

# Theoretical studies on the properties of uracil and its dimer upon thioketo substitution

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**Abstract** The influences of thioketo substitution on the properties of uracil monomer and dimer and their interactions with  $Zn^{2+}$  have been systematically investigated at the B3LYP/6-311+G\* level of theory. Those properties include the structural characteristics, acidities, ionization potentials, and singlet–triplet energy gaps of SU monomers and their dimers, where SU=2-thiouracil, 4-thiouracil, and 2,4-dithiouracil, respectively. Computational results suggest that thioketo substitution leads to an increase in the acidities of the N–H groups for both uracil and its dimer, where the  $N_1$ –H group is still the most acidic site relative to that of  $N_3$ –H group. However, the opposite behaviors are true for the ionization potentials and the singlet–triplet energy gaps of uracil monomer and its dimer, suggesting that thiouracils are more susceptible to radiation damage relative to the unsubstituted uracil. For uracil and 2-thiouracil, the corresponding triplet excited-state geometries are predicted to be highly nonplanar compared with the planar geometries of the ground state as well as 4-thiouracil and 2,4-dithiouracil upon triplet excitation. As a rule, the intermolecular H-bonds involving the sulfur atom directly have been influenced more significant than those the oxygen atom directly involved for U::U and SU::SU base pairs upon ionization and excitation. Additionally,  $Zn^{2+}$

binding is expected to lead to an increase in the stability of U::U and SU::SU base pairs.

**Keywords** Uracil · Thioketo substitution · Acidities · Ionization potential (IP) · Singlet–triplet energy gap

## 1 Introduction

In recent years, uracil and its thio derivatives have already become the intense focus of research due to their biological, pharmacological, and spectroscopic activities [1–6]. For example, a series of thiouracils, such as 2-thiouracil, 4-thiouracil, and 2,4-dithiouracil, have been identified as minor components of t-RNA and peptide nucleic acids, where the former two thiouracils have also been used as anticancer and antithyroid drugs and for the treatment of heart disease [7–18]. The inappropriate replacement of uracil by a thiouracil can be responsible for the misrecognition in m-RNA [19]. Moreover, their various tautomeric forms seem to be important for explaining the mutation occurring during DNA duplication [20–22]. Meanwhile, the complexes of thiouracil derivatives with transition metal ions of biological importance have been shown to exhibit anticancer and antimicrobial activity [23, 24]. Additionally, thiouracils have also been proposed to be efficient metal corrosion inhibitors [25]. Thus, the properties and characteristics of thiouracils are of great interest in pharmaceutical researches and applications.

Obviously, to better understand the different roles of thiouracils in biological activity, it is necessary to gain a detailed knowledge of the properties and characteristic changes induced by the bulkier and more polarizable sulfur atom. However, limited information is known regarding their intrinsic reactivity although the acidic–basic behaviors and the interactions with alkali metal and copper ions have been

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reported for the thiouracil monomers [26–29]. The selected properties, such as ionization potentials, singlet–triplet energy gaps, and the interactions of thiouracils with  $\text{Zn}^{2+}$ , have not been systematically investigated to the best of our knowledge. Especially, there is an almost complete lack of information about the influences of thio keto substitution on the U::U base pairs despite their existences in several RNAs [30–33].

Thus, in the present study, theoretical calculations have been systematically carried out to investigate the influences of thio keto substitution on the geometries, acidities, ionization potentials, singlet–triplet energy gaps, reaction activities, and uracil· · · uracil base pairing energies. In view of the fact that  $\text{Zn}^{2+}$  is involved in the deamination of cytosine to uracil in yeast cytosine deaminase [34], the interactions of  $\text{Zn}^{2+}$  with uracil and its dimer have also been explored before and after thio keto substitution. Expectedly, a detailed theoretical study of the aforementioned properties can fill a void in the available data for the property changes upon thio keto substitution of uracil and its dimer. Moreover, it can help to reveal further clues to their different fundamental roles in biological, pharmacological, and spectroscopic activities.

## 2 Computational details

All the geometries of the different species have been fully optimized using the B3LYP method within the framework of density functional theory (DFT) in conjunction with the 6-311+G\* basis set, i.e., Becke's three parameter non-local hybrid exchange potential with the non-local correlation functional of Lee, Yang, and Parr [35,36]. The B3LYP method has been shown to yield reliable geometries for a wide variety of systems also including the relevant uracil species [27–29,37–43], where the reliability of the level of theory used has been confirmed through comparison with the available experimental and theoretical results summarized in the Supporting Information for reference. However, for the systems in which dispersion energy plays a dominant role, it should be noted that DFT fails to locate the corresponding stacked structures due to the lack of London dispersion term [44–49]. This point has been further supported by the present study. As displayed in Figs. S1–S4 of Supporting Information, all the attempts to obtain the stacked or sandwiched structures have failed. Namely, the characteristics of the stacked or sandwiched structures have disappeared during the geometry optimization processes at the B3LYP/6-311+G\* level of theory. Fortunately, in the present study, none of the stacked or sandwiched structures exists and all the dimers are characterized by the intermolecular H-bonds. Additionally, vibrational frequency analysis for each optimized geometry is also performed at the same level of theory in order to identify whether the optimized

structures correspond to a true local minimum or not, as well as to obtain the corresponding thermodynamic parameters.

The acidity of uracil and thiouracils is obtained as the following reaction by losing either the proton of the  $\text{N}_1\text{-H}$  or  $\text{N}_3\text{-H}$  group.



For the following chemical process,



The ionization potential (IP) can be calculated as [50],

$$\begin{aligned} \text{IP} = \Delta E_{\text{elec}} + \Delta(\text{PV}) + \Delta(\text{ZPVE}) + \Delta E_{\text{vib}(298)} + \Delta E_{\text{rot}(298)} \\ + \Delta E_{\text{trans}(298)} = \Delta E_{\text{elec}} + \Delta E_{\text{therm}(298)} \end{aligned}$$

where  $\Delta E_{\text{elec}}$  is the variation in internal energy obtained from the corresponding level of theory;  $\Delta E_{\text{therm}(298)}$ , the variation in thermal energy at 298.15 K, is derived from the calculated harmonic vibrational frequencies, where the vibrational, rotational, and translational corrections have been included. For simplicity, the zero-point vibrational energy (ZPVE) corrections are also included in  $\Delta E_{\text{therm}(298)}$  term. The neutral and charged states correspond to the optimized geometries and consequently the calculated IP refers to the adiabatic ionization potential (AIP). Similarly, the vertical ionization potential (VIP) corresponds to the energy difference between the cationic state in the geometry of neutral state and the optimized neutral state.

