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Design, synthesis, and structural analysis of inhibitors of influenza neuraminidase containing a 2,3-disubstituted tetrahydrofuran-5-carboxylic acid core

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Abstract—(±)-(2*R*,3*R*,5*R*)-[2-(1′-*S*-Acetamido-3′-methyl)butyl-3-methoxycarbonyl]tetrahydrofuran-5-carboxylic acid (**9**) and (±)-(2*R*,3*R*,5*R*)-[2-(1′-*S*-acetamido-3′-methyl)butyl-3-(4′-imidazolyl)]tetrahydrofuran 5-carboxylic acid (**14**) were synthesized as inhibitors of influenza neuraminidase (NA). Both compounds **9** and **14** inhibit influenza NA A with an IC_{50} of about 0.5 μM and NA B with an IC_{50} of 1.0 μM.

Influenza neuraminidase (NA) has been proven as a valid therapeutic target for influenza viral infection due to its essential role in the viral replication cycle.[1–3] Two compounds, zanamavir (GG167) and oseltamivir (an ethyl ester pro-drug of GS4071), have been approved for treatment and prevention of influenza in the United States. Several other classes of potent NA inhibitors have been reported in recent years.[1]

A-315675 (**1**, Fig. 1) has been reported to be a highly potent inhibitor of both NA A and NA B, demonstrates potent anti-viral activity against various strains of influenza virus and has good oral bioavailability.[4,5] Compound **2** (Fig. 1) is a predecessor of A-315675 and represents an important milestone toward the eventual discovery of A-315675 (**1**).[6] In comparison with compound **3**, reported earlier from our laboratories,[7] the ligands of compound **2** projecting into each of the four main binding sites of the NA active site have been rearranged, and the positively charged $-NH_2$ group of **3**, typical as a S2 site ligand, is replaced with a neutral methyl ester moiety. This structural change has been

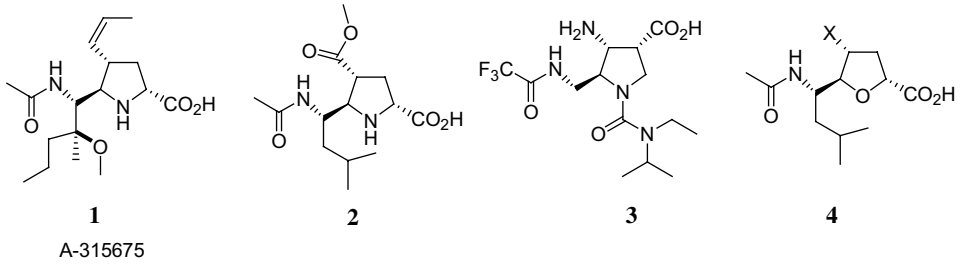

Figure 1.

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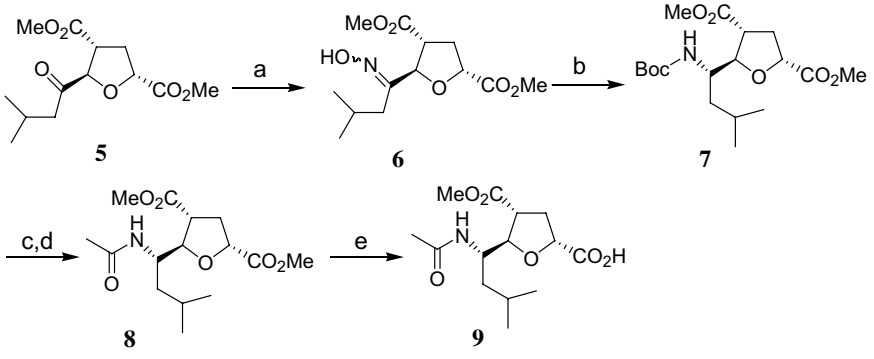
shown to improve the overall potency and render **2** and its analogs equally active against both NA A and NA B.

