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Synthesis and evaluation of novel chloropyridazine derivatives as potent human rhinovirus (HRV) capsid-binding inhibitors

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ABSTRACT

Human rhinovirus (HRV) is the most important etiologic agent causing common colds. No effective anti-HRV agents are currently available. In this paper we describe the synthesis and the evaluation of novel chloropyridazine derivatives (compounds **5a**–**g**) as potent human rhinovirus (HRV) capsid-binding inhibitors. Results showed that compound **5e** and **5f** exhibited effective anti-HRV activity against HRV-2 and HRV-14. In addition, compound **5e** and **5f** showed lower cytotoxicity than Pirodavir.

1. Introduction

Human rhinovirus (HRV) is the most important etiologic agent causing common colds and belongs to the family of picornaviruses. Although HRV infections are generally self-limiting, they are also associated with several serious upper and lower respiratory tract complications such as otitis media, chronic bronchitis, and asthma.[1–3] No effective anti-HRV agent is currently available for the control of HRV and the large number of different serotypes (−105) makes the development of vaccines unlikely.[4,5]

During the past several decades, HRV capsid-binding inhibitors have been studied as promising anti-HRV agents.[4,5] HRV capsid-binding inhibitors block viral infection by inhibiting viral uncoating and/or viral attachment to cellular receptors on host cells. The binding site of capsid-binding inhibitors appears to be a hydrophobic pocket inside VP1 located under the canyon floor.[6–8] The most studied and advanced HRV capsid-binding inhibitors are Pleconaril and Pirodavir (Fig. 1). Pleconaril has been shown to shorten the duration of upper respiratory illness in two large Phase III clinical studies in adults, but unfortunately, the development of Pleconaril for the treatment of HRV was ceased due to safety and efficacy concerns.[9–11] Pirodavir could inhibit 80% of all HRV strains at a concentration of 64 ng/ml and it could decrease rhinovirus infection and reduce the virus shedding in clinical experiment.[12,13] According to the known research results, we chose Pirodavir as the lead compound and synthesized a series of its derivatives with the aim of gaining more effective HRV capsid-binding inhibitors with lower toxicity. In our previous work, we synthesized 70 compounds and these compounds were evaluated initially. Based on the initial evaluation results, we chose some of them for further study. In this paper we mainly describe several compounds that showed potent anti-HRV activity against HRV-2 and HRV-14 and their cytotoxicity.

2. Chemistry

The protocol for the synthesis of compound **5a–f** is shown in Scheme 1. The 4-chloro group of **1a–b** was substituted by **2a–b** in the presence of Na_2CO_3, yielding **3a–c**, then the intermediates **3a–c** were coupled with **4a–f,** which were chosen from different 5-substituted pyridin-2-ol or 4-substituted phenol, yielding the compound **5a–f** by Mitsunobu reaction. Compound **5g** was synthesized via the route outlined in Scheme 2. The hydroxyl group of **3a** was converted to chloro group in the presence of $SOCl_2$ afforded the intermediate **6**. The intermediate **6** was reacted with **4g** in the presence of K_2CO_3 gave compound **5g**.

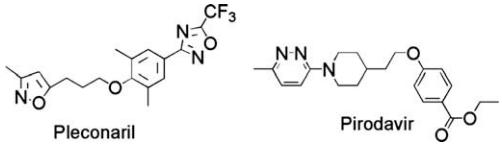

Figure 1. Structure of Pleconaril and Pirodavir.

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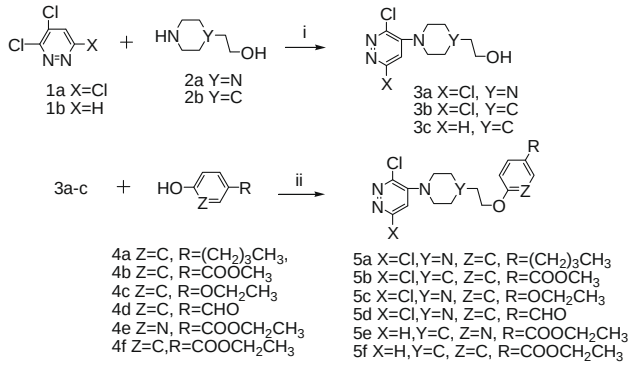
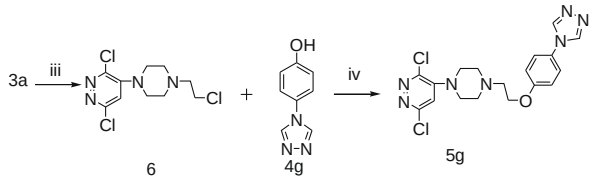


Scheme 1. Synthesis of compound **5a–f**. Reagents and conditions: (i) Na_2CO_3, DMA, rt; (ii) Ph_3P, DEAD, THF, rt.



