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Carbocyclic Influenza Neuraminidase Inhibitors Possessing a C_3-Cyclic Amine Side Chain: Synthesis and Inhibitory Activity

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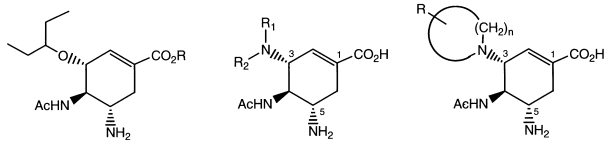
Abstract—As part of our continuing work in the area of influenza neuraminidase inhibitors, a series of C_3-aza inhibitors possessing a cyclic amine side chain was synthesized and evaluated for influenza neuraminidase inhibitory activity. Analogues possessing a six-, seven- and eight-membered ring, **4c–e**, respectively, at the C_3 position exhibited excellent influenza B neuraminidase inhibition.

In recent years, there has been significant efforts in the design of inhibitors of influenza neuraminidase as potential therapeutics for influenza virus infection.[1] Our earlier work led to the discovery of **1** (GS 4071), which has demonstrated potent in vitro and in vivo inhibitory activity against influenza A and B.[2] The corresponding prodrug **2** (GS 4104, oseltamivir, Tamiflu™) has been recently approved by the U.S. Food and Drug Administration for use as the first orally administered neuramindase inhibitor for the treatment of influenza virus infection. From previous structure–activity relationship studies we have demonstrated that replacement of the C_3-ether oxygen with a nitrogen atom **3** provides inhibitors with excellent to moderate inhibitory activity against influenza neuraminidase.[3] As part of ongoing efforts we have elaborated the C_3-aza series with the synthesis of substituted and unsubstituted cycloalkyl amine analogues **4**.

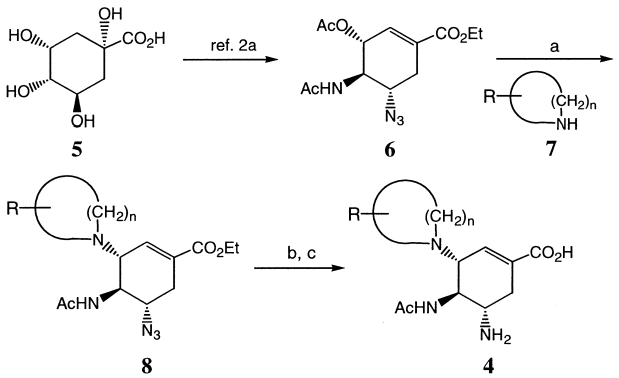


1 R = H; X = Me GS 4071
2 R = Et; X = Me GS 4104

3

4

Analogues **4a–m** were prepared according to the previously described procedure utilizing a palladium(0) catalyzed coupling of acetate **6** with the corresponding amine **7** (Scheme 1).[3] Commercially available (−)-quinic acid (**5**) was converted to the desired acetate **6** in good yield.[2a] Coupling of **6** with an excess of amine **7** with 5 mol% tetrakis(triphenylphosphine)palladium(0) in refluxing THF provided the desired product **8** in isolated yields ranging from 30–65%. Reduction of the azide group to the corresponding amine with triphenylphosphine and subsequent saponification of the ethyl ester with aqueous KOH provided the amino acids **4a–m** in yields of 60–90% after reverse phase column chromatography.



Scheme 1. Reagents: (a) 5 mol% $Pd(PPh_3)_4$, THF 30–65%; (b) PPh_3, THF, H_2O; (c) aq KOH, THF 60–90%.

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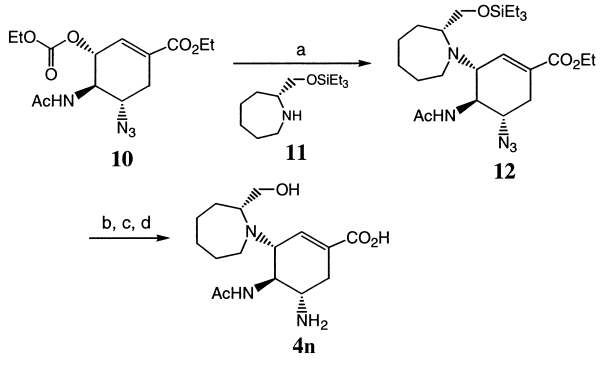
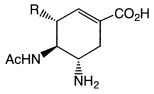
The synthesis of hydroxy methyl analogue **4n** was carried out according to Scheme 2. The requisite *O*-protected amino alcohol **11** was prepared by lithium aluminum hydride reduction of the corresponding (*R*)-amino acid[4] and subsequent protection of the free hydroxyl group with triethylsilyl triflate. Coupling of amine **11** and ethyl carbonate **10** with 5 mol% tetrakis(triphenylphosphine) palladium(0) in refluxing THF provided **12** in 60% yield after flash chromatography. Reduction of the azide group followed by acidic hydrolysis of the triethylsilyl ether and saponification with aqueous KOH furnished the desired amino acid **4n** in 26% overall yield for three steps after reverse phase column chromatography.

