

Mechanisms of Formation of 8-Oxoguanine Due To Reactions of One and Two OH• Radicals and the H₂O₂ Molecule with Guanine: A Quantum Computational Study

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Mechanisms of formation of the mutagenic product 8-oxoguanine (8OG) due to reactions of guanine with two separate OH• radicals and with H₂O₂ were investigated at the B3LYP/6-31G**, B3LYP/6-311++G**, and B3LYP/AUG-cc-pVDZ levels of theory. Single point energy calculations were carried out with the MP2/AUG-cc-pVDZ method employing the optimized geometries at the B3LYP/AUG-cc-pVDZ level. Solvent effect was treated using the PCM and IEF-PCM models. Reactions of two separate OH• radicals and H₂O₂ with the C2 position of 5-methylimidazole (5MI) were investigated taking 5MI as a model to study reactions at the C8 position of guanine. The addition reaction of an OH• radical at the C8 position of guanine is found to be nearly barrierless while the corresponding adduct is quite stable. The reaction of a second OH• radical at the C8 position of guanine leading to the formation of 8OG complexed with a water molecule can take place according to two different mechanisms, involving two steps each. According to one mechanism, at the first step, 8-hydroxyguanine (8OHG) complexed with a water molecule is formed, while at the second step, 8OHG is tautomerized to 8OG. In the other mechanism, at the first step, an intermediate complexed (IC) with a water molecule is formed, the five-membered ring of which is open, while at the second step, the five-membered ring is closed and a hydrogen bonded complex of 8OG with a water molecule is formed. The reaction of H₂O₂ with guanine leading to the formation of 8OG complexed with a water molecule can also take place in accordance with two different mechanisms having two steps each. At the first step of one mechanism, H₂O₂ is dissociated into two OH• groups that react with guanine to form the same IC as that formed in the reaction with two separate OH• radicals, and the subsequent step of this mechanism is also the same as that of the reaction of guanine with two separate OH• radicals. At the first step of the other mechanism of the reaction of guanine with H₂O₂, the latter molecule is dissociated into a hydrogen atom and an OOH• group which become bonded to the N7 and C8 atoms of guanine, respectively. At the second step of this mechanism, the OOH• group is dissociated into an oxygen atom and an OH• group, the former becomes bonded to the C8 atom of guanine while the latter abstracts the H8 atom bonded to C8, thus producing 8OG complexed with a water molecule. Solvent effects of the aqueous medium on certain reaction barriers and released energies are appreciable. 5MI works as a satisfactory model for a qualitative study of the reactions of two separate OH• radicals or H₂O₂ occurring at the C8 position of guanine.

1. Introduction

The endogenous production of 8-oxoguanine (8OG) (or its tautomer 8-hydroxyguanine, 8OHG) due to the reaction of guanine (G) in DNA with reactive oxygen species (ROS)^{1–5} or reactive nitrogen oxide species (RNOS)^{6–8} has been a subject of great interest due to the mispairing ability of 8OG with adenine (A) and cytosine (C) that leads to mutation. The mutations thus caused mostly involve GC → AT, GC → TA, and GC → CG base pair substitutions, where T stands for thymine.^{9,10} Such mutations occur at the time of DNA replication^{9,11} and can be lethal, as they may even cause cancer.^{12,13} Further, 8OG can be formed during the normal cellular metabolic activities or due to external causes, e.g., different types of radiation¹⁴ and chemicals.¹⁵ The ROS or RNOS preferentially attack guanine in DNA probably as its ionization potential is least among those of all the bases.^{16,17} Among the various ROS, the OH• radical is most reactive.^{5,18} It is produced by a large number of enzymatic¹⁹ and nonenzymatic reactions^{20,21} and can

react with almost all biomolecules leading to DNA strand break,^{8,22,23} DNA-protein cross-link,³ base pair mutation,^{9,10} base oxidation,^{24,25} and lipid peroxidation,²⁶ etc. The OH• radical is involved in addition, hydrogen abstraction, and electron-transfer reactions.^{8,27} Thus it can produce a wide variety of chemicals and can create genome instability and cellular damage.^{13,28} The OH• radical is shown to attack the C4, C5, and C8 positions of guanine, the C8 position being most favored for the reaction.^{29–31} When an OH• radical attacks 2'-deoxyguanosine, hydrogen abstraction from the C4' position of deoxyribose or formation of the C4'-OH• adduct takes place beside the formation of the C8-OH• adduct.³²

Bruskov et al.³³ have suggested that 8OG can be formed due to heat-mediated generation of ROS and their reactions with DNA. Akaike et al.³⁴ have shown that 8OHG can be produced inside DNA by infection of viruses, bacteria, and parasites that can ultimately lead to cancer. Weitzman et al.³⁵ have shown that 8OG can alter the methylation pattern of the adjacent cytosine that is linked to mutation. It has been found that 8OG is more susceptible to attack by ROS³⁶ than guanine itself, and thus its formation prevents further damage to guanine in DNA

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due to ROS.³⁷ This property of 8OG appears to be due to the fact that it has a lower ionization potential than guanine.^{38,39} It is known that the antioxidant enzymes, e.g., superoxide dismutase (SOD), catalase, glutathione peroxidase, and reductase, do not have complete control over the formation of 8OG.² Further, nonenzymatic antioxidants, e.g., vitamins A (β -carotene), C (ascorbic acid), and E (α -tocopherol), have a limited inhibiting power on the formation of 8OG.^{40,41} Misincorporation of 8OG into DNA is prevented by 8-oxo-dGMP formed due to degradation of 8-oxo-dGTP by the mutT protein of *Escherichia coli* or the related mammalian protein MTH1.⁴²

Reactions of the OH• radical with organic molecules have been extensively studied.^{43–46} Theoretical investigations of relative stabilities of the different tautomers of 8OG have been carried out by Venkateswarlu and Leszczynski, Venkateswarlu et al., and Cysewski.^{29–31} It is found that stabilities of the 6,8-diketo and 6-enol, 8-keto forms of 8OG are comparable, the former being more stable in the gas phase than the latter while the reverse is true in aqueous media.³⁰ The mechanism of OH• radical addition to imidazole and subsequent water elimination has been studied by Liano and Eriksson using density functional and ab initio methods.⁴⁷ As such, H₂O₂ does not react with any of the nucleotides efficiently but it can produce the highly reactive OH• radical and an OH[–] anion through the Fenton and Haber–Weiss mechanisms that involve metal cations.^{5,19–21,48} It has been observed that H₂O₂ in the presence of Fe₂(SO₄)₃ induces chromosome fragmentation.⁴⁹ The reaction of H₂O₂ with cysteine in the absence of any metal has been found experimentally to be a two-step process.⁵⁰ A theoretical study of the reaction of H₂O₂ with organic sulfides in the absence of any metal has also been found to involve two steps.⁵¹

To the best of our knowledge, formation of 8OG due to the reactions of OH• radicals or H₂O₂ with guanine has not been studied theoretically previously. In view of the great importance of 8OG from the biological point of view, we have addressed the following questions: (i) What are the relative binding energies of the adducts of an OH• radical with the C2, C4, C5, and C8 positions of guanine, what are the reaction barrier energies of their formation, and why is the formation of 8OG most prominently observed? (ii) What are the mechanisms of reactions of two separate OH• radicals, one after the other, with guanine leading to the formation of 8OHG and 8OG? (iii) What are the relative stabilities of the tautomers, 8OHG and 8OG, and what is the tautomerization reaction barrier between them? (iv) What are the mechanisms of formation of 8OHG and 8OG through the reaction of H₂O₂ with guanine and how do the reaction barriers in this case compare with those involved in the reaction of guanine with two separate OH• radicals?

2. Computational Methodology

Geometries of guanine, H₂O₂, and OH• radical were optimized at the B3LYP/6-31G**, B3LYP/6-311++G**, and B3LYP/AUG-cc-pVDZ levels of theory.^{52,53} The reaction of an OH• radical with the C8 position of guanine studied with these methods appeared to be barrierless. Therefore, to obtain qualitative information in this context with the help of another model system, the corresponding reaction was investigated with the C2 position of 5-methylimidazole (5MI). Equilibrium geometries and transition states involved in the formation of guanine-OH• adducts at the C2, C4, and C5 positions of guanine were obtained at all three above-mentioned levels of theory. Equilibrium geometries of reactant complexes (RC), product complexes (PC), and transition states (TS) involved in the reactions of two OH• radicals, one after the other, with the C8 position

of guanine leading to formation of the 8OG-H₂O complex were also investigated at all three above-mentioned levels of theory. The reactions of H₂O₂ with the C8 position of guanine were studied employing all three above-mentioned methods, except for one TS corresponding to dissociation of H₂O₂ in the presence of guanine that could not be located with the B3LYP/AUG-cc-pVDZ method. In that case, a single-point energy (SPE) calculation was performed employing the B3LYP/AUG-cc-pVDZ method and the geometry obtained with the B3LYP/6-311++G** method.

The reactions of two separate OH• radicals, one after the other, and H₂O₂ with 5MI leading to formation of the 2-oxo-5MI-H₂O complex were investigated at two levels of theory, i.e., B3LYP/6-31G** and B3LYP/AUG-cc-pVDZ. In all the above-mentioned cases involving guanine or 5MI, SPE calculations were performed employing the MP2/AUG-cc-pVDZ method^{54,55} using the geometries obtained with the B3LYP/AUG-cc-pVDZ method, except for one TS relating to the guanine-H₂O₂ reaction mentioned above for which the MP2/AUG-cc-pVDZ (SPE) calculation was performed using the geometry obtained with the B3LYP/6-311++G** method. For visualization of the optimized structures, the GaussView program (versions 2.1 and 3.09)⁵⁶ was used. All the calculations were performed employing the Windows versions of the Gaussian 98 (G98W)⁵⁷ and Gaussian 03 (G03W)⁵⁸ programs. The reactions involving two separate OH• radicals or H₂O₂ with guanine or 5MI were investigated considering two different schemes in each case. All the RC, PC, and TS involving guanine and 5MI were solvated in aqueous media with use of the polarized continuum model (PCM)^{59–61} of the self-consistent reaction field (SCRF) theory as implemented in the G98W program.⁵⁷ The solvation calculations in aqueous media were also carried out on the reactions involving 5MI, using the integral equation formalism of the PCM model (IEF-PCM)^{62,63} as implemented in the G03W program.⁵⁸ However, the IEF-PCM calculations were not successful for the TS of certain reaction steps involving 5MI.