For the singlet–triplet state energy gaps, the vertical one is calculated as the energy difference between the total energies of the ground singlet state and triplet state at the optimized singlet geometry. The corresponding adiabatic energy gap is evaluated as the energy difference between the singlet and triplet states at their respective relaxed geometries.

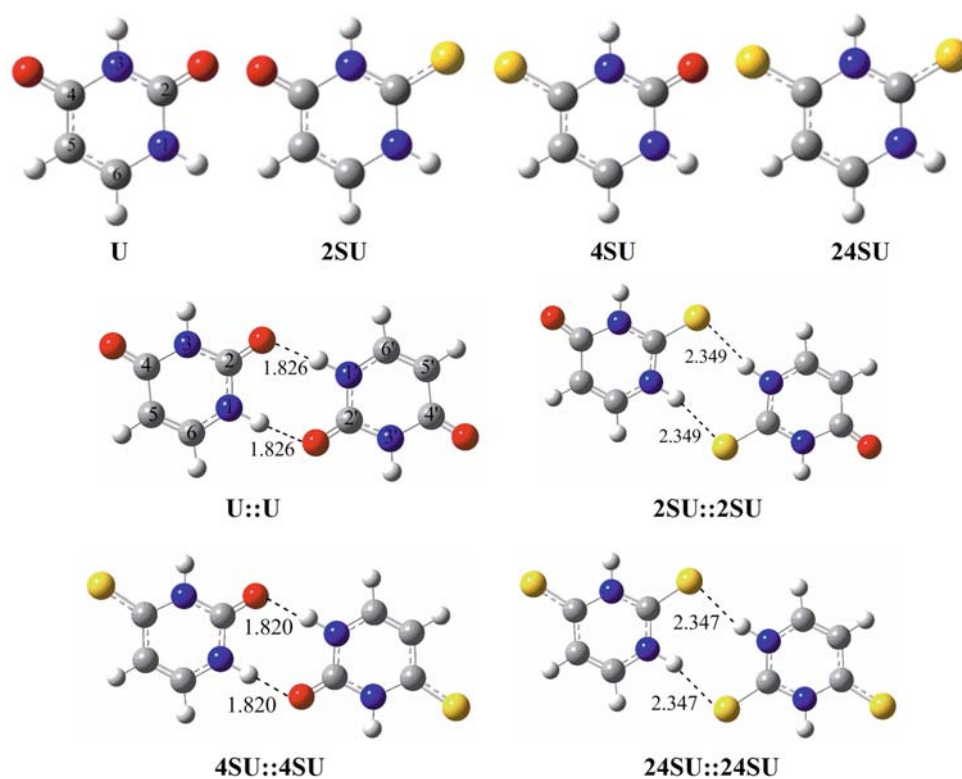
All the calculations have been completed using Gaussian 03 program [51].

## 3 Results and discussion

### 3.1 Influence of thio keto substitution on the structures of uracil monomer and its dimer

As expected, all of the C=S bonds are longer than the C=O bonds due to the larger size of sulfur atom than that of oxygen, where the calculated C=S and C=O bond lengths in U and SU are in the range of 1.685–1.662 and 1.211–1.215 Å, respectively. Dipole moment of uracil is also enhanced upon thio keto substitution (e.g., 4.61 D for U, 4.78 D for 2SU, 4.99 D for 4SU, 4.97 D for 24SU), where the calculated dipole moment of uracil at the B3LYP/6-311+G\* level of theory is well consistent with the available experimental and theoretical results as mentioned in the Supporting Information.

**Fig. 1** The optimized structures of the uracil and its dimers before and after thioketo substitution



**Table 1** The calculated base pairing energies and  $\text{Zn}^{2+}$  binding energies (in kcal/mol)

Complexes	Pairing energy $\Delta E$	Binding energies(X=O, S)	
		X <sub>4</sub> binding	X <sub>2</sub> binding
U::U	15.59(15.9) [53] [19.04] [52]	–	–
2SU::2SU	11.7	–	–
4SU::4SU	15.6	–	–
24SU::24SU	11.6	–	–
$\text{Zn}^{2+}$ (U)	–	173.6	164.1
$\text{Zn}^{2+}$ (2SU)	–	184.6	189.8
$\text{Zn}^{2+}$ (4SU)	–	199.7	178.7
$\text{Zn}^{2+}$ (24SU)	–	202.7	195.8
$\text{Zn}^{2+}$ (U::U)	47.5	205.6	–
$\text{Zn}^{2+}$ (2SU::2SU)	37.6	210.5	–
$\text{Zn}^{2+}$ (4SU::4SU)	47.9	232.1	–
$\text{Zn}^{2+}$ (24SU::24SU)	39.6	230.8	–

Moreover, thioketo substitution at the 4-position leads to a larger increase in dipole moment than that at the 2-position. The calculated HOMO–LUMO energy gaps of U, 2SU, 4SU, and 24SU are found to be 5.63, 4.62, 3.96, and 3.90 eV, respectively, implying the enhancement of the reaction activity upon thioketo substitution.

