Electronic substitution effect on the ground and excited state properties of indole chromophore: A computational study

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Abstract

Indole, being the main chromophore of amino acid tryptophan and several other biologically relevant molecules like serotonin, melatonin, has prompted considerable theoretical and experimental interest. The current work focuses on the investigation of photophysical and photochemical properties of indole and indole derivatives e.g. tryptophan, serotonin and melatonin using theoretical and computational methodologies. Having three close-lying excited electronic states, the vibronic coupling effect becomes extremely important yet challenging for the photophysics and photochemistry of indole. Here, we have used density functional theory (DFT) extensively and evaluated the performance of DFT in compared to available experimental and *ab initio* results from literature. The benchmarking of the method is followed by investigation of the chemical and geometrical effects of ring substitution in indole. A bathochromic shift has been observed in the HOMO-LUMO gap as well as vertical excitation energy from

indole to melatonin. While the contribution of the in-plane small adjacent groups increases the electron density of the indole ring, the out-of-plane long substituent groups have minor effect. The comparison of singlet-triplet gaps suggests highest probability of inter-system crossing for tryptophan which is in line with previous experiment. The absorption spectra calculated including the vibronic coupling are in good agreement with experiment. These results can be used to estimate the error in photophysical observables of indole derivatives calculated considering indole as prototypical system. This study also demonstrates the merits and demerits of using DFT functionals to compute photophysical properties of indole derivatives.

Keywords: Indole derivatives, chromophore, photophysics, electronic substitution, vibrationally resolved spectra, excited states

Introduction

Indole is one of the most abundant biomolecules in nature. Indole derivatives such as melatonin and serotonin serve crucial roles in several biological processes, including neurotransmitter, pigments, sleep, mood, aggression and learning.[1–3] On the other hand, another indole derivative, tryptophan is one of the essential amino acids that are the building blocks of protein.[4] Anomalies in their functions can lead to depression, anxiety, infantile autism, insomnia, sleep-related issues, and protein malfunction.[5–7] Moreover, the indole group is the backbone of many existing drugs and alkaloids.[8]

Study of photophysics and photochemistry of indole and its derivatives builds the foundation of understanding light-induced phenomena of indole chromophore. Several experimental and theoretical studies have been performed on indole and its derivatives.[9–12] The aromatic ring of indole acts as a chromophore, and the photochemical and photophysical properties of its derivatives vary with the adjacent substituent groups.[10] The versatility and abundance of indole derivatives have drawn chemists' attention for decades.

The excited state properties of indole have been extensively studied using different methods.[13–18] According to Platt's scheme, indole type chromophores have four $\pi - \pi^*$ singlet excited states.[19] Two low-lying states are called L_a and L_b, and the other two higher excited states are B_a and B_b. The energy difference between the L_a and L_b states was reported to be 1400 cm^{-1} in a polarized fluorescence excitation and dispersed fluorescence spectroscopy in solid argon at 20K.[20] In general, the L_a state is red-shifted with higher transition dipole moment (TDM) and therefore more sensitive to the environment compared to L_b state.[21,22] Often, the inversion of these two states occurs by the influence of the substituents, solvent or symmetry.[23–26] Several theoretical and experimental studies focused on the topic with a common agreement that the TDM in the L_b and L_a states has a positive and negative angle, respectively with the inertial axis.[16,17,27]

The current work focuses on indole and indole derivatives like tryptophan, serotonin and melatonin not only for their biological importance, but also for their interesting photophysical character. Hence, the complexity of these aforementioned two nearly degenerate states demands more scientific ventures. For example, indole has been extensively studied via experiments as well as computations as a biological building block but quantum mechanical studies on these indole derivatives are scarce in the literature. UV-visible spectroscopy is an important tool to characterize the presence of indole chromophore in biological system. The position of peak and its intensity depend on the substitution in the five-membered and six-membered rings. Therefore, in order to detect the exact molecular unit, it is important to quantify the effect of substituion on the UV-visible spectroscopic features of indole ring.

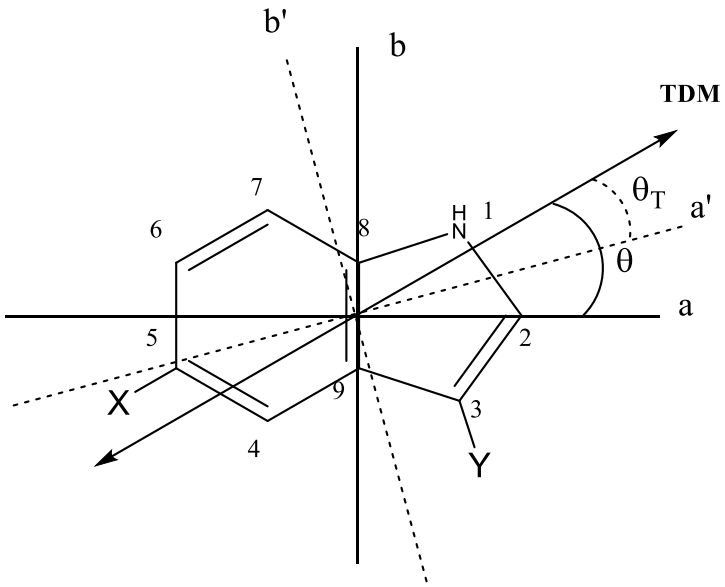
As it is difficult to treat large systems (e.g., indole ring with several substitutions) with high-level quantum chemical methods, properly benchmarked density functional theory (DFT) can be a suitable alternative. Moreover, a suitable DFT functional would also facilitate applying the methodology to an extended system with the same building block. The available data

on the excited state properties of indole allowed us to benchmark the method which is later applied to the other indole derivatives. Different adjacent groups of the indole ring show different chemical properties. It has been seen that N-H bond in indole is acidic in nature and thus takes part in N-substitution reactions.[28] This characteristic makes a molecule with indole ring bioactive. The protonated indole derivatives are ready for substitution which makes this indole scaffold so versatile. These functionalities also depend on their pk_a values.