Vibrational frequency analyses were carried out in all the cases at the different levels of geometry optimization and zero-point energy corrections (ZPEC) were made to the corresponding total energies. ZPEC in aqueous media were taken to be the same as those in the gas phase. Vibrational frequency analyses were not performed and ZPEC were not made at the MP2/AUG-cc-pVDZ (SPE) level of calculations. In all cases, the calculated transition states corresponded to a single imaginary vibrational frequency each while the RC, PC, and intermediate complexes (IC) all had real vibrational frequencies. The genuineness of the calculated TS in each case was ensured by visually examining the vibrational mode corresponding to the imaginary vibrational frequency and applying the condition that it properly connected the reactants and products.

3. Results and Discussion

3.1. Formation and Stability of Guanine-OH• Adducts. Let XH8 stand for guanine, the hydrogen atom H8 being bound to C8. In this notation, 5MI would be denoted by XH2. X stands for the remaining portions of guanine or 5MI after leaving out H8 or H2, respectively. Let us consider the following addition reaction at the C8 position of guanine (Figure 2)



where ΔE is barrier energy of the reaction and $\Delta E'$ is released energy following the reaction. Binding energies (defined as total energy of product – sum of total energies of reactants) for

TABLE 1: Binding Energies (BE, kcal/mol) of Open (OP) and Closed (CL) Ring Structures of Different Guanine-Hydroxyl Radical Adducts G2-OH•, G4-OH•, G5-OH•, and G8-OH•^a

method	G2-OH• ^d		G4-OH• ^d		G5-OH• ^d		G8-OH• ^d
	BE(OP)	BE(CL)	BE(OP)	BE(CL)	BE(OP)	BE(CL)	BE(CL)
B3LYP/6-31G** ^b	-40.42	-23.62	-14.06	-17.58	-10.31	-13.73	-32.34
B3LYP/6-311++ G** ^b	-37.26	-19.43	-8.78	-15.09	-7.64	-11.29	-29.29
B3LYP/AUG-cc-pVDZ ^b	-36.28	-20.15	-9.12	-16.07	-7.67	-12.10	-30.36
MP2/AUG-cc-pVDZ ^c	-29.93	-13.03	-6.01	-11.24	-1.82	-8.70	-25.14

^a Structures of the different adducts are shown in Figure 1. ^b Geometry optimization calculations. ^c Single-point energy calculations with B3LYP/AUG-cc-pVDZ optimized geometry. ^d Binding energies obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC.

different guanine-OH• adducts, the OH• radical being bound at C2 (G2-OH•), C4 (G4-OH•), C5 (G5-OH•), and C8 (G8-OH•) positions of guanine, are presented in Table 1. Structures of the different guanine-OH• adducts and the corresponding TS, except the TS relating to formation of the G8-OH• adduct, as well as the 5MI-OH• adduct obtained at the B3LYP/AUG-cc-pVDZ level of theory are presented in Figure 1.

When an OH• radical is added to the C2, C4, or C5 position of guanine, two types of structures are produced. First, where both the five- and six-membered rings remain closed (CL) and second where one of the rings becomes opened (OP) (Figure 1). We find that addition of the OH• radical to the C8 position of guanine or the C2 position of 5MI does not cause opening of its five-membered ring. While the open ring structure of the G2-OH• adduct is more stable than the corresponding closed ring structure, the reverse is true for the G4-OH• and G5-OH• adducts. The calculated binding energies presented in Table 1 reveal that stabilities of the various Gn-OH• ($n = 2, 4, 5, 8$) adducts, if we consider only the more stable one in each case, obtained by the different calculations follow the order $G2 > G8 > G4 > G5$. Further, the binding energies of G4-OH• and G5-OH• adducts obtained at the various levels of theory are much smaller than those of the G8-OH• and G2-OH• adducts (Table 1).

The calculated reaction barrier energies for addition of an OH• radical at each of the C2, C4, C5, and C8 positions of guanine obtained by different methods are presented in Table 2. As it was not possible to obtain RC with an OH• radical bound only to a specific C2, C4, or C5 position of guanine, the barrier energy was calculated in each of these cases with respect to the isolated reactants. We find that according to all the B3LYP calculations with different basis sets, the reaction barriers for the addition of an OH• radical at the C4 and C5 positions of guanine are negative while those for the addition reaction at the C2 position are small positive or negative depending on the basis set used (Table 2). Effectively, a negative reaction barrier would mean that the reaction under consideration is barrierless. However, the barrier energies obtained by MP2/AUG-cc-pVDZ (SPE) calculations performed with B3LYP/AUG-cc-pVDZ optimized geometries for addition of an OH• radical at each of the C2, C4, and C5 positions of guanine are positive having different magnitudes. Experimental, quantum Monte Carlo simulated, and B3LYP calculated barrier energies for the reaction $\text{OH}^\bullet + \text{H}_2 \rightarrow \text{H}_2\text{O} + \text{H}$ were found to be 0.17, 0.22, and 0.03 kcal/mol, respectively.⁶⁴ Here the B3LYP calculated reaction barrier is appreciably less than the experimental value. The H abstraction reaction $^3\text{O}_2 + \text{HOCH}_2\text{CH}_2^\bullet \rightarrow \text{OOH}^\bullet + \text{HOCHCH}_2^\bullet$ was studied by Ghigo and Tonachini⁶⁵ and the reaction $\text{Cl}^- + \text{CH}_3\text{Z} \rightarrow \text{ClCH}_3 + \text{Z}^-$ ($\text{Z} = \text{Cl}, \text{Br}$) was studied by Glukhovtsev et al.⁶⁶ In both these studies,^{65,66} different quantum chemical methods were used and it was found that the B3LYP method underestimated the reaction barrier energies. In view of this previous finding, it is quite likely that

the barrier energies for the OH• addition reactions at the C2, C4, and C5 positions of guanine are negative due to their underestimation by the B3LYP method, and thus the corresponding MP2/AUG-cc-pVDZ (SPE) calculated barrier energies appear to be more reliable. According to the MP2/AUG-cc-pVDZ (SPE) results, the addition of an OH• radical at each of the C2, C4, and C5 positions of guanine would require activation energies lying approximately in the range 1–10 kcal/mol, following the order $G2 > G4 > G5$.

The barrier energy in the case of the G8-OH• adduct was found to be too small to be computed. Several attempts with various approaches provided in the Gaussian program,^{57,58} the B3LYP method, and different basis sets (6-31G**, 6-311++G**, and AUG-cc-pVDZ) were made to locate the transition state corresponding to formation of the G8-OH• adduct. In all these cases, the OH• radical was found to move to larger distances than 3 Å from the C8 atom of guanine, eventually leading either to convergence failure or to formation of a transition state not belonging to the reaction in question, as shown by the vibrational mode of the imaginary vibrational frequency. Thus it appears that the barrier energy corresponding to formation of the G8-OH• adduct is very small. Addition of an OH• radical to another molecule, namely dimethylsulfide, was also found to be barrierless.⁶⁷ Further, addition of an OH• radical to the carbon atoms of CH_2CO has a barrier energy of only about 1 kcal/mol.⁶⁸ Thus it is not surprising that the reaction barrier for addition of an OH• radical at the C8 position of guanine is very small. This combined with the fact that the binding energy of the G8-OH• adduct is quite appreciable suggests that formation of this adduct would be highly favored in comparison to that of the other Gn-OH• ($n = 2, 4, 5$) adducts.

Structures of the different Gn-OH• ($n = 2, 4, 5$) adducts and the corresponding transition states are presented in Figure 1. Here, in (i), (ii), and (iii), the structures labeled as “a” are more stable than those labeled as “b” by about 16.1, 6.9, and 4.4 kcal/mol at the B3LYP/AUG-cc-pVDZ level and by about 16.9, 5.2, and 6.9 kcal/mol at the MP2/AUG-cc-pVDZ(SPE) level, respectively (Table 1). In view of a substantial difference between the stabilities of the structures labeled as “a” and “b”, normally the former structures would only be observed. It is noted that ring opening takes place due to breaking of a single bond in each of the three cases, i.e., (i)a, (ii)b, and (iii)b. In the structure of the G2-OH• adduct given in Figure 1(i)a, the hydrogen atom of the OH• radical has moved away from the oxygen atom and become attached to the N1 atom of guanine. Thus a CO group bonded to the C2 atom is formed. Further, in this case, a hydrogen bond ($\text{O10}\cdots\text{H10}$) exists between the oxygen atom of the CO group and the hydrogen atom that has moved away from the oxygen atom and become bonded to N1. In the structure of the G4-OH• adduct given under Figure 1(ii)b, after opening of the six-membered ring, a strong rotation of the imidazole ring has occurred about the C5C6 bond and a hydrogen bond is formed between the hydrogen atom of the

