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SYNTHESIS AND EVALUATION OF 1,4,5,6-TETRAHYDROPYRIDAZINE DERIVATIVES AS INFLUENZA NEURAMINIDASE INHIBITORS

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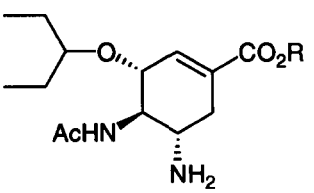
Abstract: 1,4,5,6-Tetrahydropyridazine derivative **15** and its C-5 epimer **19**, which possessed side chains similar to GS4071, were synthesized via a hetero Diels–Alder reaction, and evaluated as influenza neuraminidase inhibitors. Compounds **15** and **19** exhibited a μM range of influenza neuraminidase inhibitory activity.

Influenza infection is a serious health concern causing substantial morbidity and mortality. Current options for the treatment and prevention have severe limitations, underscoring the need for new, effective antiinfluenza agents. Influenza neuraminidase (NA) is one of the two major surface glycoproteins expressed by both influenza A and B viruses. NA catalyzes the cleavage of the terminal sialic acid residues attached to glycoproteins



1 R = H (GS4071)

2 R = Et (GS4104)

and glycolipids.[1] This process is believed to be necessary for the release of newly formed virus from infected cells and for efficient spread of virus in the respiratory tract.[2] Therefore, NA is recognized as a potential target for developing agents against influenza infection. Using structure-based drug design, GS4071 (**1**) was identified as a potent NA inhibitor.[3] GS4104 (**2**), the ethyl ester prodrug of GS4071, was found to be highly orally bioavailable in several animal species and efficacious in both mouse and ferret models of influenza infection by oral administration.[4] In clinical trials, oral efficacy of GS4104 has been demonstrated both in prophylaxis and treatment of human influenza infection.[5]

