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Synthesis Optimization of Some Nucleoside Analogues Derivatives and Their Antiviral Activity

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Abstract: To prepare the target nucleosides, the branched chain sugars was converted to their active form (1-bromosugar derivatives). This conversion included the hydrolysis of bromination with hydrobromic acid (HBr) in glacial acetic acid, forming 1-bromo sugars (4,6-di-O-Acetyl-2,3-dideoxy-3,3-di-C-nitro methyl-Darabino-hexopyranosyl bromide), which were subjected to condensation with theophylline mercury salt to give acetylated nucleosides (7(4',6'-di-O-Acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexo- pyranosyl) theophylline). Deblocking of these nucleosides with sodium methoxide in methanol afforded our target the free nucleoside analogues type of 7-(2',3'-Dideoxy-3',3'-di-C-nitromethyl-β-D-arabinohexopyranosyl) theophylline (V). In a similar manner Bis (Indole-1-yl) mercury were condensed with 1-bromo sugar derivatives mentioned above to give acetylated nucleoside analogues 1-(4',6'-di-O-Acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexo pyranosyl) indole, (VI) which upon hydrolysis afforded the target free nucleoside analogues as 1-(2',3'-Dideoxy-3',3'-di-C-nitromethyl-β-D-arabinohexo pyranosyl) Indole (VII). The prepared compounds were identified by melting point, IR spectroscopy. The two synthesized nucleoside V and VIIshowed antiviral activity against both HSV-1 and HAV.

Key words: Synthesis · Nucleoside Analogues · Antiviral Activity

INTRODUCTION

Branched-chain sugar nucleosides are present in a wide range of both naturally occurring and synthetic products, some having biological activities such as antitumor, antiviral or antibacterial. The key step in the total synthesis of these nucleosides is the stereo-controlled formation of a new C-C bond at the branching point. Many nucleosides occur in nature in minor amounts either in nucleic acids or as the nucleosides themselves [1]. Many of these are just methylated derivatives of the normal nucleosides, but a significant number have more complex modification in the nitrogen bases [1]. Nucleosides, both of natural and synthetic origin have at least some biological activity. A much smaller, but nevertheless significant number of nucleosides are either in use as or have the potential based upon extensive biological evaluation to be employed as chemotherapeutic agents [2], Such as potential anti-viral, fungicidal and anti-cancer agents [3-5]. So the major purpose for the syntheses of nucleosides is, of course, the development of new compounds of chemotherapeutic interest. Chemical modifications of naturally occurring nucleosides have been of interest for over 50 years and numerous nucleoside analogues were synthesized in order to selectively interfere with DNA and RNA [6].

Structurally modified nucleosides represent an important class of medicinal compounds which have been found to behave as therapeutic agents and are currently used in pharmaceuticals as antitumour [7], antiviral [8] and antibiotic [9] agents [10, 11]. Structural modifications include the ribose, as well as the base moieties. The general synthetic protocols involve the use of monosaccharide chirons which are coupled to hetero cycles employing a key glycosylation step which is often not stereo selective.

During the last decade Lee-Ruff *et al*. [12], have developed a photochemical glycosylation method based on the photo isomerization of cyclobutanones to transient tetrahydrofuranylidenes and their insertion into alcohols or weakly acidic N-H functions [12-16].

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Nucleoside analogs of reverse transcriptase in use an essential position in treatment of HIV and AIDS [17]. Different derivatives of nucleoside analogues derived were synthesized from cyclobutanone [18].

The novel derivatives of Schiff bases of sulfone amide and were examined against bacteria and fungi and were all shown good antimicrobial activities [19]. The antitumor activities of N-(1-phenyl-2-hydroxy-2-phenyl ethyledine)-2′,4′dinitrophenyl hydrazine; N-(1-phenyl,2-hydroxy-2-phenyl ethylidine)-2′hydroxy phenyl imine and N-(2-hydroxy benzylidine)-2′-hydroxy phenyl imine against Ehrlich ascities carcinoma cells have been studied [20] and they all found to be active.

In this study some nucleoside analogues were synthesized and study their antiviral activity.

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents used throughout the investigation were of reagent grade (BDH, England). The infrared spectra (IR) were recorded from KBr discs using Perkin-Elmer 1430 Infrared spectrophotometer. Melting points were determined in an electric melting point apparatus (FSA Laboratory Supplies, Leics, UK) with sample contained in open capillary glass tube in an electrically heated metal block apparatus and are uncorrected. Column chromatography was carried out with silica gel 60 (Merck). The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel 60 GF254 foils (Merck) plates of 0.5 mm thickness using Chloroform: Ether (1:1, v/v) and Chloroform: Benzene (1:1, v/v) as a solvent system. The spots were detection by UV light and sulfuric acid. All synthesized compounds were purified by column chromatography by using silica-gel 60 (Fluka). 1,4,6-tri-O-Acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-Darabino-hexopyranose, Bis (indole-1-yl) mercury, Bis (thepophylline-7-yl) mercury.

