DFT AND SPECTROSCOPIC ANALYSIS OF P-BROMO-DL-PHENYLALANINE

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ABSTRACT:

The Spectroscopic profile of p-Bromo-dl-phenylalanine was examined using FT-IR, FT-Raman, UV, ^{1}H, and ^{13}C NMR techniques. The geometrical parameters and energies attained from DFT/B3LYP method with 6–311++G (d,p) basis sets calculations. The geometry of the molecule was fully optimized, vibrational spectra were calculated, and assigned the fundamental vibrations based on the total energy distribution (TED) of the vibrational modes, calculated with the scaled quantum mechanics (SQM) method. The XRD data obtained from the computed geometric parameters shows that there is little deviation in the structure due to the substitution of the COOH group in the molecule. Using the NBO study, the delocalization of the electron and the corresponding attraction between the orbitals shows that the lone pair transition has higher stabilization energy when compared with the remaining atoms. The electronic properties, HOMO and LUMO energies, are performed with TD-DFT reproduces well with the experimental findings. Besides, frontier molecular orbitals (FMO), the highly reactive nature of the molecule is identified with MEP and global reactivity descriptor analysis is performed. In addition, molecular docking was also performed for the different receptors.

Keywords:

DLPA, Alanine, MEP, FT-IR, FT-Raman, NMR, UV-Visible, Molecular Docking.

INTRODUCTION:

The first description of phenylalanine was made in 1879 when Schulze and Barbieri identified a compound in yellow lupine seedings. In 1882, Erlenmeyer and Lipp first synthesized phenylalanine from phenylacetaldehyde, hydrogen, cyanide, and ammonia [1]. The molecular formula of p-Bromo-dl-phenylalanine is C9H10BrNO2 and the IUPAC name is 2-amino-3-(4-bromophenyl) propanoic acid [2]. P-Bromo-DL-phenylalanine inhibited the growth of the test organism to a greater degree [3]. Phenylalanine is an essential α-amino acid found in many foods and used by your body to produce proteins and other

important molecules. It is used as a building block of proteins in your body [4]. Phenylalanine is a precursor for tyrosine, the monoamine neurotransmitters dopamine, norepinephrine, and epinephrine, and the skin pigment melanin [1]. This molecule exists in two forms of arrangements: L-phenylalanine and D-phenylalanine. They are nearly identical but have a slightly different molecular structure. The L-form is found in foods and used to produce proteins in your body, while the D-form can be synthesized for use in certain medical applications [5,6]. The stereoisomer D-phenylalanine (DPA) can be produced by conventional organic synthesis, either as a single enantiomer or as a component of the racemic mixture. L-phenylalanine is a competitive antagonist at the glycine binding site of the NMDA receptor. DL-phenylalanine (DLPA) is marketed as a nutritional supplement for its purported analgesic and antidepressant activities [1]. Phenylalanine has been studied as a treatment for several medical conditions, including skin disorders, depression, and pain [4]. Phenylalanine may be useful in treating the skin disorder vitiligo, but research on its effects on depression, pain, or other conditions is limited.

Experimental Details

The compound Pioglitazone was purchased in commercial tablet form and the FT-IR spectrum was recorded in Bruker IFS 66V spectrometer in the range of 4000-500 cm^{-1}. The spectral resolution is $\pm$ 2cm^{-1} and the FT-Raman spectrum of compound was also recorded in the same instrument with FRA 106 Raman module equipped with Nd: YAG laser source operating at 1.064 µm line widths with 200 mW power in the range of 4000–400 cm^{-1} with a scanning speed of 30 cm^{-1} min^{-1} and spectral width 2 cm^{-1}. The frequencies of all bands are accurate to ± 1 cm^{-1}. The UV-Vis spectra were recorded in 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.

Theoretical Details

All the quantum chemical computations in the present work are performed using the Gaussian 09 software programs on a Pentium IV/3.02GHz personal computer [7]. The geometrical parameters were computed using B3LYP functional with 6-311++G (d, p) basis set. The UV-Visible spectrum, electronic transition such as HOMO-LUMO excitation energies, and oscillator strength were calculated using the time-dependent TD-SCF-B3LYP method. The NMR chemical shift was carried out by the GIAO method along with B3LYP and 6-311G++ (d, p) basis set. The natural bonding orbital (NBO) computations were done at B3LYP /6-311++G (d, p) level using second-order perturbation theory. In addition, 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.

