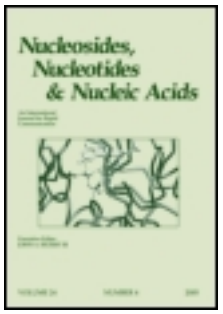

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Synthesis and Biological Evaluation of Some α-[6-(1′-Carbamoylalkylthio)-1 H-Pyrazolo[3,4-D]Pyrimidin-4-yl]Thioalkylcarboxamide Acyclonucleosides

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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME α-[6-(1′-CARBAMOYLALKYLTHIO)-1 *H*-PYRAZOLO[3,4-D]PYRIMIDIN-4-YL]THIOALKYLCARBOXAMIDE ACYCLONUCLEOSIDES

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□ *The reaction of 1*H*-pyrazolo[3,4-d]pyrimidin-4,6-dithione 11 with compounds 12a–c produces ethyl α-[6-(1′-carboethoxyalkylthio)-1* H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylates 13a–c, respectively. These heterocycles were alkylated, separately, with alkylating agents 14, 15, and 16 to afford, predominately, the N_1-acyclic nucleosides (17–19)a–c, which were deprotected to give the desired products (20–22)a–c. All synthetic compounds were characterized on the basis of their physical and spectroscopic properties. The acyclic nucleosides (20–22)a–c were evaluated for their inhibitory effects against the replication of varicella-zoster virus, human cytomegalovirus and M. tuberculosis. No marked biological activity was found.*

Keywords Acyclic nucleosides; disubstituted pyrazolo[3,4-d]pyrimidines

INTRODUCTION

Nucleoside analogues have acquired an important role as therapeutic agents in the treatment of patients with devastating infections with viruses such as human immunodeficiency virus, hepatitis B virus (HBV) and herpes

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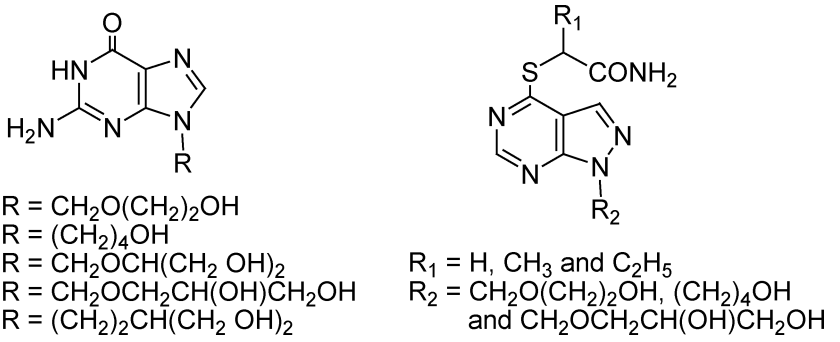


FIGURE 1

viruses. A promising class of these analogues for antiviral chemotherapy belongs to a group in which the cyclic carbohydrate moiety is replaced with open-chain (acyclic) sugar moieties.[1,2] Among purine acyclic nucleosides, Acyclovir **1** and its triphosphate form, HBG **2**, Ganciclovir **3**, iNDG **4**, and Penciclovir **5** (Figure 1) are active against herpes simplex viruses, varicella-zoster virus, HBV, and/or cytomegalovirus.[3–8]

