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An Efficient 4-Step Synthesis of Favipiravir with Industrial Potential

KIRAN KUMAR VUNNAM[1], NARESH KUMAR KATARI[1,*], SURYA PRAKASH RAO PYDIMARRY[2], VISHNU MURTHY MARISETTI[3] and RAMBABU GUNDLA[1]

[1]Department of Chemistry, GITAM School of Science, GITAM deemed to be University, Hyderabad-502329, India
[2]Research & Development, Hikma Pharmaceuticals USA Inc., Columbus, Ohio-43064, USA
[3]Research and Development, ScieGen Pharmaceuticals Inc., 89 Arkay Drive, Hauppauge, New York-11788, U.S.A.

*Corresponding author: E-mail: nkatari@gitam.edu; dr.n.k.katari@gmail.com

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An efficient, economical synthetic route is developed for the antiviral drug favipiravir using a four-steps instead of a six-step protocol. Starting with 6-bromo-3-hydroxypyrazine-2-carboxamide the method offers an overall 65% molar yield. The prime intermediate 3,6-difluoropyrazine-2-carbonitrile was synthesized by reacting 3,6-dichloropyrazine-2-carbonitrile with potassium fluoride using tetrabutylammonium bromide (TBAB) as a phase transfer catalyst in toluene and DMSO medium at reflux temperature (120 ºC). A simple purification method from fluoro intermediate was developed by making dicyclohexylamine salt and a neutral compound using hydrogen peroxide. The prepared pure favipiravir is fully characterized using NMR Mass and IR techniques. Validated analytical HPLC methods assessed the purity of the drug. The quality of the drug synthesized in terms of assay and impurities is well within the ICH and pharmacopeia standards.

Keywords: Favipiravir, 6-Bromo-3-hydroxypyrazine-2-carboxamide, 3,6-Difluoropyraine-2-carbonitrile, Potassium fluoride.

INTRODUCTION

Influenza is caused by one of the most virulent human pathogens and is the leading cause of severe respiratory disease in the elderly, pregnant women and infants with weakened immune systems [1,2]. The influenza virus is to be blamed for approximately 45 million human deaths worldwide (between 290,000 and 650,000 deaths per year) as well as significant economic losses [3-5].

Three classes of anti-influenza drugs are available for the treatment of flu diseases such as: (i) neuraminidase inhibitors (zanamivir, laninamivir octanoate, peramivir, oseltamivir) [6], (ii) M2 ion-channel blockers (amantadine and rimantadine) [7] and (iii) RNA polymerase inhibitor (favipiravir) [8-10]. The M2 ion-channel blockers and neuraminidase inhibitors are not recommended for long-term treatment of influenza due to several limitations in clinical practice, severe side effects and drug resistance [11-13]. However, new RNA polymerase inhibitors with novel mechanisms of action are being discovered and the existing drugs are in high demand. Favipiravir is one such selective and potent inhibitor of influenza viral RNA polymerase by chemical modification of a pyrazine analogue [8-10], which is chemically known as 6-fluoro-3-hydroxypyrazine-2-carboxamide. The drug was discovered, developed and manufactured by Toyama Chemical (a subsidiary of Fujifilm) in Japan [14] and approved in 2014 as an antiviral medication used for the treatment of the influenza virus under the brand name 'Avigan'.

The mechanism of the action of favipiravir differs from other influenza antiviral agents that inhibit viral genome replication. Favipiravir has shown antiviral activity as a pro-drug [15]. The ability of favipiravir to effectively inhibit all subtypes and strains of influenza viruses, including those sensitive to marketed neuraminidase and M2 inhibitors, endorses it as ideal drug against influenza. This drug substance was recently approved under emergency provisions in several countries, including India, China, Japan, Russia, Serbia, Turkey and Thailand, to treat COVID-19 pandemic disease. As a result, favipiravir consumption has increased rapidly (nearly 2.5 times) and researchers have focused on developing efficient and cost-effective routes for preparing favipiravir for global supply. In recent years, numerous synthetic routes for the preparation of favipiravir

