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Synthesis and evaluation of 4-*O*-alkylated 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid derivatives as inhibitors of human parainfluenza virus type-3 sialidase activity

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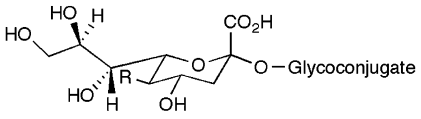
Abstract—The X-ray crystal structure of the paramyxoviral surface glycoprotein haemagglutinin-neuraminidase (HN) from Newcastle Disease virus was used as a template to design inhibitors of the HN from human parainfluenza virus type-3 (hPIV-3). 4-*O*-Alkylated derivatives of 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en), accessed from 8,9-*O*-isopropylidenated-Neu5Ac2en1Me, were found to inhibit the sialidase (neuraminidase) activity of hPIV-3 (strain C243) in the range of 3–30 μM. This is comparable or improved activity compared to the parent 4-hydroxy compound.

The paramyxoviridae family of viruses includes a number of respiratory pathogens that cause epidemics of major medical and veterinary importance.[1] Among these, the human parainfluenza viruses (hPIV), types 1 to 3, are leading causes of respiratory disease in infants and young children, the immunocompromised, the chronically ill and the elderly (reviewed in Refs. 2 and 3[2,3]). While a number of agents show in vitro activity against parainfluenza viruses (reviewed in Ref. 2[2]), there are currently no antiviral drugs with proven clinical efficacy against hPIV.[2] The wide incidence of infection, and the severity of disease in immunocompromised patients,[2] suggests that work should be directed towards the development of chemotherapeutic agents to treat parainfluenza virus infections.

Human parainfluenza viruses 1 and 3, and a number of other *paramyxoviridae* including Newcastle Disease virus (NDV) possess two membrane proteins, a fusion protein and a multifunctional glycoprotein, haemagglutinin-neuraminidase (HN).[4] HN has three roles in the pathogenesis of the virus; it mediates the attachment of the virus to the host cell by interaction with sialic acids (neuraminic acids) associated with cell-surface glycoconjugates (e.g., **1**);[5,6] it promotes fusion activity enabling viral entry into the cell;[7] and it acts through its sialidase (neuraminidase) function to cleave sialic acids from the surface of nascent virion particles to prevent self-agglutination and promote virion elution from host cells.[8] The multifunctional role of HN in the viral life-cycle makes it an attractive target for the development of therapeutics to treat hPIV infection. In particular, inhibition of sialidase activity may potentially reduce degradation of epithelial cell coating thereby hindering secondary bacterial infection.[9,10]

The first three-dimensional structure of a haemagglutinin-neuraminidase protein, the HN from Newcastle



1 R = NHAc
N-acetylneuraminic acid (Neu5Ac) glycoconjugate

Keywords: Parainfluenza virus; Sialidase; Sialic acids; Inhibition; Computational chemistry; Inhibitor design; Carbohydrates.
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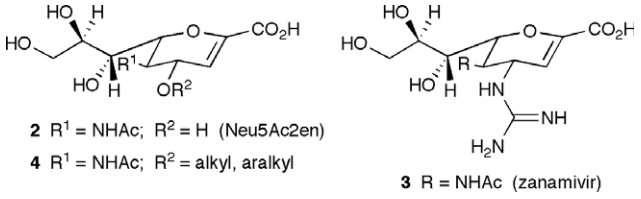
Disease virus, was reported in 2000.[11] This structure showed the presence of a single 'pliable' active site that could encompass both the sialic acid receptor-binding and sialoside hydrolysis functions of the HN, with the selection of function moderated by a conformational switch in the protein.[11] An X-ray crystal structure of the HN protein of hPIV-3 reported in 2004 showed similar structural flexibility of the active site.[12] The location of both the receptor-binding and sialidase functions in a single site[11,12] suggests that compounds designed to bind to this site could minimise these functions, and block both viral infection and virus spread.

In comparison to the sialidases from influenza virus, and from bacterial sources, there have been relatively few studies of the inhibition of the paramyxovirus HN sialidase activity, and in particular of hPIV-3 HN activity. In the case of hPIV-3 HN, the unsaturated N-acetylneuraminic acid derivative 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en, **2**), a naturally occurring sialidase inhibitor, and the potent influenza virus sialidase inhibitor 4-deoxy-4-guanidino-Neu5Ac2en (zanamivir) **3** have been evaluated for their inhibition of hPIV-3 sialidase activity in vitro,[13] with IC_{50} values reported in the low millimolar range for both compounds. Interestingly, zanamivir has also been shown to inhibit the receptor-binding and fusion processes of hPIV-3,[13,14] supporting the premise that an inhibitor binding to the active site could interfere with both the receptor-binding and sialidase functions of the HN. Herein we report the synthesis of 4-*O*-alkylated Neu5Ac2en derivatives **4**, designed based on the three-dimensional structure of NDV-HN,[15] and their evaluation as inhibitors of hPIV-3 HN sialidase activity.