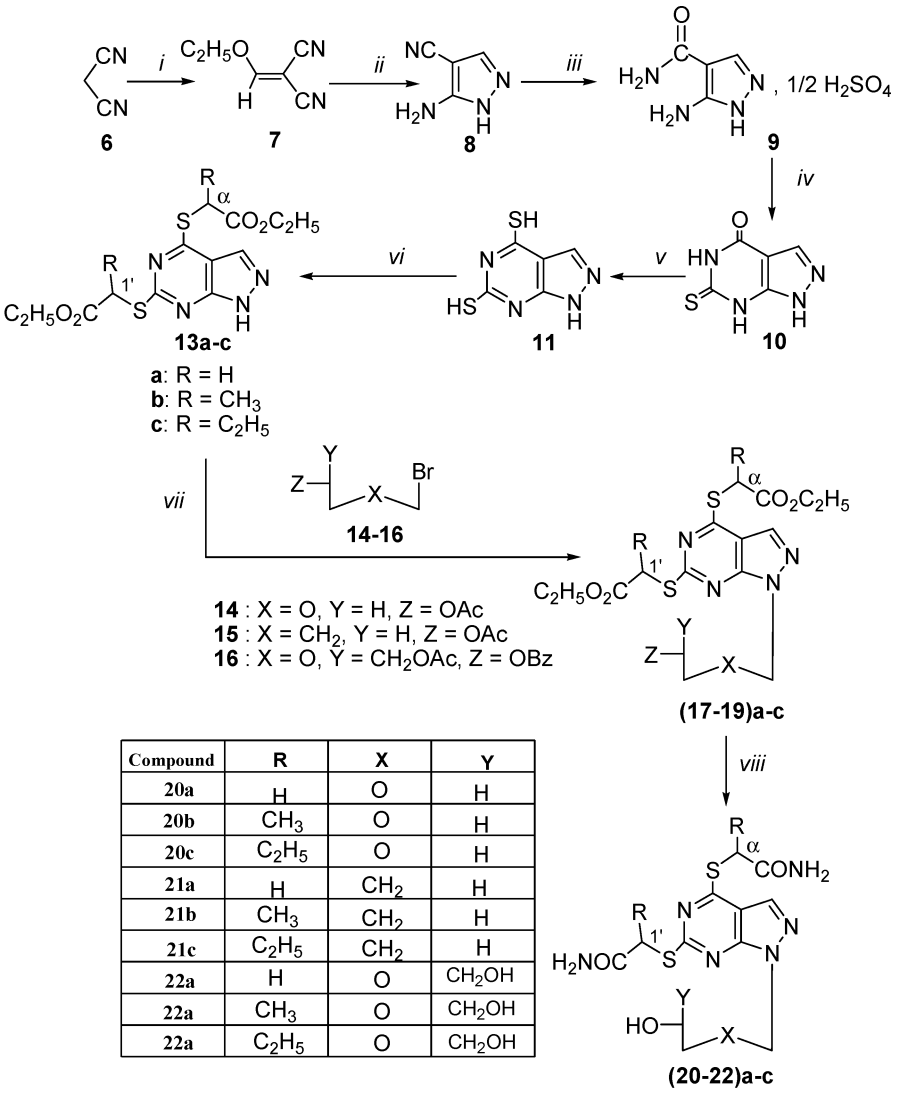
In spite of extensive research performed on acyclic purine and pyrimidine nucleosides analogues in recent years, relatively less is known on the same analogues containing 4,6- substituted pyrazolo[3,4-d]pyrimidine ring counterparts. This work is a continuation of the structure-activity relationship study in the series of acyclic α-[1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylcarboxamide nucleosides[9] (Figure 1) which concerns the effect of the substitution at C_6 position on the biological activity in this series.

RESULTS AND DISCUSSION

Chemistry

The starting heterocycle 1*H*-pyrazolo[3,4-d]pyrimidin-4,6-dithione **11** depicted in scheme 1 was readily prepared from commercially available malononitrile **6** and triethyl orthoformate following a synthetic pathway previously described by Robins et al.[10] The C_4 and C_6 sulfur atoms of compound **11** were alkylated with ethyl bromoacetate **12a**, (dl)-ethyl-2-bromopropionate **12b**, or (dl)-ethyl-2-bromobutyrate **12c** in a sodium hydroxide solution at room temperature to give ethyl α-[6-(1′-carboethoxyalkylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylates **13a–c** (Scheme 1), respectively, in good yield.

Several alkylations and glycosylations of pyrazolo[3,4-d]pyrimidines[11–13] 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, for the preparation of the acyclic nucleosides (**17–19**)**a–c**, we



Compound	R	X	Y
20a	H	O	H
20b	CH_3	O	H
20c	C_2H_5	O	H
21a	H	CH_2	H
21b	CH_3	CH_2	H
21c	C_2H_5	CH_2	H
22a	H	O	CH_2OH
22a	CH_3	O	CH_2OH
22a	C_2H_5	O	CH_2OH

(i): triethylorthoformate/acetic anhydride/ reflux; (ii): H_2NNH_2, r.t.; (iii): H_2SO_4; (iv): thiourea/ reflux; (v): P_2S_5 / pyridine; (vi): $BrCH_2CO_2C_2H_5$ (**12a**), (DL)-$BrCH(CH_3)CO_2C_2H_5$ (**12b**) or (DL)-$BrCH(C_2H_5)CO_2C_2H_5$ (**12c**) in NaOH (1N), r.t.; (vii): $KOBu^t$/18-crown-6/THF; (viii): CH_3OH/NH_3

