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REGULAR ARTICLE

A quantum chemical study of reactions of DNA bases with sulphur mustard: a chemical warfare agent

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Abstract Reactions of the sulphonium ion of sulphur mustard (SM^{+1}) at the N7, N3 and O6 sites of guanine, N7, N3 and N1 sites of adenine, O2 and N3 sites of cytosine and O2 and O4 sites of thymine were studied theoretically in gas phase and aqueous media employing density functional theory (DFT) and second order Møller–Plesset perturbation (MP2) theory. The B3LYP, B3PW91 and B1B95 functionals of DFT and the 6-31+G* and AUG-cc-pVDZ basis sets were used in the calculations. Basis set superposition error was treated using the counterpoise method by single point energy calculations at the B3LYP/6-31+G* level in gas phase. The present study explains the mechanism of alkylation of the DNA bases and shows that SM^{+1} would form stable adducts at the endocyclic nitrogen sites of the DNA bases, and at the O6 site of guanine and the O2 site of cytosine. Formation of adducts at the N7 site of guanine and N3 site of adenine are found to be most favored and next most favored respectively, which agrees with experimental observations.

Keywords Chemical warfare agent · Sulphur mustard · Sulphonium ion · DNA bases · Chemical reactions

1 Introduction

Sulphur mustard (SM), also known as mustard gas and by other names, is a highly toxic vesicant and has been frequently used for many years as an effective chemical weapon in several military conflicts and terrorist attacks [1–3]. It was mainly developed and employed as a chemical weapon for the first time in the World War I and was also recently used on a large scale in the Iran–Iraq war [1–3]. Exposure of human victims to SM can cause a wide variety of deleterious consequences such as eye and skin injury, respiratory tract damage, reproductive and developmental toxicity, gastrointestinal effects, hematological disorders, chromosomal aberrations, delayed bone marrow depression and sometimes even death [1–6]. It has also been reported to be mutagenic and carcinogenic [1, 7]. SM can enter the human body through many routes including inhalation and skin contact [1, 8]. Damages caused by SM exposure have been found to be dose, time and route-dependent [1, 8]. There are also a few reports in the literature on the use of SM for therapeutic purposes [1, 2]. Here, it may be mentioned that some analogues of SM, e.g., nitrogen mustards (NM) have been widely used as drugs for the treatment of a number of human cancers [9, 10].

It is easy and cheap to manufacture SM on a large scale and large stockpiles of SM are still present in many countries in spite of international agreements like Chemical Weapons Convention (CWC) that prohibit development, production, stockpiling and use of such chemically hazardous materials [1, 2, 4]. Thus SM, as a powerful chemical weapon, continues to be a serious threat to humanity [1, 2, 4]. Due to this reason, toxicological aspects of SM have been studied for a long time with the aim to evaluate complications caused by it and to develop effective therapy and preventive measures to deal with the same [1–8, 11–16]. However, until now no effective therapy against the various complications caused by SM has been found [1, 8, 11–16].

SM, like other mustards, is a potent alkylating agent that can react with nucleophilic centers of almost all the classes

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of biomolecules such as DNA, RNA and proteins [1, 6, 8, 11]. Alkylation of DNA by SM is well-documented and is considered to be the principal cause of its observed toxicity [1, 8, 17]. Alkylation by SM induces several types of lesions including formation of monoadducts due to its single binding to the DNA bases and biadducts due to inter- and intra-strand crosslinking of the same [1, 6, 8, 17]. The major alkylation site of SM is the N7 atom of guanine [17, 18]. Thus, its main reaction products with DNA are either monoadducts involving the N7 site of single guanines or biadducts involving the N7 sites of two guanines through crosslinking [17, 18]. Alkylation of the N3 site of adenine and O6 site of guanine by SM has also been reported [18–20].

SM before reacting with the target molecules, e.g., the DNA bases, undergoes a cyclization process to form its sulphonium ion by spontaneously losing a chloride ion from one of its 2-chloroethyl side chains in aqueous media [1, 21]. This sulphonium ion is a reactive intermediate and is capable of alkylating different sites of the DNA bases [1, 21]. Following monoalkylation, there are two main possibilities depending on how the second 2-chloroethyl group of SM behaves: (1) it can also cyclize to form the sulphonium ion following removal of the chloride ion that can alkylate another base site to produce a biadduct, e.g., bis[2-(guanin-7-yl)ethyl] sulphide [18], or (2) it can react with water to yield 2-(hydroxy-ethyl) monoadducts, e.g., N7-[2-[(2-hydroxy-ethyl)thio]ethyl]guanine [18].

In spite of the various previous studies on SM [1–8, 11–21], it has not yet been explained as to why the N7 site of guanine is the most preferred one for its reaction among the different sites of the DNA bases. It would require a detailed study of reactions of SM with all the DNA bases. A detailed study of this problem may also be useful in unraveling the mechanisms of observed toxicity of SM, in developing effective therapies against it, and to find explanation for differences between the observed properties of nitrogen and sulphur mustards. With this objective, we have studied here the addition reactions of the sulphonium ion at the N7, N3 and O6 sites of guanine, N7, N3 and N1 sites of adenine, O2 and N3 sites of cytosine and O2 and O4 sites of thymine bases of DNA in gas phase and aqueous media.

2 Computational details

Geometries of SM and its sulphonium ion (SM^{+1}) formed by the loss of one chloride ion and those of the DNA bases (guanine, adenine, cytosine and thymine) were fully optimized using the B3LYP, B3PW91 and B1B95 hybrid functionals of the density functional theory (DFT) and the 6-31+G* basis set in gas phase [22–25]. Geometries of transition states and adducts involved in the reactions of SM^{+1} at the N7, N3 and O6 sites of guanine, N7, N3 and N1 sites of adenine, O2 and N3 sites of cytosine and O2 and O4 sites of thymine were also fully optimized at all the above-mentioned levels of theory in gas phase. While the B3LYP functional is a commonly used functional of DFT, it has been shown that the B1B95 functional gives reliable barrier energies for nucleophilic substitution, unimolecular and association reactions [26, 27]. The B3PW91 functional has been used as an additional choice for the calculations. The different transition states and adducts involved in the reactions studied here have been denoted as X–Y_Z where X stands for a transition state (TS) or adduct (Ad), Y stands for any of the four DNA bases (G, A, C or T) and Z stands for the site of alkylation of the molecule under consideration. Single point (SP) energy calculations were performed for all the gas phase B3LYP/6-31+G* optimized geometries at the B3LYP/AUG-cc-pVDZ and MP2/6-31+G* levels of theory [28, 29] in gas phase. Basis set superposition error (BSSE) was obtained by single point energy calculations with the counterpoise correction approach [30, 31] at the B3LYP/6-31+G* level of theory in gas phase.

