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An efficient synthesis of 3-fluoro-5-thio-xylofuranosyl nucleosides of thymine, uracil, and 5-fluorouracil as potential antitumor or/and antiviral agents

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Abstract—1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose by the sequence of mild oxidation, reduction, fluorination, periodate oxidation, borohydride reduction, and sulfonylation gave 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-xylofuranose (**5**). Tosylate **5** was converted to thioacetate derivative **6**, which after acetolysis gave 1,2-di-*O*-acetyl-5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-D-xylofuranose (**7**). Condensation of **7** with silylated thymine, uracil, and 5-fluorouracil afforded nucleosides 1-(5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-β-D-xylofuranosyl) thymine (**8**), 1-(5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-β-D-xylofuranosyl) uracil (**9**), and 1-(5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-β-D-xylofuranosyl) 5-fluorouracil (**10**). Compounds **8**, **9**, and **10** are biologically active against rotavirus infection and the growth of tumor cells.

1. Introduction

Although an upsurge in the search for new antitumor agents has recently been observed, the survival rate of patients still remains low and one of the reasons is the poor sensitivity of tumors to drugs. Therefore, new agents capable of suppressing tumor growth would contribute greatly to a better prognosis and current therapy.

Thymidine 5′-monophosphate (TMP), which is essential for cell proliferation, can be furnished either from deoxyuridine 5-phosphate (dUMP) *via* the de novo pathway of biosynthesis or from exogenous thymidine. Today, several drugs are effective in blocking this de novo pathway.[1] An alternate route to TMP involves transfer of phosphate from a nucleoside 5-triphosphate to thymidine, a reaction which is catalyzed by thymidine kinase (TK).[1–5] Two are the major forms of TK, which are identified in mammalian tissues, the mitochondrial thymidine kinase (M-TK) and cytoplasmic thymidine kinase (C-TK).[2–7] The C-TK is described as the essential precursor in human tumor cell lines and is believed to play a significant role in the biosynthesis of TMP.[8–12] These data suggest that the use of a drug, which blocks de novo TMP biosynthesis and then selectively inhibits the C-TK, might offer a possibility for effective antineoplastic chemotherapy.[6,7] Among several nucleosides that have been synthesized, 5-alkylthio-5-deoxythymidine derivatives were found to be non-competitive inhibitors of C-TK.[5–7] Some other 5-alkylthionucleosides have also been found to be potent antitumor or antiviral agents.[13–16]

On the other hand, a number of fluorine-substituted furanosyl nucleoside analogues have demonstrated a substantial antiviral and anticancer potency.[17–29] This has been partly attributed to the small size and high electronegativity of fluorine, which is also capable of participating in hydrogen bonding.[30,31] It appears that the high strength of the C–F bond may hinder metabolic pathways and may increase the effective lifetime of the active molecule. Moreover, the presence of fluorine

