6' in phenyl), 8.59 (s, 1H, H-8 in purine), 8.85 (d, J = 8 Hz, 2H, H-3,5 in phenyl), 9.13 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 423.8 (80%) (M+H). Anal. Calcd for: $C_{18}H_{10}F_4N_4O_2S$: C, 51.19; H, 2.39; N, 13.27; S, 7.59. Found C, 51.37; H, 2.26; N, 13.54; S 7.60.

9-(4-Trifluoromethylphenylsulfonyl)-6-(4-trifluoromethylphenyl)-9*H*-purine (26)

Yield 140 mg (31%) mp 240–241 °C. ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 7.88 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 8.50 (d, J = 8 Hz, 2H, H-2',6' in phenyl), 8.60 (s, 1H, H-8 in purine), 8.84 (d, J = 8 Hz, 2H, H-3',5' in phenyl), 9.14 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 265.6 (100%) [M+H–(4-F-Ph-SO_2)]. Anal. Calcd for: $C_{19}H_{10}F_6N_4O_2S \cdot 0.6CH_2Cl_2$: C, 44.98; H, 2.16; N, 10.70; S, 6.13. Found C, 45.26; H, 2.28; N, 11.10; S 6.38.

9-(4-*tert*-Butylphenylsulfonyl)-6-(4-trifluoromethylphenyl)-9*H*-purine (27)

Yield 100 mg (22%), mp 193–195 °C. ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9H, CH_3), 7.61 (d, J = 9.2 Hz, 2H, H-3',5' in phenyl), 7.79 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 8.25 (d, J = 9.2 Hz, 2H, H-2',6' in phenyl), 8.61 (s, 1H, H-8 in purine), 8.86 (d, J = 8.4 Hz, 2H, H-3,5 in phenyl), 9.16 (s, 1H, H-2 in purine). ^{13}C NMR (DMSO-d_6) δ 30.88 (CH_3), 35.53 (C in *tert*-butyl), 125.61 (q) (CF_3), 126.75, 128.60, 130.14, 130.86, 131.66, 132.73, 133.49, 138.04 (C in phenyl), 141.97 (C-5), 151.34 (C-8), 153.96 (C-6), 154.25 (C-2), 160.04 (C-4). MS (ESI+) *m/z*: 461.8 (100%) (M+H). Anal. Calcd for: $C_{22}H_{19}F_3N_4O_2S \cdot 0.1$Hexane: C, 57.87; H, 4.38; N, 11.94; S, 6.84. Found C, 58.31; H, 4.55; N, 11.62; S 6.52.

9-(4-Fluorophenylsulfonyl)-6-(4-*tert*-butylphenyl)-9*H*-purine (28)

Yield 180 mg (44%), mp: 240–242 °C. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H, CH_3), 7.27 (t, J = 8.8 Hz, 2H, H-3',5' in phenyl), 7.56 (d, J = 8.8 Hz, 2H, H-3,5 in phenyl), 8.38 (dd, J_1 = 4.8 Hz, J_2 = 8.8 Hz, 2H, H-2',6' in phenyl), 8.55 (s, 1H, H-8 in purine), 8.59 (d, J = 8.4 Hz, 2H, H-2,6 in phenyl), 9.10 (s, 1H, H-2 in purine). ^{13}C NMR (DMSO-d_6) δ 31.13 (CH_3), 35.01 (C in *tert*-butyl), 115.32, 117.20, 125.82, 127.06, 128.38, 129.68, 131.13, 131.87 (C in phenyl), 140.83 (C-5), 150.85 (C-8), 153.96 (C-6), 155.30 (C-2), 156.38 (C-4). MS (ESI+) *m/z*: 411.8 (100%) (M+H). Anal. Calcd for: $C_{21}H_{19}FN_4O_2S$: C, 61.45; H, 4.67; N, 13.65; S, 7.81. Found C, 61.09; H, 4.89; N, 13.22; S 7.69.

