PCCP

Cite this: *Phys. Chem. Chem. Phys.*, 2011, **13**, 7178–7188

www.rsc.org/pccp

PAPER

On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxyl radicals†

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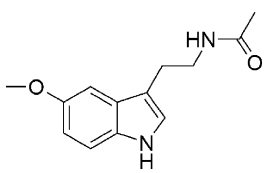
Received 7th December 2010, Accepted 11th February 2011
DOI: 10.1039/c0cp02801k

The reactions of melatonin (MLT) with hydroxyl and several peroxyl radicals have been studied using the Density Functional Theory, specifically the M05-2X functional. Five mechanisms of reaction have been considered: radical adduct formation (RAF), Hydrogen atom transfer (HAT), single electron transfer (SET), sequential electron proton transfer (SEPT) and proton coupled electron transfer (PCET). It has been found that MLT reacts with OH radicals in a diffusion-limited way, regardless of the polarity of the environment, which indicates that MLT is an excellent OH radical scavenger. The calculated values of the overall rate coefficient of MLT + $^{\bullet}$OH reaction in benzene and water solutions are 2.23×10^{10} and 1.85×10^{10} M^{-1} s^{-1}, respectively. MLT is also predicted to be a very good $^{\bullet}OOCCl_3$ scavenger but rather ineffective for scavenging less reactive peroxyl radicals, such as alkenyl peroxyl radicals and $^{\bullet}$OOH. Therefore it is concluded that the protective effect of MLT against lipid peroxidation does not take place by directly trapping peroxyl radicals, but rather by scavenging more reactive species, such as $^{\bullet}$OH, which can initiate the degradation process. Branching ratios for the different channels of reaction are reported for the first time. In aqueous solutions SEPT was found to be the main mechanism for the MLT + $^{\bullet}$OH reaction, accounting for about 44.1% of the overall reactivity of MLT towards this radical. The good agreement between the calculated and the available experimental data, on the studied processes, supports the reliability of the results presented in this work.

Introduction

Melatonin (MLT, *N*-acetyl-5-methoxytryptamine, Scheme 1) is a neurohormone, mainly secreted by the pineal gland and the retina.[1] In addition to its physiological functions related to circadian and seasonal rhythms, it has also been proposed as an efficient free radical scavenger.[2] Since MLT is highly soluble in lipids and partially soluble in water, it has the ability to cross physiologic barriers and quickly transport into the cells,[3] which is a desirable characteristic for a good antioxidant.[4–9] The capability of MLT to efficiently trap free radicals is very important since the damage caused by these very reactive species has been associated with a large variety of health disorders.[10]



Scheme 1

Tan *et al.*[2] were the first to report that MLT function as a free radical scavenger. This was an *in vitro* study, focused on the very reactive hydroxyl ($^{\bullet}$OH) radical. After this pioneering work abundant information on such ability of MLT has been gathered. It was proven that MLT reduces molecular damage associated with massive free radical generation *in vivo*.[5,6] Escames *et al.*[7] found that melatonin reduces NO-induced lipid peroxidation in a dose-dependent manner. The results reported by Matuszak *et al.*[8] support that MLT is an excellent OH radical scavenger. This has been confirmed by different independent studies, using various and reliable methodologies.[9] Khaldy *et al.*[11] found that the protective effect of MLT against dopamine autoxidation was significantly higher than those of deprenyl and vitamins E and C. Sofic *et al.*[12] have proposed that MLT acts as an efficient antioxidant against peroxyl and $^{\bullet}$OH radicals. They have also shown that MLT is twice as powerful as vitamin E, and four times more efficient than vitamin C and glutathione, for scavenging free radicals.

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† Electronic supplementary information (ESI) available: Free energies in gas phase; optimized geometries in cartesian coordinates, thermochemistry data from Gaussian03 outputs, and Bader topological analyses. See DOI: 10.1039/c0cp02801k

In contrast, Zang *et al.*[13] reported that melatonin (up to 1.2 mM) did not exhibit significant effect toward OH radical, produced by the Fenton reaction.

Regarding the reactions of MLT with peroxyl radicals, Antunes *et al.*[14] have shown that MLT is not a peroxyl scavenger. On the contrary, Mekhloufi *et al.*[15] proposed that MLT may compete with fatty acids to scavenge lipid peroxyl radicals. Sofic *et al.*,[12] Scaiano,[16] Mayo *et al.*,[17] and Livrea *et al.*[18] also supported the role of MLT as a peroxyl scavenger. Pieri *et al.*[19] suggested that melatonin is a better peroxyl scavenger than vitamin E, while Marshall *et al.*[20] arrived at the opposite conclusion. Therefore, the efficiency of MLT as a direct peroxyl scavenger may be disputed. However, apparently there is no doubt on its role to inhibit lipid peroxidation.[21]

In addition, there are two theoretical reports on the reaction of melatonin with OH radicals. Turjanski *et al.*[22] studied one hydrogen atom transfer (HAT) channel and one radical adduct formation (RAF) channel, from a thermochemical point of view, and found that both of them are viable in vacuum and in water solution. Stasica *et al.*[23] studied five RAF channels, using a low level theory (AM1 semi-empirical calculations), and proposed that all of them should occur easily and with low selectivity. However there are no previous studies including all possible mechanisms and sites of reaction, and quantifying their contributions to the overall reactivity of MLT towards OH radicals.

In summary, based on all the overwhelming experimental evidence described above it may be concluded that MLT can efficiently fight oxidative stress. Most of the published works on its $^{\bullet}$OH scavenging activity agree on the conclusion that MLT is excellent for this purpose. However there is no general agreement yet on the efficiency of MLT as a direct peroxyl scavenger. Therefore it is essential to investigate the mechanism, or mechanisms, involved in such processes in a systematic way. Accordingly it is the main goal of the present work to study all the alternative mechanisms usually associated with the free radical scavenging activity of antioxidants, at all possible reaction sites, and at a reliable level of theory for the reactions of MLT with $^{\bullet}$OH and with a series of peroxyl radicals. The studied mechanisms are: radical adduct formation (RAF), Hydrogen atom transfer (HAT), single electron transfer (SET), sequential electron proton transfer (SEPT) and proton coupled electron transfer (PCET). Since it has been previously demonstrated that the nature of free radicals plays an important role on the relative importance of competing mechanisms,[24] different free radicals have been considered: $^{\bullet}$OH, $^{\bullet}$OOH, $^{\bullet}OOCCl_3$, $^{\bullet}$OO–CH=CH_2, and $^{\bullet}$OO–CH_2–CH=CH_2. Thermodynamic and kinetic data are provided, as well as a quantitative assessment of the contributions of the different mechanisms and channels of reaction to the overall reactivity of MLT towards the above mentioned radicals.

Computational details

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional and the 6-31+G(d) basis set. The energies were improved by single point calculations at M05-2X/6-311++G(d,p) level of theory. The M05-2X functional has been recommended for kinetic calculations by their developers,[25] and it has been also successfully used by independent authors.[26–36] It also seems worth noticing that the reliability of other functionals to accurately describe the energetics of radical reactions have been recently challenged.[37] Unrestricted calculations were used for open shell systems and local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). All the electronic calculations were performed with Gaussian 03 package of programs.[38] Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. The stationary points were first modeled in gas phase (vacuum), and solvent effects were included *a posteriori* by single point calculations, at M05-2X/6-311++G(d,p) level of theory, using polarizable continuum model, specifically the integral-equation-formalism (IEF-PCM)[39–41] and RADII = UAHF. For the studied systems this approach should be accurate enough since the studied reactions involve non-charged species. It has been successfully used before for describing radical–molecule reactions in solution.[42] Benzene and water have been chosen as solvents, to mimic non polar and polar environments, respectively. The reference state has been changed from 1 atm to 1 M. The solvent cage effects have been included according to the corrections proposed by Okuno,[43] taking into account the free volume theory.[44] These corrections are in good agreement with those independently obtained by Ardura *et al.*[45] and have been successfully used by other authors.[46–52] This correction is important because the packing effects of the solvent reduce the entropy loss associated with any chemical reactions with molecularity equal to, or larger than, two.

