Original Article

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Synthesis and crystal structure of 6-fluoro-3-hydroxypyrazine-2-carboxamide

Fangyuan Shi[1], Zongtao Li[2], Lingjin Kong[2], Yuanchao Xie[1], Tao Zhang[2,*], Wenfang Xu[1,*]

[1] *Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Ji'nan, Shandong, China;*
[2] *Shandong Qidu Pharmaceutical Co., Ltd., Zibo, Shandong, China.*

Summary **As a RNA polymerase inhibitor, 6-fluoro-3-hydroxypyrazine-2-carboxamide commercially named favipiravir has been proved to have potent inhibitory activity against RNA viruses *in vitro* and *in vivo*. A four-step synthesis of the compound is described in this article, amidation, nitrification, reduction and fluorination with an overall yield of about 8%. In addition, we reported the crystal structure of the title compound. The molecule is almost planar and the intramolecular O−H···O hydrogen bond makes a 6-member ring. In the crystal, molecules are packing governed by both hydrogen bonds and stacking interactions.**

Keywords: Synthesis, crystal structure, hydrogen bond, π-π stacking interactions

1. Introduction

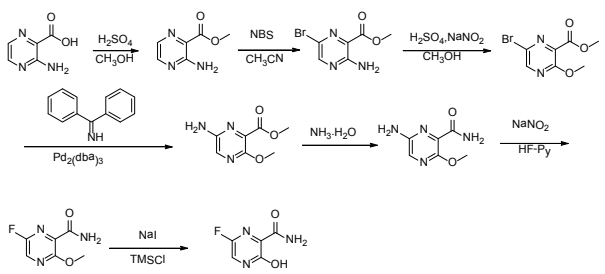
There is much interest in RNA polymerase inhibitors for their potential contributions in the treatment of the influenza (*1-3*). One inhibitor, 6-fluoro-3-hydroxypyrazine-2-carboxamide named favipiravir was first prepared by Y. Furuta and coworkers (*4*), and has been proved to have potent inhibitory activity against RNA viruses *in vitro* and *in vivo* (*5-8*). The previously reported synthetic procedure is shown in Scheme 1 involving seven steps starting from 3-aminopyrazine-2-carboxylic acid. However, in the ammoniated step, it involves the catalysis of tris(dibenzylideneacetone) dipalladium and (*S*)-(-)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP), which are very expensive. The last step proceeds poorly with a low (4.3%) isolated yield, making the overall yield approximately 0.44%. This sythesitc process is hardly suitable for large-scale production. Therefore, in order to improve the yield and reduce the cost, we report a modified procedure that boosts the overall yield over 20-fold with four steps in this article. We also report the crystal structure of the compund for the first time.

**Address correspondence to:*
Dr. Wenfang Xu, Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, Ji'nan, Shandong 250012, China.
E-mail: wenfxu@gmail.com

2. Materials and Methods

2.1. *Materials*

Solvent for anhydrous reaction should be processed before use. ^{1}H-NMR spectra were determined on a Brucker Avance 300 spectrometer or 600 using tetramethylsilane (TMS) as an internal standard. The solvents for NMR were DMSO-d^6 (δ 2.5 for ^{1}H), CD_3Cl_3 (δ 7.3 for ^{1}H). HRMS analysis was provided by Agilent 6520 Q-TOF LC/MS spectrometer (Agilent, Germany). All reactions were monitored by thin-layer chromatography (TLC) on 25.4 × 76.2 mm silica gel plates (GF-254). Silica gel used for column chromatography was 200~300 mesh. Melting points were determined on an electrothermal melting point apparatus and were uncorrected.



Scheme1. Synthesis of 6-fluoro-3-hydroxypyrazine-2-carboxamide reported in the reference.

2.2. *Synthesis*

2.2.1. *Preparation of 3-hydroxypyrazine-2-carboxamide*

To a suspension of 3-hydroxypyrazine-2-carboxylic acid (1.4 g, 10 mmol) in 150 mL MeOH, $SOCl_2$ was added dropwise at 40°C with magnetic stirring for 6 h resulting in a bright yellow solution. The reaction was then concentrated to dryness. The residue was dissolved in 50 mL 25% aqueous ammonia and stirred overnight to get a suspension. The precipitate was collected and dried. The solid yellow-brown crude product was recrystallization with 50 mL water to get the product as pale yellow crystals (1.1 g, 78%). mp = 263-265°C. ^{1}H-NMR (300 MHz, DMSO): δ 13.34 (brs, 1H, OH), 8.69 (s, 1H, pyrazine H), 7.93-8.11 (m, 3H, pyrazine H, $CONH_2$). HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_5H_6N_3O_2^+$: 140.0460; found: 140.0457.