The purpose of the current article is two-fold. First, to assess the performance of DFT functional on quantitative reproduction of photophysical properties indole and its derivatives and second, to investigate the electronic effect of substituting group(s) on the excited state properties of the indole ring using the benchmarked method. The article is organized as follows. First, the computational methods, used for the calculation of ground and excited state properties, are discussed. The results are reported and discussed in the next section. In that section, the ground and excited state electronic structures of indole and indole derivatives are discussed which is followed by the energetics of the excited states as well as vibrationally resolved spectra. Finally, the work is summarized and concluded.

Computational Methods

To study the electronic substitution effect on the ground and excited state properties of indole the DFT method is used in this work. The validity and efficiency of the DFT method in molecules having indole moiety is also investigated. The structures of indole and its derivatives are optimized at the ground state with the DFT method using Gaussian09 software.[29] B3LYP was used as the DFT functional (unless stated otherwise) along with 6-31G(d) and 6-311+G(d,p) basis sets.[30,31]



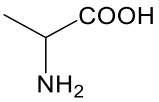
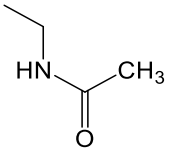
	X	Y
Indole	H	H
Tryptophan	H	
Serotonin	OH	
Melatonin	OCH_3	

Figure 1: Two-dimensional structure of indole and its derivatives. The substitutions are noted as X and Y and explained in the table on the right-hand side. The orthogonal straight lines on the figure are a and b inertial axes. The angles θ/θ_T are the angle between the transition dipole moment and a/a' axes.

Time dependent DFT (TDDFT) is a suitable choice for the excited state calculations especially when an excited state optimization is necessary for a reasonably large molecule which is the case in the present work. Furthermore, the hybrid functional like B3LYP represents the low-lying excited states reasonably well. As the number of ring increases, TDDFT fails to measure properties for upper excited states.[22] However, the molecules studied in this work are comparatively smaller in size and bicyclic. Therefore, B3LYP functional can be an optimum choice especially for the low-lying excited states. For excited state calculations, the combination of B3LYP functional and 6-311+G(d,p) basis set is chosen along with the TDDFT method. Long range corrected functional LC-BLYP has also been used for comparison. Along with several important ground and excited state properties, vibrationally

resolved absorption and emission spectra have been calculated using TDDFT method. For that purpose, the ground state optimized structures of indole and its derivatives are also optimised at the first excited state using the B3LYP or LC-BLYP functionals[32–34] and 6-311+G(d,p) basis sets.

Calculation of pK$_a$ values

The pK$_a$ values for the NH proton of indole and indole derivatives are calculated using equation 1 deduced from the thermodynamic cycle of the protonation reaction.[35,36]

$$pK_a = \frac{\Delta G_{aq}}{k_B T ln(10)} \tag{1}$$

where

$$\Delta G_{aq} = \Delta G_{gas} + \Delta\Delta G_S$$
$$\Delta G_{gas} = G_{gas}(H^+) + G_{gas}(A^-) - G_{gas}(HA)$$
$$\Delta\Delta G_S = G_S(H^+) + G_S(A^-) - G_S(HA)$$

The values of $\Delta G_{gas}(H^+)$ and $\Delta G_S(H^+)$ are taken as -0.27 eV and -11.56 eV.[36,37]

Calculation of vibrationally resolved spectra

As the electronic transitions at the Franck-Condon (FC) region are often dipole-forbidden or weakly allowed, the usual UV-visible spectra calculated from the vertical excitation is incomplete. The agreement between experimental and computational spectra is poor if the absorption spectrum is calculated considering the vertical excitation only. Hence, one-

photon vibronic absorption and emission spectra, which includes the real broadening, are better choice. In this case, the details of the vibrational wavefunctions are needed along with the coordinates of the ground and the excited states. The intensity of a vibronic spectra also depends on the overlap between the vibrational levels of both ground and excited electronic states. Normal modes of indole and its derivatives for both ground and excited states are calculated using similar procedure. For the calculation of the absorption spectra, the transition moment integral $\boldsymbol{\mu_{if}}$ is taken as a function of normal coordinates. The initial and final vibronic states are defined as ψ_i and ψ_f respectively. According to Fermi's golden rule, the spectral intensity can be written as

$$I(\omega) = \sum_{f,i} \rho_i |\langle \Psi_f | \mu | \Psi_i \rangle|^2 \delta(\Delta E - \omega) \tag{2}$$

where Ψ_i or Ψ_f are expanded as the product of electronic ($|e\rangle$) and vibrational ($v\rangle$) wavefunctions.

$$\langle \Psi_f | \mu | \Psi_i \rangle = \mu_{if} = \mu_{if}(Q'_0) < v|v' > + \sum_{k=1}^{N} (\frac{\partial \mu_{if}}{\partial Q'_k})_0 < v|Q'_k|v' > + \frac{1}{2} \sum_{k=1}^{N} \sum_{l=1}^{N} (\frac{\partial^2 \mu_{if}}{\partial Q'_k \partial Q'_l})_0 < v|Q'_k Q'_l|v' > \tag{3}$$

Further details of this method can be found in Ref.[38] and references therein. B3LYP/6-311+G(d,p) is used to compute the vibrationally resolved spectra and the full width at the half maxima is set as 250 cm^{-1} to calculate the broadening of the spectra.

Results and discussion

To understand the effect of substitution in the five- and six-membered ring of indole chromophore, the ground and excited state properties for indole, tryptophan, serotonin and melatonin are reported and discussed in the following. First, the DFT functional and basis set are benchmarked for indole with respect to the available experimental and computational

results and then the same have been applied to the other indole derivatives. The effect of substitution are discussed for the ground and excited state geometries, acidity constants, partial charges, vertical excitation energies, HOMO-LUMO gap as well as vibrationally resolved absorption spectra.