Natural population analysis (NPA)[53] have been carried out, for the transition states associated with H transfers, to obtain the atomic spin densities (ASP) on the sites relevant to the H transfer and the charge (Q) carried by the H atom that is transferred. This analysis has been performed to differentiate between HAT and PCET mechanisms.

The rate constants (k) were calculated using Conventional Transition State Theory (TST)[54–56] and 1 M standard state as:

$$k = \sigma\kappa\frac{k_B T}{h}e^{-(\Delta G^{\neq})/\mathrm{RT}} \quad (1)$$

where k_B and h are the Boltzmann and Planck constants, $\Delta G^{\neq}$ is the Gibbs free energy of activation, σ represents the reaction path degeneracy accounting for the number of equivalent reaction paths, and κ accounts for tunneling corrections. The tunneling corrections defined as the Boltzmann average of the ratio of the quantum and the classical probabilities were calculated using the Eckart barrier.[57]

For the mechanisms involving electron transfers (ET) the Marcus theory[58] was used. It relies on the transition state formalism and defines the ET activation barrier ($\Delta G^{\neq}_{ET}$) as:

$$\Delta G^{\neq}_{ET} = \frac{\lambda}{4}\left(1 + \frac{\Delta G^{0}_{ET}}{\lambda}\right)^2 \quad (2)$$

where ΔG^{0}_{ET} is the free energy of reaction and λ is a reorganization term. In this work a very simple approximation has been made in order to calculate λ:

$$\lambda \approx \Delta E_{ET} - \Delta G^{0}_{ET} \quad (3)$$

where ΔE_{ET} has been calculated as the non-adiabatic energy difference between reactants and vertical products, *i.e.* $MLT^{\bullet +}$ and R^- in the geometries of MLT and R. This approach is similar to that previously used by Nelsen and co-workers[59,60] for a large set of self-exchange reactions, and has been successfully used in previous studies.[61]

Some of the calculated rate constants (k) of the reaction MLT + $^\bullet$OH are close to the diffusion-limit. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work we have used the Collins–Kimball theory for that purpose:[62]

$$k_{app} = \frac{k_D k_{act}}{k_D + k_{act}} \quad (4)$$

where k_{act} is the thermal rate constant, obtained from TST or Marcus theory (eqn (1) or (2)), and k_D is the steady-state Smoluchowski[63] rate constant for an irreversible bimolecular diffusion-controlled reaction:

$$k_D = 4\pi R D_{AB} N_A \quad (5)$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of the reactants A (OH radical) and B (MLT). D_{AB} has been calculated from D_A and D_B according to ref. 64 D_A and D_B have been estimated from the Stokes–Einstein approach:[65]

$$D = \frac{k_B T}{6\pi\eta a} \quad (6)$$

where k_B is the Boltzmann constant, T is the temperature, η denotes the viscosity of the solvent, in our case water ($\eta = 8.91 \times 10^{-4}$ Pa s) and benzene ($\eta = 6.04 \times 10^{-4}$ Pa s); and a the radius of the solute ($a_{OH} = 4.79$ Å and $a_{MLT} = 8.95$ Å).

Results and discussion

Melatonin (MLT) has been modeled in its neutral form, since its pK_a has been determined to be 12.3 ± 0.1.[66] Therefore at physiological pH (7.4) neutral MLT is by far the dominant form of this compound. Its antioxidant activity can take place through different mechanisms:

Radical Adduct formation (RAF):

$$MLT + {}^\bullet R \rightarrow MLT\text{–}R^\bullet$$

Hydrogen atom transfer (HAT):

$$MLT + {}^\bullet R \rightarrow MLT(\text{–}H)^\bullet + HR$$

Single electron transfer (SET):

$$MLT + {}^\bullet R \rightarrow MLT^{\bullet +} + R^-$$

Additionally SET can occur rapidly followed by, or simultaneously with, proton transfer, which are known as Sequential Electron Proton Transfer (SEPT) and Proton Coupled Electron Transfer (PCET), respectively:

Sequential Electron Proton Transfer (SEPT):

$$\text{(a) } MLT + {}^\bullet R \rightarrow MLT^{\bullet +} + R^- \rightarrow MLT(-H)^\bullet + HR$$

$$\text{(b) } MLT + {}^\bullet R \rightarrow MLT(-H^+)^- + R^\bullet \rightarrow MLT(-H)^\bullet + HR$$

Proton Coupled Electron Transfer (PCET):

$$MLT + {}^\bullet R \rightarrow MLT(\text{–}H)^\bullet + HR$$

It should be noticed that in the particular case of SEPT the sequential transfer can take place in two different ways: (a) a SET process followed by deprotonation of the formed radical cation, or (b) a deprotonation followed by a SET process from the formed anion.

Even though SEPT and PCET yield the same products as HAT the influence of the solvent on their feasibility is expected to be different. While SET and SEPT are likely to be favored by polar environments that promote solvation of the intermediate ionic species, the PCET might be also viable in non-polar media since the transfer of the proton and the electron occurs simultaneously in this case, and therefore no charged intermediaries are formed. Benzene and water have been chosen to mimic polar and non-polar environments in the present study.

The structure of MLT and the site numbers assigned to each atom in this work are shown in Fig. 1. The reaction sites considered for the RAF processes are: C5, C6, C7, C8, C9, C10, C11 and C12. For the HAT/PCET processes sites C1, C3, C4, C13, N1 and N2 have been taken into account. Even though radical additions to sites C2, N1 and N2 were initially considered as possible RAF channels, any attempt to locate the corresponding products invariably led to structures that correspond to weak-bonded complexes rather than to proper radical adducts. Therefore these channels have been ruled out as viable products of reaction.

The free radicals that have been considered in this work are:

$$R1 = {}^\bullet OH \quad R2 = {}^\bullet OOH$$

$$R3 = {}^\bullet OO\text{–}CH{=}CH_2 \quad R4 = {}^\bullet OO\text{–}CH_2\text{–}CH{=}CH_2$$

$$R5 = {}^\bullet OOCCl_3$$

Hydroxyl radical ($^\bullet$OH) is the most electrophilic,[67] and reactive of the oxygen-centered radicals, with a half-life of $\sim 10^{-9}$ s.[68] This high reactivity is logically accompanied by a low selectivity. Compared to $^\bullet$OH, peroxyl radicals are less reactive species capable of diffusing to remote cellular locations.[69] Their half-lives are of the order of seconds.[70] Peroxyl radicals do not have

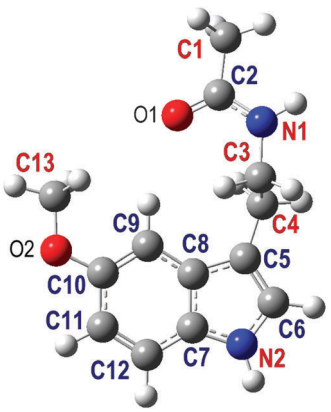

Fig. 1 Structure and site numbers of MLT. Blue labels represent RAF sites of reaction and red labels HAT/PCET sites of reaction.

as high electrophilicity as •OH radicals.[67] Among the selected peroxyl radicals R5 is the most electrophilic, and R3 and R4 have been used to mimic lipid peroxyl radicals.

The thermochemical viability of the different mechanisms and channels of reaction has been investigated first since it will determine if a chemical process can actually take place. To test the reliability of the methodology used to compute ΔG values in solution additional calculations were performed for the HAT/PCET channel from C1 and for the RAF channel at C6, both involving the OH radical. The methodology used for all the reported data consist on IEF-PCM (radii = UAHF) single point calculations at M05-2X/6-311++G(d,p) level of theory. The two other approaches used for the above mentioned channels are IEF-PCM (radii = UAHF) single point calculations at HF/6-311++G(d,p) level of theory and IEF-PCM (radii = UAKS) single point calculations at M05-2X/6-311++G(d,p) level of theory. The resulting Gibbs free energies of solvation are reported in Table 1. As the figures in this table show the results are very similar regardless of the used methodology. In fact the deviations are smaller than 0.5 kcal mol^{-1}, *i.e.* lower than the currently accepted computational accuracy.

