A semi-empirical SCF-MO study on the base-pairing properties of 8-oxopurines: significance for mutagenicity

Divi Venkateswarlu,[a] R. H. Duncan Lyngdoh[b] and Manju Bansal[a]

[a] *Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India*
[b] *Department of Chemistry, North-Eastern Hill University, Shillong 793 003, India*

C^8-Oxidised purines like 7-hydro-8-oxoguanine (8OG) and 7-hydro-8-oxoadenine (8OA) are known as products of oxidative DNA damage. Semiempirical molecular orbital calculations at the PM3 SCF-MO level are used to investigate the base-pairing properties of these bases in an attempt to understand their mutagenic properties. A detailed analysis of the base-pairing properties of these bases leads to an identification of the most probable pairing schemes involved in mutagenic base-mispairing. It is suggested that both bases are capable of inducing transversional as well as transitional mutations *via* base-mispairing. The results presented are largely in consonance with available experimental reports.

Introduction

The reactive oxygen species generated by *in vivo* oxidative metabolism or by exogenous agents such as ionising radiation or chemical oxidants have been widely implicated for C^8 modification of DNA purine bases.[1–3] There has been wide interest in the properties of these oxidised bases owing to the appreciable incidence of cellular damage by oxidative agents[4] (*ca*. 10^4 oxidative hits per day). It is widely believed that C^8-oxidation of purines could play a key role in the aging process as well as in degenerative diseases such as cancer.[5–9] Among the various oxidation products of DNA bases, 7-hydro-8-oxoguanine (8OG) and 7-hydro-8-oxoadenine (8OA) have received wide attention owing to their mutation inducing properties.[10–12]

Tautomers of 8-oxopurines

These modified bases as monomers can adopt several tautomeric structures. Fig. 1 shows the predominant and the most stable minor tautomeric forms of 8OA and 8OG. Under physiological conditions, the 6,8-dioxo species (8OG in Fig. 1) is reported to predominate[13] over the others. However, NMR spectral evidence by Kouchakdjian *et al*. indicates the presence of *ca*. 15% of the minor tautomers in the case of 8OG.[14] The significance of minor tautomers, particularly the enol forms, in mutagenic base-mispairing therefore cannot be ruled out. Gas-phase *ab initio* studies at STO-3G level also indicated that the 6,8-dioxo form predominates in 8OG with the minor 6-enol-8-keto tautomer (8OG* in Fig. 1) being energetically very close to the major tautomer.[15] While not much is known about the tautomeric preferences of 8OA, ^{15}N NMR studies on 8OA suggest that while the 8-keto form (8OA in Fig. 1) predominates under physiological conditions, the minor 8-enol tautomer (8OA* in Fig. 1) may also exist at high pK_a values.[16]

Template properties of 8-oxopurines

The DNA template properties of 8OdG indicate *in vivo*[17–19] and *in vitro*[20,21] mutagenic properties. Translesional synthesis can proceed past 8OdG in primed template reactions catalysed by DNA polymerase, in which case dA and/or dC is inserted opposite the lesion. The 8OdG : dA pair is readily extended by DNA polymerase and does not appear to be subject to the editing function of this enzyme.[21]

However, the base-mispairing and mutagenic specificity of 8OdG is not clearly known. The *in vivo* study by Cheng *et al*. of complementary bacteriophage plaque colour assays, using 8OpGTP and DNA polymerase, illustrated the mutagenic properties of 8OG as a template causing G ⟶ T substitutions, while misincorporation of 8OG as substrate caused A ⟶ C substitutions. Both are believed to be caused by 8OG : A mispairs.[19]

While these studies have shown the possibility of only G to T type transversional mutations, *in vivo* studies on the hot spots of c-Ha-ras genes raised the possibility of other types of mutations as well.[22,23] While G to T type mutations were induced in

