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A comparison between 5-fluorouracil and 5-fluorocytosine: Structure, tautomerism, vibrational spectra, and WC pairs

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Two important compounds, 5-fluorouracil (5-FU) and 5-fluorocytosine (5-FC) are widely used today for cancer treatment. In this study, the two biomolecules were compared with respect to their molecular structure, tautomerism, relative stability, NBO atomic charge, dipole moment, solid state arrangement, and spectroscopic properties. The *ketonic* form is the most stable tautomeric form of 5-FU but it does not occur in 5-FC. The experimental FT-IR and Raman spectra of 5-FU in solid state were compared to the theoretical scaled ones in the 400-4000 cm^{-1} range. The observed spectral signatures were assigned to different normal modes of vibration through a detailed comparison with the theoretical ones determined by different DFT methods. The theoretical values were improved with scaling and dimer simulations. The WC stability of both molecules was also analyzed.

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1 Introduction

Nucleobases and their derivatives play an important role in basic biological processes. Halogenated pyrimidines were synthesized in the 1957-1959's as potential anti-tumor agents after the successful discovery that certain tumors preferentially incorporated uracil rather than thymine into the DNA [1]. Therefore, the substitution of uracil/thymine by 5-XU in RNA/DNA perturbs the replication of nucleic acids. Transformation of uracil into 5-X uracil (X = halogen) significantly changes its chemical and spectroscopic properties, as well as its *in vivo* activity by inducing various biological phenomena [2]. The halogenated pyrimidines have been found to exert profound effects in a variety of microbial and mammalian agents. These compounds have also served as repair system models to study damaged DNA [3] and were tested against HIV [4]. The biology and chemistry of these halogenated uracils have been also explored as a possible means of sensitizing DNA to ionizing radiation [5]. Although ther radiosensitization properties of 5-X uracils are less effective in double-stranded than in single-stranded DNA, their electron affinities are greater than those of other nucleic acid bases, and thereby, they can capture electrons and dehalogenate [5].

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One of the most accessible, oldest and abundant substances for chemotherapy of most cancer forms is 5-fluorouracil (5-FU). It was first synthesized in 1957 [6] and has been used since for the treatment of solid tumors [7,8]. It was introduced in the early 1960s as a topical chemotherapeutic agent, and has become increasingly accepted because of its high efficacy, low cost, and relative moderate side effects in treating many pre-cancerous conditions, certain benign and malignant tumors, and dermatoses. Therefore, it is used in palliation of inoperable malignant neoplasms, especially those of the gastrointestinal tract, breast, liver and pancreas.

5-FU chemical acts by binding to the target thymidylate synthase protein and interrupting the synthesis of pyrimidine thymidine, which is required for DNA replication, and thus inhibits cell growth [9,10]. The first evaluation of 5-FU as an antineoplastic compound in Heidelberg`s laboratory [6,11] was based on several rationales. First, hepatoma cells use uracil for RNA synthesis at a rate higher than that of normal liver cells. Next, fluorine-substituted organic compounds are generally more toxic than the corresponding unsubstituted ones. Last, the 5-position in the pyrimidine ring is that of normal methylation to thymine. These features were found to facilitate the use of 5-FU as antitumor agent.

Despite its broad antitumor activity, 5-FU also has pharmacological limitations, especially in case of cancer relapse and metastasis, for which many patients show multiple drug resistance [12]. The most common side effect is the suppression of hematopoiesis [13]. To reduce the toxicity of 5-FU and to improve the pharmacological effectiveness of 5-FU, several methods were proposed [10,14]. These include the combination of 5-FU with other drugs such as leucovorin [15], isoflavone genistein [16], or others [17], and the transport improvements of 5-FU through the cell membrane by different cavities [18,19]. Another challenge of 5-FU is its poor solubility in water (ca. 1 mg/mL [20]). Therefore, its solutions are often prepared in strongly basic media, which also increase the side effects of the treatment [21]. 5-FU is a highly toxic drug. Cytotoxic drugs have been widely used for the treatment of cancer. However, they act not only on cancer cells, but also on rapidly growing normal cells. Consequently, such cytotoxic drugs could not be prescribed frequently for long time periods or at a dose level sufficiently high to cure cancer [22]. To avoid it, an alternative process with 5-fluorocytosine (5-FC) has been carried out. In this process, reagents are incorporated with 5-FC, which can transform it into 5-FU within the cell, so that chemotherapy can be introduced into the tumor and suppress it more easily and with less toxicity. This natural conversion of 5-FC to 5-FU can occur by means of deamination within the fungal cells [23].

Recent cancer research with 5-FC involving suicide gene therapy is considered an experimental approach to tumor cell death. This method activates 5-FC to the anticancer agent 5-FU inside the tumor, where 5-FU can carry out its mechanism of thymidylate synthase inhibition and leads to the subsequent impediment of DNA synthesis and tumor growth [24]. In other similar study, replicating retrovirus vectors have been used and enhanced by the addition of prodrugs such as 5-FC, which have led to more effective tumor-selective cell death [25].

For all the above reasons, 5-FC was selected as an anticancer drug model in this study. Although, it is the single antifungal agent available that can act as an antimetabolite drug, 5-FC (also known as Ancobon drug) has been much less studied than 5-FU [26]. Its mechanism of action has been debated over the years [27]. It is a combination of two or more processes that interfere with pyrimidine metabolism [28].

Nevertheless, 5-FU and 5-FC have not been evaluated yet with respect to their similarities and different structural, spectroscopic, tautomeric and WC pairs properties, which is the aim of the present manuscript. Thus, the present study will focus on: (i) comparing the geometric structure and NBO charges of 5-FU and 5-FC molecules, (ii) determining the influence of the fluorine atom in the fifth position of the ring on the tautomerism of the uracil and cytosine molecules, (iii) analyzing the dipole moment and molecular

properties of 5-FU and 5-FC, (iv) studying the different solid state arrangement of these molecules, (v) interpreting the IR and Raman spectra of their solid state, and (vi) determining the effect of the fluorine atom in the WC base pair deformations as compared to the canonical one, and the mechanism by which it could block the tumor growing.

2 Calculations

Quantum chemical calculations were carried out by using the MP2 *ab initio* method and several Density Functional Theory (DFT) methods. Both approaches were implemented in the GAUSSIAN 16 program package [29]. A UNIX version of standard parameters for this package was run on the "Brigit" computer of the Computational Center at University Complutense of Madrid, Spain. DFT methods provide a very good overall description of medium-size molecules, and represent an adequate compromise between the desired chemical accuracy and heavy demands on computer time and power. Thus, DFT [30] emerged as the appropriate tool for this work. Among the DFT methods, the Becke's three-parameter exchange functional (B3) [31] in combination with the correlation functional of Lee, Yang and Parr (LYP) [32], i.e. B3LYP, has been used satisfactoryly in many studies of drug designing [33,34], the analysis of nucleosides and nucleic bases [35,36], and in vibrational wavenumber calculations [37,38].

The Minnesota hybrid meta functional M06-2X [39] method was also used in the WC pair optimizations because it appears as one of the best options among the meta-generalized gradient functionals for analyzing dispersion–bound systems. It also shows good results for a broad range of interactions, including non-covalent interactions of biological systems [40]. The 6-31G(d,p) and 6-311++(3df,pd) basis sets were mainly selected due to their accuracy. All optimized geometries corresponded to stationary points (no negative eigenvalue), from which the IR and Raman theoretical spectra were obtained.

3 Results and Discussion

3.1 Molecular Structure

The most important optimized geometric parameters in 5-FU and 5-FC are summarized in Table 1 at the MP2/6-31G(d,p) level. For comparison purposes, the values obtained in uracil and cytosine molecules are also included. The most stable *keto* tautomer (**T1**) in uracil and 5-FU, and the most stable *enol* tautomer in 5-FC were also included together with the *keto* one (**C1**). The optimized molecular structure is shown in Fig 1. The notation used for labelling the atoms is the standard one.

The molecular structure of 5-FU has been reported using different theoretical methods [41-43]. In these studies, 5-FU is considered a flexible molecule, with a planar geometry in the isolated state, and with a non-planar ring form in the first hydration shell [43]. The water molecules slightly affect the planarity of the uracil ring with change in its torsional angles in general lower than 7º. A high conformational flexibility of the pyrimidine ring represents one of the possible ways of geometry relaxation for the creation of the most favourable conditions for inter- and intra-molecular interactions with other molecules.

The molecular structure of 5-FC has been much less studied. It also has a flexible pyrimidine ring, but in addition, it possesses an amino NH_2 group that appears out-of the ring plane (*tilt* angle, ε) and with a C2-N-C4-N torsional angle of 177.0º in the *keto* **C1** tautomer. The amino hydrogens of this NH_2 group are also out-of-plane (inversion angle, ω). The non-planarity of this amino NH_2 group has been shown to be an important factor in stabilizing the structure of DNA base pairs [44]. The large flexibility of this group facilitates the intermolecular H-bonds in the DNA helix. This flexibility in 5-FC is higher than in 5-FU with two rigid C=O groups, and, therefore, it can establish three intermolecular H-bonds in the DNA helix. This means that 5-FU presents flexibility in its pyrimidine ring, while 5-FC is flexible in both the pyrimidine ring and the amino NH_2 group.

