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Synthesis and Antitumor Activity of 5′-deoxy-4′-thio-L-nucleosides

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A series of novel 5′-deoxy-4′-thio-L-nucleosides was designed and synthesized. The absolute configuration of the target compound 23α was confirmed by X-ray crystallography. The antitumor activities of the target compounds were tested against the growth of human carcinoma of colon (LOVO), human leukemia cell line (CEM) and human breast cancer cell line (MDA-MB-435) cells *in vitro*. 6-cyclopentylamino and 6-cyclohexylamino purine compounds 26 and 27, both in α-configuration and in β-form, exhibited strong inhibition to CEM.

Key words: antitumor activity, L-thionucleosides, X-ray crystallography

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4′-Thionucleosides may be considered as the analogues by the replacement of the lactol oxygen of nucleosides with a sulfur atom and exhibit many biological activities, for example antiviral (1) and antitumor (2). Moreover, they have some inherent advantages over 4′-oxonucleosides that show a more stable glycosyl bond and increased metabolic stability against various viral and cellular enzymes (3).

Some 5′-Deoxy nucleosides (e.g., doxifluridine and capecitabine) posses superior antitumor activity against various experimental tumors in animals and cause considerably less host toxicity compared to the well-known clinically used 5-FU, 5-fluorodeoxyuridine (FdUrs), and Ftorafur (Ft) (4,5).

So far, no reports on 5′-deoxy-4′-thionucleosides have been found in literatures, but it is logical to assume that such nucleosides of both purines and pyrimidines would have stable glycosyl bond and superior antitumor activity. Here, we report the synthesis and the antitumor activities in cell culture of a series of 5′-deoxy-4′-thio-L-α-nucleosides and 5′-deoxy-4′-thio-L-β-nucleosides (Figure 1).

Materials and Methods

Reagents and analysis

Melting points were determined on an Electrothermal Melting Point Apparatus and were uncorrected. ^{1}H NMR spectra were recorded on 400 MHz instruments in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), b (broad).

Synthesis

(2S,3S,4R)-2-tert-butyldimethylsilyloxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene (3)

A solution of dimesylate **2** (32.1 g, 70 mmol) and $Na_2S{\cdot}9H_2O$ (20 g, 80 mmol) in DMF (700 mL) was stirred at 100 °C for 5 h. After being cooled to room temperature, H_2O was added. The mixture was extracted with Et_2O (5 × 200 mL). The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **3** as the syrup (13.8 g, yield = 65%).

$[\alpha]_D^{25}$ = +194 (C = 1.0, MeOH), ESI-MS m/z 305 (M^++1), ^{1}H NMR ($CDCl_3$, 400 MHz, δ*ppm*); 0.89 (s, 9H), 0.08 (s, 6H), 1.29 (s, 3H), 1.41 (s, 3H), 2.86 (t, *J* = 2.0, *J* = 1.6, 2H), 3.32–3.37 (m, 1H), 3.78 (dd, *J* = 7.2, *J* = 10.0, 1H), 4.03 (dd, *J* = 7.6, *J* = 10.4, 1H), 4.69–4.72 (m, 1H), 4.84–4.87 (m, 1H).

(2S,3S,4R)-2-hydroxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene (3′)

Compound **3** (1 g, 3.3 mmol) was dissolved in tetrahydrofuran (THF) (10 mL), and then TBAF·$3H_2O$ (1.3 g, 4.1 mmol) was added. The mixture was stirred at room temperature for 3 h. After the solvent was removed under reduced pressure, the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with

5′-deoxy-4′-thio-L-α-nucleosides **5′-deoxy-4′-thio-L-β-nucleosides**

B = pyrimidines or N^6-substituented-2-chloro-purines

Figure 1: Designed compounds of 5′-deoxy-4′-thio-L-nucleosides.

H_2O followed by brine. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The residue was crystallized from acetonitrile to obtain crystal **3′**.

$[\alpha]_D^{25}$ = +62.2 (C = 1.0, MeOH), mp 70–72 °C, ESI-MS m/z 191 (M^++1), ^{1}H NMR ($CDCl_3$, 400 MHz, *δppm*); 1.28 (s, 3H), 1.47 (s, 3H), 2.62–2.89 (m, 2H), 3.30–3.34 (m, 1H), 3.82–3.86 (m, 1H), 3.89–3.92 (m, 1H), 4.76–4.79 (m, 1H), 4.83–4.87 (m, 1H).

Crystal data: $C_8H_{14}O_3S$, monoclinic, space group P21, unit cell dimensions a = 12.6283(5), b = 5.7460(2), c = 13.2081(5) Å, $\alpha = \gamma = 90.00°$, $\beta = 94.112(2)°$, V = 955.94(6) Å^3, Z = 2, 1790 reflections were measured, and 1782 independent reflections were used for the structure solution. Final *R* values are as follows: wR_2 = 0.1035, R_1 = 0.0389, S = 1.004.

(2S,3S,4R)-2-tert-butyldimethylsilyloxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene-1-oxide (4)

Ozone gas was bubbled through a clear solution of **3** (7.4 g, 24.3 mmol) in CH_2Cl_2 (100 mL) at −78 °C. The reaction was completed in 30 min, as indicated by persistence of a blue color. Nitrogen gas was bubbled through the solution to remove excess ozone until the blue color vanished. The reaction mixture was allowed to warm to room temperature and concentrated under reduced pressure. The residue was purified by column chromatography to give sulfoxide **4** as a white solid (3.97 g, 51%).

$[\alpha]_D^{25}$ = +153 (C = 1.0, MeOH), ESI-MS m/z 343 (M^++23), ^{1}H NMR ($CDCl_3$, 400 MHz, *δppm*); 0.93 (s, 9H), 0.12 (s, 6H), 1.30 (s, 3H), 1.49 (s, 3H), 2.85 (dd, *J* = 6.0, *J* = 13.6, 1H), 3.12–3.17 (m, 1H), 3.64 (dd, *J* = 1.6, *J* = 13.6, 1H).