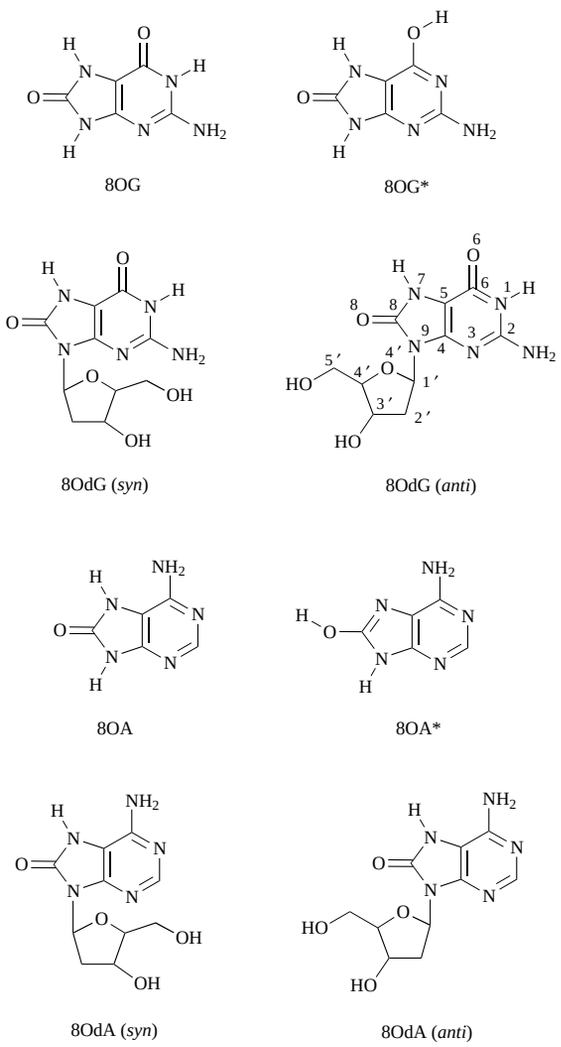

Fig. 1 Structural formulae for various 8-oxobases and nucleosides studied

the first positions of codons 12 and 61, the DNA lesion at the second position of codon 12 induced a G to A transition in addition to a G to T transversion, thus demonstrating the possibility of transitional mutations too from 8OG.[22] The question then arises as to which tautomeric form of 8OG is responsible for these G to A transitions. It may be argued that 8OG can itself mispair with thymine in its native form leading to such mutations, while the possible involvement of a minor enol tautomer of 8OG in stabilising such mispairs cannot be ruled out. Here, a detailed structural study on the base-mispairing specificities and underlying pairing energies of these oxidised bases would throw much light on the understanding of 8-oxopurine induced mutagenesis.

In contrast to 8OG, 8-oxoadenine (8OA) is not particularly mutagenic, being at least an order of magnitude less mutagenic than 8-oxoguanine.[24] Evans and co-workers have shown that 8OG and 8OA have strong structural similarities.[13,16,25] Both these lesions predominate at physiological pH in the native 8-keto form and appear to adopt the *syn* conformation about the glycosyl bond. X-Ray studies on a dodecanucleotide duplex have shown that the most likely alternative base-pair G : 8OA is asymmetric, and is similar to a purine–pyrimidine mismatch,[26] whence it may be argued that this could be an easy target for repair enzymes. On the other hand, the reported crystal structure containing 8OG has shown that it forms a stable pair with cytosine and is found to exist in the normal *anti* form.[27] However, there has so far been no other report of the base-mispairing properties of 8OA and model studies on possible mispairs could help to clear the picture.

The present work, utilising a semi-empirical molecular orbital model, is aimed at understanding mutation-inducing properties of 8-oxopurines, as well as seeking out the structural rationale behind the mutagenic potential of various base-pairing motifs. This could yield insight into the types of mutation and structural forms of the lesions involved in the free-radical induced mutagenesis.

Theoretical methodology

The structural and energetic characteristics of various base-pair motifs adopted by 8OG and 8OA are studied here using the PM3 SCF-MO method.[28] This method has been used widely to study the hydrogen-bonded complexes of both nucleic acid bases and small polar molecules by various workers and shown to be superior over other semi-empirical SCF-MO methods.[29–32] Recently, it has been substantiated to be the only semi-empirical methodology (using the NDDO scheme) with any ability to properly reproduce experimentally observed hydrogen bonding between nucleotide base pairs.[33]

The thermodynamics of base-pair formations was gauged from the enthalpy of base-pairing E_p, obtained from the heats of formation of the pair and of the individual bases. Note was taken of the number and lengths R_{HX} or R_{XY} of the hydrogen bonds formed. This could allow for the possibility of correlating the magnitude of the pairing energy E_p with the number of hydrogen bonds observed, and also with their length. All the bases here are methylated at the N^1 (in case of pyrimidines) or N^9 position (in the case of purines) in order to mimic the sugar moiety in DNA.

