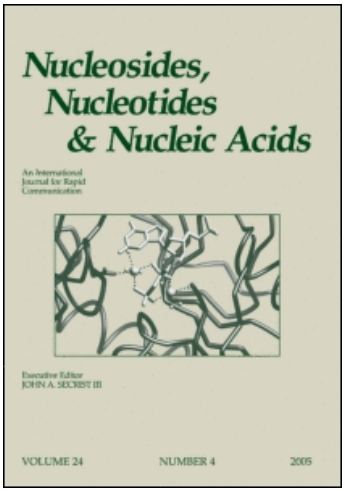

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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME ACYCLIC α-(1*H*-PYRAZOLO[3,4-d]PYRIMIDIN-4-YL)THIOALKYLAMIDE NUCLEOSIDES

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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME ACYCLIC α-(1*H*-PYRAZOLO-[3,4-d]PYRIMIDIN-4-YL)THIOALKYLAMIDE NUCLEOSIDES

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ABSTRACT

The chemical synthesis of some acyclic α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thioalkylamide nucleosides (**10–12**)**a–c** is described. The treatment of 1*H*-pyrazolo[3,4-d]pyrimidin-4-thione **1** with compounds **2a–c** gave, regioselectively, ethyl α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thioalkylates **3a-c**, respectively. These heterocycles were alkylated, separately, with alkylating agents **4**, **5** and **6** to give, regioselectively, the N_1-acyclic nucleosides (**7-9**)**a-c** which were deprotected to afford the desired products (**10-12**)**a-c**. All synthetic compounds were characterized on the basis of their physical and spectroscopic properties. The products (**10-12**)**a–c** were evaluated for their inhibitory effects against the replication of HIV-1 (III_B), HIV-2 (ROD), various DNA viruses, a variety of tumor-cell lines and M. tuberculosis. No marked biological activity was found.

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INTRODUCTION

Recently, significant progress has made in the development of antiviral chemotherapy due to the discovery of nucleosides analogues with potential activities such as ribavirin[1], acyclovir (ACV)[2], ganciclovir (DHPG)[3] (Fig. 1), etc.

Acyclovir has played an important role as a lead compound of acyclonucleoside chemistry since its inception. Thus, major efforts have been directed by the nucleoside researchers toward the synthesis of acyclic nucleosides with varying side chains and aglycons[4–7]. However, one group of acyclic nucleosides that have not been explored extensively are the acyclic pyrazolo[3,4-d]pyrimidine nucleosides.

In connection with our recent work[8,9], we present in this paper the detailed synthesis and biological evaluation of some acyclic α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thioalkylamide nucleosides (**10–12**)**a–c** (Sch. 1) with the alkyl chains of ACV, HBG[10] and iso-DHPG[11] (Fig. 1).

RESULTS AND DISCUSSION

The 1*H*-pyrazolo[3,4-d]pyrimidin-4-thione **1**, depicted in Scheme 1, was prepared in five steps according to the literature procedure[12,13] from malononitrile and triethyl orthoformate as starting materials. The C_4 sulfur atom of the base was alkylated with ethyl bromoacetate **2a**, (DL)-ethyl-2-bromopropionate **2b** or (DL)-ethyl-2-bromobutyrate **2c** in a sodium hydroxide solution at room temperature to give ethyl α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thioalkylates **3a–c** (Sch. 1), respectively, in good yield.

The preparation of the protected acyclic nucleosides (**7–9**)**a–c** (Sch. 1) was achieved using the same conditions previously described for the synthesis of some N_1-acyclic 4-substituted pyrazolo[3,4-d]pyrimidine nucleosides[14,15]. Thus, the alkylation of heterocycles **3a–c**, separately, with alkylating agents **4**[16], **5**[15,17] or **6**[18], using solid-liquid phase transfer catalysis method in which

