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A Theoretical Study on the Enthalpies of Homolytic and Heterolytic N–H Bond Cleavage in Substituted Melatonins in the Gas–Phase and Aqueous Solution

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Abstract

In this paper, the study of melatonin and 60 *meta*– and *ortho*–substituted melatonins is presented. The reaction enthalpies related to the hydrogen atom transfer (HAT), single electron transfer – proton transfer (SET–PT) and sequential proton loss electron transfer (SPLET) have been calculated using DFT/B3LYP method in gas–phase and water. Results show that electron–withdrawing substituents increase the bond dissociation enthalpy (BDE), ionization potential (IP) and electron transfer enthalpy (ETE), while electron–donating ones cause a rise in the proton dissociation enthalpy (PDE) and proton affinity (PA). In *ortho* position, substituents show larger effect on reaction enthalpies than in *meta* position. In comparison to gas–phase, water attenuates the substituent effect on all reaction enthalpies. Results show that IP and BDE values can be successfully correlated with the indolic N–H bond length after electron abstraction, $R(\text{N–H}^{+\bullet})$, and the partial charge on the indolyl radical nitrogen atom, $q(\text{N})$. Furthermore, calculated IP and PA values for *meta* and *ortho* substituted melatonins show linear dependence on the energy of the highest occupied molecular orbital (E_{HOMO}) of studied molecules in the two environments. SPLET represents the thermodynamically preferred mechanism in water.

Keywords: Natural Antioxidant, Melatonin, Solvent Effect, Reaction Enthalpy, Substituent, HOMO.

1. Introduction

In recent years, the border between chemistry and biochemistry has become even more diffuse. Computational chemistry is one of the most rapidly expanding and exciting areas of scientific endeavor in the medical and dental material sciences.[1] Natural antioxidants can avoid or at least significantly reduce the peroxidation of lipids by free radicals, which are related to a variety of disorders and diseases.[2] The indole moiety represents a medicinally relevant structure and has become a privileged structure or pharmacophore.[3] Indole unit is present in thousands of isolated natural products and also in medicinal (synthetic) compounds with diverse therapeutic activities. In animals, the most significant indolic compound is melatonin. Melatonin (N–acetyl–5–methoxytryptamine), Figure (1A), is an animal and plant hormone.[4] In 1993, its ability to scavenge hydroxyl radicals was discovered.[5] Several reviews summarize evidences that melatonin shows high antioxidant effect.[6–8] Its strong antioxidant activity against lipid peroxidation and DNA damage induced by many ox-

idative systems were reported.[9,10] Melatonin scavenges hydroxyl radicals, peroxynitrites, singlet oxygen, hydrogen peroxide, and probably peroxy radicals generated during the oxidation of unsaturated lipids[11] too. The high reactivity of melatonin towards pro–oxidants may be caused by the electron–rich aromatic system. This allows the indoleamine to act as an electron donor and to form the melatoninyl radical cation.[12,13] Melatonin also affects ageing and age–related diseases as a free radical scavenger,[14] and it may show an antioxidant effect in the brain. Hydroxy radicals and peroxy radicals *in vitro* are scavenged more effectively by melatonin than by glutathione and vitamin E.[15] Due to its antioxidant properties, it protects against lesions induced by ischemia–reperfusion.[16] The reaction between free radicals and antioxidants can follow different mechanisms.[17,18] The basic concept of antioxidant activity is encompassed by a redox transition involving the donation of a hydrogen radical, single electron or proton to the free radicals. There are two mechanisms by which indolic antioxidants (generally ArNH), analogously to the phenolic ones, can inhibit oxidation by transferring their indolic H atom to free radicals.[19] The first mechanism involves a direct hydrogen atom transfer (HAT) from the antioxidant to the free radical ($ROO^{\bullet}$) and yields a non–radical product (ROOH) that cannot propagate the chain reaction

$$ROO^{\bullet} + ArNH \rightarrow ROOH + ArN^{\bullet} \quad (1)$$

For HAT, the N–H bond dissociation enthalpy (BDE) represents one of the important parameters in the evaluation of antioxidant action. The second mechanism,[19–21] single electron transfer followed by proton transfer (SET–PT), takes place in two steps

$$ROO^{\bullet} + ArNH \rightarrow ROO^{-} + ArNH^{+\bullet} \quad (2.1)$$

$$ArNH^{+\bullet} \rightarrow ArN^{\bullet} + H^{+} \quad (2.2)$$

In the first step, $ArNH^{+\bullet}$ radical cation is formed (eq. 2.1). In the second one, deprotonation of $ArNH^{+\bullet}$ occurs (eq. 2.2). Here, melatonin acts as an electron donor and yields an indolyl radical cation.[22–25] Ionization potential (IP) and proton dissociation enthalpy (PDE),[26,27] describe energetic of SET–PT mechanism. Recently, third mechanism of primary antioxidants action has been discovered.[28–31] This two–step mechanism was named sequential proton loss electron transfer (SPLET)

$$ArNH \rightarrow ArN^{-} + H^{+} \quad (3.1)$$

$$ArN^{-} + ROO^{\bullet} \rightarrow ArN^{\bullet} + ROO^{-} \quad (3.2)$$

Mahal et al.[32] using experimental techniques, have indicated that melatonin can react with free radicals by means of SPLET mechanism. The reaction enthalpy of the first step, eq. 3.1, corresponds to the proton affinity, PA, of the ArN^{-} anion.[33–35] In the second step, eq. 3.2, electron transfer from ArN^{-} anion to $ROO^{\bullet}$ occurs and the indolyl radical is formed. The reaction enthalpy of this step is denoted as electron transfer enthalpy, ETE. From the antioxidant action viewpoint, the net result of all three mechanisms is the same, the transfer of the hydrogen atom to the free radical. Reaction enthalpies (BDE, IP, PA) characterizing first steps of three mechanisms are of importance in evaluating the antioxidant action.[36] Understanding the role of different structural features and preparation of new compounds with enhanced antioxidant property is of great interest. Therefore, melatonin molecule (Figure 1A) represents the basic structure. The melatonin structure has two N–H bonds and it can form the two radical isomer: the indole and amide type radical. The spin density value of the radical related nitrogen atom is important in order to investigate the stabilization of two radicals. The difference between them is very important because the unpaired electron in indole–type is delocalized to aromatic ring, whereas in amide–type it is just affected by carbonyl group. Thus, the indole–type radical is more stable than amide–type. Various substituents such as electron–withdrawing groups (EWG) and electron–donating groups (EDG) were located in three available positions on the aromatic ring (Figure 1B). For melatonin and substituted melatonins, energetics of the antioxidant action have not been studied, yet. Therefore, we have systematically investigated the substituent effect on reaction enthalpies of homolytic (HAT mechanism) and heterolytic two–step (SPLET and SET–PT) mechanisms of N–H bond cleavage for mono–substituted melatonins in gas phase and water. Because melatonin is a powerful water–soluble antioxidant, water as the main cell environment was chosen in order to assess the substituent effect on above mentioned enthalpies in solution–phase. Besides, the two–step

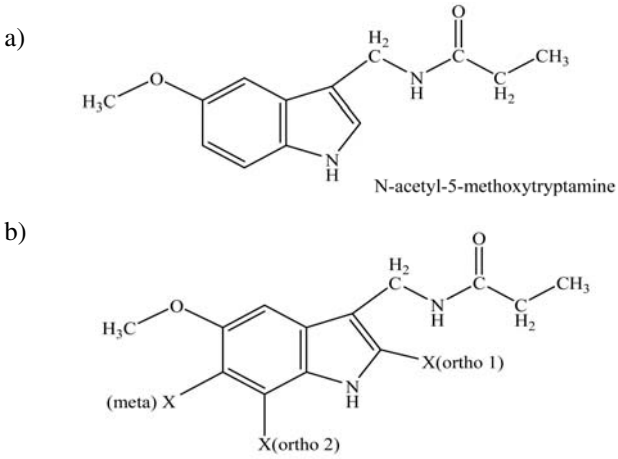

Figure 1. (a) Structure of melatonin. (b) Studied molecules: X = Br, Ethyl, CH = CH_2, CCH, CF_3, Me, Cl, CN, COMe, COH, COOH, F, NMe_2, NHMe, NH_2, NO_2, OMe, OH, Ph, t–Bu.

mechanisms are relevant in solvents with high polarity.[20,29,30] Finally, thermodynamically preferred mechanism in gas–phase and water were identified.

2. Computational Details

The geometry optimization of the molecules and respective radicals, radical cations and anions was performed using DFT method with B3LYP functional,[37–39] and the 6–31G(d, p) basis set,[37,38] in the gas–phase and water. Single point calculations were performed for 6–311++G(2d, 2p) basis set.[40,41] The optimized structures were confirmed to be real minima by frequency calculations. For the species having more conformers, all conformers were investigated. The conformer with the lowest electronic energy was used in this work. All reported enthalpies were zero–point (ZPE) corrected with non–scaled frequencies. The ground–state geometries of molecules were optimized at restricted B3LYP level and the geometry of the indolyl radicals, radical cations and anions were optimized at the restricted B3LYP open shell (half electron) level. Solvent contribution to the total enthalpies was computed employing integral equation formalism IEF–PCM method.[42–48] Since Gaussian 03 allows solution–phase geometry optimization, this approach was used for all studied species. IEF–PCM calculations were performed using default settings of Gaussian 03 program package.[49] Total enthalpies were calculated for 298.15 K and 1.0 atmosphere pressure.

