

# Improved Synthesis of the Anti-SARS-CoV-2 Investigational Agent (*E*)-*N*-(4-Cyanobenzylidene)-6-fluoro-3-hydroxypyrazine-2-carboxamide (Cyanorona-20)

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**Abstract:** Medicinal chemistry scientists' efforts and trials to discover a very potent anticoronaviral medicine specifically effective against the current frightening virus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), are not over yet. Synthetic organic chemistry will remain one of the most important branches in the entire drug discovery science. (*E*)-*N*-(4-Cyanobenzylidene)-6-fluoro-3-hydroxypyrazine-2-carboxamide (Cyanorona-20), a newly-discovered favipiravir analog/derivative, is one of the promising synthetic organic compounds that displayed very strong nanomolar potencies against this fatal coronavirus, reaching an anticoronaviral-2  $EC_{50}$  of nearly 450 nM or 0.45  $\mu$ M. This compound was found to act against the SARS-CoV-2 mainly through the powerful inhibition of the coronaviral RNA-dependent RNA polymerase (RdRp), via competitively occupying and locking this enzyme's major catalytic active site pocket (the suggested primary mechanism of action). Cyanorona-20 is still under progressive investigation as an attempt to continue developing it as a prospective remedy for the coronavirus disease 2019 (COVID-19). However, the previous literature synthetic procedures of Cyanorona-20 were criticized for several reasons like the harsh handling, difficult separation, small yield, and low purity. Herein in this short-communication or technical-note article, more reproducible and efficient novel synthetic method for Cyanorona-20 compound is presented, in an effort to address almost all of the problems which were accompanying the preceding methods.

**Keywords:** Anti-COVID-19 Medicine, SARS-CoV-2, Coronaviral-2 RNA-dependent RNA Polymerase (RdRp), Favipiravir, Drug Discovery, Cyanorona-20, Novel Synthesis, Optimized Synthetic Procedure, Monkeypox Virus (MPV) Inhibitor

## 1. Introduction

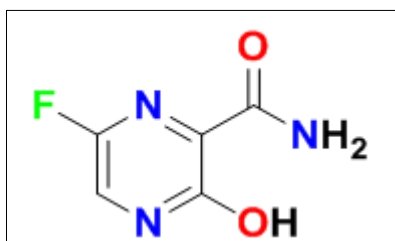
The spectacular coronavirus disease 2019 (COVID-19) with its causative extremely-pathogenic virus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), will remain one of the top dangerous microbial infections humans faced in this new century, the 21<sup>st</sup> century. The continuous efforts (which have started since about 33 months) of multinational pharmaceutical/drug companies and medical research institutions/centers to reach an effective anti-COVID-19 drug will never stop until finding such drug, which should be efficacious against all the different types of COVID-19 (including the severe/very severe cases). No specific anticoronaviral-2 therapy has been officially discovered and announced for the treatment of the severe and intensive-care cases of the COVID-19 disorder [1-3].

With the exception of molnupiravir and nirmatrelvir remedies for mild COVID-19 cases, there is not any medicine present on the shelves in pharmacies worldwide to successfully deactivate SARS-CoV-2 pathogenicity and cure COVID-19 in significantly acceptable degrees. In this scarcity of very potent and efficacious broad-scope anti-COVID-19 remedy, some scientists have proposed the repositioning of the old potent antiinfluenza/antiviral medicine favipiravir (Figure 1) to battle this newly-evolved RNA virus, coronavirus 2 [2, 4]. However, these favipiravir studies did not reach good or acceptable outcomes [4, 5]. In addition, some countries that produce favipiravir, e.g., Russia, China, and Japan, are still keeping many of its therapeutic details and appropriate relevant doses secret. Consequently, to overcome all of

