Synthesis and Anti-Hepatitis B Virus Activity of Some 2,3-Dihydroxyprop-1-yl Unnatural Hetaryls

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Summary

The sodium salts of some hetaryls of the quinoxalin-2-ones 2–4, phthalazine-1,4-dione 5, phthalazin-1-one 6, and pyridazin-6-ones 7 and 8 were alkylated with (±) 2,3-O-isopropylidene-1-O-(4-toluencesulfonyl)glycerol (1) to give the respective tetraseco-nucleosides 9–15. Their deisopropylideneation with 70% acetic acid in water gave the corresponding 2,3-dihydroxyprop-1-yl hetaryls 16–22. Compounds 16–22 showed varying inhibition activity against Hepatitis B virus (HBV) with low to moderate cytotoxicity, where 18 and 21 showed the highest replication inhibition and low cytotoxicity.

Introduction

Hepatitis B virus (HBV) infection has a great degree of prominence due to the wide prevalence of the disease and the lack of an ideal drug to combat the virus, especially in chronic infections which can lead to cirrhosis of the liver and/or hepatocellular carcinoma. In such carriers vaccination is not an effective therapy and alpha interferon has demonstrated some promise[1–6]. Nucleoside analogues and particularly the unnatural L-configuration have emerged as potential anti-HBV agents with more promising pharmacological and toxicological profiles[3,7–13] than their D-counterparts. Thus, 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil (B-L-FMAU) is considered as a clinical candidate for treatment of chronic HBV infections[3,6,14] and is undergoing preclinical toxicology studies[15]. The 2′,3′-dideoxy-β-L-cytidine (β-L-ddc) and its 5-fluoro analogue (β-L-FddC) demonstrated equally potent activity against HBV in vitro, having the same ED50 value of 0.01 µM. The unusual group of nucleosides such as L-SddC[(–)-BCH-189] in which the 3′-CH2 group has been replaced by a hetero-atom[7,16–22] exhibits potent anti-HBV and HIV activity in vitro. The compounds are undergoing clinical trials in patients with AIDS and AIDS related complex. The L-like BCH-189 is more potent than the D-like counterpart[7,10,12].

Considerable effort has been devoted to the synthesis of acyclo-nucleoside analogues which possess potent selective antiviral activity[12,23]. The S-enantiomer of 9-(2,3-dihydroxypropyl)adenine[25] (S-DHPA) was found to have inhibitory activity towards a number of DNA and RNA viruses. More recently, the phosphonate derivative 9-(3-hydroxy-2′-(S)-phosphonylethoxymethyl)adenine (S-HPMPA) and its analogues have been found to exhibit potent antiviral effects[26]. Since the trend for anti-HBV activity was similar to that for anti-HIV activity[12], and there is a great demand for the development of novel compounds with anti-HBV activity and minimal adverse effects on the host, the present work deals with the synthesis and biological activity of the 2,3-dihydroxypropyl derivatives of some hetaryls.

Introduction

Hepatitis B virus (HBV) infection has a great degree of prominence due to the wide prevalence of the disease and the lack of an ideal drug to combat the virus, especially in chronic infections which can lead to cirrhosis of the liver and/or hepatocellular carcinoma. In such carriers vaccination is not an effective therapy and alpha interferon has demonstrated some promise[1–6]. Nucleoside analogues and particularly the unnatural L-configuration have emerged as potential anti-HBV agents with more promising pharmacological and toxicological profiles[3,7–13] than their D-counterparts. Thus, 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil (B-L-FMAU) is considered as a clinical candidate for treatment of chronic HBV infections[3,6,14] and is undergoing preclinical toxicology studies[15]. The 2′,3′-dideoxy-β-L-cytidine (β-L-ddc) and its 5-fluoro analogue (β-L-FddC) demonstrated equally potent activity against HBV in vitro, having the same ED50 value of 0.01 µM. The unusual group of nucleosides such as L-SddC[(–)-BCH-189] in which the 3′-CH2 group has been replaced by a hetero-atom[7,16–22] exhibits potent anti-HBV and HIV activity in vitro. The compounds are undergoing clinical trials in patients with AIDS and AIDS related complex. The L-like BCH-189 is more potent than the D-like counterpart[7,10,12].

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(1) gave the (±)-2,3-isopropylidene-dihydroxypropyl derivatives of the quinoxalinones 9–11, phthalazinedione 12, phthalazine 13, and the pyridazinones 14 and 15 (Scheme 1). Deprotection of the isopropylidene groups was effected with 70% acetic acid in water to give the corresponding (±)-2,3-dihydroxypropyl hetaryls 16–22, respectively. The isopropylidene derivatives of phthalazine and some quinoxalinone analogues were not isolated, but converted immediately to the respective 2,3-dihydroxypropyl nucleosides. The structures of the isopropylidene derivatives have been confirmed by analyzing their 1H-NMR spectral data. They showed the presence of two singlets appearing at the high magnetic field in the range 1.26–1.40 and 1.34–1.49 ppm, respectively, corresponding to the two methyl groups of the isopropylidene residue. The difference in their chemical shifts (Δδ) lies in the range 0.08–0.14 ppm, which is in agreement with that required (Δδ > 0.05) for α-terminal isopropylidene derivatives. The higher value of Δδ in such terminal derivatives is due to the interaction of the bulky substituent on the dioxolane ring with only one of the two methyl groups. The deprotected derivatives showed the absence of the signals corresponding to the isopropylidene residue. The presence of signals due to the propyl residue and the hetaryl groups confirmed the successful nuclophilic displacement of the tosylxy group in 1.