***4,6-di-O-Acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-Darabino-hexopyranosyl bromide (III)*:** The1,4,6-tri-O-Acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-Darabino-hexopyranose (II) (1.0 g) was treated with 50% hydrogen bromide in acetic acid (3 mL). The solution was kept at 0°C until TLC indicated reaction completion then poured into an ice-cold chloroform (35 mL), washed with iced water (3×25 mL) and then with saturated aqueous solution of $NaHCO_3$ to remove the remaining acid. After a final wash with iced water (25 mL) the organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure.

***7(4',6'-di-O-Acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexopyranosyl)theophylline (IV)*:** The Bis (thepophylline-7-yl) mercury (0.5 g) was finely powdered suspended in (75 mL) sodium-dried xylene in the presence of celite (1.0 g) and the solvent was partially distilled off to remove trace of water azeotropically. When the temperature of the mixture was raised to 137°C, the residual suspension was allowed to cool. The 4,6-di-O-Acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-Darabino-hexopyranosyl bromide (III) (0.5 g) in dried xylene was then added and refluxed with vigorous stirring for 1 h. TLC ($CHCl_3$: ether, 9:1) Indicating the presence of unreacted trace of compound which was filtered from the hot xylene suspension and washed with dichloromethane (5 mL). The organic layer was washed with (2×5 mL) of 20% KI to remove the remaining trace of the mercuric salt, washed with water (2×5 mL) dried over Na_2SO_4 and the solvent was removed to give, after purification with silica gel column chromatography, the acetylated nucleoside.

***7-(2',3'-Dideoxy-3',3'-di-C-nitromethyl-β-D-arabinohexopyranosyl) theophylline (V)*:** A solution of (0.2 g) of 7(4',6'-di-O-Acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexopyranosyl) theophylline (IV) in (7 mL) of 0.1M methanolic sodium methoxide was refluxed with stirring for 0.5 h. TLC (DCM: ethanol, 8:2) showed that the reaction was complete, the mixture was neutralized with acetic acid and evaporated to dryness, the residue was partitioned between water and chloroform and the aqueous phase was evaporated to dryness *in vacuo*. The residue was dissolved in methanol (3 mL) and then purified through silica gel Column chromatography by using DCM-methanol (9.5:0.5) as mobile phase to afford the nucleoside. The product crystallized readily and was filtered and washed with ether, re-crystallization from chloroform-ether [21].

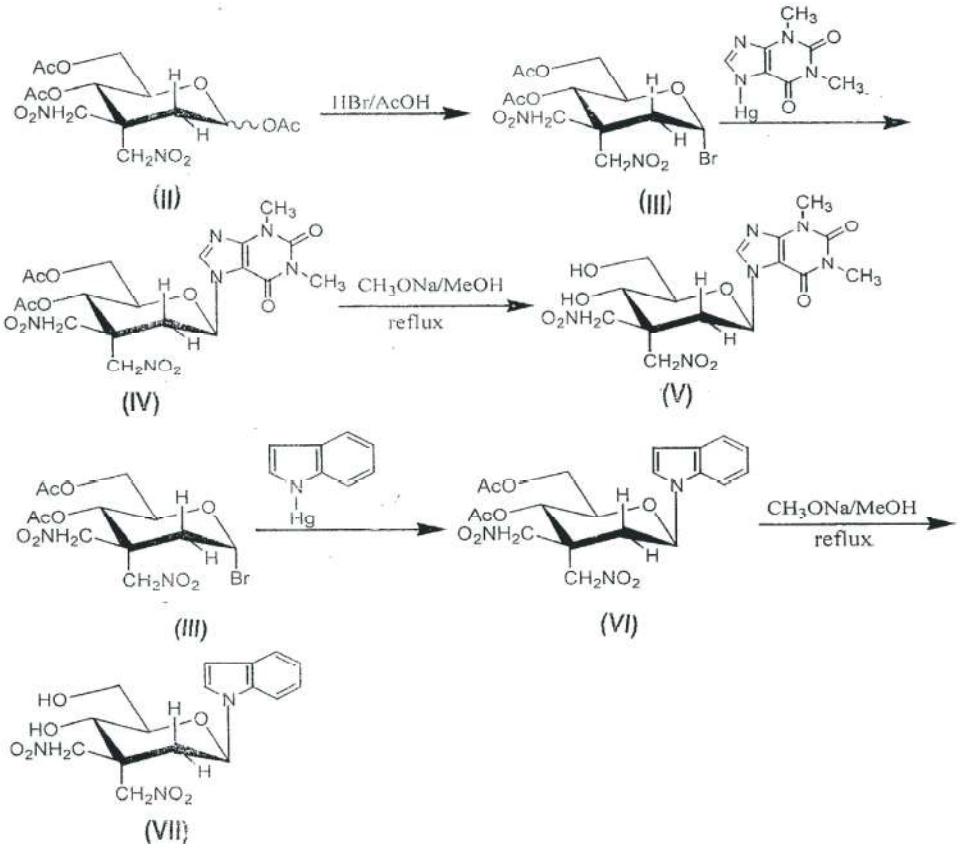
***1-(4',6'-di-O-Acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexopyranosyl) indole (VI)*:** The Bis (indole-1-yl) mercury (0.5 g) was finely powdered suspended in (75 mL) sodium-dried xylene in the presence of celite (1.0 g) and the solvent was partially distilled off to remove trace of water azeotropically. When the temperature of the mixture was raised to 137°C, the residual suspension was allowed to cool. The 4,6-di-O-Acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-Darabino-hexopyranosyl bromide (III) (0.5 g) in dried xylene was then added and refluxed with vigorous stirring for 1 h. TLC ($CHCl_3$- ether 9:1) indicating the presence of unreacted of compound which was filtered from the hot xylene suspension and washed with dichloro methane