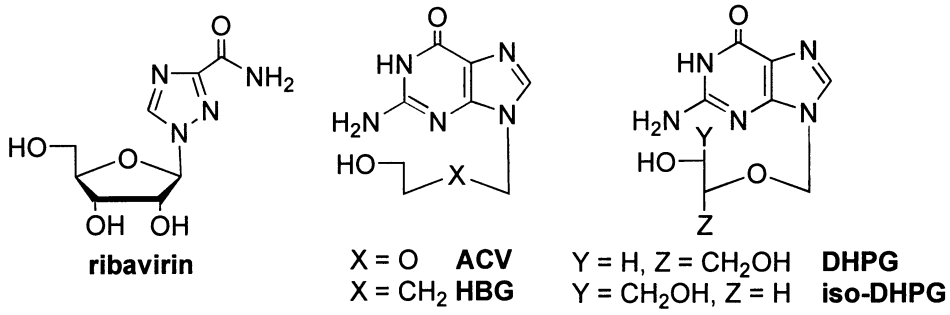
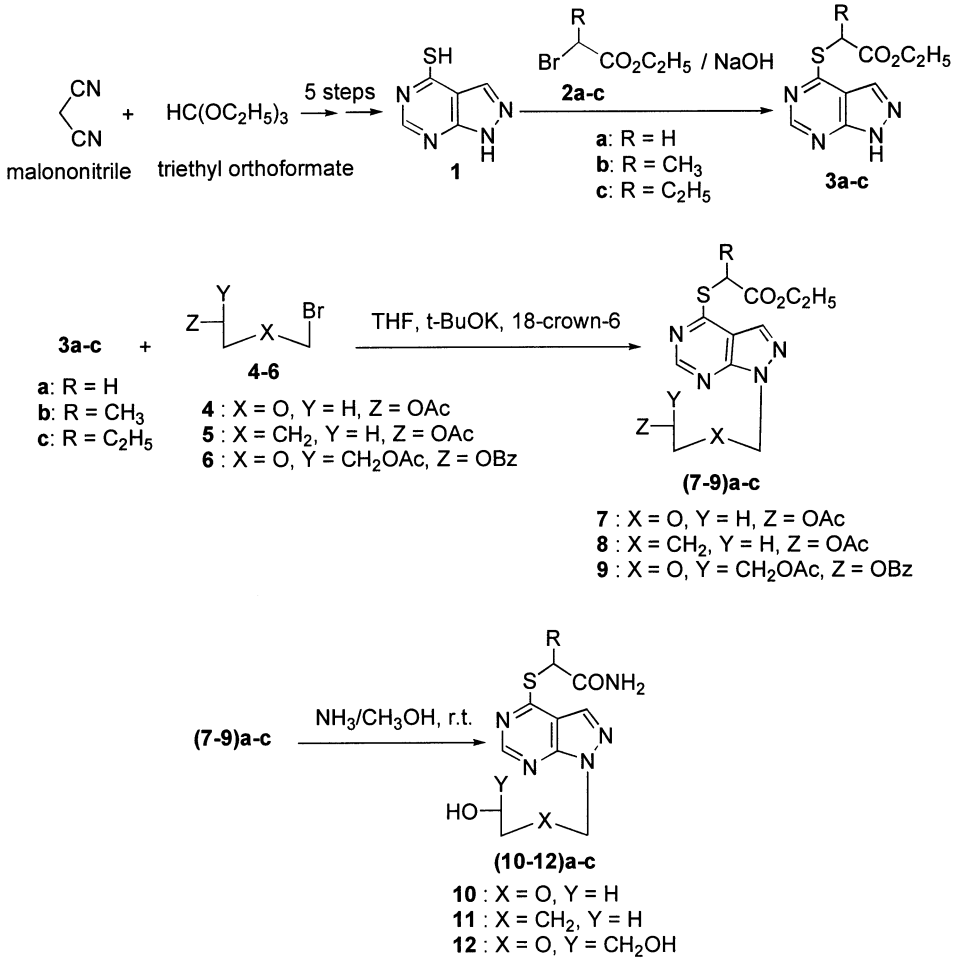


Figure 1.



Scheme 1.

potassium tert-butoxide was used as alkali, tetrahydrofuran as solvent and 18-crown-6 as catalyst, afforded regioselectively the N_1-regioisomers (**7–9**)**a–c**, respectively, in good yield.

Several alkylations and glycosylations of pyrazolo[3,4-d]pyrimidines[19–26] have been reported using various conditions as for example DMF/NaH, trimethylsilylation and phase transfer catalysis. Mainly, these conditions lead to a mixture of N_1- and N_2-regioisomers. In our case, the presumed N_2-regioisomers of (**7–9**)**a–c** were detected in only trace amounts but not isolated.

Finally, the treatment of N_1-protected acyclic nucleosides (**7–9**)**a–c** with a solution of methanolic ammonia at room temperature gave the acyclic nucleosides (**10–12**)**a–c** (Sch. 1) in quantitative yield, through deprotection of the acetyl and benzoyl groups and concomitant conversion of the esters into the amide moieties.

The site of alkylation in compounds **3a–c** was established to be at N_1 by a direct comparison of the UV spectra of the compounds (**10–12**)**a–c** with the UV spectra of the corresponding N_1-pyrazolo[3,4-d]pyrimidine nucleosides[22,27].

All structures of the synthetic products were identified by ^{1}H NMR, mass spectra, UV and/or elemental analysis.