**Scheme 1**

![Scheme 1](image)

### Biological Activities

Compounds 16–22 were tested for their activity against Hepatitis B virus (HBV) in Hep G2 2.2.15 cell. The concentrations for the tested compounds were 10 M. Compounds 18 and 21 showed high inhibition activity (Table 1) and low cytotoxicity. Compounds 17, 19, and 22 showed high inhibition activity with moderate cytotoxicity, while compounds 16 and 20 showed moderate viral replication inhibition and low cytotoxicity.

![Image](image)

**Table 1. Inhibition of HBV replication by 10 µM of selected compounds.**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>% Inhibition 1 Week</th>
<th>% Inhibition 2 Weeks</th>
<th>% Inhibition 3 Weeks</th>
<th>Cytotoxicity</th>
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</thead>
<tbody>
<tr>
<td>16</td>
<td>30.9</td>
<td>30.3</td>
<td>30.1</td>
<td>7.7</td>
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<tr>
<td>17</td>
<td>80.9</td>
<td>80.3</td>
<td>71.3</td>
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<td>83.9</td>
<td>80.5</td>
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<tr>
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<td>81.3</td>
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<td>72.1</td>
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<td>31.1</td>
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<td>77.7</td>
<td>70.5</td>
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</tr>
</tbody>
</table>

### Acknowledgments

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### Experimental Part

Melting points were determined with a Meltemp apparatus 76-mm immersion thermometer and are uncorrected. TLC was performed on Baker flex silica gel 60 F-254 precoated plates. Column chromatography was performed on Merck silica gel (0.040–0.063). 1H-NMR spectra were recorded on Bruker AC 250 and 200 MHz spectrometers, in CDCl3 using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in ppm relative to TMS and described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or b (broad singlet). Microanalyses were performed by the unit of Microanalysis, Faculty of Science, Cairo University.

2,3-O-Isopropylidene-1-O-(4-toluenesulfonyl)glycerol (1)

To a stirred ice-cooled solution of glycerol-1,2-acetonide (7.2 g, 0.05 mol) in pyridine (30 ml), 4-toluenesulfonyl chloride (10.4 g, 0.06 mol) was added portionwise. After standing for 16 h, the solution was diluted with ether (30 ml). The resulting mixture was washed successively with 1 N HCl, water and then saturated aqueous NaHCO3. The ether layer was dried over anhydrous Na2SO4 and concentrated to give 1 (13.1 g, 84% yield), ref [22] mp 40 °C, mp 41 °C.

**General Procedure**

(±)-2,3-Isopropylidene-2,3-dihydroxyprop-1-yl Hetaryls (9–15)

To a stirred solution of the hetaryls (5.0 mmol) in anhydrous DMF (10 ml) was added NaH (60% dispersed in mineral oil, 0.20 g, 5.0 mol). After complete evolution of hydrogen, the mixture was heated at 100 °C for 1 h, then 1 (1.43 g, 5.0 mmol) was added. The reaction mixture was stirred for 12 h at 100 °C, cooled to room temperature, and filtered. The solvent was evaporated to dryness under reduced pressure and 10 ml of water was added to the residue and the product was extracted with ether (3 × 20 ml). The ether layer was dried and evaporated under reduced pressure to give a dark brown gum which was purified by column chromatography using 1% MeOH/CHCl3.

(±)-1-(2,3-O-Isopropylidene-2,3-dihydroxyprop-1-yl)(1H)-Quinoxalin-2-one (9)

Syrup (40% yield). 1H-NMR (CDCl3): δ = 1.26 (s, 3 H, CH3), 1.34 (s, H, CH3), 3.84, 4.10 (2 m, 2H, CH2), 4.27 (m, 1 H, CH), 4.51 (m, 2 H, CH2), 7.29 (m, 1 H, Ar-H), 7.55 (m, 2 H, Ar-H), 7.85 (d, 1 H, J = 9.6 Hz, Ar-H), 8.24 (s, 1 H, Ar-H).

1.33 (s, 3 H, CH₃), 1.46 (s, 3 H, CH₃), 3.95 (m, 2 H, CH₂), 4.11 (m, 2 H, ±7.22 (m, 10 H, Ar-H).

15H, CH₃), 3.95, 4.16 (2 m, 2 H, CH₂), 4.31 (m, 1 H, CH), 4.54 (m, 1 H, CH₂), 7.11 (m, 5 H, Ar-H).– C₁₁H₁₂N₂O₃

Syrup (30% yield). 1H-NMR (CDCl₃): δ = 0.95 (t, 3 H, CH₃), 1.33 (s, 3 H, CH₃), 1.46 (s, 3 H, CH₃), 3.95 (m, 2 H, CH₂), 4.11 (m, 2 H, CH₂), 4.28 (m, 1 H, CH), 4.48 (m, 1 H, CH₂), 7.23 (m, 10 H, Ar-H). General Procedure: (±)-2,3-Dihydroxyprop-1-yl Hetaryls (329)

The isopropylidenes were added to the cell culture (Hep G 2 2.2.15 together with the tested compounds (concentration = 10 μM). The supernatant media were collected out in three steps: extraction of DNA from supernatant, amplification of DNA by using thermal cycler and finally detection by DIG-ELISA technique. The percent inhibition was calculated by the relation % Inhibition = \left(\frac{Inhibition of compd without compds}{Inhibition of control}ight) \times 100

The percentage cytotoxicity could be estimated by the relation between the number of the living and dead cells after three weeks counted by the hemocytometer.

References