



Original Research Article

## Reaction between Thiouracil derivatives and Chloroacetic acid in gas and soluble phases: A theoretical study

Aram Ghaempanah<sup>1,\*</sup>, Mahdi Babaie<sup>1,2</sup>, Nader Mosavari<sup>1</sup>

<sup>1</sup>Reference Laboratory for Bovine Tuberculosis, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

<sup>2</sup>Young Researchers and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, Iran.

\*Corresponding author Fax number: +98(26)34552194; Tel.: +98 (26) 34570038

\*E-mail: agh.chem@yahoo.com

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### ABSTRACT

Thiouracil is a historically relevant anti-thyroid preparation. Because of its structure you can find it in various chemical reactions differently. In this study, the reaction of Thiouracil with Chloroacetic acid and the formation of their additive products has been investigated. This reaction is a concerted process, and it has not been determined yet by exhaustive mechanisms. From the potential energy profile, two possible mechanisms as well as two NH bonds dissociations are examined. Density Functional Theory (DFT) was used to compare these mechanisms. Calculation results for comparing these two pass ways were indicated by B3LYP/6-311g (d,p) levels of theory. The activation energies to 2-(6-oxo-1,6-dihydropyrimidin-2-ylthio) acetic acid and 2-(4-oxo-1,4-dihydropyrimidin-2-ylthio) acetic acid formation were obtained 55.78 and 72.9 kcal.mol<sup>-1</sup>, respectively. These calculations were also carried out for ethyl and

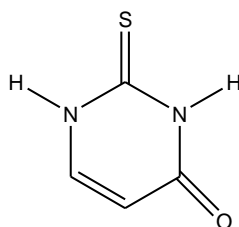
methyl Thiouracil derivatives. The calculation results indicate that removal of hydrogen from nitrogen to sulfur group at the ortho position is more favorable.

**Keywords:** Thiouracil, Chloroacetic acid, DFT, B3LYP, activation energy

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## Introduction

Thiouracil is a specific molecule that consists of sulfated uracil and its derivatives (Fig. 1). This is a suitable anti-thyroid drug. For the first time Astwood, E.B. (1943) used it as therapy of Grave's disease [1, 2]. Thiouracil blocks the thyroid peroxidase enzyme in order to inhibit thyroid activity. Thiouracil is a minor chemical component of natural tRNA that is Thio analogue of RNA base uracil. Researchers have used derivatives of Thio as melanoma seekers (tumor derived from melanin forming cells); they have covalent bond to the growing melanin through sulfur. Other derivatives of Thiouracil have been used as a diagnosis and chemotherapy for metastatic melanoma. [2].



**Fig 1.** Thiouracil scheme, it consists of sulfated uracil (C<sub>4</sub>H<sub>4</sub>N<sub>2</sub>OS)

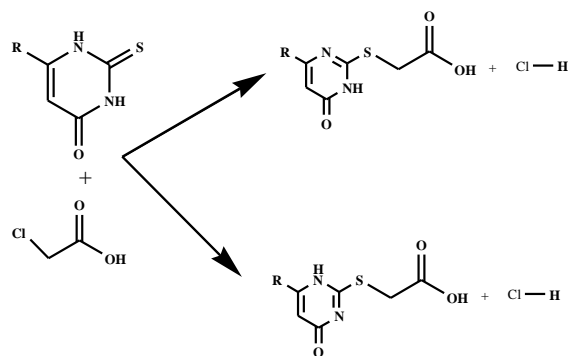
Thiouracil is produced via the condensation of Ethyl Feormylacetate and Thiourea. In 1943 it was marketed as the first anti-thyroid Ethionamide product.

Due to the high number of side effects, it has been discontinued in favor of other drugs that are less toxic, such as propyl. Currently, this product is not used as a Thyrostatic drug in humans and Tetracycline compounds is used as an active drug; also cattle men give it orally to their cattle for gaining weight before slaughter. These compounds cause livestock gain weight through the water retention in tissues [3]. In fact, Thiouracils contain nucleoside analogues which replace sulfur with one or more oxygen atoms. These compounds play important roles in molecular biology and pharmacology because of their specific structure. Naturally they are present in T-RNA structure, and have been used as fluorescent probes for thyroid disease treatment in DNA

and RNA. Their structure is similar to the canonical nucleobases structures [4]. Thiobases in the biological environment have been attracted the attention of researchers to understanding the difference of photo physics in canonical nucleobases. These molecules contain a Thiocarbonyl group that causes a red-shift in the UV absorption. Also because of their specific structure they raise intersystem crossing [5].

