Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1692–1695
Bioorganic & Medicinal Chemistry Letters

Synthesis of potent pyrrolidine influenza neuraminidase inhibitors

A. Chris Krueger,* Yibo Xu, Warren M. Kati, Dale J. Kempf, Clarence J. Maring, Keith F. McDaniel, Akhteruzzaman Molla, Debra Montgomery and William E. Kohlbrenner

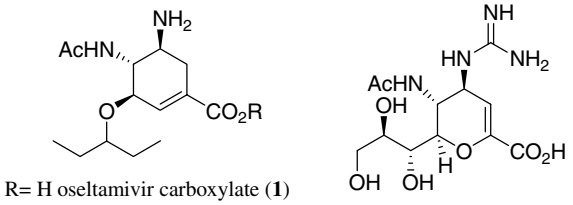
Infectious Disease Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 200 Abbott Park Road, AP-52N, Abbott Park, IL 60064, USA

Received 10 October 2007; revised 11 January 2008; accepted 14 January 2008
Available online 18 January 2008

Abstract—The synthesis of several pyrrolidine inhibitor analogs is described that possess nanomolar in vitro potencies against the neuraminidase enzymes expressed by the B/Memphis/3/89 and A/N1/PR/8/34 influenza strains.

Mortality due to the influenza virus continues to be a serious problem throughout the world.[1–6] Periodically a worldwide epidemic, or pandemic, occurs which can have disastrous consequences. One of the worst examples was the pandemic of 1918 which was estimated to have caused 40–50 million worldwide deaths.[7] Recently many world countries have begun stockpiling the influenza drug Tamiflu (**2**) to protect their populations against a possible bird flu pandemic.[8,9] Bird flu is caused by an avian influenza virus which caused at least 50 human fatalities in Asia during the winter of 2004–2005.[9] It is feared that if this influenza H5N1 virus strain becomes efficiently transmissible from human-to-human, another global pandemic could ensue. Thus there is a continuing need for development of new anti-influenza therapies.

The viral enzyme neuraminidase (NA) has been an active research area for anti-influenza therapy.[10] The structures of two currently marketed NA inhibitor drugs are shown in Figure 1. We have previously reported a series of tri-substituted pyrrolidine NA inhibitors that have potent activities (**4**, $K_i = 30$ nM).[11] One attempt to create even more potent analogs based upon this scaffold is described herein. We believed that the isobutyl group in pyrrolidine **4** was not fully optimized and began an effort to modify this hydrophobic group (Fig. 2).



R= H oseltamivir carboxylate (**1**)
R= Et oseltamivir (Tamiflu) (**2**)

Zanamavir (**3**)

Figure 1. Anti-influenza neuraminidase inhibitors.

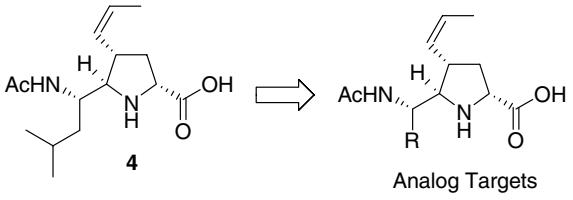
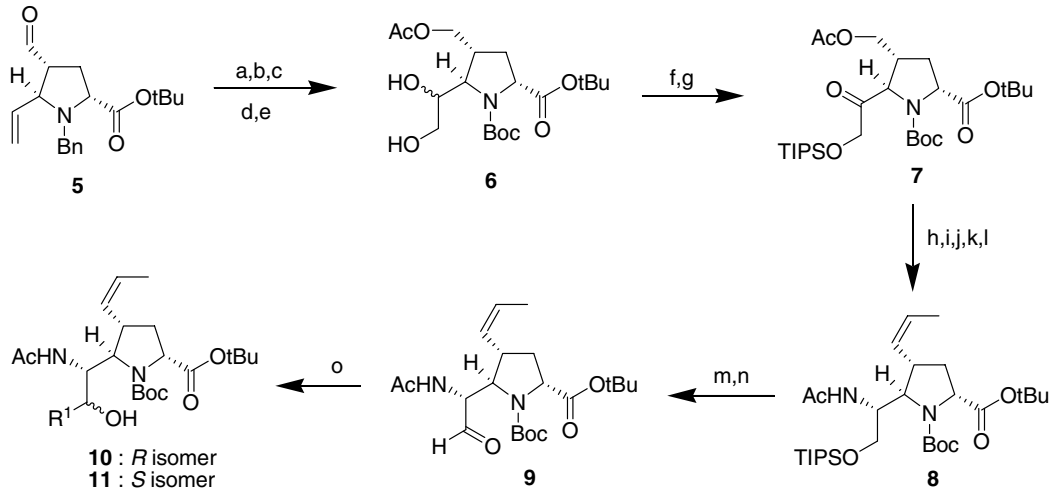