Ground and excited state geometry-effect of substitution

Table 1: Comparison of bond lengths of indole in the ground state obtained using B3LYP/6-31G(d) and B3LYP/6-311+G(d,p) methods, from previous calculations (ANO-L type basis set C,N [4s,3p,1d]/H[2s1p], CASPT2/CASSCF(10,9))[39] and from experiments.[40]

Bond	GS (6-31G(d))	GS (6-311+G(d,p))	GS (CASPT2/CASSCF)[39]	Experimental[40]
N1-H	1.008	1.006	0.99	—
N1-C2	1.383	1.383	1.376	1.377
C2-C3	1.370	1.368	1.363	1.344
C3-C9	1.437	1.437	1.441	1.451
C9-C4	1.406	1.405	1.405	1.412
C4-C5	1.389	1.387	1.384	1.397
C5-C6	1.411	1.409	1.411	1.386
C6-C7	1.390	1.388	1.385	1.399
C7-C8	1.399	1.397	1.400	1.400
C8-N1	1.381	1.380	1.372	—
C8-C9	1.424	1.421	1.404	—

To validate the method and basis sets (B3LYP functional with 6-31G(d) and 6-311+G(d,p) basis sets) used in this work for the ground state calculations, the calculated bond lengths of the indole ring at the optimized ground state are compared with the values obtained from previous CASSCF/CASPT2 calculations as well as with the experimental values. Table 1 shows the comparison of bond lengths of an indole ring in three different theoretical methods with an experimental one. DFT methods with B3LYP functional using 6-31G(d) and 6-311+G(d,p) produce similar bond lengths to that of CASPT2/CASSCF method using ANO-L type basis set C,N [4s,3p,1d]/H[2s1p] as well as the experimental values which has been taken from the X-Ray study of crystalline Tryptophan by Takigawa *et al.*[39,40] The calculated bond lengths are in reasonable agreement with the previously obtained result by

Walden and Wheeler.[41] This validates the use of DFT for the ground states of other indole derivatives too. The slight difference between the experimental values and computed values can be attributed to the effect of substitution at the C3 position for the experimental sample molecule.

To elucidate the change in geometry due to electronic substitution and/or photo-excitation, the ground and excited state optimized geometries of indole, tryptophan, serotonin and melatonin at the B3LYP/6-311+G(d,p) level of theory are shown and compared below. Table 2 shows the comparison of bond lengths for the optimized structures at the ground state of indole, tryptophan, serotonin and melatonin using the B3LYP/6-311+G(d,p) level of theory. The values indicate that N1-H, N1-C2, C2-C3, C3-C9 and C7-C8 bond length has no significant change while going from indole to substituted indoles. On the other hand, C9-C4, C4-C5, C6-C7, C8-N1 bond lengths decrease and C5-C6, C8-C9 bond length increases due to electronic substitutions. This indicates that while the long chain-like substitution in the 5-membered ring has no significant effect, the small substitution in the 6-membered ring of indole has significant effect on the ground state electronic structure of the indole ring. This can be attributed to the fact that unlike the out-of-plane longer substitution, the shorter substitution, being in the same plane, can directly take part in resonance and thereby affects the electronic and geometric properties of the ring.

Table 2: Bond lengths in the ground state of indole, tryptophan, serotonin and melatonin calculated using B3LYP/6-311+G(d,p) level of theory.

Bond	Indole	Tryptophan	Serotonin	Melatonin
N1-H	1.006	1.006	1.005	1.005
N1-C2	1.383	1.382	1.380	1.383
C2-C3	1.368	1.371	1.373	1.373
C3-C9	1.437	1.444	1.442	1.444
C9-C4	1.426	1.405	1.407	1.400
C4-C5	1.430	1.387	1.386	1.391
C5-C6	1.383	1.408	1.409	1.410
C6-C7	1.445	1.388	1.386	1.392
C7-C8	1.408	1.397	1.396	1.391
C8-N1	1.421	1.380	1.382	1.382
C8-C9	1.399	1.420	1.418	1.419

Table 3: Bond lengths at the first excited (S_1) state of indole, tryptophan, serotonin and melatonin calculated using B3LYP/6-311+G(d,p) level of theory.

Bond	Indole	Tryptophan	Serotonin	Melatonin
N1-H	1.010	1.008	1.010	1.009
N1-C2	1.341	1.337	1.352	1.401
C2-C3	1.429	1.429	1.421	1.396
C3-C9	1.431	1.419	1.452	1.442
C9-C4	1.426	1.408	1.419	1.405
C4-C5	1.428	1.389	1.432	1.437
C5-C6	1.382	1.401	1.380	1.400
C6-C7	1.444	1.405	1.437	1.426
C7-C8	1.406	1.377	1.407	1.408
C8-N1	1.417	1.405	1.404	1.364
C8-C9	1.396	1.422	1.396	1.424

The picture of electronic substitution effect in the excited state is quite different than that in the ground state. However, except a little twist in the adjacent long chain of tryptophan, there is no visual difference between their structure or orientation. Table 3 reports the comparison of bond lengths for the optimized structures at the excited state of indole, tryptophan, serotonin and melatonin at the B3LYP/6-311+G(d,p) level of theory. For all the molecules, no significant change in the N1-H bond length is noticed at the first excited state which is a $\pi - \pi^*$ state. Furthermore, C5-C6 and C8-C9 bond lengths are almost same

for all the indole and indole derivatives discussed in this work.

The C3-C9, C9-C4, C6-C7 bond lengths decreases from indole to tryptophan at the first excited state due to electronic substitution.These bonds again elongate for serotonin and get shortened for melatonin at the first excited state. N1-C2 and C4-C5 and C7-C8 follow the aforementioned trend for indole, tryptophan and serotonin but the bond lengths increase from serotonin to melatonin instead. The C2-C3 and C8-N1 bonds, in contrast, are shorter in melatonin compared to those of indole, tryptophan and serotonin. However, those bond distances are similar for indole, tryptophan and serotonin.

The observation on the excited state bond lengths indicates that the longer chain adjacent to the five member ring contributes to the electronic properties of the aromatic ring of tryptophan. However, this electronic effect is neutralized by the OH group in the C5 position. When the +R effect increases due to the OCH_3 group in melatonin the bond length again changes. Whether the change leads to elongation or shortening of the bond length depends on the amount of electron cloud over the bond.