(3R,4S,5R)-2-acetyloxy-3,4-dihydroxy-3,4-*O*-isopropylidene-5-iodiomethyl-tetrahydrothiophene (7)

The stirred mixture of sulfoxide **4** (28.9 g, 90 mmol) and Ac_2O (50 mL) was heated at 100 °C for 3 h. The reaction mixture was concentrated in vacuo to get **5** (22 g, 60 mmol). The crude **5** was dissolved in THF (500 mL), and then TBAF·$3H_2O$ (23 g, 72 mmol) was added. The mixture was stirred at room temperature for 3 h. After the solvent was removed under reduced pressure, the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with H_2O followed by brine. Then, the organic layer was dried with $MgSO_4$, filtered and concentrated to afford **6**. The crude **6** (12 g, 50 mmol), triphenylphosphine (32 g, 125 mmol), iodine (25 g, 100 mmol) and imidazole (10.2 g, 150 mmol) were stirred in toluene (500 mL) under reflux for 20 min. When the reaction mixture was cooled, saturated aqueous solution of $NaHCO_3$ was added, and the mixture was stirred for 5 min. Excess of iodine was removed by the addition of $Na_2S_2O_3$. The organic layer was washed with water, dried with $MgSO_4$, filtered and concentrated. Triphenylphosphine oxide was precipitated in diethyl ether, and the filtrate was concentrated and then subjected to column chromatography to give **7** as yellow oil (13 g, yield 42% for three steps).

$[\alpha]_D^{25}$ = −179.2 (C = 1.0, MeOH), ESI-MS m/z 381 (M^++23), ^{1}H NMR ($CDCl_3$, 400 MHz, *δppm*); 1.33 (s, 3H), 1.50 (s, 3H), 2.05 (s, 3H), 3.29–3.33 (m, 1H), 3.46 (t, *J* = 10.0, *J* = 9.6, 1H), 3.95–3.97 (m, 1H), 4.84–4.89 (m, 2H), 6.00 (s, 1H).

(3R,4S,5S)-2-acetyloxy-3,4-dihydroxy-3,4-*O*-isopropylidene-5-methyl-tetrahydrothiophene (8)

Compound **7** (6.2 g, 17.3 mmol) and AIBN (1.42 g, 8.65 mmol) were dissolved in toluene (100 mL) in a three-necked flask. N_2 was bubbled through the reaction mixture for 15 min, and tributhyltrin hydride (0.72 mL, 26.0 mmol) was added. The reaction mixture was heated in an oil bath at 80 °C for 30 min. The reaction was cooled to room temperature, washed with water, dried by $MgSO_4$ and filtered. The filtrate was evaporated to dryness, and the residue was purified by silica gel column chromatography to afford **8** as white solid (2.67 g, yield = 67%).

$[\alpha]_D^{25}$ = −212.6 (C = 1.0, MeOH), mp 44–46 °C, ESI-MS m/z 255 (M^++23), ^{1}H NMR ($CDCl_3$, 400 MHz, *δppm*); 1.32 (s, 3H), 1.50 (s, 3H), 1.38 (d, *J* = 6.8, 3H), 2.05 (s, 3H), 3.69–3.72 (m, 1H), 4.70–4.73 (m, 1H), 4.77–4.78 (m, 1H), 5.94 (s, 1H).

2,6-dichloro-9-((3R,4S,5S)-3,4-dihydroxy-3,4-*O*-isopropylidene-5-methyl-tetrahydrothiophene-2-yl)purine (9)

To a stirred solution of compound **8** (3 g, 12.9 mmol) and 2,6-dichloro-purine (6.11 g, 32.3 mmol) in dried acetonitrile (30 mL) was dropped $EtAlCl_2$ (17.7 mL, 15.9 mmol) at 0 °C. The reaction mixture was heated at 60 °C for 3 h, cooled to 0 °C and then saturated aqueous solution of $NaHCO_3$ was added. The mixture was extracted with methylene dichloride three times. The combined organic layer was washed with H_2O and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give **9** (4.5 g). It was used in the next substitution without purification.

2-Chloro-6-amino-9-((3R,4S,5S)-3,4-dihydroxy-3,4-*O*-isopropylidene-5-methyl-tetrahydrothiophene-2-yl)purine (10)

Ammonia was bubbled through a solution of **9** (2.0 g, 5.6 mmol) in ethanol (20 mL) at 60 °C until TLC showed **9** disappeared. The reaction mixture was concentrated under reduced pressure to give crude product **10** (2.2 g). It was used in the next deprotection without purification.

2-Chloro-6-methylamino-9-((3R,4S,5S)-3,4-dihydroxy-3,4-*O*-isopropylidene-5-methyl-tetrahydrothiophene-2-yl) purine (11)

9 (2.0 g, 5.6 mmol) and 30% aqueous methylamine (1 mL) was stirred in THF at room temperature until TLC showed compound **9** disappeared. The reaction mixture was concentrated under reduced pressure to give crude product **11** (2.5 g).

Compounds **12–20** were obtained by the same procedure.

1-[(3R,4S,5S)-3,4-dihydroxy-3,4-*O*-isopropylidene-5-methyl-tetrahydrothiophene-2-yl]-5-fluorouracil (32)

A mixture of 5-fluorouracil (500 mg, 3.8 mmol) and Hexamethyl disilazane (3 mL) was stirred at 120 °C for 5 h. The mixture was concentrated under reduced pressure to afford protected 5-fluorouracil as white solid. Dried acetonitrile (5 mL), compound **8** (357 mg, 1.5 mmol) in dried acetonitrile (5 mL) were added at room temperature. Then the TMSOTf (0.70 mL. 3.8 mmol) was dropped at 0 °C. The reaction mixture was stirred at room temperature for 30 min, and then saturated aqueous solution of $NaHCO_3$ was added. The mixture was extracted with methylene dichloride three times. The combined organic layer was washed with H_2O and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give crude product **32**. It was used in the next deprotection without further purification.

Compounds **33** and **34** were obtained by the same procedure.

2-Chloro-6-amino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (21α) and 2-Chloro-6-amino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (21β)

A solution of **10** (2.5 g, 7.3 mmol) in 85% aqueous formic acid (3 mL) was stirred at room temperature for 12 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel chromatography to give **21**α and **21**β.

21α: Yield = 47% (based on compound **8**), white solid, mp 167 °C (dec.), $[\alpha]_D^{25}$ = −113 (C = 1.0, DMSO), ESI-MS m/z 302 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.35 (d, J = 7.2, 3H), 4.09–4.11 (m, 1H), 4.17–4.19 (m, 1H), 4.83–4.86 (m, 1H), 6.06 (d, J = 8.0, 1H), 8.46 (s, 1H).

