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Synthesis of Aldehydo-sugar Derivatives of Pyrazoloquinoline as Inhibitors of Herpes Simplex Virus Type 1 Replication

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Synthesis of a novel series of structurally related pyrazoloquinoline nucleosides is described. All the newly synthesized compounds were examined for their *in vitro* antiviral activity against herpes simplex type-1 as shown by two different bioassays, namely; crystal violet staining or the MTS tetrazolium dye measurement. The acute toxicity (LD_{50}) values of the biologically active compounds were determined.

Keywords: Pyrazoloquinoline nucleosides; cytotoxicity; anti-HSV-1; acute toxicity

INTRODUCTION

Herpes simplex virus (HSV) infection is an ancient disease, with descriptions of orolabial herpes appearing in records from the fifth century BC.[1] HSV remains a common cause of ulcerative mucocutaneous diseases even today.[2–4] Due to the high prevalence of HSV infection worldwide, most persons infected with HIV are also infected with one or both HSV types.[5] Initial infection results in painful ulcerative mucosal and cutaneous lesions. Following resolution of the initial infection, HSV establishes a latent infection in sensory ganglia, from which symptomatic recurrences may develop periodically. In individuals who are co-infected with HIV and HSV, the frequency and extent of HSV recurrences may vary from mild to severe. It was postulated that there is an association between increased frequency and increased severity of HSV recurrences and advanced immunosuppression.[6] Antiviral drugs provide safe and effective therapy for many HSV infections. Three intravenous medications (acyclovir, vidarabine, and foscarnet) were found to be effective in severe HSV infections, and may be lifesaving in the immunocompromised host. Oral acyclovir[7] is useful in the treatment of mild to moderate HSV infections, and allows for successful outpatient management. Two newer oral antiviral agents, famciclovir[8] and valacyclovir,[9] were found to be effective in HSV infected patients, although neither drug has been extensively studied. Furthermore, dendrimers are macromolecules with broad-spectrum antiviral activity and minimal toxicity, being effective in animal models in preventing transmission of HSV infection. In an attempt to explain the mechanism of action and toxicity profiles of the dendrimer SPL-2999 against HSV, it was postulated that SPL-2999 inhibits both HSV entry into susceptible cells and the late stages of HSV replication. Data indicate that SPL-2999 is a potent inhibitor of both HSV-1 and HSV-2.[10] Moreover, *N*-methanocarbathymidine [(N)-MCT], a thymidine analog incorporating a pseudosugar with a fixed Northern conformation, was found to exhibit potent antiherpetic activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2). These results provide a biochemical rationale for the highly selective

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and effective inhibition of HSV-1 by (N)-MCT.[11] Moreover, it was discovered that the antiviral agent 5-chloro-1,3-dihydroxyacridone interferes with the assembly and maturation of herpes simplex virus.[12] Analysis of a large compound library in a high throughput virus infection assay screen identified the benzothiophene PD146626 as a potent and specific inhibitor of HSV-1 replication. PD146626 was found to possess an EC_{50} and EC_{90} against HSV-1 of 0.1 and 1 μM, respectively, and mediated no detectable cytotoxicity in cells at concentrations up to 1 μM.[13] Motivated by the high chemical diversity of inhibitors of HSV-1 replication and as a continuation of our research program in the field of designing antivirals[14–16] as well as developing the chemistry of pyrazoloquinoline,[17] it became of interest to carry out the screening of the newly synthesised pyrazoloquinoline nucleosides as inhibitors of HSV-1 replication.

EXPERIMENTAL

Chemistry

All chemicals used in this study were purchased from E. Merck (Darmstadt, Germany), Fluka AG (Buchs, Switzerland) and Aldrich (St. Louis, MO, USA). Melting points were determined in open glass capillaries using a Thomas capillary melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on a 470-Shimadzu infrared spectrophotometer using the KBr disc technique. The ^{1}H NMR spectra were recorded on a Jeol-400 MHz spectrometer (DMSO-d_6), and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s; singlet; d: doublet; m: multiplet. Elemental analyses were performed on a Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.4% of the theoretical values. Follow up of the reactions and checking of the homogenity of the compounds was made using TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) with chloroform:methanol (1:1 v/v) as a mobile phase and the spots were detected by exposure to a UV-lamp at 254 nm.

