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## Nucleosides, Nucleotides and Nucleic Acids

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### SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME 4-SUBSTITUTED 1-[1-(4-HYDROXYBUTYL)-1,2,3-TRIAZOL-(4 & 5)-YLMETHYL]-1H-PYRAZOLO-[3,4-d]PYRIMIDINES

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**SYNTHESIS AND BIOLOGICAL EVALUATION  
OF SOME 4-SUBSTITUTED  
1-[1-(4-HYDROXYBUTYL)-1,2,3-TRIAZOL-  
(4 & 5)-YLMETHYL]-1H-PYRAZOLO-  
[3,4-d]PYRIMIDINES**

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**ABSTRACT**

The synthesis of 1-[1-(4-hydroxybutyl)-1,2,3-triazol-(4 and 5)-ylmethyl]-1H-pyrazolo[3,4-d]pyrimidines **11a,b**, **12a,b** and **13–17** as carboacyclic nucleosides is described. The compounds **8a,b** were condensed, separately, with compound **7** via 1,3-dipolar cycloaddition reaction to afford, after separation and deprotection, 1,4-regioisomers **11a,b** and 1,5-regioisomers **12a,b**. The deprotected carboacyclic nucleosides **11a** served as precursor for the preparation of 4-amino **13**, 4-methylamino **14**, 4-benzylamino **15**, 4-methoxy **16** and 4-hydroxy **17** analogues. All deprotected carboacyclic nucleosides were evaluated for their inhibitory effects against the replication of HIV-1(III<sub>B</sub>), HIV-2(ROD), various DNA

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viruses, a variety of tumor-cell lines and tuberculosis. No marked biological activity was found.

## INTRODUCTION

Up to now, the synthesis of unnatural nucleosides play a significant role in the search of new antiviral and anti-tumoral agents. Following the discovery of acyclovir<sup>1</sup> **1** (Fig. 1) as potent and selective anti-herpetic agent, the preparation of modified nucleosides with structural alterations in either the heterocyclic ring or the sugar moiety or both has become a very interesting research area<sup>2-4</sup>. In this respect, some carboacyclic nucleosides, such as HBG<sup>3</sup> **2**, penciclovir<sup>3</sup> **3** and its oral form famciclovir<sup>3,5</sup> **4** (Fig. 1), which inhibit replication of herpes viruses (HSV), have been reported. Their mode of antiviral action against HSV is similar to that of acyclovir in that these compounds are phosphorylated to a greater extent in virus-infected cells than in uninfected cells. The triphosphates of these acyclic nucleosides then act as potent and selective inhibitors of the virus-encoded DNA polymerase<sup>6-8</sup>. Previously, we reported the synthesis of some carboacyclic pyrazolo[3,4-d]pyrimidine nucleosides **5a-h**<sup>9</sup> (Fig. 1) in attempts to develop novel compounds with cytostatic and/or antiviral properties, some of them show modest *in vitro* antiviral activity. In connection with this work, and based on our interest in the insertion of 1,2,3-triazol-(4 and 5)-ylmethyl part between heterocyclic rings and acyclic chains<sup>10,11</sup>, we present the synthesis of some 4-substituted 1-[1-(4-hydroxybutyl)-1,2,3-triazol-(4 and 5)-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidines **11a,b**, **12a,b** and **13-17** in order to determine the influence on biological evaluation of the 1,2,3-triazol-(4 or 5)-ylmethyl moiety as a spacer arm between the 4-substituted pyrazolo[3,4-d]pyrimidines and 4-hydroxybutyl. The synthesis and biological activity are described herein.