21β**:** Yield = 15% (based on compound **8**), white solid, mp 87 °C (dec.), $[\alpha]_D^{25}$ = −41 (C = 1.0, DMSO), ESI-MS m/z 302 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.55 (d, J = 7.2, 3H), 3.48–3.51 (m, 1H), 3.98–4.00 (m, 1H), 4.56–4.58 (m, 1H), 5.85 (d, J = 4.0, 1H), 8.45 (s, 1H).

The other target compounds **22–31** (α and β) and **35–37** (α and β) were obtained by the same procedure.

2-Chloro-6-methylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (22α)

Yield = 60% (based on compound **8**), white solid, mp 140 °C (dec.), $[\alpha]_D^{25}$ = −85 (C = 1.0, DMSO), ESI-MS m/z 338 (M^++23), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.22 (d, J = 6.8, 3H), 2.93 (s, 3H), 3.95–3.97 (m, 1H), 4.04 (d, J = 3.6, 1H), 4.79 (d, J = 6.0, 1H), 5.37 (d, J = 4.8, 1H), 5.51 (d, J = 6.8, 1H), 5.88 (d, J = 8.4, 1H), 8.20 (b, 1H), 8.43 (s, 1H).

2-Chloro-6-methylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (22β)

Yield = 21% (based on compound **8**), white solid, mp 200 °C (dec.), $[\alpha]_D^{25}$ = −23 (C = 1.0, DMSO), ESI-MS m/z 338 (M^++23), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.48 (d, J = 7.2, 3H), 2.94 (s, 3H), 3.29–3.38 (m, 1H), 3.97 (d, J = 3.2, 1H), 4.62 (d, J = 3.6, 1H), 5.30 (d, J = 4.4, 1H), 5.56 (d, J = 5.6, 1H), 5.78 (d, J = 5.6, 1H), 8.20 (b, 1H), 8.40 (s, 1H).

2-Chloro-6-ethylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (23α)

Yield = 56% (based on compound **8**), white solid, mp 180 °C (dec.), $[\alpha]_D^{25}$ = −108 (C = 1.0, MeOH), ESI-MS m/z 330 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.19 (d, J = 7.2, 3H), 1.12–1.16 (m, 3H), 3.38–3.44 (m, 2H), 3.90–3.95 (m, 1H), 3.99 (s, 1H), 4.76 (d, J = 7.6, 1H), 5.39 (b, 1H), 5.54 (b, 1H), 5.84 (d, J = 8.8, 1H), 8.31 (b, 1H), 8.45 (s, 1H).

Crystal data: $C_{12}H_{16}O_2N_5Cl_1S_1$, triclinic system, space group P1, unit cell dimensions a = 8.7829(4), b = 9.2313(4), c = 9.8800(5) Å, α = 94.110(3), β = 99.528(3), γ = 109.544(2)°, V = 737.49(6) Å^3, Z = 1, 1955 reflections were measured, and 1942 independent reflections were used for the structure solution. Final R values are as follows: wR_2 = 0.0903, R_1 = 0.0341, S = 1.056.

2-Chloro-6-ethylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (23β)

Yield = 18% (based on compound **8**), white solid, mp 197 °C (dec.), $[\alpha]_D^{25}$ = −15 (C = 1.0, MeOH), ESI-MS m/z 330 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.46 (d, J = 7.2, 3H), 1.13 (t, J = 7.2, J = 7.6, 3H), 3.41 (t, J = 7.2, J = 6.4, 2H), 1.55–1.61 (m, 2H), 3.30–3.33 (m, 1H), 3.93 (d, J = 3.6, 1H), 4.55 (d, J = 3.2, 1H), 5.34 (d, J = 4.8, 1H), 5.59 (d, J = 6.0, 1H), 5.75 (d, J = 6.0, 1H), 8.34 (b, 1H), 8.42 (s, 1H).

2-Chloro-6-propylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (24α)

Yield = 59% (based on compound **8**), white solid, mp 173 °C (dec.), $[\alpha]_D^{25}$ = −93 (C = 1.0, DMSO), ESI-MS m/z 342 (M^+-1), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.22 (d, J = 6.8, 3H), 0.87–0.92 (m, 2H), 1.57–1.64 (m, 2H), 3.37 (b, 2H), 3.94–3.97 (m, 1H), 4.03 (d, J = 2.8, 1H), 4.78 (b, 1H), 5.30 (b, 1H), 5.46 (d, J = 6.0, 1H), 5.88 (d, J = 8.4, 1H), 8.22 (b, 1H), 8.44 (s, 1H).

2-Chloro-6-propylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (24β)

Yield = 20% (based on compound **8**), white solid, mp 166 °C (dec.), $[\alpha]_D^{25}$ = −31 (C = 1.0, DMSO), ESI-MS m/z 342 (M^+-1), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.46 (d, J = 7.2, 3H), 0.87 (t, J = 7.2, J = 7.6, 3H), 1.55–1.61 (m, 2H), 3.33–3.36 (m, 2H), 3.29–3.33 (m, 1H), 3.39–3.96 (m, 1H), 4.59 (d, J = 3.2, 1H), 5.23 (d, J = 4.8, 1H), 5.55 (d, J = 5.6, 1H), 5.75 (d, J = 5.6, 1H), 8.23 (b, 1H), 8.38 (s, 1H).

2-Chloro-6-cyclopropylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (25α)

Yield = 60% (based on compound **8**), white solid, mp 168 °C (dec.), $[\alpha]_D^{25}$ = −89.5 (C = 1.0, MeOH), ESI-MS m/z 342 (M^++1), ^{1}H NMR

(CD_3OD, 400 MHz, *δppm*); 1.32 (d, *J* = 6.8, 3H), 0.61–0.66 (m, 2H), 0.84–0.90 (m, 2H), 3.04 (b, 1H), 4.03–4.09 (m, 1H), 4.14–4.16 (m, 1H), 4.79–4.82 (m, 1H), 6.02 (d, *J* = 8.0, 1H), 8.27 (s, 1H).