The bond angles adjacent to the nitrogen in the ground state for all the molecule are of similar values. But in the excited state of indole, two bond angles $\angle C8-N1-H$ and $\angle C2-N1-H$ decrease almost by 1.5° to 1.7°. However, $\angle C2-N1-C8$ increases by 2.2°. When the molecules are in the excited state, the angles get closer to 120° which is the ideal angle for an sp^2 planar configuration. The same trend is observed in tryptophan, serotonin and melatonin. The said angles tend to converge to 120°. It has been observed that $\angle C2-N1-C8$ is increasing in excited state for every molecule and $\angle C8-N1-H$ and $\angle C2-N1-H$ decrease. The change in the angle from the ground state to the excited state is becoming larger in serotonin and melatonin. This indicates that the influence of the smaller substituent groups attached to the six member ring is significant in these molecules. The ESP-fitted partial charges on the ring atoms of indole, tryptophan, serotonin and melatonin at the ground and excited state are compared and discussed in details in the supporting information (SI). Both the longer and shorter substituion play important role in partial

charge altering for the atoms in the five and six-membered ring, respectively in the ground and excited state. For individual chromophore, while going from the ground state to the excited state, the electronic effect is significant only for the atoms in the five-membered ring.

Benchmark of the DFT functional for the excited state of indole

Despite the involvement of several computational works on the photophysics of indole since decades, few points still remained debatable in this field. One of them is whether DFT functional is adequate to accurately describe the excited states of indole and its derivatives. To shed more light on this, we compared excited state results of indole for two different functionals and compared with experimental and previously reported quantum chemical results. Previous studies report calculations on indole, serotonin using B3LYP/6-31G(d) level of theory.[42,43] We have mainly focused on the first two excited states (S_1 and S_2) of $\pi - \pi^*$ character which are known as L_b and L_a states in the literature.

Table 4: Energy (E) and wavelength (λ_{max}) of the first two singlet excited states for indole calculated using B3LYP functional and 6-311+G(d,p) basis sets.

Molecule	State	E(eV)	λ_{max} (nm)	λ_{max}^{exp} (nm)[44]
Indole	S_1	4.71	263.19	283.8
Indole	S_2	4.85	255.63	273

Table 4 shows the S_1 and S_2 excited state energies for indole and compares with the experimental and previously calculated absorption wavelengths. The vertical excitation energies for indole S_1 and S_2 excited states are calculated at the B3LYP/6-311+G(d,p) level of theory. The calculated wavelength for the S_1 and S_2 states are $\sim$ 20 nm and $\sim$ 18 nm red shifted than the experimental one.[10] The calculated difference in energy between the first two excited states is 0.14 eV which is in good agreement with the experimental difference of 0.17 eV.[20] Overall, the results from B3LYP/6-311+G(d,p) level of theory compares well

with the experimental results for the excited states. Therefore, the same functional and basis sets were chosen to be used for the excited state calculations of other indole derivatives.

Excited states of indole derivatives- electronic substitution effect

Table 5: Energy (E), wavelength (λ_{max}), oscillator strength (f) and dipole moment (μ) of the first three singlet excited states for indole, tryptophan, serotonin and melatonin calculated using B3LYP functional and 6-311+G(d,p) basis sets.

Molecule	State	E(eV)	λ_{max}(nm)
Tryptophan	S_1	4.56	271.46
Tryptophan	S_2	4.65	266.86
Serotonin	S_1	4.31	287.42
Serotonin	S_2	4.59	269.7
Melatonin	S_1	4.25	292.01
Melatonin	S_2	4.58	270.93

Table 5 shows the energy, wavelength, oscillator strength and dipole moments of the first two excited states for tryptophan, serotonin and melatonin calculated at the B3LYP/6-311+G(d,p) level of theory which is benchmarked for indole excited state against experimental and previously reported theoretical values. It should be noted that our aim in this work is to investigate the electronic substitution effect on the excited states and we are comparing few similar systems. Therefore, the consistency of method for all the system is more important than the quantitative accuracy of individual system. Both the Table 5 and Figure 2 suggest that the S_1-S_0 gap decreases in the order of indole>tryptophan>serotonin>melatonin indicating a bathochromic shift due to electronic substitution. While going from indole, the gap between the S_2 and S_1 state decreases in case of tryptophan and then increases for serotonin and further for melatonin. From indole to tryptophan, one adjacent group at C3 has lesser impact on the ring than the shorter substituent adjacent to the six membered ring in serotonin and melatonin. The lone pairs on the oxygen of OH/OCH_3 in serotonin/melatonin, respectively, interacts with the π orbitals of the ring and destabilizes the HOMO. From tryp-

tophan to serotonin, p_x orbital of O interacts with the ring π orbitals which destabilize the HOMO and HOMO-1 yielding smaller S_1-S_0 gap for serotonin. As OCH_3 shows more induction and even mesomeric effect than OH, the gap is even smaller for melatonin in compared to serotonin. These results are also in line with experimental results by Livingstone *et al.*[44]

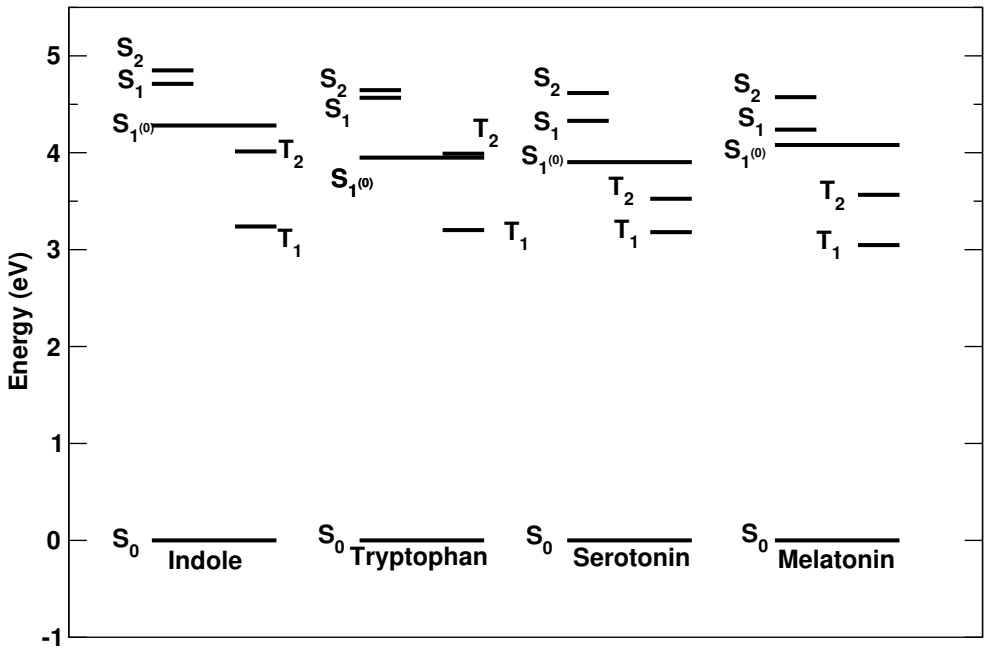


Figure 2: Jablonski type diagram for indole, tryptophan, serotonin and melatonin including S_0, S_1, S_2, T_1, T_2 states. To illustrate the fate of S_1 state, the $S_1^{(0)}$ (the minimum of S_1) state is also shown. For each case, S_0 energy is taken as reference.