9-(4-Trifluoromethylphenylsulfonyl)-6-(4-*tert*-butylphenyl)-9*H*-purine (29)

Yield 120 mg (26%), mp 199–201 °C. ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, CH_3), 7.57 (d, J = 8.8 Hz, 2H, H-3,5 in phenyl), 7.87 (d, J = 8.4 Hz, 2H, H-2',6' in phenyl), 8.50 (d, J = 8 Hz, 2H, H-2,6 in phenyl), 8.55 (s, 1H, H-8 in purine), 8.61 (d, J = 8.4 Hz, 2H, H-3',5' in phenyl), 9.08 (s, 1H, H-2 in purine). ^{13}C NMR (DMSO-d_6) δ 31.12 (CH_3), 35.03 (C in *tert*-butyl), 124.89, 125.84, 126.78 (q) (CF_3), 129.38, 129.70, 131.12, 131.78, 135.38, 137.07 (C in phenyl), 140.61 (C-5), 150.87 (C-8), 154.12 (C-6), 155.40 (C-2), 156.60 (C-4). MS (ESI+) *m/z*: 461.9 (100%) (M+H). Anal. Calcd for: $C_{22}H_{19}F_3N_4O_2S \cdot 0.3$Hexane: C, 58.77; H, 4.80; N, 11.52; S, 6.59. Found C, 58.84; H, 4.55; N, 11.21; S 6.24.

9-(4-Fluorophenylsulfonyl)-6-(4-phenoxyphenyl)-9*H*-purine (30)

Yield 300 mg (66%), mp 178–180 °C. ^{1}H NMR ($CDCl_3$) δ 7.04–7.13 (m, 4H, H-3,5 in phenyl, H-2,6 in O-phenyl), 7.17 (t, J = 8.4 Hz, 1H, H-4 in O-phenyl), 7.27 (t, J = 8.4 Hz, 2H, H-3,5 in O-phenyl), 7.38 (t, J = 8.4 Hz, 2H, H-3',5' in phenyl), 8.38 (dd, J_1 = 4.8 Hz, J_2 = 9.2 Hz, 2H, H-2',6' in phenyl), 8.54 (s, 1H, H-8 in purine), 8.71 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 9.06 (s, 1H, H-2 in purine). ^{13}C NMR (DMSO-d_6) δ 115.33, 117.21, 118.05, 119.95, 124.31, 127.10, 128.47, 129.02, 129.96, 130.83, 131.87, 132.62 (C in phenyl), 140.83(C-5), 150.88 (C-8), 153.85 (C-6), 155.84 (C-2), 160.91 (C-4). MS (ESI+) *m/z*: 447.7 (100%) (M+H). Anal. Calcd for: $C_{23}H_{15}FN_4O_3S \cdot 0.5H_2O$: C, 60.65; H, 3.54; N, 12.30; S, 7.04. Found C, 60.89; H, 3.45; N, 11.90; S 7.42.

9-(4-Trifluoromethylphenylsulfonyl)-6-(4-phenoxyphenyl)-9*H*-purine (31)

Yield 110 mg (23%), mp 184–186 °C. ^{1}H NMR ($CDCl_3$) δ 7.07–7.12 (m, 4H, H-3,5 in phenyl, H-2,6 in O-phenyl), 7.17 (t, 1H, H-4 O-phenyl), 7.38 (t, J = 8.4 Hz, 2H, H-3,5 in O-phenyl), 7.87 (d, J = 8.8 Hz, 2H, H-2',6' in phenyl), 8.49 (d, J = 9.2 Hz, 2H, H-3',5' in phenyl), 8.53 (s, 1H, H-8 in purine), 8.71 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 9.04 (s, 1H, H-2 in purine). MS (ESI+) *m/z*: 497.8 (100%) (M+H). Anal Calcd for: $C_{24}H_{15}F_3N_4O_3S \cdot 0.35$Hexane: C, 59.53; H, 3.80; N, 10.64; S, 6.09. Found C, 59.66; H, 3.41; N, 10.36; S 5.94.

