Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2177–2180

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL QUINOLIZIDINE SALICYLAMIDE INFLUENZA FUSION INHIBITORS

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Received 28 March 1999; accepted 21 June 1999

Abstract: A novel series of quinolizidine salicylamides was synthesized as specific inhibitors of the H1 subtype of influenza A viruses. These inhibitors inhibit the pH-induced fusion process, thereby blocking viral entry into host cells. Compound **16** was the most active inhibitor in this series with an EC_{50} of 0.25 μg/mL in plaque reduction assay. The synthesis and the SAR of these compounds are discussed.

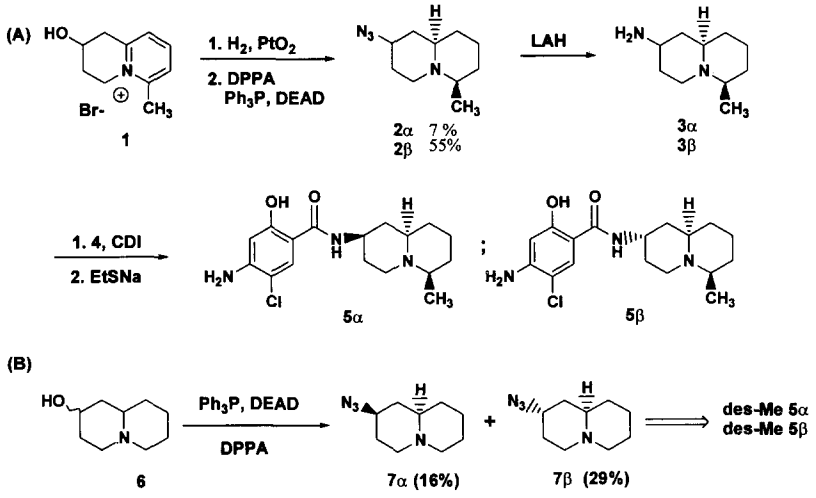
Influenza (also known as flu) is a highly contagious, acute respiratory tract infection characterized by abrupt onset of fever, cough, myalgias, headache, and inflammation of respiratory tract mucous membranes. Acute and uncomplicated influenza usually lasts for only a few days and is self-limiting. However, for the young and elderly populations the infection sometimes can be devastating, causing severe complications that lead to fatal viral pneumonia or secondary bacterial infections. Influenza is caused by a group of enveloped RNA viruses that belong to the *Orthomyxoviridae* family. There are three types of influenza viruses: A, B, and C. The first two types are responsible for annual epidemics and pandemic outbreaks, while influenza C is endemic. The most severe pandemic influenza outbreak of recent times occurred in 1918–1919, when the "Spanish" influenza killed more than 20 million people worldwide.[1] The search for therapeutic agents that treat or prevent influenza infections has, therefore, been of great interest to both academia and the pharmaceutical industry.

Although a significant effort has been devoted to influenza research in the last several decades, there has, until recently, been only limited success in identifying drugs capable of preventing the infection.[2] Currently, a multivalent vaccine is the most common means of prophylaxis. The antiviral agents, Amantadine and Rimantadine are the only two drugs approved for the prophylactic treatment of influenza A.[3] Recently, a novel series of neuraminidase inhibitors, exemplified by Zanamivir and GS4071, has emerged.[4] These agents are effective against both type A and B influenza infections and their success has spurred a new wave of research in fighting the infection. As the result of a broad-based screening program, **5β** was identified as an inhibitor of influenza A virus replication. Compound **5β** has a marked specificity for the H1 and H2 subtypes, with an IC_{50} in a plaque reduction assay of 3–8 μM.[5] Based on a series of mechanistic studies, **5β** has been postulated to interact with hemagglutinin and to repress the low pH-induced conformational change of the protein, thus blocking the fusion process that is essential for entry of the virus into host cells.[6] As the initial steps of a campaign to delineate the pharmacophore inherent to **5β** and to identify compounds with a broader spectrum of influenza inhibitory activity, a series of derivatives was synthesized that focussed on structural variation of the salicylic acid moiety. In this paper we report the synthesis and biological activity of these compounds, which has resulted in representatives with enhanced potency.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00361-3

The quinolizidine moiety of **5β** was prepared using the procedure described by Hadley et al.[7] (Scheme 1A). Hydrogenation of pyridinium bromide **1** followed by Mitsunobu reaction of the resulting alcohols with diphenylphosphoryl azide (DPPA) gave α- and β-azidoquinolizidines, **2α** and **2β**, in 7% and 55% yield, respectively. The azides were reduced to the amines with $LiAlH_4$ and then coupled to 4-amino-5-chloro-2-methoxybenzoic acid (**4**) using 1,1'-carbonyldiimidazole (CDI). Deprotection of the phenolic methyl ether with EtSNa in DMF at 100–125 °C completed the synthesis, yielding **5α** and its stereoisomer **5β**, respectively. When the same procedure was applied to alcohol **6**,[8] **des-Me 5α** and **des-Me 5β** were obtained (Scheme 1B).