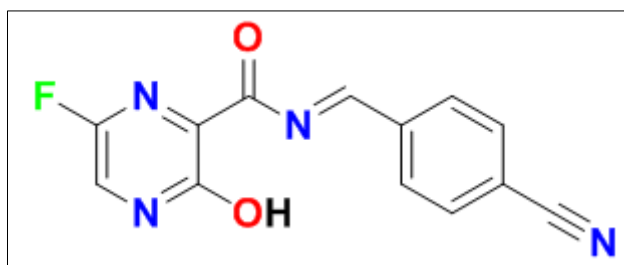
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these obstacles and more, a new derivative of favipiravir was theoretically planned, effectively synthesized, and successfully evaluated as a very potent anti-SARS-CoV-2 compound of the coronaviral-2 RNA-dependent RNA polymerase (RdRp) ligands/inhibitors class (Figure 2) [6, 7]. This new derivative or analog has very high potential to biologically act as a pseudonucleoside (i.e., a nucleoside alternative or nucleoside analog) for the viral genome and it demonstrated one of the lowest (best) anticoronaviral-2 EC<sub>50</sub> values known ever (0.45 μM) up to the publication of this present report. This novel favipiravir/nucleoside analog was generically named Cyanorona-20 to distinguish it from other similar favipiravir derivatives (Figure 2) [6, 7]. This drug is still a mystery because its inventor has kept some secrets of its ideal manufacture since 2020. That is why Cyanorona-20 clinical trials against COVID-19 are yet to start by multinational pharmaceutical companies until 100% making sure of the drug's valid synthesis to obtain a proper ultrapure powder of this compound (valid for clinical investigation in humans).

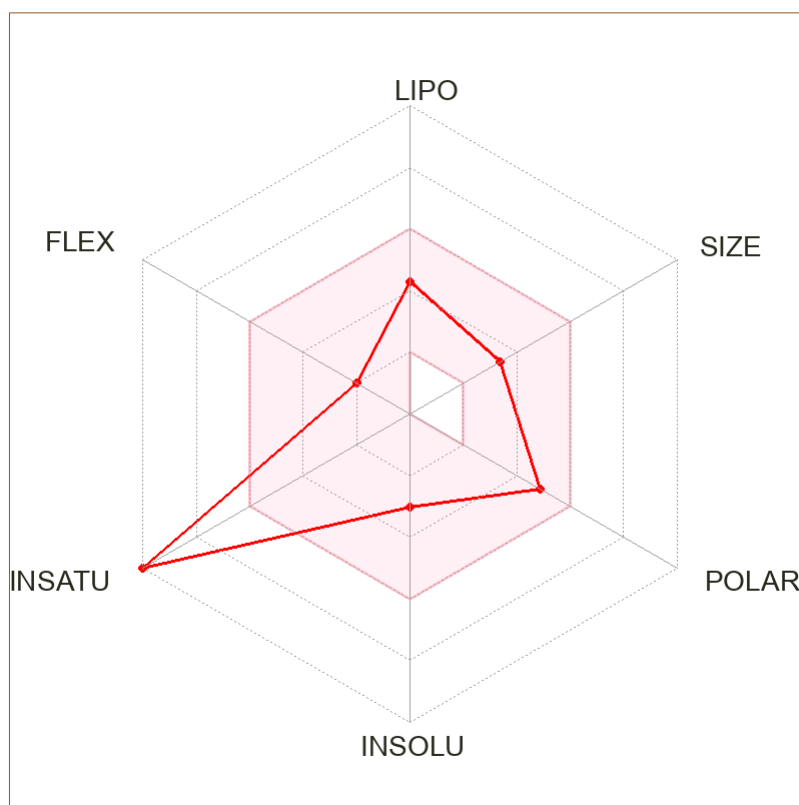


**Figure 1.** The molecular structure of the antiinfluenza drug favipiravir (IUPAC name: 6-fluoro-3-hydroxypyrazine-2-carboxamide)