Result and Discussions

STRUCTURE ANALYSIS P-BROMO-DL-PHENYL ALANINE:

The optimized molecular geometry of the compound P-Bromo-DL-Phenyl alanine is shown in Fig 1. The internal coordinates describe the position of the atoms in terms of distances, angles, and dihedral angles to an origin atom. The optimized bond length and bond angle of this compound are calculated by DFT methods with 6-311++G (d,p) are listed in table 1. This molecule has nine C-C, seven C-H, two C-O, one C-Br, one C-N, one O-H, two N-H bond lengths. The bond distance is calculated by the B3LYP/6-311++G (d,p) method. The structural parameters have been presented in table 1.

The C-C bonds in the mainframe of Phenyl rings are usually ranged between 1.38Å-1.39 Å. Here the C-C bond lengths inside the phenyl ring were observed to be very well within the expected range, except around C1 where Br is attached and C4 where the alanine group is attached, a small deviation is observed around these two atoms, the bond lengths at these points are1.40Å. In the case of alanine group, the C4-C12, C12-C13 and C13-C14 bonds are found to have the length of 1.51Å, 1.54Å, and 1.54 Å, this shows the charge distribution around these carbon atoms are almost equal. This value is higher than the expected range for even a CC single bond, which may due to the presence of an oxygen atom in the ring. The CO bonds are usually ranged between 1.20Å for double bonds and 1.35Å [8] for a single bond. Here the C14-O16 is thus proven to be a double bond and C14-O17 as a single bond. The C-H bonds are usually ranged 1.08 Å. Here the C-H bond lengths were observed to be 1.08Å-1.09 Å. The C12-H18, C12-H19, and C13-H23 bonds are found to have a length of 1.09 Å. This clear distinction between 1.08Å and 1.09 Å values among the aromatic and aliphatic CH values indicates the influence of O and N atoms present in the alanine group.
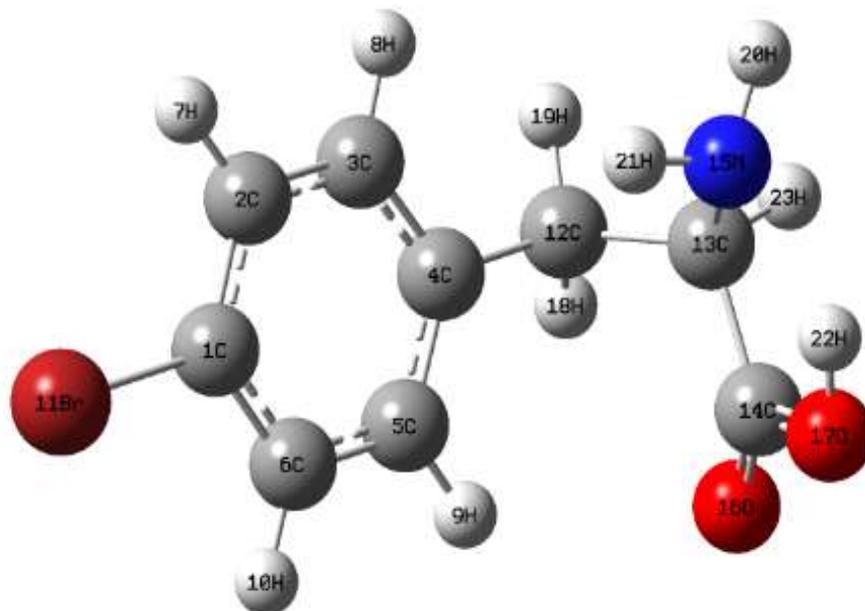
Fig. 1 Optimised molecular structure of P-Bromo-DL-Phenyl alanine

The bond angle around each carbon atom is expected to be 120Å. In the present molecule, all are in the expected range, except C1-C2-C3, C2-C3-C4, C3-C4-C5, C5-C4-C12, C4-C5-C6, C1-C6-C5, C4-C12-C13, and C12-C13-C14, these bond angles are varying between 118Å - 121Å which means the bond angles are deviated from the expected value due to the influence of O & N atoms present in the alanine group. The C-C-H bond angles also show deviation from the expected range. In C12-C13-N15 and C14-C13-N15 these values are varying between 116Å and 109Å. The deviations in these values are naturally due to the presence of an N atom in the amino group, which indicates the presence of N changes the hybridization of C from SP^2 to SP^3 with clear deviation.