It is well known that uracil dimer can form a number of H-bonded structures of biological importance [30–33]. In this study, the most stable uracil dimer possessing two equivalent  $\text{C}_2 = \text{O} \cdots \text{H}-\text{N}_1$  hydrogen bonds has been considered

[52–54], where its higher stability relative to those of stacked forms has been confirmed previously [55–58]. Correspondingly, the optimized structures of the U::U and SU::SU base pairs and the calculated base pairing energies have been presented in Fig. 1 and Table 1, respectively. Here, for the U::U dimer, the calculated pairing energy of 15.5 kcal/mol is well consistent with the result of 15.9 kcal/mol at the MP2/6-31G\*(0.25)//HF/6-31G\*\* level of theory [53]. Compared with the benchmark value of 19.04 kcal/mol at the CCSD(T) level [52], B3LYP method underestimates the pairing energies

**Table 2** The calculated gas-phase acidities (in kcal/mol), IPs, reorganization energies (REs), and singlet-triplet energy gaps (in eV)\*

Complexes	Acidity <sup>a</sup>	IPs <sup>b</sup>	REs	$\Delta E(s-t)^c$
U	330.0/343.3[332.5/345.9] [59] [329.1/340.9] [26] [333 ± 4/347 ± 4] [60] [333 ± 5] [61]	9.49/9.27 [9.46/9.22] [39] [9.45/9.20] <sup>d</sup> [9.50 ± 0.03/9.4 ± 0.1] <sup>d</sup>	0.22 [0.24] [39] [0.25] <sup>d</sup>	3.64/2.99 [3.62/3.00] [39] [3.65] [66]
2SU	323.2/335.5 [326.1/334.8] [59]	8.90/8.65	0.25	3.30/2.83
4SU	322.1/336.4 [325.0/339.0] [59]	8.53/8.37	0.16	2.75/2.25
24SU	317.0/330.0 [320.0/332.8] [59]	8.37/8.32	0.05	2.68/2.21
U::U	332.2	8.85/8.74	0.11	4.41/4.04
2SU::2SU	324.7	8.68/7.85	0.83	3.39/3.13
4SU::4SU	325.3	7.88/7.81	0.07	3.20/2.94
24SU::24SU	319.3	7.96/7.88	0.08	2.73/2.49

The available experimental and theoretical results are listed in brackets

<sup>a</sup> The data before and after slash refer to the acidity of N1 and N3 site, respectively. For the dimers, only the acidity at N3 site has been calculated since the N1 site is involved in the formation of intermolecular H-bonds

<sup>b</sup> The data before and after slash refer to the vertical and adiabatic IPs, respectively

<sup>c</sup> The data before and after slash refer to the vertical and adiabatic s-t energy gaps, respectively

<sup>d</sup> Values taken from the NIST standard reference database: <http://webbook.nist.gov/>

slightly, which is consistent with the previous reports [37]. Structurally, the  $C_2=O \cdots H-N_1$  hydrogen bond lengths are similar to each other in the U::U (1.826 Å) and 4SU::4SU (1.820 Å) base pairs. This point is not surprising since the sulfur substituent in the U::U and 4SU::4SU base pairs is not directly involved in the H-bonding interaction, and therefore, small changes occur in the geometry of the base pairs upon thioketo substitution. Compared with the H-bonds in the unsubstituted U::U base pair, the  $C_2=S \cdots H-N_1$  H-bonds have been elongated by 0.523 and 0.521 Å in the 2SU::2SU and 24SU::24SU base pairs, respectively, which can be understood due to the weaker electronegativity and the larger size of S atom relative to that of O atom. As shown in Table 1, the pairing energies have been affected more or less depending on the different sites of thioketo substitution. Here, for the 4SU::4SU base pair, the base pairing energy only changes a little relative to U::U base pair. At the same time, there are decreases of about 2.7 and 2.9 kcal/mol for 2SU::2SU and 24SU::24SU base pairs. Additionally, similar to the monomer, the reaction activities of the base pairs should also be enhanced upon thioketo substitution, where the HOMO-LUMO energy gaps of U::U, 2SU::2SU, 4SU::4SU, and 24SU::24SU are about 5.59, 4.74, 3.99, and 3.94 eV, respectively.

### 3.2 Influence of thioketo substitution on the acidities of uracil monomer and its dimer

It is well known that uracil is important for its properties as a base among the five nucleobases. However, uracil has acidic characters as well, such as the two acidic protons in the  $N_1-H$  and  $N_3-H$  groups. To explore the influence of thioketo

substitution on the acidity of uracil, both of the acidities for  $N_1-H$  and  $N_3-H$  groups have been investigated. The calculated gas-phase acidities are shown in Table 2 as well as the available experimental and theoretical results. Obviously, the calculated results are similar to those of the previous studies [26, 59–61], which confirms the reliability of the B3LYP/6-311+G\* level of theory employed. Here, in the U monomer, the  $N_1-H$  group is more acidic than the  $N_3-H$  group by about 13.3 kcal/mol. The acidity of the  $N_1-H$  ( $N_3-H$ ) group increases by about 6.8 (7.8), 7.9 (6.9), and 13.0 (13.3) kcal/mol for 2SU, 4SU, and 24SU compared with uracil, respectively. In more detail, for 2SU, thioketo substitution leads to an increase in the acidity of the  $N_3-H$  group, being slightly more than  $N_1-H$  group. Correspondingly, the acidity gap between them decreases by only 1.0 kcal/mol. However, the opposite behaviors are true for 4SU, where the corresponding acidity gap increases by 0.9 kcal/mol. For 24SU, the acidity gap between them is similar to that of uracil though thioketo substitution results in a much larger increase in acidity. As a result, the calculated acidities of the  $N_1-H$  groups increase according to the order of  $U < 2SU < 4SU < 24SU$  versus  $U < 4SU < 2SU < 24SU$  for the  $N_3-H$  groups in the gas phase. Thus, the  $N_1-H$  group is still the most acidic site and thioketo substitution leads to an increase in the overall acidities of the  $N_1-H$  and the  $N_3-H$  groups.

Furthermore, in the most stable dimer, only the acidities of the  $N_3-H$  groups have been considered since the  $N_1-H$  groups have been involved in the formation of the H-bonds. As shown in Table 2, the acidities of the  $N_3-H$  groups increase by about 11.1, 10.8, 11.1, and 10.7 kcal/mol for the U::U, 2SU::2SU, 4SU::4SU, and 24SU::24SU base pairs compared with the respective monomer. In more detail,

once thioketo substitution occurs for U::U base pair, the acidities of the N<sub>3</sub>–H group increase by about 7.5, 6.9, and 12.9 kcal/mol for the 2SU::2SU, 4SU::4SU, and 24SU::24SU base pairs, respectively, which is similar to the trend in the monomer upon thioketo substitution. As a result, the calculated acidities of the N<sub>3</sub>–H groups increase according to the order of U::U < 4SU::4SU < 2SU::2SU < 24SU::24SU. Thus, thioketo substitution leads to an increase in the acidities of the N–H groups for both uracil monomer and its dimer in the gas phase.