SCHEME 1

have used the same conditions as previously described for the synthesis of some N_1-acyclic pyrazolo[3,4- d]pyrimidine nucleosides.[9,14,15] Thus, the alkylation of heterocycles **13a–c**, separately, with the alkylating agents used in the synthesis of Acyclovir **14**,[16] HBG **15**,[15] and iNDG **16**[17] using solid-liquid phase transfer catalysis method in which potassium *tert*-butoxide was used as alkali, tetrahydrofuran as solvent and 18-crown-6 as catalyst,

afforded regioselectively the N_1-regioisomers (**17–19**)**a–c**, respectively, in good yield. The presumed N_2-regioisomers of these protected acyclic nucleosides were detected in only trace amounts but not isolated.

Finally, the treatment of **(17–19)a–c** with a solution of methanolic ammonia at room temperature gave the acyclic nucleosides (**20–22**)**a–c** (Scheme 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 **13a–c** was established to be at N_1 by a direct comparison of the UV spectra of the compounds (**20–22**)**a–c** with the UV spectra of the corresponding N_1-pyrazolo[3,4-d]pyrimidine derivative.[18]

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

Biological Studies

The acyclic nucleosides (**20–22**)**a–c** were tested against cytomegalovirus (CMV) and varicella-zoster (VZV) in a wide variety of assay systems: AD-169 and Davis strains, TK^- VZV (YS strain and OKA strain) and TK^+ VZV (07/1 strain and YS/R strain). Data for DHPG, HPMPC, ACV and BVDU are shown for comparison. No significant antiviral activity or cytotoxicity was noted at the concentrations up to 50 μg/ml.

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 antituberculosis activity was noted at concentrations up to 6.25 μg/ml.

In summary, we have regioselectively synthesized some acyclic α-[6-(1′-carbamoyl-alkylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkyl carboxamide nucleosides equipped with the alkyl chains of Acyclovir, HBG and iNDG. No significant anti-CMV, anti-VZV or anti-tuberculosis activity was witnessed.

EXPERIMENTAL PROCEDURES

General

Melting points (m.p.) 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. Signal multiplicities are reported by: 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 separations were carried out on silica gel 60 (70–230 mesh, Merck). Elemental analysis was determined by the French Microanalytical Central Service (Montpellier, France).

General Procedure for the Synthesis of Compounds 13a–c

The 1*H*-pyrazolo[3,4-d]pyrimidin-4-thione **11** (20 mmol) was dissolved in 1 N sodium hydroxide solution (40 ml). To this solution were added 40 mmol of **12a**, **12b**, or **12c** 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 coevaporated 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-[6–carboethoxymethylthio-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetate (13a): Yield: 86%. R_f: 0.30 ($CHCl_3$:CH_3OH, 95:5, v:v). m.p. = 122–123°C (water). UV (Methanol) $\lambda_{max} = 280$ nm ($\varepsilon = 14\,200$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.20 (m, 6 H, 2 OCH_2CH_3), 4.13 (m, 4H, 2 OCH_2CH_3), 4.07 (s, 2H, C_6-SCH_2), 4.21 (s, 2H, C_4-SCH_2), 8.28 (s, 1H, H_3), 13.91 (br s, 1H, NH). MS (FAB^+, NBA) *m/z*: 357 $[M + H]^+$.

Ethyl α-[6-(1′-carboethoxyethylthio)-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thiopropionate (13b): Yield: 84%. R_f: 0.34 ($CHCl_3$:CH_3OH, 95:5, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 281$ nm ($\varepsilon = 14\,800$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.12 (m, 6H, 2 OCH_2CH_3), 1.53 (m, 6H, 2 $SCHCH_3$), 4.09 (m, 4H, 2 OCH_2CH_3), 4.49–4.75 (2m, 2H, 2 $SCHCH_2$), 8.19 (s, 1H, H_3), 13.91 (br s, 1H, NH). MS (FAB^+, NBA) *m/z*: 385 $[M + H]^+$.