Figure 2. Pyrrolidine analogs.

Aldehyde **9** proved to be an important and versatile intermediate for the synthesis of novel analogs. The racemic synthesis of **9** is shown in Scheme 1. An acid catalyzed [3+2]-dipolar cycloaddition as previously described yielded a 2,4,5-tri-substituted pyrrolidine **5** as the predominant diastereomer.[11,12] Sodium borohydride reduction and acetylation of the resulting primary alcohol produced the desired acetate. Dihydroxylation of the exocyclic olefin provided a 1:1 mixture of diols followed by an exchange of the *N*-benzyl to *N*-Boc

Keywords: Influenza; Neuraminidase; Pyrrolidine.
* Corresponding author. Tel.: +1 847 938 2944; fax: +1 847 938 2756; e-mail: a.chris.krueger@abbott.com

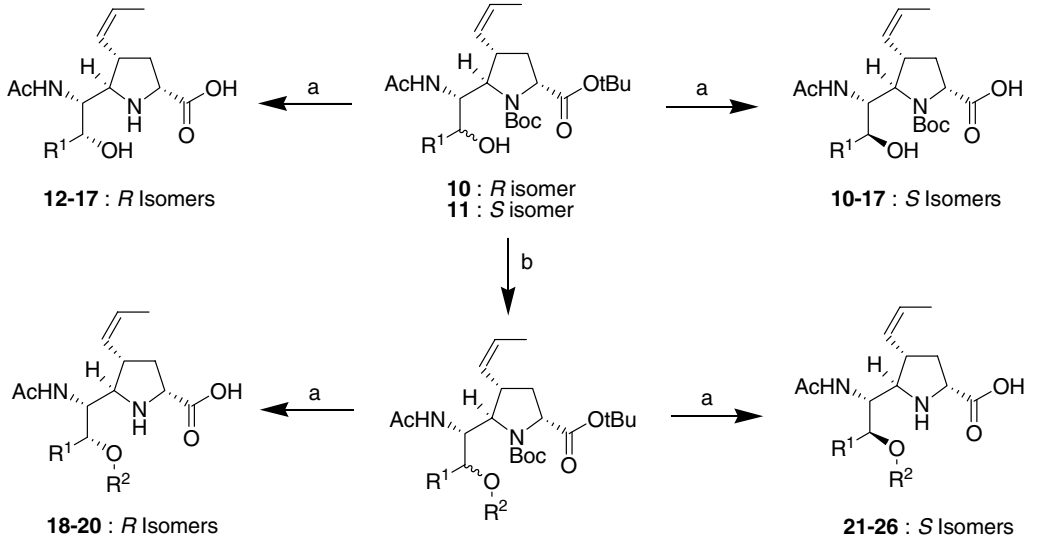
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.048



