On the Origin of Photodynamic activity of Perylene Quinone Framework

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Abstract: The basic skeleton of perylenequinone is surprisingly ubiquitous in several naturally occurring pigments, such as Hypocrellins, Cercosporin, etc. to name a few. Several of these molecules and their derivatives are also experimentally characterized as potent candidates for photodynamic therapy and are predicted to be aiding the formation singlet Oxygen. Theoretical calculations that unravel the mystery behind the perylenequinone motif in these bio-molecules. Perylenequinone framework has a unique frontier MOs that aid in facile intersystem crossing of the $\pi-\pi^*$ excitation. The resulting triplet state remarkably resists phosphorescence that presumably leads to high quantum yield of singlet oxygen production. The excitation assisted change in the nature of conjugation and the attendant out-of-plane distortion of the perylene framework is found to be the general characteristic of all these systems and the substituents at the bay region favourably assist the excited state behavior as shown by time dependent/independent DFT calculations.

1. Introduction:
Photodynamic therapy (PDT) is an effective noninvasive exogenous clinical procedure[1]. Introduction of Photosensitizers (PS) to living system is sundry. The advantage of the PDT is the indolence of the drugs in the absence of the light source. PS accumulated close the targeted area obliterate target selectively. PDT attributes treatment with minimal drug oriented adverse effects. Delicate surgery and lengthy recuperation periods is avoided along with minimal disfigurement and surgical scar. Introduction to PDT was led by Finsen in the late 1890’s[2]. Clinical applications of Hematoporphyrin derivatives in PDT are immense[3] with non-avoidable issues[4]. This lead to the search of alternative photosensitizers[5-9]. The basic skeleton of perylenequinone is surprisingly ubiquitous in several naturally occurring pigments[14]; such as Hypocrellins, Cercosporin, etc. Several of these molecules[15] and their derivatives are also experimentally characterized[16] as potent candidates for photodynamic therapy(Figure 1) and are predicted to be aiding the formation singlet Oxygen[17]. The perylenequinonoid pigments[16] provide a practical platform from which numerous clinical applications[19] with low toxicity, selective accumulation in targeted cells or tissues retention time absorption[20-21] in the phototherapeutic window between 600 to 900nm[21,34] are achieved. Strong light source absorption, high triplet state quantum yield, slow decay[20-21] low cytotoxicity, easy biological removal are the basis of an effective photosensitizer[18]

2.1. Structural features
Naturally occurring perylenequinonoid pigments consists of planner perylene backbone with four oxygen atoms to the sp$^2$-hybridised carbons of the perylene with intramolecular as well as intermolecular hydrogen bonding.
2.2 Mechanism of PS:
Several theories have been put to explore the mechanistic approach of the photodynamic therapy. Firstly, irradiated PS form slow decaying excited triplet state via intersystem crossing which transfer the energy to the triplet ground state of oxygen resulting highly reactive singlet oxygen[11-13].

In a second way, irradiated PS with hydrogen(s) at the bay region or any labile Hydrogen(s) on the structure (eg: Hypericin) either transfer hydrogen atom or hydrogen radical to the medium having activity similar to reactive singlet oxygen[10]. Hydrogen atom transfer is solvent independent. Transfer is effected by the bulky substituents at the bay region of the perylenequinones and viscosity of the medium. Experimental Validation is confirmed through hydrogen labeling. Alternatively, radiation excited PS acidify the medium by means of hydrogen bonding interaction and subsequent removal of protons form the medium [23-25]. Induced pH change impacts localized anti-viral[20,22,24], apoptosis[1,26-27] and anti-tumour activity[28-29]. Computational investigations on some selected perylenequinone molecules establishes oxygen dependent reactions[32].

![Natural pigments with perylenequinone skeleton. All the structures have a common framework marked in Blue colour. The Red colored region in each structure is the Bay Region.](image)

2.3 Present Work
Current works deals with DFT[30-31] and TDDFT studies on perylene and perylene based systems with most structural resemblance with respect to the naturally occurring perylene quinonoid pigments. The project emphasizes on the qualitative investigation of phototherapeutic potential of some naturally occurring PS having structural similarity.

3. Research Methodology
In Gaussian 09[32], Geometrical optimization and subsequent absorption spectra of all molecular structures are investigated with DFT and TDDFT respectively using B3LYP functional and 6-31G(d)[31] basis set. Molecular Orbit Analysis is done using CACAO[36], based on EHMO[35].