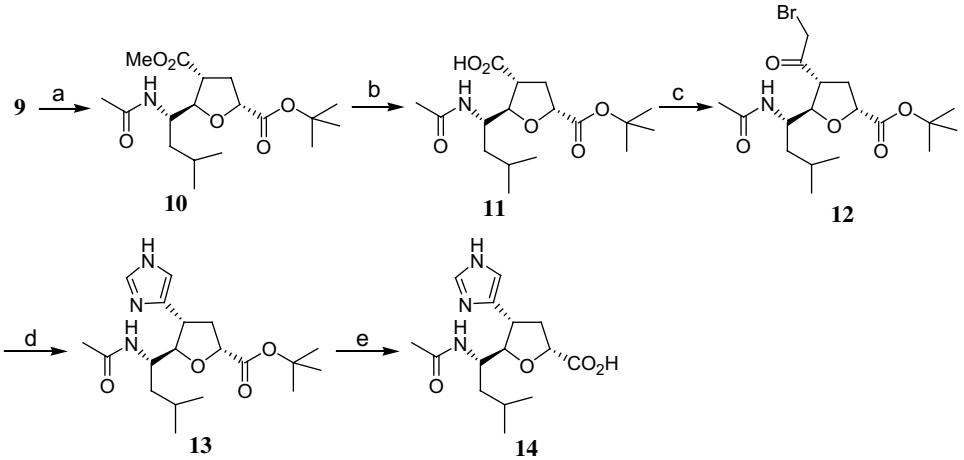
Compounds **1** and **2** contain the pyrrolidine core and are presumed to be zwitterionic, which could potentially render these molecules poorly orally bioavailable. Thus, we felt that it was important to assess the contribution of the ring nitrogen to binding of **1** or **2** with NA. Earlier studies of saccharide-based NA inhibitors indicated that weak inhibitory activity was observed for compounds possessing a tetrahydrofuran ring.[8] We therefore considered analogs of **2** in which the ring nitrogen was replaced by oxygen. In this paper, we report the synthesis and NA inhibitory activity of analogs of **2** containing the tetrahydrofuran core represented by structure **4** (Fig. 1).

We have previously described the synthesis of (±)-(2*R*,3*R*,5*R*)-tetrahydrofuran-3,5-dicarboxylic acid dimethyl ester **5**,[9] which served as a starting point of the synthesis of **4**. As shown in Scheme 1, the ketone moiety of **5** was converted to the corresponding oxime **6** (mixture of *cis*- and *trans*-isomers) in high yield in the presence of $(n\text{-Bu})_4NI$. Reduction of **6** by catalytic hydrogenation in the presence of Boc-anhydride gave two separatable Boc-amino derivatives (**7** and its diastereomer) in a ratio of 1:1.3. These two compounds were separately carried through the Boc-deprotection and acylation to give the dimethyl ester **8** and its diastereomer. Treatment of **8** with 1 equiv of LiOH at 0 °C selectively hydrolyzed the more base-labile 5-methyl ester, giving the desired mono-acid **9**.[10–13] The relative stereo-configuration of **9** was confirmed by single-crystal X-ray analysis (not shown) as well as the X-ray structure of **9** complexed to NA (vide infra). Similar treatment of the diastereomer of **8** gave the diasteromeric mono-acid, (±)-(2*R*,3*R*,5*R*)-[2-(1′-*R*-acetamido-3′-methyl)butyl-3-methoxycarbonyl]tetrahydrofuran-5-carboxylic acid, the structure of which was also confirmed by single-crystal X-ray analysis (not shown).

Since the 4-imidazolyl moiety has been shown to be a suitable ligand of the S2-binding site of NA active site in the pyrrolidine series of NA inhibitors,[4] we also decided to synthesize the analog of compound **9** in which the ester was replaced by an imidazole moiety. As shown in Scheme 2, the 5-carboxylate group of **9** was protected as the *tert*-butyl ester, which allowed the hydrolysis of the 3-carbomethoxyl group, giving compound **11**. The 3-carboxylate of **11** was converted into the bromomethyl ketone moiety via diazomethyl ketone,



Scheme 1. Reagents and conditions: (a) $H_2NOH{\cdot}HCl$, DIEA, MeOH, $(n\text{-Bu})_4NI$, 1 h, 90%; (b) H_2, Boc_2O, Raney-Ni, *iso*-propanol, 39.1%; (c) TFA, 2 h; (d) Ac_2O, DCM, 2 h, 100%; (e) LiOH (1 equiv), THF, 71%.



