Research Article

A computer-assisted systematic search for melatonin derivatives with high potential as antioxidants

Miguel Reina,[1] Romina Castañeda-Arriaga,[1] Adriana Pérez-González,[2] Eduardo Gabriel Guzmán-López,[1] Dun Xian Tan,[3] Russel J. Reiter,[3] Annia Galano,[1*]

[1] Departamento de Química. Universidad Autónoma Metropolitana-Iztapalapa. San Rafael Atlixco 186, Col. Vicentina. Iztapalapa. C. P. 09340. México D. F. México
[2] CONACYT - Universidad Autónoma Metropolitana - Iztapalapa. San Rafael Atlixco 186, Col. Vicentina. Iztapalapa. C. P. 09340. México D. F. México
[3]Department of Cell Systems and Anatomy, UT Health, San Antonio, Texas, TX 78229, USA
*correspondence: agalano@prodigy.net.mx, agal@xanum.uam.mx; Tel: +52 55 58044600

Running Title: New melatonin-derivatives with antioxidant potential

Received: September 16, 2018; Accepted: October 28, 2018

ABSTRACT

A systematic rational search for newly designed melatonin derivatives, was performed using a computer-assisted protocol. A total of 116 derivatives were generated by adding functional groups (i.e., -OH, -NH_2, -SH and -COOH) to the melatonin structure. A selection score (S^S) was built to sample the search space, simultaneously considering ADME (absorption, distribution, metabolism, excretion) properties, toxicity and manufacturability (i.e., synthetic accessibility). The search characterized the whole set of designed melatonin derivatives and allowed the selection of a reduced subset of 20 melatonin derivatives that are expected to be the most promising, regarding drug-like behavior. For this subset, several reactivity indices were estimated, as well as their pKa values. According to the gathered data, 5 melatonin derivatives have been identified as the most likely candidates to act as chemical antioxidant (directly scavenging free radicals, by electron transfer and/or H transfer). All of them are predicted to be better for that purpose than melatonin itself or trolox (a water-soluble vitamin E analog). The findings from this work are expected to motivate further investigations on these molecules, using both theoretical and experimental approaches.

Keywords: free radical scavenger; computer-aided design; electron transfer; hydrogen transfer; reaction mechanisms; reactivity; ADMET.

1. INTRODUCTION

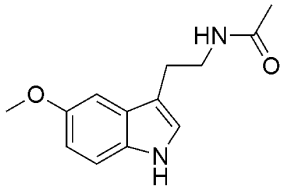
Oxidative stress (OS) and chemical agents against it are currently the focus of numerous investigations. The interest in both, oxidative damage to biomolecules and its prevention by chemical species (usually referred to as antioxidants) is well justified. There is ample evidence that OS compromises human health leading to various diseases such as different kinds of cancers,

cardiovascular and neurodegenerative disorders (1-6). At the same time, there is also abundant data supporting the protective effects of varied chemicals against OS and associated diseases. Although naturally occurring molecules have been the most widely studied in this context, there is an increased interest in designing molecules capable of exerting such protection.

Among natural antioxidants, melatonin (*N*-acetyl-5-methoxytryptamine, Scheme 1) is one of the most comprehensively studied. The role of melatonin and its metabolites for fighting OS has been extensively documented (7-41).Moreover, it has been proposed that one of the main functions of melatonin is to protect living organisms from OS,(42) where it acts as a mitochondria-targeted antioxidant (43). Some of the melatonin's features make this molecule a particularly appealing antioxidant:

- Melatonin is ubiquitous, and has been found not only in the pineal gland but in numerous organs (44-54) as well as in the plant kingdom (47, 55).
- Melatonin can easily cross physiological barriers due to its amphiphilicity and medium size (56-60).
- Melatonin has very low toxicity. Melatonin intake doses up to 1g daily have been proven to be safe (61-66).
- Melatonin's protection against OS is a continuous process that is not diminished by its metabolism since many of its metabolites are also antioxidants (32, 46, 67-69). Moreover, the combined effects of melatonin and its metabolites are expected to deactivate several equivalents of oxidants (70, 71).
- Melatonin and its metabolites are versatile antioxidants, acting as free radical scavengers and metal chelators, mediating enzymatic protection and boosting the DNA repair machinery (45, 71-75).

These features are in line with those described as required for ideal antioxidants (76). Therefore, it is only logical that, inspired for the appealing features of melatonin, the design and synthesis of melatonin derivatives is an emerging research area (68, 77-87). There is already evidence that some of these new compounds can be efficient antioxidants (68, 77-86). However, to the best of our knowledge there are no previous systematic searches for designing antioxidants derived from melatonin's framework.



Scheme 1. Melatonin structure

A rational way to perform such a systematic search is starting by using computer-assisted protocols, which significantly reduce costs and expedite the process. Thus, that is the strategy followed here. The search mainly consisted on modifying the melatonin structure through the inclusion of different functional groups, specially chosen to potentiate versatile antioxidant activity, into all the available positions of the indole ring. Then using absorption, distribution, metabolism, excretion and toxicity (ADMET) properties a subset of the most promising candidates was chosen. For that reduced space, several reactivity indices were estimated using electronic structure calculations within the framework of the Density Functional Theory (DFT). They were used to select a few molecules, among the generated pool, which are novel molecules proposed for the first time as melatonin derivatives with high probabilities of being excellent multipurpose

antioxidants. The presented results are expected to motivate further investigations on these molecules, using both theoretical and experimental approaches.

2. MATERIALS AND METHODS

2.1. Physicochemical parameters

Several physicochemical parameters that are considered relevant for absorption, distribution, metabolism and excretion (ADME) properties were estimated for all the designed melatonin derivatives, using Molinspiration Property Calculation Service (www.munilnsiration.com) and DruLiTo software (http://www.niper.gov.in/pi_dev_tools/DruLiToWeb/DurLiTo_index.html). The estimated parameters are those necessary to investigate if the designed melatonin derivatives satisfy Lipinski's rule of five (88), Ghose's rules (89) and the Veber criteria (90). According to the Lipinski's rule, orally active drugs should have no more than 5 hydrogen bond donors (HB^D), no more than 10 (5x2) hydrogen bond acceptors (HB^A), a molecular weight (MW) under 500 (5x100) g/mol, and an octanol/water partition coefficient (logP) lower than 5. Compounds violating more than one of these rules may have difficulties with bioavailability. According to Ghose's rules, for preventing orally active drugs from having low permeation or absorption issues, they must have logP values ranging from -0.4 to 5.6, molar refractivity (^MR) from 40 to 130, MW from 160 to 480, and a number of non-hydrogen atoms (^XAt) from 20 to 70. On the other hand, according to the Veber criteria, chemicals with 10 or fewer rotatable bonds (RB) and a polar surface area (PSA) ≤ 140 $Å^2$ (or 12 or fewer H-bond donors and acceptors) would have better chances of good oral bioavailability.

At this point it seems worthwhile to emphasize that all these criteria are empirical and intended to be general guidelines, not strict regulations. In fact, viable drugs must also fulfil other important requirements, including manufacturability and safety (91). Therefore, these features were also investigated here.

2.2. Toxicity

Two toxicity descriptors were used in this work, namely:

- LD_{50}: amount of the investigated chemical per body weight (mg/kg) leading to 50% death of rats, after oral ingestion.
- M: usually referred to as Ames mutagenicity. A chemical is positive if it increases (in a reproducible, dose-related manner) the number of revertant colonies in one or more strains of *Salmonella*.

To calculate both indices, the Toxicity Estimation Software Tool (T.E.S.T.), version 4.1, was used. This software makes predictions based on quantitative structure activity relationships (QSAR), which are intended for screening untested compounds. The LD_{50} and M descriptors were computed with the consensus method, which makes predictions as the average of the toxicities predicted from several QSAR methodologies, considering the applicability domain of each of them (92). There is a general agreement that the consensus method usually provides higher accuracy and coverage than other methodologies.

2.3. Synthetic accessibility

The synthetic accessibility (SA) of the designed compounds was estimated with the SYLVIA-XT 1.4 program (Molecular Networks, Erlangen, Germany) (93). It uses several contributing criteria, including the similarity to commercially available compounds, the complexity of the molecular structure and of ring systems, and the number of stereo centers. These criteria are scaled and weighted to provide a value between 1 and 10. The larger the value, the more difficult to synthesize is the compound. The SYLVIA program has been validated for ranking virtual compounds during drug discovery processes (94).

2.4. Electronic calculations

In addition to the physicochemical parameters, toxicity and synthetic accessibility, there are specific reactivity indices that are expected to indicate antioxidant behavior. To estimate them, electronic calculations are necessary. Such calculations were all performed with Gaussian 09 package of programs (95). Local minima were identified by the absence of imaginary frequencies. Unrestricted calculations were used for open shell systems. Geometry optimizations and frequency calculations were carried out using the Density Functional Theory (DFT), in particular the M05-2X functional (96) in conjunction with 6-311+G(d,p) basis set and the solvation model density (SMD) (97) using water to mimic a polar environment. M05-2X is a global hybrid exchange-correlation GGA functional designed for thermochemistry, kinetics and noncovalent interactions (96), it has also been recommended for calculating reaction energies involving free radicals (98). Furthermore, the M05-2X functional has been widely used for estimating the pKa values, the bonding dissociation energies and, in general, the free radical scavenging activity of several antioxidant molecules (99-111). SMD is considered a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known (97). In all cases, the absence of imaginary frequencies was identified to assure that structures found were local minima.

2.5. Reactivity indices

Several global reactivity indices were estimated to analyze the chemical behavior of the designed melatonin derivatives (Table 1). IE and EA values were calculated in the framework of the electron propagator theory (EPT) (112, 113), because this approach usually produces values closer to those derived from experiments than other strategies. In particular, the partial third-order quasiparticle theory (P3) (114) was chosen because it has lower mean errors than other methods (115).

Table 1. Reactivity indices, their acronyms, calculation method and interpretation

	Acronym	Calculation*	Interpretation
First (vertical) ionization energy	IE	P3, EPT	Directly related to the capability of donating one electron. The lower the IE the most likely the antioxidant protection, via electron transfer.
First (vertical) electron affinity	EA	P3, EPT	Directly related to the capability of accepting one electron. The higher the

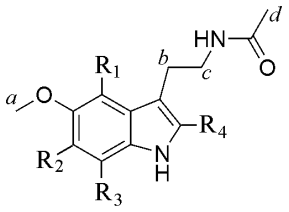
			EA the more likely the antioxidant protection, by converting $O_2^{\bullet -}$ into $^3O_2$, via electron transfer.
Electrophilicity	ω	$\frac{(IE+EA)^2}{8(IE-EA)}$	In a chemical reaction involving two molecules, that with the higher ω is expected to act as the electrophile, while the other will behave as the nucleophile (116, 117).
Electrodonating power	ω^-	$\frac{(3IE+EA)^2}{16(IE-EA)}$	Measures the capability of a chemical system to donate a fractional amount of charge. The lower the ω^- the more likely the molecule would act as an electron donor during weak interactions with other species (118, 119).
Electroaccepting power	ω^+	$\frac{(IE+3EA)^2}{16(IE-EA)}$	Measures the capability of a chemical system to accept a fractional amount of charge. The higher the ω^+ the more likely the molecule would act as an electron acceptor during weak interactions with other species (118, 119).
Chemical potential	μ	$-\left(\frac{IE+EA}{2}\right)$	Electrons will flow from regions of high μ to regions of low μ. The number of electrons that flow would be proportional to differences in μ, while the associated stabilization energy would be proportional to its μ^2.
Chemical hardness	η	$\frac{IE-EA}{2}$	Measures the resistance to change in electron number, or to deformation of the electron cloud. It rules the Pearson's hard and soft acids and bases and maximum hardness principles (120, 121).
Bond dissociation energies	BDE	$E(D)+E(H)-E(DH)$	Measures the energy necessary for breaking donor(D)-H bonds. The lower the BDE, the higher the antioxidant activity, via H transfer.

*The expressions for ω, μ and η correspond to the commonly used finite difference approximation.

Since many of other calculated reactivity indices are estimated from IE and EA values, for reliability purposes it is important that these two magnitudes are as accurate as possible. However,

it is important to keep in mind that for the EPT approximations (including P3) to be valid, the values of the pole strength (PS) should be larger than 0.80-0.85 (122, 123). This has been confirmed to be the case for all the calculations performed in this work (Table S1).

In the BDE case, all sites that are likely to act as H donors were taken into account (Scheme 2). They correspond to those already present in the melatonin's framework (*a* to *d*), and also the new possibilities arising from incorporating functional groups (-OH, -NH_2, -SH and -COOH) in sites R_1 to R_4.