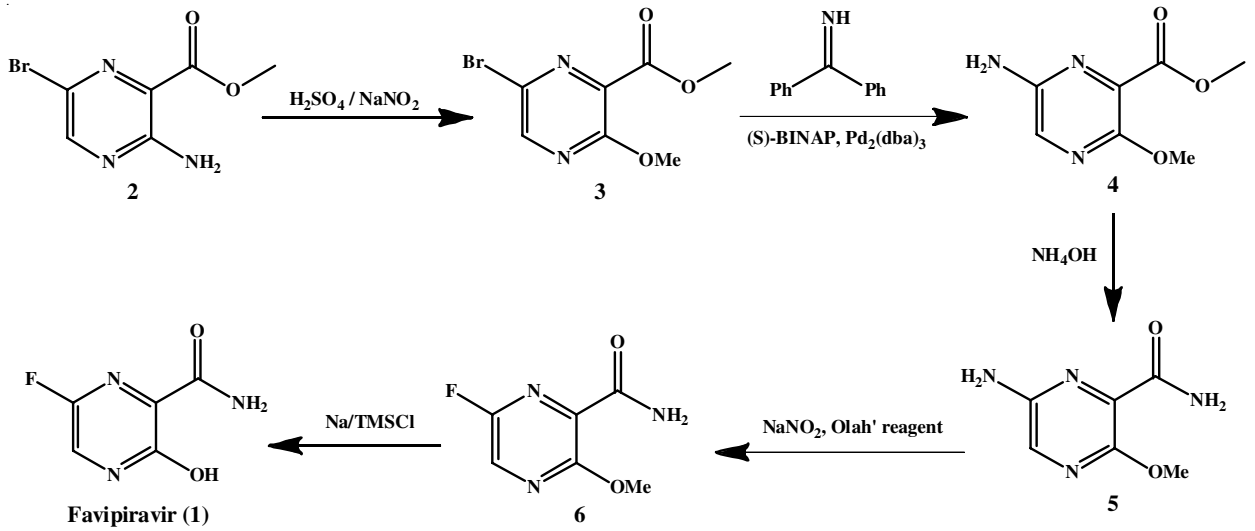
have been reported. In year 2000, Toyoma Chemical Company reported the first-generation synthetic method using methyl 3-amino-6-bromopyrazine-2-carboxylate (**2**) [14] as shown in **Scheme-I**.

The targeted compound was accomplished by (i) substituting hydroxyl group at amine through deamination with methoxylation using $NaNO_2$ followed by its hydrolysis, (ii) substitution of fluorine at bromine using chemical conversions like amination with expensive reagents *tris*(dibenzylideneacetone)dipalladium/benzophenone imine and then deamination with fluorine by treatment with $NaNO_2$/Olah reagent and then (iii) amidation of the methyl ester with aqueous ammonia. In addition to the poor yields in all stages, resulting in a meagre overall product (0.44%), the other deficiencies of the procedure are reproducibility, availability of starting material, expensive reagents and use of $NaNO_2$, a nitrosamine source.

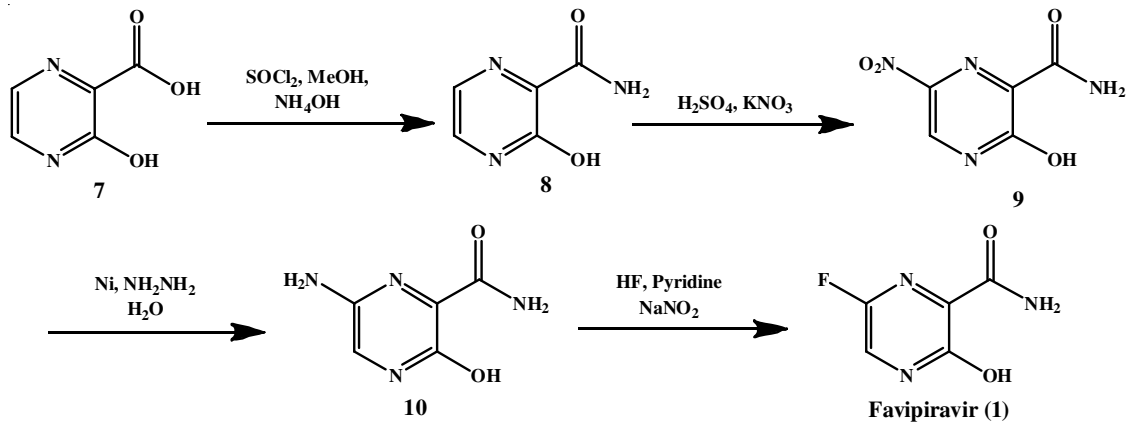
Shi *et al.* [16] reported a slightly improved synthetic route from 3-hydroxypyrazine-2-carboxylic acid, in which amidation of carboxylic acid was followed by nitrification, reduction and fluorination (**Scheme-II**) with an overall yield of about 8%. Lower yields were observed in the nitration (48%) and fluorination stages (30%) in this route (**Scheme-II**). Another improved process (overall yield 22%) was reported by Zhang *et al.* [17], from 3-amino-2-pyrazinecarboxylic acid by esterification, bromination, diazotization and then amination to accomplish precursor intermediate, 6-bromo-3-hydroxypyrazine-2-carboxamide. The process involves chlorination and then fluorine insertion, followed by a hydroxyl group (**Scheme-III**).