The Gibbs free energies of reaction (ΔG), in solution, for all the studied channels of reaction are reported in Table 2. Additionally the gas-phase data are provided in ESI† (Table S1).

Table 1 Gibbs free energies of solvation, at 298.15 K, in kcal mol^{-1}, for channels C1 (HAT/PCET) and C6 (RAF), from different approaches

	C1	C6
Benzene		
radii = UAHF, M05-2X/6-311++G(d,p)	−0.5	1.0
radii = UAHF, HF/6-311++G(d,p)	−0.6	1.0
radii = UAKS, M05-2X/6-311++G(d,p)	−0.7	0.9
Water		
radii = UAHF, M05-2X/6-311++G(d,p)	−1.9	1.5
radii = UAHF, HF/6-311++G(d,p)	−1.7	1.8
radii = UAKS, M05-2X/6-311++G(d,p)	−2.1	1.3

Table 2 Gibbs free energies of reaction, at 298.15 K, in kcal mol^{-1}

	Benzene					Water				
	R1[a]	R2	R3	R4	R5	R1[a]	R2	R3	R4	R5
SET	59.6	88.3	74.0	83.9	53.3	−5.9	33.4	25.4	32.3	8.8
HAT/PCET										
C1	−20.0	11.9	12.7	13.7	6.3	−21.4	8.3	7.6	9.3	−0.0
C3	−21.4	10.6	11.4	12.3	4.9	−22.0	7.7	7.0	8.7	−0.5
C4	−29.8	2.2	3.0	3.9	−3.4	−31.4	−1.7	−2.4	−0.7	−9.9
C13	−21.4	10.5	11.3	12.3	4.9	−22.2	7.5	6.8	8.5	−0.7
N1	−9.0	22.9	23.7	24.7	17.3	−6.6	23.2	22.5	24.2	15.0
N2	−27.2	4.7	5.5	6.5	−0.9	−27.5	2.3	1.6	3.3	−5.9
RAF										
C5	−13.0	12.9	14.0	16.4	5.1	−10.3	14.6	14.3	17.6	5.0
C6	−26.2	−1.6	2.3	3.4	−4.9	−25.7	−0.7	0.3	2.8	−6.5
C7	−7.6	19.2	20.2	21.9	12.1	−5.1	21.7	20.6	23.3	11.8
C8	1.8	25.7	25.6	28.9	17.2	3.7	27.9	26.3	30.8	17.2
C9	−17.9	8.4	8.7	10.5	1.1	−15.6	10.7	9.6	12.4	0.8
C10	−14.8	12.7	13.3	17.2	6.7	−14.2	13.9	13.4	17.8	6.3
C11	−16.4	10.0	10.9	13.9	2.6	−16.9	9.5	8.7	12.6	2.3
C12	−17.5	8.6	9.8	11.9	2.5	−17.1	9.2	7.6	11.1	2.1

[a] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$.

The SET reaction, and therefore the first step of the SEPT mechanism, was found to be endergonic in non-polar media for all the studied radicals. In aqueous solution such mechanism is also endergonic for the peroxyl radicals and exergonic, by about 5.9 kcal mol^{-1}, for the MLT + •OH reaction. Accordingly, this mechanism has been ruled out for the possible peroxyl scavenging activity of melatonin. Since the pK_a value of the MLT radical cation has been estimated to be 4.7 ± 0.1,[66] it is expected to immediately deprotonate in aqueous solution, at physiological pH. As demonstrated by Mahal *et al.*[66] this deprotonation yields the same product that the one formed by HAT/PCET from site N2. The overall process then corresponds to the SEPT mechanism. According to the results in Table 2, the exergonicity of the HAT/PCET from site C4 is larger than that of N2. The fact that the latter one has been identified as the deprotonation site can be rationalized based on kinetics. It is known that N and O sites deprotonate faster than C sites in water solution.

For the •OH (R1) reaction with MLT all the HAT/PCET channels were found to be viable, from a thermochemical point of view, regardless of the polarity of the environment. The hydrogen transfer from site C4 was found to lead to the largest energy release, while that involving N1 was found to be the least exergonic. The RAF channels were also found to be viable, with the exception of •OH additions to C8. Accordingly this channel has been ruled out, and will not be considered in the rest of this work. The largest exergonicity in this case corresponds to the •OH addition to C6, while the smallest exergonicity is associated with the •OH addition to C7.

For the MLT + •OOH (R2) reaction, only two channels were found to be exergonic. They are the HAT process from site C4, which is predicted to be thermochemically viable only in aqueous solution; and the RAF process on site C6, which is exergonic in both modeled environments. However, even for these two channels the exergonicity is small, and significantly lower than those of the equivalent reactions involving •OH radicals. This is in line with the relative reactivity of these two radicals. When the radicals involved are •OO–CH=CH_2 (R3) and •OO–CH_2–CH=CH_2 (R4) most of the HAT/PCET and RAF reactions were found to be endergonic. Three exceptions were found, all of them in aqueous solution. They are the H transfers from site C4, for both radicals, and radical additions to C6 for •OO–CH=CH_2.

Among the studied peroxyl radicals the most reactive one, from a thermochemical point of view is •$OOCCl_3$ (R5). In non-polar media H transfers from sites C4 and N2 and RAF on site C6 are predicted to be exergonic. In aqueous solution the number of thermochemically viable processes increases. In addition to those found feasible in non-polar environments, HAT/PCET from sites C1, C3, and C13 also become exergonic. However since their Gibbs free energies of reaction are very close to zero, they are actually better described as isoergonic processes.

The channels of reaction described as endergonic are not longer considered in this work since, even if they take place at a significant rate, they would be reversible and therefore the formed products will not be observed. However, it should be noticed that they might still represent significant channels if

Table 3 Distance (Å) of the forming C–O bond in the transition states corresponding to the RAF mechanism

	•OH	•OOH	•$OOCCl_3$
C5	2.042		
C6	2.243	1.944	2.030
C7	2.027		
C9	2.179		
C10	2.044		
C11	2.086		
C12	2.079		

their products rapidly react further. This would be particularly important if these later stages are sufficiently exergonic to provide a driving force, and if their barriers of reactions are low.

The most relevant geometrical parameter of the RAF transition states (TS) is the C···O distance, corresponding to the forming bond. The values of *d*(C···O) for the RAF TSs are reported in Table 3. The longest distance corresponds to TS–C6, when MLT reacts with •OH, which indicates that this is the earliest TS, and suggests this channel as the most favored one, among all the studied RAF channels. On the contrary, the shortest C···O distance corresponds to TS–C7, which suggests that this would be the least favored of the RAF paths, involving •OH. The *d*(C···O) distance in the transition state TS–C6, for the MLT + •$OOCCl_3$ reaction, is similar to that of TS–C7 for MLT + •OH reaction. The TS with the shortest *d*(C···O) distance is TS–C6(R2), which suggests that this would be the RAF TS with the highest relative energy among the studied ones. These findings are in line with the thermochemical data discussed above. They are also supported by the Hammond postulate and in agreement with the Bell–Evans–Polanyi principle.

