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5–Fluorouracil: Computational Studies of Tautomers and NMR Properties

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Abstract: Chemical computations were performed to investigate stabilities and properties for tautomers of 5–fluorouracil (5FU). In addition to optimized properties, nuclear magnetic resonance (NMR) parameters were calculated for all atoms of the stabilized structures. Di–keto form of 5FU is the most stable structure and keto–enol and di–enol structural forms are tautomeric structures. According to the results, the polar and non-polar solvents media and tautomeric forms are both important in characterizing 5FU structures.

Keywords: *5–Fluorouracil; Tautomer; Chemical computations; Density functional theory; Chemical shift.*

1. Introduction

5–Flourouracil (5FU), as an anticancer drug, is a fluorinated derivative of uracil nucleobase with the fluorination of carbon number five of pyrimidine ring [1]. 5FU has been used for therapies of several types of cancers for years; however, the side effects are still a considerable problem for this popular anticancer drug [2, 3]. Formations of tautomeric structures commonly for heterocyclic structures could be one of the reasons for appearing the side effects [4]. Tautomers are formed by the exchange of hydrogen atoms between nitrogen and oxygen atoms of the heterocyclic ring making high energetic unstable structures ready to destroy the neighborhood systems [5, 6]. Tautomers are also origins of mutations in genetics yielding several defects to living systems [7]. Considerable efforts have been dedicated to characterize and identify various aspects of tautomers especially for biological related counterparts up to now [8 – 11]. Computations are one of the proper techniques for systematic investigations of stabilities and properties for tautomeric systems at the atomic and molecular scales [12]. Characterizations of tautomers of 5FU and other uracil derivatives are interesting for the scientists due to their importance in the living systems [13 – 16]. Within this work, we have performed quantum chemical computations to investigate the stabilities and nuclear magnetic resonance (NMR) properties of tautomers of 5FU in different solvent systems. According to the results of earlier works, 5FU could participate in tautomerization process similar to uracil nucleobase, in which the di–keto form is the most stable structure. Tautomers could be in keto–enol and di–enol forms according to the exchange of hydrogen positions between nitrogen and oxygen atoms. Although the di–keto form has been seen as the most stable one, but the existence of keto–enol and di–enol tautomers are still possible (Fig. 1) [17]. Chemical environments could employ effects on the initial properties of matters especially presence of hydrophobic or hydrophilic solvents. Hereby, effects of five solvents including water, methanol, ethanol, chloroform, and carbon

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tetrachloride have been investigated on the properties of 5FU and its tautomers within current research. In fact, the major question of this work is to investigate the properties of 5FU and tautomers in the conventional and mostly used solvent media.

2. Computational Details

Density functional theory (DFT) calculations have been performed employing the B3LYP exchange–correlation functional and the 6–31G* standard basis set as implemented in the Gaussian 98 package [18]. First, the investigated molecular structures of 5FU including di–keto (Fig. 1, Panel a), keto–enol (Fig. 1, Panels b – e), and di–enol (Fig. 1, Panel f), totally six forms, have been optimized to achieve the optimized structures corresponding to minimum energies. Next, the presence of five conventional and mostly used solvents including water (H2O), methanol (MeOH), ethanol (EtOH), chloroform (CHCl3), and carbon tetrachloride (CCl4) have been considered in the calculations of atomic and molecular properties. The molecular properties including total energies, dipole moments, and energies for the highest occupied and the lowest unoccupied molecular orbitals (HOMO and LUMO) have been evaluated in different solvent systems (Table 1). Furthermore, chemical shielding (σiso) tensors have been calculated for the atoms of optimized structures based on the gauge–included atomic orbital (GIAO) approach [19] and they have been converted to chemical shifts (δ /ppm) using equation of $\delta = \sigma_{iso,\,reference} - \sigma_{iso,\,sample}$ (Tables 2 – 6). To obtain magnitudes of σiso, reference , tetramethylsilane (TMS) has been used for C and H atoms, ammonia (NH3) has been used for N atoms, and water (H2O) has been used for O atoms, details of evaluations are described elsewhere [20]. Nuclear magnetic resonance (NMR) spectroscopy is among the most versatile techniques to investigate the properties of matters especially in living systems [21]. Chemical shielding tensors are originated from the electronic sites of atoms capable of detecting any perturbations employed to these sites. It is worth noting that, the molecular properties (Table 1) are not enough to recognize the characteristics of matters whereas NMR properties could reveal insightful information at the atomic scale to better achieve the purpose [22, 23]. Due to the complexity of experiments, computations could predict or interpret the characteristics of matters, especially for unstable tautomeric structures. The combinations of results of molecular (Table 1) and atomic (Tables 2 – 6) parameters could very well describe the properties of investigated 5FU models (Fig. 1).