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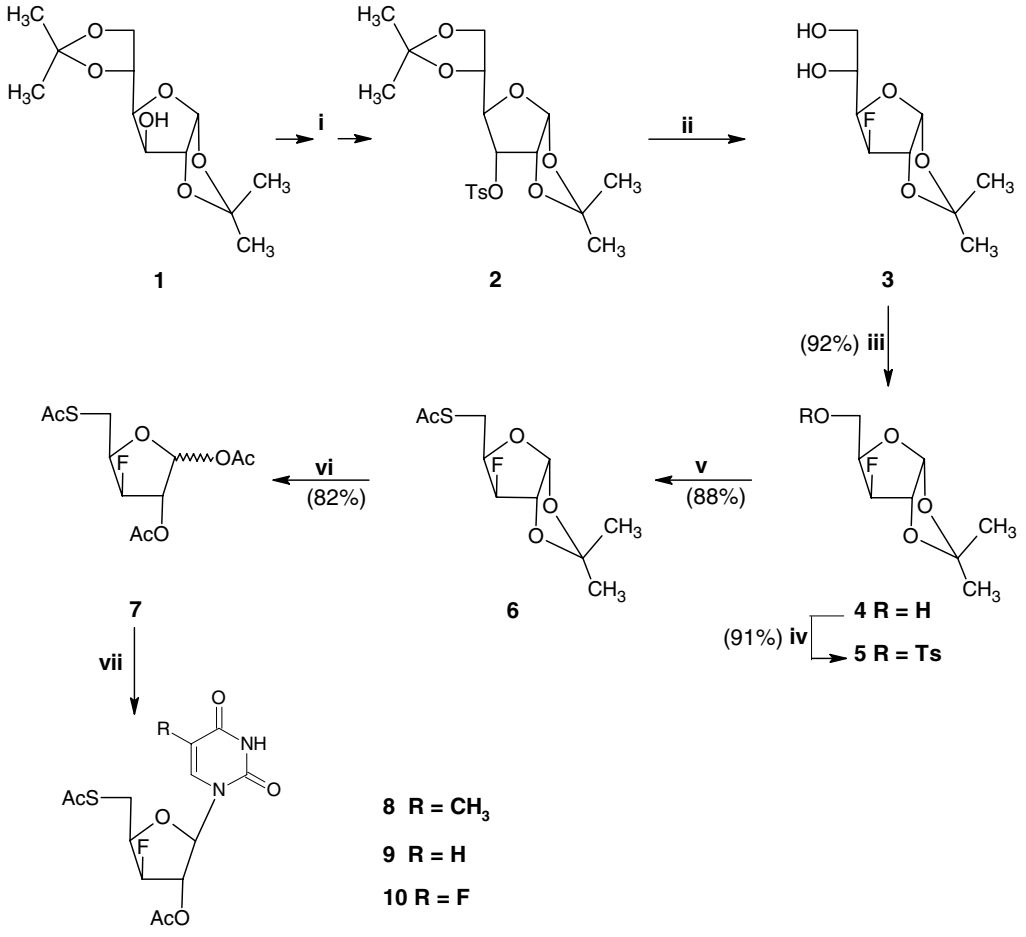
enhances lipophilicity and makes the penetration of the drug through the cell membrane easier.[32–35] The introduction of fluorine into the sugar moiety, especially in the C-2 and/or C-3 positions, induces antiviral selectivity, as in the case of 2-fluoroarabinothymidine, which demonstrates selective inhibition of Herpes Simplex viruses (HSV-1 and HSV-2).[21–23] Moreover, 3-fluoro-3-deoxy-thymidine was proven to be very active against human immunodeficiency viruses (HIV) and also a better inhibitor of HIV replication than 3-azido-3-deoxy-thymidine (AZT).[23–25]

The above observations and the continuous demand for new antitumor and antiviral agents prompted us to design and synthesize a series of 1-(5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-β-D-xylofuranosyl) nucleosides of thymine, uracil, and 5-fluorouracil (5FU), which we report herein.

2. Results and discussion

2.1. Synthesis

Oxidation of the commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) with pyridinium dichromate and acetic anhydride in dichloromethane, followed by stereoselective reduction with sodium borohydride in methanol and tosylation with *p*-toluenesulfonyl chloride in pyridine, gave the corresponding 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-allofuranose (**2**).[26–28] (Scheme 1) Displacement of the tosyloxy group at C-3 of **2** by a fluorine atom was effected by reacting **2** with potassium fluoride in acetamide. Selective removal of the 5,6-*O*-isopropylidene group afforded the 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (**3**),[29] which upon periodate oxidation, followed by borohydride reduction of the resulting aldehyde (one pot), gave 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranose (**4**). Sulfonylation of **4** with *p*-toluenesulfonyl chloride in pyridine proceeded readily, giving the tosyl derivative **5** as a white solid. Tosylate **5** was in turn treated with potassium thioacetate in hot *N*,*N*-dimethylformamide[36] to give the corresponding thioacetate **6**, the infrared spectrum of which showed a characteristic absorption at 1697 cm^{-1} (*S*-acetyl group); the NMR spectrum and chemical analysis were also consistent with structure **6**. Acetolysis[37] of **6** in the presence of acetic acid, acetic anhydride, and sulfuric acid afforded a mixture of the anomeric diacetates **7** (ratio of α to β anomer, 1:2). Compound **7** showed strong IR absorptions at 1750 cm^{-1} (OAc) and 1697 cm^{-1} (SAc), while in its ^1H NMR spectrum appeared prominent 3-proton methyl signals, belonging to the acetylthio moiety (α:δ 2.36,



Scheme 1. Reagents and Conditions: (i) a—PDC/Ac_2O/CH_2Cl_2; b—$NaBH_4$/MeOH; c—TsCl/pyridine; (ii) a—KF/acetamide; b—70% AcOH; (iii) $NaIO_4$/MeOH/$NaBH_4$; (iv) TsCl/pyridine; (v) KSAc/DMF/100 °C; (vi) Ac_2O/CH_3COOH/H_2SO_4; (vii) silylated base/$Me_3SiOSO_2CF_3$.