**Figure 2.** The molecular structure of the modernly-designed targeted anti-SARS-CoV-2 agent Cyanorona-20 (preferred IUPAC name: (*E*)-*N*-(4-cyanobenzylidene)-6-fluoro-3-hydroxypyrazine-2-carboxamide; the compound can also be informally named, away from the predominant IUPAC rules, as a benzonitrile derivative)

From the chemical point of view, Cyanorona-20 as an ideally-designed molecule characterized by being comparatively very rich in heteroatoms, mainly nitrogen and oxygen atoms, since it has four nitrogens and two oxygens. Moreover, the abundances of both atoms, nitrogen and oxygen, in this molecule is in the ratio of 2:1 (respectively), which is, historically, one of the most favorable ratios of these two atoms together to give the most active and potent compounds in biological environments. Clinically, the Cyanorona-20 molecule is predicted to exhibit very good and temperate degrees of most *in vivo* pharmacokinetic characteristics (e.g., relatively low molecular size, favorable molecular flexibilities, highly required balanced lipophilic/hydrophilic properties, extremely excellent gastrointestinal (GI) absorption, convenient solubilities, and efficient diffusion/distribution into lungs; as presented in the physicochemical radar of Figure 3) [8] that considerably support the compound's druglikeness, pharmaceutical formulation, and clinical administration potentials (even more than the corresponding properties of its parent drug molecule, the favipiravir molecule).



**Figure 3.** The *in silico* physicochemical radar of the Cyanorona-20 molecule (LIPO = Lipophilicity, INSOLU = Insolubility, INSATU = Insaturation, and FLEX = Flexibility), generated using the validated SwissADME webserver

Synthesis of organic chemicals always begins with suggested theoretical ideas (mind proposals), written logic reaction pathways (good knowledge of relevant chemical reactions), and planned affordable and feasible synthetic procedures (full awareness of proper experimental instrumentation capabilities). Effective reproducible synthesis of any new organic chemical compound is not less important than its successful affirmative biological evaluation [9]. The previous synthesis of Cyanorona-20 in both its original procedure and the modified one faced several issues and troubles including the harsh handling/conditions, difficult separation, small yield, low purity, weak reproducibility in different environments, and other problems [6, 7]. Therefore, a completely new synthetic method was suggested, designed, performed, and assessed in the current work, aiming to try to solve some or even almost all of the hindering problems of the preceding synthetic steps of the promising anticoronaviral agent Cyanorona-20. The new preparatory scheme, experimental details, and brief discussion of the currently-suggested effective synthesis of Cyanorona-20 compound are presented in the next sections below.

## 2. Materials and methods

### 2.1. Materials and general considerations

The designed chemical reactions were carried out using almost commercially available reagents and solvents. All used chemicals, including 4-cyanobenzaldehyde, were purchased from Merck (through a local distributor of chemicals in our area), unless otherwise stated in the experimental procedure. Traditional thin-layer chromatography (TLC) was employed to keep an eye on the progress of reactions. It was performed on good-quality TLC silica gel 60 F<sub>254</sub> plates utilizing a preferred mixture of *n*-hexane/EtOAc/absolute EtOH (5:2:1, v/v/v) as the eluent (the emerged chromatogram spots were visualized and observed under the used UV light of 254 nm). Reaction mixture concentration and solvent removal purposes were properly performed in a rotavap apparatus under reduced pressure. Rough melting point (M.P., °C) of the solid powder of Cyanorona-20 was registered (as a range) in appropriate



