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# STRUCTURE–ACTIVITY RELATIONSHIPS OF CARBOCYCLIC INFLUENZA NEURAMINIDASE INHIBITORS

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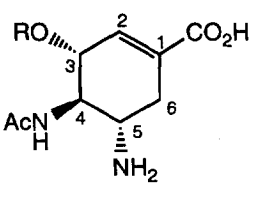
**Abstract.** The structure–activity relationships (SAR) for a new class of potent inhibitors (**1**) of influenza neuraminidase are described. Systematic modifications of substituents at the C-3, C-4, and C-5 positions of the carbocyclic ring were performed to establish fundamental SAR to assist in the design of potent inhibitors with activity against both of influenza A and B viruses. © 1997 Elsevier Science Ltd.

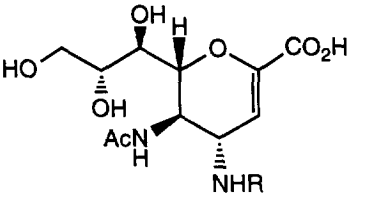
Inhibition of viral neuraminidase has been demonstrated to be viable approach to the treatment of influenza virus infection.[1] While the exact role of influenza virus neuraminidase (NA) in the life cycle of the virus remains a subject of continued interest,[2] inhibition of the viral neuraminidase has been shown to be effective in reducing viral replication and has been validated as a strategy for treating influenza virus infection.[3]

Analogues such as **2b**, derived from *N*-acetylneuraminic acid (sialic acid), were shown to be potent inhibitors of both influenza A and B neuraminidases.[4] The polar nature of these analogues, however, precludes their use as oral agents.[5] The convenience of administering an oral anti-influenza agent was viewed as an important attribute when considering the potential use of such an agent, both for the treatment and prophylaxis of influenza infection. In order to develop an orally bioavailable drug, we designed an entire new class of potent neuraminidase inhibitors **1** based on a carbocyclic scaffold.[6] A proposed mechanism for the neuraminidase enzyme catalyzed cleavage of sialic acid implicates a sialosyl cation intermediate in the transition state.[7] We expected the cyclohexene ring of **1** would serve as a suitable mimic of the transition state, and also increase the lipophilicity of the molecule, which may help to increase oral bioavailability.




**1:** R = alkyl

**1a:** R = 3-pentyl




**2a:** R = H

**2b:** R = 


Compound **1a** also incorporates additional lipophilic character by replacing the polar glycerol side chain found in analogues **2a** and **2b** with a lipophilic 3-pentyloxy group. As shown by the X-ray crystal structure of **1a** bound to the enzyme, the 3-pentyl group of **1a** makes several hydrophobic contacts in the region of the active site, which is normally the glycerol binding region for sialic acid. The overall gain in binding energy from these hydrophobic interactions appears to be substantial since **1a** does not require a guanidino group to achieve low

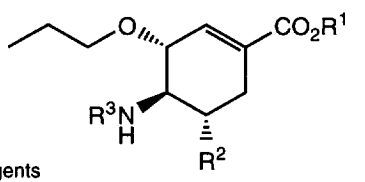
nanomolar inhibition of influenza neuraminidases.[6] Recently, several accounts of derivatives of **2a** with carboxamide side chains having lipophilic subunits have been reported.[8]

Two carbocyclic analogues based on a cyclohexene scaffold with a hydroxymethyl substituent in place of the putative glycerol side chain were synthesized and reported to be active against influenza neuraminidase.[9] For the class of compounds based on the carbocyclic scaffold depicted in **1**, structure–activity relationships have only been reported for a series of linear and branched alkyl side chains.[6] Thus, the systematic modification of the substituents at the C-3, C-4, and C-5 ring positions of **1** was undertaken to understand contributions of the substituents to inhibitory activity.

**Synthesis of Analogues**

A representative experimental procedure for the synthesis of C-3 side-chain analogues **1a** and **3–9** (Table 1) has been described.[6] The syntheses of 4-amino analogues are described in Scheme 1. Amide compounds **3**, **13**, and **14** were prepared by acylation of a common 4-amino intermediate **10** followed by azide reduction and ester hydrolysis. Introduction of the trifluoroacetyl group was performed on carboxylic acid precursor **11** since hydrolysis of the methyl ester in the later steps of the synthesis would also remove the base labile trifluoroacetamide group. The trifluoroacetylated product was then subjected to azide reduction to provide amino acid **12**.

