Theoretical insight into the antioxidant properties of melatonin and derivatives†

Jeffrey R. Johns* and James A. Plattsb

Density functional theory calculations on melatonin, metabolites and synthetic derivatives thereof, and a range of other biological antioxidant molecules are presented, with a view to understanding the antioxidant ability of these molecules. After testing of the necessary calculations, we show that melatonin lies close to vitamin E on a donor–acceptor map, indicating that it should be an excellent electron donor but a poor acceptor. The neutral radical metabolite of melatonin is predicted to be an even better donor, whereas other metabolites and synthetic derivatives should retain antioxidant ability but are less powerful than the parent. QSAR models of antioxidant activity, measured in two different assays, are presented. We show that octanol–water partition coefficient is an excellent predictor of activity in lipophilic media, while properties related to electron donor/acceptor power give good fits against activity in aqueous media.

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring molecule, biosynthesized from the precursor amino acid tryptophan, primarily by the pineal gland of vertebrates.1 Melatonin has been extensively reported as a potent antioxidant, both in vitro2 and in vivo.3–5 Much of its effectiveness in vivo may be attributed to the cascade of melatonin antioxidant metabolites produced.6,7 Unlike most small-molecule biological antioxidants such as vitamin C (ascorbic acid), α-tocopherol (vitamin E), lipoic acid etc., melatonin does not redox-cycle. It undergoes molecular rearrangement, effectively removing the free electron from the system – a so-called suicidal antioxidant (Fig. 1). Each of these products of rearrangement is also a potent antioxidant in its own right.2,8,9 Furthermore, most of these processes involve more than one reactive oxygen species (ROS) per step, so that one melatonin molecule could scavenge up to 10 radical species before the final metabolite is eliminated form the body.10 Additionally, the relative position of melatonin and its metabolites in the antioxidant “pecking order” (electrochemical potential) may contribute greatly to its utility in biological systems.11 Melatonin is finding great utility in preventing diseases related to oxidative damage including cancer12 and neuro‐degenerative diseases13,14 as well as its well known role in treatment for reducing insomnia, jet lag, migraine, headache, etc.13,15,16 It is being widely investigated for a large number of other diseases in a large number of clinical trials.17 In addition, consumption of tropical fruits containing melatonin has been shown to reduce antioxidant levels in humans.5,18

The antioxidant radical scavenging properties of melatonin and its metabolites cyclic-3-hydroxymelatonin (cyclic-3OHM),10 N(1)-acetyl-N(2)-formyl-5-methoxykynuramine (AFMK), N(1)-acetyl-5-methoxykynuramine (AMK)7 and 6-hydroxymelatonin (6-OHmel)19 occurs mainly via the one electron transfer process.8,20,21 The first ionization potential (IP) and the electron affinity (EA) are properties of a system that allow measurement of its propensity to donate or accept one electron. The best antioxidants present low IP values, because the lower the IP, the easier the electron abstraction, and vice versa for EA and electron acceptance (antireductant).

Gazquez et al.21 have presented an elegant model to explain relative scavenging activity and antioxidant power of compounds using these two properties. Quantum chemical density functional theory (DFT) calculations can be used to obtain accurate ionization potentials, electron affinities, electrodonating, and electroaccepting power indexes (with respect to internal standards, such as fluorine and sodium atoms). These values can then be used to construct a donator acceptor map (DAM), indicating whether molecules are good electron donors or acceptors. The DAM is a powerful representation of these key properties, helping to reveal the antiradical capacity of any substance and allowing qualitative comparison between substances, alongside quantitative measures obtained from experiment or theory. Previous DAM studies have included linear polyene-conjugated molecules,23 carotenoids,24 a large series of carotenoids,25 carotenoids, melatonin and vitamins,26 and psittacofulvins and anthocyanins.27
Vitamin E or α-tocopherol has been described the “last line of defense” in a multicomponent endogenous antioxidant system.28 It appears that under conditions of stress, depletion of cellular ascorbic acid occurs first, followed by glutathione, then α-tocopherol, resulting in initiation of lipid peroxidation. When glutathione is depleted, ascorbic acid plays a vital role in maintaining cellular α-tocopherol levels and survival of the cell.29 One might expect that melatonin should be depleted after α-tocopherol, particularly in membranes,30 as it is higher in the electrochemical series at 700 mV,8 compared to 500 mV for α-tocopherol.11 Melatonin may therefore truly be the last line of defense against oxidative damage.31 The role of melatonin in this multicomponent antioxidant is still unclear, although there is evidence that melatonin cause upregulation of superoxide dismutase, glutathione reductase and catalase.32,33 Several melatonin derivatives that were substituted on the indole nitrogen (Fig. 2) have been previously reported for in vitro antioxidant effects and anti-inflammatory activities.34 Their synthesis and characterization is described in this ref. 34.

