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Ab initio study of gas phase and water-assisted tautomerization of maleimide and formamide

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Abstract. Maleimide serves as an important starting material in the synthesis of drugs and enzyme inhibitors. In the present paper, knowing the importance of tautomerization in maleimide for its drug action, potential energy surface of maleimide is studied and its tautomerization has been discussed and compared with tautomerization of formamide. Gas phase tautomerization of maleimide requires large amount of energy (23·21 kcal/mol) in comparison to formamide (15·05 kcal/mol) at HF/6-31+G* level. Thus making the proton transfer reaction a difficult process in gas phase. Water molecule lowers the energy barrier of tautomerization thus facilitating the tautomerization of maleimide to 5-hydroxy-pyrrol-2-one. Water assisted tautomerization of maleimide requires 19·60 kcal/mol energy at HF/6-31+G* and 17·63 kcal/mol energy at B3LYP/6-31+G* level, a decrease of 3·61 and 5·96 kcal/mol over gas phase tautomerization. Whereas, tautomerization of formamide requires 14·16 and 12·84 kcal/mol energy, a decrease of 0·89 and 2·01 kcal/mol energy over gas phase tautomerization at HF/6-31+G* and B3LYP/6-31+G* level, respectively. Water-assisted tautomerization in maleimide and formamide showed that difference in energy barrier reduces to 2·83 kcal/mol from 10·41 kcal/mol (in gas phase) at B3LYP level, which resulted that maleimide readily undergoes tautomerization in water molecule.

Keywords. *Ab Initio* calculations; maleimide; formamide; tautomerization.

1. Introduction

Maleimide has been found to be blockbuster antidiabetic compound, which is available as drugs. Several drugs and lead compounds with this moiety have been reported. Maleimide is a drug known to possess extensive pharmacological properties, and also serves as an important starting material in the synthesis of drugs and enzyme inhibitors. Glycogen synthease kinase-3 (GSK-3) is a regulatory serine/threonine kinase, which is being targeted by the maleimide for the treatment of a number of human diseases.

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease[1] which is characterized by insulin resistance associated not only with hyperinsulinaemia and hyperglycaemia but also with atherosclerosis, hypertension and abnormal lipid profile, collectively called syndrome X. NIDDM accounts for 90–95% of the diagnosed cases of the disease.[2] There is no single approach to treat this disease and usually a combination therapy is adopted from different approaches.[3,4] Various targets are being considered for drug development to treat NIDDM and insulin resistance. Of these, GSK-3 is emerging as an important target owing to the available knowledge on this enzyme.[5] Glycogen Synthase is a terminal enzyme in the insulin-signaling pathway and is defined as the rate-limiting enzyme of glycogen biosynthesis. One of the major characteristics of diabetic muscles is severe inhibition of Glycogen Synthase (GS) and hence loss of glycogen synthesis.[6,7] GSK-3 is a kinase, which phosphorylates and inactivates GS.[8,9] In response to insulin stimulation in the phosphatidylinositide pathway, GSK-3 becomes inhibited by PKB mediated phosphorylation of N terminus,[10,11] facilitating the dephosphorylation and activation of glycogen synthase. GSK-3 was recently implicated in several human diseases such as cancer,[12] chronic inflammatory processes,[13] and neurological diseases

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such as bipolar disorders[14] or Alzheimer's disease[15] apart from NIDDM diabetes.[7,16]

Presently, three distinct regions on the GSK-3 molecule are being targeted to suppress enzyme activity: (i) metal ion (Mg^{2+}) binding site; (ii) substrate interaction domain and (iii) ATP-binding pocket.[17,18]

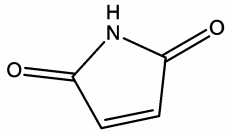
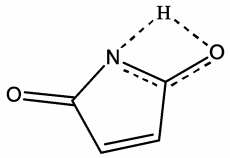
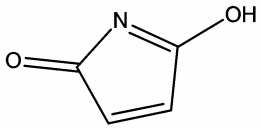
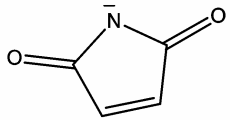
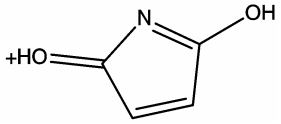
The present study is undertaken to gain understanding of potential energy surface of maleimide. The tautomerization of maleimide in gas phase as well as with water molecule has been discussed using *ab initio* calculations. Experimental determination of these parameters is not easy[19] and with the phenomenal growth in computer power, in recent years, much attention has been given to the possibility of calculating these parameters by quantum chemical methods. *Ab initio* approaches are successful in providing reliable values of proton affinities and gas phase basicities for small molecules even at lower levels of theory.[20] However, due to computational expense, application of *ab initio* methods to evaluate large molecules is still impractical. Semi-empirical methods such as AM1, MNDO and PM3 are not consistently reliable in calculations.[21] We have used *ab initio* methods for our study.