**Scheme 1.** Synthesis of 4-Amino Analogues




Reagents
(a) i.$CH_3COCl$, pyridine ii. $Ph_3P$, $H_2O$ iii. KOH
(b) i. $CF_3CO_2Me$, $Et_3N$ ii. $Ph_3P$, $H_2O$
(c) i. $CH_3CH_2COCl$, pyridine ii. $Ph_3P$, $H_2O$ iii. KOH
(d) i. MsCl, pyridine ii. $Ph_3P$, $H_2O$ iii. KOH

a: **10:** $R^1 = CH_3$, $R^2 = N_3$, $R^3 = H$ → **3:** $R^1 = H$, $R^2 = NH_2$, $R^3 = CH_3CO$

b: **11:** $R^1 = H$, $R^2 = N_3$, $R^3 = H$ → **12:** $R^1 = H$, $R^2 = NH_2$, $R^3 = CF_3CO$

c: **10:** $R^1 = CH_3$, $R^2 = N_3$, $R^3 = H$ → **13:** $R^1 = H$, $R^2 = NH_2$, $R^3 = CH_3CH_2CO$

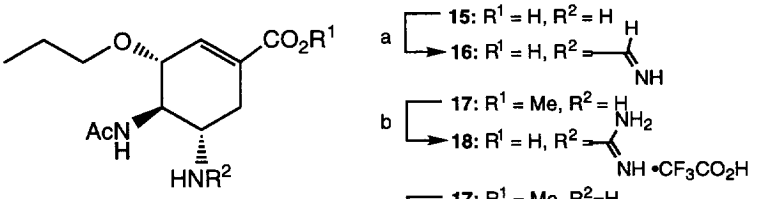
d: **10:** $R^1 = CH_3$, $R^2 = N_3$, $R^3 = H$ → **14:** $R^1 = H$, $R^2 = NH_2$, $R^3 = CH_3SO_2$

The syntheses of 5-modified amino derivatives are shown in Scheme 2. Amidine derivative **16** was prepared by treatment of amine precursor **15** with benzylformimidate[10] under basic conditions. Guanidine analogue **18** was prepared by first introducing the guanidine group in a protected form using *N,N'*-bis-*tert*-butoxycarbonylthiourea.[11] Guanidine acid **18** was obtained after ester hydrolysis and removal of the Boc protecting groups with trifluoroacetic acid. *N*-methyl compound **19** was synthesized by a similar route described for **18**, using *N*-methyl-*N'*-*tert*-butoxycarbonylthiourea.[11]

**Biological Activity**

We previously reported the structure–activity relationships for linear and branched alkyl substituents for compound **1**.[6] In our preliminary investigation of side-chain activity, decreases in the enzymatic $IC_{50}$ value were seen along a linear series as the carbon-chain length increased, with no gain in potency observed in going from *n*-propyl to *n*-butyl.[12] Therefore, propyl analogue **3** ($IC_{50}$ = 130 nM) served as a convenient reference point for exploring other modified linear analogues (Table 1).

**Scheme 2.** Synthesis of 5-Amino Analogues



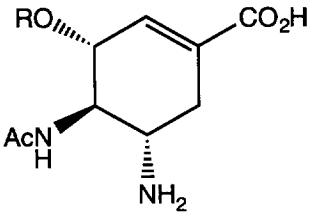

Reagents:
(a) i. benzylformimidate, NaOH
(b) i. *N,N'-tert*-butoxycarbonylthiourea, $Et_3N$, $HgCl_2$ ii. KOH iii. $CF_3CO_2H$
(c) i. *N*-methyl-*N'-tert*-butoxycarbonylthiourea, $Et_3N$, $HgCl_2$ ii. KOH iii. $CF_3CO_2H$

The importance of the side chains' hydrophobic contribution to binding affinity was demonstrated by methoxymethyl analogue **4**, which maintains a three atom extension but removes a single methylene group β to the oxygen atom, showing a 15-fold reduction in NA inhibitory activity. The addition of a terminal trifluoromethyl group, which was expected to retain lipophilic character of the side chain while adding a remote group at the γ-position capable of hydrogen-bonding, gave the less potent analogue **5** ($IC_{50}$ = 225 nM).

