Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 14, pp. 1847-1850, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00331-4

SYNTHESIS AND ACTIVITY OF C_2-SUBSTITUTED ANALOGS OF INFLUENZA NEURAMINIDASE INHIBITOR GS 4071

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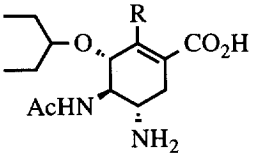
Abstract: The influence of C_2-substitution of GS 4071 on the influenza neuraminidase inhibitory activity was investigated. The introduction of lipophilic substituents (chloro, methyl, and methylthio) at the C_2 position resulted in a significant decrease of the activity. This result indicates that at the enzyme active site there is limited hydrophobic pocket for a group at the C_2 position of GS 4071. © 1997 Elsevier Science Ltd.

Influenza infection is the most serious respiratory illness both in term of morbidity and mortality.[1] In the search for a new anti-influenza agent, a potent influenza neuraminidase (NA) inhibitor GS 4071 (**1**) has recently been identified in our laboratory.[2] In addition, on the basis of in vitro and in vivo results, the ethyl ester of GS 4071 has been selected as a clinical trial candidate for the oral treatment and propylaxis of influenza infection.[3]

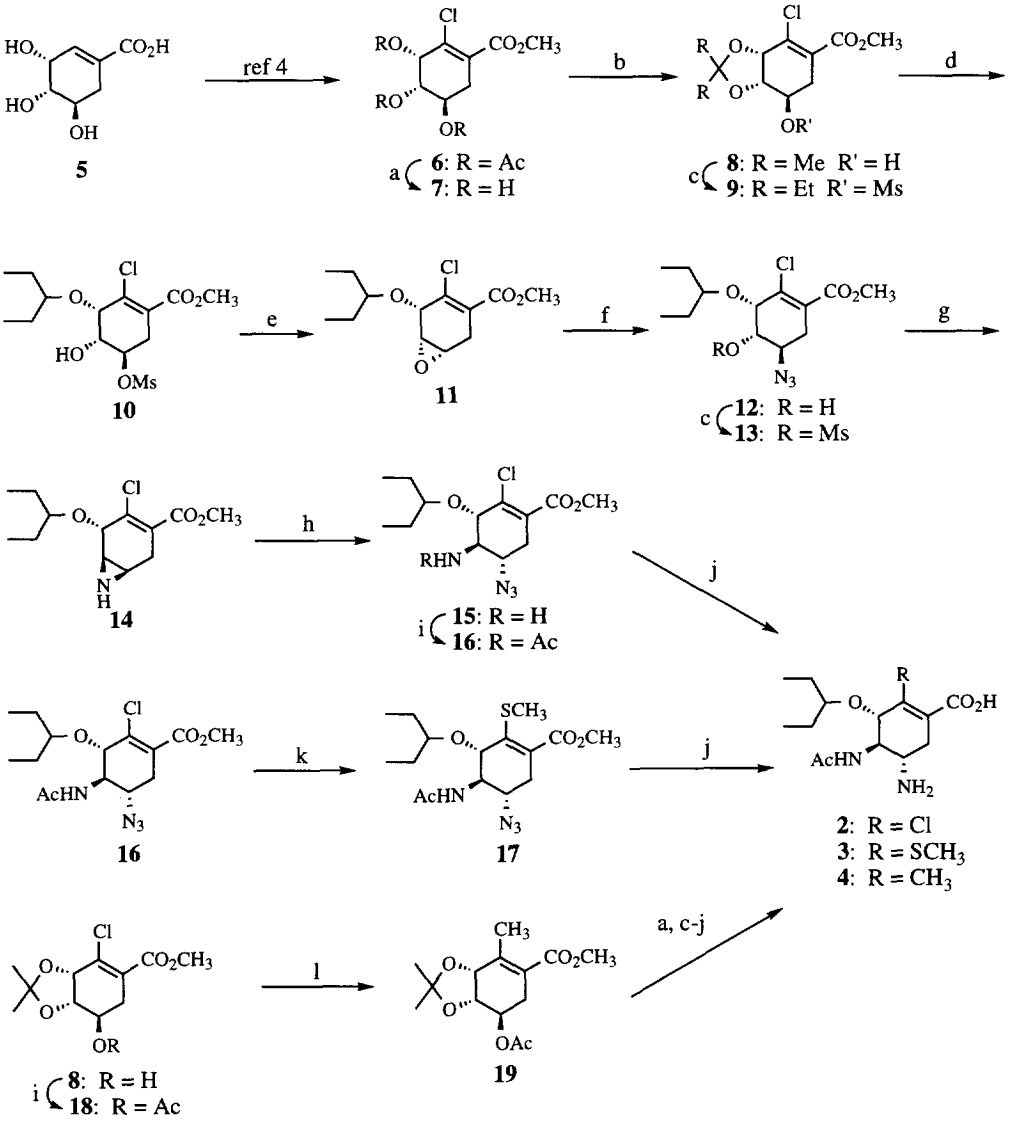


1: R = H (GS 4071)
2: R = Cl
3: R = SCH_3
4: R = CH_3

As part of the study of the structure activity relationship of this series, C_2-substituted analogs of GS 4071 were investigated. Despite the presence of the highly lipophilic 3-pentyloxy group at the C_3 position, GS 4071 has a negative partition coefficient value (log P = -1.1), indicating that it is still a rather polar molecule. Therefore, we investigated the possibility of making the molecule less polar and then improving its pharmacological properties by introducing lipophilic functional groups at the C_2 position. To this end, C_2-chloro (**2**), C_2-methylthio (**3**), and C_2-methyl (**4**) analogs were prepared.