Proton-transfer reactions are important in many chemical and biological systems.[22–24] *Ab initio* molecular orbital (MO) calculation shows that gas phase tautomerization of maleimide is energetically expensive than tautomerization in aqueous solution in which a single water molecule directly assists the tautomerization of maleimide by acting as a bridge for proton transfer from the donor (–NH) to the acceptor (=O) site. These water molecules stabilize the transition state and therefore substantially lower the classical energy barrier to proton transfer. Such phenomena have been postulated in the action of enzymes (e.g. carbonic anhydrase)[25] as well as in other tautomerization reactions.[26,27]

2. Methods of calculation

Ab initio method was used for the study of tautomerization of Maleimide and Formamide. This work was carried out using the Gaussian 98, ChemOffice, Molekel, a computational chemistry programs.

In order to investigate their electronic features and mode of their action, it is requisite to first optimize the molecule. To study the interaction of maleimide drug with GSK-3 the first step was to optimize the following structures:

Molecular system	Description
	Maleimide
	Transition state between maleimide and 5-hydroxy-pyrrol-2-one
	5-Hydroxy-pyrrol-2-one (tautomer of maleimide)
	Anion of maleimide
	5-Hydroxy-pyrrol-2-ylidene-oxonium (cation of maleimide)

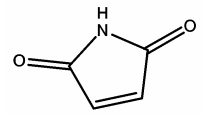
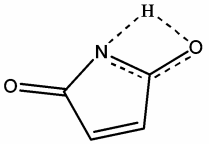
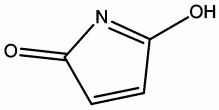
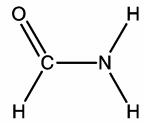
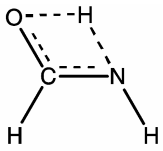
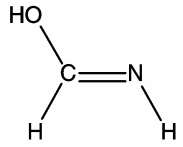
After optimization, their relative energies were compared with that of formamide. This gives the contribution of tautomerization of maleimide towards its mode of action for interacting and inhibiting GSK-3. The tautomerization of maleimide was also compared with tautomerization in formamide using water molecule and again optimization of all the corresponding structures were carried out. Activation energy required to facilitate tautomerization in all the cases was calculated. All the optimizations at various levels were performed using Gaussian 98 program.

3. Results and discussion

The potential energy (PE) surface of maleimide was studied and its tautomerization has been discussed. Complete optimization of all the structures i.e. 5-Hydroxy-pyrrol-2-one (tautomer of maleimide) has been performed using HF(E), B3LYP(E) and MP2 (full)(E) methods at the 6-31+G* basis set. Also the higher accuracy G2MP2 method was applied for the optimization.

Proton transfer reactions are important in many chemical and biological systems. Here, we have discussed tautomerization of maleimide to 5-hydroxy-pyrrol-2-one in gas phase as well as with water molecule.

Table 1. Relative energies (kcal/mol, ZPE corrected values that have been scaled by a factor of 0·9153, 0·9806 and 0·9661 for HF, B3LYP, and MP2(full) levels, respectively using 6-31+G* basis set) of various conformers of maleimide and formamide.[a]

Name of the structure	HF(E)	B3LYP(E)	MP2(E)	G2MP2(G)
Maleimide				
 Maleimide	0·00	0·00	0·00	0·00
 Maleimide-transition state	73·11	56·97	73·24	54·78
 5-Hydroxy-pyrrol-2-one	23·21	22·54	23·05	19·62
Formamide				
 Formamide	0.00	0·00	0·00	0·00
 Formamide-transition state	61·44	46·56	61·63	44·90
 Formimidic acid	15·05	14·85	15·22	12·33

