Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1921–1924

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Anti-Influenza Virus Activity of 4-Guanidino-7-substituted Neu5Ac2en Derivatives

Takeshi Honda,[a,*] Takeshi Masuda,[a] Shuku Yoshida,[b] Masami Arai,[a] Yoshiyuki Kobayashi[a] and Makoto Yamashita[b]

[a]*Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[b]*Biological Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

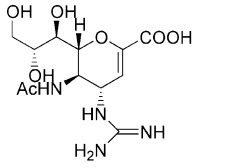
Received 27 March 2002; accepted 6 May 2002

Abstract—Substitution of 7-OH by small hydrophobic groups on zanamivir resulted in the retaining of low nanomolar inhibitory activities against not only influenza A virus sialidase but also influenza A virus in cell culture. These compounds were prepared by treatment of the corresponding 7-substituted sialic acids derived from 4-modified *N*-acetyl-D-mannosamine (ManNAc) using enzyme-catalyzed aldol condensation. © 2002 Elsevier Science Ltd. All rights reserved.

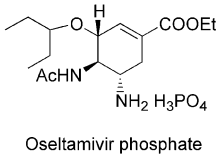
Influenza virus sialidase promotes virus release from infected cells and facilitates virus spread within the respiratory tract.[1] Zanamivir[2] and oseltamivir phosphate[3] in Figure 1 have been approved for human use as specific sialidase inhibitors for anti-influenza drugs. Since the discovery of zanamivir, syntheses of 2-deoxy-2,3-dideoxy-*N*-acetylneuraminic acid (Neu5Ac2en) analogues, which are thought to be transition-state analogues[4] of the enzyme reaction, have drawn considerable attention for sialidase inhibitors over the past decade. Several groups[5] reported the synthesis and sialidase inhibitory activities of analogues related to zanamivir and demonstrated that four substituents of guanidine, carboxylic acid, acetamide, and a glycerol side chain (especially the 8- and 9-hydroxyl groups) on the dihydropyran ring made important contributions to their binding activities to the sialidase. However, there has been only one report of the biological properties of 4-guanidino-7-substituted-Neu5Ac2en analogues, in which there was no detailed evaluation.[6] X-ray studies with sialidase have shown that the 8- and 9-hydroxyl groups of sialic acid and inhibitors such as zanamivir make hydrogen bonding interactions with the acidic sites, but the 7-hydroxyl group does not form direct hydrogen bonds with sialidase.[7]

Therefore, we were interested in the influence on sialidase binding of the replacement of the hydroxyl group at the C-7 position of Neu5Ac2en by lipophilic substituents. However, we have found that the chemical synthesis of 7-substituted-Neu5Ac derivatives from *N*-acetylneuraminic acid (Neu5Ac) is particularly difficult.

In fact, little chemistry has been reported at this position.[8] In our attempts to find a reasonable solution to this problem, we focused our attention on the enzyme-catalyzed aldol condensation[9] between 4-modified-*N*-acetylmannosamines and sodium pyruvate in the presence of Neu5Ac aldolase. Herein, we now report the synthesis of 4-guanidino-7-modified Neu5Ac2en derived from 4-modified-*N*-acetylmannosamines and their biological activities.



Zanamivir



Oseltamivir phosphate

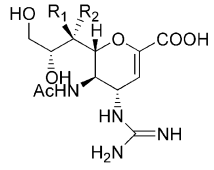


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Figure 1.

*Corresponding author. Tel.: +81-3-3492-3131; fax: +81-3-5436-8563; e-mail: thonda@shina.sankyo.co.jp

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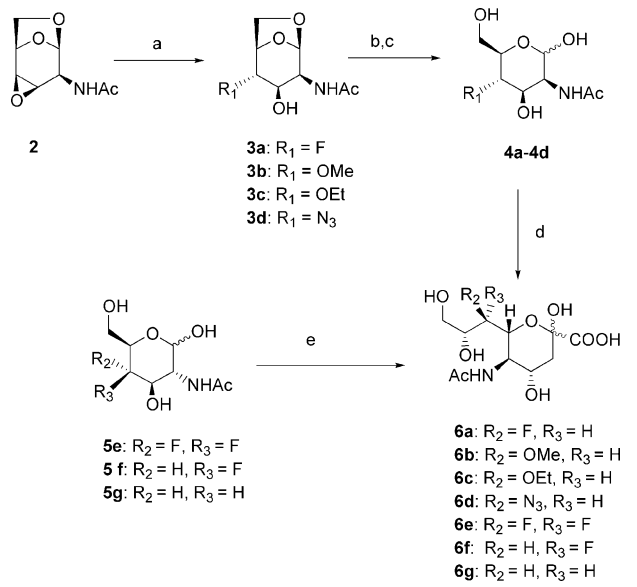
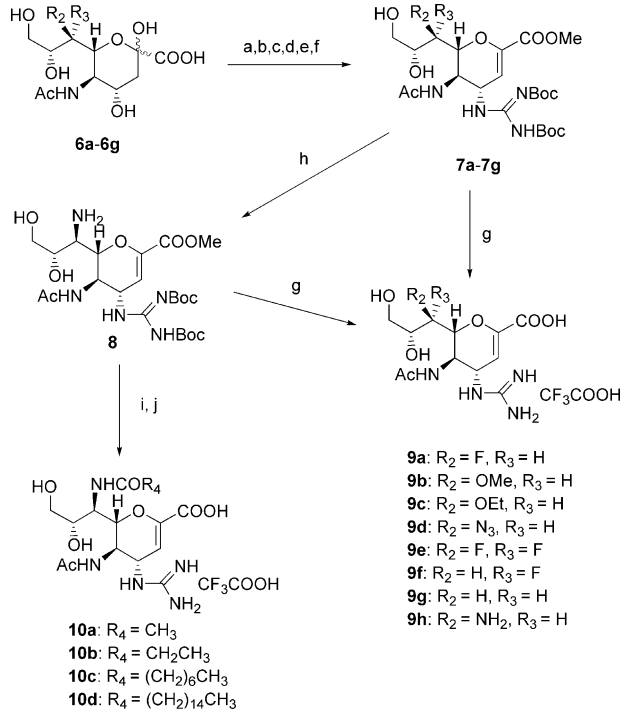
PII: S0960-894X(02)00328-1



