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Experimental and quantum chemical studies on the structure and vibrational spectra of Cearoin (a neoflavonoid)

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Abstract

In the present investigation, the natural product Cearoin is studied by both experimental and theoretical methods. It is classified as a family of neoflavonoid and is widely used as local anti-inflammatory, antibiotic and antiallergic substance. To obtain the detailed information about the discussed molecule a number of experiments has been performed by various techniques including IR, Raman and UV-Visible spectroscopy. Quantum chemical calculations were also performed for a clear interpretation and analysis of the results. HOMO-LUMO energy band gap gives a valuable understanding of the reactivity and some of the structural and physical properties of the theme molecule. Molecular electrostatic potential surface (MEPS), global and local reactivity descriptors are used to study the chemical reactivity of the molecule. Natural Bond Orbital (NBO) analysis is followed to figure out the stability of the molecule arising from hyperconjugative interactions and charge delocalization. As cearoin is utilized as an antibiotic material, molecular docking simulations has also been done on bacterial proteins in order to investigate the ligand-protein interaction.

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1. Introduction

Cearoin (2,5-dihydroxy-4-methoxyphenyl)-phenylmethanone) is a natural compound, that fall into the category of neoflavonoids (a class of polyphenolic compounds). Recently for the first time Maurya et al.[1-2] extracted it from *dalbergia sissoo*, which is a large deciduous perennial tree, belonging to the legume family (Fabaceae), and sub family (Faboideae) [2]. Structural information of cearoin (CRN) was obtained from NMR (carbon and proton), mass spectrometry and vibrational spectroscopy and compared with already reported data [3]. CRN has recently gain much attention of the medicinal research as it is very useful in the treatment of problems such as Osteoporosis, allergy, inflammation, bacterial and microbial infections [2-10]. It is also proved by various clinical and epidemiological studies that CRN can have significant influence in the inhibition of various diseases, at primary as well as secondary level [4,11-13]. To further aid that research, the present investigation is on CRN isolated from bark of *dalbergia sissoo*.

Spectroscopic and quantum chemical techniques are significant procedures to understand the molecular structure, conformational analysis, composition, dynamical behaviour and intramolecular interactions of complex molecules. The substantial characterization of CRN is experimentally done by a variety of methods comprising vibrational spectroscopy (IR and Raman), and UV-Visible spectroscopy. Since intramolecular and charge transfer interactions play a vital role in binding drug-protein complex. Hence, spectroscopic studies have been performed to analyse the intramolecular interactions. The molecular structure, energies and vibrational frequencies of the optimized geometries of CRN were computed using the density functional theory (DFT) method. Potential energy distribution (PED) is used as a base for the understanding of the energy distribution. Vibrational assignment of the normal modes was done on the basis of PED. Molecular electrostatic potential surface (MEPS) of CRN has been interpreted for determining the structure-activity relationship, which gives valuable information for the quality control of medicines and drug-receptor interactions. Natural bond

orbital (NBO) analysis has been done to investigate stability of CRN arising from intramolecular charge transfer interactions. Molecular docking studies were performed for understanding the biological activity of CRN with the protein.

59

60 **2. Experimental details**

61 *2.1. Infrared spectroscopy*

Infrared spectra was recorded on a Bruker TENSOR 27 FT-IR spectrometer with a spectral resolution of 4 cm^{-1} in the region $400\text{--}3700\text{ cm}^{-1}$. KBr pellets of solid samples were prepared from mixtures of the sample and KBr in 1:200 ratio using a hydraulic press.

65 *2.2. FT-Raman spectroscopy*

The FT-Raman spectrum of the CRN was recorded on a Bruker IFS 55 EQUINOX with Raman setup which uses a 1064 nm Nd-YAG laser line as the excitation line for recording the Raman spectra in the region $100\text{--}3700\text{ cm}^{-1}$. The samples are measured in the hemispheric bore of an aluminium sample holder. The spectral resolution of this instrument was also 4 cm^{-1} . Typical spectra are acquired with 512 scans and a laser power of 500 mW at the sample location.

72 *2.3. UV-visible spectroscopy*

The UV-visible absorption spectrum of CRN was recorded in the range 300–500 nm using a Varian-Cary 50 Bio, UV-visible Spectrophotometer equipped with a 10 mm quartz cell. The UV spectrum is taken from a $1\times 10^{-5}\text{ M}$ solution of CRN dissolved in DMSO solvent at 30° C .

77

78 **3. Computational details**

The electronic structure and optimized geometry of CRN were computed by the DFT method using the Gaussian 09 program [14] package employing 6-311++G(d,p) basis set and Becke's three parameter (local, nonlocal, Hartree-Fock) hybrid exchange

functionals with Lee–Yang–Parr correlation functional (B3LYP)[15-18]. The optimized structures were compared using Chem3D software by superimposing them and minimizing the root square distance between selected atoms[19]. Electronic transitions of the molecule were calculated using time-dependent density functional theory (TD-DFT) for both gaseous as well as for solvent phase [20,21]. The solvent effects were taken into account by means of IEF-PCM solvation model[22-23]. Visualization and confirmation of calculated data were done by using the ChemCraft[24] and GaussView [25] program. Gar2ped program was used for the vibrational assignment of the modes[26]. For this purpose a complete set of 84 internal coordinates was defined using Pulay's recommendations[27-28]. To account for the antibiotic activity of the CRN, molecular docking (ligand-protein) simulations have been performed using AutoDock1.5.4 software [29]. The active sites were examined for detailed interactions in Discovery Studio Visualizer 4.5 software[30].

4. Results and discussion

4.1. Conformational studies

The ground state optimized energy of CRN was calculated as -841.8282644 Hartree. In order to reveal all possible conformers and to get the most stable conformer of CRN, detailed 1D (one dimensional) potential energy scans were performed for the dihedral angles $\phi_1(\text{C12-C6-C5-O3})$, $\phi_2(\text{C8-C4-C5-O3})$, $\phi_3(\text{H25-O2-C7-C10})$, $\phi_4(\text{H30-O29-C11-C8})$, $\phi_5(\text{C17-O1-C9-C10})$ and $\phi_6(\text{H26-C17-O1-C9})$ using B3LYP/6-311++G(d,p) basis set. Around these bonds, the variation of torsion angles was carried out at a step of 10° in the range of $0-360^\circ$ rotation. The potential energy scans of these bonds, obtained by changing the potential energy as a function of dihedral angles are given in Fig.1. Conformers are considered on the basis of global minima. In total four conformers were obtained and the optimized structures of all the

conformers along with their relative energies are presented in Fig.S1. Amongst these, the conformer I (CRN I) and conformer II (CRN II) have minimum energy of -841.828264 and -841.824109 Hartree, respectively compared to all the other conformers, which implies that CRN I is the most stable conformer. The optimized energies and the energy difference of all the conformers with respect to the most stable conformer are given in Table S1.