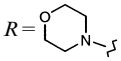
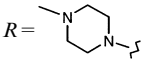
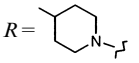
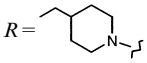
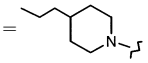
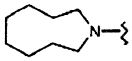
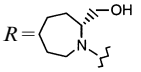
The influenza neuraminidase inhibitory activities of **4a–n** were evaluated in an enzymatic assay[5] with the results summarized in Table 1. As shown in Table 1, when the ring size of the C_3 side chain is increased, compounds **4a–f**, good inhibitory potency is maintained up to analogue **4e**. Most notably are compounds **4c**, **4d** and **4e** which exhibit potent inhibitory activity against influenza B neuraminidase comparable to that of GS 4071 (**1**). This increase in potency is likely due to increased hydrophobic interactions of the cyclic amine side chain with enzyme active site amino acid residues. This observation is analogous to the C_3-oxa series of compounds in which hydrophobic interactions of lipophilic alkyl side chains with active site amino acid residues were shown to play a significant role in binding affinity.[2a,6a] Since inhibitory activity began to decrease with the larger side chain of **4f**, we decided to prepare several analogues of **4a** and **4c** in order to examine the possibility of optimizing hydrophobic interactions of the smaller azetidine and piperidine side chain. Unfortunately, introduction of a heteroatom or alkyl substitution to the cyclic amine side chain, namely compounds **4g–m**, resulted in reduced inhibitory activity. This result is not entirely unexpected however, since it has been shown that the enzyme active site is sensitive to the steric bulk of binding groups of an inhibitor.[6] This decrease in inhibitory activity of compounds **4g–m** are probably a result of unfavorable steric and electrostatic interactions within the enzyme binding site.



Scheme 2. Reagents: (a) 5 mol% $Pd(PPh_3)_4$, THF 60%; (b) PPh_3, THF, H_2O 67%; (c) AcOH, THF, H_2O 52%; (d) KOH, MeOH 75%.

Table 1. Influenza neuraminidase inhibition



				Enzyme IC_{50} (nM)	
			Compound[a]	Flu A[b]	Flu B[c]
4a	*R* = 	**4h**	*R* = 	**4a**	1000	99
				4b	310	30
4b	*R* = 	**4i**	*R* = 	**4c**	25	4
				4d	35	7
4c	*R* = 	**4j**	*R* = 	**4e**	26	14
				4f	75	41
4d	*R* = 	**4k**	*R* = 	**4g**	265	890
				4h	260	30
4e	*R* = 	**4l**	*R* = 	**4i**	1900	23,000
				4j	51	64
4f	*R* = 	**4m**	*R* = 	**4k**	52	62
				4l	40	87
4g	*R* = 	**4n**	*R* = 	**4m**	32	30
				4n	8	14
				1 (GS 4071)	1	4

[a]All compounds gave satisfactory spectral and analytical data.
[b]A/PR/8/34 (H1N1).
[c]B/Lee/40.

In fact, X-ray crystal structures of the several inhibitors complexed with neuraminidase confirm this hypothesis. As shown in Figure 1A, the X-ray crystal structure reveals that the piperidine of compound **4c** adopts the low energy boat conformation and binds exclusively in the hydrophobic pocket formed by the hydrocarbon side chains of Ile 222, Arg 224, and Ala 246. Based on the X-ray crystal structure, molecular modeling suggested that this pocket could accommodate a larger ring. Consistent with this hypothesis, compounds **4d** and **4e** exhibited excellent inhibitory activities against both influenza A and B neuraminidase.

Compound **4h** exhibited about a 10-fold decrease in inhibitory activity compared to that of **4c** while **4i** exhibited an even larger decrease in inhibitory activity. Analysis of the X-ray crystal structure of **4h** complexed with neuramindase (Fig. 1B) indicated that the polar oxygen of the morpholine ring is situated between the hydrophobic sidechains of Ile 222 and Ala 246, which results in unfavorable polar–hydrophobic interactions. Molecular modeling calculations of interactions of compound **4i** and neuraminidase suggests that the *N*-methyl portion of the piperazine ring binds in this hydrophobic pocket in a similar manner to that of **4h** and could result in even stronger unfavorable interactions since the tertiary amine is very likely charged under physiological conditions. This is consistent with the observed inhibitory activities of **4i** shown in Table 1.