2-Chloro-6-cyclopropylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (25*β*)

Yield = 18% (based on compound **8**), white solid, mp 168 °C (dec.), $[\alpha]_D^{25}$ = −23 (C = 1.0, MeOH), ESI-MS m/z 342 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, *δppm*); 1.57 (d, *J* = 6.8, 3H), 0.64–0.68 (m, 2H), 0.87–0.92 (m, 2H), 3.04 (b, 1H), 3.49–3.55 (m, 1H), 4.00–4.02 (m, 1H), 4.57–4.58 (m, 1H), 5.86 (d, *J* = 4.0, 1H), 8.33 (s, 1H).

2-Chloro-6-cyclopentylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (26*α*)

Yield = 42% (based on compound **8**), white solid, mp 143 °C (dec.), $[\alpha]_D^{25}$ = −89 (C = 1.0, MeOH), ESI-MS m/z 368 (M^+-1), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.21 (d, *J* = 6.8, 3H), 1.55–1.94 (m, 8H), 4.42 (b, 1H), 3.94–3.98 (m, 1H), 4.03 (d, *J* = 3.2, 1H), 4.76 (b, 1H), 5.29 (d, *J* = 4.4, 1H), 5.45 (d, *J* = 6.8, 1H), 5.88 (d, *J* = 8.0, 1H), 8.13 (d, *J* = 8.0, 1H), 8.43 (s, 1H).

2-Chloro-6-cyclopentylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (26*β*)

Yield = 13% (based on compound **8**), white solid, mp 158 °C (dec.), $[\alpha]_D^{25}$ = −26.5 (C = 1.0, MeOH), ESI-MS m/z 368 (M^+-1), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.48 (d, *J* = 6.8, 3H), 1.54–1.93 (m, 8H), 4.42 (b, 1H), 3.30–3.36 (m, 1H), 3.96 (d, *J* = 3.2, 1H), 4.61 (d, *J* = 3.6, 1H), 5.27 (d, *J* = 5.2, 1H), 5.53 (d, *J* = 5.2, 1H), 5.77 (d, *J* = 5.6, 1H), 8.18 (d, *J* = 7.6, 1H), 8.40 (s, 1H).

2-Chloro-6-cyclohexylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (27*α*)

Yield = 57% (based on compound **8**), white solid, mp 127 °C (dec.), $[\alpha]_D^{25}$ = −85.6 (C = 1.0, MeOH), ESI-MS m/z 384 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.22 (d, *J* = 6.8, 3H), 1.22–1.87 (m, 10H), 3.95–3.99 (m, 1H), 4.03 (b, 1H), 4.78 (b, 1H), 5.28 (d, *J* = 2.8, 1H), 5.45 (b, 1H), 5.88 (d, *J* = 8.8, 1H), 8.02 (d, *J* = 7.6, 1H), 8.44 (s, 1H).

2-Chloro-6-cyclohexylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (27*β*)

Yield = 22% (based on compound **8**), white solid, mp 125 °C (dec.), $[\alpha]_D^{25}$ = −24 (C = 1.0, MeOH), ESI-MS m/z 406 (M^++23), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.48 (d, *J* = 6.8, 3H), 1.23–1.68 (m, 10H), 3.14–3.20 (m, 1H), 4.03 (d, *J* = 3.6, 1H), 4.60 (b, 1H), 5.26 (d, *J* = 5.2, 1H), 5.52 (d, *J* = 5.2, 1H), 5.77 (d, *J* = 5.6, 1H), 8.04 (d, *J* = 8.4, 1H), 8.39 (s, 1H).

2-Chloro-6-benzylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (28*α*)

Yield = 50% (based on compound **8**), white solid, mp 163 °C (dec.), $[\alpha]_D^{25}$ = −103 (C = 1.0, DMSO), ESI-MS m/z 414 (M^++23), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.22 (d, *J* = 5.6, 3H), 7.21–7.35 (m, 5H), 4.65 (d, J = 1.6, 2H), 3.95–3.97 (m, 1H), 4.02–4.04 (m, 1H), 4.78 (b, 1H), 5.29 (d, *J* = 4.4, 1H), 5.76 (d, *J* = 6.8, 1H), 5.89 (d, *J* = 8.4, 1H), 8.76 (b, 1H), 8.47 (s, 1H).

2-Chloro-6-benzylamino-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (28*β*)

Yield = 17% (based on compound **8**), white solid, mp 166 °C (dec.), $[\alpha]_D^{25}$ = −56 (C = 1.0, DMSO), ESI-MS m/z 414 (M^++23), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.48 (d, *J* = 7.2, 3H), 7.21–7.35 (m, 5H), 4.65 (b, 2H), 3.95–3.99 (m, 1H), 5.26 (d, *J* = 4.8, 1H), 5.53 (d, *J* = 6.0, 1H), 5.79 (d, *J* = 6.0, 1H), 8.80 (b, 1H), 8.43 (s, 1H).

2-Chloro-6-(α-methylbenzylamino-yl)-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (29*α*)

Yield = 45% (based on compound **8**), white solid, mp 162 °C (dec.), $[\alpha]_D^{25}$ = −53 (C = 1.0, DMSO), ESI-MS m/z 404 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.22 (d, *J* = 6.8, 3H), 7.18–7.45 (m, 5H), 5.29 (b, 1H), 1.54 (d, *J* = 6.8, 3H), 3.93–3.96 (m, 1H), 4.03 (b, 1H), 4.77 (b, 1H), 5.29 (d, *J* = 3.2, 1H), 5.45 (d, *J* = 6.4, 1H), 5.88 (d, *J* = 8.8, 1H), 8.68 (d, *J* = 8.0 1H), 8.47 (s, 1H).

2-Chloro-6-(α-methylbenzylamino-yl)-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (29*β*)

Yield = 14% (based on compound **8**), white solid, mp 150 °C (dec.), $[\alpha]_D^{25}$ = −20 (C = 1.0, DMSO), ESI-MS m/z 404 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.48 (d, *J* = 6.4, 3H), 7.20–7.45 (m, 5H), 5.42 (b, 1H), 1.54 (d, *J* = 5.6, 3H), 3.96 (b, 1H), 4.62 (b, 1H), 5.24 (b, 1H), 5.51 (d, *J* = 4.8, 1H), 5.77 (d, *J* = 4.8, 1H), 8.71 (d, *J* = 7.2 1H), 8.43 (s, 1H).