BIOLOGICAL STUDIES

The acyclic nucleosides (**10–12**)**a–c** were evaluated for their antiviral activity in a wide variety of assay systems: herpes simplex virus type 1 (HSV-1) (KOS) and (HSV-2) (G), vaccinia virus, vesicular stomatitis virus (VSV), thymidine kinase-deficient (TK-) strain of HSV-1 (B2006 and VWM1837) in human embryonic skin muscle fibroblasts (E_6SM), Coxackie virus B4 virus in Hela cell cultures, parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus and Punta Toro virus in Vero cell cultures. No significant antiviral activity or cytotoxicity was noted at concentrations up to 400 μg/ml. No activity was observed against either HIV-1 (strain III_B) or HIV-2 (strain ROD) in MT-4 cells.

The compounds were also evaluated for their anti-tumor activity using a series of tumor cell lines (leukemia, colon cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer, brain cancer and non-small cell lung cancer). However, none of the compounds showed appreciable anti-tumor activity at concentrations lower than 10^{-4} M.

All above mentioned acyclic nucleosides were also evaluated for their inhibitory activity against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium. No significant anti-*M. tuberculosis* activity was noted at concentrations up to 6.25 μg/ml.

In conclusion, we have regioselectively synthesized some N1-acyclic α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thioalkylamide nucleosides with the alkyl chains of acyclovir, HBG and iso-DHPG. No significant antiviral, antitumor or anti-*M. tuberculosis* activity was witnessed.

EXPERIMENTAL

Melting points (mp) were determined on a Electrothermal digital melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a HP 845x spectrophotometer. The ^{1}H-NMR spectra were recorded using a Bruker AC 250 spectrometer. The chemical shifts were reported as parts per million (δ ppm) from $(CH_3)_4Si$ (TMS) as an internal standard. Key: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Mass spectra were obtained with a JOEL JMS DX 300 instrument using fast atomic bombardment (FAB positive). Thin-layer chromatography

(tlc) was performed on plates of Merck Kieselgel 60 F_{254} and short wavelength UV light (254 nm) was used to detect the UV-absorbing spots. Column chromatography separation were obtained on silica gel 60 (70–230 mesh, Merck). Elemental analysis was determined by the French microanalytical central service.

General Preparation Procedure of 3a–c

The 1*H*-pyrazolo[3,4-d]pyrimidin-4-thione 1 (15 mmol) was dissolved in 1N sodium hydroxide solution (15 mL). To this solution were added 18 mmol of **2a**, **2b** or **2c** at 0°C and the mixture was stirred at room temperature for 3 hours. The reaction was monitored by thin-layer chromatography and was shown to be complete at this time. The excess of the solvent was removed in vacuo. The residue was coevapored with benzene (3×20 mL) and chromatographed on a silica gel column, using chloroform:methanol (98:2) as eluent, to furnish the expected heterocyclic base.

Ethyl α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thioacetate 3a. Yield: 3.03 g (85%); R_f 0.40 (95:5, $CHCl_3$:CH_3OH); mp 134–135°C (Water); UV (Methanol) λ_{max} 279 nm ($\varepsilon = 13\,900$); ^{1}H NMR (DMSO-d_6) δ: 1.18 (t, $J = 7.10$ Hz, 3H, OCH_2CH_3), 4.10 (q, $J = 7.10$ Hz, 2H, OCH_2CH_3), 4.26 (s, 2H, SCH_2), 8.36 and 8.70 (2s, 2H, H_3 and H_6), 14.15 (br s, 1*H*, NH); MS, m/z: $[M+H]^+ = 239$.

Ethyl α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thiopropionate 3b. Yield: 3.13 g (83%); R_f 0.45 (95:5, $CHCl_3$:CH_3OH); mp 109–110°C (Ethanol); UV (Methanol) λ_{max} 280 nm ($\varepsilon = 14\,000$); ^{1}H NMR (DMSO-d_6) δ: 1.10 (t, 3H, $J = 7.09$ Hz, OCH_2CH_3), 1.55 (d, 3H, $J = 7.30$ Hz, $SCHCH_3$), 4.08 (q, 2H, $J = 7.10$ Hz, OCH_2CH_3), 4.79 (q, 1H, $J = 7.30$ Hz, $SCHCH_3$), 8.27 and 8.66 (2s, 2H, H_3 and H_6), 14.15 (br s, 1H, NH); MS, m/z: $[M+H]^+ = 253$.