3. Results and Discussion

Total enthalpies of a species X, H(X), at the temperature T, are estimated from equation (4).[20,26,50,51]

$$H(\mathrm{X}) = E_0 + \mathrm{ZPE} + \Delta H_{\mathrm{trans}} + \Delta H_{\mathrm{rot}} + \Delta H_{\mathrm{vib}} + RT \quad (4)$$

where E_0 is the calculated total electronic energy, ZPE stands for zero–point energy, $\Delta H_{\mathrm{trans}}$, ΔH_{rot}, and ΔH_{vib} are the translational, rotational, and vibrational contributions to the enthalpy, respectively. Finally, RT represents PV–work term and it is added to convert the energy to enthalpy. From the calculated total enthalpies we have determined the following quantities:

$$\mathrm{BDE} = \mathrm{H(ArN^{\bullet})} + \mathrm{H(H^{\bullet})} - \mathrm{H(ArNH)} \quad (5)$$

$$\mathrm{IP} = \mathrm{H(ArNH^{+\bullet})} + \mathrm{H(e^{-})} - \mathrm{H(ArNH)} \quad (6)$$

$$\mathrm{PDE} = \mathrm{H(ArN^{\bullet})} + \mathrm{H(H^{+})} - \mathrm{H(ArNH^{+\bullet})} \quad (7)$$

$$\mathrm{PA} = \mathrm{H(ArN^{-})} + \mathrm{H(H^{+})} - \mathrm{H(ArNH)} \quad (8)$$

$$\mathrm{ETE} = \mathrm{H(ArN^{\bullet})} + \mathrm{H(e^{-})} - \mathrm{H(ArN^{-})} \quad (9)$$

The calculated gas–phase enthalpies of proton, $H(H^+)$, and electron, $H(e^-)$, are 6.197 and 3.145 kJ mol^{-1},[52] respectively. IEF–PCM method has been widely adopted in recent years, especially in the description of the thermodynamic characteristics of solvation.[42–57] In previous works,[44,45,48,58–72] IEF–PCM method was applied in the study of phenols and substituted phenylenediamines. Though various PCM approaches do not consider intermolecular hydrogen bonds between solute and solvent molecules explicitly, it was confirmed, that IEF–PCM provides reliable values of solution–phase BDEs, IPs and PAs for substituted phenols, tocopherols, chromans, polyphenols and thiophenols in water, DMSO or benzene.[44, 45,56, 73–87]

For mono–substituted phenols in water or DMSO, Guerra et al.[88] and Fu et al.[89] performed calculations also using model structures with hydrogen–bonded solvent molecules (1–4 molecules). Obtained results were in agreement with both, experimental data and BDEs obtained using IEF–PCM approach in our previous papers.[44,60] For thiophenols, IEF–PCM approach gave calculated bond dissociation enthalpies in accordance to available experimental solution–phase values[84] too. Very recently, PCM calculations were successfully applied in the investigation of antioxidant action of bioactive natural polyphenols and their metabolites in solution–phase.[90,91] Since the enthalpy of electron hydration, $\Delta_{\mathrm{hydr}}H(e^-)$, could not be found in the literature, B3LYP/6–311++G** computed electron hydration enthalpy, $\Delta_{\mathrm{hydr}}H(e^-) = -105$ kJ mol^{-1}, was employed.[45]

3. 1. Bond Dissociation Enthalpies in Gas Phase and Water

Knowledge of BDEs has been accumulating substantially for the past 15–20 years owing to the development of both experimental and quantum chemical techniques.[44,54–63,88–91] In previous studies, substituent and solvent effects on O–H BDEs of substituted phenols and chromans have been investigated in gas– and solution–phase.[44,57–62] These works confirmed that DFT/B3LYP method describes the substituent effect in very good agreement with available experimental results. Klein et al.[63] investigated N–H BDEs of several *para*– and *meta*–substituted anilines using B3LYP and semi–empirical PM3 and AM1 quantum chemical methods. Few N–H BDEs in,[63] were computed also using MP2 method. It was found that B3LYP method describes substituent induced changes in BDEs and IPs in accordance to experimental values.

In this study, all conformers have been optimized and the conformer with highest stability has been used in each case. Melatonin structure has two N–H bonds; therefore it is necessary to calculate the spin density on the radical related atom in order to investigate the stabilization of radicals. Low spin density means that the unpaired electron in

the radical is dispersed perfectly to another part in the molecule, resulting in a more stable radical formation. In previous study, Zhao et al.[64] implicated that melatonin can form two kinds of radicals by N–H bond cleavage – indole– and amide–type of radicals. Their calculations showed that the spin density on N atom in indole–type is 0.3702, while in radical of amide–type it is 0.7369. The unpaired electron in indole–type is delocalized to aromatic ring, whereas in amide–type it is just affected by carbonyl group. Thus, the indole–type radical is more stable than amide–type. The calculated BDE for the basic structure, melatonin, in gas–phase reached 361.8 kJ mol^{-1}. The computed gas–phase BDE and ΔBDE values, where ΔBDE = BDE (X–ArNH) – BDE (ArNH), for substituents placed in *ortho* 1, *ortho* 2 and *meta* positions (Figure 1B) are reported in Tables 1 and 1s (in Supporting material). All tables and figures with denotation "s" are compiled in the Supporting Material. The N–H BDEs of melatonin with strong electron–withdrawing NO_2 group in *ortho* and *meta* positions were higher by ca 27 and 16 kJ mol^{-1} in comparison to BDE of basic structure, respectively. For *ortho 1*, *ortho 2* and *meta*–substituted melatonins with strongest electron–donating NMe_2, NH_2 and NHMe groups, BDE values are by 37, 32 and 22 kJ mol^{-1} lower in comparison to the basic structure, respectively. For *ortho 1* substituted melatonins with halogens the BDE values are by 6.5 kJ mol^{-1} lower in comparison to the basic structure. On the contrary, halogens in *ortho 2* and *meta* positions induce the rise in BDE by 7.5 and 3.5 kJ mol^{-1}, respectively. The N–H BDEs of melatonins with the OH group in *ortho* and *meta* positions were lower by ca 22 and 11 kJ mol^{-1} in comparison to non–substituted melatonine, respectively. For *ortho* and *meta*–substituted melatonins with OMe, the BDE values are by 14 and 9 kJ mol^{-1} lower in comparison to melatonin, respectively. For electron–withdrawing COH, COOH and COMe groups in *ortho 1*, *ortho 2* and *meta* positions, BDE values are higher by 15, 20 and 9 kJ mol^{-1}. The difference between the highest and lowest BDE values for *ortho 1*–, *ortho 2*– and *meta*–substituted melatonins were 64.5, 63.0 and 39.3 kJ mol^{-1}, respectively. These results can be interpreted with a known fact that electron–withdrawing groups in *ortho* and *meta* positions stabilize the parent molecule and destabilize the radical; hence, this results in the increased N–H BDE. However, electron–donating groups show opposite effect, and therefore, their presence leads to a decrease in the N–H BDEs. In water, calculated BDE for the melatonin is 356.6 kJ mol^{-1}. This value is lower than gas–phase one by 6 kJ mol^{-1}.

The computed BDEs and ΔBDEs in the water are also reported in Tables 1 and 1s (in Supporting material). For *ortho 1*–, *ortho 2*– and *meta*–substituted melatonins with NMe_2, NH_2 and NHMe groups, the BDE values are by 25, 29 and 21 kJ mol^{-1} lower in comparison to the basic structure, respectively. For *ortho 1*–substituted melatonins with halogens, the BDE values are by 6 kJ mol^{-1} lower in comparison to the basic structure. For halogens in *ortho 2* and *meta* positions, the BDEs are by 3 and 5 kJ mol^{-1} higher than that of melatonin, respectively. For *ortho 1*–, *ortho 2*– and *meta*–substituted melatonins with COH, COOH and COMe groups, the BDEs are by 11, 13 and 2.5 kJ mol^{-1} higher than BDE of melatonin, respectively. An inspection of the N–H BDE values in Table 1 shows that in water BDE values are lower than the gas–phase ones. Water causes certain changes in enthalpies of studied molecules and radicals. Similar to the gas–phase, electron–donating groups cause a decrease in BDE, whereas electron–withdrawing groups cause an increase in BDE. In water, found differences between the highest and lowest BDEs for *ortho 1*, *ortho 2* and *meta* positions are 44, 48 and 35 kJ mol^{-1}, respectively. In comparison to gas–phase, the effect of substituents on the BDEs is decreased in water. The fundamental reason for obtained results stems from unequal stabilization/destabilization of the parent molecules and the respective radicals in water. Therefore, a decrease in BDEs (negative ΔBDEs) for EDG–substituted melatonins is a combined result of the radical stabilization and the parent molecules destabilization. However, increased BDEs (positive ΔBDEs) for EWG–substituted melatonins seem to be a combination of both, the parents and the radicals destabilization. Obtained BDEs indicate that EWG–substituted melatonins with higher BDEs may exhibit weaker antioxidant activity in comparison to EDG ones in the two studied environments. In *ortho* positions, substituents exert significantly stronger influence upon N–H BDE than in *meta* position. These results are in agreement with previous studies on the *meta*– and *ortho*–substituted phenols and chromans.[19,44,61,65,66]

Table 1. Calculated BDEs (kJ mol^{-1}) of ortho1, ortho2 and meta–substituted melatonins in the gas phase and water.