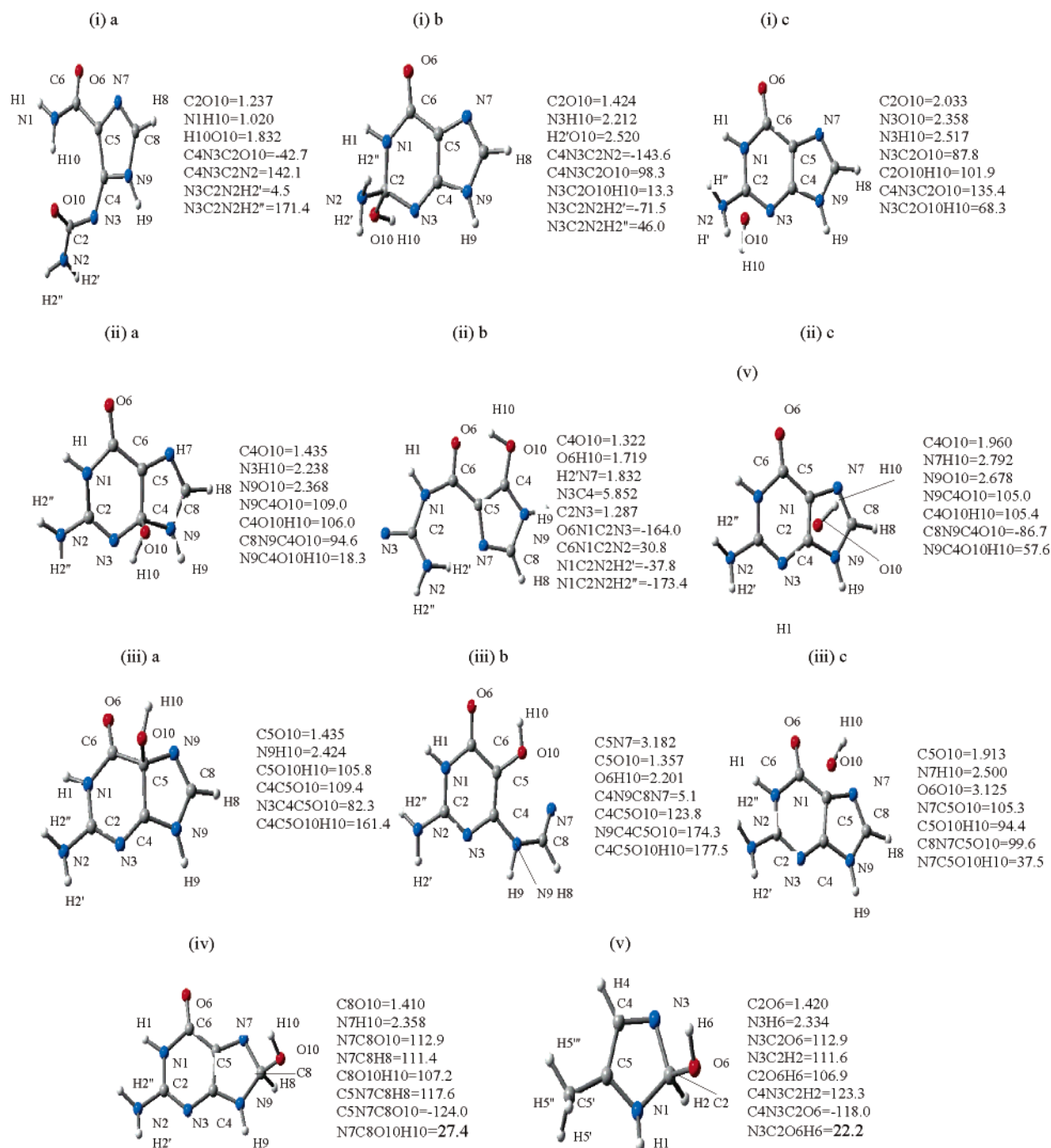


Figure 1. Structures of different guanine-OH• adducts. In structures (i), (ii), and (iii), the OH• radical is bonded to C2, C4, and C5 positions of guanine, respectively. The structures shown in (a) and (b) are in decreasing order of stability, and (c) is the transition state corresponding to the formation of (a) in each case. There is only one structure of G8-OH• as shown in (iv), and the barrier energy in this case is nearly zero. Structure (v) is the 5MI-OH• adduct. The optimized geometrical parameters were obtained at the B3LYP/AUG-cc-pVDZ level of theory.

OH• group and the O6 atom of guanine. In the structure of Figure 1(iii)b, after addition of the OH• radical at the C5 position, the imidazole ring is opened. At each of the transition states (i)c, (ii)c, and (iii)c of Figure 1, the oxygen atom of the OH• radical is located above the ring plane, near the carbon atom where the addition reaction takes place. Further, at these transition states, the distances between the corresponding carbon and oxygen atoms lie approximately in the range 1.9–2 Å, following the order G5 > G4 > G2.

To study reactions taking place at the C8 position of guanine, 5MI was taken as a model since the imidazole ring is common between the two molecules. Substitution of the methyl group in imidazole provides the required asymmetry and the C5

position was considered better than the C4 position for this purpose since the O6 atom is present near the region in guanine that would be occupied by a methyl group if the substitution is made at the C4 position, producing nearly an opposite type of electronic environment to that provided by the O6 atom (Figure 1, (iv) and (v)). We had the following advantages from the use of 5MI as a model: (i) Transition state calculations could be completed for 5MI more easily than for guanine due to the much smaller size of the former molecule than that of the latter. Further, it helped determine the starting structures for the transition states of reactions involving guanine. (ii) As the imidazole ring is common to many purines, it would be useful to compare the computed barrier energies for reactions involving

TABLE 2: Reaction Barrier Energies (kcal/mol) for the Formation of the More Stable Adduct of the Two for Each of G2-OH•, G4-OH•, G5-OH•, and G8-OH•^a

method	G2-OH• ^d	G4-OH• ^d	G5-OH• ^d	G8-OH• ^d
B3LYP/6-31G** ^b	1.68	−0.88	−4.18	~0
B3LYP/6-311++G** ^b	−0.58	−0.89	−3.90	~0
B3LYP/AUG-cc-pVDZ ^b	−0.97	−1.39	−4.32	~0
MP2/AUG-cc-pVDZ ^c	10.34	8.95	0.91	

^a Structures of the different adducts are shown in Figure 1. ^b Geometry optimization calculations. ^c Single-point energy calculations with B3LYP/AUG-cc-pVDZ optimized geometry. ^d Reaction barrier energies obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC. These energies are given with respect to the sum of total energies of the isolated guanine molecule and OH• radical.

TABLE 3: Binding Energies (kcal/mol) of Reactant Complex (RC) and Adduct with Addition of an OH• Radical at the C2 Position of 5MI (5MI-OH•) and the Corresponding Reaction Barrier Energy (ΔE)^a

method	binding energy ^d		barrier energy ^{d,e}
	RC	adduct	ΔE
B3LYP/6-31G** ^b	−8.67	−29.69	2.96
B3LYP/AUG-cc-pVDZ ^b	−7.47	−27.78	2.64
MP2/AUG-cc-pVDZ ^c	−8.66	−27.11	4.20

^a Structures are shown in Figures 1v and 2a,c. ^b Geometry optimization calculations. ^c Single-point energy calculations with B3LYP/AUG-cc-pVDZ optimized geometry. ^d Binding and barrier energies obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC. ^e With respect to the corresponding RC. The structure of the TS is shown in Figure 2b.

5MI and guanine as it would also throw light on the orders of magnitudes of the corresponding barriers for other purines. (iii) As discussed below, it was possible to confirm that the barrier energy for the addition reaction of an OH• radical at the C2 position of 5MI corresponding to the C8 position of guanine is indeed low.

Binding energies of RC and adduct corresponding to addition of an OH• radical at the C2 position of 5MI (5MI-OH•) obtained at different levels of theory are presented in Table 3. Structures of the RC of 5MI and an OH• radical, the 5MI-OH• adduct, and the transition state corresponding to formation of the adduct along with the barrier (ΔE) and released (ΔE') energies are shown in Figure 2. The corresponding structures of RC and adduct relating to guanine are also shown in this figure. We note that the structures of RC and adduct in the cases of 5MI and guanine are quite similar (Figure 2). The barrier energy obtained by Liano and Eriksson⁴⁷ in the gas phase for the addition of an OH• radical at the C2 position of imidazole at the B3LYP/6-31G** level of theory with ZPEC was 2.6 kcal/mol. At the same level of theory, in the present study, the barrier energy for the addition of an OH• radical at the C2 position of 5MI in the gas phase was found to be about 3 kcal/mol, which is close to that obtained by Liano and Eriksson⁴⁷ for imidazole, as mentioned above (Table 3). Further, the barrier energy obtained by Liano and Eriksson⁴⁷ by MP2/6-311G** SPE calculation, using MP2/6-31G** optimized geometry for the addition of an OH• radical at the C2 position of imidazole, was found to be 5.1 kcal/mol. It is also close to the barrier energy of 4.2 kcal/mol obtained by us for the addition of an OH• radical at the C2 position of 5MI by MP2/AUG-cc-pVDZ (SPE) calculation using the geometry obtained employing the B3LYP/AUG-cc-pVDZ method. We note that with ZPEC, the 5MI-

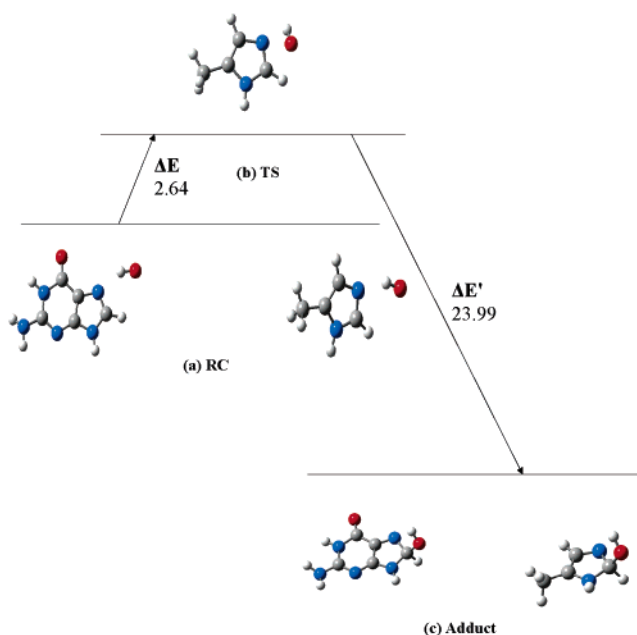
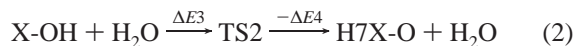
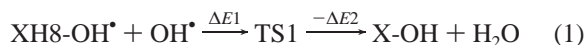


Figure 2. Reactant complexes (RC) and adducts of reactions of one OH• radical with guanine at the C8 position and with 5MI at the C2 position. TS is the transition state for the addition of an OH• radical to the C2 position of 5MI. TS could not be located in the case of guanine. Barrier and released energies (kcal/mol) with ZPEC and the different structures related to guanine and 5MI obtained at the B3LYP/AUG-cc-pVDZ level of theory are shown. The locations of different structures in terms of energy are not to scale.

OH• adduct is more stable than the corresponding RC by different amounts of energy lying approximately in the range 18.5–21 kcal/mol according to the calculations performed at the different levels of theory (Table 3).

