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Investigation of reactive and spectroscopic properties of oxobutanoic acid derivative:
Combined spectroscopic, DFT, MD and docking study

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Abstract

The optimized geometry and wavenumbers of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid (APAMOB) have been calculated by density functional theory (DFT) using Becke's three-parameters hybrid functional (B3LYP) with 6-311++G(d) (5D, 7F) basis set. The theoretically predicted vibrational wavenumbers were compared with the observed FT-IR and FT-Raman spectra. The NH stretching wavenumber is red shifted in the IR spectrum from the computed wavenumber, which indicates the weakening of the NH bond. The ring breathing mode of the para substituted phenyl ring is assigned at 836 cm⁻¹ experimentally and at 835 cm⁻¹ theoretically. Molecular electrostatic potential map has also been plotted for predicting the reactive sites in the molecule. Using natural bond orbitals analysis hyperconjugative interactions and second order stabilization energies are calculated. Nonlinear optical properties of the molecule are predicted. In order to determine molecule sites that could be sensitive towards electrophilic attacks we have calculated ALIE values and mapped them to electron density surface. In the same way we have also visualized Fukui functions, which helped us to further gain an insight into the local reactivity properties of title molecule. Bond dissociation energies (BDE) have been calculated in order to determine if

autoxidation mechanism is possible, while the potential influence of hydrolysis was investigated to calculations of radial distribution functions (RDF) obtained after molecular dynamics (MD) simulations. Molecular docking study suggests that the title compound shows the inhibitory activity against 2-deoxy-d-gluconate 3-dehydrogenase.

Keywords: DFT; oxobutanoic; ALIE; BDE; RDF; Molecular docking.

1. Introduction

Substituted 4-oxobut-2-enoic acids and their derivatives exhibit very promising pharmacological properties such as antiulcer, cytoprotective, kynurenine-3-hydroxylase, human cytomegalovirus protease inhibiting activities [1-3]. Also several acylacrylic acids isolated from natural sources show antibiotic activities [4]. EL-Hashash et al., reported the utility of 4-(4-acetamidophenyl)-4-oxobut-2-enoic acid as a key starting material for the preparation of a novel series of pyridazinones, thiazole derivatives and other heterocycles via interaction with nitrogen, sulfur, and carbon nucleophiles under Michael addition conditions and studied their antimicrobial activities [5]. These heterocycles also possessed analgesic and anti-inflammatory activities [6]. Removal of organic pharmaceutical molecules is great challenge for scientists due to great stability of these structures, for which degradation natural conditions are not effective [7]. Due to very frequent usage and improper handling pharmaceutical molecules enter the environment and accumulate, especially in water resources. This is great concern since conventional water purification methods are not efficient for their removal [8,9]. Forced degradation of pharmaceuticals by advanced oxidation could be the right solution for the pollution by organic pollutants, however still there are no wide commercial applications of this approach [8, 10-15]. Forced degradation studies are great challenge, however rationalization and optimization of complex and time consuming activities is achieved by employment of molecular modeling principles, mainly DFT calculations and MD simulations [16-19].

2. Experimental details

3-Methylidenedihydrofuran-2,5-dione (0.112 g, 1 mmol) and 30 ml acetone were mixed together and stirred at ambient temperature. To this mixture, 4-aminoacetophenone (0.135 g, 1 mmol) in 20 mL acetone was added over 30 mins. After stirring for 1.5 h the slurry was filtered, washed with acetone and dried to give the title compound. The FT-IR (Fig. S1-supporting material) and FT-Raman spectra (Fig.S2-supporting material) were obtained on a DR/Jasco FT-IR spectrometer and on a Bruker RFS 100/s, Germany.

3. Computational details

Calculations of the title compound were carried out with the Gaussian09 program [20] using B3LYP/6-311++G(d) (5D, 7F) basis set to predict the molecular structure and wavenumbers and a scaling factor of 0.9613 had to be used for obtaining a considerably better agreement with experimental data [21]. Structural parameters with XRD data corresponding to the optimized geometry of the title compound (Fig.1) are given in Table S1 (supporting material). The assignments of the calculated wavenumbers are done using Gaussview [22] and GAR2PED [23] software. Jaguar 9.0 program [24] and Desmond [25-28] programs, as implemented in Schrödinger Materials Science Suite 2015-4, have been utilized for DFT calculations and MD simulations, respectively. DFT calculations were performed with B3LYP exchange-correlation functional [29] and with 6-311++G(d,p), 6-31+G(d,p) and 6-311G(d,p) basis sets for the calculations of ALIE, Fukui functions and BDEs, respectively. In case of MD simulations OPLS 2005 force field [30] was used with simulation time set to 10 ns, temperature set to 300 K, pressure to 1.0325 bar, cut off radius set to 12 Å, within isothermal–isobaric (NPT) ensemble class. Simple point charge (SPC) model [31] was used for the description of solvent. MD simulation system was modelled by placing of one APAMOB molecule into the cubic box with ~3000 water molecules. To analyze the electron density a method of Johnson et al. [32,33] was applied. Maestro GUI [34] was used for the preparation of input files and analysis of results when Schrödinger Materials Science Suite 2015-4 was used