3. Results and Discussion

The models of this work include various forms of 5FU including the initial di–keto form and the keto–enol and di–enol tautomers (Fig. 1). For a quick description of models, nitrogen atoms numbers one and three have their original hydrogen atoms in the initial di–keto form (Panel a, Fig. 1). To make the tautomers, first the position of hydrogen atom number one has been exchanged to oxygen atom number two then atom number four to make the keto–enol forms (Panels b and c, Fig. 1). Afterwards, the hydrogen atom number three has been exchanged to oxygen atom number two then atom number four to make the second set of keto–enol forms (Panels d and e, Fig. 1). For the di–enol form (Panel f, Fig. 1), both of hydrogen atoms have been exchanged to oxygen atoms to make the third set of tautomers for the investigated 5FU.

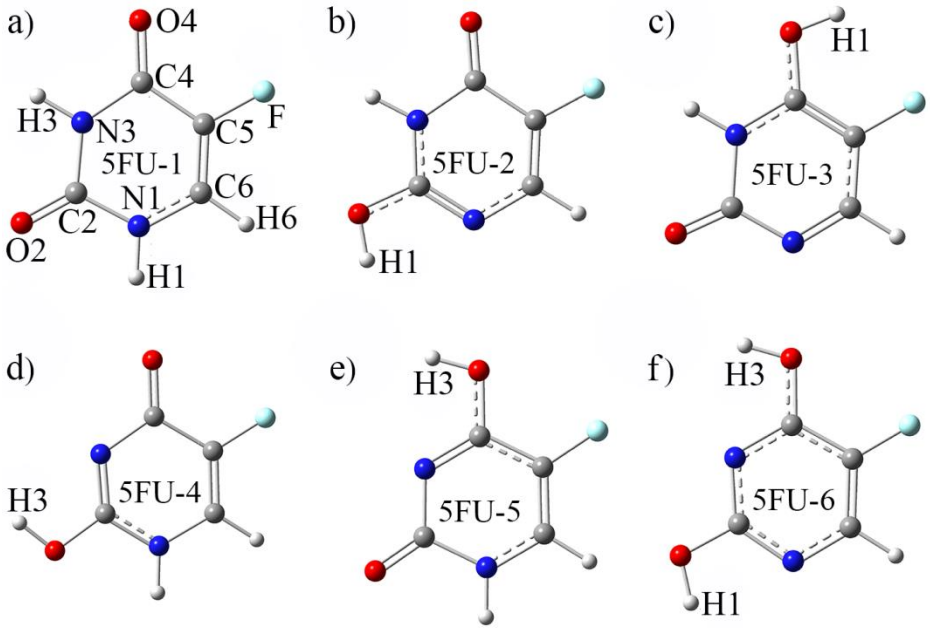

Figure 1. (a) Di–keto, (b) – (e) keto–enol, and (f) di–enol forms of 5FU

The optimization processes indicated that the magnitudes of energies for di–keto forms of 5FU are smaller than other tautomeric forms among different solvents, which shows the best stability of this structure among available tautomers (Table 1). However, the results show that the differences between the energy magnitudes are not significant, which is a clue for participation of the initial di–keto form in the tautomerization processes without a major energy barrier. The results indicate that the polarities of tautomers are changed in different solvents as indicated by dipole moments. It is known that the electronic properties of matters could detect different electrical effects employed by solvents media. 5FU–4 and 5FU–6 have respectively the largest and the smallest magnitudes of dipole moments in all solvent systems. Comparing the effects of solvents reveals that the structures in H2O solvent have the largest magnitude of dipole moments whereas the magnitudes in CCl4 solvents are the smallest ones. The trends of dipole moments properties could be explained because of different charge distributions in each of tautomers and solvents. The energies for the highest occupied and the lowest unoccupied molecular orbitals (HOMO and LUMO) also demonstrate that the conducting properties of structures are changed among the investigated tautomers and solvents. The HOMO and LUMO properties are important for several electronic characteristics of matters especially towards other matters. Moreover, the electronic properties could define the reactivity of chemical substances, which are important to define their characteristic roles in chemical or biochemical systems. The exact energy levels of HOMO and LUMO and the magnitudes of differences between the two levels are mainly due to changes happened to initial properties of matters. It is noted that the di–keto form (5FU–1) is the evidence for tracking the changes of other structures among the models of this work. As an overview of this section, it could be mentioned that the molecular properties of tautomers are different from the evidence molecule.