The synthesis of the C_2-chloro analog **2** was accomplished starting from the C_2-chloro shikimic acid derivative **6**, which was prepared from shikimic acid **5** by literature method[4] (Scheme 1). Tetraester **6** was treated with a catalytic amount of sodium methoxide in methanol to give triol **7** in quantitative yield. Although the 3-pentyl ketal could be prepared directly from triol **7** in one step, a two-step procedure via acetonide **8** gave a better yield. Thus, **7** was first treated with 2,2-dimethoxypropane in acetone in the presence of toluenesulfonic acid to give acetonide **8**. Mesylation and transketalization of **8** in the presence of a catalytic amount of perchloric

Scheme 1[a]



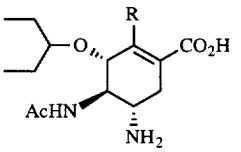
[a]Reagents: (a) CH_3ONa (5 mol %), CH_3OH, rt; (b) 2,2-dimethoxypropane, aceton, p-TsOH (2 mol %), reflux; (c) MsCl, Et_3N, CH_2Cl_2, rt; 3-pentanone, $HClO_4$ (2 mol %), 40 °C; (d) $BH_3{\cdot}Me_2S$, TMSOTf, CH_2Cl_2, -20-0 °C; (e) $KHCO_3$, CH_3OH/H_2O, 50 °C; (f) NaN_3, NH_4Cl, CH_3OH/H_2O, reflux; (g) Ph_3P, THF, rt; Et_3N/H_2O, rt; (h) NaN_3, NH_4Cl, DMF, 70 °C; (i) Ac_2O, pyridine, rt; (j) Ph_3P, THF/H_2O, 50 °C; KOH; Dowex (H^+); (k) CH_3SNa, CH_3CN, rt; (l) Me_2CuLi, Et_2O, -78 °C.

acid gave 3-pentyl ketal **9** in 94% yield. Reductive acetal cleavage of **9** resulted in a mixture of **10** and its regio-isomer in 70% yield (3:2 ratio), which could not be separated by chromatography on silica gel. When the mixture of **10** and its regio-isomer was treated with potassium hydrogen carbonate in methanol-water, the regio-isomer remained unreacted, and **10** reacted to give epoxide **11**, which was isolated by silica gel chromatography. Nucleophilic ring opening of epoxide **11** with sodium azide resulted in azido alcohol **12**. Mesylation of **12** gave **13**, which was then treated with triphenylphosphine in anhydrous tetrahydrofuran followed by triethylamine and water to give aziridine **14** in 75% yield. The ring opening of aziridine **14** with sodium azide gave **15** in 54% yield. Acetylation of **15** followed by reduction of the azido group and saponification of the methyl ester gave the C_2-chloro analog **2**.

The preparation of C_2-methylthio analog **3** was accomplished starting with intermediate **16**. Michael addition of the methylthio group to **16** followed by elimination of the chloro group resulted in **17**. Reduction of **17** followed by the saponification gave C_2-methylthio analog **3**.

The synthesis of C_2-methyl analog was also accomplished starting from intermediate **8**.[5] Acetylation of **8** gave acetonide **18**, which was then treated with methyl cuprate[6] to give a mixture (1:1 ratio) of **19** and the reduced product in 70% yield. After removal of the acetyl group, **19** was converted to final product **4** through the same procedures used for the preparation of compound **2.**

Table 1. Influenza Neuraminidase Inhibition[7]



Compound	R	IC_{50} (nM)
1	H	1.4
2	Cl	3100
3	SCH_3	3400
4	CH_3	2300

The enzymatic neuraminidase inhibitory activity of the three compounds is shown in Table. The results show that the introduction of a lipophilic chloro, methyl or methylthio group at the C_2 position of GS4 071 resulted in an approximately 2000-fold decrease in the inhibitory activity. This result indicates that the enzyme active site contains a limited hydrophobic pocket capable of accommodating modifications at the C_2 position of GS 4071.

Neuraminidase enzyme assay: Neuraminidase enzyme activity was determined using minor modifications to the literature method.[7] Influenza A/PR/8/34 (H1N1), purified on sucrose density gradients, was used as the source of enzyme and 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid was used as substrate in a reaction buffer containing 33 mM MES, pH 6.5, and 4 mM calcium chloride. Virus was mixed with various inhibitor concentrations and incubated at room temperature for 30 min. before substrate was added to a final concentration of 10 μM. Reactions were stopped after 8 min. at 37 °C with the addition of 1.5 volumes of 14 mM sodium hydroxide in 83% ethanol. Fluorescence was quantitated in a Perkin-Elmer fluorimeter (Model LS50B) with an excitation wavelength of 360 nm, emission wavelength of 448 nm, and slit widths of 2.5 nm.

Acknowledgment: We would like to thank Dr. John Rohloff and Mr. Ken Kent for providing reaction conditions for the conversion of **8** to **11.**

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(*Received in USA* 1 *May* 1997; *accepted* 11 *June* 1997)