Scheme 2. Reagents and conditions: (a) TBTA, $BF_3{\cdot}Et_2O$; (b) LiOH, THF, 40% for two steps; (c) *i*-BuOCOCl, CH_2N_2, then HBr, 60.0%; (d) formamidine, NH_3, 45 °C, O.N., 20.0%; (e) 6 N HCl, 4 h, 95.8%.

giving compound **12**. Condensation of **12** with formamidine in liquid ammonia in a sealed tube gave the imidazole derivative **13**, which, upon deprotection, afforded the desired compound **14**.[14–18]

The NA inhibitory activities of compounds **9** and **14**, as well as **2**, are shown in Table 1. The IC_{50} values of compounds **9** and **14** against NA A (A/Tokyo/3/67) were 410 and 580 nM, respectively, about 10-fold less potent than the pyrrolidine analog **2**. Against NA B (B/Memphis/3/89), compounds **9** and **14** were about 20-fold less potent than **2** (IC_{50} 960 and 1000 nM, respectively).

To understand the reduced potency of **9** and **14** in comparison to **2**, an X-ray crystallographic structure of compound **9** complexed with NA A was obtained (Fig. 2).[19] Compound **9** binds to NA A as expected for this class of compounds.[6] Thus, the carboxylate interacts with the positively charged S1 site consisting of Arg292, Arg371, and Arg118. The carbomethoxyl group makes a hydrophobic and π-sacking interaction with the hydrophobic S2 site consisting of Glu119 and Leu 135, similar to compound **2**.[6] The side-chain acetamide interacts with the hydrophobic S3 site consisting of Trp178 and Ile222, and, finally, the side-chain *iso*-butyl group makes a hydrophobic interaction with the rotated Glu276. The overlay of the structures of compound **9** and compound **2** shows very similar binding mode for these two compounds (Fig. 2). The 10-fold decrease in potency in **9**, relative to **2**, must therefore be due to the most obvious difference between the two compounds: the ring oxygen in **9** replaced by the ring nitrogen in **2**. Since this ring heteroatom does not make a direct H-bond with the protein, the obvious difference between compounds **2** and **9** is the change in polarity that resulted from the nitrogen-to-oxygen switch. Polar factors, such as desolvation or electrostatic interactions, would be expected to be different for these two compounds, since compound **2** is expected to be zwitterionic under the assay condition (pH 7.5) with a net charge of zero in contrast to **9** with a net charge of −1. Our observations indicate that the net effect of these polar factors resulted in diminished binding for the tetrahydrofuran furan derivatives.

Table 1. Inhibition of influenza neuraminidase

Compound	IC_{50}, NA A/nM (A/Tokyo)	IC_{50}, NA B/nM (B/Memphis)
2	41	56
9	410	960
14	580	1000

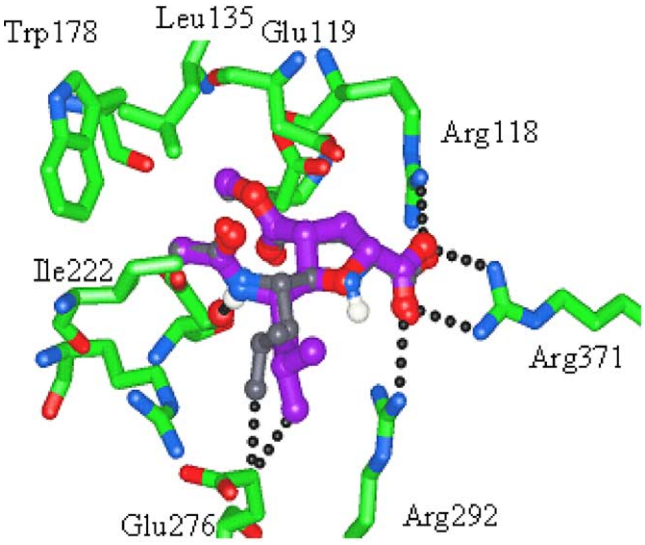


Figure 2. Overlay of the X-ray crystallographic structure of compound **2** (black) and **9** (purple) complexed to NA A.

