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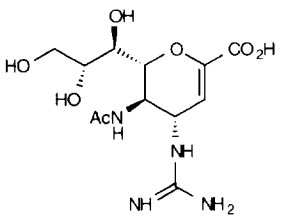
SYNTHESIS AND INFLUENZA NEURAMINIDASE INHIBITORY ACTIVITY OF AROMATIC ANALOGUES OF SIALIC ACID

Matthew Williams, Norbert Bischofberger, S. Swaminathan and Choung Un Kim*

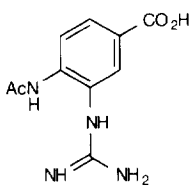
Gilead Sciences Inc.,
353 Lakeside Drive, Foster City, CA 94404

Abstract: Aromatic analogues of sialic acid (**2** and **3**) have been synthesized as potential influenza neuraminidase inhibitors. Whereas compound **2** exhibited good neuraminidase inhibitory activity, compound **3**, possessing the glycerol side chain at the C_5 position, was not a neuraminidase inhibitor.

Influenza virus expresses two envelope glycoproteins: hemagglutinin and neuraminidase. Neuraminidase has at least two critical roles in facilitating the process of infection and in aiding the elution of newly formed viruses from the infected cell by cleaving terminal sialic acid residues of mucous glycoproteins.[1,2] Therefore, neuraminidase plays a pivotal role in the influenza virus replication. Recently, an extremely potent influenza neuraminidase inhibitor, 2,3-didehydro-2,4-dideoxy-4-guanyl-N-acetylneuramic acid (GG167, **1**) has been reported.[3,4,7] GG167 is a transition state analog, representing the optimal conformational state imposed on the sialic acid unit by the enzyme during the catalytic cleavage of the glycosidic linkage.[4] The presence of the C_4 guanidino functionality in GG167 is critically important for increased potency and selectivity against influenza neuraminidase.[3]



1



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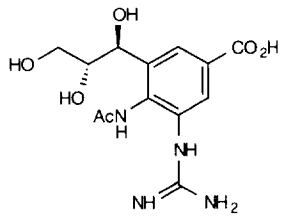


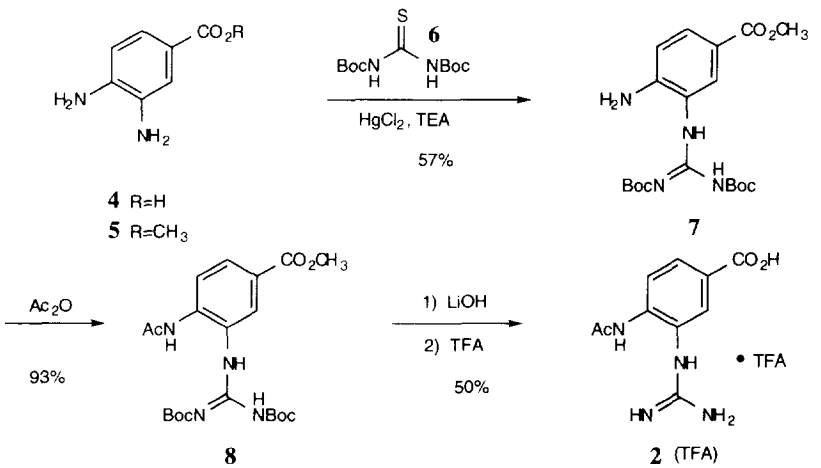
3

Clinical efficacy of GG167 against influenza infection has been demonstrated when it is administered by the intranasal route.[5] GG167 is , however, not effective if given systemically due to rapid excretion of drug and poor tissue penetration.[6] Therefore, an objective of the present research was to develop new, pharmacologically improved neuraminidase inhibitors for the systemic treatment of influenza infection. Such compounds with good oral bioavailability would be highly desirable. Inspection of the X-ray structure of the neuraminidase-GG167 complex revealed that the half-chair conformation of the dihydropyran ring in GG167 is almost flat and all equatorial C_4, C_5, and C_6 substituents on the ring lay on the same flat plane.[4] Therefore, in designing a new