Table 1. Optimized properties*

Atom	Solvent	5FU–1	5FU–2	5FU–3	5FU–4	5FU–5	5FU–6
E_{Total} /keV	H_2O	–13.988	–13.987	–13.987	–13.987	–13.987	–13.987
	MeOH	–13.988	–13.987	–13.987	–13.987	–13.987	–13.987
	EtOH	–13.988	–13.987	–13.987	–13.987	–13.987	–13.987
	$CHCl_3$	–13.988	–13.987	–13.987	–13.987	–13.987	–13.987
	CCl_4	–13.988	–13.987	–13.987	–13.987	–13.987	–13.987
D_{Moment} /Debye	H_2O	5.056	5.123	7.528	8.798	4.589	0.647
	MeOH	5.021	5.093	7.475	8.732	4.554	0.644
	EtOH	5.002	5.077	7.447	8.697	4.537	0.642
	$CHCl_3$	4.705	4.826	7.009	8.149	4.252	0.620
	CCl_4	4.391	4.548	6.541	7.568	3.949	0.603
E_{HOMO} /eV	H_2O	–6.593	–6.483	–6.332	–6.630	–6.492	–6.739
	MeOH	–6.598	–6.483	–6.333	–6.629	–6.493	–6.739
	EtOH	–6.601	–6.483	–6.334	–6.629	–6.494	–6.739
	$CHCl_3$	–6.647	–6.485	–6.340	–6.628	–6.502	–6.737
	CCl_4	–6.698	–6.487	–6.347	–6.624	–6.509	–6.734
E_{LUMO} /eV	H_2O	–1.185	–1.138	–1.468	–0.869	–1.347	–1.073
	MeOH	–1.191	–1.139	–1.471	–0.870	–1.352	–1.074
	EtOH	–1.194	–1.140	–1.472	–0.869	–1.354	–1.074
	$CHCl_3$	–1.239	–1.152	–1.498	–0.869	–1.393	–1.077
	CCl_4	–1.289	–1.165	–1.528	–0.869	–1.435	–1.080

* See Fig. 1 for the model structures.

Moreover, the polarities as detected by the magnitudes of dipole moments are also different for the investigated structures among the tautomeric forms and solvents media. Although the stabilities are not very different, but the type of solvent has a remarkable effect on the initial properties of 5FU model structures. The molecular orbital energy levels and their corresponding electronic properties are mainly dependent on tautomeric forms and solvents media.

NMR Properties

To better investigate the considered systems at the atomic levels, chemical shifts (δ /ppm) for atoms of the optimized 5FU structures are listed in Tables 2 – 6 based on the atoms types in NMR measurements. The first set of NMR data belongs to three hydrogen atoms of 5FU in different solvents (Table 2). Hydrogen atoms numbers one and three (H1 and H3) participate in tautomerization processes but hydrogen atom number six (H6) is kept fixed. Interestingly, the properties for H6 are changed in tautomeric structures meaning that in–direct effects detection of tautomerization by the electronic site of this atom. For H1, which is in its original position in 5FU–1, 5FU–4, and 5FU–5, different results are seen. When the position of H3 is changed, the effects of tautomerization on the properties of this atom are still recognized. For H3, which is in its original position in 5FU–1, 5FU–2, and 5FU–3, different results are also achieved parallel to results of H1. Indeed, the hydrogen atom plays the major role in tautomerization process, in which its own properties are changed among tautomeric structures. Moreover, the largest magnitudes of shifts are seen in H2O solvent and the smallest magnitudes are seen for the CCl4 solvent. In fact, the hydrogen atom has a small magnitude of electron at the atomic site but it is still enough to detect the effects of any employed perturbations revealing the importance of NMR properties in materials characterizations.

