

Structural, Vibrational, NBO –UV, NMR and Molecular Docking Studies on 2,4 -Dichloro phenoxy acetic acid using DFT Methods

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Abstract

FT-IR (4000-500 cm^{-1}) and FT-Raman (4000-100 cm^{-1}) spectra of 2,4 -Dichloro phenoxy acetic acid in the liquid phase were recorded and vibrational modes were discussed. ^{13}C and ^1H NMR spectra were recorded in the CDCl_3 (deuterated chloroform) solvent phase and the UV-Visible spectrum (400-200 nm) in the Ethanol phase and the results are analyzed based on the charge distribution and donor-acceptor molecular orbitals respectively. To study the molecular geometries and vibrational frequencies the optimized molecular structure was used which was predicted by the B3LYP/6-311++G (d,p) level. The electronic properties arising due to the interaction of frontier orbital emphasizes the modification of the chemical properties of the compound through the reaction path. Charge distributions using Mulliken population, NBO analysis helps to locate the intermolecular electronic interactions.

Keywords: FT-IR, FT-Raman, NMR, UV analysis, B3LYP

Introduction

2,4-Dichlorophenoxy acetic [1] acid is an organic compound with the chemical formula $\text{C}_8\text{H}_6\text{Cl}_2\text{O}_3$ which is usually referred to by its common name 2,4-D. 2,4-D is primarily used as a selective herbicide that kills many terrestrial and aquatic broadleaf weed plants, pastures, orchards, and cereal crops such as corn, oats, rice, and wheat. 2,4-D can be found in commercial lawn herbicide mixtures, which often contain other active ingredients including mecoprop and dicamba. Over 1,500 herbicide products contain 2,4-D as an active ingredient. Along waterways, it is used to control aquatic weeds that might interfere with boating, fishing, and swimming, or clog irrigation and hydroelectric equipment. It is often used by government agencies to control the spread of invasive, noxious, and non-native weed species and prevent them from crowding out native species, and also control many poisonous weeds such as poison ivy and poison oak. 2,4-D has been used in laboratories for plant research as a supplement in plant cell culture media.

The International Agency for Research on Cancer certifies 2,4-D as a possible carcinogen to humans. Ingestion, skin contact and inhalation are the three main pathways of human exposure to 2,4-D herbicides. 2,4-D was the first successful selective herbicide ever developed and its commercial

release came in 1946. The overuse of 2,4-D by both the farmers and manufacturers in the tropics has greatly damaged the health of the local ecosystem because it is deleterious to both terrestrial and aquatic living organisms [2]. As one of the most widely used herbicides in the world, it continues to be the most studied pesticide, both in animals and humans. A previous study also reported that 2,4-D could induce deleterious pathological effects on the vital organs including preneoplastic changes in the liver of Sprague-Dawley rats [3-4].

The literature survey indicates the DFT computations combined with experimental spectral analysis was not carried out so far. Hence, the present study is undertaken to do the complete structural, vibrational, NBO, NMR and UV analyses on this molecule.

Experimental details

The compound 2,4 -Dichloro phenoxy acetic acid was purchased in the powder form from Sigma-Aldrich Chemicals, Chennai. The FT-IR spectrum of the compound was recorded using a Bruker IFS 66V spectrometer in the range of 4000–400 cm^{-1} . The spectral resolution is $\pm 2 \text{ cm}^{-1}$. The FT-Raman spectrum of the same compound was also recorded using the same instrument with an FRA 106 Raman module equipped with an Nd: YAG laser source operating at 1.064 μm line widths with 200 mWatt power, in the range of 4000–400 cm^{-1} with a scanning speed of 30 $\text{cm}^{-1} \text{ min}^{-1}$ and spectral width 2 cm^{-1} . The frequencies of all bands are accurate to $\pm 1 \text{ cm}^{-1}$. The high-resolution ^1H NMR and ^{13}C NMR spectra were recorded using 400 MHz and 100 MHz NMR spectrometer respectively. The UV-Vis spectra were recorded in the liquid phase dissolved in ethanol in the range of 200 nm to 400 nm, with the scanning interval of 0.5 nm, using the UV-1700 series instrument.

Computational details

The entire quantum chemical computations are performed using the Gaussian 09 software on a Pentium IV/3.02GHz personal computer [5]. The wavenumbers and geometrical parameters are computed using B3LYP methods in combination with 6-311++G (d,p) basis sets. The geometry of the title molecule 2,4 -Dichloro phenoxy acetic acid is fully optimized using B3LYP functional with 6-311++G (d,p) basis set, and the electronic properties, such as NBO and HOMO-LUMO were calculated using time-dependent TD-SCF - B3LYP functional with 6-311+G (d,p) basis set. Similarly, the NMR chemical shifts are also carried out by the GIAO method in combination with B3LYP/6-311+G (2d, p). The Fukui function, the dipole moment, linear polarizability, and the first-order hyperpolarizability of the title molecule are also computed using the B3LYP method and 6-311++G (d,p) basis set.

Results and discussion

Molecular Analysis:

The calculated bond length and bond angles obtained using B3LYP methods with 6-311++G (d, p) basis sets for 2,4 -Dichloro phenoxy acetic acid with experimental values are presented in Table 1. The CC bond lengths of the aromatic ring are expected to be 1.39-1.40 Å. All the CC bonds within

the benzene ring in the present molecule also lie in the expected range, except C1-C6 where the bond length 1.40 Å, this is because of the attachment of highly positive acetic acid functional groups at this point. In the case of the C-Cl bond lengths, C1-Cl10 is found to be 1.747 Å and C3-Cl11 1.7566 Å, though they are within the expected region, there is a difference between them, which is naturally due to the substitution acetic acid that causes an asymmetry in charge distribution around the benzene ring.

Among the bond lengths of CO, C16-O14 is 1.36 Å, C6-O12 1.36 Å, and C15 – O12 is 1.40 Å. All of them are single bond CO, the first two are identical in electron density while the third one is longer than the other two, which means the electron density here is lesser, this may be due to the presence of double bond CO, O13-C16 (1.19 Å) at this point. All the C-H bonds in the benzene ring are found to have equal values 1.08 Å, and in the acetic acid group 1.09 Å, which indicate the CH bonds in the benzene ring are not affected by the substitution acetic acid, the increase in the bond length value in acetic acid is due to the presence O atoms in this group. The bond lengths of CO, CH and OH are observed with the literature [6].

In the case of the bond angles, the bond angles C2-C1-C6 & C4-C5-C6 have values of exactly 120 degrees. But remaining all the bond angles vary between 118° & 122°, particularly at places where substitutions are attached. At C6, where the acetic acid is attached, the bond angles are observed to be C1-C6-C5 (116°) and C5-C6-O12 (124°). At C1 at which one of the Cl atoms is substituted, the angles are found to be 119° at C2-C1-Cl10 and 119° at C6-C1-Cl10. The same angle 119° is subtended at C3 where the other Cl atom is substituted. This shows that structurally the influence of the Cl atom is relatively less when compared to that of the acetic acid group [7]. In the case of acetic acid, the deviations from the SP² and SP³ hybridization are large, for example, the angle at H17-C15-H18 is 107°, at O13-C16-C15 is 124° and at O14-C16-C15 is 113°, all these deviations imply the presence of O atoms heavily influence the charge distribution around the carbon atom and hence the structure of the molecule as a whole.