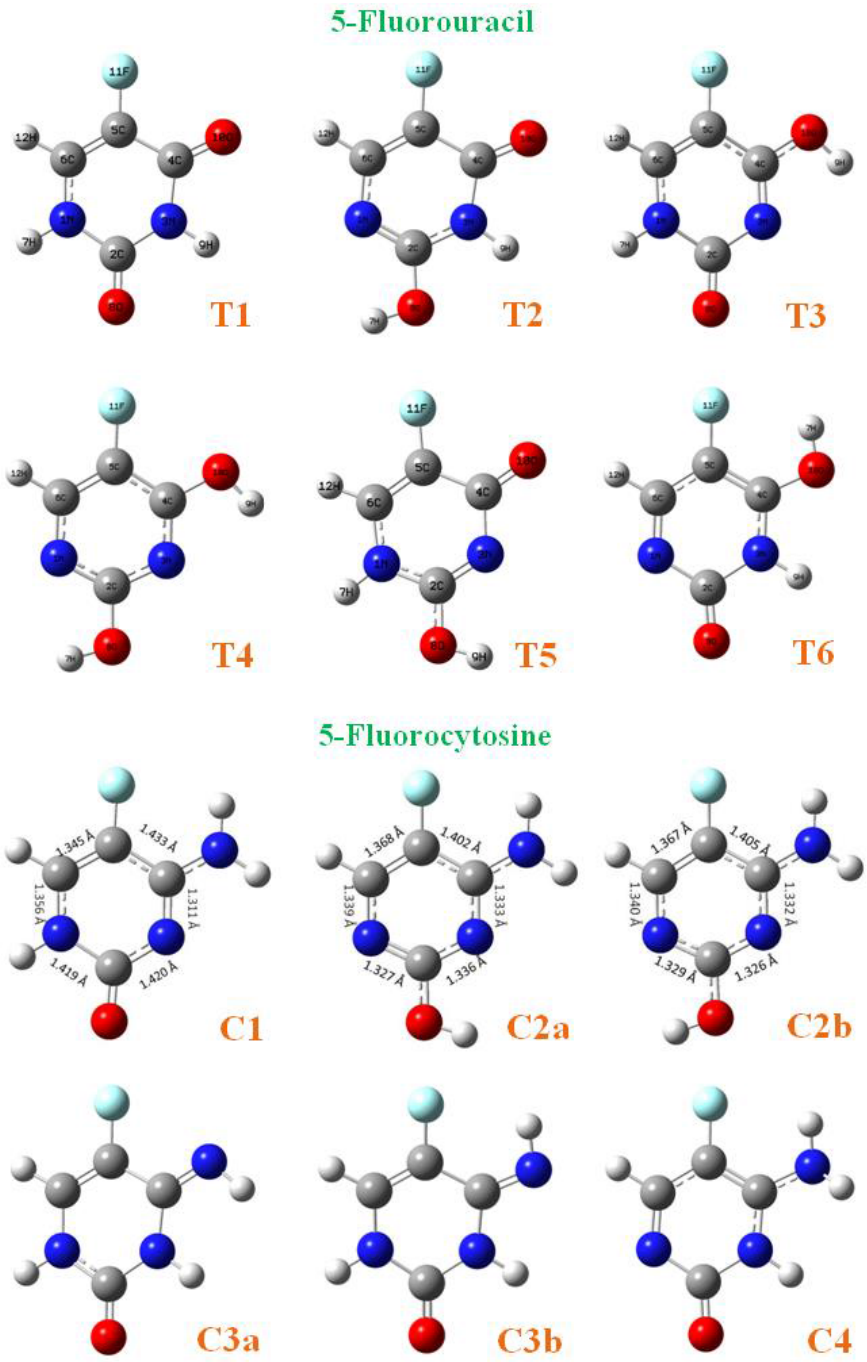

Fig 1. Optimized geometrical structures of the six main tautomers of 5-fluorouracil (5-FU) and 5-fluorocytosine (5-FC) at the MP2/6-31G(d,p) level. The name of the different tautomers is as follows: (**T1**) 2,4-dioxo 5-fluorouracil, (**T2**) 2-hydroxy-4-oxo 5-fluorouracil, (**T3**) 2-oxo-4-hydoxy 5-fluorouracil, (**T4**) 2,4-dihydroxy 5-fluorouracil, (**T5**) 1H-2-hydroxy-4-oxo 5-fluorouracil, (**T6**) 3H-2-oxo-4-hydroxy 5-fluorouracil, (**C1**) oxo 5-fluorocytosine, (**C2a**, **C2b**) hydroxy 5-fluorocytosine, (**C3a**, **C3b**) imino 5-fluorocytosine, and (**C4**) 3H-oxo 5-fluorocytosine.

The carbonyl C=O groups of 5-FU with a high negative charge on the oxygen atoms facilitate the binding to metal ions such as Mn(II) and Co(II) [45], but this cannot be achieved by 5-FC. Therefore, 5-FU like other 5-halogenated uracil derivatives can bind metals and is an antimetabolite that can be incorporated into nucleic acids.

Table 1. Optimized geometrical parameters at the MP2/6-31G(d,p) level in the main tautomer forms of uracil, cytosine, 5-fluorouracil (5-FU), and 5-fluorocytosine (5-FC).

Molecule:	Uracil	5-FU	Cytosine	5-FC	
Tautomer:	T1	T1	C1	C1	C2b
Bond lengths (Å)					
N1-C2	1.390	1.384	1.418	1.415	1.334
C2-N3	1.384	1.392	1.382	1.387	1.339
N3=C4	1.408	1.401	1.318	1.315	1.336
C4-C5	1.457	1.462	1.437	1.435	1.407
C5=C6	1.351	1.348	1.359	1.352	1.373
N1-C6	1.376	1.381	1.358	1.365	1.351
C2=O	1.224	1.223	1.226	1.226	1.353
C4=O/ C4-N10	1.227	1.224	1.369	1.359	1.366
C5-F	-	1.345	-	1.359	1.358
Bond angles (°)					
N-C2-N	112.4	112.5	115.9	116.1	128.5
C2-N3-C4	128.9	129.4	119.9	120.4	115.9
N3=C4-C5	113.2	111,7	124.6	122.7	120.2
C4-C=C6	119.9	121,9	116.0	118.8	119.0
N1-C6=C5	121,8	120.3	119.6	117.7	121.2
N3-C2=O	124.4	123.8	125.2	124.8	114.4
N3-C4=O /N10	120.6	122.4	116.8	119.4	118.9
C4-C5-F / H	118.5	117.0	122.4	119.0	118.8
C6=C5-F /H	122.5	122.2	121.6	122.2	122.2
Torsional angles (°)			-		
C2-N-C4=O/N10	180.0	180.0	176.7	177.0	176.7

The main effect of the fluorine atom in 5-FC as compared to cytosine is the lengthening of the N1-C6 bond (ca. 0.01 Å) and the shortening of the C4-N10 bond, 0.01 Å. There is also an opening of the C4-C5=C6 and N3=C4-N10 angles (ca. 2º), and closing of the N3-C4-C5, N1-C6=C5 and especially C4-C5-F, ca. 3º. These changes appear to bring the negative fluorine atom closer to the positive amino NH_2 hydrogens. The small shortening of the C4-N10 bond slightly increases the NH_2 planarity.

3.2 Tautomerism

Tautomers induce alterations in the normal base pairing, leading to the possibility of spontaneous mutations in the DNA or RNA helices. Thus, tautomerism in nucleic acid bases and their derivatives has a role in mutagenesis of DNA and raises significant scientific interest [46]. The occurrence of rare tautomeric

forms might lead to a point mutation during DNA replications. Halogenated bases produce specific mutations similarly to canonical bases when incorporated into double-helical DNA. This occurs during their occasional mispairing with adenine/guanine during genetic replication. Similarly, 5-FU/5-FC nucleobases also produce coding errors in messenger RNAs. Whether this ability is higher or not in 5-FU or 5-FC and when compared to the related canonical ones is analyzed in the present manuscript.

3.2.1 Molecular structure

Generally, the *keto* form (**T1**) of uracil exists as the main form in the double helix. The formation of specific AU Watson-Crick (WC) hydrogen bonds is responsible for the maintenance of the genetic code. If uracil is replaced by another type of base, it may lead to the introduction of a wrong genetic code. However, mutations in the genetic code are observed, and one of the mutation mechanisms is the tautomerism. Different tautomers were optimized in the 5-FU molecule using different theoretical methods [42,43,47]. The six most stable ones are plotted in Fig 1 in their optimized form. The other possible tautomers, not included in this figure, are remarkably less stable and thus, they were omitted. The notation used for these tautomers is the standard one. Only one of these tautomers (*diketo* C=O/C=O form **T1**) is exclusively present in isolated state [43,47], solid state [48], and aqueous solutions [49,50]. This tautomer is only active as a chemotherapeutic agent [51,52].

The main effect occurring in tautomer **T2** of 5-FU due to the displacement of the proton on N1 is shortening of the N1-C2 bond (1.291 Å in **T2** vs. 1.385 Å in **T1**), a shortening of the N1-C6, C2-N3, C4-C5 bonds and a lengthening of the C2-O, N3-C4, C5-C6 bonds. The small lengthening of the C5-F bond (1.336 Å in **T2** vs. 1.334 Å in **T1**) is counteracted by closing of the C4-C5-F angle (116.9º in **T2** vs. 117.4º of **T1**). The proton of the OH group remains in the ring plane.

