Exploring synthetic ureidyl carbohydrates for potential human adenovirus inhibition

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Abstract

Adenoviruses is a common pathogen associated to multiple diseases. Carbohydrates containing a diversely substituted urea moiety were synthesized from the corresponding isocyanate and used on Human adenovirus as target. The structure with phenyl ring as substituent provides a potential anti-Ads with an inhibition about 50%.

Keywords

Human Adenovirus, urea, carbohydrates.

Introduction

Nowadays, adenoviruses (Ads) are common pathogens in patients with compromised immunity being a potentially life-threatening disease. Human Ads are usually associated with respiratory infections, gastroenteritis or viral conjunctivitis among others with significant clinical impact.[1]

These adenoviruses are non-enveloped showing an icosahedral morphology. In this context, the structure is formed by three capsid proteins named hexon, penton and fibre. In particular, both penton and fibre proteins are involved in the interactions between virus and cell surface.[2]

Carbohydrates are essential in everyday life being keys in several biological processes as molecular recognition by protein. There are a number of natural and synthetic carbohydrates with different applicability. For instance, chitosan can be used in several fields due to its sole properties such as drug delivery system, polar sensing biomaterial,[3] molecular NLO properties[4] or heterogeneous catalyst.[5] Additionally, sugar-peptide hybrids have the capability to acts as foldamers.[6] Finally, it is known that nucleotide and nucleoside analogues as ribavirin (1-β-ribofuranosyl-1,4,6-triazole-3-carboxamide)[7] act

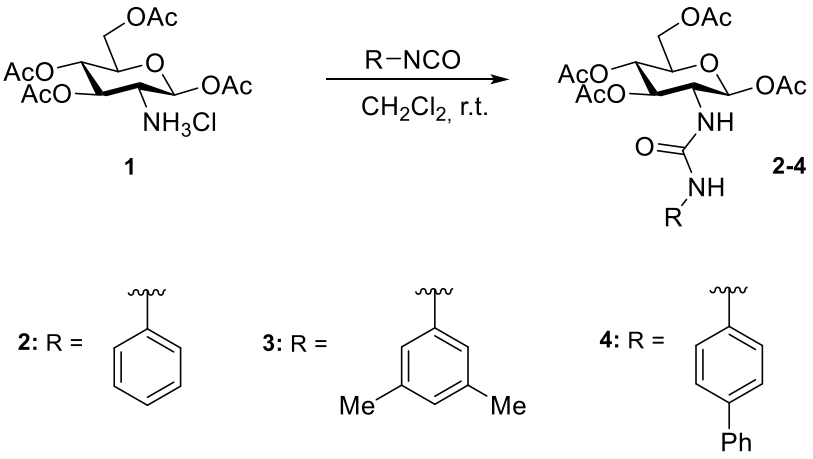
as DNA polymerase inhibitor being potential compounds for the treatment of adenovirus infections.

Herein, we report an easy synthesis of both mono and disaccharides ureidyl-glucosamine derivatives. All new compounds were characterized by means of ^{1}H, ^{13}C NMR as well as mass spectrometry. Finally, a biological screening of synthesized compounds on human epithelial cell was carried out.

Results and discussion

Novel ureidyl-carbohydrates (**2**-**11**) derived from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose (**1**) after reaction with the suitable isocyanate were obtained (Scheme 1). Compounds **2**-**6** are considered as lipophilic ureidyl derivatives whereas their corresponding deacetylated compounds (**7**-**11**) have an amphiphilic behaviour.

As expected for electrophilic properties of isocyanate reagents, anhydrous solvents were required for these reactions. Under anhydrous conditions, the yields are good in the range of 79-89% (for compounds **2** and **4**, respectively).



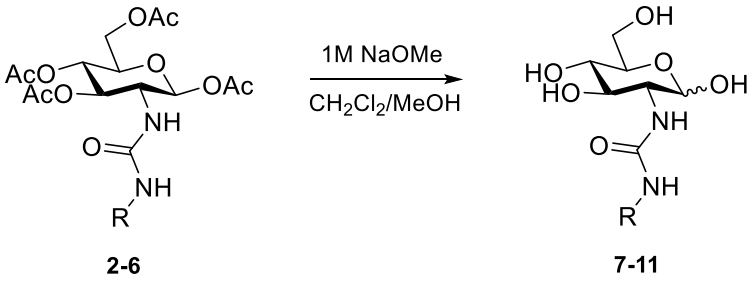
Scheme 1. Synthesis of mono ureidyl derivatives **2**-**4**

^{1}H NMR Spectra for these ureidyl derivatives typically show the new two NH protons after the reaction approximately at 7.00 ppm (conjugated with aromatic ring) and 5.20 ppm. Additionally, ^{13}C NMR spectra reveal the presence of ureidyl quaternary carbon (C=O) at 155.0 ppm. IR Spectra also corroborate the presence of the urea function by showing a peak at 1675 cm^{-1} for C=O bond.