	ortho1		ortho2		meta	
Substituent	**gas**	**water**	**gas**	**water**	**gas**	**water**
NMe_2	324.6	329.5	330.6	327.5	338.4	336.6
NHMe	322.6	331.4	327.6	326.8	339.3	335.5
NH_2	327.2	333.9	329.8	328.1	338.8	333.8
OH	340.2	334.5	338.1	347.9	350.7	350.9
OMe	348.2	339.8	345.7	348.7	353.2	350.8
t–Bu	360.2	355.6	356.9	356.0	364.3	358.9
Me	360.0	354.4	358.2	354.3	363.9	358.7
Ethyl	360.1	356.3	356.2	354.0	364.0	358.9
$CH=CH_2$	359.6	359.0	354.5	351.0	361.2	355.2
Ph	356.4	350.2	359.4	355.8	363.3	359.0
F	354.3	349.2	369.8	359.0	365.5	361.3
CCH	358.2	358.5	372.2	360.8	364.7	362.8
Cl	355.3	350.0	371.0	360.0	365.1	360.6
Br	356.8	351.1	370.1	359.1	365.2	362.7
COH	375.4	366.5	382.2	372.6	371.6	357.8
COOH	377.0	367.1	385.4	370.4	370.1	360.6
COMe	379.9	369.3	380.6	369.7	371.7	359.5
CF_3	373.5	365.9	376.2	363.4	376.3	365.8
CN	377.1	366.2	376.2	364.4	376.5	364.9
NO_2	387.1	373.4	390.6	374.5	378.1	368.6

In this paper, we also investigated substituent and

solvent effects on indolic nitrogen charge, *q*(N). For DFT optimized geometries, the partial charges (Mulliken population analysis) were obtained for 6–31(d,p) basis set in gas–phase and water. For melatonin, *q*(N) charge reached values of –0.479 and –0.527 in gas–phase and water, respectively. Computed *q*(N) values for investigated molecules are summarized in Table 2. In the presence of electron–donating groups, partial charge on nitrogen becomes more negative. The values in Table 2 show that in the two environments, melatonins with substituents in *meta* position have more negative *q*(N) values in comparison to the *ortho*–substituted ones. In water, *q*(N) values are higher than corresponding gas–phase values.

Table 2. Calculated partial charge on indolyl radical nitrogen, q(N) of ortho1, ortho2 and meta–substituted melatonins in the gas phase and water.

	ortho1		ortho2		meta	
Substituent	**gas**	**water**	**gas**	**water**	**gas**	**water**
NMe_2	–0.644	–0.677	–0.549	–0.586	–0.498	–0.561
NHMe	–0.625	–0.670	–0.546	–0.577	–0.500	–0.578
NH_2	–0.606	–0.668	–0.544	–0.580	–0.502	–0.571
OH	–0.585	–0.657	–0.505	–0.585	–0.492	–0.540
OMe	–0.584	–0.635	–0.488	–0.549	–0.491	–0.540
t–Bu	–0.538	–0.573	–0.497	–0.525	–0.482	–0.530
Me	–0.519	–0.566	–0.488	–0.536	–0.477	–0.532
Ethyl	–0.539	–0.581	–0.496	–0.536	–0.477	–0.531
$CH{=}CH_2$	–0.551	–0.588	–0.499	–0.537	–0.481	–0.529
Ph	–0.546	–0.578	–0.503	–0.536	–0.479	–0.527
F	–0.512	–0.562	–0.478	–0.531	–0.478	–0.526
CCH	–0.510	–0.557	–0.489	–0.539	–0.478	–0.524
Cl	–0.504	–0.556	–0.471	–0.522	–0.473	–0.523
Br	–0.501	–0.558	–0.473	–0.524	–0.474	–0.526
COH	–0.494	–0.557	–0.458	–0.518	–0.473	–0.520
COOH	–0.501	–0.559	–0.460	–0.518	–0.468	–0.521
COMe	–0.487	–0.551	–0.460	–0.516	–0.471	–0.514
CF_3	–0.510	–0.533	–0.479	–0.529	–0.468	–0.515
CN	–0.497	–0.534	–0.482	–0.534	–0.468	–0.512
NO_2	–0.484	–0.535	–0.461	–0.516	–0.465	–0.510

Previous studies,[48,61] showed that O–H BDE and ΔBDE values of mono–substituted phenols and chromans depend on calculated phenoxy radical oxygen, *q*(O), charge linearly. Therefore, we tried to correlate BDEs and ΔBDEs with charge on indolyl radical nitrogen, *q*(N). For *meta*–substituted molecules, BDEs are plotted against *q*(N) in Figure 2. The correlation coefficients reached 0.96 and 0.98 for gas–phase and water, respectively. Obtained equations are as follows:

$$\text{BDE (kJ mol}^{-1}) = 814.5 \times q(\text{N}) + 754.4 \quad \text{(gas)} \quad (10)$$

$$\text{BDE (kJ mol}^{-1}) = 691.0 \times q(\text{N}) + 725.2 \quad \text{(water)} \quad (11)$$

Analogous BDE = *f*(*q*(N)) dependences for the *ortho 1*– and *ortho 2*– substituted melatonins are plotted in Figures 1s and 2s (in Supporting material), respectively. For *ortho 1*–substituted melatonins, the correlation coefficients reached values of 0.91 and 0.90 in gas– and solution–phase, respectively. From the linear regressions, we obtained these equations:

$$\text{BDE (kJ mol}^{-1}) = 348.3 \times q(\text{N}) + 544.7 \quad \text{(gas)} \quad (12)$$

$$\text{BDE (kJ mol}^{-1}) = 253.7 \times q(\text{N}) + 501.4 \text{ (water)} \quad (13)$$

For *ortho 2*–substitued melatonins, the correlation coefficients reached 0.93 and 0.92 in gas–phase and solution phase, respectively. Obtained equations are as follows

$$\text{BDE (kJ mol}^{-1}) = 765.9 \times q(\text{N}) + 742.3 \quad \text{(gas)} \quad (14)$$

$$\text{BDE (kJ mol}^{-1}) = 821.8 \times q(\text{N}) + 798.9 \text{ (water)} \quad (15)$$

These results show that N–H BDE values grow with increasing *q*(N). Therefore, there is a good agreement between calculated BDEs with *q*(N) for, *ortho 1– ortho 2–* and *meta*–substituted melatonins.

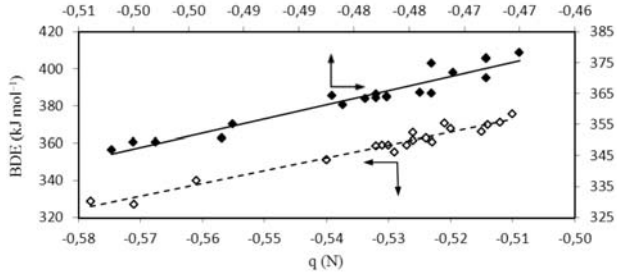

Figure 2. Dependence of BDE on q(N) for meta substituted melatonins in gas phase (solid quares, solid line, top x–axis, right y–axis) and water (open squares, dashed line, bottom x–axis, left y–axis).

3. 2. Ionization Potentials in Gas Phase and Water

IP represents the reaction enthalpy of the first step in the SET – PT mechanism (eq. 2.2). Previously, the substituent effect on gas–phase IPs in the case of substituted phenols and chromans have been investigated employing B3LYP approach.[44,59,62] Klein et al.[63] also investigated IP values of several substituted anilines using various quantum chemical methods. This paper represents the first theoretical systematic study of substituted melatonins IPs. Martinez et al.[25] reported experimental gas–phase value of 658 kJ mol^{-1} for melatonin. In this paper, the calculated gas–phase IP for non–substituted melatonin reached 668.2 kJ mol^{-1}. The computed gas–phase IPs and ΔIP values, where ΔIP = IP(X–ArNH) – IP(ArNH), are reported in Tables 3 and 2s (in Supporting material), respectively. For *ortho 1–*, *ortho 2–* and *meta*–substituted melatonins with strong EDGs – NMe_2, NH_2 and NHMe, IP values are

by 77, 68 and 54 kJ mol^{-1} lower in comparison to melatonin, respectively. Decrease in IP of the molecules with OH group in *ortho* and *meta* positions are ca 50 and 28 kJ mol^{-1}, respectively. For *ortho*– and *meta*–substituted melatonins with OMe, decrease in IP reached 20 kJ mol^{-1}. Halogens in *ortho 1*, *ortho 2* and *meta* positions induce 23, 11 and 22 kJ mol^{-1} rise in IP, respectively. For *ortho 1*–, *ortho 2*– and *meta*–substituted melatonins with COH, COOH and COMe, IP values are by ca 25, 28 and 35 kJ mol^{-1} higher, respectively. The IP values of melatonins with the strongest electron–withdrawing NO_2 group in *ortho 1*, *ortho 2* and *meta* positions were higher by ca 75, 67 and 55 kJ mol^{-1} in comparison to non–substituted melatonin, respectively. Found differences between the highest and lowest IP values for *ortho 1*, *ortho 2* and *meta* positions are 157.7, 138.2 and 112.7 kJ mol^{-1}, respectively. Highest IP values were found for strong EWG substituents (NO_2, CF_3 and CN), while the lowest ones were obtained for strong EDG substituents (NMe_2, NH_2, and NHMe). The decrease in IPs (negative ΔIPs) in EDG–substituted molecules is a combined result of the radical cations stabilization and the parent molecules destabilization. However, increased IPs (positive ΔIPs) of EWG–substituted structures may stem from the combination of both, the molecules and the radical cations destabilization.

