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# A new free radical scavenging cascade involving melatonin and three of its metabolites (3OHM, AFMK and AMK)

Miguel Reina, Ana Martínez *

*Departamento de Materiales de Baja Dimensionalidad, Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Circuito Exterior S.N. Ciudad Universitaria, P.O. Box 70-360, Coyoacán, C.P. 04510 Ciudad de México, Mexico*

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ABSTRACT

Melatonin and three of its metabolites [cyclic 3-hydroxymelatonin (3OHM), $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK) and $N^1$-acetyl-5-methoxykynuramine (AMK)] are investigated to analyze their antioxidant capacity. The sequential scavenging action of melatonin and these metabolites has been named an antioxidant cascade, which greatly increases capacity of melatonin to scavenge free radicals and, therefore, decrease oxidative damage. In this theoretical study, we identified a new antioxidant cascade for melatonin and its metabolites by studying different antioxidant mechanisms: hydrogen atom transfer (HAT), radical adducts formation (RAF) and single electron transfer (SET). We demonstrate that melatonin and its metabolites 3OHM, AFMK and AMK are capable of scavenging free radicals through successive dehydrogenations, formation of stable radical adducts and by increasing electron donor-acceptor properties. The main conclusion in this report is that melatonin and its metabolites participate in a new antioxidant cascade and therefore scavenge many free radicals. These are new findings and help to explain the impressive ability of melatonin to reduce oxidative stress.



## 1. Introduction

Free radicals are highly reactive chemical species with one or more unpaired electrons [1]. They are produced in all aerobic organisms and are also a product of certain anthropogenic activities [2,3]. Free radicals react with biomolecules and produce oxidative stress [4]. Oxidative stress is harmful to all molecules [5] and contributes to numerous health disorders, such as Parkinson's and Alzheimer's diseases, and also depression and cancer [6–11]. Antioxidants are molecules, capable of scavenging free radicals and limiting oxidative stress [12]. Among the main mechanisms for trapping free radicals [13,14], we have studied the hydrogen atom transfer (HAT), the radical adduct formation (RAF) and the single electron transfer (SET) mechanisms [15]. Melatonin (MEL, *N*-acetyl-5-methoxytryptamine) is a tryptophan derivative secreted by the pineal gland and produced in many other organs [16–18]. MEL is a highly efficient free radical scavenger [19–24]. Its capacity for preventing oxidative stress is widely recognized and it is considered a more potent antioxidant than carotenoids, vitamins E and C [25–36]. Three principal metabolites of MEL include cyclic 3-hydroxymelatonin (3OHM), $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK) and $N^1$-acetyl-5-methoxykynuramine (AMK). These metabolites are generated from MEL, and all of them manifest powerful antioxidant properties [37–58]. The sequential scavenging by MEL and its metabolites is referred to as the free radical scavenging cascade and distinguishes MEL from other antioxidant molecules [40]. Additionally, experimental studies indicate that AFMK exhibits a considerably lower reactivity compared to MEL and other metabolites [52–54]. Fig. 1 illustrates the chemical structures of MEL, 3OHM, AFMK and AMK.

It was reported before that HAT is a major mechanism for scavenging free radicals by MEL, 3OHM, AFMK, and AMK [19,39,49]. As these molecules posses several H atoms, the possibility of a single dehydrogenated molecule losing a second H atom and thus deactivating another free radical becomes relevant. Therefore, one of the purposes of this work is to investigate successive dehydrogenations, which may enable MEL and its derivatives the scavenging of several free radicals. Successive dehydrogenations represent another type of antioxidant cascade. Herein, we investigate successive dehydrogenation reactions for MEL and its metabolites. In Fig. 2, we present a schematic representation for successive dehydrogenations of ANTI molecules (MEL, 3OHM, AFMK and AMK) and the HAT mechanism with the ·OOH free radical. This is the first stage in this new antioxidant cascade.

The RAF mechanism is not thermodynamically feasible for MEL and its metabolites [19,39,49]. However, the reactivity of

* Corresponding author.
*E-mail address:* martina@unam.mx (A. Martínez).

