Favipiravir tautomerism: a short theoretical report

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Abstract:

There is no experimental data about the tautomerism of Favipiravir (T-705). Therefore its tautomeric state was predicted by using density functional theory in gas phase and in solution (toluene, acetonitrile and water). The solvent effect was described by means of the Polarizable Continuum Model. The results have shown that the enol form is strongly dominating in both gas phase and solution. In order to validate the theoretical predictions, 2-hydroxy pyridine and 2-hydroxy pyrazine were also included in the set of studied compounds. The available experimental data for their tautomerism are in very good agreement with the theoretical predictions, which validate the conclusions made for T-705.

Keywords: T-705, Avigan, Favilavir, Favipiravir, COVID-19, tautomerism

Introduction:

According to the UIPAC definition[1], tautomerism is "Isomerism of the general form:

G-X-Y=Z $\rightleftarrows$ X=Y-Z-G

where the isomers (called *tautomers*) are readily interconvertible; the atoms connecting the groups X, Y, and Z are typically any of C, N, O, or S, and G is a group that becomes an electrofuge or nucleofuge during isomerization. The commonest case, when the electrofuge is H^+, is also known as *prototropy*."

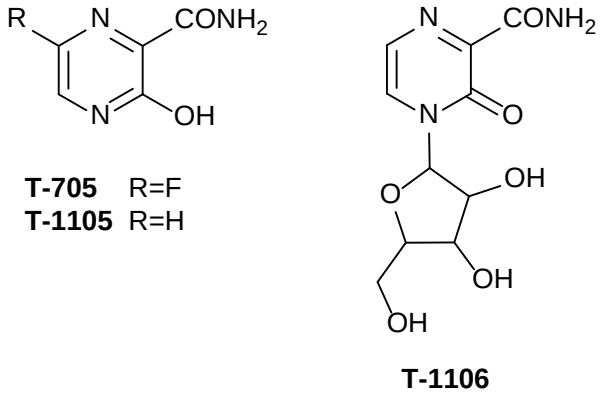
Although the prototropic tautomerism can occurs (really or potentially) in relatively limited number of molecules, it is one of the important phenomena in organic chemistry in respect of the properties and reactivity[2]. The interconvertivity is the major difference with the other types of isomers: enantiomers, or *cis* and *trans* isomers, for instance, also possess a formulaic identity just as tautomers do, but are difficult to interconvert, which allows physically to be isolated. Tautomers have chameleonic nature in most of the cases. They are able to switch from one, well known, structure to another following the changes in the local environment, and then to return back, when original conditions are restored. The corresponding transfer of the proton from one place of the molecule to another dramatically changes the electronic structure and, hence, variety the properties. As a result, the tautomeric forms differ in shape, functional groups, surface, and hydrogen-bonding pattern.

Biological activity is one of the properties that are heavily affected by the proton transfer. The vital importance of knowing the tautomeric state in various aspects the drug design has been underlined by many authors[3–7]. Martin[4] wrote recently that about 21% of molecules in various drug discovery databases are potentially tautomeric[8–10] and it is crucially important to know the exact tautomeric state in the different stages of the drug design.

Favipiravir (also known as T-705, Avigan, Favilavir, Scheme 1) is an antiviral drug[11–14], developed by FUJIFILM Toyama Chemical Co (http://fftc.fujifilm.co.jp/en/di/pipeline/index.html)[15], belongs to the pyrazine carboxamide family along with other experimental antiviral drugs (T-1105 and T-1106)[11]. According to the results of studies, T-705 revealed activity against influenza viruses, West Nile virus, yellow fever virus, foot-and-mouth disease virus as well as other flaviviruses, arenaviruses, bunyaviruses and alphaviruses[11,12]. Recently the same drug has shown promising results in the treatment of coronavirus disease 2019 (COVID-19) in China[16–21].