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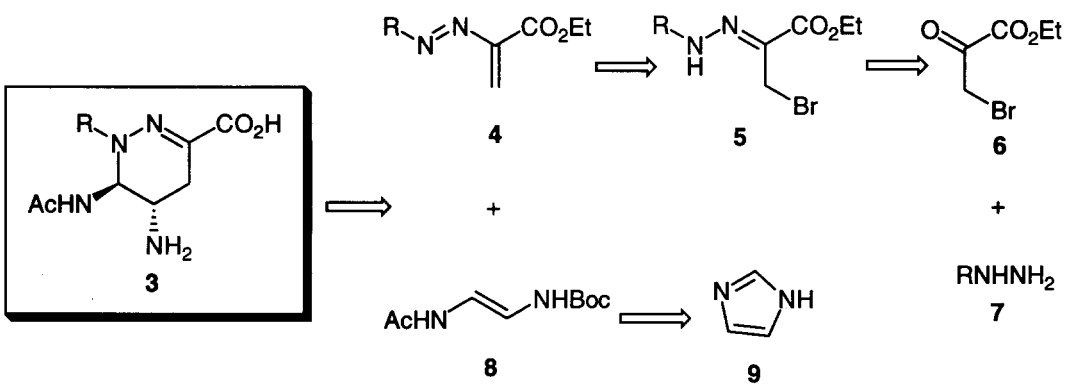
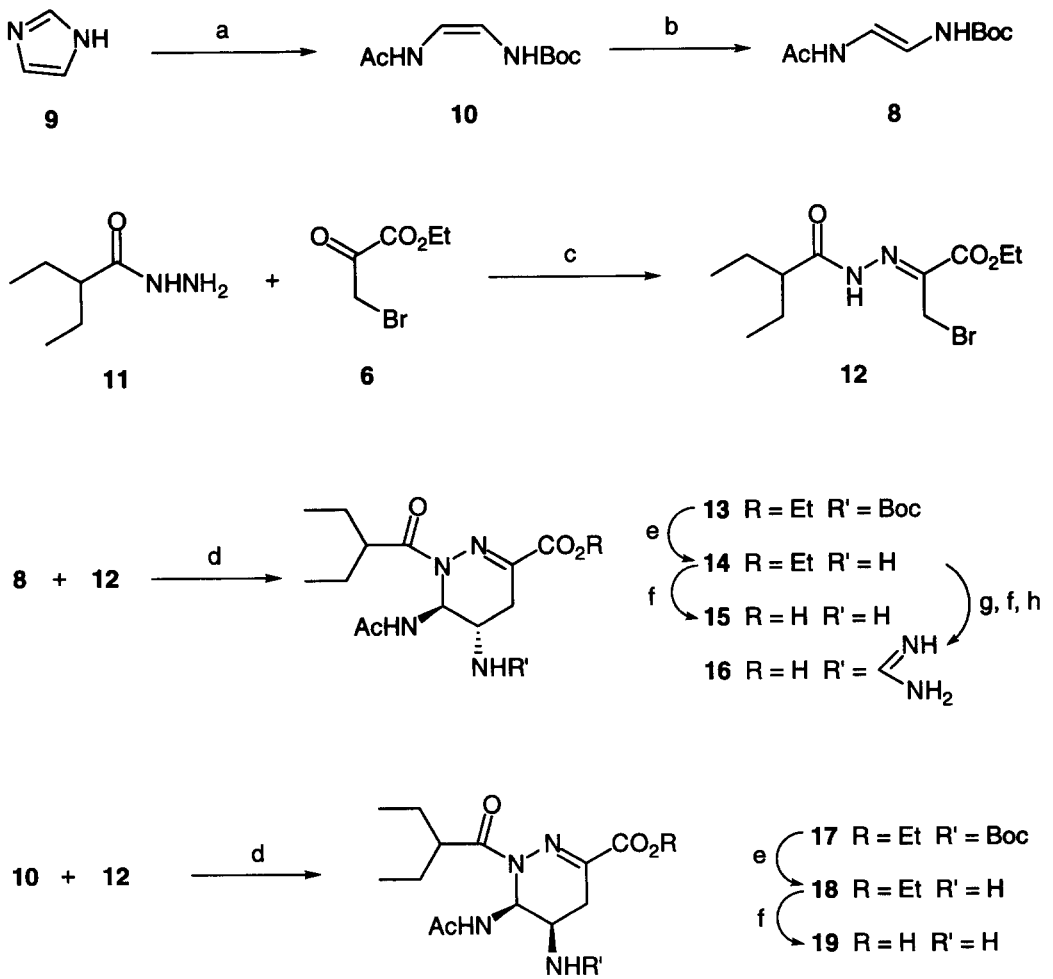
As a continuation of our influenza project, 1,4,5,6-tetrahydropyridazine derivatives **3** were targeted as a new series of potential influenza neuraminidase inhibitors. We envisioned that this highly functionalized ring system could be rapidly assembled via a hetero Diels–Alder reaction[6] of heterodiene **4** and alkene **8** (Scheme 1). The heterodiene **4** could be generated from hydrazone **5** which is readily available from ethyl bromopyruvate **6** and hydrazine **7**. The alkene **8** could be obtained from imidazole **9**.