3.2. Formation of 8OG and 2-oxo-5MI by Reactions of Two OH• Groups. Formation of 8OG complexed with a water molecule due to the reaction of an OH• radical with the adduct XH8-OH• i.e., G8-OH•, can take place according to the following scheme (Scheme 1, Figure 3):



It is to be noted that in other reaction schemes and figures also, the same abbreviations TS1 and TS2 would be used for the first and second transition states, respectively. Therefore, the meanings of TS1 and TS2 would be different for the various reaction schemes and figures. The above-mentioned reaction is a two-step one. In step 1, with XH8 = guanine, an OH• radical reacts with XH8-OH• i.e., G8-OH•, abstracts the hydrogen atom H8 bound to C8 of guanine, leading to the formation of X-OH, i.e., 8OHG and a water molecule (Figure 3). The reaction step 2 corresponds to enol (X-OH i.e., 8OHG) to keto (H7X-O i.e., 8OG) tautomerism. In this process, the hydrogen atom of the OH group bound to C8 of guanine moves and becomes attached to the N7 atom of the molecule, where it is labeled as H7. The binding energies of 8OHG and 8OG calculated with respect to the isolated reactants, i.e., guanine and two separate OH• radicals, with ZPEC, at the B3LYP/AUG-cc-pVDZ level of theory, were found to be −124.4 and −134.2 kcal/mol, respectively (Table 4). For the formation of 8OHG in step 1, at the B3LYP/AUG-cc-pVDZ level with ZPEC, the required

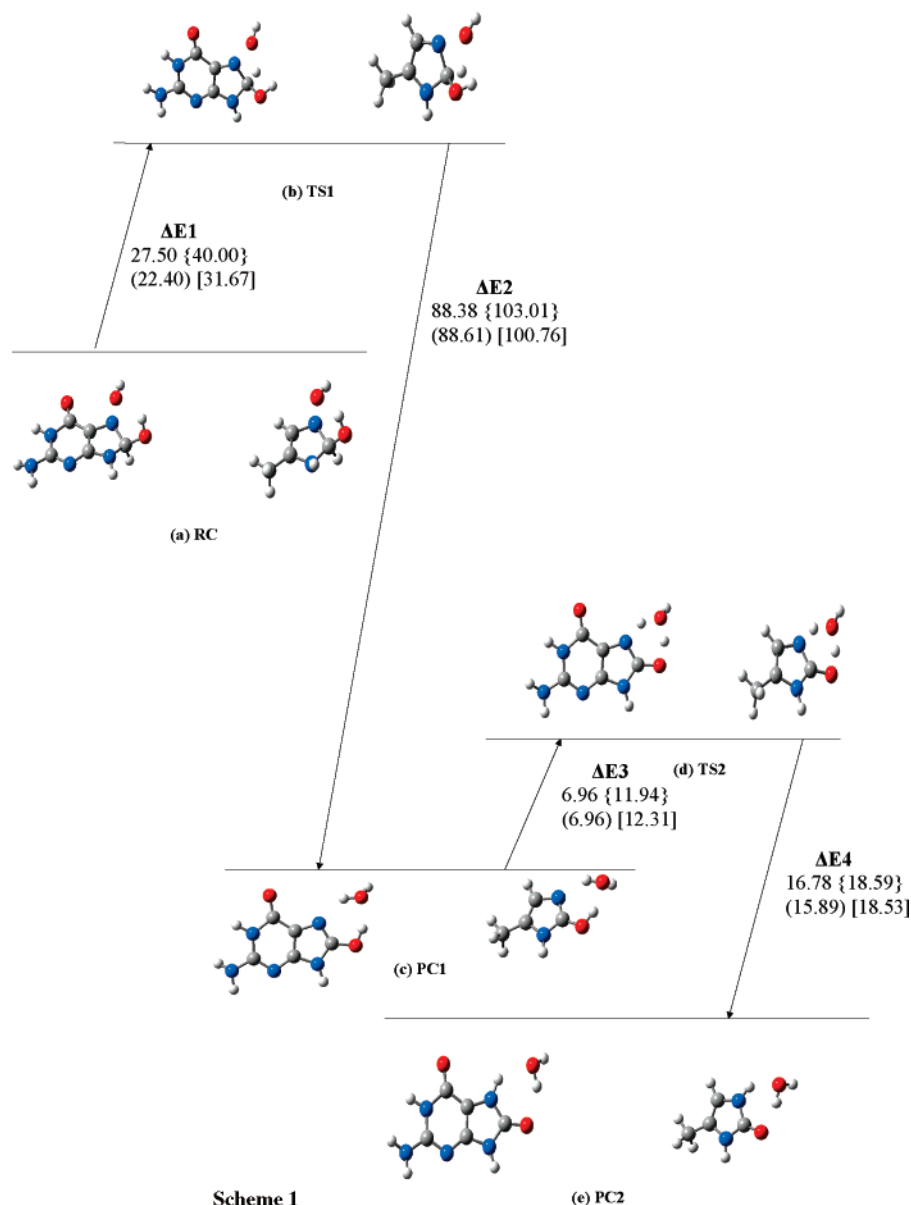
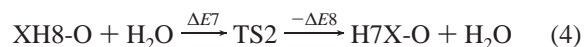
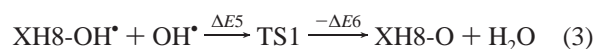


Figure 3. Reactant complex (RC), product complexes (PC1 and PC2), and transition states (TS1 and TS2) involved in the reaction of two OH• radicals with guanine at the C8 position and with 5MI at the C2 position. Barrier and released energies (kcal/mol) with ZPEC and the different structures related to guanine and 5MI (in parentheses, second line) obtained at the B3LYP/AUG-cc-pVDZ level of theory are shown. The corresponding MP2/AUG-cc-pVDZ (SPE) calculated energies without ZPEC related to guanine and 5MI are given in curly and square brackets, respectively. The locations of different structures in terms of energy are not to scale.

barrier energy ($\Delta E1$) is found to be about 27.5 kcal/mol while the energy released consequent to the reaction ($\Delta E2$) is found to be about 88.4 kcal/mol. The MP2/AUG-cc-pVDZ (SPE) calculation performed with the geometry obtained by the B3LYP/AUG-cc-pVDZ method yielded the corresponding energies, without ZPEC as about 40 and 103 kcal/mol, respectively (Figure 3). In step 2, at the B3LYP/AUG-cc-pVDZ level with ZPEC, the required barrier energy ($\Delta E3$) is about 7 kcal/mol while the energy released after the reaction ($\Delta E4$) is found to be about 16.8 kcal/mol (Figure 3, Table 4). The corresponding energies ($\Delta E3$ and $\Delta E4$) obtained by MP2/AUG-cc-pVDZ (SPE) calculation without ZPEC were found to be about 11.9 and 18.6 kcal/mol, respectively. As 8OG is appreciably more stable than 8OHG, the former would be formed preferentially. However, as the binding energy of 8OHG is also large and it is separated from 8OG by a substantial barrier, it would also be fairly stable in the gas phase.

An alternative mechanism for the formation of 8OG with the same reactants as those involved in step 1 of Scheme 1 is as follows (Scheme 2, Figure 4):



where the symbols have the same meanings as in steps 1 and 2. The above reaction is also a two-step process. In step 3, an OH• radical reacts with XH8-OH•, i.e., G8-OH• radical, abstracting the hydrogen atom of the OH• group bonded to the C8 atom, leading to the formation of an IC (XH8-O), the five-membered ring of which is open, and a water molecule (Figure 4). The binding energy of the IC calculated with respect to the isolated reactants guanine and two separate OH• radicals, with ZPEC,

TABLE 4: Binding Energies (kcal/mol) of Reactant Complex (RC) and Product Complexes (PC1 and PC2) and Reaction Barrier Energies ($\Delta E1$ and $\Delta E3$) Corresponding to Transition States Involved in the Formation of 8OG or 2-oxo-5MI Complexed with a Water Molecule Each When Two OH \cdot Radicals React with the C8 Position of Guanine or the C2 Position of 5MI According to Scheme 1

method	binding energy ^a			barrier energy ^{a,d}	
	RC	PC1	PC2	$\Delta E1$	$\Delta E3$
B3LYP/ 6-31G** ^b	-69.06	-129.73	-138.61	35.32	5.81
	[-62.12]	[-128.04]	[-135.86]	[31.14]	[6.06]
B3LYP/ 6-311++G** ^b	-61.67	-124.22	-134.51	26.59	7.90
B3LYP/ AUG-cc-pVDZ ^b	-63.53	-124.42	-134.23	27.50	6.96
	[-56.33]	[-122.54]	[-131.47]	[22.40]	[6.96]
MP2/ AUG-cc-pVDZ ^c	-77.91	-141.93	-148.59	38.99	11.94
	[-70.39]	[-139.47]	[-145.70]	[31.67]	[12.31]

^a Scheme 1 and related structures are shown in Figure 3. Binding energies corresponding to the C8 position of guanine obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC. The corresponding results related to the C2 position of 5MI are given in brackets. ^b Geometry optimization calculations. ^c Single-point energy calculations with B3LYP/AUG-cc-pVDZ optimized geometry. ^d $\Delta E1$ and $\Delta E3$ correspond to TS1 and TS2 of Figure 3 and reaction steps 1 and 2, respectively.

at the B3LYP/AUG-cc-pVDZ level of theory, is found to be about -83.5 kcal/mol (Table 5). Thus this IC is fairly stable.

TABLE 5: Binding Energies (kcal/mol) of Reactant Complex (RC), Intermediate Complex (IC), and Product Complex (PC) and Barrier Energies ($\Delta E5$ and $\Delta E7$) Corresponding to Transition States Involved in the Formation of 8OG or 2-oxo-5MI Complexed with a Water Molecule Each When Two OH \cdot Radicals React with the C8 Position of Guanine or the C2 Position of 5MI According to Scheme 2

method	binding energy ^a			barrier energy ^{a,d}	
	RC	IC	PC	$\Delta E5$	$\Delta E7$
B3LYP/ 6-31G** ^b	-69.06	-89.22	-138.61	6.44	18.43
	[-62.12]	[-92.97]	[-135.86]	[3.87]	[7.89]
B3LYP/ 6-311++G** ^b	-61.67	-84.04	-134.51	5.60	22.40
B3LYP/ AUG-cc-pVDZ ^b	-63.53	-83.46	-134.23	6.34	20.41
	[-56.33]	[-90.25]	[-131.47]	[4.51]	[28.01]
MP2/ AUG-cc-pVDZ ^c	-77.91	-92.56	-148.59	15.00	23.12
	[-70.39]	[-99.45]	[-145.70]	[8.68]	[26.84]

^a Scheme 2 and related structures are shown in Figure 4. Binding energies corresponding to the C8 position of guanine obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC. The corresponding results related to the C2 position of 5MI are given in brackets. ^b Geometry optimization calculations. ^c Single-point energy calculations with the B3LYP/AUG-cc-pVDZ optimized geometry. ^d $\Delta E5$ and $\Delta E7$ correspond to TS1 and TS2 of Figure 4 and reaction steps 3 and 4, respectively.