4. Results and discussion

4.1 Geometrical parameters

For the title compound, the CC bond lengths in the phenyl ring lie between 1.3822-1.4068 Å (DFT) and 1.3802-1.3962 (XRD) which are in agreement with literature [35]. In the present case, the C=O bond lengths are 1.2164, 1.2043, 1.2186 Å (DFT), 1.2222, 1.2492, 1.2162 (XRD) and C-O bond length is 1.3720 Å (DFT), 1.2882 (XRD) which are in agreement with reported values [36]. The C-N distances (DFT/XRD) are $C_8-N_6 = 1.3756/1.3544$ Å, $C_{17}-N_6 = 1.4056/1.4202$ Å and these values indicate that the CN bonds show partial double bond character in this fragment. Also the CN bond distances were found to be in between the single and double bond character of C-N bond [36], suggesting some multiple bond character is present. The C=C bond length (DFT/XRD) is 1.3369/1.3282 Å and the C-C bond lengths (DFT/XRD) lie in the range 1.4922-1.5388/1.4852-1.5232 Å which are in agreement with reported literature [36]. At C_{22} position, the bond angles (DFT/XRD) $C_{20}-C_{22}-C_{27} = 118.7/118.7$ Å and $C_{23}-C_{22}-C_{27} = 123.2/123.1$ Å and this asymmetry in angles reveal the interaction between O_5 and H_{21} atoms. Similarly at C_{17} position, the bond angle $C_{18}-C_{17}-$

C_{25} and $C_{18}-C_{17}-N_6$ are reduced by $0.8/0.3^\circ$ and 2.9° while the bond angle $C_{25}-C_{17}-N_6$ is increased by $3.7/2.6^\circ$ from 120° which reveals the interaction between the phenyl ring and the $NHC=O$ moiety. The bond angles (DFT/XRD) around C_8 atom are, $C_9-C_8-N_6 = 114.1/115.1$, $C_9-C_8-O_1 = 121.3/121.4^\circ$ and $N_6-C_8-O_1 = 124.7/123.5^\circ$ which is due to the interaction between the carbonyl group and neighbouring units.

4.2 IR and Raman spectra

The calculated scaled wavenumbers, observed IR, Raman bands and vibrational assignments of the title compound are given in table 1.

C=OCH₃ modes

The methyl stretching modes are expected in the region $3050-2900\text{ cm}^{-1}$ [37] and for the title compound methyl stretching vibrations are observed at $2970, 2922\text{ cm}^{-1}$ in the Raman spectrum and at $3024, 2974, 2920\text{ cm}^{-1}$ theoretically with PEDs around 100%. The CH_3 deformation modes are expected in the regions $1480-900\text{ cm}^{-1}$ [37] and for the title compound these modes are assigned at $1433, 1338, 965, 940\text{ cm}^{-1}$ (IR), $1434, 1350, 963\text{ cm}^{-1}$ (Raman) and at $1440, 1431, 1346, 965, 940\text{ cm}^{-1}$ theoretically with average IR intensities and for the mode at 940 cm^{-1} the PED is 42% while for all other modes the PED lies between 84 and 95%. The carbonyl stretching mode exhibits a strong band at $1750 \pm 20\text{ cm}^{-1}$ in acetates [37] and in the present case, the stretching mode of $C_{27}=O_5$ is assigned at 1720 cm^{-1} in the IR spectrum, 1725 cm^{-1} in the Raman spectrum and at 1737 cm^{-1} theoretically with high IR intensity of 298.25 and a PED of 75%.

C=OOH modes

The $C_{13}=O_2$ stretching vibration in the spectra of carboxylic acids give rise to a band in the region $1600-1700\text{ cm}^{-1}$ [37] and the band observed at 1680 cm^{-1} in the IR spectrum, 1688 cm^{-1} in the Raman spectrum and at 1694 cm^{-1} (DFT) is assigned at $C_{13}=O_2$ stretching mode (IR intensity, 228.75, Raman activity 72.21 and PED 72%) and the reported values are 1680 cm^{-1} (IR) and 1675 cm^{-1} (DFT) [36]. The OH in-plane deformation and the C-O stretching mode are expected in the regions $1390 \pm 55\text{ cm}^{-1}$ and $1250 \pm 80\text{ cm}^{-1}$ [37] and the band observed at 1312 cm^{-1} in the IR spectrum and at 1308 cm^{-1} (DFT) is assigned as the in-plane bending of OH and $C_{13}-O_3$ stretching mode is assigned at 1238 cm^{-1} theoretically and at 1240 cm^{-1} experimentally. The OH deformation and C-O stretching has high IR intensities and average Raman activities with PEDs, 47 and 36%. The reported values of OH deformation is at 1321 cm^{-1} experimentally, at 1318 cm^{-1} theoretically and the C-O stretching vibration is at 1235 cm^{-1} experimentally, at 1238 cm^{-1} theoretically [36].