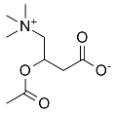
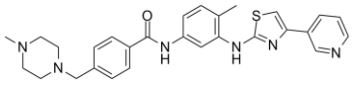
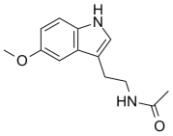
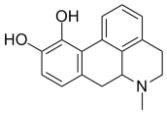
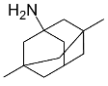
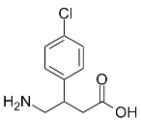
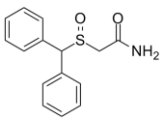


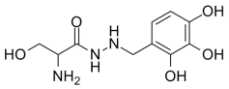
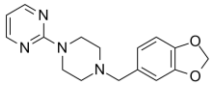
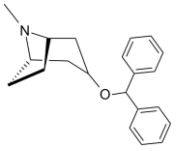
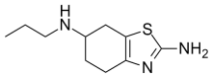
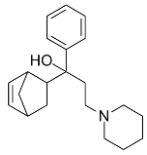
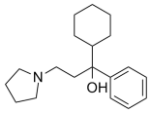
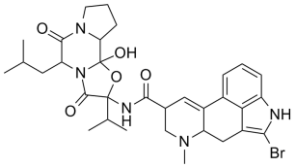
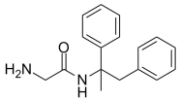
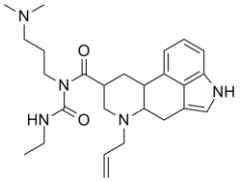
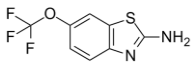
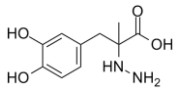
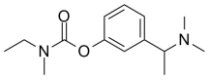
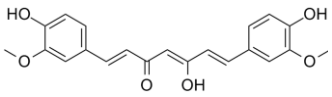
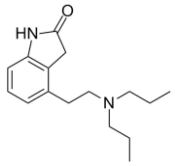
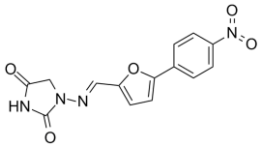
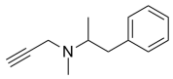
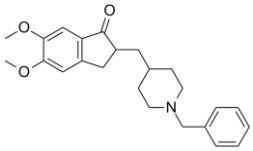
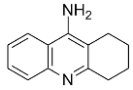
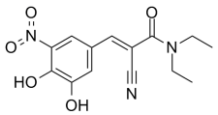
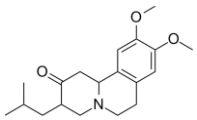
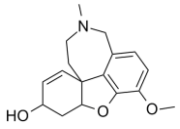
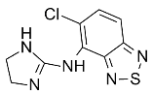
Scheme 2. Sites considered in the BDE calculations

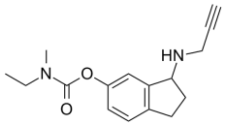
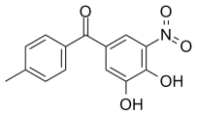
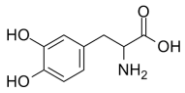
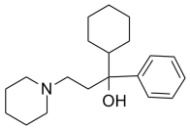
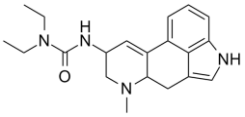
2.6. Reference set

To put into perspective the data obtained for the newly designed melatonin derivatives, a reference set of molecules was used. It consists of 35 chemicals already clinically used as neuroprotectors. Their names and structures are shown in Table 2, while their properties are provided as Supporting Information (Tables S2 and S3). Neuroprotectors were used to construct this set because, as previously mentioned, neurodegenerative disorders are among the diseases that are attributed (at least partially) to oxidative stress. Thus, efficient antioxidants might also play a protective role in this context. However, it should be noted that the ways of action, identified so far, for the neuroprotectors in Table 2 do not necessary involves antioxidant protection.

Table 2. Reference set of molecules, used to compare the estimated properties of melatonin's derivatives

Compound (CAS)	Structure	Ref.*	Compound		Ref.*
Acetylcarnitine (3040-38-8)		(124, 125)	Masitinib (790299-79-5)		(126, 127)
Amantadine (768-94-5)		(128-130)	Melatonin (73-31-4)		(131, 132)
Apomorphine (58-00-4)		(133, 134)	Memantine (19982-08-2)		(135)
Baclofen (1134-47-0)		(130, 136, 137)	Modafinil (68693-11-8)		(138, 139)

Benserazide (14919-77-8)		(140, 141)	Piribedil (3605-01-4)		(129, 142)
Benztropine (86-13-5)		(143, 144)	Pramipexole (104632-26-0)		(129, 140, 145)
Biperiden (514-65-8)		(128, 144, 146)	Procyclidine (77-37-2)		(144)
Bromocriptine (25614-03-3)		(129, 147)	Remacemide (128298-28-2)		(148)
Cabergoline (81409-90-7)		(129, 149, 150)	Riluzole (1744-22-5)		(151)
Carbidopa (28860-95-9)		(152, 153)	Rivastigmine (123441-03-2)		(142, 154)
Curcumin (458-37-7)		(155, 156)	Ropinirole (91374-21-9)		(129, 140, 145)
Dantrolene (7261-97-4)		(157-159)	Selegiline (14611-51-9)		(128, 129, 145, 147, 160)
Donepezil (120014-06-4)		(142, 154, 161)	Tacrine (321-64-2)		(162, 163)
Entacapone (130929-57-6)		(140, 152, 164, 165)	Tetrabenazine (58-46-8)		(130, 166)
Galantamine (357-70-0)		(154, 167-169)	Tizanidine (51322-75-9)		(170)

Ladostigil (209349-27-4)		(171, 172)	Tolcapone (134308-13-7)		(140)
L-DOPA (59-92-7)		(140, 141, 147, 149, 152, 153)	Trihexyphenidyl (144-11-6)		(130, 144, 145)
Lisuride (18016-80-3)		(173, 174)			

*The references correspond to reports of their use as neuroprotectors.

2.6. pKa calculations

Acid constants, expressed as pKa, were calculated for the subset of the most promising melatonin derivatives. This is an important feature of molecules intended to be used as the medicinal drugs, since drug absorption across the gastrointestinal lining mainly takes place via passive diffusion (175). This is ruled by passing across lipid membranes; thus, neutral species are more likely to do so than charged ones. The proportion of neutral vs. charged species for molecules with acid-base bahaviour is ruled by the pKa-pH relationship. It is evident that for newly designed chemicals, pKa values have not been experimentally estimated. Fortunately, theoretical predictions can be used instead. Although this is usually a very challenging task, there is at least one approach that is easy to use and reliable enough. It is usually referred to as the fitted parameters approach (FPA). It involves using experimental pK_a values of a set of small reference molecules to obtain two parameters (m and C_0) from linear fittings:

$$pKa_{\text{exp}} = m\Delta G_{BA} + C_0$$

In this equation ΔG_{BA} represents the difference in Gibbs energy between the conjugated base and the corresponding acid ($G_{\text{calc(A}-\text{)}} - G_{\text{calc(HA)}}$). The m and C_0 parameters are currently available at numerous levels of theory, for phenols, amines, carboxylic acids and thiols (100, 101), i.e., the functional groups used here to construct melatonin derivatives. The values of m and C_0 at the level of calculation used here are shown in Table 3.

Table 3. Values of the *m* and C_0 parameters, at M05-2X/6-311+G(d,p) level of theory, for different functional groups

Functional group	*m*	C_0	Ref.
Phenol	0.316	-81.497	(101)
Carboxylic acid	0.356	-94.380	(101)
Amine	0.464	-121.000	(101)
Thiol	0.357	-94.639	(100)

It has been demonstrated that for all of them, the pK_a values calculated with the FPA approach deviate from experiments by less 0.5 pK_a units, in terms of mean unsigned errors. Therefore, that is the approach used in this work to estimate the pK_a values of the designed melatonin derivatives.

3. RESULTS

Computer-based design of drug-like molecules is a challenging task. There are at least three important issues that should be properly addressed: (i) building the candidate species; (ii) sampling the search space in an efficient way; and (iii) evaluating their potential for the intended purpose (176). The detailed criteria on each of them are provided next.

3.1. Building melatonin derivatives

The melatonin derivatives designed here were conceived to promote multifunctional antioxidant activity. Antioxidants can be classified as primary (Type I, or chain breaking), secondary (Type II, or preventive) and tertiary antioxidants (Type III, or repairing) (177, 178). Type I are molecules that directly react with free radicals, thus they are usually referred to as free radical scavengers. Type II de-active free radicals otherwise, for example acting as $^{\bullet}$OH-inactivating ligand (OIL) (179, 180) through metal chelation. Type III restore oxidative damaged biomolecules to their original structure (mainly through H or electron transfer). Multifunctional antioxidants are molecules that exhibit more than one of these types of protection.

To take advantage of the desirable features of melatonin as much as possible, the new molecules were built by moderate structural modifications. To that purpose four functional groups (i.e., -OH, -NH_2, -SH and -COOH) were used. They were chosen considering that, because of their chemical nature, they are expected to play, at least, one of the following roles:

- They can influence the acid-base behavior, thus modulating the proportion of neutral species at specific pH values, which is important for drugs passing across lipid barriers via passive diffusion (175).
- They may contribute to increase free radical scavenging activity (i.e., antioxidant activity, types I and III) via H donation, or electron donation.
- They may contribute to increase metal chelating capability (i.e., antioxidant activity type II, OIL behavior).

Although the thiol group might be considered as an unwanted functionality in drug discovery because of its high reactivity (181), it has been included in this investigation. The reason is just that reactivity, since the compounds designed in this work mainly act as chemical (antioxidant) agents. In addition, the thiol functionality is expected to increase metal chelating abilities and has been identified as crucial for the free radical scavenging activity of widely recognized antioxidants such as glutathione.(182, 183)

Placing the above-mentioned functional groups in all the available positions of the indole ring (R_1 to R_4, Scheme 2), 116 melatonin derivatives were constructed (Table S4, Supporting Information). Sixteen of them with only one functional group (all possible species within the used substitution scheme), 96 of them with two functional groups (using any possible combination) and 4 with three functional groups. The latter were built from the most promising bi-functionalized species (details on this are provided in the next section). Melatonin itself was included in the set of molecules for comparison purposes.

3.2. Sampling the search space, using extended ADME properties

As previously mentioned, in addition to ADME (absorption, distribution, metabolism, excretion) properties, toxicity (T) and manufacturability (i.e., synthetic accessibility, SA) are also important features for viable medicinal drugs. Therefore, a selection score (S^S) including all these aspects has been constructed. It was used to characterize the whole set of designed melatonin derivatives (116 species) and choosing a reduced subset of 20 molecules that are expected to be the most promising, regarding drug-like behavior.

The selection score was constructed in such a way that the higher the value of S^S the more likely that a melatonin derivative (dM) has a drug-like behavior:

$$S^S = S^{ADME} + S^T + S^{SA}$$

where

$$S^{ADME} = \frac{S^{logP} + S^{HB^D} + S^{HB^A} + S^{MW} + S^{^M R} + S^{^X A} + S^{RB} + S^{PSA}}{8}$$

$$S^T = \frac{S^{LD_{50}} + S^M}{2}$$

with

$$S^{logP} = \begin{cases} 1, & \text{if } -0.4 \le logP \le 5.0 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{HB^D} = \begin{cases} 1, & \text{if } HB^D \le 5 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{HB^A} = \begin{cases} 1, & \text{if } HB^A \le 10 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{MW} = \begin{cases} 1, & \text{if } 160 \le MW \le 480 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{^M R} = \begin{cases} 1, & \text{if } 40 \le {}^M R \le 130 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{^X A} = \begin{cases} 1, & \text{if } {}^X A \le 70 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{RB} = \begin{cases} 1, & \text{if } RB \le 10 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{PSA} = \begin{cases} 1, & \text{if } PSA \le 140 \\ 0, & \text{otherwise} \end{cases}$$

$$S^{LD_{50}} = 1 + \log\left(\frac{LD_{50}^{dM}}{LD_{50}^{\overline{RefSet}}}\right)$$

$$S^{M} = 1 + \log\left(\frac{M^{\overline{RefSet}}}{M^{dM}}\right)$$

$$S^{SA} = 1 + \log\left(\frac{SA^{\overline{RefSet}}}{SA^{dM}}\right)$$

In the particular case of S^{XA}, only the upper limit of the Ghose's rules was used because many of the currently used drugs have $S^{XA} < 20$. For the reference set used here 16 out of 35, i.e., ~45%, including the parent molecule (melatonin). They are acetylcarnitine (S^{XA}=14), amantadine (S^{XA}=11), baclofen (S^{XA}=14), benserazide (S^{XA}=18), carbidopa (S^{XA}=16), L-DOPA (S^{XA}=14), melatonin (S^{XA}=17), memantine (S^{XA}=13), modafinil (S^{XA}=19), pramipexole (S^{XA}=14), riluzole (S^{XA}=15), rivastigmine (S^{XA}=18), ropinirole (S^{XA}=19), selegiline (S^{XA}=14), tacrine (S^{XA}=15) and tizanidine (S^{XA}=16).

