Synthesis and Antiviral Evaluation of 5-(1,2,3-Triazol-1-ylmethyl)-uridine Derivatives

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Some 5-(1,2,3-triazol-1-ylmethyl)uridine derivatives were synthesized via the 1,3-dipolar cycloaddition of a 5-azidomethyluridine derivative with substituted acetylenes. The antiviral activities of these compounds against hepatitis A virus (HAV, MBB cell culture-adapted strain) and *Herpes simplex* virus type-1 (HSV-1) were tested.

Key words: 1,2,3-Triazoles, Uridine Derivatives, Anti-Hepatitis A Virus

Introduction

A varity of pyrimidine nucleosides have shown interesting biological activities including antitumour activity (Vince, 1981; De Napoli *et al.*, 1986), antiviral activity (Heredewijn, 1992), virucidal activity against the herpes virus (Shealy and O'Dell, 1985) and strain HF of the *Herpes simplex* virus type-1 (HSV-1) (Shealy and Clayton, 1988). Various analogues possess effective antibacterial, antifungal, insecticidal, and miticidal activities (Cheng, 1969). The chemistry of azides has attracted the attention of many chemists, since several of these compounds play an important role in organic chemistry (Scriven and Turnbull, 1988; Patai, 1971; Ridois, 1984). One of the most useful applications of azides is the preparation of 1,2,3-triazoles via 1,3-dipolar cycloaddition reactions of azides with substituted acetylene compounds (Gilchrist *et al.*, 1974; Patei and Smalley, 1984; Loubinoux *et al.*, 1984). 1,2,3-Triazoles have also attained much attention because of their chemotherapeutical value (Sanghvi *et al.*, 1990). Moreover 1,2,3-triazole derivatives show significant antimicrobial, cytostatic, virostatic and anti-inflammatory activities (Chen *et al.*, 2000; Sherement *et al.*, 2004; Banu *et al.*, 1999). The versatile biological properties of pyrimidine nucleosides and 1,2,3-triazoles prompted us to investigate the synthesis and the antiviral activity of uridine modified with an 1,2,3-triazolylmethyl moiety at position 5 of the pyrimidine moiety. Ribavirin, a powerful antiviral nucleoside having a broad spectrum of activities against RNA and DNA viruses (De Clercq, 1997), is representative of 1,2,4-triazole nucleosides and exhibits pronounced biological activities. Also, 1,2,3-triazole analogues (Alvarez *et al.*, 1994) have potent anti-HIV-1 activities. Both findings attracted attention toward the synthesis of their analogues. 1,3-Dipolar cycloaddition of azides with acetylenes is an efficient method to obtain 1,2,3-triazole rings of acyclo- and carboacyclonucleosides (Chafiq *et al.*, 2001 a; Lazrak *et al.*, 2001; El Ashry *et al.*, 2006). It is known that the reaction is controlled by electronic and steric factors (Alvarez *et al.*, 1994). In general, such an addition reaction tends to give mainly the isomer with electron-withdrawing groups at the 4-position and electron-donating groups at the 5-position. On the other hand, the sterically less hindered isomer tends to be the common one (Chafiq *et al.*, 2001 b; Lazrak *et al.*, 2001; El Ashry *et al.*, 2006).

Results and Discussion

The reaction of 5-azidomethyl-2′,3′-*O*-isopropylidene-uridine (**1**) (Scheit, 1966; Fromageot *et al.*, 1967; Seio *et al.*, 1998) and the monoacetylene derivatives **2a**–**c** refluxing in toluene for 48 h gave only the sterically less hindered regioisomers **4a**–**c** in 55–62% yield, rather than **5a**–**c** (Fig. 1). The structures of **4a**–**c** were established by their ^{1}H NMR spectra, which showed a singlet signal for H-5 at δ 8.32–8.38 ppm in agreement

with the formation of the 4-substituted 1,2,3-triazole derivatives **4a**–**c** (Alvarez *et al.*, 1994; Lazrak *et al.*, 1997 b). On the other hand, the reaction of **1** with the disubstituted acetylenes **3a**–**c** in toluene refluxing for 48 h afforded the 4,5-disubstituted 1,2,3-triazoles **6a**–**c** in lower yield (40–42%). Deprotection of compounds **4a**–**c** and **6a**–**c** was carried out by using 70% AcOH and refluxing for 2 h. The crude products were purified on a silica gel column using 10% MeOH in CH_2Cl_2 to afford **7a**–**c** and **8a**–**c** in 85–88% and 80–83% yields, respectively. The structures of the deprotected derivatives were confirmed by ^{1}H NMR and mass spectra which showed the disappearance of the isopropylidene group in all cases. Elemental analyses of these compounds were in agreement with the assigned structures.

The plaque infectivity assay (Farag *et al.*, 2004) was carried out to test the prepared compounds for their antiviral activity. The test was performed to include three possibilities of antiviral activity: virucidal effect, virus adsorption, and effect on virus replication for both hepatitis A virus (HAV-27) and HSV-1.

For the antiviral activity against HAV-27 it has to be noted, that at both concentrations tested, 10 and 20 μg/10^5 cells, compounds **7a** and **7b** revealed the highest antiviral activity in this series of compounds, and compounds **7c** and **8a** revealed high activity at 10 μg/10^5 cells using amantadine (C*) as a control. Compound **8b** showed moderate activity, while at 20 μg/10^5 cells compound **8c** revealed little antiviral activity.