In summary, we have synthesized two 2,3-disubstituted tetrahydrofuran-5-carboxylic acid derivatives as influenza neuraminidase inhibitors. These compounds have reduced inhibitory potency in comparison to the corresponding pyrrolidine analogs.

References and notes

1. Wang, G. T. *Expert Opin. Ther. Pat.* **2002**, *12*, 845.
2. Lew, W.; Chen, X.; Kim, C. U. *Curr. Med. Chem.* **2000**, *7*, 663.
3. Kim, C. U.; Chen, X.; Mendel, D. B. *Antiviral Chem. Chemother.* **1999**, *10*, 141.
4. Maring, C.; McDaniel, K.; Krueger, A.; Zhao, C.; Sun, M.; Madigan, D.; DeGoey, D.; Chen, H.-J.; Yeung, M. C.; Flosi, W.; Grampovnik, D.; Kati, W.; Klein, L.; Stewart, K.; Stoll, V.; Saldivar, A.; Montgomery, D.; Carrick, R.; Steffy, K.; Kempf, D.; Molla, A.; Kohlbrenner, W.; Kennedy, A.; Herrin, T.; Xu, Y.; Laver, W. G. Presented at 14th International Conference on Antiviral Research, *Antiviral Res.* **2001**, *50*, A76; Abstract 129.
5. 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.
6. Stoll, V.; Stewart, K. D.; Maring, C. J.; Muchmore, S.; Giranda, V.; Gu, Y-G.; Wang, G. T.; Chen, Y.; Sun, M.; Zhao, C.; Kennedy, A.; Madigan, D. L.; Xu, Y.; Saldivar, A.; Kati, W.; Laver, G.; Sowin, T.; Sham, H. L.; Greer, J.; Kempf, D. *Biochemistry* **2003**, *42*, 718.
7. Wang, G. T.; Chen, Y.; Wang, S.; Gentles, R.; Sowin, T.; Kati, W.; Muchmore, S.; Giranda, V.; Stewart, K.; Sham, H. L.; Kempf, D.; Laver, G. *J. Med. Chem.* **2001**, *44*, 1192.
8. THF-based NA inhibitors isomeric to sialic acid have been reported: Yamamoto, T.; Kumazawa, H.; Inami, K.; Teshima, T.; Shiba, T. *Tetrahedron Lett.* **1992**, *33*, 5791.
9. Wang, G. T.; Wang, S.; Chen, Y.; Gentles, R.; Sowin, T. *J. Org. Chem.* **2001**, *66*, 2052.
10. Preparation of **6**: A solution of compound **5** (0.3 g, 1.09 mmol), hydroxyamine hydrochloride (0.23 g, 3.3 mmol), DIEA (0.57 mL, 3.3 mmol), and *n*-Bu_4 NI in MeOH (10 mL) was stirred at 40 °C for 2 h. The solution was concentrated and purified directly on a 10-g silica gel cartridge, eluting with 30% EtOAc in hexane, giving **6** as a mixture of *cis* and *trans* oxime. 0.28 g, 90%. MS (DCI–NH_3): *m/z* 287 (M+H), 305 ($M+NH_4$). ^1H NMR ($CDCl_3$): 0.95–1.0 (m, 6H), 2.19 (m, 1H), 2.40–2.60 (m, 4H,), 3.45 (m, 1H), 3.65–3.80 (4s, 6H), 4.50–4.60 (br s, 1H), 4.70 (m, 1H), 5.0 (br s, 1H).
11. Preparation of **7**: A solution of oxime **6** (0.27 g, 0.94 mmol) and Boc_2O (2.11 mL, 10×) in isopropanol (70 mL) was hydrogenated with Raney-Ni at 4 atm for 15 h. After