In this paper, effect of water as a highly polar solvent on IPs and ΔIPs of substituted melatonins has been investigated too. In water, determination of IP requires the value of electron hydration enthalpy, $\Delta_{hydr}H(e^-)$. In accordance to our previous papers,[45,60–62,84] we used electron hydration enthalpy $\Delta_{hydr}H(e^-) = -105$ kJ mol^{-1} found using B3LYP/6–311++G** approach described in.[45] Electron affinity of water EA = 125 kJ mol^{-1} (as the energy released by electron attachment to neutral water molecule) was experimentally determined in.[92] This value is in fair accordance with employed calculated value.[45] Recently, Fifen et al.[93] also calculated hydration enthalpy of electron and obtained value of –104.4 kJ mol^{-1}. Potential inaccuracies related to employed electron hydration enthalpy value will be canceled when the substituent effect is expressed in terms of ΔIPs. Partly due to the negative enthalpy of electron hydration in water, calculated melatonin IP in water is lower than the gas – phase value by ca 240 kJ mol^{-1}.

All computed IPs and ΔIPs in water are compiled in Tables 3 and 2s (in Supporting material), respectively. Water causes considerable changes in the enthalpies of molecules and radical cations of studied structures. For strong EDGs, i.e. NMe_2, NH_2 and NHMe, in *ortho 1*, *ortho 2* and *meta* positions, found drops in IP values are 70, 60 and 47 kJ mol^{-1}, respectively. For OH group in *meta* and *ortho* positions, decrease in IP reached ca 18 and 38 kJ mol^{-1}, respectively. For halogens in *ortho 1* position, IPs are by 15 kJ mol^{-1} higher than in non–substituted melatonin. For halogens in *ortho 2* and *meta* position increase in IP reached 12 kJ mol^{-1}. For *ortho*– and *meta*–substituted melatonins with strong electron–withdrawing NO_2 group, the rise in IP value is ca 40 and 34 kJ mol^{-1}, respectively. In water, substituent induced changes in IPs are in 83.9 (*meta*), 106.7 (*ortho 1*) and 103.1 kJ mol^{-1} (*ortho 2*) ranges. Highest IP values were found for strong EWG–substituents and lowest ones for strong EDG–substituents Therefore, EDG–substituted melatonins should release an electron easily. The EWG–substituents stabilize the parent molecule and destabilize the radical cation. On the other hand, EDG–substituents have an opposite effect. Water causes ca 12 kJ mol^{-1} attenuation of substituent effect in terms of narrower ΔIP range. Again, substituents in *ortho* positions exert stronger influence upon IP than the same substituents in *meta* position. Values in Table 3 also reveal that IPs in water are considerably lower than corresponding gas–phase ones.

Table 3. Calculated IPs (kJ mol^{-1}) of ortho1, ortho2 and meta–substituted melatonins in the gas phase and water.

	ortho1		ortho2		meta	
Substituent	**gas**	**water**	**gas**	**water**	**gas**	**water**
NMe_2	593.1	350.9	601.9	360.4	613.6	368.2
NHMe	585.3	343.7	597.3	356.9	615.7	371.7
NH_2	593.9	352.6	603.9	359.9	610.6	372.9
OH	617.6	379.2	618.0	380.6	639.6	400.1
OMe	646.9	390.8	649.0	394.9	649.2	404.7
t–Bu	657.6	411.4	658.0	405.0	640.7	414.6
Me	657.8	410.8	656.5	408.8	645.9	417.1
Ethyl	652.6	414.2	659.5	409.2	641.9	415.2
$CH{=}CH_2$	661.5	408.4	662.7	409.4	655.2	413.8
Ph	649.6	415.2	654.4	412.3	660.7	423.3
F	692.9	434.5	678.4	429.6	688.2	430.9
CCH	679.2	430.2	677.3	427.2	681.8	422.0
Cl	690.0	432.6	680.6	430.0	692.0	428.8
Br	688.7	432.3	679.4	430.5	690.8	430.6
COH	690.8	440.4	698.8	439.5	702.7	436.7
COOH	696.9	437.3	694.0	440.1	705.1	432.7
COMe	692.1	433.0	696.0	438.8	702.9	437.4
CF_3	718.5	448.3	720.3	446.8	705.7	439.2
CN	731.9	452.4	726.8	453.7	719.7	444.8
NO_2	743.0	457.6	735.5	460.0	723.3	452.1

In the recent study,[67] it was found that IP values can be successfully correlated with phenolic O–H bond lengths, denoted as R(O–H$^{+\bullet}$), in radical cations formed from *meta*–substituted chromans, both in gas–phase and water. In this paper we tried to correlate calculated IP values with N–H bond lengths in indolyl radical cations, R(N–H)$^{+\bullet}$. The values of R(N–H)$^{+\bullet}$ corresponding to *ortho 1*–, *ortho 2*– and *meta*–substituted melatonins in two environments are compiled in Table 4.

In Figure 3, IP values for *meta*–substituted melatonins are plotted against R(N–H)$^{+\cdot}$ The correlation coefficients reached 0.93 and 0.97 in gas–phase and water, respectively. From the linear regressions, we obtained these

Table 4. Calculated R(N–H$^{+\bullet}$) bond lengths (Å) of ortho1, ortho2 and meta–substituted melatonins in the gas phase and water.

	ortho1		ortho2		meta	
Substituent	**gas**	**water**	**gas**	**water**	**gas**	**water**
NMe_2	1.00803	1.00868	1.00715	1.00793	1.00803	1.00931
NHMe	1.00814	1.00952	1.00778	1.00858	1.00891	1.00930
NH_2	1.00819	1.01020	1.00982	1.01082	1.00926	1.00969
OH	1.01067	1.01139	1.01259	1.01262	1.01051	1.01196
OMe	1.01119	1.01113	1.01184	1.01224	1.01030	1.01160
t–Bu	1.01181	1.01249	1.01014	1.01074	1.01133	1.01273
Me	1.01295	1.01348	1.01225	1.01277	1.01146	1.01263
Ethyl	1.01240	1.01377	1.01174	1.01267	1.01133	1.01234
$CH{=}CH_2$	1.01060	1.01169	1.01123	1.01195	1.01007	1.01128
Ph	1.01006	1.01149	1.01214	1.01271	1.01064	1.01254
F	1.01337	1.01478	1.01311	1.01343	1.01286	1.01351
CCH	1.01224	1.01354	1.01319	1.01371	1.01158	1.01301
Cl	1.01322	1.01394	1.01328	1.01374	1.01215	1.01370
Br	1.01284	1.01369	1.01398	1.01414	1.01201	1.01368
COH	1.01663	1.01481	1.01803	1.01585	1.01348	1.01449
COOH	1.01677	1.01523	1.01852	1.01642	1.01320	1.01436
COMe	1.01684	1.01565	1.01896	1.01634	1.01274	1.01445
CF_3	1.01592	1.01376	1.01377	1.01367	1.01378	1.01458
CN	1.01563	1.01394	1.01494	1.01445	1.01362	1.01481
NO_2	1.01613	1.01527	1.01646	1.01515	1.01265	1.01484

equations:

$$\text{IP (kJ mol}^{-1}) = 22662 \times R(\text{N–H, Å})^{+\bullet} - 22255 \quad \text{(gas)} \tag{16}$$

$$\text{IP (kJ mol}^{-1}) = 16977 \times R(\text{N–H, Å})^{+\bullet} - 16776 \quad \text{(water)} \tag{17}$$

Analogous dependences for substituents in *ortho 1* and *ortho 2* positions are plotted in Figures 3s and 4s (in Supporting material), respectively. For *ortho 1* position, the correlation coefficients reached values of 0.90 and 0.92 in gas–phase and water, respectively. Equations obtained from the linear regressions are as follows:

$$\text{IP (kJ mol}^{-1}) = 13600 \times R(\text{N–H, Å})^{+\bullet} - 13105 \quad \text{(gas)} \tag{18}$$

$$\text{IP (kJ mol}^{-1}) = 15456 \times R(\text{N–H, Å})^{+\bullet} - 15241 \quad \text{(water)} \tag{19}$$

For *ortho 2* position, correlation coefficients reached values of 0.90 and 0.91 in gas–phase and water, respectively. Using linear regression, we found these dependencies:

$$\text{IP (kJ mol}^{-1}) = 10342 \times R(\text{N–H, Å})^{+\bullet} - 10292 \quad \text{(gas)} \tag{20}$$

$$\text{IP (kJ mol}^{-1}) = 11614 \times R(\text{N–H, Å})^{+\bullet} - 11348 \quad \text{(water)} \tag{21}$$

IP values grow with the increasing $R(\text{N–H})^{+\bullet}$. The linearity of found dependences can be considered satisfactory and corresponding equations may be used for rough estimation of IPs from $R(\text{N–H})^{+\bullet}$ in substituted melatonins or vice versa. $R(\text{N–H})^{+\bullet}$ bond lengths may be also applied as a criterion of a proposed compound suitability, since they correlate with IP values relatively well.

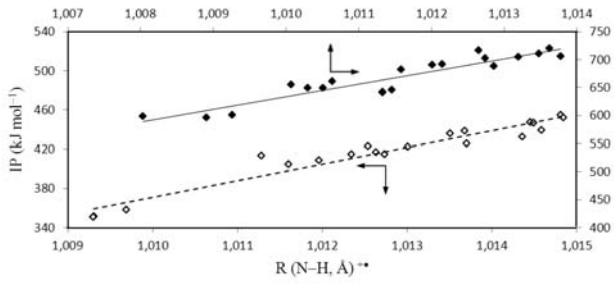

Figure 3. Dependence of IP on R(N–H$^{+\bullet}$) for meta substituted melatonins in gas–phase (solid squares, solid line, top x–axis, right y–axis) and water (open squares, dashed line, bottom x–axis, left y–axis).