β:δ 2.37) and 6-proton methyl peaks assigned to the acetoxyl group (δ: 2.06–2.16). Condensation of the anomeric mixture **7** with the appropriate silylated nucleic acid bases in the presence of trimethylsilyl trifluoromethanesulfonate afforded solely the protected β-nucleosides, that is the title 1-(5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-β-D-xylofuranosyl) nucleosides of thymine (**8**), uracil (**9**), and 5FU (**10**), respectively.

2.2. Antiviral activity

The results from the antiviral assays on the newly synthesized compounds are summarized in Table 1 and compared to AZT. In the neutralization assay all three compounds exhibited much higher efficacy (IC_{50}) than AZT. Compound **8** showed the highest direct antiviral effect on rotavirus as the virus vanished at a very low concentration (0.002 mg/mL), while slightly higher concentrations were necessary in the case for compounds **9** and **10** (0.006 mg/mL), in order to achieve the same result. Compound **8** exhibited the highest selectivity as the corresponding CC_{50}/IC_{50} value reached an optimum level. All compounds were also capable of inhibiting the rotavirus infection in Caco-2 cells, as strong inhibition of infectivity was observed after rotavirus attachment to the cells. Inhibition of rotavirus infectivity following virus attachment of all tested compounds was comparable to that of AZT or even slightly better, while their CC_{50}/IC_{50} values were almost of the same magnitude.

2.3. Cytotoxic and growth inhibition activity

The cytotoxicity of compounds **8**–**10** was measured on H4 normal human intestinal cells and on a series of other human tumor cells, such as human colonic adenocarcinoma derived Caco-2 cells, skin melanoma cells, and epithelial breast cancer derived MCF-7 cell line, and is expressed by the CC_{50} values. The growth inhibition of Caco-2 cells induced by the new compounds was measured by determining the minimal inhibitory concentration (IC_{50}). The results are summarized in Table 2 and compared with the values obtained for 5FU.

The tested compounds exhibited higher cytotoxicity in tumor cells than in the normal H4 cell line, with the exception of compound **8**, which was 2-fold more toxic (*vide* TSI values) in skin melanoma and MCF-7 cells than in normal cells. Compounds **9** and **10** were highly selective for malignant cells. Comparison with 5FU revealed that these molecules were 2.5-fold Caco-2 cells selective and as selective as 5FU in skin melanoma and MCF-7 cells; compound **10** did not show any MCF-7 cells selectivity. This selective activity of the new compounds is noteworthy and merits further investigation.

The effect of compounds **8**–**10** on cell growth was determined using Caco-2 cells. As determined by colony numbers after 10 days of incubation, it was found that the new compounds are capable of inhibiting the growth of these cells in a concentration-dependent manner. Compound **8** exhibited growth inhibitory activity (IC_{50} 1.9 μM) similar to 5FU (IC_{50} 1.5 μM), but 4-fold less tumor selectivity (TSI 2.5 vs 10). Compounds **9** and **10** were also found to be strong inhibitors of cell growth, but although their effect was somewhat less pronounced than that of 5FU, they were highly selective in malignant cells, as they exhibited a 2.5-fold higher TSI values than 5FU.