The main advantage of this process was that it used all commercially available raw materials and reagents and achieved moderate to good yields in all stages. Still, it has the disadvantage of several synthetic steps to achieve the desired product. Liu *et al.* [18] research group synthesized favipiravir in six steps, beginning with 2-aminopyrazine-2-carboxylic acid (**Scheme-IV**). Even though some of the conversions were efficient, there were some limitations regarding starting material availability, poor overall yields and more steps, making the route unsuitable for large-scale synthesis.

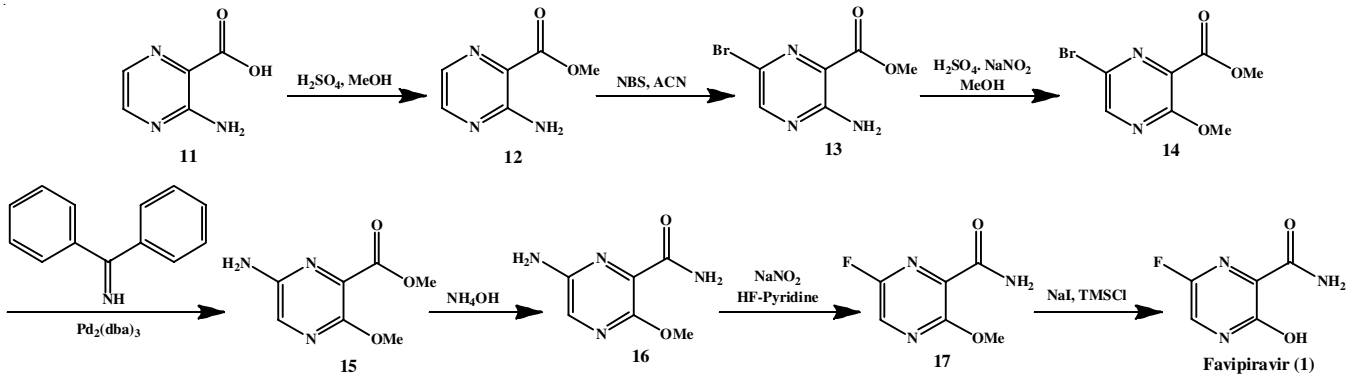
The literature search indicates that the existing synthetic routes do not meet the need for production of the favipiravir in high demand due to real-time synthesis issues. Therefore, there is a compulsion for an efficient and cost-effective drug process involving fewer steps. In current work, we focused on developing such process with additional features of robust, scalable and easy operations in the favipiravir preparation. The developed process achieves the desired level of quality by utilizing a commercially available key starting material, 6-bromo-3-hydroxypyrazine-2-carboxamide.



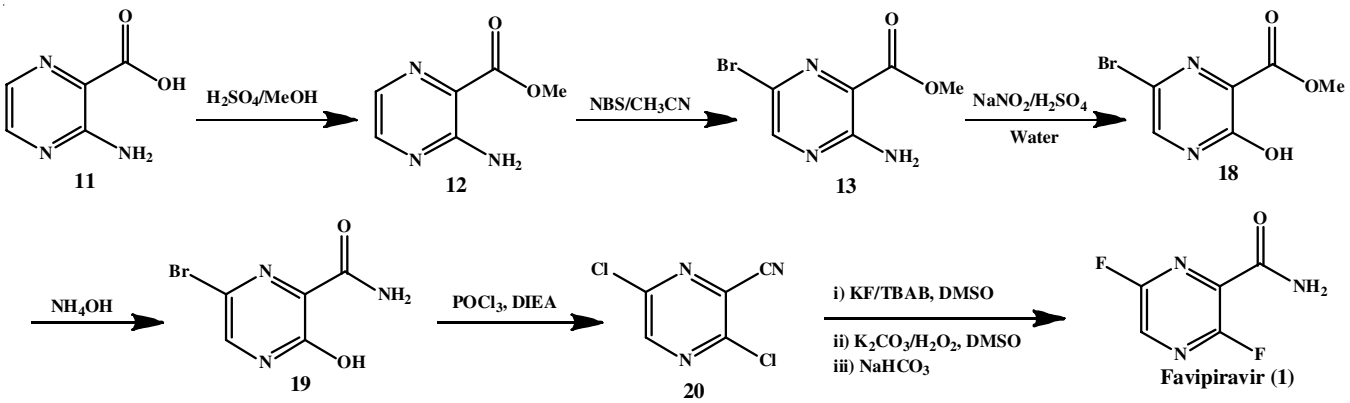
Scheme-I: Synthetic route developed by the innovator