Qualitatively, to further explore the nature of the enhancement of the acidity in the uracil monomer and dimer upon thioketo substitution, natural bond orbital (NBO) analyses have been performed on the basis of the optimized geometries [62–65]. Calculated results show that the degrees of the polarization of the N–H bonds have decreased more or less upon thioketo substitution, especially for the dithio-substituted cases, where the hybrid coefficients  $n$  for  $sp^n$  of N atom have been increased by about 0.22 upon dithio-substitution. Thus, in the thioketo substituted uracil monomers and dimers, the protons attached to the N atoms can be easily lost.

### 3.3 Influence of thioketo substitution on the ionization potentials of uracil monomer and its dimer

The ionization potential (IP) is fundamental not only in assessing the electron donating ability but also in understanding the electron-transfer process that occurs in the gas phase. In this study, the ionization behaviors for (thioketo substituted) uracil and U::U base pair have been investigated to gain insights into the influence of thioketo substitution on the electron detachment in the gas phase. The calculated AIPs, VIPs, and reorganization energies (REs) have been summarized in Table 2 together with the available experimental and theoretical results [39].<sup>1</sup> Obviously, there is good agreement between the calculated IPs of uracil and the available experimental and theoretical data. The HOMO and spin density contour plots for the neutral and cationic bases and base pairs are depicted in Figs. 2 and 3, respectively.

For uracil monomer and its thio-derivatives, all of the main molecular skeletons have not been distorted and the approximately planar structures are still kept upon ionization. Certainly, some obvious geometrical changes can be also observed. For example, the C5–C6 bond in the uracil monomer U, which is a typical double bond (1.35 Å) in the ground state, becomes almost a single bond (1.40 Å) upon ionization due to the removal of certain electron away from this bond bearing bonding orbital characteristics in HOMO as displayed in Fig. 2. Similarly, the following geometrical changes induced by ionization for thioketo substituted uracil

monomers and dimers can be understood from the diagrams of the relevant HOMOs.

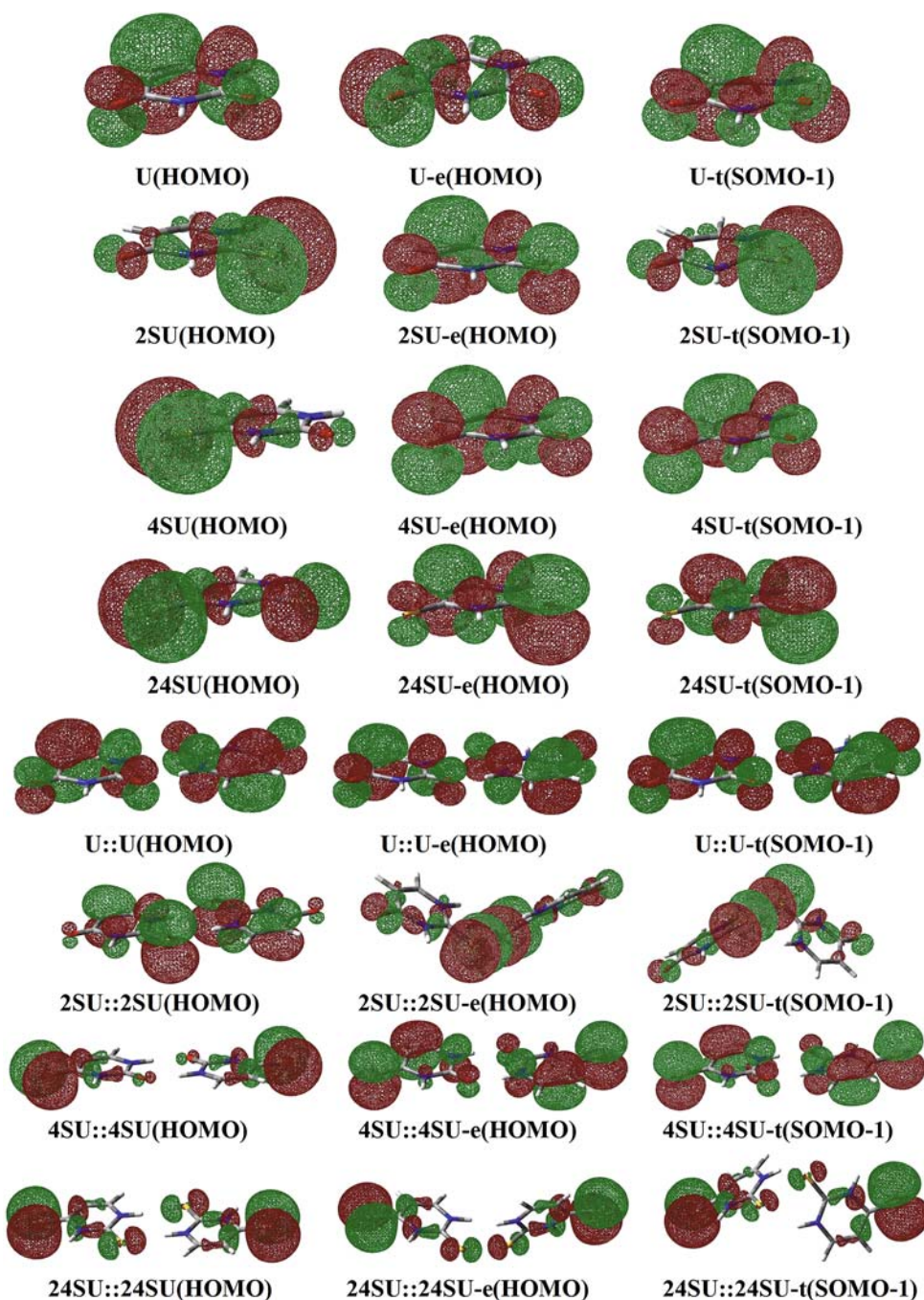
As listed in Table 2, all the relaxation energies (REs) are in the range of 0.05–0.25 eV, indicating that slight nuclear relaxation should occur for uracil and its thio-derivatives upon ionization. At the same time, charge redistribution should occur accompanying the loss of an electron, which can be reflected from the different HOMO shapes between the neutral and cationic molecules as displayed in Fig. 2. Compared with uracil, the calculated AIPs (VIPs) are reduced by about 0.62(0.59), 0.90(0.96), 0.95(1.12) eV for 2SU, 4SU, and 24SU, respectively. As a result, the calculated IPs of the bases decrease according to the order of U > 2SU > 4SU > 24SU in the gas phase. Therefore, it is much easier to lose an electron for uracil upon thioketo substitution, which should be related to the larger size and lower electronegativity of S atom. Moreover, this point can be further supported by the spin density distributions, where the single electron distributions almost concentrate on sulfur atoms as displayed in Fig. 3.