2-Chloro-6-(2-fluorobenzylamino-yl)-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (30*α*)

Yield = 51% (based on compound **8**), white solid, mp 164 °C (dec.), $[\alpha]_D^{25}$ = −91 (C = 1.0, DMSO), ESI-MS m/z 408 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, *δppm*); 1.22 (d, *J* = 6.8, 3H), 7.11–7.37 (m, 4H), 4.71 (b, 2H), 3.94–3.97 (m, 1H), 4.02–4.04 (m, 1H), 4.77 (b, 1H), 5.28 (d, *J* = 4.4, 1H), 5.44 (d, *J* = 6.8, 1H), 5.89 (d, *J* = 8.8, 1H), 5.89 (d, *J* = 8.8, 1H), 8.78 (b, 1H), 8.46 (b, 1H)., 8.48 (s, 1H).

2-Chloro-6-(2-fluorobenzylamino-yl)-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (30*β*)

Yield = 16% (based on compound **8**), white solid, mp 162 °C (dec.), $[\alpha]_D^{25}$ = −55 (C = 1.0, DMSO), ESI-MS m/z 408 (M^++1), ^{1}H NMR

(DMSO-d_6, 400 MHz, δppm); 1.49 (d, J = 7.2, 3H), 7.13–7.39 (m, 4H), 4.71 (b, 2H), 3.33–3.39 (m, 1H), 3.98 (d, J = 3.6, 1H), 4.64 (d, J = 3.2, 1H), 5.27 (d, J = 5.2, 1H), 5.54 (d, J = 6.0, 1H), 5.80 (d, J = 5.2 1H), 8.79 (b, 1H), 8.46 (s, 1H).

2-Chloro-6-(4-fluorobenzylamino-yl)-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (31α)

Yield = 51% (based on compound **8**), white solid, mp 173 °C (dec.), $[\alpha]_D^{25}$ = −89 (C = 1.0, DMSO), ESI-MS m/z 408 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.22 (d, J = 6.4, 3H), 7.10–7.14 (m, 2H), 7.36–7.39 (m, 2H), 4.62 (b, 2H), 3.94–3.97 (m, 1H), 4.03 (b, 1H), 4.77 (b, 1H), 5.30 (d, J = 3.2, 1H), 5.46 (b, 1H), 5.88 (d, J = 8.8, 1H), 8.77 (b, 1H), 8.47 (s, 1H).

2-Chloro-6-(4-fluorobenzylamino-yl)-9-((2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine (31β)

Yield = 18% (based on compound **8**), white solid, mp 154 °C (dec.), $[\alpha]_D^{25}$ = −50 (C = 1.0, DMSO), ESI-MS m/z 408 (M^++1), ^{1}H NMR (DMSO-d_6, 400 MHz, δppm); 1.48 (d, J = 7.6, 3H), 7.10–7.17 (m, 2H), 7.36–7.40 (m, 2H), 4.60–4.64 (m, 1H), 5.25 (d, J = 4.8, 1H), 5.51 (d, J = 5.6, 1H), 5.78 (d, J = 5.2, 1H), 8.80 (b, 1H), 8.43 (s, 1H).

1-[(2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl]-5-fluorouracil (35α)

Yield = 56% (based on compound **8**), white solid, mp 196 °C (dec.), $[\alpha]_D^{25}$ = −112 (C = 1.0, MeOH), ESI-MS m/z 285 (M^++23), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.30 (d, J = 6.4, 3H), 3.93–3.95 (m, 1H), 4.06–4.08 (m, 1H), 4.27–4.30 (m, 1H), 6.24 (d, J = 8.4, 1H), 8.23 (d, J = 6.8, 1H).

1-[(2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl]-5-fluorouracil (35β)

Yield = 15% (based on compound **8**), white solid, mp 180 °C (dec.), $[\alpha]_D^{25}$ = −28 (C = 1.0, MeOH), ESI-MS m/z 285 (M^++23), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.51 (d, J = 7.2, 3H), 3.38–3.41 (m, 1H), 3.85–3.87 (m, 1H), 4.24–4.26 (m, 1H), 6.03 (d, J = 5.2, 1H), 8.13 (d, J = 7.2, 1H).

1-[(2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl]-uracil (36α)

Yield = 47% (based on compound **8**), white solid, mp 167 °C (dec.), $[\alpha]_D^{25}$ = −152 (C = 1.0, MeOH), ESI-MS m/z 245 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.30 (d, J = 6.4, 3H), 3.92–3.94 (m, 1H), 4.07–4.09 (m, 1H), 4.30–4.33 (m, 1H), 5.81 (d, J = 8.0, 1H), 6.24 (d, J = 8.4, 1H), 8.02 (d, J = 8.0, 1H).

1-[(2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl]-uracil (36β)

Yield = 16% (based on compound **8**), white solid, mp 154 °C (dec.), $[\alpha]_D^{25}$ = −47 (C = 1.0, MeOH), ESI-MS m/z 245 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.50 (d, J = 7.2, 3H), 3.37–3.42 (m, 1H), 3.85–3.87 (m, 1H), 4.23–4.25 (m, 1H), 5.80 (d, J = 8.4, 1H), 6.22 (d, J = 5.6, 1H), 8.02 (d, J = 8.4, 1H).

1-[(2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl]-thymine (37α)

Yield = 55% (based on compound **8**), white solid, mp 158 °C (dec.), $[\alpha]_D^{25}$ = −150 (C = 1.0, MeOH), ESI-MS m/z 259 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.30 (d, J = 6.4, 3H), 1.95 (s, 3H), 3.93–3.96 (m, 1H), 4.07–4.08 (m, 1H), 4.32–4.35 (m, 1H), 6.25 (d, J = 9.2, 1H), 7.83 (s, 1H).

1-[(2S,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl]-thymine (37β)

Yield = 19% (based on compound **8**), white solid, mp 167 °C (dec.), $[\alpha]_D^{25}$ = −55 (C = 1.0, MeOH), ESI-MS m/z 259 (M^++1), ^{1}H NMR (CD_3OD, 400 MHz, δppm); 1.44 (d, J = 7.2, 3H), 1.87 (s, 3H), 3.30–3.34 (m, 1H), 3.82–3.84 (m, 1H), 4.20–4.23 (m, 1H), 6.00 (d, J = 6.0, 1H), 7.69 (s, 1H).