Table: 1

Optimized Geometrical parameter for P-Bromo-dl-phenyl alanine Computed at B3LYP/6-311++G (d,p)

Bond Length	B3LYP/ 6-311++G (d,p) Å	Bond Angle (°)	B3LYP/ 6-311++G (d,p) Å	Dihedral Angle (°)	B3LYP/ 6-311++G (d,p)
C1-C2	1.3898	C2-C1-C6	120.9681	C6-C1-C2-C3	-0.2864
C1-C6	1.3921	C2-C1-Br11	119.4652	C6-C1-C2-H7	179.2607
C1-Br11	1.9177	C6-C1-Br11	119.5653	Br11-C1-C2-C3	-179.8553
C2-C3	1.395	C1-C2-C3	118.9588	Br11-C1-C2-H7	-0.3081
C2-H7	1.0824	C1-C2-H7	120.5089	C2-C1-C6-C5	0.2692
C3-C4	1.3995	C3-C2-H7	120.5307	C2-C1-C6-H10	-179.4649
C3-H8	1.0857	C2-C3-C4	121.5054	Br11-C1-C6-C5	179.8376
C4-C5	1.4017	C2-C3-H8	118.7974	Br11-C1-C6-H10	0.1036
C4-C12	1.5136	C4-C3-H8	119.6856	C1-C2-C3-C4	-0.0211
C5-C6	1.3927	C3-C4-C5	118.102	C1-C2-C3-H8	178.7347
C5-H9	1.0839	C3-C4-C12	120.7071	H7-C2-C3-C4	-179.5682
C6-H10	1.0824	C5-C4-C12	121.1885	H7-C2-C3-H8	-0.8123
C12-C13	1.5489	C4-C5-C6	121.1514	C2-C3-C4-C5	0.3333
C12-H18	1.0919	C4-C5-H9	119.6107	C2-C3-C4-C12	-179.1228
C12-H19	1.0937	C6-C5-H9	119.2376	H8-C3-C4-C5	-178.4117
C13-C14	1.5478	C1-C6-C5	119.3127	H8-C3-C4-C12	2.1322
C13-N15	1.4703	C1-C6-H10	120.3851	C3-C4-C5-C6	-0.3508
C13-H23	1.0955	C5-C6-H10	120.3017	C3-C4-C5-H9	179.4362
C14-O16	1.2042	C4-C12-C13	114.2391	C12-C4-C5-C6	179.1026
C14-O17	1.3371	C4-C12-H18	110.2527	C12-C4-C5-H9	-1.1104
N15-H20	1.0128	C4-C12-H19	109.3545	C3-C4-C12-C13	99.1192

N15-H21	1.0165	C13-C12-H18	107.3876	C3-C4-C12-H18	-139.8533
O17-H22	0.9838	C13-C12-H19	107.9186	C3-C4-C12-H19	-21.93
		H18-C12-H19	107.4448	C5-C4-C12-C13	-80.32
		C12-C13-C14	111.7885	C5-C4-C12-H18	40.7075
		C12-C13-N15	116.0413	C5-C4-C12-H19	158.6308
		C12-C13-H23	107.3848	C4-C5-C6-C1	0.0569
		C14-C13-N15	109.4427	C4-C5-C6-H10	179.7912
		C14-C13-H23	103.9702	H9-C5-C6-C1	-179.7308
		N15-C13-H23	107.3954	H9-C5-C6-H10	0.0035
		C13-C14-O16	123.0054	C4-C12-C13-C14	73.8196
		C13-C14-O17	113.9371	C4-C12-C13-N15	-52.6431
		O16-C14-O17	123.0186	C4-C12-C13-H23	-172.7528
		C13-N15-H20	112.3259	H18-C12-C13-C14	-48.7821
		C13-N15-H21	111.5208	H18-C12-C13-N15	-175.2449
		H20-N15-H21	108.1103	H18-C12-C13-H23	64.6454
		C14-O17-H22	105.3208	H19-C12-C13-C14	-164.3393
				H19-C12-C13-N15	69.198
				H19-C12-C13-H23	-50.9117
				C12-C13-C14-O16	38.5766
				C12-C13-C14-O17	-143.6335
				N15-C13-C14-O16	168.5545
				N15-C13-C14-O17	-13.6557
				H23-C13-C14-O16	-76.9562
				H23-C13-C14-O17	100.8336
				C12-C13-N15-H20	-88.9826
				C12-C13-N15-H21	32.5614
				C14-C13-N15-H20	143.3854
				C14-C13-N15-H21	-95.0705