pure open glass capillaries in the specialized *Fisher-Johns* melting point apparatus (a new edition of it is already available in our laboratory). IR spectrum of the proposed Cyanorona-20 sample was registered on *Nicolet™ iS™ 10* Mid-Infrared (Thermo Fisher Scientific) FT-IR spectrometer of high quality ( $\nu$  in  $\text{cm}^{-1}$ ) utilizing the relevant potassium bromide (KBr) disks (abbreviations used in the IR analysis results are as follows: aliph for aliphatic, arom for aromatic, br for broad, fluhetcyc for fluoroheterocyclic, phen for phenolic, and str for strong).  $^1\text{H}$ -NMR spectrum of the Cyanorona-20 sample was registered on *Varian Gemini-300* spectrometer (Mercury-300BB "NMR300") at 300 MHz employing the conventional standard tetramethylsilane ( $\text{Si}(\text{CH}_3)_4$ , abbreviated as TMS) as an internal standard, and its chemical shift values ( $\delta$ ) were expressed in ppm downfield from TMS at a temperature of about  $30^\circ\text{C}$ , utilizing  $\text{DMSO-}d_6$  as a solvent for the powder of the compound's sample. Similarly,  $^{13}\text{C}$ -NMR spectrum of the Cyanorona-20 sample was also registered on the same apparatus used for generating the  $^1\text{H}$ -NMR spectrum but at 75 MHz using also TMS as an internal standard, and its chemical shift values ( $\delta$ ) were expressed, as usual, in ppm downfield from TMS at a temperature of about  $30^\circ\text{C}$ , utilizing the same strong solvent,  $\text{DMSO-}d_6$ . Mass spectrometry (MS) analysis of Cyanorona-20 was performed on the available *Shimadzu Qp-2010 Plus* apparatus at 70 eV and the resultant outcomes were represented by  $m/z$  (relative intensity "rel. int." in %) as usual. In addition, elemental analyses (elem. anal.) of Cyanorona-20 were done (at a private pharmaceutical research center) so as to determine the content % of the C, H, and N atoms, which are the major atoms of the Cyanorona-20 molecule. All of the above-mentioned analytical and measurement procedures were carried out in temperate environmental conditions (i.e., under the requested standard reference conditions).

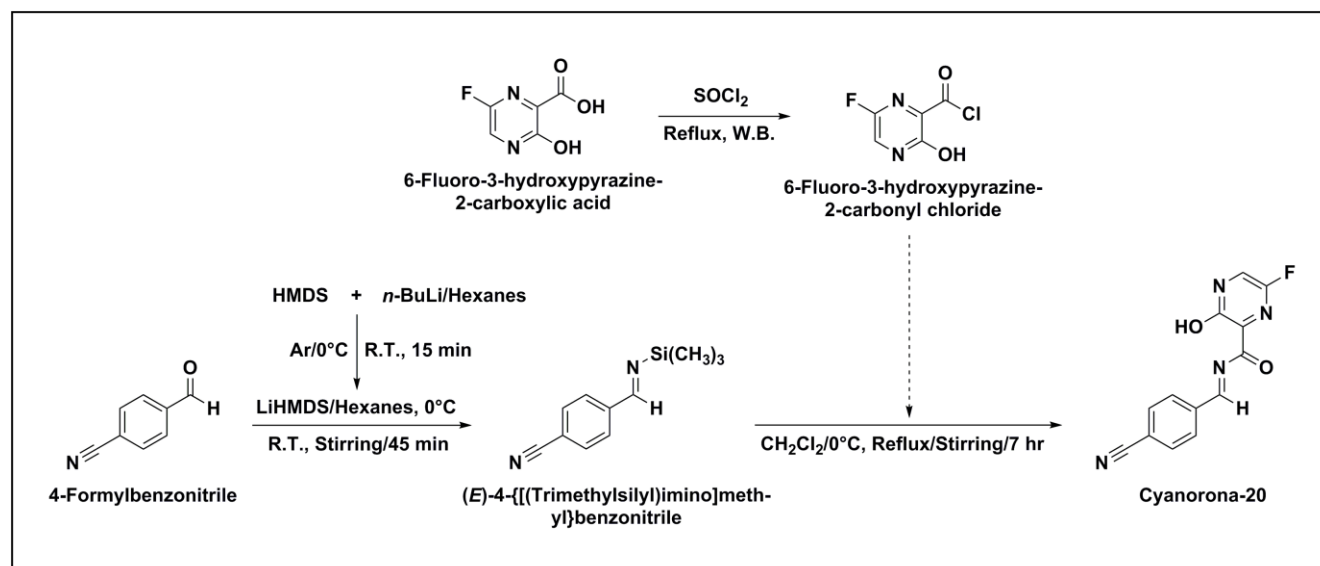
## 2.2. The newly-designed synthetic procedure for the preparation of Cyanorona-20