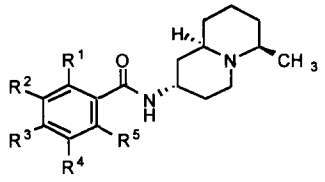
Scheme 1



Coupling of the amine **3β** to a series of O-methylated salicylic acids using the same procedure described above (CDI followed by EtSNa, Method A) provided several of the analogs presented in Table 1. In some cases $(COCl)_2$/DMF (Method B) was used for the coupling reaction. In the case of 3,5-dichlorosalicylate analog, **31**, deprotection of the methyl ether with NaSEt produced a gross mixture of products, presumably due to displacement of the chlorines by the sulfur nucleophile during the reaction. As an alternative, the phenol was protected with a MOM-group and the resulting acid was coupled to amine **3β** using the mixed anhydride method (isobutyl chloroformate/Et_3N). Subsequent hydrolysis of the MOM group gave **31** (Method C).

In further attempts to use commercially available salicylates as the starting materials, several coupling procedures described in the literature, including CDI, were surveyed. However, most of the procedures gave either a complicated mixture of products or no reaction occurred. Activation of the carboxylic acid using $SOCl_2$ (Method D) provided some analogs, but a modification of this approach in which the phenol of the salicylate was first selectively protected with an acetate, using Ac_2O/H_2SO_4 or P_2O_5, proved to be more advanteageous. The acetylated salicylic acid was converted to the acid chloride with $SOCl_2$ or $(COCl)_2$/DMF and then coupled to amine **3β** (Method E). The acetate protecting group proved to be very labile and was frequently cleaved during workup or readily removed by treating the crude reaction product with K_2CO_3 in MeOH. Compound **34** was prepared by reacting isatoic anhydride with **3β** in DMF at 66 °C for 3 h. Treatment of **34** with acetic anhydride provided **35**.

Table 1. Influenza inhibitory activity associated with variation of the aromatic substitution pattern of quinolizidine derivatives.



No.	R^1	R^2	R^3	R^4	R^5	Method[a]	EC_{50}[b]	CC_{50c}
5β	OH	H	NH_2	Cl	H	A	1-3	>100
8	OCH_3	H	NH_2	Cl	H	A	NA	>100
9	H	H	NH_2	Cl	H	A	NA	ND
10	OH	H	H	H	H	A	1.6–3.1	>100
11	OH	H	NH_2	H	H	A	NA	>100
12	OH	H	H	F	H	D	0.4–0.8	>100
13	OH	H	H	Cl	H	A	0.8	>100
14	OH	H	H	Br	H	E	0.8	>100
15	OH	H	H	I	H	E	1	100
16	OH	H	H	CH_3	H	E	0.2–0.3	100
17	OH	H	H	OCH_3	H	A	0.6	100
18	OH	H	H	OH	H	E	NA	50-100
19	OH	H	H	CF_3	H	B	NA	>100
20	OH	H	H	$CH=CH_2$	H	A	2	>100
21	OH	H	H	N_3	H	D	0.5	>100
22	OH	H	H	*tert*-Bu	H	B	NA	50
23	OH	H	H	Ph	H	B	NA	30
24	OH	H	H	PhCO	H	C	NA	>100
25	OH	H	CH3	H	H	E	4	>100
26	OH	H	N3	Cl	H	A	NA	ND
27	OH	H	-CH=CH-CH=CH-		H	A	NA	>100
28	OH	H	H	-CH=CH-CH=CH-		A	NA	>100
29	OH	H	$-(Me)_2CCH_2CH_2C(Me)_2-$		H	A	NA	8
30	OH	CH_3	H	H	H	E	8	>100
31	OH	Cl	H	Cl	H	C	10–20	>100
32	OH	OH	H	H	H	E	NA	10–20
33	OH	H	H	H	F	D	10	>100
34	NH_2	H	H	H	H	--	5–10	>100
35	CH_3CONH	H	H	H	H	--	NA	>100

Note: [a]See text for preparation conditions.
[b]50% inhibition of A/WSN/33 strain influenza virus in cell protection assay in MDBK cells in μg/mL.
[c]Cytotoxicity in the plaque reduction assay in μg/mL.
[d]NA = inactive. ND = not determined.