To accelerate the discovery of novel antioxidants, considerable effort has been devoted to investigating the structure–activity relationships (SARs) for antioxidants. Furthermore, rational design strategies for antioxidants have been proposed and applied in research. It was shown that IPs determined using the DFT computational approach are sufficiently accurate to characterize the electron–donating ability of antioxidants.[68,69] The energy of

the highest occupied molecular orbital (E_{HOMO}) represents an alternative parameter to assess the electron–donating ability of antioxidants. This is widely used in antioxidant study,[71,72] because of the simple calculation procedure, where only calculation for parent molecule is required. Besides, according the Koopmans' theorem,[70] vertical IP_v value can be estimated from E_{HOMO}, $IP_v = -E_{HOMO}$. Therefore, HOMO energy represents an applicable parameter for prediction of antioxidant activity,[73,74] and oxidant scavenging ability,[75] *via* SET–PT mechanism. In previous studies,[76,77] gas–phase E_{HOMO} for melatonin molecule was calculated using semi–empirical (AM1) and DFT methods. Obtained values were –5.4 (AM1) and –5.1 eV (DFT). In this paper, found E_{HOMO} for melatonin in gas–phase and water are –5.11 and –5.26 eV, respectively. As a general rule, the higher the E_{HOMO}, the more active the compound is as an antioxidant.[75,78] The computed E_{HOMO} values of investigated melatonins in gas–phase and water are summarized in Table 5.

Table 5. Calculated E_{HOMO} (eV) of ortho1, ortho2 and meta–substituted melatonins in the gas phase and water.

	ortho1		ortho2		meta	
Substituent	**gas**	**water**	**gas**	**water**	**gas**	**water**
NMe_2	–4.55	–4.72	–4.85	–4.97	–5.04	–5.03
NHMe	–4.25	–4.56	–4.87	–4.99	–4.96	–5.07
NH_2	–4.52	–4.50	–4.80	–4.90	–4.82	–4.97
OH	–4.61	–4.95	–4.89	–5.06	–5.04	–5.27
OMe	–5.13	–5.06	–4.85	–5.04	–5.14	–5.26
t–Bu	–4.99	–5.21	–5.04	–5.20	–5.21	–5.31
Me	–4.96	–5.20	–5.03	–5.19	–5.19	–5.32
Ethyl	–4.96	–5.22	–5.03	–5.19	–5.17	–5.31
$CH{=}CH_2$	–5.07	–5.28	–5.08	–5.34	–5.19	–5.34
Ph	–5.06	–5.28	–5.07	–5.23	–5.35	–5.44
F	–5.30	–5.41	–5.20	–5.32	–5.49	–5.54
CCH	–5.26	–5.41	–5.20	–5.35	–5.45	–5.55
Cl	–5.36	–5.46	–5.33	–5.43	–5.60	–5.62
Br	–5.33	–5.45	–5.33	–5.42	–5.58	–5.62
COH	–5.41	–5.52	–5.52	–5.58	–5.61	–5.60
COOH	–5.39	–5.49	–5.42	–5.53	–5.68	–5.70
COMe	–5.33	–5.45	–5.40	–5.50	–5.65	–5.67
CF_3	–5.45	–5.50	–5.50	–5.54	–5.72	–5.69
CN	–5.69	–5.61	–5.67	–5.64	–5.88	–5.69
NO_2	–5.84	–5.73	–5.82	–5.81	–5.97	–5.87

These reveal that in the case of EWG–substituents, E_{HOMO} values become more negative, while the presence EDG–substituents results in less negative E_{HOMO} values. Therefore, melatonins with strong electron–donating groups are better electron donors, i.e. they enter SET–PT mechanism more easily. In our previous study of mono–substituted anilines, phenols and thiophenols, we found that B3LYP/6–311+G(2d,2p) method significantly (by ca 2 eV) underestimates vertical gas–phase ionization potentials obtained from E_{HOMO} according to Koopmans' theorem.[63] However, the trends in ionization potentials, in terms of ΔIPs, are described reliably. Therefore, we decided to find expected linear dependence between calculated IPs and corresponding $-E_{HOMO}$ values (Figure 4). For *meta*–substituted molecules, correlation coefficients in gas–phase and water reached 0.95 and 0.92, respectively. Obtained equations are as follows:

$$IP\ (kJ\ mol^{-1}) = 1.28 \times (-E_{HOMO})\ (kJ\ mol^{-1}) + 74 \quad (gas) \tag{22}$$

$$IP\ (kJ\ mol^{-1}) = 1.06 \times (-E_{HOMO})\ (kJ\ mol^{-1}) - 84 \quad (water) \tag{23}$$

Analogous IP= $f(-E_{HOMO})$ dependences for melatonins with substituents in *ortho 2* and *ortho 1* positions are depicted in Figures 5s and 6s (in Supporting material), respectively. For groups in *ortho 1* position, the correlation coefficients reached 0.97 and 0.98 in gas phase and water, respectively. Equations obtained from the linear regressions are as follows:

$$IP\ (kJ\ mol^{-1}) = 1.25 \times (-E_{HOMO})\ (kJ\ mol^{-1}) + 110 \quad (gas) \tag{24}$$

$$IP\ (kJ\ mol^{-1}) = 1.14 \times (-E_{HOMO})\ (kJ\ mol^{-1}) - 110 \quad (water) \tag{25}$$

For substituents in *ortho 2* position, correlation coefficients reached 0.94 (gas–phase) and 0.96 (water) and following equations were found

$$IP\ (kJ\ mol^{-1}) = 1.5 \times (-E_{HOMO})\ (kJ\ mol^{-1}) + 184 \quad (gas) \tag{26}$$

$$IP\ (kJ\ mol^{-1}) = 1.4 \times (-E_{HOMO})\ (kJ\ mol^{-1}) - 220 \quad (water) \tag{27}$$

Obtained equations enable fast IP estimations for *meta* and *ortho*–substituted melatonins from the computed E_{HOMO} values. This can be useful in the selection of suitable candidates for the synthesis of novel melatonin derivatives with enhanced antioxidant properties.

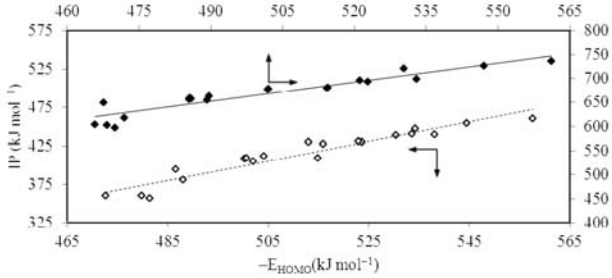

Figure 4. Dependence of IP on $-E_{HOMO}$ for meta substituted melatonins in gas–phase (solid squares, solid line, top x–axis, right y–axis) and water (open squares, dashed line, bottom x–axis, left y–axis).

3. 3. Proton Affinities in gas–Phase and Water

PA represents the reaction enthalpy of the first step in SPLET mechanism (eq. 3.1). For substituted melatonins, SPLET was not studied, yet. Substituent effect on PAs was theoretically studied only for mono–substituted chromans, phenols and thiophenols in gas–phase and several solvents.[33,44,50,61,66,84] Chandra et al.[79] calculated PA values also for substituted pyridinethiols in the gas phase. Found gas–phase PA for the basic structure (melatonin) is 1446 kJ mol^{-1}. The computed PA and ΔPA values for the substituents in *ortho 1, ortho 2* and *meta* positions in gas–phase are reported in Tables 6 and 3s (in Supporting material), respectively. Strongest electron–donating groups induce the largest rise in PAs. Compared to non–substituted melatonin, for melatonins with NMe_2, NH_2 and NHMe groups in *ortho 1*, *ortho 2* and *meta* positions, PA values are by 22, 20 and 14 kJ mol^{-1} higher, respectively. Alkyl substituents in the three positions induce 5–7.5 kJ mol^{-1} increase in PA. Halogens in *ortho 1*, *ortho 2* and *meta* positions cause decrease in PA by 21, 15 and 8 kJ mol^{-1}, respectively. For *ortho 1*, *ortho 2* and *meta*–substituted melatonins with COH, COOH and COMe PAs are by 29, 23 and 19 kJ mol^{-1} lower than PA of melatonin, respectively. For strong electron–withdrawing NO_2 substituent in *meta*, *ortho 1* and *ortho 2* positions, drops in PA reached largest values: ca 70, 62 and 52 kJ mol^{-1}, respectively. The differences between the highest and lowest gas–phase PA values for *ortho 1*, *ortho 2* and *meta*–substituents were 94.1, 83.7 and 68.2 kJ mol^{-1}, respectively.