Compounds **5** and **6** being symmetrically ureidyl-derived disaccharides have been obtained starting from per-*O*-acetylated glucosamine (**1**) and 4,4'-methylenebis(phenyl

isocyanate) or hexamethyldiisocyanate, respectively. For compound **5**, ^{1}H NMR spectrum allows us to differentiate protons of methylene moiety at 3.75 ppm.

In order to access the biological screening, the acetyl groups deprotection is necessary. For this purpose, compounds **2-6** were treated with a 1M methanolic solution of NaOMe, in CH_2Cl_2, to give the corresponding amphiphilic carbohydrates (**7**-**11**, Scheme 2). In general, this reaction provides excellent to quantitative yields. For these compounds, in ^{1}H NMR spectra different doublets and a triplet for hydroxyl groups were observed by using of DMSO-d_6 as solvent.



2-6 **7-11**

Scheme 2. Synthesis of amphiphilic ureidyl derivatives **7-11**

Biological screening was carried out in Human A549 and 293β5 cell lines. These cells were modified with HAd5-GFP by using a cytomegalovirus promoter. In this case, the compounds obtained were evaluated in a plaque assay with a concentration of 10 µM. The outcomes reveal that compound **7** (R = Ph) is the best hit of compounds studied (Figure 1).

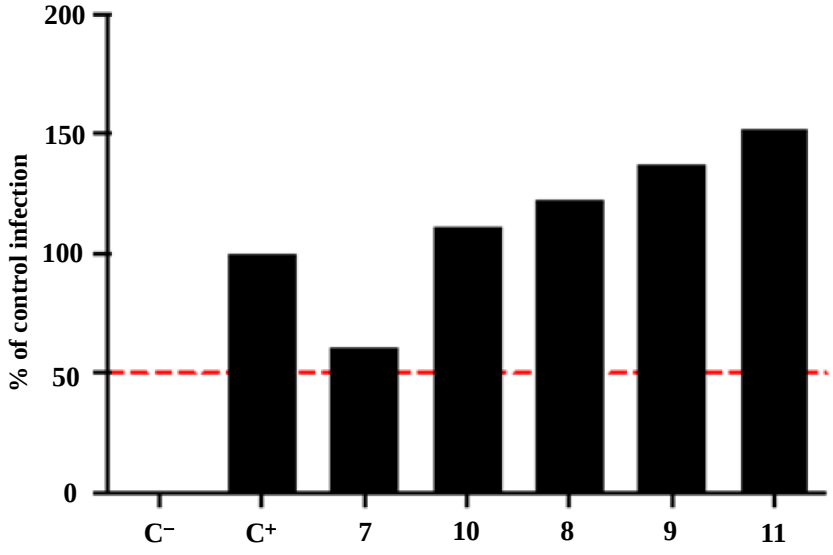


Figure 1. Plaque assay for amphiphilic uredyl-carbohydrates

In summary, new ureidyl derivatives from carbohydrates were easily synthesized and characterized. Biological assays provide a good candidate as hit to be modified in order to improve the biomedical activity.

Experimental Methods

All chemicals were purchased and used without further purification. Evaporations were conducted under reduced pressure. TLC was performed on silica gel plates (MN ALUGRAM Xtra SIL G/UV 254). All new compounds were synthesized following the below general procedure. Detection of compounds was accomplished with UV light (254 nm) and by charring with H_2SO_4 and characterization by ^{1}H and ^{13}C NMR spectroscopy and Mass spectrometry.

*General procedure for the synthesis of ureidyl-glucosamine derivatives (**2**-**6**)*

To a heterogeneous mixture of compound **1** (1 equiv.) in toluene/H_2O $CaCO_3$ was added. After 1 h, the mixture was filtered and extracted with EtOAc (3 × 3 mL). The organic layers were evaporated to dryness and then, was dissolved in CH_2Cl_2 and treated with the corresponding isocyanate (1 equiv.) at room temperature. Reaction monitoring was made by TLC. The new compounds were purified by column chromatography.

Data for compound **2**[7]:

^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.34-7.28 (4H, m, Ph), 7.10 (1H, m, Ph), 6.77 (1H, s, PhN*H*CO), 5.79 (1H, d, $J_{1,2}$ = 8.7 Hz, H-1), 5.25 (1H, t, $J_{3,4}$ = $J_{3,2}$ = 9.3 Hz, H-3), 5.14 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, H-4), 5.07 (1H, d, $J_{NH,2}$ = 9.3 Hz, N*H*CO), 4.28 (1H, dd, $J_{6a,5}$ = 4.7 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.13 (2H, m, H-6b and H-2), 3.85 (1H, m, H-5), 2.12, 2.09, 2.05 and 2.03 (3H, s, C*H*$_3$)

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