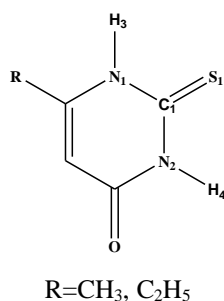
To find out how the nature of substituent's along the purine and pyrimidine heterocyclic act [6,7], a set of topography of the excited state potential energy surfaces (PESs) have been used to the photo physics properties of nucleobases. Researchers have shown that specific nucleobases are fragment of basic RNA macromolecules [8, 9]. These systems are currently valuable prototypes for photosensitizes to use in pharmacological applications. They have light-sensitive properties and similar structure with canonical nucleobases that cause these DNA parent molecules attribute acceptably. Some bacteria in their transfer ribonucleic acid (t-RNA) have 2-thiouracil (2t-Ura) and 4-thiouridinedine such as Yeasts, *Escherichia coli*, and Bacillus [10, 11]. Thiouracil and their compounds have unique structures. Reaction of Thiouracil by Chloroacetic acid and formation of their concentration products were investigated in the study presented here. In this reaction a hydrogen atom must be removed when a carbon atom from the Chloroacetic acid molecule attaches to a sulfur atom from the Thiouracil. The two NH groups in Thiouracil are the best candidates for losing this hydrogen atom.

The molecule structure shows two groups of NH in different positions (Fig. 1), each of them can lose a hydrogen atom to complete the reaction. For this additional reaction, two mechanisms were considered. These mechanisms illustrates as follow (Fig. 2).



**Fig 2.** Probable reaction pathways of concentration reaction between Thiouracil and Chloroacetic acid

In the first step, two NH groups were investigated to find out which groups lose the hydrogen atom. As follows, reaction of Thiouracil with Chloroacetic acid derivatives was considered. Thiouracil derivatives were also divided into two groups (Fig .3).



**Fig 3.** Methyl and Ethyl derivations of Thiouracil

In order to estimate the effect of different groups on the reaction mechanism and examining the possible changes in presence of these groups, density functional theory (DFT) was used. The interaction of Thiouracil with Chloroacetic acid was investigated theoretically. Computing the chemical reactions modeling is the key step for geometry optimization.

To achieve this goal the geometric structures of the reactants, products, and transition states were optimized by the theory of quantum mechanics. Subsequently, a negative frequency was confirmed by calculating the transition state frequencies. To determine the accurate molecular structures; the electronic energy calculations of reactants and the products were done. Furthermore it connects them together. The stationary points of reactant structure and the products are less important, but the transition mode is a saddle point with one negative-curvature direction on the molecular potential energy surface.

### Calculation method

In recent years, many methods have been developed to find stationary points on potential energy surfaces (PESs). Also these studies have shown that density functional theory (DFT) predicts molecular structures and harmonic vibrational frequencies. Significantly higher than those obtained by SCF calculations and have the same precision as predictions of MP2 calculations. [12]. Becke's three-parameter exchange functional nonlocal and Lee-Yang-Parr correlation functional were used in DFT calculations [13]. For this purpose, all calculations were performed

with Gaussian program 2003 [14]. B3LY hybrid was used in conjunction with the 6-311G (d, p) basis for optimizing all stationary points and transition states in the gas and soluble phases.

These basis sets are assembled based on the initial Gaussian primitives contractions have been designed for the third-row elements from K to Zn. Six initial Gaussians have been used for orbitals 1s, 2s, 2p, 3s and 3p, which are 4s and 5p for atoms K and Ca, and 4s, 4p, and 3d for atoms Sc to Zn [15-16].

First of all, the structure of each reactant and its products were optimized. These optimized structures defined the appropriate transition state structure for each mechanism, also optimal transition state structures were obtained with the Qst2 and Qst3 approaches. These calculations were done to find the minimum-energy paths. Moreover normal vibration frequencies (Hessian force constant matrices) computation confirms that every point on each stationary point is a transition structure or not. One of the methods for identifying the transition state structure is the existence of a negative frequency. In the next step, the NBO [16] analyses were subjected to find out the broken and formed bonds.

