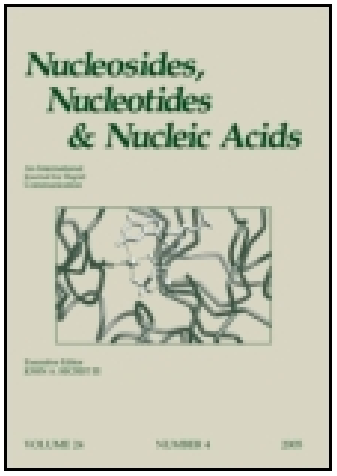

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DESIGN AND SAR STUDY OF A NOVEL CLASS OF NUCLEOTIDE ANALOGUES AS POTENT ANTI-HCMV AGENTS.

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ABSTRACT: We have developed a novel class of 2-phosphonate 1,3-dioxolane nucleotide analogues, from which the guanine derivative displayed weak anti-HCMV activity. Further SAR studies led to the identification of both *cis* and *trans* guanine derivatives of tetrahydrofuran analogues as potent anti-HCMV agents, both *in vitro* and *in vivo,* compared to ganciclovir and HPMPC.

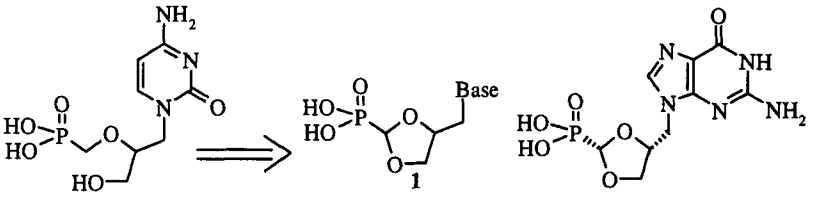
INTRODUCTION

Human Cytomegalovirus (HCMV) infections pose a serious problem for individuals whose immune system has been compromised by disease, such as AIDS or by medication as in organ transplant recipients[1]. Although the current therapies for treatment of HCMV are effective, they all suffer from serious toxic effects such as nephrotoxicity (Foscarnet and HPMPC) and myelotoxicity (Ganciclovir); there is therefore a need for better HCMV drugs.

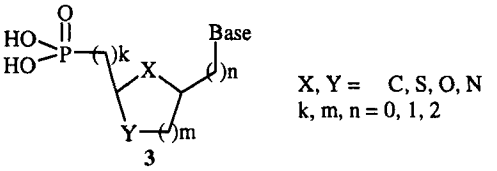
We and others have described novel classes of acetal and thioacetal nucleoside analogues such as dioxolane guanine (DXG) and 3TC™ as potent anti-HIV and HBV agents by replacement of C-3' of 2',3'-dideoxynucleosides by a heteroatom such as oxygen or sulfur[2]. In fact, 3TC™ has been approved for treatment of HIV infection in combination with other anti-HIV agents. However, these compounds were found to be inactive against HCMV, possibly due to the lack of phosphorylation.

The success of the acetal and thioacetal nucleosides in combination with HPMPC inspired us to design a novel class of nucleotides in which the oxygen moiety of the terminal hydroxyl group is tethered to the carbon α to the phosphonate. The outcome is a

series of 1,3-dioxolane nucleotide analogues **1**, where the phosphonate group is directly attached to C-2 of the ring and the base is separated from the ring by a methylene group. The series was completed with the synthesis of pyrimidine and purine derivatives in racemic form and one of them, the guanine analogue displayed weak anti-HCMV activity. Chiral synthesis of both enantiomers revealed that the antiviral activity (IC_{50} = 45 μg/mL) resided in the (+)-enantiomer **2** ($[\alpha]_D$ +35.7° (*c* 0.26, H_2O)) having *2S,4R* configuation[3].

