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Structure–Activity Relationships for a Series of Thiobenzamide Influenza Fusion Inhibitors Derived from 1,3,3-Trimethyl-5-hydroxy-cyclohexylmethylamine

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Abstract—The anti-influenza activity of a series of thiobenzamide fusion inhibitors derived from 1,3,3-trimethyl-5-hydroxy-cyclohexylmethylamine is profiled. Axial disposition of the thioamide moiety is essential for potent influenza inhibitory activity.

Influenza virus is an etiologic cause of widespread epidemics of acute respiratory tract infections that occur annually during the winter season. Less frequent pandemic episodes of influenza are associated with greater mortality and morbidity, usually a consequence of genetic recombination of viral strains. Indeed, the Spanish influenza outbreak of 1918 was the most devastating outbreak of infectious disease since the infamous plague of the 14th century, being responsible for upwards of 20 million deaths.[1] Currently, control of influenza infection relies mainly on a preventative strategy dependent on the annual identification, production and distribution of a multivalent vaccine designed to predict the predominant epidemic viral strains.[2] Until recently, anti-influenza drugs were restricted to the M2 channel blockers amantadine and rimantadine, both of which are approved for the prophylactic and therapeutic treatment of the influenza viruses within the A family. However, the licensing of the neuraminidase inhibitors zanamavir and oseltamivir has provided the clinician with antiviral agents that effectively prevent and treat both type A and B influenza infections.[3]

Influenza virus penetrates host cells via a multi-step process that relies upon an initial attachment of the virus to sialylated proteins on the surface of susceptible cell types. Following endocytosis of virus into endosomes, the lowered pH induces an acid-catalyzed conformational rearrangement of the viral hemagglutinin surface protein. This event results in the fusion of the viral and endosomal membranes, thereby releasing the viral genome into the cytoplasm. The genome segments then migrate to the nucleus and initiate infection.[4] We have previously described the discovery of BMY-27709, an influenza inhibitor that selectively inhibits the fusion process by stabilizing hemagglutinin and preventing the essential conformational rearrangement.[5–7] Initial structure–activity studies focussed on the salicylamide and quinolizidine moieties and revealed the elements essential for antiviral activity.[8,9] However, a second avenue of exploration with this chemotype examined a parallel synthesis strategy designed to identify scaffolds structurally simpler and synthetically more accessible than the quinolizidine moiety found in BMY-27709. Moreover, it was hoped that this strategy would provide potent fusion inhibitors that encompassed inhibition of the influenza A H3 subtype in addition to the H1 and H2 subtypes that characterized the prototype. Two new series of fusion inhibitors, **1** and **2**, in which the quinolizidine moiety is replaced by 1,3,3-trimethyl-5-

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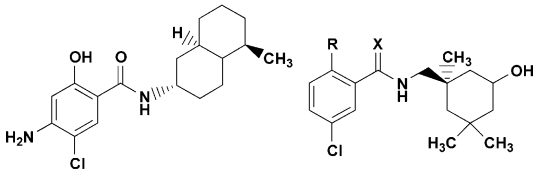
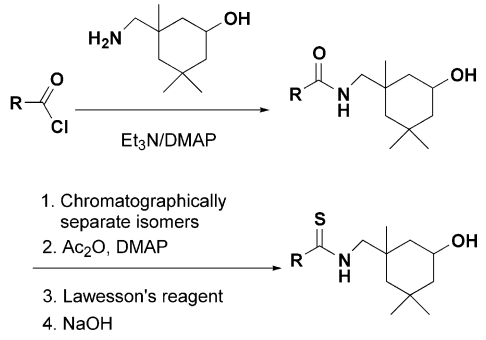
hydroxycyclohexylmethylamine, emerged from this work. These prototypes demonstrated an interesting and complementary dependence on the conformation of the cyclohexanol moiety.[10] For the salicylamide series **1**, the isomer in which the hydroxyl and amide are *cis* oriented and the amide moiety adopts an equatorial conformation is about 4 times more potent than the corresponding *trans* isomer, in which the amide moiety is axially disposed. In contrast, in the thioamide series, **2**, the *trans* isomer, in which the thioamide element is axial, is almost equipotent with the *cis* isomer of **1**, while the *cis* thioamide (thioamide equatorially disposed) is completely inactive.[10] As part of an effort to further probe this intriguing SAR, a series of thioamides, in which the substitution pattern of the aromatic group was varied, was synthesized and evaluated for inhibition of influenza virus in cell culture.