9-(4-*tert*-Butylphenylsulfonyl)-6-(4-phenoxyphenyl)-9*H*-purine (32)

Yield 210 mg (43%), mp 157–159 °C. ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9H, CH_3), 7.07–7.12 (m, 3H, H-2,4,6 in O-pheny), 7.37 (t, J = 8.8 Hz, 2H, H-3,5 in O-phenyl), 7.54 (d, J = 8.8 Hz, 2H, H-3,5 in phenyl), 7.82 (d, J = 8.8 Hz, 2H, H-3',5' in phenyl), 8.22 (d, J = 8.8 Hz, 2H, H-2',6' in phenyl), 8.53 (s, 1H, H-8 in purine), 8.71 (d, J = 8.8 Hz, 2H, H-2,6 in phenyl), 9.07 (s, 1H, H-2 in purine). ^{13}C NMR (DMSO-d_6) δ 30.89 (CH_3), 35.50 (C in *tert*-butyl), 118.07, 119.89, 124.22, 126.19, 126.68, 127.69, 128.52, 129.38, 129.93, 130.91, 131.77, 133.69 (C in phenyl), 141.12 (C-5), 151.02 (C-8), 153.93 (C-6), 155.38 (C-2), 160.70 (C-4). MS (ESI+) *m/z*: 485.9 (100%) (M+H). Anal. Calcd for: $C_{27}H_{24}N_4O_3S$: C, 66.92; H, 4.99; N, 11.56; S, 6.62. Found C, 66.63; H, 4.78; N, 11.84; S 6.29.

2. 2. Cytotoxic Activity

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 in 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 (5-FU, Fludarabine and Cladribine) used as positive controls were from Calbiochem.

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

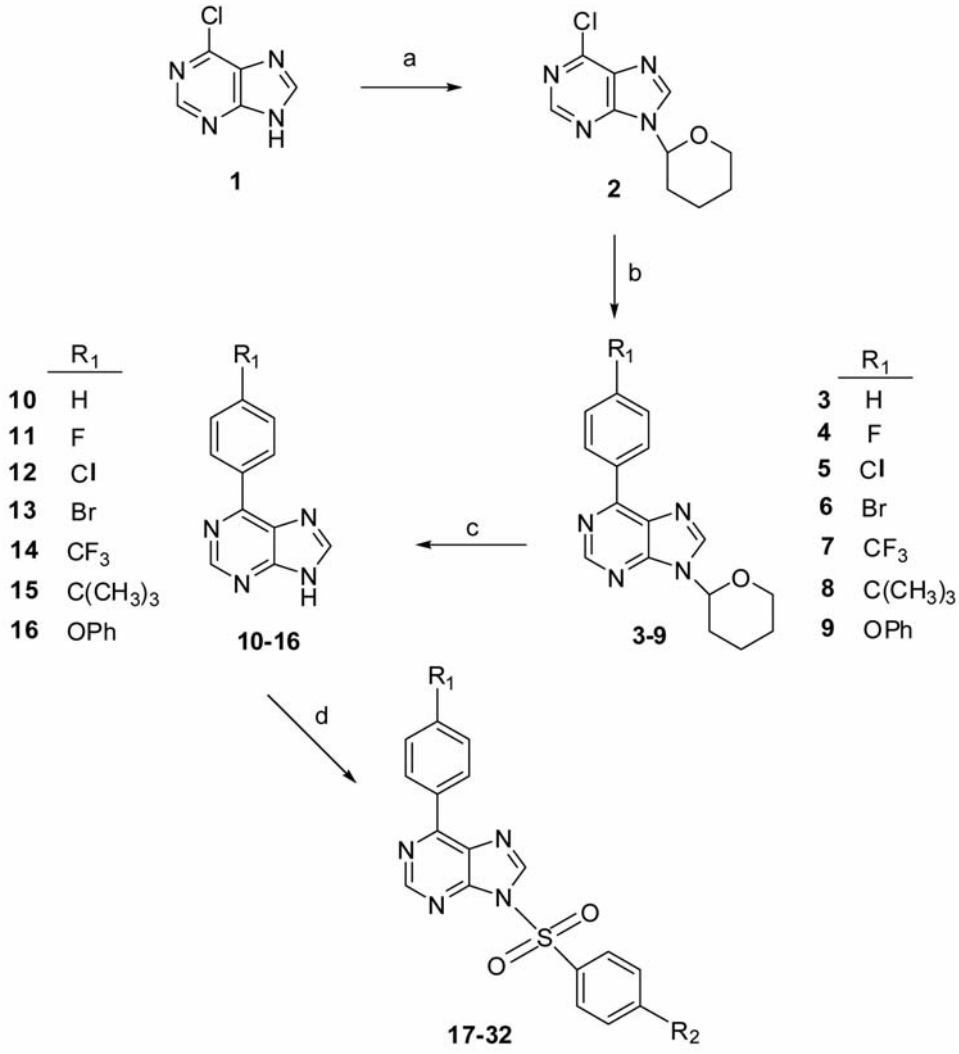
Huh7, HCT116, MCF7, HepG2, Mahlavu, and FOCUS cells were inoculated (2000-10000 cells/well in 200 µL) in 96-well plates. The next day, the media were refreshed and the compounds dissolved in DMSO were applied in concentrations between 1 and 40 µM in parallel with DMSO-only treated cells as negative controls. At the 72nd hour of treatment with compounds **3**–**32** 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 the dark for one hour. TCA fixation was terminated by washing the wells with ddH_2O five times. Air-dried plates were stained with 0.4% sulphorhodamine B (SRB) dissolved in 1% acetic acid solution for 10 min in the dark and at room temperature. 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 were 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 phenyl)-9-[(4-substituted phenyl)sulfonyl]purine derivatives **17**–**32** were prepared as shown in Scheme 1. The *N*-9 position in the starting compound 6-chloropurine (**1**) was protected as the tetrahydropyran-2-yl (THP) derivative **2**[30] by reacting **1** with