concentration of the solution, separation on a silica gel column with 10% EtOAc in hexane gave **7** (137 mg, 39%) and 176 mg of diastereomeric dimethyl (±)-(2*R*,3*R*,5*R*)-{2-[1′-*R*-(butoxycarbonyl)amino-3′-methyl]butyl}tetrahydrofuran-3,5-dicarboxylate. MS (DCI–NH_3): *m*/*z* 374, 391, 317, 335. ^{1}H NMR ($CDCl_3$): 0.9–0.95 (2d, 6H), 1.22–1.31 (m, 1H), 1.49 (s, 9H), 1.50–1.55 (m, 1H), 1.62–1.75 (m, 1H), 2.40–2.60 (m, 2H), 2.95–3.10 (m, 1H), 3.70 (s, 3H), 3.75 (s, 3H), 4.20 (t, 1H), 4.36, 4.38 (br d, 1H), 4.60 (dd, 1H).

12. Preparation of **8**: A solution of **7** (40 mg, 0.11 mmol) in 2 mL of 50% TFA/DCM was stirred at rt for 1 h and then evaporated to dryness. The residue was dissolved in DCM (0.5 mL). Acetic anhydride (104 μL, 10.0 equiv) and DIEA (192 μL, 10.0 equiv) was added. After stirring for 2 h, the mixture was directly loaded on a 5-g silica gel cartridge and eluted with 60% EtOAc in hexane, giving a solid (34 mg, 100%). MS (DCI–NH_3): *m*/*z* 316 (M+H), 333 $(M+NH_4)^+$. ^{1}H NMR ($CDCl_3$): 0.9–0.95 (2d, 6H), 1.25–1.35 (m, 1H), 1.50–1.70 (m, 2H), 1.99 (s, 3H), 2.40–2.60 (m, 2H), 2.95–3.10 (m, 1H), 3.70 (s, 3H), 3.75 (s, 3H), 4.20–4.40 (m, 2H), 4.60 (dd, 1H), 5.30 (br d, 1H).
13. Preparation of **9**: To a solution of **8** (32 mg, 0.1 mmol) in THF (1.0 mL) cooled to 0 °C was added a solution of LiOH (0.1 M, 1.0 mL) slowly. The solution was then stirred for 15 min and quenched with HOAc (1.0 mL). After extracting with EtOAc (3 × 1 mL), the organic solution was dried ($MgSO_4$) and purified on a 5-g silica gel cartridge, eluting with 0–20% MeOH/EtOAc containing 5% HOAc. The product obtained was further purified by recrystallization from EtOAc and *iso*-propanol (22 mg, 71%). HRMS: $C_{14}H_{24}O_6N$, calcd: 302.1604, found: 302.1592. ^{1}H NMR ($CDCl_3$): 0.9–0.95 (2d, 6H), 1.25–1.35 (m, 1H), 1.50–1.70 (m, 2H), 1.99 (s, 3H), 2.40–2.50 (m, 1H), 2.60–2.70 (m, 1H), 2.95–3.10 (m, 1H), 3.70 (s, 3H), 4.10–4.40 (m, 2H), 4.60 (dd, 1H), 5.30 (br d, 1H). The relative geometry was confirmed by the X-ray structure of a single crystal of **9**.
14. Preparation of **10**: To a solution of **9** (25 mg, 0.08 mmol) in DCM (1.0 mL) was added a solution of *tert*-butyl 2,2,2-trichloroacetimidate (54 mg, 0.24 mmol) in cyclohexane (0.3 mL). After cooling to 0 °C, boron trifluoride etherate (three drops) was added. The mixture was stirred for another 40 min and quenched with 5% $NaHCO_3$ solution (2 mL). After diluting with DCM (10 mL), the mixture was filtered to remove insoluble by-product and the organic phase was further washed with 5% $NaHCO_3$ solution, dried, and concentrated. The residue was purified on a 5-g silica gel cartridge to give 26 mg of an oil, which was used directly for the next step.