Chemistry

The synthesis of the series of thioamides that constitute this study was straightforward, as illustrated in Scheme 1. The amides were obtained by reacting a commercially available acid chloride with a mixture of *cis*- and *trans*-1,3,3-trimethyl-5-hydroxycyclohexylmethylamine and separating the resulting mixture of isomers by flash chromatography over silica gel (Scheme 1). In general, the *cis* isomers were found to be less mobile than the corresponding *trans* isomer. The stereochemistry of the individual isomers was confirmed by ^{1}H NMR with the signal for the CH_2N protons proving to be diagnostic. For the isomers in which the OH and CH_2N moieties are *trans* disposed, the CH_2N moiety is axial and the protons are diastereotopic, providing a well separated pattern of resonance. In contrast, in the *cis* isomers the signal for the equatorially disposed NCH_2 protons resonates as a complex multiplet.

To complete the synthesis of the thioamides, the cyclohexane hydroxyl moiety was first protected as an acetate, the amide treated with Lawesson's reagent[11] and the acetate hydrolyzed using 1 N NaOH in MeOH, as depicted in Scheme 1. Conversion of the salicylamides to their thioamide derivatives relied upon the same synthetic methodology, with the exception that the phenol moiety was carried through the sequence as a methyl ether and deprotected using BBr_3 in CH_2Cl_2 as the final step (Scheme 2).



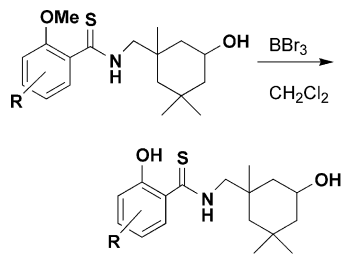
Scheme 1.

Results and Discussion

The compounds prepared as part of this study were evaluated in a cell protection assay in which MDCK cells were infected with the A/WSN/33 (H1N1 subtype) strain of influenza virus.[5] The compounds of interest were also evaluated for inhibition of influenza virus-mediated hemolysis of erythrocytes, an assay which more specifically focuses on inhibition of hemagglutinin-induced membrane fusion.[5,6] The results are compiled in Table 1 along with data for compounds **1e**, **1a**, **2e**, **2a**, **15e**, **15a** that were disclosed previously.[10] With the specific exception of the salicylamide derivatives, the chemotype discovered in the parallel synthesis exercise,[10] all amides, whether *cis* or *trans* disposed with respect to the cyclohexane ring hydroxyl, were found to be inactive ($EC_{50} > 10$ μM) influenza inhibitors. As a consequence, data for these compounds is not included in Table 1. This strict requirement for an *ortho* hydroxyl moiety as the mediator of influenza inhibitory activity is analogous to the SAR observed for quinolizidine-based salicylamide derivatives where the salicylamide element is postulated to function as a carboxylic acid is isostere.[8] However, a significant difference discovered here is that a CH_3 or Cl substituent at the 5-position of the aromatic ring is not required for manifestation of potent activity.

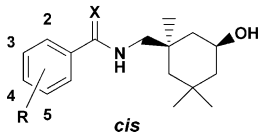
The prototype **3e** is equipotent with the 3-substituted homologues **2e** and **5e**, which contrasts with results for the quinolizidine-based series[8] and suggests that cyclohexanol-based salicylamide derivatives bind to hemagglutinin in a similar but nevertheless distinct fashion that allows the phenyl ring to adopt a slightly different orientation in the binding site.[7]

With the notable exception of the *ortho*-hydroxy derivatives **14e**, **16e** and **17e**, all of the thioamides in which this moiety is *cis* to the hydroxyl and thus equatorially disposed are inactive in the cell culture environment, although several show weak inhibition of hemolysis. However, the antiviral activities associated with **14e**, **16e**

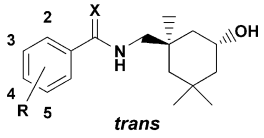


Scheme 2.

Table 1. Anti-influenza activity evaluated using cell protection assay in MDCK cells infected with A/WSN/33 (H1N1 subtype) strain of influenza virus