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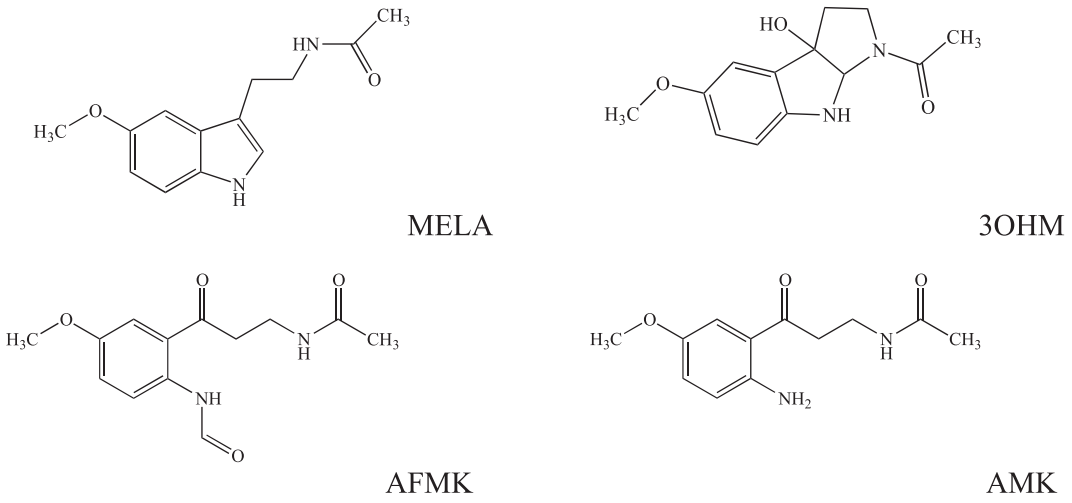


**Fig. 1.** Schematic representation of melanin (MEL), cyclic 3-hydroxymelatonin (3OHM), $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK) and $N^1$-acetyl-5-methoxykynuramine (AMK).

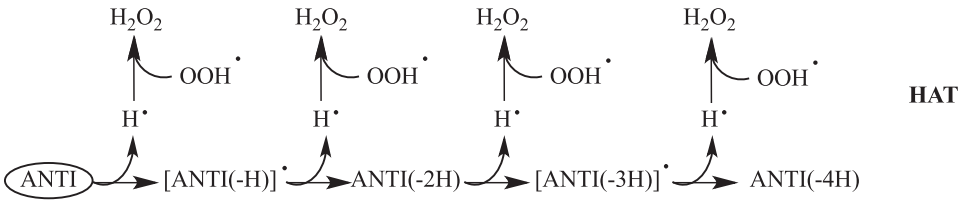


**Fig. 2.** Successive dehydrogenation reactions and schematic representation of HAT mechanism. ANTI(−xH) (x = 1–4) represents the dehydrogenated ANTI.

dehydrogenated molecules is unknown. In this investigation, we studied the RAF of dehydrogenated molecules to facilitate analysis of their antioxidant properties. This is the second stage for the antioxidant cascade shown in Fig. 3, where we report the schematic representation for RAF reactions with the ·OOH free radical, in the case of dehydrogenated molecules.

Dehydrogenated and radical adduct molecules may also stabilize free radicals by the SET mechanism. Fig. 4 presents the complete new antioxidant cascade, which is summarized in the current report. $[ANTI]^{+/-\cdot}$, $[ANTI(-xH)]^{+/-\cdot}$ and $[ANTI(-xH)\text{-}OOH]^{+/-}$ ($x = 1–4$) represent ANTI, ANTI(−xH) and [ANTI(−xH)-OOH] molecules that have either donated or accepted an electron with which to scavenge a free radical. We thus propose this new antioxidant cascade, where MEL and its metabolites participate in several antioxidant processes and scavenge many free radicals.

In our study of the antioxidant cascade, we have theoretically investigated the capacity of MEL and its metabolites to scavenge ·OOH, following the scheme presented in Fig. 4. Subsequently, it becomes clear that MEL and its derivatives may constitute a new effective antioxidant cascade. These results may be useful for future experimental studies and help explain the impressive ability of MEL to reduce oxidative stress.

## 2. Computational details

Electronic calculations were performed with Gaussian09 code [59]. All geometry optimizations were carried out with M06 functional and 6-31+G(d,p) basis set [60–64], in conjunction with the continuum solvation model density (SMD) using water to mimic a polar environment [65]. This methodology has been previously employed for this type of system and has been proven to be adequate [66–68]. Harmonic analyses were calculated to verify local minima (zero imaginary frequencies). The free radical scavenger properties were studied by analyzing the hydrogen atom transfer (HAT), the radical adduct formation (RAF) and the single electron transfer (SET) mechanisms and using the ·OOH molecule. The