Like cytosine 5-FC may exist in various tautomeric forms differing from each other by the position of the proton, which may be bound to either ring nitrogen atoms or oxygen atoms. The six most stable tautomers in 5-FC according to the MP2/6-31G(d,p) calculation level were also included in Fig 1. The optimized bond length values in the three most stable tautomers were also included. Thus, it can be observed that the main effect of the proton transfer on N1 to the O2 oxygen atom in the **C2a** and **C2b** enolic forms is a strong shortening of the N1-C2 bond length (1.419 Å in **C1** vs. 1.327 Å in **C2a** and 1.329 Å in **C2b**). A shortening of the N1-C6, C2-N3 and C4-C5 bonds and a lengthening of the C=O, N3=C4, and C5=C6 bonds were also observed. The OH and NH_2 groups of cytosine tautomers are more strongly bonded with the heterocyclic ring than with the benzene ring when compared to the phenol and aniline molecules. This is clearly illustrated by the decrease in the C-OH and C-NH_2 bond distances at CCSD level (by ca. 0.025 and 0.04 Å, respectively), and by the decrease in NH_2 pyramidalization. It may also be noted that in the **C2** tautomers the ring structure is less perturbed by the substitution. These geometric changes in tautomerization appear somewhat larger in the cytosine tautomers than the uracil tautomers. This makes the **C2b** tautomer most stable in cytosine (as well as in 5-FC) even more stable than the *keto* one **C1**.

3.2.2. Relative stabilities

The gas-phase relative energies of 5-FU tautomers were calculated at different theoretical levels. They are shown in Table 2 by comparison with those corresponding to the uracil molecule. The fluorine atom does not change the stability order of tautomers, which is **T1** > **T2** > **T4** > **T3** > **T5** > **T6** at all theoretical levels.

The dioxo form **T1** in 5-FU is the most stable form similarly to the uracil molecule, while tautomer **T2** is the most stable *enol* form. All *enol* and di-*enol* forms are energetically much less stable than the canonical *keto* tautomer. The relative energy of the *enol* tautomers is large enough to confirm that **T1** is the only important form in the isolated state of 5-FU and in others halo derivatives, as previously reported both theoretically [42,43,47] and experimentally [49,50]. The planarity of the heterocyclic ring in the isolated state has a noticeable influence on this tautomer stability of **T1**, and the two carbonylic C=O groups bounded to

the ring are responsible for this stability.The fluorine atom slightly stabilizes the *keto-enol* form **T2**, which is 34.74 kJ/mol above **T1** in 5-FU and 43.30 kJ/mol in uracil at the MP2/6-31+G(d,p) level. The calculated value at this level is very close to that reported at the sophisticated CCSD/6-31G(d,p) level, 46.77 kJ/mol. Figure 2 shows how the proton transfer changes the tautomer stability in the four most stable tautomers. The proton transfer to the neighbouring oxygen atom leads to more stable tautomers when it is on N1 (**T2**) rather than on N3 (**T3**, **T5**).

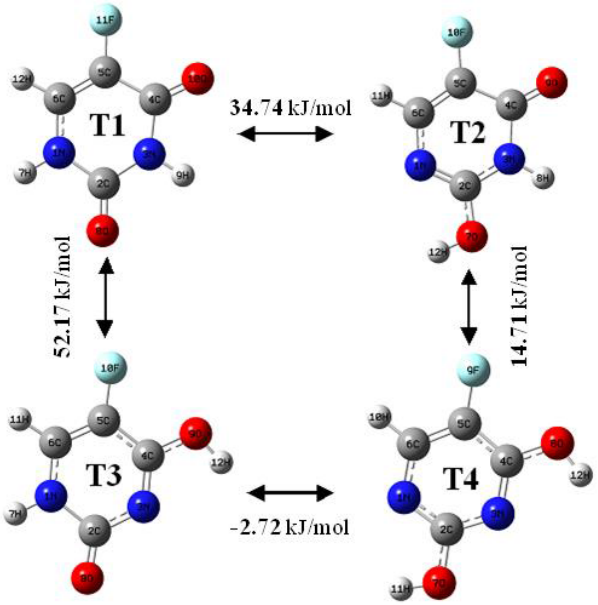

Fig 2. Tautomerism in 5-fluorouracil (5-FU). The energies were calculated at the MP2/6-31+G(d,p) level. Tautomer **T3** is the least stable of the four tautomers plotted.

Table 2. Relative energies (in kJ mol^{-1}) calculated in the six most stable tautomers of uracil, 5-fluorouracil (5-FU), cytosine, and 5-fluorocytosine (5-FC) at different theoretical levels.

Molecule	Level	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Uracil	B3LYP/6-311++G(3df,pd)	0	46.48	48.20	53.88	78.96	85.02
	MP2/6-31G(d,p)	0	45.48	51.54	47.74	80.34	90.25
	MP2/6-31+G(d,p)	0	43.30	48.57	43.56	78.29	90.66
	CCSD/6-31G(d,p)	0	46.77	53.84	51.79	82.01	94.47
	Experimental	0			79 ± 25	92 ±4	
5-FU	B3LYP/6-311++G(3df,pd)	0	38.50	51.29	48.45	68.59	82.43
	MP2/6-31G(d,p)	0	37.95	55.22	43.60	71.27	89.08
	MP2/6-31+G(d,p)	0	34.74	52.17	49.45	68.68	87.28
		C1	**C2a**	**C2b**	**C3a**	**C3b**	**C4**
Cytosine	MP2/6-31G(d,p) + ZPE	6.66	2.92	0.0[b]	16.39	10.26	34.79
	MP2/cc-pvtz	8.74	2.93	0.0[c]	20.27	13.38	38.37
	MP4/6-31G(d,p)//MP2/6-31G(d,p)	2.35	2.94	0.0[d]	9.26	3.07	30.86
	CCSD(T)/cc-pvdz//MP2/cc-pvtz	9.11	2.68	0.0[e]	13.84	7.40	35.49

	MP2/6-31G(d,p)+ZPE	13.29	3.29	0.0[f]	31.26	16.42	47.00
	Gibbs energy	12.14	3.27	0.0[g]	29.54	14.78	45.68
5-FC	MP2/cc-pvtz + ZPE	16.01	3.14	0.0[h]	35.68	20.94	50.61
	MP4/6-31G(d,p)//MP2/6-31G(d,p)	8.66	3.33	0.0[i]	23.01	8.03	42.43
	CCSD(T)/cc-pvdz//MP2/cc-pvtz	15.84	3.02	0.0[j]	27.77	12.73	47.88

The gas-phase relative energies of the six most stable tautomers of 5-FC calculated at different theoretical levels are also presented in Table 2 together with the corresponding cytosine values. Imino-*enol* forms are much higher in energy and are of no practical significance. Therefore, they were not included in Table 2. It was observed that tautomerism is more favored in cytosine than in uracil. Like in 5-FU, the fluorine atom in 5-FC does not change the stability order of tautomers, which is **C2b** > **C2a** > **C1** > **C3b** > **C3a** > **C4** at the MP2 level but remarkably increment the destabilization of all tautomers as compared with the most stable one, **C2b**.

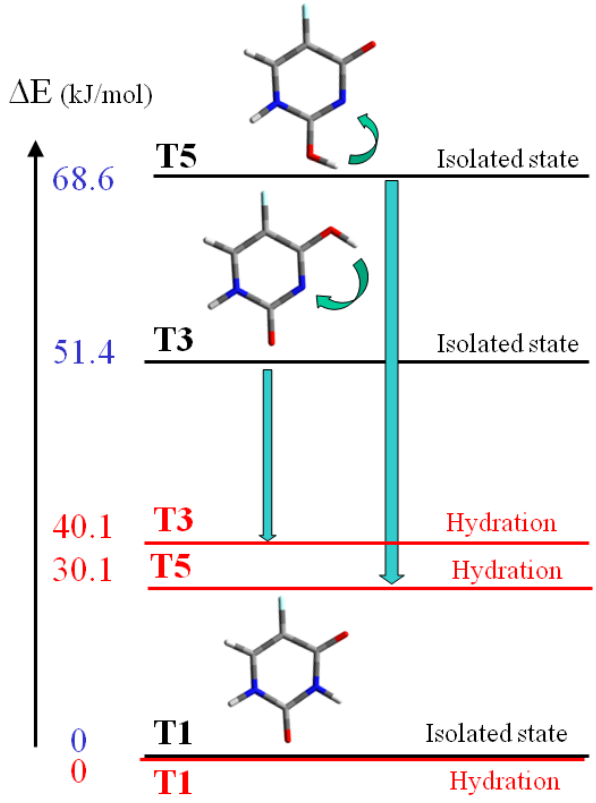

Fig 3. Effect of hydration with 10 water molecules on the energies of tautomers **T3** and **T5** of 5-FU when compared to the tautomer **T1** at the B3LYP/6-311++G(3df,pd) level.

