Nucleosides, Nucleotides, and Nucleic Acids, 26:1269–1271, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701528354

Taylor & Francis
Taylor & Francis Group

SULFUR CONTAINING ACYCLOVIR DERIVATIVES: SYNTHESIS, CYTOTOXIC ACTIVITY, AND CELL PHENOTYPE STUDIES

Martins Ikaunieks, Irina Shestakova, Ilona Domracheva, Sergey Belyakov, and Marina Madre □ *Latvian Institute of Organic Synthesis, Riga, Latvia*

□ *New 2-amino-6-oxo-8-thioxo-9-substituted purine derivatives were prepared and assayed for the in vitro cytotoxic activity. Some products exhibited moderate activity on HT-1080 cells and rather high activity on MG-22A cells.*

Keywords Sulfur; acyclovir; antiherpetic drugs

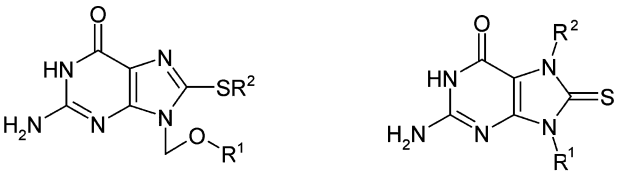
INTRODUCTION

Acyclovir is a well-known highly potent and selective antiherpetic drug. At the same time, it has been found to have the growth inhibitory activity against murine leukemia L 1210,[1a] but some of its 8-substituted or/and 1,N-2-bridged (tricyclic) analogues have demonstrated moderate activity and remarkable cytotoxic selectivity against KB and HeLa tumour cells.[1b] This article describes the synthesis and the in vitro cytotoxic activity of a series of new 2-amino-6-oxo-8-thioxo-9-(2-hydroxyethoxymethyl)purine derivatives as well as several 2-amino-6-oxo-8-thioxopurines bearing an alternative substituent at position 9 of the heterocycle.

RESULTS AND DISCUSSION

The synthesized derivatives of 2-amino-6-oxo-8-thioxopurine are listed in Figure 1. Products **2a–c** were prepared and described previously.[2a] Compounds **1a–d** were obtained by N, O- or N-deacetylation of the corresponding 2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurines.[2a] The interaction of 8-(2-hydroxyethyl)thiopurine **1b** with adipic anhydride led to the formation of dicarboxylic acid **1f.** The N,N-dimethylaminomethylene protecting group removal in 8-(ethoxycarbonylmethyl)thiopurine

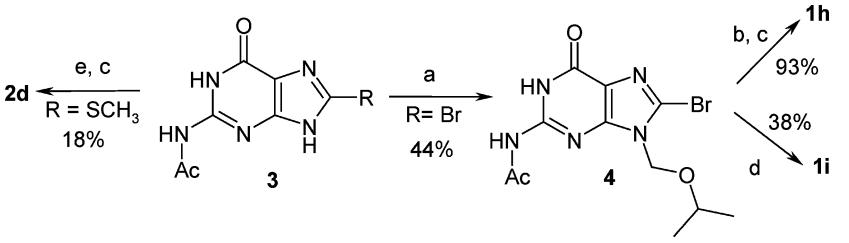
Address correspondence to M. Ikaunieks, Latvian Institute of Organic Synthesis, Riga, Latvia. E-mail: ikaunieks@osi.lv



1a $R^1=(CH_2)_2OH$, $R^2= CH_2CH=CH_2$; **1b** $R^1=R^2=(CH_2)_2OH$; **1c** $R^1=(CH_2)_2OH$, $R^2=CH_2C_6H_3$-3,4-diCF_3; **1d** $R^1=(CH_2)_2OAc$, $R^2=CH_2OC_{18}H_{37}$; **1e** $R^1=(CH_2)_2OAc$, $R^2=C_6H_5$; **1f** $R^1=R^2=(CH_2)_2OCO(CH_2)_2COOH$; **1g** $R^1=(CH_2)_2OH$, $R^2= CH_2CONHNH_2$; **1h** $R^1=CH(CH_3)_2$, $R^2=H$; **1i** $R^1=CH(CH_3)_2$, $R^2=C_6H_4$-3-OCH_3; **2a** $R^1=CH_2O(CH_2)_2OH$, $R^2=CH_2OC_8H_{17}$; **2b** $R^1=CH_2O(CH_2)_2OH$, $R^2=CH(CH_3)OC_4H_9$; **2c** $R^1=CH_2O(CH_2)_2OCH(CH_3)OC_4H_9$, $R^2=CH(CH_3)OC_4H_9$; **2d** $R^1=R^2= CH_2C_6H_5$

FIGURE 1 Structural formulas of 2-amino-6-oxo-8-thioxopurine derivatives.