Scheme-II: Improved synthetic route developed by Shi *et al.* [16]



Scheme-III: Alternative improved synthetic route developed by Zhang *et al.*



Scheme-IV: Improved synthetic route developed by Liu *et al.* [18]

EXPERIMENTAL

Chemicals and reagents phosphorous oxychloride, sodium carbonate, sodium bicarbonate, sodium chloride, triethyl amine, potassium fluoride, potassium acetate, tetrabutylammonium bromide, dicyclohexylamine and hydrogen peroxide were procured from LR grade (Sigma Aldrich & S.D. fine chemicals). Solvents, toluene, dimethyl sulfoxide, acetic acid, acetone and ethyl acetate were procured from Spectrochem, India.

The melting point of the synthesized intermediates was recorded using calibrated open capillary apparatus and are uncorrected. The isolated drug substance was characterized using an NMR spectrometer (Ascend TM Bruker 400 instrument). The proton (^{1}H) NMR was performed at 400 MHz and ^{13}C NMR was performed at 100 MHz in DMSO-d_6 and tetramethylsilane (TMS) was used for both ^{1}H and ^{13}C NMR spectra as internal standards. The mass spectrophotometry with an electrospray ionization (ESI) source used to determine the molecular mass of the compounds. The infrared (IR) spectroscopy data was accomplished on a Shimadzu IR affinity FT-IR spectrophotometer by pressed pellet method using KBR disc. The progress of all reactions and the purity of crystallized intermediates and active pharmaceutical ingredient, favipiravir, were monitored by ultrahigh-performance liquid chromatography (Shimadzu Nexera X2) with an isocratic reverse-phase column (Zorbax RX C-8, 250 mm 94.6 mm, 10.0 µL) eluted with 0.1% orthophosphoric acid (aq.) and acetonitrile in 230 nm.

Synthesis of 3,6-dichloropyrazine-2-carbonitrile (14): To a 2 L vessel, toluene (400 mL) and $POCl_3$ (281.0 g, 1.83 mmol) were charged under nitrogen condition at 25-30 ºC. 6-Bromo-3-hydroxypyrazine-2-carboxamide (100 g, 0.458 mmol) was loaded into the vessel and the reaction mass was agitated for 15 min at ambient temperature. Triethylamine (139.5 g, 1.38 mmol) was slowly added to the reaction mass for about 1 h at below 50 ºC. The reaction mass was warmed to 90-95 ºC and was agitated for 10 h. After completion of the reaction that HPLC monitored, the mixture was cooled to 40-45 ºC and slowly added to the water (1000 mL in 3 L vessel) at 40-45 ºC and then aged for 30 min at the same temperature. The organic layer was separated and the aqueous layer was extracted with toluene (300 mL). The combined organic layer was washed with aqueous Na_2CO_3 (300 mL), brine solution (200 mL) and then water (200 mL). The solvent was removed under vacuum at below 50 ºC and the reaction mass was degassed for 2 h at 50 ºC to afford the product as brown colour liquid. Yield 86.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 113.2, 128.0, 146,6, 148.1, 149.1; ESI Mass: 175.02 (M+1).

Synthesis of 6-fluoro-3-hydroxypyrazine-2-carbonitrile dicyclohexylamine salt (16): To a 2 L vessel equipped with an azeotropic distillation system with N_2 blanketing, toluene (600 mL), KF (200 g, 3.44 mmol) and TBAB (44.2 g, 0.137 mmol) were charged. The reaction mass was warmed to reflux (110-115 ºC) and the water was removed azeotropically from the reaction mass. The water content of the reaction mass was checked by Karl-Fischer titration method and it complied with the desired limit of not more than 0.1% w/w. The reaction mass was cooled to 45-50 ºC and then 3,6-dichloropyrazine-2-carbo-

nitrile (100 g, 0.574 mmol) in toluene (200 mL) was charged into the reaction mass at 45-50 ºC. The reaction mass was heated to 55-60 ºC and agitated for 10 h. HPLC monitored the progress of the reaction. After completion of the reaction, it was cooled to ambient temperature, stirred for 30 min and the reaction mass was filtered by filtration to remove salts.