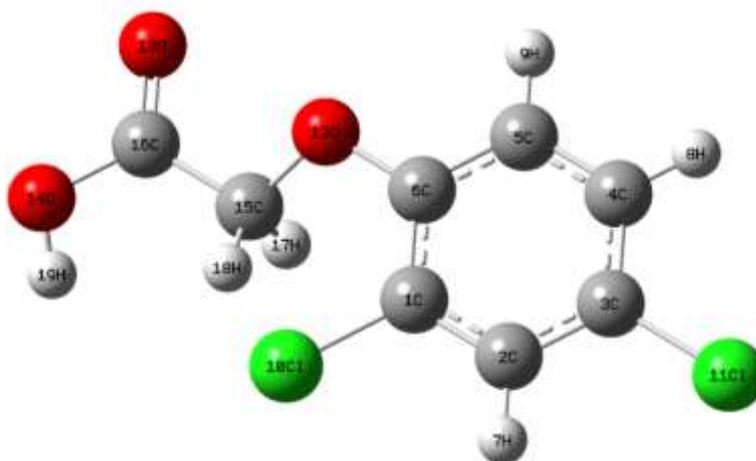


Fig.1 The optimized geometry of the molecule 2,4 –Dichloro phenoxy acetic acid

Table: 1. Optimized Geometrical parameter for 2,4-Dichloro phenoxy acetic acid Computed at B3LYP/6-311++G(d,p).

Bond Length (Å)	B3LYP/6-311++G (d,p)	Bond Angle (°)	B3LYP/6-311++G (d,p)
C1-C2	1.3883	C2-C1-C6	120.9925
C1-C6	1.4037	C2-C1-C110	119.2237
C1-C110	1.7475	C6-C1-C110	119.7832
C2-C3	1.3915	C1-C2-C3	119.2757
C2-H7	1.0813	C1-C2-H7	119.8696
C3-C4	1.3874	C3-C2-H7	120.8543
C3-C111	1.7566	C2-C3-C4	120.812
C4-C5	1.3934	C2-C3-C111	119.2801
C4-H8	1.0819	C4-C3-C111	119.9077
C5-C6	1.3946	C3-C4-C5	119.478
C5-H9	1.0813	C3-C4-H8	120.3841
C6-O12	1.3651	C5-C4-H8	120.133
O12-C15	1.4035	C4-C5-H9	118.4969
O13-C16	1.1926	C6-C5-H9	120.6148
O14-C16	1.3612	C1-C6-C5	118.5738
O14-H19	0.9661	C1-C6-C5	116.4689
C15-C16	1.5338	C5-C6-O12	124.957
C15-H18	1.0941	C6-O12-C15	119.9833
		C16-O14-H19	111.7484
		O12-C15-C16	113.5906
		O12-C15-H17	111.6051
		O12-C15-H18	105.4221
		C16-C15-H17	109.7572
		C16-C15-H18	108.386
		H17-C15-H18	107.7875
		O13-C16-O14	121.0696
		O13-C16-C15	124.9847
		O14-C16-C15	113.9455

Atomic charge analysis

Atomic charge prediction plays an important role as they cause the dipole moment, molecular polarizability, electronic structure, and molecular reactivity of the system. The charges on the atoms of the present compound are calculated by two methods; Mullikan Population analysis (MPA) method and natural atomic charge (NAC) methods, using B3LYP method with 6-311++ G (d, p) basis set, the graphical representation of the results is presented in Fig. 2.

In the benzene ring, all carbon atoms are expected to be equally negative as they share the charges of the H atoms. In the present case except for C1, C3, C6 where the substitutions are attached and found to positive in NAC, MPA predicts C1 & C3 positive while C6 where acetic acid is attached as extremely negative. Other carbon atoms C2, C4, C5 are found equally negative as expected in both methods. Among C15 and C16, in the acetic acid group, C15 is negative as two hydrogen atoms are attached with this carbon, C16 where O atoms are attached are found to be extremely positive in both the methods. C1 and C3 are positive in MPA with the values 1.189 and 0.573 respectively. In both cases, the changes predicted by the NAC method are seen to be much probable than that of MPA, this can also be verified from the NMR chemical shift values which are based on the charge distribution of these atoms, as discussed in the following chapter.

All hydrogen atoms are found to be almost equally positive in both the methods MPA and NCA, as the electrons from these atoms are pulled towards the bonded C atoms, but H19 which is attached with O atoms is found to be relatively highly positive as expected. In the benzene ring, all the hydrogen atoms are equally positive, whereas they differ in magnitude with those hydrogen atoms attached with O atoms in acetic groups. This difference is a clear indication of the electron-pulling power difference between C and O atoms.

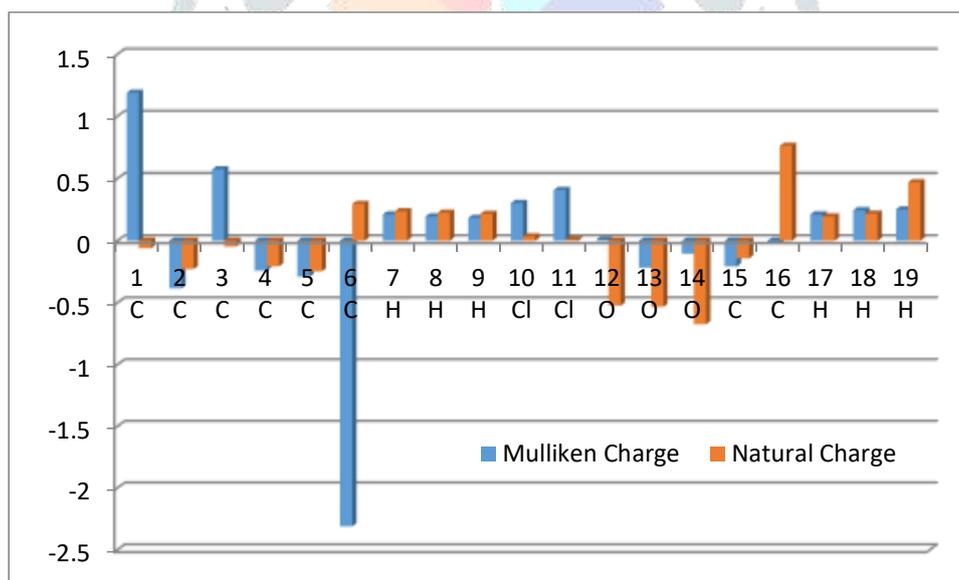


Fig.2 The graphical representation of the atomic charges

Table: 2.

Mulliken and Natural Charges for 2,4 -Dichloro phenoxy acetic acid Computed at B3LYP/6-311++G(d,p) basis set.

Atoms	Mullikan Atomic Charge	Natural atomic Charge
1 C	1.189442	-0.06049
2 C	-0.38393	-0.22827
3 C	0.573742	-0.0475
4 C	-0.24002	-0.20646
5 C	-0.28846	-0.24695
6 C	-2.31041	0.29643
7 H	0.210914	0.23701
8 H	0.195023	0.22451
9 H	0.184099	0.21495
10 Cl	0.303248	0.0339
11 Cl	0.407545	0.00832
12 O	0.006492	-0.52259
13 O	-0.21739	-0.53292
14 O	-0.10525	-0.67251
15 C	-0.20744	-0.14193
16 C	-0.02505	0.76344
17 H	0.210647	0.19443
18 H	0.245614	0.21668
19 H	0.25119	0.46997

NMR Analysis:

The chemical shifts for H and C atoms of the compound are computed for optimized structure by the GIAO method in combination with the B3LYP functional and 6-311+ G (2d, p) basis set. The computed values in the gas and solvent phase are presented in Table.3. The experimental and computational CNMR and HNMR spectrum of the compound is shown in Fig 3(a), (b) respectively.