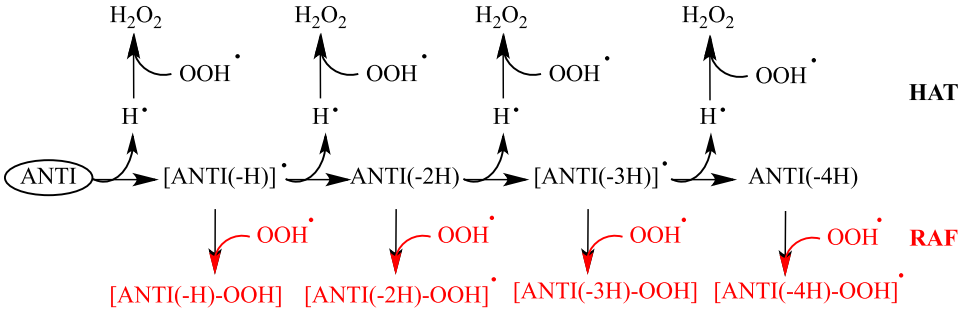


**Fig. 3.** Schematic representation of RAF reactions for dehydrogenated molecules. [ANTI(−xH)-OOH] (x = 1–4) represents the adduct formed by the reaction of ANTI(−xH) and ·OOH.

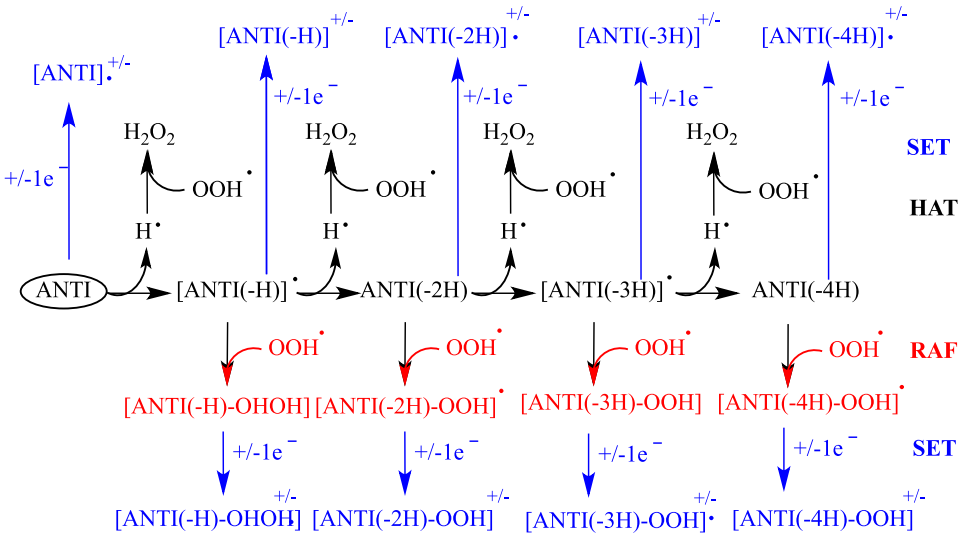


**Fig. 4.** Schematic representation of the new antioxidant cascade.

hydroperoxyl radical (·OOH) has been selected to perform this study since it has an important biological relevance, a moderate reactivity and not too short half-lives. These features are important to measure accurately the antioxidant capacity of a scavenger molecule. [69,70]. Although it has been reported that MEL is not a very efficient ·OOH scavenger [40], we have selected it since most of computational studies make comparisons with this molecule. For the HAT mechanism, we have investigated successive dehydrogenation of four H atoms of each molecule. ΔG was calculated for each dehydrogenation. Gibbs free energies at 298 K were computed according to the next reaction scheme:

$$\mathrm{ANTI} + x\ \cdot\mathrm{OOH} \rightarrow \mathrm{ANTI}(\text{-}x\mathrm{H}) + x\mathrm{H_2O_2} \quad (1)$$

$$\Delta G_{HAT} = [G(\mathrm{ANTI}(\text{-}x\mathrm{H}) + x{*}G(\mathrm{H_2O_2})] - [G(\mathrm{ANTI}) + x{*}G(\cdot\mathrm{OOH})]$$

ANTI represents the molecules under study (MEL, 3OHM, AFMK, AMK) and ANTI(−*x*H) the successive dehydrogenated ANTI systems (*x* = 1–4). We also calculated the Gibbs free energy of the global process. The global reaction can be represented by the next scheme:

$$\mathrm{ANTI} + 4\ \cdot\mathrm{OOH} \rightarrow [\mathrm{ANTI}(\text{-}4\mathrm{H})] + 4\ \mathrm{H_2O_2} \quad (2)$$

$$\Delta G_{Total} = [G[(\mathrm{ANTI}(\text{-}4\mathrm{H})] + 4{*}\ G(\mathrm{H_2O_2})] - [G(\mathrm{ANTI}) + 4{*}\ G(\cdot\ \mathrm{OOH})]$$