The aim of this study was to investigate the antioxidant radical scavenging properties of melatonin and its metabolites cyclic-3OHM, AFMK, AMK and 6-hydroxymelatonin, and several N-indole substituted derivatives via the one electron transfer process, using a donor–acceptor map. Other classical antioxidants and vitamins are modeled for comparison. QSAR relationships of some N-indole substituted derivatives between in vitro antioxidant properties experimentally measured by lipid peroxidation of rat brain homogenate using thio-barbituric acid reacting substances (TBARS IC50)35,36 and Oxygen Radical Absorbance Capacity Assay (ORAC) data, and a number of derived electronic properties e.g. HOMO/LUMO energies, donor and acceptor power (Rd, Ra), hardness, electronegativity and log P were investigated. The ORAC assay is based on the scavenging of peroxyl radicals generated by 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) in aqueous media, which prevent the degradation of the fluorescein probe and, consequently, prevent the loss of fluorescence of the probe.37,38 The antioxidant activity was calculated from the integrated area under the fluorescence curve (AUC) for each antioxidant.

Methods

B3LYP/DFT as implemented in Gaussian09-RevC.0139 software was used for all IP and EA calculations with complete optimizations, without symmetry constraints. Geometries were first minimized in Molecular Operating Environment MOE.40 Calculations were performed on the ARCCA/Raven Supercomputer at Cardiff University. Both vertical IE and EA, where energies for the cation and anion were computed at the optimized geometry of the ground state (single point), and relaxed (adiabatic) IE and EA for optimized cation and anion geometries were calculated. Harmonic frequency analysis was used to verify optimized minima using Molden.41

To determine the accuracy of DFT for predicting IP/EA of indoleamines, calculations in gas phase using different basis sets
were compared to previously reported photoelectron spectroscopy measurements. IP and bond-dissociation energies for many antioxidant systems do not follow the same trends in gas and solution phases, such that major differences with respect to vacuum are found when water computations are performed.\textsuperscript{42} On the basis of the computed BDE and IP values, to more realistically model antioxidant activity \textit{in vivo}, calculations were therefore performed using the polarizable continuum model (PCM water) \textit{i.e.} placing the solute in a cavity within the solvent reaction field.

The validity of using B3LYP for calculating EA has been raised, due to most DFT functionals (including B3LYP) being incapable of binding the whole excess electron.\textsuperscript{43} This may not be revealed when using standard basis sets, even with multiple diffuse functions, since they artificially constrain the electron density to remain near the nuclei. The error due to this constraint depends on the magnitude of the EA, which could render trends in EA unreliable, especially for low EAs. Thus a range-separated DFT method, CAM-B3LYP,\textsuperscript{44} was used to investigate the problem of fractional EA. A positive energy for the HOMO of an anion species is an easy diagnostic for the fractional EA problem.\textsuperscript{43}

The melatonin molecule contains three freely rotatable bonds in its imidazole side chain. To investigate the effect of conformation, MOE\textsuperscript{46} was used to select a range of typical conformers \textit{via} stochastic search of rotatable bonds, using the MMFF94 forcefield. 10,000 conformers were generated then sorted into clusters based on dihedral angles of the freely rotatable bonds. Vertical IP and EA of the lowest energy conformers from each cluster were then calculated, following geometry optimization of the neutral molecule. No significant changes in geometry on DFT optimization were noted, indicating that conformers remained in their local energy minima.

Donor–acceptor maps were calculated following the method of Martinez \textit{et al.},\textsuperscript{27} using the same experimental values of IE and EA for sodium of 5.140 and 0.540 eV and for fluorine of 17.540 and 3.400 eV respectively, taken from the literature. This set reference points on the map of sodium, and for fluorine atom, and for fluorine atom. Values calculated using Gaussian 09/RB3LYP/6-31+G* gas phase were 5.406 and 0.596 eV for sodium and 21.405 and 3.513 eV for fluorine respectively.