The optimized geometries of the HAT/PCET transition states are shown in Fig. 2 and 3. With the exceptions of TS–C3(R1), TS–N1(R1), and TS–N2(R1), the transition states, involving OH radicals, present H bond like interactions

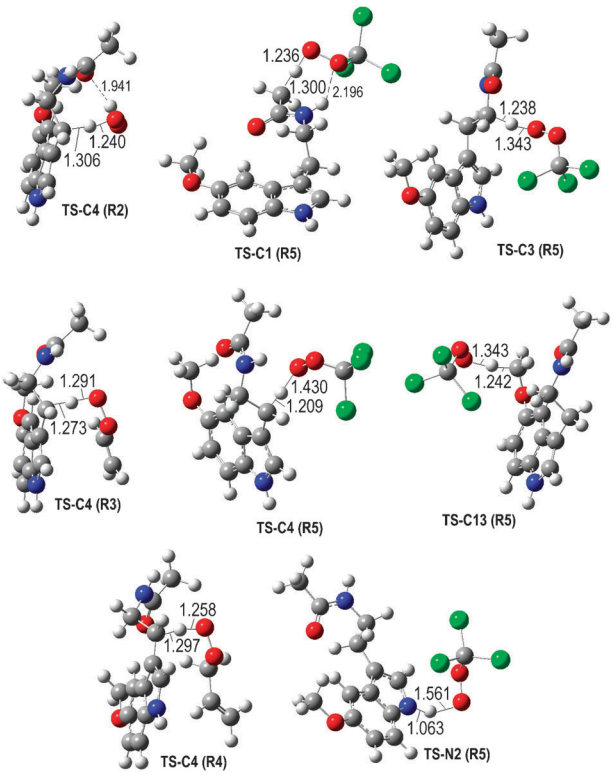

Fig. 3 Optimized geometries of the transition states corresponding to the HAT mechanism, involving peroxyl radicals. R1 = •OH, R2 = •OOH, R3 = •OO–CH═CH_2, R4 = •OO–CH_2–CH═CH_2, R5 = •$OOCCl_3$.

(Fig. 2). Such interactions are expected to lower the energies of these TSs, *i.e.* the associated barriers of reactions. The strongest interaction was found for TS–C13(R1), and involves the H atom in the OH radical and the atom O1 in MLT. The interaction distance was found to be 1.93 Å, in this case. The interactions

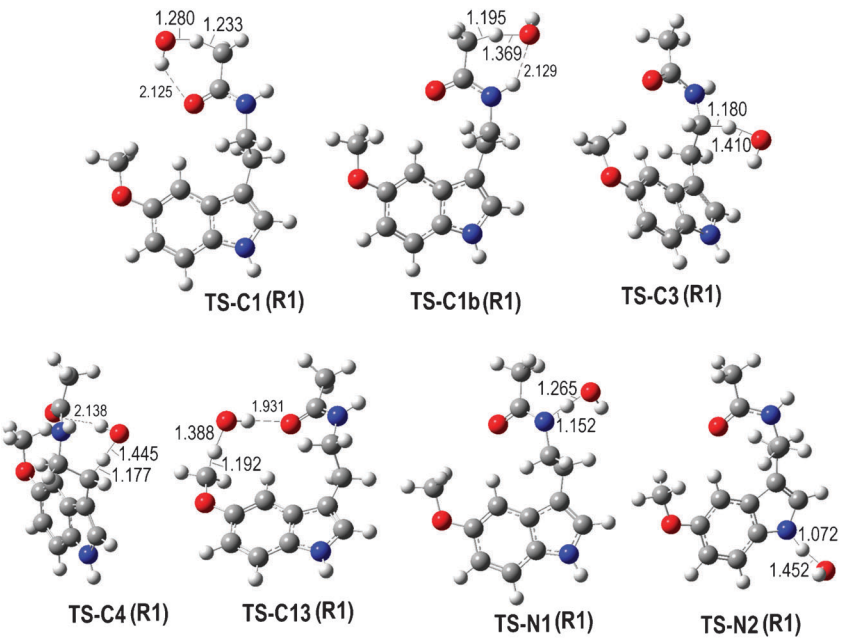

Fig. 2 Optimized geometries of the transition states corresponding to the HAT mechanism, involving OH radicals.

in transition states TS–C1(R1) and TS–C4(R1) also involve these two particular atoms, while the interaction in TS–C1b(R1) takes place between the O atom in the OH radical and the H atom bonded to N1 in MLT. The interaction distance for these three TSs is about 2.13 Å.

Among all the studied TSs, involving peroxyl radicals (Fig. 3), the only one presenting H bond like interaction are TS–C4(R2) and TS–C1(R5). For TS–C4(R2) the interaction takes place between the H atom in the OOH radical and the O1 atom in MLT. The interaction distance was found to be equal to 1.94 Å. For TS–C1(R5) the interaction takes place between the H atom bonded to N1 and the O atom bonded to the CCl3 moiety in the radical, with the interaction distance equal to 2.196 Å.

The presence of the above described interactions was confirmed by Bader topological analyses[71] of the M05-2X/6-31+G(d) wave functions, which were computed with the Aim2000 program.[72,73] Bond critical points were identified, and have been characterized through the corresponding electronic charge density $\rho(r)$ and its Laplacian, $\nabla^2\rho(r)$ (Table S3, ESI†). A regular trend was found between the interaction distance and these two descriptors. The absence of bond critical points for the other TSs confirms the lack of interactions in these structures.

For the H transfer reactions three different analyses have been performed to identify if the corresponding TS structures actually correspond to HAT or PCET mechanisms. They are based on atomic spin densities (ASP) on the sites relevant to the studied reactions, the charge (Q) carried by the H atom that is transferred, and the electronic density of the singly occupied molecular orbital (SOMO). The ASP and Q values were obtained from natural population analysis (NPA) calculations and are reported in Tables 4 and 5. As the coefficients of the natural orbital carrying the unpaired electron indicate, the spin population is concentrated on the two atoms which undergo the H exchange, while a small negative value is found on the H atoms being transferred. This is consistent with a three-center three-electron bond and usually corresponds to HAT processes.[74] This behavior is the same for all the analyzed TS structures, regardless of the involved free radical, with the exception of TS–N2(R1) and TS–N2(R5). For these two TS, on the other hand, the spin population is mainly located on the MLT fragment, suggesting a PCET mechanism.

Regarding the charge carried by the H atom that is being transferred, it has been stated that migrating Hs with substantial positive charge are typical of proton migrations.[75,76] The Q values obtained for transition states corresponding to H transfer from C sites are about 0.3. This value is similar to those reported for other systems undergoing HAT processes.[26] Therefore the charge criterion also seems to support that these TSs correspond to the HAT mechanism. Regarding TSs where the H transfer takes place from N2 sites the charge on the migrating H is higher, and about 0.5. This suggests a PCET mechanism. However the comparison is not straightforward since the site from which the H atoms are being transferred are different in nature (carbon and nitrogen).

Since it is commonly accepted that the PCET mechanism can be defined as that in which the proton and the electron are transferred between different sets of orbitals,[75] the analysis of the singly occupied molecular orbital (SOMO) of the TS seems to be the most reliable, among the used criteria, to differentiate between HAT and PCET processes. The SOMO of TSs corresponding to the HAT mechanism is expected to have significant density in atomic orbitals oriented along, or nearly along, the transition vector (donor···H···acceptor). The SOMO of TSs corresponding to the PCET mechanism, on the other hand, involve p orbitals that are orthogonal to the transition vector.[75] As the plots in Fig. 4 and 5 show the SOMO of all transition states corresponding to H transfer from C atoms, has a node at the migrating H and is mostly localized on the donor···H···acceptor vector, which corresponds to HAT transition states. Therefore it can be stated that they correspond to the HAT mechanism. On the contrary, the SOMO in TS–N2(R1) and TS–N2(R5), show that the p orbital in the H acceptor is aligned with the transition vector,

Table 4 Atomic spin densities (ASD) from natural orbital population analyses in aqueous solution

	R1[a]	R2	R3	R4	R5
TS-C1					
C1	0.456				0.529
H	−0.044				−0.024
O	0.612				0.437
TS–C1b					
C1	0.365				
H	−0.041				
O	0.685				
TS–C3					
C3	0.305				0.360
H	−0.035				−0.007
O	0.673				0.442
TS–C4					
C4	0.288	0.410	0.346	0.376	0.260
H	−0.033	−0.023	−0.014	−0.020	−0.004
O	0.632	0.361	0.352	0.355	0.421
TS–C13					
C13	0.299				0.341
H	−0.036				−0.005
O	0.658				0.436
TS–N1					
N1	0.436				
H	−0.035				
O	0.516				
TS–N2					
N2	0.367				0.243
H	−0.013				−0.007
O	0.149				0.046

[a] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$.