3. Conclusion

In conclusion, the newly synthesized 1-(3-deoxy-3-fluoro-5-*S*-acetyl-5-thio-β-D-xylofuranosyl) nucleosides

Table 1. Antiviral activity of nucleosides **8**–**10** and **AZT** against rotavirus RF strain on Caco-2 cells (IC_{50})

Compound	Treatment A[a]			Treatment B[a]		
	IC_{50}		CC_{50}/IC_{50}[b]	IC_{50}		CC_{50}/IC_{50}
	(mg/mL)	(μM)		(mg/mL)	(μM)	
8	0.002	5.55	10	0.006	16.65	3.33
9	0.006	17.32	3.33	0.006	17.32	3.33
10	0.006	16.47	3.33	0.006	16.47	3.33
AZT	0.020	74.84	0.75[c]	0.006	22.45	2.5

CC_{50}/IC_{50} ratios were calculated from CC_{50} values given in Table 2.
[a] Treatment A, Neutralization of the virus in the solution before its attachment. Treatment B, Inhibition of infectivity following virus attachment.
[b] CC_{50}/IC_{50} values were calculated using CC_{50} values in Table 2.
[c] CC_{50} for AZT on Caco-2 cells = 56.1 μM.

Table 2. Cytotoxic effect (CC_{50}, μM) of compounds **8**–**10** and 5-fluorouracil (5FU) on Caco-2, H4, MCF 7, and skin melanoma cells, and growth inhibition (IC_{50} μM) on Caco-2 cells

Compound	Cytotoxic effect (CC_{50} μM)				TSI[a]			Growth inhibition (IC_{50} μM)
	H4	Caco-2	Skin melanoma	MCF-7	Caco-2	Skin melanoma	MCF-7	Caco-2
8	138.7	55.5	277.5	277.5	2.5	0.5	0.5	1.9
9	1443.7	57.8	17.3	288.7	25	83.5	5	5.8
10	1372.4	54.9	16.5	1372.4	25	83.2	1	16.5
5FU	3843.8	384.4	46.1	768.8	10	83.4	5	1.5

[a] TSI, tumor selectivity index (CC_{50} on H4 cells/CC_{50} on the specified host cells).

8–**10** are good candidates for the development of potential antiviral agents, as significantly lower concentrations of these agents with respect to AZT were needed to neutralize rotavirus infectivity. Further investigation will enable us to elucidate potential mechanisms of their activity and application. The most promising antitumor activity was observed in the case of colon carcinoma treatment, where growth inhibition and cytotoxic effect were achieved at low concentration in comparison to 5FU, although the antitumor activity of the new compounds was in most cases cell type depended.

4. Experimental

4.1. General procedure

Solutions were removed in vacuo below 40 °C under reduced pressure. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Flash chromatography was performed with Silica Gel 60 (220–440 mesh, Merck). TLC was carried out on Silica gel (240–400 mesh, Merck), and the developing solvents were as specified. NMR spectra were recorded at room temperature with a Brucker 400 MHz spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts (δ) were given in ppm measured downfield from TMS, and spin–spin coupling constants are in Hz. Infrared spectra were obtained with a Perkin–Elmer Model 1600 FT-IR spectrophotometer. Optical rotations were measured using a Schmidt and Haensch polarimeter. All reactions were carried out in dry solvents. CH_3CN was distilled from calcium hydride and stored over 3E molecular sieves. *N*,*N*-dimethylformamide (DMF) was also stored over 3E molecular sieves, and pyridine over potassium hydroxide pellets.

4.1.1. 3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranose (4). The diol **3**[26–29] (5.0 g, 22.5 mmol) was added to a stirred solution of $NaIO_4$ (5.09 g, 23.8 mmol) in H_2O (79 mL) and MeOH (79 mL) leading to immediate precipitation of $NaIO_3$. After 1 h at room temperature, any residual periodate was destroyed with a drop of ethylene glycol. The reaction mixture was stirred for 1 h at room temperature with $NaBH_4$ (2.0 g, 52.3 mmol), neutralized with aqueous $NaHCO_3$, and then extracted with ethyl acetate (EtOAc) (4× 500 mL). The organic layer was washed with $NaHSO_4$, dried over anhydrous sodium sulfate, evaporated to dryness, and purified by column chromatography with EtOAc/hexane (2:8) to give compound **4** (4.0 g, 92%, R_f = 0.3 in EtOAc/hexane, 2:8).