It has been found in many cases that solvent effect significantly influences the stability, structure, spectra and properties of different molecules [32–43]. The solvent effect is generally accounted for in two different ways, i.e., by using the ab initio or classical dynamics involving a large number of specific solvent molecules, or by using a reaction field continuum model where a bulk of the solvent medium instead of specific solvent molecules is considered. The polarizable continuum model (PCM) has been reported to be a successful method for the study of solvent effect [32, 34, 42, 43]. In order to take into account the solvent effect in the present study, we have employed the second approach, i.e., the PCM as implemented in the Gaussian98 package [44]. Thus single point energy calculations in aqueous media were performed employing the PCM at the B3LYP/AUG-cc-pVDZ and MP2/6-31+G* levels of theory using the B3LYP/6-31+G* optimized geometries and at the B3PW91/AUG-cc-pVDZ level using the B3PW91/6-31+G* optimized geometries. Geometry optimization calculations in aqueous media using the PCM were also performed at the B3LYP/6-31+G* level of theory for all the cases and at the B3PW91/6-31+G* level for some selected cases.

Vibrational frequency analysis was performed for all the optimized structures at the B3LYP/6-31+G*, B1B95/6-31+G* and B3PW91/6-31+G* levels of theory in gas phase and at the B3LYP/6-31+G* level of theory in aqueous media to ensure that each total energy minimum had all real frequencies and each transition state had only one imaginary frequency. Genuineness of the calculated transition states was ensured by visually examining the

vibrational modes of the imaginary frequencies and applying the condition that these connected the corresponding reactants and adducts properly. As the reactions involved a single step each and the vibrational modes corresponding to the imaginary frequencies at the transition states clearly showed formation of the products and their dissociation into the reactants in each case, intrinsic reaction coordinate (IRC) calculations [45] usually carried out to ensure genuineness of transition states, were not considered to be necessary.

Gibbs free energies at 298.15 K were obtained for all the cases at the B3LYP/6-31+G*, B1B95/6-31+G* and B3PW91/6-31+G* levels of theory in gas phase and at the B3LYP/6-31+G* level of theory in aqueous media. As an approximation, the thermal energy correction giving Gibbs free energy obtained at the B3LYP/6-31+G* level of theory in gas phase for each species was applied to the total energies obtained at the B3LYP/AUG-cc-pVDZ and MP2/6-31+G* levels of theory also, in both gas phase and aqueous media, and this correction was also applied to the corresponding counterpoise-corrected total energy obtained at the B3LYP/6-31+G* level of theory in gas phase. To obtain Gibbs free energies at the B3PW91/AUG-cc-pVDZ level in aqueous media, thermal energy corrections obtained at the B3PW91/6-31+G* level of theory were used. The reaction barrier energy in each case was obtained as the difference between the Gibbs free energy of the transition state and the sum of the corresponding Gibbs free energies of the isolated reactants. Similarly, the binding energy of each adduct was obtained as the difference between the Gibbs free energy of the adduct and the sum of the Gibbs free energies of the corresponding isolated reactants. All the calculations were performed using the Windows version of Gaussian 98 (G98W) package [44]. The GaussView program was used for visualization of optimized structures and vibrational modes [46].

3 Results and discussion

Structures of SM and its sulphonium ion (SM^{+1}) along with the values of certain optimized geometrical parameters obtained at the B3LYP/6-31+G* level of theory in aqueous media are presented in Fig. 1. The distances between the two chlorine atoms Cl1 and Cl2 in SM were found to be 7.54 and 6.98 Å in gas phase and aqueous media respectively at the B3LYP/6-31+G* level of theory. The corresponding distance in gas phase was obtained as 7.32 and 6.67 Å at the B3PW91/6-31+G* and B1B95/6-31+G* levels of theory respectively. These distances in both gas phase and aqueous media are appreciably less than the length required for crosslinking the N7 sites of