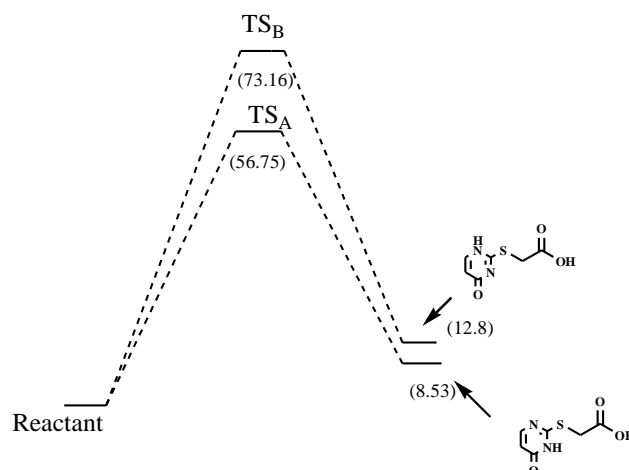
Experiment should start as a continuation to introduction on the same page. All important materials used along with their source shall be mentioned. The main methods used shall be briefly described, with references. New methods or substantially modified methods may be described in sufficient detail. The statistical method and the level of significance chosen shall be clearly stated.

## Results and discussion

Given the calculated results, the comparison of two proposed paths for the reaction between Thiouracil and its derivatives with Chloroacetic acid and different products formations as well as studying the effect of solvent on these reactions as follows.

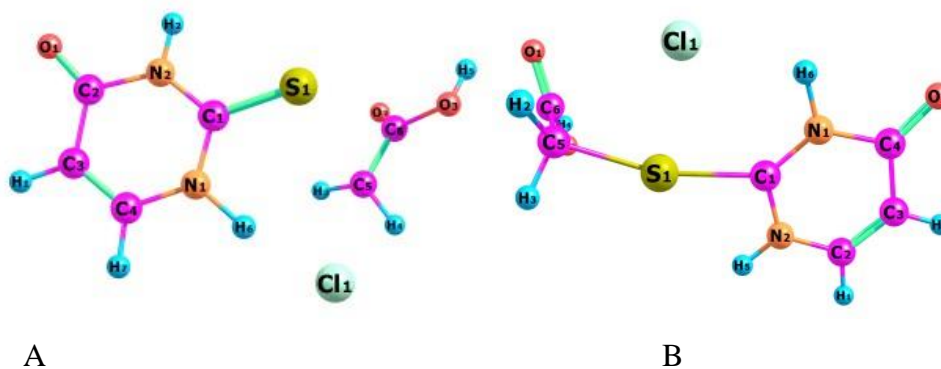
### 1-Investigation of the thermochemistry parameters for the thiouracil and chloroacetic acid reaction in the gas phase

These reaction mechanisms were optimized by all molecular structure. The activation energies of the reactions were calculated. The barrier energy of the transition state in path A and B was calculated 56.75 and 73.16 Kcal.mol<sup>-1</sup>, respectively (Fig. 4).



**Fig 4.** Energy profiles for mechanism A and B (values in parentheses are Gibbs free energies in  $\text{kcal.mol}^{-1}$ ).

It was shown that the reaction is endothermic and the calculated energy of reaction equals to 8.53 and 12.8  $\text{kcal.mol}^{-1}$ .



**Fig 5.** Optimized B3LYP/6-311 G (*d, p*) geometrical structures of transition states in Path A and B

The C1-S1 bond length in reactant and product was changed from  $1.68 \text{ \AA}$  to  $1.77 \text{ \AA}$  (Table 1). NBO calculations also indicated that the C1-S1 bond order in the reactant equals 2 and becomes 1 in the product, which indicates the formation of the C5-S1 bond.

The calculation results of or for H3-N1 bond length in reactant, product and transition state were obtained 1.01, 1.03,  $4.44 \text{ \AA}$  respectively. The NBO calculations showed that the population bond in the reactant and product bond was 1.98389 to 0 which indicates the bond is broken (Fig. 5).