				H23-C13-N15-H20	31.1213
				H23-C13-N15-H21	152.6654
				C13-C14-O17-H22	3.4089
				O16-C14-O17-H22	-178.8015

MULLIKEN AND NATURAL ATOMIC CHARGE ANALYSIS:

The study of charges around each atom is considered important, as only they determine the bond strength, bond length, and bond angle, dipole moment, polarizability, reactive sites, electronic transitions, etc. which in turn determine the physical, chemical, and biological properties and applications of the molecule. The charges of the atoms are computed using the same functional and basis set B3LYP/6-311++G (d,p) in two different methods Mulliken and Natural methods, they are shown graphically in fig 11 and numerically in table 2

For Carbon atoms in the benzene ring, the charges are expected to be equal because of the conjugation around -0.35 in Mulliken and -0.22 in the Natural (NAC) method respectively. The 1C where Br is attached is found to be positive in Mulliken whereas less negative in NAC, in this case, the Mullikan prediction may not be correct as Br relatively has not much electron-withdrawing tendency when compared to carbon. 4C where the alanine group is attached has the highest positive value in Mulliken, slightly negative in NAC. In this case, NAC prediction may be correct as there at least two carbon atoms in between, from N and O atoms in this group. The carbon atoms 2C, 3C, 5C, and 6C show negative in both Mulliken and Natural charge in the benzene ring as expected. C14 which is attached to O atoms in the acid group is found to be negative in Mulliken but highly positive in NAC, here also the prediction by NAC would be correct as O atoms can withdraw charges from this carbon atom. The H atoms in the molecules are found to be equally positive 0.22 whether they are in the benzene ring or methyl group. With an exception of 22H is attached with O atoms which shows an extremely high positive charge value of 0.50 among the other H atoms.
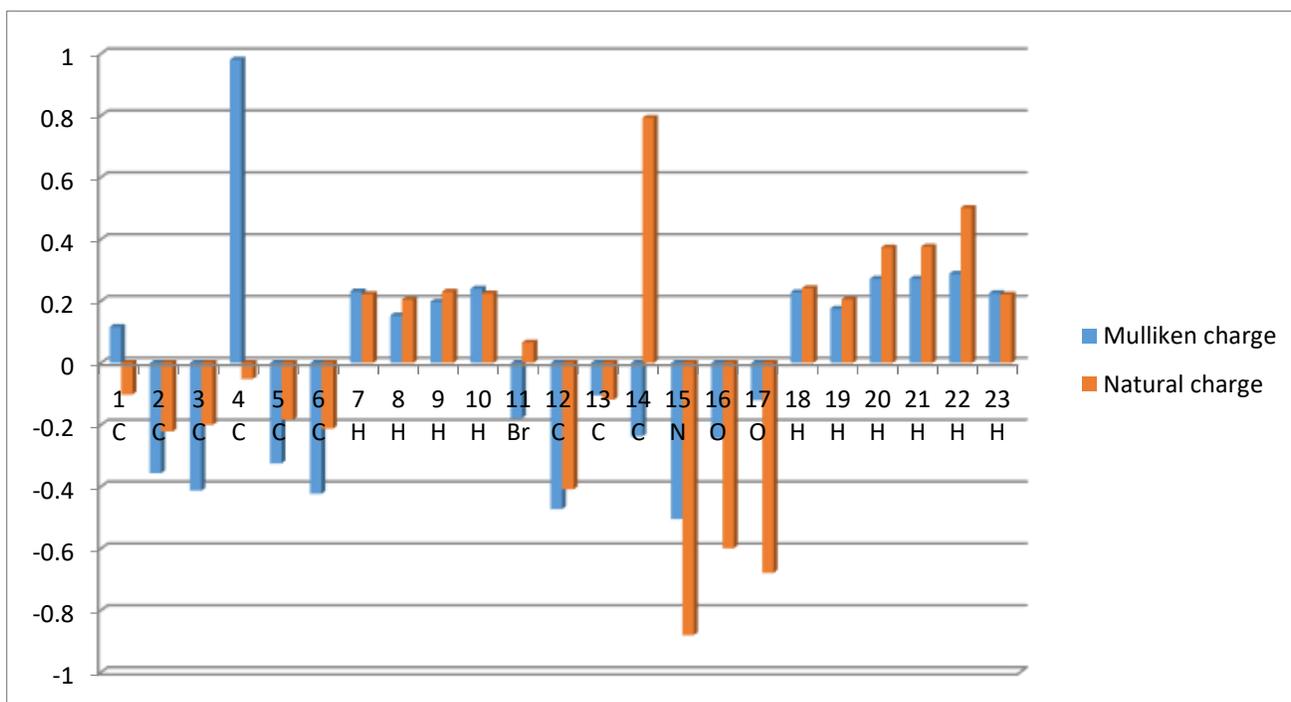
Fig 2. Graphical Representation Of Atomic Charge Analysis Of P-Bromo-Dl-Phenylalanine