the carbocation formed *in situ* from 3,4-dihydro-2*H*-pyran and catalytic amount of *p*-TSA in refluxing THF. We prepared the 6-(substituted phenyl)purines **3–9** by Suzuki coupling reaction. This coupling with 4-substituted phenyl boronic acids in toluene catalyzed by $Pd(PPh_3)_4$ gave compounds **3–9**. The THP derivatives **3–9** were deprotected using wet Dowex 50 × 8 (H+) in methanol to obtain 6-(4-substituted phenyl)purines **10–16**. Compounds **10–16** were *N*-sulfonylated with complete regioselectivity applying the same set of reaction conditions as



	R_1	R_2		R_1	R_2
17	H	F	**25**	CF_3	F
18	H	CF_3	**26**	CF_3	CF_3
19	H	$C(CH_3)_3$	**27**	CF_3	$C(CH_3)_3$
20	F	F	**28**	$C(CH_3)_3$	F
21	F	CF_3	**29**	$C(CH_3)_3$	CF_3
22	F	$C(CH_3)_3$	**30**	OPh	F
23	Cl	F	**31**	OPh	CF_3
24	Br	F	**32**	OPh	$C(CH_3)_3$

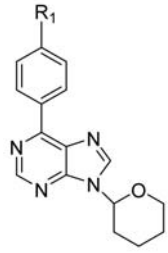
Scheme 1. (a) 3,4-dihydro-2*H*-pyran, *p*-TSA, THF; (b) $R_1PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene; (c) Dowex 50 × 8 (H+), MeOH, H_2O; (d) (4-substituted benzene)sulfonyl chloride, pyridine, CH_2Cl_2

reported for the sulfonylation of adenine. This reaction took place only at the *N*-9 atom, without the simultaneous *N*-7 sulfonylation .[29,32] Treatment of 6-(4-substituted phenyl)-9*H*-purines **10**–**16** with (4-substituted phenyl)sulfonyl chlorides in CH_2Cl_2 and pyridine on an ice bath gave the corresponding N^9-sulfonylated purines **17**–**32**.