Scheme 2. Synthesis of compound **5g**. Reagents and conditions: (iii) $SOCl_2$, CH_2Cl_2, reflux; (iv) K_2CO_3, DMF, 80 °C.

3. Biology

The anti-HRV activity and the cytotoxicity of the compounds **5a–g** on the two test strains HRV-2 and HRV-14 were evaluated by using cell culture cytopathic effect (CPE) assays. The procedure of the biological evaluation was previously reported,[12,14] and the results are listed in Tables 1 and 2.

4. Results and discussion

The anti-HRV-2 activity and the cytotoxicity of **5a–g** are shown in Table 1, the compounds **5a–d** have weak activity for inhibition of HRV-2 in both visual assay and neutral assay (EC_{50}: 0.31–56 and 0.1–46 μg/ml, respectively), and **5g** has no inhibition for HRV-2 (EC_{50}: >100 μg/ml). The compounds **5e** and **5f** have high potency for anti-HRV-12 (EC_{50}: <0.032 μg/ml) and lower cytotoxicity (CC_{50}: >100 μg/ml). Although **5e** and **5f** have 10-fold lower potency than Pirodavir, their cytotoxicity also 10-fold lower than Pirodavir, meaning that they have the same toxicity/antiviral activity selectivity number (CC_{50}/EC_{50}: 3125).

The anti-HRV-14 activity and the cytotoxicity of **5a–g** were shown in Table 2. The compounds **5a**, **5b**, **5d**, and **5g** have moderate activity for anti-HRV-14 (EC_{50}: 0.15–43 μg/ml). The **5c**, **5e**, and **5f** have higher potency of inhibition of HRV-14 than Pirodavir (EC_{50}: 0.036, 0.032, and 0.032–0.081 μg/ml, respectively) and lower cytotoxicity (CC_{50}: >100 μg/ml). Their toxicity/antiviral activity selectivity numbers are much higher than Pirodavir (CC_{50}/EC_{50}: 3125–123, respectively).

Pirodavir is a capsid-binding, anti-picornaviral agent against most HRV serotypes. The goal of the current study was to extend the structure–activity relationships by using Pirodavir as a lead compound. According to the biological evaluation results, if a 3,6-dichloropyridazine replaced the 6-methylpyridazine in Pirodavir, the derivative compounds **5a–d** and **5g** have lower or have no activity for inhibition of HRV-12 and HRV-14. The 3-chloropyridazine derivatives **5e** and **5f** have more anti-HRV activity and lower cytotoxicity. The **5e** and **5f** showed similar inhibition potency and cytotoxicity, suggesting that the change from phenyl ring to pyridine ring had little effect on the anti-HRV activity and cytotoxicity. The fact that compounds **5a**, **5c**, and **5d** exhibited obviously different activity indicated that the substituted group on the aryl ring played a most important role on the anti-HRV activity of the compounds.

In conclusion, a novel series of chloropyridazine derivatives were synthesized as potent human rhinovirus capsid-binding inhibitors. The anti-HRV activity of them was evaluated in vitro by using cell culture cytopathic effect assays. Among newly synthesized compounds, **5e** and **5f** exhibited excellent anti-HRV activity and low cytotoxicity that suggest they are very promising candidates for further development as anti-HRV agents.

5. Experimental

^{1}H NMR spectra are recorded on JNM-ECA-400 400 MHz instrument in the solvent indicated below. Chemical shift values are reported in parts per million (ppm) relative to those for tetramethylsilane used as an internal reference standard. Spectral splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Melting points were determined using a RY-1 apparatus and are uncorrected. Mass spectra were obtained from Micromass ZabSpec high-resolution magnetic mass spectrometer. HRESIMS spectra were recorded on Apex-Qe-FTMS instrument. Thin-layer chromatography (TLC) was carried out on silica gel GF/UV 254 and the chromatograms were performed on silica gel (200–300 mesh) visualized under UV light at 254 and 365 nm. All purchased starting materials and reagents were used without further purification or purified by standard methods prior to use. All moisture- and air-sensitive reactions and reagent transfers were carried out under dry nitrogen. The reported yields were for purified materials but were not optimized.