Ethyl α-[6-(1′-carboethoxypropylthio)-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutyrate (13c): Yield: 80%. R_f: 0.38 ($CHCl_3$:CH_3OH, 95:5, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 280$ nm ($\varepsilon = 16\,100$). ^{1} H NMR (Me_2 SO-d_6) δ: 1.04 (m, 6H, 2 $SCHCH_2CH_3$), 1.20 (m, 6H, 2 OCH_2CH_3), 1.98 (m, 4H, 2 $SCHCH_2CH_3$), 4.17 (m, 4H, 2 OCH_2CH_3), 4.54 (t, $J = 7.11$ Hz, 1H, C_6-$SCHCH_2CH_3$), 4.73 (t, $J = 7.11$ Hz, 1H, C_4-$SCHCH_2CH_3$), 8.27 (s, 1H, H_3), 13.99 (br s, 1H, NH). MS (FAB^+, NBA) *m/z*: 413 $[M + H]^+$.

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). The heterocycle **13a**, **13b**, or **13c** (10 mmol) was added and the reaction mixture was stirred at room temperature for 15 minutes.

At this time the reaction mixture was cooled to 0°C and 10 mmol of compound **14**, **15**, or **16** in 10 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-6-carboethoxymethylthio-1H-pyr azolo[3,4-d]pyrimidin-4-yl]thioacetate (17a): Yield: 86%. R_f: 0.60 ($CHCl_3$:CH_3OH, 98:2, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 249$ nm ($\varepsilon = 20\,200$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.13 (m, 6 H, 2 OCH_2CH_3), 1.90 (s, 3H, CH_3CO), 3.70 et 4.03 (2 m, 4H, OCH_2CH_2 O), 4.05 (s, 2H, C_6-SCH_2), 4.11 (m, 4H, 2 OCH_2CH_3), 4.19 (s, 2H, C_4-SCH_2), 5.64 (s, 2H, OCH_2 N), 8.37 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 473 $[M + H]^+$.

Ethyl α-[1-(2-acetoxyethoxy)methyl-6-(1′carboethoxyethylthio)-1 H-pyr-azolo [3,4-d-]pyrimidin-4-yl]thiopropionate (17b): Yield: 80%. R_f: 0.63 ($CHCl_3$:CH_3OH, 98:2, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 249$ nm ($\varepsilon = 18\,800$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.12 (m, 6 H, 2 OCH_2CH_3), 1.53 (m, 6 H, 2 $SCHCH_3$), 1.90 (s, 3H, CH_3CO), 3.70 et 4.03 (2 m, 4H, OCH_2CH_2 O), 4.09 (m, 4H, 2 OCH_2CH_3), 4.49–4.75 (2 m, 2H, 2 $SCHCH_3$), 5.64 (s, 2H, OCH_2 N), 8.19 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 501 $[M + H]^+$.

Ethyl α-[1-(2-acetoxyethoxy)methyl-6-(1′-carboethoxypropylthio)-1 H-pyrazolo [3,4-d]pyrimidin-4-yl]thiobutyrate (17 c): Yield: 78%. R_f: 0.68 ($CHCl_3$:CH_3OH, 98:2, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 250$ nm ($\varepsilon = 21\,100$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.04 (m, 6 H, 2 $SCHCH_2CH_3$), 1.20 (m, 6 H, 2 OCH_2CH_3), 1.90 (s, 3H, CH_3CO), 1.98 (m, 4H, 2 $SCHCH_2CH_3$), 3.70 et 4.03 (2 m, 4H, OCH_2CH_2 O), 4.17 (m, 4H, 2 OCH_2CH_3), 4.54 (t, $J = 7.11$ Hz, 1H, C_6-$SCHCH_2CH_3$), 4.73 (t, $J = 7.11$ Hz, 1H, C_4-$SCHCH_2CH_3$), 5.64 (s, 2H, OCH_2 N), 8.27 (s, 1 H, H_3). MS (FAB^+, NBA) *m/z*: 529 $[M + H]^+$.

Ethyl α-[1-(4-acetoxybutyl)-6-carboethoxymethythio-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetate (18a): Yield: 80%. R_f: 0.55 ($CHCl_3$:CH_3OH, 99:9, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 250$ nm ($\varepsilon = 19\,200$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.20 (m, 6 H, 2 OCH_2CH_3), 1.52 (m, 2H, $AcOCH_2CH_2$), 1.92 (m, 2H, CH_2CH_2 N), 1.95 (s, 3H, CH_3CO), 4.00 (t, $J = 6.48$ Hz, 2H, $AcOCH_2CH_2$), 4.07 (s, 2H, C_6-SCH_2), 4.13 (m, 4H, 2 OCH_2CH_3), 4.21 (s, 2H, C_4-SCH_2), 4.32 (t, $J = 6.71$ Hz, 2H, CH_2 N), 8.28 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 471 $[M + H]^+$.