Table 5 Charge (Q) carried by the migrating H atom from natural orbital population analyses in aqueous solution

	R1[a]	R2	R3	R4	R5
TS–C1	0.347				0.366
TS–C1b	0.301				
TS–C3	0.314				0.351
TS–C4	0.323	0.392	0.381	0.390	0.352
TS–C13	0.283				0.321
TS–N1	0.475				
TS–N2	0.506				0.502

[a] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$.

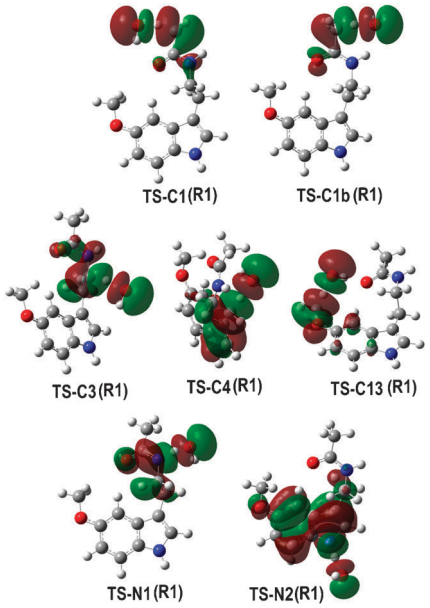

Fig. 4 SOMO density surfaces of the HAT and PCET transition states, involving OH radicals, computed with an isodensity value of 0.02 au.

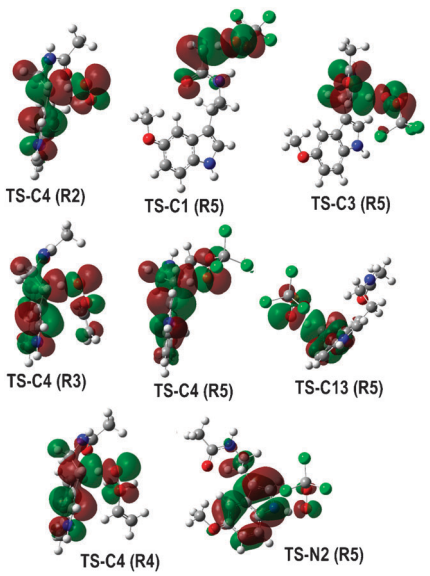

Fig. 5 SOMO density surfaces of the HAT and PCET transition states, involving peroxyl radicals, computed with an isodensity value of 0.02 au. R2 = $^{\bullet}$OOH, R3 = $^{\bullet}$OO–CH=CH_2, R4 = $^{\bullet}$OO–CH_2–CH=CH_2, R5 = $^{\bullet}OOCCl_3$.

while the p orbital in the H donor (N) is orthogonal to it, indicating that the H and the electron transfers take place from different orbitals, *i.e.* they correspond to the PCET mechanism.

According to all previously discussed criteria it seems that H transfers from the N2 atom in MLT, to $^{\bullet}$OH and $^{\bullet}OOCCl_3$ radicals take place by PCET, while H atoms in the alkyl sites are transferred by HAT.

The barriers of reaction, in terms of Gibbs free energy ($\Delta G^{\neq}$), at room temperature, are reported in Table 6. The gas-phase data are provided in ESI† (Table S2). In general

Table 6 Gibbs free energies of activation, at 298.15 K, in kcal mol^{-1}

	Benzene					Water				
	R1[a]	R2	R3	R4	R5	R1[a]	R2	R3	R4	R5
HAT										
C1	6.4				21.5	10.0				24.4
C1b	6.8					11.6				
C3	4.2				10.3	7.6				8.8
C4	2.3	16.9	17.4	18.1	12.9	5.3	21.0	18.6	20.2	12.7
C13	1.1				15.2	4.3				15.5
N1	8.9					15.3				
N2	2.7				7.9	5.6				5.9
RAF										
C5	1.0					2.9				
C6	0.2	14.5			6.0	0.6	16.1			5.0
C7	3.9					6.4				
C9	0.5					2.2				
C10	2.5					3.3				
C11	2.8					3.3				
C12	2.6					4.6				

[a] R1 = $^{\bullet}$OH, R2 = $^{\bullet}$OOH, R3 = $^{\bullet}$OO–CH=CH_2, R4 = $^{\bullet}$OO–CH_2–CH=CH_2, R5 = $^{\bullet}OOCCl_3$.

the barriers of RAF channels were found to be lower than those of the HAT channels. It was also found that the barriers are systematically higher in aqueous solution than in benzene solution for radicals $^{\bullet}$OH, $^{\bullet}$OOH, $^{\bullet}$OO–CH=CH_2, and $^{\bullet}$OO–CH_2–CH=CH_2. For the channels of reaction involving the $^{\bullet}OOCCl_3$ radical the opposite trend was found, with two exceptions. They are the barriers corresponding to HAT from C1 and C13. For these two channels the $\Delta G^{\neq}$ values are higher in aqueous solution than in non-polar media.

For MLT + $^{\bullet}$OH, the lowest value of $\Delta G^{\neq}$ corresponds to RAF at C6, both in non-polar and in polar environments. The highest barrier was found to be that of H transfer from site N1. Among the studied HAT channels, that corresponding to H transfers from site C13 is predicted to be the one with the lowest barrier. For MLT + $^{\bullet}OOCCl_3$ the lowest value of $\Delta G^{\neq}$ also corresponds to RAF at C6, both in non-polar and in polar environments. Regarding the HAT mechanism, the lowest barrier corresponds to channel N2.

The rate constants for the different channels of reaction, in aqueous and benzene solutions are reported in Table 7 and 8, together with the overall rate coefficients. It has been assumed that neither mixing nor crossover between different pathways occurs and therefore the overall rate coefficient (k) has been calculated as the sum of the rate coefficients of each channel. For example for the MLT + $^{\bullet}$OH reaction in aqueous solution:

$$k = k_{app}^{SET} + k_{app}^{HAT} + k_{app}^{RAF} \quad (7)$$

where:

$$k_{app}^{HAT} = k_{app}^{Cl} + k_{app}^{C1b} + k_{app}^{C3} + k_{app}^{C4} + k_{app}^{C13} + k_{app}^{N1} + \underset{app}{k^{N2}} \quad (8)$$

$$k_{app}^{RAF} = k_{app}^{C5} + k_{app}^{C6} + k_{app}^{C7} + k_{app}^{C9} + k_{app}^{C10} + k_{app}^{C11} + \underset{app}{k^{C12}} \quad (9)$$

The MLT + $^{\bullet}$OH reaction was found to be diffusion limited, in agreement with the available experimental data. In fact the agreement between calculated and experimental

Table 7 Apparent rate constants of the different channels, and overall rate coefficient (M^{-1} s^{-1}), at 298.15 K, in aqueous solution

	R1[a]	R2	R3	R4	R5
SET	8.16×10^{9}				
HAT					
C1	9.37×10^{6}				3.81×10^{-4}
C1b	1.87×10^{4}				
C3	3.84×10^{7}				5.31×10^{6}
C4	1.11×10^{9}	1.24×10^{-2}	7.34×10^{-1}	4.26×10^{-2}	1.39×10^{4}
C13	1.40×10^{9}				9.28×10^{1}
N1	1.41×10^{2}				
N2	7.39×10^{8}				5.88×10^{8}
RAF					
C5	1.17×10^{9}				
C6	1.19×10^{9}	1.99×10^{1}			8.11×10^{8}
C7	2.11×10^{8}				
C9	1.18×10^{9}				
C10	1.16×10^{9}				
C11	1.16×10^{9}				
C12	9.73×10^{8}				
Overall	1.85×10^{10}	1.99×10^{1}	7.34×10^{-1}	4.26×10^{-2}	1.40×10^{9}
Exp.	1.25×10^{1} [b]				
	2.7×10^{1} [c]				

[a] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$. [b] Ref. 66. [c] Ref. 8.