The NMR properties for four carbon atoms are listed in Table 3. Since the carbon atoms make the skeleton of heterocyclic ring, their properties are very important in definitions of their structural properties. Changes of the hydrogen atom position around the ring could make effects to the initial properties of carbon atoms.Different magnitudes of

shifts for each of carbon atoms in different tautomers and solvents show that the properties are very sensitive to environment. Although C5 and C6 do not directly participate in tautomerization, but the results indicate that the properties are changed during this process. Polarities of solvents are also important for the properties of carbon atoms as could be seen by the changes of chemical shifts in different environments.

Table 2. ^{1}H Chemical shifts (δ /ppm)*

Atom	Solvent	5FU–1	5FU–2	5FU–3	5FU–4	5FU–5	5FU–6
H_1	H_2O	5.876	5.636	5.839	6.552	6.595	5.808
	MeOH	5.852	5.619	5.814	6.519	6.572	5.795
	EtOH	5.839	5.610	5.801	6.502	6.560	5.788
	$CHCl_3$	5.630	5.464	5.587	6.227	6.366	5.674
	CCl_4	5.398	5.292	5.350	5.931	6.152	5.544
H_3	H_2O	6.370	7.308	6.750	5.340	5.750	5.635
	MeOH	6.364	7.295	6.731	5.328	5.741	5.622
	EtOH	6.360	7.288	6.722	5.321	5.736	5.614
	$CHCl_3$	6.302	7.171	6.561	5.207	5.652	5.493
	CCl_4	6.225	7.036	6.379	5.064	5.542	5.350
H_6	H_2O	6.946	7.318	8.201	6.870	7.208	7.841
	MeOH	6.929	7.311	8.201	6.850	7.192	7.835
	EtOH	6.920	7.308	8.201	6.839	7.184	7.833
	$CHCl_3$	6.780	7.248	8.199	6.672	7.047	7.788
	CCl_4	6.627	7.181	8.190	6.492	6.898	7.739

* See Fig. 1 for the model structures.

Table 3. ^{13}C Chemical shifts (δ /ppm)*

Atom	Solvent	5FU–1	5FU–2	5FU–3	5FU–4	5FU–5	5FU–6
C_2	H_2O	138.112	144.356	142.311	144.616	142.258	152.386
	MeOH	138.072	144.310	142.249	144.553	142.196	152.388
	EtOH	142.249	144.286	142.160	144.519	142.163	152.388
	$CHCl_3$	137.713	143.886	141.682	143.976	141.625	152.393
	CCl_4	137.328	143.433	141.085	143.365	141.020	152.386
C_4	H_2O	148.562	145.648	145.639	154.594	154.088	151.672
	MeOH	148.509	145.594	145.562	154.513	154.083	151.668
	EtOH	145.562	145.566	145.522	154.470	154.080	151.666
	$CHCl_3$	148.037	145.109	144.883	153.785	154.016	151.619
	CCl_4	147.553	144.602	144.210	153.026	153.905	151.546
C_5	H_2O	136.181	142.429	128.608	142.174	129.569	136.239
	MeOH	136.202	142.456	128.536	142.214	129.555	236.229
	EtOH	128.536	142.471	128.498	142.236	129.547	136.225
	$CHCl_3$	136.390	142.710	127.909	142.581	129.431	136.154
	CCl_4	136.582	142.980	127.310	142.959	129.313	136.086
C_6	H_2O	121.671	133.101	148.045	117.887	126.850	140.538
	MeOH	121.528	133.039	148.054	117.695	126.721	140.504
	EtOH	148.054	133.008	148.058	117.594	126.653	140.486
	$CHCl_3$	120.275	132.492	148.091	116.025	125.592	140.204
	CCl_4	119.040	131.926	148.053	114.406	124.490	139.912

* See Fig. 1 for the model structures.