The carbon atoms in benzene generally have shifted in the range of 120-130 ppm [8]. In the present case, among the aromatic carbons in 2,4 -Dichloro phenoxy acetic acid, C2, C4, and C5 where no substitution is attached are found to have a chemical shift in the expected range, but the C5 value is slightly less, 115 ppm, the usual value. Among all the carbon atoms in the benzene C6 relatively has a larger value of 152.1ppm where the acetic acid group is attached, which shows charges are drained from this carbon atom because of the O atoms in the acid group. The carbon atom in the acid group C16 possesses a very high shift value of 172.4 ppm, it is connected directly to double O atoms in this group, hence might have led to large deshielding. Thus, it may be concluded that all the biological activities of the molecule can only be due to the C6 and C16 carbon atoms, due to the drastic variation in charge distribution.

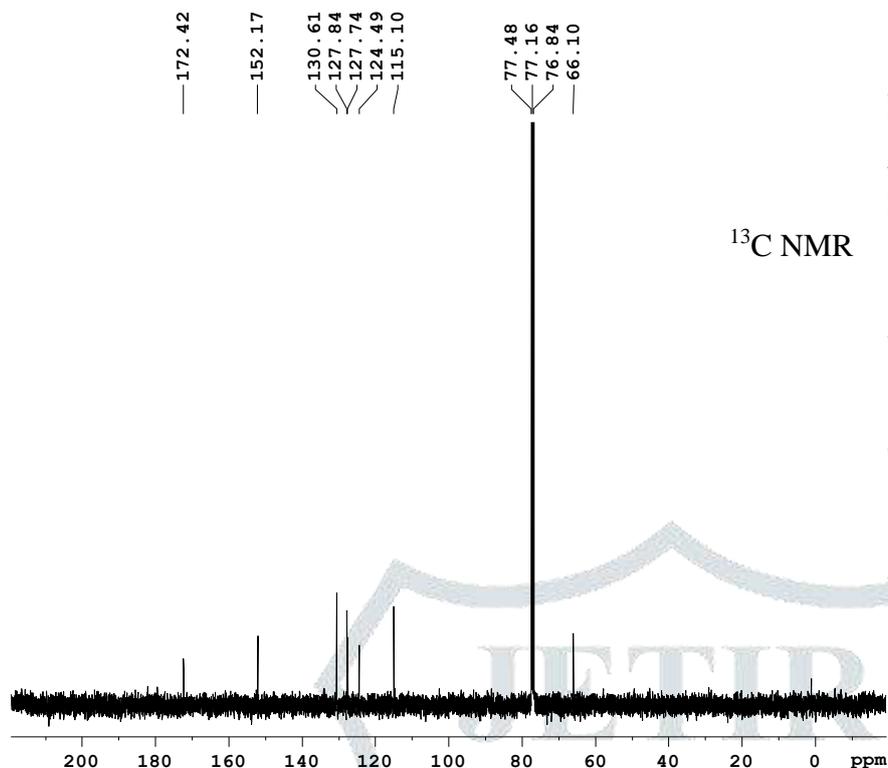
The chemical shifts of the hydrogen atoms are found almost below 7-8 ppm [9], which shows that the chemical environment of the hydrogen atoms is unaffected by oxygen atoms. Among these the acetic group proton is heavily shielded having values between 4.7-5.6 ppm, this trend is in tune with the literature [10]. There is an appreciable difference observed in the chemical shifts in different solvents phases. Hence the impact of the solvents on the chemical shifts of the compound for various atoms is negligibly small.

Table. 3.

Calculated both ^1H and ^{13}C NMR chemical shifts (ppm) of 2,4 -Dichloro phenoxy acetic acid

Atom	Gas	CDCl_3	Experimental
1C	136.6		127.8
2C	135.5		127.7
3C	140.0		130.6
4C	131.1		124.4
5C	114.4		115.1
6C	158.2		152.1
15C	65.0		66.1
16C	167.9		172.4
7H	7.4		7.4
8H	7.0		7.1
9H	6.2		6.8
17H	4.0		4.7
18H	4.5		5.6
19H	5.3		5.6

C13CPD CDC13 {D:\CIF} CIF_NMR 1



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FIDRES        0.366798 Hz
AQ            1.3631988 sec
RG            406
DW            20.800 usec
DE            6.00 usec
TE            297.0 K
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SF            2.00000000 sec
L1            0.03000000 sec
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rD0           1

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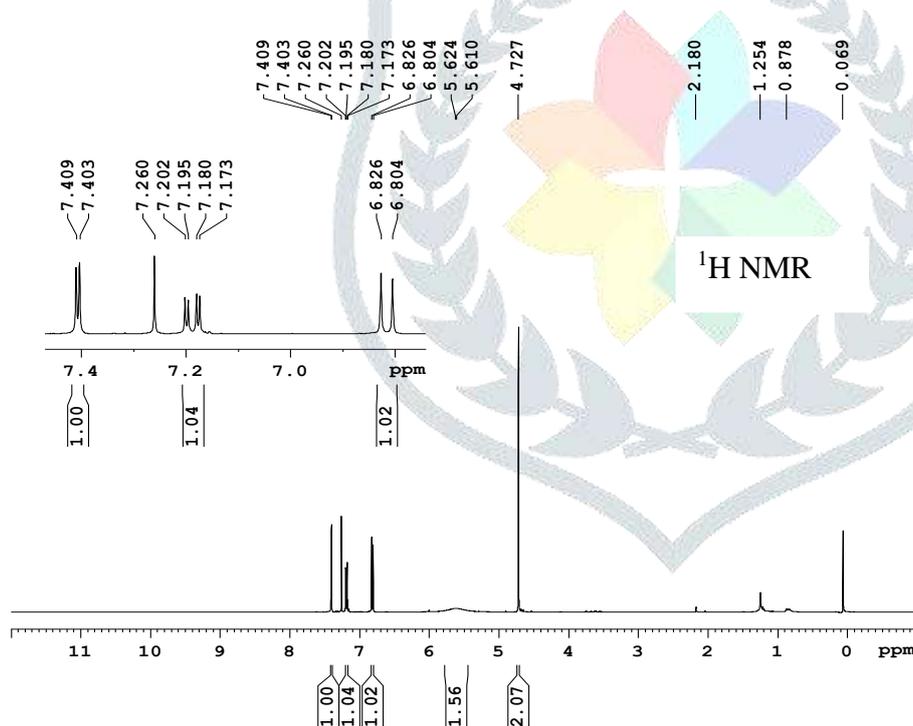
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PL12          14.95 dB
PL13          120.00 dB
PL2           -1.00 dB
SFO2          400.1316005 MHz
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SOLVENT       CDC13
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DS            2
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FIDRES        0.125483 Hz
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RG            575
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DE            6.00 usec
TE            296.7 K
SI            1
SF            1.00000000 sec
D1            1.00000000 sec
TD0           1

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===== CHANNEL f1 =====
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PL1           -1.00 dB
SFO1          400.1324710 MHz
SI            32768
SF            400.1300094 MHz
WDW           EM
SSB           0
LB            0.30 Hz
GB            0
PC            1.00

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Fig.3. (a) & (b) Theoretical ¹³C and ¹H NMR chemical shift the analysis of 4-Chloro phenyl acetyl chloride

Vibrational Analysis:

Vibrational wave numbers for all the fundamental modes of the titled compound are computed using B3LYP functional, and 6-311++G (d, p) basis sets and the values along with the experimental data are presented in Table 4. The theoretical FT-IR and FT-Raman spectra of the titled compound are

shown in Figure 4 (a) & (b) and Experimental FT-IR and FT-Raman spectra of the titled compound is shown in Figure 5 (a) & (b). The molecule 2,4 -Dicholoro phenoxy acetic acid has 19 numbers of atoms and hence 51 fundamental modes.