The results for S^S are shown in Fig. 1, and the values of the individual properties used to calculate S^S are reported in Table S5 (Supporting Information). In general, in this figure the molecules with higher S^S values are expected to have lower toxicity, better synthetic accessibility, and better ADME properties. Based on this criterion, 20 melatonin derivatives (Scheme 3) were selected for the next stage of the investigation, i.e., to evaluate their potential as antioxidants based on reactivity indices.

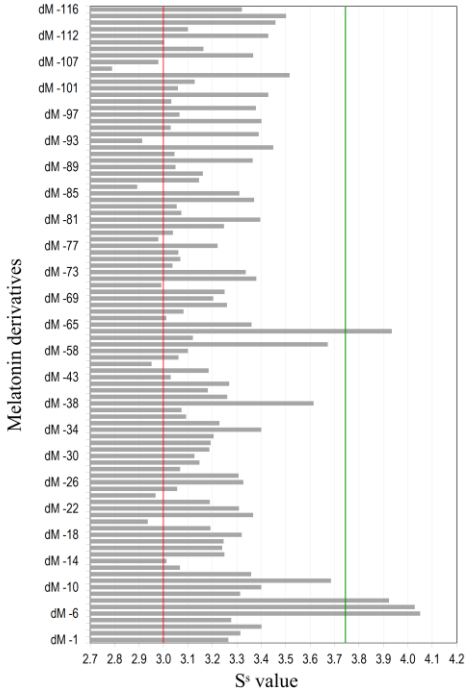
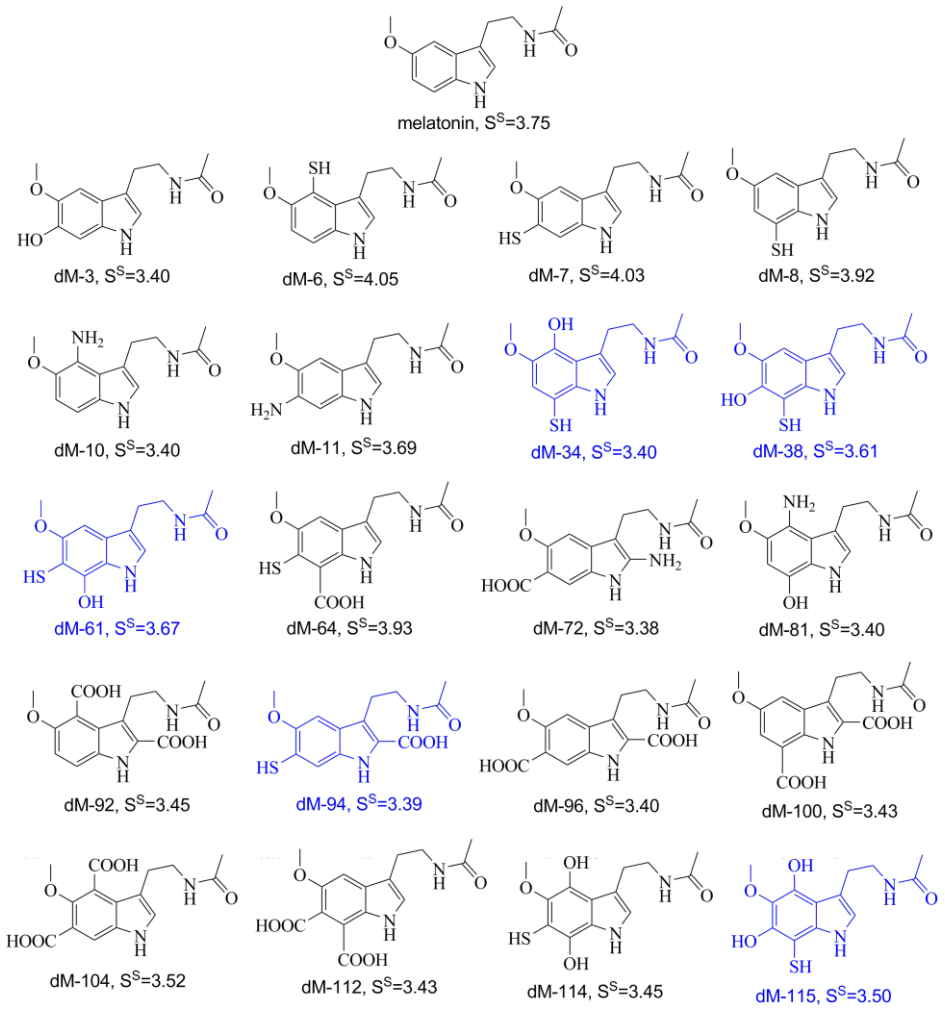


Figure 1. Selection score (S^S) for the melatonin derivatives designed in this work. Vertical lines mark the arithmetic mean of the reference set (red) and the value for the parent molecule (melatonin, green).



Scheme 3. Structure and S^S values of melatonin and the derivatives selected for the next stage of the investigation. The molecules printed in blue are the most likely candidates to act as chemical antioxidants (See section 4.2).

However, the selection score is rather general and might mask a particular case with high S^S but a failure for a particular property. Therefore, in addition to S^S, exclusion scores (S^E) were also used, inspired by previous proposals (184, 185), to double-check if any molecule in the selected subset significantly deviates (in any of its properties) from the average value of the reference set (described in section 2.6). Four exclusion scores were used for that purpose. The first one, here referred to as $S^{E,ADME2}$ is identical to that previously proposed (184, 185):

$$S^{E,ADME2} = \left| \frac{\log P_{\overline{RefSet}} - \log P_{dM}}{SD_{\log P}} \right| + \left| \frac{MW_{\overline{RefSet}} - MW_{dM}}{SD_{MW}} \right|$$

The second one, $S^{E,ADME8}$, uses the same kind of analysis but including 8 terms, one per each ADME property evaluated here:

$$S^{E,ADME8} = S^{E,ADME2} + \left|\frac{PSA_{\overline{RefSet}} - PSA_{dM}}{SD_{PSA}}\right| + \left|\frac{{}^{X}A_{\overline{RefSet}} - {}^{X}A_{dM}}{SD_{{}^{X}A}}\right| + \left|\frac{HB^{A}{}_{\overline{RefSet}} - HB^{A}{}_{dM}}{SD_{HB^{A}}}\right|$$
$$+ \left|\frac{HB^{D}{}_{\overline{RefSet}} - HB^{D}{}_{dM}}{SD_{HB^{D}}}\right| + \left|\frac{RB_{\overline{RefSet}} - RB_{dM}}{SD_{RB}}\right| + \left|\frac{{}^{M}R_{\overline{RefSet}} - {}^{M}R_{dM}}{SD_{{}^{M}R}}\right|$$

The third one, $S^{E,ADMET}$, includes two other terms, related to toxicity; and the fourth ($S^{E,ADMETSA}$) also includes a term for synthetic accessibility:

$$S^{E,ADMET} = S^{E,ADME8} + \left|\frac{LD_{50\,\overline{RefSet}} - LD_{50\,dM}}{SD_{LD_{50}}}\right| + \left|\frac{M_{\overline{RefSet}} - M_{dM}}{SD_{M}}\right|$$

$$S^{E,ADMETSA} = S^{E,ADMET} + \left|\frac{SA_{\overline{RefSet}} - SA_{dM}}{SD_{SA}}\right|$$

These exclusion scores (Fig. 2 and Table S6) measure deviations from the average values of already used oral drugs. $S^{E,ADME2}$ values have been reported to be 1.5 and 1.2, when considering 152 and 1791 oral drugs, respectively (184, 185). For the reference set used here (35 molecules), average $S^{E,ADME2}$ = 1.3, with individual values ranging from 0.15 to 4.49. On the other hand, for the 20 melatonin derivatives with highest selection scores, average $S^{E,ADME2}$ = 0.7 with individual values ranging from 0.4 to 1.1 (Table S6, Supporting Information). Therefore, according to $S^{E,ADME2}$ they seem to be suitable as drug-like molecules.

The $S^{E,ADME2}$ score, however, only accounts for 2 of the analyzed properties and has rather similar values for all the analyzed molecules (Fig. 2). That is the reason the other 3 elimination scores were also implemented. They account for more properties and span the scales. Average $S^{E,ADME8}$ = 4.1, with individual values ranging from 1.4 to 7.2; $S^{E,ADMET}$ = 6.5, with individual values ranging from 2.7 to 14.7; and $S^{E,ADMETSA}$ = 6.8, with individual values ranging from 3.1 to 14.8. It is important to consider, though, that high values of these scores may arise from either worse or better behavior (as oral drug-like species) than the average of the reference drugs.

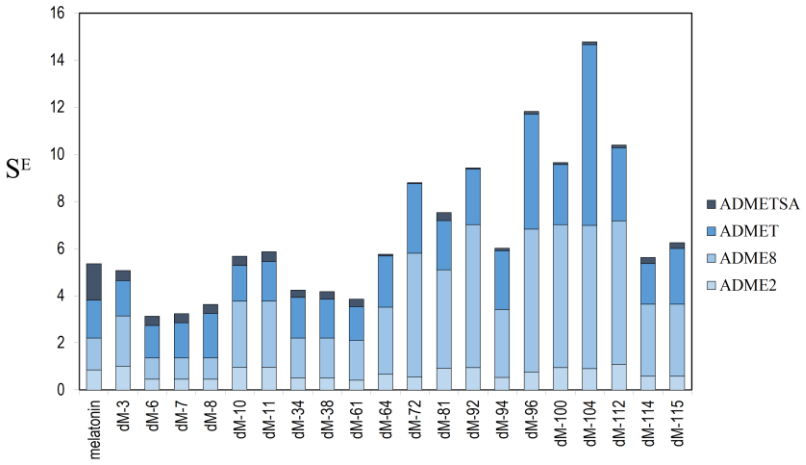


Figure 2. Elimination score (S^{E}) for the most promising melatonin derivatives, according to S^{S}. Columns are divided to show the influence of the new contributions included in each score, with respect to the previous one.

3.3. Evaluating antioxidant likeliness, using reactivity indices

Reactivity indices were estimated for the subset of molecules chosen in the previous section. Since their involvement in acid-base equilibria may be relevant to the target behavior, their *p*Ka values were estimated (Table 4) and the associated deprotonation routes elucidated (Figure S1, Supporting Information). The corresponding distribution diagrams are also provided as Supporting Information (Figure S2), while the molar fractions (^{M}f) of the different acid-base species, at physiological *p*H, are reported in Table 4. The reactivity indices for the acid-base species with non-negligible population ($^{M}f > 0.1\%$), at *p*H=7.4, are reported in Table 5.