Ethyl α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)thiobutyrate 3c. Yield: 3.27 g (82%); R_f 0.50 (95:5, $CHCl_3$:CH_3OH); Appearance: syrup; UV (Methanol) λ_{max} 279 nm ($\varepsilon = 14\,900$); ^{1}H NMR (DMSO-d_6) δ: 0.99 (t, 3H, $J = 7.30$ Hz, $SCHCH_2CH_3$), 1,15 (t, 3H, $J = 7.09$ Hz, OCH_2CH_3), 1.90–2.10 (m, 2H, $SCHCH_2CH_3$), 4.13 (q, 2H, $J = 7.09$ Hz, OCH_2CH_3), 4.80 (t, 1H, $J = 6.91$ Hz, $SCHCH_2CH_3$), 8.32 and 8.69 (2s, 2H, H_3 and H_6), 14.15 (br s, 1H, NH); MS, m/z: $[M+H]^+ = 267$.

General Alkylation Procedure

To a solution of 0.66 g (2.5 mmol) of 18-crown-6 in 140 mL of anhydrous tetrahydrofuran (THF) was added 1.13 g (10 mmol) of potassium tert-

butoxide (t-BuOK). Then 10 mmol of heterocycle **3a**, **3b** or **3c** was added and the reaction mixture was stirred at room temperature for 15 mn. At this time the reaction mixture was cooled to 0°C and 10 mmol of compound **4**, **5** or **6** in 20 mL of anhydrous THF was added dropwise with stirring. When the addition was finished, the reaction mixture was stirred for 1 hour at room temperature. The reaction mixture was then filtrated and the filtrate was evaporated to dryness in vacuo. The residue was chromatographed on a silica gel column, using chloroform as eluent, to give the N_1-protected acyclic nucleoside.

Ethyl α-[1-(2-acetoxyethoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl] thioacetate 7a. Yield: 3.15 g (89%); R_f 0.50 (50:50, ethyl ether:hexane); mp 71–72°C (Ethanol); ^{1}H NMR (DMSO-d_6) δ: 1.12 (t, 3H, $J = 7.14$ Hz, OCH_2CH_3), 1.96 (s, 3H, CH_3CO), 3.70 and 4.15 (2m, 4H, OCH_2CH_2O), 4.13 (s, 2H, SCH_2), 4.18 (q, 2H, $J = 7.14$ Hz, OCH_2CH_3), 5.80 (s, 2H, OCH_2N), 8.09 and 8.68 (2s, 2H, H_3 and H_6); MS, m/z: $[M+H]^+ = 355$; Elem. Anal. Calcd for $C_{14}H_{18}N_4O_5S$ (354.37): C 47.45, H 5.12, N 15.80, Found: C 47.53, H 5.14, N 15.94.

Ethyl α-[1-(2-acetoxyethoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl] thiopropionate 7b. Yield: 2.94 g (80%); R_f 0.52 (50:50, ethyl ether:hexane); Appearance: syrup; ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3H, $J = 7.10$ Hz, OCH_2CH_3), 1.68 (d, 3H, $J = 7.35$ Hz, $SCHCH_3$), 1.93 (s, 3H, CH_3CO), 3.75 and 4.19 (2m, 4H, OCH_2CH_2O), 4.23 (q, 2H, $J = 7.10$ Hz, OCH_2CH_3), 4.94 (q,1H, $J = 7.35$ Hz, $SCHCH_3$), 5.82 (s, 2H, OCH_2N), 8.03 and 8.66 (2s, 2H, H_3 and H_6); MS, m/z: $[M+H]^+ = 369$; Elem. Anal. Calcd for $C_{15}H_{20}N_4O_5S$ (368.40): C 48.90, H 5.47, N 15.20, Found: C 49.21, H 5.50, N 15.30.

Ethyl α-[1-(2-acetoxyethoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl] thiobutyrate 7c. Yield: 3.05 g (80%); R_f 0.54 (50:50, ethyl ether:hexane); Appearance: syrup; ^{1}H NMR ($CDCl_3$) δ: 1.00 (t, 3H, $J = 7.30$ Hz, $SCHCH_2CH_3$), 1.15 (t, 3H, $J = 7.09$ Hz, OCH_2CH_3), 1.92 (s, 3H, CH_3CO), 1.98–2.10 (m, 2H, $SCHCH_2CH_3$), 3.70 and 4.10 (2m, 4H, OCH_2CH_2O), 4.15 (q, 2H, $J = 7.09$ Hz, OCH_2CH_3), 4.80 (t, 1H, $J = 6.91$ Hz, $SCHCH_2CH_3$), 5.80 (s, 2H, OCH_2N), 8.10 and 8.68 (2s, 2H, H_3 and H_6); MS, m/z: $[M+H]^+ = 383$; Elem. Anal. Calcd for $C_{16}H_{23}N_4O_5S$ (382.43): C 50.25, H 6.06, N 14.64, Found: C 50.42, H 6.23, N 14.82.