4.2. Geometry optimizations and energies

The geometrically optimized structures of CRN I and CRN II in addition to the atom number scheme are shown in Fig. 2. A comparison of the structural parameters (bond lengths, bond angles and dihedral angles) of CRN I and CRN II obtained by geometry optimization by DFT methods are presented in Table S2. The optimized structure of both conformers CRN I and CRN II was compared by nearly superimposing them using a least squares algorithm that minimizes the distances of the corresponding non hydrogen atoms as shown in Fig.S2. It is clear from Fig.S2 that both the conformers nearly overlapped but, the methoxy group, hydroxyl group and ring R2 shows some differences; this is due to the difference in their direction of orientation. The bond lengths of CRN I and CRN II are almost same. The bond angles $C5-C4-C7$, $C2-C7-C4$, $O1-C9-C11$, $O1-C9-C10$ and $O1-C9-C11$ showed a difference of 1.3741° , 6.5146° , -5.5912° , -8.2831° , and 9.3485° . The other bond angles in both conformers are almost the same and do not show significant differences. The dihedral angles $O3-C5-C6-C13$, $C8-C4-C5-O3$, $H25-O2-C7-C10$, $C8-C11-H29-H30$, $C9-O1-C17-H26$ and $C17-O1-C9-C10$ showed a difference of 10.5266° , -11.7135° , 176.6143° , -6.4727° and -129.4928° between both conformers. The two rings R1 and R2 are essentially planar in both the conformers.

4.3. Vibrational Spectra and Assignments

CRN has 30 atoms; hence it gives $84(3N-6)$ normal modes of vibrations. The vibrational spectrum is mainly determined by the modes of the free molecule observed at higher wavenumbers, together with the lattice (translational and vibrational) modes in the low wavenumber region.

DFT calculations yield Raman scattering amplitudes which cannot be taken directly to be the Raman intensities. The Raman scattering cross section, $\partial\sigma_j/\partial\Omega$, which is proportional to Raman intensity may be calculated from the Raman scattering amplitude and predicted wavenumbers for each normal mode using the relationship [31,32]

$$\frac{\partial\sigma_j}{\partial\Omega} = \left(\frac{2^4\pi^4}{45}\right) \left(\frac{(\nu_0 - \nu_j)^4}{1 - \exp\left[\frac{-h\nu_j}{kT}\right]}\right) \left(\frac{h}{8\pi^2 c \nu_j}\right) S_j$$

Where S_j and ν_j are the scattering activities and the predicted wavenumbers, respectively of the j^{th} normal mode, $\partial\Omega$ is the solid angle, ν_0 is the wavenumber of the Raman excitation line and h , c and k are universal constants. The calculated Raman and IR intensities were used to convolute each predicted vibrational mode with a Lorentzian line shape (FWHM = 8 cm^{-1}) to produce simulated spectra. Assignments have been made on the basis of relative intensities, energies, line shape and PED. All the vibrational bands have been assigned adequately. The detailed assignment of the wavenumbers of the vibrational modes together with their PED is given in Table 1.

4.4. Vibrational wavenumber

The comparison of the wavenumbers calculated at B3LYP level with the observed spectra reveals an overestimation of the wavenumbers of the vibrational modes. This is due to the anharmonicity present in a real system which was not taken into consideration. The

vibrational wavenumbers were scaled down by the wavenumber linear scaling procedure (WLS) [$v_{\text{obs}} = (1.0087 - 0.000016 \cdot v_{\text{cal}})v_{\text{cal}}$][33], as the wavenumbers obtained from the DFT calculations are higher than the observed wavenumbers. The vibrational wavenumbers calculated with appropriate functional are often found in good agreement with the observed wavenumbers when the calculated wavenumbers are uniformly scaled with only one scaling factor [34,35]. All the calculated vibrational wavenumbers for both the conformers reported in this study are the scaled values and were comparatively the same. The evaluation of observed and calculated (scaled) IR and Raman spectra of CRN I and CRN II are shown in Fig. 3 and Fig.4, respectively. There are two significant rings in the CRN and hence we discuss the assignment of these two rings individually.

4.4.1. Ring R1 Vibrations

The R1 ring vibrations are mainly of two types, one is predominantly C-H stretching vibrations and another is C-C stretching vibration. The CH stretching mode of the ring is calculated at 3036/3032 cm^{-1} in CRN I/II and assigned to the IR/Raman peak at 3020/3025 cm^{-1} . The ring CC stretching band is calculated at 1630/1625 cm^{-1} in CRN I/II with medium intensity and observed at 1638/1634 cm^{-1} in IR/Raman spectra. The C-H in-plane deformation is observed at 1283 cm^{-1} in the IR spectra and matches well with the calculated wavenumber at 1286 cm^{-1} for both the conformers [36-37]. The torsional deformation mode of ring R1 is calculated at 1009/1007 cm^{-1} corresponding to the observed IR mode at 1011 cm^{-1} . The peaks at the wavenumber 885 cm^{-1} and 906 cm^{-1} in both IR and Raman spectra and at the wavenumber 900/891 cm^{-1} in CRN I/II are assigned to the C-H out-of-plane bending vibration. Puckering mode calculated at 703 cm^{-1} and 709 cm^{-1} for CRN I and CRN II corresponds to the observed peaks at 705 and 700 cm^{-1} in both Raman and IR spectra.

4.4.2. Ring R2 Vibration

The CH stretching modes are observed at 3076/3075 cm^{-1} in the IR/Raman spectra and are predicted at 3059/3060 cm^{-1} and 3053/3054 cm^{-1} for CRN I/II. The C-C stretching vibrations bond occurs at 1572 cm^{-1} in the IR spectrum and at 1570 cm^{-1} in the Raman spectrum. They are calculated at 1589/1578 cm^{-1} for CRN I/II and observed at 1587/1570 cm^{-1} in the IR/Raman. The C-H in plane deformation modes are calculated at 1171 cm^{-1} and 1192 cm^{-1} in CRN II whereas it is calculated at 1169/1205 cm^{-1} in CRN I. The CH out of plane bending mode is calculated at 999/1006 cm^{-1} in CRNI/II and assigned to the observed IR/Raman band at 1001/1001 cm^{-1} , respectively.

4.4.3. Carbonyl and methoxy group vibration mode

Usually carboxyl group shows its characteristic bands. The carbonyl C=O stretching vibration absorbs strongly in the region 1670 -1820 cm^{-1} [38-40]. The strong IR band observed at 1638 cm^{-1} and its Raman counterpart at 1634 cm^{-1} are assigned to the C=O stretching mode and calculated at 1676 and 1668 cm^{-1} for CRN I and CRN II. Medium intense bands at 2980, 2953 and 2911 cm^{-1} in the Raman spectrum are assigned to C-H stretching asymmetric and symmetric vibrations of methoxy group. The counterpart occurred at 2993, 2951 and 2910 cm^{-1} in the IR spectrum matches well with predicted CRNI/II value at 3008/3010 cm^{-1} , 2949/2979 cm^{-1} and 2892/2900 cm^{-1} .