For U::U base pair, the double intermolecular C<sub>2</sub> = O ··· H–N<sub>1</sub> H-bonds have been enhanced upon ionization, which is contrary to that of 4SU::4SU. Here, the corresponding H-bonding distances have been shortened and elongated by about 0.03 and 0.02 Å for the former and the latter, respectively. Moreover, for both base pairs, all of the main molecular skeletons have not been distorted and the geometrical changes are not significant upon ionization. In contrast, as displayed in Fig. 3, for 2SU::2SU and 24SU::24SU base pairs, the molecular skeletons have been distorted significantly. Here, the dihedral angles D(N<sub>1</sub>C<sub>2</sub>O<sub>2</sub>H<sub>1</sub>) have been changed about 58° and 56° in 2SU::2SU and 24SU::24SU, respectively. Correspondingly, the C<sub>2</sub> = S ··· H–N<sub>1</sub> H-bond has been elongated by about 0.217 and 0.221 Å, respectively. Overall, the influence of ionization on the H-bond involving the sulfur atom directly is more significant than that of the oxygen directly involved.

Similar to the results as mentioned above, as shown in Table 2, the trivial nuclear relaxation should occur for U::U, 4SU::4SU, and 24SU::24SU base pairs upon ionization. On the other hand, for 2SU::2SU base pair, the RE of 0.83 eV indicates that larger nuclear relaxation occurs for it upon ionization. Compared with U::U base pair, the calculated AIPs (VIPs) are reduced by about 0.89(0.17), 0.93(0.97), and 0.86(0.89) eV for 2SU::2SU, 4SU::4SU, and 24SU::24SU base pairs, respectively. As a result, the calculated AIPs decrease according to the order of U::U > 24SU::24SU > 2SU::2SU > 4SU::4SU. Thus, the IP of U::U base pair has been decreased upon thioketo substitution, suggesting that SU::SU base pairs are more susceptible to radiation damage through the loss of an electron. Here, as displayed in Fig. 3, the single electron almost localizes on sulfur atoms for the dimers upon thioketo substitution, further supporting the readily oxidation of the sulfur site.

<sup>1</sup> Values taken from the NIST standard reference database: <http://webbook.nist.gov/>.

**Fig. 2** Diagrams of HOMO for the neutral, ionized, and excited monomer and dimer of uracil and thiouracils, where the middle HOMO in every row refers to the case of ionized state

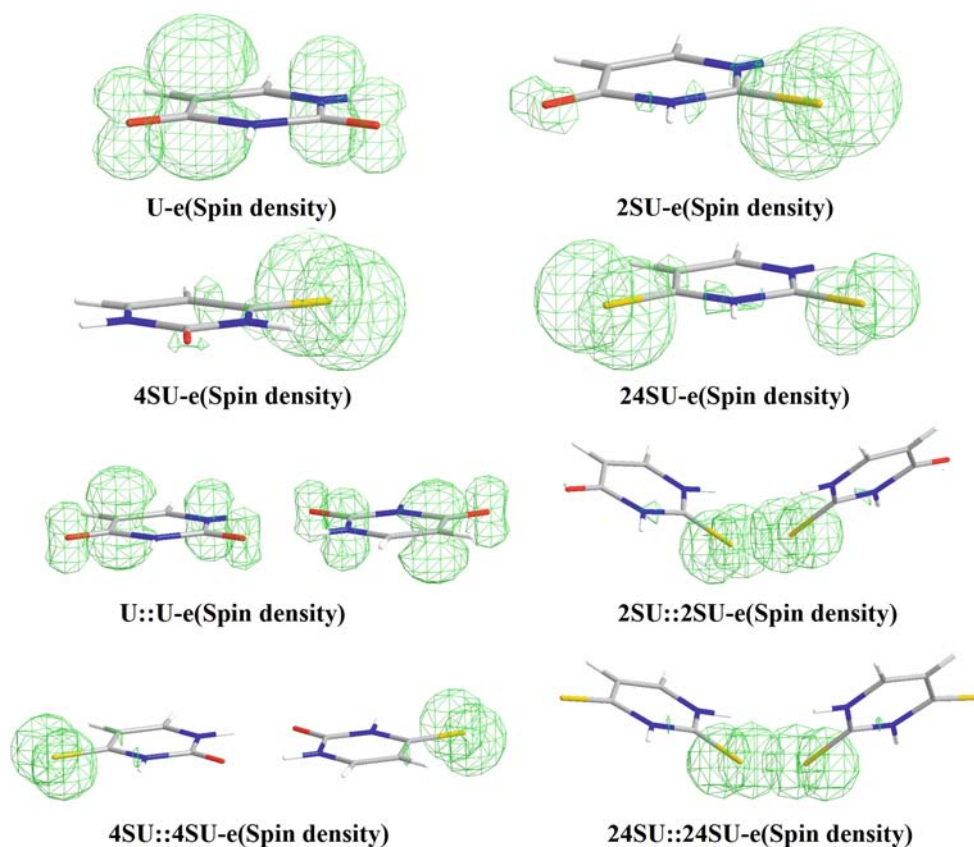


### 3.4 Influence of thio keto substitution on the triplet excitation of uracil monomer and its dimer

Accurate information on the triplet nucleic bases is important in understanding the intermolecular energy transfer for the photoexcitation of DNA. Thus, the relevant properties of the uracil, thiouracil monomers, and their dimers in lowest lying triplet states have been investigated. The singlet triplet–energy gaps and the selected structural parameters have been presented in Tables 2 and 3, respectively. Here, for the sake

of simplicity, the symbols “s” and “t” denote the singlet and triplet states, respectively. The corresponding single occupied molecular orbital (SOMO-1) associated with the original HOMO before triplet excitation has been displayed in Fig. 2.