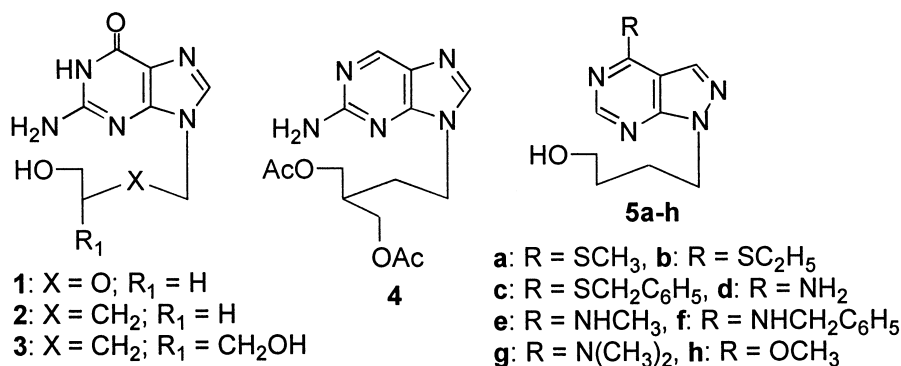
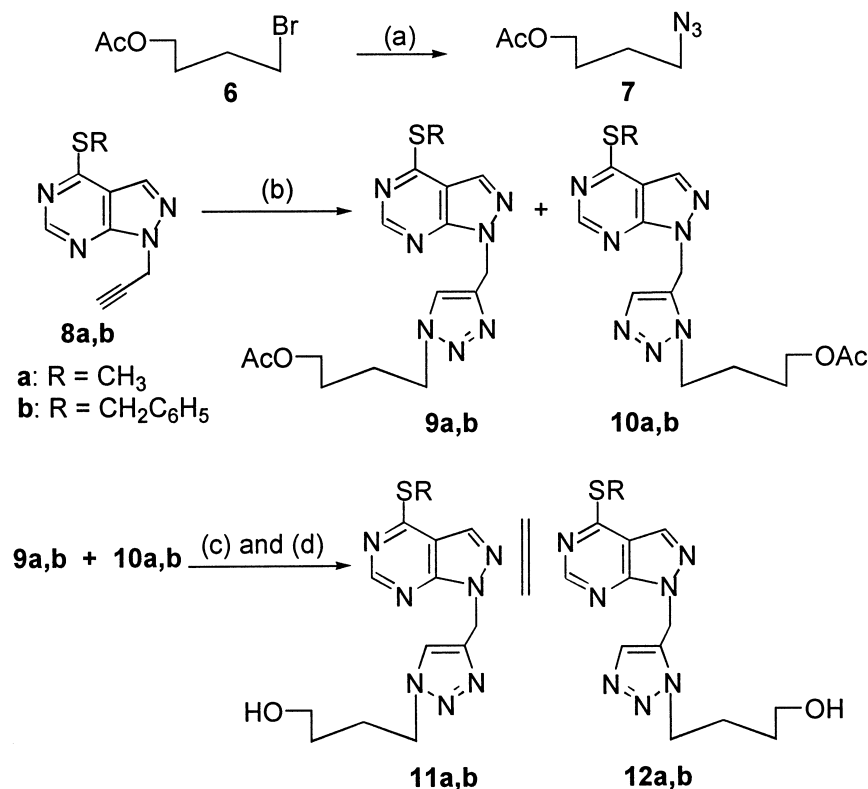


Figure 1.

## RESULTS AND DISCUSSION

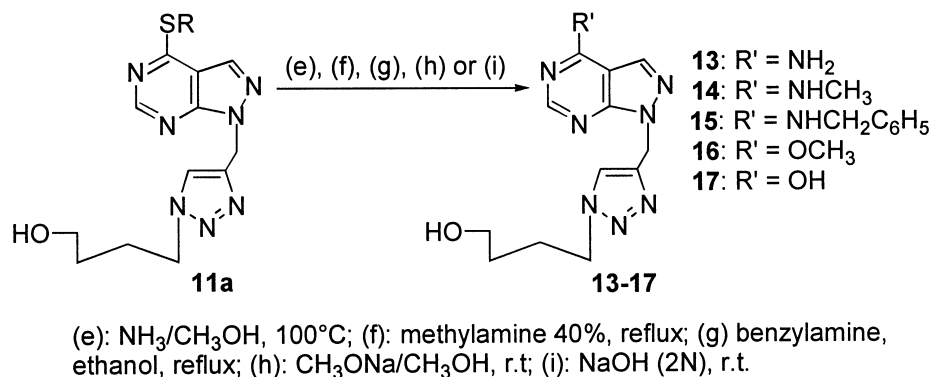
Treatment of 4-acetoxybutylbromide<sup>9,12</sup> **6** with sodium azide in anhydrous *N,N*-dimethylformamide (DMF) gave 4-acetoxybutylazide **7** in 96% yield (Scheme 1). The 4-(methyl and benzyl)thio-1-(prop-2-ynyl)-1*H*-pyrazolo[3,4-*d*]pyrimidines **8a,b** (Scheme 1) which have been described before<sup>11</sup> served as starting materials. The latter were reacted with azido-compound **7** via 1,3-dipolar cycloaddition reaction, in anhydrous toluene under reflux, to afford a mixture of two possible regioisomers (**9a** + **10a**) and (**9b** + **10b**) in 89% and 87% overall yield, respectively (Scheme 1). It is well known from the literature<sup>13</sup> that the addition of azides to unsymmetrical acetylenes is determined by steric and electronic factors. In general, such addition tends to give mainly the isomers with electron-withdrawing groups at the 4-position and electron-releasing groups at 5-position. Thus, after separation of



Ac: acetyl group

(a): NaN<sub>3</sub>, DMF, 90°C; (b): compound **7**, toluene, reflux; (c): separation by initial fractional crystallization and followed by chromatography on silica gel column; (d): NH<sub>3</sub>/CH<sub>3</sub>OH, r.t.

Scheme 1.