Scheme 1



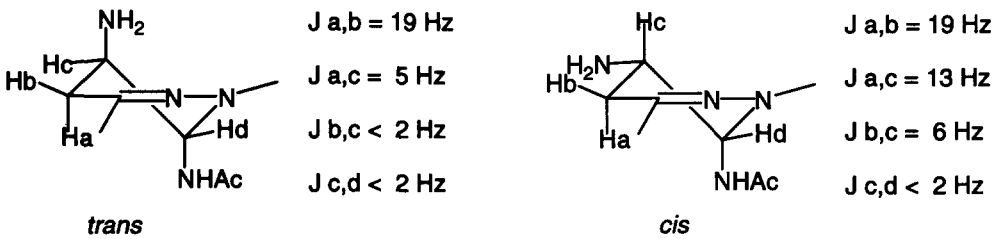
Thus, using a modified literature procedure,[7] imidazole **9** was treated with di-*tert*-butyl dicarbonate and acetic anhydride in ethyl acetate followed by sodium hydroxide in THF to give *cis*-alkene **10** (Scheme 2). Treatment of *cis*-isomer **10** with catalytic amount of iodine in THF afforded the *trans*-isomer **8**. Following a procedure similar to that found in the literature,[6a] hydrazine **11** was condensed with ethyl bromopyruvate **6** to give hydrazone **12**. In the presence of sodium carbonate, reaction of hydrazone **12** and alkene **8** in acetonitrile afforded a 1:3 mixture of desired cycloadduct **13** and its regio-isomer in 87% yield,[8] which could not be separated by chromatography. After removal of the Boc group, however, the desired amino compound **14** was separated from its regio-isomer by chromatography on silica gel. Saponification of **14** furnished the desired product **15**. The guanidino analog **16** was prepared from amino **14** in the same fashion we reported before.[9] The *cis* product **19** was obtained from the *cis*-alkene **10**. The stereochemistry assignment of **15** and **19** was based on NMR studies of **14** and **18**. In the ROESY NMR experiment (Figure 1), strong ROE between Ha and NHAc and no ROE between Ha and Hd were observed in both sample **14** and **18**. This result indicated that the NHAc group is in pseudo-axial and Hd in pseudo-equatorial position in both cases. This preference is presumably due to the anomeric effect. Therefore, compound **14**, which has smaller *J*a,c, is *trans*, and compound **18**, which has bigger *J*a,c, is *cis*.

Scheme 2[a]



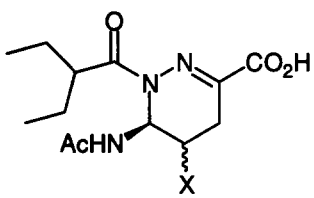
[a]Reagents: (a) Boc_2O, Et_3N (cat), CH_3CO_2Et, 0 °C-rt, 2h; Ac_2O, $NaHCO_3$, CH_3CO_2Et, 6h; NaOH, THF, 3h, 54%; (b) I_2 (cat.), THF, 15%; (c) AcOH (cat.), Et_2O, rt, 16h, 91%; (d) Na_2CO_3, CH_3CN, rt-50 °C /16h (87%); (e) HCl / CH_3CO_2Et, 94%; (f) KOH, CH_3OH, H_2O; Dowex (H^+), 98%; (g) BocNHCSNHBoc, $HgCl_2$, Et_3N. DMF, 96%; (h) CF_3CO_2H, CH_2Cl_2, 80%.

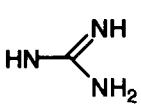
Figure 1.



The influenza neuraminidase inhibitory activity of compound **15**, **16** and **19** is shown in the Table. Compound **15**, which had similar side chain and stereochemistry (*trans*) to GS4071, exhibited IC_{50} of 6 μM against influenza A and 62 μM against influenza B. As expected, the *cis* isomer **19** shown much reduced inhibitory activity. Significantly increased activity was resulted from converting the amino group of **15** into the guanidino

Table. Influenza Neuraminidase Inhibitory Activity of tetrahydropyridazine derivatives[a]



Compounds	X	*cis* / *trans*	IC_{50} (μM) A / PR (H1N1)	IC_{50} (μM) B / Lee / 40
1			0.0014	0.0036
15	NH_2	*trans*	6	62
16		*trans*	0.14	22
19	NH_2	*cis*	160	> 1000