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH; (b) Ac_2O, pyridine (72%, 2 steps); (c) OsO_4, NMO, 72%; (d) ammonium formate, Pd/C, EtOH; (e) Boc_2O, MeOH, H_2O (78%, 2 steps); (f) TIPSCl, imidazole, DMF, 79%; (g) Swern oxidation, 77%; (h) ammonium acetate, $NaCNBH_3$, MeOH; (i) Ac_2O, Et_3N, DMAP, CH_2Cl_2 (41%, 2 steps); (j) K_2CO_3, MeOH, H_2O, 86%; (k) Swern oxidation, 82%; (l) $Ph_3P^+EtBr^-$, *t*-BuOK, THF, 85%; (m) TBAF, THF, 89%; (n) Dess–Martin oxidation, 96%; (o) R^1MgX, THF, 50–73%.

protection groups to provide diols **6**. A number of attempts were made to find reagents or conditions to control the diastereoselectivity of the dihydroxylation reaction, and all failed to impart any significant improvement in diastereoselectivity. Silylation of the terminal C-5 side-chain hydroxyl with triisopropylsilyl chloride (TIPSCl) followed by Swern oxidation produced ketone **7**. Reductive amination with ammonium acetate and sodium cyanoborohydride in refluxing methanol again yielded a 1:1 mixture of diastereomeric amines that was acetylated and readily separated by chromatography. All attempts to control the diastereoselectivity of the reductive amination in favor of the desired *R*-isomer were unsuccessful.

Hydrolysis of the *O*-acetyl group of the *R*-isomer followed by Swern oxidation of the resulting alcohol produced the aldehyde. Wittig olefination of the aldehyde gave exclusively the *Z*-propenyl substituted pyrrolidine **8**. Fluoride deprotection of the TIPS group afforded the corresponding primary alcohol which was oxidized with Dess–Martin periodinane to provide aldehyde **9** without epimerization of the adjacent acetylamino-substituted center. Addition of the aldehyde **9** to a variety of Grignard reagents produced both diastereomeric alcohols **10** and **11**, with the *R*-diastereomer (**10**) predominating. For example, the reaction of ethyl magnesium bromide with aldehyde **9** generated a 5.5:1 ratio of **13a**–**b**, where R_1 = ethyl. The diastereoselectivity of this reaction appears to be governed by the Cram chelation model. Finally, many of the alcohol and ether NA inhibitors were generated as shown in Scheme 2. Trifluoroacetic acid deprotection of the diastereomers **10** and **11** provided the corresponding secondary alcohol inhibitors **12a**–**17a** and **12b**–**17b**, respectively, as TFA salts. Alkylation of the various secondary alcohols **10** and

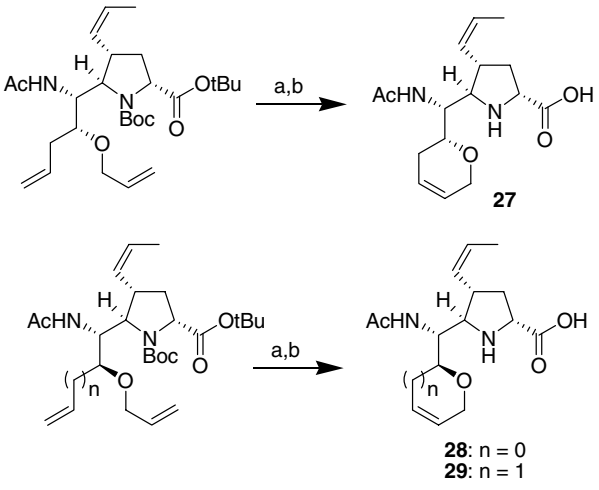


Scheme 2. Reagents and conditions: (a) trifluoroacetic acid, CH_2Cl_2, 98–100%; (b) MeI, EtI, or allyl bromide, DMF, KOH, 18-crown-6, 16–80%.