Scheme 2.

protected carboacyclic nucleosides **9a,b** and **10a,b** by initial fractional crystallization and followed by chromatography, the compounds **9a,b** were obtained as the major regioisomers in 70% yield. The structures of the two regioisomers **9a,b** and **10a,b** were established by comparison of the chemical shift values for the triazole ring protons with those available from a known pair 4 and 5-substituted 1,2,3-triazole derivatives<sup>11,13</sup>. The 1,4-regioisomers **9a** and **9b** showed an H-5 resonance at lower field (8.06 and 8.09 ppm, respectively). For 1,5-regioisomers **10a,b**, the H-4 resonance is at higher field (7.61 and 7.63 ppm, respectively).

The acetyl groups were subsequently removed from the protected carboacyclic nucleosides **9a,b** and **10a,b**, by treatment with a solution of methanol saturated with ammonia at  $25^\circ\text{C}$ , affording the required deacetylated products **11a,b** and **12a,b** in good yield (Scheme 1).

When compound **11a** was treated with methanol saturated with ammonia in a sealed reacting vessel at  $100^\circ\text{C}$ , the carboacyclic nucleoside **13** was obtained in 90% yield (Scheme 2). Condensation of the compound **11a** with primary amines in aqueous or alcohol solution afforded the compounds **14** and **15** in good yield (Scheme 2). The carboacyclic nucleosides **16** and **17** were synthesized in 73% and 72% yield, respectively, *via* treatment of **11a** with  $\text{CH}_3\text{ONa}/\text{CH}_3\text{OH}$  and  $\text{NaOH}$  solutions at room temperature (Scheme 2).

Structure identification of the synthetic products was done by  $^1\text{H}$  NMR, mass spectra and elemental analysis.

## BIOLOGICAL STUDIES

The carboacyclic nucleosides **11a,b**, **12a,b** and **13–17** 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), vaccina virus, vesicular stomatitis (VSV), thymidine kinase-deficient (TK<sup>-</sup>) strain of HSV-1 (B2006 and VWM1837) in human embryonic skinmuscles fibroblasts (E<sub>6</sub>SM), 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 and toxicity were noted at concentration up to 400 µg/ml. Only compounds **11a** and **16** have a low activity, *in vitro*, against parainfluenza virus type 3 and reovirus type 1, respectively (MIC<sub>50</sub> = 240 µg/ml, MCC > 400 µg/ml). Neither anti-HIV-1(III<sub>B</sub>) nor anti-HIV-2(ROD) activity was observed 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 compound concentrations lower than 10<sup>-4</sup> M.

Primary screening of compounds **11a,b**, **12a,b** and **13–17** against *Mycobacterium tuberculosis* H<sub>37</sub>Rv (ATCC 27294) in BACTEC 12B medium shows that only compounds **11b** and **14** have a low anti-mycobacteriane activity (42% and 8% of inhibition, respectively, MIC = 12.5 µg/ml).

In conclusion, we showed that the 1,3 dipolar cycloaddition reaction is an efficient method to obtain the carboacyclic 1,2,3-triazole nucleosides with acyclic chain of HBG, in good yield, from easily accessible starting materials.

## EXPERIMENTAL

Melting points (mp) were determined on a Electrothermal digital melting point apparatus and were uncorrected. The <sup>1</sup>H NMR spectra were recorded using a Bruker AC 250 (250 MHz) spectrometer. The chemical shifts were reported as parts per million (δ ppm) from (CH<sub>3</sub>)<sub>4</sub>Si (TMS) as an internal standard. Key: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Mass spectra (MS) were obtained with a JOEL JMS DX 300 instrument using fast atomic bombardment (FAB positive). Infrared (IR) spectra were recorded with a Perkin-Elmer 580 spectrometer. Thin-layer chromatography (tlc) was performed on plates of Merck Kieselgel 60 F<sub>254</sub> and short wavelength UV light (254 nm) was used to detect the UV-absorbing spots. *R<sub>f</sub>*<sup>2</sup> is *R<sub>f</sub>* after two migrations in hexane:ethyl ether (50:50). Hexane:ethyl ether (50:50) was used as solvent for fractional crystallization of compounds **9a,b** and **10a,b**. Column chromatography separation were obtained on silica gel 60 (70–230 mesh, Merck). Elemental analysis were determined by the French microanalytical central service.