Scheme 1. Reagents and conditions: (a) **3a**: $Bu_4NFH_2F_3$, KHF_2, Dowex 50W (H^+) (28%); **3b**: MeOH, Dowex 50W (H^+) (95%); **3c**: EtOH, Dowex 50W (H^+) (82%); **3d**: NaN_3, Dowex 50W (H^+), DMF (72%); (b) (i) CF_3COOH, Ac_2O, (ii) 3 N-HCl (60–70%); (c) NaOAc, Ac_2O (80–90%); (d) Neu5Ac aldolase, sodium pyruvate, pH 7.5, **6a**: (72%); **6b**: (73%); **6c**: (30%); **6d**: (80%); (e) Neu5Ac aldolase, sodium pyruvate, pH 10 (20%).



Scheme 2. Reagents and conditions: (a) MeOH, Dowex 50W (H^+) (80–90%); (b) Ac_2O, AcOH, cat. H_2SO_4 (10:10:1, v/v) (80–90%); (c) NaN_3, Dowex 50W (H^+) DMF (80–90% accompanied by their epimer at C-4 position of 5–7%); (d) Lindlar cat. H_2 EtOH (70–80%); (e) *N*, *N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxyamidine (95%); (f) NaOMe, MeOH (95%); (g) (i) NaOH; (ii) CF_3COOH–CH_2Cl_2 (90–100%); (h) Lindlar catalyst, H_2, EtOH (70–80%); (i) **10a**: AcCl, TEA (95%); **10b**: CH_3CH_2COCl, TEA (95%); **10c**: $CH_3(CH_2)_6COCl$, TEA (82%); **10d**: $CH_3(CH_2)_{14}COCl$, TEA (80%); (j) (i) NaOH; (ii) CF_3COOH–CH_2Cl_2 (90–100%).

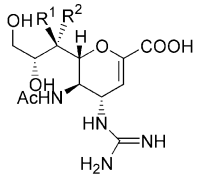
Chemistry

The synthetic route for 4-guanidino-Neu5Ac2en is outlined in Schemes 1 and 2. Compounds **3a**–**3d** were prepared by treatment of **2**[11] with $Bu_4NFH_2F_3$–KHF_2,[10] MeOH, EtOH, and NaN_3, respectively, in the presence of Dowex 50W (H^+), without formation of a 3-substituent, followed by acetylation, acidic hydrolysis of all the acetyl groups and re-acetylation of the 2-amino group gave the compounds **4a**–**4d** as previously described.[11] Chemoenzymatic reaction of 4-modified-*N*-acetyl-D-mannosamines **4a**–**4d** with 2.0 equiv of sodium pyruvate in the presence of *N*-acetylneuraminic acid aldolase[12] gave the corresponding 7-modified sialic acids **6a**–**6d**. Compounds **6e**–**6g** could be prepared using Ohira's method.[13] Thus, the enzyme-catalyzed condensation of **5e**,[14] **5f**,[13] and **5g**[15] were performed under in situ epimerization[16] of 2-acetamide group (epimerization of D-GlcNAc to D-ManNAc). The synthesis of **7a**–**7g** from **6a**–**6g** were accomplished using conventional methodology[17] and these compounds were transformed into **9a**–**9g** by deprotection of the *N*-Boc group with CF_3COOH–CH_2Cl_2. Compound **7d** was converted to **8** by reduction of azide using Lindlar catalyst under H_2 atmosphere, followed by amidation with AcCl, CH_3CH_2COCl, $CH_3(CH_2)_6COCl$, or $CH_3(CH_2)_{14}COCl$ providing the corresponding amides, and their deprotection afforded **10a**–**10d**, respectively. Compound **9h** was prepared from **8** by ester hydrolysis and deprotection of the *N*-Boc group with CF_3COOH–CH_2Cl_2.