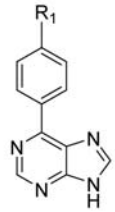
3. 2. Cytotoxic Activity and Structure-Activity Relationship (SAR)

The *in vitro* cytotoxicity of the compounds **3**–**32** were initially analyzed on human cancer cells (liver Huh7, colon HCT116, breast MCF7), using a sulforhodamine B (SRB) assay. The IC_{50} values for each compound were also calculated in comparison with the known cell growth

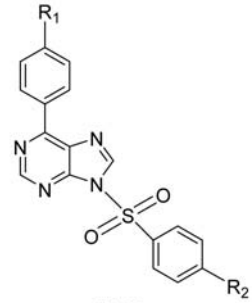
Table 1. *In vitro* cytotoxicity of the compounds **3**–**32** on different human cancer cell lines (Huh7, HCT116, MCF7)



3–9



10–16



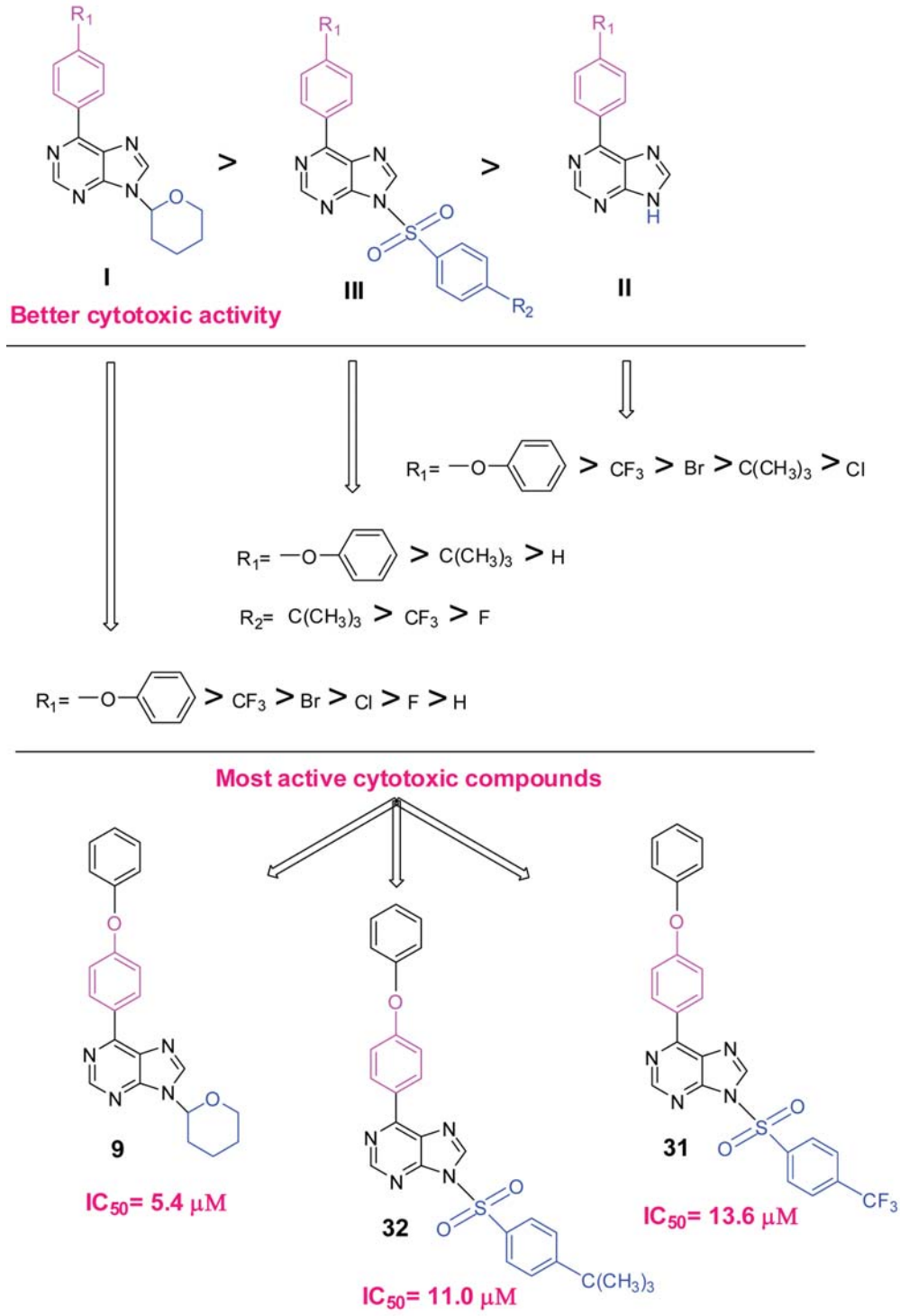
17–32

Compound	R_1	R_2	Cancer cell lines, IC_{50} (μM)[a] Huh7	HCT116	MCF7
3	H	–	69.8 ± 12.1	NI	NI
4	F	–	49.6 ± 1.9	NI	NI
5	Cl	–	29.2 ± 7.2	NI	NI
6	Br	–	27.3 ± 12.6	NI	NI
7	CF_3	–	22.2 ± 6.9	NI	NI
8	$C(CH_3)_3$	–	NI	NI	NI
9	OPh	–	5.4 ± 0.7	15.9 ± 9.3	7.4 ± 1.3
10	H	–	NI	NI	NI
11	F	–	NI	NI	NI
12	Cl	–	>100	78.8 ± 21.1	NI
13	Br	–	56.4 ± 16.7	NI	NI
14	CF_3	–	44.1 ± 17.5	NI	NI
15	$C(CH_3)_3$	–	>100	NI	NI
16	OPh	–	16.0 ± 1.2	44.8 ± 1.1	24.0 ± 0.1
17	H	F	NI	>100	NI
18	H	CF_3	42.1 ± 5.5	NI	54.9 ± 5.7
19	H	$C(CH_3)_3$	NI	NI	NI
20	F	F	NI	65.2 ± 25.8	NI
21	F	CF_3	NI	53.1 ± 41.6	NI
22	F	$C(CH_3)_3$	NI	NI	NI
23	Cl	F	NI	78.2 ± 59.9	NI
24	Br	F	NI	NI	NI
25	CF_3	F	NI	NI	NI
26	CF_3	CF_3	NI	NI	NI
27	CF_3	$C(CH_3)_3$	NI	NI	NI
28	$C(CH_3)_3$	F	NI	NI	NI
29	$C(CH_3)_3$	CF_3	16.0 ± 1.2	30.2 ± 4.9	27.1 ± 0.3
30	OPh	F	14.3 ± 1.6	14.5 ± 2.1	22.7 ± 0.5
31	OPh	CF_3	13.6 ± 0.9	13.1 ± 4.6	17.0 ± 0.7
32	OPh	$C(CH_3)_3$	11.0 ± 0.8	18.2 ± 3.3	21.1 ± 1.6