Table 8 Apparent rate constants of the different channels, and overall rate coefficient (M^{-1} s^{-1}), at 298.15 K, in benzene solution

	R1[a]	R2	R3	R4	R5
SET					
HAT					
C1	1.48×10^{9}				5.43×10^{-2}
C1b	6.63×10^{7}				
C3	1.92×10^{9}				4.20×10^{5}
C4	2.27×10^{9}	1.15×10^{1}	5.22×10^{0}	1.72×10^{0}	9.88×10^{3}
C13	2.28×10^{9}				1.39×10^{2}
N1	6.44×10^{6}				
N2	2.25×10^{9}				3.10×10^{7}
RAF					
C5	1.75×10^{9}				
C6	1.75×10^{9}	3.00×10^{2}			4.09×10^{8}
C7	1.59×10^{9}				
C9	1.75×10^{9}				
C10	1.73×10^{9}				
C11	1.73×10^{9}				
C12	1.73×10^{9}				
Overall	2.23×10^{10}	3.11×10^{2}	5.22×10^{0}	1.72×10^{0}	4.40×10^{8}

[a] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$.

values is excellent, with the calculated value in between the two experimental values reported in aqueous solution. The calculated overall rate coefficient is only 1.5 times larger and 1.5 times smaller than those reported by Mahal *et al.*[66] and Matuszak *et al.*,[8] respectively. For the MLT reaction with the •$OOCCl_3$ there are also two experimental values available. One reported by Mahal *et al.*[66] (6×10^{8} M^{-1} s^{-1}), and the other by Marshall *et al.*[20] (2.7×10^{8} M^{-1} s^{-1}). In this case the comparison with the calculated values is not straightforward since the experiments were conducted in a mixture of solvents. However the calculated values, in non-polar and polar environments, are close enough to those obtained from experiments. Moreover the value reported by Mahal *et al.*[66] is intermediate with respect to the calculated values in benzene and water solutions. Therefore the general agreement between calculated and observed overall rate coefficients supports the reliability of the results presented in this work and of the kinetic and mechanistic data proposed here for the first time.

The finding that the MLT + •OH reaction is diffusion-limit controlled indicates that MLT is an excellent •OH radical scavenger. Regarding the efficiency of MLT as peroxyl radicals scavenger, it is predicted that it would be strongly influenced by the nature of the particular radical. It was found that MLT is a very good •$OOCCl_3$ scavenger, while it is rather ineffective for scavenging •OOH, •OO–CH=CH_2, and •OO–CH_2–CH=CH_2. Since the two latter radicals were chosen to mimic lipid peroxyl radicals it is proposed that MLT cannot scavenge these radicals either. Therefore it seems that MLT can only scavenge very reactive peroxyl radicals, such as those with strong electron-withdrawing substituents. This does not mean that MLT is futile to prevent lipid peroxidation. On the contrary since lipid peroxidation can occur by O_2 addition to radical sites arising from reactions with very reactive radicals, such as •OH, MLT is proposed to be efficient in inhibiting lipid peroxidation by trapping the free radicals that initiate the degradation process. However, the reported results strongly indicate that MLT cannot directly scavenge alkenyl peroxyl or hydroperoxyl radicals.

The branching ratios of the different channels of reaction in aqueous and benzene solutions are reported in Table 9 and 10, respectively. In aqueous solution the electron transfer is predicted to be the most important mechanism for the MLT + •OH reaction, accounting for the 44.1% of the overall reaction. Since in aqueous solution, at physiological pH, $MLT^{\bullet+}$ is expected to immediately deprotonate from site N2,[66] this corresponds to the SEPT mechanism, which is identified as the main mechanism in this case. RAF and H transfer have contributions of 17.8% and 38.1%, respectively. For the MLT + •$OOCCl_3$ reaction RAF accounts for 57.7% and H transfer for 42.3%.

In non-polar environments the RAF mechanism is predicted to be the one contributing the most to the overall

Table 9 Branching ratios (Γ^a) of the different channels of reaction, at 298.15 K, in aqueous solution

	R1[b]	R2	R3	R4	R5
SET	44.13				
HAT					
C1	0.05				~0
C1b	~0				
C3	0.21				0.38
C4	5.99	0.06	100	100	~0
C13	7.57				~0
N1	~0				
N2	4.00				41.89
RAF					
C5	6.33				
C6	6.42	99.94			57.73
C7	1.14				
C9	6.39				
C10	6.25				
C11	6.25				
C12	5.26				

[a] $\Gamma_i = \frac{k_i}{k_{overall}} \times 100$. [b] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$.

Table 10 Branching ratios (Γ) of the different channels, at 298.15 K, in benzene solution

	R1[a]	R2	R3	R4	R5
SET					
HAT					
C1	6.64				~0
C1b	0.30				
C3	8.61				0.10
C4	10.17	3.69	100	100	~0
C13	10.20				~0
N1	0.03				
N2	10.08				7.03
RAF					
C5	7.85				
C6	7.85	96.31			92.87
C7	7.13				
C9	7.85				
C10	7.78				
C11	7.74				
C12	7.77				

[a] R1 = •OH, R2 = •OOH, R3 = •OO–CH=CH_2, R4 = •OO–CH_2–CH=CH_2, R5 = •$OOCCl_3$.

MLT + •$OOCCl_3$ reaction, with a contribution of 92.9%. For this reaction H transfer can be considered a minor channel accounting only for 7.1% of the overall reactivity of MLT. For the MLT + •OH reaction, on the other hand, both mechanisms are important (RAF 54% and H transfer 46%).

According to the estimated branching ratios a wide product distribution is expected for the MLT + •OH reaction. In non-polar environments significant populations of products formed by HAT at sites C1, C3, C4, C13 and N2 are expected, as well as those corresponding to RAF at sites C5 to C12. In aqueous media the main product is predicted to be that arising from the SEPT mechanism, with the deprotonation taking place at N2. The other products that are predicted to be formed to significant proportions are those from HAT at C4, C13 and N2 and from RAF at C5, C6, C9, C10, C11 and C12. Even though the branching ratio is reported for the SET mechanism, the formed radical cation is predicted to spontaneously and rapidly deprotonate from site N2, yielding the same product that the one arising from HAT at this site.

For the MLT + •$OOCCl_3$ reaction only two products are predicted to be observed, when the reaction takes place in non-polar environments. They are those formed from RAF on C6 site (92.9%) and from H transfer from N2 (~7%). In aqueous solution these two products are also predicted to be the main ones. However, according to the calculated branching ratios, they would be formed in a more similar proportion in this case (57.7% and 41.2%, respectively).

For the MLT reactions with the other studied peroxyl radicals, only that involving •OOH is predicted to yield more than one product. In this case the radical adduct formed by •OOH addition to site C6 in MLT is expected to be the main product of reaction, regardless of the polarity of the environment.

Conclusions

MLT was found to react with OH radicals in a diffusion-limited way, regardless of the polarity of the environment. This supports previous findings indicating that MLT is an excellent OH radical scavenger. The calculated values of the overall rate coefficient of MLT + •OH reaction in benzene and water solutions are 2.23×10^{10} and 1.85×10^{10} M^{-1} s^{-1}, respectively.

MLT is also predicted to be a very good •$OOCCl_3$ scavenger. The calculated overall rate coefficients in benzene and water solutions are 4.40×10^{8} and 1.40×10^{9} M^{-1} s^{-1}, respectively.

However MLT was found to be rather ineffective for scavenging less reactive peroxyl radicals, such as alkenyl peroxyl radicals and •OOH. Therefore it is concluded that the protective effect of MLT against lipid peroxidation does not take place by directly trapping peroxyl radicals, but rather by scavenging more reactive radicals, such as •OH, which can initiate the degradation process.