Biological assay

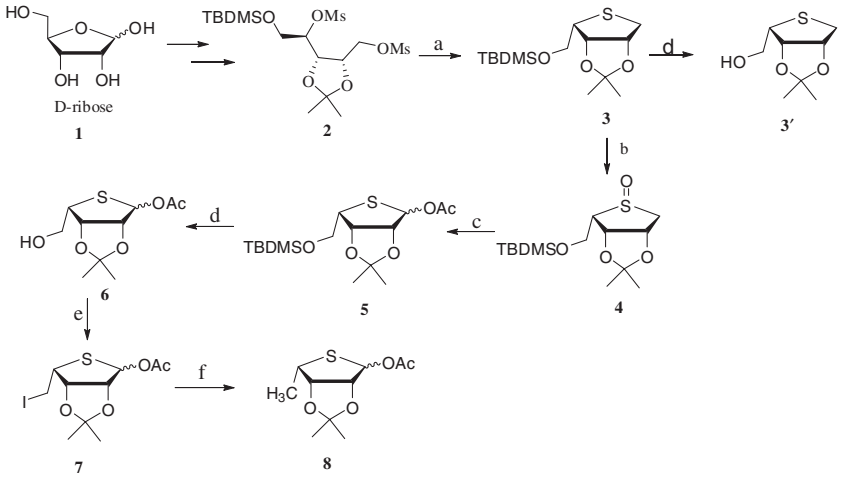
Cell viability was measured by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to assess the chemosensitivity of tumor cells. The growth inhibitory activity of target compounds was determined on cell lines LOVO (human carcinoma of colon), MDA-MB-435 (human breast cancer cell line) and CEM (human leukemia cell line) using a modified version of the microculture tetrazolium assay. All the cell lines tested were cultured in our pharmacology department.

Once the cells reached 90% confluency, a cell suspension was prepared by trypsinization of monolayer cultures. Cell counts were performed, and the suspensions were diluted accordingly to give 4–5 × 10^4 cells/mL with the appropriate medium. Aliquots (10 μL) of the cell suspension were added to each well in a 96-well microtitre plate. The cells were incubated for 24 h (37 °C, 5% CO_2). Stock solutions of the test compound were prepared in dimethyl sulfoxide (DMSO), and serial dilutions were made with medium to over a 100-fold concentration range. Not more than 1% DMSO (final concentration) was present in each well. The test sample was incubated with the cells for 72 h. Wells without cells and those with cells in culture medium/DMSO were examined in parallel. At the end of the incubation period, the medium was decanted and replaced with 20 μL MTT solution (5 mg/mL). The cells were incubated for another 4 h, after which the medium was removed from each well by pipetting. DMSO (100 μL) was added to each well to lyse the cells. The OD values were measured within 30 min at 570 nm on an elisa reader (WellscanMK-2). IC50 values were determined from logarithmic plots of the % absorbance versus concentration generated.

Results and Discussion

Synthesis

Our strategy to the desired 5′-deoxy-4′-thio-L-nucleosides is to synthesize the glycosyl donor and then to condense the donor with the



Scheme 1: Reagents and conditions: (a) Na2S, DMF, heat, 65%. (b) m-CPBA, CH2CL2, −78, 51%. (c) Ac2O, heat. (d) TBAF, THF, r.t. (e) Ph3P, imidazole, I2, toluene, heat, 42% (three stepts) (f) Bu3SnH, azobisisobutyronitrile, toluene. heat, 67%.

nucleosidic bases. The synthesis of the key L-glyxosyl donor **8** started from D-ribose, as shown in Scheme 1.

D-ribose **1** was converted to 5-*O*-tert-butyldimethylsilyl-1,4-*O*-dimesyl-2,3-*O*-isopropylidene-D-ribitol (**2**) in four steps according to the published procedures (6–8). The cyclization of **2** with sodium sulfide smoothly proceeded upon heating in N, N-dimethylformamide (DMF) to yield the (2S,3S,4R)-2-tert-butyldimethylsilyloxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene (thioether **3**) in a yield of 65%. To verify the absolute configuration of the thioether **3**, it was deprotected with tetrabutylammonium fluoride trihydrate (TBAF·3H_2O) to obtain (2S,3S,4R)-2-hydroxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene (**3′**). Compound 3′ was crystallized from acetonitrile, and its X-ray crystallographic analysis showed that the configuration is 2S, 3S, 4R. The three-dimensional structure of **3′** was illustrated in Figure 2.

To achieve the synthesis of the nucleosidic base, we chose to synthesize an acetate derivative through the Pummerer reaction. Initially,thioether **3** was treated with 1.0 eq. *m*-chloroperoxybenzoic acid (*m*-CPBA) at −78 °C as reported by Jeong *et al.* (9), but the sulfoxide (**4**) was obtained only in a low yield (15%), while the major problem was shown by the over-oxidation to give sulfone. Even when the quantity of m-CPBA was decreased to 0.9 eq., the reaction was not improved. We therefore decided to replace *m*-CPBA with ozone (10,11). Ozonization of the thioether **3** at −78 °C gave the desired (2S,3S,4R)-2-tert-butyldimethylsilyloxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene-1-oxide (sulfoxide **4)** in 78% yield, followed by heating the sulfoxide **4** with acetic anhydride afforded (3R,4S,5S)-2-acetyloxy-5-tert-butyldimethylsilyloxymethyl-3,4-dihydroxy-3,4-*O*-isopropylidene-tetrahydrothiophene (**5)**. Treatment of **5** with tetrabutylammonium fluoride trihydrate gave (3R,4S,5S)-2-acetyloxy -3,4-dihydroxy-3,4-*O*-isopropylidene-5-hydroxymethyl-tetrahydrothiophene (**6**). Iodination was performed on the 5-position by heating **6** with imidazole, triphenylphosphine and iodine in toluene produced (3R,4S,5R)-2-acetyloxy-3,4-dihydroxy-3,4-*O*-isopropylidene-5-iodiomethyl-tetrahydrothiophene (**7**), which was converted to the key intermediate, the glycosyl donor, (3R,4S,5S)-2-acetyloxy-3,4-dihydroxy-3,4-*O*-isopropylidene-5-methyl-tetrahydrothiophene (**8**) by treating with tributyltin hydride and azobisisobutyronitrile (AIBN) in toluene at 100 °C in 67% yield.