The internal configuration of a base-pair was gauged by various markers, *viz*. the distance R_{NN} between the two glycosidic nitrogen atoms, the distance R_{CC} between the carbon atoms of the two methyl groups attached to N^1 (for pyrimidine bases) or N^9 (for purine bases) ring atoms, the buckle and propeller twist between the two base planes, the angles, θ_1 and θ_2, between the two glycosidic and C1′–C1′ vectors. Comparison of the values of these configurational markers for a particular base-pair with those for the standard Watson–Crick base-pairs could lead to a proper evaluation of the degree with which the given base-pair resembled or departed from the standard

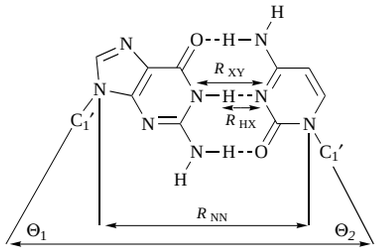

Fig. 2 Schematic representation of various configurational markers monitored in the present study

double-helical configuration. The schematic representation of various configurational markers observed in the present study are shown in Fig. 2.

All the structures were fully optimised using the eigenvector following (EF) method,[34] with the PRECISE option in effect, as incorporated into the MOPAC 6.1 package,[35] and were characterised as true minima, with all Hessian eigenvalues being positive. Various configurational parameters obtained in the present work were calculated using a modified version of NUPARM[36] program.

Results and discussion

Base-pairing properties of 8-oxoguanine

In principle, 8OG can pair with other bases to form four different types of motifs, which may lead to various types of mutagenic or non-mutagenic events: (1) 8OG can pair with cytosine in normal Watson–Crick fashion leading to a non-mutagenic pairing situation; (2) 8OG can pair with thymine either in native or tautomeric form leading to a G ⟶ A type transitional mutagenic event; (3) 8OG can form a base pair with adenine in either *syn* or *anti* fashion, *i.e.* using either its Hoogsteen face or Watson–Crick respectively, leading to G ⟶ T type transversional mutagenic event; and (4) 8OG can also pair with guanine, leading to G ⟶ C type substitutions. This wide range of pairing possibilities offers an interesting opportunity to study the stability of various pairs in the context of the *in vivo* and *in vitro* mutagenicity reported for 8OG, particularly so since the precise structural details of mutation induction by 8OG are not well understood. A schematic representation of some of the mispairs studied in the present work are shown in Fig. 3.

Table 1 presents the calculated data for various base-pairing schemes adopted by 8OG at the PM3 SCF-MO level. The results obtained in the present work compare qualitatively with the previous conformational studies on DNA duplexes containing 8OG and using JUMNA classical potential energy algorithm.[37] The sequences examined were $d(A_5XA_5)$-$d(T_5YT_5)$ and $d(G_5XG_5)$ $d(C_5YC_5)$ with X or Y being 8OG. Within the limited pairing combinations studied, they observed the following order of stability in both duplexes, when one of the bases is 8OG, eqn. (1).

$$8OG_a : C_a \approx G_a : C_a > 8OG_s : G_a \approx 8OG_a : T_a \approx 8OG_s : A_a \quad (1)$$

Thus, the classical potential function is only able to differentiate between the Watson–Crick pairs and the mismatches but not among the various mismatches. The present calculations using a more accurate quantum mechanical method leads to a clear differentiation in the pairing energies among hydrogen bonded base-pairing motifs in the various possible mismatches. The following order of pairing energies for various base-pairing combinations between 8OG and other nucleic acid bases is obtained, eqn. (2).

$$\begin{aligned} 8OG_a : C_a > G_a : C_a > 8OG^*_a : T_a > G^*_a : T_a \approx \\ 8OG_a : A_a > 8OG_s : 8OG_a > G_a : A_a > 8OG_a : T_a > \\ 8OG_s : G_a > G_a : A_s > G_a : T_a \approx 8OG_s : A_s > 8OG_a : A_s > \\ 8OG_s : A_a > 8OG_s : T_a > 8OG_a : G_s \approx G_a : G_s \end{aligned} \quad (2)$$