At the same level of theory, the barrier energy ($\Delta E5$) required for the formation of this IC is about 6.3 kcal/mol while the

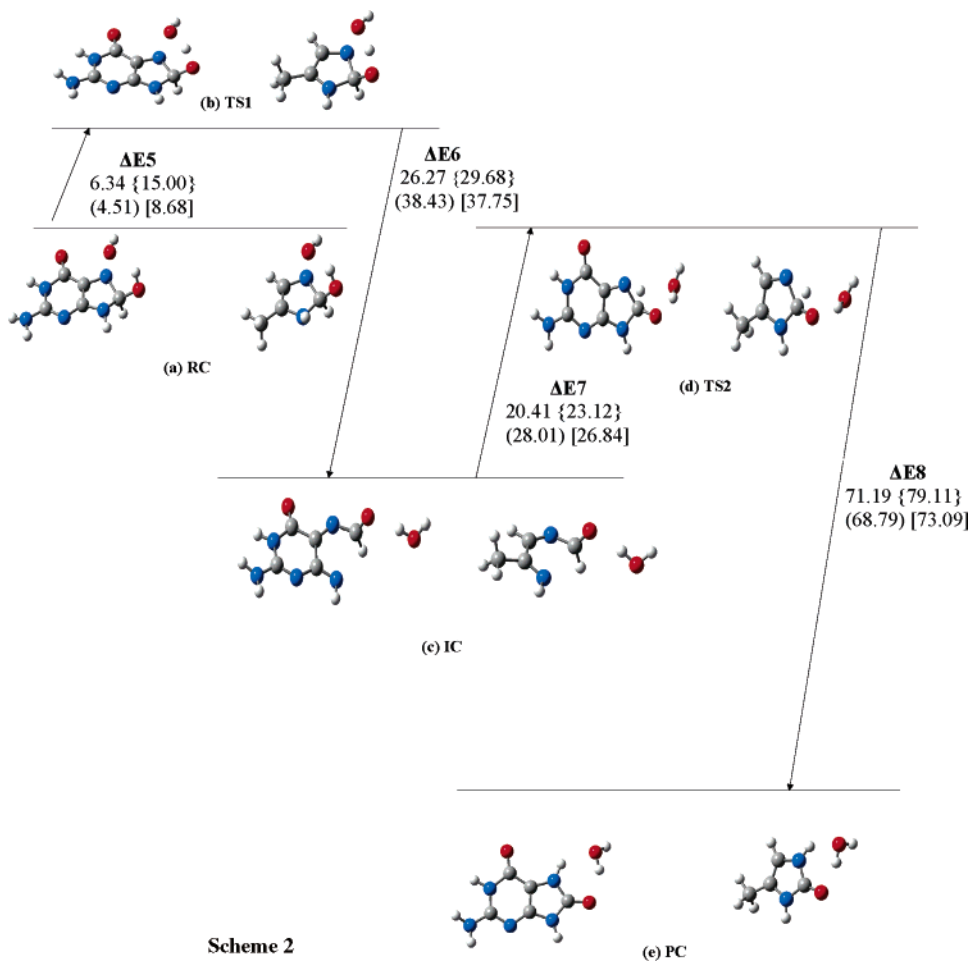


Figure 4. Reactant complex (RC), intermediate complex (IC), product complex (PC), and transition states (TS1 and TS2) formed due to reaction of two OH \cdot radicals with guanine at the C8 position and with 5MI at the C2 position. Barrier and released energies (kcal/mol) with ZPEC and the different structures related to guanine and 5MI (in parentheses, second line) obtained at the B3LYP/AUG-cc-pVDZ level of theory are shown. The corresponding MP2/AUG-cc-pVDZ (SPE) calculated energies without ZPEC related to guanine and 5MI are given in curly and square brackets, respectively. The locations of different structures in terms of energy are not to scale.

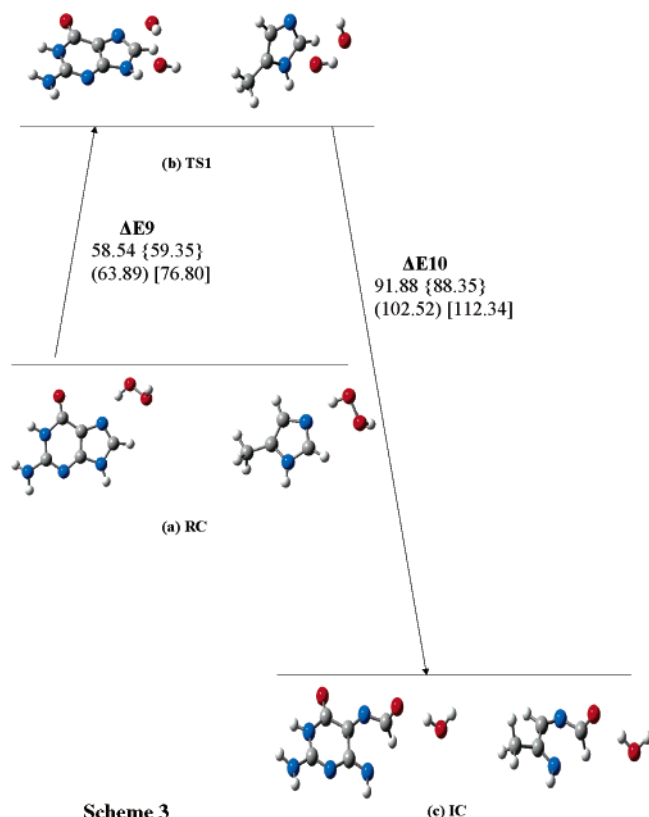


Figure 5. Reactant complex (RC), intermediate complex (IC), and transition state (TS1) involved in the reaction of H_2O_2 with the C8 position of guanine or the C2 position of 5MI. The reaction step that follows the formation of the PC is the same as that in Figure 4 and is not shown here. Barrier and released energies (kcal/mol) with ZPEC and the different structures obtained at the B3LYP/6-311++G** level for guanine and the B3LYP/AUG-cc-pVDZ level for 5MI (in parentheses, second line) are shown. The corresponding MP2/AUG-cc-pVDZ (SPE) calculated energies without ZPEC related to guanine and 5MI are given in curly and square brackets, respectively. The locations of different structures in terms of energy are not to scale.

energy released consequent to the reaction ($\Delta E6$) is about 26.3 kcal/mol. The values of $\Delta E5$ and $\Delta E6$ obtained by MP2/AUG-cc-pVDZ (SPE) calculation were found to be about 15 and 29.7 kcal/mol, respectively. In step 4, the IC undergoes a rearrangement and is converted to 8OG (H7X-O). In this process, the hydrogen atom H8 moves and becomes attached to the N7 atom of guanine, where it is labeled as H7. The barrier energy ($\Delta E7$) required for this rearrangement is found to be about 20.4 kcal/mol while the energy released ($\Delta E8$) after the reaction is found to be about 71.2 kcal/mol at the B3LYP/AUG-cc-pVDZ level of theory (Figure 4, Table 5). The MP2/AUG-cc-pVDZ (SPE) calculation yielded the values of $\Delta E7$ and $\Delta E8$ as about 23.1 and 79.1 kcal/mol, respectively. It is found that in the above two alternative Schemes 1 and 2 involving two steps each, if the first reaction barrier is low, the second one is high, but if the first barrier is high, the second one is low. On the whole, the various barrier energies ($\Delta E1$, $\Delta E3$, $\Delta E5$ and $\Delta E7$) suggest that Scheme 2 may be somewhat more probable than Scheme 1 in the gas phase (Figure 3 and 4; Tables 4 and 5).

The barrier and released energies related to the reactions of 5MI with two separate OH^\bullet radicals, one after the other, leading to the formation of 2-oxo-5MI according to the above two schemes (steps 1–4), and binding energies of RC, IC, and PC are qualitatively similar to those of the corresponding reactions involving guanine (Tables 4 and 5; Figures 3 and 4). Further, the vibrational modes corresponding to the imaginary frequen-

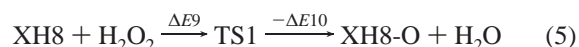
TABLE 6: Binding Energies (kcal/mol) of Reactant Complex (RC), Intermediate Complex (IC), and Product Complex (PC) and Barrier Energy ($\Delta E9$) Corresponding to Formation of the IC Complexed with a Water Molecule When H_2O_2 Reacts with the C8 Position of Guanine or the C2 Position of 5MI According to Scheme 3

method	binding energy ^a			barrier energy ^{a,d}
	RC	IC	PC	$\Delta E9$
B3LYP/6-31G** ^b	−9.66	−38.33	−90.49	56.51
	[−10.20]	[−44.85]	[−87.74]	[58.43]
B3LYP/6-311++G** ^b	−8.14	−41.49	−91.97	58.54
B3LYP/AUG-cc-pVDZ ^b	−8.14	−39.43	−90.21	64.34 ^e
	[−8.90]	[−46.22]	[−87.44]	[63.89]
MP2/AUG-cc-pVDZ	−12.57	−41.57	−97.56	59.35 ^e
	[−12.88]	[−48.42]	[−94.67]	[76.80]

^a Scheme 1 and related structures are shown in Figure 5. Binding energies corresponding to the C8 position of guanine obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC. The corresponding results relating to the C2 position of 5MI are given in brackets. ^b Geometry optimization calculations. ^c Single-point energy calculations taking the B3LYP/AUG-cc-pVDZ optimized geometry. ^d $\Delta E9$ corresponds to TS1 of Figure 5 and reaction step 5. ^e Obtained by single-point energy calculations with the B3LYP/6-311++G** optimized geometry.

cies of transition states connecting the reactants and products were also found to be similar in the two cases.

3.3. Formation of 8OG and 2-oxo-5MI by Reactions Involving H_2O_2 . There are two alternative schemes of reaction between guanine or 5MI and H_2O_2 leading to the formation of 8OG or 2-oxo-5MI complexed with one water molecule each. These schemes involving a two-step reaction each are as follows (Scheme 3, Figure 5):



where the symbols have the same meanings as in steps 1–4. The next step that follows step 5 is the same as step 4 (Figure 4) and, therefore, is not given here. The above step 5 corresponds to dissociation of H_2O_2 into two OH^\bullet groups which is followed by binding of the oxygen atom of one of the OH^\bullet groups to the C8 atom of guanine leading to the formation of a water molecule and an IC (XH8-O), the five-membered ring of which is open (Figure 5c; Figure 4c). This IC is the same as that obtained in the reaction of guanine with two OH^\bullet radicals in step 3. The values of the barrier energy ($\Delta E9$) with ZPEC, required to dissociate H_2O_2 into two OH^\bullet groups in the presence of guanine according to different calculations performed with the B3LYP method and different basis sets, lie in the range 56.51–64.34 kcal/mol (Table 6). Experimentally measured barrier energy for the dissociation of H_2O_2 into two OH^\bullet groups has been found to be 51 ± 1 kcal/mol.⁶⁹ The present result obtained at the B3LYP/6-311++G** level with ZPEC is larger than this value by about 15%. The amount of energy released ($\Delta E10$) consequent to the reaction is found to be quite large, i.e., about 91.9 kcal/mol at the B3LYP/6-311++G** level of theory (Figure 5).