5.1. 2-(4-(3,6-Dichloropyridazin-4-yl) piperazin-1-yl) ethanol (3a)

To a stirred solution of **1a** (9.18 g, 0.05 mol) in *N*,*N*-dimethyl acetylamide (DMA, 20 ml) at room temperature was added Na_2CO_3 (5.30 g, 0.05 mol) followed by the solution of **2a** (6.54 g, 0.05 mol) in DMA (10 ml) dropwise. Ice water (100 ml) was added after stirring for 12 h and the reaction mixture was stirred for another 30 min. Compound 3a (10.20 g, 73.6%) was obtained by filtration as a white solid. Mp 139–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.88 (s, 1H), 3.71–3.68 (t, 2H, J = 5.0 Hz), 3.38–3.36 (t, 4H, J = 4.5 Hz), 2.76–2.73 (t, 4H, J = 4.8 Hz), 2.68–2.66 (t, 2H, J = 5.1 Hz); FAB-MS (m/z): 277.1 [M+1]; HRESIMS ($C_{10}H_{14}Cl_2N_4O$ + H): calcd 277.06174, found 277.06141.

5.2. 2-(1-(3,6-Dichloropyridazin-4-yl) piperidin-4-yl) ethanol (3b)

To a stirred solution of **1a** (9.18 g, 0.05 mol) in DMA (20 ml) at room temperature was added Na_2CO_3 (5.30 g, 0.05 mol) followed by the solution of **2b** (6.54 g, 0.05 mol) in DMA (10 ml) dropwise. Ice water (100 ml) was added after stirring for 12 h and the reaction mixture was extracted with CH_2Cl_2 (3× 50 ml). The combined organic phases were washed with brine (3× 50 ml), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel afforded **3b** (6.2 g, 44.9%) as a white solid. Mp 90–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.35 (s, 1H), 4.42–4.40 (t, 1H, J = 5.3 Hz), 3.73–3.70 (d, 2H, J = 12.6 Hz), 3.49–3.44 (q, 2H, J = 6.4 Hz), 2.87–2.81 (q, 2H,

Table 1
The anti-HRV activity and the cytotoxicity of **5a**–**g** on HRV-2

Compound	Visual assay			Neutral red assay		
	EC_{50} (μg/ml)	CC_{50} (μg/ml)	SI	EC_{50} (μg/ml)	CC_{50} (μg/ml)	SI
5a	5.7	10	1.8	>1.9*	>1.9	0
5b	0.31	30	97	0.1	5.8	58
5c	>35*	35	0	>12*	12	0
5d	>56*	56	0	46	50	1.1
5e	<0.032	>100	>3125	<0.032	>100	>3125
5f	<0.032	>100	>3125	<0.032	>100	>3125
5g	>100	>100	0	>42*	42	0
Pirodavir	<0.0032	>10	>3125	<0.0032	>10	>3125

SI (selectivity index) = CC_{50} (toxicity)/EC_{50} (antiviral activity).
* Cytopathic effect was indistinguishable from drug cytotoxicity.

Table 2
The anti-HRV activity and the cytotoxicity of **5a**–**g** on HRV-14

Compound	Visual assay			Neutral red assay		
	EC_{50} (μg/ml)	CC_{50} (μg/ml)	SI	EC_{50} (μg/ml)	CC_{50} (μg/ml)	SI
5a	0.15	18	120	0.55	1.9	3.5
5b	7.1	56	8	>4.7*	4.7	0
5c	0.036	35	972	<0.032	12	>370
5d	43	>100	>2	>43*	43	0
5e	<0.032	>100	>3125	<0.032	>100	>3125
5f	<0.032	>100	>3125	<0.032	>100	>3125
5g	12	35	3	15	39	2.5
Pirodavir	0.081	>10	>123	0.01	>10	>1000

SI (selectivity index) = CC_{50} (toxicity)/EC_{50} (antiviral activity).
* Cytopathic effect was indistinguishable from drug cytotoxicity.

J = 10.4 Hz), 1.78–1.75 (d, 3H, J = 12.4 Hz), 1.43–1.38 (q, 2H, J = 6.7 Hz), 1.27–1.24 (m, 2H); FAB-MS (m/z): 276.0 [M+1]; HRESIMS ($C_{11}H_{15}Cl_2N_3O$ + H): calcd 276.06649, found 276.06684.

5.3. 2-(1-(3-Chloropyridazin-4-yl) piperidin-4-yl) ethanol (3c)

The compound was prepared with a 45.6% yield according to the method for **3b** using **1b** and **2b**. Mp 79–80 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.48–7.46 (d, 1H, J = 9.8 Hz), 7.38–7.36 (d, 1H, J = 9.5 Hz), 4.39 (br s, 1H), 4.32–4.29 (d, 2H, J = 13.4 Hz), 3.48–3.45 (m, 2H), 2.91–2.89 (m, 2H), 1.74–1.69 (m, 3H), 1.39–1.35 (q, 2H, J = 6.4 Hz), 1.13–1.10 (m, 2H); FAB-MS (m/z): 242.0 [M+1]; HRESIMS ($C_{11}H_{16}ClN_3O$ + H): calcd 242.10547, found 242.10558.