The strongest change observed in Table 2 is that the *amino-keto* form **C1** (also called *amino-oxo*) is not the most stable one, although it is close in energy to the most stable *enol* **C2b** form. Another change observed is that the three most stable tautomers in cytosine/5-FC molecules have relative energies that are much lower than those of their counterparts in uracil/5-FU molecules. Two features can explain this by comparing the *keto* tautomer in 5-FC with the one in 5-FU: (i) the N1-H bond length is slightly longer in

5-FC than in 5-FU. (ii) The C=O bond length is slightly longer in 5-FC than in 5-FU (1.226 vs. 1.223 Å, respectively). Both features favor tautomerism in 5-FC. As described above, the *keto* tautomer **T1** in uracil/5-FU and the enol tautomer **C2b** in cytosine/5-FC are the most stable forms in the isolated state. However, it has been experimentally observed by X-ray diffraction (XRD) [53] and neutron diffraction [54] that in those crystals of cytosine molecule in the solid state only occurs the **C1** tautomeric form. In aqueous solutions [49,50] the tautomerism is favored, and the *keto* tautomer is the only form present.

Figure 3 shows how the first hydration shell noticeably favors the tautomerism in the **T3** and **T5** *enol* forms of 5-FU rather than **T1**. These tautomers, **T3** and **T5,** were selected in this study [43] because they are the only ones present in the corresponding 5-fluoro uridine nucleoside. Therefore, they are the only ones that can appear in DNA helices. The *keto* form continues being the most stable form despite the reduction in the energy gap between **T3** and **T5** as compared to **T1**. Similar behavior is also expected in the 5-FC molecule and the *keto* form ("canonical") is the only one found in DNA and RNA helices, which are surrounded by water. Because the biological functions of nucleic acids depend on their interactions with water in the medium, the volume and electronic effects of the fluorine atom make it difficult for water molecules to enter into the N-C4-C5-F region. Thus, replacing the hydrogen atom in the 5-position by the fluorine atom, the protection exerted by water molecules in this region is lost. Consequently, the possibility of tautomerism occurring is much more favored and is much higher than in the cytosine/5-FC molecules.

Table 3 summarizes the natural NBO atomic charges on the main tautomers forms of 5-FU and 5-FC. These values are shown by comparison with those corresponding to uracil and cytosine in the isolated state at the MP2/6-31G(d,p) level.

Table 3. Calculated natural NBO atomic charges (*e*, where *e* is the electron charge) at the MP2/6-31G(d,p) level in the main tautomers of uracil, 5-fluorouracil (5-FU), cytosine, and 5-fluorocytosine (5-FC).

Parameters	Uracil	5-FU	Cytosine	5-FC		
	T1	T1	C1	C1	C2a	C2b
N1	–0.65	–0.735	–0.73	–0.73	–0.63	–0.68
C2	0.81	1.01	0.98	0.98	0.83	0.90
N3	–0.70	–0.78	–0.71	–0.70	–0.70	–0.65
C4	0.64	0.77	0.59	0.54	0.50	0.50
C5	–0.38	0.28	–0.47	0.26	0.25	0.25
C6	0.02	0.02	0.17	0.07	0.09	0.09
O2	–0.60	–0.726	–0.74	–0.73	–0.75	–0.75
O4 / N10	–0.56	–0.67	–0.89	–0.89	–0.89	–0.89
F	-	–0.37	-	–0.39	–0.39	–0.39

When the hydrogen atom in position five of the ring with +0.25*e* is substituted by the electron-withdrawing fluorine atom, it causes a redistribution of all atomic charges in the molecule. As expected, the most affected atom is C5 to which the fluorine atom is bonded. It drastically shifts from –0.38*e* in uracil to +0.28*e* in 5-FU, and from –0.47*e* in cytosine to +0.26*e* in 5-FC. This shift in the atomic charge on C5 is higher in 5-FC than in 5-FU and leads to an opposite effect in the charges on the neighbor C4 atom. Thus, the positive charges on C4 are increased from uracil toward 5-FU and decreased from cytosine toward 5-FC. The negative charge on the fluorine atom is slightly higher in 5-FC than in 5-FU.

In the *enolic* tautomers of 5-FU, the negative charge on O2 is slightly increased to –0.75*e* as compared to –0.73*e* in **T1**. The positive charge on **C2** is significantly reduced to 0.83*e* as compared to 1.01*e*

in **T1**. By contrast, the negative charge on N1 is reduced. The negative charge on the fluorine atom is little affected by tautomerism.

In the *keto* tautomers of 5-FU and 5-FC, the atomic charges on O2 and N1 have almost the same values. Therefore, the transfer of the hydrogen proton onto N1 to O2 to form the corresponding T2/C2b *enol* tautomers is not facilitated in 5-FU/5-FC. However, this occurs in uracil/cytosine with a considerable higher negative charge on O2 in cytosine (–0.74*e*) than in uracil (–0.60*e*) facilitating the formation of the most stable **C2b** tautomer. This can only be explained by the greater barrier height experienced by 5-FU than 5-FC in the proton transfer. In 5-FU, the negative charge on O2 is higher than on O4 and, therefore, it is expected that O2 will be more susceptible to be attacked by a proton than O4. However, hydration with few water molecules [43] increases the negative charge on O4 more than on O2, and thus with 10 water molecules the negative charge on O4 is like the one on O2.

3.2.4 Dipole moments

The prediction of accurate dipole moments (μ) is a very important issue, because its magnitude is strongly related to the tautomeric stability in polar environments. The value in **T1** of uracil is larger than that of 5-FU, Table 4. Different tautomers have different electronic structures and in principle, they may display significant variations in their physical properties (i.e., very different values of μ). In the six most stable tautomers of 5-FU, the calculated μ lies in a wide range of values (1 - 7 D) [43,47], increasing in the order **T4 < T2 < T1 < T3 < T5 < T6** in uracil, and in the order **T4 < T3 < T1 < T2 < T6 < T5** in 5-FU. The smallest one corresponds to **T4**, while **T5** and **T6** possess the largest μ. Because of the proximity of μ values in several tautomers, **T2** and **T3** as well as **T5** and **T6** appear in reverse order in 5-FU related to uracil. The canonical *diketo* form possesses a noticeable high μ. Large values of μ indicate considerable stabilization when these tautomers are exposed to a polar solvent like water. As μ increases, the hydration experienced by the molecule is greater. The fluorine atom increases μ in the **T2** and **T5** tautomers, and decreases it in the **T1, T3, T4** and **T6** tautomers. By contrast, the fluorine atom reduces noticeably the value of μ in all 5-FC tautomers. The largest value appears in **C4** followed by **C1**, similarly to the cytosine molecule. Thus, the tautomer **C1** will be remarkably hydrated and stabilized when compared to the **C2b** tautomer of the lowest μ value. This large stabilization of C1 in water defines it as the most stable form. 5-FC has a larger μ than 5-FU in the canonical *keto* form. Hence, 5-FC is favored over 5-FU, and 5-FC may have larger anticancer effect when it is used in this environment.

Table 4. Calculated dipole moments (μ, Debye) at the **(a)** B3LYP/6-311++G(3df,pd) and **(b)** MP2/6-31G(d,p) levels in the main tautomers of uracil, 5-fluorouracil (5-FU), cytosine**,** and 5-fluorocytosine (5-FC) biomolecules.

	Uracil		5-FU			Cytosine	5-FC	
Tautomer	(a)	(b)	(a)	(b)	Tautomer	(b)	(a)	(b)
T1	4.48	5.02	4.12	4.52	C1	6.42	5.220	5.723
T2	3.33	3.66	4.36	4.79	C2a	4.48	3.920	3.804
T3	4.88	5.52	3.60	4.04	C2b	3.32	1.983	2.047
T4	1.18	1.57	0.64	0.96	C3a	2.46	2.256	2.368
T5	6.58	7.05	7.05	7.55	C3b	4.72	3.820	4.260
T6	7.20	7.81	5.87	6.14	C4	7.71	7.108	7.089

3.3 Molecular structure in the solid state

5-FU may exhibit tautomerism in the isolated state. The absence of OH band in the 3700-3500 cm^{-1} spectral range and the appearance of C=O strong vibrational modes in the 1750-1600 cm^{-1} region of 5-FU [48] indicate that 5-FU exist only in the *keto* form in the solid state. Moreover, only the **T1** *keto* form has been

found in the 5-FU crystal [55]. The optimized crystal unit cell with the most stable tetramer form is depicted in Fig 4. A planar arrangement is found with intermolecular H-bonds through the amino hydrogen and carboxylic oxygen atoms. The fluorine atom does not participate in the crystal net due to the small negative charge. Although X-ray values have not been reported in 5-FC, but an optimized form of the crystal unit cell has been completed (Fig 5) at the B3LYP/6-31G(d,p) level according to that reported experimentally in the cytosine crystal [56]. Herein, the molecules are tilted ~27.5º and the intermolecular H-bonds are formed by all the three available protons.

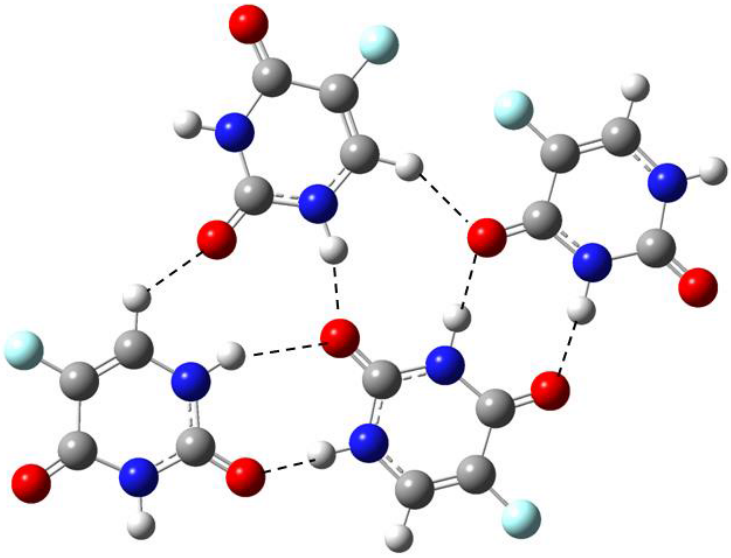

Fig 4. Optimized crystal unit cell of 5-fluorouracil with the most stable tetramer form.

