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Sialidase Inhibitors Related to Zanamivir. Further SAR Studies of 4-Amino-4*H*-pyran-2-carboxylic Acid-6-propylamides

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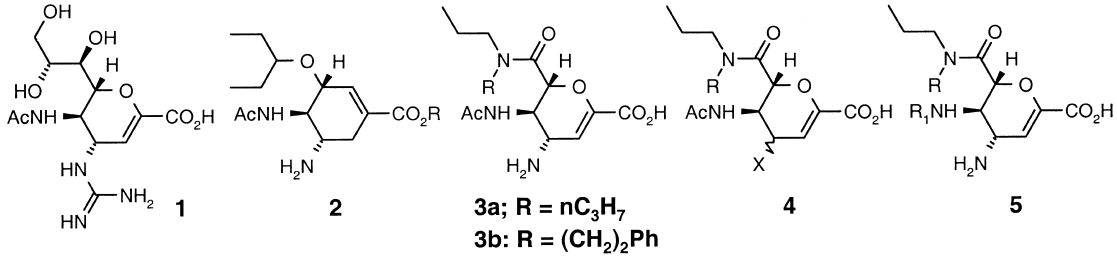
Abstract—SAR investigations of the 4- and 5-positions of a series of 4-amino-4*H*-pyran-2-carboxylic acid 6-carboxamides are reported. Potent inhibitors of influenza A sialidase with marked selectivity over the influenza B enzyme were obtained when the basic 4-amino substituent was replaced by hydroxyl or even deleted. Modifications at the 5-position exhibited a tight steric requirement, with trifluoroacetamide being optimal.

Introduction

Zanamivir **1**[1] and GS 4071 **2** (R = H)[2,3] are potent selective inhibitors of influenza A and B sialidases and influenza A and B in vitro. The effectiveness of both zanamivir and the prodrug GS 4104 **2** (R = Et) during clinical trials has demonstrated the utility of sialidase inhibitors as anti-influenza agents.[4] Previously, as part of the SAR investigations into zanamivir, we reported on a series of 4*H*-pyran carboxamide influenza sialidase inhibitors **3**.[5,6] In these compounds the glycerol side chain of zanamivir was replaced by a lipophilic carboxamide moiety. Although some of this series were more potent inhibitors of influenza A sialidase than zanamivir, they were significantly less potent against the influenza B enzyme. In this paper we describe further development of the SAR of the carboxamide series, looking at potency and selectivity. During these studies we have focused on the 4- and 5-positions on the pyran ring, structures **4** and **5**, respectively. The in vitro and in vivo antiviral properties of representatives of these series are reported.

Results and Discussion

Although some of the previously described 4-amino-4*H*-pyran-2-carboxylic acid 6-carboxamides are potent inhibitors of influenza A sialidase, they are significantly



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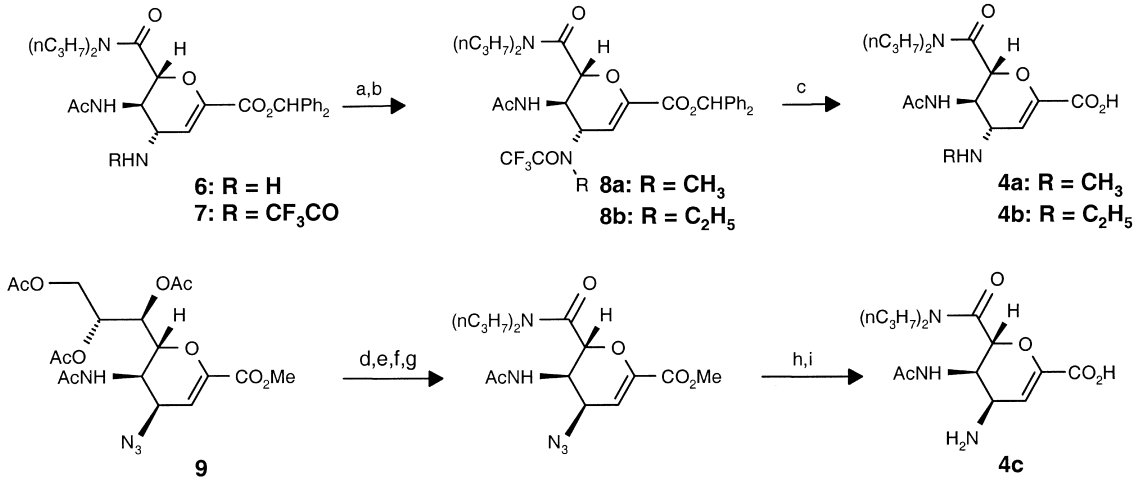
less inhibitory of influenza B sialidase.[5,6] To expand the SAR studies of the carboxamide series, the 4- and 5-substituents were modified to investigate the effect on potency and selectivity.