Table 2. Mulliken And Natural Atomic Charges For P-Bromo-Dl-Phenylalanine

Atom	Mulliken Atomic Charge	Natural Atomic Charge
1C	0.11631	-0.1024
2C	-0.3565	-0.2218
3C	-0.4128	-0.2008
4C	0.98062	-0.0518
5C	-0.3248	-0.183
6C	-0.423	-0.2125
7H	0.23144	0.22208
8H	0.15355	0.20509
9H	0.19753	0.23029

10H	0.2399	0.22451
11BR	-0.1787	0.06575
12C	-0.473	-0.4084
13C	-0.1049	-0.119
14C	-02376	0.79214
15N	-0.5048	-0.8805
16O	-0.2451	-0.5993
17O	-0.1204	-0.679
18H	0.2283	0.24196
19H	0.17538	0.20577
20H	0.27187	0.37298
21H	0.27247	0.3755
22H	0.2885	0.50113
23H	0.22576	0.22125

NMR CHEMICAL SHIFT ANALYSIS:

The chemical shift analysis helps to identify the carbon atoms which are responsible for the medical and chemical applications of the molecule. The carbons which have a chemical shift greater than 150 ppm are generally observed to be contributing to the biological activities of the molecule. The chemical shift of the carbon and hydrogen atoms in the present molecule is computed with B3LYP/6-311++G (d,p) in combination with GIAO functional. The shift values are shown graphically in Fig 3 and Table 3.

The chemical shift values for the carbon atoms in the phenyl ring are expected between 120-130ppm, here in this molecule, in the benzene ring, the carbon chemical shifts are C1 (142.5) C2 (133.8), C3 (133.84), C4 (149.2), C5 (136.01), and C6 (136.49). The higher shift for C1 (142.5) proves the lessening negative charge prediction by NAC is correct rather than that of completer positive charge by Mulliken. The shift for C4 (149.2) where the alanine group is attached, also show that the charge withdrawal is not as high as predicted by Mullikan, it almost coincides with the charge prediction by NAC, it shows the impact of N and O atoms on this carbon atomC4 is less, due to the presence of C14, C13, and C12 on the way.

The most active site of the molecule is found in 14C where the chemical shift values are found to be 167 ppm. This is an extremely high value which indicates it is made highly positive due to the presence of double oxygen atoms linked directly to this carbon atom. The chemical shift for aromatic H atoms is expected at values between 7 to 8ppm, in this molecule also, they are present within this limit, which indicates they are not influenced by the presence of substitutional groups. However, the H atoms shifts in the methyl group are found enhanced, which is naturally due to the presence of ester [13]. The shift of 22H which is present OH group is 6.7 ppm and that of 20H which is present in the NH group is 1.7 ppm, which indicates the electronegative power of O and N respectively.
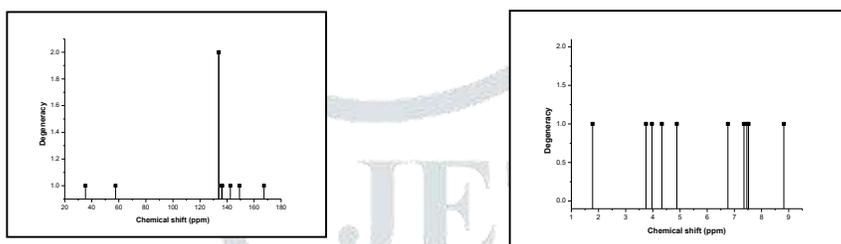