^{1}H NMR ($CDCl_3$): δ 5.91 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1), 4.92 (dd, $J_{3,F}$ = 50.4 Hz, $J_{3,4}$ = 2.3 Hz, 1H, H-3), 4.62 (dd, $J_{2,F}$ = 11.1 Hz, $J_{2,1}$ = 3.8 Hz, 1 H, H-2), 4.28 (m, 1H, H-4), 3.82 (m, 2H, H-5a and H-5b), 1.41 (s, 3H, CH_3), 1.25 (s, 3H, CH_3).

Found: C, 49.67; H, 7.00; F, 9.63. Calcd for $C_8H_{13}FO_4$: C, 50.00; H, 6.82; F, 9.89.

ESI-MS *m*/*z* (relative intensity, %): 193.3 [(M+H$^+$), 100].

4.1.2. 3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-xylofuranose (5). To a solution of compound **4** (4.0 g, 20.7 mmol) in dry pyridine (53.1 mL) was added *p*-toluenesulfonyl chloride (5.2 g, 27.6 mmol) and kept overnight at room temperature. After neutralization ($NaHCO_3$) and extraction with EtOAc (4× 500 mL), the combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. Purification of the residue by flash chromatography with EtOAc/hexane (1:9) yielded the title compound (6.5 g, 91%, R_f = 0.2 in EtOAc/hexane, 1:9) as a white solid. mp 61 °C.

^{1}H NMR ($CDCl_3$): δ 7.4–7.8 (m, 4H, tosyl group), 5.93 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.96 (dd, $J_{3,F}$ = 50.1 Hz, $J_{3,4}$ = 1.8 Hz, 1H, H-3), 4.67 (dd, $J_{2,F}$ = 10.5 Hz, $J_{2,1}$ = 3.7 Hz, 1H, H-2), 4,35-4,49 (m, $J_{4,F}$ = 28.1 Hz, $J_{4,3}$ = 1.8 Hz, 1H, H-4), 4.18–4.29 (m, 2 H, H-5a and H-5b), 2.47 (s, 3H, $ArCH_3$), 1.48 (s, 3H, CH_3), 1.33 (s, 3H, CH_3).

Found: C, 52.23; H, 5.69; F, 5.72. Calcd for $C_{15}H_{19}FO_6S$: C, 52.01; H, 5.53; F, 5.48.

ESI-MS *m*/*z* (relative intensity, %): 347.4 [(M+H$^+$), 100].

4.1.3. 3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-5-*S*-acetyl-5-thio-α-D-xylofuranose (6). The tosylate **5** (6.5 g, 18.8 mmol) was heated with potassium thioacetate (2.9 g, 25.5 mmol) in DMF (71 mL) at 100 °C for 1 h. The reaction mixture was neutralized with aqueous $NaHCO_3$. After that, the mixture was concentrated under high vacuum pump to eliminate the DMF. The residue was partitioned between water and EtOAc, the organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography using EtOAc/hexane (1:9) as eluent to give compound **7** (4.1 g, 87%, R_f = 0.3 in EtOAc/hexane, 1:9) as a yellow syrup.

IR (Nujol): 1697 (SAc) cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 5.96 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.88 (dd, $J_{3,F}$ = 50.1 Hz, $J_{3,4}$ = 2.1 Hz, 1H, H-3), 4.68 (dd, $J_{2,F}$ = 10.8 Hz, $J_{2,1}$ = 3.8 Hz 1H, H-2), 4,19-4,34 (m, $J_{4,F}$ = 28.4 Hz, $J_{4,3}$ = 2.1 Hz, 1H, H-4), 3.12–3.26 (m, 2H, H-5a and H-5b), 2.36 (s, 3H, SAc), 1.47 (s, 3H, CH_3), 1.31 (s, 3H, CH_3).

Found: C, 48.27; H, 5.89; F, 7.74. Calcd for $C_{10}H_{15}FO_4S$: C, 47.99; H, 6.04; F, 7.59.

ESI-MS *m*/*z* (relative intensity, %): 251.4 [(M+H$^+$), 100].

4.1.4. 1,2-Di-*O*-acetyl-5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-D- xylofuranose (7). Compound **6** (4.1 g, 16.5 mmol) was acetolyzed with 350 mL of a mixture of acetic anhydride–acetic acid–sulfuric acid (70:30:1, v/v). After 3 days, anhydrous ether (600 mL) was added, followed by sodium acetate (25 g). The mixture was filtered and the residue was washed with ether (2× 500 mL). The combined solutions were co-evaporated with toluene and the residue was purified by flash chromatography

on a silica gel column using EtOAc/hexane (2:8) as eluent. Pure compound **7** was collected as a thick syrup (4.0 g, 82%, $R_f = 0.3$ in EtOAc/hexane, 2:8).