**Table 1.** Activity of C-3 Side-Chain Analogues

| R | compd | enzyme[a] $IC_{50}$(nM) |
|---|---|---|
| $CH_3CH_2CH(CH_3CH_2)$– | **1a** | 1 |
| $CH_3CH_2CH_2$– | **3** | 130 |
| $CH_3OCH_2$– | **4** | 2000 |
| $CF_3CH_2CH_2$– | **5** | 225 |
| $CH_2$=$CHCH_2$– | **6** | 2200 |
|  | **7** | 22 |
|  | **8** | 60 |
|  | **9** | 530 |

[a]influenza neuraminidase A/PR/8/34 (H1N1)




The importance of the substituent at the β-position of the alkyl side chain was also evident when the terminal ethyl group of **3** was replaced by a vinyl group. The unsaturated derivative **6** ($IC_{50}$ = 2200 nM) showed approximately a 15-fold decrease in the enzymatic $IC_{50}$ value compared to propyl analogue **3** ($IC_{50}$ = 130 nM).

Cyclic analogues combining lipophilic character with potential entropic benefits were also investigated. Cyclopentyl derivative **7** was quite active ($IC_{50}$ = 22 nM) but was not as potent as its acyclic counterpart **1a**. Enlargement of the ring to the more sterically demanding cyclohexyl analogue **8** gave an approximate threefold

decrease in NA inhibition. X-ray crystallographic studies of the complex of **8** with influenza neuraminidase (Figure 1) shows the important interactions of the cyclohexyl ring in the enzyme active site. Primary hydrophobic interactions are seen with Ala246 and a methylene group of Arg224. Additional hydrophobic contributions are derived from a methyl group of Ile222. Comparing this crystal structure to that of 3-pentyl analogue **1a**[6] (Figure 1) reveals a key difference in binding. In the binding of sialic acid based analogues, the carboxylate of Glu276 forms a bidentate interaction with two hydroxyl groups of the glycerol side chain.[13] When a lipophilic group is placed in this region of the active site, the methylene backbone of Glu276 is positioned for optimal hydrophobic interactions with the 3-pentyl group. In contrast with the cyclic pentyl and hexyl analogues, the 3-pentyl group can maximize hydrophobic contacts because of its extended conformation. However, the active site will accommodate lipophilic groups, such as a cyclohexyl ring, which do not induce the Glu276 carboxylate shift.

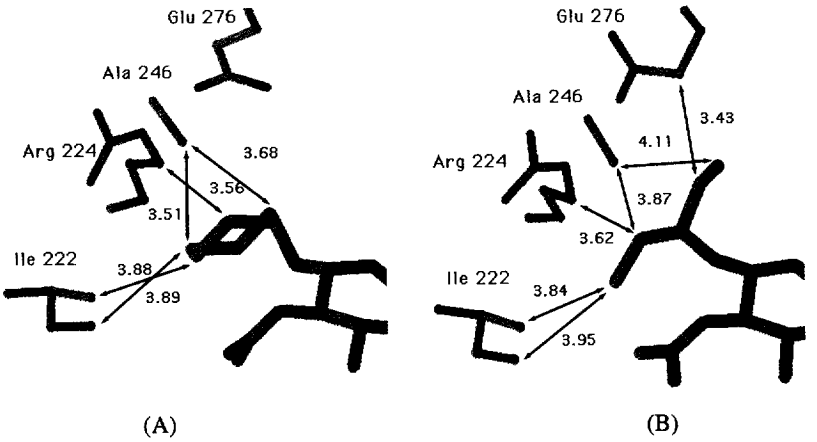



**Figure 1.** X-ray crystal structure of influenza neuraminidase bound to compound **8** (A) and compound **1a** (B) showing hydrophobic interactions

In view of the binding of cyclohexyl analogue **8** in the active site, the reason for reduced activity of phenol derivative **9** ($IC_{50}$ = 530 nM) becomes readily apparent. In addition to removing all interactions of the cyclohexyl $CH_2$-units, the planar arrangement of the phenol ring will not permit an orientation similar to that of the fully unsaturated cyclohexyl ring.