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] IC_{50} 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

inhibitors 5-fluorouracil (5-FU), fludarabine and cladribine and the results are summarized in Table 1.

Among the molecules synthesized in this study, analogues accommodate substituted tetrahydropyran moiety at their *N*-9 position **3**–**9**, and the one with a promising IC_{50} value against Huh7 (5.4 μM) is 6-(4-phenoxyphenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (**9**). Analyzing the data presented in Table 1, highlights the 4-phenoxyphenyl substitution as the group at C-6 as the most responsible for the anti-cancer activity against Huh7. When we compared their IC_{50} values with the nucleobase analogue 5-FU and nucleoside analogue Fludarabine, we observed that our compounds **9**, **16**, **30**, **31** and **32** had showed lower values in micromolar concen-



Scheme 2. Structure-activity relationship (SAR) of substituted purines against Huh7 (**3**–**32**)

trations and these molecules had a better cytotoxic activity on Huh7 cells (5.4, 16.0, 14.3, 13.6 and 11.0 vs 30.6 μM and 28.4 for 5-FU and Fludarabine). Compound **29**, bearing a 4-*tert*-butylphenyl substituent at C-6 position of the purine, was active derivative with greater potency against Huh7 cell line than 5-FU and Fludarabine. The

Table 2. IC_{50} values of **5**–**9**, **14**, **16**, **18**, **28**–**32** against hepatocellular carcinoma (HCC) cell lines: Huh7, HepG2, MAHLAVU, FOCUS.