Compared with the ground state of uracil, the  $C_5$ – $C_6$  bond is elongated (by about 0.135 Å) and twisted in the triplet state. As mentioned above, this point should be due to the excitation of certain electron away from this bond bearing bonding orbital characteristics in HOMO of uracil as displayed



**Fig. 3** Diagrams of spin density distributions for ionized monomer and dimer of uracil and thiouracils

**Table 3** The calculated relative values of bond lengths (in Å), bond angles, and dihedral angles (in degrees) between triplet and singlet states

Complexes	Bond lengths (X=O, S)				Bond angles				
	B(C <sub>4</sub> –C <sub>5</sub> )	B(C <sub>5</sub> –C <sub>6</sub> )	B(C <sub>2</sub> –X <sub>2</sub> )	B(C <sub>4</sub> –X <sub>4</sub> )	A(C <sub>2</sub> N <sub>1</sub> C <sub>6</sub> )	A(N <sub>3</sub> C <sub>4</sub> C <sub>5</sub> )	$\alpha$	$\beta$	$\gamma$
U	–0.020	0.135	0.001	0.011	1.5	0.7	51.1	31.8	–
2SU	–0.034	0.132	0.017	0.012	1.4	0.6	8.5	23.6	–
4SU	–0.066	0.058	0.003	0.089	2.0	3.9	0.1	0.1	–
24SU	–0.068	0.061	0.007	0.090	1.9	3.5	0.0	0.1	–
U::U	–0.027	0.060	0.030	0.013	1.4	1.0	0.0	0.0	0.0
2SU::2SU	–0.008	0.018	0.050	0.002	2.8	8.4	2.5	2.1	74.0
4SU::4SU	–0.032	0.018	0.001	0.054	0.5	2.1	0.0	0.0	0.0
24SU::24SU	–0.066	0.059	0.052	0.009	1.1	4.0	1.4	1.4	66.6

The positive results denote the larger values in triplet states relative to those in singlet states and vice versa.  $\alpha$ ,  $\beta$ , and  $\gamma$  represent the dihedral angles D(H<sub>6</sub>C<sub>6</sub>C<sub>5</sub>C<sub>4</sub>), D(H<sub>5</sub>C<sub>5</sub>C<sub>6</sub>N<sub>1</sub>), and D(N<sub>1</sub>C<sub>2</sub>O<sub>2</sub>H<sub>1</sub>'), respectively

in Fig. 2. As a result, the double bond characters of C<sub>5</sub>–C<sub>6</sub> bond have been significantly weakened and the protons H<sub>5</sub> and H<sub>6</sub> localize on the two opposite sides of the approximate ring plane. Especially, as shown in Table 3, the six-membered ring of uracil is nonplanar in triplet state contrary to the planar ring in the ground state. Similarly, the same holds for 2SU upon triplet state formation although the elongation and twist of the C<sub>5</sub>–C<sub>6</sub> bond is less significant than that in U-t. For

4SU and 24SU, the C<sub>4</sub>–S<sub>4</sub> bonds in the corresponding triplet state are elongated by about 0.089 and 0.090 Å, respectively, becoming almost single bonds. As shown in Fig. 2, these phenomena should be due to the fact that the HOMOs associated with the excitation of an electron are mainly composed of sulfur orbitals. It is worth noting that the six-membered rings in both 4SU and 24SU are approximately planar in the triplet state. Overall, the planar geometries of uracil and

**Table 4** The calculated bond lengths (in Å), bond angles, and dihedral angles (in degrees) for the uracil and thio-substituted uracil complexes upon  $\text{Zn}^{2+}$  binding

Complexes	X <sub>4</sub> bonding(X=O,S)						X <sub>2</sub> bonding(X=O, S)				
	B(C <sub>4</sub> –X)	B(Zn–X)	A(CXZn)	$\alpha$	$\beta$	$\gamma$	B(C <sub>2</sub> –X)	B(Zn–X)	A(CXZn)	$\alpha$	
Zn <sup>2+</sup> (U)	1.308	1.850	141.2	86.4	–99.3	–	1.295	1.876	141.4	70.9	
Zn <sup>2+</sup> (2SU)	1.291	1.879	141.7	–176.4	3.78	–	1.786	2.264	107.2	78.4	
Zn <sup>2+</sup> (4SU)	1.789	2.249	107.7	80.6	–108.5	–	1.274	1.908	157.1	143.5	
Zn <sup>2+</sup> (24SU)	1.777	2.260	108.8	127.9	–60.3	–	1.766	2.304	109.4	97.9	
Zn <sup>2+</sup> (U::U)	1.290	1.890	141.9	–169.7	10.8	2.5	–	–	–	–	
Zn <sup>2+</sup> (2SU::2SU)	1.278	1.923	143.7	171.8	–8.6	116.0	–	–	–	–	
Zn <sup>2+</sup> (4SU::4SU)	1.755	2.318	107.2	–138.4	46.6	–6.2	–	–	–	–	
Zn <sup>2+</sup> (24SU::24SU)	1.747	2.332	107.4	–142.0	42.7	–149.6	–	–	–	–	

$\alpha$ ,  $\beta$ , and  $\gamma$  represent the dihedral angles of  $\text{D}(\text{N}_3\text{CXZn})$ ,  $\text{D}(\text{C}_5\text{C}_4\text{XZn})$ , and  $\text{D}(\text{C}_2\text{N}_1\text{H}_1\text{X}'_2)$ , respectively

2-thiouracil have been distorted significantly upon triplet excitation. On the other hand, the opposite behaviors are true for 4-thiouracil and 2,4-dithiouracil.