As seen from Scheme 1, **T-705**, as well as its analogues (**T-1105** and **T-1106**), belong to the potentially tautomeric 2-hydroxy pyrazine family. At that stage, it could be highly speculative and unfair to pretend that their activity is related to their tautomerism – there is no experimental evidences about. However, at this moment of urgent need for COVID-19 treatment solutions any additional information, including tautomeric one, could be useful. The experimental investigations of the tautomerism of **T-705** will be performed in future for sure,

but in the current communication the potential of theoretical chemistry will be used to predict the tautomeric state of favipiravir and the effects of substitution in the pyrazine ring. Such study is not unrelated to reality, because there is sufficient number of experimental and theoretical data for the tautomerism of structurally similar compounds, which will be used to validate the theoretical predictions.



T-705 R=F
T-1105 R=H

T-1106

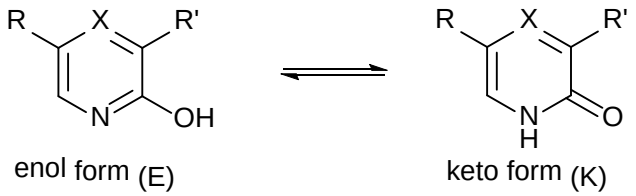
Scheme 1. Compounds in the current study.

Methodology:

Quantum-chemical calculations were performed using the Gaussian 09 D.01 program suite [22]. The M06-2X functional[23,24] was used with def2TZVP[25] basis set. This fitted hybrid meta-GGA functional with 54% HF exchange is specially developed to describe main-group thermochemistry and non-covalent interactions, showing very good results in prediction of the position of tautomeric equilibria[26–31]. In addition, a specially optimized to predict the tautomerism in azodyes and Schiff bases, B3LYP based functional TautLYP[32] was used with 6-31++Gdp basis set. All structures were optimized in ground state without restrictions, using tight optimization criteria and ultrafine grid in the computation of two-electron integrals and their derivatives. TD-DFT was used for excited state optimizations again without restrictions using normal optimization criteria and ultrafine grid in the computation of two-electron integrals and their derivatives. Solvent effects are described using the Polarizable Continuum Model (PCM, the integral equation formalism variant, IEFPCM, as implemented in Gaussian 09)[33]. The true minima were verified by performing frequency calculations in the corresponding environment. The vertical excitation energies were calculated using the TD-DFT formalism. TD-DFT calculations were carried out at the same functional and basis set, which is in accordance with conclusions about the effect of the basis set size and the reliability of the spectral predictions[34,35].

Results and discussion:

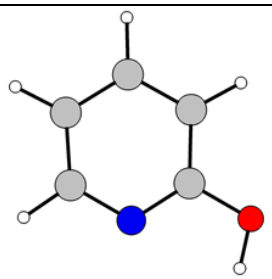
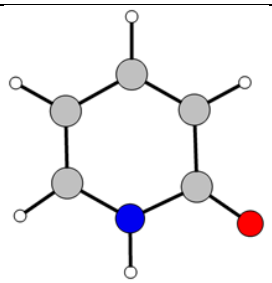
The tautomeric equilibrium in the studied compounds is sketched in Scheme 2. The corresponding stabilities of the tautomers are collected in Table 2 in gas phase and in three solvents with different polarity. Taking into account that implicit solvation is used to describe the solvent effect, the results in the tables has to be considered only in the light of the relative stabilization of the more polar tautomer, i.e. the expected specific solute-solvent interactions are not accounted in water. Logically, the increased dielectric constant of the solvents, from toluene to water, leads to relative stabilization of the more polar keto form in each of the listed compounds.

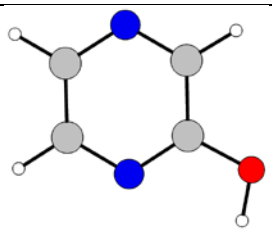
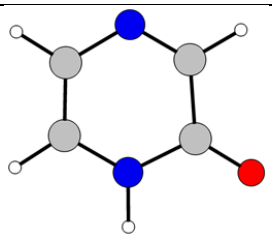
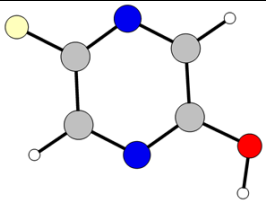
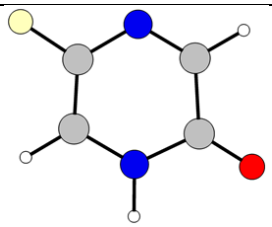
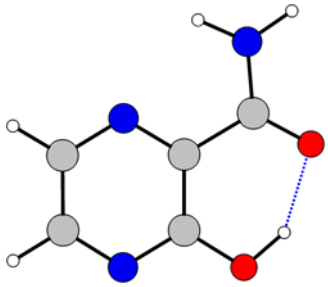
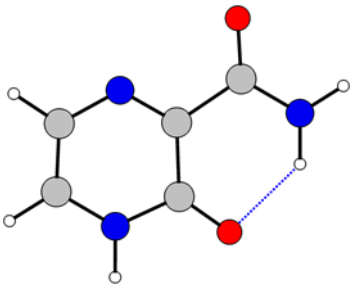