Based on molecular modeling, compound **4n** was designed to introduce potential hydrogen bonding interactions, via the hydroxy methyl group, with amino acid residue Glu 276 in the enzyme active site. Although inhibitory activity against influenza A was improved relative to the unsubstituted analogue **4d**, inhibitory activity against influenza B was decreased 2-fold. Analysis of the X-ray crystal

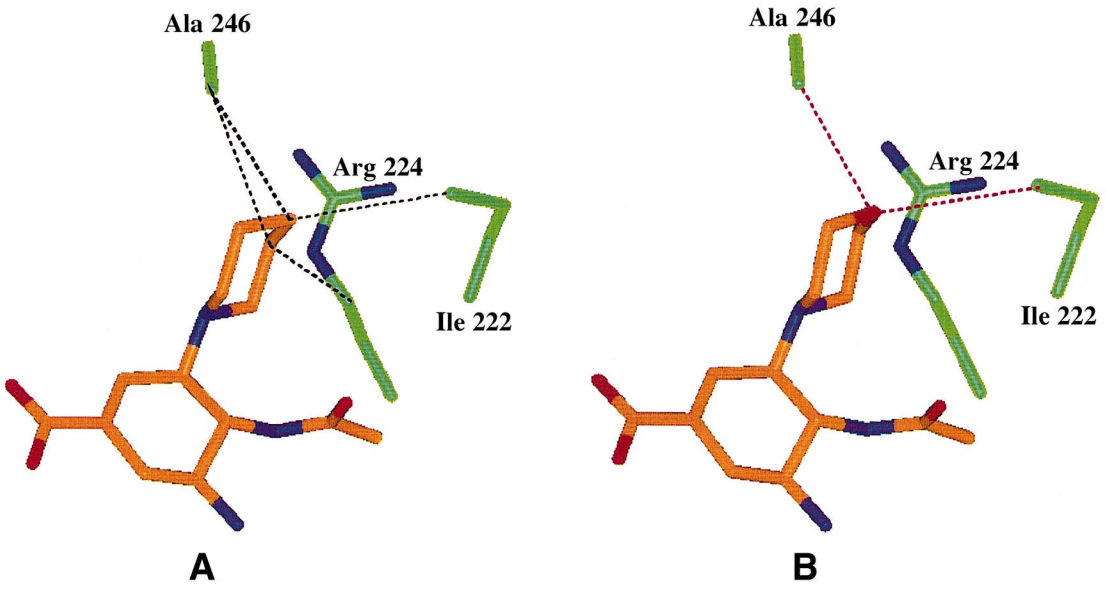


Figure 1. X-ray crystal structures of **4c** and **4h** complexed with neuraminidase, **A** and **B**, respectively. For clarity, only the side chains of pocket 2 residues of neuraminidase are shown. Black dashes indicate hydrophobic interactions, red dashes highlight unfavorable polar–hydrophobic contacts. Oxygen is colored in red, nitrogen in blue, neuraminidase carbon in green, and inhibitor carbon in brown.

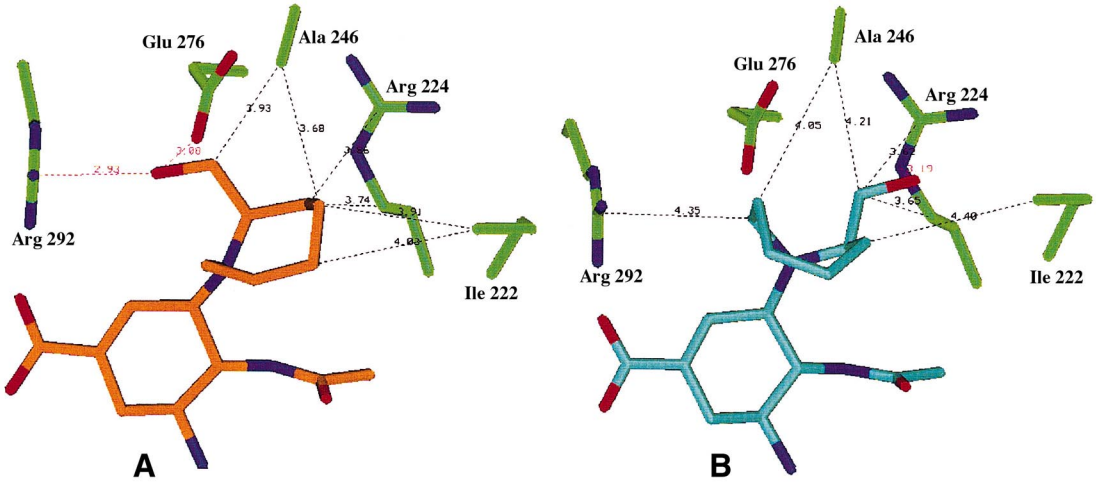


Figure 2. X-ray crystal structures of **4n** complexed with neuraminidase. For clarity, only the side chains of some neuraminidase active site residues are shown. Color scheme is as in Figure 1 except that the carbon of **4n** is highlighted in brown for structure **A** and cyan for structure B.

structure of **4n** complexed with neuraminidase indeed revealed that the hydroxy methyl group of **4n** interacts with Glu 276 as modeled (Fig. 2A). However, it also revealed that **4n** can adopt an alternate second binding mode in which the hydroxy methyl group does not interact with Glu 276 but insteads protrudes out of the enzyme active site into solvent (Fig. 2B).

In conclusion, a series of C_3-aza analogues related to the potent influenza neuraminidase inhibitor GS 4071 were prepared and evaluated in vitro as potential influenza neuraminidase inhibitors. Of these, **4c–e** and **4n** exhibited very good overall inhibitory activity for both influenza A and B neuraminidase. The observed inhibitory activities of this series of compounds is consistent with information derived from X-ray crystallographic and molecular modeling studies.

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References and Notes

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