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Salicylamide Inhibitors of Influenza Virus Fusion

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Abstract—Structural variation of the quinolizidine heterocycle of the influenza fusion inhibitor BMY-27709 was examined by several topological dissections in order to illuminate the critical features of the ring system. This exercise resulted in the identification of a series of synthetically more accessible decahydroquinolines that retained the structural elements of BMY-27709 important for antiviral activity. The 2-methyl-*cis*-decahydroquinoline **6f** was the most potent influenza inhibitor identified that demonstrated an EC_{50} of 90 ng/mL in a plaque reduction assay.

Influenza is responsible for over 20,000 deaths annually in the US despite the annual seasonal vaccination campaigns that have become familiar. Vaccination provides limited protection based on the ability to predict the exact strains of influenza that will predominate a year in advance of the influenza season. The antiviral agents amantadine and rimantadine have been available for some time but do not protect against influenza B infections and resistance to these agents has been reported to develop quickly.[1,2] More recently, a new class of drugs that inhibit influenza neuraminidase has been licensed for use in the US and European countries.[3,4]

We have recently described a new class of influenza inhibitor that interferes with the membrane fusion activity of the virus surface protein hemagglutinin (HA)[5–7] and have disclosed fundamental aspects of the structure–activity relationships associated with the salicylic acid moiety of this new chemotype.[8] Mechanistic studies with the prototype, BMY-27709 (**1**), indicate that the compound prevents the low pH-induced conformational rearrangement of HA that occurs in the endosomal compartment after endocytosis of the virion. Variation of the substitution pattern of the aromatic ring of BMY-27709 (**1**) led to the identification of the 5-methyl and 5-chloro-salicylamide derivatives, (**1b–c**) as optimal.[8] In this article, we describe complementary studies that provide insight into the SARs associated with the quinolizidine heterocycle of BMY-27709 (**1**) as part of a broader effort to identify structurally simpler elements that would facilitate optimization of potency and spectrum of activity. Conceptually, the quinolizidine ring was systematically simplified using the bond disconnections and ring addition depicted in Figure 1. Breaking bond ***c*** identified a series of 4-aminopiperidines **2** which could be elaborated into the tropanes **3a–b**, examined in an effort to provide some insight into the active conformation of the heterocyclic element. The double disconnection of ***a*** and ***c*** produced the dialkyaminoethyl derivatives **4** as targets while breaking only the carbon–carbon bond defined by **a** led to a series of 2,6-dimethylpiperidines **5**. Since the presence and orientation of the methyl group of BMY-27709 (**1**) was known to be of importance,[8] a series of decahydroquinolines **6** and **7**, in which this alkyl group is projected from a heterocyclic ring with less conformational flexibility, was identified based on combining bond disconnection ***a*** with the ring addition defined by ***b***.[9]

Chemistry

1,1-*N,N*-Diisopropylpropylenediamine was prepared by alkylation of diisopropylamine with 3-bromopropionitrile

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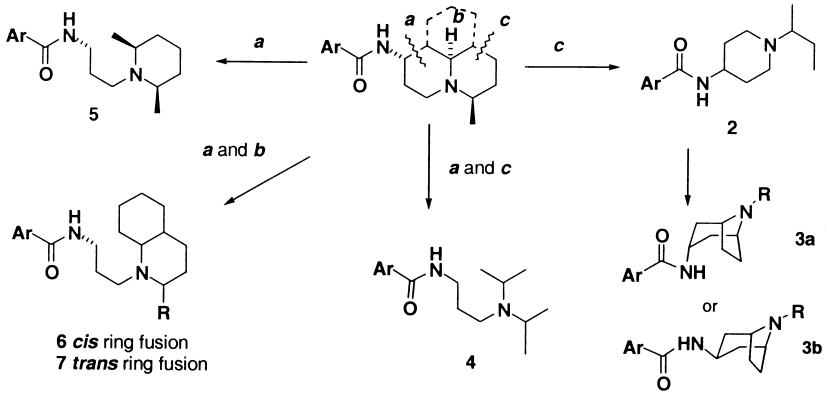
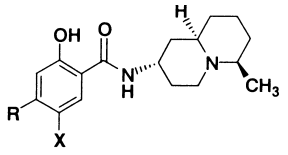