Modification of the 4-position

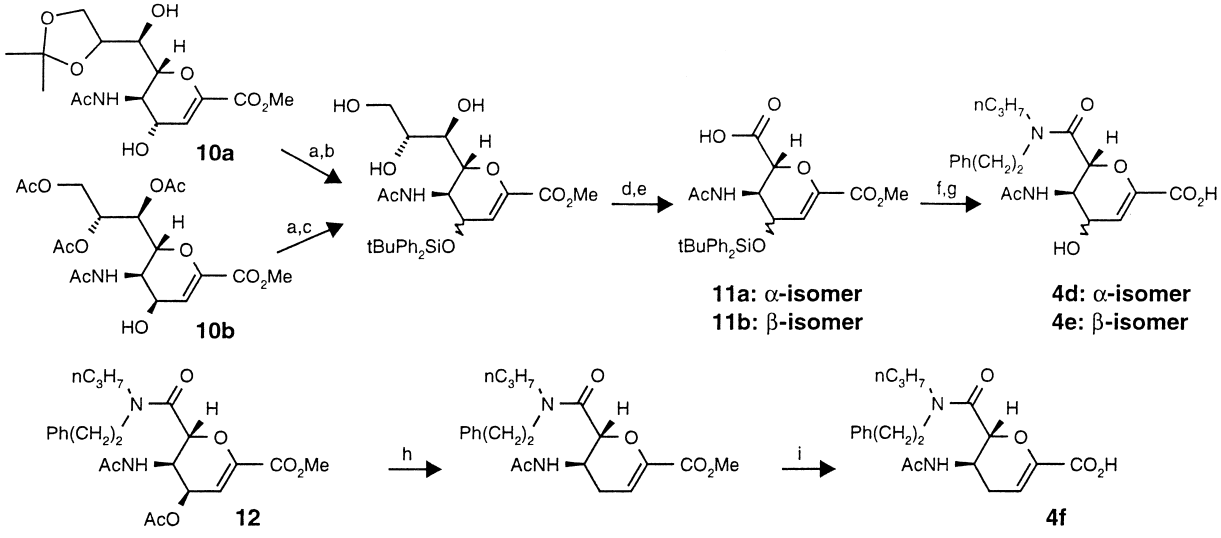
Previous studies of the SAR of the 4-position of zanamivir suggested that modification of the 4-guanidino substituent was not tolerated. Also taking in consideration the potency of some of the 4-amino carboxamides[14] so far described, we decided not to investigate 4-guanidino derivatives but to use amines **3a**,**b** as our starting point. 4-Alkylamino derivatives **4a**,**b** were synthesised using the route shown in Scheme 1. Whereas alkylation of the 4-amino derivative **6**[5] afforded gross mixtures, caesium carbonate mediated alkylation of the 4-trifluoroacetamide **7** gave good yields of the 4-*N*-alkylamino derivatives **8a**,**b**. Deprotection under standard conditions[5] yielded **4a**,**b**.[7] Using established chemistry[5] β-azide **9**[8] was converted to the β-amino derivative **4c**.