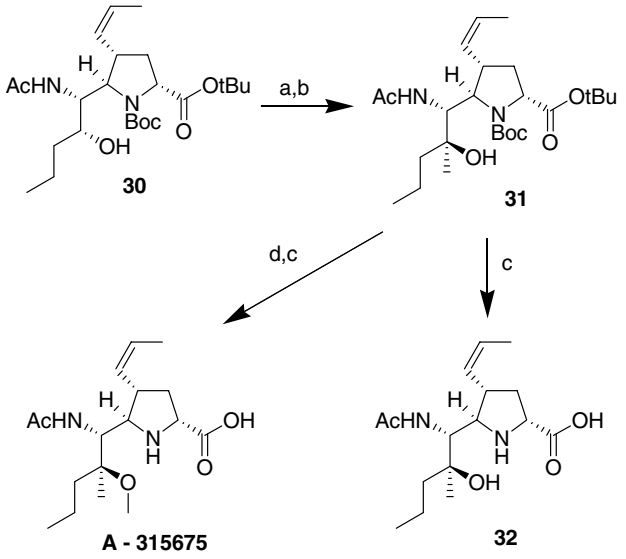
11 with methyl iodide, ethyl iodide, or allyl bromide in the presence of potassium hydroxide and 18-crown-6 followed by standard TFA deprotection, generated the desired ether inhibitors **18**–**26**. The cyclic secondary ether inhibitors were generated by an olefin metathesis, followed by a TFA deprotection protocol of the requisite alkene precursors as shown in Scheme 3.

Preparation of tertiary alcohol inhibitor **32** was accomplished by the oxidation of alcohol **30** to the corresponding ketone followed by reaction with six equivalents of Grignard reagent then TFA deprotection. The methyl ether analog of inhibitor **32** was also constructed by reaction of the tertiary alcohol in **31** with sodium bis-(trimethylsilyl)amide and methyl iodide followed by TFA deprotection to provide inhibitor **A-315675** as shown in Scheme 4.

We targeted potent in vitro antiviral potency against the medically relevant strains of influenza virus (types A and B) with the hope of achieving uniform activity. The two NA viral strains we routinely tested our inhibitors against were B/Memphis/3/89 and A/N1/PR/8/34. The NA A strain was selected because of its similarity to the highly pathogenic avian H5N1 virus mentioned earlier. The secondary alcohol analogs with the *S*-configuration (**12b**–**17b**) universally were more potent than the corresponding *R*-configuration analogs (**12a**–**17a**) (Table 1).[13] Additionally, all of the inhibitors were more potent against the B virus strain. The most potent analogs in Table 1 contained a 3-carbon chain with the *S*-isomer configuration (**15b** and **16b**). In an effort to gain more potency, the secondary alcohols were transformed into ethers. We reasoned that the more hydrophobic alkyl ethers might provide more binding energy, somewhat analogous to the isopentyl ether in oseltamivir carboxylate (**1**). Table 2 displays our results. Again as in the secondary alcohol cases, the corresponding *S*-isomers were more potent than the *R*-isomers. Against the NA B strain, a loss in potency ensued when progressively larger ether alkyl groups were employed, as evidenced from the methyl, ethyl, and allyl data (**18**–**20**, **21**–**23**, and **24**–**26**). Against the NA A strain however, the most potent ether analogs contained the allyl functionality (**20**, **23**, and **26**). The two most potent ethers against NA B (**21** and **24**) were roughly 2- to 3-fold more potent than the corresponding alcohols (**14b** and **16b**). However against the NA A strain, these two methyl ether analogs (**21** and **24**) were approximately equal to 2-fold less potent verses the corresponding alcohols (**14b** and **16b**). Thus the potency selectivity of methyl ether **24** against the NA B strain verses the NA A strain was approxi-

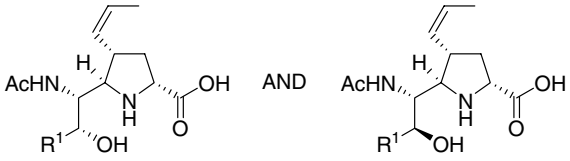


Scheme 3. Reagents and conditions: (a) Grubbs I catalyst, 53–90%; (b) trifluoroacetic acid, CH_2Cl_2, 98–100%.