OH Vibrations

The OH stretching vibrational bands are expected in the range of $3700 - 3300 \text{ cm}^{-1}$ [11], particularly when it is present on the benzene ring and form an intermolecular hydrogen bonding. Hence, the OH group vibrations are considered to be very sensitive to the environment. The band is present very weak at 3450 cm^{-1} in this molecule in FT-IR. The OH group vibrations are observed theoretically at wave number 3736 cm^{-1} . The presence of very weak intensity and lower wavenumber indicate that no hydrogen bonding is present in this molecule which may due to the presence of the C=O group adjacent to this group, this fact is also verified in molecular docking analysis. The OH in-plane bending band is expected at $1450-1350 \text{ cm}^{-1}$ [12], this is observed at 1262 cm^{-1} respectively and these are observed experimentally at 1250 cm^{-1} in the FT-Raman spectrum. Similarly, the out-of-plane bending modes are expected between $710 - 517 \text{ cm}^{-1}$, but these are observed at 797 cm^{-1} theoretically and at 794 cm^{-1} experimentally. Hence all the bending modes agree with the experimental values while there is a considerable deviation in stretching modes.

CH Vibrations

The titled molecule has five CH stretching vibrations, three aromatics and two aliphatics. The aromatic structure needs the presence of C-H stretching vibration in the characteristic region $3100-3000 \text{ cm}^{-1}$ [13-14], while that of aliphatic between $3000-2900 \text{ cm}^{-1}$. The Experimental values in this molecule are observed at $3122, 3073 \text{ cm}^{-1}$ in FT-IR and 3167 cm^{-1} FT-Raman spectrum. All the three lie within the expected range and two of them are slightly higher than the expected range which may be due to the slight alteration in the conjugation pattern due to the substitutions. The last two aliphatic values 2977 and 2450 cm^{-1} are in both FT-Raman and FT-IR spectra also are within the expected range, which shows the CH bonds are not much affected in this molecule. The corresponding theoretical values are $3170, 3168, 3155, 3028, 2958 \text{ cm}^{-1}$; these are exactly what is expected without the influence of the COOH groups, hence a necessary correction factor has to be introduced in the Hamiltonian of the functionals for the influence of these functional groups.

The C-H in-plane bending mode usually occurs as strong to weak bands in the region of 1300 to 1200 cm^{-1} [15]. Experimental study of the title compound manifested C-H in-plane bending vibrations in the region of $1228, 1092 \text{ cm}^{-1}$ in FT-IR and $1210, 1200, 1170,$ and 1112 cm^{-1} in FT-Raman spectrum. Their corresponding computed bands appeared $1257, 1159, 1115, 1103, 1088 \text{ cm}^{-1}$. The C-H out-of-plane bending vibrations are expected to occur as strong to weak intensity bands in the region of $1000-600 \text{ cm}^{-1}$ [16]. The recorded FT-IR spectrum of the titled molecule showed

bands at 645, 582, 570, 562 cm^{-1} in the FT-IR spectrum. Their corresponding computed values were noted at 668, 643, 580, 563, 553 cm^{-1} respectively. Both the bending modes have slightly deviated from the expected range, which indicates that they are not pure like stretching which means a lot of influence of other modes (CC & CO) which occur in this region.

CC VIBRATIONS

The aromatic ring CC stretching vibrations occur generally in the region 1600-1450 cm^{-1} [17]. In the present study, there are six aromatic and one aliphatic CC vibrations, these are observed at 1603, 1579, 1478, 1448, 1392, 1320, 1310 cm^{-1} . The computed wave numbers are observed at 1600, 1584, 1486, 1454, 1395, 1379, 1322 cm^{-1} respectively. The first three bands conventionally represent the CC double bond vibrations which are relatively less compared to the expected range, which naturally represents the altered conjugation pattern within the ring due to substitutional groups. The only aliphatic CC mode is observed at 1310 cm^{-1} which is a slightly enhanced value for the aliphatic group and can only be due to the presence of O atoms in this group.

C-O and CCl VIBRATIONS

For the CO double and single bond stretching vibrations are usually observed around 1850-1800, and 1260-1000 cm^{-1} [18]. The Stretching vibration for this CO double bond in the present case is observed at 1703 cm^{-1} in FT-IR single bond in the present case is observed at 1309 and 1276 cm^{-1} experimentally and double and single bond observed theoretically at 1846, 1282 and 1265 cm^{-1} theoretically. These vibrations are in the expected range which indicates these groups are not much affected by phenyl ring. The CCl stretching vibrations are usually observed around. The present molecule CCl bands are observed at 836 and 826 cm^{-1} in FT-Raman and FT-IR spectrum respectively. These vibrations are also lie in the expected range.