C=ONH modes

For the title compound the stretching mode of $C_8=O_1$ is assigned at 1668 cm^{-1} in the IR spectrum, 1650 cm^{-1} in the Raman spectrum and at 1672 cm^{-1} theoretically which is in agreement with literature [37, 38]. This stretching mode has a high IR intensity of 248.49, Raman activity of 123.29 and PED of 78%. The reported value of the carbonyl stretching mode is at 1654 cm^{-1} in the IR spectrum and at 1659 cm^{-1} (theoretically) [36]. In the present study, the NH stretching band is observed at 3339 cm^{-1} in the IR spectrum and the DFT calculations give this mode at 3434 cm^{-1} which is expected in the range $3500\text{--}3300\text{ cm}^{-1}$ [37, 39]. The NH stretching wavenumber is red shifted by 95 cm^{-1} in the IR spectrum from the computed frequency, which indicates the weakening of the NH bond [36]. In the present case the bands observed at 1507 cm^{-1} in the IR spectrum, 1506 cm^{-1} in the Raman spectrum and at 1508, 1234, 661 cm^{-1} (DFT) were assigned as NH deformations modes which are expected around 1500, 1250 cm^{-1} and $600\text{--}700\text{ cm}^{-1}$ [37, 38]. These deformation modes possess PEDs, 39, 46 and 34% and has high IR intensities of 532.15, 241.68 and 80.84. For the mode at 661 cm^{-1} the Raman activity is low and other two modes has high Raman activities. Mary et al. [40] reported the NH bands at 1537, 1250, 650 cm^{-1} in the IR spectrum and at 1580, 1227, 652 cm^{-1} theoretically. The CN stretching modes of the title compound are assigned at 1228, 1205 cm^{-1} (IR), 1225 cm^{-1} (Raman) and at 1231, 1205 cm^{-1} theoretically as expected [37,38] with PEDs, 33 and 42%. The mode at 1231 cm^{-1} has high IR intensity and Raman activity. The NH deformation modes are reported at 1541, 1256 cm^{-1} in IR and 1551, 1255 cm^{-1} theoretically [36] and the CN stretching modes are reported at 1277 cm^{-1} (IR), 1277, 1202 cm^{-1} (DFT) [36].

C=C, C-C and CH_2 modes

The C-C stretching modes of the title compound are assigned at 1011, 940 cm^{-1} in the IR spectrum, 1012, 734 cm^{-1} in the Raman spectrum and at 1052, 1011, 940, 739 cm^{-1} theoretically as expected [37,41]. For all these modes the IR intensity is low. For the title compound, C=C stretching mode is observed at 1610 cm^{-1} experimentally and at 1617 cm^{-1} theoretically. The CH_2 group vibrations are expected in the regions, $3050\text{--}2900\text{ cm}^{-1}$ (stretching modes) and $1475\text{--}900\text{ cm}^{-1}$ (deformation modes) [37,38] and for the title compound these modes are assigned at 3010, 2958 cm^{-1} (IR), 3008, 2954 cm^{-1} (Raman), 3032, 3031, 3012, 2956 cm^{-1} (DFT) (stretching modes) and at 1275, 1240, 1113, 1080 (IR), 1274, 1240, 1113, 1080 (Raman), at 1440, 1386, 1277, 1238, 1110, 1082, 932, 911 cm^{-1} (DFT) (deformation modes). All the deformation modes possess average IR intensity and PEDs are also high.

Phenyl ring modes

The calculated values of CH stretching modes of the phenyl ring are at 3122, 3121, 3068 cm^{-1} and 3066 cm^{-1} and bands are observed experimentally at 3115, 3064 cm^{-1} in the IR spectrum and at 3114, 3080, 3067 cm^{-1} in the Raman spectrum as expected in literature [37]. The phenyl ring C-C stretching modes of the title compound are assigned at 1588, 1300 cm^{-1} in the IR spectrum, 1480, 1393, 1303 cm^{-1} in the Raman spectrum and at 1586, 1574, 1483, 1389, 1300 cm^{-1} theoretically with PEDs in the range 47-64% and for the mode at 1483 cm^{-1} the IR intensity is less than 10.00 while all other modes possess high IR intensity and these modes are expected in the range 1610-1280 cm^{-1} [37, 42]. For the title compound the ring breathing mode of the para substituted phenyl ring is assigned at 836 cm^{-1} experimentally and at 835 cm^{-1} theoretically, with 43% PED and IR intensity and Raman activity are less than 10.00. The reported value of this ring breathing mode is at 873 cm^{-1} (IR), at 861 cm^{-1} (DFT) [43] and at 849 cm^{-1} (IR), 852 cm^{-1} (DFT) [44]. The CH deformation modes of the phenyl ring are assigned at 1161 cm^{-1} in IR, 1165, 988 cm^{-1} in Raman spectrum (in-plane) and at 825 cm^{-1} in IR spectrum, 824 cm^{-1} in Raman spectrum (out-of-plane) which are expected above and below 1000 cm^{-1} [37]. The DFT calculations give these modes at 1288, 1159, 1105, 996 cm^{-1} (in-plane) and at 943, 941, 826, 807 cm^{-1} (out-of-plane).