The binding energies of the RC and IC of step 5 calculated with respect to the isolated reactants guanine and H_2O_2 were found to be about −8.1 and −39.4 kcal/mol, respectively, at the B3LYP/AUG-cc-pVDZ level of theory with ZPEC (Table 6). Several hydrogen bonded complexes of uracil and adenine with H_2O_2 have been studied^{70,71} while no report is available regarding the study of hydrogen bonded complexes of guanine with H_2O_2 . The binding energy of one of the complexes of

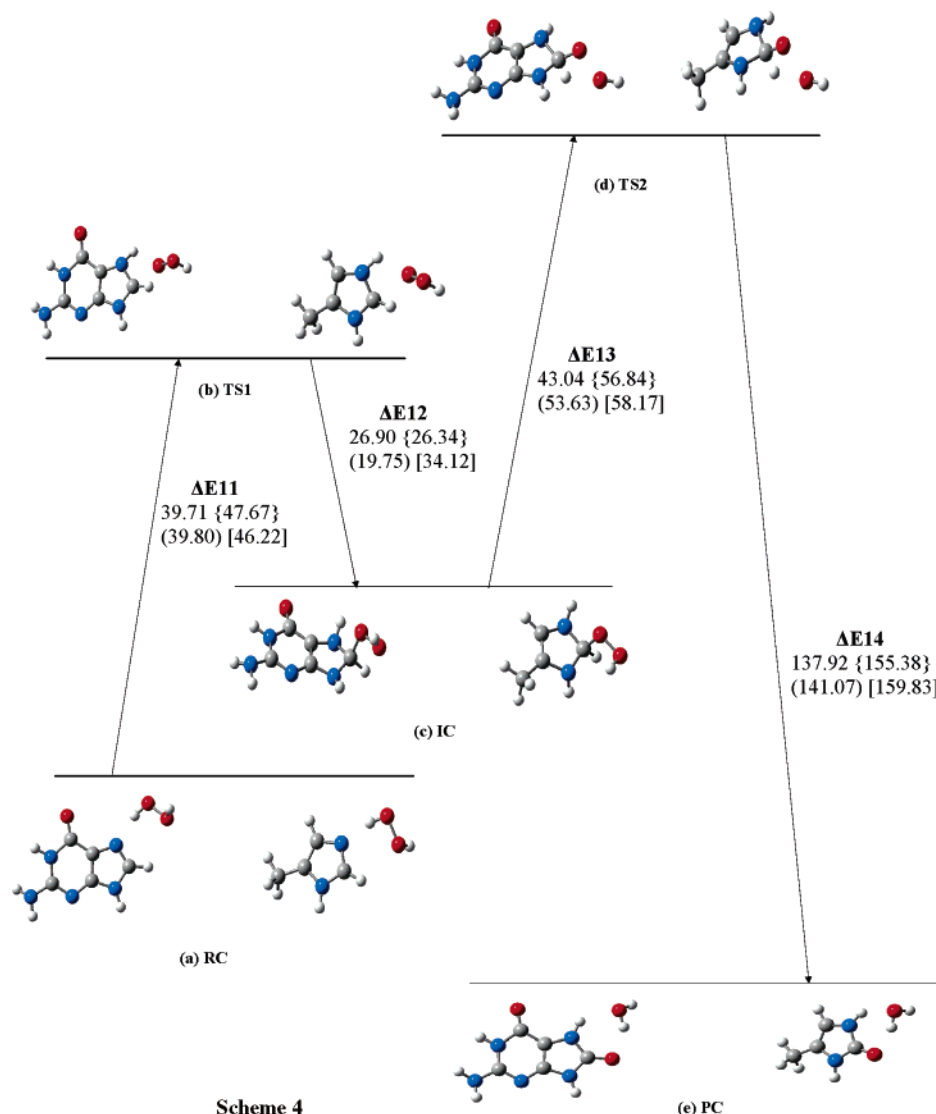
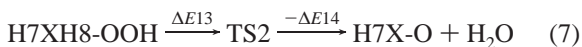
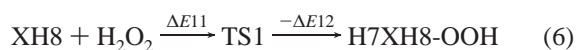


Figure 6. Reactant complex (RC), intermediate complex (IC), product complex (PC), and transition states (TS1 and TS2) involved in the reaction of H_2O_2 with guanine at the C8 position and with 5MI at the C2 position. Barrier and released energies (kcal/mol) with ZPEC and the different structures related to guanine and 5MI (in parentheses, second line) obtained at the B3LYP/AUG-cc-pVDZ level of theory are shown. The corresponding MP2/AUG-cc-pVDZ (SPE) calculated energies without ZPEC related to guanine and 5MI are given in curly and square brackets, respectively. The locations of different structures in terms of energy are not to scale.

adenine with H_2O_2 having two hydrogen bonds, one of which involves the N7 atom of the former molecule, has been found to be about -14.1 kcal/mol at the B3LYP/6-31G** level of theory without ZPEC.⁷¹ In the present work, the binding energy of the RC between guanine and H_2O_2 with ZPEC was found to be about -9.7 kcal/mol (Table 6). The above-mentioned binding energy of the complex of adenine and H_2O_2 obtained in the earlier work⁷¹ is larger in magnitude than that of the RC of guanine and H_2O_2 obtained by us. It is justified in a qualitative sense as there is only one hydrogen bond in the RC studied in the present work (Figure 5a), while there were two hydrogen bonds in the complex of adenine and H_2O_2 .⁷¹

An alternative two-step reaction scheme to that represented by step 5 followed by step 4 for the formation of 8OG or 2-oxo-5MI complexed with a water molecule each when guanine or 5MI reacts with H_2O_2 is as follows (Scheme 4, Figure 6):



where the symbols have the same meanings as in the previous steps 1–5. In step 6 of this scheme, at the transition state, H_2O_2 is dissociated into an OOH^\bullet group and a hydrogen atom, the former and latter becoming bonded to the C8 and N7 atoms of guanine, respectively. The calculated barrier energy of this reaction for guanine (ΔE_{11}) was found to be about 39.7 kcal/mol at the B3LYP/AUG-cc-pVDZ level of theory with ZPEC and about 47.7 kcal/mol at the MP2/AUG-cc-pVDZ (SPE) level without ZPEC (Figure 6; Table 7). Two experimental dissociation energies of H_2O_2 for dissociation into a hydrogen atom and an OOH^\bullet group in the gas phase have been reported.^{69,72} In one case,⁶⁹ the dissociation energy has been found to be 90 ± 2 kcal/mol while in the other it has been found to be 86.7 ± 0.8 kcal/mol.⁷² The large difference between any one of these two experimental gas-phase values and the present B3LYP/AUG-cc-pVDZ or MP2/AUG-cc-pVDZ (SPE) calculated value can be explained as due to a catalytic effect arising due to the interaction of guanine with H_2O_2 during dissociation of the latter. In step 6 the dissociated OOH^\bullet group becomes attached to the C8 atom of guanine leading to the formation of an IC (H7XH8-OOH), the energy released (ΔE_{12}) in this process being about

TABLE 7: Binding Energies (kcal/mol) of Reactant Complex (RC), Intermediate Complex (IC), and Product Complex (PC) and Barrier Energies (ΔE_{11} and ΔE_{13}) for the Formation of 8OG or 2-oxo-5MI Complexed with a Water Molecule Each When H_2O_2 Reacts with the C8 Position of Guanine or the C2 Position of 5MI According to Scheme 4

method	binding energy ^a			barrier energy ^{a,d}	
	RC	IC	PC	ΔE_{11}	ΔE_{13}
B3LYP/ 6-31G** ^b	-9.66	-3.69	-90.49	41.85	49.53
	[-10.20]	[-8.67]	[-87.74]	[42.30]	[55.73]
B3LYP 6-311++G** ^b	-8.14	-5.57	-91.97	39.91	46.92
B3LYP/ AUG-cc-pVDZ ^b	-8.14	-4.67	-90.21	39.71	43.04
	[-8.90]	[-11.15]	[-87.44]	[39.80]	[53.63]
MP2/ AUG-cc-pVDZ ^c	-12.57	-0.98	-97.56	47.67	56.84
	[-12.88]	[-6.99]	[-94.67]	[46.22]	[58.17]

^a Scheme 4 and related structures are shown in Figure 6. Binding energies corresponding to the C8 position of guanine obtained by the B3LYP method employing different basis sets include ZPEC while those obtained by the MP2/AUG-cc-pVDZ method do not include ZPEC. The corresponding results relating to the C2 position of 5MI are given in brackets. ^b Geometry optimization calculations. ^c Single-point energy calculations with the B3LYP/AUG-cc-pVDZ optimized geometry. ^d ΔE_{11} and ΔE_{13} correspond to TS1 and TS2 of Figure 6 and reaction steps 6 and 7, respectively.

26.9 kcal/mol at the B3LYP/AUG-cc-pVDZ level of theory with ZPEC and about 26.3 kcal/mol according to the MP2/AUG-cc-pVDZ (SPE) calculation without ZPEC. The binding energy of the IC related to guanine is only about -4.7 and -1 kcal/mol according to B3LYP/AUG-cc-pVDZ and MP2/AUG-cc-pVDZ (SPE) calculations (Table 7). Obviously, this IC would be short-lived.

In the next step 7 of the reaction involving the IC, the H8 atom of guanine is detached from C8 while the OOH^\bullet group is dissociated into an OH^\bullet group and an oxygen atom. While the dissociated oxygen atom becomes bonded to C8, the OH^\bullet group and the H8 atom react to form a water molecule. Thus a hydrogen bonded complex of 8OG and a water molecule is formed (Figure 6e). The required barrier energy (ΔE_{13}) and the energy released (ΔE_{14}) in the above process were found to be about 43 and 137.9 kcal/mol at the B3LYP/AUG-cc-pVDZ level of theory with ZPEC, respectively, the corresponding energies obtained by MP2/AUG-cc-pVDZ (SPE) calculation without ZPEC being about 56.8 and 155.4 kcal/mol, respectively (Figure 6). The gas-phase dissociation energy of the OOH^\bullet group into an oxygen atom and an OH^\bullet group has been found experimentally to be 64.3 ± 0.8 kcal/mol.⁷² The present MP2/AUG-cc-pVDZ (SPE) value of ΔE_{13} is about 12% less than this experimental value, while the B3LYP/AUG-cc-pVDZ value is much less. The difference between the experimental and calculated values for ΔE_{13} can be ascribed mainly to the interactions of the OOH^\bullet group and its fragments with guanine or 5MI during the process of dissociation.