As shown in Table 2, the calculated vertical and adiabatic single–triplet (s–t) energy gaps of uracil are 3.64 and 2.99 eV, respectively. Obviously, they are well consistent with the previous theoretical results (3.62 versus 3.00 eV) and experimental data (3.65 eV for vertical value) [39, 66], which further confirms the reliability of the B3LYP/6-311+G\* level of theory employed. Compared with uracil, the vertical (adiabatic) s–t energy gaps are decreased by about 0.34(0.16), 0.89(0.74), and 0.96(0.78) eV for 2SU, 4SU, and 24SU, respectively. For the triplet states of uracil and thiouracils, a geometry relaxation from the vertical to equilibrium position results in an energy gain of about 0.47–0.65 eV. Correspondingly, the shapes of the original orbitals associated with the triplet excitation have been modified more or less depending on the selected species, which can be further reflected by comparisons of the HOMO orbitals as shown in Fig. 2. Thus, both the vertical and adiabatic s–t energy gaps of uracil have been decreased upon thioketo substitution, suggesting the easier triplet excitations of thiouracils relative to uracil.

As for U::U and 4SU::4SU base pairs, all the atoms are in a plane and the main molecular skeletons have not been distorted in the triplet state. Compared with the corresponding ground state, the C<sub>4</sub>–C<sub>5</sub> bonds in U::U-t and 4SU::4SU-t have been shortened by about 0.027 and 0.032 Å, respectively. In contrast, the C<sub>5</sub>–C<sub>6</sub> and C<sub>4</sub>–S<sub>4</sub> bonds have been elongated by about 0.060 and 0.054 Å in the former and the latter, respectively. On the other hand, for 2SU::2SU and 24SU::24SU base pairs, the molecular skeletons have been distorted significantly and the planar structures have been destroyed upon triplet excitation. For example, the dihedral angle  $\text{D}(\text{N}_1\text{C}_2\text{O}_2\text{H}'_1)$  has been changed to about 74° and 67° in 2SU::2SU and 24SU::24SU, respectively. The corresponding intermolecular C<sub>2</sub> = S ··· H–N<sub>1</sub> H-bond is elongated by about 0.08 and 0.11 Å, respectively. As a result, for U::U

and SU::SU base pairs, the geometrical changes upon triplet excitation are similar to those upon ionization, which can be further reflected from the similar orbital shapes between SOMO-1 and HOMO upon triplet excitation and ionization (in Fig. 2). Moreover, the influence of triplet excitation and ionization on the intermolecular H-bond involving the sulfur atom directly is more significant than that of the oxygen atom directly involved.

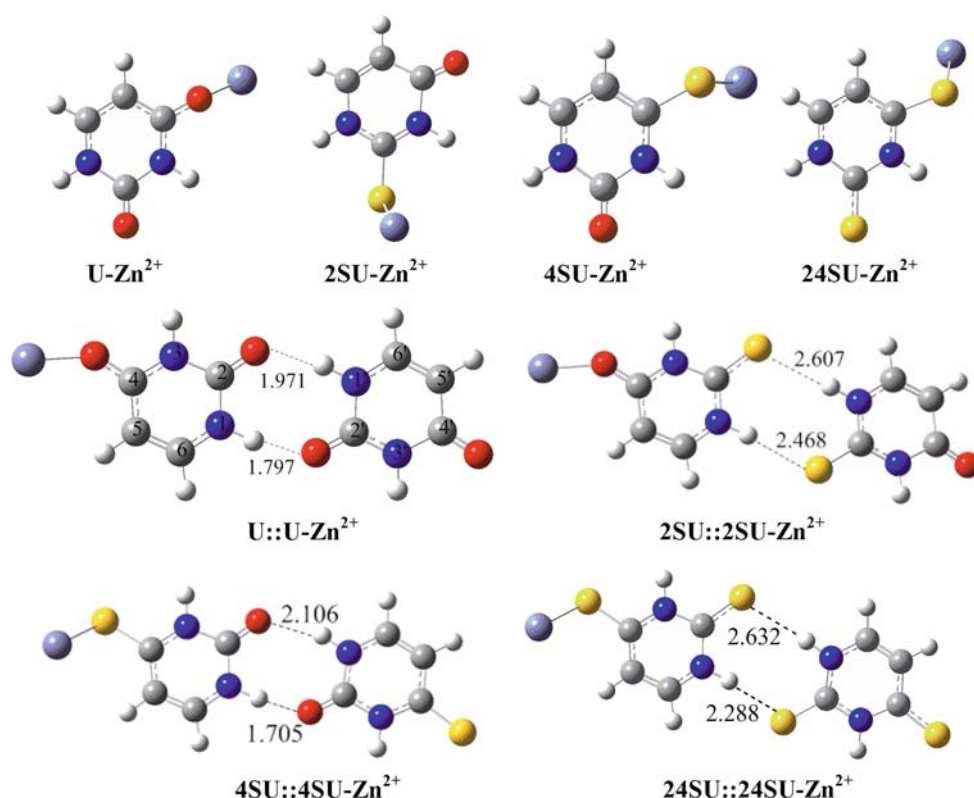
As shown in Table 2, the calculated vertical and adiabatic s–t energy gaps of U::U are 4.41 and 4.04 eV, respectively. Moreover, further thioketo substitution leads to significant reductions of them. Here, compared with U::U, the vertical (adiabatic) s–t energy gaps are reduced by about 1.02(0.91), 1.39(1.10), and 1.68(1.55) eV for 2SU::2SU, 4SU::4SU, and 24SU::24SU, respectively. Additionally, a geometry relaxation from the vertical to equilibrium position results in an energy gain of 0.24–0.37 eV in triplet U::U and SU::SU base pairs.