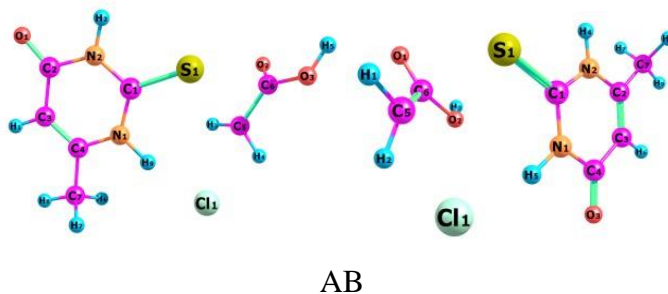
**Table 1.** The bond length and NBO results for Thiouracil, transition state and product

R=H	Reactant		Ts1		Ts2		Product 1		Product 2	
	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies
C5-S1	5.06262	–	2.82506	1.79219	1.93997	1.78857	1.83057	1.97868	1.82595	1.97776
C1-S1	1.68307	1.98321 1.97632	1.7066	1.97586	2.11726	1.80502	1.77195	1.97156	1.78306	1.97113
N1-H3	1.01048	1.98389	1.03094	1.9806	–	1.98117	4.44897	–	1.01087	1.98467
N2-H4	1.01273	1.98261	1.01213	1.98297	1.03084	1.97966	1.01213	1.98443	3.40122	–
C5-Cl1	1.79617	1.98925	2.98202	1.82813	2.94432	1.83183	6.02338	–	3.19992	–

## 2-Investigation of the thermochemistry parameters for the thiouracilderivatives and chloroacetic acid reaction in the gas phases

### 2-1- CH<sub>3</sub>

Here, the methyl group is located on C6 from Thiouracil. In pathway A, H3 atom was been leaving group, at N1 position, but hydrogen was leaved at N4 molecule position in path B (Fig. 6).



**Fig 6.** Optimized B3LYP/6-311 G (*d, p*) geometrical structures of transition states in Path A and B

Also, results indicated that the energy of the transition states at path A and B were 56.28 and 62.58 kcal.mol<sup>-1</sup> higher than the reactants, respectively. In this reaction, the sulfur atom in Thiouracil has a bond length of 1.66 Å<sup>0</sup> which for paths A and B becomes 1.77 and 1.78 Å<sup>0</sup> in product. In path A, the N1-H3 bond length increasing was calculated 1.00 Å<sup>0</sup> to 3.79 Å<sup>0</sup>. In B path, the length of the N2-H4 bond in reactant varies to the largest extent (from 1.00 to 2.95 Å).

The required energy for endothermic mechanisms A and B was obtained 56.28 and 62.56 kcal mol<sup>-1</sup>, respectively (Table 2).

Table 2: The bond length and NBO results for CH<sub>3</sub> derivation reactant, transition state and product

R=CH3	Reactant		Ts1		Ts2		Product 1		Product 2	
	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies
C5-S1	7.55649	-	2.77046	1.78191	2.60738	1.83235	1.83112	1.97836	1.8257	1.9772
C3-S1	1.66491	1.98457	1.70988	1.97623	1.70846	1.97687	1.77578	1.97168	1.78357	1.9698
		1.97807								
N1-H3	1	1.98459	1.03285	1.97915	1.01026	1.98368	3.79001	-	1.00158	1.9852
N2-H4	1	1.98401	1.00121	1.98356	1.04761	1.97899	1.00285	1.98418	2.95585	-
C5-Cl1	1.76	1.98678	2.71042	1.79813	2.70485	1.8126	5.08912	-	3.41639	-

## 2-2- C<sub>2</sub>H<sub>5</sub>

Ethyl thiouracil derivation reaction was also studied. In this reaction, two paths were investigated (Fig. 7).