Table 4. ^{15}N Chemical shifts (δ /ppm)*

Atom	Solvent	5FU–1	5FU–2	5FU–3	5FU–4	5FU–5	5FU–6
N_1	H_2O	120.616	185.498	247.807	110.656	142.896	228.624
	MeOH	120.398	185.482	248.454	110.329	142.7007	228.685
	EtOH	120.284	185.474	248.793	110.159	142.599	228.718
	$CHCl_3$	118.493	185.369	254.214	107.496	141.003	229.280
	CCl_4	116.622	185.314	249.905	104.745	139.356	229.979
N_3	H_2O	157.261	153.487	141.543	214.289	218.059	211.600
	MeOH	157.233	153.405	141.370	214.492	218.341	211.719
	EtOH	157.218	153.362	141.279	214.598	218.489	211.781
	$CHCl_3$	156.966	152.671	139.844	216.255	220.826	212.752
	CCl_4	156.670	151.911	138.318	217.969	223.303	213.768

* See Fig. 1 for the model structures.

Table 5. ^{17}O Chemical shifts (δ /ppm)*

Atom	Solvent	5FU–1	5FU–2	5FU–3	5FU–4	5FU–5	5FU–6
O_2	H_2O	265.784	123.014	289.433	123.430	282.438	130.720
	MeOH	266.368	122.931	290.613	123.308	283.495	130.845
	EtOH	266.675	122.888	291.234	123.245	284.052	130.909
	$CHCl_3$	271.707	122.183	301.344	122.251	293.154	131.952
	CCl_4	277.430	121.416	312.666	121.228	303.443	133.079
O_4	H_2O	298.320	307.439	99.897	274.180	134.099	120.311
	MeOH	297.095	308.440	99.658	272.196	134.178	120.378
	EtOH	296.452	308.966	99.534	271.153	134.219	120.413
	$CHCl_3$	286.201	315.708	97.592	254.416	134.810	120.936
	CCl_4	275.097	306.338	95.601	236.075	135.316	121.443

* See Fig. 1 for the model structures.

The magnitudes of chemical shifts of nitrogen atoms (Table 4) are significantly changed from the initial di–keto form to keto–enol and di–enol forms. The solvent effects are also observed for the NMR properties of nitrogen atoms.

O2 and O4 are two different types of oxygen atoms, a urea type and an amide type, respectively. The magnitudes of chemical shifts for O2 and O4 (Table 5) also demonstrate different chemical properties for these atoms according to their own types. The keto and enol forms (oxo and hydroxy forms) are very important to be considered for each oxygen atom. The oxygen atoms are also similar to nitrogen atoms due to excess of electrons in the valance shells; therefore, the effects are significant on their properties. The fluorine atom, which is the characteristic atom of 5FU, also shows the detections of effects through tautomerization. The major effects are especially seen for 5FU–3, in which the hydrogen atom has been oriented to fluorine atom. The effects of solvents on the properties of fluorine atom are also observed. As an overview of atomic scale NMR properties, it could be mentioned that the properties of all atoms could undergo significant effects through tautomerization processes, in which the type of solvent media and the form of tautomeric structure are both important for chemical characterizations. From H_2O to CCl_4, polar to non-polar solvents, the influences are detected by the NMR properties of atoms. Since the electrical properties of solvents are different, the corresponding electronic properties are also different for atoms in different solvent media. It could be mentioned that the NMR properties, which are originated from the electronic sites, could well detect the atomic scale properties of 5FU tautomers in different media. The potential reader can find here that choosing the solvent media is very important for chemical substances characterizations especially at the atomic levels.

4. Conclusion

The performed quantum chemical computations on the possible forms of 5FU during tautomerization could reveal some remarkable trends. First, the molecular properties are not good enough to well describe the characteristics of matters and the atomic scale properties are needed for the purpose. Second, the small magnitudes of energy differences among the initial di–keto form and the keto–enol and di–enol forms indicated that the tautomeric structures of 5FU could be formed without any significant energy barriers. Third, the type of solvent media and the type of tautomeric could influence on the properties of 5FU structures as indicated by the magnitudes of dipole moments and HOMO / LUMO properties. Fourth, atomic scale NMR properties could well describe the electronic properties of 5FU structures as indicated by the magnitudes of chemical shifts in different tautomers and solvents media. And finally, due to specific electrical properties for each solvent, it is important to select the type of solvent for the studies. Polar solvents like H2O, MeOH and EtOH are almost similar but there are significant differences between the polar solvents and the non-polar solvents, $CHCl_3$ and CCl_4; therefore, the solvent shod be carefully chosen for the desired investigations.

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