Charged the above-collected filtrate into the 2 L vessel and cooled it to 5 ºC. Acetic acid (58 g, 0.965 mmol) and then triethylamine (97.20 g, 0.960 mmol) were slowly added to the reaction mass at 5-10 ºC. The mass temperature was raised to 25-30 ºC and stirred for 2 h. After completion of the reaction that HPLC monitored, aq. NH_3 (5 mL), water (280 mL) and then activated carbon into the reaction mass and then it was agitated for 30 min at 25-30 ºC. The slurry mass was filtered through the Celite bed to remove activated carbon and then the residue bed was washed with water (120 mL). The combined filtrate mass was charged into a cleaned 3 L vessel and then acetone (280 mL), toluene (160 mL) and potassium acetate (50 g, 0.51 mmol) were added, followed by agitated the reaction mass for 2 h at 20-25 ºC. Charged dicyclohexylamine (94.0 g, 0.518 mmol) into the reaction mass after cooling it to 15-20 ºC. The reaction mass was warmed to ambient temperature, agitated for 3 h at 25-30 ºC and charged water (280 mL) into the reaction mass. The temperature of the reaction mass was decreased to 5-10 ºC and then agitated for 2 h. The slurry mass was filtered, the residue mass was washed with water (150 mL) followed by acetone (100 mL) and then the wet material was dried under vacuum at 45-50 ºC for 8 h to obtain 6-fluoro-3-hydroxy-pyrazine-2-carbonitrile dicyclohexylamine salt with optimum yield and desirable quality as white solid. Yield: 91.5%, quality (HPLC purity): 99.5%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.61 (d, 1H, 9 NH), 1.07-1.58 (m, 12H, 5,6,7H), 1.73-1.98 (m, 8H, 4, 8H), 3.09 (m, 2H, 3H), 8.48 (S, 1H, OH), 8.01-8.04 (d, 1H, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 106.6, 28.6, 51.2, 166.8, 118.8, 136.3, 146.5; ESI Mass: 183 (M+Na).

Synthesis of 6-fluoro-3-hydroxy pyrazine-2-carboxamide (12): Water (400 mL), sodium hydroxide (25 g, 0.625 mmol), toluene (200 mL) and 6-fluoro-3-hydroxypyrazine-2-carbo-nitrile dicyclohexylamine salt (**16**) (100 g, 0.312 mmol) were charged in to a 2 L vessel at 20-30 ºC. The reaction mass was agitated for 30 min at 25-30 ºC and the toluene layer was removed after settling the reaction mass for 30 min followed by washing the aqueous layer with toluene (150 mL). Hydrogen peroxide (70 g, 0.617 mmol) was added to the aqueous layer at 15-30 ºC and then the reaction mass was agitated for 3 h at 25-30 ºC. After completion of the reaction as monitored by HPLC and cooled to 5-10 ºC. The pH of mass was adjusted to 2-3 using aqueous dil. HCl. The slurry mass was agitated for 3 h at 5-10 ºC, the solid was separated by filtration and the residual solid was washed with water (150 mL) followed by the wet solid was dried for 45 min under vacuum.

Ethyl acetate (1500 mL) and the above obtained wet product were charged into a 3 L vessel, warmed the reaction mass to 55-60 ºC and then agitated for 2 h. The aqueous layer was separated from the reaction mass after settling for 30 min and then the organic layer was washed with brine solution (200 mL) followed by water (100 mL). Activated carbon (5 g) was added to the organic layer at 55-60 ºC, agitated for 45 min and then the mass was filtered through celite to remove carbon. The distillation recovered the solvent under vacuum at > 50 ºC and water was added to the residue. The slurry reaction mass was stirred for 3 h at 5-10 ºC, the solid was separated by filtration and the solid residual mass was washed with water (150 mL) followed by drying process under vacuum at 55-60 ºC for 15 h to attain favipiravir as a white solid with an excellent yield (yield: 85.6%) and quality (HPLC purity: 99.89%). IR spectra (cm^{-1}): 3353, 1113, 1183, 1603, 1734. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.50-8.52 (d, 2H), 8.70 (s, 1H), 13.30 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6):135.60, 136.0, 151.3, 159.9; ESI Mass: 158.11 (M+1).