Ethyl α-[1-(4-acetoxybutyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thio-acetate 8a. Yield: 2.78 g (79%); R_f 0.50 (98:2, $CHCl_3$:CH_3OH); Appearance: syrup; ^{1}H NMR (DMSO-d_6) δ: 1.21 (t, 3H, $J = 7.10$ Hz, OCH_2CH_3), 1.52 (m, 2H, $AcOCH_2CH_2$), 1.92 (m, 2H, CH_2CH_2N), 1.95 (s, 3H, CH_3CO), 4.00 (t, 2H, $J = 6.49$ Hz, $AcOCH_2CH_2$), 4.19 (q, 2H,

$J = 7.10$ Hz, OCH_2CH_3), 4.30 (s, 2H, SCH_2), 4.47 (t, 2H, $J = 6.78$ Hz, CH_2N), 8.43 and 8.76 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 353$; Elem. Anal. Calcd for $C_{15}H_{20}N_4O_4S$ (352.40): C 51.12, H 5.72, N 15.89, Found: C 51.38, H 5.85, N 15.98.

Ethyl α-[1-(4-acetoxybutyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiopropionate 8b. Yield: 2.78 g (76%); R_f 0.56 (98:2, $CHCl_3$:CH_3OH); Appearance: syrup; ^{1}H NMR (DMSO-d_6) δ: 1.17 (t, 3H, $J = 7.08$ Hz, OCH_2CH_3), 1.47 (m, 2H, $AcOCH_2CH_2$), 1.61 (d, 3H, $J = 7.30$ Hz, $SCHCH_3$), 1.90 (m, 2H, CH_2CH_2N), 1.97 (s, 3H, CH_3CO), 3.98 (t, 2H, $J = 6.51$ Hz, $AcOCH_2CH_2$), 4.15 (q, 2H, $J = 7.08$ Hz, OCH_2CH_3), 4.44 (t, 2H, $J = 6.80$ Hz, CH_2N), 4.89 (q, 1H, $J = 7.30$ Hz, $SCHCH_3$), 8.37 and 8.75 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 367$; Elem. Anal. Calcd for $C_{16}H_{22}N_4O_4S$ (366.43): C 52.44, H 6.05, N 15.28, Found: C 52.67, H 6.21, N 15.37.

Ethyl α-[1-(4-acetoxybutyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutyrate 8c. Yield: 3.04 g (80%); R_f 0.63 (98:2, $CHCl_3$:CH_3OH); Appearance: syrup; ^{1}H NMR (DMSO-d_6) δ: 0.99 (t, 3H, $J = 7.37$ Hz, $SCHCH_2CH_3$), 1.15 (t, 3H, $J = 7.09$ Hz, OCH_2CH_3), 1.47 (m, 2H, $AcOCH_2CH_2$), 1.90 (m, 2H, CH_2CH_2N), 1.94 (m, 2H, $SCHCH_2CH_3$), 1.95 (s, 3H, CH_3CO), 3.95 (t, 2H, $J = 6.49$ Hz, $AcOCH_2CH_2$), 4.16 (q, 2H, $J = 7.09$ Hz, OCH_2CH_3), 4.41 (t, 2H, $J = 6.82$ Hz, CH_2N), 4.80 (t, 1H, $J = 6.92$ Hz, $SCHCH_2CH_3$), 8.34 and 8.72 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 381$; Elem. Anal. Calcd for $C_{17}H_{24}N_4O_4S$ (380.45): C 53.66, H 6.35, N 14.72, Found: C 53.97, H 6.41, N 14.81.

Ethyl α-[1-(3-acetoxy-2-O-benzoyl-1-propoxy)methyl-1*H*-pyrazolo[3,4-d]-pyrimidin-4-yl]thioacetate 9a. Yield: 3.75 g (77%); R_f 0.48 (98:2, $CHCl_3$/CH_3OH); Appearance: syrup; ^{1}H NMR (DMSO-d_6) δ: 1.14 (t, 3H, $J = 7.10$ Hz, OCH_2CH_3), 1.86 (s, 3H, CH_3CO), 3.81 (d, 2H, $J = 4.98$ Hz, OCH_2CH), 4.12 (q, 2H, $J = 7.09$ Hz, OCH_2CH_3), 4.23 (s, 2H, SCH_2), 4.30 (distorted d, 2H, CH_2OAc), 5.19 (m, 1H, CH_2CHOBz), 5.76 (s, 2H, OCH_2N), 7.40–7.96 (m, 5H, aromatic protons of phenyl group), 8.39 and 8.70 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 489$; Elem. Anal. Calcd for $C_{22}H_{24}N_4O_7S$ (488.51): C 54.09, H 4.95, N 11.46, Found: C 54.39, H 5.14, N 11.58.

