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Design, Synthesis, and Neuraminidase Inhibitory Activity of GS-4071 Analogues that Utilize a Novel Hydrophobic Paradigm

Stephen Hanessian,[a,*] Jianchio Wang,[a] Debra Montgomery,[b] Vincent Stoll,[b] Kent D. Stewart,[b] Warren Kati,[b] Clarence Maring,[b] Dale Kempf,[b] Charles Hutchins[b] and W. Graeme Laver[c]

[a]*Department of Chemistry, Université de Montréal, PO Box 6128, Station Centre-ville, Montréal, QC, Canada H3C 3J7*
[b]*Abbott Laboratories, Abbott Park, IL 60064, USA*
[c]*The Australian National University, Canberra 260, Australia*

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Abstract—Structure-based design has led to the synthesis of a novel analogue of GS-4071, an influenza neuraminidase inhibitor, in which the basic amino group has been replaced by a hydrophobic vinyl group. An X-ray co-crystal structure of the new inhibitor ($K_i = 45$ nM) bound to the active site shows that the vinyl group occupies the same subsite as the amino group in GS-4071.

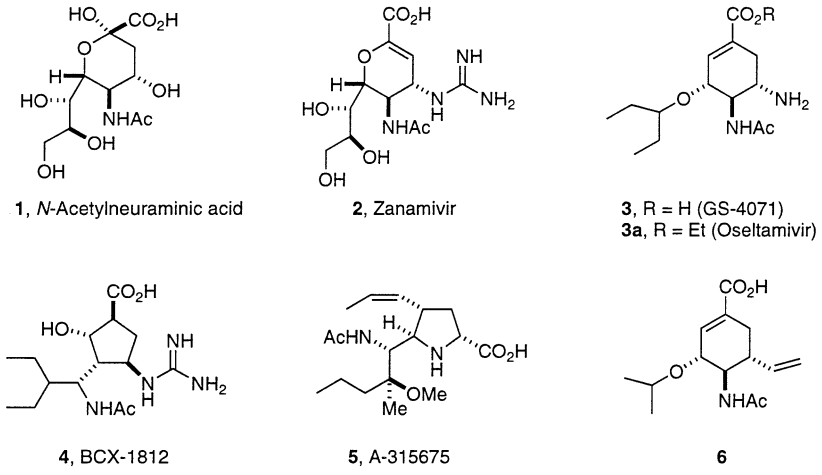

Figure 1.

The catalytic action of influenza neuraminidase[1] is responsible for infectivity and propagation of the influenza RNA virus.[2] This enzyme cleaves the terminal *N*-acetylneuraminic acid residues of epithelial cell glycoconjugates in the upper respiratory tract, a process that spares the virus from being entrapped by aggregation, and prolongs its survival in the host. The development of inhibitors of neuraminidase continues to be an active area of research as this strategy has been clinically validated in efforts to treat respiratory influenza infection.[3] Utilizing X-ray crystallographic data on the enzyme[4] and *N*-acetylneuraminic acid (NANA) **1** as a weak inhibitor, extensive research has already produced two drugs: zanamivir (**2**),[5] a guanidino analogue of dehydro-NANA, and oseltamivir carboxylate (**3**),[6] a carbocyclic analogue of dehydro-NANA (GS-4071) administered as an ethyl ester prodrug (**3a**) (Fig. 1). Elegant X-ray studies

*Corresponding author. Tel.: +1-514-343-6738; fax: +1-514-343-5728; e-mail: stephen.hanessian@umontreal.ca

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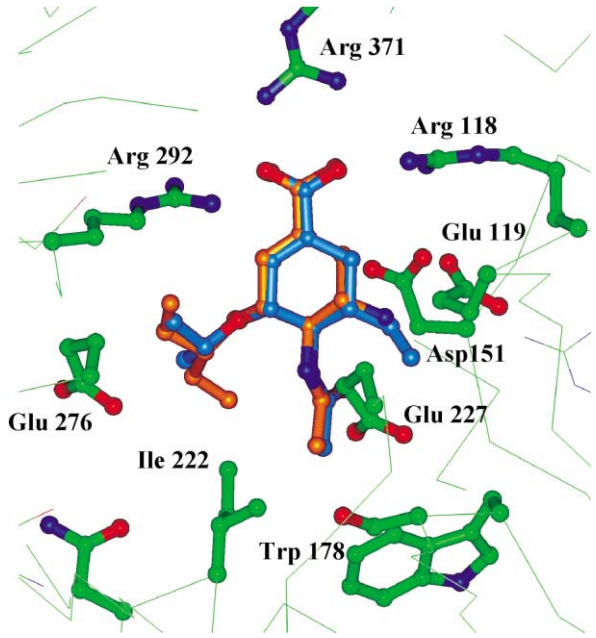