The RAF mechanism was studied for dehydrogenated systems according to the following reaction scheme:

$$[\mathrm{ANTI}(\text{-}x\mathrm{H})] + \cdot\ \mathrm{OOH} \rightarrow [\mathrm{ANTI}(\text{-}x\mathrm{H})\ \text{-}\ \mathrm{OOH}] \quad (3)$$

$$\Delta G_{RAF} = G[\mathrm{ANTI}(\text{-}x\mathrm{H})\ \text{-}\ \mathrm{OOH}] - [G[\mathrm{ANTI}(\text{-}x\mathrm{H})] + G(\cdot\mathrm{OOH})]$$

[ANTI(−*x*H)] represents dehydrogenated systems (*x* = 1–4) and [ANTI(−*x*H)-OOH], the radical adduct. To investigate the SET mechanism, vertical ionization energy (*I*) and vertical electron affinity (*A*) were obtained from single point calculations of the corresponding cationic and anionic molecules, using the optimized structure of the neutrals with the improved 6-311+G(d,p) basis set. The Full Electron Donor-Acceptor Map (FEDAM) is a graphic useful previously defined tool [71,72]. In this map, *I* and *A* (Fig. 5) are plotted and allow us to classify substances as either donors or acceptors of electrons. Electrons are transfer from good donor systems (down to the left of the map) to good electron acceptor systems (up to the right of the map). The SET mechanism was investigated for ANTI molecules, [ANTI(−*x*H)] and [ANTI(*x*H)-OOH] systems (*x* = 1–4), in order to determine if further antirradical processes could continue.

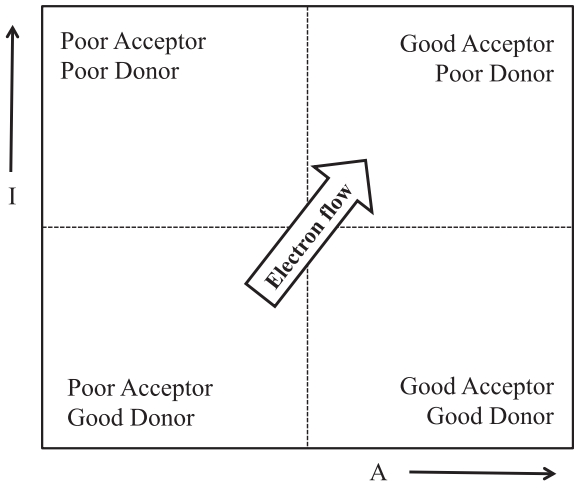


**Fig. 5.** Full Electron Donor-Acceptor Map.

## 3. Results and discussion

### 3.1. Optimization structures

In Fig. 6, we present the optimized geometries of MEL and its metabolites 3OHM, AFMK and AMK. Some representative bond distances (in Å) are also indicated. These structures constitute the reagents in our study. Based on these structures, antioxidant capacity was investigated.

Fig. 7 present the optimized geometries and schematic representations of the most stable dehydrogenated molecules; MEL (−*x*H) and the radical adduct structures; [MEL(−xH)-OOH]. For these optimized structures, we report representative bond distances. The optimized geometries of ANTI(−*x*H) and the radical adduct structures [ANTI(−*x*H)-OOH]; (ANTI: 3OHM, AFMK and AMK) can be found in Figs. S1–S3 of the Supplementary Material