General Procedure for the Synthesis of Aldehydo-sugar {1H-pyrazolo[3,4- b] Quinoline-3-yl} Imines 4–18

A solution of the selected aldohexose (D-glucose, D-mannose or D-galactose) or aldopentose (D-arabinose or D-xylose) (10 mmol) in water (0.5 ml) was added to a solution of the appropriate 3-amino-1H-pyrazolo[3,4-b]quinoline **1–3** in dioxane (10 ml) and the mixture was heated under reflux for 3 h on a boiling water bath. The solid product separated at room temperature was filtered, dried and crystallised from ethanol. Physical and analytical data of compounds **4–18** are recorded in Table III.

IR (cm^{-1}) of compounds **4–18**: 3414 − 3232 (OH), 3154 − 3138 (NH), 1634 − 1622 (C═N).

ALDEHYDO-D-GLUCOSE{1H-PYRAZOLO[3,4-B]QUINOLINE-3-YL}IMINE **4**

δ ^{1}H-NMR (DMSO-d_6): δ 3.56 (s, 1H, OH, D_2O exchangeable), 4.26 (s, 1H, OH, D_2O exchangeable), 4.45–4.49 (d, J = 9 Hz, 1H, OH, D_2O exchangeable), 4.61 (d, 1H, OH, D_2O exchangeable), 4.80–4.84 (m, 2H, alditolyl-H), 4.85 (d, 1H, alditolyl-H), 4.91 (m, 1H, OH, D_2O exchangeable), 5.95 (d, J = 4.4 Hz, 1H, OH, D_2O-exchangeable), 7.26 (d, J = 9.52 Hz, 1H, C*H*═N), 7.37(t, J = 7.3 Hz, 1H, py-quin C-6-H), 7.69 (t, J = 7.3 Hz, 1H, py-quin C-7-H), 7.85 (d, J = 7.3 Hz, 1H, py-quin C-5-H), 8.00 (d, J = 8.0 Hz 1H, py-quin C-8-H), 8.75 (s, 1H, py-quin C-4-H), 11.76 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

ALDEHYDO-D-GALACTOSE{1H-PYRAZOLO[3,4-B]QUINOLINE-3-YL}IMINE **5**

δ 3.77 (t, J = 6.6 Hz, 1H, OH, D_2O exchangeable), 4.26 (s, 1H, OH, D_2O exchangeable), 4.47 (d, J = 9 Hz, 1H, OH, D_2O exchangeable), 4.91 (d, J = 6.0 Hz, 1H, OH, D_2O-exchangeable), 6.09–6.11 (m, 1H, OH, D_2O exchangeable), 7.37 (t, J = 7.3 Hz, 1H, py-quin C-6-H), 7.69 (2s,2H, py-quin C-7-H & C*H*═N), 7.85 (d, J = 7.32 Hz, 1H, py-quin C-5-H), 8.00 (d, J = 8.0 Hz, 1H, py-quin C-8-H), 8.76 (s, 1H, py-quin C-4-H), 11.77 (s, 1H, NH, D_2O exchangeable), other protons were congregated with solvent absorption at δ = 3.5 ppm.

ALDEHYDO-D-MANNOSE{1H-PYRAZOLO[3,4-B]QUINOLINE-3-YL}IMINE **6**

δ 3.66–3.70 (m, 1H, OH, D_2O exchangeable), 4.26 (s, 1H, OH, D_2O exchangeable), 4.48 (d, J = 9 Hz, 1H, OH, D_2O exchangeable), 4.91 (d, J = 5 Hz, 1H, OH, D_2O exchangeable), 6.12 (d, J = 4.4 Hz, 1H, OH, D_2O exchangeable), 7.31 (d, J = 9.52 Hz, 1H, C*H*═N), 7.40 (t, J = 7.8 Hz,1H, py-quin C-6-H), 7.72 (t, J = 6.9 Hz 1H, py-quin C-7-H), 7.84 (d, J = 7.7 Hz 1H, py-quin C-5-H), 8.01 (d, J = 8.06 Hz,1H, py-quin C-8-H), 9.05 (s, 1H, py-quin C-4-H), 12.07 (s, 1H, NH, D_2O exchangeable), other protons were gathered with solvent absorption at δ = 3.5 ppm.