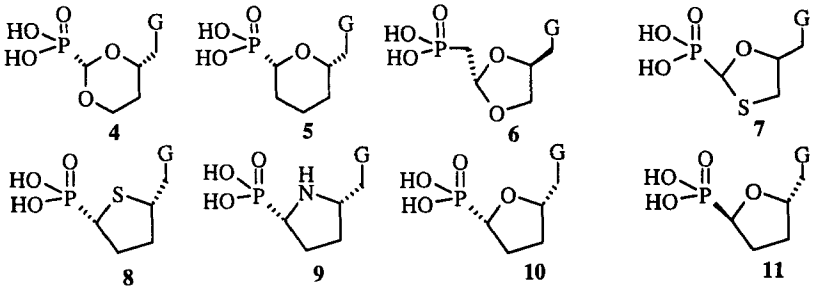


The finding of **2** gave us impetus to undertake a systematic SAR study to assess the role of the heteroatom in dioxolane ring, the ring size and the distance between the phosphonate moiety and the base as shown in the generic structure **3**.

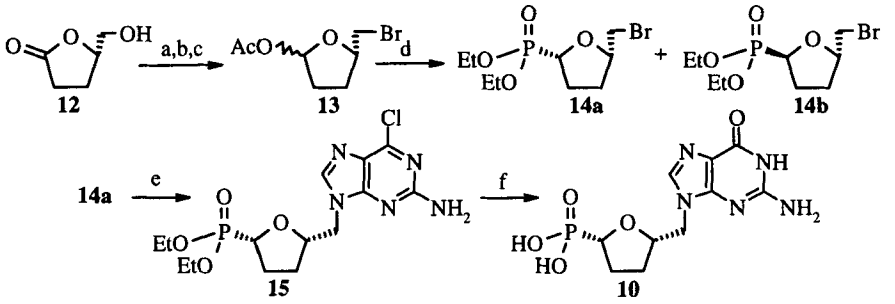


RESULTS AND DISCUSSION

SAR Study: An antiviral activity was abolished by enlargement of ring size by one carbon unit as in the cases of 1,3-dioxane **4** and pyran **5** analogues. On the contrary, insertion of one carbon unit between phosphonate moiety and the C-2 of dioxolane ring increased antiviral activity as in the case of **6** (IC_{50} of 1.5 μg/mL). It is interesting to note that **6** has a *trans* configuration.



Scheme 1



a) CBr_4, Ph_3P, MeCN, 84%; b) DIBAL-H, toluene, -78°C, 77%; c) Ac_2O, pyridine, DMAP, CH_2Cl_2, 86%; d) $TiCl_4$, $P(OEt)_3$, CH_2Cl_2, -30°C, 86%; e) Cs_2CO_3, 2-amino-6-chloropurine, DMF, 95°C, 45%; f) TMSBr, CH_2Cl_2, then H_2O, reflux, 66%.

Furthermore, modification of heteroatoms in the 1,3-dioxolane ring resulted in loss of antiviral activity in most cases, for examples in 1,3-oxathiolane **7** or tetrahydrothiophene **8** or pyrrolidine **9** analogues. However, a fruitful result was obtained by replacing O-3 of dioxolane analogue **2** with carbon to give a series of tetrahydrofuran analogues[4]. In this series the *cis* and *trans* isomers of guanine derivatives **10** and **11** displayed significant *in vitro* activity against Human Cytomegalovirus comparable to ganciclovir and HPMPC.

Tetrahydrofuran Analogues: The synthesis of target molecules **10** and **11** is described in **Scheme** 1. The commercially available (Aldrich) (-)-(5S)-5-hydroxymethyl-tetrahydrofuran-2-one **12** was first converted to the corresponding bromide[5], followed by reduction and acetylation under standard conditions gave the acetate **13** in 66% yields as a 1:1 mixture of isomers. The phosphonate group was then introduced into the tetrahydrofuran moiety using Arbuzov reaction, catalyzed by a Lewis acid to give the corresponding phosphonates **14a,b** in 80% yield as a 1:1 mixture of *cis* and *trans* isomers. The mixture was readily separable by flash chromatography (10-20% acetone in hexanes) and each isomer was then treated independently. Displacement of bromide **14a** with 2-amino-6-chloropurine in the presence of Cs_2CO_3 in DMF at 95°C gave the corresponding 2-amino-6-chloropurine nucleotide analogue **15** in 45% yield[6]. Removal of phosphonate ethyl ester was achieved by treatment with excess bromotrimethylsilane (TMSBr) , followed by conversion of 2-amino-6-chloropurine derivative to corresponding guanine derivative **10** ($[\alpha]_D$+52° (*c* 0.10, H_2O)), by