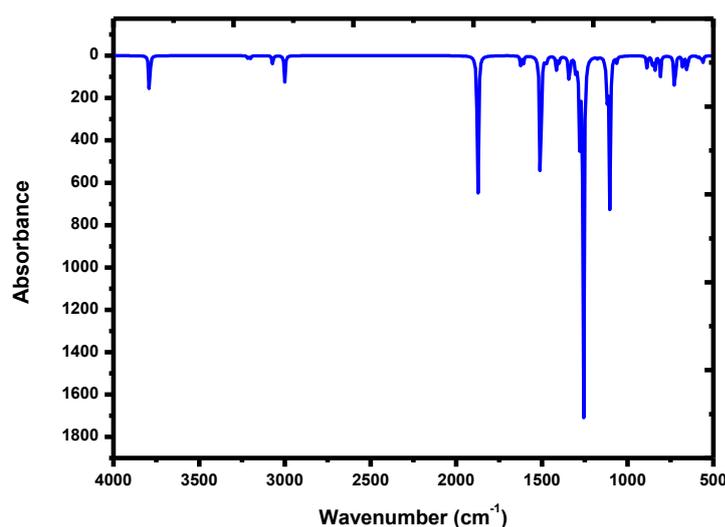


Fig.4. (a) Theoretical FT-IR analysis of 4-Chloro phenyl acetyl chloride

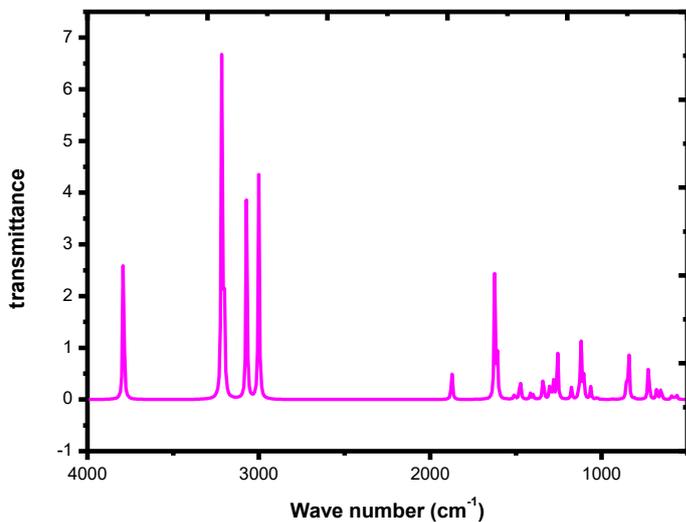


Fig.4. (b) Theoretical FT-Raman analysis of 4-Chloro phenyl acetyl chloride

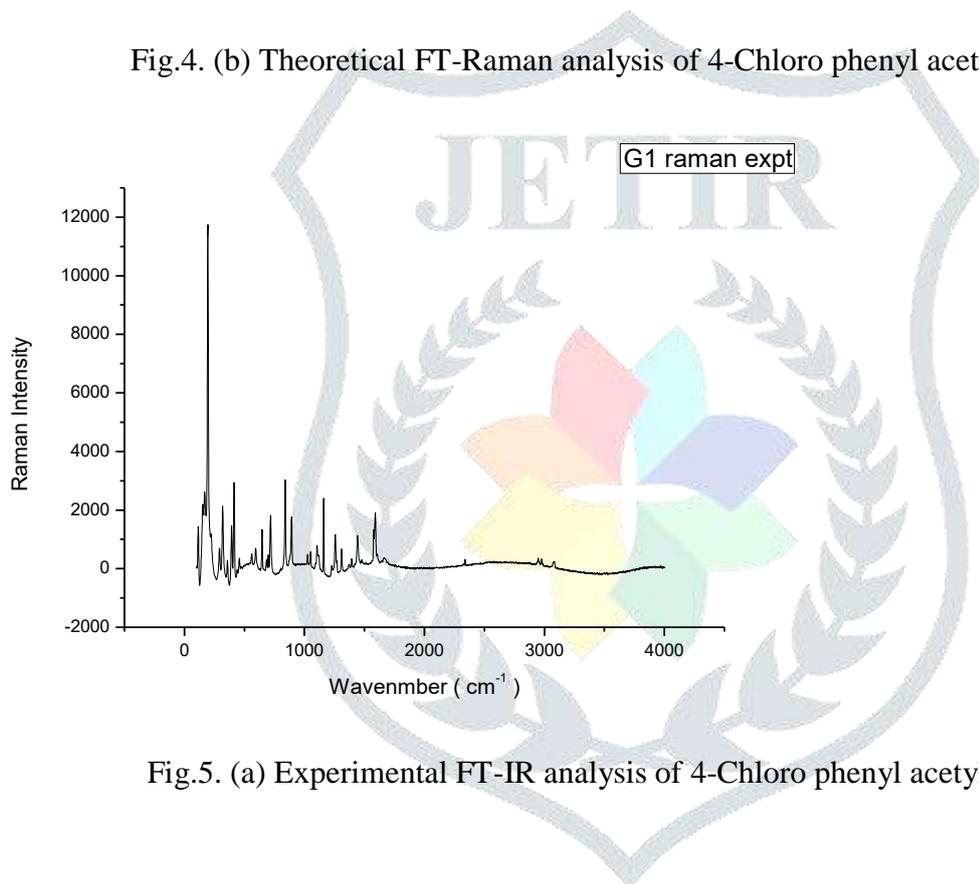


Fig.5. (a) Experimental FT-IR analysis of 4-Chloro phenyl acetyl chloride

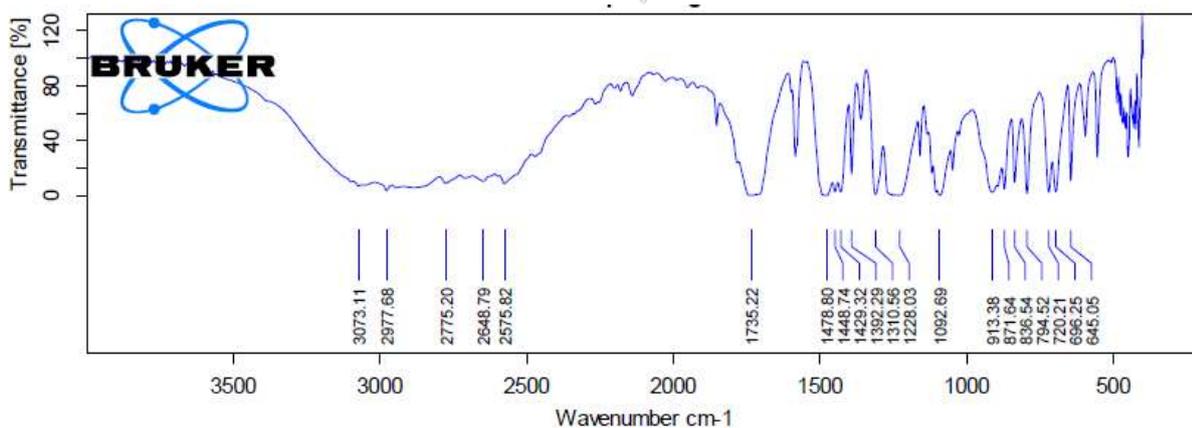


Fig.5. (b) Experimental FT-Raman analysis of 4-Chloro phenyl acetyl chloride

Table: 4.

Observed method B3LYP/6-311++G (d,p) level calculated Vibrational frequencies of 2,4 -Dichloro phenoxy acetic acid

Experimental frequency (cm ⁻¹)		B3LYP/6-311++G (d,p)		Assignment	PED %
FT-IR	FT-RAMAN	Unscaled (cm ⁻¹)	Scaled (cm ⁻¹)		
3450		3790	3736.94	vOH	vOH 100
	3167	3216	3170.976	vCH	vCH 95
3122		3213	3168.018	vCH	vCH 38+CH 57
3073		3200	3155.2	vCH	vCH 39+CH 60
2977		3071	3028.006	vCH	vCH 91
	2450	3000	2958	vCH	vCH 91
1736		1873	1846.778	vOC	vOC 38
	1603	1623	1600.278	vCC	vCC 27+CC 14+CC11
1579		1607	1584.502	vCC	vCC 29+CC 24
1478		1508	1486.888	vCC	βHCC 19+11+22
1448		1475	1454.35	vCC	vCC 27
1392		1415	1395.19	vCC	vCC 16+CC 25+βHCC18
1320		1399	1379.414	vCC	vCC 77
1310		1341	1322.226	vCC	vOC 11
	1309	1301	1282.786	vOC	vOC 13
	1276	1283	1265.038	vOC	vCC 10+βHCC24+27
	1250	1280	1262.08	βOH	βHOC 27+HCC14
1228	1210	1257	1239.402	βCH	vOC 20+βHOC31
	1200	1176	1159.536	βCH	vCC 16
	1170	1131	1115.166	βCH	vOC19+27+βHOC18
	1112	1119	1103.334	βCH	vCC21+CLC15+HCC16
1092		1104	1088.544	βCH	vOC37+βHOC11
	1056	1063	1048.118	βCO	vOC10+βCCC28
	1035	1027	1012.622	βCO	βCO+HCC16
913	902	938	924.868	βCO	βCO10+βHCC24+27
871	862	886	873.596	τOH	τOH+HCC16
836		856	844.016	vCLC	vCC49+OC10
	826	842	830.212	vCLC	vOC11+CLC10+CLC11
794		809	797.674	τ CH	τ CH10+βHCC24+27
696		728	717.808	βCLC	vCLC11
	709	720	709.92	vCLC	vCLC+HCC16
	651	678	668.508	τ CH	vOC14
645		653	643.858	τ CH	vCLC19
582	574	589	580.754	τ CH	τ CH+HCC16
570	563	572	563.992	τ CH	τ CH16+CC 25+βHCC18
562	554	561	553.146	τ CO	τ CO+HCC16
450	446	452	445.672	τ CO	τ CO10+βHCC24+27
	432	440	433.84	τ CO	τ CO16+CC 25+βHCC18