2 R^1 = NHAc; R^2 = H (Neu5Ac2en)

4 R^1 = NHAc; R^2 = alkyl, aralkyl

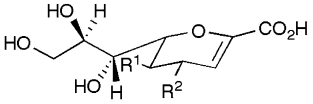
3 R = NHAc (zanamivir)

At the beginning of this work, the sole three-dimensional structure of a paramyxoviral HN was that from NDV.[11] The crystal structure of NDV HN (1E8V)[11] containing Neu5Ac2en (**2**) shows a large cavity around the C-4 position of bound Neu5Ac2en, the hydroxyl group of which makes no interactions with the protein.[11] A GRID[16] study of the active site showed an area of hydrophobic interaction within the large C-4 cavity and directed our molecular modelling studies to the design of a series of Neu5Ac2en derivatives substituted at C-4 with hydrophobic groups that were envisaged to bind well into the large active site. These derivatives of Neu5Ac2en were anticipated to have enhanced binding to NDV HN, and potentially to other paramyxoviral HNs such as those from the human parainfluenza viruses.

The target 4-*O*-alkylated Neu5Ac2en derivatives **4a–e** were synthesised using the approach shown in Scheme 1. *N*-Acetylneuraminic acid (**5**, Neu5Ac) was converted, via elimination of HCl from the β-chloride derivative **6**, to the unsaturated derivative **7** in ~75% yield over three steps. De-*O*-acetylation, followed by isopropylidenation of the C-8 and C-9 hydroxyl groups, gave derivative **8**[17] in high yield. Methods for the selective alkylation of the C-4 hydroxyl group of neuraminic acid derivatives have been reported in the literature.[17,18] In this work, selective 4-*O*-alkylation of **8** in the presence of sodium hydride gave the products **9a–e** in moderate yield. Alternatively, 4-*O*-alkylation could be carried out using silver(I) oxide as catalyst, however the yields for the straight alkyl derivatives **9c–e** were considerably lower using this method. Subsequent two-step deprotection of compounds **9a–e** gave the target 4-*O*-alkylated Neu5Ac2en derivatives **4a–e** in good yields.[19]

The 4-*O*-alkylated Neu5Ac2en derivatives **4a–e** were evaluated for their ability to inhibit the sialidase action of hPIV-3 (strain C243) in a whole virus assay[20,21] using 4-methylumbelliferyl *N*-acetyl-α-D-neuraminide as the fluorogenic enzyme substrate. The IC_{50} values of **4a–e** and the parent compound Neu5Ac2en **2**, as well as 4-deoxy-4-guanidino-Neu5Ac2en **3** (zanamivir), are given in Table 1. The 4-*O*-alkylated Neu5Ac2en derivatives with the largest hydrophobic groups [**4a** (benzyl) **4b** (2-phenyl-benzyl), and **4e** (decyl)] showed comparable activity to the parent 4-hydroxy derivative **2**, while derivatives with the smaller hydrophobic groups [**4e** (ethyl) and **4d** (hexyl)] showed an order of magnitude improvement in inhibition over Neu5Ac2en. These data indicate that hydrophobic groups [even large groups such as the 2-phenylbenzyl substituent (**4b**)] introduced onto the C-4 hydroxyl of Neu5Ac2en can indeed bind into the active site of hPIV-3 HN without detriment to binding affinity. Analysis of the subsequently published 3D structure of hPIV-3 HN[12] shows that the active site cavity accommodating the C-4 hydroxyl group of Neu5Ac or Neu5Ac2en is in fact larger than that of NDV HN (used as the design template in this study), although the environment of the pocket is slightly less hydrophobic than that observed in NDV HN.