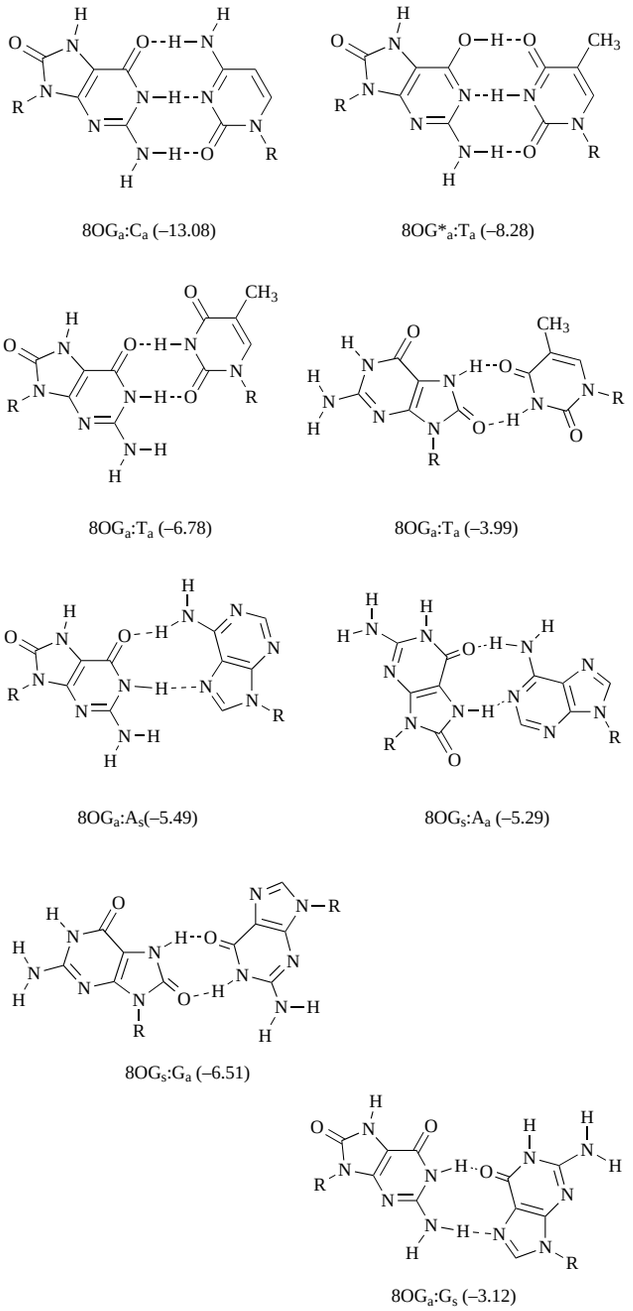

Fig. 3 Schematic representation of some plausible base-mispairing schemes that can be adopted by 8-oxoguanine, pairing energies (E_p/ kcal mol^{-1}) are given in parentheses

It is immediately obvious from the data that 8OG shows a clear preference to pair with cytosine in the normal Watson–Crick fashion, the pairing energy of this pair being 1.2 kcal mol^{-1} (1 cal = 4.184 J) more favourable than the normal G : C pair. The only reported crystal structure containing 8OG has shown that it forms a stable pair with cytosine and is in the normal *anti* form.[27] It may be also noted that the 8OG : C pair is very similar to the standard G : C Watson–Crick configuration, as is evident from all the configurational markers listed in Table 1.

The 6-enol-8-keto tautomer of 8OG (8OG* in Fig. 1) has been shown by *ab initio* study[15] to be energetically very close to the 6,8-diketo species and may exist in significant population *in vivo*. Table 1 reveals that 8OG* can pair with thymine in the normal *anti* form with a good pairing energy (−8.3 kcal mol^{-1}), and this pairing scheme may be held responsible for G ⟶ A type transitional mutations. 8OG in its normal diketo tautomeric form can compete in pairing with thymine leading to the same kind of transitional mutation though the resultant base-pair is relatively less stable (−6.8 kcal mol^{-1}). The other pairing scheme between 8OG in *syn* configuration and thymine in *anti* form may be a rather unlikely event, as reflected by the unfavourably short glycosidic distances and low pairing energy (3.7 kcal mol^{-1} lower than for the 8OG* : T pair). In the absence of structural data on this type of mutagenic lesion, the base-mispairing motifs 8OG*$_a$: T$_a$ and 8OG$_a$: T$_a$, may both be proposed as competing possibilities leading to a G ⟶ A type transitional mutation.