[a]*E* is the total energy, *G* is the free energy

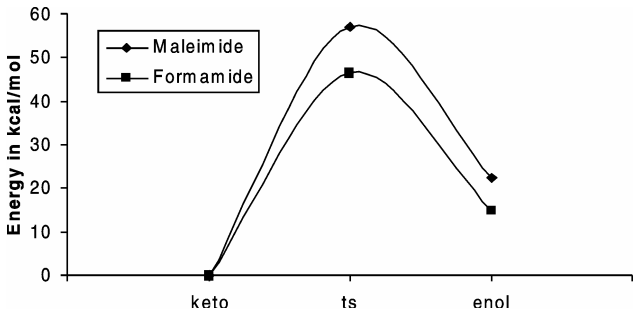


Figure 1. Energy difference between tautomerization of maleimide and formamide.

4. Gas phase tautomerization

Ab initio MO calculation has been shown that gas phase tautomerization in maleimide requires 23·21 kcal/mol energy at HF/6-31+G* level. This energy shows little change after including the electron correlation to 22·54 kcal/mol at the B3LYP/6-31+G* level and 23·05 kcal/mol at the MP2(full)/6-31+G* level. Higher accuracy G2MP2 method showed a further decrease in energy of about 3·43 kcal/mol i.e. 19·62 kcal/mol from MP2(full)/6-31+G* level (table 1). This value is much larger than that in formamide (12·33 kcal/mol).

This shows that the gas phase tautomerization of maleimide requires at least 19–20 kcal/mol of energy which is a large amount of energy requirement, thus making proton transfer reaction a difficult process in gas phase.

Further, when we compare this gas phase tautomerization with formamide using same methods, it is reported that tautomerization in maleimide requires 7·69 kcal/mol higher energy than that required to tautomerize formamide at B3LYP level. This energy difference becomes 7·29 kcal/mol at higher accuracy G2MP2 level (tautomerization energies of formamide at various levels are given in table 1). Also, it has to cross 10·41 kcal/mol greater energy barrier as compared to formamide at B3LYP level (figure 1).

The energy barrier in case of maleimide was 73·11, 56·97, 73·24 kcal/mol at HF/6-31+G*, B3LYP/6-31+G* and MP2 (full)/6-31+G* levels, respectively. This barrier becomes 54·78 kcal/mol at higher accuracy G2MP2 level. In case of formamide the energy barrier was 61·44, 46·56, 61·63 and 44·90 kcal/mol at HF/6-31+G*, B3LYP/6-31+G* and MP2 (full)/6-31+G* levels, respectively (table 1). The energy difference between tautomerization of maleimide and formamide is shown in figure 1. It shows that the tautomerization in maleimide is difficult as compare to simple formamide tautomerization. The activation barrier for maleimide is more than that of formamide because for tautomerization of maleimide in gas phase, maleimide molecule must undergo large structural change that is energetically expensive.

It has been reported that the addition of water molecule can lower the energy barrier of tautomerization, thus facilitating the tautomerization easily. Therefore in our further study, we examined the water-assisted tautomerization of maleimide to 5-Hydroxy-pyrrol-2-one.

5. Water-assisted tautomerization

A particularly interesting type of proton transfer in aqueous solution is one in which one or more solvent water molecules can mediate the process by serving as a bridge that connects the donor and acceptor sites. These water molecules stabilize the transition state and therefore substantially lower the classical energy barrier to proton transfer. Such phenomena have been postulated in the action of enzymes (e.g. carbonic anhydrase)[25] as well as in other tautomerization reactions.[26,27] In the present study, we examined the water-assisted tautomerization of maleimide to 5-hydroxy-pyrrol-2-one. This class of reactions has importance in protein and pharmaceutical chemistry and provides the simplest model for peptide linkage.[26,28–30] The N–C=O backbone of maleimide is involved in the proton transfer reaction. Theoretical studies on the water complex have determined that the preferred mechanism to form tautomer is via stable cyclic double hydrogen bonded transition state.[31–34]

By *ab initio* calculations using Gaussian 98, it was found that the tautomerization of maleimide to 5-hydroxy-pyrrol-2-one has a classical barrier of 56·97 kcal/mol (table 1) in the gas phase at B3LYP level using 6-31+G* basis set. A single water molecule directly assists the tautomerization of maleimide by acting as a bridge for proton transfer from the donor (–NH) to the acceptor (=O) site and consequently, lowers the barrier to 23·52 kcal/mol (table 2). Total decrease of 33·45 kcal/mol which is a very significant decrease (figure 2a). Furthermore, it was determined that this water-assisted tautomerization proceeds via a concerted double proton-transfer mechanism. Also the B3LYP water-assisted classical barrier of 23·52 kcal/mol gives the better agreement