Ethyl α-[1-(3-acetoxy-2-O-benzoyl-1-propoxy)methyl-1*H*-pyrazolo[3,4-d]-pyrimidin-4-yl]thiopropionate 9b. Yield: 3.81 g (76%); R_f 0.50 (98:2, $CHCl_3$:CH_3OH); Appearance: syrup; ^{1}H NMR (DMSO-d_6) δ: 1.17 (t, 3H, $J = 7.08$ Hz, OCH_2CH_3), 1.61 (d, 3H, $J = 7.30$ Hz, $SCHCH_3$), 1.86 (s, 3H, CH_3CO), 3.81 (d, 2H, $J = 4.98$ Hz, OCH_2CHOBz), 4.19 (q, 2H, $J =$ 7.08 Hz, OCH_2CH_3), 4.30 (distorted d, 2H, CH_2OAc), 4.89 (q, 1H, $J =$ 7.30 Hz, $SCHCH_3$), 5.19 (m, 1H, CH_2CHOBz), 5.76 (s, 2H, OCH_2N),

7.50–8.00 (m, 5H, aromatic protons of phenyl group), 8.39 and 8.77 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 503$; Elem. Anal. Calcd for $C_{23}H_{26}N_4O_7S$ (502.53): C 54.97, H 5.21, N 11.14, Found: C 55.28, H 5.33, N 11.36.

Ethyl α-[1-(3-acetoxy-2-O-benzoyl-1-propoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutyrate 9c. Yield: 3.92 g (76%); $R_f = 0.60$ (98:2, $CHCl_3$:CH_3OH); Appearance: syrup; ^{1}H NMR (DMSO-d_6) δ: 0.89 (t, 3H, $J = 7.32$ Hz, $SCHCH_2CH_3$), 1.00 (t, 3H, $J = 7.21$ Hz, OCH_2CH_3), 1.89 (s, 3H, CH_3CO), 1.97–2.01 (m, 2H, $SCHCH_2CH_3$), 3.88 (d, 2H, $J = 4.98$ Hz, OCH_2CH), 4.15 (q, 2H, $J = 7.21$ Hz, OCH_2CH_3), 4.30 (distorted d, 2H, CH_2OAc), 4.80 (t, 1H, $J = 6.94$ Hz, $SCHCH_2CH_3$), 5.31 (m, 1H, CH_2-CHOBz), 5.79 (s, 2H, OCH_2N), 7.46–7.95 (m, 5H, aromatic protons of phenyl group), 8.40 and 8.74 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 517$; Elem. Anal. Calcd for $C_{24}H_{28}N_4O_7S$ (516.78): C 55.78, H 5.46, N 10.84, Found: C 55.88, H 5.53, N 10.95.

General Deprotection Procedure

To 90 mL of dry methanol saturated with ammonia at −5°C was added 1 mmol of the protected acyclic nucleoside **(7–9)a–c**. The flask was stopped tightly and the solution was stirred for 16–20 hours at room temperature. Thin-layer chromatography indicated that complete deprotection of protected product had occurred. Volatile materials were evaporated in vacuo. The residue was purified by column chromatography on silica gel, using chloroform:methanol (98:2) as eluent, to obtain the expected acyclic nucleoside.

α-[1-(2-Hydroxyethoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetamide 10a. Yield: 0.26 g (94%); $R_f = 0.20$ (90:10, $CHCl_3$:CH_3OH); mp 160–161°C (Ethanol); UV (Methanol) λ_{max} 282 nm (ε = 5 200); ^{1}H NMR (DMSO-d_6) δ: 3.45 (m, 4H, OCH_2CH_2O), 4.30 (s, 2H, SCH_2), 4.55 (t, 1H, $J = 5.00$ Hz, HO, D_2O exchangeable), 5.74 (s, 2H, OCH_2N), 7.21 and 7.67 (2 br s, 2H, NH_2, D_2O exchangeable), 8.39 and 8.70 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 284$; Elem. Anal. Calcd for $C_{10}H_{13}N_5O_3S$ (283.30): C 42.39, H 4.62, N 24.71, Found: C 42.47, H 4.68, N 24.84.

α-[1-(2-Hydroxyethoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiopropionamide 10b. Yield: 0.28 g (94%); $R_f = 0.22$ (90:10, $CHCl_3$:CH_3OH); mp 129–130°C (Ethanol); UV (Methanol) λ_{max} 282 nm (ε = 5 400); ^{1}H NMR (Dme_2O-d_6) δ: 1.55 (d, 3H, $J = 7.02$ Hz, $SCHCH_3$), 3.40 (m, 4H, OCH_2CH_2O), 4.55 (t, 1H, $J = 5.00$ Hz, HO, D_2O exchangeable), 4.85 (q, 1*H*, $J = 7.02$ Hz, $SCHCH_3$), 5.74 (s, 2H, OCH_2N), 7.23 and 7.73 (2 br s, 2H, NH_2, D_2O exchangeable), 8.31 and 8.75 (2s, 2H, H_3 and H_6); MS, m/z:

$[M+H]^+ = 298$; Elem. Anal. Calcd for $C_2H_{15}N_5O_3S$ (297.32): C 44.43, H 5.08, N 23.55, Found: C 44.54, H 5.11, N 23.81.