In aqueous solutions SEPT was found to be the main mechanism for the MLT + •OH reaction, accounting for the 44.1% of the overall reactivity of MLT towards OH radicals. RAF and H transfer have contributions of 17.8% and 38.1%, respectively. In addition a wide product distribution is predicted for the MLT + •OH reaction.

The good agreement between the calculated and the available experimental data, on the studied processes, supports the reliability of the results presented in this work.

Acknowledgements

The author thanks Laboratorio de Visualización y Cómputo Paralelo at UAM-Iztapalapa for the access to its computer facilities.

References

1 (*a*) S. F. Pang and A. E. Allen, *Pineal Res. Rev.*, 1986, **4**, 55; (*b*) R. J. Reiter, *Endocrinol. Rev.*, 1991, **12**, 151.
2 D. X. Tan, L. D. Chen, B. Poeggeler, L. C. Manchester and R. J. Reiter, *Endocr. J.*, 1993, **1**, 57.
3 (*a*) A. Menendez-Pelaez and R. J. Reiter, *J. Pineal Res.*, 1993, **15**, 59; (*b*) L. Ceraulo, M. Ferrugia, L. Tesoriere, S. Segreto, M. A. Livrea and V. Turco-Liveri, *J. Pineal Res.*, 1999, **26**, 108; (*c*) A. Menendez-Pelaez, B. Poeggeler, R. J. Reiter,

L. Barlow-Walden, M. I. Pablos and D. X. Tan, *J. Cell. Biochem.*, 1993, **53**, 373; (*d*) J. Leon, D. Acuña-Castroviejo, R. M. Sainz, J. C. Mayo, D. X. Tan and R. J. Reiter, *Life Sci.*, 2004, **75**, 765.

4 M. Karbownik and R. J. Reiter, *Proc. Soc. Exp. Biol. Med.*, 2000, **225**, 9.

5 D. Melchiorri, R. J. Reiter, A. M. Attia, M. Hara, A. Burgos and G. Nistico, *Life Sci.*, 1994, **56**, 83.

6 D. X. Tan, R. J. Reiter and L. D. Chen, *Carcinogenesis*, 1994, **15**, 215.

7 G. Escames, J. M. Guerrero, R. J. Reiter, J. J. Garcia, A. Munoz-Hoyos, G. G. Ortiz and C. S. Oh, *Neurosci. Lett.*, 1997, **230**, 147.

8 Z. Matuszak, K. J. Reszka and C. F. Chignell, *Free Radical Biol. Med.*, 1997, **23**, 367.

9 See for example: (*a*) P. Stasica, P. Ulanski and J. M. Rosiak, *J. Pineal Res.*, 1998, **25**, 65; (*b*) H. Ebelt, D. Peschke, H. J. Brömme, W. Mörke, R. Blume and E. Peschke, *J. Pineal Res.*, 2000, **28**, 65; (*c*) D. Bandyopadhyay, K. Biswas, U. Bandyopadhyay, R. J. Reiter and R. K. Banerjee, *J. Pineal Res.*, 2000, **29**, 143; (*d*) H. J. Brömme, W. Mörke, E. Peschke, H. Ebelt and D. Peschke, *J. Pineal Res.*, 2000, **29**, 201; (*e*) X. J. Li, J. Gu, S. D. Lu and F. Y. Sun, *J. Pineal Res.*, 2002, **32**, 47; (*f*) Z. A. Velkov, Y. Zh. Velkov, B. T. Galunska, D. N. Paskalev and A. V. Todjer, *Eur. J. Med. Chem.*, 2009, **44**, 2834.

10 See for example: (*a*) M. T. Lin and M. F. Beal, *Nature*, 2006, **443**, 787; (*b*) P. H. Reddy, *J. Neurochem.*, 2006, **96**, 1; (*c*) C. Schoeneich, *Biochim. Biophys. Acta, Proteins Proteomics*, 2005, **1703**, 111; (*d*) B. I. Giasson, H. Ischiropoulos, V. M. Y. Lee and J. Q. Trojanowski, *Free Radical Biol. Med.*, 2002, **32**, 1264; (*e*) M. Y. Aksenov, M. V. Aksenov, D. A. Butterfield, J. W. Geddes and W. R. Markesbery, *Neuroscience*, 2001, **163**, 373; (*f*) G. Perry, A. K. Raina, A. Nunomura, T. Wataya, L. M. Sayre and M. A. Smith, *Free Radical Biol. Med.*, 2000, **28**, 831; (*g*) B. S. Berlett and E. R. Stadtman, *J. Biol. Chem.*, 1997, **272**, 20313.

11 H. Khaldy, G. Escames, J. León, F. Vives, J. D. Luna and D. Acuña-Castroviejo, *J. Pineal Res.*, 2000, **2**, 100.

12 E. Sofic, Z. Rimpapa, Z. Kundurovic, A. Sapcanin, I. Tahirovic, A. Rustembegovic and G. Cao, *J. Neural Transm.*, 2005, **112**, 349.

13 L. Y. Zang, G. Cosma, H. Gardner and V. Vallyathan, *Biochim. Biophys. Acta*, 1998, **1425**, 469.

14 F. Antunes, L. R. C. Barclay, K. U. Ingold, M. King, J. Q. Norris, J. C. Scaiano and F. Xi, *Free Radical Biol. Med.*, 1999, **26**, 117.

15 J. Mekhloufi, D. Bonnefont-Rousselot, S. Yous, D. Lesieur, M. Couturier, P. Therond, A. Legrand, D. Jore and M. Gardes-Albert, *J. Pineal Res.*, 2005, **39**, 27.

16 J. C. Sciano, *J. Pineal Res.*, 1995, **19**, 189.

17 J. C. Mayo, D. X. Tan, R. M. Sainz, S. Lopez-Burillo and R. J. Reiter, *Free Radical Res.*, 2003, **37**, 543.

18 M. A. Livrea, L. Tesoriere, D. D'arpa and M. Morreale, *Free Radical Biol. Med.*, 1997, **23**, 706.

19 C. Pieri, F. Moroni, M. Marra, F. Marcheselli and R. Recchioni, *Arch. Gerontol. Geriatr.*, 1994, **20**, 159.

20 K. A. Marshall, R. J. Reiter, B. Poeggeler, O. I. Aruoma and B. Halliwell, *Free Radical Biol. Med.*, 1996, **21**, 307.

21 See for example: (*a*) I. B. Zavodnik, A. V. Domanski, E. A. Lapshina, M. Bryszewska and R. J. Reiter, *Life Sci.*, 2006, **79**, 391; (*b*) E. Gitto, S. Pellegrino, P. Gitto, I. Barberi and R. J. Reiter, *J. Pineal Res.*, 2009, **46**, 128; (*c*) B. Longoni, M. G. Salgo, W. A. Pryor and P. L. Marchiafava, *Life Sci.*, 1998, **62**, 853; (*d*) S. Taysi, M. Koc, M. E. Buynkuroglu, K. Altinkaynak and Y. N. Sahin, *J. Pineal Res.*, 2003, **34**, 173.

22 A. G. Turjanski, R. E. Rosenstein and D. A. Estrin, *J. Med. Chem.*, 1998, **41**, 3684.

23 P. Stasica, P. Paneth and J. M. Rosiak, *J. Pineal Res.*, 2000, **29**, 125.

24 A. Galano, R. Alvarez-Diduk, M. T. Ramirez-Silva, G. Alarcon-Angeles and A. Rojas-Hernández, *Chem. Phys.*, 2009, **363**, 13.

25 Y. Zhao, N. E. Schultz and D. G. Truhlar, *J. Chem. Theory Comput.*, 2006, **2**, 364.

26 C. Zavala-Oseguera, J. R. Alvarez-Idaboy, G. Merino and A. Galano, *J. Phys. Chem. A*, 2009, **113**, 13913.

27 E. Velez, J. Quijano, R. Notario, E. Pabón, J. Murillo, J. Leal, E. Zapata and G. Alarcón, *J. Phys. Org. Chem.*, 2009, **22**, 971.