4.4.4. OH group vibration

The non-hydrogen bonded or free hydroxyl group absorbs strongly in the region 3600-3550 cm^{-1} , while the hydrogen bonding lowers the wavenumbers to the region 3550-3200 cm^{-1} along with increase in intensity and width of IR spectrum[41]. In the FT-IR spectra the bands due to the stretching modes of O2H and O29H group are assigned at 3325 cm^{-1} and are

calculated at 3637/3629 cm^{-1} and 3592/3573 cm^{-1} in CRN I/II, respectively. A difference in wavenumber is observed in the calculated and observed IR mode of CRN. This lowering of wavenumber from calculated to experimental value may be attributed to the hydrogen bonding present in the experimental spectrum (solid state), whereas calculated spectrum is plotted for the isolated molecule free from hydrogen bonds. Raman and IR peak at 632 cm^{-1} and 651 cm^{-1} could be assigned to the out of plane bending of OH₂₉. It is in excellent agreement with calculated value at 631/636 cm^{-1} .

212

213 *4.5.Natural Bond Orbital Analysis*

The NBO analysis has been performed in order to investigate the resonance structure contributions to molecules and also provides an efficient method to study intramolecular charge transfer interactions, rehybridization and delocalization of electron density (ED) within the molecule. The interaction of donor atom and acceptor will be stronger if $E^{(2)}$ value is more [42-43]. When donation tendency from electron donors to electron acceptors is more, the extent of conjugation of the whole system is greater [44]. The hyperconjugative interaction energy was deduced from the second-order perturbation approach[45,46].

$$221 \quad E^{(2)} = -q_i \frac{(F_{ij})^2}{\epsilon_j - \epsilon_i} (2)$$

where, q_i is the population of donor orbital or donor orbital occupancy; ϵ_i , ϵ_j are orbital energies of donor and acceptor NBO orbitals, respectively; F_{ij} is the off-diagonal Fock or Kohn-Sham matrix element between i and j NBO orbitals. NBO analysis allowed us to investigate which orbital interactions are mainly involved in the stability of the molecule. The different types of donor-acceptor interactions and their stabilization energies are determined by second order perturbation analysis of Fock matrix of CRN compound is presented in Table 2.

The following different interactions responsible for the conjugation of the respective π -bonds in the rings R1 and R2 of CRN have been computed with an interaction energy of about 22 kcal/mol: $\pi(\text{C13-C15}) \rightarrow \pi^*(\text{C14-C16})$, $\pi(\text{C14-C16}) \rightarrow \pi^*(\text{C6-C12})$, $\text{LP}(2)\text{O1} \rightarrow \pi^*(\text{C9-C10})$, $\text{LP}(2)\text{O2} \rightarrow \pi^*(\text{C4-C7})$, $\pi(\text{C4-C7}) \rightarrow \pi^*(\text{C8-C11})$, $\pi(\text{C8-C11}) \rightarrow \pi^*(\text{C9-C10})$, $\pi(\text{C9-C10}) \rightarrow \pi^*(\text{C4-C7})$. The $\pi(\text{O3-C5}) \rightarrow \pi^*(\text{C6-C12})$ interaction has maximum interaction energy of 58.33 kcal/mol which is responsible for the stability of the carbonyl group.

Selected Lewis orbitals (occupied bond orbital) of CRN with percentage ED over bonded atoms (ED_x , ED_y in %), hybrid NBOs with s and p character are listed in Table3. The valence hybrids analyses of NBOs show that all the O-C bond orbitals are polarized towards the Oxygen (72.69% at O) atoms, however the O29 -H30 bond orbitals are polarized towards the Oxygen atom (78.82% at O). The electron density distribution (occupancy) about the C=O group mainly affects the polarity of the compound. Consequently, they consist with the maximum electron density on the oxygen, Carbon atom and responsible for the polarity of the molecule.

4.6. Chemical reactivity

The chemical reactivity of the molecule has been described in two ways: (i) MEPS map, (ii) chemical reactivity descriptors.

4.6.1. Molecular electrostatic potential surface

The molecular electrostatic potential surface (MEPS)[47,48] at a point r in the space around a molecule (in atomic units) can be expressed as:

$$V(r) = \sum_A \frac{Z_A}{|\vec{R}_A - \vec{r}|} - \int \frac{\rho(\vec{r}') d\vec{r}'}{|\vec{r}' - \vec{r}|} \quad \text{----(3)}$$

where Z_A is the charge on nucleus A, located at R_A and $\rho(r')$ is the electronic density function for the molecule. The first and second terms represent the contributions to the potential due to nuclei and electrons, respectively. $V(r)$ is the resultant at each point r , which is the net electrostatic effect produced at the point r by both the electrons and nuclei of the molecule. MEPS are used to predict thereactive sites for electrophilic attack and nucleophilic attack. Three-dimensional MEPS superimposed onto the total electron density provides useful information for the interpretation of long-range interaction between molecules, which helps to understand how a ligand binds to its receptor. It provides a clear picture to understand the relative polarity of a molecule and also gives the clear picture of the reactive sites of the molecule. MEP surface are very helpful in which negative regions are regarded as nucleophilic centres, whereas regions with positive electrostatic potential are region of electrophilic sites. Moreover, the electrostatic potential makes the polarization of the electron density visible. Furthermore, they provide useful information on the shape and size of the molecules [49].

MEP surface of CRN I and CRN II is presented in Fig.5. The red and blue region in the MEPS refers to the electron rich and electron deprived region while green region suggests the neutral region. The MEP surface shows that the most electronegative region is visible over C=O group attached to ring R1 and R2 in both CRN I and CRN II. The most electropositive region is localised on both OH group of ring R1 in case of CRN I whereas it is localised only on O29H group in CRN II. The orientation of O2H group is changed in case of CRN II, as H atom of O2H group is turned inwards. As a result no electropositive charge is localized there. These are the reactive sites which are responsible for the chemical reactivity of CRN.

274

275 *4.6.2. Electronic absorption and Frontier Molecular orbitals (FMOs)*

In order to understand electronic transitions in terms of energies and oscillator strengths, the TD-DFT/6-311++G(d,p)[20,21] calculations involving configuration interaction between the singly excited electronic states were performed in the gaseous phase and in the DMSO solution incorporating IEF-PCM model [22,23]. The UV-Vis spectrum of CRN is shown in Fig. 6. Absorption band at 373 nm is observed in DMSO solvent. Both the frontier molecular orbitals, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are the main orbitals taking part in chemical reaction. The electron delocalization between HOMO and LUMO is the principle factor in determining the easiness of a chemical reaction and the stereoselective path, irrespective of intra- and intermolecular process.

