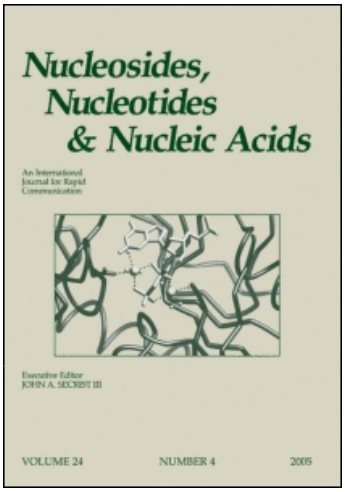

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O. Moukha-chafiq[a]; M. L. Taha[ab]; A. Mouma[a]; H. B. Lazrek[c]; J. J. Vasseur[d]; E. De Clercq[e]
[a] Faculté des Sciences, Laboratoire de Chimie Bio-Organique, Agadir, Morocco [b] Département de Chin, Faculté des Sciences, Laboratoire de Chimie Bio-Organique, Agadir, Morocco [c] Faculté des Sciences I, Laboratoire de Chimie Bio-Organique, Marrakech, Morocco [d] Laboratoire de Chimie Organique Biomoléculaire de Synthèse, U. S. T., Montpellier II, France [e] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

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Synthesis and Biological Evaluation of Some Acyclic 4,6-Disubstituted 1*H*-Pyrazolo[3,4-d]pyrimidine Nucleosides

O. Moukha-chafiq,[1] M. L. Taha,[1,*] A. Mouma,[1] H. B. Lazrek,[2] J. J. Vasseur,[3] and E. De Clercq[4]

[1]Laboratoire de Chimie Bio-Organique, Faculté des Sciences, Agadir, Morocco
[2]Laboratoire de Chimie Bio-Organique, Faculté des Sciences I, Marrakech, Morocco
[3]Laboratoire de Chimie Organique Biomoléculaire de Synthèse, U. S. T. Montpellier II, France
[4]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

ABSTRACT

The chemical synthesis and biological evaluation of some acyclic α-[6-(1′-carbamoylalkylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylamide nucleosides are described.

Key Words: Acyclic nucleosides; Pyrazolo[3,4-d]pyrimidines; Biological evaluation.

*Correspondence: M. L. Taha, Département de Chin, Faculté des Sciences, Laboratoire de Chimie Bio-Organique, BP 28/5, Agadir 80000, Morocco; Fax: +212 48 22 0100; E-mail: labd1@caramail.com.

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INTRODUCTION

The pyrazolo[3,4-d]pyrimidines are of considerable chemical and pharmacological importance as purine analogues due to their anti-tumor effects[1–3] and their strong therapeutic activity against various diseases.[4] However, only few acyclic pyrazolo[3,4-d]pyrimidine nucleosides have been reported in the literature. In this respect, we recently reported the synthesis of some acyclic α-(1*H*-pyrazolo[3,4-d]pyrimidin-4-yl) thioalkylamide nucleosides[5,6] equipped with the acyclic chains of the acyclovir,[7] HBG[8] and iso-DHPG[9] (Fig. 1). Some of these compounds showed modest activity against some DNA viruses. Along these lines, we attempted at synthesizing, through modification at the C_6 position of pyrazolo[3,4-d]pyrimidine ring, some acyclic α-[6-(1′-carbamoylalkylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylamide nucleosides (Schs. 1, 2 and 3) and determined their biological activity.

RESULTS AND DISCUSSION

The 1*H*-pyrazolo[3,4-d]pyrimidin-4,6-dithione **6**, depicted in Sch. 1, was prepared in five steps according to the literature procedure[10,11] from malononitrile and triethyl orthoformate as starting materials. The C_4 and C_6 sulfur atoms of the heterocycle **6** were alkylated with ethyl bromoacetate **7**, (DL)-ethyl-2-bromopropionate **8** or (DL) -ethyl-2-bromobutyrate **9** in a sodium hydroxide solution at room temperature to give regioselectively ethyl α-[6-(1′-carboethoxyalkylthio) -1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylates **10a–c** (Sch. 1) in good yield.