Compound	HCC Cancer cell lines, IC_{50} (μM)[a]			
	Huh7	HepG2	Mahlavu	FOCUS
5	29.2 ± 7.2	39.7 ± 17.7	NI	NI
6	27.3 ± 12.6	38.4 ± 13.9	NI	82.6 ± 43.3
7	22.2 ± 6.9	NI	NI	NI
8	NI	NI	NI	NI
9	5.4 ± 0.7	NI	54.9 ± 69.4	6.2 ± 1.6
14	44.1 ± 17.5	44.9 ± 23.6	54.1 ± 4.9	45.0 ± 14.6
16	16.0 ± 1.2	23.4 ± 0.6	30.2 ± 1.7	25.4 ± 4.8
18	42.1 ± 5.5	NI	NI	90.0 ± 39.8
28	NI	NI	NI	NI
29	16.0 ± 1.2	47.1 ± 19.5	17.4 ± 0.9	NI
30	14.3 ± 1.6	34.4 ± 9.5	16.6 ± 2.1	17.3 ± 1.5
31	13.6 ± 0.9	23.4 ± 1.5	21.0 ± 0.3	27.0 ± 5.4
32	11.0 ± 0.8	14.5 ± 0.9	23.5 ± 0.4	22.2 ± 3.0
5-FU	30.6 ± 1.8	5.1 ± 0.8	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] IC_{50} 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

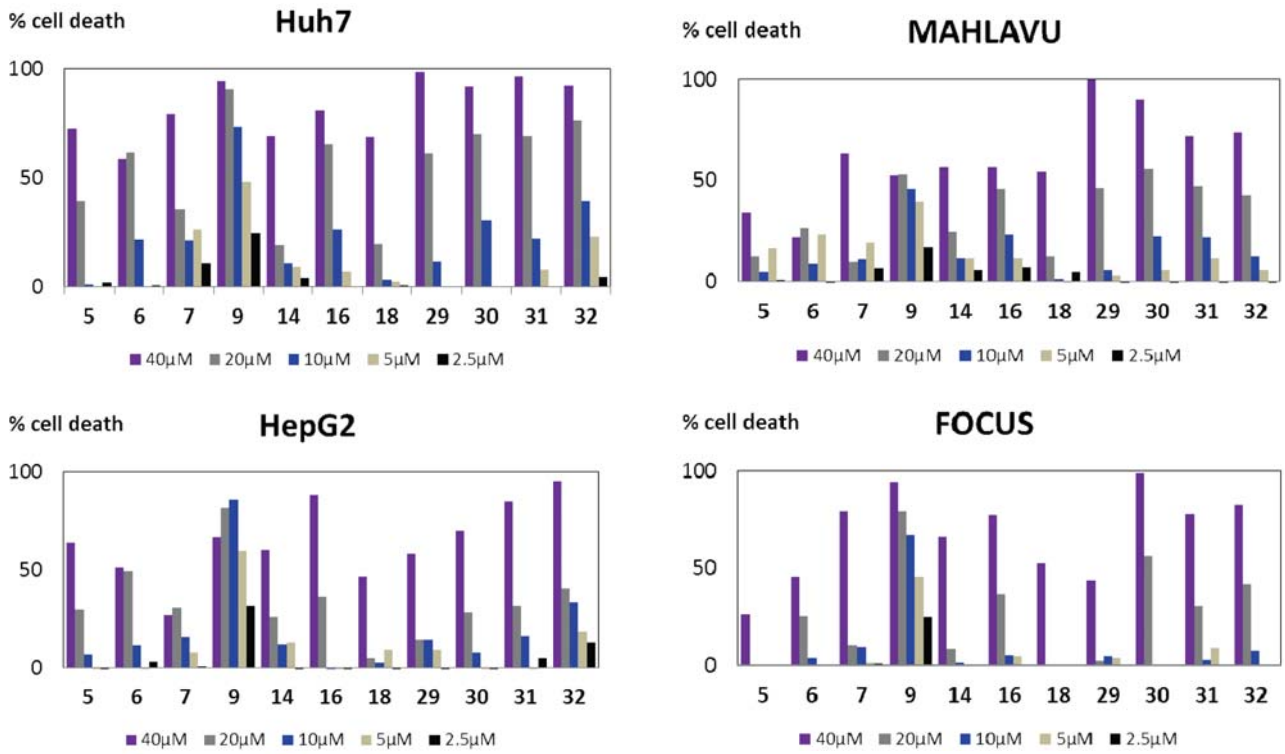


Figure 4. Percent cell death in the presence of the most active compounds. Huh7, HepG2, Mahlavu and FOCUS cells were inoculated in 96-well plates. All molecules and their DMSO controls were administered to the cells in triplicate with five different concentrations: 40, 20, 10, 5, and 2.5 μM. After 72 h of incubation, SRB assays were generated and the cell death percentages were calculated in comparison with DMSO-treated wells.

structure-activity relationship (SAR) results are summarized in Scheme 2.