Pairing of 8OG with adenine would be responsible for a G ⟶ T type transversion. Table 1 presents four possibilities where 8OG in either *syn* or *anti* conformation pairs with adenine. Out of these, 8OG(*anti*) pairing with adenine (*anti*) is energetically more favourable than the other three possible motifs. Even though, the configurational features of this pair suggest that it may not be easily accommodated into a DNA double-helix because of a much wider separation between glycosidic nitrogens (N^9–N^9 distance of *ca*. 11 Å), a similar base-mispair of the type G$_a$: A$_a$ has in fact been observed in NMR and crystallographic studies of oligonucleotides.[38,39] The next possible base-pair 8OG$_s$: A$_s$ has a pairing energy *ca*. 1.8 kcal mol^{-1} less than 8OG$_a$: A$_a$. Despite being a very symmetric base-pairing configuration, this structure may be ruled out in a duplex owing to the very short glycosidic bond distance. The next possible base mispair 8OG$_s$: A$_a$ is *ca*. 2.2 kcal mol^{-1} less favourable than 8OG$_a$: A$_a$. Interestingly, this pair is conformationally very stable, with minimal perturbation to the duplex stability as reflected by various configurational parameters. The base-pair 8OG$_s$: A$_a$ is also energetically and conformationally very close to 8OG$_a$: A$_s$. However, despite the close structural similarity between these base-pairs, only the former base-pair is generally believed to be responsible for the experimentally observed G ⟶ T transversional mutations. Taking a cue from the close structural similarity between these two base-pairs, it may be argued that there exists a strong possibility of competition between these two pairs leading to purine–purine mismatches.

DNA duplexes containing 8OG$_s$: A$_a$ base-mispair have been shown to cause minimal distortion to the global conformation by various structural and theoretical studies.[14,36] These types of purine–purine mismatches among normal DNA bases have also been identified and characterised by various X-ray and NMR studies.[38–41,43–46] The observed base-pairing preference of 8OG(*syn*) for adenine(*anti*), despite low pairing energy, may be attributed to sequence-specific stabilisation of this mismatch basepair, as has been observed for normal G : A base mispairs in oligonucleotide duplexes. In summary, it may be concluded that the three mismatches 8OG$_a$: A$_a$, 8OG$_s$: A$_a$ and 8OG$_a$: A$_s$ may all exist in DNA duplexes, depending on the sequence context of the duplex.

Lastly, 8OG can pair with guanine leading to a G ⟶ C type transversion, in three different ways. Though only of theoretical interest, the 8OG$_s$: 8OG$_a$ pair is energetically favoured over the other two possibilities, *viz*. the 8OG$_s$: G$_a$ and 8OG$_a$: G$_s$ pairs. Excluding the possible occurrence of this mispair *in vivo*, 8OG may be expected to pair in the other two possible ways. The data predict that the 8OG$_s$: G$_a$ pair is 3.4 kcal mol^{-1} more favourable than the 8OG$_a$: G$_s$ pair. Since the pairing energy of the 8OG$_s$: G$_a$ mispair is slightly more favourable than that of the mutagenic lesion 8OG$_s$: A$_a$ (by *ca*. 1.2 kcal mol^{-1}) and in view of its close structural homology with the normal Watson–Crick configuration, it may be expected that this mispair may compete with G : A type mismatches.

Base-pairing properties of 8-oxoadenine

Unlike the case of 8OG, the mutagenic role of 8OA is much less studied. Comparative studies on the genetic effects of 8OG and 8OA have indicated that 8OA is at least an order of magnitude less mutagenic than 8OG in *E. coli* cells with normal DNA repair capabilities.[24] 8OA can adopt mispairing schemes leading

Table 1 PM3 data for various base-pairing motifs between 8OG and nucleic acid bases (N^1/N^9 positions of all bases are methylated)