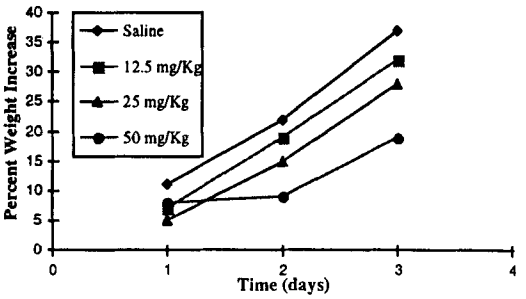


FIG. 1: Tolerance of 10 in mice.

refluxing in water in 66% yield. The corresponding *trans* analogue **11** ($[\alpha]_D$ -9° (*c* 0.26, H_2O)) was similarly obtained starting from **14b**. The optical antipodes of both compounds described were also prepared in order to compare their biological activity and to ascertain that the active entities also have the same configuration as dioxolane **2**.

***In Vitro* Activity**: The anti-HCMV activity of the tetrahydrofuran phosphonates **10, 11** and their optical antipodes was measured by a plaque reduction assay in the Wi-38 cell line and the cytotoxicity was determined by inhibition of cell proliferation in the same cell line. These results were compared with that of ganciclovir (GCV) and HPMPC. The *cis* isomer **10** had an IC_{50} of 0.5-1 μg/mL and a CC_{50} of 10-50 μg/mL and is equipotent to GCV (IC_{50} of 0.3 μg/mL and a CC_{50} of 12.5 μg/mL) whereas the *trans* isomer **11** with an IC_{50} of 0.1-1μg/mL and a CC_{50} of 10-100μg/mL is comparable to that of HPMPC (IC_{50} of 0.1-1 μg/mL and a CC_{50} of 10-50 μg/mL). The antipodes of **10** and **11** were found to be inactive. It is apparent that activity was limited to the analogues with the similar configuration as **2** and the centre bearing the guanine moiety is crucial for antiviral activity whereas the relative position of the phosphonic acid is not important.

***In Vivo* Efficacy:** Both active tetrahydrofuran guanine compounds **10** and **11** were selected for evaluation of *in vivo* efficacy in mice infected with Murine Cytomegalovirus (MCMV). Since no convenient animal model for HCMV exists, the murine model serves as a useful surrogate for determination of *in vivo* activity. Tolerance studies as a function of body weight were performed prior to compounds administration.

10 was well tolerated in CRH mice at doses of 12.5 and 25 mg/kg/day 3 times per day for 4 days when administered *i.p.* (**FIG. 1**), but at 50 mg/kg the rate of weight increase was less than placebo indicating presence of sub-acute toxicity. Complete