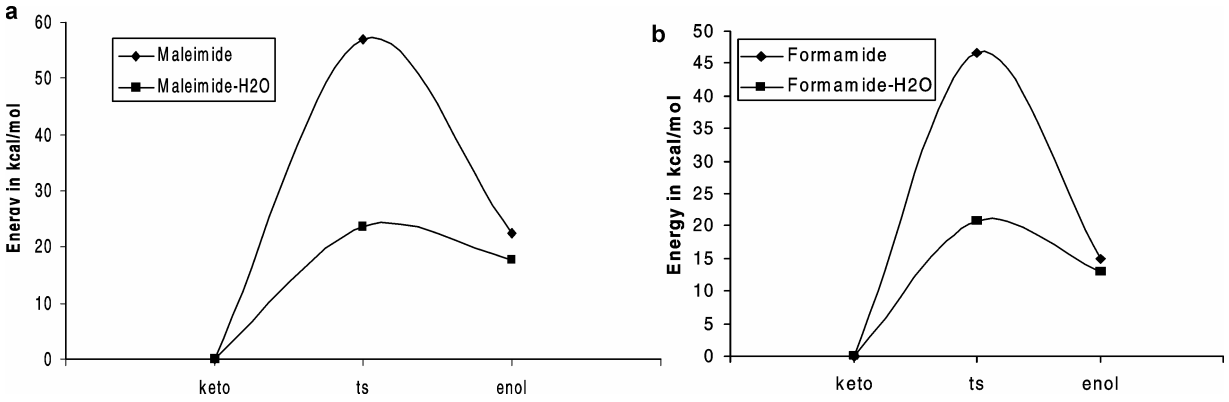
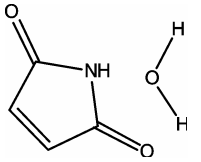
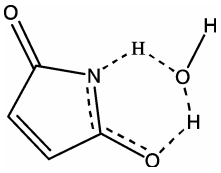
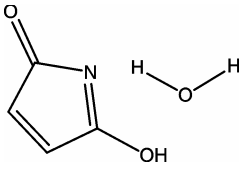
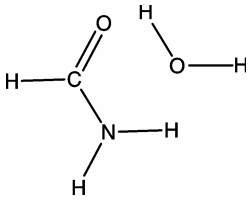
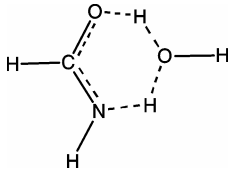
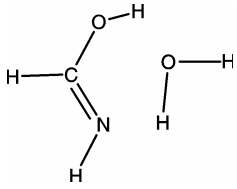


Figure 2. Energy difference between gas phase tautomerization and water assisted tatutomerization of (**a**) maleimide and (**b**) formamide.

Table 2. Relative energies (Kcal/mol, ZPE corrected values that have been scaled by a factor of 0·9153, 0·9806, and 0·9661 for HF, B3LYP, and MP2 (full) levels, respectively using 6-31+G* Basis set) of maleimide and formamide with water.[a]

Name of the structure	HF(E)	B3LYP(E)	MP2(E)	G2MP2(G)
Maleimide with water				
 Maleimide-H_2O	0·00	0·00	*	*
 Maleimide-H_2O-transition state	39·07	23·52	*	*
 5-Hydroxy-pyrrol-2-one-H_2O	19·60	17·63	*	*
Formamide with water				
 Formamide-H_2O	0·00	0·00	0.00	0.00
 Formamide-H_2O-transition state	37·55	20·69	36·85	21·82
 Formimidic acid-H_2O	14·16	12·84	14·21	10·83

[a]E is the total energy, G is the free energy
Due to higher computational cost, optimization at MP2 (full)/6-31+G and G2MP2 level is not done for these structures

over the HF barrier of 39·07 kcal/mol. Due to decrease in energy barrier, water-assisted tautomerization of maleimide requires 19·60 kcal/mol energy at HF/6-31+G* level, a decrease of 3·61 kcal/mol over gas phase

tautomerization. This energy difference increases after including the electron correlation to 4·91 kcal/mol at the B3LYP/6-31+G* level.