derivative[2b] with hydrazine hydrate brought about simultaneous amidation of the ester fragment yielding product **1g**. For the incorporation of a phenylthio substituent at position 8 of the purine cycle (**1e**) the reaction of 2-acetamido-9-(2-acetoxyethoxymethyl)-8-bromo-6-oxopurine with PhSH/NaOAc/EtOH system was used. Simultaneous splitting of the N-acetyl protecting group occurred during this reaction yielding **1e** in one step. Scheme 1 presents the synthesis of products **1i, h** via intermediate **4** prepared by alkylation of purine **3** (R = Br) with isopropoxymethyl chloride. The formation of the 9- and 7-alkoxyalkylated products in equal ratio and in good overall yield (88%) was observed in this reaction. The transformation of **4** into compound **1i** was carried out via routine thionation and deprotection.[2c] To obtain product **1h** intermediate **4** was treated with 3-methoxyphenyl thiol similar to the synthesis of **1e**. 7,9- Dibenzyl-8-thioxopurine **2d** was obtained by alkylation of **3** (R = SCH_3) with benzyl bromide and isolated alongside with the corresponding 7-benzyl- and 9-benzyl-8-methylthiopurine. The structures of compounds **1, 2** were supported by ^1H NMR spectra and elemental analysis data as well as by single crystal X-ray analysis for product **2d**.[3] The cytotoxic activity of



SCHEME 1 Reagents: (a) $ClCH_2OCH(CH_3)_2$, Et_3N, THF; (b) $Na_2S_2O_3$, $AlCl_3$, H_2O; (c) $MeNH_2$, H_2O (d) ArSH, NaOAc, MeOH, H_2O; (e) BnBr, K_2CO_3, DMF.

TABLE 1 In vitro cytotoxicity of 8-thioxopurine derivatives **1**, **2** on monolayer tumor cell lines HT-1080, MG-22A, and on normal mouse fibroblasts cells (NIH 3T3)

	HT-1080		MG-22A		NIH 3T3	
Cmpd.	TD_{50}[a]	NO, 100%	TD_{50}	NO, 100%	TD_{50}	LD_{50} mg/kg
1d	0.024	367	0.003	275	0.127	1000
1e	0.099	75	NA[b]	9	2.664	2403
1i	0.019	650	NA	20	0.278	872
2a	0.200	28	NA	17	2.503	2517
2d	0.048	233	0.018	300	0.036	360

[a]TD_{50}-Concentration (mole/l $\times$ 10^3) providing 50% cell killing effect [(CV+ MTT)/2]; NO Concentration (%).
[b]NA-inactive.

products **1, 2** as well as their influence on cell morphology were tested in vitro on monolayer tumour cell lines HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma) and on normal mouse fibroblasts cells (NIH 3T3). The results obtained are summarized in Table 1. Compound **1a–c**, **f; 2b, c** were inactive in both test systems (data not shown). However, the inspection of the cell morphology demonstrated that one of these compounds, namely **1h** dramatically increased the speed of fibrosarcoma cell growth. Products **1d,e,i** and **2a,d** exhibited moderate cytotoxic effect on HT-1080 cell line but derivatives **1d, 2d** had rather high activity also on MG-22A cells. All cytotoxic products showed low acute toxicity except for **2d** that had similar values on the three cell lines.

REFERENCES

1. a) Nishimaki, J.; Miyazawa, K.; Gotoh, A.; Yoshikawa, O.; Ohyashiki, K.; Toyama, K. Inhibitory effect of a nucleoside analog, acyclovir, on leukemia cells. *Leukemia Res.* **1996**, 20, 415–420; b) Hladon, B.; Goslinski, T.; Laskowska, H.; Baranowski, D.; Ostrowski, T.; Zeidler, J.; Ruskowski, P.; Golankiewicz, B. *In vitro* cytostatic activity of 8-substituted and tricycylic analogues of acyclovir. *Pol. J. Pharmacol.* **2002**, 54, 45–53.
2. a) Ikaunieks, M.; Madre, M. Purine nucleoside analogues. 12. Synthesis of new 8,9-disubstituted guanine derivatives by S-alkylation of 8-thio-9-(2-acetoxyethoxymethyl)-N^2-acetylguanine. *Chem. Heterocycl. Comp.* (Engl. Ed.) **2003**, 2, 274–280; b) Ikaunieks, M.; Madre, M. Efficient synthesis of 8-thiosubstituted guanine derivatives as potential tools for biochemical and biological studies. *Nucleosides, Nucleotides Nucleic Acids* **2003**, 22, 755–758; c) Ikaunieks, M.; Madre, M. Reinvestigation of the reaction of 8-bromoguanine derivatives with sodium thiosulfate. *J. Chem. Res.* (S) **2002**, 226–227.
3. Selected data for the synthesized compounds. **1e**: m.p. 215–217°C. ^{1}H-NMR (if not stated otherwise, 200 MHz, DMSO-d_6, δ): 1.91 (s, 2H, CH_3); 6.64 (s, 2H, NH_2); 7.26–7.38 (m, 5H, ArH), **1h**: m.p. >250°C, ^{1}H-NMR: 1.07 (d, 6H, 2 $\times$ CH_3, J = 6.8 Hz); 3.99 (septet, 1H, CH, J = 6.8 Hz); 5.36 (s, 2H, CH_2); 6.70 (s, 2H, NH_2); 10.93 (bs, 1H, NH); 12.85 (bs, 1H, NH), **1i**: m.p. 218–220°C. ^{1}H-NMR: 3.71 (s, 3H, CH_3); 6.67 (s, 2H, NH_2); 6.80–6.90 (m, 3H, ArH); 7.22–7.29 (m, 1H, ArH), **2d**: m.p. 259–260°C, ^{1}H-NMR: 5.28 (s, 2H, CH_2); 5.50 (s, 2H, CH_2), 6.76 (s, 2H, NH_2); 7.20–7.42 (m, 10H, Ar-H); 10.82 (bs, 1H, NH). X-ray crystal structure CCDC deposition number 289468.