Ethyl α-[1-(4-acetoxybutyl)-6-(1′-carboethoxyethylthio)-1 H-pyrazolo[3,4-d]pyrim-idin-4-yl]thiopropionate (18b): Yield: 79%. R_f: 0.58 ($CHCl_3$:CH_3OH, 99:1, v:v). Appearance: liquid. UV (Methanol) $\lambda_{max} = 250$ nm ($\varepsilon = 14\,800$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.12 (m, 6 H, 2 OCH_2CH_3), 1.50–1.54 (m, 6H, 2 $SCHCH_3$ and 2H,$AcOCH_2CH_2$), 1.92 (m, 2H, CH_2CH_2 N), 1.95

(s, 3H, CH_3CO), 4.00 (t, J = 6.48 Hz, 2H, $AcOCH_2CH_2$), 4.12 (m, 4H, 2 OCH_2CH_3), 4.32 (t, J = 6.71 Hz, 2H, CH_2 N), 4.49–4.75 (2 m, 2H, 2 $SCHCH_3$), 8.19 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 499 $[M + H]^+$.

Ethyl α-[1-(4-acetoxybutyl)-6-(1′-carboethoxypropylthio)-1 H-pyrazolo [3,4-d]pyrimidin-4-yl]thiobutyrate (18c): Yield: 78%. R_f: 0.61 ($CHCl_3$:CH_3OH, 99:1, v:v). Appearance: liquid. UV (Methanol) λ_{max} = 250 nm (ε = 20 100). ^1H NMR (Me_2 SO-d_6) δ: 1.04 (t, J = 7.33 Hz, 6 H, 2 $SCHCH_2CH_3$), 1.17 (m, 6 H, 2 OCH_2CH_3), 1.53 (m, 2H, $AcOCH_2CH_2$), 1.89 (m, 2H, CH_2CH_2 N), 1.91 (m, 4H, 2 $SCHCH_2CH_3$), 1.91 (s, 3H, CH_3CO), 3.97 (t, J = 6.48 Hz, 2H, $AcOCH_2CH_2$), 4.15 (m, 4H, 2 OCH_2CH_3), 4.32 (t, J = 6.71 Hz, 2H, CH_2 N), 4.45 (m, 1H, C_6-$SCHCH_2CH_3$), 4.68 (m, 1H, C_4-$SCHCH_2CH_3$), 8.27 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 527 $[M + H]^+$.

Ethyl α-[1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-6-carboethoxy-methylthio)-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetate (19a): Yield: 76%. R_f: 0.53 ($CHCl_3$:CH_3OH, 98:2, v:v). Appearance: liquid. UV (Methanol) λ_{max} = 251 nm (ε = 22 200). ^1H NMR (Me_2 SO-d_6) δ: 1.20 (m, 6 H, 2 OCH_2CH_3), 1.86 (s, 3H, CH_3CO), 3.81 (d, J = 4.98 Hz, 2H, OCH_2CH), 4.07 (s, 2H, C_6-SCH_2), 4.13 (m, 4H, 2 OCH_2CH_3), 4.21 (s, 2H, C_4-SCH_2), 4.30 (distorted d, 2H, CH_2 OAc), 5.19 (m, 1H, CH_2CHOBz), 5.76 (s, 2H, OCH_2 N), 7.40–7.96 (m, 5 H, C_6H_5), 8.28 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 607 $[M + H]^+$.

Ethyl α-[1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-6-(1′-carboethoxyethylthio) -1 H-pyr azolo[3,4-d]pyrimidin-4-yl]thiopropionate (19b): Yield: 75%. R_f: 0.56 ($CHCl_3$:CH_3OH, 98:2, v:v). Appearance: liquid. UV (Methanol) λ_{max} = 250 nm (ε = 21 300). ^1H NMR (Me_2 SO-d_6) δ: 1.19 (m, 6 H, 2 OCH_2CH_3), 1.50 (m, 6 H, 2 $SCHCH_3$), 1.97 (s, 3H, CH_3CO), 3.91 (m, 2H, OCH_2CH), 4.09 (m, 4H, 2 OCH_2CH_3), 4.15 (distorted d, 2H, CH_2 OAc), 4.49–4.75 (2m, 2H, 2 $SCHCH_3$), 5.30 (m, 1H, CH_2CHOBz), 5.76 (s, 2H, OCH_2 N), 7.50–8.02 (m, 5H, C_6H_5), 8.34 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 635 $[M + H]^+$.