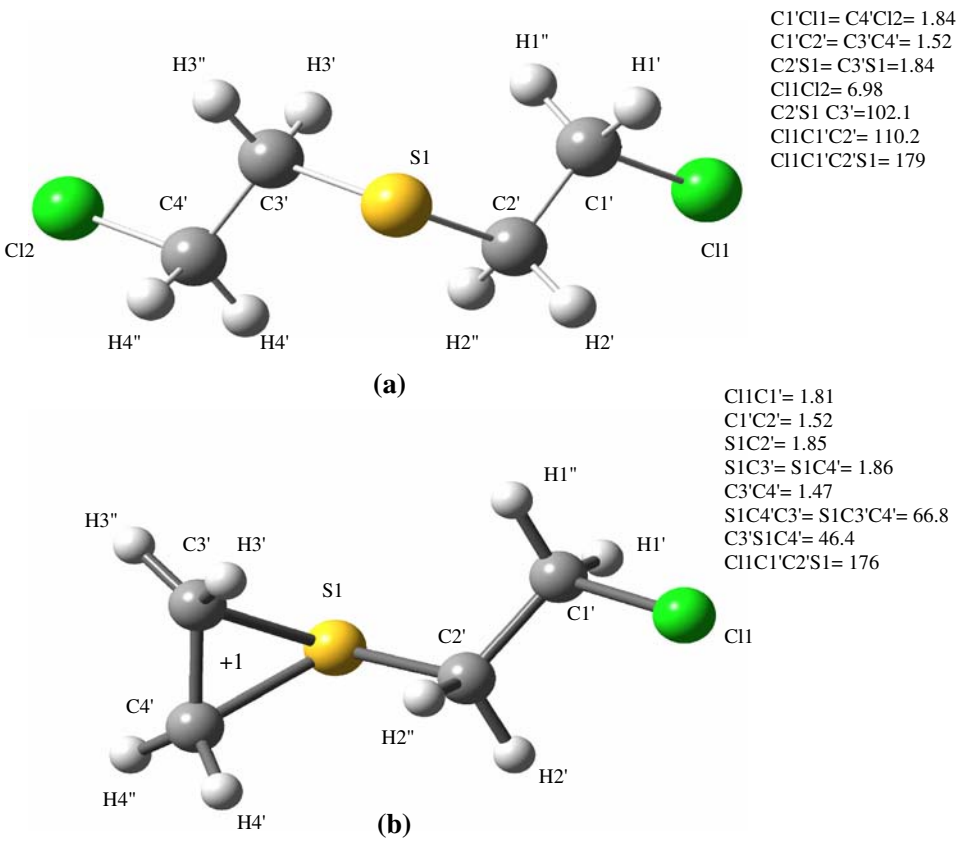


Fig. 1 **a** Structure of sulphur mustard and **b** structure of its sulphonium ion (SM^{+1}). Some optimized geometrical parameters (Å, deg.) obtained at the B3LYP/6-31+G* level of theory in aqueous media are given

guanines located on the opposite strands of B DNA (8.9 Å) [47] which suggests that SM would not easily form interstrand crosslinked products involving the N7 sites of guanines. However, it is observed that SM does form interstrand crosslinked products involving the N7 sites of guanines [17, 18]. This discrepancy can be explained by considering a bend in the DNA structure that may be associated with cross linking. This possibility is supported by the fact that the nitrogen mustard mechlorethamine (chlorine–chlorine distance 7.04 Å) [9] is involved in crosslinking of the N7 sites of guanines on the opposite DNA strands where a bend of ~12.4–16.8° in DNA has been found [47].

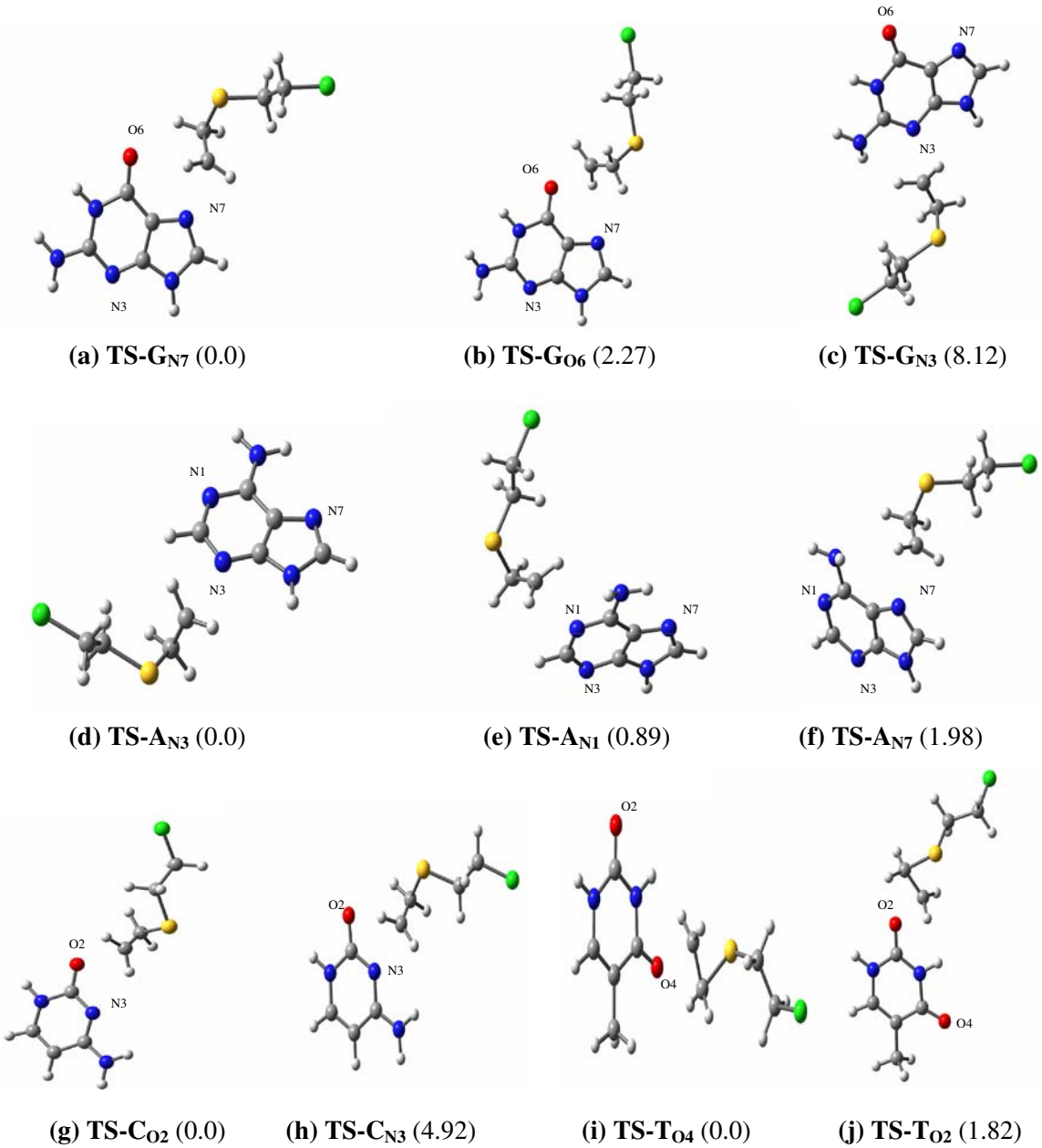
3.1 Barrier energies

We found that the alkylation reactions of SM^{+1} at the N7, O6 and N3 sites of guanine, N7, N3 and N1 sites of adenine, O2 and N3 sites of cytosine and O2 and O4 sites of thymine at different levels of theory in gas phase and aqueous media involve a transition state each and the nature of the transition states was found to be of S_N2 type. Optimized structures of the various transition states involved in alkylation at the different sites of guanine, adenine, cytosine and thymine obtained at the B3LYP/6-31+G* level of theory in aqueous media along with the relative Gibbs free energies at 298.15 K obtained at the MP2/6-31+G* level of theory in aqueous media with respect to those of the transition states TS–G_{N7}, TS–A_{N3}, TS–C_{O2} and TS–T_{O4} respectively are shown in Fig. 2. The barrier energies involved in the reactions of SM^{+1} at the different sites of guanine, adenine, cytosine and thymine obtained at the different levels of theory in gas phase and aqueous media are presented in Table 1. In going from B3LYP/6-31+G* to B3LYP/AUG-cc-pVDZ(SP) level of theory in gas phase, the calculated barrier and binding energies do not change significantly (Tables 1, 2). Further, all the calculated binding energies and seven out of the ten calculated barrier energies have changed by less than 10% in going from B3LYP/6-31+G* to B3LYP/AUG-cc-pVDZ(SP) level of theory in gas phase (Tables 1, 2). Thus, the change in the basis set does not affect the calculated results significantly.