Base-pair[a] Motif	E_p	H-bond	$R_{H\cdots X}$	$R_{X\cdots Y}$	R_{NN} R_{CC}	Prop.[b] Buck.	θ_1 θ_2
G : C type							
$8OG_a : C_a$	−13.08	$N^2G : O^2C$	1.84	2.84	9.05	3.07	53.58
		$N^1G : N^3C$	1.78	2.79	10.79	19.43	53.15
		$O^6G : N^4C$	1.80	2.81			
$G_a : C_a$	−11.87	$N^2G : O^2C$	1.84	2.85	9.04	4.59	52.98
		$N^1G : N^3C$	1.78	2.80	10.79	17.33	52.36
		$O^6G : N^4C$	1.80	2.81			
G : T type							
$8OG^*_a : T_a$	−8.28	$N^2G : O^2T$	1.86	2.87	9.14	1.98	53.16
		$N^1G : N^3T$	1.75	2.79	10.93	−11.01	50.84
		$O^6G : O^4T$	1.80	2.76			
$G^*_a : T_a$	−7.71	$N^2G : O^2T$	1.86	2.87	9.12	2.69	50.98
		$N^1G : N^3T$	1.76	2.79	10.96	−9.64	51.08
		$O^6G : O^4T$	1.80	2.76			
$8OG_a : T_a$	−6.78	$N^1G : O^2T$	1.79	2.80	9.09	3.35	43.20
		$O^6G : N^3T$	1.81	2.82	10.74	−17.68	65.84
$G_a : T_a$	−5.86	$N^1G : O^2T$	1.79	2.81	9.06	−0.14	40.06
		$O^6G : N^3T$	1.80	2.82	10.76	−7.29	66.21
$8OG_s : T_a$	−4.58	$N^7G : O^2T$	1.81	2.82	7.25	29.56	38.36
		$O^6G : N^3T$	1.81	2.82	8.57	−14.28	81.68
$8OG_s : T_a$	−3.99	$N^7G : O^4T$	1.82	2.78	7.44	−16.48	64.32
		$O^8G : N^3T$	1.80	2.82	9.19	−11.22	40.06
G : A type							
$8OG_a : A_a$	−7.65	$N^1G : N^1A$	1.76	2.79	11.03	−1.56	48.59
		$O^6G : N^6A$	1.82	2.83	13.04	2.44	44.50
$G_a : A_a$	−7.11	$N^1G : N^1A$	1.77	2.80	11.01	−5.94	44.89
		$O^6G : N^6A$	1.82	2.83	13.08	7.86	44.61
$G_a : A_s$	−6.34	$N^1G : N^7A$	1.78	2.80	8.99	20.49	51.76
		$O^6G : N^6A$	1.81	2.79	10.99	11.75	41.28
$8OG_s : A_s$	−5.82	$O^6G : N^6A$	1.84	2.77	6.77	−6.34	49.23
		$N^7G : N^7A$	1.79	2.79	8.67	−10.37	48.70
$8OG_a : A_s$	−5.49	$N^1G : N^7A$	1.79	2.82	9.07	18.72	53.15
		$O^6G : N^6A$	1.81	2.79	11.05	17.02	40.69
$8OG_s : A_a$	−5.29	$O^6G : N^6A$	1.82	2.81	9.03	0.06	42.98
		$N^7G : N^1A$	1.80	2.82	10.99	−9.01	51.16
G : G type							
$8OG_s : 8OG_a$	−7.43	$N^7G : O^6G$	1.81	2.79	9.01	−10.93	53.80
		$O^8G : N^1G$	1.78	2.80	10.96	8.92	40.07
$8OG_s : G_a$	−6.51	$N^7G : O^6G$	1.82	2.79	8.96	−10.89	55.91
		$O^8G : N^1G$	1.79	2.81	10.93	18.73	37.05
$8OG_a : G_s$	−3.12	$N^1G : O^6G$	1.82	2.84	9.79	−19.46	68.09
		$N^2G : N^7G$	1.83	2.82	11.59	−15.37	28.91
$G_a : G_s$	−3.03	$N^1G : O^6G$	1.83	2.84	9.80	−19.75	66.13
		$N^2G : N^7G$	1.84	2.83	11.66	−16.77	28.10

[a] *represents minor tautomeric form 8OG* (see Fig. 1); all pairing energies (E_p) are in kcal mol^{-1}, distances (Å) angles (°). [b] Propeller and buckle parameters between the two base planes are calculated using the NUPARM[37] program.

to either transitional or transversional mutagenic events. We put forward three types of mispairing schemes, within the normal Watson–Crick configuration, possible in the case of 8OA: (1) 8OA pairs in either native or tautomeric form with thymine, leading to a non-mutagenic base-pairing situation; (2) 8OA pairs with guanine, leading to an A ⟶ C transversion; and (3) 8OA pairs with cytosine, leading to an A ⟶ G transition. Fig. 4 illustrates the structural forms of various mispairs that can be adopted by 8OA.

The data in Table 2 presents the pairing possibilities of 8OA with all other nucleic acid bases in either the *syn* or *anti* conformation. Of these, the 6-amino-8-enol form of 8-oxoadenine (8OA* in Fig. 1) in the *syn* conformation pairs very favourably with thymine, with the formation of three hydrogen-bonds, and is *ca*. 5 kcal mol^{-1} more stable than the normal A : T pair. This, however, is not a mutagenic event. Significant participation of this base-mispair would be critically dependent on the pH of the environment since ionisation of N^7 has been observed only at a pH above 8.7 and the base-pair is also geometrically very difficult to accommodate in a duplex. Thus, under physiological conditions, it may be ruled out as a competing base mispair. However, 8OA itself can pair with thymine in both the *anti* and *syn* conformation. These basepairs, $8OA_a : T_a$ and $8OA_s : T_a$, have pairing energies very similar to an $A_a : T_a$ pair, but only the former has a near normal base-pair configuration. Thus, it may be expected that the pair $8OA_a : T_a$ may compete with normal base-pairs, leading, of course, to a non-mutagenic situation.