Ethyl α-[1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-6-(1′-carboethoxy propylthio) -1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutyrate (19c): Yield: 75%. R_f: 0.59 ($CHCl_3$:CH_3OH, 98:2, v:v). Appearance: liquid. UV (Methanol) λ_{max} = 251 nm (ε = 22 900). ^1H NMR (Me_2 SO-d_6) δ: 1.04 (m, 6 H, 2 $SCHCH_2CH_3$), 1.20 (m, 6 H, 2 OCH_2CH_3), 1.94 (s, 3H, CH_3CO), 1.98 (m, 4H, 2 $SCHCH_2CH_3$), 3.91 (m, 2H, OCH_2CH), 4.15 (distorted d, 2H, CH_2 OAc), 4.18 (m, 4H, 2 OCH_2CH_3), 4.54 (t, J = 7.11 Hz, 1H, C_6-$SCHCH_2CH_3$), 4.73 (t, J = 7.11 Hz, 1H, C_4-$SCHCH_2CH_3$), 5.30 (m, 1H, CH_2CHOBz), 5.76 (s, 2H, OCH_2 N), 7.50–8.02 (m, 5 H, C_6H_5), 8.27 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 663 $[M + H]^+$.

General Deprotection Procedure

To 80 ml of dry methanol saturated with ammonia at -5°C was added 1 mmol of the protected acyclic nucleoside **(17–19)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-6-carbamoylmethylthio-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetamide (20a): Yield: 83%. R_f: 0.23 ($CHCl_3$:CH_3OH, 80:20, v:v). UV (Methanol) $\lambda_{max} = 249$ nm ($\varepsilon = 20\,200$). ^{1}H NMR (Me_2 SO-d_6) δ: 3.45 (m, 4H, OCH_2CH_2 O), 4.05 (s, 2H, C_6-SCH_2), 4.19 (s, 2H, C_4-SCH_2), 4.55 (t, $J = 5.00$ Hz, 1H, HO, D_2O exchangeable), 5.74 (s, 2H, OCH_2 N), 7.23, 7.32, 7.65 et 7.75 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.37 (s, 1H, H_3). MS (FAB$^+$, NBA) *m/z*: 373 $[M + H]^+$. Elem. Anal. Calcd for $C_{12}H_{16}N_6O_4S_2$ (372.41): C 38.70%, H 4.33%, N 22.56%, Found: C 38.90%, H 4.50%, N 22.89%.

α-[1-(2-hydroxyethoxy)methyl-6-(1′-carbamoylethylthio)-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thiopropanamide (20b): Yield: 80%. R_f: 0.27 ($CHCl_3$:CH_3OH, 80:20, v:v). UV (Methanol) $\lambda_{max} = 249$ nm ($\varepsilon = 18\,800$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.54 (m, 6 H, 2 SCHCH_3), 3.45 (m, 4H, OCH_2CH_2 O), 4.55 (t, $J = 5.00$ Hz, 1H, HO, D2 O exchangeable), 5.74 (s, 2H, OCH_2 N), 7.23, 7.32, 7.65 et 7.75 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 4.81 (2 m, 2H, 2 SC$H$$CH_3$), 5.75 (s, 2H, OCH_2 N), 8.19 (s, 1H, H_3). MS (FAB$^+$, NBA) *m/z*: 401 $[M + H]^+$. Elem. Anal. Calcd for $C_{14}H_{20}N_6O_4S_2$ (400.46): C 41.98%, H 5.03%, N 20.98%, Found: C 42.21%, H 5.30%, N 21.19%.

α-[1-(2-hydroxyethoxy)methyl-6-(1′-carbamoylpropylthio)-1 H-pyrazolo [3,4-d]pyrimidin-4-yl]thiobutanamide (20c): Yield: 80%. R_f: 0.31 ($CHCl_3$:CH_3OH, 80:20, v:v). UV (Methanol) $\lambda_{max} = 250$ nm ($\varepsilon = 21\,100$). ^{1}H NMR (Me_2 SO-d_6) δ: 1.04 (m, 6 H, 2 SCHCH_2CH_3), 1.98 (m, 4H, 2 SCHC$H_2$$CH_3$), 3.50 (m, 4H, OCH_2CH_2 O), 4.51 (t, $J = 7.11$ Hz, 1H, C_6-SC$H$$CH_2CH_3$), 4.55 (t, $J = 5.00$ Hz, 1H, HO, D_2O exchangeable), 4.73 (t, $J = 7.11$ Hz, 1H, C_4-SC$H$$CH_2CH_3$), 5.74 (s, 2H, OCH_2 N), 7.22, 7.32, 7.64 et 7.75 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.27 (s, 1H, H_3). MS (FAB$^+$, NBA) *m/z*: 429 $[M + H]^+$. Elem. Anal. Calcd for $C_{16}H_{24}N_6O_4S_2$ (428.52): C 44.84%, H 5.64%, N 19.61%, Found: C 45.10%, H 5.80%, N 19.89%.