Fig 3. Theoretical ^{13}C and ^{1}H NMR Chemical Shift Analysis Of 3-Bromo-Dl-Phenylalanine

Table 3. Calculated 1H and 13C NMR Chemical Shifts (Ppm) Of 3-Bromo-Dl-Phenylalanine

Atom	GasB3LYP/6-311++G(d,p)GIAO(ppm)	SOLB3LYP/6-311++G(d,p)GIAO(ppm)
1C	143.956	142.521
2C	133.666	133.872
3C	133.014	133.843
4C	148.403	149.256
5C	135.669	136.018
6C	136.796	136.494
12C	35.1369	35.3472
13C	57.2014	57.5691
14C	164.106	167.334
7H	7.3583	7.4608
8H	8.6246	8.8222
9H	7.2335	7.3532
10H	7.4642	7.5234

18H	4.068	3.9673
19H	3.6851	3.7495
20H	1.3108	1.7792
21H	4.2994	4.3307
22H	6.1299	6.7645
23H	4.7913	4.8838

Vibrational Analysis:

The title molecule consists of 23 atoms and therefore the numbers of fundamental modes of vibrations, based on the 3N-6 formula for non-linear molecule, are 63. They are all predicted theoretically and the values are presented in Table 4 along with the experimental values from IR and Raman spectra. The wavenumbers are calculated using B3LYP methods with 6-311++G (d,p) basis sets. The experimental IR and Raman spectra of the title molecule are presented in Figure 4.

C-H Vibration

Generally, all the aromatic CH stretching vibrations are identified at 3100-3000 cm^{-1} [11] whereas all the aliphatic CH vibrations are identified at 3000-2900 cm^{-1}. In the case of P-Bromo-DL-Phenyl alanine, seven CH modes of vibrations are expected. The frequencies observed are 3260, 3199, 3163, 3150, 3070, 3061, and 3022 cm^{-1}; this indicates all the values are in the aromatic range. This is purely due to the influence of O atoms in the alanine group. This variation is in agreement with the increase in CH bond length in this group.

C-C VIBRATIONS

In the case of P-Bromo-DL-Phenyl alanine, eight cc modes of vibrations are expected. The CC stretching modes are normally found in the region between $1600 - 1400$ cm^{-1} [10 &12]. In the title molecule, CC stretching vibrations were observed as 1686, 1643, 1592, 1524, 1491, 1470, 1443, and 1417 cm^{-1} both in IR and Raman spectra, almost close values are also obtained in computations. The observation of all these values clearly shows high values for both double bond and single bond, as well as aliphatic CC stretching, in comparison with the literature values. This indicates that the occupancy in all these bonds is increased due to the impact of the alanine group.