RESULTS AND DISCUSSION

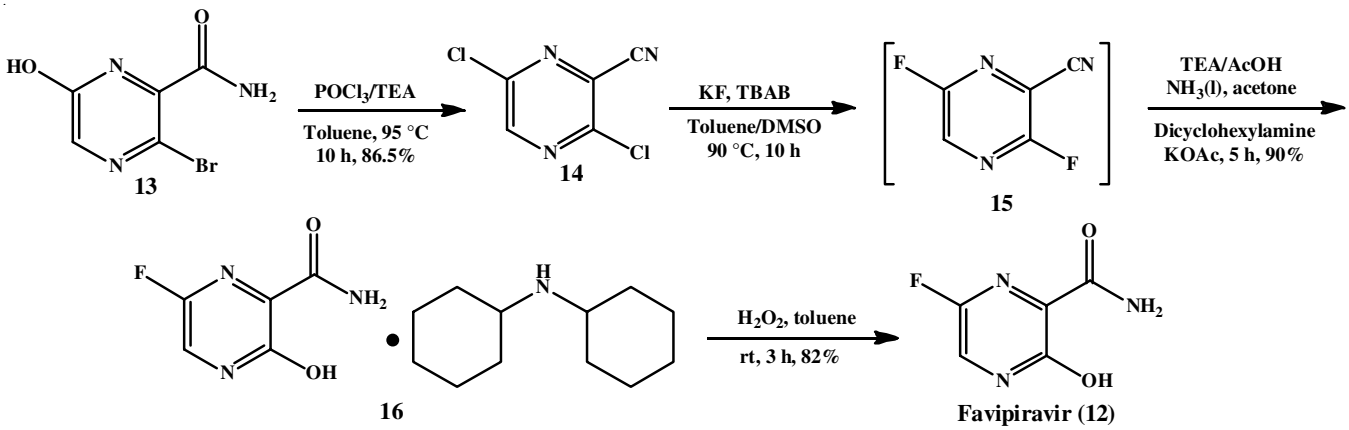
The synthesis started with 6-bromo-3-hydroxypyrazine-2-carboxamide (**13**) and after the initial stabilization experiments, it was found that using $POCl_3$ in toluene at 95 ºC gave 3,6-dichloropyrazine-2-carbonitrile (**14**) with 86.5% isolated yield. The chloro intermediate (**14**) was converted into intermediate 3,6-difluoropyrazine-2-carbonitrile (**15**). To achieve this conversion, TBAB in DMSO/toluene was tried at various experimental conditions to improve the yields and isolation without column chromatography (Table-1).

Most of these conditions offered poor yields due to the decomposition of fluoro intermediate. With a slight modification in the reaction conditions using a 1:6 ratio of TBAB *versus* potassium fluoride (KF) in DMSO/toluene solvent mixture at 90 ºC, the reaction gave excellent yields (~90%) in 10 h (Table-1, entry 4). Without further purification, intermediate (**15**) was successfully converted into its dicyclohexylamine salt (**16**) with excellent purity (> 99%) and good isolated yield. Dicyclohexyl-amine salt was finally converted into its pure form of favipiravir (**12**) using hydrogen peroxide in toluene and isolated in water at room temperature (**Scheme-V**).

It is noteworthy that all the existing protocols use sodium nitrite to convert the amine group to a hydroxy moiety. Due to the disadvantages associated with this method, notably the formation of nitroso impurities, it is imperative to avoid using sodium nitrite. Present approach achieved the synthesis without

TABLE-1
OPTIMIZATION OF 3,6-DIFLUOROPYRAINE-2-CARBONITRILE (**15**)

Entry	TBAB:KF	Solvent	Temperature (°C)	Reaction time (h)	Yield (%)
1	1:1	Toluene and DMSO	90	24	10
2	1:4	Toluene and DMSO	90	24	20
3	1:5	Toluene and DMSO	RT	24	No reaction
4	1:6	Toluene and DMSO	90	10	90



Scheme-V: Improved process for synthesis of favipiravir

using $NaNO_2$ and circumvents the formation of nitroso impurities in the process.

Conclusion

An efficient, commercially viable new synthetic route using inexpensive raw materials starting from 6-bromo-3-hydroxypyrazine-2-carboxamide (**13**) is developed. Further, an optimized chromatographic free method to isolate favipiravir in the form dicyclohexylamine salt (**16**). This stable intermediate was successfully converted into the target product with a 100% yield. The four-step synthesis offers an overall 65% molar yield. This protocol is ideal for large-scale production.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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