Figure 2 shows the comparison of the singlet and triplet excited state energies too. The difference between S_1 and T_2 states for tryptophan is much smaller than the same for the other molecules which indicates that the probability of inter-system crossing which is significant in case of tryptophan where the T_2 state lies in between the vertical excitation energy of S_1 state and the S_1 minimum ($S_1^{(0)}$). This is in line with the experimental observation of

phosphorescence for tryptophan.[45,46]

The HOMO-LUMO energy gap - effect of substitution

The HOMO-LUMO energy gaps for indole, tryptophan, serotonin and melatonin are compared with the vertical excitation energies calculated at the same level of theory.

The reduction of HOMO-LUMO energy gap while going from indole, tryptophan, serotonin and melatonin can be explained in the light of substitution effect. While going from indole to melatonin, the +R effect of the substitutions increase the π conjugation in the ring which in turn reduces the HOMO-LUMO energy gap (see Fig. 3) and thereby inducing bathochromic shift. The HOMO-LUMO energy gap calculated with two different functionals (B3LYP and LC-BLYP) shows the same decreasing trend in the order of indole > tryptophan > serotonin > melatonin which explains the bathochromic shift with respect to the vertical excitation energy. There is a direct correlation between the HOMO-LUMO energy gap and VEE (see Table 6) i.e. bathochromic shift is observed with respect to ΔE as well as VEE while going from indole to melatonin. The trend in the change of calculated vertical excitation energy is in good agreement with experimental absorption energy which is given in column 4 of Table 6. The experimental absorption energy value is calculated by the conversion of experimental absorption wavelength to the energy. The comparison of experimental and calculated vertical and adiabatic excitation energies is discussed in Table S1 of the supporting information.

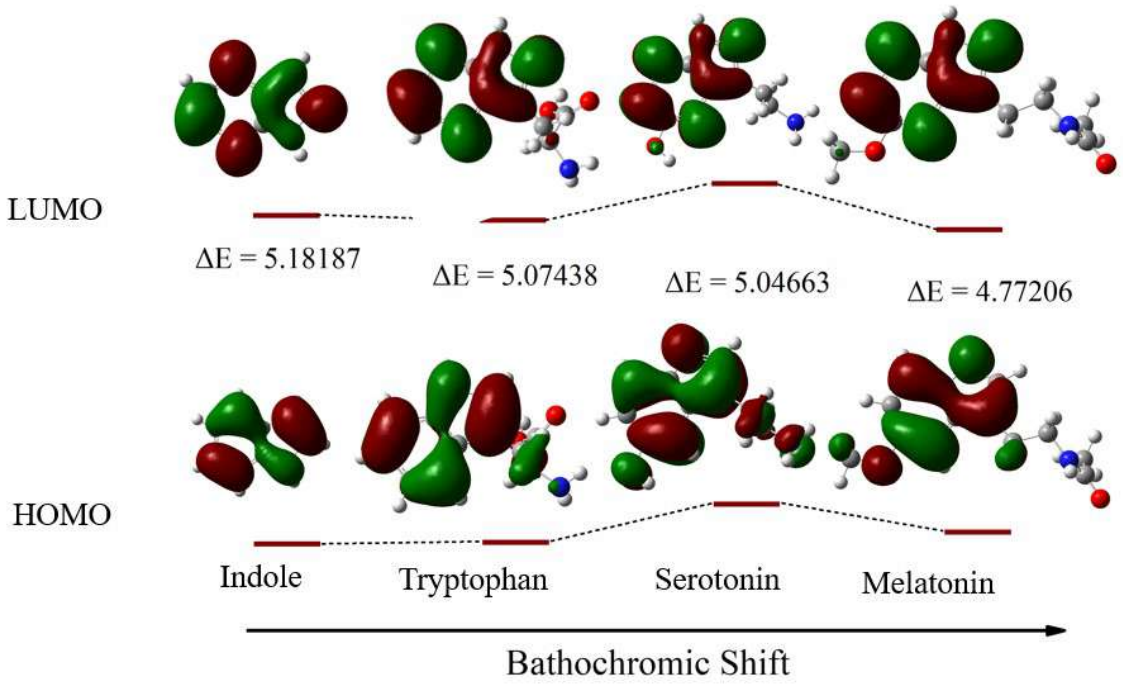


Figure 3: Comparison of HOMO-LUMO energy gaps of indole, tryptophan, serotonin and melatonin. ΔE refers to the HOMO-LUMO gap in eV.