IR (Nujol): 1697 (SAc), 1750 (OAc) $cm^{-}1$.

^1H NMR ($CDCl_3$): δ 6.45 and 6.11 (α: d, $J_{1,2} = 4.8$ Hz, 0.3H, H-1; β: s, $J_{1,2} = 0.7$ Hz, 0.7H, H-1), 5.29–5.33 (m, 1H, H-2), 4.97 (dd, $J_{3,F} = 50.3$ Hz, $J_{3,4} = 3.7$ Hz 1H, H-3), 4.31–4.48 (m, $J_{4,F} = 25.8$ Hz, $J_{4,3} = 3.7$ Hz, 1H, H-4), 3.14–3.34 (m, 2H, H-5a and H-5b), 2.36 and 2.37 (β and α, s, 3H, SAc), 2.06–2.16 (m, 6H, OAc).

Found: C, 45.08; H, 5.29; F, 6.31. Calcd for $C_{11}H_{15}FO_6S$: C, 44.89; H, 5.14; F, 6.46.

ESI-MS *m/z* (relative intensity, %): 295.4 [($M+H^+$), 100].

4.1.5. 1-(5-*S*-Acetyl-3-deoxy-3-fluoro-5-thio-β-D- xylofuranosyl) thymine (8). A mixture of thymine (2.4 g, 18.9 mmol), hexamethyldisilazane (4.9 mL, 23.3 mmol), and saccharine (0.115 g, 0.63 mmol) in anhydrous CH_3CN (65 mL) was refluxed at 120 °C for 20 min. To this were added diacetylated 3-deoxy-3-fluoro-5-*S*-acetyl-5-thio-D-xylofuranose **7** (4.0 g, 13.5 mmol) and trimethylsilyl trifluoromethane-sulfonate (3.4 mL, 19 mmol). The reaction mixture was refluxed at 80 °C for 2 h. The mixture was neutralized with aqueous $NaHCO_3$, then diluted with water and extracted with EtOAc (1000 mL). The extract was dried over sodium sulfate, filtered, and evaporated to a syrup, which was purified by column chromatography using EtOAc/hexane (8:2) to afford the title compound (2.7 g, 56%, $R_f = 0.35$ in EtOAc/hexane, 8:2).

$[\alpha]_D^{22} + 28.9$ (*c* 0.25, $CHCl_3$)

IR (Nujol): 1697 (SAc), 1750 (OAc) cm^{-1}.

^1H NMR ($CDCl_3$): δ 8.67 (br s, NH), 7.26 (s, 1H, H-6′), 6.06 (d, $J_{1,2} = 2.2$ Hz, 1H, H-1), 5.23 (d, $J_{2,F} = 16.9$ Hz, $J_{2,1} = 2.2$ Hz, 1H, H-2), 4.99 (dd, $J_{3,F} = 50.2$ Hz, $J_{3,4} = 2.3$ Hz, 1H, H-3), 4.11–4.26 (m, $J_{4,F} = 28.7$ Hz, $J_{4,3} = 2.3$ Hz, 1H, H-4), 3.29–3.33 (m, 2H, H-5a and H-5b), 2.40 (s, 3H, SAc), 2.14 (s, 3H, OAc), 1.94 (s, 3H, 5′-CH_3).

Found: C, 46.91; H, 5.13; F, 5.46; N, 7.52. Calcd for $C_{14}H_{17}FN_2O_6S$: C, 46.66; H, 4.75; F, 5.27; N, 7.77.

ESI-MS *m/z* (relative intensity, %): 361.4 [($M+H^+$), 100].