Table 4. **Estimated *p*Ka values and molar fractions of the protonated ($^{M}f_{prot}$), neutral ($^{M}f_{neutral}$), anionic ($^{M}f_{anion}$) dianionic ($^{M}f_{dian}$) and trianionic ($^{M}f_{trian}$) species of melatonin and its derivatives, at *p*H=7.4**

	pKa$_1$	pKa$_2$	pKa$_3$	$^{M}f_{prot}$	$^{M}f_{neutral}$	$^{M}f_{anion}$	$^{M}f_{dian}$	$^{M}f_{trian}$
dM-3	10.25	-	-	-	0.999	0.001	-	-
dM-6	7.28	-	-	-	0.431	0.569	-	-
dM-7	7.98	-	-	-	0.792	0.208	-	-
dM-8	5.46	-	-	-	0.011	0.989	-	-
dM-10	6.16	-	-	0.054	0.946	-	-	-
dM-11	5.20	-	-	0.006	0.994	-	-	-
dM-34	5.87	11.47	-	-	0.029	0.971	$<10^{-4}$	-
dM-38	5.90	12.12	-	-	0.031	0.969	$<10^{-4}$	-
dM-61	5.65	13.41	-	-	0.017	0.983	$<10^{-6}$	-
dM-64	3.27	8.46	-	-	$<10^{-4}$	0.920	0.080	-
dM-72	0.92	5.03	-	$<10^{-8}$	0.004	0.996	-	-
dM-81	6.59	10.05	-	0.134	0.864	0.002	-	-
dM-92	3.43	4.34	-	-	$<10^{-7}$	0.001	0.999	-
dM-94	4.03	7.60	-	-	$<10^{-3}$	0.613	0.387	-
dM-96	3.35	4.31	-	-	$<10^{-7}$	$<10^{-3}$	0.999	-
dM-100	3.27	4.01	-	-	$<10^{-7}$	$<10^{-3}$	$\approx$1.000	-
dM-104	3.95	4.65	-	-	$<10^{-6}$	0.002	0.998	-
dM-112	3.02	4.39	-	-	$<10^{-7}$	$<10^{-3}$	0.999	-
dM-114	4.89	11.16			0.003	0.997	$<10^{-3}$	-
dM-115	6.14	10.46	13.41	-	0.052	0.947	0.001	$<10^{-9}$

Table 5. First ionization energy (IE, eV) and electron affinities (EA, eV), electrophilicity (ω), electrodonating (ω^-), electroaccepting (ω^+) powers, chemical potential (μ, eV), chemical hardness (η, eV), and bond dissociation energies (BDE, kcal/mol) for melatonin and the selected subset of derivatives

	IE	EA	ω	ω^-	ω^+	μ	η	BDE
Protonated								
dM-10	11.17	2.09	2.42	8.72	2.09	-6.63	9.08	90.48
dM-11	10.89	2.91	2.98	9.91	3.01	-6.90	7.98	89.45
dM-81	10.84	2.05	2.36	8.49	2.05	-6.44	8.79	80.62
Neutral								
Melatonin	7.49	-0.97	0.63	3.41	0.15	-3.26	8.46	89.33
dM-3	7.19	-0.97	0.59	3.25	0.14	-3.11	8.16	77.76
dM-6	7.09	-0.81	0.62	3.31	0.17	-3.14	7.90	74.33
dM-7	7.11	-0.91	0.60	3.25	0.15	-3.10	8.02	73.24
dM-8	7.23	-0.92	0.61	3.31	0.15	-3.16	8.15	73.46
dM-10	6.44	-0.98	0.50	2.83	0.10	-2.73	7.42	86.75
dM-11	6.81	-1.02	0.53	3.01	0.11	-2.89	7.83	88.18
dM-34	6.92	-0.94	0.57	3.13	0.13	-2.99	7.85	66.68
dM-38	7.17	-0.93	0.60	3.26	0.15	-3.12	8.10	71.51
dM-61	7.26	-0.93	0.61	3.32	0.15	-3.16	8.18	69.14
dM-72	7.12	0.54	1.12	4.56	0.73	-3.83	6.58	89.15
dM-81	6.26	-1.02	0.47	2.71	0.09	-2.62	7.28	63.65
dM-114	6.91	-0.77	0.61	3.24	0.17	-3.07	7.68	68.32
dM-115	7.06	-0.75	0.64	3.34	0.19	-3.16	7.81	67.54
Anionic								
dM-3	2.17	-3.18	0.02	0.13	0.63	0.50	5.35	87.37
dM-6	1.98	-3.19	0.04	0.09	0.70	0.61	5.17	84.91
dM-7	1.72	-2.68	0.03	0.09	0.57	0.48	4.40	87.48
dM-8	2.12	-2.73	0.01	0.17	0.47	0.30	4.85	88.56
dM-34	1.98	-2.90	0.02	0.12	0.58	0.46	4.88	61.88
dM-38	1.95	-2.70	0.02	0.13	0.51	0.38	4.65	68.31
dM-61	1.99	-2.68	0.01	0.14	0.49	0.35	4.67	70.75
dM-64	3.88	-2.56	0.03	0.80	0.14	-0.66	6.44	78.19
dM-72	3.65	-2.46	0.03	0.74	0.14	-0.60	6.11	88.21
dM-81	1.61	-2.90	0.05	0.05	0.70	0.65	4.51	87.86

dM-92	4.51	-3.13	0.03	0.89	0.19	-0.69	7.64	93.98
dM-94	4.23	-3.11	0.02	0.78	0.22	-0.56	7.35	73.87
dM-104	4.46	-2.30	0.09	1.14	0.06	-1.08	6.77	90.17
dM-114	2.08	-2.53	0.01	0.19	0.41	0.22	4.61	69.83
dM-115	1.72	-2.53	0.02	0.10	0.50	0.40	4.25	63.09
Di-anionic								
dM-64	-1.46	-4.11	1.47	1.70	4.49	2.79	2.65	88.35
dM-92	0.96	-5.26	0.37	0.06	2.21	2.15	6.22	88.16
dM-94	-0.54	-4.58	0.81	0.59	3.15	2.56	4.04	87.70
dM-96	1.33	-4.49	0.21	0.003	1.58	1.58	5.82	88.83
dM-100	1.33	-4.50	0.21	0.003	1.59	1.58	5.84	88.81
dM-104	1.03	-4.68	0.29	0.03	1.86	1.83	5.71	85.91
dM-112	0.64	-4.03	0.31	0.06	1.76	1.70	4.66	89.13
dM-115	-1.99	-4.69	2.07	2.63	5.98	3.34	2.70	59.50

4. DISCUSSION

4.1. Elimination scores

Some discussions on the different contributions to the elimination scores seem worthwhile. Figure 2 clearly shows that synthetic accessibility and the two properties included in $S^{E,ADME2}$ (logP and MW) both have rather small (and similar contributions) to the deviations from the reference molecules. On the contrary, the new properties included in $S^{E,ADME8}$ and the toxicity indices have the largest contribution to $S^{E,ADMETSA}$.

To analyze the individual contributions of the different properties to the $S^{E,ADMETSA}$ elimination score, a more detailed plot was constructed (Fig. 3). It was found that the largest deviations from the average value of the reference set of molecules arise from LD_{50}, M, PSA, HB^D and HB^A. Regarding LD_{50}, the derivatives deviating the most from the average (dM-104, dM-96, dM-72, dm-112, dM-96 and dM-100) are less toxic to rats than the reference average (LD_{50} = 960.8), with values 6960.5, 4733.5, 2892.9, 2861.8, 2399.7 and 2303.2, respectively. Thus, these large deviations mean a more desirable behavior than that of the references and, consequently, these derivatives were not excluded from the subset selected as the most promising, based on ADMETSA properties.

A similar trend was found for the Ames mutagenicity, i.e., the compounds predicted as the least mutagenic are just those that deviate the most from the reference set (M = 0.41). They are dM-6, dM-7, dM-8, dM-64, dM-38 and dM-61, all with M ≤ 0.02. Thus, it is important not just to identify the designed compounds with the largest deviation from the reference set, but also what causes such deviations. Otherwise, good candidates might be eliminated for the wrong reasons.

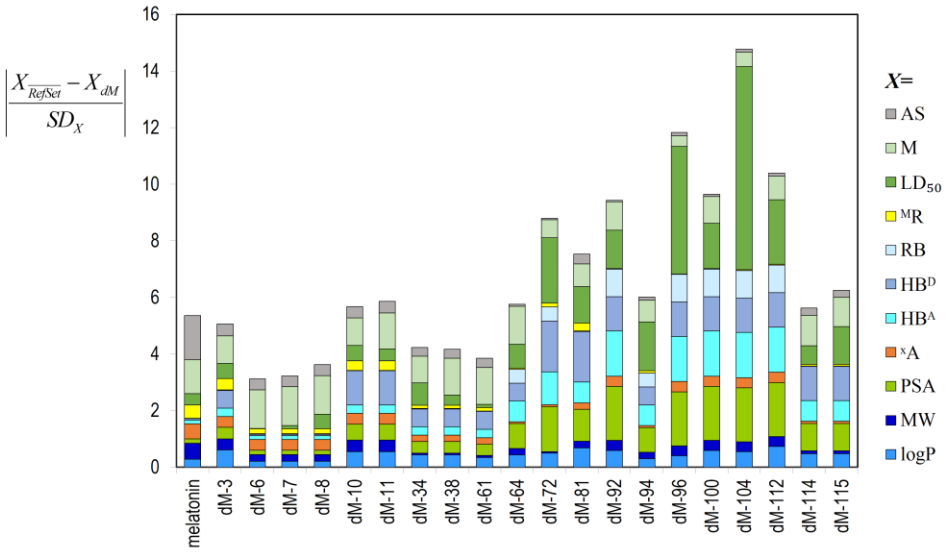


Figure 3. **Individual contributions to the elimination score (S^E), for the most promising melatonin derivatives**.

On the contrary, for the other indices (PSA, HB^D and HB^A) larger S^E values actually mean that the behavior of the investigated derivatives approaches the upper limits of the recommended range for the investigated parameters, although they still fulfil Lipinski's and Ghose's rules, as well as the Veber criteria. Regarding PSA the selected derivatives deviating the most from the reference set are dM-92, dM-96, dM-100, dM-104, dM-112 and dM-72. Their PSA values range from 128.7 to 117.5 (i.e., below the Veber's threshold, 140 $Å^2$). The largest deviations for HB^D correspond to dM-72, dM-81, dM-10, dM-11, dM-92 and dM-96, with HB^D = 5 or 4; and for HB^A correspond to dM-92, dM-96, dM-100, dM-104, dM-112 and dM-72, with HB^D = 8 or 7. Thus, they do not constitute violations of the Lipinski's rule.

Based on what has been discussed in this section, none of the 20 melatonin derivatives identified as the most likely candidates, based on the selection score, was eliminated after further screening using the elimination scores. Accordingly, reactivity indices were estimated and analyzed for all of them.

4.2. Antioxidant-like behavior

The reactivity indices estimated in this work are expected to help predicting antioxidant behavior, via free radical scavenging activity, provided that such activity involves single electron transfer (SET) and/or formal hydrogen atom transfer (HAT) mechanisms. There is graphical tool, known as the full electron donator acceptor map (FEDAM) (186, 187) that allows predicting, quickly and qualitatively, the direction of the electron flow in SET reactions (Fig. 4). It is based on the precept, that in SET reactions between two chemical species, that with the lower IE would be the electron donor, and that with the higher IE would be the electron acceptor.

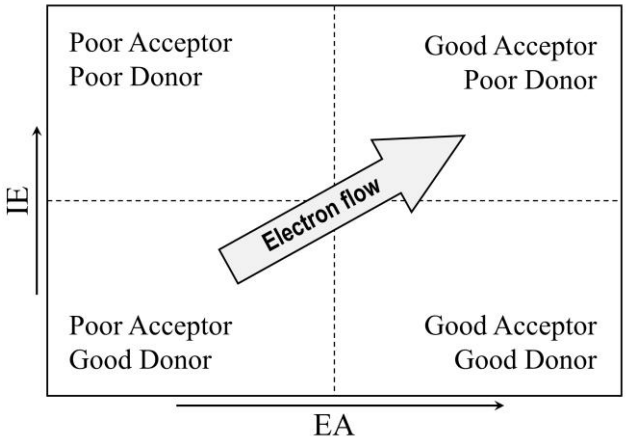


Figure 4. **Schematic representation of the Full Electron Donor-Acceptor Map (FEDAM)**

Thus, the FEDAM tool was used for the subset of melatonin derivatives selected in section 3.2 (Fig 5). Some reactive oxygen species (ROS) were also included in the map to facilitate the analyses, as well as the parent molecule for comparison purposes. The different acid-base species of the newly designed derivatives were explicitly included in Fig 5, since deprotonation is expected to play an important role on SET feasibility. In fact, this is clearly shown in the figure, where the acid-base species are located in a cluster-like way depending on their charge. According to this map, all of them except the protonated ones, are expected to donate one electron to ROS. Thus, the designed melatonin derivatives are predicted to behave as ROS scavengers, at least via SET. It is also interesting to note that some of them are also expected to be slightly more efficient for that purpose than melatonin itself. The trend obtained from the FEDAM is in line with that of the chemical potential (μ, Table 5). This is a logical result, since electrons are expected to flow from regions of high μ to regions of low μ. Moreover μ has a linear dependence with IE (Figure S3, Supporting Information).

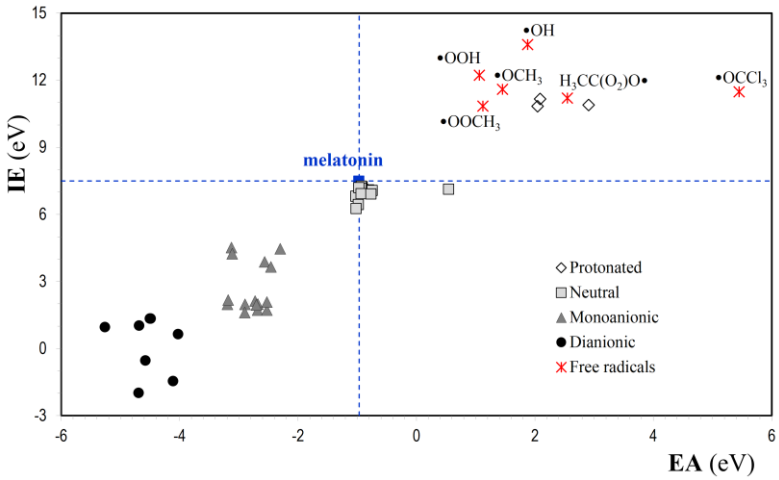


Figure 5. **FEDAM (Full Electron Donor Acceptor Map) for melatonin derivatives**

On the contrary, electrophilicity (ω) and electrodonating power (ω^-) do not have linear dependences with IE (Figure S4, Supporting Information). In fact, for species with very low IE, the ω and ω^- values increase. This behavior resembles to some extent that of the inverted region

of the Marcus parabola, which indicates that for Gibbs (ΔG) energy of reaction much lower than minus the reorganization energy, reaction barriers increase as ΔG becomes more negative (188-190). Albeit this is a counterintuitive behavior, it suggests that species with very low IE are not expected to be very efficient as free radical scavengers acting as electron donors in SET reactions. That would be the case for the dianionic species of the investigated melatonin derivatives. However, such a behavior would need further confirmation.