As formulated by Gazquez \textit{et al.}\textsuperscript{22} and applied in the study of Martinez \textit{et al.},\textsuperscript{27} the propensity to donate charge, or electro-donating power, may be defined as:

\[
\omega^- = \frac{(I + A)^2}{16(I - A)}
\]

(where \(I\) is ionization potential and \(A\) is electron affinity), whereas the propensity to accept charge, or electroaccepting power, may be defined as:

\[
\omega^+ = \frac{(I + 3A)^2}{16(I - A)}
\]

For electroaccepting power, higher values imply a greater capacity for accepting charge, whereas for electrodonating power, lower values imply a greater capacity for donating charge. It should be noted that \(\omega^-\) and \(\omega^+\) refer to fractional charges, however \(I\) and \(A\) refer to donating or accepting a single, whole electron. Thus, a simple charge transfer model, framed in terms of chemical potential and hardness is used to describe electrodonating and electroaccepting powers. The charge flow direction is measured by chemical potential, along with the capacity to donate or accept charge. More emphasis is assigned to the ionization potential than to the electron affinity in the context of the charge donation process. Likewise, more significance is assigned to electron affinity than to ionization potential for electroaccepting power. Hardness provides a measure of the resistance to the electron transfer. So that a range of substances can be compared for electrodonating and electroaccepting power, experimental values of \(I\) and \(A\) for sodium and fluorine are used a reference points to provide corresponding \(\omega^-\) and \(\omega^+\) values. Sodium represents a good electron donor and fluorine represents a good electron acceptor. For some substance \(L\), the electron acceptance index can be defined as:

\[
R_a = \frac{\omega^-_F}{\omega^-_L}
\]

When \(R_a = 1\), then \(\omega^-_L \approx \omega^-_F\) and \(L\) is as effective an electron acceptor as fluorine. When \(R_a > 1\), then \(\omega^-_L > \omega^-_F\) and \(L\) is a more effective electron acceptor than fluorine. When \(R_a < 1\), then \(\omega^-_L < \omega^-_F\) and \(L\) is a less effective electron acceptor than fluorine. Similarly, the electron donation index can be defined as:

\[
R_d = \frac{\omega^+_F}{\omega^+_L}
\]

When \(R_d = 1\), then \(L\) is as effective an electron donor as sodium, and when \(R_d > 1\), \(L\) is a less effective electron donor than sodium, whereas when \(R_d < 1\), \(L\) is a more effective electron donor. If \(R_a\) and \(R_d\) are determined, then any substance \(L\) can be characterized in terms of its electron donor–acceptor capacity, and mapped on a donor acceptor map (DAM).

Octanol–water partition coefficients (log \(P\)) were estimated using ACD/Chemsketch log \(P\) plugin (Advanced Chemistry Development, Inc., Toronto, Canada, 2012), MarvinView 5.11.3 (Chemaxon, Budapest, Hungary, 2012) and MolKa (Molecular Discovery Ltd, Perugia, Italy, 2012) and compared to literature values where available.

Data on the antioxidant capacity of melatonin derivatives \textit{in vitro}, using the widely adopted method of Callaway \textit{et al.},\textsuperscript{35} for measurement of lipid peroxidation, the thiobarbituric acid reacting substances (TBARS) and brain homogenate, was used in this study. Inhibitory effect on nitric oxide (NO) of melatonin and these derivatives has been previously reported by our group.\textsuperscript{34}

Results and discussion

DFT predictions of gas phase IP and EA for different basis sets (Fig. 3) compared favorably with the previously reported
photoelectron spectroscopy measurements and IP/EA of other workers (Table 1). Although there were some differences between values obtained with varying basis sets, these were not large. It is notable, though, that smaller basis sets like 6-31G and 6-31G* incorrectly predict positive EA, and that differences between vertical and relaxed values were small, at any particular basis set. To optimise computational time, the 6-31+G* basis set was chosen for all further calculations as using a larger basis sets did not result in significant improvement in IP or EA values.

Using the CAM-B3LYP functional did not give significantly different EA values for melatonin using any of the basis sets in this study. Furthermore, no positive energies for HOMO’s of any anion species were observed. The reactivity indices calculated for compounds in this study depend mainly on the IP values, rather than EA, as they are mostly electron donors, thus small EA errors will have negligible impact on the overall findings.