As shown in Table 2, 8OA can pair with guanine in at least three ways leading to A ⟶ C type transversions. The pair involving 8OA(*syn*) with guanine(*anti*) is an interesting one. This base-pair is 2.6 kcal mol^{-1} more stable than the normal A : T pair, and leads to a conformationally very stable base-pairing situation.[26] Despite its stability when compared with the widely studied $8OG_s : A_a$ mispair (which leads to transversional mutations both *in vivo* and *in vitro*), the $8OA_s : G_a$ pair has not been attributed much mutagenic significance in experimental studies. The $8OA_s : G_a$ pair has been shown to be an order of magnitude less mutagenic than the $8OG_s : A_a$ pair.[24] However, recent *in vivo* studies on c-Ha-ras gene NIH 3T3 cells by Kamiya *et al*. indicate that 8OA is capable of inducing both A ⟶ G type transitional and A ⟶ C transversional mutations.[22] This observation is quite noteworthy and throws further light on the structural elements through which DNA repair proteins, such as MutT, recognise base-pair mismatches. It has been argued that the pattern of hydrogen-bond donors–acceptors in the major groove regions of these mispairs might be a differentiating factor for repair enzymes to recognise the mispairing.[27] Our results support this hypothesis since the pos-

Table 2 PM3 data for various base-pairing motifs between 8OA and nucleic acid bases (N^1/N^9 positions of all bases are methylated)[a]

Base-pair[b] Motif	E_p	H-bond	$R_{H\cdots X}$	$R_{X\cdots Y}$	R_{NN} R_{CC}	Prop. Buck.	θ_1 θ_2
A : T type							
$8OA^*_s : T_a$	−10.70	$O^8A : O^2T$	1.79	2.76	6.83	−2.80	49.20
		$N^7A : N^3T$	1.73	2.76	8.50	−11.77	60.60
		$N^6A : O^4T$	1.86	2.84			
$A_a : T_a$	−5.56	$N^1A : N^3T$	1.78	2.82	9.14	−4.00	51.96
		$N^6A : O^4T$	1.82	2.83	10.99	−0.93	49.73
$8OA_a : T_a$	−5.17	$N^1A : N^3T$	1.79	2.83	10.93	9.18	55.12
		$N^6A : O^4T$	1.82	2.83	9.18	−10.31	51.34
$8OA_s : T_a$	−4.92	$O^8A : N^3T$	1.80	2.82	7.42	−6.10	66.08
		$N^7A : O^4T$	1.81	2.79	9.12	25.63	39.12
A : G type							
$8OA_s : G_a$	−8.14	$N^7A : O^6G$	1.79	2.79	8.94	7.90	50.00
		$O^8A : N^1G$	1.79	2.82	11.03	−20.82	37.62
$8OA_a : G_a$	−6.08	$N^1A : N^1G$	1.78	2.81	11.02	6.54	47.79
		$N^6A : O^6G$	1.82	2.83	13.04	−12.40	44.90
$8OA^*_s : G_a$	−2.42	$O^8A : N^2G$	1.87	2.84	9.09	3.98	40.25
		$N^7A : N^1G$	1.81	2.84	11.14	−1.93	50.42
		$N^6A : O^6G$	1.80	2.78			
A : C type							
$8OA_s : C_a$	−3.54	$N^7A : O^2T$	1.81	2.81	7.26	−26.78	30.79
		$N^6A : N^3C$	1.93	2.88	8.70	2.93	81.18

[a] See the footnotes of Table 1 for units. [b] All bases * represent minor tautomeric form 80A* (see Fig. 1).