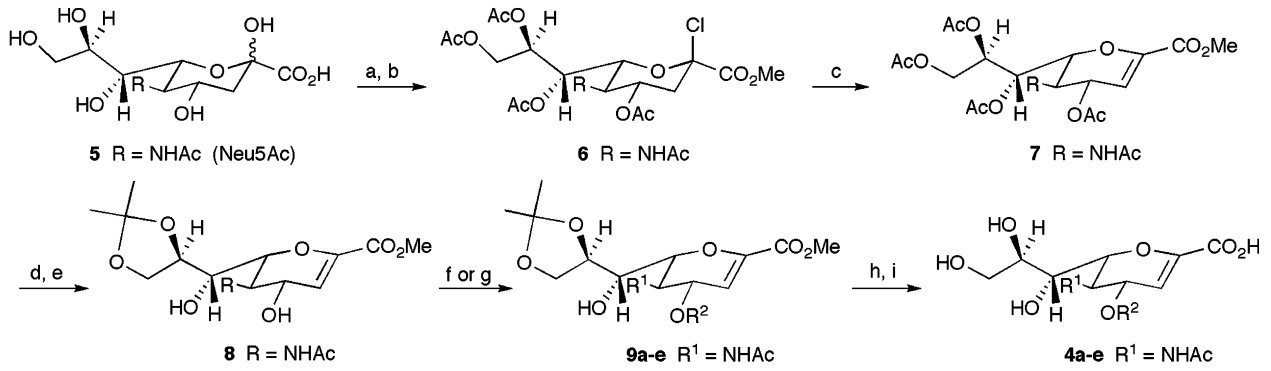
The design of hPIV inhibitors using the X-ray structure of NDV HN has also been reported by Alymova et al.[22] Two unsaturated neuraminic acid derivatives, C-4 substituted *N*-(2-methylpropionyl)-2-deoxy-2,3-didehydro-neuraminic acid derivatives BCX-2798 **10** and BCX-2855 **11**, were reported to inhibit both the haemagglutination and sialidase activities of hPIVs 1-3, and the growth of the viruses in cell culture.[22] The IC_{50} values reported for BCX-2798 **10** and BCX-2855 **11** against hPIV-3 sialidase activity were 20 and 4.3 μM, respectively. These values are comparable to those obtained with the Neu5Ac2en derivatives **4a–e** reported



R^1 = $NHC(O)CH(CH_3)_2$

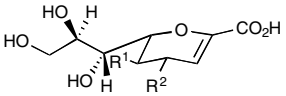
10 R^2 = N_3 (BCX-2798)

11 R^2 = $NHS(O)_2CHCl_2$ (BCX-2855)



Scheme 1. Reagents and conditions: (a) MeOH, cat. H^+ resin, rt, o/n; (b) AcCl, rt, 2 d; (c) DBU, DCM, rt, 2.5 h (75% over 3 steps); (d) NaOMe, MeOH, 0 °C, 2 h; (e) 2,2-dimethoxypropane, cat. H^+ resin, acetone, rt, o/n (92% over 2 steps); (f) RBr (1.2 equiv), DMF, NaH (1.3 equiv), 0 °C, 0.5–4 h (**9a**,[17] 65%; **9b**, 61%; **9c**, 62%; **9d** 61%; **9e**, 33%); (g) i—RBr (1.2 equiv), DCM, 3 Å mol. sieves, rt, 2 h; ii—Ag_2O (3 equiv), *n*-Bu_4NI, rt 2 d (**9a**, 62%; **9b**, 82%; **9c**,[18] 40%; **9d** 34%; **9e**, 11%); (h) 80% AcOH, 0.5–2 h; (i) 0.1 M KOH, MeOH, rt, 1 h–3 d (~90% over 2 steps).

Table 1. Inhibition (IC_{50} μM) of hPIV-3 (strain C243)[20] sialidase activity by C-4 modified Neu5Ac2en derivatives **4a**–**e**[a]



Compound	R^2	Sialidase inhibition (IC_{50} μM)
4a	$OCH_2C_6H_5$	10
4b	$OCH_2C_6H_4$-*o*-C_6H_5	30
4c	OCH_2CH_3	3
4d	$OCH_2(CH_2)_4CH_3$	5
4e	$OCH_2(CH_2)_8CH_3$	10
2 (Neu5Ac2en)	OH	24
3 (zanamivir)	$NHC(=NH)NH_2$	25

[a] Sialidase activity was measured in a fluorometric assay using 4-methylumbelliferyl *N*-acetyl-α-D-neuraminide as substrate.[20]

here, that contain significantly different substitutions at C-4.

The results of both the study described here and that reported by Alymova et al.[22] demonstrate that the crystal structure of NDV HN is suitable as a model for the design of hPIV-3 HN inhibitors. The X-ray structure of hPIV-3 HN reported[12] subsequent to this work will allow further, directed, structure-based design of potential inhibitors of hPIV-3. With regard to inhibition of other hPIVs, investigation of the 4-substituted neuraminic acid derivatives as inhibitors of hPIV-1,[22,23] for which there is as yet no crystal structure, indicates that there are differences in the C-4 binding pockets between the HN of hPIV-3 and that of hPIV-1.

In summary, derivatives of Neu5Ac2en with hydrophobic substituents on the C-4 oxygen have been shown to give comparable or improved inhibitory potency against hPIV-3 sialidase activity compared to the parent compound in vitro. This work sets the stage for the development of more potent inhibitors of infection by viruses of the Paramyxoviridae subfamily.

Acknowledgments

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20. Sialidase inhibition assay: Sialidase activity was assayed by a fluorometric assay using 4-methylumbelliferyl *N*-acetyl-α-D-neuraminic acid as previously described for hPIV-1.[21] hPIV-3 was isolated from infected LLC-MK2 cells as previously described for hPIV-1.[21] Assays were performed in duplicate and the determined values are averages and reproducible.
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