α-[1-(2-Hydroxyethoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutanamide 10c. Yield: 0.29 g (94%); R_f 0.24 (90:10, $CHCl_3$:CH_3OH); mp 109–110°C (Ethanol); UV (Methanol) λ_{max} 282 nm ($\varepsilon = 5\,800$); ^{1}H NMR (DMeSO-d_6) δ: 1.00 (t, 3H, $J = 7.30$ Hz, $SCHCH_2CH_3$), 1.70–2.10 (m, 2H, $SCHCH_2CH_3$), 3.40 (m, 4H, OCH_2CH_2O), 4.50 (t, 1H, $J = 5.00$ Hz, HO, D_2O exchangeable), 4.81 (t, 1H, $J = 6.92$ Hz, $SCHCH_2CH_3$), 5.74 (s, 2H, OCH_2N), 7.22 and 7.72 (2 br s, 2H, NH_2, D_2O exchangeable), 8.40 and 8.74 (2s, 2H, H_3 and H_6); MS, *m/z*: $[M+H]^+ = 312$; Elem. Anal. Calcd for $C_{12}H_{17}N_5O_3S$ (311.35): C 46.29, H 5.50, N 22.49, Found: C 46.45, H 5.51, N 22.61.

α-[1-(4-Hydroxybutyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetamide 11a. Yield: 0.26 g (94%); R_f 0.30 (90:10, $CHCl_3$:CH_3OH); mp 152–153°C (Ethanol); UV (Methanol) λ_{max} 282 \,nm ($\varepsilon = 5\,000$); ^{1}H NMR (DMSO-d_6) δ: 1.32 (m, 2H, OCH_2CH_2), 1.83 (m, 2H, CH_2CH_2N), 3.37 (m, 2H, OCH_2CH_2), 4.11 (s, 2H, SCH_2), 4.42 (m, 2H, CH_2N, 1H, OH, D_2O exchangeable), 7.21 and 7.67 (2 br s, 2H, NH_2, D_2O exchangeable), 8.34 and 8.72 (2s, 2H, H_3 and H_6); MS, *m/z*: $[M+H]^+ = 282$; Elem. Anal. Calcd for $C_{11}H_{15}N_5O_2S$ (281.32): C 46.96, H 5.37, N 24.89, Found: C 47.09, H 5.40, N 24.98.

α-[1-(4-Hydroxybutyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiopropionamide 11b. Yield: 0.28 g (94%); R_f 0.35 (90:10, $CHCl_3$:CH_3OH); mp 145–146°C (Ethanol); UV (Methanol) λ_{max} 282 nm ($\varepsilon = 5\,400$); ^{1}H NMR (DMSO-d_6) δ: 1.33 (m, 2H, OCH_2CH_2), 1.55 (d, 3H, $J = 7.02$ Hz, $SCHCH_3$), 1.86 (m, 2H, CH_2CH_2N), 3.35 (m, 2H, OCH_2CH_2), 4.35 (t, 2H, $J = 6.82$ Hz, CH_2N), 4.40 (t, 1*H*, $J = 5.30$ Hz, OH, D_2O exchangeable), 4.85 (q, 1H, $J = 7.02$ Hz, $SCHCH_3$), 7.23 and 7.73 (2 br s, 2H, NH_2, D_2O exchangeable), 8.31 and 8.75 (2s, 2H, H_3 and H_6); MS, *m/z*: $[M+H]^+ = 296$; Elem. Anal. Calcd for $C_{12}H_{17}N_5O_2S$ (295.35): C 48.80, H 5.80, N 23.71, Found: C 48.92, H 5.82, N 23.85.