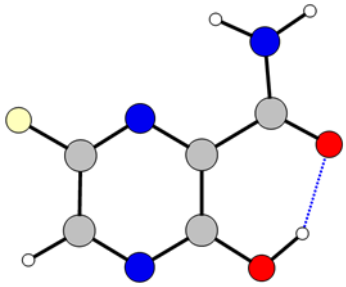
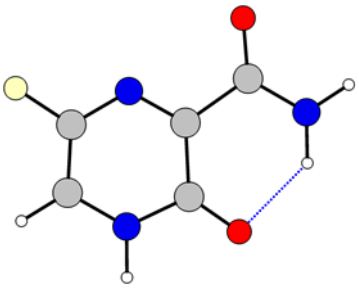
enol form (E) keto form (K)

Scheme 2. Tautomerism of the studied compounds.

Table 1. Relative energies* (M06-2X/def2TZVP, TautLYP/6-31++Gdp in the brackets) of the tautomers of the studied compounds (see Scheme 2).

Comp.	Tautomer**	μ [D]	ΔE [kcal/mol]			
			Gas phase	Toluene	Acetonitrile	Water
1 X=CH R=H R'=H	 enol	1.3	-1.26 (-1.41)	0.76 (0.84)	3.07 (3.44)	3.18 (3.57)
	 keto	4.3				

2 X=N R=H R'=H	 enol	1.4	-2.33 (-2.47)	-0.41 (-0.34)	1.84 (2.19)	1.96 (2.32)
	 keto	4.1				
3 X=N R=F R'=H	 enol	2.2	-5.82 (-5.66)	-3.82 (-3.47)	-1.49 (-0.87)	-1.37 (-0.74)
	 keto	3.2				
T-1105 X=N R=H R'=$CONH_2$	 enol	4.5	-10.2 (-10.9)	-7.25 (-7.63)	-3.73 (-3.73)	-3.55 (-3.52)
	 keto	6.0				

T-705 X=N R=F R'=$CONH_2$	 enol	3.3	-13.3 (-13.7)	-10.2 (-10.4)	-6.53 (-6.27)	-6.33 (-6.05)
	 keto	6.0				

* $\Delta E=E_E-E_K$, negative value indicates more stable enol form and *vice versa*; ** Presented as the most stable isomer.

As seen, in the case of 2-hydroxy pyridine (**1**), the enol form is more stable in gas phase (~90%), while going to condensed phase its fraction is reduced from ~20% in toluene to negligible amount in rest of the solvents. The replacement of the carbon atom with nitrogen in **2**, leads to destabilization of the keto tautomer. The results suggest that the equilibrium is almost fully shifted towards **2E** in gas phase and, following the same trend as in **1**, the increased solvent polarity stabilizes **2K**. Both, fluorine substitution in **3** and a carboxamide group in **T-1105,** lead to a further stabilization of the enol tautomers. The effects are accumulated in **T-705** suggesting that the keto tautomer is not likely to be experimentally observed. There is a detail in **T-1105** and **T-705**, which should be taken into account. The tautomeric proton in the enol form is a part of a strong intramolecular hydrogen bonding, while the NH proton in the keto form is available for interaction with proton acceptor solvents like water. Additional stabilization of the keto tautomer, where two neighbor carbonyl groups are present, can be achieved also by a complexation with metal ions. This could change the tautomeric state, but only the further experiments can prove or not such expectation. It should be noted that some recent cases of tautomerism of drugs (curcumin[27] and Piroxicam[28]) show that theoretically unfavoured tautomers can be stabilized in water as a result of specific interactions.