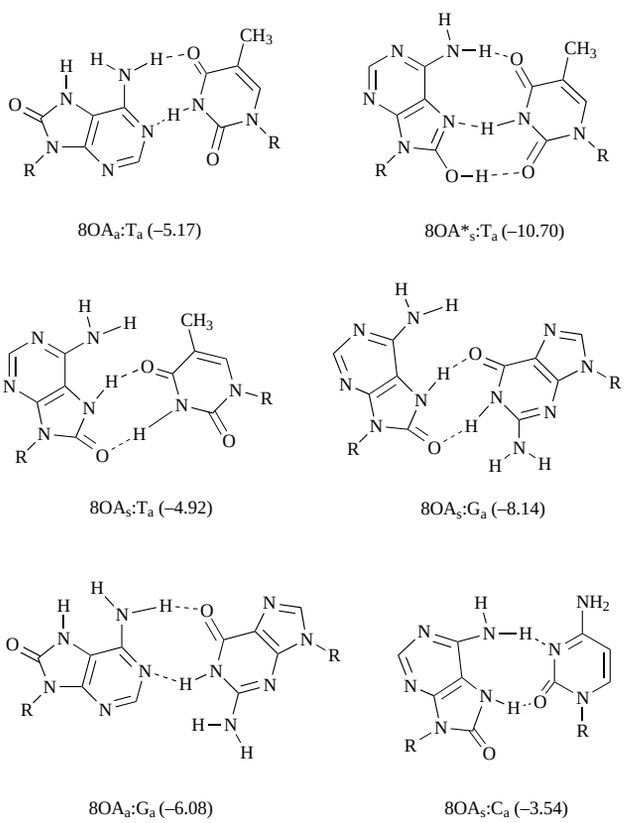

Fig. 4 Various plausible base-mispairing schemes that can be adopted by 8-oxoadenine, pairing energies (E_p in kcal mol^{-1}) are indicated in parentheses

sible base-mispairs adopted by both 8OA and 8OG are conformationally very similar, causing little perturbation to the DNA double-helix and leading to similar base-mispairing situations from structural considerations. Another interesting pair that 8OA can adopt is with guanine, both being in the normal *anti* conformation. Such mispairs between normal adenine and guanine were observed in various structural studies as discussed before.[38,39] Note that this pair is more stable than the normal $A_a : T_a$ pair (by *ca.* 0.5 kcal mol^{-1}) and less stable than $8OA_s : G_a$. It is interesting to note that despite being of the normal Watson–Crick type, this pair is *ca.* 2 kcal mol^{-1} less stable than the Hoogsteen type pair $8OA_s : G_a$. Nonetheless, being a symmetric Watson–Crick type structure, there is a fair possibility that this pair may compete with $8OA_s : G_a$, leading to an A ⟶ C type transversional mutation, if not recognised by repair enzymes such as MutT. The third possible mispair between 8OA*(*syn*) and G(*anti*) has very weak binding as indicated by its very low pairing energy and as discussed above such a mispair, of course, depends critically on the ionisation of 8OA.

The other interesting base-mispairing situation that 8OA can adopt is by pairing with cytosine. The analogous base-mispair between adenine and cytosine is not possible under normal physiological conditions, though under high acidic conditions, protonated adenine can mispair with cytosine.[47] Despite unfavourable configurational parameters (extremely short C1′–C1′ distance), this mispair is the only plausible structural motif, that can be attributed for A ⟶ G type transitional mutations under physiological conditions which have been reported recently.[22]

Conclusions

The detailed studies on the base-pairing properties of 8-oxoguanine and 8-oxoadenine at PM3 SCF-MO level indicate that both adducts can indeed lead to base-misincorporations during replication. In the case of 8-oxoguanine, the oxoadduct in *anti* form shows a preference to pair with cytosine(*anti*), a pairing which is energetically more stable than all other pairing combinations. The competing mispairs $8OG^*_a : T_a$ and $8OG_a : T_a$ may be held responsible for G ⟶ A type transitional mutations, while $8OG_a : A_s$ and $8OG_s : A_a$ may mutually compete with both leading to G ⟶ T type transversional mutagenic events. Similarly, $8OA_s : T_a$ and $8OA_a : T_a$ might compete, both being non-mutagenic base-pairing schemes for 8-oxoadenine. While $8OA_s : G_a$ and $8OA_a : G_a$ might be held responsible for A ⟶ C type transversional mutations. Though it is structurally rather difficult to accommodate in a duplex, the $8OA_s : C_a$ mispair may be the only possible motif to which an A ⟶ G type transitional mutagenic event can be attributed under normal physiological conditions.

Thus, the present study though giving only approximate numbers for the relative energies for isolated base-pairs clearly indicates the large base-mispairing potential of both 8-oxoguanine and 8-oxoadenine. This can lead to several different thermodynamically facile transversional and transitional mutations involving these bases.

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