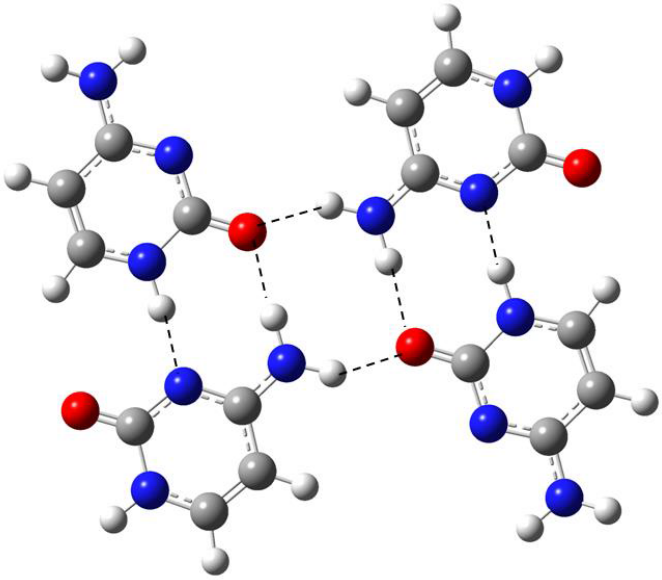

Fig 5. Crystal unit cell of 5-fluorocytosine (5-FC) optimized according to the one reported experimentally for the cytosine crystal [56].

The main difference between the 5-FU and 5-FC crystal unit cell optimizations is that the 5-FU system is full planar with strong H-bonds, while with the structure of 5-FC it is slightly out-of-plane through the amino NH_2 group and has slightly weaker H-bonds. The large flexibility of this NH_2 group facilitates the intermolecular H-bonds and, therefore, a symmetric arrangement of molecules is obtained in 5-FC. The properties of purine and pyrimidine bases are determined by their hydrogen and π-bonding systems [56]. Although, it has not been represented that the fluorine substituent affects solid state base stacking patterns, but it has been suggested that stacking interactions involving this fluorine atom may contribute to the unusual physical and biological properties of these molecules [57]. Due to the non-planarity of the 5-FC arrangement and a slightly higher aromaticity in its ring, it is expected to be largely interplanar and to exhibit π interactions in its crystal when compared to 5-FU.

3.3 Comparison of several properties

Wavenumbers have been determined to examine several properties of the main tautomers of uracil, 5-FU, cytosine, and 5-FC biomolecules, which are collected in Table 5. For example, rotational constants are slightly higher in 5-FC than in 5-FU due to the NH_2 group. The fluorine atom leads to a noticeable decrease in their values as compared to the respective non-substituted uracil/cytosine molecules. The heat capacity at constant volume (Cv) is also higher in cytosine than in uracil, and fluorine substitution increases the values by ca. 3 cal/mol. The fluorine atom also leads to a slight increase (ca. 4 $cal \cdot mol^{-1} \cdot K^{-1}$) in the vibrational contribution to the total calculated entropies. The fluorine effect on translational and rotational contributions is insignificant. The total entropy is only 1 $cal \cdot mol^{-1} \cdot K^{-1}$ higher in 5-FC than in 5-FU.

Table 5. Theoretically computed rotational constants, heat capacity (Cv), entropies, HOMO and LUMO energies at the MP2/6-31G(d,p) level in the main tautomers of uracil, 5-fluorouracil (5-FU), cytosine, and 5-fluorocytosine (5-FC) biomolecules.

	Uracil	5-FU	Cytosine	5-FC	
Parameters	T1	T1	C1	C1	C2b
Rotational constants (GHz)	3.879	3.190	3.861	3.203	3.227
	1.999	1.392	2.011	1.403	1.405
	1.319	0.969	1.323	0.976	0.979
Cv (cal/mol)	23.52	26.63	25.37	28.65	28.16
Entropy ($cal \cdot mol^{-1} \cdot K^{-1}$)					
Total	79.59	84.33	80.36	85.27	83.78
Translational	40.06	40.50	40.03	40.48	40.48
Rotational	27.84	28.70	27.84	28.68	28.67
Vibrational	11.69	15.13	12.49	16.10	14.63
HOMO (eV)	–0.363	–0.366	–0.338	–0.342	–0.340
LUMO (eV)	0.099	0.087	0.107	0.094	0.109
E_g (eV)	0.462	0.453	0.445	0.436	0.449
IP (eV)	0.363	0.366	0.338	0.342	0.340
EA (eV)	–0.099	–0.087	–0.107	–0.094	–0.109
χ (eV)	0.132	0.139	0.115	0.124	0.115
η (eV)	0.231	0.226	0.222	0.218	0.224
S (eV)	0.115	0.113	0.111	0.109	0.112

HOMO (the highest occupied molecular orbital) and LUMO (the lowest unoccupied molecular orbital) values were also included in Table 5. A plot of these orbitals in 5-FU is included in Fig 6(a), which shows that the charge density is shifted towards the N3-H bond on going from HOMO to LUMO. The LUMO energy values are noticeably lower as negative values in 5-FC than in 5-FU, while in the HOMO they are slightly higher in 5-FC than in 5-FU. The fluorine atom decreases the LUMO values and increases in negative value of the HOMO. With these computed energies, several global chemical reactivity descriptors were determined according to the well-known formulae in Eqs (1-5) [58]:

$$IP = -E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

$$c = -(E_{HOMO} + E_{LUMO})/2 \quad (3)$$

$$h = (E_{LUMO} - E_{HOMO})/2 \quad (4)$$

$$S = ½\ h \quad (5)$$

The energy gap (E_g) between the HOMO and LUMO frontier orbitals (HOMO–LUMO gap), Fig 6(a), appears as one of the meaningful characteristics of molecules. It facilitates the characterization of the chemical reactivity and the kinetic stability of the molecules. The values are remarkably higher in uracil/5-FU than cytosine/5-FC. This means that uracil/5-FU are more stable than cytosine/5-FC. The fluorine atom reduces the E_g value, i.e., 5-FU/5-FC are more reactive than the corresponding uracil/cytosine.

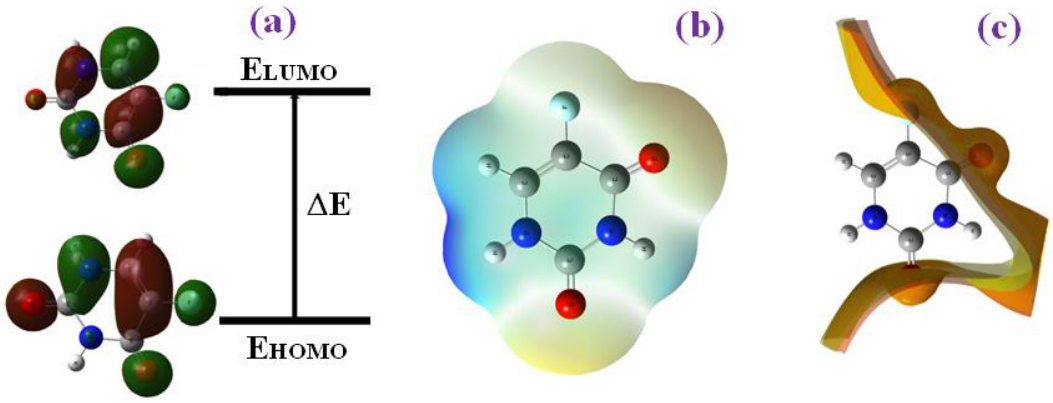

Fig 6. Calculated (a) HOMO-LUMO orbitals, (b) MEP plot, and (c) ESP plot for 5-fluorouracil (5-FU).

Electron affinity (EA) values are negative, while IP are positive and higher in uracil/5-FU than in cytosine/5-FC. The low electronegativity (χ) observed agrees with those of neutral molecules and they are slightly lower in cytosine/5-FC than uracil/5-FU.

The chemical hardness (η) and global softness (S) values indicate the opposition of the system to an alteration in its number of electrons. They are slightly higher in uracil/5-FU than in cytosine/5-FC. In general, these values are slightly small in the molecules investigated herein. When the values of η are small, the system is identified as soft. Therefore, our molecules are soft systems, with small gaps, and with an easily modified electron density.

The Molecular Electrostatic Potential (MEP) plot in 5-FU (Fig 6b) shows the charge density distribution over a single molecule. The different colors refer to the nature of the charge in the vicinity of the site. The red color indicates the site of the highest negative charge, while the blue color depicts the sites of the highest positive charge. Therefore, the strong nucleophilic substitution sites appear near the H atoms, attached to the N1 and N3 atoms, while the site of electrophilic attack is near to the oxygen atoms of the keto group, attached to the ring at positions 2 and 4.