Notably 6,9-disubstituted derivative **9** showed superior cytotoxic activity (IC_{50} 7.4 μM) compared with Fludarabine (IC_{50} 15.2 μM) against MCF7 tumor cell line. Within the tested purine analogues on HCT116 cell, compounds **9** and **31** with 4-phenoxyphenyl group at *N*-9 position, showed good cytotoxic activity (IC_{50} 15.9 and 13.1 μM, respectively).

We then screened the cytotoxic activity of the most potent purine derivatives (**5–9**, **14**, **16**, **18**, **28–32**) against further hepatocellular cancer (HCC) cells: HepG2, Mahlavu, and FOCUS (Table 2, Fig. 4). We found out that the most important cell growth inhibition was observed in the presence of 6-(4-phenoxyphenyl)-9-(tetrahydropyran-2-yl)purine derivative **9**, with IC_{50} values of 5.4–6.2 μM against Huh7 and FOCUS cell lines. Furthermore, **9** had a better cytotoxic activity than the cytotoxic drugs 5-FU and Fludarabine on Huh7 cells (Table 2). The 9-(4-(*tert*-butyl)phenylsulfonyl) analogue **32** was also very active (IC_{50} values in range of 11.0–14.5 μM) against Huh7 and HepG2 cell lines.

4. Conclusion

A series of 6-(4-substituted phenyl)-9-(tetrahydropyran-2-yl)purines **3–9**, 6-(4-substituted phenyl)purines **10–16**, and 9-(4-substituted phenylsulfonyl)-6-(4-substituted phenyl)purine analogues **17–32** were prepared and their cytotoxic activities identified. 6-(4-Phenoxyphenyl)purine derivatives **9**, **16**, **30**, **31**, **32** showed potent anticancer activity at low concentrations against Huh7 cell line when compared to 5-FU and Fludarabine as potent cytotoxic drugs. Among the 30 compounds investigated, the most potent purine derivatives **5–9**, **14**, **16**, **18**, **28–32** were further analysed for their activity on HCC cells (Huh7, HepG2, Mahlavu, FOCUS). The molecule **9** exhibited promising cytotoxic activity with IC_{50} value of 5.4 μM on Huh7 cell line.

5. Acknowledgements

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Povzetek

Pripravili smo serijo 6-(4-substituiranih fenil)-9-(tetrahidropiran-2-il)purinov **3**–**9**, 6-(4-substituiranih fenil)purinov **10**–**16** in 9-((4-substituiranih fenil)sulfonil)-6-(4-substituiranih fenil)purinov **17**–**32**. Pripravljenim spojinam smo določili njihovo *in vitro* aktivnost proti izbranim človeških rakastim celicam (jeter Huh7, debelega črevesja HCT116, dojk MCF7). 6-(4-Fenoksifenil)purinski analogi **9**, **16**, **30**–**32** so izkazali visoke citotoksične aktivnosti. Za najbolj aktivne purinske derivate **5–9**, **14**, **16**, **18**, **28**–**32** smo nadalje določili citotoksično aktivnost za hepatocelične rakaste celice. Izkazalo se je, da ima 6-(4-fenoksifenil)-9-(tetrahidropiran-2-il)-9*H*-purin (**9**) večjo citotoksično aktivnost (IC_{50} 5.4 μM) na Huh7 celice kot pa dobro znani analog nukleinskih baz 5-FU in tudi večjo kot nukleozidna učinkovina fludarabin. Iz študij odvisnosti aktivnosti od strukture lahko zaključimo, da so za delovanje proti raku pomembni zlasti substituenti na položaju C-6 purinskega jedra; 4-fenoksifenilna skupina pa se je izkazala kot najbolj učinkovita izbira.