N-H & O-H VIBRATIONS

In the case of P-Bromo-DL-Phenyl alanine, two NH modes of vibrations and one O-H mode are expected around 3200 to 3400 for NH and 3400 to 3700cm-[1] for OH respectively [13]. The OH stretching vibration is observed in this molecule at 3617 cm^{-1} even in experimental value, this is very much larger in magnitude, which may be due to the influence of double O atoms in the adjacent position in the ester group. The NH vibrations are found at 3522 and 3441 cm^{-1}. Even these values are larger than usual NH values, which also owes to the presence of the ester group in this alanine group.
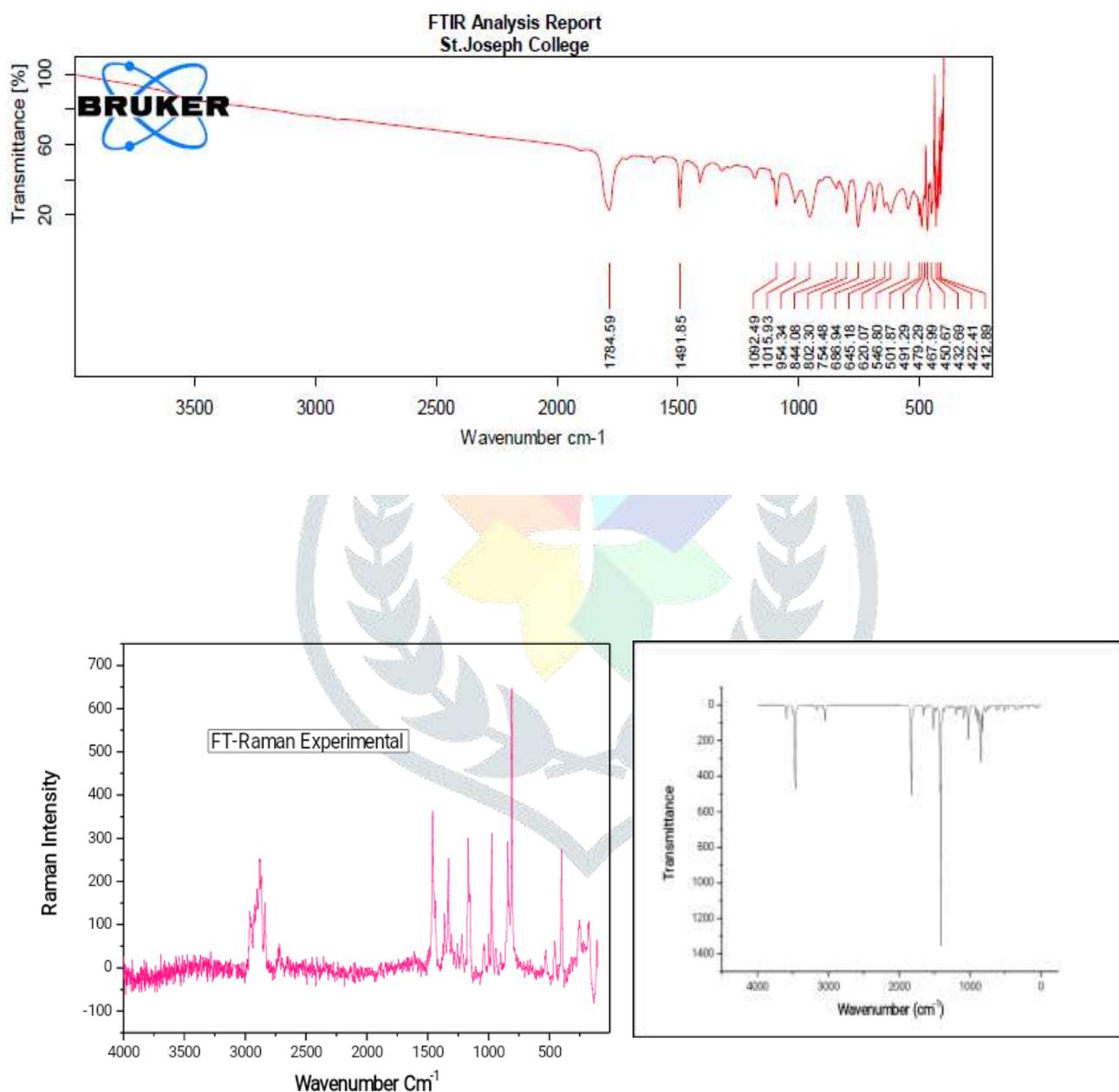

Fig. 4 The theoretical and experimental IR and Raman spectra of the molecule P-Bromo-DL-Phenyl alanine

Table: 4

Observed method B3LYP/6-311++G (d, p) level calculated Vibrational frequencies of P-Bromo-DL-Phenyl alanine

Experimental frequency (cm⁻¹)		B3LYP/6-311++G (d,p)		PED %
FT-IR	*FT-RAMAN*	*Unscaled (cm⁻¹)*	*Scaled (cm⁻¹)*	
	3617	3593	3542.698	νOH77
3535	3522	3500	3451	νNH21
	3441	3467	3418.462	νNH93
3260	3252	3203	3158.158	νCH15
	3198	3200	3155.2	νCH93
	3163	3184	3139.424	νCH84
3150	3144	3158	3113.788	νCH96
	3076	3097	3053.642	νCH78
	3061	3050	3007.3	νCH27
	3022	3037	2994.482	νCH72
1784	1875	1827	1801.422	νOC84
	1686	1658	1634.788	βHNH88
	1643	1628	1605.208	νCC19
	1592	1605	1582.53	νCC32
	1524	1517	1495.762	βHCC21
1491	1470	1485	1464.21	βHCH81
	1443	1434	1413.924	βHCC15
	1417	1415	1395.19	βHOC69
	1389	1396	1376.456	βHNC17
	1357	1365	1345.89	βHCC17