The acid-base species that, based on ω values, seem to be the most promising for deactivating free radicals via SET, acting as electron donors, are the mono-anions (Figure 6). They have the lowest values of ω for each derivative; and as previously mentioned, in a chemical reaction involving two reactants that with the lower ω is expected to act as the nucleophile (116, 117). The mono-anionic species analyzed in Figure 5, all have lower ω values than any of the investigated free radicals, thus they are expected to be efficient for scavenging free radicals via electron transfer. In addition, all the mono-anions of the subset of melatonin derivatives with better drug-like behavior have similar electrophilicity. Thus, they are probably similarly efficient as free radical scavengers via SET. To tell them apart another criterion is necessary.

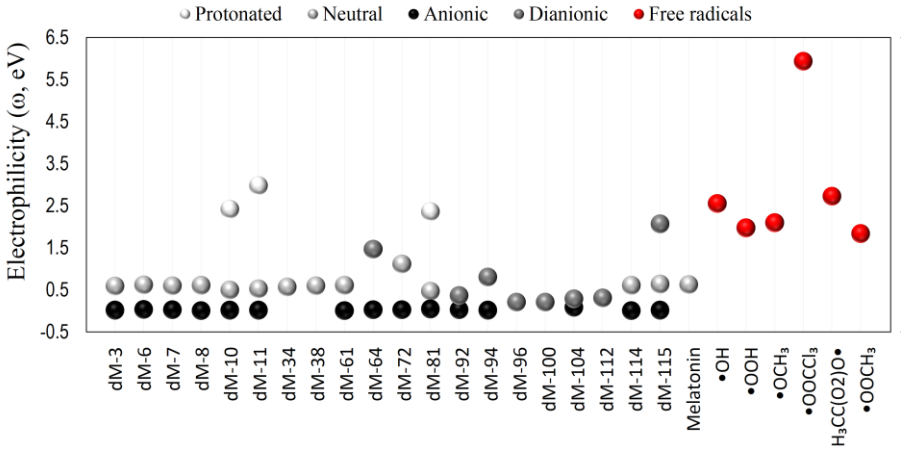


Figure 6. **Electrophilicity of the acid-base species of melatonin derivatives**.

Another graphical tool has been designed, simultaneously accounting for likeliness as electron donors (SET reactions) and H donors (formal HAT reactions). Here it is referred to as the electron and hydrogen donating ability map for antioxidants (eH-DAMA), and simultaneously includes electrodonating power (ω^-, accounting for SET feasibility) and bond dissociation energies (BDE, accounting for HAT feasibility). The BDE values for each species are provided as Supporting Information (Table S7). Figure 7 shows this map for melatonin derivatives, it also includes the parent molecule and trolox for comparison purposes and the $H_2O_2/O_2^{\bullet-}$ pair as the potential oxidant target. The later has been chosen because it is usually harder to scavenge than other reactive oxygen species, and because it has been previously found that melatonin itself is not very efficient for chemically deactivating this radical.(191)

The chemical species with lower ω^- are expected to be particularly efficient for scavenging free radicals acting as electron donors via SET, while the species with lower BDE are expected to be particularly efficient for scavenging free radicals acting as H donors via formal HAT. Therefore, the species located at the bottom and left side of the eH-DAMA are likely to act both ways, i.e., they are particularly interesting as antioxidants. This region has been highlighted in Figure 6, and shows that the species fulfilling both criteria are all mono-anions, including that of trolox.

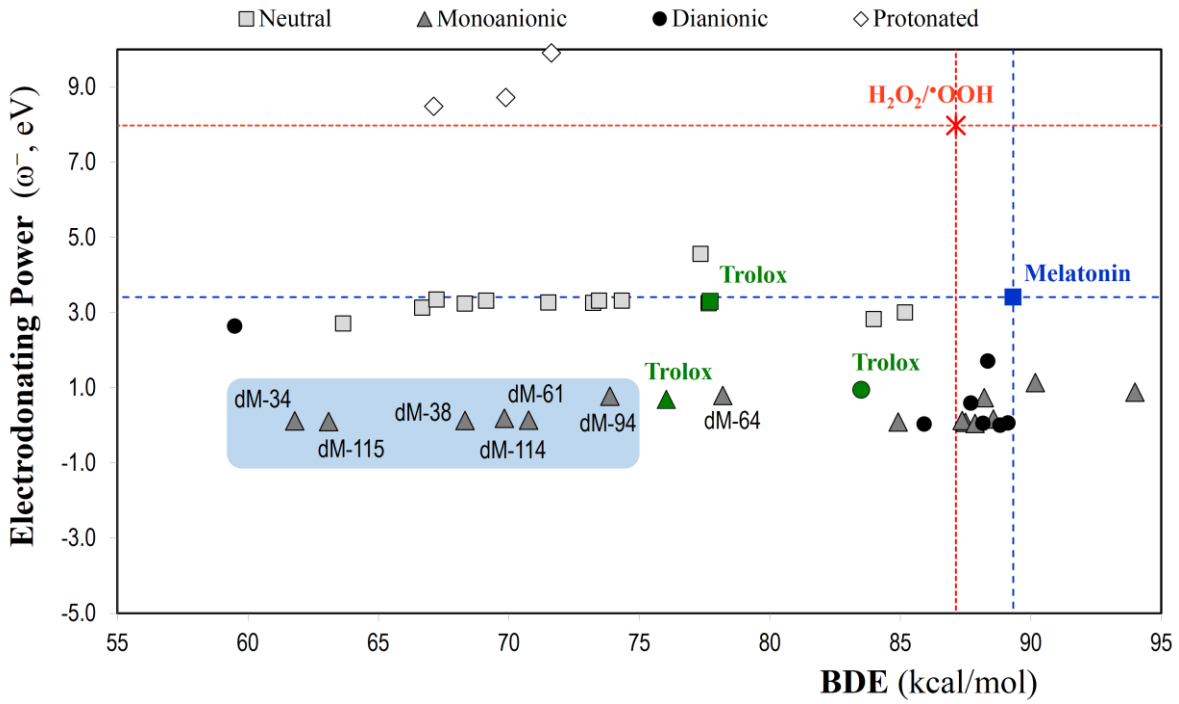


Figure 7. The electron and hydrogen donating ability map for antioxidants (eH-DAMA), including the acid-base species of melatonin derivatives, the parent molecule, trolox and the oxidant the $H_2O_2/O_2^{\bullet-}$ pair

All the species in the target region are predicted to have similar electron-donor capability, but rather different H-donating power. Based on the data summarized in Fig. 7, derivatives dM-34, dM-115, dM-38, dM-114, dM-61 and dM-94 are predicted to be better hydroperoxyl scavengers than trolox, and also than the parent molecule. On the other hand, dM-64 should be better than melatonin for that purpose, but its antioxidant activity is not expected to surpass that of trolox. Since their most active species are expected to be the mono-anions, their molecular fractions are relevant in this context. For all these derivatives, mono-anions are the most abundant acid-base species at physiological pH (Table 4). However, to cross biological barriers it is also important that the molar fractions of the neutral species are not negligible. Most of above mentioned derivatives also fulfill this requirement. The only exception is dM-114. Therefore, the melatonin derivatives proposed as the most promising antioxidants are dM-34, dM-115, dM-38, dM-61 and dM-94 (in that order). Further, more detailed and quantitative, investigations on their antioxidant action are still needed and highly desirable, to confirm or refute the proposal from this work.

4.3. Other considerations

At this point it seems worthwhile to make some comments regarding the limitations of the present study, and the necessity of further investigations on the topic of this investigation using both theoretical and experimental approaches. Antioxidant protection is a complex process that involves different chemical and non-chemical routes, thus there are several aspects on the behavior of the designed compounds, in biological systems that need to be further explored. Some of them are:

1. Quantitative estimations of the kinetics involved in the free radical scavenging activity of the designed compounds.
2. Identification of the primary products yielded from their reactions with free radicals.
3. Investigations on the possible capability of the designed compounds to chelate redox metals and act as OH inactivated ligands.
4. The possibility of pro-oxidant effects.
5. Antioxidant protection arising from melatonergic signaling, including the evaluation of the designed compounds as ligands to melatonin receptors.
6. Enzymatic metabolism of the designed compounds, and characterization of the corresponding metabolites.
7. Experimental assessments of toxicity of both the proposed compounds and their metabolites.
8. The possibility that the melatonin derivatives identified here as the most promising antioxidants may undergo transnitrosation reactions, since they all contain a sulfhydryl group.

As it is evident from these points, it is unfeasible to carry out all the necessary research on the designed compounds in a single investigation. Hopefully, the results from this work are promising enough to motivate further researches on these compounds, and help obtained a more complete picture regarding their possible use as antioxidant agents.

5. CONCLUSIONS

A systematic rational search for newly designed melatonin derivatives, performed using a computer-assisted protocol, is presented. A total of 116 derivatives were generated by adding functional groups (i.e., -OH, -NH_2, -SH and -COOH) to the melatonin structure; 16 with only one functional group (all possible species within the used substitution scheme), 96 with two functional groups (using any possible combination) and 4 with three functional groups.

A selection score (S^S) was built to sample the search space, simultaneously considering ADME (absorption, distribution, metabolism, excretion) properties, toxicity and manufacturability (i.e., synthetic accessibility). It was used to characterize the whole set of designed melatonin derivatives and allowed the selection of a reduced subset of 20 melatonin derivatives that are expected to be the most promising, regarding drug-like behavior.

For this subset, several reactivity indices were estimated, as well as their pKa values. These indices account for electron and H donor capabilities; thus, they are expected to reflect free radical scavenging behavior through single electron transfer (SET) and formal hydrogen transfer (HAT) mechanisms. According to the gathered data, 5 melatonin derivatives have been identified as the most likely candidates to act as chemical antioxidant (by directly scavenging free radicals). They are dM-34, dM-115, dM-38, dM-61 and dM-94 (Scheme 3), in that order. All of them are predicted to be better for that purpose than melatonin itself and trolox. The findings from this work are expected to motivate further investigations on these molecules, using both theoretical and experimental approaches.

AUTHORSHIP

Miguel Reina, Romina Castañeda-Arriaga, Adriana Pérez-González and Eduardo Gabriel Guzmán-López: Contributed to acquisition of data, data analysis and interpretation, drafting of the

manuscript and approval of the article. Dun Xian Tan and Russel J. Reiter: Contributed to the data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article. Annia Galano: Contributed to the conception and design of the investigation, acquisition of data, data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Laboratorio de Visualización y Cómputo Paralelo at Universidad Autónoma Metropolitana-Iztapalapa. A. P.-G. acknowledges the Program of Cátedras - CONACYT from CONACyT - UAMI (2015-2025), ID-Investigador 435. This investigation is inserted in the research project IFC-2016/1828.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

REFERENCES

1. Tekiner-Gulbas B, Westwell AD, & Suzen S (2013) Oxidative stress in carcinogenesis: New synthetic compounds with dual effects upon free radicals and cancer. *Curr. Med. Chem*.**20** (36): 4451-4459.
2. Matsuda M & Shimomura I (2014) Roles of adiponectin and oxidative stress in obesity-associated metabolic and cardiovascular diseases. Rev. Endocr. Metab. Disord. 15 (1): 1-10.
3. Eren E, et al. (2014) Heart valve disease: The role of calcidiol deficiency, elevated parathyroid hormone levels and oxidative stress in mitral and aortic valve insufficiency. *Redox Rep*. **19** (1): 34-39.
4. Halliwell B (2001) Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. *Drugs Aging* **18** (9): 685-716.
5. Pohanka M (2014) Alzheimer's disease and oxidative stress: A review. *Curr. Med. Chem*. **21** (3): 356-364.
6. Pimentel C, Batista-Nascimento L, Rodrigues-Pousada C, & Menezes RA (2012) Oxidative stress in Alzheimer's and Parkinson's diseases: Insights from the yeast Saccharomyces cerevisiae. *Oxid. Med. Cell. Longev.* 2012: 132146.
7. Ramis MR, Esteban S, Miralles A, Tan DX, & Reiter RJ (2015) Protective effects of melatonin and mitochondria-targeted antioxidants against oxidative stress: A review. *Curr. Med. Chem.* **22** (22): 2690-2711.
8. Galano A, Tan DX & Reiter RJ (2011) Melatonin as a natural ally against oxidative stress: A physicochemical examination. *J. Pineal Res.* **51** (1): 1-16.
9. Hardeland R (2005) Antioxidative protection by melatonin: Multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine* **27** (2): 119-130.
10. Tan DX, Manchester LC, Terron MP, Flores LJ, & Reiter RJ (2007) One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* **42** (1): 28-42.