4.1.6. 1-(5-*S*-Acetyl-3-deoxy-3-fluoro-5-thio-β-D- xylofuranosyl) uracil (9). Compound **9** was obtained from the 1,2-Di-*O*-acetyl-5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-D- xylofuranose (**7**) (4.0 g, 13.5 mmol), according to the general procedure as described for 1-(5-*S*-acetyl-3-deoxy-3-fluoro-5-thio-β-D-xylofuranosyl) thymine (**8**). After purification on silica gel column using EtOAc/hexane (8:2), the title nucleoside **9** was obtained (2.4 g, 51%, $R_f = 0.3$ in EtOAc/hexane, 8:2).

$[\alpha]_D^{22} + 27.6$ (*c* 0.25, $CHCl_3$)

IR (Nujol): 1697 (SAc), 1750 (OAc) cm^{-1}.

^1H NMR ($CDCl_3$): δ 7.37 (d, $J_{6',5'} = 8.2$ Hz, 1H, H-6′), 6.03 (d, $J_{1,2} = 2.0$ Hz, 1H, H-1), 5.79 (d, $J_{5',6'} = 8.2$ Hz, 1H, H-5′), 5.24 (dd, $J_{2,F} = 16.0$ Hz, $J_{2,1} = 2.0$ Hz, 1H, H-2), 4.97 (dd, $J_{3,F} = 50.0$ Hz, $J_{3,4} = 2.2$ Hz, 1H, H-3), 4.16-4.27 (m, $J_{4,F} = 28.9$ Hz, $J_{4,3} = 2.2$ Hz, 1H, H-4), 3.24-3.36 (m, 2H, 1H, H-5a and 1H, H-5b), 2.40 (s, 3H, SAc), 2.15 (s, 3H, OAc).

Found: C, 45.33; H, 4.63; F, 5.26; N, 7.82. Calcd for $C_{13}H_{15}FN_2O_6S$: C, 45.08; H, 4.37; F, 5.49; N, 8.09.

ESI-MS *m/z* (relative intensity, %): 347.5 [($M+H^+$), 100].

4.1.7. 1-(5-*S*-Acetyl-3-deoxy-3-fluoro-5-thio-β-D- xylofuranosyl) 5-fluorouracil (10). A mixture of 5FU (2.5 g, 18.9 mmol), hexamethyldisilazane (5.0 mL, 23.4 mmol), and saccharine (0.159 g, 0.87 mmol) in anhydrous CH_3CN (59 mL) was refluxed at 120 °C for 20 min. After cooling to rt, diacetylated compound **7** (4.0 g, 13.5 mmol) was added, followed by trimethylsilyl trifluoromethane-sulfonate (3.4 mL, 19 mmol). The reaction mixture was stirred at rt for 6 h, then diluted with EtOAc (1000 mL), washed with aqueous $NaHCO_3$ and finally with water. The organic layer was dried over sodium sulfate, filtered, and evaporated to syrup. The resulting material was purified by column chromatography using EtOAc/hexane (5:5) to give pure **10** (2.7 g, 55%, $R_f = 0.3$ in EtOAc/hexane, 5:5).

$[\alpha]_D^{22} + 36.8$ (*c* 0.25, $CHCl_3$)

IR (Nujol): 1697 (SAc), 1750 (OAc) cm^{-1}.

^1H NMR ($CDCl_3$): δ 7.74 (d, $J_{6,F5} = 5.8$ Hz, 1H, H-6′), 6.01 (dd, $J_{1,2} = 1.9$ Hz, $J_{1,F5} = 1.7$ Hz, 1H, H-1), 5.23 (dd, $J_{2,F} = 16.0$ Hz, $J_{2,1} = 1.9$ Hz, 1H, H-2), 4.98 (dd, $J_{3,F} = 50.0$ Hz, $J_{3,4} = 2.2$ Hz, 1H, H-3), 4.16–4.28 (m, $J_{4,F} = 28.8$ Hz, $J_{4,3} = 2.2$ Hz, 1H, H-4), 3.25–3.37 (m, 2H, 1H, H-5a and 1H, H-5b), 2.40 (s, 3H, SAc), 2.15 (s, 3H, OAc).

Found: C, 43.11; H, 3.63; F, 10.66; N, 7.42. Calcd for $C_{13}H_{14}F_2N_2O_6S$: C, 42.86; H, 3.87; F,10.43; N, 7.69.

ESI-MS *m/z* (relative intensity, %): 365.5 [($M+H^+$), 100].