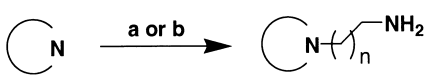
Figure 1. Carbon–carbon bond disconnections and connections used to design compounds.

followed by reduction with $LiAlH_4$. The *N*-ethyl- and *N*-propylamino-2,6-dimethyl piperidine derivatives **5a–d** were synthesized by literature methods.[10] *N*-sec-Butyl-4-aminopiperidine was prepared by reductive amination of the secondary amine with methyl ethyl ketone and $NaBH_3CN$.[11] The decahydroquinoline derivatives were obtained by a two step reduction of the corresponding quinoline.[12,13] The 3 carbon linker was introduced by Michael addition of the amine to acrylonitrile followed by reduction of the nitrile with $LiAlH_4$. The 2 carbon linker element was installed by acylation of the amine with chloroacetyl chloride followed by displacement of the chloride by azide and concomitant reduction of the azide and amide using $LiAlH_4$ (Scheme 1).



1a, BMY-27709, R = NH_2, X = Cl
1b, R = H, X = CH_3
1c, R = H, X = Cl

Direct coupling of commercially available salicyclic acid derivatives via standard methods sometimes gave low yields of the desired product along with a complex mixture of side products. In these cases, the phenol was masked as an acetate or methyl ether, the acid chloride generated and coupled with the amine. This procedure was particularly useful for hindered amines such as the axial amino tropanes **3a**. The phenol was subsequently regenerated either by mild hydrolysis of the acetate or nucleophilic demethylation of the methyl ether using sodium methanethiolate (Scheme 2, method A and B). However, the most convenient method for amide bond formation was to protect the phenol and acid elements simultaneously as an acetonide and heat this with an excess of amine in toluene,[14,15] as depicted in Scheme 2, method C.

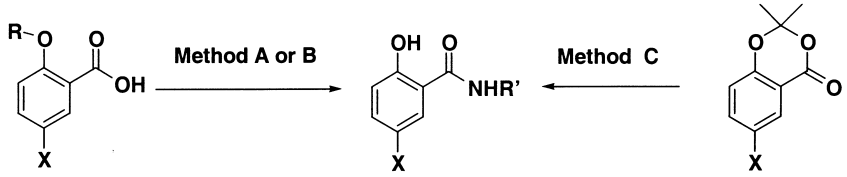


Scheme 1. (a) $n=1$; (i) $ClCH_2COCl/Et_3N$; (ii) $NaN_3/THF/H_2$; (iii) $LiAlH_4$; ether; (b) $n=2$; (i) acrylonitrile; (ii) $LiAlH_4$, ether.

The compounds prepared as part of this study are compiled in Table 1. All compounds were evaluated in a cell protection assay using MDCK cells infected with the A/WSN/33 strain of influenza virus (H1N1 subtype) and the concentration of drug affording 50% protection (EC_{50}) determined.[5]

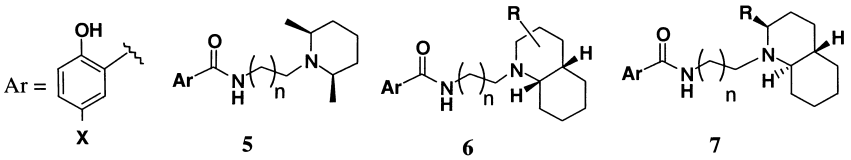
Results and Discussion

The amino piperidine derivative **2** was inactive and activity was not restored by introducing the conformational constraint afforded by the tropanes **3a–b**, examined as the individual axial and equatorial isomers (only four representatives of several examined are depicted in Table 1).[16] The simplest structural representation of the quinolizidine element, dialkyaminoalkyl derivatives **4** were also inactive with the exception of the diisopropyl analogue **4**, which possesses antiviral activity comparable to that of the prototype **1**. Further elaboration of this chemotype into the *cis*-dimethyl piperidines **5a–d** provided active compounds in which there is a preference for a 3 carbon linker (**5a** and **5b**) compared to the ethyl linkage found in **5c** and **5d**. This trend is consistent with the topological disconnection ***a*** depicted in Figure 1 and, taken together with the SAR for compounds **2–4**, suggests that non-collapsible structural elements distal to the amide moiety are required for effective expression of antiviral activity. This theme was