4.3 Nonlinear Optical properties

NLO properties like the dipole moment, polarizability, first and second order hyperpolarizabilities are calculated using B3LYP/6-311++G(d) (5D, 7F) basis set. The total molecular dipole moment of the title compound is 3.4068 Debye, polarizability is 2.714×10^{-23} esu, and the first and second order hyperpolarizabilities are 6.268×10^{-30} and -15.928×10^{-37} esu. Here the first hyperpolarizability of the title compound is 48.22 times that of the standard NLO material urea [45] and comparable with the reported values of similar derivatives [36]. Also the NLO properties are related to the energy gap between HOMO and LUMO. The energy gap of the title compound is 3.357 eV which is lower than that of urea (6.7 eV) [45]. Therefore, the investigated molecule is suitable for nonlinear optical applications.

4.4 Frontier Molecular Orbitals

The frontier orbitals are used to determine the way through which the molecule interacts with other species and the formation of a transition state is due to an interaction between them [46]. The HOMO and LUMO are related to ionization potential and electron affinity [47] and the HOMO-LUMO energy gap is a stability factor which gives the electron transport properties [48]. In the HOMO-LUMO plot (Fig.S3-supporting material), the HOMO is localized over the entire molecule, except on methyldene and carboxylic group

while the LUMO is over the entire molecule, which shows the charge transfer within the molecular system. Using the frontier molecular orbital energies, the ionization potential and electron affinity can be found as: $I = -E_{\text{HOMO}}$, $A = -E_{\text{LUMO}}$ [36]. The different chemical descriptors, the hardness (η) and chemical potential (μ) are given by the following relation $\eta = (I-A)/2$ and $\mu = -(I+A)/2$ where I and A are ionization potential and electron affinity [49]. The value of $E_{\text{HOMO}} = -8.667$, $E_{\text{LUMO}} = -5.233$, energy gap = 3.434, global hardness $\eta = 1.717$, chemical potential $\mu = -6.9507$, global electrophilicity index $\omega = \mu^2/2\eta = 14.066$ eV in the case of the title compound. The descriptor, molar refractivity (MR) is an important term used in quantitative structure property relationship and is given as, $MR = 1.333\pi N\alpha$, where N is the Avogadro number and α is the polarizability of the molecular system [50]. For the title compound, the molar refractivity is 68.41 and this is responsible for the binding property of the molecular system and can be used for the cure of different diseases [51].

4.5 Molecular Electrostatic Potential

The molecular electrostatic potential (MEP) and electronic density are related to each other and MEP is a very useful term for determining reactive sites in the molecular system [52]. The different values of the electrostatic potential are represented by different colours and potential increases in the order of red < orange < yellow < green < blue. In MEP maximum negative region represents the site for electrophilic attack indicated by red color while the maximum positive region represents nucleophilic attack indicated by blue color. As seen from the MEP map (Fig.S4-supporting material) of the title compound, regions of negative potential are over the electro negative oxygen atoms of the carbonyl groups and the regions having the positive potential are over the hydrogen atoms of the amide and carboxylic acid group.

4.6 ALIE surfaces, Fukui functions and noncovalent interactions

Molecule sites that are possibly prone to electrophilic attacks can be detected by applying the concept of average local ionization energy (ALIE). This quantity provides information about the energy required to remove an electron from the target molecule. Molecule sites where ALIE values are the lowest determine locations that are prone to electrophilic attacks. ALIE is defined as a sum of orbital energies weighted by the orbital densities according to the following equation:

$$I(r) = \sum_i \frac{\rho_i(\vec{r}) |\epsilon_i|}{\rho(\vec{r})}. \quad (1)$$

where $\rho_i(\vec{r})$ denotes electronic density of the i -th molecular orbital at the point $\vec{r}$, ε_i denotes orbital energy, while $\rho(\vec{r})$ denotes total electronic density function [53,54]. This quantity was introduced by Sjöberg et al. [55,56] for the studies of reactivity of aromatic molecular systems. ALIE values mapped to electron density surface of APAMOB molecule are presented in Fig.2. Analysis of Fig.2 indicates that there are several locations possibly prone to electrophilic attacks. Beside benzene ring, the areas characterized with red color (ALIE values of ~ 209 kcal/mol) are located in the near vicinity of hydrogen atom H10 and oxygen atom O5. At these molecule locations the electrons are least tightly bound and therefore prone to electrophilic attacks to the greatest extent. On the other side, there are two molecule sites with electrons most tightly bound to molecule, hydrogen atoms H4 and H15, characterized by ALIE value of ~ 384 kcal/mol.