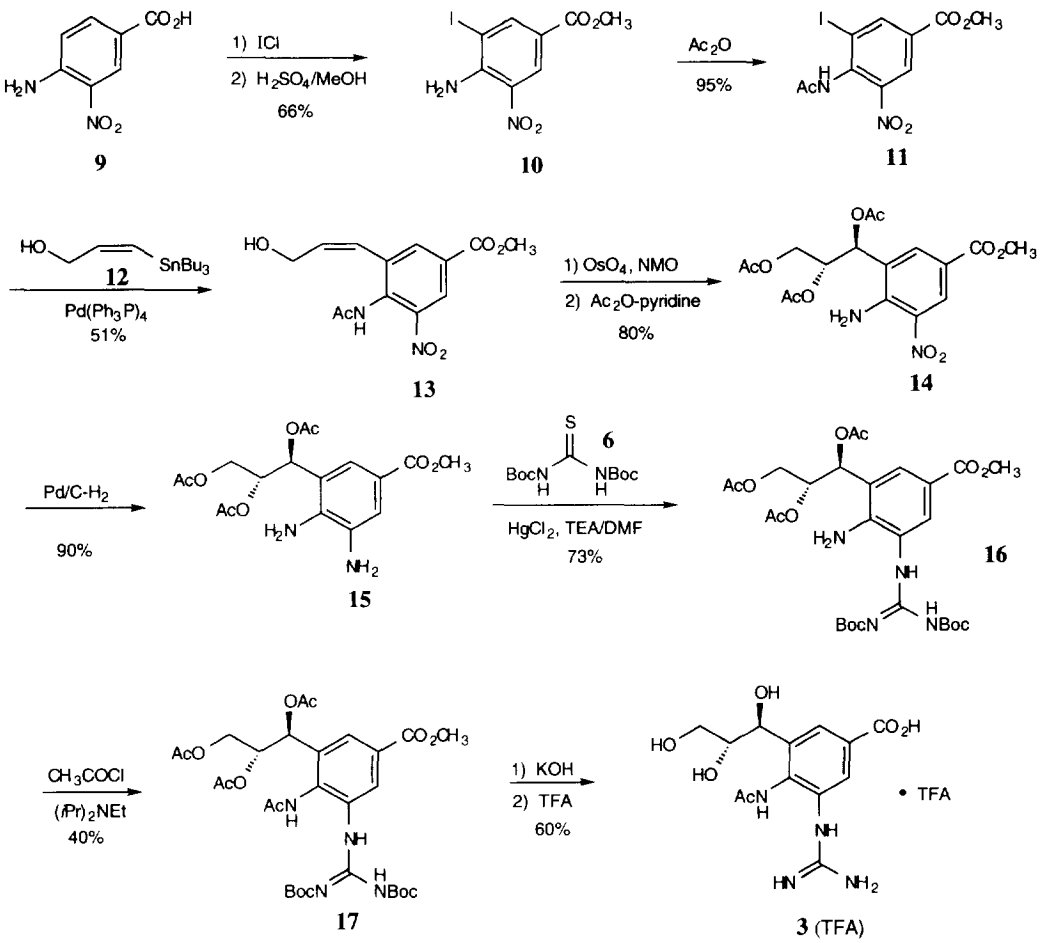
series of neuraminidase inhibitors, it is determined to mimic this planar arrangement of substituents by using a benzene ring as a replacement for the dihydropyran ring in GG167. The benzene ring scaffold has advantages of non-chirality, chemical and metabolic stability and increased lipophilicity compared with the dihydropyran ring. These factors may be important for improving the deficient pharmacokinetic profiles observed for GG167. Recently simple aromatic influenza neuraminidase inhibitors based on similar rational described herein have been reported.[8]

In order to test the validity of our structural modification, a simple aromatic analogue **2** was prepared as illustrated in Scheme I. The methyl ester **5**, prepared from commercially available 3,4-diaminobenzoic acid (**4**), was reacted with N-N'-di(*tert*-butoxycarbonyl)thiourea (**6**) in the presence of mercury chloride[9] to provide the C_3 guanylated intermediate **7**. Obviously, the selectivity observed in this reaction arises from the decreased nucleophilicity of the C_4 amino group due to the electron withdrawing para carbomethoxy functionality. Acylation of **7** followed by saponification and acidic removal of *tert*-butoxycarbonyl groups gave **2** as a trifluoroacetic acid salt. Having completed the synthesis of **2**, our next objective became the preparation of **3** (Scheme III). Iodination of 4-amino-3-nitrobenzoic acid (**9**) with iodine monochloride followed by esterification gave iodide **10**. Acylation of **10** with hot acetic anhydride provided amide **11**. Palladium catalyzed coupling of **11** with Z-vinylstanne (**12**)[10] generated cis olefin **13**. Treatment of **13** with osmium tetraoxide followed by acetylation with acetic anhydride in pyridine gave triol **14**. During this two step sequence, however, the acetamide group, highly activated by the ortho nitro functionality was cleaved to the amine. Catalytic hydrogenation of triacetate **14** produced the diamine **15**. Selective guanidylation of **15** to **16** was accomplished by the same procedure described for conversion of **5** to **7**. Finally, acylation of the amine **16** with acetyl chloride in the presence of N,N-diisopropylethylamine followed by saponification and acidic removal of the *tert*-butoxycarbonyl groups completed the synthesis of **3** as a trifluoroacetic acid salt.

Scheme I



Scheme II



The neuraminidase inhibitory activities and the 50% effective inhibitory concentration (EC_{50}) against influenza A are shown in Table I. Compound **2** exhibited good neuraminidase activity (Ki=8μM, IC_{50}=20μM) and good cell culture inhibitory activity (EC_{50}=65μM), albeit much weaker than those of GG167 (**1**). Interestingly, compound **3**, with the glycerol side chain at the C_5 position, did not show the enzyme inhibitory activity up to 100μM. As demonstrated by many X-ray crystallographic structures of neruaminidase-inhibitor complexes,[3,4,11] the binding pocket of the influenza neuraminidase is very shallow and dictates that good inhibitors must meet stringent structural requirements. Clearly, modification of the dihydropyran ring in GG167 to a benzene ring does not allow correct orientation of the ring substituents in the enzyme active site. More structure-activity relationship information is needed to understand how aromatic derivatives fit in the active site.

Nevertheless, our preliminary findings discussed in this paper should be useful for designing more potent influenza neuraminidase inhibitors.

Table I

Inhibition of Influenza A[a] Neuraminidase and Influenza Virus Inhibitory Activity in MDCK Cells.

Compound	Enzyme Assay Ki(μM)	Enzyme Assay IC_{50}(μM)	Cell Assay EC_{50}(μM)[b]
2	8	20	65
3	>100	>100	nd[c]
1[d]	0.001	0.005	0.04

a Influenza A/Shandong/09/93 (H3N2)

b The concentration at which the average viral cytopathic effect is reduced to 50% of that seen in the virus control.

c nd: not determinded.

d References 6 and 7

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