Scheme 4. Reagents and conditions: (a) Dess–Martin oxidation, 93%; (b) 6 equiv MeMgBr, THF, 70%; (c) trifluoroacetic acid, CH_2Cl_2, 98–100%; (d) NaHMDS, MeI, THF, −78 to 0 °C, 67%.

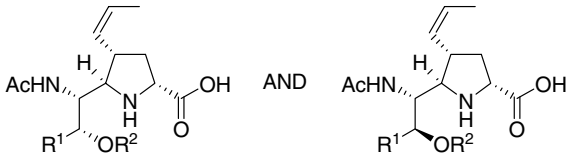
Table 1. Biochemical potency of secondary alcohol analogs **12a**–**17b**



Compound	R^1=	Isomer	NA B K_i[a] (nM)	NA A K_i[a] (nM)
12a	Methyl	*R*	85	303
12b	Methyl	*S*	6.5	59
13a	Ethyl	*R*	29	108
13b	Ethyl	*S*	2.8	11.5
14a	Vinyl	*R*	21	—
14b	Vinyl	*S*	2.0	25
15a	*n*-Propyl	*R*	28	53
15b	*n*-Propyl	*S*	1.3	5.3
16a	2-Propenyl	*R*	49	212
16b	2-Propenyl	*S*	1.6	10
17a	*n*-Butyl	*R*	86	94
17b	*n*-Butyl	*S*	12	14

[a] The K_i values for all compounds are given in nanomolar units and refer to their activities as racemates. The K_i values in all tables are also means of at least two independent determinations, standard deviation ±10%. Detailed protocols can be found in Supplementary material.

Table 2. Biochemical potency of ether analogs **18**–**26**



R Isomer *S* Isomer

Compound	R^1=	R^2	Isomer	NA B K_i (nM)	NA A K_i (nM)
18	2-Propenyl	Methyl	*R*	38	145
19	2-Propenyl	Ethyl	*R*	46	60
20	2-Propenyl	2-Propenyl	*R*	110	23
21	Vinyl	Methyl	*S*	0.6	19.8
22	Vinyl	Ethyl	*S*	4.8	21
23	Vinyl	2-Propenyl	*S*	9.3	11.3
24	2-Propenyl	Methyl	*S*	0.67	17.3
25	2-Propenyl	Ethyl	*S*	10.6	18.4
26	2-Propenyl	2-Propenyl	*S*	17.5	12.7

Table 3. Biochemical potency comparisons of pyrrolidine and marketed drug inhibitors

Compound	NA B K_i (nM)	NA A K_i (nM)
27	10.3	56
28	24.2	180
29	0.3	55
32	1.2	1.3
A-315675	0.14	0.21
1 (oseltamivir carboxylate)	1.1	0.1
3 (Zanamivir)	0.1	0.06
4	30	210
15b	1.3	5.3
24	0.67	17.3

mately 25–1 (0.67 vs 17.3 nM). In an effort to improve the absolute potency against the NA A strain while maintaining a sub-nanomolar potency against the NA B strain, a few cyclic ether inhibitors were constructed. The *R*-dihydropyran (**29**) was the most potent of the three analogs at 0.3 nM against the NA B strain (Table 3). Unfortunately its potency against the NA A strain decreased to 55 nM though. Upon conversion of the secondary alcohol inhibitor **15b** into the tertiary alcohol inhibitor **32** a modest enhancement in potency (4-fold) against the NA A strain was realized. However when the tertiary alcohol group in analog **32** was converted to a methyl ether functionality to provide **A-315675**,[14] a roughly 10-fold potency improvement was realized. This resulted in **A-315675** having sub-nanomolar potency against both NA strains (Table 3). The potency of some of the pyrrolidine analogs as compared to oseltamivir carboxylate (**1**), the active component of the marketed prodrug Tamiflu (**2**), and Zanamivir (**3**) in our assay are also shown in Table 3.