Study of electron density within APAMOB molecule reveals the existence of two intramolecular noncovalent interactions, Fig.3. Both of these two noncovalent interactions are formed between oxygen and hydrogen atoms and both of them are having the same strength (-0.017 electron/bohr³). They are precisely located between atoms O1–H26 and O3–H7. Thanks to the concept of Fukui functions, further insight into the local reactive properties of APAMOB molecule can be located. Fukui functions in the finite difference approximation, which is the case with Jaguar program, are calculated according to the following equations:

$$f^+ = \frac{(\rho^{N+\delta}(r) - \rho^N(r))}{\delta}, \quad (2)$$

$$f^- = \frac{(\rho^{N-\delta}(r) - \rho^N(r))}{\delta}. \quad (3)$$

where N stands for the number of electrons in reference state of the molecule, while δ stands for the fraction of electron which default value is set to be 0.01 [57]. In this work the values of Fukui functions have been also mapped to the electron density surface, Fig.4.

In the case of Fukui functions in this work the following color coding has been adopted. Positive color is purple color, while negative color is red color. Since Fukui functions indicate change in electron density after addition (Fukui f^+ function) or removal (Fukui f^- function) of charge, positive color in Fig.4a indicate the molecule locations where electron density increases after the addition of charge, while negative color in Fig.4b indicate molecule locations where electron density decreases after the charge removal. This approach helps in determining electrophilic and nucleophilic molecule sites. According to the results presented in Fig.4a it can be stated that after charge addition electron density increases in the

near vicinity of hydrogen atom H15. On the other side results presented in Fig.4b indicate that red color is delocalized over atoms O3, C9, C12, C13 and H10, indicating that at these locations electron density decreases after the charge removal.

4.7 Natural Bond Orbital analysis

The natural bond orbitals (NBO) calculations were performed using NBO 3.1 program [58] as implemented in the Gaussian09 software package and the important interactions are given in tables S2 and S3 (supporting material). The strong interactions in the molecular system are: $n_2(O_1) \rightarrow \sigma^*(N_6-C_8)$, O_2 of $n_2(O_2) \rightarrow \sigma^*(O_3-C_{13})$, O_3 of $n_2(O_3) \rightarrow \pi^*(O_2-C_{13})$, $n_1(O_5) \rightarrow \sigma^*(C_{22}-C_{23})$ and $n_1(N_7) \rightarrow \pi^*(O_1-C_8)$ with stabilization energies, 26.22, 35.85, 38.78, 167.12 and 58.75 kJ/mol. The 100% p-character was observed in π -bonding of O_1-C_8 , O_2-C_{13} , O_5-C_{27} , $C_{12}-C_{14}$, $C_{17}-C_{25}$, $C_{22}-C_{23}$ and nearly 100% p-character was observed in lone pairs of O_1 , O_2 , O_3 , O_5 and N_6 . Thus, a very close to pure p-type lone pair orbital participates in the electron donation to the $\pi^*(O-C)$, $\pi^*(N-C)$ and $\pi^*(C-C)$ orbitals in the compound.

4.8 Reactive and degradation properties based on autoxidation and hydrolysis

DFT calculations and MD simulations are widely used in order to rationalize and optimize different experimental procedures. Understanding of forced degradation studies greatly depends on the understanding of reactive properties of organic molecules that are being decomposed. In this regard it is important to mention that calculations of BDE can be very helpful for the prediction of molecule sites that are sensitive towards autoxidation mechanism. On the other side, radial distribution functions (RDF) after MD simulations are effective tool for determination of atoms with pronounced interactions with water and thus can be helpful in predicting the sensitivity towards the mechanism of hydrolysis [59-62]. Autoxidation mechanism can start only at certain molecules sites; the ones where it is possible to abstract hydrogen atoms. At these molecule sites BDE values for hydrogen abstraction lie in the specific interval. Drug candidate molecule is the most sensitive towards the mechanism of autoxidation if the BDE values for hydrogen abstraction lie in the interval of 70 to 85 kcal/mol [63,64]. According to Gryn'ova et al. [65] BDE values between 85 and 90 kcal/mol are questionable, however could be important for the autoxidation mechanism. On the other side, BDE values lower than 70 kcal/mol are not suitable for autoxidation mechanism [63, 66, 67]. BDE values for all single acyclic bonds of APAMOB molecule are given in Fig.5. APAMOB molecule could be sensitive towards autoxidation mechanism, since there is one representative value for hydrogen abstraction. That is bond denoted with number 3 in Fig.5 (C_9-H_{10} bond) and in this case BDE has the value of ~87 kcal/mol. This value is higher than desired 85 kcal/mol, but it is located in the range of questionable and

possibly important BDE values, 85-90 kcal/mol, according to the Gryn'ova et al. [65]. All other BDE values for hydrogen abstraction are too high to be considered as interesting for the autoxidation mechanism. Of the BDE values for the rest of the single acyclic bonds the lowest has been calculated for bond denoted with number 11 in Fig.5 (C8–C9 bond), which is adjacent to the bond denoted with number 3, which has the lowest BDE for hydrogen abstraction. These results indicate that degradation could start by abstraction of hydrogen atoms H10 (or H11) and or by breaking of C8–C9 bond.

