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NOVEL INHIBITORS OF INFLUENZA SIALIDASE RELATED TO GG167
Synthesis of 4-Amino and Guanidino-4H-Pyran-2-Carboxylic Acid-6-Propylamides; Selective Inhibitors of Influenza A Virus Sialidase

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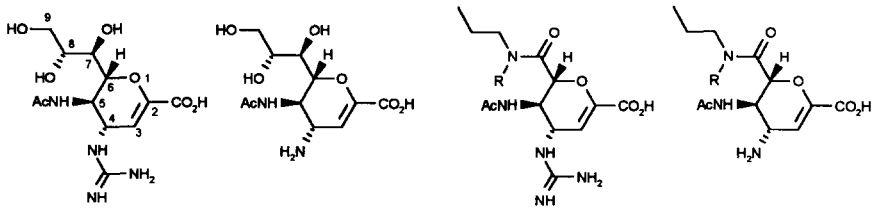
Abstract: N-Propylcarboxamides **1a,b-3a,b** have been synthesised from 2,3-didehydro-2,4-dideoxy-4-amino-N-acetylneuraminic acid **4**. The tertiary amides **2a,b-3a,b** are highly potent but selective inhibitors of influenza A sialidase. The exceptional inhibitory activity of the dipropylamides **3a** and **3b** against influenza A shows that the 6-dipropylcarboxamide substituent is preferable to the polar glycerol sidechain found in the related sialidase inhibitors GG167 and **4**.

Introduction

Influenza sialidase plays a crucial role in the life cycle of the virus and it has long been postulated that inhibitors of this enzyme would have potential in the treatment of influenza.[1] GG167 is the most potent reported inhibitor of both influenza A and B virus sialidases and is currently undergoing clinical evaluation.[2] It was discovered through a rational drug design approach based on the crystal structure of influenza A sialidase and using computational chemistry techniques.[3,4] In recent studies we have examined the contribution to sialidase binding made by each of the dihydropyran substituents of GG167,[4-9] whilst others have reported weak aromatic inhibitors.[10,11] The present study is concerned with the identification of new replacements for the polar 6-glycerol substituent in GG167. Previous X-ray studies, with both influenza A and B sialidases, have shown that the 8- and 9-hydroxyl groups of both sialic acid and sialidase inhibitors such as GG167 make important hydrogen bonding interactions with these enzymes.[3,12] Furthermore, the poor activity of analogues with truncated glycerol sidechains confirmed the major contribution of these interactions towards inhibitor binding.[7] We now report that the propylamides **2-3** are also potent inhibitors of influenza sialidases. Some of these analogues show even better activity against influenza A sialidase than the corresponding 6-glycerol analogues GG167 and **4**.