**4-Acetoxybutylazide 7.** To a solution of finely ground  $\text{NaN}_3$  (5.49 g, 84.46 mmol) in 240 ml of anhydrous DMF were added 11 g (56.41 mmol) of freshly distilled compound **6** and the resulting mixture was heated at  $90^\circ\text{C}$  under stirring for 4 hours. The solid was removed and washed with DMF ( $3 \times 30$  ml). To a combined filtrate and washings were added 150 ml of water. The mixture was then extracted with ether ( $4 \times 60$  ml). The extracts were combined, dried ( $\text{MgSO}_4$ ) and then evaporated *in vacuo* to leave a pale yellow oil. The oil was chromatographed on silica gel column with hexane as eluent to give compound **7** (8.14 g, 96%) as a clear oil. IR ( $\text{CCl}_4$ ):  $2095\text{ cm}^{-1}$  ( $\text{N}_3$ ),  $1745\text{ cm}^{-1}$  (CO).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.61 (m, 4H,  $\text{AcOCH}_2\text{CH}_2\text{CH}_2$ ), 2.10 (s, 3H,  $\text{CH}_3\text{CO}$ ), 3.40 (t, 2H,  $J=6.42$  Hz,  $\text{AcOCH}_2$ ), 4.14 (t, 2H,  $\text{CH}_2\text{N}_3$ ,  $J=7.00$  Hz). Anal. calcd. for  $\text{C}_6\text{H}_{11}\text{N}_3\text{O}_2$  (157.17): C 45.85, H 7.05, N 26.73; found: C 45.95, H 7.13, N 26.97.

#### General Procedure of the Preparation of Compounds **9a,b** and **10a,b**

A mixture of propargylated heterocycle **8a** or **8b** (1 mmol) and azide derivative **7** (1.5 mmol) in anhydrous toluene (60 ml) was refluxed for 30 hours. The reaction was monitored by thin-layer chromatography with formation of two regioisomers. The solution was evaporated to dryness. The mixture of regioisomers was separated by fractional crystallization followed by chromatography on silica gel column, using hexane:ethyl ether (50:50) as eluent, to give the expected carboacyclic nucleoside.

**1-[1-(4-Acetoxybutyl)-1,2,3-triazol-4-ylmethyl]-4-methylthio-1H-pyrazolo [3,4-d]pyrimidine 9a.** Yield: 252 mg (70%).  $R_f^2$  0.51. mp:  $93\text{--}94^\circ\text{C}$  (ethanol).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.44–1.85 (2m, 4H,  $\text{AcOCH}_2\text{CH}_2\text{CH}_2$ ), 1.99 (s, 3H,  $\text{CH}_3\text{CO}$ ), 2.73 (s, 3H,  $\text{SCH}_3$ ), 3.98 (t, 2H,  $J=6.54$  Hz,  $\text{AcOCH}_2$ ), 4.34 (t, 2H,  $J=7.10$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 5.70 (s, 2H,  $\text{CCCH}_2$ ), 8.06 (s, 1H, aromatic proton of triazole group), 8.37 and 8.82 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 362$ . Anal. calcd. for  $\text{C}_{15}\text{H}_{19}\text{N}_7\text{O}_2\text{S}$  (361.41): C 49.85, H 5.29, N 27.12; found: C 49.96, H 5.31, N 27.25.

**1-[1-(4-Acetoxybutyl)-1,2,3-triazol-5-ylmethyl]-4-methylthio-1H-pyrazolo [3,4-d]pyrimidine 10a.** Yield: 68 mg (19%).  $R_f^2$  0.59. Appearance: syrup.  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 0.76–1.66 (2m, 4H,  $\text{AcOCH}_2\text{CH}_2\text{CH}_2$ ), 1.92 (s, 3H,  $\text{CH}_3\text{CO}$ ), 2.66 (s, 3H,  $\text{SCH}_3$ ), 3.86 (t, 2H,  $\text{AcOCH}_2$ ,  $J=6.42$  Hz), 4.37 (t, 2H,  $J=7.25$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 5.79 (s, 2H,  $\text{CCCH}_2$ ), 7.61 (s, 1H, aromatic proton of triazole group), 8.35 and 8.78 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 362$ . Anal. calcd. for  $\text{C}_{15}\text{H}_{19}\text{N}_7\text{O}_2\text{S}$  (361.41): C 49.85, H 5.29, N 27.12; found: C 49.93, H 5.30, N 27.23.

**1-[1-(4-Acetoxybutyl)-1,2,3-triazol-4-ylmethyl]-4-benzylthio-1H-pyrazolo [3,4-d]pyrimidine 9b.** Yield: 306 mg (70%).  $R_f^2$  0.58. mp: 123–124 °C (ethanol).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.51 (m, 2H,  $\text{AcOCH}_2\text{CH}_2$ ), 1.84 (m, 2H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 2.00 (s, 3H,  $\text{CH}_3\text{CO}$ ), 4.00 (t, 2H,  $\text{AcOCH}_2$ ,  $J=6.50$  Hz), 4.35 (t, 2H,  $J=7.06$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 4.72 (s, 2H,  $\text{SCH}_2$ ), 5.71 (s, 2H,  $\text{CCCH}_2$ ), 7.33 and 7.52 (2m, 5H,  $\text{C}_6\text{H}_5$ ), 8.09 (s, 1H, aromatic proton of triazole group), 8.36 and 8.89 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 438$ ,  $[\text{C}_6\text{H}_5\text{CH}_2]^+ = 91$ . Anal. calcd. for  $\text{C}_{21}\text{H}_{23}\text{N}_7\text{O}_2\text{S}$  (437.51): C 57.65, H 5.29, N 22.40; found: C 57.81, H 5.36, N 22.66