4.2. Methods for measurement of biological activity

4.2.1. Cells and culture conditions. The human colonic adenocarcinoma Caco-2 cells were a generous gift of dr. Rene L'Harridon, INRA, VIM, Jouy-en-Josas, France; human fetal small intestine cell line H4, breast carcinoma cell line MCF 7, and skin melanoma cell line were used. Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich, Grand Island, USA), supplemented with 5% fetal calf serum (Cambrex, Verviers, Belgium), L-glutamine (2 mmol/L, Sigma, St. Louis, USA), penicillin (100 Us/mL, Sigma, St. Louis, USA), and streptomycin (1 mg/mL, Fluka, Buchs, Switzerland) at 37 °C in 5% CO_2 atmosphere in tissue

culture flasks until confluent. Cell culture medium was regularly changed.

4.2.2. Fluoro-thiofuranosyl nucleosides. Stock drug solutions were freshly prepared in sterile dimethylsulfoxide (DMSO) at the concentration of 0.5 mg/mL. The final concentration of DMSO in the cell culture medium was less than 0.1%. All solutions were protected against light.

AZT (Retrovir®) GlaxoSmithKline,USA, a drug used for antiretroviral therapy (ART) was used as a standard compound in antiviral experiments and 5FU as a standard compound in antitumor experiments. Solutions were prepared in the same way as those of fluoro-thiofuranosyl nucleosides.

4.2.3. Virus propagation. *Rotavirus RF* strain was propagated on Caco-2 monolayers in the presence of trypsin (1 μg per mL of DMEM) as described previously.[38] Supernatant containing the virus was collected from the flasks when cytopathic effect (CPE) was observed (24–48 h at 37 °C) by microscopy and clarified by centrifugation. Virus was stored at −70 °C until used. For the antiviral assay, virus with 1.5 tissue culture infective dose 50% units per mL ($TCID_{50}$/mL) was used (100 μL per well).

4.2.4. Antiviral assay. The potential antiviral activity of the newly synthesized compounds was tested against rotavirus by investigating:

(a) The inhibition of infectivity following virus attachment: Washed monolayer Caco-2 cells were first incubated with rotavirus for 1 h at 37 °C in the presence of 5% CO_2 (time for virus to attach to cell membrane receptors). After incubation, the remaining virus was washed off with DMEM without supplements and monolayer was treated immediately with the nucleoside added in 3-fold serial dilutions (initial concentration of 0.5 mg/mL). After 72 h of incubation for rotavirus, the plates were stained with Crystal Violet in ethanol, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) compared to those in control (only virus-infected) wells ($n = 6$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

(b) The neutralization of the virus in solution before attachment: Threefold dilutions of the tested compound (initial concentration of 0.5 mg/mL) were first pre-incubated with rotavirus in DMEM supplemented with trypsin for 12 h prior to the infection of cell monolayer at 37 °C and 5% CO_2. Residual viral infectivity was measured after 72-h of infection for rotavirus. Rotavirus alone was treated in the same way as the control. After 72-h of incubation, the plates were stained with Crystal Violet in ethanol, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) in comparison to those in control (only virus-infected) wells ($n = 6$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

4.2.5. Growth inhibition assay. It was performed on Caco-2 cell line by modified method described previously.[39] Briefly, in 96-well plates, six wells of 3-fold dilutions of compound (initial concentration of 0.5 mg/mL) were applied to monolayers of 10 cells/well in DMEM/10% fetal bovine serum. Incubation was performed at 37 °C in the humidified incubator for 10 days. The colonies were counted in each well and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of colony number in treated wells ($n = 2$) in contrast with those in control wells ($n = 24$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

4.2.6. Cytotoxicity assay. Caco-2, H4, MCF 7, and skin melanoma cells (6×10^6 cells per plate) were seeded in P 96 plate and treated with the compounds at 3-fold serial dilutions of each compound (initial concentration of 0.5 mg/mL). Then, the cells were incubated at 37 °C in the humidified incubator for 72 h. The plates were stained with Crystal Violet in ethanol, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in treated wells ($n = 2$) compared to those in control wells ($n = 24$). The minimal inhibitory concentration (CC_{50}) of the tested compounds was obtained from the concentration–effect curve.

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