ALDEHYDO-D-ARABINOSE{1H-PYRAZOLO[3,4-B]QUINOLINE-3-YL}IMINE **7**

δ 4.61(d, J = 2.92 Hz, 2H, alditolyl-H), 4.82–4.84 (m, 2H, alditolyl-H), 4.85 (d, J = 5.16 H, 1H, alditolyl-H), 4.89–4.91(m, 1H, OH, D_2O exchangeable), 5.95 (s, 1H, OH, D_2O exchangeable), 7.26 (d, J = 9.5 Hz, 1H, C*H*═N), 7.37 (t, J = 7.7 Hz, 1H, py-quin C-6-H), 7.71 (t, J = 7.7 Hz 1H, py-quin C-7-H), 7.87 (d, J = 8.8 Hz, 1H, py-quin C-5-H), 8.03 (d, J = 7.32 Hz, 1H, py-quin

C-8-H), 8.87 (s, 1H, py-quin C-4-H), 11.90 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Xylose{1H-pyrazolo[3,4-b]quinoline-3-yl}imine **8**

δ 4.60 (d, J = 5.9 Hz, 1H, OH, D_2O exchangeable), 4.85–4.87 (m, 2H, 2OH, D_2O exchangeable), 4.89 (d, J = 5.2 Hz, 1H, alditolyl-H), 7.25 (d, J = 9.52 Hz, 1H, C*H*═N), 7.39 (t, J = 6.6 Hz, 1H, py-quin C-6-H), 7.71 (t, J = 7.7 Hz, 1H, py-quin C-7-H), 7.86 (d, J = 8.8 Hz, 1H, py-quin C-5-H), 8.01 (d, J = 7.2 Hz, 1H, py-quin C-8-H), 8.86 (s, 1H, py-quin C-4-H), 11.97 (s, 1H, NH D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Glucose{7-methyl-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **9**

δ 2.49 (s, 3H, CH_3), 4.52 (t, J = 5.8 Hz, 1H, OH, D_2O exchangeable), 4.64 (d, J = 5.1 Hz, 1H, alditolyl-H), 4.77 (d, J = 5.1 Hz, 1H,OH, D_2O exchangeable), 4.84–4.88 (m, 1H, alditolyl-H), 4.94 (t, J = 4.76 Hz, 1H, alditolyl-H), 5.02 (d, J = 6.0 Hz, 1H, alditolyl-H), 6.20 (d, J = 5.2 Hz, 1H, OH, D_2O exchangeable), 6.58 (d, J = 5.8 Hz, 1H, OH, D_2O exchangeable), 7.15–7.21 (m, 1H, alditolyl-H), 7.63 (d, J = 9.5 Hz, 1H, C*H*═N), 7.90 (d, J = 8.03 Hz, 1H, py-quin C-6-H), 8.02 (d, J = 7.32 Hz, 1H, py-quin C-5-H), 8.38 (s, 1H, py-quin C-8-H), 8.82 (s, 1H, py-quin C-4-H), 11.88 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Galactose{7-methyl-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **10**

δ 2.50 (s, 3H, CH_3), 4.43 (d, J = 5.8 Hz, 1H, OH, D_2O exchangeable), 4.48 (t, J = 5.88 Hz, 1H, OH, D_2O exchangeable), 4.78-4.87 (m, 1H,OH, D_2O exchangeable), 4.91 (t, J = 5 Hz, 1H, alditolyl-H), 6.11 (d, J = 5.4 Hz, , 1H, OH, D_2O exchangeable), 6.49 (d, J = 6.6 Hz, 1H, OH, D_2O exchangeable), 7.22 (d, J = 8.8 Hz, 1H, C*H*═N), 7.16 (d, J = 8 Hz, 1H, py-quin C-6-H), 7.63 (s, 1H, py-quin C-8-H), 7.88 (d, J = 8.8 Hz, 1H, py-quin C-5-H), 8.84 (s, 1H, py-quin C-4-H), 11.88 (s, 1H, NH, D_2O exchangeable), other protons were gathered with solvent absorption at δ = 3.5 ppm.