To understand the electronic transitions, positions of experimental absorption peaks, calculated wavelengths ($\lambda_{\max}$), vertical excitation energies, oscillator strengths (f), dipole moments, and excitation transition with spectral assignments for vacuum and solvent environment are carried out. There was a difference observed in the transition orbital on going from gaseous to solvent phase of CRN I. The transition observed in the UV spectrum is $\pi \rightarrow \pi^*$ and $\sigma \rightarrow \sigma^*$. The experimental absorption peak is observed at 373 nm corresponding the theoretically calculated peak at 379 and 376 nm (H $\rightarrow$ L) in CRN I and CRN II with oscillator strength of 0.0969 and 0.0951 in solvent phase and in gaseous phase it is calculated at 355 and 376 nm (H $\rightarrow$ L) with oscillator strength of 0.0686 and 0.0651 (Table 4). This shows that the calculations done in solvent phase are closer to the experimental results especially in case of CRN I.

The HOMO and LUMO are the main orbitals that contribute towards the chemical stability [50-51]. The energy gap between the HOMO and LUMO molecular orbitals characterizes the chemical reactivity and kinetic stability along with spectroscopic properties [52]. Fig.7 shows the energy gap between HOMO and LUMO for CRN I and II. In CRN I in case of HOMO,

the charge density is largely on the R2 and carbonyl group while in case of CRN II HOMO charge density is mainly accumulated on carbonyl group and partially on R1 and R2. On the other hand in case of the LUMO more charge density moves to the R1, R2 and carbonyl group for both CRN I and CRN II. The energy gap of HOMO and LUMO shows the chemical strength of the molecule [53-55]. CRN I is chemically more active than CRN II, as its band gap is less than the band gap of CRN II, which implies chemical stability of CRN II is more than the chemical stability of CRN I.

4.6.3. Electronic Reactivity descriptors

Global reactivity descriptor helps in the prediction of electrophilic and nucleophilic reagent, whereas local reactivity descriptors help in the prediction of the site selectivity of electrophilic and nucleophilic attack as well as the order of preference.

4.6.3.1. Local Reactivity descriptors

The local reactivity parameters can be used to analyse the specific reactive sites within the molecule. Fukui function $f(r)$ (FF) is widely used local reactivity parameter to identify the chemical reactivity and site selectivity of the molecule and is defined as the derivative of the electron density with respect to the total number of electrons N in the system, at constant external potential acting on an electron due to all the nuclei in the system. Thus, for an atom k in a molecule, three kinds of condensed FF, namely, f_k^+ , f_k^- and f_k^0 can be used to describe the electrophilic, nucleophilic and radical reactivity, respectively, which are defined by equations in a finite difference approximation [56]. According to Parr and Yang [57], the sites which have highest values of Fukui function $f(r)$ are more reactive centres in chemical species.

For nucleophilic attack $f_k^+(r) = [q_k(N+1) - q_k(N)]$ (4)

326 For electrophilic attack $f_k^-(r) = [q_k(N) - q_k(N-1)]$ (5)

327 For radical attack $f_k^0(r) = 1/2[q_k(N+1) - q_k(N-1)]$ (6)

328 Where q_k is the gross electronic population of atom k in the molecule. Equations 4, 5 and 6
 329 were employed to calculate the Fukui function values. In this case atomic charges derived
 330 from Hirshfeld are used for the Fukui function calculation (Table5). The most susceptible site
 331 for an electrophilic and nucleophilic attack is O3 and O29, respectively in both the
 332 conformers. For a free radical attack the most reactive site is C17 in cases of CRN I and C5 in
 333 CRN II.

334

335 4.6.3.2. Global reactivity descriptors

336 On the basis of Koopman's theorem [58], electrophilicity encompasses both the ability of an
 337 electrophile to acquire additional electronic charge and the resistance of the system to
 338 exchange electronic charge with the environment. It contains information about both electron
 339 transfer (chemical potential) and stability (hardness) and is the better descriptor of global
 340 chemical reactivity. Global reactivity descriptors such as electronegativity (χ), chemical
 341 potential (μ), global hardness (η), global softness (S) and global electrophilicity index (ω) are
 342 calculated using the energies of frontier molecular orbitals ϵ_{HOMO} , ϵ_{LUMO} and given by
 343 eqs.[59–63].

344
$$\chi = -\frac{1}{2}(\epsilon_{HOMO} + \epsilon_{LUMO}) \quad (7)$$

345
$$\mu = -\chi = \frac{1}{2}(\epsilon_{HOMO} + \epsilon_{LUMO}) \quad (8)$$

346
$$\eta = \frac{1}{2}(\epsilon_{LUMO} - \epsilon_{HOMO}) \quad (9)$$

347
$$\omega = \frac{\mu^2}{2\eta} \quad (10)$$

348
$$S = \frac{1}{2\eta} \quad (11)$$

According to Parr et al [64], ω is a global reactivity index similar to μ and η . This is positive and definite quantity. This new reactivity index measures the stabilization in energy when the system acquires an additional electronic charge (ΔN) from the environment. The direction of the charge transfer is completely determined by the electronic chemical potential of the molecule because an electrophile is a chemical species capable of accepting electrons from the environments. Therefore its energy must decrease upon accepting electronic charge and its electronic chemical potential must be negative. The energies of frontier molecular orbitals (ϵ_{HOMO} , ϵ_{LUMO}), energy band gap ($\epsilon_{\text{HOMO}} - \epsilon_{\text{LUMO}}$), χ , μ , η , S and ω for CRN I and CRN II are listed in Table 6. The energy band gap ($\epsilon_{\text{HOMO}} - \epsilon_{\text{LUMO}}$), for CRN I and II are 3.87165 eV and 4.10308 eV respectively. In addition the value of global softness is more in CRN I which signifies that it is softer than CRN II.

4.7. Molecular docking

Molecular docking is a significant tool in structural molecular biology and computer-assisted drug design. The aim of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure [65,66]. Peroxisome Proliferator-active Protein is used for the prevention and treatment of inflammation-related diseases. Crystal structure of Peroxisome Proliferator-active (anti-inflammatory) and DCD-1 (anti-microbial) bacterial protein is downloaded from RSCB PDB website (PDB code: 4XTA-B and 2YMK-C [67]. The active site of the enzyme was defined to include residues of the active site within the grid size of $60\text{\AA} \times 60\text{\AA} \times 60\text{\AA}$. The protein was prepared by removing the waters, co-crystallized ligands, co-factors and Lamarckian Genetic Algorithm (LGA) available in AutoDock was employed for docking [68]. Out of all the docked conformations, one which bound well at the active site was examined for detailed interactions in Discovery Studio Visualizer 4.5 software[28] and shown in Fig. 8 and Fig. 9. Two proteins of E. coli

were studied. Protein (Peroxisome Proliferator-active)-ligand interaction shows that there are three conventional H-bond with amino acids: Asp-B:441(1.70 Å), Lys-B:(2.21 Å) interact with OH group of ring R1 and Thr-B:440(2.56 Å) interact with C=O group attached to ring R1 and R2 of CRN (Fig. 8). Other interactions were also observed such as pi-cation (Asp-A:396, 4.96 Å), pi-Anion (Arg-A:443, 3.55 Å), and pi-alkyl (Ile-A:391, 4.98 Å, Met-A:439, 4.08 Å).