The barrier and released energies in the reactions of 5MI and H_2O_2 leading to the formation of 2-oxo-5MI complexed with a water molecule in accordance with Schemes 3 and 4 discussed above (steps 5–7), binding energies of RC, IC, and PC (Tables 6 and 7; Figures 5 and 6), as well as the vibrational modes corresponding to the imaginary frequencies of transition states connecting the reactants and intermediates or products, were found to be similar to those obtained for the corresponding reactions involving guanine. The correlation coefficient of the magnitudes of binding energies of all RC, IC, and PC with and without ZPEC obtained by B3LYP/6-31G**, B3LYP/AUG-cc-pVDZ, and MP2/AUG-cc-pVDZ (SPE) calculations for the

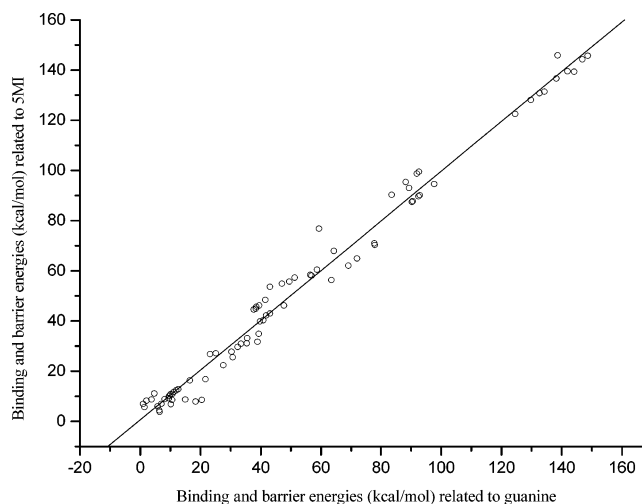


Figure 7. Correlation of magnitudes of binding energies of RC, IC, and PC and barrier energies with and without ZPEC relating to formation of the IC and PC by reactions of one and two OH^\bullet radicals and H_2O_2 at the C8 position of guanine with the magnitudes of corresponding energies relating to reactions at the C2 position of 5MI. The straight line was obtained by the least-squares fitting.

reactions of one and two OH^\bullet radicals and H_2O_2 at the C8 position of guanine with the magnitudes of corresponding energies at the C2 position of 5MI was found to be 0.995 while the correlation coefficient for barrier energies for the formation of the IC and PC relating to guanine and 5MI was found to be 0.974. The overall correlation coefficient considering all the above-mentioned data was found to be 0.993. The correlation curve obtained considering all the data points is presented in Figure 7. All these correlation coefficients were significant at the level of less than 0.5%, which shows that the correlation is meaningful. Thus study of reactions at the C2 position of 5MI can serve as a good model for a qualitative study of those at the C8 position of guanine.

3.4. Comparison of Reactions Involving 2OH^\bullet Radicals and H_2O_2 . We have discussed above reactions leading to the formation of the same product, i.e., 8OG or 2-oxo-5MI complexed with a water molecule each, starting with two different reactants, i.e., guanine or 5MI, and two OH^\bullet radicals or an H_2O_2 molecule. There are interesting differences and similarities between the two situations as follows. In the case of H_2O_2 , after its dissociation, two weakly bound OH^\bullet radicals become available for reaction with guanine or 5MI simultaneously while in the other case, two OH^\bullet radicals react with guanine or 5MI, one after the other, as discussed earlier. We find that the same intermediate complex (IC) (Figures 4c and 5c) is formed in both situations, and subsequently the two reactions proceed in the same direction, as discussed earlier. This shows that even in the case of H_2O_2 , after its dissociation, the two weakly bound OH^\bullet radicals react with guanine or 5MI in the same way, one after the other, as two separate OH^\bullet radicals. As discussed earlier, for the reactions of two separate OH^\bullet radicals with the C8 position of guanine, one after the other, the first and second barrier energies were found to be nearly 0 and 6.3 kcal/mol, respectively, at the B3LYP/AUG-cc-pVDZ level of theory with ZPEC, and an IC is formed after the reaction of the second OH^\bullet group (Figures 2 and 4c; Tables 2 and 5). However, in the case of H_2O_2 , at the same level of theory, there is only one, but high, barrier with a barrier energy of about 64.3 kcal/mol leading to formation of the same IC (Figure 5c). It is also noted that guanine reacts with H_2O_2 producing 8OG without the formation of 8OHG (Figures 5 and 6), but in its

TABLE 8: Solvent Effect of Aqueous Medium on the Different Barrier and Released Energies (kcal/mol) with ZPEC Involved in the Reactions of Guanine or 5MI with One and Two OH• Radicals or H₂O₂, Obtained, Unless Otherwise Specified, at the B3LYP/AUG-cc-pVDZ Level of Theory with the PCM and IEF-PCM Methods

energy ^a	guanine		5MI		
	gas	aqueous medium	gas	aqueous medium	
		PCM		PCM	IEF-PCM
barrier energies					
ΔE			2.64	1.30	1.15
$\Delta E1$	27.50	20.11	22.40	11.98	11.26
$\Delta E3$	6.96	8.67	6.96	7.83	
$\Delta E5$	6.34	−0.40	4.51	−1.08	−1.80
$\Delta E7$	20.41	9.91	28.01	20.83	
$\Delta E9^b$	58.54	57.84	63.89	57.85	56.77
$\Delta E11$	39.71	39.89	39.80	32.79	30.24
$\Delta E13$	43.04	42.55	53.63	34.51	37.42
released energies					
$\Delta E'$			23.99	26.90	22.40
$\Delta E2$	88.38	76.38	88.61	74.98	76.40
$\Delta E4$	16.78	17.41	18.89	21.55	
$\Delta E6$	26.27	15.58	38.43	32.32	35.32
$\Delta E8$	71.19	50.53	68.79	58.27	
$\Delta E10^b$	91.88	90.93	102.52	96.13	94.27
$\Delta E12$	29.90	25.90	19.75	14.98	7.30
$\Delta E14$	137.92	131.93	141.07	127.40	136.24

^a ΔE and $\Delta E'$ correspond to the addition reaction of one OH• radical with 5MI (Figure 2). The other barrier and released energies are defined in Figures 3–6. ^b Obtained at the B3LYP/6-311++G** level of theory (Figure 5).

reaction with two separate OH• radicals, the latter is a precursor to the former according to Scheme 1 (Figure 3).

3.5. Solvent Effects of Aqueous Medium on Reaction Barrier and Released Energies. Reaction barrier and released energies corresponding to the various reaction steps involving guanine and 5MI in aqueous medium calculated with the PCM and IEF-PCM models are presented in Table 8 where the corresponding gas-phase energies are also given for comparison. We make the following observations: (i) Changes in the reaction barrier and released energies in going from the gas phase to aqueous medium as obtained by the PCM and IEF-PCM models, where results calculated by both the methods are available, are qualitatively similar, with the exception that while the released energy $\Delta E'$ consequent to the addition of one OH• radical to 5MI increases by a small amount according to the PCM calculation, it decreases by another small amount according to the IEF-PCM calculation. (ii) The solvent effects on the reaction barrier and released energies are also similar for guanine and 5MI, except that, according to the PCM model, two barrier energies $\Delta E11$ and $\Delta E13$ for guanine are almost unchanged while the corresponding energies are substantially decreased for 5MI in going from the gas phase to aqueous medium. These results show that even after including the solvent effect, reactions at the C2 position of 5MI would usually serve as a reliable model to study reactions at the C8 position of guanine. (iii) The different barrier and released energies are appreciably decreased in going from the gas phase to aqueous medium with the exception of one barrier ($\Delta E3$) and one release ($\Delta E4$) energy that are somewhat increased as obtained with the PCM model. The barrier energy $\Delta E5$ in the gas phase for both guanine and 5MI is small, but after inclusion of the solvent effect, it becomes negative. This implies that the reaction would occur in aqueous medium in a barrierless manner. Thus it appears that in going from the gas phase to aqueous medium, the second mechanism of the reaction of two separate OH• radicals with guanine or

5MI (Scheme 2) would become substantially more favorable than the first one (Scheme 1). Further, the barrier energies $\Delta E9$, $\Delta E11$, and $\Delta E13$ show that 8OG would be very unlikely to be formed in aqueous media through the reactions of guanine with H₂O₂ as in the gas phase.

3.6. Biological Significance. It would be valuable to know which mechanism of formation of 8OG is most likely to be followed in biological media. The aqueous medium may be taken to represent biological media approximately. Considering the solvent effect discussed above, we find that in aqueous media the formation of 8OG through the reaction of two separate OH• radicals with guanine according to Scheme 2 would have only one reaction barrier ($\Delta E7$) of about 10 kcal/mol while there would be two reaction barriers in the other mechanism (Scheme 1) with barrier energies of about 20.1 and 8.7 kcal/mol. As discussed above, 8OG is not likely to be formed through the reaction of guanine with H₂O₂ directly, i.e., without the involvement a catalyst, e.g., a metal cation. As OH• radicals formed by various enzymatic and nonenzymatic processes^{19–21} occur in biological media, it seems that the mechanism represented by Scheme 2 of the reaction of two separate OH• radicals with guanine would be most likely to be followed for the formation of 8OG in biological systems. The above-mentioned reaction barrier energy $\Delta E7$ (~10 kcal/mol) cannot be overcome by thermal fluctuations. It may be modified due to environmental effects, e.g., interaction with specific solvent molecules or anions and cations present in the medium. This point deserves a separate study.

3.7. Structures of Transition States and Intermediates.

Some important structural details of all the transition states relating to the reactions of guanine with two separate OH• radicals, one after the other, and with H₂O₂ are presented in Figure 8. The structures of the two intermediates involved in these reactions are also included in this figure. Some interesting features of these structures are discussed below. The structures of Figure 8a,b correspond to formation of 8OHG + H₂O and 8OG + H₂O due to the reaction of guanine with two separate OH• radicals, respectively. While the C8H8 bond is only somewhat elongated in the former case, it is broken in the latter. Further, in the latter case (Figure 8b), the H8 atom is nearly equally shared by the N7 atom of guanine and the O11 atom of the second OH• group, and also the H10 atom that is bonded to O10 in 8OHG (Figure 8a) is nearly equally shared by the O10 atom of the CO group and the O11 atom of the second OH• group. The structure of the transition state presented in Figure 8c corresponds to formation of the IC shown in Figure 8d due to the reaction of two OH• radicals with guanine (Figure 4). In isolated guanine, the C8N9 bond length is about 1.37 Å while it is elongated to about 1.54 Å at the transition state (Figure 8c), and the corresponding bond is broken in the IC (Figure 8d).