Table 6: Correlation between HOMO-LUMO energy gaps (ΔE), VEEs and comparison to experimental absorption energy (E_{abs}^{exp}) of indole, Tryptophan, Serotonin, Melatonin)

Molecule	$\Delta E(eV)$	VEE_{S1} (eV)	E_{abs}^{exp} (eV)	$\Delta E_{S_2-S_1}$ (eV)
Indole	5.18	4.70	4.77[10,24,44]	0.14
Tryptophan	5.07	4.43	4.7686[47]	0.09
Serotonin	5.04	4.33	4.5085[48]	0.36
Melatonin	4.77	4.25	4.27[49]	0.49

Vibrationally resolved spectra of indole and indole derivatives

Theoretical calculations at the Frank Condon region often lacks the effect of subtle characteristics like vibronic coupling etc. of high-resolution absorption spectra of a molecule. These effects are reflected when the vibrational modes are taken into account in the calculation of the vibrationally resolved spectra which involves coupled vibronic wavefunctions to generate

vibronic Hamiltonian.[35,38]

In Figure 4, vibrationally resolved spectra for the first excited state of indole calculated using the method, which is described in the computational method section, are shown. The vibrationally resolved spectra for the S_1 state obtained with B3LYP functional are in line with the experimental spectra. The local maxima represent excitation to different vibrational states and the highest peak for indole is nearly at 280 nm. The vertical excitation energy for the S_1 state shows the peak at 263.19 nm. Therefore, the peak maxima of the vibrationally resolved absorption spectra for indole shows a redshift of is $\sim$ 17 nm from the Frank Condon region. The simulated spectra are in line with the experimental ones.[44] The vibrationally resolved spectra using the LC-BLYP functional for both the excited states do not correspond well with the experimental absorption band both in terms of position of the mean peak as well as position of the highest peak and therefore are not discussed in details. Following similar procedure, the vibrationally resolved absorption spectra for tryptophan, serotonin and melatonin are also calculated using B3LYP functional (see Figure 5).

The calculated vibrationally resolved absorption spectra for indole and tryptophan are quite similar both in terms of position of peak maximum and broadening. But for serotonin and melatonin, the peak maxima are shifted towards higher wavelength. This indicates that the effect of smaller substitution plays important role in the absorption wavelength showing a bathochromic shift which was already reflected in the order of HOMO-LUMO energy gap and vertical excitation energy.

In case of indole, the position of the mean peak, highest peak and broadening of the calculated spectra agrees very well to the experimental spectra. For tryptophan, the calculated spectra is 15 nm blue-shifted than the experimental spectra.[50] However, the inclusion of vibronic coupling reproduces the broadening of the spectra correctly. The experimental absorption spectra of serotonin and melatonin are much broader than the calculated one. This is probably because excitation to two close-lying excited states are considered in the experimental spectra. The peak maxima of the calculated spectra is situated at the middle of the

experimental spectra.

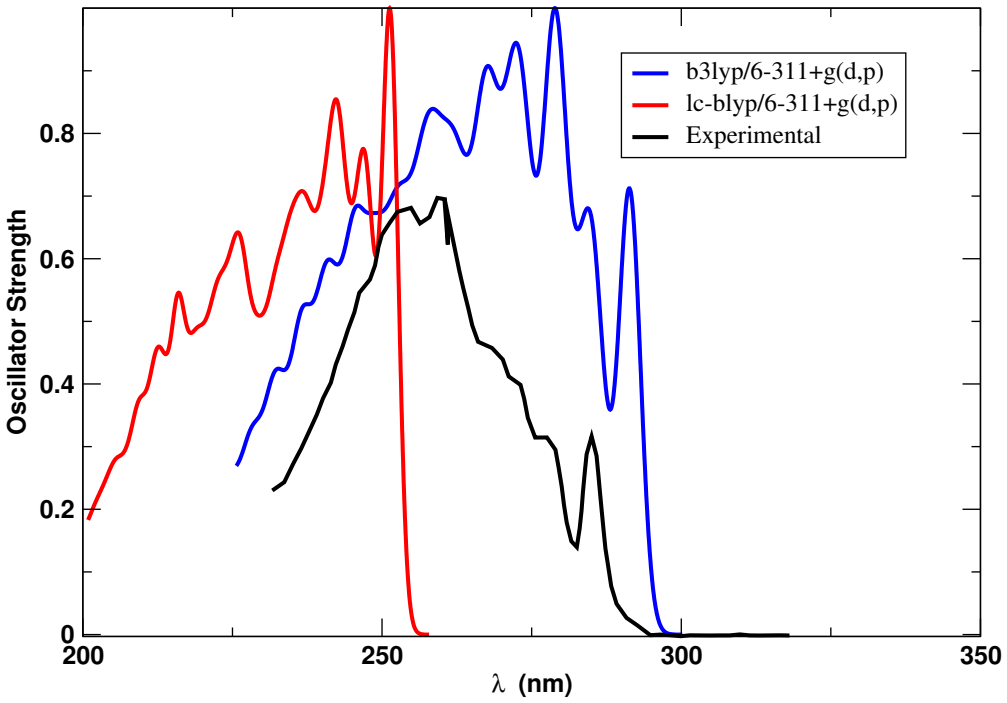


Figure 4: Comparison of calculated vibrationally resolved absorption spectra of Indole using B3LYP/6-311+G(d,p)(blue) and LC-BLYP/6-311+G(d,p) (red) with the experimental one (black)[44]

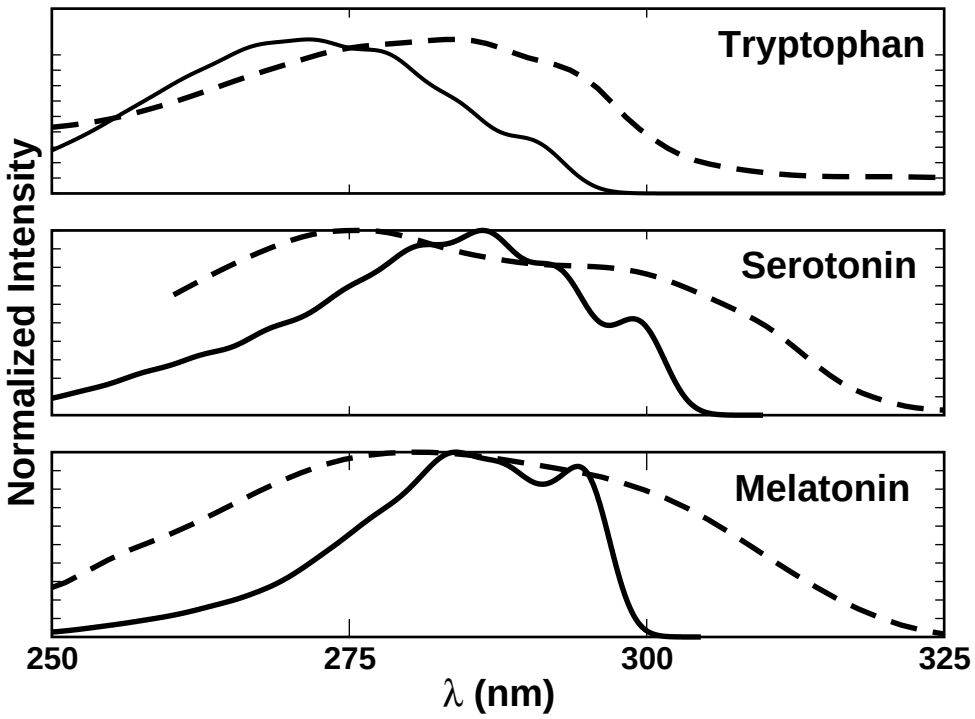


Figure 5: Comparison of experimental[50,51] and calculated vibrationally resolved absorption spectra of Tryptophan, Serotonin and Melatonin using B3LYP/6-311+G(d,p) level of theory.