The compounds synthesized as part of this study are compiled in Table 1 along with their biological properties in the cell protection assay following the A/WSN/33 strain (an H1N1 subtype) infection of MDBK cells.[5] For the quinolizidine-modified derivatives, both α-isomers, **5**α and **des-Me 5**α, were inactive in the assay. Although the active conformation of **5**β remains the subject of conjecture,[7] these results indicate that the relative stereochemistry of the quinolizidine moiety is critical for the expression of potent antiviral activity. The

des-Me 5β is sixfold less potent than the prototype, consistent with the suggestion that this methyl group is involved in an important hydrophobic interaction with hemagglutinin.[6]

The data presented in Table 1 clearly indicate the importance of the phenolic hydroxyl, since methylation (**8**) or removal (**9**) completely eliminates antiviral activity. Interestingly, the amine analog **34** retains activity, although this compound is seven-fold less potent than **5β**. The data presented in Table 1 also indicate the preferred pattern of substitution of the salicylic acid ring with substituents at both the 3- and 4-positions leading to diminished antiviral activity while the single compound bearing a substituent at the 6-position, **28**, is inactive. For substituents at the 5-position, small and nonpolar hydrophobic groups are preferred, with optimal activity residing in the 5-halo (**12–15**) and 5-methyl (**16**) derivatives. The azide **21** retains excellent antiviral properties, providing an opportunity to synthesize a photo-affinity probe that has led to an enhanced understanding of the mode of action of **5β**.[9] Larger hydrophobic groups or polar substituents at the 5-position markedly reduce antiviral activity. Interestingly, the inactive 5-phenyl derivative **23** antagonizes the inhibitory activity of **5β**,[6] suggesting that this compound may still be able bind to the same site of hemagglutinin without establishing all of the key interactions necessary for expression of inhibitory activity. Of the representatives presented in Table 1, compound **16** is the most active inhibitor, with an EC_{50} of 0.25 μg/mL, which is five fold more potent than **5β**. All of the compounds prepared were evaluated as inhibitors of an H3-subtype of influenza A virus but, unfortunately, none demonstrated significant inhibitory activity.

In conclusion, we have demonstrated that **5β** and several of its salicylamide analogs are specific inhibitors against H1 subtype influenza A virus. The SAR study indicates that the stereochemistry of the quinolizidine and the substitution pattern of the aromatic ring of **5β** are important for its antiviral activity. In this study, compound **16** is the most potent fusion inhibitor, with an EC_{50} of 0.25 μg/mL. The use of 5-methylsalicylic acid for the preparation of libraries will be presented in due course.

References and Notes

1. Taubenberger, J. K.; Reid, A. H.; Krafft, A. E.; Bijwaard, K. E. and Fanning T. G. *Science* **1997**, *275*, 1793.
2. Meanwell, N. A.; Krystal, M. *Drug Discovery Today*, **1996**, *1*, 316; *ibid*, **1996**, *1*, 388.
3. Hay, A. J. *Semin. Virology* **1992**, *3*, 21.
4. Bethell, R. C.; Smith, P. W. *Drugs of the Future* **1998**, *23*, 1099.
5. All the compounds in the Table 1 were screened for both H1 and H3 influenza viruses using the procedure described by Luo, G.; Colonno, R.; Krystal, M. *Virology* **1996**, *226*, 66.
6. Luo, G; Torri, A.; Harte, W. E.; Danetz, S.; Cianci, C.; Tiley, L.; Day, S.; Mullaney, D.; Yu, K.-L.; Quellet, C.; Dextraze, P.; Meanwell, N. A.; Colonno, R.; Krystal, M. *J. Virol.* **1997**, *71*, 4062.
7. Hadley, M. S.; King, F. D.; McRitchie, B; Turner, D. H.; Watts, E. A. *J. Med. Chem.* **1985**, *28*, 1843.
8. This starting material was purchased from Ryan Scientific but has been recently discontinued.
9. Cianci, C; Yu, K.-L.; Dischino, D. D.; Harte, W.; Deshpande, M.; Luo, G.; Colonno, R. J.; Meanwell, N. A.; Krystal, M. *J. Virol.* **1999**, *73*, 1785.