The syntheses of the 4-α- and β-hydroxy epimers **4d** and **4e**, from the glycerol-protected 4-α- and β-hydroxy derivatives **10a**[9] and **10b**,[10] respectively, are outlined in Scheme 2. The acid-stable *t*-butyldiphenylsilyl ether was used to protect the 4-hydroxy moiety of **10a** and **10b**, allowing the synthesis of acids **11a** and **11b**. TBTU[11] mediated coupling of acids **11a**,**b** with phenethyl-propylamine gave the corresponding amides in higher yields than the previously used two-step process via the pentafluorophenyl esters.[5]

The 4-desoxy derivative **4f** was synthesised using a sodium borohydride reduction of the palladium complex of β-acetate **12** (Scheme 2).[12] Acetate **12** was obtained in 92% yield by acetylating the methyl ester of **4e** using acetic anhydride in pyridine.



Scheme 1. (a) $(CF_3CO)_2O$ (93%); (b) Cs_2CO_3, RBr, DMF (85% R = CH_3); (c) (i) TFA, CH_2Cl_2; (ii) K_2CO_3, MeOH, H_2O (85% R = CH_3); (d) NaOMe, MeOH (94%); (e) (i) $NaIO_4$ MeOH, H_2O; (ii) $NaClO_2$, *t*BuOH (58%); (f) $CF_3CO_2C_6F_5$, pyridine; (g) $(nC_3H_7)_2NH$, THF (30% over 2 steps); (h) PPh_3, THF, then Et_3N, H_2O; (i) Et_3N, H_2O (74% over 2 steps).



Scheme 2. (a) *t*$BuPh_2SiCl$, DMAP, imidazole, DMF (77% 4-β-OH); (b) 80% aq AcOH (83% from **10a**); (c) NaOMe, MeOH (99%); (d) (i) $NaIO_4$, MeOH, H_2O; (ii) $NaClO_2$, *t*BuOH (89% 4-β-OH); (e) TBTU, $[Ph(CH_2)_2](nC_3H_7)NH$, THF (42% 4-β-OH); (f) TBAF, THF (90% 4-β-OH); (g) Et_3N, H_2O (43% 4-β-OH); (h) $Pd(Ph_3)_4$, $NaBH_4$, THF (41%); (i) Et_3N, H_2O (69%).

The 4-*N*-α-methyl- and ethylamino derivatives **4a**,**b** (Table 1) exhibited at least a 10-fold loss of activity when compared to **3a**. These results mirrored the findings in the 6-glycerol series.[15] The 4-β-amino derivative **4c** proved to be approximately 10-fold less active than the corresponding 4-α-amino epimer **3a** both against influenza A and B sialidase.

To investigate whether the need for a basic group in the 4-position for potent activity still held for the carboxamide series, we synthesised the epimeric 4-α- and β-hydroxy derivatives **4d** and **4e**, respectively. Unexpectedly, the compounds exhibited similar activity against influenza A sialidase to **3b**, indicating this group was not as important for binding in this series as for the 6-glycerol series. More surprisingly, the 4-desoxy derivative **4f** had similar activity to **3b** and **4d**. This was an important finding that could aid the design of further influenza sialidase inhibitors. Even against influenza B sialidase, where the carboxamide moiety is less favourably bound, removal of the basic amine of **3b** did not significantly affect the binding, as **4f** had a similar potency to **3b**. Overall, the modifications carried out did not reduce the difference in potency against influenza A and B and in some cases increased it.

Comparison of the crystal structures of **3b** and **4e** bound into influenza A sialidase (Fig. 1) or influenza B sialidase (data not shown) did not give any obvious rationale for the above observations, as the whole crystal

Table 1. Sialidase inhibitory and plaque reduction activities of **4a**–**f**. Comparison with **1** and **3a**,**b**[a]

	X	R	Sialidase inhibition IC_{50}		Plaque reduction IC_{50}	
			A Aichi (μM)	B Victoria (μM)	Flu A (μg/mL)	Flu B (μg/mL)
1			0.002	0.004	0.005	0.002
3a	α-NH_2	*n*-Propyl	0.012	2.0	0.002	0.7
3b	α-NH_2	$Ph(CH_2)_2$	0.003	3.6	0.03	0.28
4a	α- NHMe	*n*-Propyl	0.24	140		
4b	α-NHEt	*n*-Propyl	0.42	4.5		
4c	β-NH_2	*n*-Propyl	0.14	7.4		
4d	α-OH	$Ph(CH_2)_2$	0.007	51	0.005	>100
4e	β-OH	$Ph(CH_2)_2$	0.017	>400		
4f	H	$Ph(CH_2)_2$	0.007	14		