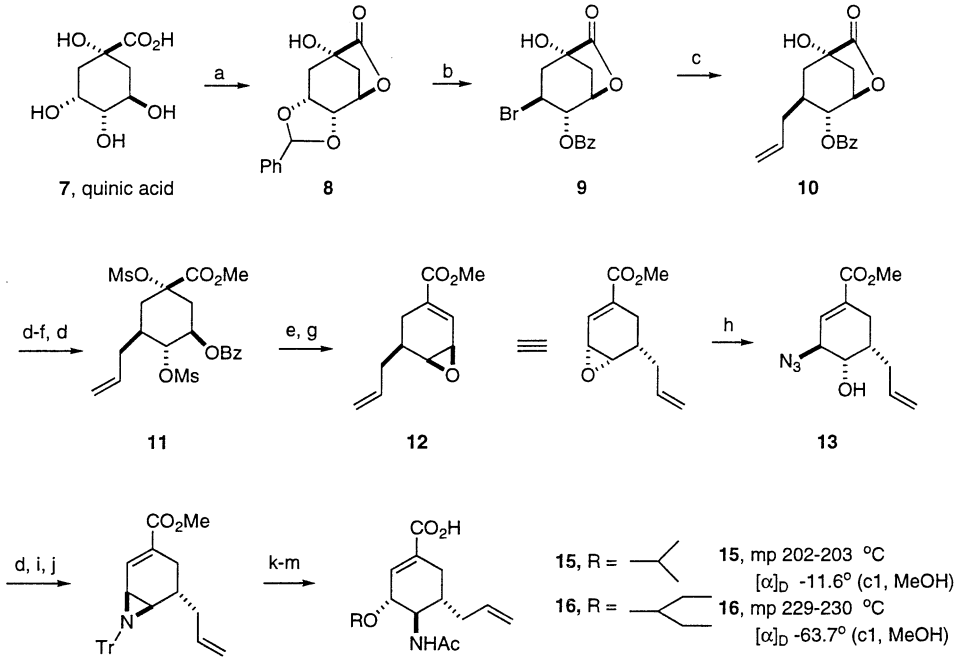
Figure 2. Crystal structure of compound **6** (blue) in neuraminidase A/Tern/75 (green) overlaid with GS-4071 (orange, PDB entry 2QWK). The side chains of Arg 118, Arg 371, and Arg 292 form subsite 1. The side chains of Glu 119 and Glu 227 form subsite 2. The side chains of Trp178 and Ile 222 form subsite 3. Subsites 4 and 5 form a contiguous region with the more solvent exposed site 4 near Ile 222 side chain and the hydrophobic (in this case) site 5, generated by Glu 276 in its bent conformation.

have also facilitated the discovery of potent carbocyclic[7,8] and heterocyclic[9] inhibitors.

The active site of neuraminidase can be divided into five subsites into which polar and hydrophobic groups project from inhibitor core structures. A carboxyl group and an acetylamino group are required for effective binding within subsites 1 and 3.[4–7] The replacement of a hydrophilic interaction in subsite 5 of the enzyme generated by the triol group of **1** with a hydrophobic 3-pentyl ether substituent projecting into subsites 4 and 5 from the carbocyclic core of **3** exploited the first new hydrophobic paradigm in neuraminidase inhibitor design resulting in orally bioavailable inhibitors.[6] Utilizing similar binding interactions as the six-membered inhibitor **3**, a five-membered inhibitor **4**, BCX-1812, has been described.[7] Recently, a new class of inhibitors exploiting a novel second hydrophobic paradigm, exemplified by **5**, has replaced the hydrophilic/charged interaction in subsite 2 that is usually occupied by a basic group such as a primary amine or a guanidine. A-315675 (**5**), a potent inhibitor discovered by Abbott scientists[9] revealed that a basic group normally required to interact with the amino acid residues Glu119, Glu227, and Asp151 in neuraminidase could be replaced by a hydrophobic *cis*-propenyl group. Total syntheses of **5** have been reported by the Abbott group,[10] and independently by Hanessian and coworkers.[11]

The X-ray co-crystal structure of the proline-based inhibitor A-315675 with neuraminidase[12] led us to consider alternative core structures that also capitalized on a hydrophobic interaction. Molecular modeling studies indicated that six-membered analogues of GS-4071 could be designed with hydrophobic olefinic substituents which would replace the primary amino group. With this premise in mind, we proceeded to synthesize **6** (Fig. 2), and closely related compounds to test this paradigm shift in our understanding of subsite 2 interactions.