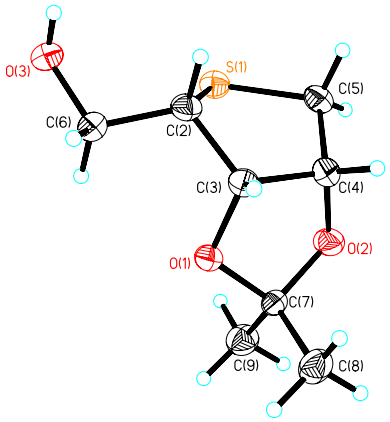
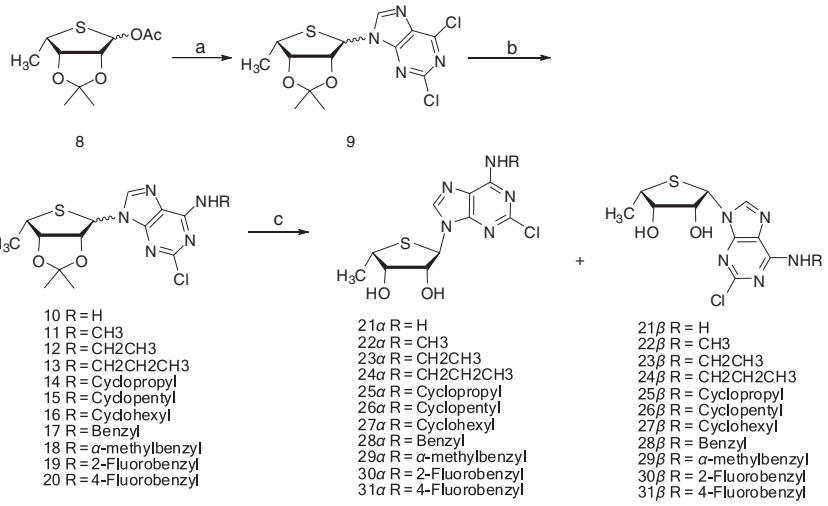


Figure 2: X-ray crystal structure of **3′**.

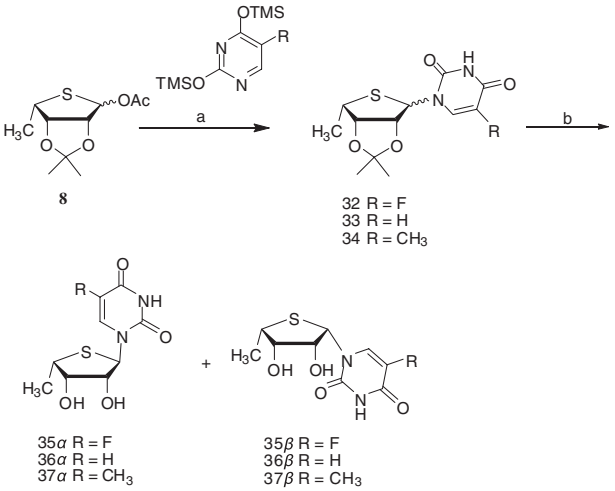
The gylcosyl donor **8** was condensed with 2,6-dichloropurine in the presence of $EtAlCl_2$ in acetonitrile at room temperature to give the nucleoside derivative **9**. ^{1}H NMR of **9** showed two sets of peak. For example, there were two singlets, respectively, at 8.20 ppm and 8.38 ppm corresponding to H(8) in purine. The ratio of the two singlets was approximately 3:1. So, the nucleoside derivative **9** might be the mixture of α-isomer and β-isomer with the approximate ratio of 3:1. They were used in next substitution without separation. Substitution of N^6-position of **9** with various amines gave the isomeric N^6-substituted purine nucleoside derivatives **10–20** (Scheme 2).

The treatment of **10–20** with 85% aqueous formic acid at room temperature offered the deprotection products **21–31** (α and β), and the separation of the isomers was achieved by column chromatography on silica gel. The ratio of the isomers obtained was approximately 3:1 as confirmed the by ^{1}HMR analysis of compound **9**.

Condensation of the acetate **8** with persilylated pyrimidine derivatives under Vorbrüggen conditions with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst was depicted in Scheme 3.



Scheme 2: (a) 2,6-dichloropurine, EtAlCl2, CH3CN, r.t. (b) Appropriate amine, r.t. THF. (c) 85% aqueous HCOOH, r.t.



Scheme 3: (a) trimethylsilyl trifluoromethanesulfonate, CH3CN, r.t. (b) 85% aqueous HCOOH, r.t.

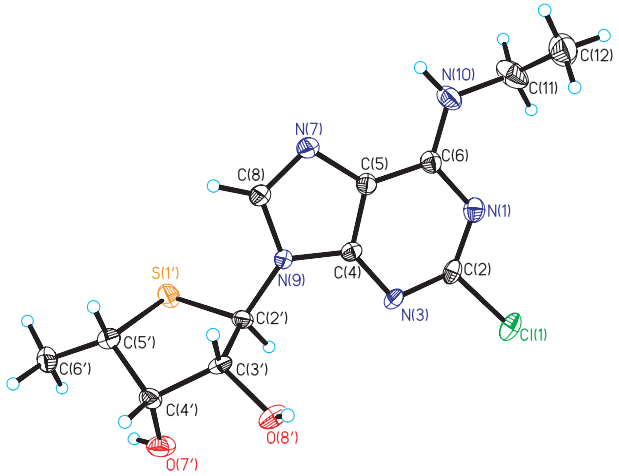


Figure 3: X-ray crystal structure of **23**α.

The isomeric mixtures of **32–34** were formed in moderate yield. After deprotection with 85% aqueous formic acid at room temperature, the isomers (**35**α**–37**α and **35**β**–37**β) were separated by chromatography on silica gel.