Concerning the interactions of APAMOB's atoms with water molecules, obtained results according to the calculated RDFs after MD simulations indicate interesting results, Fig.6. Namely, there are four carbon, two oxygen and two hydrogen atoms with $g(r)$ curve profiles that indicate significant interactions with water molecules. $g(r)$ indicates the probability of finding a particle in the distance r from another particle [68]. Carbon atoms of interest, from the aspect of hydrolysis, are C8, C12, C14 and C28. The highest maximal $g(r)$ value has been obtained for atom C12, while in the same time the peak distance is located at the highest distance, of almost 5 Å. Carbon atoms C8, C14 and C28 are very similar in terms of peak distance, namely their peak distances are located at around 3.5 Å, while their maximal $g(r)$ values are in the range from around 1.1 to around 1.4. Non-carbon atoms have much more significant interactions with water molecules. Two oxygen atoms, O1 and O3, are very similar in terms of both maximal $g(r)$ value and peak distance, but RDF profile of oxygen atom O1 is much sharper indicating stronger interactions with water molecules comparing with O3. Certainly the strongest interactions with water molecules, according to RDFs, have been determined in case of hydrogen atoms H4 and H7. H7 has the peak distance located at around 1.8 Å with corresponding maximal $g(r)$ value of almost 0.9. The sharpest RDF, with the lowest peak distance and the highest maximal $g(r)$ value of all the APAMOB's atoms, has been obtained in the case of hydrogen atom H4. This hydrogen atom has the most pronounced interactions with water molecules since its maximal $g(r)$ value is almost 1.9, while its peak distance is located at just 1.6 Å.

4.9 Molecular docking studies

AutoDock Vina [69] software is used to identify the binding modes of the molecule responsible for the activity. The docking grid box was set at the size of 40×40×40 points with the spacing of 0.375 Å and genetic algorithm is used as search parameter. PASS (Prediction of Activity Spectra) server [70] predicts different types of activities based on the structure of the molecule. In the present case the PASS prediction list of the title compound is tabulated in table S4 (supporting material) and Gluconate 2-dehydrogenase with probability to active

value 0.782. Gluconate 5-dehydrogenase (Ga5DH) is an NADP(H)-dependent enzyme that catalyzes a reversible oxido reduction reaction between d-gluconate and 5-keto-d-gluconate, thereby regulating the flux of this important carbon and energy source in bacteria. d-gluconate metabolism are found only in certain bacterial species, and consequently, these proteins are attractive targets for inhibition and drug therapy in the case of *S. suis* infections [71]. Literature survey suggests the antibacterial activity of oxabutanonic acid derivatives [5, 72]. Thus we choose the title compound as ligand and 2-deoxy-d-gluconate 3-dehydrogenase as target and is downloaded from RCSB protein data bank with PDBID is 4hp8. After optimization with B3LYP/6-31G(d,p) method, the molecular docking studies were performed to gain insight into the interaction with molecule and target protein. The protein-ligand complex is visualized by Discovery studio visualizer4.0 software. The docked ligand form stable complexes (Fig.7 and Fig.S5-supporting material) with the protein and got the binding affinity value is -6.4 kcal/mol(table 2). Thus title compound shows the inhibitory activity against 2-deoxy-d-gluconate 3-dehydrogenase. The inhibition mainly depends on the binding modes and binding affinities of the small molecules to the enzyme [73]. Key residues were also considered to be a significant factor that can affect binding activities. The nearest amino acid residues and shortest intermolecular contacts between ligand and receptor and the most of the interactions are electrostatic interaction, hydrophobic interaction and hydrogen bonding. There are two strong H-bond interaction with C=O group and the amino acids Gly20 and Asn188. The phenyl ring forms π -cation, π -anion and π -alkyl interaction with polar residues His214, Glu216 and Ala24 respectively. Though the results shows the validate result for biological test.

5. Conclusions

A detailed interpretation of the vibrational spectra has been carried out for the biologically important 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid. The HOMO and LUMO energies and its plot show that charge transfer occurs within the molecule. The electron density changes in the antibonding orbitals and excitation energies have been calculated using NBO analysis and this gives a clear evidence for stabilization energy originating from molecular interactions. The most sensitive locations towards electrophilic attacks are benzene ring and near vicinities of hydrogen atom H10 and oxygen atom O5, as determined by ALIE surface. Other possibly important molecule sites include near vicinities of atoms H15, O3, C9, C12, C13 and H10, as determined by Fukui functions. According to the calculated BDE values there is one interesting molecule site, possibly suitable for the start of autoxidation mechanism. That is C9–H10 bond, characterized by BDE value for hydrogen

abstraction of ~87 kcal/mol. It is also important to note that H10 is also interesting molecule site from the aspect of ALIE surface. In the same time, bond C8–C9 adjacent to bond C9–H10 has the lowest BDE value of all (~69 kcal/mol) indicating that degradation could start here. From the aspect of hydrolysis mechanism, the most important molecule site is certainly hydrogen atom H4, with very sharp RDF profile indicating strong interactions with water molecules. This indicates that autoxidation mechanism for this molecule is possible, because two mechanisms do not compete at the position of H10 atom, the one the lowest BDE value for hydrogen abstraction. The docked ligand form stable complexes with the protein with a binding affinity value of -6.4 kcal/mol and the two strong H-bond interaction with C=O group and the amino acids Gly20 and Asn188.