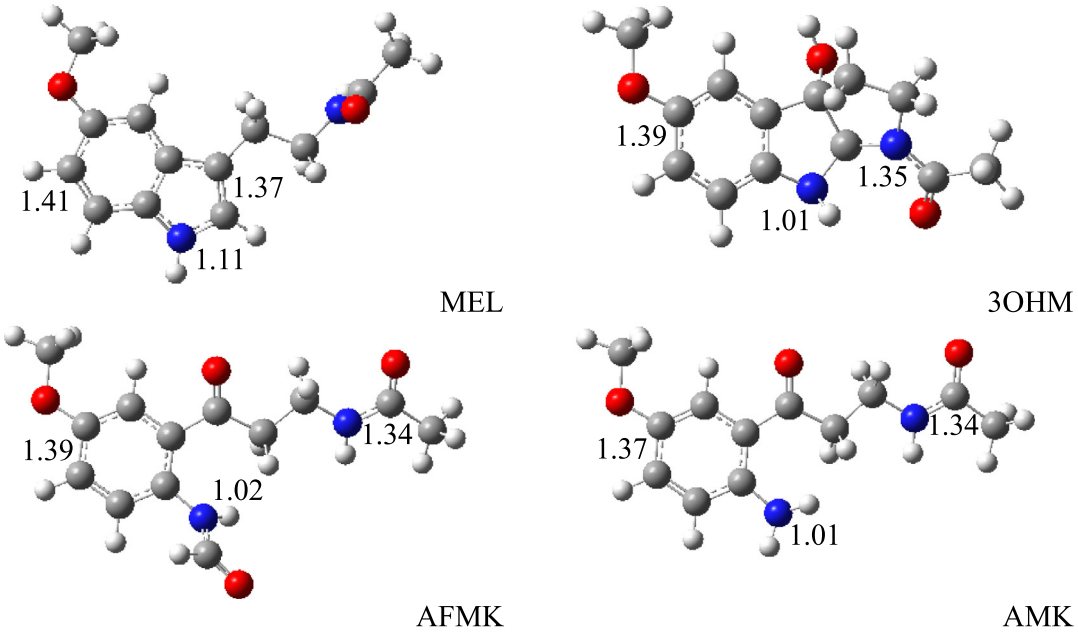


**Fig. 6.** Optimized structures of compounds under study (MEL, 3OHM, AFMK and AMK) (Bond distances are in Å).

section. The first stage of the antioxidant cascade is the successive dehydrogenation of the molecules. To investigate the HAT mechanism, all hydrogen atoms were removed one by one. We considered the most stable dehydrogenated molecules; [ANTI(−*x*H)], (*x* = 1–4), in order to analyze the RAF mechanism with ·OOH. The ·OOH free radical was bonded to all C atoms forming double bonds and to the atoms that had lost the H atom, as a result of the HAT mechanism. Only the most stable [ANTI(−*x*H)-OOH] structures are considered in what follows. The reaction of dehydrogenated molecules with ·OOH is the second stage of the antioxidant cascade presented in Fig. 3. [MEL(−3H)-OOH] and [MEL(−4H)-OOH]· optimized structures present intramolecular hydrogen bonds with the ·OOH free radical. These interactions were reported as important for stabilizing systems for the HAT mechanism [73,74]. Intramolecular hydrogen bond distances are indicated in Fig. 7. The other optimized structures do not exhibit these interactions, as the H atom of ·OOH is not bonded to N or O atoms of molecules.

### 3.2. HAT mechanism

Table 1 presents Gibbs free energies for the HAT study of the most stable structures. Negative ΔG values imply that reactions are exergonic and ·OOH is scavenged. For MEL, all successive dehydrogenated reactions are thermodynamically feasible and MEL (−2H) is the most stable molecule. For 3OHM, three reactions are exergonic. The only endergonic reaction corresponds to the third dehydrogenated molecule, [3OHM(−3H)]·. Conversely, 3OHM (−4H) is the most stable molecule. For AFMK, HAT analysis shows that all studied reactions are exergonic and AFMK(−2H) is the most stable dehydrogenated molecule. For AMK, three reactions are exergonic and the reaction to form AMK(−2H) and $H_2O_2$ is thermodynamically preferred. These results indicate that MEL and AFMK are better ·OOH scavengers than 3OHM and AMK, as they present negative $\Delta G_{HAT}$ values in all reactions studied. For all systems analyzed, AFMK(−2H) is the most stable dehydrogenated molecule ($\Delta G_{HAT}$ = −58.5 kcal/mol). According to data from Table 1, $\Delta G_{Total}$ values are negative, indicating that successive dehydrogenated molecules are capable of scavenging at least four ·OOH. In this regard, AFMK is the best antioxidant, as it presents the most negative values, when compared to MEL, 3OHM and AMK. Notably, some reactions are endergonic (ΔG > 0), but the overall reaction is exergonic. These findings are interesting as all molecules that were investigated have the capacity to transfer more than one H atom.

According to these results, AFMK could be a good antioxidant molecule for the second and fourth dehydrogenated compounds. However, it was reported an experimental study indicating that AFMK exhibits a considerably lower experimental reactivity compared to MEL and other metabolites [52]. Authors explained that potent antioxidant effects by AFMK in biological systems cannot be taken as an indicator of chemical properties, since the protection may be mediated by its metabolite AMK, which had turned out to be much more reactive in various oxidation systems [53,54]. The possible reasons are several, for example, their abundance, half-life range of action and specific relativities. Further studies are necessary to determine the antioxidant capacity of this metabolite.