**1-[1-(4-Acetoxybutyl)-1,2,3-triazol-5-ylmethyl]-4-benzylthio-1H-pyrazolo [3,4-d]pyrimidine 10b.** Yield: 74 mg (17%).  $R_f^2$  0.66. Appearance: syrup.  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.40–1.61 (2m, 4H,  $\text{AcOCH}_2\text{CH}_2\text{CH}_2$ ), 1.93 (s, 3H,  $\text{CH}_3\text{CO}$ ), 3.89 (t, 2H,  $\text{AcOCH}_2$ ,  $J=6.35$  Hz), 4.37 (t, 2H,  $J=7.13$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 4.66 (s, 2H,  $\text{SCH}_2$ ), 5.67 (s, 2H,  $\text{CCCH}_2$ ), 7.22–7.50 (m, 5H,  $\text{C}_6\text{H}_5$ ), 7.63 (s, 1H, aromatic proton of triazole group), 8.35 and 8.85 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 438$ ,  $[\text{C}_6\text{H}_5\text{CH}_2]^+ = 91$ . Anal. calcd. for  $\text{C}_{21}\text{H}_{23}\text{N}_7\text{O}_2\text{S}$  (437.51): C 57.65, H 5.29, N 22.40; found: C 57.87, H 5.38, N 22.71

### General Deprotection Method

To 45 ml of anhydrous methanol saturated with  $\text{NH}_3$  at  $-5^\circ\text{C}$  was added 1 mmol of the acetylated product **9a,b** or **10a,b**. The flask was stoppered tightly and the solution was stirred for 16 hours at room temperature. Thin-layer chromatography (tlc) indicated that complete deprotection of acetylated product had occurred. Volatile materials were evaporated *in vacuo* and the resulting solid was recrystallized or, if necessary, purified on a column of silica gel, using chloroform:methanol (98:02) as eluent, to obtain the expected deacetylated carboacyclic nucleoside.

**1-[1-(4-Hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-4-methylthio-1H-pyrazolo [3,4-d]pyrimidine 11a.** Yield: 0.30 g (94%).  $R_f$  0.44 (90:10,  $\text{CHCl}_3/\text{MeOH}$ ). mp: 104–105 °C (ethanol).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.31 (m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 1.77 (m, 2H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 2.69 (s, 3H,  $\text{SCH}_3$ ), 3.36 (m, 2H,  $\text{HOCH}_2$ ), 4.28 (t, 2H,  $J=7.14$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 4.44 (t, 1H,  $J=5.17$  Hz, HO,  $\text{D}_2\text{O}$  exchangeable), 5.66 (s, 2H,  $\text{CCCH}_2$ ), 8.03 (s, 1H, aromatic proton of triazole group), 8.33 and 8.79 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 320$ . Anal. calcd. for  $\text{C}_{13}\text{H}_{17}\text{N}_7\text{OS}$  (319.38): C 48.88, H 5.36, N 30.69; found: C 48.92, H 5.38, N 30.83.

**1-[1-(4-Hydroxybutyl)-1,2,3-triazol-5-ylmethyl]-4-methylthio-1H-pyrazolo [3,4-d]pyrimidine 12a.** Yield: 0.30 g (94%).  $R_f$  0.46 (90:10,  $\text{CHCl}_3:\text{MeOH}$ ).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.01–1.57 (2m, 4H,  $\text{HOCH}_2\text{CH}_2\text{CH}_2$ ), 2.75 (s, 3H,



SCH<sub>3</sub>), 3.38 (m, 2H, HOCH<sub>2</sub>), 4.42 (t, 2H,  $J=7.40$  Hz, CH<sub>2</sub>CH<sub>2</sub>N), 5.35 (t, 1H,  $J=5.17$  Hz, HO, D<sub>2</sub>O exchangeable), 5.86 (s, 2H, CCCH<sub>2</sub>), 7.68 (s, 1H, aromatic proton of triazole group), 8.44 and 8.87 (2s, 2H, H<sub>3</sub> and H<sub>6</sub>). MS,  $m/z$ :  $[M+H]^+ = 320$ . Anal. calcd. for C<sub>13</sub>H<sub>17</sub>N<sub>7</sub>OS (319.38): C 48.88, H 5.36, N 30.69; found: C 48.94, H 5.40, N 30.92.