11. Tan DX, *et al.* (2002) Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr. Top. Med. Chem.* **2** (2): 181-197.
12. Miller E, Walczak A, Majsterek I, & Kedziora J (2013) Melatonin reduces oxidative stress in the erythrocytes of multiple sclerosis patients with secondary progressive clinical course. *J. Neuroimmunol.* **257** (1-2): 97-101.
13. Manchester LC, *et al.* (2015) Melatonin: an ancient molecule that makes oxygen metabolically tolerable. *J. Pineal Res.* **59** (4): 403-419.
14. Kaya Y, Savas K, Sarikcioglu L, Yaras N, & Angelov DN (2015) Melatonin leads to axonal regeneration, reduction in oxidative stress, and improved functional recovery following sciatic nerve injury. *Curr. Neurovasc. Res.* **12** (1): 53-62.
15. Colín-González AL, *et al.* (2015) On the relationship between the light/dark cycle, melatonin and oxidative stress. *Curr. Pharm. Des.* **21** (24): 3477-3488.
16. Joshi N, Biswas J, Nath C, & Singh S (2015) Promising Role of Melatonin as Neuroprotectant in Neurodegenerative Pathology. *Mol. Neurobiol.* **52** (1): 330-340.
17. Rosales-Corral SA, Reiter RJ, Tan DX, Manchester LC, & Liu X (2014) Antioxidant and anti-inflammatory role of melatonin in Alzheimer's neurodegeneration. *Aging: Oxidative stress and dietary antioxidants*), pp 177-193.
18. Rosales-Corral SA, *et al.* (2012) Alzheimer's disease: Pathological mechanisms and the beneficial role of melatonin. *J. Pineal Res.* **52** (2): 167-202.
19. 19. Paterniti I, Cordaro M, Esposito E, & Cuzzocrea S (2016) The antioxidative property of melatonin against brain ischemia. *Expert Rev. Neurother.* **16** (7): 841-848.
20. García JJ, *et al.* (2001) N-acetylserotonin suppresses hepatic microsomal membrane rigidity associated with lipid peroxidation. *Eur. J. Pharmacol.* **428** (2): 169-175.
21. Oxenkrug G, Requintina P, & Bachurin S (2001) Antioxidant and antiaging activity of N-acetylserotonin and melatonin in the in vivo models. *Ann. NY Acad. Sci.* **939**: 190-199.
22. Bachurin S, *et al.* (1999) N-acetylserotonin, melatonin and their derivatives improve cognition and protect against β-amyloid-induced neurotoxicity. *Ann. NY Acad. Sci.* **890**: 155-166.
23. Jiang J, *et al.* (2014) N-acetyl-serotonin protects HepG2 cells from oxidative stress injury induced by hydrogen peroxide. *Oxid. Med. Cell. Longev.* 2014: 310504.
24. Qi W, *et al.* (2000) Increased levels of oxidatively damaged DNA induced by chromium(III) and H2O2: Protection by melatonin and related molecules. *J. Pineal Res.* **29** (1): 54-61.
25. Oxenkrug G (2005) Antioxidant effects of N-acetylserotonin: Possible mechanisms and clinical implications. in *Ann. N. Y. Acad. Sci.*, pp 334-347.
26. López-Burillo S, *et al.* (2003) Melatonin and its derivatives cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine and 6-methoxymelatonin reduce oxidative DNA damage induced by Fenton reagents. *J. Pineal Res.* **34** (3): 178-184.
27. Tan DX, Hardeland R, Manchester LC, Galano A, & Reiter RJ (2014) Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicals and suppresses oxidative reactions. *Curr. Med. Chem.* **21** (13): 1557-1565.
28. Galano A, Tan DX, & Reiter RJ (2014) Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. *RSC Adv.* **4** (10): 5220-5227.
29. Tan DX, *et al.* (2001) N1-acetyl-N2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. *FASEB J.* **15** (12): 2294-2296.

30. Burkhardt S, *et al.* (2001) DNA oxidatively damaged by chromium(III) and H2O2 is protected by the antioxidants melatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, resveratrol and uric acid. *Int. J. Biochem. Cell Biol.* **33** (8): 775-783.
31. Manda K, Ueno M, & Anzai K (2007) AFMK, a melatonin metabolite, attenuates X-ray-induced oxidative damage to DNA, proteins and lipids in mice. *J. Pineal Res.* **42** (4): 386-393.
32. Galano A, Tan DX, & Reiter RJ (2013) On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. *J. Pineal Res.* **54** (3): 245-257.
33. Maharaj DS, *et al.* (2002) The identification of the UV degradants of melatonin and their ability to scavenge free radicals. *J. Pineal Res.* **32** (4):257-261.
34. Ressmeyer AR, *et al.* (2003) Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): Scavenging of free radicals and prevention of protein destruction. *Redox Rep.* **8** (4): 205-213.
35. Schaefer M & Hardeland R (2009) The melatonin metabolite N1-acetyl-5-methoxykynuramine is a potent singlet oxygen scavenger. *J. Pineal Res.* **46** (1): 49-52.
36. Guenther AL, *et al.* (2005) Reactions of the melatonin metabolite AMK (N1-acetyl-5-methoxykynuramine) with reactive nitrogen species: Formation of novel compounds, 3-acetamidomethyl-6-methoxycinnolinone and 3-nitro-AMK. *J. Pineal Res.* **39** (3): 251-260.
37. Hardeland R, Backhaus C, & Fadavi A (2007) Reactions of the NO redox forms NO+, •NO and HNO (protonated NO-) with the melatonin metabolite N1-acetyl- 5-methoxykynuramine. *J. Pineal Res.* **43** (4): 382-388.
38. Hardeland R, Backhaus C, Fadavi A, & Hess M (2007) N1-acetyl-5-methoxykynuramine contrasts with other tryptophan metabolites by a peculiar type of NO scavenging: Cyclization to a cinnolinone prevents formation of unstable nitrosamines. *J. Pineal Res.* **43** (1): 104-105.
39. Maharaj DS, Walker RB, Glass BD, & Daya S (2003) 6-Hydroxymelatonin protects against cyanide induced oxidative stress in rat brain homogenates. *J. Chem. Neuroanat.* **26** (2): 103-107.
40. Maharaj DS, *et al.* (2005) 6-Hydroxymelatonin protects against quinolinic-acid-induced oxidative neurotoxicity in the rat hippocampus. *J. Pharm. Pharmacol.* **57** (7): 877-881.
41. Pérez-González A, Galano A, Alvarez-Idaboy JR, Tan DX, & Reiter RJ (2017) Radical-trapping and preventive antioxidant effects of 2-hydroxymelatonin and 4-hydroxymelatonin: Contributions to the melatonin protection against oxidative stress. *Biochim. Biophys. Acta* 1**861** (9): 2206-2217.
42. Tan DX, *et al.* (2010) The changing biological roles of melatonin during evolution: From an antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev. Camb. Philos. Soc.* **85** (3): 607-623.
43. Reiter RJ, *et al.* (2017) Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. *Cell. Mol. Life Sci.* **74** (21): 3863-3881.
44. Acuña-Castroviejo D, *et al.* (2014) Extrapineal melatonin: Sources, regulation, and potential functions. *Cell. Mol. Life Sci.* **71** (16): 2997-3025.
45. Reiter RJ, Tan DX, & Fuentes-Broto L (2010) Melatonin: A multi-tasking molecule. *Prog. Brain Res.* **181**: 127-151.
46. Reiter RJ, Tan DX, & Galano A (2014) Melatonin: Exceeding expectations. *Physiology (Bethesda)* **29** (5):325-333.
47. Reiter RJ, *et al.* (2015) Phytomelatonin: Assisting plants to survive and thrive. *Molecules* **20** (4): 7396-7437.

48. Wiechmann AF & Sherry DM (2013) Role of melatonin and its receptors in the vertebrate retina. *Int. Rev. Cell Mol. Biol.* **300**: 211-242.
49. Kim TK, *et al.* (2013) Metabolism of melatonin and biological activity of intermediates of melatoninergic pathway in human skin cells. *FASEB J.* **27** (7):2742-2755.
50. Pinato L, *et al.* (2013) Selective protection of the cerebellum against intracerebroventricular LPS is mediated by local melatonin synthesis. *Brain Struct. Funct.* **220** (2):1-14.
51. Venegas C, *et al.* (2012) Extrapineal melatonin: Analysis of its subcellular distribution and daily fluctuations. *J. Pineal Res.* **52** (2): 217-227.
52. Cruz MHC, Leal CLV, Cruz JF, Tan DX, & Reiter RJ (2014) Essential actions of melatonin in protecting the ovary from oxidative damage. *Theriogenology* **82** (7): 925-932.
53. Peschke E (2008) Melatonin, endocrine pancreas and diabetes. *J. Pineal Res.* **44** (1): 26-40.
54. Suofu Y, *et al.* (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc. Natl. Acad. Sci. U. S. A.* **114** (38): E7997-E8006.
55. Wang L, *et al.* (2017) Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. *J. Pineal Res.* **63** (3). doi: 10.1111/jpi.12429.
56. Reiter RJ, Tan DX, Rosales-Corral S, & Manchester LC (2013) The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. *Mini-Rev. Med. Chem.* **13** (3): 373-384.
57. Hevia D, *et al.* (2015) Melatonin uptake through glucose transporters: A new target for melatonin inhibition of cancer. *J. Pineal Res.* **58** (2): 234-250.
58. Huo X, *et al.* (2017) Human transporters, PEPT1/2, facilitate melatonin transportation into mitochondria of cancer cells: An implication of the therapeutic potential. *J. Pineal Res.* **62** (4)., doi: 10.1111/jpi.12390.
59. Ceraulo L, *et al.* (1999) Interactions of melatonin with membrane models: Portioning of melatonin in AOT and lecithin reversed micelles. *J. Pineal Res.* **26** (2): 108-112.
60. Bonnefont-Rousselot D & Collin F (2010) Melatonin: Action as antioxidant and potential applications in human disease and aging. *Toxicology* **278** (1): 55-67.
61. Jahnke G, *et al.* (1999) Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. *Toxicol. Sci.* **50** (2): 271-279.
62. Andersen LPH, Gögenur I, Rosenberg J, & Reiter RJ (2016) The safety of melatonin in humans. *Clin. Drug Investig.* **36** (3):169-175.
63. Vijayalaxmi, Meltz ML, Reiter RJ, & Herman TS (1999) Melatonin and protection from genetic damage in blood and bone marrow: Whole-body irradiation studies in mice. *J. Pineal Res.* **27** (4):221-225.
64. Vijayalaxmi, Meltz ML, Reiter RJ, Herman TS, & Kumar K S (1999) Melatonin and protection from whole-body irradiation: Survival studies in mice. *Mutat. Res.* **425** (1): 21-27.
65. Kaya H, Delibas N, Serteser M, Ulukaya E, & Özkaya O (1999) The effect of melatonin on lipid peroxidation during radiotherapy in female rats. *Strahlenther. Onkol.* **175** (6):285-288.
66. Nordlund JJ & Lerner AB (1977) The effects of oral melatonin on skin color and on the release of pituitary hormones. *J. Clin. Endocrinol. Metab.* **45** (4): 768-774.
67. Tan DX, *et al.* (2000) Significance of melatonin in antioxidative defense system: Reactions and products. *Biol. Signals Recept.* **9** (3-4): 137-159.
68. Gurer-Orhan H & Suzen S (2015) Melatonin, its metabolites and its synthetic analogs as multi-faceted compounds: Antioxidant, prooxidant and inhibitor of bioactivation reactions. *Curr. Med. Chem.* **22** (4):490-499.