aldehydo-D-mannose{7-methyl-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **11**

δ 2.50 (s, 3H, CH_3), 3.66 (d, J = 5.2 Hz, 1H, OH, D_2O exchangeable), 3.83 (d, J = 6 Hz, 1H, OH, D_2O exchangeable), 4.40 (d, J = 5.4 Hz, 1H, OH, D_2O exchangeable), 4.78 (d, J = 7.1 Hz, 1H, OH, D_2O exchangeable), 5.24 (d, J = 9.1 Hz, 1H, alditolyl0-H), 6.62 (d, J = 9.5 Hz, 1H, C*H*═N), 7.22 (d, J = 8 Hz, 1H, py-quin C-6-H), 7.63 (s, 1H, py-quin C-8-H), 7.87 (d, J = 7.3 Hz, 1H, py-quin C-5-H), 8.95 (s, 1H, py-quin C-4-H), 11.98 (s, 1H, NH, D_2O exchangeable), other protons were gathered with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Arabinose{7-methyl-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **12**

δ 2.50 (s, 3H, CH_3), 4.59 (d, J = 5 Hz, 1H, alditolyl 0-H), 4.83 (d, J = 8 Hz, 1H, OH, D_2O exchangeable), 4.85 (s, 1H, alditolyl-H), 4.89 (d, J = 8 Hz, H, OH, D_2O exchangeable), 7.22 (m, 1H, C*H*═N), 7.33 (d, J = 8.4 Hz,1H, py-quin C-6-H), 8. 02 (d, J = 8.0 Hz, 1H, py-quin C-5-H), 8.38 (s, 1H, py-quin C-8-H), 8.87 (s, 1H, py-quin C-4-H),11.90 (s, 1H, NH D_2O exchangeable), other protons were congregated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Xylose{7-methyl-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **13**

δ 2.50 (s, 3H, CH_3), 4.59 (d, J = 5 Hz, 1H, alditolyl-H), 4.83 (d, J = 8 Hz, 1H, OH, D_2O exchangeable), 4.85 (s, 1H, alditolyl-H), 4.89 (d, J = 7.6 Hz, 1H, 1OH, D_2O exchangeable), 7.20 (d, J = 7.4 Hz, 1H, C*H*═N), 7.22 (d, J = 8.4 Hz, 1H, py-quin C-6-H), 7.64 (s, 1H, py-quin C-8-H), 7.90 (d, J = 8 Hz, 1H, py-quin C-5-H), 8.78 (s, 1H, py-quin C-4-H), 11.90 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Glucose{7-methoxy-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **14**

δ 3.91 (s, 3H, OCH_3), 4.55 (t, J = 5.5 Hz, 1H, OH, D_2O exchangeable), 4.64 (d, J = 5.12 Hz, 1H, alditolyl-H), 4.85 (t, J = 8.4 Hz, 1H, alditolyl-H), 4.95 (d, J = 5.1 Hz,1H, alditolyl-H), 5.05 (d, J = 5.9 Hz, 2H, alditolyl-H), 6.20 (d, J = 5.2 Hz, 1H, OH, D_2O exchangeable), 6.58 (d, J = 5.8 Hz, 1H, OH, D_2O exchangeable), 7.15–7.21 (m, 1H, alditolyl-H), 7.03 (d, J = 8.8 Hz, 1H, C*H*═N), 7.13 (d, J = 8.8 Hz, 1H, py-quin C-6-H), 7.18 (s, 1H, py-quin C-8-H), 7.89 (d, J = 8.7 Hz, 1H, py-quin C-5-H), 8.77 (s, 1H, py-quin C-4-H), 11.89 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Galactose{7-methoxy-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **15**