cis



trans

Compd	X	R	Hemolysis IC_{50} (μg/mL)	EC_{50} (μg/mL)	CC_{50} (μg/mL)	Compd	X	R	Hemolysis IC_{50} (μg/mL)	EC_{50} (μg/mL)	CC_{50} (μg/mL)
1e	O	2-OH, 5-Cl	0.025	0.06	80	**1a**	O	2-OH, 5-Cl	0.15±0.07	0.2	>100
2e	S	3-Cl	2.5	NA	8.5	**2a**	S	3-Cl	0.047	0.02	20
3e	S	H	3.5	NA	50	**3a**	S	H	0.15	0.05	10
4e	S	2-Me	2.4±2	NA	2	**4a**	S	2-Me	0.14	0.065±0.035	70
5e	S	3-Me	7.5	NA	20	**5a**	S	3-Me	0.03	0.015±0.007	35
6e	S	3-F	5	NA	20	**6a**	S	3-F	0.19	0.038	70
7e	S	3-CF_3	23	NA	5	**7a**	S	3-CF_3	0.3	0.09	15±7
8e	S	4-Me	NA	NA	25±9	**8a**	S	4-Me	7±6	NA	40
9e	S	2,4-diMe	NA	NA	30	**9a**	S	2,4-diMe	0.45	0.15	35
10e	S	2,5-diMe	1.5	NA	30	**10a**	S	2,5-diMe	0.08	0.023±0.011	45
11e	S	3,5-diMe	0.5	NA	40	**11a**	S	3,5-diMe	0.52	0.03±0.07	18
12e	S	2-OMe	NA	NA	45	**12a**	S	2-OMe	0.5	1.0	43
13e	O	2-OH	0.15	0.05	70	**13a**	O	2-OH	0.8	25	90
14e	S	2-OH	2.4	4.5	40	**14a**	S	2-OH	2.4	2	40
15e	O	2-OH, 5-Me	0.039±0.025	0.03	50±25	**15a**	O	2-OH, 5-Me	0.015±0.07	0.15±0.071	70
16e	S	2-OH, 5-Me	0.16±0.08	0.4	15	**16a**	S	2-OH, 5-Me	0.33	0.5	20
17e	S	2-OH, 5-Cl	2.8±1.8	0.9	5.5	**17a**	S	2-OH, 5-Cl	1	1.8	4
						18a	S	3-CN	NA	2.5	22
						19a	S	3-NO_2	1.8	NA	15
						20a	S	3-CO_2Me	NA	NA	50±35

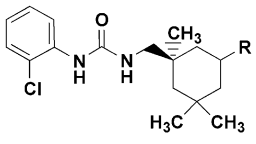
NA, not active, IC_{50} or EC_{50} > 10 μg/mL; data presented are the average of two determinations with STD indicated for ranges >2-fold ($n=2$). CC_{50} refers to the concentration of drug causing a 50% reduction in cell viability in the absence of influenza infection.

and **17e** are an order of magnitude or more weaker than the analogous amides, reflecting the different physical properties of amides and thioamides. In this context, the thioamide moiety appears to act as a poor surrogate for an amide, a finding consistent with the proposed binding mode of amides.[7] The amide carbonyl oxygen is postulated to engage hemagglutinin as a hydrogen bond acceptor, combining with the hydrogen bond-donating *ortho* hydroxyl in constellation to function as a carboxylic acid isostere, complementing the guanidine of Arg-106. In addition to possessing a longer C=S bond, the thioamide moiety is a weaker hydrogen bond acceptor than an amide[12a,b] and engages the donor hydrogen with an altered directionality that reflects a more rectangular topological disposition.[12c]

Substitution patterns examined in the axial thioamide series illuminate key aspects of structure–activity correlates and reveal, remarkably, that the 3-Cl derivative **2a** discovered from the original parallel synthesis strategy is essentially optimal. Lipophilic substituents incorporated at the 3-position, CH_3 (**5a**), F (**6a**), or CF_3 (**8a**), afforded potent antiviral agents with EC_{50}'s correlating with inhibition of hemolysis. However, more polar functional groups at the 3-position, specifically CN (**18a**), NO_2 (**19a**) and CO_2Me (**20a**), afforded analogues considered to be inactive.

An *ortho* hydroxyl (**14a**, **16a**, and **17a**) or MeO (**12a**) significantly attenuates the antiviral activity of the prototype **3a** but this effect appears not be steric in origin since the 2-CH_3 derivative, **4a**, exhibits excellent potency. The activity expressed by analogues **14a**, **16a** and **17a** may represent a confluence of SARs between the amide and thioamide chemotypes.

A methyl subsituent at the 4-position (**8a**), is sufficient to abolish the activity of the prototype **3a** but combination with a 2-CH_3 (**9a**) restores potency to a modest level. The optimal patterns of substituent combinations reflect individual contributions. Thus, the 2,4- and 3,5-dimethyl derivatives, (**9a**) and (**11a**), respectively, are the most potent analogues. In data not shown, the replacement of the phenyl ring of both amides and thioamides with simple alkyl or cycloalkyl (e.g., cyclohexyl) groups or heterocyclic rings (e.g., furan) resulted in inactive compounds as did an attempt to replace the amide and thioamide moieties with a sulfonamide surrogate. However, a urea has been found to be an acceptable bridging functionality between the gem-dimethylated cyclohexanol moiety and the aromatic element of the pharmacophore.[13] However, intriguingly and in contrast to the findings disclosed herein, these ureas exhibited little or no dependence on relative stereochemistry with the *cis* and *trans* isomers of **21** equiactive inhibitors of H1 influenza infectivity in cell culture.