Comparison of reported values for IP and EA reported in the literature is made with those calculated using B3LYP 6-31+G* basis set and gas phase in Table 1.

Table 1 Reported photoelectron spectroscopy measurements (PES) and DFT calculated IP/EA values for melatonin

| IP (eV) | EA (eV) |
|---------|---------|
| 7.76    | 0.65    |
| 7.03    | 0.70    |
| 7.7     | 0.88    |
| 6.83    | 0.70    |
| 7.07    | 0.65    |

Fig. 3 Gas phase ionization potentials and electron affinities using different basis sets.

Table 2 Vertical IP and EA of selected conformers of melatonin at B3LYP/6-31+G* PCM (water)

| MOE conformer energy (kcal mol⁻¹) | Vertical IP/eV | Vertical EA/eV |
|-----------------------------------|----------------|----------------|
| 20.573446                         | 5.544          | -0.886         |
| 20.573452                         | 5.603          | -0.739         |
| 21.250622                         | 5.338          | -0.879         |
| 21.250629                         | 5.338          | -0.879         |
| 21.694929                         | 5.429          | -0.947         |
| 21.694931                         | 5.429          | -0.947         |
| 21.724112                         | 5.443          | -0.952         |
| Mean                              | 5.5036         | -0.8898        |
| S.D.                              | 0.0694         | 0.0745         |

Effect of conformation

Effects of conformation of melatonin on calculated IP and EA in PCM (water) are shown in Table 2. Values of IP and EA calculated in PCM are quite different from those in gas phase due to the effects of solvent polarization. However, conformation changes resulted in less that 1.3% difference in IP and 8.4% difference in EA values in the PCM model. This is not unsurprising, as removal or addition of an electron to the neutral molecule would be expected to affect the extensively delocalized rigid indole moiety only, such that the conformation of the imidazole side chain would have little impact on these processes. Therefore, subsequent DFT calculations reported below use the global energy minimum conformation found from the stochastic search.

Donor acceptor map

Electron acceptance (\(R_a\)) and electron donation (\(R_d\)) indexes were calculated from IP and EA, using fluorine and sodium as references as previously described in the methods section. The donor acceptor for melatonin, its metabolites and some classical antioxidants is show in Fig. 4.

The donor/acceptor maps shows melatonin to be a very good electron donor, along with its metabolites 6-hydroxymelatonin and cyclc-3-hydroxymelatonin and the melatoninyl neutral radical, and several classical antioxidants such as vitamin E (\(\alpha\)-tocopherol), epigallocatechin gallate (EGCG), resveratrol, xanthurenic acid and quercetin (a typical flavenoid). Other melatonin metabolites (AFMK, AMK) and melatonin derivatives showed weaker electron donor strength, similar to other classical antioxidants such as vitamin A, vitamin C, beta-carotene and \(\alpha\)-lipoic acid. The 4-nitro derivative of melatonin is not shown on the DAM and appears off the top right quadrant at \(R_a = 4.35\) and \(R_d = 5.62\), being a much poorer electron donor and better electron acceptor than melatonin, due to its strongly withdrawing nitro group. Interestingly, the melatoninyl neutral radical that results from a 1-electron 1-proton donation from melatonin is an even more powerful electron donor than melatonin itself.

These results are supported by a large number of experimental observations where melatonin acts as a direct scavenger of free radicals with the ability to detoxify both reactive oxygen and reactive nitrogen species, and indirectly by...
increasing the activity of the antioxidative defense systems.\textsuperscript{9,47} Researchers have reported that the peroxyl radical scavenger ability of melatonin is better than \( \alpha \)-tocopherol, vitamin C and reduced glutathione (GSH),\textsuperscript{48} and more potent than xanthurenic acid, resveratrol, EGCG, vitamin C and \( \alpha \)-lipoic acid in inhibiting \( \cdot \text{OH} \)-induced oxidative DNA damage generated by oxygen-derived free radicals from Fenton reaction.\textsuperscript{49} Melatonin has been demonstrated to reduce the formation of 8-hydroxy-2'-deoxyguanosine, a product of damaged DNA repair, 60 to 70 times more effectively than ascorbate or \( \alpha \)-tocopherol.\textsuperscript{50} Melatonin also plays an important role in protecting cellular membranes against lipid peroxidation.\textsuperscript{51}

It should be noted that most other dietary antioxidants lie outside the scale of this donor–acceptor map, towards the top right quadrant, including carotenoids, psittacofulvins and anthocyanins, flavonoids and polyphenols. These tend to be electron acceptors rather than electron donors \( \textit{i.e.} \) antireductants.