Electrostatic potential (ESP) in 5-FU is shown in Fig 6(c). It provides information about the potential interaction sites with other molecules in the form of ESP. The Iso-surface plot fully covers the C=O bonds and creates a well between the two C=O bonds due to the negative charge on oxygen atoms.

3.4 Vibrational spectra

The vibrational spectra of 5-FU have been extensively studied by IR, FT-Raman, and SERS [41,42,47,48,59-61]. The characteristic vibrational modes of 5-FU are summarized in Table 6 and assigned according to this literature. The existence of H-bonding, C=O···H-N, in the solid state of uracil and 5-FU significantly changes the magnitudes and intensities of the wavenumbers appearing in the IR and Raman spectra. Therefore, significant deviations in the scaled wavenumbers are observed, especially for those involved in H-bonds. To correct these theoretical deviations, the crystal unit cell of 5-FU was simulated using a dimer form and their wavenumbers were calculated [47]. This is the typical procedure for the simulation of dimer, trimer, tetramer, etc. [62].

The computed wavenumbers were improved by using a scaling procedure [63]. Among them, one of the best is the linear scaling equation (LSE) procedure [37], which was used for the calculated values of the first column in Table 6. The scaled wavenumbers were included in the fourth column. With the scaling, the assignments proposed for the experimental wavenumbers can be considered accurate. The second column provides their relative infrared intensities (A) in %, which were obtained by normalizing the computed value to the intensity of the strongest band. Similarly, the relative Raman intensities (S) were obtained and included in the third column. The definition of the uracil ring normal modes was implemented for the assignments shown in the last column [64], whose values were included in the previous column. The calculated displacement vectors of the 5-FU normal modes were from ref [48].

Table 6. Calculated harmonic vibrational wavenumbers (ν, cm^{-1}) in 5-FU at the B3LYP/6-31G(d,p) level, relative infrared (A, %) and Raman (S, %) intensities, scaled wavenumbers and experimental (exp.) IR and Raman data (cm^{-1}) obtained for 5-FU in solid state.

Calculated			Scaled[a]	Exp. IR	Exp. Raman		Ring No.[b]	Vibrational assignment
ν	A	S		ref. [60]	SERS [61]	ref. [48]		
3659	9	37	3491	3141 vs	3146 sh	3142.9	30	ν(N1–H)
3258	100	100	3112	3069 vs	3073 w	3066.5	29	ν(N3-H)
3243	0	28	3098	3001 vs	3001 vw	3002	27	ν(C6-H)
1849	39	3	1781	1722 s	1725 vw	1724.5	26	ν(C2=O) + ν(C4=O) + δ(N-H)
1766	63	22	1703	1671 vs	1672 m	1670.4	25	ν(C4=O) + ν(C2=O) + δ(N3-H)
1730	3	9	1669	1662 vs	1659 w	1658.3	24	ν(C5=C6) + ν(ring)
1520	4	3	1471	1502 m	1506 vw	1503.6	23	δ(N1-H) + ν(ring)
1479	2	9	1432	1430 s	1425 w	1424.1	22	δ(N3-H) + δ(N1-H) + δ(ring)
1429	4	1	1385	1349 m	1350 vs	1348.5	20	ν(ring) + δ(N1-H)
1286	18	3	1249	1225 m	1225 m	1224	28	ν(C-F) + ν(ring)
1218	2	2	1185	1181 m	1187 vw	1186.8	18	δ(C6-H) + δ(N3-H) + δ(ring)
975	3	2	956	949 w	950 vw	949.5	14	δ(N3-H) + δ(C6-H) + δ(ring)
937	9	0	920		937 vw	936	9	γ(N3-H)
892	1	1	877	880 m	887 vw	894.8	15	γ(C6-H)
823	3	1	812	813 s	810 vw	809.1	17	δ(ring)
762	1	6	754	771 w	769 m	767.9	12	δ(ring)

747	1	0	740	740 m	738 vw	734.7	11	γ(C2=O) + γ(C4=O) + γ(ring)
639	1	2	638	643 m	639 w	638.3	7	δ(ring)
541	2	1	546	546 s	546 w	545.2	5	6b, δ(ring)
462	1	1	471	469 s	471 w	470	16	δ(C-F) + δ(C=O) + δ(ring)
408	3	0	420	419 s	414 vw	414	3	δ(OCNCO) + δ(ring)
346	1	0	361		372 vw	371	13	γ(NC=CF) + γ(ring)
321	0.2	0	338		333 vw	331	6	δ(OCCF) + δ(ring)
177	0	0	202		207 vw	207	1	γ(C=O) + γ(N3-H) + γ(ring)
117	0.2	0	145		167 vw	167	2	γ(C=O)+ γ(C-F)+ γ(N1-H)+ γ(ring)
80	0	1	110		110 ms	109		lattice modes
65	0.2	0	96		92	91		lattice modes

[a] Scaling equation was $\nu^{scaled} = 34.6 + 0.9447 \cdot \nu^{calculated}$ from ref [37,62]. [b] Notation of the uracil ring modes from ref [64].

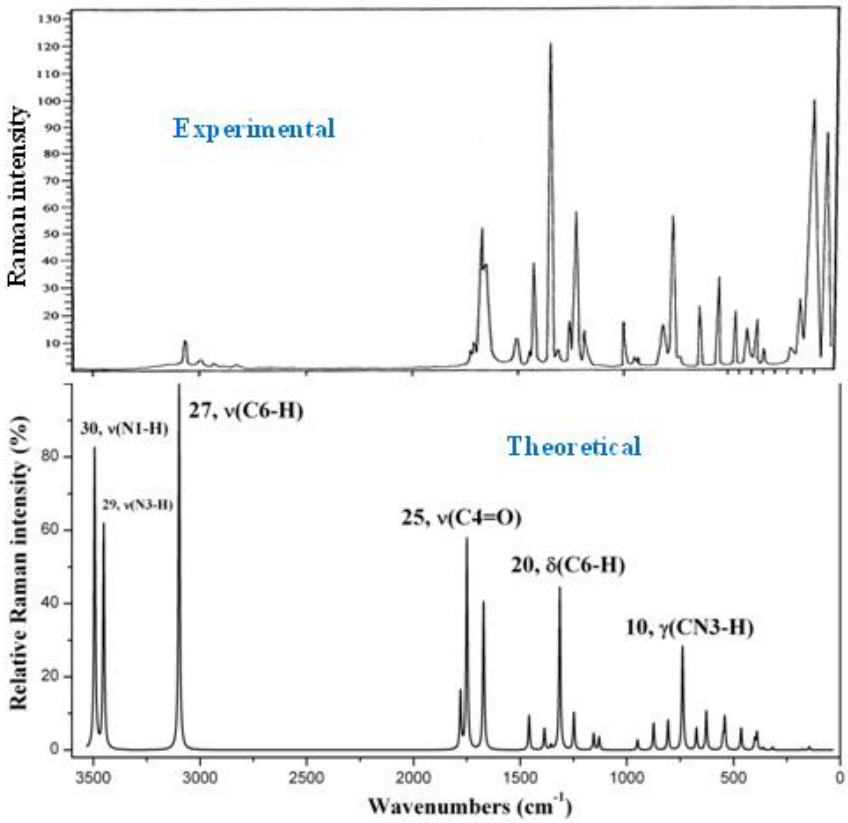

Fig 7. Comparison of the experimental Raman spectra of 5-FU with the theoretical scaled spectrum at the B3LYP/6-31G(d,p) level. The LSE scaling procedure was based on the established equation: $\nu^{scal} = 34.6 + 0.9447 \cdot \nu^{cal}$.

The scaled Raman spectrum is shown in Fig 7. The calculated wavenumber positions of the characteristic vibrations of 5-FU are close to the experimental ones. This can be observed in the comparison of the scaled wavenumbers in column 4th with the experimental values in the next three columns. As mentioned

above, the larger deviations correspond to the modes involved in intermolecular H-bonds, mainly to the stretching of N1-H, N3-H, and C4=O.

As expected, the Raman spectrum of 5-FU noticeably differs of that found for 5-FC, which is displayed in Fig 8. The main differences correspond to the presence of an amino NH_2 group in 5-FC, whose bands are in general strongly active in both Raman and IR. For simplicity, the theoretical scaled spectrum was not included, as well as the corresponding IR ones.

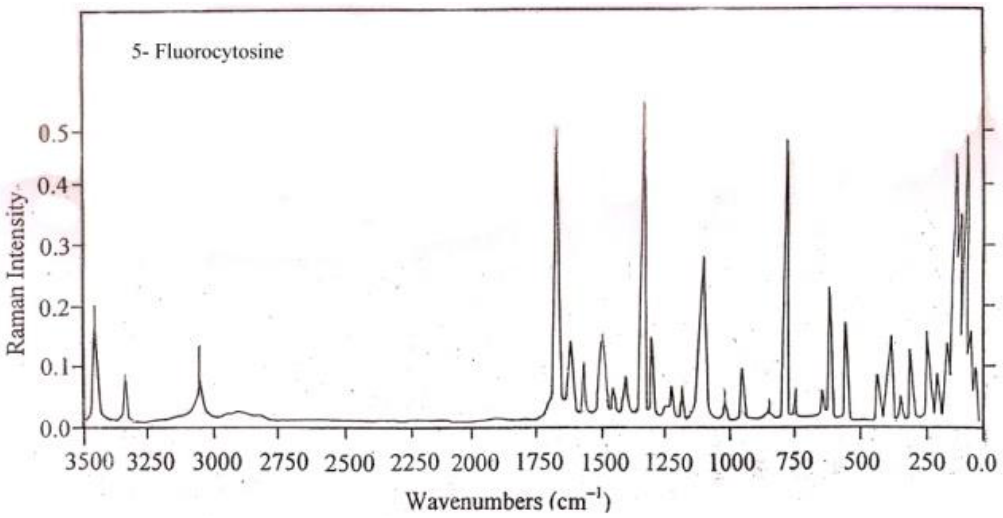

Fig 8. Experimental Raman spectrum of 5-FC in the solid state.