DCD-1 protein shows antimicrobial activity thereby limiting skin infection diseases. Second protein (DCD-1) - ligand interaction reveals that protein interacts with amino acids through four conventional H-bonds. Lys-B:34, (2.08 Å, 2.13 Å) interacts with C=O group and OH group of ring R1. Glu-A:30, (2.05 Å) interact with OH group of ring R1, Lys-A:34(1.91 Å) interact with OH group present in R1 (Fig. 9). Pi-alkyl (LysA:4, 5.02 Å) interactions are also observed. The ligand CRN forms a stable complex with Peroxisome Proliferator-active and DCD-1 having binding energy -6.55 and -3.73 kcal/mol, respectively. Peroxisome Proliferator-active has larger binding energy than DCD-1 which shows that it has strong interactions with the ligand. The result of docking as well as local reactivity descriptors are same having O-H and C=O as prominent reactive sites.

5. Conclusions

In the present study the experimental and theoretical analysis of the possible conformations of the cearoin (CRN) has been reported. Comprehensive assignments of the vibrational spectra of the most stable conformers have been made with the aid of theoretically predicted vibrational wavenumbers. Finally, the calculations were done for the two most stable conformers that are similar in geometry except for the orientation of the methoxy group, OH group and ring 2 of. Further the calculated results of both the conformers are used to simulate infrared and Raman spectra which showed good agreement with observed spectra. NBO

analysis shows that O3 (oxygen) atom has the most intensive interaction in between the acceptor and the donor. From the HOMO-LUMO energy gap and global reactivity parameters, it is clear that cearoinI (CRN I) is more reactive and having high value of global softness than cearoin II (CRN II). MEPS was plotted for predicting the electronic properties. The absorption peaks are recorded at 373 nm in UV-Vis spectrum which has transition from $H \rightarrow L$ ($\pi \rightarrow \pi^*$). Fukui calculations show that O3 and O29 are susceptible for nucleophilic and electrophilic attack for both the conformers where as for free radical attack the most reactive site is C17 (carbon) for CRN I and C5 for CRN II. Molecular docking simulation shows protein-ligand interactions by means of H-bond. The C=O and OH groups binding the protein conventionally.

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Figure captions:

Fig. 1 Potential energy surface scan with varying dihedral angle ϕ_1 , ϕ_2 , ϕ_3 , ϕ_4 , ϕ_5 and ϕ_6 of CRN.

Fig. 2 Optimized structure for CRN I and CRN II with the atoms number.

Fig. 3 Observed and calculated infrared spectra of CRN in the region $400\text{-}1000\text{cm}^{-1}$, $1001\text{-}1720\text{cm}^{-1}$ and $2800\text{-}3700\text{cm}^{-1}$.

Fig. 4 Observed and a calculated Raman spectra of CRN in the region $100\text{-}900\text{cm}^{-1}$, $900\text{-}1800$ and $2801\text{-}3800\text{cm}^{-1}$.

Fig. 5 Molecular electrostatic potential (MESP) formed by mapping of total density over electrostatic potential of CRN I and CRN II.

Fig. 6 Experimental UV–Visible spectra of CRN in DMSO solvent.

Fig. 7 HOMO-LUMO plots for CRN I and CRN II.

Fig. 8 Docking of CRN with the molecular target Peroxisome Proliferator-active.

Fig. 9 Docking of CRN with the molecular target DCD-1.

Fig.S1 List of all the conformers with their optimized structure along with their energies.

Fig.S2 Comparison of the experimental and the optimized structure of CRN I and CRN II (hydrogen atoms are not shown).

Table 1 Theoretical and experimental vibrational wavenumbers (cm^{-1}) of CRN with potential energy distribution (PED).

449 **Table 2** Second order perturbation theory analysis of Fock matrix in NBO Basis for CRN.

450 **Table 3** Selected Lewis orbitals (occupied bond orbital) with percentage ED over bonded
 451 atoms (ED_X , ED_Y in %), hybrid NBOs with s and p character in % for CRN.

452 **Table 4** Electronic transitions, absorption wavelength λ_{max} (nm), excitation energy (eV) and
 453 oscillator strengths (f) of CRN I and CRN II.

454 **Table 5** Calculated local reactivity properties of the selected atoms using Hirshfeld
 455 [B3LYP/6-311++G(d,p)] derived charges of CRN I and CRN II.

456 **Table 6** Calculated ϵ_{HOMO} , ϵ_{LUMO} , energy band gap ($\epsilon_H - \epsilon_L$), chemical potential (μ),
 457 electronegativity (χ), global hardness (η), global softness (S) and global electrophilicity index
 458 (ω) at 298.15 K of CRN I and CRN II.

459 **Table S1** Ground state optimized energy and energy difference of all the four conformers of
 460 CRN.

461 **Table S2** Comparison of bond length Å, bond angle ($^\circ$) and dihedral angle ($^\circ$) of the two
 462 conformers of CRN by DFT method using 6-311++g(d,p).
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Potential energy surface scan with varying dihedral angle ϕ_1 , ϕ_2 , ϕ_3 , ϕ_4 , ϕ_5 and ϕ_6 of CRN.

32x13mm (600 x 600 DPI)

Optimized structure for CRN I and CRN II with the atoms number
24x8mm (600 x 600 DPI)

Observed and calculated infrared spectra of CRN in the region 400-1000cm⁻¹, 1001-1720cm⁻¹ and 2800-3700 cm⁻¹.

41x14mm (600 x 600 DPI)

Observed and a calculated Raman spectra of CRN in the region 100-900 cm⁻¹, 900-1800 and 2801-3800cm⁻¹.

42x16mm (600 x 600 DPI)

Molecular electrostatic potential (MESP) formed by mapping of total density over electrostatic potential of CRN I and CRN II.

28x36mm (600 x 600 DPI)

Experimental UV–Visible spectra of CRN in DMSO solvent.

21x10mm (600 x 600 DPI)

HOMO-LUMO plots for CRN I and CRN II.

38x18mm (600 x 600 DPI)

Docking of CRN with the molecular target Peroxisome Proliferator-active.

15x9mm (600 x 600 DPI)

Docking of CRN with the molecular target DCD-1.

14x8mm (600 x 600 DPI)

Table 1

Theoretical and experimental vibrational wavenumbers (cm⁻¹) of CRN with potential energy distribution (PED).