First, 1,1,1,3,3,3-hexamethyldisilazane "HMDS" (16 mL, 75.8 mmol) was added into a 100-mL flame-dried round bottom flask (equipped with stir bar) under argon (Ar) and cooled to  $0^\circ\text{C}$ . To which, *n*-BuLi (1.6 M in hexanes, 45.2 mL, 72.3 mmol) was added slowly using an air-tight syringe, and the reaction mixture was warmed to room temperature (R.T.) for 15 min. The resultant reaction solution was cooled to  $0^\circ\text{C}$  and 4-cyanobenzaldehyde "4-formylbenzotrile" powder (9.022 g, 68.8 mmol) was gradually and slowly added. The reaction mixture was again warmed to R.T. and stirred for 45 min. The remaining hexanes were adequately rotavapped under continuous reduced pressure, leaving a very concentrated pale yellow to buff solution/residue (which is supposed to be a very concentrated solution of the silyl imine intermediate (*E*)-4-[[trimethylsilyl]imino]methyl}benzotrile) which is directly dissolved in  $\text{CH}_2\text{Cl}_2$  (75 mL) and cooled to  $0^\circ\text{C}$ . To this solution, 6-fluoro-3-hydroxypyrazine-2-carbonyl chloride (11.53 g, 65.3 mmol; which was obtained ready for use, directly prior to performing the experiment, from a local chemicals distributor through custom synthesis from 6-fluoro-3-hydroxypyrazine-2-carboxylic acid, which was purchased firstly from Biosynth Carbosynth "Carbosynth Ltd., Berkshire, U.K.", CAS Registry Number: 1079990-21-8, Product Code: FF177937, Purity:  $\geq 98\%$ ) was added and the reaction mixture was refluxed with stirring for about 7 hr. Finally, the reaction mixture was cooled and the solvent  $\text{CH}_2\text{Cl}_2$  along with trimethylsilyl chloride (TMSCl) were removed in rotavap under reduced pressure to afford the target acyl imine Cyanorona-20 as crude yellow solid (15.35 g, 87% yield) after drying. Optionally, to get a highly pure form of Cyanorona-20, the previously-afforded crude solid was put on a filtration apparatus and fully washed with cold distilled water ( $3 \times 330$  mL), cold absolute EtOH ( $3 \times 280$  mL), and lastly with cold pure hexanes ( $3 \times 230$  mL), respectively. Finally, the washed Cyanorona-20 solid was again appropriately dried, then, additionally, extrapurified by being recrystallized one to two (preferred) times from a triple solvent mixture consisting of EtOH 75%/EtOAc/ $\text{CHCl}_3$  (200 mL:300 mL:400 mL, i.e., 2:3:4, v/v/v), and left for complete dryness to obtain the pure Cyanorona-20 (pale whitish to yellowish buff solid). For the detailed data of the physico-chemical properties, spectral analyses, and elemental measurements of this end product Cyanorona-20, see the Results and discussions section.