R = ◄OH **21-*cis*** IC_{50} (A/Kaw) = 0.1 μg/mL)
R = ıllOH **21-*trans*** IC_{50} (A/Kaw) = 0.1 μg/mL)

A common biochemical pharmacological profile that has emerged for inhibitors of influenza fusion is the

restriction of antiviral activity to virus subtypes expressing hemagglutinin H1 and H2.[5–10,13–15] All of the compounds disclosed above were evaluated as inhibitors of a representative H3 virus and found to be inactive with EC_{50}'s in excess of 20 μg/L.

In summary, we have identified the key structural elements required for H1 influenza inhibitory activity of a series of salicylamide- and thiobenzamide-based fusion inhibitors derived from 1,3,3-trimethyl-5-hydroxycyclohexylmethylamine and demonstrated that activity is specifically dependent on the thioamide element and its conformational disposition on the cyclohexanol ring. The contrasting SARs between the salicylamide and thioamide series leads to the conclusion that these two types of influenza fusion inhibitor bind to the hemagglutinin protein in subtly different fashions.

References and Notes

1. Reid, A. H.; Fanning, T. G.; Hultin, J. V.; Taubenberger, J. K. *Proc. Natl. Acad. Sci.* **1999**, *96*, 1651.
2. Treanor, J. J. in *Principles and Practice of Infectious Diseases*; Mandell, G. L; Bennett, J. E.; Dolin, R., Eds.; Churchill Livingstone: Philadelphia, 2000; 5th Ed., Vol. 2, pp 1823–1848.
3. Meanwell, N. A.; Krystal, M. *Drug Disc. Today* **1996**, *1*, 316; **1996**, *1*, 388.
4. Carr, C. M.; Kim, P. S. *Science* **1994**, *266*, 234.
5. Luo, G.-X.; Colonno, R.; Krystal, M. *Virology* **1996**, *226*, 66.
6. Luo, G.-X.; 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. Cianci, C.; Yu, K.-L.; Dischino, D. D.; Harte, W.; Deshpande, M.; Luo, G.-X.; Colonno, R. J.; Meanwell, N. A.; Krystal, M. *J. Virol.* **1999**, *73*, 1785.
8. Yu, K.-L.; Ruediger, E.; Luo, G.; Cianci, C.; Danetz, S.; Tiley, L.; Trehan, A. K.; Monkovic, I.; Pearce, B.; Martel, A.; Krystal, M.; Meanwell, N. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2177.
9. Combrink, K. D.; Gulgeze, H. B.; Yu, K.-L.; Pearce, B. C.; Trehan, A. K.; Wei, J.; Deshpande, M.; Krystal, M.; Torri, A.; Luo, G.; Cianci, C.; Danetz, S.; Tiley, L.; Meanwell, N. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1649.
10. Deshpande, M.; Wei, J.; Luo, G.-X.; Cianci, C.; Danetz, S.; Tiley, L.; Pearce, B. C.; Krystal, M.; Yu, K.-L.; Meanwell, N. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2393.
11. (a) Scheibye, S.; Pedersen, B. S.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 229. (b) Thomsen, I.; Clausen, K.; Scheibye, S.; Lawesson, S.-O. *Org. Synth.* **1984**, *62*, 158.
12. (a) Laurence, C.; Berthelot, M.; Le Questel, J.-Y.; El Ghomari, M. J. *J. Chem. Soc. Perkin Trans. 2* **1995**, 2075. (b) Min, B. K.; Lee, H.-J.; Choi, Y. S.; Park, J.; Yoon, C.-J.; Yu, J.-A. *J. Mol. Struct.* **1998**, *471*, 283. (c) Steiner, T. *Angew. Chem. Int. Ed* **2002**, *41*, 48.
13. (a) Fritz, J. E. Kaldor, S. W.; Kyle, J. A.; Munroe, J. E. WO 9741846 to Eli Lilly and Company. (b) Munroe, J. E.; Kyle, J. A.; Tang, J.; Sachs, R. S.; Kaldor, S. W.; Fritz, J. E. 218th ACS National Meeting, New Orleans, LA, Aug. 22–26, 1999; MEDI-278.
14. Plotch, S. J.; O'Hara, B.; Morin, J.; Palant, O.; LaRocque, J.; Bloom, J. D.; Lang, S. A.; Digrandi, M. J.; Bradley, M.; Nilakantan, R.; Gluzman, Y. *J. Virol.* **1999**, *73*, 140.
15. Yoshimoto, J.; Kakui, M.; Iwasaki, H.; Fujiwara, T.; Sugimoto, H.; Hattori, N. *Arch. Virol.* **1999**, *144*, 865.