The preparation of the protected acyclic nucleosides **(14–16)a–c** (Sch. 2) was achieved using the same conditions as previously described for the synthesis of some N_1-acyclic 4-substituted pyrazolo[3,4-d]pyrimidine nucleosides.[12,13] Thus, the alkylation of heterocycles **10a–c**, separately with alkylating agents **11**,[14] **12**[13,15] or **13**,[16] using solid-liquid phase transfer catalysis method in which potassium tert-butoxide

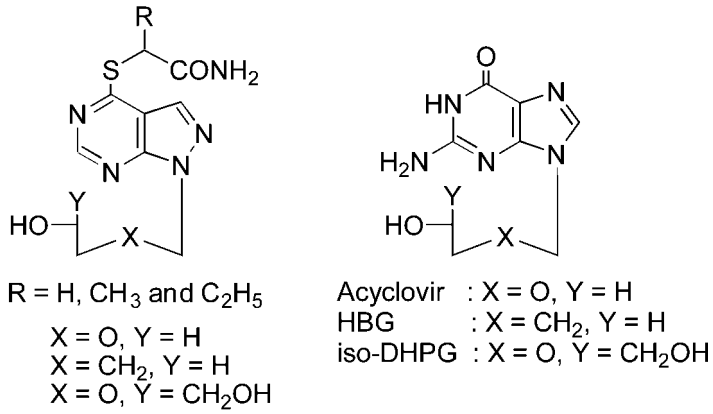

Figure 1.

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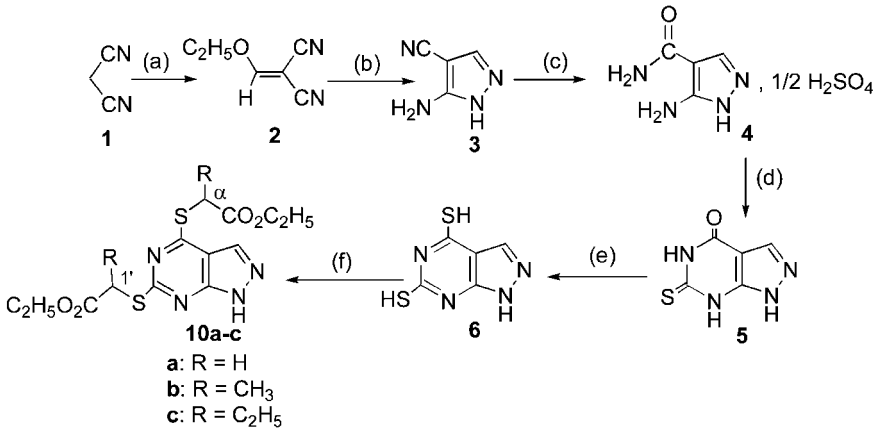


(a) : triethylorthoformate/acetic anhydride/ reflux; (b) : H_2NNH_2, r.t.; (c) : H_2SO_4; (d) : thiourea / reflux; (e) : P_2S_5 / pyridine; (f) : $BrCH_2CO_2C_2H_5$: **7**, (DL)-$BrCH(CH_3)CO_2C_2H_5$: **8** or (DL)-$BrCH(C_2H_5)CO_2C_2H_5$: **9** in NaOH (1N), r.t.

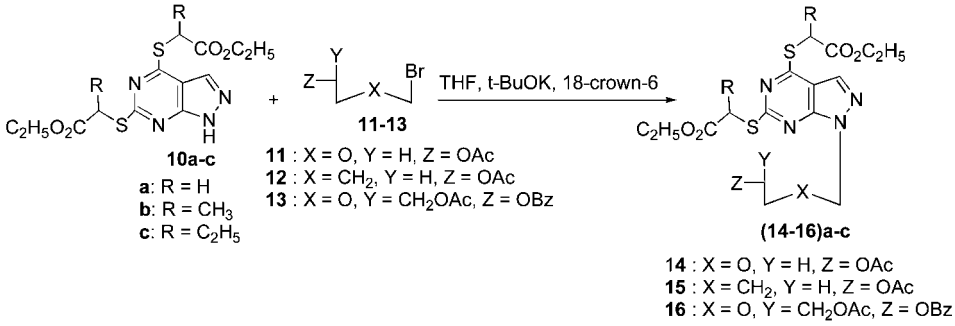
Scheme 1.

was used as alkali, tetrahydrofuran as solvent and 18-crown-6 as catalyst, afforded regioselectively the N_1-regioisomers **(14–16)a–c**, respectively, in good yield.