	423	416	410.176	β CCC	vCLC19+HCC16
	407	406	400.316	β CCC	vCLC23+ β CCC11
	378	387	381.582	β CCC	vCLC23+ β CCC11
	361	354	349.044	β CCC	β CCC+HCC16
	282	288	283.968	β CCO	β CCO39+CH 60
	245	250	246.5	β CCO	β CCO+HCC16
	210	199	196.214	β CCCL	β CCCL16+CC
	172	175	172.55	β CCCL	β CCCL39+CH 60
	167	169	166.634	β COH	β COH16+CC
	123	113	111.418	β CCCC	β CCCC+HCC16+HCC16
	57	58	57.188	β CCCC	β CCCC39+CH 60
	43	45	44.37	β CCCC	β CCCC16+CC
	20	24	23.664	β CCCC	β CCCC+HCC16

NBO Analysis:

The electronic characteristics of the present compound 2,4 -Dichloro phenoxy acetic acid is simulated using TD-SCF functional along with B3LYP &6-311+G (d, p) combination. The NBO output parameters such as occupancy, donors and acceptors, stabilization energy, polarization energy, etc., calculated using B3LYP/6-311+ G (d, p) method, are presented in Table 5. The table shows the various possible donors and acceptors in the molecule with their occupancy value in each position, similarly the various possible transitions among these donors and acceptors. The stabilization energy E2 for these transitions give a measure of the probabilities of these transitions; according to which the highly probable top seven electronic transitions in this molecule are O14 to O13- C16 ($n-\pi^*$, 41.2 Kcal/mol), O13 to O14-C16 ($n-\pi^*$, 34.2 Kcal/mol), O12 to C5-C6 ($n-\pi^*$, 27.55 Kcal/mol), C5 - C6 to C3-C4 ($\pi-\pi^*$, 21.67 Kcal/mol), C5-C6 to C3-C4 ($\pi-\pi^*$, 21.45Kcal/mol), C1-C2 to C5-C6($n-\pi^*$, 19.83Kcal/mol) and C5-C6 to C1-C2($\pi-\pi^*$, 18.06Kcal/mol). The first three most probable transitions are $n-\pi^*$ transition taking in the acetic acid group. The remaining four transitions are the usual $\pi-\pi^*$ transitions in the benzene ring. Of all the transitions only a few transitions will be favored by the selection rules which can be identified from HOMO – LUMO and UV analyses as discussed in the following section.

Table: 5: Second order perturbation theory of Fock matrix in NBO basis of 2,4 -Dichloro phenoxy acetic acid

Donor	Type of bond	Occupancy	Acceptor	Type of bond	Occupancy	Energy e(2) Kcal/mol	E(j)-e(i)	F(i,j)
O 14	n	1.83422	O 13 - C 16	π^*	0.19113	41.2	0.35	0.109
O 13	n	1.82712	O 14 - C 16	π^*	0.1046	34.28	0.6	0.13
O 12	n	1.8446	C 5 - C 6	π^*	0.38713	27.55	0.35	0.093
C 3 - C 4	σ	1.69068	C 1 - C 2	π^*	0.38402	21.85	0.28	0.071
C 5 - C 6	π	1.66427	C 3 - C 4	π^*	0.39578	21.67	0.29	0.071
O 13	n	1.82712	C 15 - C 16	π^*	0.09196	21.45	0.59	0.103
C 1 - C 2	π	1.70818	C 5 - C 6	π^*	0.38713	19.83	0.29	0.07
C 5 - C 6	π	1.66427	C 1 - C 2	π^*	0.38402	18.06	0.28	0.064

C 3 - C 4	σ	1.98817	C 5 - C 6	π^*	0.38713	17.25	0.29	0.064
C 1 - C 2	π	1.70818	C 3 - C 4	π^*	0.39578	16.84	0.29	0.064
Cl 10	n	1.92467	C 1 - C 2	π^*	0.38402	12.78	0.32	0.062
Cl 11	n	1.92467	C 3 - C 4	π^*	0.39578	12.23	0.33	0.062
O 12	n	1.8446	C 15 - C 16	σ^*	0.09196	8.21	0.65	0.066
O 12	n	1.95957	C 5 - C 6	σ^*	0.0273	6.63	1.1	0.076
Cl 10	n	1.96768	C 1 - C 6	σ^*	0.03799	5.23	0.84	0.059
C 5 - C 6	σ	1.97246	C 1 - C 6	σ^*	0.03799	5.18	1.26	0.072
C 4 - C 5	σ	1.96632	C 3 - Cl 11	σ^*	0.03044	5.15	0.86	0.059
O 14 - H 19	σ	1.9842	O 13 - C 16	σ^*	0.01706	4.72	1.41	0.073
O 14	n	1.97444	C 15 - C 16	σ^*	0.09196	4.49	0.96	0.06
C 4 - C 5	σ	1.96632	C 6 - O 12	σ^*	0.0298	4.47	1.05	0.061
C 5 - C 6	σ	1.97246	C 1 - Cl 10	σ^*	0.02687	4.42	0.88	0.056
C 1 - C 2	σ	-1.972	C 3 - Cl 11	σ^*	0.03044	4.4	0.88	0.056
Cl 11	n	1.92467	C 2 - C 3	σ^*	0.02864	4.33	0.87	0.055
C 2 - C 3	σ	1.97308	C 1 - Cl 10	σ^*	0.02687	4.3	0.88	0.055
C 15 - H 18	σ	1.96947	O 13 - C 16	π^*	0.1046	4.29	0.55	0.045
C 4 - H 8	σ	1.97811	C 2 - C 3	σ^*	0.02864	4.27	1.09	0.061
C 1 - C 6	σ	1.9735	C 5 - C 6	σ^*	0.0273	4.24	1.28	0.066
C 2 - H 7	σ	1.9755	C 1 - C 6	σ^*	0.03799	4.14	1.06	0.059
Cl 11	n	1.92467	C 3 - C 4	σ^*	0.02709	4.06	0.88	0.053
O 12	n	1.8446	C 15 - H 17	σ^*	0.02398	4.04	0.67	0.048
C 2 - H 7	σ	1.9755	C 3 - C 4	σ^*	0.02709	4.02	1.1	0.059
C 15 - H 17	σ	1.9761	O 13 - C 16	π^*	0.19113	3.79	0.56	0.043
C 5 - H 9	σ	1.97428	C 1 - C 6	σ^*	0.03799	3.74	1.07	0.057
Cl 10	n	1.96768	C 1 - C 2	σ^*	0.02419	3.71	0.87	0.051
C 4 - H 8	σ	1.97811	C 5 - C 6	σ^*	0.0273	3.68	1.08	0.056
C 2 - C 3	σ	1.97308	C 1 - C 2	σ^*	0.02419	3.65	1.29	0.061
C 15 - H 18	σ	1.96947	C 6 - O 12	σ^*	0.0298	3.62	0.9	0.051
C 2 - C 3	σ	1.97308	C 3 - C 4	σ^*	0.02709	3.6	1.3	0.061

UV-Visible spectral analysis

The UV-Vis absorption spectrum of the titled compound recorded in the range 200-400 nm in the ethanol phase is shown in Fig 6. Theoretical simulation of the same is carried out both in gas and ethanol phases for comparison by TD-SFT method, to compare and discuss with the theoretical spectrum. The UV theoretical computation predicts for the above top seven transitions the absorption wavelengths, oscillator strengths, and H-L orbital contributions of the titled compound [19]. The probable seven electronic transitions and the corresponding excitation energies for these two phases are presented in Table 6.