Similarly in water-assisted tautomerization of formamide, it was found that water-assisted tautomerization lowers the energy barrier to 20·69 kcal/mol from 46·56 kcal/mol of gas phase at B3LYP level (figure 2b), a total decrease of 25·87 kcal/mol which is a large amount of decrease in energy. Therefore, the required energy for water-assisted tautomerization of formamide was reduced to 14·16 and 12.84 kcal/mol, which is 0·89 and 2·01 kcal/mol less than the gas phase tautomerization at HF/6-31+G* and B3LYP/6-31+G* level respectively (table 2).

The comparison of water-assisted proton transfer in maleimide and formamide shows that now the difference in energy barrier reduced to 2·83 kcal/mol from 7·99 kcal/mol (in gas phase) at B3LYP level (figure 3). This is quite significant decrease and clearly shows that maleimide readily undergo proton transfer reaction in the presence of H_2O.

6. Geometries

We have discussed here maleimide and maleimide-water geometries at the B3LYP level. These structures (taken from B3LYP level optimized geometries) shown here are in over all agreement with the highest level of theoretical or available experimental data. The B3LYP (Density Functional Theory method) is better than Hartree-Fock (HF) method because it adds correlation corrections to the basis Hartree–Fock model. For H-bonded complexes (figure 4), this method yields better results for active bonds,

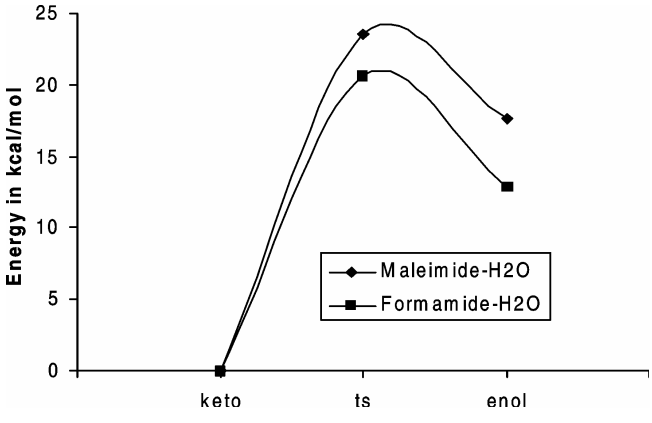


Figure 3. Energy difference between water-assisted tatutomerization of maleimide and formamide.

bonds which are either being formed or broken in the course of the reaction.

The potential energy along with the corresponding structural changes in the maleimide–water complex is illustrated in figure 4c. This system reveals a concerted two-stage mechanism in qualitative agreement with that for the maleimide–water system. From an examination of the structural changes from reactant to product, it can be seen that the N–C=O bond angle starts to lengthen. Tautomerization in the maleimide water system requires the N–C=O bond angle to be compressed by 2·94 or 2·33% from the equilibrium value then the N–H bond is stretched from an equilibrium value of 1·021–1·438 Å at the transition state, an increase of 40·84%.

For tautomerization to occur, the maleimide (gas phase) molecule must undergo large structural changes that are energetically expensive. The N–C=O bond angle was compressed from an equilibrium value of 126·25° to 109·57°, a decrease of 13·21%. The N–H bond was then stretched from 1·011 Å to 1.370 Å, an increase of 35·51% (figure 4a), to reach the transition state. The large reduction in the classical energy barrier from the gas phase to the water-assisted case is attributed to the water acting as a catalyst through the stabilization of the transition state. Most of the energy savings was achieved because the N–C=O angle has to be decreased by only 2·94° when compared with that for the gas phase which has to be decreased by 16·67° (figure 4c).

Similarly, in formamide the N–C=O bond angle was compressed from 124.77 to 108.55 and then N–H bond was stretched from 1·012 Å to 1·356 Å, a decrease of 13·0% in N–C=O bond angle and increase of 33·99% in N–H bond length to reach transition state (figure 4b). Whereas in water-assisted tautomerization N–C=O angle was compressed by 3·14° or 2.51% from equilibrium value then the N–H bond was stretched from 1·021 Å to 1·299 Å, an increase of 27·23% at transition state (figure 4d).

This shows that the water-assisted tautomerization reduces the large structural changes which reduces the N–C=O bond angle compression by 10·0 to 10·50%, thus lowering the large amount of energy requirement for tautomerization.