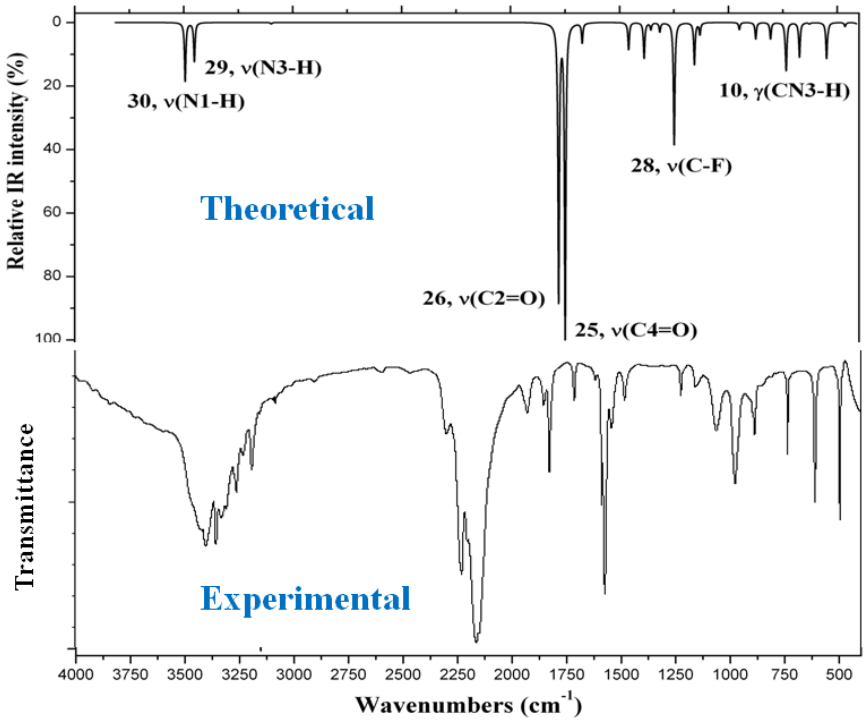

Fig 9. Comparison of the theoretical scaled IR spectrum of 5-flurouracil (5-FU) with the experimental one [47].

The scaled IR spectrum obtained is shown in Fig 9. Analogously, the larger deviations correspond to the modes involved in intermolecular H-bonds. No band was observed above 3500 cm^{-1} due to the stretching *ν*(O-H) mode. Therefore, only the *keto* **T1** form exists in the solid-state sample of 5-FU.

For simplicity, a short description of the characteristic or strongest vibrations observed in the IR/Raman spectra of the solid-state sample were included, as follows:

N-H modes:

No bands were detected in the 3500-3200 cm^{-1} range of the IR and Raman experimental spectra. This indicates that all N1-H and N3-H groups are involved in an intermolecular H-bond, as is observed in the crystal study [55]. In this net, weak C-H···O=C intermolecular H-bonds can be identified shifting the ν(N1-H), ν(N3-H) and ν(C-H) stretching modes to lower wavenumbers. The N-H stretching region exhibits a broad band, Fig 9, which originates from the hydrogen-bonded network. Nevertheless, it is difficult to determine the fundamentals modes. In our simulation with the dimer form, only the N3-H group is involved in H-bonds and appears shifted ca. 300 cm^{-1} to lower wavenumbers. Moreover, this *ν*(N3-H) mode has the highest IR and Raman intensities on the scaled wavenumber at 3112 cm^{-1} and is in good agreement with the observed bands at 3069 cm^{-1} (IR) and 3067 cm^{-1} (Raman). The small spectral difference (40-90 cm^{-1}) could indicate a stronger H-bond in the crystal form than in our model.

The in-plane bending δ(N3-H) mode is also involved in strong H-bonds and thus, it was scaled at 1432 cm^{-1}. It is related to the Raman band at 1424 cm^{-1} and experiences a blue-shift of 58 cm^{-1}. The out-of-plane γ(N3-H) mode appears to move to higher wavenumbers at 220-250 cm^{-1} in accordance to the red-shift observed in its stretching band. The weak Raman band at 936 cm^{-1} was assigned to this vibrational mode.

Our simplified model [47] did not consider the N1-H···O=C intermolecular H-bonds present in the crystal of the solid state sample [55] and, therefore, a large difference was observed between the scaled value at 3491 cm^{-1} and the experimental ones at 3141 cm^{-1} (IR) and 3143 cm^{-1} (Raman) for the ν(N1-H) stretching mode. The ν(N1-H) mode exhibited a similar spectral shift of ca. 300 cm^{-1}, in good agreement with the observed experimental value.

C=O stretching modes:

In the experimental spectra of uracil and 5-halouracil, one can expect two main bands due to C=O stretching modes. The fluorine atom has a significant influence on the positions of the ν(C=O) modes. The halogen effect on the C4=O group is stronger than on the C2=O group, which is located farther from the fluorine atom. Moreover, it is surrounded by the two N-H groups, which buffer it from possible influences of the other molecular moieties.

The ν(C2=O) and ν(C4=O) stretchings appear slightly coupled with the δ(N-H) does. Both oxygen atoms are involved in H-bonds, and both ν(C2=O) and ν(C4=O) modes appear at lower wavenumbers in the experimental spectrum than in our theoretical predictions. Thus, the two lines observed in the IR and Raman spectra at 1722 and 1725 cm^{-1}, respectively, were assigned to the C2=O and C4=O stretching vibrations.

C-F modes:

The ν(C-F) stretching mode usually appears in the 1000-1450 cm^{-1} range and has strong intensity, in both the Raman and IR spectra. Therefore, it was assigned at ~1160 cm^{-1} in fluorothymines [65]. The C-F moiety is expected to be little affected by the H-bonds. Hence, the medium intense band appearing at 1225 cm^{-1} in IR and at 1224.0 cm^{-1} in Raman was assigned to this vibrational mode. The in-plane bending δ(C-F) mode was assigned to the observed experimental band at 469 (IR) and at 470 cm^{-1} (Raman) according to literature values [41,42].

3.5. Watson-Crick (WC) *base-pairs*

The optimized Watson-Crick (WC) base-pairs of 5-FU with adenine and 5-FC with guanine are shown in Fig 10. The pair of 5-FU with adenine was labeled as 5-FU:A, while the 5-FC with guanine pair

was denoted as 5-FC:G. Similarly, the canonical pair of uracil with adenine was labeled as U:A, while the pair of cytosine with guanine was named as C:G. The labeling of the atoms is the standard one. The subscripts (U, A, C, G) on the labeled atoms correspond to uracil, adenine, cytosine and guanine, respectively. The intermolecular H-bond values were obtained at the M06-2X/6-31G(d,p) level.

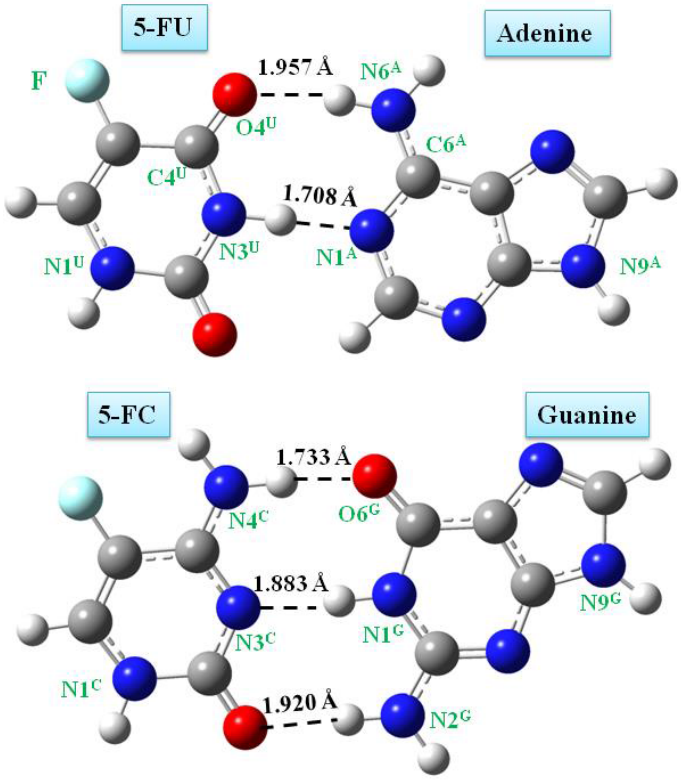

Fig 10. Comparison of the optimized WC base pairs with 5-FU and 5-FC molecules at the M06-2X/6-31G(d,p) level.