For the antiviral activity against HSV-1 the results revealed that compounds **7a**–**c** and **8a** showed the highest effect at 10 μg/10^5 cells, while compounds **8b** and **8c** showed moderate activity.

In conclusion, new 5-(1,2,3-triazol-1-ylmethyl)-uridine derivatives were synthesized in order to increase the number of compounds screened for antiviral activity. Some of them displayed promising activities.

Experimental

General

Melting points were determined using a Büchi apparatus. ^{1}H NMR spectra were recorded with

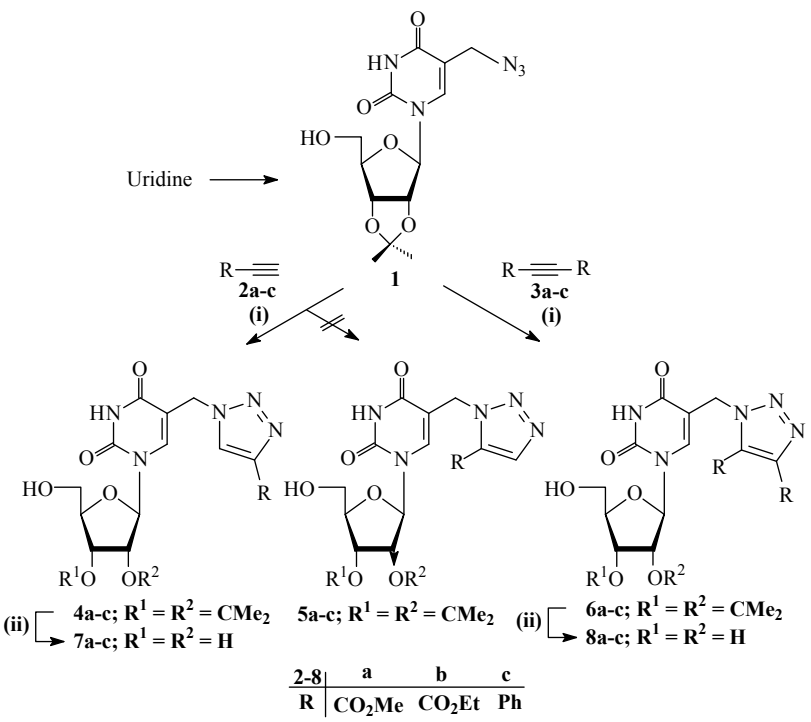

Fig. 1. Preparation of the compounds. Reaction conditions: (i) toluene/reflux, 48 h; (ii) 70% AcOH/reflux, 2 h

a Varian Gemini spectrometer at 300 MHz and 200 MHz with TMS as internal standard. Chemical shifts are reported in δ scale (ppm) relative to TMS as internal standard; the coupling constants (J values) are given in Hz. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA). Antiviral activities were tested at the Liver Institute, Menoufia University, Egypt.

Preparation of the compounds for the bioassay

100 mg of the compounds were dissolved in 1 ml of 10% DMSO in water. The final concentration was 100 μg/ml (stock solution). The dissolved stock solutions were decontaminated by addition of 50 μg/ml antibiotic-antimycotic mixture (10000 U penicillin G sodium, 10000 μg streptomycin sulfate, and 250 mg amphotericin B; PAA Laboratories GmbH, Pasching, Austria).

Cell culture

African green monkey kidney-derived cells (Vero; Egyptian Organization of Biological Products and Vaccines) and human hepatoma cell line (HepG2; Egyptian Organization of Biological Products and Vaccines) were used. Cells were propagated in Dulbeccos' Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic mixture. The pH value was adjusted to 7.2–7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through a 0.2 mm pore size nitrocellulose membrane.

Viruses

Herpes simplex virus type-1 (HSV-1) and hepatitis-A virus (HAV, MBB cell culture-adapted strain) were obtained from Environmental Virology Laboratory, Department of Water Pollution Research, National Research Centre, Cairo, Egypt.

Cytotoxicity assay

The cytotoxicity was assayed for both DMSO and the test compounds. Serial dilutions were prepared and inoculated on Vero cells grown in 96-well tissue culture plates. The maximum tolerated concentration (MTC) for each compound was determined by both cell morphology and cell viability by staining with trypan blue dye.

Plaque reduction infectivity assay

A 6-well plate was cultivated with cell culture (10^5 cell/ml) and incubated for 2 d at 37 °C. HSV-1 and HAV were diluted to give 10^4 PFU/ml final concentrations for each virus and mixed with the test compound at the previous concentration and incubated overnight at 4 °C. The growth medium was removed from the multiwell plate and the virus-compound mixture was inoculated (100 ml/well). After 1 h contact time, the inoculum was aspirated and the cell sheets were overlaid with 3 ml of MEM with 1% agarose. The plates were left to solidify and incubated at 37 °C until the development of virus plaques. Cell sheets were fixed in 10% formaline solution for 2 h and stained with crystal violet stain. Control virus and cells were treated identically without compound. Virus plaques were counted and the percentage of reduction was calculated (Farag *et al.*, 2004).

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