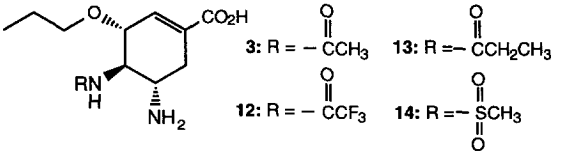
Previous studies have demonstrated the importance of the *N*-acetyl group for biological activity in the sialic acid based analogues. When 4-guanidino-Neu5Ac2en was synthesized lacking the acetylamino group at the 5-position of the dihydropyran ring a large reduction in inhibitory activity was observed.[14] The substituents of the 5-amino group have been extensively modified.[15] This region of the active site places limitations on the functionality with respect to sterics and polarity. Although we assumed that the behavior of these analogues would be similar to the sialic acid based analogues, subtle changes introduced by the cyclohexene scaffold and the lipophilic substitution at C-3 may influence the binding in the *N*-acetyl region of the active site. To address this a concise series of analogues was prepared and are shown in Table 2. Substitution of the *N*-acetyl group at C-4 with a trifluoroacetyl (**12**) affords little improvement in measured enzymatic $IC_{50}$ value. The plaque reduction assay was more sensitive to this substitution affording $IC_{50}$ values of 44 μM and 220 μM for the acetyl and trifluoroacetyl compounds, respectively. The steric restrictions within this region of the active site were clearly evident based on

the observation that propionamide derivative **13** had an approximate tenfold increase in $IC_{50}$ value relative to **3**. Changing the acetyl group to a methanesulfonamide group (**14**) resulted in almost 200-fold increase in the $IC_{50}$ value. This result is in notable contrast to the 5-methanesulfonamide analogue of 4-amino-Neu5Ac2en, which was reported to have an $IC_{50}$ value fivefold higher than reported for the 5-acetyl derivative.[15]

**Table 2.** Activity of 4-Substituted Analogues

| compound | enzyme[a] $IC_{50}$ (nM) | plaque[b] $IC_{50}$ (nM) |
|---|---|---|
| **3** | 130 | 44000 |
| **12** | 100 | 220000 |
| **13** | 1500 | ND[c] |
| **14** | 25000 | ND[c] |

[a]influenza neuraminidase A/PR/8/34 (H1N1)
[b]influenza A/WS/33 (H1N1)
[c]not determined

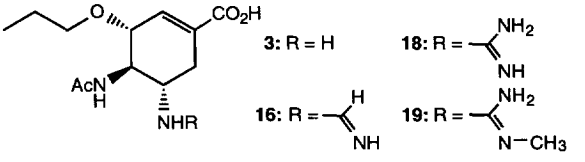



In the sialic acid based series, the amine (**2a**) to guanidine (**2b**) modification gave a 100-fold increase in inhibitory activity.[13] The increase in binding affinity of the guanidine analogue is primarily attributed to interactions with two active site carboxylates. Whereas the amino group normally interacts with Glu119, the extension of a guanidine group permits interaction with Glu 227. In a similar fashion, modification of the amine group at the 5-position of **3** was expected to further enhance inhibitory activity. An amindine group would provide the necessary extension and enhanced basicity analogous to a guanidine group. Conversion of **3** to the corresponding amidine analogue **16**, however, did not yield any enhancement in potency (Table 3). Guanidine derivative **18** was substantially more active than its amino precursor with an $IC_{50}$ value of 1.8 nM. In an effort to increase the hydrophobicity of **18**, *N*-methyl derivative **19** was prepared. While the inhibitory activity of **19** was similar to **18**, the plaque reduction assay indicated a significant reduction in the inhibitory concentration resulting from the addition of a methyl group to the guanidine of **18**.

**Table 3.** Activity of 5-Substituted Analogues

| compound | enzyme[a] $IC_{50}$ (nM) | plaque[b] $IC_{50}$ (nM) |
|---|---|---|
| **3** | 130 | 44000 |
| **16** | 140 | ND[c] |
| **18** | 1.8 | 44 |
| **19** | 4.6 | 1.6 |

[a,b]influenza neuraminidase A/PR/8/34 (H1N1)
[b]influenza A/WS/33 (H1N1)
[c]not determined



Although there was a general trend toward increased activity upon conversion of the 5-amino to the corresponding 5-guanidino derivative, the magnitude of the increase was quite varied. For example, the guanidine derivative of *n*-propyl analogue **3** was >70-fold more potent than **3**, whereas guanidine modification of 3-pentyl analogue **1a** was only twofold more potent than **1a**.[16] These results suggest that the individual substituent contributions to overall binding affinity may not be purely additive.

In summary, a series of analogues were prepared to establish fundamental structure-activity relationships for the design of potent inhibitors of influenza neuraminidase. These efforts culminated in the identification of **1a** as a potent inhibitor with activity against both influenza A and B viruses. The ethyl ester prodrug of **1a** (designated GS 4104) is currently a clinical candidate for the oral treatment and prophylaxis of influenza infection.

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16. A full account of structure-activity relationships of carbocyclic neuraminidase inhibitors related to **1a** will be published separately.

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