### 3.5 Influence of thioketo substitution on $\text{Zn}^{2+}$ binding affinities of uracil monomer and its dimer

It is well known that the presence of metal ions may influence the conformational behaviors and functions of DNA and RNA [23, 24]. Moreover, the thioketo groups of thiouracils are good ligands for transition metal ions (e.g.,  $\text{Zn}^{2+}$ ). As shown in Table 4 and Fig. 4, the structures of uracil and thiouracils change little upon  $\text{Zn}^{2+}$  binding. The Zn–O and Zn–S bonds are in the ranges of 1.850–1.908 and 2.249–2.304 Å, respectively. Here, the  $\text{Zn}^{2+}$  in uracil- $\text{Zn}^{2+}$  complexes is nonplanar to the six-membered ring due to the repulsive interactions with the H atom attached to the adjacent N or C atom, resulting in the favorable attack of  $\text{Zn}^{2+}$  to the oxygen atoms from the direction out of the plane. Compared with the angle  $\text{A}(\text{COZn})$ , the  $\text{A}(\text{CSZn})$  has been bent to 37.1° upon thioketo substitution.



**Fig. 4** The optimized complexes formed between  $Zn^{2+}$  and uracil, thiouracils, and their dimers



As shown in Table 1, the calculated  $Zn^{2+}$  binding energy at the 4-position is higher by 9.5 kcal/mol than that at the 2-position, suggesting that the most basic center for  $Zn^{2+}$  attachment is at the 4-position. However, the situation is completely different for thiouracils. Computational results suggest that  $Zn^{2+}$  prefers to attack sulfur site in 2- and 4-thiouracil, which should be due to the more polarizable of S atom partially relative to O atom. As a result, the  $Zn^{2+}$  binding energies at S site in 2- and 4-thiouracil are larger than those at O atom by 5.2 and 21.0 kcal/mol, respectively. Consistent with the results of uracil, for 2,4-dithiouracil, the most basic center for  $Zn^{2+}$  attachment is still at the 4-position, where the  $Zn^{2+}$  binding energy at the 4-position is higher by 6.9 kcal/mol than that of the 2-position. Additionally, in all the complexes, the net positive charge of  $Zn^{2+}$  has been reduced to about 0.8 and 1.0 when  $Zn^{2+}$  approaches the S and O atoms, indicating the charge transfer of almost one electron from neutral system to  $Zn^{2+}$  accompanying the charge redistribution in the system. In summary, the order of the ability to interact with  $Zn^{2+}$  is 2,4-dithiouracil > 4-thiouracil > 2-thiouracil > uracil.

Additionally, attempts to obtain the stacked structure of uracil with the  $Zn^{2+}$  on the top of the ring have also been made. As displayed in Fig. S2 of Supporting Information, the starting stacked geometry has been optimized into non-stacked structure with  $Zn^{2+}$  bonded to both C and O atoms,

where the calculated  $Zn^{2+}$  binding energy is smaller by about 4.6 kcal/mol than that of the O-binding at the 4-position.

Furthermore, the  $Zn^{2+}$  binding behaviors at  $O_4(S_4)$  sites in  $U::U(SU::SU)$  have been investigated since the  $O_2$  or  $S_2$  site is directly involved in the H-bonding interactions. For  $U::U$  and  $4SU::4SU$ , the coordination of  $Zn^{2+}$  does not cause significant structural changes. On the contrary, the structural changes in  $2SU::2SU$  and  $24SU::24SU$  are relatively larger upon coordination of  $Zn^{2+}$ . As presented in Table 4, for  $2SU::2SU$ , the association with  $Zn^{2+}$  leads to the change of the dihedral angles  $D(C_2N_1H_1S'_2)$  from about  $180.0^\circ$  to  $116.0^\circ$  and the elongation of two intermolecular H-bonds by about 0.258 and 0.119 Å, respectively. Thus, the intermolecular H-bonds in  $U::U - Zn^{2+}$  system have been weakened significantly upon thioketo substitution.

As shown in Table 1, the pairing energies have been increased by about 32.0 and 25.9–32.3 kcal/mol for the  $U::U$  and  $SU::SU$  base pairs, respectively, implying the enhancement of the stability of  $U::U$  and  $SU::SU$  base pairs upon  $Zn^{2+}$  binding. Similarly, the binding energies between  $Zn^{2+}$  and base pairs have also been increased relative to those between  $Zn^{2+}$  and monomers. Moreover, the  $Zn^{2+}$  binding energy at  $S_4$  site in  $4SU::4SU$  and  $24SU::24SU$  is larger by about 23.4 kcal/mol than that at  $O_4$  site in  $U::U$  and  $2SU::2SU$ . Thus,  $Zn^{2+}$  should prefer to approach the sulfur atom in thioketo substituted derivatives. Additionally, the possible

structures with  $\text{Zn}^{2+}$  sandwiched between two uracil rings have been systematically investigated. As displayed in Fig. S4 of Supporting Information, all the characteristics of sandwiched structures have disappeared during the geometry optimization processes. In other words, the present level of theory (B3LYP/6-311+G\*) fails to localize the sandwiched structures.

#### 4 Conclusions

In this study, the influences of thioketo substitution on the structures, acidities, IPs, singlet–triplet energy gaps, U::U and SU::SU base pairing energies of uracil monomer and its dimer and their interactions with  $\text{Zn}^{2+}$  have been systematically investigated at the B3LYP/6-311+G\* level of theory. Computational results suggest that thioketo substitution leads to a significant increase in the acidity of the N–H groups for both uracil monomer and its dimer, where the  $\text{N}_1$ –H group is still the most acidic site relative to the  $\text{N}_3$ –H group. The uracil monomer and its dimer are much easier to lose an electron upon thioketo substitution accompanying the decrease of the corresponding ionization potentials. Moreover, the single electron distribution is almost on the sulfur atom in thio-uracil monomers and dimers, suggesting that the sulfur site in thio-uracils is more readily ionized and oxidized than the oxygen site. Similarly, thioketo substitution also results in a decrease in both the vertical and adiabatic s–t energy gaps of uracil monomer and its dimer. For uracil and 2-thiouracil, the corresponding triplet state geometries are predicted to be highly nonplanar relative to the planar ground state geometries. In contrast, the geometries are still planar upon triplet excitation for 4-thiouracil and 2,4-dithiouracil. As a rule, the intermolecular H-bonds involving the sulfur atom directly have been influenced more significant than those of the oxygen atom directly involved for U::U and SU::SU base pairs upon ionization and excitation. Additionally,  $\text{Zn}^{2+}$  binding is expected to lead to an increase in the stability of U::U and SU::SU base pairs.

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