The calculated equilibrium OO bond length of H₂O₂ at the B3LYP/AUG-cc-pVDZ level of theory was found to be about 1.46 Å. It is in reasonable agreement with the experimental crystallographic value⁷³ 1.49 Å of the same. At TS1 of Figure 5b, structural details of which are presented in Figure 8e, the OO interatomic distance of H₂O₂ is elongated to about 2.31 Å. Thus the structure of the transition state presented in Figure 8e corresponds to dissociation of H₂O₂ into two OH• groups in the presence of guanine. The H10O11 distance in Figure 8e is about 1.76 Å. It shows that at TS1 of Figure 5b, one of the OH• groups becomes reoriented and makes a strong hydrogen bond with the oxygen atom O11 of the other group that is also involved in a strong hydrogen bond with the H9 atom bonded

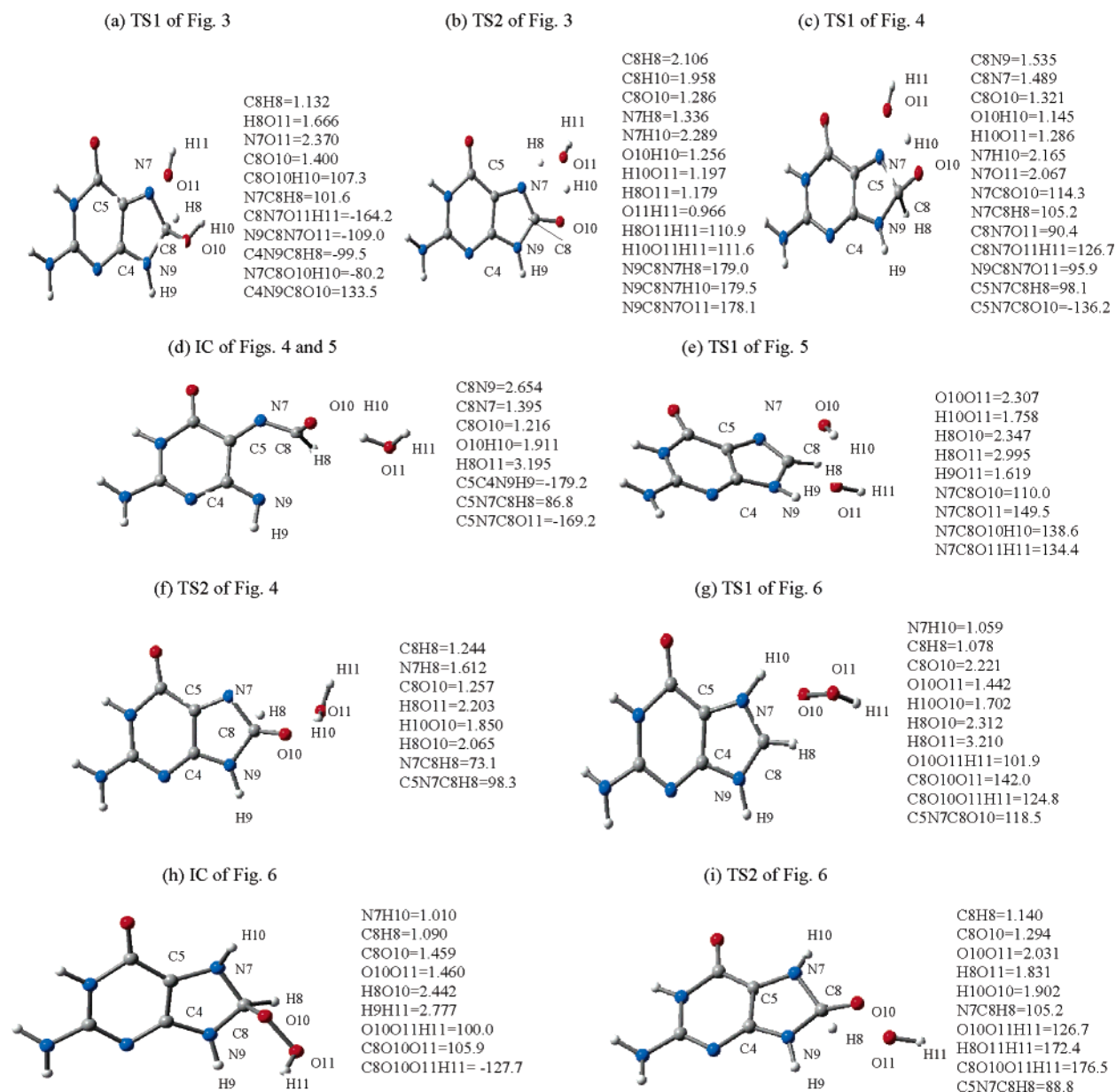


Figure 8. Structures of transition states and intermediates involved in reactions of two OH• radicals or H₂O₂ with guanine according to Schemes 1–4 and Figures 3–6, respectively. The optimized geometrical parameters were obtained at the B3LYP/AUG-cc-pVDZ level of theory except for the structure in (e) where the B3LYP/6-311++G** method was used.

to N9. It is desirable to examine changes in charge distribution in H₂O₂ in going from one situation to another including its dissociation. For this purpose, CHelpG point charges^{74,75} were calculated on the atoms of H₂O₂ in (i) free H₂O₂, (ii) RC of Figure 5a, and (iii) TS1 of Figure 5b. The CHelpG point charges are based on the fitting of molecular electrostatic potentials and are quite reliable for polar systems.⁷⁶ The sums of net CHelpG charges at the four atoms of H₂O₂ in the above-mentioned situations (i), (ii), and (iii) considering the interaction of H₂O₂ with guanine were found (in the unit of the magnitude of electronic charge) to be 0.0, -0.127, and -0.511, respectively. The sums of net CHelpG charges on the corresponding oxygen and hydrogen atoms in situation (ii) were found to be -0.084 (for the group that is involved in hydrogen bonding with N7) and -0.042 (located near C8) (Figure 5a), while the corresponding sums in situation (iii) were found to be -0.127 (for O10H10) and -0.384 (for O11H11), respectively (Figure 8e). These charge distributions reveal the following information: (a) asymmetry is caused in charge distribution between the two

OH components of H₂O₂ due to its interaction with guanine, but the two components still look like OH• radicals with some negative charge on each of them, and (b) the process of dissociation of H₂O₂ is accompanied by an appreciable amount of electronic charge transfer from guanine to the H₂O₂ moiety.

In the transition state structure presented in Figure 8f that corresponds to formation of 8OG consequent to a rearrangement in the IC of Figure 8d, the C8H8 bond length is increased to about 1.24 Å, and the H8 atom has also moved a bit toward N7. Further, in this case, the O10 atom bonded to C8 makes a hydrogen bond with the H10 atom of the water molecule. The transition state structure of Figure 8g corresponds mainly to dissociation of the O10H10 bond of H₂O₂. It is accompanied by binding of the dissociated H10 atom of H₂O₂ to the N7 atom of guanine and a strong hydrogen bonding between the O10 atom of the OOH• group and the dissociated hydrogen atom. The O10O11 bond length of the OOH• group is somewhat less than the equilibrium value of the OO bond length in H₂O₂ (1.46 Å) at this transition state (Figure 8g). The IC presented in Figure

8h is formed following the binding of the O10 atom of the OOH[•] group to the C8 atom of guanine (Figure 6c). At this IC, the O10O11 bond length of the OOH[•] group is increased slightly with respect to that at the transition state (Figure 8g). A rearrangement in the IC of Figure 8h occurs at the transition state presented in Figure 8i such that the O10 atom of the OOH[•] group remains bonded to C8 while the OH[•] part of the OOH[•] group abstracts the H8 atom bonded to C8 making a water molecule. At the transition state presented in Figure 8i, the C8O10 and O10O11 interatomic distances are about 1.29 and 2.03 Å, respectively, showing that the OO bond of the OOH[•] group is broken and the C8O10 bond is formed. This results in the formation of 8OG complexed with a water molecule (Figure 6e).

4. Conclusion

We arrive at the following conclusions from this study:

1. The barrier energies for addition reactions of an OH[•] radical at the C2, C4, C5, and C8 positions of guanine follow the order C2 > C4 > C5 > C8, the reaction at the C8 position being nearly barrierless, while the binding energies follow the order C2 > C8 > C4 > C5. Thus the addition reaction at the C8 position would be highly favored. The six-membered ring of guanine on addition of the OH[•] radical at the C2 position is broken. This and the fact that the barrier energy of the addition reaction is high may be the reason the G2-OH[•] adduct has not been observed.

2. The reaction of a second OH[•] radical at the C8 position of guanine can take place according to two different mechanisms, involving two steps each. According to one mechanism, at the first step, 8OHG complexed with a water molecule is formed while at the second step, 8OHG is tautomerised to 8OG, the water molecule remaining complexed with it. In the other mechanism, at the first step, an IC hydrogen bonded with a water molecule is formed, the five-membered ring of which is open while at the second step, the five-membered ring becomes closed and 8OG complexed with a water molecule is formed.

3. The reaction of H₂O₂ with guanine leading to the formation of 8OG complexed with a water molecule can also take place in accordance with two different mechanisms, each of which involves two steps. According to one mechanism, at the first step, H₂O₂ is dissociated into two OH[•] groups which react with guanine to form the same IC as that mentioned in conclusion 2 above. The subsequent step in this case is also the same as that of the reaction of two separate OH[•] radicals. According to the other mechanism, at the first step, H₂O₂ is dissociated into a hydrogen atom and an OOH[•] group, the two fragments becoming bonded to the N7 and C8 atoms of guanine, respectively. At the second step of this mechanism, the OOH[•] group is dissociated into an oxygen atom and an OH[•] group, the former becomes bonded to the C8 atom of guanine and the latter abstracts the H8 atom bonded to C8, thus producing 8OG complexed with a water molecule.

4. Formation of 8OG through the reaction of guanine with H₂O₂ appears to be much less probable than when guanine reacts with two separate OH[•] radicals, one after the other, since in the former case the barrier energy required to dissociate H₂O₂ into two OH[•] groups or an OOH[•] group and a hydrogen atom is much larger than each of those involved in the latter. Guanine would react with H₂O₂ producing 8OG without the formation of 8OHG but 8OHG would be formed according to one scheme of reaction of guanine with two separate OH[•] radicals.

5. Certain barrier and released energies relating to reactions of two separate OH[•] radicals with guanine or 5MI are ap-

preciably modified in going from the gas phase to aqueous medium. Consequently, it appears that one mechanism (Scheme 2) of the reaction of two separate OH[•] radicals with guanine or 5MI would become appreciably more favorable than the other (Scheme 1). The barrier and released energies involving reactions of H₂O₂ with guanine or 5MI are usually not modified appreciably in going from the gas phase to aqueous medium. Therefore, even in aqueous media, formation of 8OG or 2-oxo-5MI through the reactions of H₂O₂ with guanine or 5MI would continue to be unfavorable as in the gas phase.

6. Study of reactions of one or two OH[•] radicals or an H₂O₂ molecule at the C2 position of 5MI serves as a qualitatively satisfactory model for the corresponding reactions occurring at the C8 position of guanine.

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