**4-Benzylthio-1-[1-(4-hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-1H-pyrazolo [3,4-d]pyrimidine 11b.** Yield: 0.37 g (93%).  $R_f$  0.48 (90:10, CHCl<sub>3</sub>:MeOH). mp: 141–142 °C (ethanol). <sup>1</sup>H NMR (Me<sub>2</sub>SO-d<sub>6</sub>)  $\delta$ : 1.37 (m, 2H, HOCH<sub>2</sub>CH<sub>2</sub>), 1.83 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N), 3.42 (m, 2H, HOCH<sub>2</sub>), 4.34 (t, 2H,  $J=7.14$  Hz, CH<sub>2</sub>CH<sub>2</sub>N), 4.50 (t, 1H,  $J=5.17$  Hz, HO, D<sub>2</sub>O exchangeable), 4.74 (s, 2H, SCH<sub>2</sub>), 5.72 (s, 2H, CCCH<sub>2</sub>), 7.30–7.54 (2m, 5H, C<sub>6</sub>H<sub>5</sub>), 8.09 (s, 1H, aromatic proton of triazole group), 8.38 and 8.90 (2s, 2H, H<sub>3</sub> and H<sub>6</sub>). MS,  $m/z$ :  $[M+H]^+ = 396$ . Anal. calcd. for C<sub>19</sub>H<sub>21</sub>N<sub>7</sub>OS (395.47): C 57.70, H 5.35, N 24.79; found: C 57.84, H 5.39, N 24.86.

**4-Benzylthio-1-[1-(4-hydroxybutyl)-1,2,3-triazol-5-ylmethyl]-1H-pyrazolo [3,4-d]pyrimidine 12b.** Yield: 0.37 g (93%).  $R_f$  0.50 (90:10, CHCl<sub>3</sub>:MeOH). Appearance: syrup. <sup>1</sup>H NMR (Me<sub>2</sub>SO-d<sub>6</sub>)  $\delta$ : 1.30 (m, 2H, HOCH<sub>2</sub>CH<sub>2</sub>), 1.65 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N), 3.38 (m, 2H, HOCH<sub>2</sub>), 4.42 (t, 2H,  $J=7.14$  Hz, CH<sub>2</sub>CH<sub>2</sub>N), 4.45 (t, 1H,  $J=5.17$  Hz, HO, D<sub>2</sub>O exchangeable), 4.72 (s, 2H, SCH<sub>2</sub>), 5.86 (s, 2H, CCCH<sub>2</sub>), 7.28–7.51 (2m, 5H, C<sub>6</sub>H<sub>5</sub>), 7.68 (s, 1H, aromatic proton of triazole group), 8.41 and 8.91 (2s, 2H, H<sub>3</sub> and H<sub>6</sub>). MS,  $m/z$ :  $[M+H]^+ = 396$ ,  $[C_6H_5-CH_2]^+ = 91$ . Anal. calcd. for C<sub>19</sub>H<sub>21</sub>N<sub>7</sub>OS (395.47): C 57.70, H 5.35, N 24.79; found: C 57.90, H 5.38, N 24.96.

**4-Amino-1-[1-(4-hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-1H-pyrazolo[3,4-d]pyrimidine 13.** Solution of **11a** (0.16 g, 0.50 mmol) in 30 ml of methanol saturated with ammonia (previously saturated at –5 °C) was heated at 100 °C for 20 hours in a sealed reacting vessel. After removal of the solvent, the resulting solid was recrystallized from 95% ethanol to provide pure compound **13** (0.13 g, 90%).  $R_f$  0.30 (90:10, CHCl<sub>3</sub>:MeOH). mp: 172–173 °C (ethanol). <sup>1</sup>H NMR (Me<sub>2</sub>SO-d<sub>6</sub>)  $\delta$ : 1.33 (m, 2H, HOCH<sub>2</sub>CH<sub>2</sub>), 1.78 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N), 3.38 (m, 2H, HOCH<sub>2</sub>), 4.30 (t, 2H,  $J=7.14$  Hz, CH<sub>2</sub>CH<sub>2</sub>N), 4.46 (t, 1H,  $J=5.19$  Hz, HO, D<sub>2</sub>O exchangeable), 5.53 (s, 2H, CCCH<sub>2</sub>), 7.70 (br s, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.97 (s, 1H, aromatic proton of triazole group), 8.09 and 8.20 (2s, 2H, H<sub>3</sub> and H<sub>6</sub>). MS,  $m/z$ :  $[M+H]^+ = 289$ . Anal. calcd. for C<sub>12</sub>H<sub>16</sub>N<sub>8</sub>O (288.30): C 49.99, H 5.29, N 38.86; found: C 50.10, H 5.61, N 38.93.