Inversion of L_a and L_b states-performance of DFT functional

Table 7 shows the comparison of energy, wavelength, oscillator strength and dipole moment for the first two singlet states of indole calculated using B3LYP and LC-BLYP functional with 6-311+G(d,p) basis sets. It is interesting to note that the values of the dipole moments and the oscillator strengths of the S_1 and S_2 states are of opposite order when calculated with these two functional. The values of dipole moment and oscillator strength indicate that while the S_1 and S_2 states calculated using LC-BLYP functional is of L_b and L_a type, respectively, the characteristic of the S_1 and S_2 states calculated using B3LYP functional is inverted. Therefore, if we consider that the Platt's scheme (which was initially applied to benzene) is also applicable to indole, the excited state characteristics of L_a and L_b type

is in better agreement when calculated using LC-BLYP functional rather than the same calculated with B3LYP functional.

However, the calculated λ_{max} for the S_1 at the LC-BLYP/6-311+G(d,p) level of theory is $\sim$ 43 nm redshifted than the experimental one. But all other properties e.g. dipole moment, oscillator strengths, transition dipole angles agrees well with previously reported data to accurately represent the L_a and L_b states and their order. Therefore, we have also used LC-BLYP functional along with the B3LYP functional for tryptophan, serotonin and melatonin to calculate their excited state properties which are shown in Table 7.

Table 7: Energy (E), wavelength (λ_{max}), oscillator strength (f) and dipole moment (μ) of the first two singlet excited states for Indole, tryptophan, serotonin and melatonin calculated using B3LYP/LC-BLYP functional and 6-311+G(d,p) basis sets at the B3LYP/6-311+G(d,p) optimized geometry.

Molecule	Functional	State	E(eV)	λ(nm)	f	μ (D)
Indole	B3LYP	S_1	4.71	263.19	0.076	4.15
Indole	B3LYP	S_2	4.85	255.63	0.035	2.56
Indole	LC-BLYP	S_1	5.15	240.66	0.040	2.20
Indole	LC-BLYP	S_2	5.29	234.17	0.139	3.64
Tryptophan	B3LYP	S_1	4.56	271.46	0.061	3.32
Tryptophan	B3LYP	S_2	4.65	266.86	0.002	14.14
Tryptophan	LC-BLYP	S_1	5.11	242.18	0.043	2.08
Tryptophan	LC-BLYP	S_2	5.21	237.85	0.11	3.25
Serotonin	B3LYP	S_1	4.31	287.42	0.0562	3.54
Serotonin	B3LYP	S_2	4.59	269.7	0.002	3.99
Serotonin	LC-BLYP	S_1	4.80	258.07	0.0986	2.55
Serotonin	LC-BLYP	S_2	5.16	240.19	0.13	4.67
Melatonin	B3LYP	S_1	4.25	292.01	0.0683	6.0464
Melatonin	B3LYP	S_2	4.58	270.93	0.00	10.8221
Melatonin	LC-BLYP	S_1	4.83	256.48	0.1113	6.3329
Melatonin	LC-BLYP	S_2	5.32	233.07	0.0972	6.6414

Acidity of N-H proton-effect of substitution

To elucidate the reactivity of the indole ring with respect to the N-H bond at the ground state and the effect of substitution on that, pK_a value for the N-H group is calculated using the method described in the method section. Indole has one acidic side i.e. N-H group

in the five-member ring. But indole derivatives have more than one specific acidic sites. Tryptophan, serotonin and melatonin has 2,3 and 2 acidic sites, respectively. For example, serotonin has the hydroxyl end and aliphatic NH_2 acidic site along with the N-H group. As a consequence, indole derivatives possess more than one pK_a values with the other acidic site having lower pK_a value than the N-H group. Therefore, it is difficult to measure the pK_a of indole derivatives with respect to the N-H proton. Computational calculations can help to predict the acidity of these sites.

Serotonin is known to have pk_a of 9.97 and 10.73 for amino group and the hydroxyl group, respectively.[52] Similarly, two pK_a values available in literature for melatonin are 16.9 and -0.69.[53] The pk_a values of tryptophan are 2.36 and 9.42 for acidic and basic nature respectively.[54] However, in the current work we are focusing on the pK_as of the N-H site only. As it is challenging to measure the experimental pK_a of that region, the acid-base reactions of the adjacent groups are neglected in implicit medium. The *in silico* measurements have been performed to check the effect of the substitution to the ring. The calculated pK_a values for indole, tryptophan, serotonin and melatonin are 24.7, 24.4, 74.5, 14.9, respectively.