### 3.3. RAF mechanism

In Table 2, we present Gibbs free energies for RAF reactions. $\Delta G_{RAF}$ negative values indicate that the formation of adducts is thermodynamically feasible and ·OOH is bonded to ANTI. Most radical adducts are stable, as reactions are exergonic. The only exception is the reaction to form [AFMK(−2H)-OOH]·, which presents a positive $\Delta G_{RAF}$ value. For the first dehydrogenated molecules, ([ANTI(−H)]·, the best antioxidant is [AFMK(−H)]· as $\Delta G_{RAF}$ value is the most negative, ($\Delta G_{RAF}$ = −43.3 kcal/mol). AMK(−2H) is the best antioxidant for scavenging ·OOH among ANTI(−2H) dehydrogenated molecules and [3OHM(−4H)-OOH]· is the most favored compound compared to [ANTI(−4H)-OOH]· radical adducts. Finally, [MEL(−4H)-OOH]· is the most stable molecule within [ANTI(−4H)-OOH]· systems. These results suggest that it may be possible for the RAF mechanism to occur once the molecules are dehydrogenated.

### 3.4. SET mechanism

The single electron transfer mechanism was analyzed for ANTI, [ANTI(−*x*H)] and for [ANTI(*x*H)-OOH] (*x* = 1–4) molecules. We