Unscaled DFT CRN I	Scaled		Experimental		Assignment
	CRN I	CRN II	IR	RAMAN	
3844	3637	3629	3325	-	v(O2H)(93)+v(O29H)(7)
3794	3592	3573		-	v(O29H)(93)+v(O2H)(7)
3204	3064	3066	3096	3090	R2[v _s (CH)](93)
3198	3059	3060	3076	3075	R2[v _a (CH)](99)
3192	3053	3054		3063	R2[v _a (CH)](96)
3182	3045	3046	3042		R2[v _a (CH)](94)
3180	3043	3038			R1[v _s (CH)](99)
3172	3036	3032	3020	3025	R1[v _a (CH)](99)
3161	3026	3031	2993	2994	R2[v _a (CH)](94)
3141	3008	3010		2980	[v _a (CH ₃)](99)
3076	2949	2979	2951	2953	[v _a (CH ₃)](99)
3013	2892	2900	2910	2911	[v _s (CH ₃)](99)
1708	1676	1668	1638	1634	v(C=O)(75)+δ _{in} (C5C6)(5)
1660	1630	1625			R1[v(CC)(63)+δ _a ⁺ (O1C9)(10)]
1638	1608	1605	1599		R2[v(CC)(57)+δ _{in} (C12H)(7)]+R1[δ _a (O1C17)(9)]
1634	1604	1585		1601	R1[v(CC)(56)+δ _a (O1C9)(10)]
1618	1589	1578	1572	1570	R2[v(CC)(66)+δ _{in} (C16H)(10)]+R1[δ _a ⁺ (O1C)(9)]
1543	1517	1511	1512	1503	R1[δ _{in} (CH)(28)+v(C9C)(27)+v(O1C)(9)]
1520	1495	1493			R2[δ _{in} (C15H)(60)+v(C15C)(37)]
1505	1482	1486		1503	δ _a (CH ₃)(80)+δ _a ⁺ (CH ₃)(9)+ρ(CH ₃)(5)+ρ ⁺ (CH ₃)(5)
1493	1470	1465			δ _a ⁺ (CH ₃)(79)+δ _a (CH ₃)(8)
1480	1457	1460	1460	1460	δ _s (CH ₃)(54)+R2[δ _{in} (C16H)(6)]
1478	1455	1452	1450		δ _s (CH ₃)(20)]+R1[δ _{in} (CH)](41)+v(CC)(19)
1473	1450	1441	1433	1439	R1[v(CC)](33)+δ _s (CH ₃)(12)+δ(C11H30O)(7)+v(O2C)(13)
1396	1377	1354	1385	1383	R1[v(CC)(64)+v(OC)(13)]+δ(C11H30O)(7)
1351	1333	1333	1342	1344	R2[δ _{in} (CH)(54)+v(CC)(37)]
1335	1318	1315	1317	1317	R2[v(CC)(70)+δ _{in} (CH)(16)]
1317	1300	1303		1290	R1[v(CC)(49)+v(CO)(32)+δ _{in} (CH)(5)]
1304	1287	1286	1283		R1[δ _{in} (CH)](42)+δ(C7H25O)(17)+δ(C11HO)(16)
1280	1265	1265	1269		v(CC)(45)+R1(δ _{trig})(11)+δ(C7HO)(8)+v(OC)(6)
1242	1228	1228	1238	1237	R2[v(CC)(18)+δ _{in} (CH)(8)]+R1[v(C11O)(16)+δ _{trig} (10)]
1226	1212	1209	1209	1213	ρ ⁺ (CH ₃)(33)+R1[v(O2C)(16)+δ _{in} (C10H)(12)+ρ(CH ₃)(8)+v(C4C)(5)
1218	1205	1192	1194	1196	R2[δ _{in} (CH)(64)+v(CC)(7)+ρ ⁺ (CH ₃)(5)]
1198	1185	1185			R1[δ _{in} (C10H)](19)+δ(C11HO)(18)+v(O1C)(7)
1187	1174	1172			δ(C7HO)(30)+R1[δ _{in} (C8H)(17)+v(C4C)(7)+v(O2C)(5)]
1182	1169	1171	1161	1167	R2[δ _{in} (CH)(74)+v(C15C)(17)]
1170	1158	1158			ρ(CH ₃)(73)+ρ ⁺ (CH ₃)(20)+δ _a (CH ₃)(5)
1169	1157	1139		1166	δ(C11HO)(20)+R1[δ _{trig} (O1C)(14)+v(C8C)(19)+v(C11O)(14)]+δ(C7HO)(13)
1121	1110	1120	1128	1125	R1[v(C4C)(15)+v(OC)(22)+v(O2H)(7)+v(C7C)(7)+δ _{trig} (12)]+R2[v(C6C13)(5)]
1101	1091	1092	1082		R2[v(CC)(48)+δ _{in} (CH)(31)]
1052	1043	1039	1028		R2[v(CC)(49)+δ _{in} (CH)(22)]+R1[δ _{trig} (OC)](10)
1042	1033	1011	1011	1034	v(O1C17)(45)+R1(δ _{trig})(25)
1017	1009	1007			R1[(O1C)(δ _{trig})(35)+v(OC)(13)]+R2[v(CC)(44)]
1007	999	1006	1001	1001	R2[δ _{oop} (CH)](78)+R1(puck)(16)
993	985	991	977	966	R2[δ _{oop} (CH)](90)+R1[(τ _a ⁺)(8)]
952	945	949	933	934	R2[δ _{oop} (CH)(78)+δ _{oop} (CC)(5)]+R1[τ _a (6)+puck(5)]
939	933	927	918		δ(C5OC)(15)+R1[v(OC)(21)+δ _{oop} (C8H)(5)]+R2[v(CC13)(6)]
906	900	891	885	906	R1[δ _{oop} (CH)(74)+τ _a (8)]