**1-[1-(4-Hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-4-methylamino-1H-pyrazolo [3,4-d]-pyrimidine 14.** A solution of 0.25 g (0.62 mmol) of **11a** in 40% aqueous methylamine (6.3 ml) was refluxed. The reaction was monitored by tlc, and was

shown to be complete after 10 minutes. After cooling, the residue was coevaporated with benzene ( $5 \times 4$  ml) and the resulting solid was recrystallized from ethanol to provide crystalline **14** (0.15 g, 79%).  $R_f$  0.36 (90:10,  $\text{CHCl}_3$ :MeOH). mp: 175–176°C (ethanol).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.32 (m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 1.78 (m, 2H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 2.97 (d, 3H,  $J = 5.28$  Hz,  $\text{HNCH}_3$ ), 3.38 (m, 2H,  $\text{HOCH}_2$ ), 4.29 (t, 2H,  $J = 7.14$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 4.45 (t, 1H,  $J = 5.15$  Hz, HO,  $\text{D}_2\text{O}$  exchangeable), 5.53 (s, 2H,  $\text{CCCH}_2$ ), 7.96 (s, 1H, aromatic proton of triazole group), 8.07 and 8.29 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ), 8.27 (br s, 1H, HN,  $\text{D}_2\text{O}$  exchangeable). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 303$ . Anal. calcd. for  $\text{C}_{13}\text{H}_{18}\text{N}_8\text{O}$  (302.33): C 51.64, H 6.00, N 37.06; found: C 51.74, H 6.11, N 37.13.

**4-Benzylamino-1-[1-(4-hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-1H-pyrazolo [3,4-d]-pyrimidine 15.** To a solution of **11a** (0.20 g, 0.62 mmol) in 7 ml of absolute ethanol were added 2.42 ml of freshly distilled benzylamine and the mixture was refluxed overnight. The solvent was removed *in vacuo*, the residue was purified on a column of silica gel and then recrystallized from ethanol to give compound **15** (0.19 g, 80%).  $R_f$  0.38 (90:10,  $\text{CHCl}_3$ :MeOH). mp: 146–147°C (ethanol).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.36 (m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 1.81 (m, 2H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 3.41 (m, 2H,  $\text{HOCH}_2$ ), 4.33 (t,  $J = 7.14$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 4.49 (t, 1H,  $J = 5.18$  Hz, HO,  $\text{D}_2\text{O}$  exchangeable), 4.78 (d, 2H,  $J = 5.93$  Hz,  $\text{HNCH}_2$ ), 5.58 (s, 2H,  $\text{CCCH}_2$ ), 7.22–7.40 (m, 5H,  $\text{C}_6\text{H}_5$ ), 8.01 (s, 1H, aromatic proton of triazole group), 8.19 and 8.32 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ), 8.84 (t,  $J = 5.93$  Hz, HN,  $\text{D}_2\text{O}$  exchangeable). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 379$ ,  $[\text{C}_6\text{H}_5\text{CH}_2]^+ = 91$ . Anal. calcd. for  $\text{C}_{19}\text{H}_{22}\text{N}_8\text{O}$  (378.43): C 60.30, H 5.86, N 29.60; found: C 60.10, H 5.88, N 29.69.

**1-[1-(4-Hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-4-methoxy-1H-pyrazolo [3,4-d]pyrimidine 16.** To a solution of 0.2 g (8.6 mmol) of sodium in 50 ml of anhydrous methanol were added 1.71 g (5.38 mmol) of compound **11a**. The mixture was stirred at room temperature for 5 hours. The resulting clear solution was neutralized with Amberlite IRN 77. The resin was removed by filtration and washed with hot methanol ( $3 \times 50$  ml). The filtrate and washings were combined and evaporated under diminished pressure to provide a white solid. The latter was recrystallized from ethanol to furnish deacetylated product **16** (1.2 g, 73%).  $R_f$  0.44 (90:10,  $\text{CHCl}_3$ :MeOH). mp: 94–95°C (ethanol).  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.25 (m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 1.74 (m, 2H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 3.33 (m, 2H,  $\text{HOCH}_2$ ), 4.06 (s, 3H,  $\text{OCH}_3$ ), 4.26 (t, 2H,  $J = 7.14$  Hz,  $\text{CH}_2\text{CH}_2\text{N}$ ), 4.41 (t, 1H,  $J = 5.16$  Hz, HO,  $\text{D}_2\text{O}$  exchangeable), 5.62 (s, 2H,  $\text{CCCH}_2$ ), 8.00 (s, 1H, aromatic proton of triazole group), 8.18 and 8.58 (2s, 2H,  $\text{H}_3$  and  $\text{H}_6$ ). MS,  $m/z$ :  $[\text{M} + \text{H}]^+ = 304$ . Anal. calcd. for  $\text{C}_{13}\text{H}_{17}\text{N}_7\text{O}_2$  (303.31): C 51.47, H 5.64, N 32.32; found: C 51.35, H 5.69, N 32.43.