In this case, the energy gaps in the solvent phase for the seven transitions are found to be 4.75, 5.19, 5.34, 5.466, 5.72, 5.96, 6.00 eV respectively. The transitions should take place only when they have the minimum energy difference between the donor and acceptor levels, but the intensity of the transition is based on the oscillator strength value. The respective oscillator strengths, which theoretically predict the intensity of the bands, are 0.0522, 0.0205, 0.0147, 0.1296, 0.0029, 0.0425, 0.0135 respectively. These oscillator strength values indicate the intensity of the peak will be very high

only for first and fifth transitions, but since the HOMO to LUMO, contributions are very less for these transitions, only the first transition with oscillator strength value 0.0522 and HOMO to LUMO contribution 84% will be very prominent in UV-Visible transition. The calculated absorption wavelengths for these ten transitions are 260, 238, 231, 226, 216, 207, 206, 204, 203, 201 nm respectively. There are two peaks in the theoretical spectrum which are assigned to the first and fifth transitions as per the oscillator strength values. The wavelength for the first transition is 260 nm theoretically, experimentally peak is obtained at 275 nm and that of the fifth transition is theoretically 226 and experimentally 225 nm. According to the NBO analysis, these transitions are O14 to O13-C16 ($n-\pi^*$, 41.2 Kcal/mol) and C5-C6 to C3-C4 ($\pi-\pi^*$, 21.45 Kcal/mol) transitions. The first one is the acetic acid group while the fifth one is in the benzene ring.

In the gas phase, the energy gaps for these transitions are found to be 4.72, 4.96, 5.060, 5.393, 5.478, 5.757, and 5.814 respectively. The respective oscillator strengths are 0.038, 0.019, 0.007, 0.005, .089, 0.002, and 0.006 respectively. The calculated absorption maximum values corresponding to these are 261, 249, 244, 229, 226, 214, and 213nm respectively. The comparison of these values with values in the solvent phase indicates that the same transitions will be favored in the gas phase also, the only difference is the slight decrease in values which can be accounted for by the solvent effect.

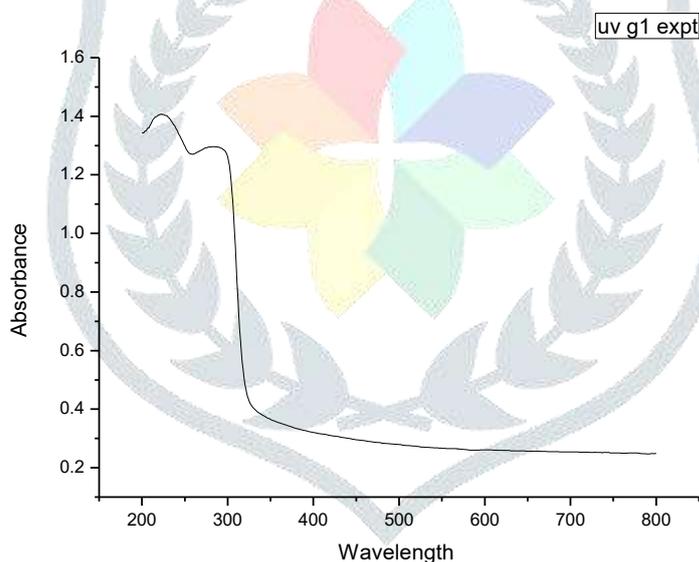


Fig.6. (a) Experimental UV-Visible analysis of 4-Chloro phenyl acetyl chloride

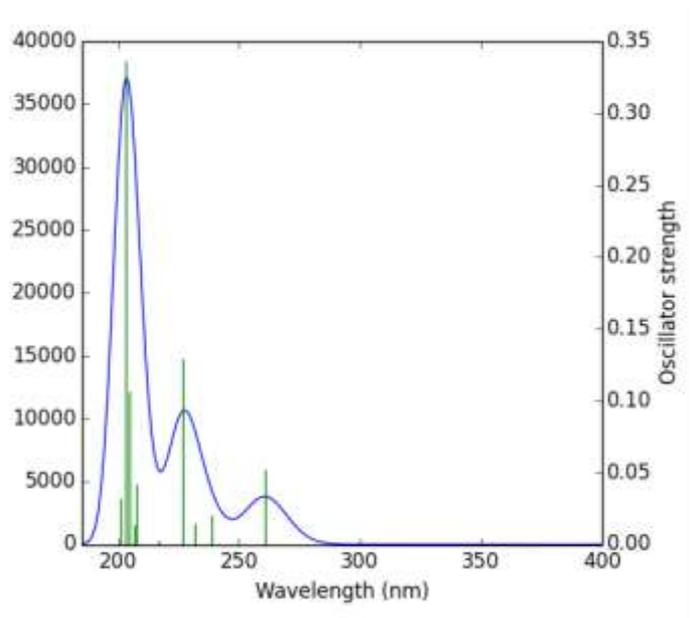


Fig.6. (b) Theoretical UV-Visible analysis of 4-Chloro phenyl acetyl chloride

Table: 6.

Theoretical electronic absorption spectra of 2,4-Dichloro phenoxy acetic acid absorption wavelength λ (nm), excitation energies E (eV), and oscillator strengths (f) using TD-DFT/B3LYP/6-311++G(d,p) method.

λ (nm)	Experimental	E (eV)	(f)	Major contribution
GAS				
261.1		4.729	0.0384	HOMO→LUMO (81%)
249.5		4.967	0.0196	HOMO→L+1 (95%)
244.9		5.060	0.0072	HOMO→L+2 (79%)
229.6		5.398	0.0058	HOMO→L+4 (88%)
226.3		5.478	0.0891	HOMO→L+3 (60%)
214.9		5.767	0.0023	H-2→L+2 (44%)
213.2		5.814	0.0069	H-1→L+1 (90%)
260.9	275	4.7518	0.0522	HOMO→LUMO (84%)
238.7		5.1934	0.0205	HOMO→L+1 (42%)
231.9		5.3452	0.0147	HOMO→L+3 (59%)
226.8	225	5.4666	0.1296	HOMO→L+2 (37%)
216.6		5.7218	0.0029	HOMO→L+4 (61%)
207.7		5.9690	0.0425	H-2→L+2 (32%)
206.4		6.0051	0.0135	HOMO→L+5 (60%)

HOMO-LUMO:

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital are computed with B3LYP functional with 6-311+ G (d, p) basis set and the pictorial diagram of the HOMO-LUMO are shown in Fig. 7. And the energies of the HOMO-LUMO, energy gap and different reactivity descriptors of molecule in both optimized and electronic transition levels are presented in Table. 7. The energy gap of the optimized ring is -0.273 eV, which shows the possibility of a high flow of energy from HOMO to LUMO. Similarly, other descriptors of the molecule do vary from the

optimized to transition state and the values are presented in the same Table. The electronegativity, which is a measure of the attraction of an atom for electrons in a covalent bond, has 0.151eV. The chemical hardness of benzene is 0.136 eV, which shows that the present molecule is less stable compared to the benzene ring. The electrophilicity index is a measure of lowering of total energy due to the maximal electron flow between the donors and the acceptors. The chemical potential of 2,4 - Dichloro phenoxy acetic acid is 0.083 eV.



Fig.7 The pictorial diagram of the HOMO-LUMO

Table: 7

HOMO, LUMO, global electronegativity, global hardness and softness, global electrophilicity index of 2,4 -Dichloro phenoxy acetic acid

Parameters	Gas
E_{HOMO} (eV)	-0.287
E_{LUMO} (eV)	-0.014
$\Delta E_{\text{HOMO-LUMO gap}}$ (eV)	-0.273
Electronegativity (χ)	0.151
Global hardness (η)	0.136
Global softness (S)	0.546
Chemical Potential (μ)	0.083

MEP:

The molecular electrical potential surfaces from Fig.7. illustrate the charge distributions of molecules three-dimensionally. This map allows us to visualize variably charged regions of a molecule. The knowledge of the charge distributions can be used to determine how molecules interact with one another and it is also used to determine the nature of the chemical bond. The molecular electrostatic potential is calculated at the B3LYP/6-311++G(d,p) optimized geometry.^[37,38] The figure shows the negative charges are more concentrated at the bottom of the acetic acid group, whereas the blue region is spread over at the top of the hydrogen atoms of the hydroxyl group, and other regions are found to be almost neutral. The color code of these maps is in the range between -8.877a.u. (deepest red) and 8.877a.u. (deepest blue) in the compound. The positive (blue) regions of MEP are related to electrophilic reactivity and the negative (red) regions to nucleophilic reactivity.