[a]Sialidase inhibitory and plaque reduction activities of compounds were determined by previously reported methods.[5,16]

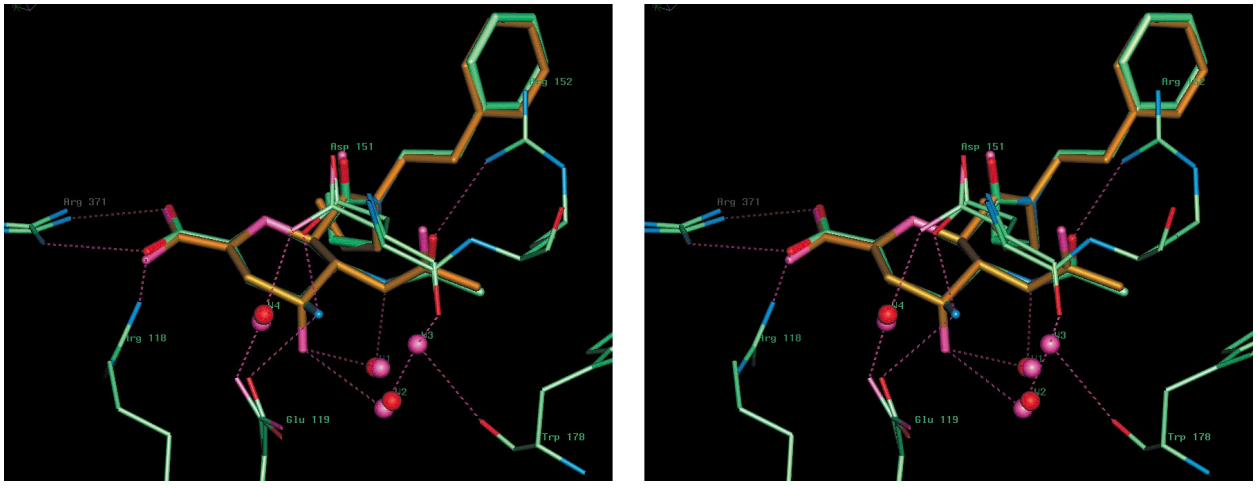
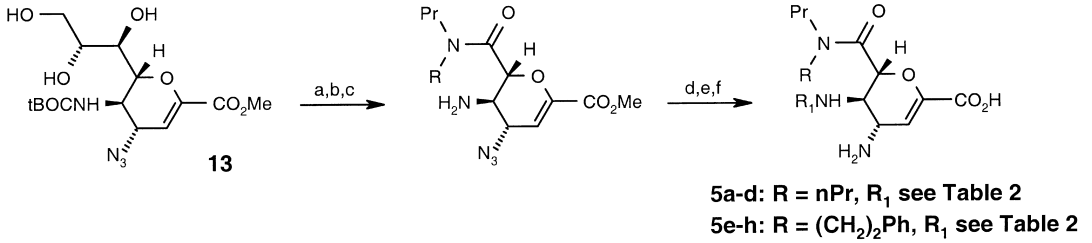


Figure 1. Stereo figure showing a superimposition of the crystal structure of the active site of influenza sialidase with the inhibitors **3b** and **4e**. Only the residues involved in interactions around the 4-position of the ligands are shown. Protein and ligand are in atom colours for **3b**, ligand in yellow for **4e** with oxygens of protein and water molecules in pink. Hydrogen bonds are shown as dotted lines.



5a-d: R = nPr, R_1 see Table 2
5e-h: R = $(CH_2)_2$Ph, R_1 see Table 2

Scheme 3. (a) (i) $NaIO_4$, MeOH, H_2O; (ii) $NaClO_2$, *t*BuOH; (b) TBTU, $Pr(R_1)NH$, THF; (c) 4 M HCl in dioxan; (d) RCOCl or CH_3SO_2Cl, pyridine; (e) PPh_3, THF, then Et_3N, H_2O; (f) Et_3N, H_2O, 50 °C.