**1-[1-(4-Hydroxybutyl)-1,2,3-triazol-4-ylmethyl]-4-hydroxy-1H-pyrazolo [3,4-d]pyrimidine 17.** Compound **11a** (0.20 g, 0.62 mmol) was stirred in 2N NaOH (50 ml) at room temperature for three hours. The reaction was monitored by tlc and was shown to be completed at this time. After neutralization using a 2N HCl solution and filtration, the solvent was removed *in vacuo*. The residue was coevaporated with benzene (5 × 4 ml) and the resulting solid was chromatographed on a silica gel column to give crystalline **17** (0.13 g, 72%).  $R_f$  0.26 (90:10, CHCl<sub>3</sub>:MeOH). mp: 181–182 °C (ethanol). <sup>1</sup>H NMR (Me<sub>2</sub>SO-d<sub>6</sub>) δ: 1.38 (m, 2H, HOCH<sub>2</sub>CH<sub>2</sub>), 1.84 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N), 3.44 (m, 2H, HOCH<sub>2</sub>), 4.36 (t, 2H,  $J$  = 7.15 Hz, CH<sub>2</sub>CH<sub>2</sub>N), 4.49 (br s, 1H, HOCH<sub>2</sub>, D<sub>2</sub>O exchangeable), 5.59 (s, 2H, CCCH<sub>2</sub>), 8.08 (s, 1H, aromatic proton of triazole group), 8.12 and 8.16 (2s, 2H, H<sub>3</sub> and H<sub>6</sub>), 13.60 (br s, 1H, OH, D<sub>2</sub>O exchangeable). MS,  $m/z$ :  $[M + H]^+ = 290$ . Anal. calcd. for C<sub>12</sub>H<sub>15</sub>N<sub>7</sub>O<sub>2</sub> (289.29): C 49.82, H 5.22, N 33.89; found: C 49.75, H 5.23, N 33.99.

## BIOLOGICAL ASSAYS

The cytostatic and antiviral assays were carried out according to previously published procedures<sup>14,15</sup>.

The anti-tumor experiments were realized in the National Cancer Institute using the procedure published in Seminars in Oncology<sup>16</sup>.

The anti-tuberculosis assay is as described previously<sup>17</sup>.

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## REFERENCES

1. Shaeffer, H.J.; Beauchamp, L.; De Miranda, P.; Elion, G.B.; Bauer, D.B.; Collins, P. *Nature* (London) **1978**, 272, 583–585.
2. Chu, C.K.; Cutler, S.J. *J. Heterocycl. Chem.* **1986**, 23, 289–319.

3. Agrofoglio, L.A.; Challand, S.R. *Acyclic, Carbocyclic and L-Nucleosides*; Kluwer Academic Publishers, 1998.
4. El Ashry, E.S.H.; El Kilany, Y. *Advan. Heterocycl. Chem.* **1996**, *67*, 391–438; **1997**, *68*, 1–88; **1998**, *69*, 129–215.
5. Vere Hodge, R.A. *Antiviral Chemistry & Chemotherapy* **1993**, *4* (2), 67–84.
6. Larsson, A.; Alenius, S.; Johansson, N.-G.; Öberg, B. *Antiviral Research* **1983**, *3*, 77–86.
7. Harnden, M.R. *Drugs of the Future* **1989**, *14*, 347–358; and references cited therein.
8. De Miranda, P.; Good, S.S. *Antiviral Chemistry & Chemotherapy* **1992**, *3* (1), 1–8.
9. Taha, M.L.; Lazrek, H.B. *Bull. Soc. Chim. Belg.* **1995**, *104* (11), 647–652.
10. Lazrek, H.B.; Taourirte, M.; Oulih, T.; Lebtoumi, M.; Barascut, J.L.; Imbach, J.L. *Nucleosides & Nucleotides* **1997**, *16* (7–9), 1115–1118.
11. Moukha-chafiq, O.; Taha, M.L.; Lazrek, H.B.; Pannecouque, C.; Witvrouw, M.; De Clercq, E.; Barascut, J.L.; Imbach, J.L. *Nucleosides & Nucleotides* (accepted).
12. Lazrek, H.B.; Taourirte, M.; Barascut, J.L.; Imbach, J.L. *Bull. Soc. Chim. Belg.* **1996**, *105* (7), 391–395.
13. Alvarez, R.; Velasquez, S.; San-félix, A.; Aquaro, S.; De Clercq, E.; Perno, C.F.; Karlsson, A.; Balzarini, J.; Camarasa, M.J. *J. Med. Chem.* **1994**, *37*, 4185–4194 and references cited therein.
14. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309–321.
15. Witvrouw, M.; Balzarini, J.; Pannecouque, C.; Jhaumar-Laullo, S.; Esté, J.; Schols, D.; Cherepanov, P.; Schmit, J.-C.; Debyser, Z.; Vandamme, A.-M.; Desmyter, J.; Ramadas, R.; De Clercq, E. *Antimicrob. Agents Chemother.* **1997**, *41*, 262–268.
16. Grever, M.R.; Schepartz, S.A.; Chabner, B.A. *Seminars in Oncology* **1992**, *19*, 622–653.
17. Collin, L.; Franzblau, S.G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.

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