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Figure captions

- Fig.1 Optimized geometry of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid
- Fig.2 ALIE surface of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule
- Fig.3 Intramolecular noncovalent interactions of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule
- Fig.4 Fukui functions a) f^+ and b) f^- of the 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule
- Fig.5 BDEs of all single acyclic bonds of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule
- Fig.6 Representative RDFs of atoms of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule
- Fig.7 The docked ligand (pink) at the active site of the receptor

Table 1

Calculated wavenumbers (scaled), observed IR and Raman bands and vibrational assignments

B3LYP/ 6-311++G(d) (5D, 7F)			IR	Raman	Assignments ^a
$\nu(\text{cm}^{-1})$	IRI	Raman	$\nu(\text{cm}^{-1})$	$\nu(\text{cm}^{-1})$	-
3587	64.58	89.28	-	-	$\nu\text{OH}(100)$
3434	109.65	206.61	3339	-	$\nu\text{NH}(99)$
3122	2.49	46.28	-	-	$\nu\text{CH}(98)$
3121	0.41	60.93	3115	3114	$\nu\text{CH}(100)$
3078	3.18	79.24	-	3080	$\nu\text{CH}(97)$
3066	8.29	67.41	3064	3067	$\nu\text{CH}(98)$
3032	3.01	93.85	-	-	$\nu\text{CH}_2(98)$
3031	18.37	76.90	-	-	$\nu\text{CH}_2(97)$
3024	17.50	114.53	-	-	$\nu\text{CH}_3(99)$
3012	4.01	51.41	3010	3008	$\nu\text{CH}_2(99)$
2974	10.45	42.85	-	2970	$\nu\text{CH}_3(100)$
2956	7.93	99.43	2958	2954	$\nu\text{CH}_2(99)$
2920	3.68	161.22	-	2922	$\nu\text{CH}_3(100)$
1737	298.25	16.87	1720	1725	$\nu\text{C=O}(75)$
1694	228.75	72.21	1680	1688	$\nu\text{C=O}(72)$
1672	248.49	123.29	1668	1650	$\nu\text{C=O}(78)$
1617	39.39	93.92	1610	1610	$\nu\text{C=C}(65)$, $\nu\text{Ph}(15)$
1586	56.71	471.10	1588	-	$\nu\text{Ph}(64)$, $\delta\text{NH}(10)$
1574	283.32	191.25	-	-	$\nu\text{Ph}(62)$, $\delta\text{NH}(12)$
1508	532.15	199.90	1507	1506	$\nu\text{Ph}(41)$, $\delta\text{NH}(39)$
1483	2.25	3.23	-	1480	$\nu\text{Ph}(50)$, $\delta\text{CH}(18)$
1440	35.95	11.77	-	-	$\delta\text{CH}_2(91)$
1440	12.30	10.45	-	-	$\delta\text{CH}_3(94)$
1431	19.60	5.29	1433	1434	$\delta\text{CH}_3(90)$
1389	47.32	3.90	-	1393	$\nu\text{Ph}(47)$, $\delta\text{CH}_2(14)$, $\delta\text{CH}(26)$
1386	10.73	32.62	-	-	$\nu\text{Ph}(10)$, $\delta\text{CH}_2(54)$
1346	52.42	1.75	1338	1350	$\delta\text{CH}_3(84)$

1308	132.98	26.25	1312	-	δ OH(47), ν CC(24)
1300	71.16	94.20	1300	1303	ν Ph(56), δ CH(18)
1288	23.54	7.36	-	-	ν Ph(17), δ CH(68)
1277	3.01	5.69	1275	1274	δ CH ₂ (85)
1238	67.45	12.85	1240	1240	δ CH ₂ (35), ν CO(36)
1234	241.68	42.56	-	-	δ NH(46), ν CO(10)
1231	159.64	153.77	1228	1225	ν CN(33), ν Ph(22)
1205	5.90	21.51	1205	-	ν CN(42), ν CO(14)
1159	121.53	128.74	1161	1165	δ CH(68)
1110	246.84	12.14	1113	1113	δ CH ₂ (57), ν CN(11), ν CO(18)
1105	101.29	0.97	-	-	δ CH(47), ν Ph(19)
1082	116.66	4.15	1080	1080	δ CH ₂ (60)
1052	2.09	69.76	-	-	ν CC(38), ν Ph(25)
1011	0.89	0.29	1011	1012	ν CC(64)
990	0.80	12.19	-	988	δ CH(53), ν Ph(14)
965	34.86	4.10	965	963	δ CH ₃ (95)
943	1.33	0.20	-	-	γ CH(86)
941	0.23	0.17	-	-	γ CH(83), τ Ph(14)
940	11.51	4.15	940	-	ν CC(33), δ CH ₃ (42)
932	18.45	4.66	-	-	ν CC(17), δ CH ₂ (47)
924	34.81	19.39	-	-	ν CC(44), δ CH ₂ (11), δ C=C(10)
911	6.60	2.13	-	-	δ CH ₂ (51), ν CC(19)
835	5.00	8.70	836	836	δ NH(15), ν Ph(43)
826	48.28	0.29	825	824	γ CH(71)
807	34.38	0.50	-	-	γ CH(66)
801	21.74	2.30	-	-	γ C=O(40), τ C=C(18), γ CH(15)
775	2.48	14.09	-	780	γ C=O(27), τ C=C(21), δ CH ₂ (25)
739	8.25	4.58	-	734	ν CC(42), δ C=O(13)