Table 2. Sialidase inhibitory and plaque reduction activities of **5a**–**h**. Comparison with **3a**,**b**[a]

	R	R_1	Sialidase inhibition IC_{50}		Plaque reduction IC_{50}	
			A Aichi (μM)	B Victoria (μM)	Flu A (μg/mL)	Flu B (μg/mL)
3a	*n*-Propyl	CH_3CO	0.012	2.0	0.002	0.7
5a	*n*-Propyl	C_2H_5CO	0.005	7.0	0.0002	3.0
5b	*n*-Propyl	$(CH_3)_2CHCO$	17	>540		
5c	*n*-Propyl	CF_3CO	0.0003	0.2	0.00001	0.3
5d	*n*-Propyl	CH_3SO_2	3.0	92		
3b	$Ph(CH_2)_2$	CH_3CO	0.003	3.0	0.03	0.3
5e	$Ph(CH_2)_2$	C_2H_5CO	0.0007	6.0		
5f	$Ph(CH_2)_2$	C_3H_7CO	43	>500		
5g	$Ph(CH_2)_2$	cC_3H_5CO	0.05	90		
5h	$Ph(CH_2)_2$	CF_3CO	0.003	0.1	0.004	0.056

[a]Sialidase inhibitory and plaque reduction activities of compounds were determined by previously reported methods.[5,16]

structures including the bound ligands were essentially superimposable. As can be seen in Figure 1 the only differences observed were slight movements of aspartic acid 151 and glutamic acid 119 and the accompanying structural water molecules near to the 4-position. These side-chain movements are due to the loss of hydrogen bonds to the amino group of **3b**. The small shifts of the water molecules occur as a consequence of the replacement of the α-amino group of **3b** with the β-hydroxy of **4e**.

The similarity of the activity of **3b** compared to **4c**–**f** suggests that within the carboxamide series the overall binding of the compounds is dominated by the lipophilic interactions of the carboxamide moiety. Therefore, any binding energy gained from hydrogen bonding of the 4-substituent with the enzyme and the structural water molecules is balanced by the desolvation of the native enzyme and unbound ligand.[17]

Modification of the 5-position

The synthesis of 5-modified derivatives **5a**–**f** from **13**[13] is outlined in Scheme 3.

The activities of a range of 5-modified compounds are summarised in Table 2. Consistent with previous studies[13] there was an extremely tight steric requirement for the 5-substituent. Replacement of acetamide with propionamide **5a**,**e** was tolerated with little change in activity against influenza A or B sialidase. However, further homologation to either methylpropionamide **5b** or 2-butyramide **5f** resulted in a 1000-fold loss of activity. Remarkably, the cyclopropyl derivative **5g** was only 25-fold less active than the acetamide **3b**. The trifluoroacetamide derivative of the dipropylamide **5c** was 100-fold more potent against influenza A in both enzyme and plaque assays. The 10-fold increase in potency in the influenza B enzyme assay did not translate into significantly improved influenza B plaque activity.

The trifluoroacetamide of the phenethyl-propylamide **5h** did not exhibit the large increase in influenza A activity shown by **5c**. However, the increase in the influenza B activity made **5h** the most potent 6-carboxamide derivative against influenza B. Replacement of the amide moiety with a sulphonamide group **5d** gave a 300-fold loss of activity, whereas this modification was well tolerated in the 6-glycerol series.[13]

Conclusion

In contrast to the 6-glycerol substituted series related to zanamivir, the 6-carboxamide series does not require a basic group in the 4-position for potent sialidase activity. In fact, no functionality at all is required for nanomolar activity against influenza A sialidase. This surprising finding could be used to design further influenza sialidase inhibitors. Modifications at the 5-position demonstrated a very tight steric requirement for this substituent; trifluoroacetamide was optimal. As with the previous examples of the carboxamides, a significant selectivity for influenza A over influenza B sialidase was observed for all the compounds synthesised.

Acknowledgements

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