The optimized geometrical values of the canonical WC pairs, as well those of 5-FU and 5-FC are provided in Table 7. One can notice that the fluorine atom of the WC pair shortened the N1-C2 and N3-C4 bond lengths and lengthened the C2-N3 bond when compared to the canonical pairs. The effect of the fluorine atom is almost the same in both molecules. The shortening of the C4=O bond length by the fluorine atom in 5-FU (from 1.226 Å to 1.222 Å) is similar to one on the C4-N4 bond length in 5-FC (from 1.330 Å to 1.325 Å). In the WC pairs, the effect of the fluorine atom is almost the same on the C4=O bond length as on C4-N4 bond. As expected, the fluorine atom does not significantly impact the C2=O bond length and the N1-C2-N3 bond angle.

The effect of the fluorine atom is also the same on the C2-N3-C4 angles with an opening of 0.5º in both 5-FU and 5-FC. The pyrimidine ring in the canonical WC pairs, as well as those in 5-FU and 5-FC, are almost fully planar with torsional angles N1-C-N3-C, C2-N3-C-C, and C-N1-C-N3 of nearly 0.0º.

The presence of the fluorine atom in the 5-FU:A pair drastically shortens the intermolecular $O4^{U}\cdots H6^{A}$ H-bond (0.03 Å) when compared to the canonical forms. It also lengthenes the $O4^{U}\cdots H6^{A}$-bond by a similar value. This fluorine effect is small for the 5-FC:G pair, especially in the $N1^{C}\cdots H1^{G}$ H-bond with a length reduction of 0.006 Å. As a result, it will modify the DNA/RNA helix and blocks the tumor growing processes.

Table 7. Optimized geometrical parameters at the M06-2X/6-31G(d,p) level in the WC pairs: uracil:adenine, 5-FU:adenine, cytosine:guanine, and 5-FC:guanine. The values correspond to the pyrimidine ring, except for those indicated by a subscript letter.

	Bond lengths (Å)					Bond angles (º)		Intermolecular H-bonds	
WC pair	N1-C2	C2=O	C2-N3	N3-C4	C4=O /C4-N4	N1-C2-N3	C2-N3-C4	$H3^{U}\cdots N1^{A}$ $N1^{C}\cdots H1^{G}$	$O4^{U}\cdots H6^{A}$ $H4^{C}\cdots O6^{G}$
U:A	1.392	1.214	1.373	1.385	1.226	113.8	127.2	1.734	1.935
5-FU:A	1.387	1.214	1.379	1.379	1.222	113.9	127.7	1.708	1.957
C:G	1.403	1.227	1.357	1.332	1.330	117.2	121.0	1.889	1.752
5-FC:G	1.400	1.227	1.363	1.327	1.325	117.3	121.5	1.883	1.733

Table 8 collects several intermolecular geometrical parameters of interest. The WC pairs are planar with $C4\text{-}N3^{U}\cdots N1\text{-}C6^{A}$, $C2\text{-}N3^{C}\cdots N1\text{-}C2^{G}$ and $N1\text{-}C1^{C}\cdots N1\text{-}C1^{G}$ intermolecular torsional angles of 0.0º. The only exception is the $C4\text{-}N3^{C}\cdots N1\text{-}C6^{G}$ torsional angle with a value of –1.0º in the C:G pair. The intermolecular H-bonds, $H3^{U}\cdots N1^{A}$ and $N3^{C}\cdots N1^{G}$, are almost linear, with a bond angle close to 180º. The linearity is slightly higher in the U:A and 5-FU:A pairs than in the C:G and 5-FC:G pairs. The fluorine atom marginally reduces the linearity of this H-bond. This effect may also affect the DNA/RNA helix growing process. Because the $H3^{U}\cdots N1^{A}$ intermolecular H-bond is shorter in the presence of the fluorine atom than in the related canonical form, the distance between the $N1^{U}\cdots N9^{A}$ atoms is remarkably reduced. This means that the 5-FU molecule is closer to guanine than the canonical form of uracil.

Table 8. Several intermolecular geometrical parameters in the WC pairs under study.

WC pair	$O2^{C}\cdots H2^{G}$	$N1^{U}\cdots N9^{A}$ $N1^{C}\cdots N9^{G}$	$N3\text{-}H3^{U}\cdots N1^{A}$ $N3^{C}\cdots H1\text{-}N1^{G}$	$C4\text{-}N3^{U/C}\cdots N1\text{-}C6^{A/G}$
U:A	--	8.799	178.8	0.0
5-FU:A		8.767	178.6	0.0
C:G	1.897	8.988	177.4	–1.0
5-FC:G	1.920	8.994	177.1	–0.1

Table 9 collects the calculated NBO atomic charges of the WC pairs described above. The fluorine atom noticeably reduces the negative charge on the O4 atom of 5-FU, but the effect is very small on the N4 atom of 5-FC. As expected, the fluorine effect is very small on N1 of both molecules.

Table 9. Calculated NBO atomic charges in the WC pairs.

WC pair	N1	O2	N3	O4/ N4	$N1^{A}$
U:A	–0.666	–0.647	–0.718	–0.654	–0.628
5-FU:A	–0.664	–0.652	–0.717	–0.628	–0.620
C:G	–0.650	–0.692	–0.678	–0.815	–0.686
5-FC:G	–0.651	–0.689	–0.669	–0.813	–0.685

4 Summary and Conclusions

The most important findings of the present study are the following:

- 5-FU presents flexibility in its pyrimidine ring, while 5-FC shows flexibility in both pyrimidine ring and amino NH_2 group.

- The fluorine atom in the uracil/cytosine molecules does not change the stability order of the tautomers at the MP2 level.
- The dioxo tautomer **T1** is the most stable form in the isolated state at all levels of computation. However, the water molecules stabilize the *enol* forms more than the *keto* ones. The hydration reduces the relative energy among the tautomers. Although the water molecules can protect **T1** from tautomerization, they also reduce the energy gap between the *keto* and the *enol* tautomers and increase the reactivity of the oxygen atoms.
- Tautomerism does not change the planarity of 5-FU. However, the hydration significantly changes it. The changes are greater in **T1** and lower in **T2** and **T4**.
- The higher change in the atomic charge on C5 on going from cytosine to 5-FC than from uracil to 5-FU leads to an opposite trend in the charges on the neighboring C4 atom. The negative charge on the fluorine atom is slightly higher in 5-FC than in 5-FU.
- The fluorine atom increases the dipole moment, μ, in **T2** and **T5** tautomers, while decreases it in **T1**, **T3**, **T4** and **T6**. In contrast, the fluorine atom noticeably reduces the value of μ in all 5-FC tautomers. In the canonical *keto* tautomer, 5-FC has a larger μ than in 5-FU, indicating that 5-FC will be favored over 5-FU in a biological hydrated medium. Thus, 5-FC may have a stronger anticancer effect when it is used in this environment.
- The crystal unit cell of 5-FU is fully planar with strong H-bonds, while that of 5-FC is slightly out-of-plane through the amino NH_2 group and has slightly weaker H-bonds. The large flexibility of the NH_2 group facilitates the intermolecular H-bonds and, therefore, a symmetric arrangement of molecules is obtained in 5-FC. The dipole moment value, μ, is noticeably higher in the *keto* tautomer of 5-FC than in 5-FU. In 5-FU, the fluorine atom increases μ for the **T2** and **T5** tautomers, and decrease for the **T1**, **T3**, **T4**, and **T6** tautomers. In contrast, the fluorine atom reduces noticeably the value of μ in all 5-FC tautomers.
- The lowest μ value of the **C2b** tautomer and the highest value of the keto **C1** tautomer identify **C2b** as the lowest stabilized tautomer, and **C1** as the most stabilized one.
- The LUMO energy values are noticeably lower in their negative values for 5-FC than 5-FU, while the HOMO values are slightly higher for 5-FC than for 5-FU. The fluorine atom decreases the LUMO values and increases the negative values of the HOMO.
- The energy gap (E_g) between the HOMO and LUMO frontier orbitals are remarkably higher in uracil/5-FU than cytosine/5-FC, i.e. uracil/5-FU molecules are more stable than cytosine/5-FC. The fluorine atom reduces the E_g value, i.e. 5-FU/5-FC are more reactives than the corresponding uracil/cytosine.
- The absence of a *ν*(O-H) stretching band in the experimental IR and Raman spectra of 5-FU and 5-FC in solid state indicates that both molecules exist only in the *keto* form.
- The scaled wavenumbers in 5-FU are close to the experimental IR/Raman values. The use of an accurate scaling equation procedure reduces the error in the calculated wavenumbers and thus, the assignment of most of the vibrational fundamentals is believed to be unambiguous.
- The fluorine atom considerably shortens the intermolecular $O4^{U}\cdots H6^{A}$ H-bond in the 5-FU:A WC pair when compared to the canonical ones, and lengthens the $O4^{U}\cdots H6^{A}$ by a similar degree. In addition, it slightly reduces the linearity of the $N3^{U}$-H$\cdots N1^{A}$ H-bond. This fluorine effect is smaller in the 5-FC:G pair. Because this effect is stronger in 5-FU than in 5-FC, 5-FU is expected to modify the DNA/RNA helix growing process to a higher degree and therefore, to better block the tumor growing processes than 5-FC.

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Conflicts of Interest

The authors declare no conflict of interest.

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