δ 3.91 (s, 3H, OCH_3), 4.46 (d, J = 5.8 Hz, 1H, OH, D_2O exchangeable), 4.64 (t, J = 4.7 Hz, 1H, OH, D_2O exchangeable), 4.86-4.80 (m, 1H, OH, D_2O exchangeable), 4.91 (t, J = 4.0 Hz, 1H, alditolyl-H), 6.11 (d, J = 4.4 Hz, 1H, OH, D_2O exchangeable), 6.49 (d, J = 6.6 Hz, 1H, OH, D_2O exchangeable), 7.03 (d, J = 8.7 Hz, 1H, C*H*═N), 7.11 (d, J = 8.8 Hz, 1H, py-quin C-6-H), 7.81 (s, 1H, py-quin C-8-H), 7.87 (d, J = 8.8 Hz, 1H, py-quin C-5-H), 8.79 (s, 1H, py-quin C-4-H), 11.86 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

aldehydo-D-Mannose{7-methoxy-1H-pyrazolo-[3,4-b]quinoline-3-yl}imine **16**

δ 3.91 (s, 3H, OCH_3), 4.78 (t, J = 5.1 Hz, 1H, OH, D_2O exchangeable), 4.94 (dist.d, J = 5.1 Hz, 1H, OH, D_2O exchangeable), 5.23 (d, J = 8.2 Hz, 1H,

alditolyl-H), 6.23 (d, J = 4.3 Hz, 1H, OH, D_2O exchangeable), 6.56 (d, J = 9.2. Hz, 1H, OH, D_2O exchangeable), 7.01–7.04 (m, 1H, py-quin C-6-H and C*H*═N), 7.17 (s, 1H, py-quin C-8-H), 7.86 (d, J = 8.8 Hz, 1H, py-quin C-5-H), 8.88 (s, 1H, py-quin C-4-H), 11.94 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

ALDEHYDO-D-ARABINOSE{7-METHOXY-1H-PYRAZOLO-[3,4-b]QUINOLINE-3-YL}IMINE **17**

δ 3.91 (s, 3H, OCH_3), 4.43 (d, J = 5.0 Hz, 1H, OH D_2O exchangeable), 4.61(s, 1H, OH, D_2O exchangeable), 4.83–4.87 (m, 2H, alditolyl-H), 6.03 (d,1H,OH, D_2O exchangeable), 6.40 (d, J = 5.0 Hz, 1H, OH, D_2O exchangeable), 7.03 (d, J = 8.7 Hz, 1H, py-quin C-6-H), 7.16 (m, 1H, C*H*═N), 7.86 (d, J = 8.8 Hz, 1H, py-quin C-5-H), 7.95 (s, 1H, py-quin C-8-H), 8.73 (s, 1H, py-quin C-4-H), 11.87 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

ALDEHYDO-D-XYLOSE{7-METHOXY-1H-PYRAZOLO-[3,4-b]QUINOLINE-3-YL}IMINEC **18**

δ 3.91 (s, 3H, OCH_3), 4.43 (d, J = 5.0 Hz, 1H, OH, D_2O exchangeable), 4.58(s,1H, OH, D_2O exchangeable), 4.83–4.90 (m, 2H, alditolyl-H), 6.03 (d, 1H, OH, D_2O exchangeable), 6.40 (d, J = 6.0 Hz, OH, D_2O exchangeable), 7.03 (d, J = 8.0 Hz, 1H, C*H*═N), 7.13 (d, J = 8.0 Hz, 1H, py-quin C-6-H), 7.81 (s, 1H, py-quin C-8-H), 7.89 (d, J = 8.0 Hz, 1H, py-quin C-5-H), 8.72 (s, 1H, py-quin C-4-H), 11.86 (s, 1H, NH, D_2O exchangeable), other protons were associated with solvent absorption at δ = 3.5 ppm.

Biological Evaluation

Herpes Simplex Virus Replication and Inhibitory Activity of the Compounds

The test compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare a 50 mM stock solution. Since these compounds were all chromogenic, they were kept covered with aluminum foil until dissolved in the culture medium to be used in the experiment. Toxicity data on the host cell culture was accomplished before testing them for their antiviral activity on HSV-1. Most of these compounds did not show any cytotoxicity at a dose level of 200 μM but a few were borderline cytotoxic. We chose to use only four concentrations, which were shown to be safe for the host cells, i.e., 12.5, 25, 50 and 100 μM.