**Lipophilicity of antioxidant species**

Log \( P \) values for melatonin and its metabolites and some classical antioxidants were calculated and compared to literature values where available (Table 3). The compounds may be classified into roughly three groups – highly lipophilic compounds (\( \log P > 6 \)) like \( \alpha \)-tocopherol, vitamin A and beta-carotene that mainly protect lipid membranes; vitamin C that is very hydrophilic (\( \log P < -3 \)) and mainly protects aqueous cellular and tissue environments; and the "melatonin type" compounds.

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**Table 3** Calculated and experimental \( \log P \) values

| Antioxidant                               | ACD\textsuperscript{a} | Marvin weighted\textsuperscript{b} | MolKa\textsuperscript{c} | Literature values |
|-------------------------------------------|-------------------------|------------------------------------|--------------------------|------------------|
| Beta-carotene (vitamin E)                 | 15.51 ± 0.43            | 11.12                              | 9.0                      | 14.76            |
| \( \alpha \)-Tocopherol                   | 10.66 ± 0.28            | 8.94                               | 9.0                      | 10.51\textsuperscript{d} |
| Vitamin A (retinol)                       | 6.84 ± 0.33             | 6.07                               | 6.1                      | 4.69–6.38\textsuperscript{e} |
| Alpha-lipoic acid                         | 2.16 ± 0.29             | 2.11                               | 2.4                      | —                |
| Melatonin                                 | 0.96 ± 0.44             | 1.41                               | 1.4                      | 1.2\textsuperscript{54,55} |
| 6-OH-melatonin                            | 0.02 ± 0.83             | 0.84                               | 1.0                      | —                |
| Melatoninyl neutral radical               | 0.02 ± 0.83             | 0.88                               | —                        | —                |
| AFMK                                      | 0.82 ± 0.52             | 0.34                               | 0.0                      | 0.48\textsuperscript{56} |
| AMK                                       | 0.65 ± 0.49             | 0.33                               | 0.2                      | 0.74\textsuperscript{56} |
| Tryptophan                                | 0.87 ± 0.31             | 1.51                               | 0.6                      | 1.08\textsuperscript{57} |
| 2-Naphthyl-melatonin                     | 2.73 ± 0.46             | 3.31                               | 3.7                      | —                |
| 2-Benzoyl-melatonin                      | 1.5 ± 0.46              | 2.32                               | 2.4                      | —                |
| Cyclic-3OHmel                              | -0.64 ± 0.89            | -0.21                              | 0.8                      | —                |
| Acetyl-melatonin                          | 1.00 ± 0.87             | 0.47                               | 1.3                      | —                |
| Vitamin C                                 | -3.26 ± 0.56            | -1.98                              | -3.4                     | -1.85\textsuperscript{58} |

\textsuperscript{a} ADC/Chemsketch \( \log P \) plugin (Advanced Chemistry Development, Inc., Toronto, Canada) 2012 http://www.acdlabs.com. \textsuperscript{b} MarvinView 5.11.3 (Chemaxon, Budapest, Hungary) 2012 http://www.chemaxon.com. \textsuperscript{c} MoKa (Molecular Discovery Ltd, Perugia, Italy) 2012 http://www.moldiscovery.com. \textsuperscript{d} Human Metabolome Database Version 3.6. http://www.hmdb.ca/metabolites/HMDB00305.
pounds that may be considered “amphiphilic” (log $P$ between $-1$ and 2). This latter group should be active antioxidants in all cellular (cytosol and membrane) and tissues environments, and may be important in regenerating some of the other redox-cycling antioxidants like α-tocopherol, and mediating antioxidant reactions at aqueous-lipid membrane interfaces. Melatonin has been shown to have strong synergistic effects with α-tocopherol and vitamin C.\textsuperscript{49,53}