15. Preparation of **11**: To a solution of **10** (26 mg, 0.073 mmol) in THF (1.5 mL), cooled to 0 °C, was added a solution of 0.1 N LiOH (0.73 mL). The mixture was allowed to warm up to ambient temperature over 90 min, then acidified to pH 2–3 with 1 N HCl and extracted with EtOAc. The combined EtOAc solution was dried and concentrated. The residue was purified on a 5-g silica gel cartridge to give a solid (10 mg, 40% for two steps). MS (DCI–NH_3): *m*/*z* 344 (M+H), 361 $(M+NH_4)$. ^{1}H NMR (MeOH-d_4): 0.87, 0.89 (d, 3H), 0.92, 0.94 (d, 3H), 1.35–1.45 (m, 1H), 1.47 (s, 9H), 1.50–1.70 (m, 2H), 1.92 (s, 3H), 2.30–2.37 (m, 1H), 2.45–2.55 (m, 1H), 2.90–2.97 (m, 1H), 4.00–4.07 (m, 1H), 4.20 (t, 1H), 4.45 (dd, 1H).
16. Preparation of **12**: To a solution of **11** (10 mg, 0.029 mmol) in THF (1.0 mL), cooled to 0 °C, was added *N*-methyl morpholine (13 μL, 4×) and *iso*-butyl chloroformate (15 μL, 0.12 mmol). The mixture was stirred at 0 °C for 1 h. A solution of diazomethane generated from Diazald (0.21 g, 1.0 mmol) and KOH (0.8 g) in 2 mL of Et_2O was then added via a syringe until the yellow color persisted. The solution was then stirred for 3 h, washed with brine (2 × 2.0 mL) and dried ($MgSO_4$). Evaporation of solvent gave a yellow solid, which was redissolved in dioxane (1.5 mL). To this solution was added 48% HBr (aq) solution (12.5 μL) and the mixture was stirred for 10 min. Saturated $NaHCO_3$ solution (0.5 mL) was then added slowly and the mixture was extracted with EtOAc (5 × 1.0 mL). The combined EtOAc solution was dried ($MgSO_4$), filtered, and concentrated. Chromatography on a 5-g silica gel cartridge eluting with 60% EtOAc–hexane gave pure **12** (7.3 mg, 60%), which was used directly for the next step.
17. Preparation of **13**: To a vial containing **12** (7 mg, 0.014 mmol) and formamidine acetate (30 mg, excess) was added liquid NH_3 (~2.0 mL). The vial was sealed and stirred at 45 °C overnight. Liquid NH_3 was then allowed to evaporate slowly. The residue was taken up in 5% Na_2CO_3 (aq 1.0 mL) and extracted with EtOAc (4 × 1.0 mL). The combined EtOAc solution was dried and concentrated. The residue was chromatographed on a 2-g silica gel cartridge eluting with EtOAc to give a solid (1.0 mg, 20%). MS (APCI+): *m*/*z* 366 (M+H), 310 ($M-C_4H_9$), base peak. ^{1}H NMR (MeOH-d_4): 0.87, 0.89 (d, 3H), 0.95, 0.97 (d, 3H), 1.27–1.37 (m, 1H), 1.47 (s, 9H), 1.42–1.67 (m, 2H), 1.84 (s, 3H), 2.10–2.17 (dt, 1H), 2.77–2.87 (dt, 1H), 3.50 (q, 1H), 4.02–4.06 (m, 1H), 4.16 (t, 1H), 4.55 (t, 1H), 7.34 (s, 1H), 8.64 (s, 1H).
18. Preparation of **14**: A solution of **13** (1.0 mg, 0.0027 mmol) in 1.0 mL of 6 N aqueous HCl was stirred for 1 h. The solution was then concentrated to dryness, leaving **14** as a white solid (0.8 mg, 96%). ^{1}H NMR (D_2O): 0.81, 0.0.83 (d, 3H), 0.87, 0.89 (d, 3H), 1.30–1.58 (m, 4H), 1.84 (s, 3H), 2.10–2.22 (m, 1H), 2.85–2.95 (m, 1H), 3.58 (q, 1H), 3.90–3.94 (m, 1H), 4.00–4.04 (m, 1H), 4.20 (t, 1H), 7.32 (s, 1H), 8.64 (s, 1H).
19. The X-ray coordinates for the structures of **2** and **9** complexed with NA A/Tokyo have been deposited into Brookhaven database. PDB accession numbers are PDB ID 1XOE and PDB ID 1XOG for **2** and **9**, respectively.