Considering the carbocyclic nature and functional characteristics of the intended targets, it was obvious



Scheme 1. (a) PhCHO, TsOH, toluene, reflux, 84%; (b) NBS, AIBN, CCl_4, reflux, 80%; (c) $SnBu_3$, AIBN, benzene, reflux, 82%; (d) MsCl, Et_3N, CH_2Cl_2, 99%; (e) MeOH, K_2CO_3, 99%; (f) BzCl, pyr, 85%; (g) DBU, THF, reflux; (h) NaN_3, NH_4Cl, MeOH, H_2O, 67% (two steps); (i) Ph_3P, Et_3N, H_2O; (j) TrCl, Et_3N, CH_2Cl_2, 92% (two steps); (k) BF_3Et_2O, ROH; (l) Ac_2O, Pyr. 60% (two steps); (m) LiOH, THF, H_2O, 98%.

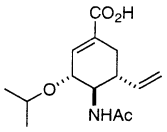
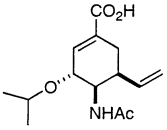
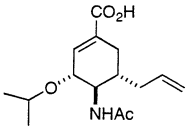
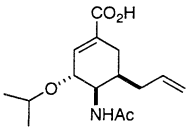
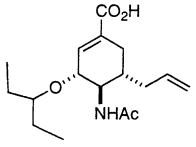
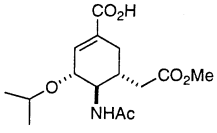
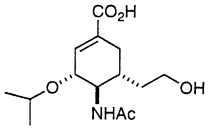
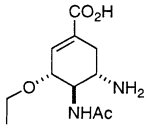
that as in the case of GS-4071,[6] quinic acid **7**[13] would be a logical chiron to build upon as shown in Scheme 1. The readily available lactone **8** was subjected to a regioselective ring opening reaction with NBS[14] to give the bromobenzoate **9** in high yield and excellent selectivity. Radical-induced allylation[15] afforded the *C*-allyl product **10** with retention of configuration, presumably as a result of a greater shielding by the axially disposed benzoate. Mesylation, hydrolysis, selective benzoylation and a second mesylation led to **11** in excellent overall yield. Treatment with potassium carbonate in methanol followed by DBU at reflux in THF gave the epoxide **12**. Upon treatment with sodium azide,[5,16] the epoxide ring was regioselectively opened to afford the azido alcohol **13**. Mesylation and treatment with triphenylphosphine led to the corresponding aziridine which was *N*-tritylated to give **14**. Following an established protocol,[6] treatment of **14** with 2-propanol or 2-pentanol in the presence of $BF_3{\cdot}Et_2O$ followed by *N*-acetylation gave the intended *C*-allyl analogues of GS-4071, **15** and **16**, respectively.

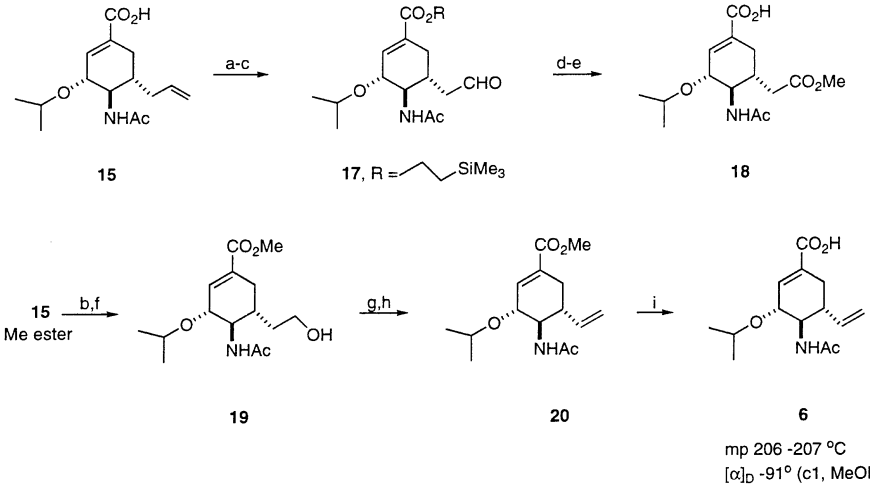
Rather than explore an alternative route to the desired *C*-vinyl analogue **6**, we decided to prepare it from **15** by a degradative protocol as shown in Scheme 2. Thus, dihydroxylation of the trimethylsilylethyl ester of **15**, followed by oxidation gave the aldehyde **17**. Further oxidation to the acid by the Pinnick procedure,[17] and functional group manipulation led to **18**, a *C*-methoxycarbonylmethyl analogue of GS-4071.