Having in mind that the relative stability of the tautomers strongly depends on the used level of theory, selected basis set and the solvent description, reference compounds, for which reliable experimental data are available, are needed. The tautomerism of both, **T-705** and **T-1105**, has never been studied experimentally before. Therefore, along with **T-705** and **T-1105**, **1** and **2**, having the same tautomeric backbone, are included in the set in order to clarify the reliability of the theoretical predictions in Table 1.

The keto-enol tautomerism of 2-hydroxy pyridine (**1**) is one of the most studied tautomeric cases, both theoretically and experimentally. Very detailed description of the effects of the used level of theory can be found in[7,36,37]. For gas phase most of the density functionals overestimate (some of them very strongly) the keto form stability, while HF and post-HF methods predict more, but not dramatically, stable **1E**. Fortunately there are experimental data to compare. The ΔG value has been estimated, in kcal/mol units, by variety of techniques and at different temperatures as follows: -0.8 (573K, IR spectroscopy[38]; 412K, UV spectroscopy[39]), -0.57 (403K, X-ray photoelectron spectroscopy)[40], -0.88 (323K, photoelectron spectroscopy)[41], -0.87 (360K, IR matrix isolated technique)[42] and -0.77 (356K, microwave spectroscopy)[43]. The decrease of the temperature leads to increase of the enol form content from 68%[44] (**E**/**K**) at 473K to 80% at 323K[41]. In this respect the prediction of 90% for **1K** at room temperature, made in Table 1, is very reasonable. Even at CCSD(T)/def2TZVP level of theory the stability of the enol form is slightly overestimated in respect of the experiment, predicting ΔE values of -1.2 kcal/mol[45]. It is worth to underline that the estimation of the relative stability of the tautomers in gas phase is based, in some of the cases, on assumption that both tautomers have equal individual responses (UV/IR individual intensity, ionization cross section, etc.). This is a common problem in spectroscopy when tautomeric equilibria are investigated, because the individual tautomers cannot be isolated and, hence, their individual responses are unknown. Details about the possible assumptions and deviations of the results can be found in [46,47]. Returning back to the classical work of Beak and Fry[39], the tautomeric ratio was determined in the range 393-412K by using gas phase UV spectroscopy. In this particular case the tautomeric fractions were determined by using the intensities of the methylated compounds (so called fixed tautomers) as individual responses of the pure tautomers, which makes the approach physically reliable. The estimated enol fractions is in the range 50-80% (2.5±1.5/1 ratio of **1E**/**1K**), which leads a ΔG value in from 0 to -1.1 kcal/mol.

The data for the tautomerism of **1** in solution are also helpful in validating the approach. Such information is available for cyclohexane[48,49], carbon tetrachlode[50] and acetonitrile[49], determined by UV spectroscopy and methylated compounds as references. The corresponding values of ΔG are 0.3, 1.3 and 2.7 kcal/mol respectively, which indicates predominance of the keto tautomer as predicted by the calculations. The results for water are approximate (based on the pKa approximation[49,51,52]) and indicate a ratio **1E**/**1K** < 1/900, i.e. the equilibrium is practically switched towards the keto tautomer.

Comparing to **1**, in the case of **2** less experimental data are available. By using IR matrix isolated technique ΔG values were estimated as follows: -1.55±0.11 (360K)[42] and -1.85±0.24 (360K)[53] kcal/mol, confirming that the enol form is dominating in gas phase. The stabilization of the enol in **2** is larger comparing to **1**, as the calculations suggest. It was shown in DMSO by NMR that the **2K** form is dominating[54].

The experimental data for **1** and **2** confirm the predicted effect of the environment and the structural changes on the tautomerism. In this respect we can assume that the theoretical

predictions should be correct in the case of the other compounds for which there are no experimental data available. It should be expected that **T-1105** and **T-705** with high probability exist as enol tautomers in solution. However, these predictions could be fully validated only by further experimental studies.

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