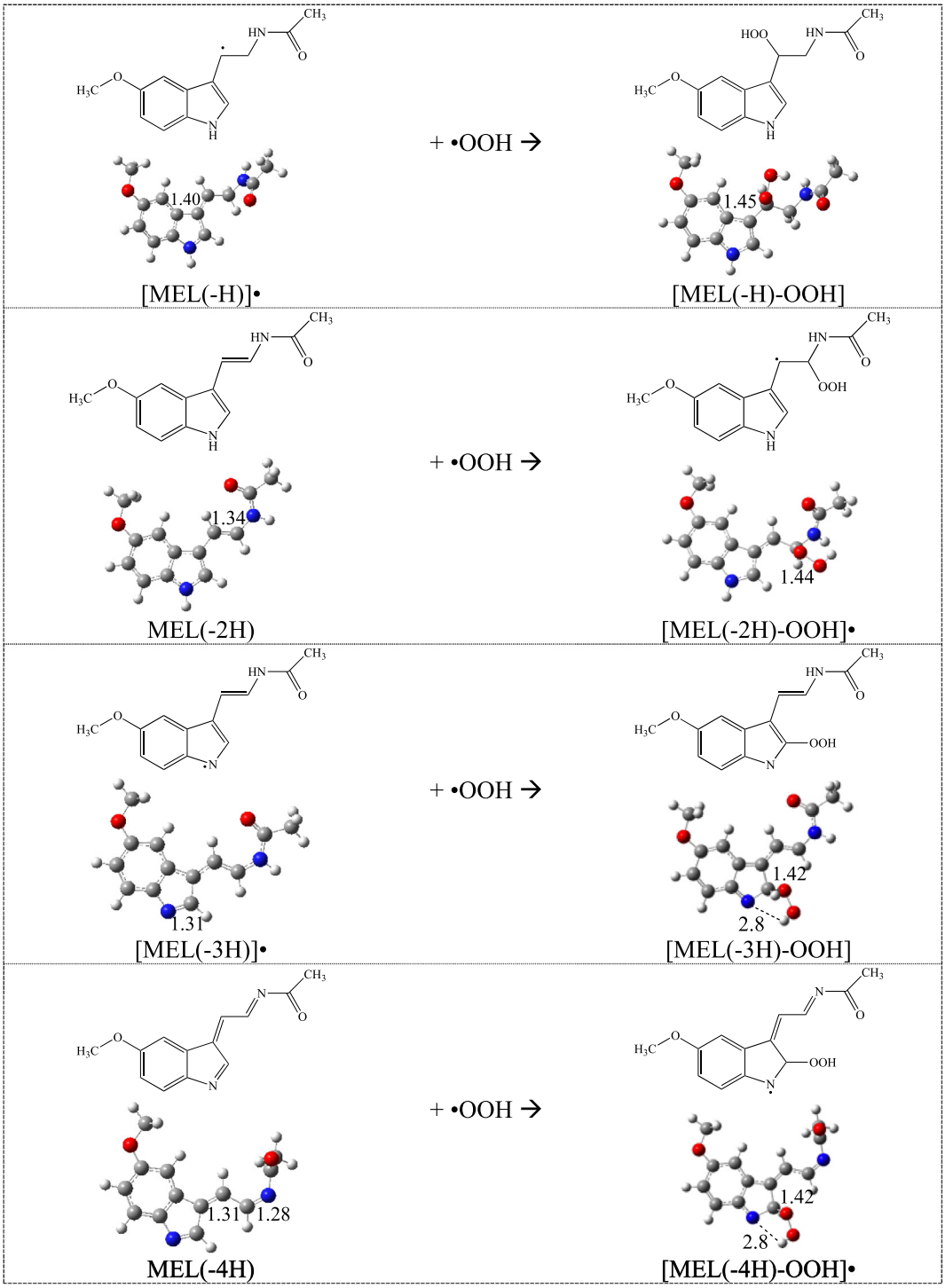


**Fig. 7.** Schematic structures and optimized geometries of MEL and dehydrogenated MEL (Bond distances are given in Å).

**Table 1**
HAT study according to Eqs. (1) and (2).

| H atoms | $\Delta G_{HAT}$ (kcal/mol) | | | |
|---|---|---|---|---|
| | MEL | 3OHM | AFMK | AMK |
| −H | −3.0 | −12.1 | −0.2 | −2.7 |
| −2H | −39.4 | −33.3 | −58.5 | −44.5 |
| −3H | −11.3 | 6.9 | −7.3 | −2.2 |
| −4H | −19.7 | −39.9 | −40.8 | 2.3 |
| $\Delta G_{Total}$ | −73.3 | −78.4 | −106.7 | −47.1 |

investigate their capacity to either donate or accept an electron and induce antioxidant processes. This analysis corresponds to the third stage, which completes the antioxidant cascade (Fig. 4). In Fig. 8, we present the FEDAM for [ANTI(−*x*H)] systems. ANTI molecules and ·OOH are included for purposes of comparison. Due to their location in the FEDAM, all ANTI molecules are able to transfer an electron to ·OOH. ANTI(−*x*H) systems generally have similar *I* values, but larger *A* values than ANTI. Their capacity to donate an electron remains practically the same, but their

**Table 2**
RAF study according to Eq. (3).

| Reaction | $\Delta G_{RAF}$ (kcal/mol) |
|---|---|
| [MEL(−H)]· + ·OOH → [MEL(−H)-OOH] | −41.6 |
| [3OHM(−H)]· + ·OOH → [3OHM(−H)-OOH] | −13.7 |
| [AFMK(−H)]· + ·OOH → [AFMK(−H)-OOH] | −43.3 |
| [AMK(−H)]· + ·OOH → [AMK(−H)-OOH] | −8.1 |
| MEL(−2H) + ·OOH → [MEL(−2H)-OOH]· | −8.5 |
| 3OHM(−2H) + ·OOH → [3OHM(−2H)-OOH]· | −6.1 |
| AFMK(−2H) + ·OOH → [AFMK(−2H)-OOH]· | 10.6 |
| AMK(−2H) + ·OOH → [AMK(−2H)-OOH]· | −46.6 |
| [MEL(−3H)]· + ·OOH → [MEL(−3H)-OOH] | −16.5 |
| [3OHM(−3H)]· + ·OOH → [3OHM(−3H)-OOH] | −52.4 |
| [AFMK(−3H)]· + ·OOH → [AFMK(−3H)-OOH] | −31.6 |
| [AMK(−3H)]· + ·OOH → [AMK(−3H)-OOH] | −14.1 |
| MEL(−4H) + ·OOH → [MEL(−4H)-OOH]· | −10.9 |
| 3OHM(−4H) + ·OOH → [3OHM(−4H)-OOH]· | −0.41 |
| AFMK(−4H) + ·OOH → [AFMK(−4H)-OOH]· | −1.3 |
| AMK(−4H) + ·OOH → [AMK(−4H)-OOH]· | −7.6 |

ability to accept an electron is increased. Except for AMK(−4H), [AFMK(−H)]· and [AFMK(−3H)]·, all dehydrogenated molecules are able to transfer an electron in order to trap ·OOH. Fig. 9 reports the FEDAM for [ANTI(*x*H)-OOH] (*x* = 1–4) molecules. [ANTI(*x*H)-OOH] generally have similar *I*, but slightly larger *A* values than ANTI. These results indicate that [ANTI(*x*H)-OOH] are better electron acceptors than ANTI. All [ANTI(*x*H)-OOH], except [AFMK(−4H)-OOH]· are able to transfer an electron to the ·OOH free radical. These results lead us to conclude that [ANTI(*x*H)-OOH] compounds may be participating in further antioxidant processes. In summary, the new antioxidant cascade is thermodynamically viable for all mechanisms studied. These findings are important, as a single antioxidant would be able to trap many ·OOH molecules.