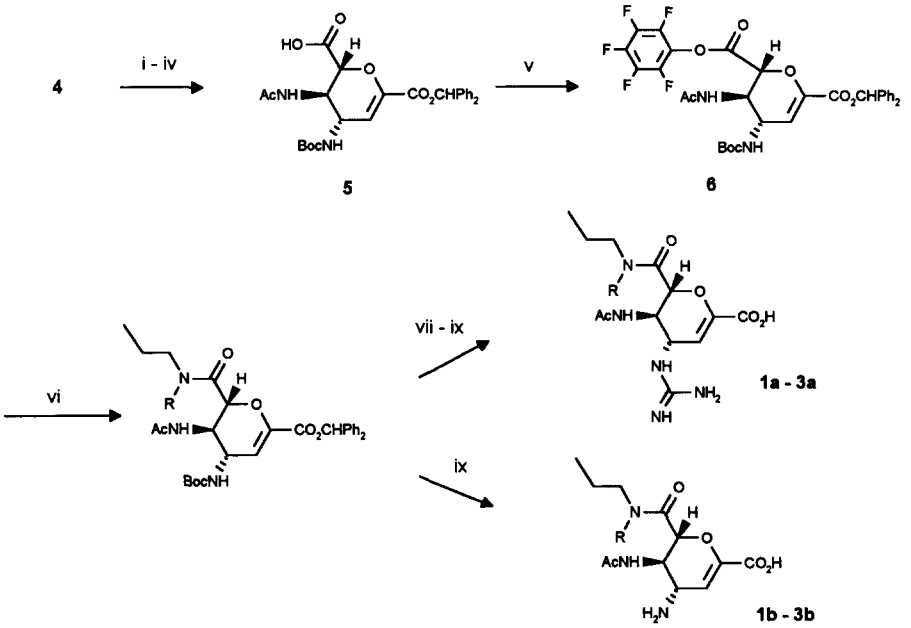


GG167 **4**

R = H:	**1a**	**1b**
R = Methyl:	**2a**	**2b**
R = n-Propyl:	**3a**	**3b**

Chemistry

2,3-Didehydro-2,4-dideoxy-4-amino-N-acetylneuraminic acid **4** [5] was converted to the protected intermediate acid **5** in 4 steps using conventional methodology (50% overall yield). None of the intermediates in this sequence required chromatographic purification and the process was routinely carried out on multi-gram scale. Various coupling methods were investigated in order to introduce the required carboxamide sidechains, but the most satisfactory route developed was *via* the pentafluorophenyl ester **6** (prepared by treating **5** with pentafluorophenyl trifluoroacetate[13]). This intermediate afforded moderate to high yields of amides when treated with both primary and secondary propylamines. Following the coupling of **6** with the appropriate amine, the acid labile protecting groups were removed by treatment with trifluoroacetic acid in dichloromethane to afford the target 4-amino derivatives **1-3b** directly. Alternatively, the N-tBoc protecting group was selectively removed in the presence of the diphenylmethyl ester by treatment with HCl in dioxan, and the guanidino group introduced using (*tert*-butoxycarbonylamino)pyrazol-1-yl-methyl carbamic acid *tert*-butyl ester ('BisBocPCH').[14] Subsequent treatment with trifluoroacetic acid in dichloromethane afforded the target 4-guanidino amides **1-3a**.[15]



i) Boc_2O, dioxan / aqueous $KHCO_3$, ii) Ph_2CN_2, CH_2Cl_2 (70% over 2 steps) iii) $NaIO_4$, 2 equiv / aq MeOH iv) $NaClO_2$, cyclohexene, tBuOH, KH_2PO_4 aq (70% over 2 steps) v) pentafluorophenyl trifluoroacetate, pyridine, DMF (100%) vi) R_1R_2NH, THF (60-80%) vii) HCl / dioxan (100%) viii) BisBocPCH, Et_3N, THF (60% over 2 steps) ix) CF_3CO_2H / CH_2Cl_2 (>90%)

Sialidase Inhibition and *in vitro* Antiviral Activity of Propylamides

Inhibition of influenza sialidase was determined in a fluorimetric assay by measuring the ability of compounds to inhibit the hydrolysis of 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUN) by whole virus (A/Aichi N2 or B Victoria) grown in hen eggs.[16,17] The IC_{50} value quoted is the

concentration of inhibitor required to reduce the enzymic activity in this preparation by 50%. *In vitro* antiviral activity was evaluated in a plaque reduction assay by the method previously reported.[17,18] The IC_{50} value is the concentration of inhibitor required to reduce the number of viral plaques by 50%.
The biological activities of propylamides **1a,b-3a,b** in these assays are shown in the table below together with the data obtained for GG167 and the corresponding 4-amino analogue **4**.[19]

R		Sialidase Inhibition IC_{50}		Plaque Reduction IC_{50}	
		A Aichi (μM)	B Victoria (μM)	Flu A (μg/ml)	Flu B (μg/ml)
H	**1 a:**	0.5	4.4	0.28	3.8
	b:	19	50	1.4	44
Methyl	**2 a:**	0.004	4.5	0.011	2.2
	b:	0.18	23	0.32	16
n-Propyl	**3 a:**	0.002	0.54	0.0001	0.32
	b:	0.012	2	0.002	0.7
	GG167	0.005	0.004	0.005	0.002
	4	0.32	0.41	0.47	0.02

Sialidase Inhibition and *in vitro* Antiviral Activity of Propylamides **1-3a,b**

The 4-amino secondary propylamide **1b** is a weak inhibitor of influenza A and B sialidases when compared to GG167. However, further substitution of this amide with methyl or propyl to give the tertiary amides **2b** and **3b** results in a dramatic improvement in activity against the influenza A enzyme and virus, but has a relatively small effect on activity against influenza B. The 4-guanidines **1a** - **3a** all show improved activity over the corresponding amines **1b** - **3b**, but the improvement is less than that seen between GG167 and **4**. The tertiary carboxamides are thus highly potent and selective inhibitors of influenza A sialidase and virus, with the dipropylamides **3b** and **3a** showing better activity than the corresponding compounds retaining the 6-glycerol sidechain of sialic acid (**4** and GG167). Evaluation of **2a** and **3a,b** against a selection of sialidases from other influenza A and B strains suggest that this high degree of selectivity for influenza A is general (data not shown). Structural and computational studies to rationalise the potent activity and high specificity of these 6-propylamides will be reported shortly.[20]

Conclusion

In summary, the propylamides **2-3a,b** were readily prepared from 2,3-didehydro-2,4-dideoxy-4-amino-N-acetylneuraminic acid **4**. They are highly potent and selective inhibitors of influenza A sialidase and display antiviral activity *in vitro*. The activity of the 4-amino dipropylamide **3b** against influenza A is comparable with GG167. This demonstrates that nanomolar inhibition of influenza A sialidase is possible for compounds which lack both a glycerol and guanidino group.

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