African green monkey kidney (Vero) cells and the HSV-1 strain, F, a DNA containing virus, were purchased from American type Culture Collection (ATCC, Manassas, VA, USA). All media, serum and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The monkey kidney cells were seeded in 96 well plates in Eagle's modified Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 2.2 g of $NaHCO_3$ per liter and penicillin and streptomycin at a concentration of 100 I.U. and 100 μg/ml respectively. After the formation of monolayer in the next 24 h, the cells were infected with HSV-1 at a multiplicity of infection of 10. The virus was adsorbed onto the cells for 90 min, the unadsorbed virus was removed and the medium containing the test compounds was added to each well. The plates were re-incubated for 24 and 48 h respectively. After incubation, the plates were examined microscopically and to one set was added physiological saline with 0.5% formaldehyde to fix the monolayer. After 6 h of fixation, the fixative was removed from the wells and 0.2 ml of crystal violet (0.5% crystal violet in saline fixative) was added to each well for 30 min. The stain was washed off under the tap water and the plates were left to dry. Ethylene glycol monomethyl ether (0.2 ml) was added to each well and left for 2 h for the complete dissociation of the dye. Plates were read at 620 nm wavelength.

In the second set, to each well in 96 well plates was added 100 μl of a tetrazolium dye [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfonphenyl)-2H-tetrazolium] i.e. MTS dye (Promega, Inc., Madison, WI, USA) and the mixture incubated for a period of 3 h and the plates then read at a wavelength of 490 nm. This test is based on the fact that MTS, a tetrazolium dye is reduced by hydrogenases in living cells to a water-soluble formazan, which is measured spectrophotometrically.[18]

Acute Toxicity

The compounds deemed to be biologically significant were further investigated for their approximate LD_{50} in male mice.[19,20] Two groups of mice each consisting of six animals, were used. The compounds were given orally in doses of 1, 10, 100, 200, 250, 500 mg/kg, respectively. After 24 h, the percent mortality in each group and for each compound was recorded.

RESULTS AND DISCUSSION

Chemistry

Synthesis of the target compounds **4–18** was performed by the reaction illustrated in Figure 1 which is by the direct condensation of the appropriate aldose with the selected 3-amino-1H-pyrazolo[3,4-b]quinoline **1–3** in aqueous dioxane (Table I).

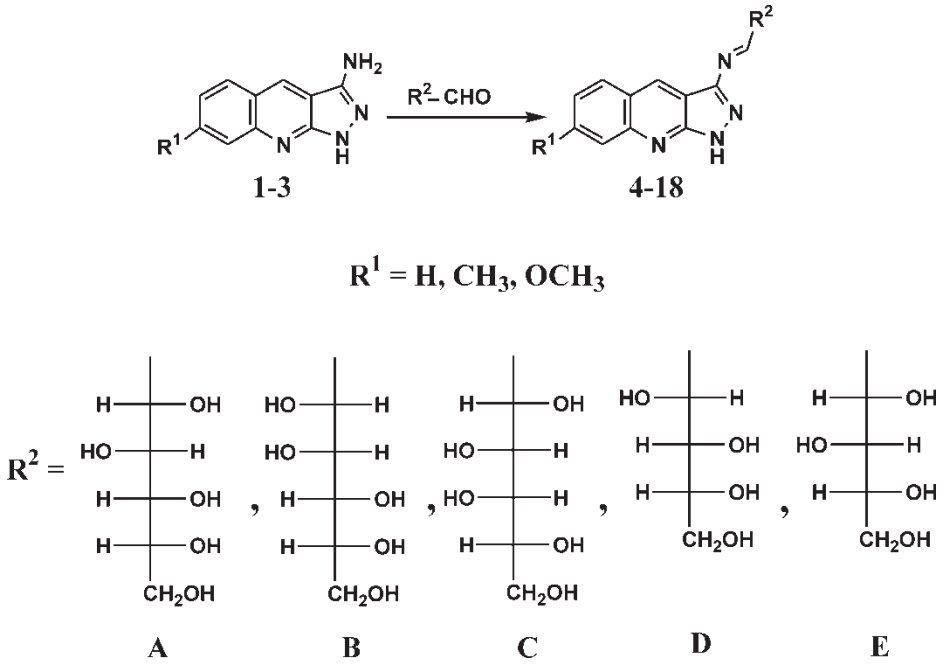

FIGURE 1 Synthesis of pyrazoloquinolone sugar derivatives.