(5 mL). The organic layer was washed with (2×5 mL) of 20 % aqueous potassium iodide to remove the remaining trace of the mercuric salt, washed with water (2×5 mL) dried over Na_2SO_4 and the solvent was removed to give 1-(4',6'-di-O-Acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexopyranosyl) indole.

***1-(2'3'Dideoxy-3'3'di-C-nitromethyl-β-D-arabinohexopyranosyl) Indole (VII)*:** A solution of (0.2 g) of 1-(4'6'di-O-Acetyl-2'3'dideoxy-3'3'di-C-nitromethyl-β-D-arabino-hexopyranosyl) indole (VI) in (7 mL) of 0.1M methanolic NaOH was refluxed with stirring for 0.5 h. TLC (DCM: ethanol 8:2) showed that the reaction was complete, the mixture was neutralized with acetic acid and evaporated to dryness, the residue was partitioned between water and chloroform and the aqueous phase was evaporated to dryness *in vacuo*. The residue was dissolved in methanol (3 mL) and then chromatographed on a column of silica using DCM-methanol (8:2, v/v). The product crystallized readily and was filtered and washed with ether, re-crystallization from chloroform-ether [21].

RESULTS AND DISCUSSION

The biological activity exhibited by the naturally occurring c-nucleosides has given rise to research directed toward their synthesis as well as toward the synthesis of many analogues containing various hetero cycles and carbohydrates. Two basic approaches have been employed for synthesis of nucleosides. In the first approach a protected and activated hetero cycle is condensed with a suitably protected carbohydrate derivative in a C-C bond forming reaction. In the second approach, which has been used in most examples, a carbohydrate is constructed that contains both the C-C bond to become the glycosidic linkage and the functionality that will be elaborated to the desired hetero cycle [1, 2]. Glycosyl bromides are most commonly prepared by treatment of 1,4,6-tri-O-acetyl-2,3-dideoxy-3-3-di-C-nitromethyl-D-arabino-hexopyranos (II) with HBr in glacial acetic acid [22]. In this reaction the anomeric acetyl moiety was converted into good leaving group by protonation. Subsequently, an oxocarbonium ion was formed by departure of acetic acid, which was substituted by bromide anion afforded 4,6-di-O-acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-α-D-arabino-hexopyrano- syl bromide (III) as a syrup in 69.2% yield. Theophylline has been used frequently as a model purine for the nucleoside synthesis because of its availability and the fact that only one of the nitrogen atoms is reactive. Moreover, unlike other purine there are no additional groups that need protecting prior to the coupling reaction [11, 23]. The reaction of theophylline with mercuric chloride in aqueous alkali afforded the mercury derivative rather than the chloro mercury one and it was assigned that the mercury derivative of theophylline was coupled with glycosyl halides and involves direct displacement of the mercury group from nitrogen by the in coming acylglycosyl halide [24-26]. The bis (theophylline-7-yl) mercury was attached with4,6-di-Oacetyl-2,3-dideoxy-3,3-di-C-nitromethyl-α-D-arabino-hexopyranosyl bromide (III) in anhydrous xylene in occurrence of celite under reflux to afford the desired theophylline nucleoside analogue 7-(4',6'-di-O-acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexo-pyranosyl) theophylline (IV) as a pale yellow solid in 59% yield. Rf, 0.25; ($CHCl_3$: ether 9:1, v/v); melting point 175 - 178°C; IR, 2940 cm^{-1} (C-H) aliphatic, 1731 cm^{-1} (C=O) group 1662 cm^{-1} (C=O) amide, 1590 cm^{-1} (C=C) bond, 1280 cm^{-1} (C-N) bond. Treatment the acetylated theophylline nucleoside 7-(4',6'-di-O-acetyl-2'3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexopyranosyl) theophylline (IV) with sodium methoxid in methanol under reflux [18, 27] gave the target nucleoside analogue 7-(2',3'-dideoxy-3',3'-di-C-nitromethyl-β-D-arabino-hexopyranosyl)theophylline (V) after silica column chromatography in 81% yield (Scheme 1). Rf 0.42 (DCM: EtOH, 8:2); melting point 218-220 °C; IR spectrum showed a stretching band at 3410 cm^{-1} for (O-H) groups indicating the complete hydrolysis of acetate groups. Further more the absorption of (C=O) group at 1665 cm^{-1} with shoulder at 1710 cm^{-1} which were characteristic for amide and 1325 cm^{-1} (NO_2) group [28]. Condensation of Bis (indole-1-yl) mercury with 4,6-di-O-Acetyl-2,3-dideoxy-3,3-di-C-nitromethyl-Darabino-hexo- pyranosyl bromide (III) in anhydrous xylene in the occurrence of celite under reflux gave 1-(4,6'-di-O-acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl- β-D-arabino-hexo pyranosyl) indole (VI) as a syrup in 51% yield (Scheme 1). Rf, 0.36 ($CHCl_3$: ether 9:1); IR spectrum showed a stretching band at 3040 cm^{-1} (C-H) aromatic and 1280 cm^{-1} for (C-N) band assigned for indole base, 1720 cm^{-1} (C=O) band, 1540 and 1350 cm^{-1} (NO_2) group [28]. Indole is an important heterocyclic material due to its industrial and medical applications [29]. Large and main compounds, their structure related to the aromatic indole alkaloids, in addition to tryptophane and serotonin were broadly distributed in nature and stimulated a variety of smooth muscles and nerves. Several drugs related to serotonin are bufotenin, psilocine and lysergic acid occurs most frequently in the some seeds and leaves [30]. Treatment of1-(4,6'-di-O-acetyl-2',3'-dideoxy-3',3'-di-C-nitromethyl-β-



Scheme 1: Synthetic way used for the preparation of the nucleoside analogues V and VII

Table 1: *In-vitro* antiviral activity of nucleoside analogues V and VII against HSV-1 & HAV

		Inhibition (%)	
Nucleoside	Concentration (µg/mL)	HSV-1	HAV
V	10	41.0	37.7
	20	53.6	45.8
	30	68.5	62.6
	40	82.4	75.3
VII	10	45.3	39.8
	20	61.5	53.6
	30	71.8	73.2
	40	89.2	84.7

D-arabino-hexo- pyranosyl) indole (VI) with sodium methoxide in dry methanol to obtain the target free nucleoside, 1-(2',2'-dideoxy-3',3'-C-nitromethyl-β-D-arabino- hexopyranosyl) indole (VI), yielding a pale yellow crystal in 53% yield (Scheme 1). Rf, 0.35 (DCM: EtOH, 9:1); melting point 167-169°C and IR spectrum showed 3402 cm^{-1} (O-H) group, 1588 cm^{-1} and 1357 cm^{-1} (NO_2) [28].

***In-vitro* Antiviral Activity:** The nucleoside (V and VII) were evaluated for their antiviral activity against HSV-I and HAV by a virus plaque reduction assay as shown in Scheme 1. The nucleoside VII elicited a marked antiviral activity; this derivative was more active than other derivatives. An explanation of these results may be indicating that the nucleoside VII has low molecular mass than nucleoside V. The conformational flexibility of this chain was for adopting a definite shape that might be required during the formation of nucleoside-virus complex. It is likely that post infection involved inhibition of early post absorption steps such as virus internalization and inhibition of cell-to-cell transmission in successive cycles of replication. The interest in 2',3'-dideoxy nucleosides has increased tremendously in recent years and the number of publication dealing with these compounds is extremely extensive and growing at a rapid rate because of the discovery that 2',3'-deoxy nucleosides are effective therapeutic agents for the treatment of AIDS and other virus diseases [31-34].

CONCLUSION

The branched chain sugars was converted to their active form (1-bromosugar derivatives).7-(2',3'-Dideoxy-

3',3'-di-C-nitromethyl-β-D-arabinohexopyranosyl) theophylline (V) and 1-(2',3'-Dideoxy-3',3'-di-C-nitromethyl-β-D-arabinohexo pyranosyl) Indole (VII) were successfully prepared. The structure of both was confirmed. Both was subjected to antiviral assay compound (V) showed lower antiviral activity than (VII) at all different used concentrations.

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