Reduction of the methyl ester of **15** to the alcohol **19** followed by elimination via the 2-nitrophenylselenate gave the ester **20** which was hydrolyzed to the desired analogue **6**. A co-crystal X-ray structure of **6** with neuraminidase validates the proposed binding mode in our initial modeling studies (Scheme 2). The vinyl group projects into the region occupied by an amino group in GS-4071.

The type B neuraminidase inhibition K_i data for **6**, **15**, and other analogues are shown in Table 1. The most

Table 1. Neuraminidase (type B/Lee) inhibitory activity

Entry	Compd	Number	K_i (μM)
1		**6**	0.045
2		(ref 18)	69
3		**15**	1.1
4		(ref 18)	14
5		**16**	1.3
6		**18**	2.6
7		**19**	4.1
8		(ref 20)	0.18



Scheme 2. (a) Me_3Si⌒⌒OH, 2-chloro-1-methylpyridinium chloride, Et_3N, 67%; (b) OsO_4, NMO, aq acetone; (c) $NaIO_4$ aq MeOH, 73% (two steps); (d) $NaClO_2$, 2-butene, pH 3–4; then CH_2N_2, 40%; (e) TBAF, CH_2Cl_2, 97%; (f) R = Me, $NaBH_4$, MeOH; (g) Bu_3P, *p*-nitrophenylselenyl cyanide, (two steps, 95%); (h) H_2O_2, THF, 89%; (i) LiOH, aq MeOH, 96%.

active compound is the *anti*/*anti* vinyl analogue **6** (Table 1, entry 1). The *anti*/*anti* allyl analogue **15** (entry 3) was significantly less active. The epimeric *anti*/*syn* *C*-vinyl and *C*-allyl analogues (entries 2 and 4) were relatively inactive.[18] Elongation of the 2-propyl ether in **6** to 3-pentyl as in **16** (entry 5) did not alter the inhibitory potency. The introduction of polar substituents such as in the ester **18** and hydroxyl analogue **19** was slightly detrimental to the activity (entries 6 and 7). The crystal structure of compound **6** bound to the neuraminidase active site was determined and confirmed that the binding mode is similar to GS4071 as shown in Figure 2. Importantly, the vinyl group of compound **6** overlays with the basic amine of GS4071 as designed. The two carbons of the vinyl group are in good Van der Waals contact (3.5–4.5 Å) with the methylenes of Asp 151 and Glu 119 and the π-system of Glu 119 carboxylate. There is a longer Van der Waals contact (4.8 Å) between the π-system of Glu 227 carboxylate and the terminal vinyl carbon.

The nanomolar activity of **6** ($K_i = 45$ nM) represents a significant finding, but falls short of the subnanomolar potencies reported previously for highly advanced and optimized compounds **2**–**5**. The 2-propyl ether substituent of **6** is likely to be suboptimal for interaction within subsites 4 and 5 and should be more properly compared to a corresponding analogue of GS-4071.[19] The replacement of the amino group in GS-4071 by a vinyl group, while maintaining its *anti*/*anti* spatial disposition, leads to an inhibitor of comparable activity to the parent series. The potential for enhancement of activity by further optimization of these two hydrophobic interactions in subsites 2 and 4 in this carbocyclic inhibitor has sufficient precedent to warrant further investigation.[20] Our results further validate the importance of hydrophobic interactions in structure-based drug design.[21]

Acknowledgements

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18. The *anti*/*syn* *C*-vinyl and *C*-allyl analogues in Table 1 were synthesized by a protocol analogous to the one in Scheme 1 starting with quinic acid. Details available upon request.

19. The ethylether/amine analogue (entry 8) has been reported to exhibit a K_i of 185 nM under identical assay conditions; see: Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem.* **1998**, *41*, 2451.
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