2.2.2. *Preparation of 3-hydroxy-6-nitropyrazine-2-carboxamide*

In the solution of 3-hydroxypyrazine-2-carboxamide (1.0 g, 7 mmol) in 6 mL concentrate sulfuric acid under ice-cooling, potassium nitrate (1.4 g, 14 mmol) was added. After stirring at 40°C for 4 h, the reaction mixture was poured into 60 mL water. The product was collected by filtration as yellow solid (0.62 g, 48%). mp = 250-252°C. ^{1}H-NMR (600 MHz, DMSO): δ 12.00-15.00 (br, 1H, OH), 8.97 (s, 1H, pyrazine H), 8.32 (s, 1H, $CONH_2$), 8.06 (s, 1H, $CONH_2$). ^{13}C-NMR (75 MHz, DMSO): δ 163.12, 156.49, 142.47, 138.20, 133.81. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_5H_5N_4O_4^+$: 185.0311; found: 185.0304.

2.2.3. *Preparation of 6-amino-3-hydroxypyrazine-2-carboxamide*

3-Hydroxy-6-nitropyrazine-2-carboxamide (0.6 g, 3.3 mmol) and a catalytic amount of raney nickel were suspended in MeOH, then hydrazine hydrate was added dropwise. The resulting solution was refluxed 2 h, cooled, filtered with diatomite, and then MeOH is evaporated in vacuo to get the crude product as dark brown solid without further purification (0.4 g, 77%). HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_5H_7N_4O_2^+$: 155.0569; found:155.0509.

2.2.4. *Preparation of 6-fluoro-3-hydroxypyrazine-2-carboxamide*

To a solution of 6-amino-3-hydroxypyrazine-2-carboxamide (0.4 g, 2.6 mmol) in 3 mL 70% HF-pyridine aqueous at -20°C under nitrogen atmosphere, sodium nitrate (0.35 g, 5.2 mmol) was added. After stirring 20 min, the solution was warmed to room temperature for another one hour. Then 20 mL ethyl acetate/water (1:1) were added, after separation of the upper layer, the aqueous phase is extracted with four 20 mL portions of ethyl acetate. The combined extracts are dried with anhydrous magnesium sulfate and concentrated to dryness to get crude product as oil. The crude product was purified by chromatography column as white solid (0.12 g, 30%). mp = 178-180°C. ^{1}H-NMR (600 MHz, DMSO): δ 12.34 (brs, 1H, OH), 8.31 (d, 1H, pyrazine H, J = 8.0 Hz), 7.44 (s, 1H, $CONH_2$), 5.92 (s, 1H, $CONH_2$). ^{13}C-NMR (75 MHz, DMSO): δ 168.66, 159.69, 153.98, 150.76, 135.68. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_5H_5FN_3O_2^+$: 158.0366; found: 158.0360.

2.3. *Single X-ray crystallography*

6-Fluoro-3-hydroxypyrazine-2-carboxamide (0.2 g) was dissolved in methanol (50 mL) at room temperature. Colorless crystals of this compound were obtained through slow evaporation after two weeks. A colorless single crystal with dimension 0.37 × 0.29 × 0.27 mm^3 was selected for indexing and data collection at 150 Kon aNonius-based Kappa Bruker diffractrometer equipped with a charge-coupled de vice (CCD) area detector and Mo Kα (k = 0.7107 Å) radiation. The structures were solved by direct methods using the program SHELXS-97 and refined by full-matrix least-square refinementon F2 using the program SHELXL-97. All H atoms were placed in geometrically calculated positions and refined using a riding model with C-H = 0.93 Å (for CH); 0.86 Å (for NH_2 groups) and 0.82 Å (for OH), their isotropic displacement parameters were set to 1.2 times (1.5 times for OH) the equivalent displacement parameter of their parent atoms. Crystal data: $C_5H_4FN_3O_2$, Mr = 157.11, orthorhombic, $Pna2_1$, Z = 4, a = 9.1106(8), b = 14.7619(14), c = 4.6910(4) Å, α = β = γ = 90º, V = 630.89 (10) $Å^3$, T = 296 K, F(000) = 320, u = 0.15 mm^{-1}, Dx = 1.654 Mg m^{-3}. 7031 reflections were corrected, 832 unique (R_{int} = 0.0195). R_1 = 0.0294, wR_2 = 0.0825. Further details of the crystallographic data can be found in the supporting information (CCDC deposition number 969968).

3. Results and Discussion

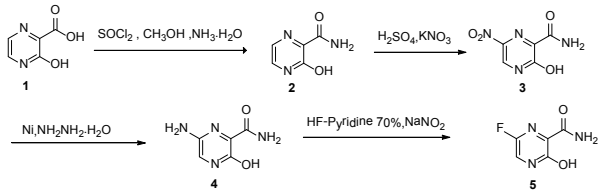
The low yield and high cost for the synthesis of favipiravir is attributed to the complicated process and expensive cytalyst in the ammoniated step. 3-Hydroxypyrazine-2-carboxylic acid (compound **1**) could be purchased or synthsized from 3-aminopyrazine-2-carboxylic acid (*9*), which is a common intermediate in orgainc synthesis. In our synthetic route, compound **1** was first esterified and amidated to give compound **2**, followed by nitration with potassium nitrate. During the reduction stept, we carried out several different methods. Hydrogen reduction proceeded poorly with a low (30%) yield and produced a large number of by-products. Reaction with zinc and

ammonia produced a large number of brown solid, which were difficult to purity and led to the failure of fluorination. The raney nickel went well with higher (77%) yield and fewer by-products. In the last fluorination step, it was found that about an hour after the reaction started, the yield decrease with reaction time. If the reaction time exceed 12 h, there would be no product existed. Overall, the target compound was prepared with a yield of about 8%, as shown in Scheme 2.