α-[1-(4-Hydroxybutyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutanamide 11c. Yield: 0.29 g (94%); R_f 0.41 (90:10, $CHCl_3$:CH_3OH); mp 117–118°C (Ethanol); UV (Methanol) λ_{max} 282 nm ($\varepsilon = 5\,900$); ^{1}H NMR (DMSO-d_6) δ: 0.92 (t, 3H, $J = 7.35$ Hz, $SCHCH_2CH_3$), 1.28 (m, 2H, OCH_2CH_2), 1.70–2.03 (m, 4H, CH_2CH_2N and $SCHCH_2CH_3$), 3.33 (m, 2H, OCH_2CH_2), 4.36 (m, 2H, CH_2N, 1H, OH, D_2O exchangeable), 4.75 (t, 1H, $J = 6.94$ Hz, $SCHCH_2CH_3$), 7.22 and 7.72 (2 br s, 2H, NH_2, D_2O exchangeable), 8.28 and 8.70 (2s, 2H, H_3 and H_6); MS, *m/z*:$[M+H]^+ = 310$; Elem. Anal. Calcd

for $C_{13}H_{19}N_5O_2S$ (309.38): C 50.46, H 6.19, N 22.63, Found: C 50.55, H 6.20, N 22.74.

α-[1-(2,3-Dihydroxy-1-propoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]-thioacetamide 12a. Yield: 0.28 g (92%); R_f 0.28 (90:10, $CHCl_3$:CH_3OH); Appearance: syrup; UV (Methanol) λ_{max} 282 nm ($\varepsilon = 5\,300$); ^{1}H NMR (DMSO-d_6) δ: 3.24–3.55 (m, 5H, OCH_2CHCH_2), 4.12 (s, 2H, SCH_2), 4.46 (t, 1H, $J = 5.65$ Hz, *H*OCH_2, D_2O exchangeable), 4.71 (d, 1H, $J = 4.84$ Hz, *H*OCH, D_2O exchangeable), 5.76 (s, 2H, OCH_2N), 7.23 and 7.73 (2 br s, 2H, NH_2, D_2O exchangeable) 8.34 and 8.67 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 314$; Elem. Anal. Calcd for $C_{11}H_{15}N_5O_4S$ (313.32): C 42.16, H 4.82, N 22.35, Found: C 42.32, H 4.82, N 22.45.

α-[1-(2,3-Dihydroxy-1-propoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]-thiopropionamide 12b. Yield: 0.30 g (92%); R_f 0.31 (90:10, $CHCl_3$:CH_3OH); mp 96–97°C (Ethanol); UV (Methanol) λ_{max} 282 nm ($\varepsilon = 5\,500$); ^{1}H NMR (DMSO-d_6) δ: 1.60 (d, 3H, $J = 7.30$ Hz, CH_3CH), 3.20–3.50 (m, 5H, OCH_2CHCH_2), 4.50 (t, 1H, $J = 5.64$ Hz, *H*OCH_2, D_2O exchangeable), 4.70 (d, 1H, $J = 4.84$ Hz, *H*OCH, D_2O exchangeable), 4.80 (q, 1H, $J = 7.30$ Hz, $SCHCH_3$), 5.79 (s, 2H, OCH_2N), 7.23 and 7.73 (2 br s, 2H, NH_2, D_2O exchangeable), 8.35 and 8.70 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 328$; Elem. Anal. Calcd for $C_{12}H_{17}N_5O_4S$ (327.35): C 44.02, H 5.23, N 21.39, Found: C 44.15, H 5.24, N 21.52.

α-[1-(2,3-Dihydroxy-1-propoxy)methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]-thiobutanamide 12c. Yield: 0.31 g (92%); R_f 0.37 (90:10, $CHCl_3$:CH_3OH); mp 76–77°C (Ethanol); UV (Methanol) λ_{max} 282 nm ($\varepsilon = 5\,700$); ^{1}H NMR (DMSO-d_6) δ: 1.00 (t, 3H, $J = 7.35$ Hz, $SCHCH_2CH_3$), 1.70–2.10 (m, 2H, $SCHCH_2CH_3$), 3.20–3.50 (m, 5H, OCH_2CHCH_2), 4.50 (t, 1H, *H*OCH_2, $J = 5.64$ Hz, D_2O exchangeable), 4.70 (d, 1H, *H*OCH, $J = 4.84$ Hz, D_2O exchangeable), 4.81 (t, 1H, $J = 6.94$ Hz, $SCHCH_2CH_3$), 5.79 (s, 2H, OCH_2N), 7.22 and 7.73 (2 br s, 2H, NH_2, D_2O exchangeable), 8.40 and 8.73 (2s, 2H, H_3 and H_6); MS, m/z:$[M+H]^+ = 342$; Elem. Anal. Calcd for $C_{13}H_{19}N_5O_4S$ (341.38): C 45.73, H 5.61, N 20.51, Found: C 45.85, H 5.64, N 20.62.

BIOLOGICAL ASSAYS

The antiviral activity assays were carried out according to previously established procedures[28,29].

The antitumor experiments were performed at the National Cancer Institute using the procedure published in Seminars in Oncology[30].

The anti-M. tuberculosis assay was carried out as described previously[31].

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