TBARS $\text{pIC}_{50}$ results were correlated with a number of derived electronic properties e.g. HOMO/LUMO energies, donor and acceptor power ($R_d$, $R_a$), hardness, electronegativity and log $P$, and results are shown in Table 4. Only lipophilicity (log $P$) correlated well, correlation with other molecular properties (donor/acceptor power, electronegativity and LUMO was poorer, with low or no correlation with hardness, HOMO and HOMO/LUMO energy difference. This may be because of the nature of the brain homogenate lipid peroxidation assay, where solubility of the antioxidant in the lipid domain is the dominant factor contributing to radical scavenging. The hydrophobicity of the antioxidant may also be an important criterion for passive transport into cells across the hydrophobic phospholipid bilayer of the cellular membranes. Furthermore, the single electron transfer mechanism for direct radical scavenging of melatonin, although the most favourable mechanism in aqueous solution, is not favourable in aprotic solvents e.g. benzene, where hydrogen atom transfer/proton coupled electron transfer or radical adduct formation are favoured.\textsuperscript{29}

By contrast, for the ORAC assay, which was performed in aqueous medium, all molecular parameters correlated highly with the ORAC AUC except for log $P$ (Table 4), as has been observed previously with indoleamines where antioxidant potency was measured for lipid peroxidation using a conjugated dienes assay\textsuperscript{60} and in phenolic compounds.\textsuperscript{61}

Some QSAR correlation plots are shown in Fig. 5, and all correlation plots are shown in the ESI.\textsuperscript{†}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Molecular parameters & Correlation ($r^2$) with TBARS $\text{pIC}_{50}$ & Correlation ($r^2$) with ORAC log AUC \\
\hline
Donor power ($R_d$) & 0.636 & 0.951 \\
Acceptor power ($R_a$) & 0.647 & 0.935 \\
Hardness ($\eta$) & 0.458 & 0.982 \\
electronegativity (X) & 0.589 & 0.987 \\
Energy HOMO (eV) & 0.331 & 0.928 \\
Energy LUMO (eV) & 0.560 & 0.995 \\
Energy HOMO–LUMO (eV) & 0.237 & 0.941 \\
Log $P$ & 0.978 & 0.554 \\
\hline
\end{tabular}
\caption{Correlation of TBARS $\text{pIC}_{50}$ and ORAC AUC for melatonin and 3 derivatives with various calculated molecular parameters}
\end{table}

Fig. 5 QSAR correlation plots of some calculated molecular parameters and TBARS or ORAC antioxidant activity.

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Conclusions

The electron donor power of melatonin and its metabolites demonstrated in this theoretical study support the experimental evidence that melatonin is a powerful biological antioxidant and radical scavenger. Our computational studies, presented above, shed light on this important biological property. We have shown that the B3LYP DFT method, along with the 6-31+G(d) basis set, satisfactorily reproduces experimental gas phase ionization potential and electron affinity, while larger basis sets do not improve performance. Importantly, calculated properties are not dependent on molecular conformation, such that data derived from a single conformation should be sufficient to capture all relevant aspects of this molecule.

This method has therefore been used to map out the donor–acceptor power of melatonin, its metabolites, some synthetic derivatives and a range of classical antioxidants. This approach clearly shows that melatonin lies in the range of good electron donors and bad electron acceptors, with similar power to vitamin E. Interestingly, the first neutral, radical metabolite of melatonin is an even better donor than the parent molecule, which will have important implications for the overall biology of the cascade process by which melatonin mops up ROS. Other metabolites, as well as most synthetic derivatives, remain in the range where substantial antioxidant ability should be expected, but a 4-nitro derivative lies well outside this region.

QSAR investigation indicates the ability of melatonin derivatives to protect against lipid peroxidation of brain homogenate strongly correlated with their lipophilicity (log P) but only weakly to other molecular properties related to donor/acceptor ability (donor/acceptor power, electronegativity, hardness, HOMO, LUMO, HOMO-LUMO energies). By contrast, these molecular parameters correlate strongly with ORAC antioxidant power measured in aqueous phase.

The range of lipophilicity of melatonin and its metabolites (log P between −1 and 2) may explain the large number of antioxidant areas where melatonin seems to play a role in protecting against ROS damage; they lie between the traditional membrane protectors (α-tocopherol, vitamin A and carotenoids) and hydrophilic compounds (vitamin C, lipoic-acid) and aqueous antioxidant enzymes.

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