69. Reiter RJ, *et al.* (2008) Biogenic amines in the reduction of oxidative stress: Melatonin and its metabolites. *Neuro Endocrinol. Lett.* **29** (4):391-398.
70. Galano A, Medina ME, Tan DX, & Reiter RJ (2015) Melatonin and its metabolites as copper chelating agents and their role in inhibiting oxidative stress: A physicochemical analysis. *J. Pineal Res.* **58** (1): 107-116.
71. Galano A & Reiter RJ (2018) Melatonin and its metabolites vs oxidative stress: From individual actions to collective protection. *J. Pineal Res.* **65** (1):e12514.
72. Galano A, Tan DX, & Reiter RJ (2018) Melatonin: A versatile protector against oxidative DNA damage. *Molecules* **23** (3): 23(3). pii: E530. doi: 10.3390/molecules23030530.
73. Romero A, *et al.* (2014) A review of metal-catalyzed molecular damage: Protection by melatonin. *J. Pineal Res.* **56** (4): 343-370.
74. Majidinia M, *et al.* (2017) Melatonin: A pleiotropic molecule that modulates DNA damage response and repair pathways. *J. Pineal Res.* **63** (1): art. e12416, DOI 12410.11111/jpi.12416.
75. Boutin JA (2016) Quinone reductase 2 as a promising target of melatonin therapeutic actions. *Expert Opin. Ther. Targets* **20** (3): 303-317.
76. Galano A (2015) The role of indoleamines in reducing free radical damage and oxidative stress: A physicochemical perspective. *Indoleamines: Sources, Role in Biological Processes and Health Effects*), pp 1-41.
77. Suzen S (2013) Melatonin and synthetic analogs as antioxidants. *Curr. Drug Del.* **10** (1): 71-75.
78. Johns JR & Platts JA (2014) Theoretical insight into the antioxidant properties of melatonin and derivatives. *Org. Biomol. Chem.* **12** (39): 7820-7827.
79. Tsia PL & Hu MK (2003) Free radical scavenging and antioxidative activity of melatonin derivatives. *J. Pharm. Pharmacol.* **55** (12): 1655-1660.
80. Ates-Alagoz Z, Coban T, & Buyukbingol E (2006) Synthesis and antioxidant activity of new tetrahydro-naphthalene-indole derivatives as retinoid and melatonin analogs. *Arch. Pharm.* **339** (4): 193-200.
81. Ateş-Alagöz Z, Coban T, & Suzen S (2005) A comparative study: Evaluation of antioxidant activity of melatonin and some indole derivatives. *Med. Chem. Res.* **14** (3): 169-179.
82. Suzen S, Bozkaya P, Coban T, & Nebioğlu D (2006) Investigation of the in vitro antioxidant behaviour of some 2-phenylindole derivatives: Discussion on possible antioxidant mechanisms and comparison with melatonin. *J. Enzyme Inhib. Med. Chem.* **21** (4):405-411.
83. Shirinzadeh H, Eren B, Gurer-Orhan H, Suzen S, & Özden S (2010) Novel indole-based analogs of melatonin: Synthesis and in vitro antioxidant activity studies. *Molecules* **15** (4): 2187-2202.
84. Yilmaz AD, Coban T, & Suzen S (2012) Synthesis and antioxidant activity evaluations of melatonin-based analogue indole-hydrazide/hydrazone derivatives. *J. Enzyme Inhib. Med. Chem.* **27** (3):428-436.
85. Gürkök G, Coban T, & Suzen S (2009) Melatonin analogue new indole hydrazide/hydrazone derivatives with antioxidant behavior: Synthesis and structureactivity relationships. *J. Enzyme Inhib. Med. Chem.* **24** (2): 506-515.
86. Suzen S, Cihaner SS, & Coban T (2012) Synthesis and comparison of antioxidant properties of indole-based melatonin analogue indole amino acid derivatives. *Chem. Biol. Drug Des.* **79**(1): 76-83.
87. Galano A (2016) Computational-aided design of melatonin analogues with outstanding multifunctional antioxidant capacity. *RSC Advances* **6** (27): 22951-22963.

88. Lipinski CA, Lombardo F, Dominy BW, & Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Del. Rev.* **46** (1-3): 3-26.
89. Ghose AK, Viswanadhan VN, & Wendoloski JJ (1999) A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J. Comb. Chem.* **1** (1): 55-68.
90. Veber DF, *et al.* (2002) Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **45** (12): 2615-2623.
91. Leeson PD & Davis AM (2004) Time-related differences in the physical property profiles of oral drugs. *J. Med. Chem.* **47** (25): 6338-6348.
92. Zhu H, *et al.* (2008) Combinatorial QSAR modeling of chemical toxicants tested against Tetrahymena pyriformis. *J. Chem. Inf. Model.* **48** (4): 766-784.
93. Boda K, Seidel T, & Gasteiger J (2007) Structure and reaction based evaluation of synthetic accessibility. *J. Comput. Aided Mol. Des.* **21** (6): 311-325.
94. Bonnet P (2012) Is chemical synthetic accessibility computationally predictable for drug and lead-like molecules? A comparative assessment between medicinal and computational chemists. *Eur. J. Med. Chem.* **54**: 679-689.
95. Frisch MJ, *et al.* (2009) Gaussian 09 (Gaussian, Inc., Wallingford, CT, USA).
96. Zhao Y, Schultz NE, & Truhlar DG (2006) Design of density functionals by combining the method of constraint satisfaction with parametrization for thermochemistry, thermochemical kinetics, and noncovalent interactions. *Journal of Chemical Theory and Computation* **2** (2):364-382.
97. Marenich AV, Cramer CJ, & Truhlar DG (2009) Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B* **113** (18):6378-6396.
98. Zhao Y & Truhlar DG (2008) How well can new-generation density functionals describe the energetics of bond-dissociation reactions producing radicals? *J. Phys. Chem. A* **112** (6): 1095-1099.
99. Galano A & Alvarez-Idaboy JR (2013) A computational methodology for accurate predictions of rate constants in solution: Application to the assessment of primary antioxidant activity. *J. Comput. Chem.* **34** (28): 2430-2445.
100. Pérez-González A, *et al.* (2017) Estimation of empirically fitted parameters for calculating pK a values of thiols in a fast and reliable way. *Theor. Chem. Acc.* **137** (1): art. 5 (10 pages).
101. Galano A, *et al.* (2016) Empirically Fitted Parameters for Calculating pKa Values with Small Deviations from Experiments Using a Simple Computational Strategy. *J. Chem. Inf. Model.* **56** (9): 1714-1724.
102. Galano A, Francisco Marquez M, & Pérez-González A (2014) Ellagic acid: An unusually versatile protector against oxidative stress. *Chem. Res. Toxicol.* **27** (5): 904-918.
103. Marino T, Galano A, & Russo N (2014) Radical scavenging ability of gallic acid toward OH and OOH radicals. Reaction mechanism and rate constants from the density functional theory. *J. Phys. Chem. B* **118** (35):10380-10389.
104. Galano A, Raul Alvarez-Idaboy J, & Francisco-Márquez M (2010) Mechanism and branching ratios of hydroxy ethers + ·oH gas phase reactions: Relevance of H bond interactions. *J. Phys. Chem. A* **114** (28):7525-7536.
105. León-Carmona JR, Martínez A, & Galano A (2014) New free radicals to measure antiradical capacity: A theoretical study. *J. Phys. Chem. B* **118** (34): 10092-10100.

106. Pérez-González A, Galano A, & Alvarez-Idaboy JR (2014) Dihydroxybenzoic acids as free radical scavengers: Mechanisms, kinetics, and trends in activity. *New J. Chem.* **38** (6): 2639-2652.
107. Pérez-González A & Galano A (2013) On the hydroperoxyl radical scavenging activity of two Edaravone derivatives: Mechanism and kinetics. *J. Phys. Org. Chem.* **26** (3): 261-268.
108. Pérez-González A & Galano A (2012) On the •oH and •oOH scavenging activity of 3-methyl-1-pyridin-2-yl-5-pyrazolone: Comparisons with its parent compound, edaravone. *Int. J. Quantum Chem* **112** (21): 3441-3448.
109. Álvarez-Diduk R, Ramírez-Silva MT, Galano A, & Merkoçi A (2013) Deprotonation mechanism and acidity constants in aqueous solution of flavonols: A combined experimental and theoretical study. *J. Phys. Chem. B* **117** (41): 12347-12359.
110. Medina ME, Galano A, & Alvarez-Idaboy JR (2014) Theoretical study on the peroxyl radicals scavenging activity of esculetin and its regeneration in aqueous solution. *Phys. Chem. Chem. Phys.* **16** (3): 1197-1207.
111. Rebollar-Zepeda AM & Galano A (2016) Quantum mechanical based approaches for predicting pKa values of carboxylic acids: evaluating the performance of different strategies. *RSC Adv.* **6** (113):112057-112064.
112. Ortiz JV (1999) Toward an Exact One-Electron Picture of Chemical Bonding. *Adv. Quantum Chem.* **35**: 33-52.
113. Ortiz JV (2013) Electron propagator theory: An approach to prediction and interpretation in quantum chemistry. *Wiley Interdisciplinary Reviews: Computational Molecular Science* **3** (2): 123-142.
114. Ortiz JV (1996) Partial third-order quasiparticle theory: Comparisons for closed-shell ionization energies and an application to the Borazine photoelectron spectrum. *J. Chem. Phys.* **104** (19): 7599-7605.
115. Pérez-González A, Galano A, & Ortiz JV (2014) Vertical ionization energies of free radicals and electron detachment energies of their anions: A comparison of direct and indirect methods versus experiment. *J. Phys. Chem. A* **118** (31):6125-6131.
116. Parr RG, Szentpály Lv, & Liu S (1999) Electrophilicity Index. *J. Am. Chem. Soc.* **121** (9): 1922-1924.
117. Chattaraj PK, Maiti B, & Sarkar U (2003) Philicity: A Unified Treatment of Chemical Reactivity and Selectivity. *J Phys. Chem. A* **107** (25):4973-4975.
118. Gázquez JL, Cedillo A, & Vela A (2007) Electrodonating and Electroaccepting Powers. *J. Phys. Chem. A* **111** (10): 1966-1970.
119. Gázquez JL (2008) Perspectives on the density functional theory of chemical reactivity. *J. Mex. Chem. Soc.* **52** (1): 3-10.
120. Pearson RG (1963) Hard and Soft Acids and Bases. *J. Am. Chem. Soc.* **85** (22):3533-3539.
121. Pearson RG (1993) The principle of maximum hardness. *Acc. Chem. Res.* **26** (5): 250-255.
122. Ortiz JV (2003) Quasiparticle Approximations and Electron Propagator Theory. *Int. J. Quantum Chem* **95** (4-5): 593-599.
123. Singh RK, Ortiz JV, & Mishra MK (2010) Tautomeric forms of adenine: Vertical ionization energies and Dyson orbitals. *Int. J. Quantum Chem* **110** (10): 1901-1915.
124. Areti A, Yerra VG, Naidu VGM, & Kumar A (2014) Oxidative stress and nerve damage: Role in chemotherapy induced peripheral neuropathy. *Redox Biol* **2** (1): 289-295.