28 A. Vega-Rodriguez and J. R. Alvarez-Idaboy, *Phys. Chem. Chem. Phys.*, 2009, **11**, 7649.

29 A. Galano and J. R. Alvarez-Idaboy, *Org. Lett.*, 2009, **11**, 5114.

30 G. Black and J. M. Simmie, *J. Comput. Chem.*, 2010, **31**, 1236.

31 T. Furuncuoglu, I. Ugur, I. Degirmenci and V. Aviyente, *Macromolecules*, 2010, **43**, 1823.

32 N. R. Pillsbury, C. W. Muller and T. S. Zwier, *J. Phys. Chem. A*, 2009, **113**, 5013.

33 S. Nangia and B. J. Garrison, *Mol. Phys.*, 2009, **107**, 831.

34 A. Galano, N. A. Macías-Ruvalcaba, O. N. M. Campos and J. Pedraza-Chaverri, *J. Phys. Chem. B*, 2010, **114**, 6625.

35 F. Wang, D. B. Cao, G. Liu, J. Ren and Y. W. Li, *Theor. Chem. Acc.*, 2010, **126**, 87.

36 T. Gao, J. M. Andino and J. R. Alvarez-Idaboy, *Phys. Chem. Chem. Phys.*, 2010, **12**, 9830.

37 E. I. Izgorodina, D. R. B. Brittain, J. L. Hodgson, E. H. Krenske, C. Y. Lin, M. Namazian and M. L. Coote, *J. Phys. Chem. A*, 2007, **111**, 10754.

38 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *GAUSSIAN 03 (Revision E.01)*, Gaussian, Inc., Wallingford, CT, 2004.

39 M. T. Cances, B. Mennucci and J. Tomasi, *J. Chem. Phys.*, 1997, **107**, 3032.

40 B. Mennucci, E. Cances and J. Tomasi, *J. Phys. Chem. B*, 1997, **101**, 10506.

41 J. Tomasi, B. Mennucci and E. Cances, *THEOCHEM*, 1999, **464**, 211.

42 See for example: (*a*) M. Belcastro, T. Marino, N. Russo and M. Toscano, *Theor. Chem. Acc.*, 2006, **115**, 361; (*b*) A. Martinez, R. Vargas and A. Galano, *Theor. Chem. Acc.*, 2010, **127**, 595; (*c*) M. Leopoldini, F. Rondinelli, N. Russo and M. Toscano, *J. Agric. Food Chem.*, 2010, **58**, 8862; (*d*) A. Pérez-González and A. Galano, *J. Phys. Chem. B*, 2011, **115**, 1306.

43 Y. Okuno, *Chem.–Eur. J.*, 1997, **3**, 210.

44 S. W. Benson, *The foundations of chemical kinetics*, Krieger, Malabar, Florida, 1982.

45 D. Ardura, R. Lopez and T. L. Sordo, *J. Phys. Chem. B*, 2005, **109**, 23618.

46 J. R. Alvarez-Idaboy, L. Reyes and J. Cruz, *Org. Lett.*, 2006, **8**, 1763.

47 J. R. Alvarez-Idaboy, L. Reyes and N. Mora-Diez, *Org. Biomol. Chem.*, 2007, **5**, 3682.

48 A. Galano, *J. Phys. Chem. A*, 2007, **111**, 1677.

49 A. Galano, *J. Phys. Chem. C*, 2008, **112**, 8922.

50 A. Galano and A. Cruz-Torres, *Org. Biomol. Chem.*, 2008, **6**, 732.

51 A. Galano and M. Francisco-Márquez, *Chem. Phys.*, 2008, **345**, 87.

52 N. Mora-Diez, S. Keller and J. R. Alvarez-Idaboy, *Org. Biomol. Chem.*, 2009, **7**, 3682.

53 (*a*) J. P. Foster and F. Weinhold, *J. Am. Chem. Soc.*, 1980, **102**, 7211; (*b*) A. E. Reed and F. Weinhold, *J. Chem. Phys.*, 1983, **78**, 4066; (*c*) A. E. Reed, L. A. Curtiss and F. Weinhold, *Chem. Rev.*, 1985, **83**, 1736; (*d*) A. E. Reed, R. B. Weinstock and F. Weinhold, *J. Chem. Phys.*, 1985, **83**, 735; (*e*) A. E. Reed, L. A. Curtiss and F. Weinhold, *Chem. Rev.*, 1988, **88**, 899.

54 H. Eyring, *J. Chem. Phys.*, 1935, **3**, 107.

55 M. G. Evans and M. Polanyi, *Trans. Faraday Soc.*, 1935, **31**, 875.

56 D. G. Truhlar, W. L. Hase and J. T. Hynes, *J. Phys. Chem.*, 1983, **87**, 2664.

57 C. Eckart, *Phys. Rev.*, 1930, **35**, 1303.

58 (*a*) R. A. Marcus, *Annu. Rev. Phys. Chem.*, 1965, **16**, 155; (*b*) R. A. Marcus, *Rev. Mod. Phys.*, 1993, **65**, 599; (*c*) R. A. Marcus, *Pure Appl. Chem.*, 1997, **69**, 13.
59 S. F. Nelsen, S. C. Blackstock and Y. Kim, *J. Am. Chem. Soc.*, 1987, **109**, 677.
60 S. F. Nelsen, M. N. Weaver, Y. Luo, J. R. Pladziewicz, L. K. Ausman, T. L. Jentzsch and J. J. O'Konek, *J. Phys. Chem. A*, 2006, **110**, 11665.
61 See for example: (*a*) A. Galano, *J. Phys. Chem. B*, 2007, **111**, 12898; (*b*) A. Galano and J. R. Alvarez-Idaboy, *Org. Lett.*, 2009, **11**, 5114; (*c*) A. Galano, N. A. Macías-Ruvalcaba, O. N. M. Campos and J. Pedraza-Chaverri, *J. Phys. Chem. B*, 2010, **114**, 6625; (*d*) A. Galano, R. Vargas and A. Martínez, *Phys. Chem. Chem. Phys.*, 2010, **12**, 193.
62 F. C. Collins and G. E. Kimball, *J. Colloid Sci.*, 1949, **4**, 425.
63 M. Smoluchowski, *Z. Phys. Chem.*, 1917, **92**, 129.
64 D. G. Truhlar, *J. Chem. Educ.*, 1985, **62**, 104.
65 (*a*) A. Einstein, *Ann. Phys.*, 1905, **17**, 549; (*b*) G. G. Stokes, *Mathematical and Physical Papers*, Cambridge University Press, Cambridge, 1903, vol. 3, (esp. Sect. IV, p. 55).
66 H. S. Mahal, H. S. Sharma and T. Mukherjee, *Free Radical Biol. Med.*, 1999, **26**, 557.
67 W. A. Pryor, *Free Radical Biol. Med.*, 1988, **4**, 219.
68 I. G. Draganic and Z. D. Draganic, *The Radiation Chemistry of Water*, Academic Press, New York, 1971.
69 L. J. Marnett, *Carcinogenesis*, 1987, **8**, 1365.
70 W. A. Pryor, *Annu. Rev. Physiol.*, 1986, **48**, 657.
71 R. F. W. Bader, *Atoms in Molecules—A Quantum Theory*, Oxford University Press, Oxford, 1990.
72 F. Biegler-Köning, J. Schönbohm and D. Bayles, *J. Comput. Chem.*, 2001, **22**, 545.
73 F. Biegler-Köning, *AIM2000, version 1.0*, University of Applied Sciences, Bielefeld, Germany, 2000.
74 S. Olivella, J. M. Anglada, A. Solé and J. M. Bofill, *Chem.–Eur. J.*, 2004, **10**, 3404.
75 J. M. Mayer, D. A. Hrovat, J. L. Thomas and W. T. Borden, *J. Am. Chem. Soc.*, 2002, **124**, 11142.
76 F. Turecek and E. A. Syrstad, *J. Am. Chem. Soc.*, 2003, **125**, 3353.