Structure analysis

The structural assignment of the target compounds were made on the basis of ^{1}H NMR studies. The magnitude of the coupling constant (*J*-value) provided the important structural information. In the target compounds of our report, the $J_{2'3'}$ = 8–9 Hz for H(2′) (δ = 5.80–6.30 ppm, d) on the main products was larger than the $J_{2'3'}$ = 4–6 Hz of minor products (δ = 5.70–6.00 ppm, d). Based on the fact that trans-configuration gave larger vicinal coupling constant than the cis-configuration, we postulated the main products were the α-isomers (2′R), and the minor products were the β-isomers (2′S).

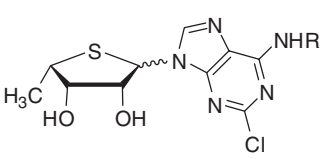
The absolute configuration of **23**α was determined on the basis of X-ray crystallography (in Figure 3). It verified that the structure of **23**α was 2-chloro-6-ethylamino-9-((2R,3R,4S,5S)-3,4-dihydroxy-5-methyl-tetrahydrothiophene-2-yl)purine. The result of X-ray crystallography confirmed the postulate from ^{1}H NMR.

Biological activity

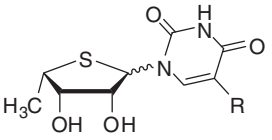
All the target compounds were tested for the inhibition of the growth of LOVO human carcinoma of colon, CEM human leukemia and MDA-MB-435 human breast adenocarcinoma cells in culture with 5-FU as the reference (Table 1).

From the data in Table 1, it was seen that purine nucleosides gave moderate or strong inhibition on the growth of CEM cells. For example, compounds **22** (α, IC_{50} = 43.01; β, IC_{50} = 40.32), **23** (α, IC_{50} = 37.20; β, IC_{50} = 43.62), **24** (α, IC_{50} = 40.52; β, IC_{50} = 36.24) and **25** (α, IC_{50} = 35.95) shown comparable activity to **5-FU** (IC_{50} = 103.4), **26–31** (α and β) shown greater inhibition than **5-FU**.

Table 1: Inhibition against human tumor cells *in vitro*



21–31



35–37

Compound No.	R	CEM		LOVO		MDA-MB-435	
		IC_{50} (μM)	IC% at 100 μg/mL	IC_{50} (μM)	IC% at 100 μg/mL	IC_{50} (μM)	IC% at 100 μg/mL
21β	H	14.8	98.22	183.3	64.96	67.50	70.17
21α	H	153.8	69.81	>240	4.17	316.8	50.58
22β	Methyl	40.32	88.10	>240	44.83	237.9	53.53
22α	Methyl	43.01	93.99	>240	19.81	219.8	58.21
23β	Ethyl	43.62	91.95	>240	30.44	>240	48.92
23α	Ethyl	37.20	96.94	≈240	49.63	254.4	52.26
24β	Propyl	36.24	96.60	280.3	51.68	247.9	52.19
24α	Propyl	40.52	90.70	>240	47.23	257.4	51.89
25β	Cyclopropyl	10.94	99.21	71.70	72.93	51.58	78.73
25α	Cyclopropyl	35.95	86.41	>240	44.93	231.4	53.41
26β	Cyclopentyl	0.4932	93.86	43.01	81.30	76.69	72.86
26α	Cyclopentyl	0.2336	99.76	52.47	90.48	89.19	71.16
27β	Cyclohexyl	0.1757	98.32	≈240	49.01	41.30	88.17
27α	Cyclohexyl	0.1875	99.64	190.47	52.41	47.94	87.43
28β	Benzyl	2.711	98.92	110.7	63.97	52.30	79.89
28α	Benzyl	2.685	99.88	43.84	100	44.07	84.92
29β	α-Methylbenzyl	10.72	89.54	>240	7.45	35.95	92.17
29α	α-Methylbenzyl	12.10	89.98	>240	46.78	>240	38.69
30β	2-Fluorobenzyl	7.506	96.44	>240	31.91	52.40	77.53
30α	2-Fluorobenzyl	7.384	98.66	>240	42.49	47.65	87.10
31β	4-Fluorobenzyl	7.873	98.89	>240	22.00	63.79	82.59
31α	4-Fluorobenzyl	8.85	98.66	>240	23.65	48.63	89.64
35β	F	>240	14.57	>240	0	>240	18.58
35α	F	>240	20.16	>240	0	>240	16.97
36β	H	>240	23.33	>240	0	>240	14.34
36α	H	>240	32.99	>240	0	>240	28.61
37β	Methyl	>240	37.36	>240	0	>240	26.49
37α	Methyl	>240	31.33	>240	0	>240	19.67
5-FU	–	103.4	60.68	>240	42.28	31.54	77.17

6-cyclopentylamino and 6-cyclohexylamino derivatives **26** (α, IC_{50} = 0.2336; β, IC_{50} = 0.4932) and **27** (α, IC_{50} = 0.1875; β, IC_{50} = 0.1757) shown the best inhibition to CEM human leukemia cells *in vitro*. But they gave weaker inhibition to LOVO and MDA-MB-435 than 5-FU. Pyrimidine nucleosides **35–37** (α and β) show no significant effect on the growth of LOVO, CEM or MDA-MB-435 cells.

Structure–activity relationship

The target compounds showed the better inhibition against CEM cell than against LOVO or MDA-MB-435 *in vitro*. The inhibitory activity of 5′-deoxy-4′-thio-L-purine nucleosides was better than that of 5′-deoxy-4′-thio-L-pyrimidine nucleosides. Among the 5′-deoxy-4′-thio-L-purine nucleosides, the best substituted group in position 6 in purine was cycloalkylamino (e.g. compounds **26** or **27**), followed by benzylamino. The alkylamino groups only gave moderate activity. Generally, the epimers at position 2′ (α or β form) had no significant difference on the antitumor activity.

Conclusion

In summary, we have synthesized a series of novel 5′-deoxy-4′-thio-L-nucleosides and verified the configuration of the target compounds by MS, ^{1}H-NMR and X-ray crystallography. The antitumor activities of the target compounds were tested against the growth of LOVO, CEM and MDA-MB-435 cells *in vitro*. It showed that 6-cyclopentylamino and 6-cyclohexylamino purine compounds **26** and **27**, both in α-configuration and in β-form, shown strong inhibition to CEM.

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