It was reported that N_2-nucleoside (N_2-acyclonucleoside) formation occurred during glycosylation (alkylation) of the pyrazolo[3,4-d]pyrimidines.[17–23]. In our case, the presumed N_2-regioisomers of **(14–16)a–c** were detected in only trace amounts but not isolated.

Then, the treatment of N_1-protected acyclic nucleosides **(14–16)a–c** with a solution of methanolic ammonia at room temperature gave the deprotected acyclic nucleosides **(17–19) a–c** (Sch. 3) in quantitative yield, through removing of the acetyl and benzoyl groups and concomitant conversion of the esters into the amide moieties.

The site of alkylation in compounds **10a–c** was established to be at N_1 by a direct comparison of the UV spectra of the compounds **(17–19)a–c** with the UV spectra of the corresponding N_1-pyrazolo[3,4-d]pyrimidine nucleosides.[24]



Scheme 2.

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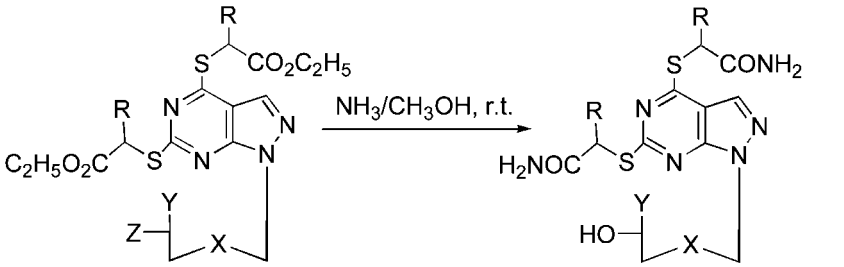

(14-16)a-c **(17-19)a-c**

14 : X = O, Y = H, Z = OAc
15 : X = CH_2, Y = H, Z = OAc
16 : X = O, Y = CH_2OAc, Z = OBz

17 : X = O, Y = H
18 : X = CH_2, Y = H
19 : X = O, Y = CH_2OH

Scheme 3.

All structures of the synthetic products were identified by ^1H NMR, mass spectra, UV and/or elemental analysis (for example, compound 18a).[a]

BIOLOGICAL STUDIES

The acyclic nucleosides **(17–19)a–c** were evaluated against cytomegalovirus [CMV (AD-169 and Davis strains)] and varicella-zoster virus [TK^+ VZV (YS and OKA strains) and TK^- VZV (07/1 and YS/R strains)] in human embryonic lung (HEL) fibroblasts. No significant antiviral activity or cytotoxicity was noted at 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 activity was noted against *M.tuberculosis* at concentrations up to 6.25 μg/mL.

In conclusion, we have regioselectively synthesized some α-[6-(1′-carbamoylalkylthio)()-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioalkylamide nucleosides with the alkyl chains of acyclovir, HBG and iso-DHPG. No significant anti-CMV; anti-VZV or anti-tuberculosis activity was witnessed. Further antiviral and anti-tumor evaluation is in progress.

[a]**α-[1-(4-hydroxybutyl)-6-carbamoylmethylthio-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]thioacetamide 18a**: Yield: 87%. R_f : 0.12 ($CHCl_3$:CH_3OH, 90:10, v:v). Appearence : liquid. **UV** (methanol) $\lambda_{max} = 250$ nm ($\varepsilon = 19\,200$). ^{1}H-NMR (Me_2SO-d_6, 250 MHz) δ : 1.35 (m, 2H, $HOCH_2CH_2$), 1.84 (m, 2H, CH_2CH_2N), 3.42 (m, 2H, $HOCH_2CH_2$), 3.92 (s, 2H, 6-SCH_2), 4.11 (s, 2H, 4-SCH_2), 4.33 (t, $J = 6.86$ Hz, 2H, CH_2CH_2N), 4.44 (t, $J = 5.17$ Hz, 1H, HO, D_2O exchangeable), 7.23, 7.32, 7.66 et 7.74 (4sl, 4H, 2 NH_2, D_2O exchangeable), 8.28 (s, 1H, H_3). MS (FAB^+, NBA) *m/z*: 371 $[M+H]^+$. elemental analysis calculated 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%.

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