5.4. 4-(4-(2-(4-Butylphenoxy) ethyl) piperazin-1-yl)-3,6-dichloro pyridazine (5a)

To a stirred solution of **3a** (0.56 g, 2.0 mmol), triphenylposphine (0.63 g, 2.4 mmol) and **4a** (0.36 g, 2.4 mmol) in anhydrous THF (15 ml) at 0 °C under nitrogen was added a solution of diethyl diazenedicarboxylate (DEAD, 0.45 ml, 2.4 mmol) in anhydrous THF (5 ml). The reaction mixture was warmed to room temperature and then stirred for 12 h. The reaction solvent was removed under reduced pressure and the resultant residue was dissolved in CH_2Cl_2 (200 ml). The organic layer was washed with water (100 ml), brine (100 ml), and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded crude product of **5a**. Column chromatography of the crude product on silica gel afforded **5a** (0.38 g, 46.6%) as a white solid. Mp 83–84 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.11–7.09 (d, 2H, J = 8.4 Hz), 6.85 (s, 1H), 6.84–6.82 (d, 2H, J = 8.4 Hz), 4.14–4.11 (t, 2H, J = 5.3 Hz), 3.37–3.35 (t, 4H, J = 4.5 Hz), 2.91–2.88 (t, 2H, J = 5.3 Hz), 2.80–2.78 (t, 4H, J = 4.5 Hz), 2.57–2.53 (t, 2H, J = 7.8 Hz), 1.58–1.53 (m, 2H), 1.37–1.33 (m, 2H), 0.94–0.90 (t, 3H, J = 7.3 Hz); EI-MS (m/z): 408.0 [M^+]; HRESIMS ($C_{20}H_{27}Cl_2N_4O$ + H): calcd 409.15564, found 409.15593.

5.5. Methyl 4-(2-(1-(3,6-dichloropyridazin-4-yl) piperidin-4-yl) ethoxy) benzoate (5b)

The compound was prepared with a 42.3% yield according to the method for **5a** using **3b** and **4b**. White solid; mp 116–117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.92–7.90 (d, 2H, J = 8.9 Hz), 7.36 (s, 1H), 7.07–7.05 (d, 2H, J = 8.7 Hz), 4.15–4.12 (t, 2H, J = 6.2 Hz), 3.81 (s, 3H), 3.75–3.72 (d, 2H, J = 12.3 Hz), 2.91–2.85 (m, 2H), 1.86–1.83 (d, 2H, J = 12.3 Hz), 1.75 (br s, 3H), 1.40–1.32 (m, 2H); EI-MS (m/z): 409.0 [M^+]; HRESIMS ($C_{19}H_{22}Cl_2N_3O_3$ + H): calcd 410.10327, found 410.10373.

5.6. 4-(4-(2-(4-Ethoxyphenoxy) ethyl) piperazin-1-yl)-3,6-dichloro pyridazine (5c)

The compound was prepared with a 54.5% yield according to the method for **5a** using **3a** and **4c**. White solid; mp 93–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.87–6.84 (m, 5H), 4.12–409 (t, 2H, J = 5.0 Hz), 4.01–3.96 (q, 2H, J = 7.0 Hz), 3.38 (br s, 4H), 2.90 (br s, 2H), 2.81 (br s, 4H), 1.41–1.38 (m, 3H); EI-MS (m/z): 396.1 [M^+]; HRESIMS ($C_{18}H_{23}Cl_2N_4O_2$ + H): calcd 397.11926, found 397.12011.

5.7. 4-(2-(4-(3,6-Dichloropyridazin-4-yl) piperazin-1-yl) ethoxy) benzaldehyde (5d)

The compound was prepared with a 52.5% yield according to the method for **5a** using **3a** and **4d**. White solid; mp 111–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.9 (s, 1H), 7.87–7.85 (d, 2H, J = 8.7 Hz), 7.04–7.02 (d, 2H, J = 8.7 Hz), 6.87 (s, 1H), 4.24 (br s, 2H), 3.38 (br s, 4H), 2.95 (br s, 2H), 2.81 (br s, 4H); EI-MS (m/z): 380.0 [M^+]; HRESIMS ($C_{17}H_{19}Cl_2N_4O_2$ + H): calcd 381.08796, found 381.08779.