The alkylation barrier energies for the N7 and O6 sites of guanine are large negative while those for the N3 site of guanine are quite appreciable and positive at all the levels of theory in gas phase (Table 1). Further, the magnitudes of barrier energies at the B1B95/6-31+G* level of theory are larger by 2.2–2.9 kcal/mol and by ~1 kcal/mol than those obtained at the B3LYP/6-31+G* and B3PW91/6-31+G* levels of theory in gas phase respectively. The counterpoise-corrected and uncorrected barrier energies for the N7, O6 and N3 sites of guanine are also similar, the extent of correction being ~1 kcal/mol at the B3LYP/6-31+G* level of theory in gas phase (Table 1). The calculated negative barrier energies show that alkylation at the N7 and O6 sites of guanine in gas phase by SM^{+1} would be barrierless. However, in going from gas phase to aqueous media, the alkylation barrier energies for all the three sites of guanine are found to be enhanced greatly at the different levels of theory. Thus all the barrier energies in aqueous media are positive and follow the order N7 < O6 < N3 at the B3LYP/AUG-cc-pVDZ(SP), B3PW91/AUG-cc-pVDZ(SP) and MP2/6-31+G*(SP) levels of theory (Table 1). The alkylation barrier energies for the N7, O6 and N3 sites of guanine are found to be 12.4, 14.7 and 20.6 kcal/mol at the MP2/6-31+G*(SP) level of theory in aqueous media respectively (Table 1).

As the alkylation barrier energies at the N7, O6 and N3 sites of guanine are increased dramatically at the single point energy level calculations in aqueous media using PCM, we performed full geometry optimization calculations in aqueous using PCM at the B3LYP/6-31+G* level for all the three sites of guanine and at the B3PW91/6-31+G* level for the N7 site of guanine. The alkylation barrier energies for the N7, O6 and N3 sites of guanine in aqueous media were thus found at the B3LYP/6-31+G* level of theory to be 12.9, 14.7 and 19.9 kcal/mol respectively (Table 1). The barrier energy in aqueous media for the N7 site of guanine at the B3PW91/6-31+G* level by full geometry optimization was found to be 14.2 kcal/mol. These barrier energies are quite similar to those obtained by single point energy PCM calculations at the different levels of theory (Table 1). It shows that the barrier energies obtained by single point energy PCM calculations in aqueous media are reliable. The above discussion also shows that for alkylation of guanine by SM^{+1}, the N7 site would be most favored while the N3 site would be least favored.

The barrier energies for alkylation at the different sites of adenine by SM^{+1} obtained at the different levels of theory in gas phase follow the order N3 ≈ N1 < N7 (Table 1). The counterpoise-corrected barrier energies at the N3, N1 and N7 sites of adenine obtained at the B3LYP/6-31+G* level in gas phase were also found to follow the same order. In going from gas phase to aqueous media, the calculated barrier energies for all the N7, N3 and N1 sites of adenine were found to be enhanced by 12–15 kcal/mol and follow the order N3 < N1 < N7, the lowest barrier energy corresponding to the N3 site being ~15 kcal/mol at the B3LYP/AUG-cc-pVDZ(SP), B3PW91/AUG-cc-pVDZ(SP) and MP2/6-31+G*(SP) levels of theory (Table 1). The alkylation barrier energies obtained by full geometry optimization in aqueous media employing PCM at the B3LYP/6-31+G* level of theory for the three sites of adenine were found to lie in the range of 14–17 kcal/mol (Table 1). We also performed full geometry optimization in aqueous media for the N3 site of adenine at the



(a) TS-G_{N7} (0.0) **(b) TS-G_{O6}** (2.27) **(c) TS-G_{N3}** (8.12)

(d) TS-A_{N3} (0.0) **(e) TS-A_{N1}** (0.89) **(f) TS-A_{N7}** (1.98)

(g) TS-C_{O2} (0.0) **(h) TS-C_{N3}** (4.92) **(i) TS-T_{O4}** (0.0) **(j) TS-T_{O2}** (1.82)

Fig. 2 Structures of transition states involved in the reactions of sulphonium ion (SM^{+1}) at the N7, O6 and N3 sites of guanine (**a**–**c**), N3, N1 and N7 sites of adenine (**d**–**f**), O2 and N3 sites of cytosine (**g**, **h**), and O4 and O2 sites of thymine (**i**, **j**) obtained at the B3LYP/6-31+G* level of theory in aqueous media. Relative Gibbs free energies (kcal/mol) of transition states (**a**–**c**), (**d**–**f**), (**g**, **h**) and (**i**, **j**), obtained at MP2/6-31+G*(SP) level of theory in aqueous media, are given in parentheses with respect to that of the transition states **a** TS–G_{N7}, **d** TS–A_{N3}, **g** TS–C_{O2}, and **i** TS–T_{O4} respectively

B3PW91/6-31+G* level and the corresponding barrier energy was found to be 16.4 kcal/mol. We note that the barrier energies obtained by single point energy PCM calculations for alkylation of the three sires of adenine are similar to those obtained by geometry optimization in aqueous media. On the basis of the above-mentioned results (Table 1) and also the fact that the N1 site of adenine would be involved in hydrogen bonding with thymine in DNA, it appears that the N3 and N7 sites of adenine in DNA would be its most and least favored sites for alkylation respectively. Thus an interesting difference between the N7 sites of guanine and adenine with regard to alkylation by SM^{+1} is brought out. That is, while the N7 site is most favored in guanine, the corresponding (N7) site is least favored in adenine for alkylation by SM^{+1}.