α-[1-(4-hydroxybutyl)-6-carbamoylmethylthio-1 H-pyrazolo[3,4-d]pyrimi-din-4-yl]thioacetamide (21a): Yield: 87%. R_f: 0.12 ($CHCl_3$:CH_3OH, 90:10, v:v). UV (Methanol) $\lambda_{max} = 250$ nm ($\varepsilon = 19\,200$). ^{1}H NMR (Me_2 SO-d_6) δ : 1.35 (m, 2H, $HOCH_2C$$H_2$), 1.84 (m, 2H, C$H_2CH_2$ N), 3.42 (m, 2H, HOCH_2CH_2), 3.92 (s, 2H, C_6-SCH_2), 4.11 (s, 2H, C_4-SCH_2), 4.33 (t, $J = 6.86$ Hz, 2H, CH_2CH_2 N), 4.44 (t, $J = 5.17$ Hz, 1H, HO, D_2O exchangeable), 7.23, 7.32, 7.66 et 7.74 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.28 (s, 1H, H_3). MS (FAB$^+$, NBA) *m/z*: 371 $[M + H]^+$. Elem. Anal. Calcd for $C_{13}H_{18}N_6O_3S_2$

(370.44): C 42.15%, H 4.89%, N 22.68%, Found: C 42.51%, H 5.00%, N 22.89%.

α-[1-(4-hydroxybutyl)-6-(1′-carbamoylethylthio)-1 H-pyrazolo[3,4-d]pyrimidin -4-yl]thiopropanamide (21b): Yield: 85%. R_f: 0.18 ($CHCl_3$:CH_3OH, 90:10, v:v). UV (Methanol) λ_{max} = 250 nm (ε = 21 800). ^{1}H NMR (Me_2 SO-d_6) δ: 1.35 (m, 2H, $HOCH_2CH_2$), 1.52 (m, 6 H, 2 $SCHCH_3$), 1.84 (m, 2H, CH_2CH_2 N), 3.42 (m, 2H, $HOCH_2CH_2$), 4.32 (t, J = 6.71 Hz, 2H, CH_2CH_2 N), 4.44 (t, J = 5.17 Hz, 1H, HO, D_2O exchangeable), 4.80 (m, 2H, 2 $SCHCH_3$), 7.23, 7.32, 7.66 et 7.74 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.19 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 399 $[M + H]^+$. Elem. Anal. Calcd for $C_{15}H_{22}N_6O_3S_2$ (398.49): C 45.21%, H 5.56%, N 21.08%, Found: C 45.51%, H 5.78%, N 21.39%.

α-[1-(4-hydroxybutyl)-6-(1′-carbamoylpropylthio)-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutanamide (21c): Yield: 85%. R_f: 0.23 ($CHCl_3$:CH_3OH, 90:10, v:v). UV (Methanol) λ_{max} = 250 nm (ε = 20 100). ^{1}H NMR (Me_2 SO-d_6) δ: 1.04 (t, J = 7.33 Hz, 6 H, 2 $SCHCH_2CH_3$), 1.35 (m, 2H, $HOCH_2CH_2$), 1.89 (m, 2H, CH_2CH_2 N), 1.91 (m, 4H, 2 $SCHCH_2CH_3$), 3.42 (m, 2H, $HOCH_2CH_2$), 4.32 (t, J = 6.71 Hz, 2H, CH_2CH_2 N), 4.44 (t, J = 5.17 Hz, 1H, HO, D_2O exchangeable), 4.51 (m, 1H, C_6-$SCHCH_2CH_3$), 4.68 (m, C_4-$SCHCH_2CH_3$), 7.23, 7.32, 7.66 et 7.74 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.27 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 427 $[M + H]^+$. Elem. Anal. Calcd for $C_{17}H_{26}N_6O_3S_2$ (426.54): C 47.86%, H 6.14%, N 19.70%, Found: C 48.11%, H 6.50%, N 19.91%.