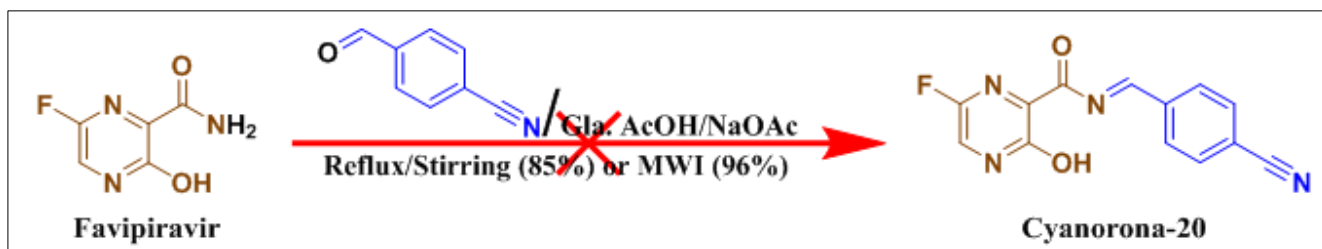
### 3. Results and discussions

The cyano-containing chemical ligand Cyanorona-20 was efficiently prepared, as presented in the schematic pathway of Figure 4, in good yield from a novel synthetic route other than the favipiravir pathway (the criticized method) which was previously suggested in the literature and hindered by some technical synthetic problems (Figure 5). The principal backbone of the present pathway consists of two major steps, a direct condensation reaction (at low temperatures) followed by a normal replacement (substitution) reaction. The reactions mostly proceed under mild temperatures and conditions. The chemical structure of the produced final product was assured and correlated with the proposed structure of Cyanorona-20 molecule *via* the several spectroscopic analyses (including IR,  $^1\text{H-NMR}/^{13}\text{C-NMR}$ , and MS analyses) along with the specific microanalyses (elem. anal. for the product sample content of the most predominant three atoms in the suggested Cyanorona-20 molecule, the C, H, and N atoms, separately). Spectral data and elemental estimations of this product were in nearly 100% agreement with the suggested molecular structure of Cyanorona-20 (i.e., the analytical spectra and measurements adequately assign to the drawn chemical structure of the Cyanorona-20 molecule).

The pure Cyanorona-20 compound was obtained as a pale whitish to yellowish beige solid (crude yield: 15.35 g, 87%). M.P.: 293-297°C (almost rough); FT-IR ( $\nu$  in  $\text{cm}^{-1}$ ): Str & br 3440 (O-H, arom), str 3010 (C-H, aliph), 2919 (C-H, arom), 2229 (C $\equiv$ N, nitrile), 1679 (C=N, aldimine), 1640 (C=O, amide), 1610 & str 1549 & 1499 & 1463 & 1359 (C=C, arom), str & br 1299 (C-F, fluhetcyc), 1270 (C-N, aliph), 1208 (C-O, phen);  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ ,  $\delta$  in ppm): 13.70 (s, 1H, 1 pyrazine phenolic OH), 9.59 (s, 1H, 1 2ry aldimine H), 8.09 (s, 1H, 1 pyrazine H), 7.89-7.71 (m, 4H, 4 benzylidenimine benzene H);  $^{13}\text{C-NMR}$  (75 MHz, DMSO- $d_6$ ,  $\delta$  in ppm): 167.40 (1C, 1 carbonyl C), 159.95 (1C, 1 pyrazine C-OH), 157.95 (1C, 1 2ry aldimine C), 153.50-151.95 (d,  $J = 124.8$  Hz, 1C, 1 pyrazine C-F), 150.40 (1C, 1 pyrazine  $\underline{\text{C}}\text{-C=O}$ ), 140.10 (1C, 1 benzene  $\underline{\text{C}}\text{-C=N}$ ), 136.40 (2C, 2 similar benzene C attached to C-C $\equiv$ N), 133.10 (1C, 1 unsubstituted pyrazine C), 129.25 (2C, 2 similar benzene C attached to C-C=N), 119.25 (1C, 1 nitrile C), 114.10 (1C, 1 benzene  $\underline{\text{C}}\text{-C}\equiv\text{N}$ ); GC-MS (EI) ( $m/z$ , rel. int. in %, molecular weight "M.Wt." = 270.22): 269.95 ( $\text{M}^+$ ); Elem. Anal. (% for  $\text{C}_{13}\text{H}_7\text{FN}_4\text{O}_2$ , *calcd* (*found*)): C: 57.78 (57.83), H: 2.61 (2.59), N: 20.73 (20.69).