Herpes Simples Virus Replication and Inhibitory Activity of 4–18

The antiviral activity of the synthesised compounds **4–18** was evaluated *in vitro* against herpes simplex type-1 by two different bioassays namely; crystal violet staining or tetrazolium dye (MTS) measurement.[18] Twenty-four hours post addition of the test compounds to the infected cells did not show a difference by crystal violet staining or the MTS dye measurement although, within this time-frame, the infected cultures were showing sufficient cytopathic effect along with those infected and treated with the synthesized compounds. Even the MTS dye measurement did not reveal any difference. Plates that were incubated for 48 h post infection and treated with the test compounds gave more encouraging results. Results from both procedures revealed concordancy with regard to compounds **12** and **18**, which contain aldopentose moieties, which were inhibiting the virus growth at a concentration above 50 μM (Tables II and III). All the other compounds were inactive.

Acute Toxicity

The biologically significant compounds **12** and **18** were further evaluated for their approximate LD_{50} in male mice using a literature method.[19,20] Results indicated that the two tested compounds proved to be non-toxic and well tolerated by the experimental animals as evidenced by their LD_{50} values (> 300 mg/Kg).

TABLE I Physical data for compounds **4–18**

Comp. No.	R^1	R^2	Yield (%)	M.p. (°C)	Mol. Formula (Mol. wt.)
4	H	A	84	204–205	$C_{16}H_{18}N_4O_5$ (346.34)
5	H	B	72	211–113	$C_{16}H_{18}N_4O_5$ (346.34)
6	H	C	78	179–181	$C_{16}H_{18}N_4O_5$ (346.34)
7	H	D	80	138–140	$C_{15}H_{16}N_4O_4$ (316.31)
8	H	E	75	182–184	$C_{15}H_{16}N_4O_4$ (316.31)
9	CH_3	A	90	197–199	$C_{15}H_{16}N_4O_4$ (316.31)
10	CH_3	B	82	221–213	$C_{15}H_{16}N_4O_4$ (316.31)
11	CH_3	C	76	219–220	$C_{15}H_{16}N_4O_4$ (316.31)
12	CH_3	D	73	185–187	$C_{16}H_{18}N_4O_4$ (330.34)
13	CH_3	E	75	151–153	$C_{16}H_{18}N_4O_4$ (330.34)
14	CH_3O	A	82	208–210	$C_{17}H_{20}N_4O_6$ (376.37)
15	CH_3O	B	79	198–200	$C_{17}H_{20}N_4O_6$ (376.37)
16	CH_3O	C	73	190–192	$C_{17}H_{20}N_4O_6$ (376.37)
17	CH_3O	D	82	220–222	$C_{16}H_{18}N_4O_5$ (346.34)
18	CH_3O	E	81	172–174	$C_{16}H_{18}N_4O_5$ (346.34)

TABLE II Screening by cytoxicity test using crystal violet in anti-HSV study on compounds

	100 μm	50 μm	25 μm	12.5 μm
	1 2 3	4 5 6	7 8 9	10 11 12
Control	0.227 ± .074			
Virus Control	0.198 ± 0.025			
Compound 12	0.243 ± .015	0.206 ± .027	0.232 ± .003	0.198 ± .008
Compound 18	0.232 ± .005	0.231 ± .022	0.206 ± .027	0.204 ± .012

TABLE III Cytotoxicity Test Using MTS Dye

	100 μm	50 μm	25 μm	12.5 μm
	1 2 3	4 5 6	7 8 9	10 11 12
Control	1.010 ± .021			
Virus Control	0.718 ± 0.06			
Compound 12	1.041 ± .009	0.865 ± .040	0.710 ± .023	0.694 ± .010
Compound 18	1.066 ± .116	0.759 ± .026	0.800 ± .039	0.715 ± .029

CONCLUSION

The results obtained from the two different bioassays, namely crystal violet staining or tetrazolium dye (MTS) measurement, were concordant in respect to compounds **12** and **18**. These two compounds possess inhibitory activity against virus growth in concentrations above 50 μM. This study revealed that the aldopentose derivatives were more promising than the aldohexose derivatives. The compounds described here represent a fruitful means of developing a new class of antiherpetic agent and deserve further investigation.

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