In the literature, there are only two experimental works devoted to gas–phase proton affinities of the mono–substituted phenols available,[34,35] therefore reliability of obtained PAs cannot be verified directly. B3LYP method, however, provided PAs of various mono–substituted phenols,[44] in very good agreement with published experimental values. In accordance with previous studies on substituted phenols and chromans,[33,34,50,76,67] it can be concluded that EDG substituents increase PA, whereas EWG ones decrease it. It is known that a charged molecule is more sensitive to the effect of substituent than its neutral counterpart. EWG substituents stabilize ArN^- but destabilize the parent molecule. EDG substituents show an opposite effect.[27,61,67,80–82]

In this work, water was employed in order to understand polar solvent and substituent effects on PA. For PA calculations in water, proton hydration enthalpy, $\Delta_{hydr}H(H^+)$, is inevitable. Therefore, we utilized $\Delta_{hydr}H(H^+)$ = –1022 kJ mol^{-1} value from.[45] Although we use H^+ denotation here, actually it represents H_3O^+ cation, as it is clear from the calculation procedure for the corresponding hydration enthalpy: proton was attached to one molecule of water (resulting in H_3O^+) and its enthalpy was computed using IEF–PCM method.[45] Although obtained value is lower than the experimental one (–1090 kJ mol^{-1}),[53] it is in good agreement with other published theoretical values. Mejías and Lago,[94] found proton hydration enthalpy of –999.10 kJ mol^{-1}. Fifen et al.[93] found $\Delta_{hydr}H(H^+)$ = –1024.3 kJ mol^{-1}. For proton hydration Gibbs free energy in,[95] theoretically determined value was $\Delta_{hydr}G(H^+)$ = –1098 kJ mol^{-1}. Potential inaccuracies (i.e. less negative $\Delta_{hydr}H(H^+)$ in comparison to experiment) stemming from the employed proton hydration enthalpy are canceled if the substituent effect is described in terms of ΔPAs. More negative $\Delta_{hydr}H(H^+)$ would result in even lower proton affinities in water. In water, PA for the basic structure reached 243 kJ mol^{-1}. For substituted melatonins, PAs and ΔPAs in water are reported in Tables 6 and 3s (in Supporting material), respectively. Water causes considerable changes in the enthalpies of anions. Calculated PA for melatonin in water is lower than gas–phase value by 1403 kJ mol^{-1}. Mainly due to the large negative enthalpy of H^+ hydration, PAs in the water are significantly lower than the gas–phase ones. However, the trends in PAs are maintained in agreement with results for mono–substituted phenols and chromans in water.[60,61,67] Again, strong electron–donating NMe_2, NH_2 and NHMe cause an increase in PA. For *ortho 1*–, *ortho 2*– and *meta*–substituted melatonins, PA values are by 17.3, 11.5 and 8 kJ mol^{-1} higher in comparison to the basic structure, respectively. The presence of OH group in *ortho 1*, *ortho 2* and *meta* positions results in 13, 8 and 5 kJ mol^{-1} PA growth, respectively. For halogens in *ortho 1*, *ortho 2* and *meta*–positions, the drops in PAs reached 17, 12 and 7.5 kJ mol^{-1}, respectively. The largest decrease in PA shows melatonin with NO_2 group in *ortho* (43 kJ mol^{-1}) or *meta* (29 kJ mol^{-1}) positions. Differences between the highest

Table 6. Calculated PAs (kJ mol^{-1}) of ortho1, ortho2 and meta–substituted melatonins in the gas phase and water.

	ortho1		ortho2		meta	
Substituent	**gas**	**water**	**gas**	**water**	**gas**	**water**
NMe_2	1470.2	259.9	1468.5	254.6	1459.2	251.7
NHMe	1469.3	261.1	1464.4	255.2	1462.5	250.3
NH_2	1469.2	262.3	1467.0	255.7	1460.8	252.9
OH	1464.0	257.3	1458.9	251.8	1459.4	248.7
OMe	1459.9	251.7	1459.5	250.3	1454.1	247.1
t–Bu	1452.2	250.1	1455.1	248.8	1453.8	248.9
Me	1453.9	249.0	1452.7	247.1	1451.4	248.1
Ethyl	1451.0	248.8	1453.4	248.1	1452.1	249.4
$CH{=}CH_2$	1437.5	242.3	1434.2	238.8	1438.9	239.8
Ph	1434.8	236.5	1434.4	244.9	1432.1	238.8
F	1424.7	224.4	1430.7	231.3	1438.7	235.2
CCH	1421.4	228.7	1438.5	234.6	1438.7	237.7
Cl	1425.7	226.7	1431.9	231.0	1437.8	236.1
Br	1424.8	228.2	1432.3	232.1	1436.4	237.1
COH	1414.2	226.4	1420.7	228.3	1425.4	229.6
COOH	1421.5	228.7	1423.1	229.9	1427.3	230.5
COMe	1418.3	230.1	1421.9	230.6	1430.2	231.7
CF_3	1400.2	213.7	1398.7	214.7	1409.5	223.1
CN	1385.5	208.6	1392.8	213.6	1404.4	224.3
NO_2	1376.1	200.6	1384.8	201.7	1394.3	215.0

and lowest PA values for three studied positions were 61.7 (*ortho 1*), 68.2 (*ortho 2*) and 37.9 kJ mol^{-1} (*meta*). It confirms that water attenuates substituent induced changes.

We have also performed linear correlation of PA values with E_{HOMO} in the two environments. Obtained dependences for substituents in *meta* position are shown in Figure 5. Correlation coefficients reached 0.96 (gas–phase) and 0.95 (water). Following regression lines were obtained

$$PA\ (kJ\ mol^{-1}) = -0.65 \times E_{HOMO}\ (kJ\ mol^{-1}) + 1700 \quad (gas) \qquad (28)$$

$$PA\ (kJ\ mol^{-1}) = -0.46 \times E_{HOMO}\ (kJ\ mol^{-1}) + 460 \quad (water) \qquad (29)$$

Dependences for substituents in *ortho 1* and *ortho 2* positions are plotted in Figures 7s and 8s (in Supporting material), respectively. For *ortho 1*, the correlation coefficients of found dependences

$$PA\ (kJ\ mol^{-1}) = -0.74 \times E_{HOMO}\ (kJ\ mol^{-1}) + 1760 \quad (gas) \qquad (30)$$

$$PA\ (kJ\ mol^{-1}) = -0.56 \times E_{HOMO}\ (kJ\ mol^{-1}) + 490 \quad (water) \qquad (31)$$

reached 0.95 (gas–phase) and 0.91 (water). In the case of *ortho 2* position, we have obtained these equations

$$PA\ (kJ\ mol^{-1}) = -0.93 \times E_{HOMO}\ (kJ\ mol^{-1}) + 1850 \quad (gas) \qquad (32)$$

$$PA\ (kJ\ mol^{-1}) = -0.68 \times E_{HOMO}\ (kJ\ mol^{-1}) + 550 \quad (water) \qquad (33)$$

with correlation coefficients 0.96 (gas–phase) and 0.95 (water). The positive line slopes reflect the fact that the EDG–substituents increase PA, as well as the absolute value of E_{HOMO}. On the other hand, EWG–substituents cause decrease in PAs and absolute E_{HOMO} values.

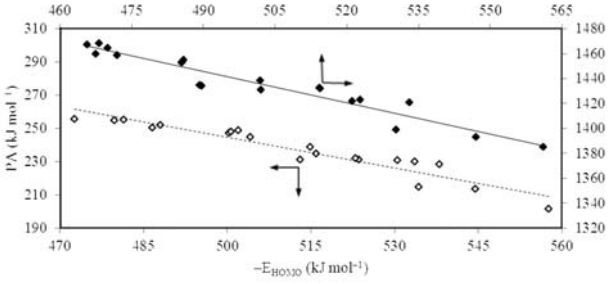

Figure 5. Dependence of PA on $-E_{HOMO}$ for meta substituted melatonins in gas–phase (solid squares, solid line, top x–axis, right y–axis) and water (open squares, dashed line, bottom x–axis, left y–axis).

Obtained equations may be used to predict PAs for substituted melatonins from their E_{HOMO}. Fundamental statistics data for all performed linear regressions, i.e. values of the line slopes and intercepts, as well as their standard deviations are compiled together with corresponding *P*–values in Table 4s (in Supporting material). We can conclude that E_{HOMO} can be employed for fast estimations of reaction enthalpies for the first steps of both investigated two–step SET–PT and SPLET mechanisms.

3. 4. Proton Dissociation Enthalpies and Electron Transfer Enthalpies in Gas–phase and Water

PDE and ETE represent the reaction enthalpies of the second steps in SET–PT and SPLET mechanisms, respectively. For the whole SET–PT and SPLET energetics knowledge, it is also important to study PDEs and ETEs and to investigate the solvent and substituent effects on these reaction enthalpies.

In previous papers,[20,27,44,61,67] the substituent effect on PDEs for substituted phenols have been theoretically investigated by DFT using B3LYP functional. In recent papers,[61,67] the PDE and ETE values of *ortho*– and *meta*–substituted chromans have been calculated in gas– and solution–phase. There are no experimental PDEs available yet. PDE values for amine–type antioxidants have not been studied previously. Calculated PDEs for the melatonin reached 1014 kJ mol^{-1} in gas–phase and 136 kJ mol^{-1} in water. PDE of melatonin in water is lower by 878 kJ mol^{-1}. Mainly, due to the large enthalpy of proton hydration, PDEs in water are significantly lower than gas–phase values. Water also causes considerable changes in the enthalpies of radicals and radical cations of studied structures.