7. Conclusions

Ab initio calculations using HF/6-31+G*, B3LYP/6-31+G*, MP2(full)/6-31+G* and G2MP2 levels of quantum chemical methods were carried out on the

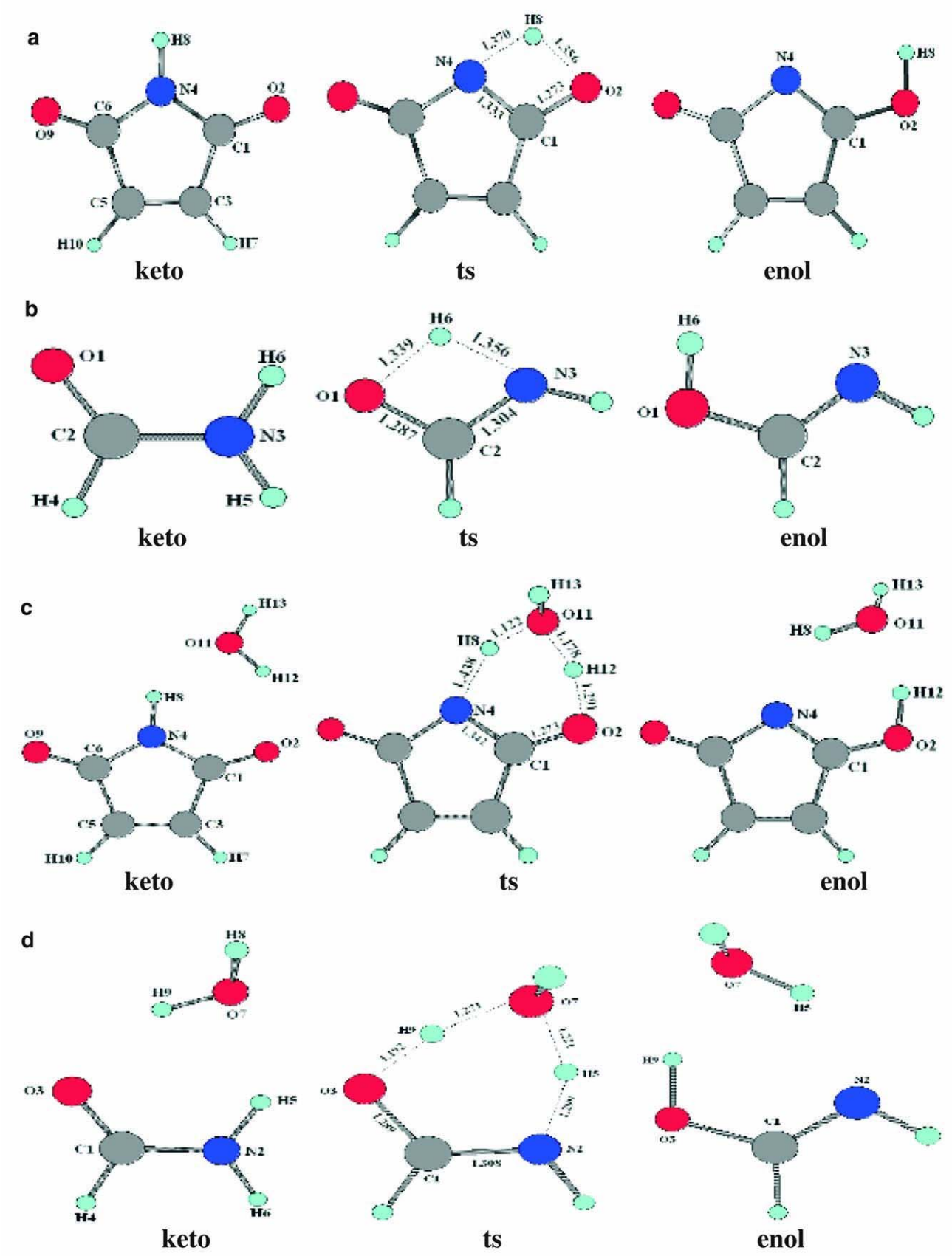


Figure 4. Keto to enol conversion of (**a**) maleimide and (**b**) formamide in gas phase. (**c**) maleimide and (**d**) formamide with water.

tautomeric process in maleimide. The 1, 3-H shift process has been found to be not favourable in gas phase but favourable in aqueous solvents because of the participation of water molecules.

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