The similar pK_as value of Indole and tryptophan indicate that there are no effects of the longer substitutions in the electronic character of the indole ring, especially, the smaller five-membered ring which is also reflected by the geometry and charge analysis. On the other hand, the increment of pK_a value from tryptophan to serotonin indicates that the smaller -OH substitution has direct contribution to the change of electronic character of the indole ring. Furthermore, serotonin has two more proton which are more acidic in nature than the N-H proton and thereby increasing the pK_a value of the N-H proton drastically. The $-OCH_3$ in melatonin shows the opposite effect and thereby reduces the pK_a value of the N-H group than the indole ring itself. However, it is well known that the calculated pK_a values are better for qualitative comparison only as the errorbar for quantitively accurate pk_a value is high.[35,36]

Reactivity of indole and indole derivatives at the $\pi - \sigma^*$ excited state-effect of substitution

To understand the reactivity of N-H bond at the excited state and the effect of substitution on the same, the indole and indole derivatives are optimized at their 3rd excited state (which is of $\pi - \sigma^*$ character) and the N-H bond length is monitored. The N-H bond lengths for indole, tryptophan, serotonin and melatonin calculated from the optimized structure at the $\pi - \sigma^*$ state are 1.76 Å, 1.009 Å, 1.007 Å and 1.007 Å, respectively. This compares with their ground state N-H bond lengths of 1.006 Å, 1.006 Å, 1.005 Å and 1.005 Å, respectively. The values indicate that a N-H bond length of 1.76 Å for indole at the $\pi - \sigma^*$ state, indicating a broken N-H bond, while the N-H bond lengths for the indole derivatives remain almost unaltered compared to geometry of the ground states.

From the optimized geometry, a schematic excited state potential energy cut along the N-H bond can be predicted for indole and indole derivatives which is shown in Figure 6. While the $\pi - \sigma^*$ excited state is dissociative along N-H bond for indole, there may be a slight barrier for the other derivatives due to substitution effect which is refraining the N-H bond to dissociate at the excited state. Further study involving the calculation of excited state potential energy surface is necessary to confirm this conjecture which is beyond the scope of this article and will be considered in our future work.

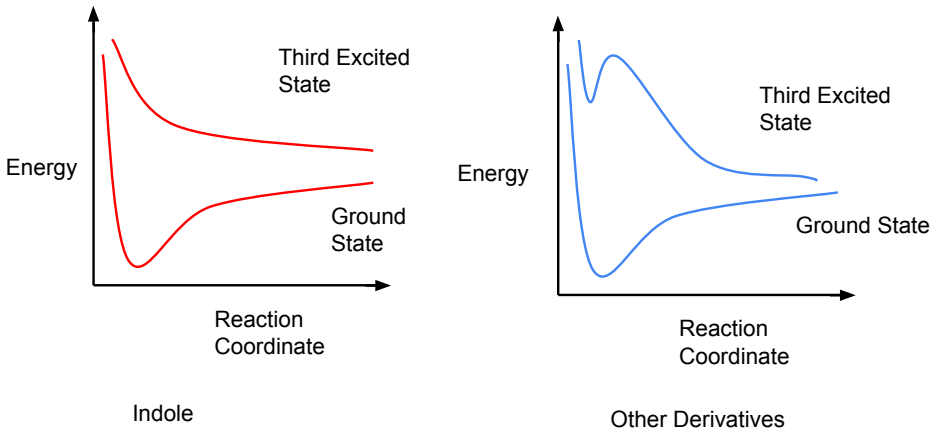


Figure 6: Schematic potential energy cut of the $\pi - \sigma^*$ excited states along N-H bond for indole and indole derivatives.

Conclusion

In this article, we have investigated the electronic substitution effect on the ground and excited state properties of indole ring for some important indole derivatives using quantum chemical calculations. The performance of DFT method for the same has been assessed. The DFT functional and basis sets are benchmarked both for the ground and the excited states of indole before applying it to the other indole derivatives. The effect of substitution on the ground and excited state geometries as well as on the spectral properties is investigated. Electronic substitution effect on the reactivity of the N-H proton at the ground and excited state has alsobeen explored. A detailed description of the excited state including the energy profile of the excited states is reported. The calculated vibrationally resolved absorption spectra for indole, tryptophan, serotonin and melatonin are in good agreement with the literature. This further demonstrates the performance of DFT for this set of chromophores.

Substitution effect plays a vital role in the photo physical properties of indole ring. Indole ring consists of two types of substitutions in its derivatives are discussed in this work. The shorter substitution at the C5 position of the six-membered ring is more active than the longer one in the five membered ring at the C3 position. The shorter substitution not only affects the geometry of the molecule in ground and excited state, but also has significant impact on the vertical and adiabatic excitation energies, HOMO-LUMO gap, peak maxima and broadening of the vibrationally resolved spectra. Bathochromic shift is observed in the absorption spectra in the order of $\lambda_{\text{indole}} < \lambda_{\text{tryptophan}} < \lambda_{\text{serotonin}} < \lambda_{\text{melatonin}}$ due to substitution effect. In case of serotonin and melatonin, the C5 position of six-membered ring consists of electron donating substituing groups which push electron due to +R effect. As a consequence, the electron density on the ring increases. Compared to serotonin, the stronger electron donating group in melatonin induces stronger effect leading to further electron dense six-membered ring. In contrast, tryptophan has no substitution in the six-membered ring. Consequently, indole and tryptophan have similar characteristics in terms of geometry and energy profiles.

While B3LYP functional shows inversion of the L_a and L_b character of the first two excited states, LC-BLYP functional reproduces the correct order of the L_a and L_b type states. On the other hand, the peak maxima and broadening of the spectra calculated using B3LYP functional is in better agreement with the experimental results. The assessment of the performance of DFT for the excited states of indole and indole derivatives paves the path to the quantum chemical study of biologically relevant larger indole derivatives like lysergic acid diethylamide, 4-((3-(ethoxycarbonyl)-1-methyl-5-(pyrrolidin-1-ylmethyl)-1H-indol-2-yl)methyl)benzenesulfinate, (Z)-4-allyl-1-(1-(morpholinomethyl)-5-nitro-2-oxoindolin-3-ylidene) thiosemicarbazide, (3Z,3E)-3-(2-(3-ethyl-4-oxothiazolidin-2-ylidene)hydrazono)-5-nitroindolin-2-one, methyl 1-((1H-indol-3-yl)methyl)-2-naphthoate and (3Z,3E)-5-nitro-3-(2-(4-oxo-3-phenylthiazolidin-2-ylidene) hydrazono)indolin-2-one etc. These molecules are used for several therapeutic ap-

plications[55] and are difficult to be treated using high-level quantum chemical methods due to the presence of large no of atoms. Furthermore, comparison of structure and properties of these molecules provides quantitative estimation of the error originated from the use of indole ring as prototypical system for the calculation of photophysical properties of indole derivatives which can be taken into account in relevant future works.

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