Computed PDE values for the substituents in *ortho* and *meta* positions in gas–phase and water are reported in Table 5s (in Supporting material). Highest PDEs were found for strong EDG substituents (NMe_2, NH_2, and NHMe), whereas lowest PDEs were obtained for strong EWG substituents (NO_2, CF_3 and CN). This trend is opposite to that observed for PAs. It is known that electron–donating groups stabilize $ArNH^{+\bullet}$ but destabilize the parent structure, while electron–withdrawing groups have an opposite effect.[27,80,81] These results are in agreement with previous papers on substituted phenols and chromans.[20,27,44,61,67]

For substituted melatonins, ETEs were not studied previously. In literature, only DFT/B3LYP ETEs of substituted phenols and chromans,[44,61,67,83] are available. Calculated ETE values for melatonin reached 236 kJ mol^{-1} in gas–phase and 310 kJ mol^{-1} in water. The computed ETEs for molecules with substituents in *ortho* and *meta* positions are compiled in Table 5s (in Supporting material). In gas–phase and water, highest ETEs were found for strong EWG substituents (NO_2, CF_3 and CN). Lowest ETEs were found in the case of strong EDG sub-

stituents (NMe_2, NH_2, NHMe). This trend corroborates to that observed for BDEs and IPs. It is known that electron withdrawing groups are favorable to stabilize ArN^-. Electron donating groups have an opposite effect. Therefore, electron withdrawing groups increase ETE values, while electron donating groups decrease ETEs.[27,79–82]

3. 5. Thermodynamically Preferred Mechanism

In general, free energy represents the criterion of the thermodynamically preferred process. However, in the case of studied reactions the absolute values of the entropic term $-T\Delta_rS$ reach few tens of kJ mol^{-1},[45,84–87] and all free energies, $\Delta_rG = \Delta_rH - T\Delta_rS$, are only shifted in comparison to corresponding enthalpies. Klein et al.[45,61] calculated reaction free energies for *p*–phenylenediamine, tetracyano–*p*–phenylenediamine and substituted chromans and compared these values with corresponding reaction enthalpies (BDE, IP and PA). To verify employed assumption that entropic term $-T\Delta_rS$ should not affect obtained results significantly; we have also calculated reaction free energies of all studied reactions, eqs. (5)–(9). Reaction free energies related to hydrogen atom transfer, eq. (1), are by 6–10 kJ mol^{-1} higher than BDEs in water. In the case of process described in eq. (2–1), free energies reached almost the same values as IPs, differences are in 2–4 kJ mol^{-1} range. For the first step of SPLET mechanism, eq. (3–1), differences between the free energies and PAs reached largest values. Gibbs free energies are higher by 15–22 kJ mol^{-1}. All observed trends are valid for free energies too. Therefore, comparison of BDEs, PAs and IPs can indicate which mechanism is thermodynamically preferred.

Calculated gas–phase IPs and PAs of substituted melatonins are significantly higher, by 280–340 and 1020–1130 kJ mol^{-1}, than BDEs, respectively. Therefore, HAT mechanism represents the most anticipated process in the gas–phase from the thermodynamic point of view. In water, PA values are lower than BDE and IP values by 80–170 and 100–250 kJ mol^{-1}, respectively. In water, IP values remain still higher than BDEs by ca 40–80 kJ mol^{-1}, respectively. Significantly lower PAs indicate that SPLET represents the thermodynamically preferred reaction pathway in water.

4. Conclusions

In this article, the reaction enthalpies of the individual steps of three antioxidant action mechanisms, HAT, SET–PT and SPLET, for various mono–substituted melatonins were calculated in gas–phase and water. The melatonin structure has two N–H bonds and it can form the two radical isomers: the unpaired electron in indole–type is delocalized to aromatic ring, whereas in amide–type it is just affected by carbonyl group. Thus, the indole–type radical is more stable than amide–type. Obtained results indicate that electron–donating substituents induce rise in PDEs and PAs, whereas electron–withdrawing groups cause increase in the reaction enthalpies of the processes, where electron (IPs and ETEs) or hydrogen atom is abstracted (BDEs). Substituents placed in *ortho* positions show larger effect on reaction enthalpies in comparison to the same groups in *meta* position. The ortho isomer can be considered as structure with the high antioxidant activity than meta isomer, because in ortho position the substituent can form intramolecular hydrogen bonds and steric effects are able to considerably stabilize the parents and radicals. Water attenuates the substituent effect on all reaction enthalpies. In the gas phase, BDEs are lower than PAs and IPs, i.e. HAT represents the thermodynamically preferred pathway. On the other hand, SPLET mechanism represents the thermodynamically favored process in water. IP and BDE values can be correlated with the length of indolic $R(N–H)^{+\bullet}$ bond length and partial charge on the indolyl radical nitrogen, q(N), of the studied molecules successfully. It has been also found that PA and IP values for substituted melatonins can be estimated from their E_{HOMO} values. This fact may be useful for the development of new melatonin based antioxidants.

4. 1. Supporting Material

The figures 1s–8s and tables 1s–5s are present in Supporting Material.

5. Acknowledgement

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6. Abbreviations

IEF–PCM – Integral Equation Formalism Polarized Continuum Model; DFT – Density Functional Theory; BDE – Bond Dissociation Enthalpy; IP – Ionization Potential; PA – Proton Affinity; SET–PT – Single Electron Transfer followed by Proton Transfer; SPLET – Sequential Proton Loss Electron Transfer; HAT – Hydrogen Atom Transfer; EDG – Electron–Donating Group; EWG – Electron–Withdrawing Group; HOMO – Highest Occupied Molecular Orbital.

7. References

1. G. W. Burton, M. G. Traber, *Annu. Rev. Nutr.* **1990**, *10*, 357–382.

2. W. A. Pryor, *Free Radic. Biol. Med.* **2000**, *28*, 141–164.
3. D. A. Horton, G. T. Bourne, M. L. Smythe, *Chem. Rev.* **2003**, *103*, 893–930.
4. M. M. Posmyk, K. M. Janas, *Acta Physiol. Plant.* **2009**, *31*, 1–11.
5. D. X. Tan, L. D. Chen, B. Poeggeler, L. C. Manchester, R. J. Reiter, *Endocr. J.* **1993**, *1*, 57–60.
6. R. J. Reiter, C. S. Oh, O. Fujimori, *Trends Endocrinol. Metab.* **1996**, *7*, 22–27.
7. R. J. Reiter, D. X. Tan, W. Qi, *Acta Pharmacol. Sinica.* **1998**, *19*, 575–581.
8. R. J. Reiter, D. X. Tan, J. Cabrera, D. D'Arpa, R. M. Sainz, J. C. Mayo, S. Ramos, *Biol. Signals Recept.* **1999**, *8*, 56–63.
9. M. Martin, M. Macias, G. Escames, J. Leon, D. Acuna–Castroviejo, *FASEB J.* **2000**, *14*, 1677–1679.
10. M. J. Jou, T. I. Peng, P. Z. Yu, S. B. Jou, R. J. Reiter, J. Y. Chen, H. Y. Wu, C. C. Chen, L. F. Hsu, *J. Pineal Res.* **2007**, *43*, 389–403.
11. C. Pieri, M. Marra, F. Moroni, R. Recchioni, F. Marcheselli, *Life Sci.* **1994**, *55*, 271–276.
12. P. Stasica, P. Ulanski, J. M. Rosiak, *J. Pineal Res.* **1998**, *25*, 65–66.
13. D. X. Tan, R. J. Reiter, L. C. Manchester, M. T. Yan, M. El–Sawi, R. M. Sainz, J. C. Mayo, R. Kohen, M. Allegra, R. Hardeland, *Curr. Top. Med. Chem.* **2002**, *2*, 181–197.
14. R. J. Reiter, D. X. Tan, B. Poeggeler, A. Menendez–Pelaez, L. D. Chen, S. Saarela, *Ann. N. Y. Acad. Sci.* **1994**, *719*, 1–12.
15. R. J. Reiter, *FASEB J.* **1995**, *9*, 526–533.
16. P. C. Konturek, S. J. Konturek, J. Majka, M. Zembala, E. G. Hahn, *Eur. J. Pharmacol.* **1997**, *322*, 73–77.
17. E. Cadenas, *Biofactors,* **1997**, *6*, 391–397.
18. P. Elia, A. Azoulay, Y. Zeiri, **2012**, *19*, 314–324.
19. J. S. Wright, E. R. Johnson, G. A. Dilabio, *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183.
20. A. P. Vafiadis, E. G. Bakalbassis, *Chem. Phys.* **2005**, *316*, 195–204.
21. M. Musialik, G. Litwinienko, *Org. Lett.* **2005**, *7*, 4951–4954.
22. R. Hardeland, R. J. Reiter, B. Poeggeler, D.X. Tan, Neurosci. *Biobehav. Rev.* **1993**, *17*, 347–357.
23. E. Migliavacca, J. Ancerewicz, P. A. Carrupt, B. Testa, *Helv. Chim. Acta* **1998**, *81*, 1337–1348.
24. A. Gozzo, D. Lesieur, P. Duriez, J. C. Fruchart, E. Teissier, *Free Radic. Biol. Med.* **1999**, *26*, 1538–1543.
25. A. Martinez, M. A. Rodríguez–Girones, A. Barbosa, M. Costas, *J. Phys. Chem. A* **2008**, *112*, 9037–9042.
26. H. Y. Zhang, H. F. Ji, *J. Mol. Struct.: (Theochem)* **2003**, *663*, 167–174.
27. H. Y. Zhang, Y. M. Sun, X. L. Wang, *J. Org. Chem.* **2002**, *67*, 2709–2712.
28. G. Litwinienko, K. U. Ingold, *J. Org. Chem.* **2003**, *68*, 3433–3438.
29. G. Litwinienko, K.U. Ingold, *J. Org. Chem.* **2004**, *69*, 5888–5896.
30. M. C. Foti, C. Daquino, C. Geraci, *J. Org. Chem.* **2004**, *69*, 2309–2314.
31. G. Litwinienko, K. U. Ingold, *J. Org. Chem.* **2005**, *70*, 8982–8990.
32. H. S. Mahal, H. S. Sharma, T. Mukherjee, *Free Radic. Biol. Med.* **1999**, *26*, 557–565.
33. R. Vianello, Z. B. Maksic, *Tetrahedron* **2006**, *62*, 3402–3411.
34. M. Fujio, R. T. McIver Jr, R. W. Taft, *J. Am. Chem. Soc.* **1981**, *103*, 4017–4023.
35. T. B. McMahon, P. Kebarle, *J. Am. Chem. Soc.* **1977**, *99*, 2222–2230.
36. P. Mulder, H. G. Korth, K. U. Ingold, *Helv. Chim. Acta* **2005**, *88*, 370–374.
37. A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652.
38. A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098–3100.
39. C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785–789.
40. E. R. Davidson, D. Feller, *Chem. Rev.* **1986**, *86*, 681–696.
41. W. J. Hehre, L. Radom, P. V. R. Schleyer, J. A. Pople, Ab Initio Molecular Orbital Theory, Wiley, New York, **1986**.
42. E. Cances, B. Mennucci, J. Tomasi, *J. Chem. Phys.* **1997**, *107*, 3032–3041.
43. E. Cances, B. Mennucci, *J. Math. Chem.* **1998**, *23*, 309–326.
44. E. Klein, V. Lukes, *J. Phys. Chem. A,* **2006**, *110*, 12312–12320.
45. J. Rimarcik, V. Lukes, E. Klein, M. Ilcin, *J. Mol. Struct.: (Theochem)* **2010**, *952*, 25–30.
46. J. Tomasi, B. Mennucci, R. Cammi, *Chem. Rev.* **2005**, *105*, 2999–3093.
47. E. S. Boes, P. R. Livotto, H. Stassen, *Chem. Phys*. **2006**, *331*, 142–158.
48. E. Klein, V. Lukes, *J. Mol. Struct.: (Theochem)* **2006**, *767*, 43–50.
49. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J .A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al–Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian 03, Gaussian, Inc., Pittsburgh, PA, **2003**.
50. A. K. Chandra, T. Uchimaru, *Int. J. Mol. Sci.* **2002**, *3*, 407–422.
51. M. Navarrete, C. Rangel, J. C. Corchado, J. Espinosa–Garcia, *J. Phys. Chem. A* **2005**, *109*, 4777–4784.
52. M. M. Bizarro, B. J. C. Cabral, R. M. B. dos Santos, J. A. M. Simons, *Pure Appl. Chem.* **1999**, *71*, 1249–1256.