4. Result and discussion:
Two naphthalene units are molecules connected by sp²-hybridized carbon-carbon bonds at the 1 and 8 positions. Kekule model for perylene of D₂h symmetry reveals that the π-conjugation in the perylene is incomplete due to single bonded carbon–carbon atoms at central region with length 1.476Å as observed on optimized structure(Figure 2 A.) Nonbonding Carbon-Carbon and Hydrogen-Hydrogen interaction at the bay region lie within van der waal’s contact. The lowest
frequency corresponding out of plane distortion at central carbon-carbon bond occurs around 27cm\(^{-1}\).

Both highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are non-degenerate at the central region. Highest occupied molecular orbital (HOMO) is antibonding and the lowest unoccupied molecular orbital (LUMO) is bonding; favouring electron transition HOMO to LUMO with band gap around 1.8eV. Ground state Perylenequinone dianion is a 1,2,7,8-tetraoxo perylene dianion. Connected oxo group may be either carbonyl linked or hydroxyl linked to perylene. Optimized Singlet perylenequinone dianion reveals forced double bond character at the central region Carbon-Carbon bond with D\(_{2h}\) symmetry. The distances of nonbonding carbon-carbon atom and nonbonding hydrogen-hydrogen at the bay region are shorter than that of perylene. Out of plane distortion central carbon-carbon bond region is observed at the lowest frequency of 25cm\(^{-1}\). Both HOMO and LUMO of high electron density are non-degenerate and anti-bonding at the central region with band gap 2.0ev.

Figure 2 A. *Kekule structure of perylene showing single bond character at the central region*

Perylenequinone dianion in triplet state pertaining planner geometry(D\(_{2h}\)) is the transition state. The computed minimum for ground state of D\(_{2}\) symmetry which exhibits out of plane distortion where two naphthalene units have rotation by an angle of 7\(^\circ\). The carbon-carbon bonds neighbouring to oxygen atoms show large elongation as corresponding oxygen atoms are antibonding. The central carbon-carbon bonds are also elongated due to increased electron density. The energy gap between singlet and triplet is 23.3KCalMol\(^{-1}\). The sp mixing results reduced energy gap(Figure 2 B). The geometry of the perylenquinone tetra anion is similar to that of the triplet state of perylenequinone dianion with more pronounced effect due to high electron density as well as out-of-plane distortion by 42cm\(^{-1}\). The rotation angle of the two naphthalene units is 11\(^\circ\) which
is 4° more than that of triplet dianion. However, there is substantial distortion within individual naphthalene units. Four Chlorine atoms are substituted at the bay region analyzed computationally for Singlet and triplet states. On substitution of four chlorine atoms at the bay region there is enhanced electron density and the steric hindrance. The individual naphthalene units have a waving type distortion. Chlorine substitution increased the distortion. The dihedral angle between the two bay carbons rises to 32° along with a geometry D₂. The computed properties are similar to that of singlet state of tetra-chloro substituted perylene quinone dianion but the extent of effect is larger. The extent of bond compression is more than that of bond elongation in the triplet state. The energy gap between the singlet and triplet is 16.3KCalMol⁻¹.

Figure 3. Dianion ground state of perylenequinone of D₂ₕ symmetry becomes non-planer D₂ geometry either by radiation or by increased electron density to attain energy minima. The naphthalene units are maneuver out-of-plane rotation. Purple spheres denotes Hydrogen atoms and Red spheres represents the oxygen atoms. Carbon atoms are marked as Grey spheres.

4.3 UV/Visible Spectra

Figure 4. UV/Visible spectra of perylenequinone based structures
Two peaks in the UV/Visible Spectra are attributed to $\pi-\pi^*$ and n-$\pi^*$ transitions from perylene quinone based structures as per computational data analysis. In case of unsubstituted perylene quinone structures, the $\pi-\pi^*$ and n-$\pi^*$ transitions are strong and both are overlapping to each other marginally. In case of tetra-chlorosubstituted perylenequinone, there is decreased absorption intensities. Both the absorptions are red shifted and fall in the phototherapeutic window. Both transitions are overlapping with each other.

**Conclusion:**
Forced single bond character at the central carbon-carbon bond plays crucial role in the photo biological activities. Perylenequinone framework has a unique frontier MOs that aid in facile intersystem crossing of the $\pi-\pi^*$ excitation. The resulting triplet state remarkably resists phosphorescence that presumably leads to high quantum yield of singlet oxygen production. Quinone framework partially removes the forced single bond character implanting rotational restriction. The twisted naphthalene units in the excited state maintains stability. Enhanced non-planarity in the triplet excited state ease energy transfer and subsequent activity of PS. Varying the size of the substituents at the bay region provides finer control to engineer the pigments and synthesis of more efficient photosensitizers.

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