Scheme 2. Method A: R = Me or Ac; (1) $SOCl_2$; (2) amine, Et_3N, CH_2Cl_2; (3) NaSEt or Na_2CO_3, respectively; Method B: R = H, EDC, HOBT, DMF, amine; Method C: amine, toluene, Δ.

Table 1. Cell protection assay of cells infected with H1N1 subtype influenza for compounds **1**–**7**



Compound no.	*n*	X	R	Synthetic method[a]	EC_{50} (μg/mL)[b]	CC_{50} (μg/mL)[c]
1c		Cl			0.80[8]	>200
2		Me		B	na[d]	100
3a–b		Me or Cl	Me, iPr, s-Bu or Bn	A	na	100
4		Me		B	0.90	>100
5a	3	Cl		A	1	100
5b	3	Me		A	0.70 (±0.1)	>100
5c	2	Cl		A	4.5 (±1.5)	30
5d	2	Me		A	1.8 (±0.2)	>100
6a	3	Me	H	A	na	30
6b	2	Me	H	A	0.65 (±0.5)	>100
6c	2	Cl	H	A	1.4 (±0.6)	43
6d	3	Me	α-2-Me	A	na	90
6e	2	Me	α-2-Me	C	0.19 (±0.05)	35
6f	2	Cl	α-2-Me	B	0.09 (±0.02)	40
6g	2	Me	β-2-Me	A	6.0 (±0.5)	>100
6h	2	Me	α-2-iPr	A	0.25	80
6i	2	Me	α-3-Me	A	na	9.5
6j	2	Me	α/β-4-Me	A	na	100
7a	2	Me	H	C	0.35 (±0.15)	60
7b	2	Cl	H	C	1.4 (±0.6)	40–45
7c	2	Me	α-Me	C	1.5 (±0.5)	20–30

[a]See Scheme 2 for the synthetic method.
[b]50% Inhibition of A/WSN/33 influenza strain-induced toxicity in a cell protection assay in MDBK cells, values are mean of duplicate experiments; standard deviation in parentheses.
[c]Cytotoxicity of the compound in MDBK cell.
[d]na = not active at 50 μg/mL.

further explored in the context of the decahydroquinolines **6** and **7**, several of which demonstrate potent influenza inhibitory activity in cell culture. Interestingly, in this series a 2 carbon linker appears to be optimal since **6a** and **6d** were found to be inactive. The two unsubstituted *cis*-decahydroquinolines **6b** and **6c** are active antiviral agents that can be improved by the introduction of a methyl group at C-2, compounds **6e** and **6f**. Activity is sensitive to the stereochemistry at C-2, since the β-methyl isomer **6g** is markedly less active, and to the relative topological location, since the 3 and 4-substituted methyl isomers **6i** and **6j**, respectively, are devoid of antiviral properties. The single alkyl homologue examined, **6h**, retains good inhibitory activity. In the *trans*-decahydroquinoline series, the activity associated with the parent structures **7a** and **7b** are comparable to that of their *cis*-configured counterparts **6b** and **6c** while the single substituted analogue **7c** is approximately 10-fold weaker than **6e**.

Whilst this study has identified potent and effective inhibitors of influenza virus that are synthetically more accessible than the quinolizidine heterocycle found in the prototype **1**, the antiviral activity resides predominantly in compounds with only limited conformational constraint. As a consequence, conclusions about the active conformation of **1** cannot be logically reached from this study.

None of the compounds presented in Table 1 demonstrated significant inhibition of an influenza H3 strain, a shortcoming inherent to **1** and structurally related series of influenza fusion inhibitors.[8,17,18] Based on this finding and the discovery of more potent influenza inhibitors within a series of cyclohexanol derivatives,[14] further examination of this series was not pursued.

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