The negative values of gas phase alkylation barrier energies corresponding to the O2 and N3 sites of cytosine obtained at all the levels of theory reveal that alkylation at

Table 1 Barrier energies (kcal/mol) for addition reactions of the sulphonium ion (SM^{+1}) at the different sites of the DNA bases obtained at various levels of theory in gas phase and aqueous media

Base and site	Gas phase					Aqueous media			
	Optimization			Single point[b]		Optimization	Single point[b]		
	B3LYP/6-31+G*[a]	B3PW91/6-31+G*	B1B95/6-31+G*	B3LYP/AUG-cc-pVDZ	MP2/6-31+G*	B3LYP/6-31+G*	B3LYP/AUG-cc-pVDZ	B3PW91/AUG-cc-pVDZ	MP2/6-31+G*
Guanine									
N7	−10.7 (−9.8)	−9.3	−8.2	−11.4	−10.9	12.9	15.2	13.7	12.4
N3	12.0 (13.2)	13.5	14.2	11.3	10.2	19.9	26.3	21.5	20.6
O6	−11.5 (−10.6)	−9.6	−8.6	−12.0	−10.4	14.7	15.6	15.7	14.7
Adenine									
N7	6.2 (7.2)	7.7	9.2	5.9	3.8	17.2	19.9	19.5	17.1
N3	2.7 (3.4)	4.0	5.5	1.8	2.0	14.3	14.7	15.6	15.2
N1	2.8 (3.6)	3.9	4.4	2.5	0.6	16.0	17.9	17.9	16.0
Cytosine									
O2	−12.5 (−11.6)	−10.9	−9.8	−12.6	−13.2	12.8	10.5	13.5	10.3
N3	−5.7 (−4.7)	−4.2	−3.6	−6.0	−5.9	15.2	14.3	15.9	15.3
Thymine									
O2	5.2 (6.0)	7.3	9.1	4.4	8.1	20.0	18.1	20.7	21.5
O4	3.3 (4.1)	5.2	6.2	2.5	6.2	20.2	16.7	19.3	19.7

[a] Counterpoise-corrected barrier energies are given in parentheses

[b] Single point energy calculations at the B3LYP/AUG-cc-pVDZ and MP2/6-31+G* levels were performed using the gas phase B3LYP/6-31+G* optimized geometry while single point energy calculations at the B3PW91/AUG-cc-pVDZ level were performed using the gas phase B3PW91/6-31+G* optimized geometry

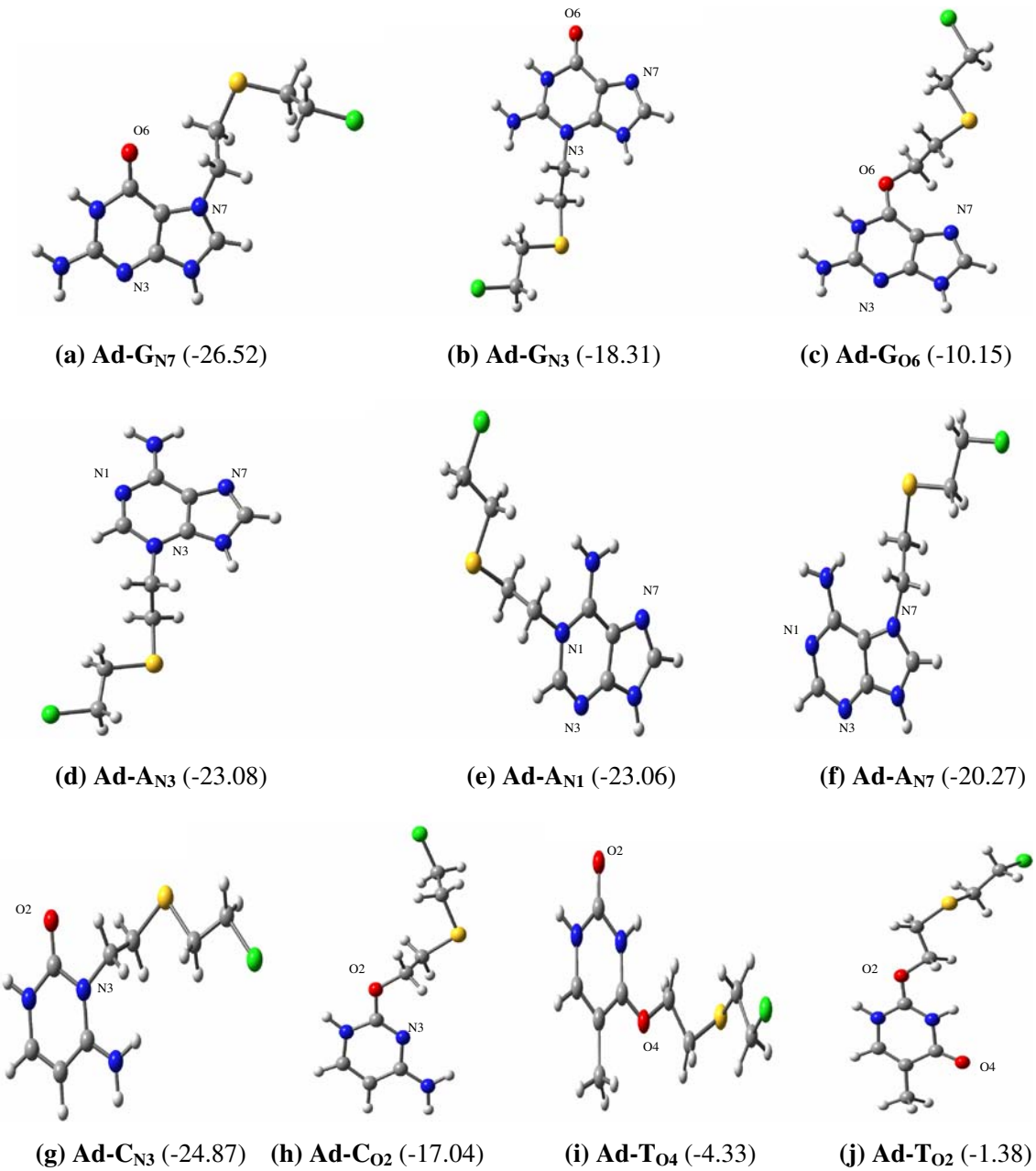
Table 2 Binding energies (kcal/mol) of adducts formed by alkylation of the DNA bases at their different sites by the sulphonium ion (SM^{+1}) obtained at various levels of theory in gas phase and aqueous media