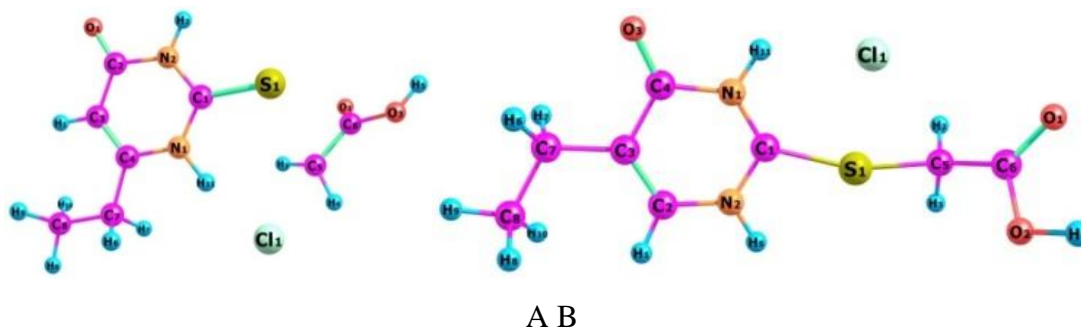


Fig 7. Optimized B3LYP/6-311 G (*d, p*) geometrical structures of transition states in Path A and B

NBO calculations indicate that in the path A, N1-H3 bond was broken and the H-Cl bond was formed but on the path B, the N2-H4 bond was broken. The activation energy barrier for paths A and B was calculated 56.72 and 74.42 kcal.mol<sup>-1</sup>, respectively (Table 3).



**Table 3.** The bond length and NBO results for C<sub>2</sub>H<sub>5</sub> derivation reactant, transition state and product

C <sub>2</sub> H <sub>5</sub>	Reactant		Ts1		Ts2		Product 1		Product 2	
	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies	Bond lengths	Occupancies
C5-S1	8.06155	-	2.7692	1.79267	2.74113	1.78756	1.83057	1.97237	1.82588	1.97762
C3-S1	1.68407	1.98745	1.71	1.97582	1.71111	1.97619	1.77195	1.9789	1.78425	1.97084
		1.97705		-				-		
N1-H3	1.01126	1.98287	1.0325	1.98041	1.01132	1.98301	4.44897	-	1.01154	1.98398
N2-H4	1.07975	1.98318	1.01358	1.98302	1.03173	1.98041	1.01213	1.98373	3.25623	-
C5-C11	1.79617	1.98977	2.7043	1.82563	2.70956	1.82546	6.02338	-	3.88957	-

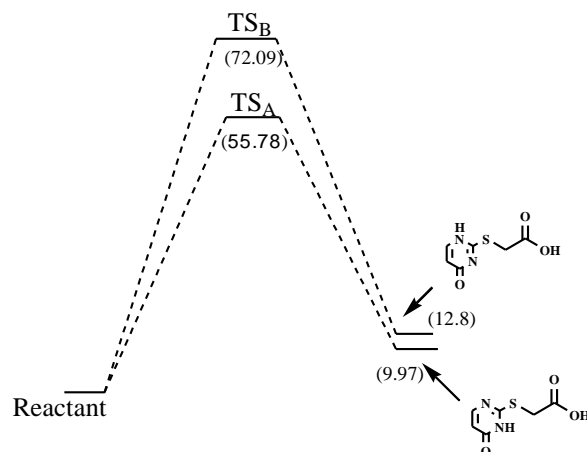
### 3- Investigation of the kinetic and thermodynamic parameters for the Thiouracil derivatives and Chloroacetic acid reaction in the soluble phase

In this section, the effect of solvent was investigated on the activation energy of the two paths. Ethanol was chosen as a solvent for this purpose. For Thiouracil and its derivatives, the duplexes (path A and B) were examined (Table 4).

**Table 4.** B3LYP/6-311G (d,p) method energy for reactant, transition state and product

R	ENERGY	Eact1	Eact2	ΔH1	ΔH2
		(kcal mol <sup>-1</sup> )	(kcal mol <sup>-1</sup> )	(kcal mol <sup>-1</sup> )	(kcal mol <sup>-1</sup> )
H		55.78	72.09	9.97	12.8
CH <sub>3</sub>		55.59	76.49	9.85	11.48
C <sub>2</sub> H <sub>5</sub>		55.72	76.02	10.47	12.36

This table shows the activation energy required for Thiouracil path A and B reaction, as the results in the gas phase, reaction in the soluble phase for two paths was endothermic and the energies were 9.97 and 12.8 kcal mol<sup>-1</sup> for the path A and B. The diagram of the geometric optimization for the Thiouracil in the two paths A and B is shown in Fig 8.



**Fig 8.** Energy profiles for mechanism A and B (values in parentheses are Gibbs free energies in kcal.mol<sup>-1</sup>).