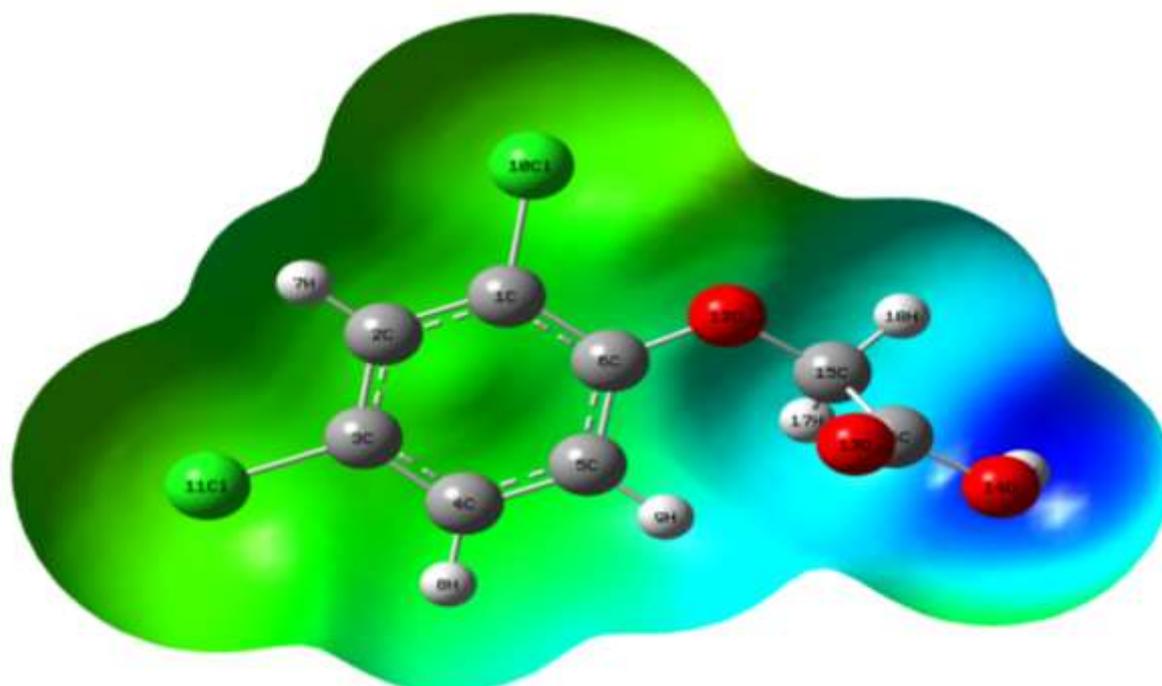


Fig.7 MEP analysis of 4-Chloro phenyl acetyl chloride

Docking Analysis

Molecular docking is a powerful approach for predicting the molecular mechanism of protein-ligand interactions, which bind to a receptor of a known three-dimensional structure. Auto Dock suite 4.0 [20] has been used to get inside into probable protein-ligand interactions and to identify the binding affinity of the molecules. For docking the ligand was prepared by minimizing its energy at B3LYP/6-311++G (d,p), Hydrogen atoms are added to the target protein and Kollaman atomic charges were calculated. It is found that the present molecule can dock with the apathogenic fungal FKBP12 whose protein structure is named 5HW6. Water molecules and other co-crystallized agents were removed. Lamarckian genetic algorithm (LGA) is used for molecular docking analysis. The binding protein of the target proton was specified using grid size 90X90X90 Å⁰ with the aid of Auto grid. Docked conformation which has the lowest binding energy was chosen to investigate the mode of binding. The result is shown in table .8. The bond lengths are shown in Fig. 8. It can be seen from the diagram the molecule docks with protein through only one hydrogen bond of length 2.2 Å with binding energy 5.48 Kcal/mol.

Table.8. The Docking binding pose for 2,4-Dichlorophenoxy acetic acid

Protein ID	Binding energy (kcal/mol)	Intermolecular energy (kcal/mol)	Bond Residue	Distance (Å)
5HW6	-5.48	-6.67	LYS 80	2.2

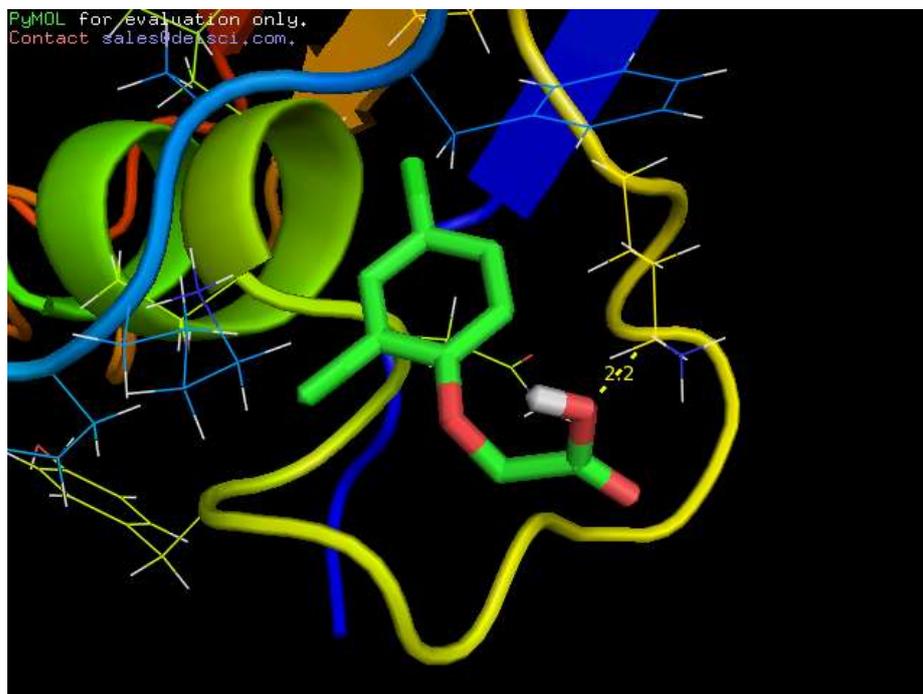


Fig.8. Docking analysis of 4-Chloro phenyl acetyl chloride

Conclusion

Comprehensive structural, vibrational, NBO, NMR, UV analyses were carried out for the title molecule. The structural analysis shows the electronic conjugation pattern within the benzene ring is mainly altered by the acetic acid substitution. The hybridizations of the carbon atoms in the acetic acid group are drastically changed by the presence of double oxygen atoms in this group. The vibrational analysis shows that the skeletal vibrations in the benzene ring are appreciably changed due to the changed conjugation pattern. The NMR chemical shift shows that carbon atoms in the carboxylic acid group and at the substitutional point of the benzene ring show a very high shift which indicates these two carbon atoms are made highly positive. The UV analysis shows that the $n-\pi^*$ electronic transition within the acetic acid group is very prominent in this molecule. This molecule docks with a pathogenic fungus through a hydrogen bond at the C=O site of the acid group.

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