53. J. E. Bartmess, *J. Phys. Chem.* **1994**, *98*, 6420–6424.
54. Q. Zhu, X. M. Zhang, A. J. Fry, *Polym. Degrad. Stab.* **1997**, *57*, 43–50.
55. E. T. Denisov, *Polym. Degrad. Stab.* **1995**, *49*, 71–75.
56. G. C. Justino, A. J. S. C. Vieira, *J. Mol. Model.* **2010**, *16*, 863–876.
57. M. Leopoldini, T. Marino, N. Russo, M. Toscano, *J. Phys. Chem. A* **2004**, *108*, 4916–4922.
58. E. G. Bakalbassis, A. T. Lithoxoidou, A. P. Vafiadis, *J. Phys. Chem. A* **2003**, *107*, 8594–8606.
59. E. G. Bakalbassis, A. T. Lithoxoidou, A. P. Vafiadis, *J. Phys. Chem. A* **2006**, *110*, 11151–11159.
60. E. Klein, J. Rimarcik, V. Lukes, *Acta Chimica Slovaca*. **2009**, *2*, 37–51.
61. M. Najafi, E. Nazarparvar, K. Haghighi Mood, M. Zahedi, E. Klein, *Comput. Theoret. Chem.* **2011**, *965*, 114–122.
62. M. Najafi, M. Zahedi, E. Klein, *Comput. Theoret. Chem.* **2011**, *978* ,16–28.
63. E. Klein, V. Lukes, Z. Cibulkova, J. Polovkova, *J. Mol. Struct.: (Theochem)* **2006**, *758*, 149–159.
64. F. Zhao, Z. Q Liu, D. Wu, *Chem. Phys. Lipids* **2008**, *151*, 77–84.
65. J. S. Wright, D. J. Carpenter, D.J. McKay, K.U. Ingold, *J. Am. Chem. Soc.* **1997**, *119*, 4245–4252.
66. R. Bosque, J. Sales, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 637–642.
67. M. Najafi, K. Haghighi Mood, M. Zahedi, E. Klein, *Comput. Theoret. Chem.* **2011**, *969*, 1–12.
68. G. A. DiLabio, D. A. Pratt, J. S. Wright, *Chem. Phys. Lett.* **1999**, *311*, 215–220.
69. G. A. DiLabio, D. A. Pratt, J. S. Wright, *J. Org. Chem.* **2000**, *65*, 2195–2203.
70. T. Koopmans, *Physica* **1933**, *1*, 104–113.
71. E. Migliavacca, P. A. Carrupt, B. Testa, *Helv. Chim. Acta* **1997**, *80*, 1613–1626.
72. H. Y. Zhang, *J. Am. Oil Chem. Soc.* **1998**, *75*, 1705–1709.
73. H. Y. Zhang, *J. Am. Oil Chem. Soc.* **1999**, *76*, 1109–1110.
74. V. D. Kanchev, L. Saso, P. V. Boranova, A. Khan, M. K. Saroj, M. K. Pandey, S. Malhotra, J. Z. Nechev, S. K. Sharma, A. K. Prasad, M. B. Georgieva, C. Joseph, A. L. DePass, R. C. Rastogi, V. S. Parmar, *Biochimie* **2010**, *92*, 1089–1100.
75. F. C. Lavarda, *Int. J. Quant. Chem.* **2003**, *95*, 219–223.
76. S. Erkoc, F. Erkos, N, Keskin, *J. Mol. Struct.: (Theochem)* **2002**, *587*, 73–79.
77. I. B. Zavodnik, A. V. Domanski, E. A. Lapshina, M. Bryszewska, R. J. Reiter, *Life Sci.* **2006**, *79*, 391–400.
78. W. Bi, Y. Bi, P. Xue, Y. Zhang, X. Gao, Z. Wang, M. Li, M. Baudy–Floch, N. Ngerebara, K. M. Gibson, L. Bi, *J. Med. Chem.* **2010**, *53*, 6763–6767.
79. P. C. Nam, M. T. Nguyen, A. K. Chandra, *J. Phys. Chem. A* **2006**, *110*, 10904–10911.
80. F. G. Bordwell, J. P. Cheng, *J. Am. Chem. Soc.* **1991**, *113*, 1736–1743.
81. T. Brinck, M. Haeberline, M. Jonsson, *J. Am. Chem. Soc.* **1997**, *119*, 4239–4244.
82. M. I. de Heer, H. G. Korth, P. Mulder, *J. Org. Chem.* **1999**, *64*, 6969–6975.
83. E. Klein, V. Lukes, M. Ilcin, *Chem. Phys.* **2007**, *336*, 51–57.
84. J. Rimarcik, V. Lukes, E. Klein, L. Rottmannova, *Comput. Theoret. Chem.* **2011**, *967*, 273–283.
85. M. J. S. Dewar, The Molecular Orbital Theory of Organic Chemistry, McGraw–Hill, New York, **1969**.
86. G. A. DiLabio, D. A. Pratt, A. D. Lofaro, J. S. Wright, *J. Phys. Chem. A* **1999**, *103*, 1653–1661.
87. E. Klein, V. Lukes, *Chem. Phys.* **2006**, *330*, 515–525.
88. M. Guerra, R. Amorati, G. F. Pedulli, *J. Org. Chem.* **2004**, *69*, 5460–5467.
89. Y. Fu, L. Liu, Q–X. Guo, *J. Phys. Org. Chem.* **2004**, *17*, 282–288.
90. D. Mikulski, M. Molski, *Comp. Theor. Chem.* **2012**, *981* , 38–46.
91. Z. S. Marković, J. M. D. Marković, D. Milenković, N. Filipović, *J. Mol. Model.* **2011**, *17*, 2575–2584.
92. R. E. Ballard, *Chem. Phys. Lett.* **1972**, *16*, 300–301.
93. J. J. Fifen, M. Nsangou, Z. Dhaouadi, O. Motapon, N. Jaidane, *Comp. Theor. Chem.* **2011**, *966*, 232–243.
94. J. A. Mejias, S. Lago, *J. Chem. Phys.* **2000**, *113*, 7306–7316.
95. C. G. Zhan, D. A. Dixon, *J. Phys. Chem. B* **2003**, *107*, 4403–4417.

Povzetek

V tem delu smo proučevali melatonin ter 60 različnih *meta* in *orto* substituiranih derivatov te spojine. Z uporabo teorije gostotnega potenciala (DFT) na B3LYP nivoju smo izračunali entalpije različnih procesov v vodi in v plinski fazi, kot so: prenos vodikovega atoma (HAT), pronos elektron-proton (SET–PT) in zaporedni prenos elektron –proton (SPLET). Dobljeni rezultati kažejo, da substituenta, ki elektrone sprejema, poveča entalpijo razcepa vezi (BDE), ionizacijski potencial (IP) in entalpijo prenosa elektronov (ETE). Substituenta, ki učinkuje kot donor elektronov, pa poveča entalpijo odcepa protona (PDE) in protonsko afiniteto (PA). Izkazalo se je, da imajo substituente, vezane na *orto* mesto večji efekt na energetiko proučevanih procesov, kot pa če so vezane na *meta* poziciji.