α-[1-(2,3-dihydroxy-1-propoxy)methyl-6-carbamoylmethylthio-1 H-pyrazolo [3, 4-d] pyrimidin-4-yl]thioacetamide (22a): Yield: 84%. R_f: 0.19 ($CHCl_3$:CH_3OH, 80:20, v:v). UV (Methanol) λ_{max} = 251 nm (ε = 22 200). ^{1}H NMR (Me_2 SO-d_6) δ: 3.20–3.54 (m, 5 H, OCH_2CHCH_2), 3.89 (s, 2H, C_6-SCH_2), 3.95 (s, 2H, C_4-SCH_2), 4.54 (t, J = 5.70 Hz, 1H, $HOCH_2$, D_2O exchangeable), 4.72 (d, J = 4.66 Hz, 1H, $HOCH$, D_2O exchangeable), 5.61 (s, 2H, OCH_2 N), 7.11, 7.25, 7.53 et 7.75 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.09 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 403 $[M + H]^+$. Elem. Anal. Calcd for $C_{13}H_{18}N_6O_5S_2$ (402.43): C 38.79%, H 4.50%, N 20.88%, Found: C 39.09%, H 4.78%, N 21.10%.

α-[1-(2,3-dihydroxy-1-propoxy)methyl-6-(1′-carbamoylethylthio)-1 H-pyrazolo [3,4-d]pyrimidin-4-yl]thiopropanamide (22b): Yield: 82%. R_f: 0.23 ($CHCl_3$:CH_3OH, 80:20, v:v). UV (Methanol) λ_{max} = 250 nm (ε = 21 900). ^{1}H NMR (Me_2 SO-d_6) δ: 1.50 (m, 6 H, 2 $SCHCH_3$), 3.20–3.54 (m, 5 H, OCH_2CHCH_2), 4.61 (m, 2H, 2 $SCHCH_3$), 4.54 (t, J = 5.70 Hz, 1H, $HOCH_2$, D_2O exchangeable), 4.72 (d, J = 4.66 Hz, 1H, $HOCH$, D_2O exchangeable), 5.62 (s, 2H, OCH_2 N), 7.11, 7.25, 7.53 et 7.75 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.14 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 431 $[M + H]^+$. Elem. Anal. Calcd for $C_{15}H_{22}N_6O_5S_2$ (430.49): C 41.85%, H 5.15%, N 19.52%, Found: C 41.98%, H 5.23%, N 19.71%.

α-[1-(2,3-dihydroxy-1-propoxy)methyl-6-(1′-carbamoylpropylthio)-1 H-pyrazolo[3,4-d]pyrimidin-4-yl]thiobutanamide (22c): Yield: 82%. R_f: 0.27 ($CHCl_3$:CH_3OH, 80:20, v:v). UV (Methanol) λ_{max} = 251 nm (ε = 22 500). ^{1}H NMR (Me_2 SO-d_6) δ: 1.04 (m, 6 H, 2 $SCHCH_2CH_3$), 1.98 (m, 4H, 2 $SCHCH_2CH_3$), 3.20–3.54 (m, 5 H, OCH_2CHCH_2), 4.50 (t, J = 7.11 Hz, 1H, C_6-$SCHCH_2CH_3$), 4.56 (t, J = 5.70 Hz, 1H, $HOCH_2$, D_2O exchangeable), 4.69 (t, J = 7.11 Hz, 1H, C_4-$SCHCH_2CH_3$), 4.75 (d, J = 4.66 Hz, 1H, $HOCH$, D_2O exchangeable), 5.67 (s, 2H, OCH_2 N), 7.12, 7.24, 7.55 et 7.74 (4 br s, 4H, 2 -$CONH_2$, D_2O exchangeable), 8.17 (s, 1H, H_3). MS (FAB$^+$, NBA) *m/z*: 459 $[M + H]^+$. Elem. Anal. Calcd for $C_{17}H_{26}N_6O_5S_2$ (458.54): C 44.52%, H 5.71%, N 18.32%, Found: C 44.85%, H 5.95%, N 18.61%.

Biological Assays

The antiviral activity assays were carried out according to previously established procedures.[19,20]

The antituberculosis assay was carried out as described previously.[21]

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