Adduct	Gas phase					Aqueous media			
	Optimization			Single point[b]		Optimization	Single point[b]		
	B3LYP/6-31+G*[a]	B3PW91/6-31+G*	B1B95/6-31+G*	B3LYP/AUG-cc-pVDZ	MP2/6-31+G*	B3LYP/6-31+G*	B3LYP/AUG-cc-pVDZ	B3PW91/AUG-cc-pVDZ	MP2/6-31+G*
Ad–G_{N7}	−36.0 (−34.3)	−36.5	−34.5	−36.5	−38.5	−22.6	−22.5	−22.7	−26.5
Ad–G_{N3}	−15.4 (−13.6)	−15.2	−14.4	−17.0	−19.7	−9.6	−10.6	−11.1	−18.3
Ad–G_{O6}	−25.3 (−24.8)	−24.4	−23.1	−25.3	−24.5	−6.1	−6.8	−5.8	−10.2
Ad–A_{N7}	−23.0 (−21.2)	−23.2	−20.6	−23.6	−25.9	−14.1	−17.6	−20.3	−12.9
Ad–A_{N3}	−28.8 (−27.1)	−28.7	−27.5	−29.6	−31.2	−16.6	−23.6	−23.1	−19.1
Ad–A_{N1}	−28.0 (−26.3)	−27.9	−26.5	−28.9	−31.6	−17.1	−20.9	−23.1	−16.9
Ad–C_{O2}	−30.2 (−28.5)	−29.4	−28.6	−29.3	−31.2	−11.5	−13.6	−17.0	−11.4
Ad–C_{N3}	−30.6 (−28.6)	−30.8	−30.4	−30.8	−35.0	−17.9	−20.2	−24.9	−18.2
Ad–T_{O2}	−4.7 (−3.2)	−3.7	−2.9	−5.1	−4.8	3.7	−0.9	−1.4	4.7
Ad–T_{O4}	−9.4 (−7.9)	−8.6	−7.7	−9.7	−9.1	0.5	−2.4	−4.3	0.5

[a] Counterpoise-corrected binding energies are given in parentheses

[b] Single point energy calculations at the B3LYP/AUG-cc-pVDZ and MP2/6-31+G* levels were performed using the gas phase B3LYP/6-31+G* optimized geometry while single point energy calculations at the B3PW91/AUG-cc-pVDZ level were performed using the gas phase B3PW91/6-31+G* optimized geometry

these sites would be barrierless in gas phase (Table 1). In going from gas phase to aqueous media, the barrier energies for these two sites of cytosine were found to be highly enhanced that follow the order O2 < N3 at the different levels of theory (Table 1). In the case of thymine, the alkylation barrier energies were also found to be strongly



(a) Ad-G_{N7} (-26.52) **(b) Ad-G_{N3}** (-18.31) **(c) Ad-G_{O6}** (-10.15)

(d) Ad-A_{N3} (-23.08) **(e) Ad-A_{N1}** (-23.06) **(f) Ad-A_{N7}** (-20.27)

(g) Ad-C_{N3} (-24.87) **(h) Ad-C_{O2}** (-17.04) **(i) Ad-T_{O4}** (-4.33) **(j) Ad-T_{O2}** (-1.38)

Fig. 3 Structures of adducts formed by the reactions of sulphonium ion (SM^{+1}) at the N7, N3 and O6 sites of guanine (**a**–**c**), N3, N1 and N7 sites of adenine (**d**–**f**), N3 and O2 sites of cytosine (**g**, **h**), and O4 and O2 sites of thymine (**i**, **j**) obtained at the B3LYP/6-31+G* level of theory in aqueous media. Binding energies (kcal/mol) of these adducts, obtained at the MP2/6-31+G*(SP) level of theory in aqueous media, are given in parentheses

enhanced in going from gas phase to aqueous media. Further, the barrier energies for alkylation of the O2 and O4 sites of thymine obtained at the different levels of theory in aqueous media are similar (Table 1).

3.2 Binding energies

Optimized structures of the adducts formed by the binding of SM^{+1} at the N7, N3 and O6 sites of guanine, N7, N3 and N1 sites of adenine, O2 and N3 sites of cytosine and O2 and O4 sites of thymine obtained at the B3LYP/6-31+G* level of theory in aqueous media along with their binding energies obtained at the MP2/6-31+G* level of theory in aqueous media are presented in Fig. 3. The calculated binding energies of various adducts obtained at the different levels of theory in gas phase and aqueous media are presented in Table 2. A negative binding energy implies that the product under consideration would be stable.

When the counterpoise correction is applied to the binding energies of the adducts at the B3LYP/6-31+G* level of theory in gas phase, the magnitudes of the binding energies are decreased by 0.5–2.0 kcal/mol but still they follow the same order as without counterpoise correction (Table 2). The magnitudes of the binding energies obtained using the B1B95 functional are found to be somewhat smaller than those obtained using the B3LYP and B3PW91 functionals in gas phase (Table 2). In going from gas phase to aqueous media, the magnitudes of the negative binding energies of adducts for all the sites of DNA bases are reduced substantially at the different levels of theory (Table 2). However, even after this reduction, the binding energies of adducts formed at the endocyclic nitrogen sites of the DNA bases in aqueous media are quite large. Further, while in aqueous media the binding energy of the adduct formed at the O2 site of cytosine is still quite large, that of the adduct formed at the O6 site of guanine is moderate. The adducts formed at the O2 and O4 sites of thymine (Ad–T_{O2}, Ad–T_{O4}) were also found to have considerable negative binding energies in gas phase. In aqueous media also, the binding energies of the adducts Ad–T_{O2} and Ad–T_{O4} at the B3LYP/AUG-cc-pVDZ(SP) and the MP2/6-31+G*(SP) levels of theory are negative but their magnitudes are much less than those in gas phase (Table 2). However, the binding energies of the adducts Ad–T_{O2} and Ad–T_{O4} are positive at the B3LYP/6-31+G* and B3PW91/AUG-cc-pVDZ(SP) levels of theory in aqueous media (Table 2). The binding energies of the adducts formed at the O6 site of guanine, O2 site of cytosine and all the endocyclic nitrogen sites of the DNA bases in gas phase are fairly large negative at the different levels of theory employed here.