719	0.18	1.22	716	712	τ Ph(66), γ CN(14), γ CC(11)
685	19.06	28.58	684	686	ν CC(18), δ Ph(46)
661	80.84	1.02	-	-	γ NH(34), τ CN(17), τ C=C(14)
659	80.14	2.12	657	657	τ C=C(25), τ CO(24), γ C=O(21), γ NH(13)
624	0.08	8.62	622	630	δ Ph(75)
613	5.24	2.71	-	611	τ CO(34), γ C=O(11), δ C=O(11)
591	59.99	3.71	593	590	τ CO(21), δ C=O(20), τ C=C(10)
579	12.59	0.46	577	-	γ C=O(37), δ CC(13), γ CC(10), τ Ph(13)
558	7.09	1.85	555	560	δ C=O(42), δ Ph(20)
519	7.35	5.97	-	-	δ C=O(25), δ CC(22)
501	8.00	2.37	502	-	δ CC(40), γ CN(10)
484	20.05	0.38	488	488	τ Ph(31), γ CC(40)
458	7.06	0.55	465	462	δ CC(20), δ Ph(32)
411	1.24	1.09	-	415	δ CC(52), δ CH ₂ (22)
404	0.05	0.08	-	-	τ Ph(81), τ CC(10)
386	19.04	2.33	-	389	γ CC(22), δ CN(11), γ C=O(22)
361	4.53	1.89	-	359	δ CH ₂ (28), δ C=C(16), δ C=O(13)
331	0.48	1.60	-	-	δ CN(28), δ CH ₃ (31)
294	0.59	1.32	-	296	τ Ph(29), γ CC(27)
284	0.22	0.59	-	-	δ C=C(16), γ CC(21), τ Ph(26)
227	0.96	3.57	-	230	δ NH(16), δ CH ₃ (23), δ Ph(10)
204	1.26	0.84	-	-	δ CH ₂ (74)
163	0.07	0.18	-	-	δ CC(78), τ Ph(12)
154	0.40	0.61	-	152	δ CH ₂ (18), γ NH(13), γ CC(29), τ CN(11)

120	9.25	0.28	-	122	$\tau\text{CH}_2(31)$, $\delta\text{CH}_3(18)$, $\delta\text{CN}(17)$, $\delta\text{NH}(10)$
103	2.77	0.76	-	105	$\delta\text{CH}_2(19)$, $\tau\text{CH}_3(37)$
72	2.18	1.28	-	-	$\tau\text{CH}_3(49)$, $\delta\text{CH}_2(11)$
64	1.85	1.20	-	65	$\tau\text{CH}_3(77)$
54	6.70	0.47	-	-	$\tau\text{CH}_2(53)$, $\gamma\text{NH}(12)$
46	4.19	3.22	-	-	$\tau\text{CH}_2(87)$
28	0.18	3.34	-	-	$\tau\text{CN}(40)$, $\tau\text{NH}(16)$
23	0.32	3.84	-	-	$\tau\text{CH}_3(95)$

^a ν -stretching; δ -in-plane deformation; γ -out-of-plane deformation; τ -torsion; Ph-phenyl ring

Table 2

The binding affinity values of different poses of the title compound predicted by Autodock Vina.

Mode	Affinity (kcal/mol)	Distance from best mode (Å)	
		RMSD l.b.	RMSD u.b.
-	-		
1	-6.4	0.000	0.000
2	-5.7	1.781	2.298
3	-5.5	12.516	13.935
4	-5.5	2.577	3.607
5	-5.4	2.583	3.498
6	-5.3	14.335	18.343
7	-5.3	14.015	17.580
8	-5.3	18.211	19.099
9	-5.2	13.823	18.021

Fig.1 Optimized geometry of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid

208.60 ALIE [kcal/mol] 384.29

Fig.2 ALIE surface of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule

Fig.3 Intramolecular noncovalent interactions of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule

Fig.4 Fukui functions a) f^+ and b) f^- of the 4-[(4-acetylphenyl)amino]-2-methyldene-4-oxobutanoic acid molecule

Fig.5 BDEs of all single acyclic bonds of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule

Fig.6 Representative RDFs of atoms of 4-[(4-acetylphenyl)amino]-2-methylidene-4-oxobutanoic acid molecule

Fig.7 The docked ligand (pink) at the active site of the receptor

Highlights

- * IR and Raman spectra were measured
- * Most reactive sites are identified
- * ALIE, BDE, RDF have been discussed in detail
- * Inhibitory activity against 2-deoxy-d-gluconate 3-dehydrogenase.