Slow evaporation of a solution of the title compound in methanol gave single crystals that were suitable for X-ray diffraction. It crystallized in the orthorhombic space group $Pna2_1$. In the title compound, $C_5H_4FN_3O_2$, the molecule is almost planar (r.m.s. deviation for the non-H atoms = 0.014 Å) and an intramolecular O–H···O hydrogen bond closes a 6-member ring (Figure 1), which can also prevent the keto-enol tautomerism of C3 position.

In the crystal, the molecules arrange in a prism structure, and are linked into chains by N–H···O hydrogen bonds and N–H···N hydrogen bonds (Figure 2). The characteristics of these bonds are given in Table 1. The molecules arrange in two nearly vertical (θ = 87.12°) orientations, forming a cavity structure. Each pyrazine ring is both a hydrogen bond donor and acceptor, and these interactions work cooperatively to lock the molecules together. All bond length and angles are in the normal range.

From the crystal structure we find that the neighboring pyrazine ring are nearly-parallel (θ = 2.81), with a vander Waals distance of about 3.23 Å. The arrangement of aromatic (π) systems predicts that between molecules, π-π stacking interactions exist. We note the structure also displays displacement with a distance of about 3.08 Å. As we all know, π-π electron interaction is an important force, which is roughly proportional to the area of π-overlap. Of course, displacement of π-systems can diminish the repulsion (*10*). These characteristics show that the crystal structure is packing governed by both hydrogen bonds and stacking interactions.



Scheme 2. Synthesis of 6-fluoro-3-hydroxypyrazine-2-carboxamide.

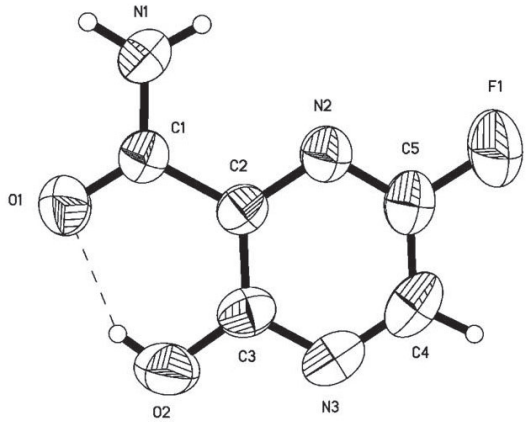


Figure 1. ORTEP plot of the 6-fluoro-3-hydroxypyrazine-2-carboxamide.

4. Conclusion

We have presented a new method for the synthesis of 6-fluoro-3-hydroxypyrazine-2-carboxamide, which is more effective and economical relative to the one previously reported. Upon crystallization, we find the molecule is almost planar and exist an intramolecular O–H···O hydrogen bond. Molecules are linked into chains by hydrogen bonds and the neighboring pyrazine ring display π-π stacking interactions (offset face-to-face). These arrangements show that the crystal structure is packing governed by both hydrogen bonds and stacking interactions, arranging in two nearly vertical orientations and forming a cavity structure.

Acknowledgements

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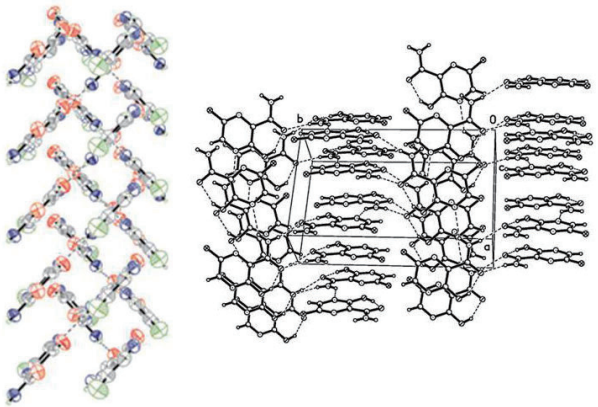

Figure 2. Crystal structure of 6-fluoro-3-hydroxypyrazine-2-carboxamide.

Table 1. Hydrogen bonds geometry (Å,°)

D–H···*A*	*D*–H	H–*A*	*D*–*A*	*D*–H···*A*
N1–H1B···N3^{i}	0.86	2.34	3.000 (2)	134
N1–H1*A*···O1^{ii}	0.86	2.06	2.9099 (18)	170
O2–H2···O1	0.82	1.88	2.591 (2)	144

Symmetry codes: (i) x−1/2, −y+1/2, z−1; (ii) −x+1, −y+1, z−1/2.

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