**Figure 4.** A schematic representation of the newly-proposed multistep synthetic pathway (quadristep synthetic avenue) of Cyanorona-20



**Figure 5.** A schematic representation of the previously-proposed conventional and microwave monostep (one-step) synthetic/preparative pathways of the derivative chemical Cyanorona-20 from the precursory procompound favipiravir

Unlike favipiravir avenue which consists of only one slow reaction, the newly-suggested method is a multistep procedure which comprises a series of successive faster reactions. As obviously shown in the scheme of Figure 4, the current synthetic strategy starts with formation of the corresponding silyl imine intermediate from its parent aldehyde-nitrile compound, 4-formylbenzonitrile, using the reagent LiHMDS which is prepared *in situ* (preferably). Thereafter, this produced imine intermediate is reacted with the acid chloride (carbonyl chloride) derivative of 6-fluoro-3-hydroxypyrazine-2-carboxylic acid (the carboxylic acid moiety or scaffold in the favipiravir molecule) to afford our targeted compound Cyanorona-20. Replacing the known path with this novel one provides us with several merits, e.g., less time of contact with drastic reaction conditions, less net conventional reaction time (i.e., faster multistep reaction), easier separation, larger yield, higher purity, and more reliable reproducibility in diverse environments. Hence, more convenient results could be obtained through the presented synthesis proposal. Promisingly, this current work may reveal the secrets of Cyanorona-20 synthesis or, at least, represents an important step in paving the way for an optimal manufacture of this therapeutically-strategic product.

#### 4. Conclusions and anticipated improvements

Finding specific and highly efficacious remedy/remedies for the infections caused by the different strains of the hurtful SARS-CoV-2 virus is still the major duty of the relevant scientific communities in 2022. The discovery of the promising antipolymerase compound Cyanorona-20, which is selective against the viral polymerase, is just an attempt among hundreds of trials to find that anticoronaviral agent. This attempt to perfectly synthesize and continue developing this pyrazine or favipiravir derivative may be a hit or a miss in the end like any other similar drug development trial. Cyanorona-20 molecule is, simply, a straightforward structural extension of the parent favipiravir molecule. Previous efforts to prepare Cyanorona-20 were not good enough and were faced by some criticisms. One of the major roles of modern synthetic organic chemistry is to solve the problems that may arise with the traditional one-step reactions/procedures. This could be achieved through, for example, tactically bypassing the hindered direct monostep avenue (which starts with the parent compound) by applying a series of successive indirect and simpler steps beginning with a smaller/much smaller primary starting compound (this also enables the use of more readily available and cheaper starting materials). In this correcting mini-article, the synthetic/preparative method of the medicinally-important compound Cyanorona-20 was updated with a new multistep procedure to avoid most of the drawbacks which appeared in the previous old original method. Furthermore, slight modifications and/or optimizations can be planned and applied to the presented method of Cyanorona-20 synthesis in the near future to simplify it into just one-pot and green reaction (i.e., faster nonconventional one-step procedure), beginning with the same smaller starting molecule (6-fluoro-3-hydroxypyrazine-2-carboxylic acid) or even with much smaller one (e.g., pyrazine-2-carboxylic acid). Being closely similar in chemical structure with the monkeypox-virus (MPV) inhibitors, Tecovirimat, Brincidofovir, and Cidofovir (these three antiviral drugs act against the reemerged scary double-stranded DNA virus MPV mainly through hitting and inhibiting the major viral envelope protein "p37" and the virus-specific DNA polymerase,



respectively) [10], Cyanorona-20 is recommended to be tried against the MPV in the current 2022 MP outbreak. Finally, an important piece of advice to all organic chemists; failing to synthetically prepare a compound properly in the laboratory once, two or three times, or even more, does not mean we should stop trying to synthesize it at all.

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