859	854	864	854	859	R2[$\delta_{\text{oop}}(\text{CH})$](99)
839	835	863	842	839	R1[$\delta_{\text{oop}}(\text{C10H})$](47)+puck(25)+ $\delta_{\text{oop}}(\text{CO})$ (13)]
815	810	821	825	815	R1[$\delta_{\text{oop}}(\text{CC})$](27)+ $\delta_{\text{oop}}(\text{CH})$ (24)+v(CO)(5)+ $\delta_{\text{irrig}}(5)$]
800	796	791	802	800	R1[puck(13)+v(C11O)(12)+ $\delta_{\text{oop}}(\text{CH})$ (8)+v(C9C)(8)+ $\delta_{\text{irrig}}(6)$])+R2[$\delta_{\text{oop}}(\text{CC})$](15)]
750	747	746	759	750	R1[puck(13)+ $\delta_{\text{oop}}(\text{CC})$ (14)+ $\delta_{\text{oop}}(\text{C7O})$ (8)+v(C9C)(5))+R2[$\delta_{\text{oop}}(\text{CH})$](14)]
736	733	735	736	736	R1[puck(13)+ $\delta_a(\text{O1C})$ (13)+ $\delta_{\text{oop}}(\text{C4C})$ (6)+ $\delta_{\text{oop}}(\text{C7O})$ (5)+v(CO)(10)]
711	708	725		711	R1[$\delta_{\text{oop}}(\text{CO})$](48)+puck(20)+ $\tau'_a(12)$]+ $\delta(\text{C9C17O1})$ (6)]
705	703	709	700	705	R1[puck(48)+ $\delta_{\text{oop}}(\text{CH})$ (34)+ $\delta_{\text{oop}}(\text{CC})$ (5)]
693	691	693	686	693	R1[puck(29)+ $\delta_a(\text{O1C})$ (18)+ $\delta_{\text{oop}}(\text{C7O})$ (11)]
688	686	676	665	688	R1[puck(39)+ $\delta_{\text{oop}}(\text{C7O})$ (9)+ $\delta_{\text{in}}(\text{C5C})$ (5)]
632	631	636	633	632	R1[$\delta'_a(\text{O1C})$ (80)+ $\delta_{\text{oop}}(\text{O29H})$ (13)]
614	613	628	592	614	R1[$\delta_{\text{in}}(\text{C11O})$ (31)+ $\delta_{\text{oop}}(\text{CO})$ (14)+ $\delta_{\text{oop}}(\text{CC})$ (5)+ $\delta_{\text{in}}(\text{C7O})$ (5)]
592	591	614		592	R1[$\delta_{\text{oop}}(\text{C7O})$ (19)+puck(14)+ $\delta(\text{C5OC})$ (12)+ $\delta_a(\text{OC})$ (15)+ $\delta_{\text{in}}(\text{CO})$ (6)]
538	537	530	540	538	R1[$\tau_a(15)$ + $\delta_{\text{in}}(\text{C5C})$ (13)+ $\delta_a(\text{O1C})$ (8)+ $\delta_{\text{oop}}(\text{C7O})$ (7)+ $\delta_{\text{in}}(\text{C7O})$ (5))+R2[$\delta_{\text{oop}}(\text{C6C5})$](11)]
484	484	490	513	484	R1[$\tau_a(26)$ + $\delta_{\text{oop}}(\text{C11O})$ (21)+ $\delta'_a(\text{O1C})$ (11)+ $\delta_a(\text{O1C})$ (7)+ $\delta_{\text{oop}}(\text{C9O1})$ (5)+ $\delta(\text{C9CO})$ (5)]
461	461	477	476	461	R1[$\tau_a(33)$ + $\delta_{\text{oop}}(\text{CO})$ (29)+ $\delta'_a(\text{O1C9})$ (9)]
458	458	426	459	458	R1[$\tau_a(\text{OC})$ (29)+ $\delta_{\text{oop}}(\text{CC})$ (8)+ $\delta_{\text{oop}}(\text{CO})$ (8)+ $\delta_a(\text{OC})$ (7))+R2[v(CO)(5)+ $\delta_{\text{oop}}(\text{CC})$](10)]
416	416	418		416	R1[$\tau_a(32)$ + $\tau'_a(11)$ + $\delta_{\text{oop}}(\text{CO})$ (5)+ $\delta'_a(5)$ + $\delta_{\text{oop}}(\text{CC})$ (5)+R2[$\delta_{\text{oop}}(\text{CC})$](10)]
414	414	411	410	414	R1[$\tau'_a(76)$ + $\delta_{\text{oop}}(\text{C14H})$ (5)]
401	401	383		401	R2[$\delta_{\text{in}}(\text{C11O})$ (17)+ $\delta'_a(\text{O1C})$ (15)]+ $\delta(\text{C5O3C4})$ (17)]
385	385	374		385	$\delta(\text{C9C17O})$ (23)+R1[$\delta_{\text{in}}(\text{C7O})$ (28)+ $\tau_a(8)$ + $\delta_{\text{oop}}(\text{C9O})$ (8)]
337	337	350	-	337	R1[$\delta_{\text{in}}(\text{CO})$ (37)]+ $\delta(\text{C5O3C})$ (11)+ $\delta_{\text{oop}}(\text{C11O})$ (8)+ $\delta_{\text{in}}(\text{C6C})$ (5)]
319	320	335	-	319	R1[$\tau(\text{O2C})$ (71)+ $\delta_{\text{in}}(\text{C9O})$ (7)]
281	282	287	-	281	R1[$\delta_{\text{in}}(\text{C9O})$ (29)+ $\tau(\text{C11O})$ (20)+ $\tau(\text{O2C})$ (14)+ $\delta_{\text{oop}}(\text{C7O})$ (5)+ $\tau(\text{O1C})$ (5)]
250	250	280	-	250	R1[$\tau(\text{C11O})$ (63)+ $\delta_{\text{in}}(\text{C9O})$ (10)]
248	249	247	-	248	R2[$\delta_{\text{in}}(\text{C6C})$ (28)]+R1[v(C4C)(15)+ $\delta'_a(11)$ +puck(5)]
217	217	233	-	217	$\delta_{\text{in}}(\text{C4C5})$ (14)+R1[$\tau(\text{O1C})$ (13)+ $\delta_{\text{oop}}(\text{C10H})$ (8)+ $\tau(\text{O1C})$ (8)+ $\delta_{\text{oop}}(\text{C9O})$ (7)]
211	212	204	-	211	R1[$\tau_a(19)$ + $\delta_{\text{oop}}(\text{CH})$ (8)+ $\delta(\text{C9CO})$ (7)+ $\delta_{\text{oop}}(\text{CC})$ (6))+R2[$\delta_{\text{in}}(\text{CC})$](25)]
191	192	180	-	191	R1[$\delta_{\text{in}}(\text{C4C})$ (26)+ $\tau_a(16)$ + $\delta_{\text{oop}}(\text{C6C})$ (15)+ $\tau(\text{C4C})$ (5)]
176	177	158	-	176	R1[$\tau'_a(20)$ + $\tau(\text{CC})$ (23)+ $\delta_{\text{oop}}(\text{C4C5})$ (13)+ $\delta_{\text{in}}(\text{C4C})$ (10)+ $\delta_{\text{oop}}(\text{C5C})$ (10)]
147	148	140	-	147	R1[$\tau(\text{O1C})$ (42)+ $\delta_{\text{oop}}(\text{C9O})$ (12)+ $\delta(\text{C9CO})$ (8)]
113	113	99	-	113	R1[$\tau'_a(10)$ + $\tau(\text{C4C})$ (9)+ $\delta_{\text{in}}(\text{C4C})$ (5)+R2[$\tau(\text{C5C})$ (23)+ $\delta_{\text{oop}}(\text{C4C})$ (16)+ $\delta_{\text{in}}(\text{CC})$ (10)]
88	88	62	-	87	R1[$\tau'_a(39)$ + $\tau(\text{C4C})$ (33)+ $\delta_{\text{oop}}(\text{C4C5})$ (8)]
48	48	58	-	48	R1[$\tau(\text{O1C})$ (6)+ $\delta_{\text{in}}(\text{C9O})$ (9)]+ $\delta(\text{C9CO})$ (8)]
43	43	46	-	43	R1[$\tau(\text{O1C})$ (56)+ $\tau(\text{C5C})$ (18)+ $\delta_{\text{in}}(\text{C9O1})$ (7)]+ $\delta(\text{C9C17O})$ (7)]
30	30	28	-	29	R1[$\tau(\text{CC})$ (53)+ $\tau(\text{O1C})$ (21)]

Table 2
Second order perturbation theory analysis of Fock matrix in NBO basis for CRN I.