125. Miyagawa T, *et al.* (2013) Effects of Oral L-Carnitine Administration in Narcolepsy Patients: A Randomized, Double-Blind, Cross-Over and Placebo-Controlled Trial. *PLoS ONE* **8** (1)**.** e53707. doi: 10.1371/journal.pone.0053707.
126. Vermersch P, *et al.* (2012) Masitinib treatment in patients with progressive multiple sclerosis: a randomized pilot study. *BMC Neurol* **12**:36.
127. Folch J, *et al.* (2015) Masitinib for the treatment of mild to moderate Alzheimer's disease. *Expert Rev Neurother* **15** (6): 587-596.
128. Bareš M, Kaňovský P, Klajblová H, & Rektor I (2003) Intracortical inhibition and facilitation are impaired in patients with early Parkinson's disease: A paired TMS study. *Eur. J. Neurol.* **10** (4): 385-389.
129. Fox SH, *et al.* (2018) International Parkinson and movement disorder society evidence-based medicine review: Update on treatments for the motor symptoms of Parkinson's disease. *Mov. Disord.* **33** (8):1248-1266.
130. Niemann N & Jankovic J (2018) Treatment of Tardive Dyskinesia: A General Overview with Focus on the Vesicular Monoamine Transporter 2 Inhibitors. *Drugs* **78** (5):525-541.
131. Koziróg M, *et al.* (2011) Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. *J. Pineal Res.* **50** (3): 261-266.
132. Chahbouni M, *et al.* (2010) Melatonin treatment normalizes plasma pro-inflammatory cytokines and nitrosative/oxidative stress in patients suffering from Duchenne muscular dystrophy. *J. Pineal Res.* **48** (3):282-289.
133. Hanaki M, Murakami K, Katayama S, Akagi KI, & Irie K (2018) Mechanistic analyses of the suppression of amyloid β42 aggregation by apomorphine. *Bioorg. Med. Chem.* **26** (8): 1538-1546.
134. Auffret M, Drapier S, & Vérin M (2018) Pharmacological Insights into the Use of Apomorphine in Parkinson's Disease: Clinical Relevance. *Clin. Drug Investig.* **38** (4): 287-312.
135. Tavakoli-Ardakani M, Abbaspour H, Farhadi Nasab A, Mazaheri Meibodi A, & Kheradmand A (2018) Study of the effect of memantine on negative sign in patients with schizophrenia and schizoaffective disorders. *Iran J. Pharm. Res.* **17** (Special Issue): 122-129.
136. Pucks-Faes E, *et al.* (2018) Eleven years' experience with Intrathecal Baclofen – Complications, risk factors. *Brain Behav.* **8** (5): e00965.
137. McLaughlin MJ, *et al.* (2018) Pharmacogenomic Variability of Oral Baclofen Clearance and Clinical Response in Children With Cerebral Palsy. *PM and R* **10** (3):235-243.
138. Porsdam Mann S, de Lora Deltoro P, Cochrane T, & Mitchell C (2018) Is the use of modafinil, a pharmacological cognitive enhancer, cheating? *Ethics and Education* **13** (2): 251-267.
139. Minzenberg MJ, Yoon JH, Soosman SK, & Carter CS (2018) Altered brainstem responses to modafinil in schizophrenia: Implications for adjunctive treatment of cognition. *Transl Psychiatry* **8**(1): 58. doi: 10.1038/s41398-018-0104-z ..
140. Politi C, Ciccacci C, Novelli G, & Borgiani P (2018) Genetics and Treatment Response in Parkinson's Disease: An Update on Pharmacogenetic Studies. *Neuromolecular Med.* **20** (1): 1-17.
141. Gasser UE, Fischer A, Timmermans JP, & Arnet I (2013) Pharmaceutical quality of seven generic Levodopa/Benserazide products compared with original Madopar® / Prolopa®. *BMC Pharmacol Toxicol* **14**: 24. doi: 10.1186/2050-6511-14-24.

142. Taddei RN, Spinnato F, & Jenner P (2017) New Symptomatic Treatments for the Management of Motor and Nonmotor Symptoms of Parkinson's Disease. *Int. Rev. Neurobiol.* **132**: 407-452.
143. Ogino S, Miyamoto S, Miyake N, & Yamaguchi N (2014) Benefits and limits of anticholinergic use in schizophrenia: Focusing on its effect on cognitive function. *Psychiatry Clin Neurosci.* **68** (1): 37-49.
144. Bergman H & Soares-Weiser K (2018) Anticholinergic medication for antipsychotic-induced tardive dyskinesia. *Cochrane Database Syst. Rev.* **2018** (1): CD000204.
145. Mizuno Y, Shimoda S, & Origasa H (2018) Long-term treatment of Parkinson's disease with levodopa and other adjunctive drugs. *J Neural Transm (Vienna)* **125** (1): 35-43.
146. Grimaldi R, *et al.* (1986) Pharmacokinetic and pharmacodynamic studies following the intravenous and oral administration of the antiparkinsonian drug biperiden to normal subjects. *Eur J Clin Pharmacol* **29** (6): 735-737.
147. Hauser RA & Holford NHG (2002) Quantitative description of loss of clinical benefit following withdrawal of levodopa-carbidopa and bromocriptine in early Parkinson's disease. *Mov Disord* **17** (5): 961-968.
148. Zaccara G, *et al.* (2017) Do antiepileptic drugs increase the risk of infectious diseases? A meta-analysis of placebo-controlled studies. *Br J Clin Pharmacol* **83** (9): 1873-1879.
149. Steiger MJ, El-Debas T, Anderson T, Findley LJ, & Marsden CD (1996) Double-blind study of the activity and tolerability of cabergoline versus placebo in parkinsonians with motor fluctuations. *J Neurol.* **243** (1): 68-72.
150. Rinne UK, *et al.* (1998) Early treatment of Parkinson's disease with cabergoline delays the onset of motor complications. Results of a double-blind levodopa controlled trial. *Drugs* **55** (SUPPL. 1): 23-30.
151. Oberstadt M, *et al.* (2018) TDP-43 self-interaction is modulated by redox-active compounds Auranofin, Chelerythrine and Riluzole. *Sci. Rep.* **8** (1): 2248. doi: 10.1038/s41598-018-20565.
152. Stocchi F, *et al.* (2010) Initiating levodopa/carbidopa therapy with and without entacapone in early Parkinson disease: The STRIDE-PD study. *Ann Neurol.* **68** (1): 18-27.
153. Hauser RA, *et al.* (2013) Extended-release carbidopa-levodopa (IPX066) compared with immediate-release carbidopa-levodopa in patients with Parkinson's disease and motor fluctuations: A phase 3 randomised, double-blind trial. *Lancet Neurol.* **12** (4): 346-356.
154. Takeda A, *et al.* (2006) A systematic review of the clinical effectiveness of donepezil, rivastigmine and galantamine on cognition, quality of life and adverse events in Alzheimer's disease. *Int J Geriatr Psychiatry* **21** (1): 17-28.
155. Epstein J, Sanderson IR, & MacDonald TT (2010) Curcumin as a therapeutic agent: The evidence from in vitro, animal and human studies. *Br. J. Nutr.* **103** (11): 1545-1557.
156. Chen M, *et al.* (2018) Use of curcumin in diagnosis, prevention, and treatment of Alzheimer's disease. *Neural Regen Res* **13** (4): 742-752.
157. Schmidt RT, Lee RH, & Spehlmann R (1976) Comparison of dantrolene sodium and diazepam in the treatment of spasticity. *J. Neurol. Neurosurg. Psychiatry* **39** (4): 350-356.
158. Katrak PH, Cole AMD, Poulos CJ, & McCauley JCK (1992) Objective assessment of spasticity, strength, and function with early exhibition of dantrolene sodium after cerebrovascular accident: A randomized double-blind study. *Arch Phys Med Rehabi l* **73** (1): 4-9.

159. Glass A & Hannah A (1974) A comparison of dantrolene sodium and diazepam in the treatment of spasticity. *Paraplegia* **12** (3): 170-174.
160. Ebadi M, Sharma S, Shavali S, & El Refaey H (2002) Neuroprotective actions of selegiline. *J. Neurosci. Res.* **67** (3): 285-289.
161. Román GC, *et al.* (2010) Randomized, placebo-controlled, clinical trial of donepezil in vascular dementia: Differential effects by hippocampal size. *Stroke* **41** (6): 1213-1221.
162. Hepnarova V, *et al.* (2018) The concept of hybrid molecules of tacrine and benzyl quinolone carboxylic acid (BQCA) as multifunctional agents for Alzheimer's disease. *Eur. J. Med. Chem.* **150**: 292-306.
163. Giacobini E (1998) Invited review. Cholinesterase inhibitors for Alzheimer's disease therapy: From tacrine to future applications. *Neurochem. Int.* **32** (5-6): 413-419.
164. Poewe WH, Deuschl G, Gordin A, Kultalahti ER, & Leinonen M (2002) Efficacy and safety of entacapone in Parkinson's disease patients with suboptimal levodopa response: A 6-month randomized placebo controlled double blind study in Germany and Austria (Celomen study). *Acta Neurol. Scand.* **105** (4): 245-255.
165. Myllylä VV, *et al.* (2001) Twelve-month safety of entacapone in patients with Parkinson's disease. *Eur. J. Neurol.* **8** (1): 53-60.
166. Scorr LM & Factor SA (2018) VMAT2 inhibitors for the treatment of tardive dyskinesia. *J. Neuro.l Sci.* 389:.43-47.
167. Erkinjuntti T, *et al.* (2002) Efficacy of galantamine in probable vascular dementia and Alzheimer's disease combined with cerebrovascular disease: A randomised trial. *Lancet* **359** (9314): 1283-1290.
168. Burns A, *et al.* (2009) Safety and efficacy of galantamine (Reminyl) in severe Alzheimer's disease (the SERAD study): a randomised, placebo-controlled, double-blind trial. *Lancet Neurol.*. **8** (1):39-47.
169. Blautzik J, *et al.* (2016) Functional connectivity increase in the default-mode network of patients with Alzheimer's disease after long-term treatment with Galantamine. *Eur. Neuropsychopharmacol.* **26** (3): 602-613.
170. Rudolf G, *et al.* (2018) A novel non-opioid protocol for medically supervised opioid withdrawal and transition to antagonist treatment. *Am. J. Drug Alcohol Abuse* **44** (3): 302-309.
171. Geldenhuys WJ & Van Der Schyf CJ (2013) Designing drugs with multi-target activity: The next step in the treatment of neurodegenerative disorders. *Expert Opin. Drug Discov.* **8** (2): 115-129.
172. Weinreb O, Amit T, Bar-Am O, & Youdim MBH (2011) A novel anti-Alzheimer's disease drug, ladostigil. neuroprotective, multimodal brain-selective monoamine oxidase and cholinesterase inhibitor. in *Int. Rev. Neurobiol.*, pp 191-215.
173. Velázquez-Pérez L, *et al.* (2012) Lisuride reduces involuntary periodic leg movements in spinocerebellar ataxia type 2 patients. *Cerebellum* **11** (4): 1051-1056.
174. Md S, Haque S, Sahni JK, Baboota S, & Ali J (2011) New non-oral drug delivery systems for Parkinson's disease treatment. *Expert Opin. Drug Deliv.* **8** (3): 359-374.
175. Zhong HA (2017) ADMET properties: Overview and current topics. *Drug Design: Principles and Applications*), pp 113-133.
176. Schneider G & Fechner U (2005) Computer-based de novo design of drug-like molecules. *Nat. Rev. Drug Discov.* **4** (8): 649-663.
177. Chaiyasit W, Elias RJ, McClements DJ, & Decker EA (2007) Role of physical structures in bulk oils on lipid oxidation. *Crit. Rev. Food Sci. Nutr.* **47** (3): 299-317.

178. Galano A & Alvarez-Idaboy JR (2018) Computational strategies for predicting free radical scavengers' protection against oxidative stress: Where are we and what might follow? *Int. J. Quantum Chem.* **118** (13): art. e25665 (25623 pages).
179. Miche H, Brumas V, & Berthon G (1997) Copper(II) interactions with nonsteroidal antiinflammatory agents. II. Anthranilic acid as a potential OH-inactivating ligand. *J. Inorg. Biochem.* **68** (1): 27-38.
180. Gaubert S, Bouchaut M, Brumas V, & Berthon G (2000) Copper-ligand interactions and physiological free radical processes. Part 3. Influence of histidine, salicylic acid and anthranilic acid on copper-driven Fenton chemistry in vitro. *Free Radic. Res.* **32** (5): 451-461.
181. Brenk R, *et al.* (2008) Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. *Chem. Med .Chem.* **3** (3): 435-444.
182. Alvarez-Idaboy JR & Galano A (2012) On the chemical repair of DNA radicals by glutathione: Hydrogen vs electron transfer. *J. Phys. Chem. B* **116** (31): 9316-9325.
183. Galano A & Alvarez-Idaboy JR (2011) Glutathione: Mechanism and kinetics of its non-enzymatic defense action against free radicals. *RSC Adv.* **1** (9): 1763-1771.
184. Gleeson MP, Hersey A, Montanari D, & Overington J (2011) Probing the links between in vitro potency, ADMET and physicochemical parameters. *Nat. Rev. Drug Discov.* **10** (3): 197-208.
185. Zhong HA, Mashinson V, Woolman TA, & Zha M (2013) Understanding the molecular properties and metabolism of top prescribed drugs. *Curr Top Med Chem* 13(11):1290-1307.
186. Martínez A, Vargas R, & Galano A (2009) What is important to prevent oxidative stress? A theoretical study on electron-transfer reactions between carotenoids and free radicals. *J. Phys. Chem. B* **113** (35):12113-12120.
187. Martínez A, Rodríguez-Girones MA, Barbosa A, & Costas M (2008) Donator acceptor map for carotenoids, melatonin and vitamins. *J. Phys. Chem. A* **112** (38):9037-9042.
188. Ulstrup J & Jortner J (1975) The effect of intramolecular quantum modes on free energy relationships for electron transfer reactions. *J. Chem. Phys.* **63** (10): 4358-4368.
189. Marcus RA & Sutin N (1985) Electron transfers in chemistry and biology. *BBA Reviews On Bioenergetics* **811** (3): 265-322.
190. Marcus RA (1993) Electron transfer reactions in chemistry: Theory and experiment (Nobel lecture). *Angew Chem Int. Ed. Engl.* **32** (8): 1111-1121.
191. Galano A (2011) On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxyl radicals. *Phys. Chem. Chem. Phys.* **13** (15): 7178-7188.