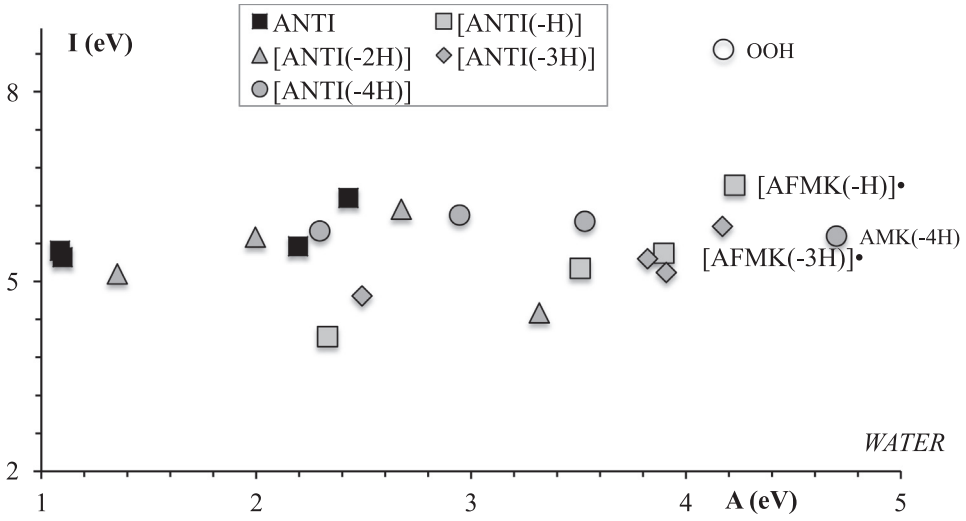


**Fig. 8.** FEDAM for the most stable ANTI(−*x*H) structures, (*x* = 1–4).

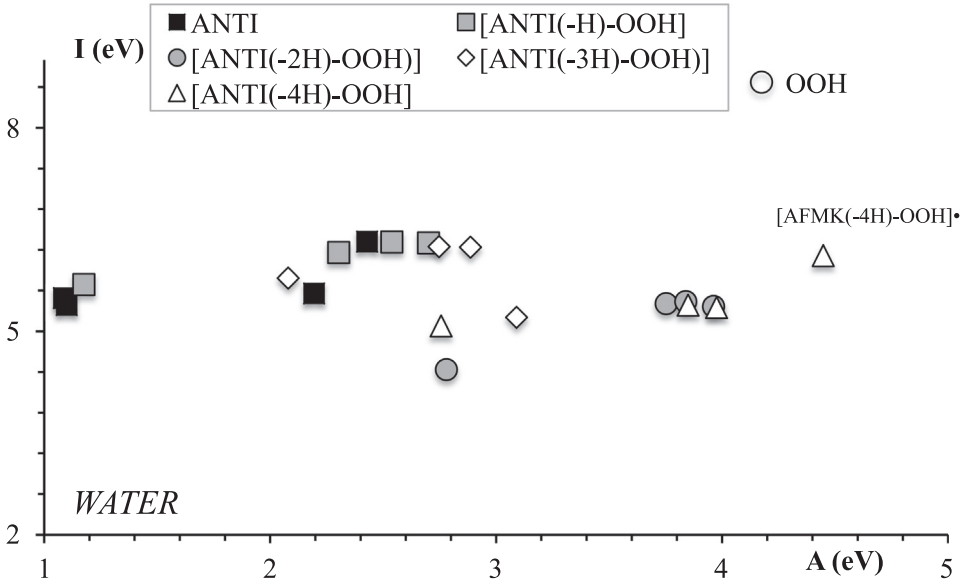


**Fig. 9.** FEDAM for the most stable [ANTI(−*x*H)-OOH] structures, (*x* = 1–4).

## 4. Concluding remarks

MEL and three of its metabolites (3OHM, AFMK and AMK), present in a new antioxidant cascade, are theoretically investigated in terms of their capacity to scavenge multiple free radicals. Results for AFMK should take with caution since they are not in agreement with some experimental investigations. For the HAT mechanism, the conclusion is that all molecules are capable of transferring at least four H atoms. The RAF study reveals that all dehydrogenated molecules efficiently scavenge ·OOH and form stable radical adducts. Some optimized adducts present intramolecular hydrogen bonds. We also studied the capacity of molecules to either donate or accept an electron via the SET mechanism. The results suggest that the electron donor facility is similar for all compounds, but the electron acceptor capacity is greaten for [ANTI(−*x*H)] and [ANTI(*x*H)-OOH], when compared to ANTI. Practically all systems studied have the capacity to transfer an electron to ·OOH. These results imply that MEL and its metabolites (3OHM, AFMK and AMK) participate in a new antioxidant cascade, capable of scavenging multiple free radicals. These results support the versatile and highly effective antioxidant capacity of MEL.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.comptc.2017.11.017.

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