Thus the above-mentioned results suggest that in aqueous media, stable adducts of SM^{+1} would be formed at all the endocyclic nitrogen sites of the DNA bases, the O2 site of cytosine and the O6 site of guanine while the adducts formed at the O2 and O4 sites of thymine may not be stable. In a previous theoretical study [9], it was found that the nitrogen mustard mechlorethamine would not alkylate any of the exocyclic oxygen sites of the DNA bases to form stable products. Thus there is an interesting difference between the reactions of the sulphur and nitrogen mustards with respect to alkylation of the O6 site of guanine and the O2 site of cytosine.

Considering both the binding and barrier energies together, we can predict the most favorable sites for alkylation by SM^{+1} among the various sites of DNA bases reliably. We may take the gas phase results obtained at the MP2/6-31+G*(SP) level to be more reliable than those obtained at the B3LYP/6-31+G* level in view of a better electron-correlation treatment at the former level than at the latter level. At the MP2/6-31+G*(SP) level of theory in aqueous media, the magnitudes of binding energies of the adducts follow the order Ad–G_{N7} > Ad–C_{N3} > Ad–A_{N3} ≈ Ad–A_{N1} > Ad–A_{N7} > Ad–G_{N3} > Ad–C_{O2} > Ad–G_{O6} > Ad–T_{O4} > Ad–T_{O2} (Table 2). The barrier energies corresponding to the first four of these adducts, i.e., Ad–G_{N7}, Ad–C_{N3}, Ad–A_{N3} and Ad–A_{N1}, obtained at the same level of theory in aqueous media, follow the order Ad–G_{N7} < Ad–A_{N3} ≈ Ad–C_{N3} < Ad–A_{N1} (Table 1). These results and the fact that the N1 site of adenine and the N3 site of cytosine would not be easily accessible for reactions due to their involvement in hydrogen bonding between the complementary bases of DNA show that the N7 site of guanine would be the most favored alkylation site and the N3 site of adenine would be the next most favored alkylation site in DNA by SM^{+1}.

With regard to alkylation of the O6 site of guanine by SM^{+1} that has been found to be highly toxic and mutagenic [48, 49], the present calculations reveal the following information. At the MP2/6-31+G*(SP) level of theory in aqueous media, the calculated barrier energy for this reaction is low which shows that this reaction would be a favored one. The calculated binding energy of the adduct Ad–G_{O6} in aqueous media at the MP2/6-31+G*(SP) level is also fairly high though the corresponding values obtained at the B3LYP/AUG-cc-pVDZ(SP) and B3PW91/AUG-cc-pVDZ(SP) levels are only moderate. Therefore, the adduct Ad–G_{O6} would occur in biological media, consequent to the reaction of SM^{+1} with guanine in DNA, at least in a moderate abundance. Alkylation of the N7 and O6 sites of guanine and the N3 site of adenine by SM, the N7 site of guanine being most favored, has been observed experimentally [17–20]. As discussed above, the present calculated results are consistent with the experimental observations [17–20].

4 Conclusions

The present study leads us to the following conclusions:

1. Alkylation reactions at the different endocyclic nitrogen and exocyclic oxygen sites of the DNA bases by SM^{+1} involve a transition state of S_N2 type each. The barrier energies for the alkylation reactions increase substantially in going from gas phase to aqueous media in all the cases. The barrier energies corresponding to alkylation of the different sites of the DNA bases lie in the range 10–22 kcal/mol at the MP2/6-31+G*(SP) level of theory in aqueous media.
2. The calculated binding energies of the adducts in aqueous media reveal that SM^{+1} would form stable adducts at all the endocyclic nitrogen sites as well as the O6 site of guanine and the O2 site of cytosine. It has been reported that stable products are not formed

when nitrogen mustard (mechlorethamine) reacts with any of the oxygen sites of the DNA bases. Thus, there is a major difference between the behaviors of SM and nitrogen mustard with regard to alkylation of the oxygen sites of guanine and cytosine.

3. The calculated barrier and binding energies in aqueous media show that the N7 site of guanine would be the most favored alkylation site by SM^{+1} while the N3 site of adenine would be the next most favored alkylation site among the various sites of the DNA bases. Alkylation at the O6 site of guanine by SM^{+1} would also occur and the adduct thus formed would be fairly stable. These calculated results agree with experimental observations.