Biological Activities

The influenza A virus sialidase inhibitory and plaque reduction activities of a range of 7-modified compounds are summarized in Table 1. Compounds **9a**, **9b**,[18] **9c**, and **9d**, C-7 modified derivatives of zanamivir possessing relatively small and lipophilic substituents, showed similar sialidase inhibitory activity to zanamivir. The result by von Itzstein's group based on X-ray crystallographic studies of the complex of zanamivir binding to influenza A virus sialidase that the C-7 hydroxyl group has no direct interaction with the enzyme supports our result that a small substituent can replace the hydroxyl group at the C-7 position without any influence on interaction with the enzyme. However, these compounds exhibited slightly increased activity (2- to 10-fold) in a plaque reduction assay relative to zanamivir. This result may suggest that the replacement of the C-7 hydroxyl group by lipophilic substituents would make it easier to access the cell membrane or active site compared to zanamivir. Compound **9h** having a basic substituent and compound **9g** having no substitution at the C-7 position exhibited slightly decreased activity against influenza A virus sialidase. Furthermore, a variation of amide derivatives influenced their activity. Thus, **10a**, **10b**, and **10c** were tolerated with little change in activity against influenza A virus sialidase. However, further homologation to **10d** resulted in significant loss of enzyme activity. Difluoro derivative **9e**, closely resembling the structure of **9a**, exhibited 20-fold

Table 1. Sialidase inhibitory and plaque reduction activities of 4-guanidino-7-substituted NeuAc5Ac2en derivatives [IC_{50} (ng/mL)]



	R^1	R^2	Sialidase inhibition assay[19]	Plaque reduction assay[20]
			A/PR/8/34	A/Yamagata/32/89
Zanamivir	OH	H	1.8–20 (1.0)[a]	1.0–20 (1.0)[a]
9a	F	H	0.8 (0.44)	1.8 (0.43)
9b	OMe	H	6.1 (1.2)	3.0 (0.15)
9c	OEt	H	14.6 (0.73)	0.7 (0.14)
9d	N_3	H	1.6 (0.5)	0.3 (0.1)
9h	NH_2	H	37.4 (4.0)	20.0 (5.7)
10a	NHAc	H	18.1 (2.0)	ND[b]
10b	$NHCOCH_2CH_3$	H	48.0 (5.2)	100 (20)
10c	$NHCO(CH_2)_6CH_3$	H	47 (5.1)	80.0 (16)
10d	$NHCO(CH_2)_{14}CH_3$	H	479 (52)	400 (80)
9e	F	F	40.0 (10)	10.0 (10)
9g	H	H	10.0 (2.5)	4.0 (4.0)

[a]Since IC_{50} values varied depending on the experiments, the relative potencies of the compounds to zanamivir are shown in the parentheses, based on the IC_{50} values of zanamivir as a reference. IC_{50} values of zanamivir in enzyme inhibition and plaque reduction were 1.8–20 and 1.0–20 ng/mL, respectively.
[b]ND, not determined.

decrease in influenza A virus activity compared to **9a**. We postulated the bulkiness and the electronegativity of the difluoro substituent of **9e** influenced the structural conformation. Further investigation into the structure activity relationship of C-7 substituted compounds is detailed in the accompanying article.

In summary, a series of zanamivir derivatives possessing C-7 substituted glycerol side chains were synthesized using enzyme-catalyzed aldol condensation and evaluated in vitro. The compounds **9a**, **9b**, **9c**, and **9d** exhibited very good inhibitory activity against influenza A virus sialidase. Furthermore, replacement of the C-7 hydroxyl group of zanamivir by small lipophilic substituents (F, OMe, OEt, N_3) improved influenza A virus plaque reduction activity.

Acknowledgements

We wish to thank Dr. Mutsuo Nakajima and Ms. Reiko Kametani for performing the sialidase assays.

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18. The compound **9b** was effectively inhibited the sialidase activity of several influenza virus strains (H1N1, H2N2, H3N2, B), furthermore, its IC_{50} against influenza B virus (B/Mie/1/93) replication was 3.9 nM which was twice as high as that of zanamivir.
19. Inhibition of influenza virus sialidase was determined in a fluorometric assay by measuring the ability of compounds to inhibit the hydrolysis of 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUN) by whole virus (A/PR/8/34) grown in hen eggs. Each IC_{50} value quoted is the concentration of inhibitor required to reduce the enzymatic activity in this preparation by 50%.
20. Confluent MDCK cells were infected with A/Yamagata/32/89 (H1N1) virus strain for 60 min. After removing the virus solution, pre-warmed agar media containing the compounds was added. The cells were cultured for 30–38 h. After that, the live cells were fixed and stained and visual plaques were counted.