#### 4- Calculation of the reaction rate constant

Using this equation, the rate constant was calculated for all paths. Table 5 shows the results.

$$k(298, H) = \left( e^{-\Delta G^\ddagger} \right) \frac{kT}{hc^2}$$

**Table 5.** The rate constant for reactions in gas and solution phases

R \ K	K <sub>A,gas</sub> (s <sup>-1</sup> )	K <sub>B,gas</sub> (s <sup>-1</sup> )	K <sub>A,soluble</sub> (s <sup>-1</sup> )	K <sub>B,soluble</sub> (s <sup>-1</sup> )
H	5.643*10 <sup>12</sup>	5.488*10 <sup>12</sup>	5.652*10 <sup>12</sup>	5.498*10 <sup>12</sup>
CH <sub>3</sub>	5.647*10 <sup>12</sup>	5.587*10 <sup>12</sup>	5.654*10 <sup>12</sup>	5.457*10 <sup>12</sup>
C <sub>2</sub> H <sub>5</sub>	5.643*10 <sup>12</sup>	5.476*10 <sup>12</sup>	5.651*10 <sup>12</sup>	5.461*10 <sup>12</sup>

#### Conclusion

After this condensation reaction, in some cases empirical studies indicate that removal of hydrogen from nitrogen at the ortho position is more possible, but in most papers it is not focused on this reaction. In this paper, we tried to guess the ultimate endpoint by examining the reaction mechanism and the optimization of the transition state. Barmaki and et al (2013)

investigated the Synthesis of 2,3-Dihydro-6-methyl-2-thiopyrimidin-4(1*H*)-one(6-Methyl thiouracil) derivatives and their reactions by IR, 1H, and 13C NMR and elemental analysis and showed that hydrogen removing course at the nitrogen ortho position in the final product [18].

With attention to bellow equation, the reactivity indices of chemical potentials  $\mu$  were calculated.

$$\mu = (E_{\text{HOMO}} + E_{\text{LUMO}})/2;$$

The Cl atom leaves carboxylic acid and also the electronic chemical potential of Thiouracil (-0.08 eV) is higher than the Chloroacetic acid (-0.14 eV) thus Thiouracil becomes a nucleophile agent. Since it has several nucleophilic centers, Thiouracil and Chloroacetic acid reaction can proceed in several ways. This choice of reagents was based on their availability and high reactivity.

According to Table 4, the reactions were endothermic and the enthalpy changes comparison is as follows.

$$\Delta H_{B,g} > \Delta H_{A,g}, \Delta H_{B,s} > \Delta H_{A,s}$$

It seems that the product of path A was more stable thermodynamically. Also, the activation energy and the rate constant results for these two paths were shown:

$$E_{a,A} < E_{b,B}, \quad K_A > K_B$$

Therefore, the reaction rate constant for path A was greater. In the next step, by comparing the results for the CH<sub>3</sub> and C<sub>2</sub>H<sub>5</sub> derivatives, it was found that:

$$\Delta H_{B,g}^{\text{CH}_3} > \Delta H_{A,g}^{\text{CH}_3}, \Delta H_{B,s}^{\text{C}_2\text{H}_5} > \Delta H_{A,s}^{\text{C}_2\text{H}_5}$$

$$\Delta H_{B,g}^{\text{CH}_3} > \Delta H_{A,g}^{\text{CH}_3}, \Delta H_{B,s}^{\text{C}_2\text{H}_5} > \Delta H_{A,s}^{\text{C}_2\text{H}_5}$$

$$E_{a,A,g}^{\text{CH}_3} < E_{a,B,g}^{\text{CH}_3}, E_{a,A,s}^{\text{CH}_3} < E_{a,B,s}^{\text{CH}_3}$$

$$E_{a,A,g}^{\text{C}_2\text{H}_5} < E_{a,B,g}^{\text{C}_2\text{H}_5}, E_{a,A,s}^{\text{C}_2\text{H}_5} < E_{a,B,s}^{\text{C}_2\text{H}_5}$$

Finally, with respect to these data, it can be said that for Thiouracil and its derivatives in the gas and soluble phase, kinetically and thermodynamically, mechanism A is more probable. On the other hand, although phase changing affects the activation energy and reduces it in general, the solvent does not change the whole mechanism and in both phases the path A is more likely.

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