In conclusion, we were successful in designing and synthesizing several potent sub-nanomolar pyrrolidine inhibitors against the NA B strain. One of these inhibitors displayed sub-nanomolar potency against both the NA A and B strains, thus being similar in potency to the two currently marketed anti-influenza drugs.

Supplementary data

Detailed biological protocols for biochemical K_i determinations are available in Supplementary data.Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.048.

References and notes

1. Murray, C. J. L.; Lopez, A. D.; Chin, B.; Feehan, D.; Hill, K. K. *Lancet* **2006**, *368*, 2211.
2. Goldrick, B. A.; Goetz, A. M. *Am. J. Infect. Control* **2007**, *35*, 7.
3. Flahault, A.; Vergu, E.; Coudeville, L.; Grais, R. F. *Vaccine* **2006**, *24*, 6751.
4. De Clercq, E.; Neyts, J. *Trends Pharmacol. Sci.* **2007**, *28*, 280.
5. Oxford, J. S.; Lambkin, R.; Elliot, A.; Daniels, R.; Sefton, A.; Gill, D. *Vaccine* **2006**, *24*, 6742.
6. Moscona, A. *N. Engl. J. Med.* **2005**, *353*, 1363.
7. (a) Poland, G. A.; Jacobson, R. M.; Targonski, P. V. *Vaccine* **2007**, *25*, 3057; (b) Nguyen-Van-Tam, J. S.; Sellwood, C. *J. Hosp. Infect.* **2007**, *65*, 10; (c) Oxford, J. S.; Novelli, P.; Sefton, A.; Lambkin, R. *Antiviral Chem. Chemother.* **2002**, *13*, 205.
8. Andrawiss, M. *Drug Discovery Today* **2005**, *10*, 811.
9. Hileman, B. *Chem. Eng. News* **2005**, *83*, 47.
10. (a) Hayden, F. G. *Antiviral Res.* **2006**, *71*, 372; (b) Brouillette, W. J.; Bajpai, S. N.; Ali, S. M.; Velu, S. E.; Atigadda, V. R.; Lommer, B. S.; Finley, J. B.; Luo, M.; Air, G. M. *Bioorg. Med. Chem.* **2003**, *11*, 2739; (c) Kim, C. U.; Lew, W.; Williams, M. A. *J. Am. Chem. Soc.* **1997**, *119*, 681; (d) von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418; (e) Wade, R. C. *Structure* **1997**, *5*, 1139.
11. Maring, C. J.; Stoll, V. S.; Zhao, C.; Sun, M.; Krueger, A. C.; Stewart, K. D.; Madigan, D. L.; Kati, W. M.; Xu, Y.; Carrick, R. J.; Montgomery, D. A.; Kempf-Grote, A.; Marsh, K. C.; Molla, A.; Steffy, K. R.; Sham, H. L.; Laver, W. G.; Gu, Y. G.; Kempf, D. J.; Kohlbrenner, W. E. *J. Med. Chem.* **2005**, *48*, 3980.
12. Gu, Y. G.; Xu, Y.; Krueger, A. C.; Madigan, D.; Sham, H. L. *Tetrahedron Lett.* **2002**, *43*, 955.
13. All compound structures were consistent by ^{1}H NMR and LC–MS analysis (>95% purity).
14. (a) Kati, W. M.; Montgomery, D.; Carrick, R.; Gubareva, L.; Maring, C.; McDaniel, K.; Steffy, K.; Molla, A.; Hayden, F.; Kempf, D.; Kohlbrenner, W. *Antimicrob. Agents Chemother.* **2002**, *46*, 1014; (b) DeGoey, D. A.; Chen, H.; Flosi, W. J.; Grampovnik, D. J.; Yeung, C. M.; Klein, L. L.; Kempf, D. J. *J. Org. Chem.* **2002**, *67*, 5445.