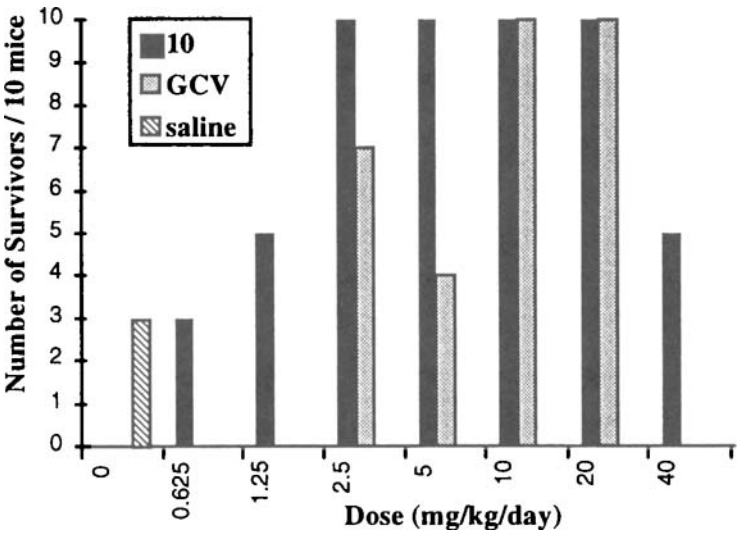


FIG. 2: *In vivo* efficacy of 10 and GCV.

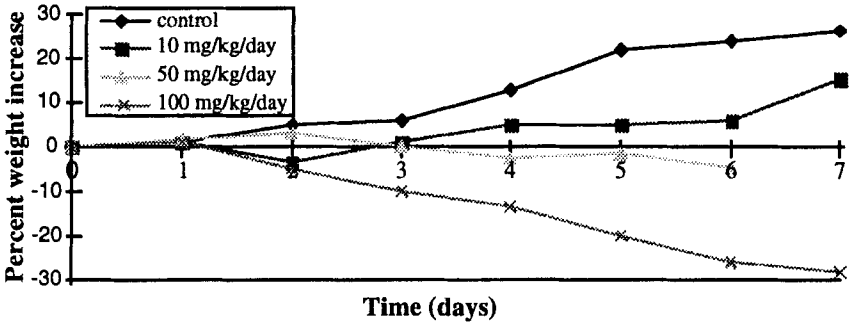


FIG. 3: Tolerance of 11 in mice.

protection from death was achieved at doses ≥ 2.5 mg/kg whereas to achieve the same degree of protection with ganciclovir, a dose of 10 mg/kg was required (**FIG. 2**). Thus **10** is approximately 3-4 times as active *in vivo* as ganciclovir. No cytotoxicity related deaths were observed at doses up to 20 mg/kg; however, at 40 mg/kg, **10** was found to be toxic to the animals.

11 was administered *i.p.* to BALB/c mice at dosages ranging from 10 to 100 mg/kg/day twice a day for 6 days (**FIG. 3**). **11** was found to have a lethal toxic effect at 100 mg/kg/day whereas a slight weight loss was observed at the 50 mg/kg/day dose. A

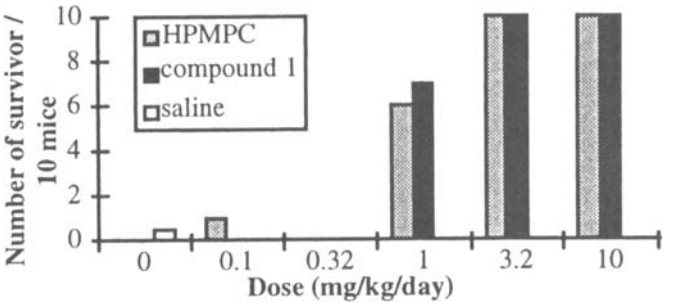


FIG. 4: ***In vivo*** **efficacy of 11 and HPMPC.**

weight gain was noticed with the 10 mg/kg/day dose which was the highest dose used in the *in vivo* efficacy study. Both compounds were found to have approximately equal anti-HCMV activity at using dosages with regard to the prevention of MCMV-induced death and prolongation of mean day to death. At 1 mg/kg/day 70% of mice were protected from death by **11** in comparison with 60% by HPMPC. Complete protection were achieved by **11** and HPMPC at 3.2 and 10 mg/kg/day (**FIG. 4**).

Summary: We have described the design and SAR study of a novel class of 1,3-dioxolane nucleotide analogues. Compounds **10** and **11** displayed *in vitro* and *in vivo* anti-HCMV activity comparable to ganciclovir and HPMPC.

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