Donor NBO (i)	ED(i)/e	Acceptor NBO(j)	ED(j)/e	E(2) ^a kcal/mol	E(j)-E(i) ^b	F(i,j) ^c
π (C4 - C7)	1.63718	π^* (O3-C5)	0.18625	13.80	0.27	0.057
	1.63718	π^* (C8-C11)	0.36169	22.61	0.28	0.071
	1.63718	π^* (C9-C10)	0.01880	17.26	0.27	0.062
π (C6-C12)	1.62449	π^* (O3-C5)	0.18625	18.04	0.26	0.064
	1.62449	π^* (C13-C15)	0.29322	20.22	0.28	0.069
	1.62449	π^* (C14-C16)	0.32959	18.95	0.27	0.065
π (C8-C11)	1.69985	π^* (C4-C7)	0.44097	15.32	0.29	0.062
	1.69985	π^* (C9-C10)	0.40014	21.52	0.28	0.071
π (C9-C10)	1.68114	π^* (C4-C7)	0.44097	23.41	0.29	0.076
	1.68114	π^* (C8-C11)	0.36169	16.01	0.29	0.061
π (C13-C15)	1.65315	π^* (C6-C12)	0.36314	18.63	0.28	0.065
	1.65315	π^* (C14-C16)	0.32959	21.99	0.28	0.070
π (C14-C16)	1.65261	π^* (C6-C12)	0.36314	21.86	0.28	0.071
	1.65261	π^* (C13-C15)	0.29322	17.60	0.29	0.064
LP (2)O1	1.85674	π^* (C9-C10)	0.40014	23.07	0.34	0.084
LP (2)O2	1.97793	π^* (C4- C7)	0.44097	24.87	0.35	0.091
LP (2)O3	1.90144	π^* (C4-C5)	0.05824	16.41	0.69	0.096
	1.90144	π^* (C5-C6)	0.05750	15.56	0.70	0.094
LP (2)O29	1.90483	π^* (C8-C11)	0.36169	21.51	0.35	0.083
π (O3-C5)	1.96690	π^* (C6-C12)	0.36314	58.53	0.02	0.065

^a E(2) means the energy of hyperconjugative interaction (stabilization energy).

^b Energy difference between donor (i) and acceptor (j) NBO orbitals.

^c F(i,j) is the Fock matrix element between i and j NBO orbitals.

Table 3

Selected Lewis orbitals (occupied bond orbital) with percentage ED over bonded atoms (ED_X , ED_Y in %), hybrid NBOs with s and p character in % for CRN I.

Bond (X-Y) (ED_{X-Y})	ED_X (%) ED_Y (%)	Hybrid NBOs	s (%)	p (%)
$\sigma(O1 - C9)$ (1.99158)	66.93% 33.07%	$0.8181(sp^{2.06})o^+$ $0.5750(sp^{2.96})c^+$	32.66% 25.21%	67.27% 74.58%
$\sigma(O1-C17)$ (1.98949)	69.37% 30.63%	$0.8329(sp^{2.67})o^+$ $0.5534(sp^{3.85})c^+$	27.26% 20.57%	72.69% 79.14%
$\sigma(O2-C7)$ (1.99356)	66.81% 33.19%	$0.8174(sp^{1.99})o^+$ $0.5761(sp^{3.14})c^+$	33.48% 24.09%	66.45% 75.70%
$\sigma(O29-C11)$ (1.99356)	66.61% 33.39%	$0.8162(sp^{2.06})o^+$ $0.5778(sp^{3.11})c^+$	32.62% 24.27%	67.31% 75.52%
$\sigma(O3-C5)$ (1.99234)	64.74% 35.26%	$0.8046(sp^{1.59})o^+$ $0.5938(sp^{2.39})c^+$	38.63% 29.45%	61.27% 70.36%
$\sigma(O2 -H25)$ (1.98760)	73.99% 26.01%	$0.8602(sp^{3.80})o^+$ $0.5100(sp^{0.00})H^+$	20.80% 99.88%	79.11% 0.12%
$\sigma(O29 -H30)$ (1.98794)	74.16% 35.26%	$0.8612(sp^{3.74})o^+$ $0.5938(sp^{2.39})H^+$	21.09% 99.88%	78.82% 0.12%

Table 4 Electronic transitions, absorption wavelength λ_{max} (nm), excitation energy (eV) and oscillator strengths (f) of CRN I and CRN II.

Excited state	Experimental	Conformers	Calculated					DMSO				
			Gas phase					Solvent Phase				
			λ_{max} (nm)	E(eV)	Oscillator strength (f)	Excitation transition	Transition type/ assignment	λ_{max} (nm)	Oscillator strength (f)	E(eV)	Excitation transition	Transition type/ assignment
1.	373	CRN I	355	3.4920	0.0686	H→L	$\pi \rightarrow \pi^*$	379	0.0969	3.269	H→L	$\sigma \rightarrow \sigma^*$
		CRN II	376	3.2935	0.0651	H→L	$\sigma \rightarrow \sigma^*$	376	0.0951	3.296	H→L	$\sigma \rightarrow \sigma^*$

Table 5

Calculated local reactivity properties of the selected atoms using Hirshfeld [B3LYP/6-311++G(d,p)] derived charges.

CRN I						CRN II					
Atom	f_k^+	Atom	f_k^-	Atom	f_k^0	Atom	f_k^+	Atom	f_k^-	Atom	f_k^0
2O	0.09385	3O	0.11990	5C	0.09578	2O	0.08044	3O	0.12622	5C	0.10012
4C	0.07259	5C	0.10285	7C	0.09760	4C	0.06687	5C	0.10946	7C	0.09228
8C	0.04873	9C	0.04933	9C	0.07226	7C	0.06964	9C	0.04574	9C	0.06368
9C	0.04967	13C	0.04099	11C	0.08280	9C	0.05488	13C	0.0450	11C	0.08003
11C	0.08950	15C	0.04139	18H	0.05080	11C	0.08299	16C	0.07526	18H	0.06332
29O	0.09567	16C	0.07288	20H	0.05928	29O	0.09979	24H	0.04035	19H	0.04103

Table 6
Calculated ϵ_{HOMO} , ϵ_{LUMO} , energy band gap ($\epsilon_{\text{H}}-\epsilon_{\text{L}}$), chemical potential (μ), electronegativity (χ), global hardness (η), global softness (S) and global electrophilicity index (ω) at 298.15 K for conformer I and conformer II of CRN.

Molecule	ϵ_{H}	ϵ_{L}	$\epsilon_{\text{H}}-\epsilon_{\text{L}}$	χ	μ	η	S	ω	ΔN_{max}
CRN I	-6.0714	-2.1997	3.8717	4.1355	-4.1355	1.9358	0.2583	4.4174	2.1363
CRN II	-5.8875	-1.7844	4.1031	3.8359	-3.8359	2.0515	0.2437	3.5862	1.8698

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