5.8. Ethyl 6-(2-(1-(3-chloropyridazin-4-yl) piperidin-4-yl) ethoxy) pyridine-3-carboxylate (5e)

The compound was prepared with a 40.4% yield according to the method for **5a** using **3c** and **4e**. White solid; mp 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.82–8.81 (d, 1H, J = 2.3 Hz), 8.17–8.15 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.4Hz), 7.19–7.17 (d, 1H, J = 9.6 Hz), 6.93–6.91 (d, 1H, J = 9.6 Hz), 6.76–6.74 (d, 1H, J = 8.0 Hz), 4.4–4.43 (t, 4H, J = 6.4 Hz), 4.38–4.35 (q, 2H, J = 7.0 Hz), 3.00–2.94 (t, 2H, J = 12.6 Hz), 1.91–1.87 (d, 2H, J = 13.7 Hz), 1.79–1.76 (m, 4H), 1.41–1.37 (m, 4H); FAB-MS (m/z): 391.0 [M+1]; HRESIMS ($C_{19}H_{24}ClN_4O_3$ + H): calcd 391.15314, found 391.15401.

5.9. Ethyl 4-(2-(1-(3-chloropyridazin-4-yl) piperidin-4-yl) ethoxy) benzoate (5f)

The compound was prepared with a 42.3% yield according to the method for **5a** using **3c** and **4f**. White solid; mp 115–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.00–7.98 (m, 2H), 7.19–7.17 (d, 1H, J = 9.6 Hz), 6.93–6.91 (m, 3H), 4.38–4.33 (m, 4H), 4.09–4.07 (q, 2H, J = 6.4 Hz), 3.00–2.95 (t, 2H, J = 12.4 Hz), 1.87–1.78 (m, 5H), 1.40–1.37 (m, 5H); FAB-MS (m/z): 390.0 [M+1]; HRESIMS ($C_{20}H_{24}ClN_3O_3$ + H): calcd 390.15790, found 390.15801.

5.10. 3,6-Dichloro-4-(4-(2-chloroethyl) piperazin-1-yl) pyridazine (6)

To a stirred solution of $SOCl_2$ (1.49 g, 12.5 mmol) in anhydrous CH_2Cl_2 (10 ml) at room temperature was added **3a** (0.69 g, 2.5 mmol) in anhydrous CH_2Cl_2 (30 ml) dropwise. The reaction mixture was refluxed for 8 h. After cooling to room temperature, the reaction was quenched by addition of a $NaHCO_3$ aqueous solution (200 ml). The aqueous layer was extracted with CH_2Cl_2 (3× 100 ml). The combined organic phases were washed with brine (3× 70 ml), dried (Na_2SO_4), and concentrated under reduced pressure afforded **6** (0.55 g, 74.3%) as a pale yellow solid. Mp 84–85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.41 (s, 1H), 3.74–3.71 (t, 2H, J = 6.4 Hz), 3.33–3.31 (t, 4H, J = 4.8 Hz), 2.73–2.70 (t, 2H, J = 6.8 Hz), 2.62–2.61 (t, 4H, J = 4.8 Hz); FAB-MS (m/z): 276.0 [M+1]; HRESIMS ($C_{10}H_{13}Cl_3N_4$ + H): calcd 295.02786, found 295.02806.

5.11. 4-(4-(2-(4-(4*H*-1,2,4-Triazol-4-yl) phenoxy) ethyl) piperazin-1-yl)-3, 6-dichloropyridazine (5g)

A solution of **6** (0.59 g, 2.0 mmol), **4g** (041 g, 2.5 mmol), and K_2CO_3 (0.70 g, 5.0 mmol) in DMF (20 ml) was heated to 80 °C for 8 h. After cooling to room temperature, the reaction was quenched by addition of water (50 ml). The aqueous layer was extracted with CH_2Cl_2 (3× 50 ml). The combined organic phases were washed with brine (3× 30 ml), dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded crude product of **5g**. Column chromatography of the crude product on silica gel afforded **5g** (0.38 g, 45.6%) as a white solid. Mp 160–161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.19 (s, 1H), 8.20 (s, 1H), 7.77–7.75 (m, 2H), 7.41 (s, 1H), 7.15–7.13 (m, 2H), 4.19–4.17 (t, 2H, J = 5.6 Hz), 3.35 (br s, 4H), 2.80–2.78 (t, 2H, J = 5.6 Hz), 2.51 (br s, 4H); EI-MS (m/z): 419.2 [M^+]; HRESIMS ($C_{18}H_{19}Cl_2N_7O$ + H): calcd 420.11009, found 420.11149.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.11.061.

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