[a]Compounds were assayed according to the published procedure.[3]

functionality as shown in compound **16**. The relatively weak neuraminidase inhibitory activity of **15** compared to that of GS4071 (**1**) can be explained from NMR and X-ray crystallography studies. As mentioned above (Figure 1), conformational analysis by 2-D NMR studies reveal that the NH_2 and NHAc groups of **15** occupy the pseudo-axial positions, rather than the normally preferred pseudo-equatorial positions which are adopted on binding to influenza neuraminidase,[3] indicating an energy penalty is incurred upon the binding. In addition, the X-ray structure of **15** bound to neuraminidase reveals a different binding mode for the 3-pentyl side chain as compared with that of GS4071 (Figure 2). In the case of GS4071, the two ethyl groups of the 3-pentyl side chain bind in two different pockets, pocket 1 and pocket 2, respectively. However, presumably owing to the partly planar nature of the amide bond, the 3-pentyl side chain of **15** is forced to bind only in pocket 1, which is too small to accommodate this large group, resulting in a poor fit and exposure of the hydrophobic group to water. In addition, there is a significant loss in hydrophobic interactions with pocket 2.

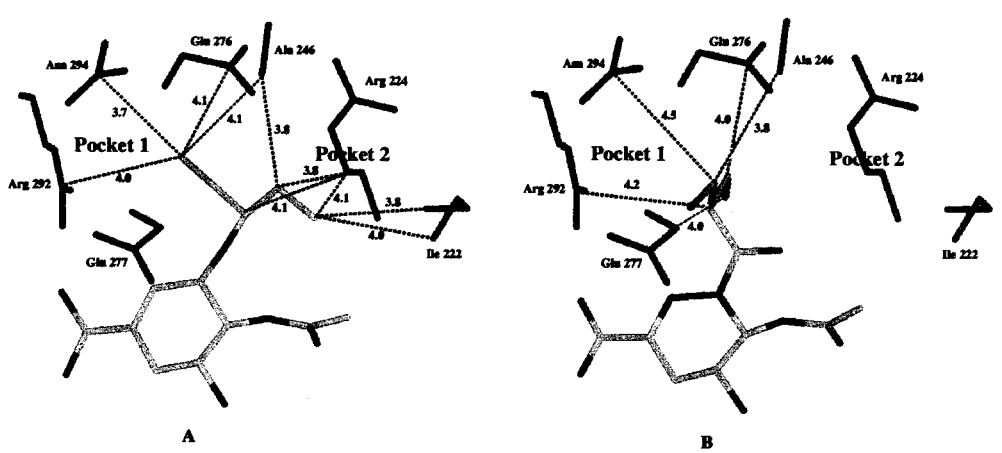


Figure 2. X-ray Crystal Structures of GS4071 (**1**) (A) and **15** (B) complexed with Type A neuraminidase. Only side chains of residues in pockets 1 and 2 are shown. Color is shown as red for oxygen, blue for nitrogen, green for neuraminidase carbon, brown for inhibitor carbon. Cyan indicates exposed carbon of **15**.

In conclusion, 1,4,5,6-tetrahydropyridazine derivatives **15**, **16**, and **19** were synthesized via a hetero Diels–Alder reaction, and evaluated as influenza neuraminidase inhibitors. A μM range of influenza neuraminidase inhibitory activity of these compounds was observed in the enzymatic assay. The low neuraminidase inhibitory activity of these compounds is consistent with the results from X-ray crystallography and NMR studies.

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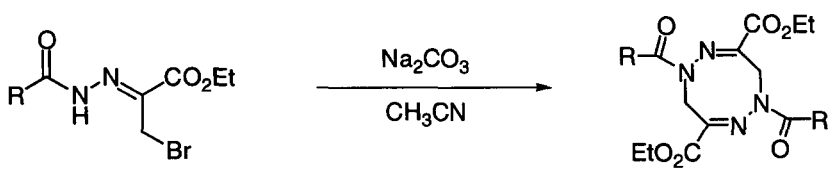
8. Four equivalents of hydrazone **12** were used in the reaction. The excess of hydrazone was converted into an 8-membered ring compound:



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