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References

1. Kehe K, Szinicz L (2005) Toxicology 214:198. doi:10.1016/j.tox.2005.06.014
2. Smith KJ, Hurst CG, Moeller RB, Skelton HG, Sidell FR (1995) J Am Acad Dermatol 32:765. doi:10.1016/0190-9622(95)91457-9
3. Dacre JC, Goldman M (1996) Pharmacol Rev 48:289
4. Graham JS, Chilcott RP, Rice P, FRCPath BM, Milner SM, Hurst GC, Maliner BI (2005) J Burns Wounds 4:1
5. Wormser U, Izrael M, van der Zee EA, Brodsky B, Yanai J (2005) Neurotoxicol Teratol 27:65. doi:10.1016/j.ntt.2004.09.006
6. Shahin S, Cullinane C, Gray PJ (2001) Chem Biol Interact 138:231. doi:10.1016/S0009-2797(01)00275-7
7. Gray PJ (1995) Nucleic Acids Res 23:4378. doi:10.1093/nar/23.21.4378
8. Rao PVL, Vijayaraghavan R, Bhaskar ASB (1999) Toxicol 139:39. doi:10.1016/S0300-483X(99)00097-9
9. Shukla PK, Mishra PC, Suhai S (2007) Chem Phys Lett 449:323. doi:10.1016/j.cplett.2007.10.072
10. Masta A, Gray PJ, Phillips DR (1996) Carcinogenesis 17:525. doi:10.1093/carcin/17.3.525
11. Lawley PD (1971) Biochem J 125:829
12. Sawyer TW, Hancock JR, D' Agostino PA (1998) Toxicol Appl Pharmacol 151:340. doi:10.1006/taap.1998.8457
13. Korkmaz A, Yaren H, Topal T, Oter S (2006) Arch Toxicol 80:662. doi:10.1007/s00204-006-0089-x
14. Anshoo G, Singh S, Kulkarni AS, Pant SC, Vijayaraghavan R (2005) Indian J Pharmacol 37:103
15. Gao X, Ray R, Xiao Y, Barker PE, Ray P (2007) BMC Cell Biol 8:17. doi:10.1186/1471-2121-8-17
16. Geraci MJ (2008) Ann Pharmacother 42:237. doi:10.1345/aph.1K445
17. Ludlum DB, Austin-Ritchie P, Hagopian M, Niu TQ, Yu D (1994) Chem Biol Interact 91:39. doi:10.1016/0009-2797(94)90005-1
18. Fidder A, Moes GWH, Scheffer AG, van der Schans GP, Bean RA, De Jong LPA, Benschop HP (1994) Chem Res Toxicol 7:199. doi:10.1021/tx00038a013
19. Ludlum DB, Kent S, Metha JR (1986) Carcinogenesis 7:1203. doi:10.1093/carcin/7.7.1203
20. Matijasevic Z, Stering A, Niu TQ, Austin-Ritchie P, Ludlum DB (1996) Carcinogenesis 17:2249. doi:10.1093/carcin/17.10.2249
21. Mozier NM, Hoffman JL (1990) FASEB J 4:3329
22. Becke AD (1993) J Chem Phys 98:5648. doi:10.1063/1.464913
23. Lee C, Yang W, Parr RG (1988) Phys Rev B 37:785. doi:10.1103/PhysRevB.37.785
24. Becke AD (1996) J Chem Phys 104:1040. doi:10.1063/1.470829
25. Perdew JP (1991) In: Ziesche P, Esching H (eds) "Unified theory of exchange and correlation beyond the local density approximation" in electronic structure of solids. Academic Verlag, Berlin, pp 11–20
26. Zhao Y, Truhlar DG (2005) J Phys Chem A 109:5656. doi:10.1021/jp050536c
27. Sousa SF, Fernandes PA, Ramos MJ (2007) J Phys Chem A 111:10439. doi:10.1021/jp0734474
28. Frisch MJ, Head-Gordon M, Pople JA (1990) Chem Phys Lett 166:275. doi:10.1016/0009-2614(90)80029-D
29. Frisch MJ, Head-Gordon M, Pople JA (1990) Chem Phys Lett 166:281. doi:10.1016/0009-2614(90)80030-H
30. Boys SF, Bernardi F (1970) Mol Phys 19:553. doi:10.1080/00268977000101561
31. Simon S, Duran M, Dnnenberg JJ (1996) J Chem Phys 105:11024. doi:10.1063/1.472902
32. Tomasi J, Persico M (1994) Chem Rev 94:2027. doi:10.1021/cr00031a013
33. Cramer CJ, Truhlar DG (1999) Chem Rev 99:2200. doi:10.1021/cr960149m
34. Tomasi J, Mennucci B, Cammi R (2005) Chem Rev 105:2999. doi:10.1021/cr9904009
35. Jalkanen KJ, Suhai S (1996) Chem Phys 208:81. doi:10.1016/0301-0104(96)00042-0
36. Deng Z, Polavarapu PL, Ford SJ, Hecht L, Baron LD, Ewig CS, Jalkanen KJ (1996) J Phys Chem 100:2025. doi:10.1021/jp951865f
37. Han W-G, Jalkanen KJ, Elstner M, Suhai S (1998) J Phys Chem B 102:2587. doi:10.1021/jp972299m
38. Jalkanen KJ, Jürgensen VW, Claussen A, Rahim A, Jensen GM, Wade RC, Nardi F, Jung C, Degtyarenko IM, Nieminen RM, Herrmann F, Knapp-Mohammady M, Niehaus TA, Frimand K, Suhai S (2006) Int J Quantum Chem 106:1160. doi:10.1002/qua.20863
39. Degtyarenko IM, Jalkanen KJ, Gurtovenko AA, Nieminen RM (2007) J Phys Chem B 111:4227. doi:10.1021/jp0676991
40. Deplazes E, van Bronswijk W, Zhu F, Barron LD, Ma S, Nafie LA, Jalkanen KJ (2008) Theor Chem Acc 119:155. doi:10.1007/s00214-007-0276-8
41. Ramnarayan K, Bohr HG, Jalkanen KJ (2008) Theor Chem Acc 119:265. doi:10.1007/s00214-007-0285-7
42. Barone V, Cossi M, Tomasi J (1997) J Chem Phys 107:3210. doi:10.1063/1.474671
43. Cossi M, Scalmani G, Rega N, Barone V (2002) J Chem Phys 117:43. doi:10.1063/1.1480445
44. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewski VG, Montgomery JA Jr, Stratmann RE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammy R, Mennucci B, Pomelli C, Adamo C, Clifford S, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Rega N, Salvodar P, Dannenberg JJ, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Cioslowski J, Ortiz JV, Baboul AG, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW,

Andres JL, Gonzalez C, Head-Gordon M, Replogle ES, Pople JA (2001) Gaussian 98, Revision A.11.2, Gaussian Inc., Pittsburgh
45. Gonzalez C, Schlegel HB (1989) J Chem Phys 90:2154. doi: 10.1063/1.456010
46. Frisch AE, Dennington RD, Keith TA, Neilsen AB, Holder AJ (2003) GaussView, Rev. 3.09, Gaussian Inc., Pittsburgh
47. Rink SM, Hopkins PB (1995) Biochemistry 34:1439. doi: 10.1021/bi00004a039
48. Bignami M, O' Driscoll M, Aquilina G, Karran P (2000) Mutat Res 462:71. doi:10.1016/S1383-5742(00)00016-8
49. Shukla PK, Mishra PC, Suhai S (2007) Int J Quantum Chem 107:1270. doi:10.1002/qua.21233