	1349	1340	1321.24	βHCC19
	1309	1320	1301.52	βHCC10
	1295	1284	1266.024	βHCC26
	1241	1236	1218.696	νCC10
	1228	1214	1197.004	νCC23
	1211	1207	1190.102	νCC11
	1207	1201	1184.186	βHOC10
	1160	1164	1147.704	βHCC13
	1147	1133	1117.138	βHCC31
1092	1066	1089	1073.754	νNC31
	1052	1083	1067.838	νNC12
	1025	1027	1012.622	βCCC18
1015	1015	1020	1005.72	νCC22
	985	997	983.042	τHCCH76
954	958	964	950.504	τHCH81
	944	921	908.106	ΥCCCH29
	876	891	878.526	ΥNHCH21
	861	866	853.876	ΥCCCH32
844	850	851	839.086	τHOCO35
	837	848	836.128	ΥCCCH33
802	822	822	810.492	ΥCCCH39
	782	778	767.108	τCCCO58
754	768	765	754.29	βCCO10
686	728	725	714.85	τCCCC18
645	633	645	635.97	βCCC19
620	620	627	618.222	τCCCC11
		585	576.81	βCCO27
546	525	531	523.566	νCC21

501		512	504.832	τCCCC21
467	458	465	458.49	τCCCC23
422	445	420	414.12	τCCCC13
412	391	391	385.526	βCCN18
		348	343.128	βCCC15
	323	331	326.366	τHNCC70
		306	301.716	τCCCBr18
	283	282	278.052	νBrC43
	256	262	258.332	βCCC28
	174	177	174.522	βCCC20
	120	140	138.04	βCCC26
	80	79	77.894	τCCCO58
		71	70.006	τCCCN40
		46	45.356	τCCCO20
		40	39.44	τCCCN36

NBO Analysis of P-Bromo-DL-phenylalanine

The bonding and non-bonding (anti-bonding) interactions can be quantitatively explained by Natural bonded orbitals (NBO) analysis, which can be predicted in terms of the second-order perturbation interaction energy $E^{(2)}$. This energy defines the estimation of the off-diagonal NBO Fock matrix elements. It can be deduced from the second-order perturbation approach by the relation

$$E^{(2)} = \Delta E_{ij} = q_i \frac{F(i,j)^2}{\varepsilon_j - \varepsilon_i}$$

Where,

q_i is the donor orbital occupancy, ε_i and ε_j are diagonal elements (orbital energies) and F(i,j) NBO Fock matrix elements of off-diagonals [14].

In this analysis, the occupancies, from bonding to anti-bonding levels, and their energy levels were calculated and presented in Table 5. The table also lists the bonding (donor) and antibonding orbitals between which the electronic transitions are highly probable in this molecule. The parameter $E^{(2)}$ represents the energy required for the stabilization of the donor and acceptor levels, which also indicates the probability of electronic transitions among the various possible transitions. The higher the $E^{(2)}$ value, the higher is the probability of electronic transition.

Therefore the top ten highly probable electronic transitions, according to their stabilization energy can be listed as follows: (1) O17 to C14-O16 (n- π^*, 41.45 kcal/mol), (2) O16 to C14-O17 (n- σ *, 30.98 kcal/mol), (3) C3-C4 to C1-C2 (π-π^*, 21.59 kcal/mol),(4) C5-C6to C1-C2(π-π^*, 21.21 kcal/mol), (5) C5-C6 to C3-C4 (π-π^*, 21.04 kcal/mol), (6) O16 to C13-C14 (n-σ *, 19.81 kcal/mol), (7) C3-C4 to C5-C6 (π-π^*, 19.47kcal/mol), (8) C1-C2 to C5-C6 (π-π^*, 19.22 kcal/mol), (9) C1-C2 to C3-C4 (π-π^*, 17.88 kcal/mol), and (10) N15 to O17-H22 (n-σ *, 11.56 kcal/mol).

Accordingly, the O17 to C14-O16 (n- π^*, 41.45 kcal/mol) is the most probable transition which happens in the ester (COOH) inside the alanine group. The other probable π-π^* transitions are taking place within the benzene ring. The highest π-π^* transitions C3-C4 to C1-C2 (π-π^*, 21.59 kcal/mol), C5-C6 to C1-C2 (π-π^*, 21.21 kcal/mol), C5-C6 to C3-C4 (π-π^*, 21.04 kcal/mol), C3-C4 to C5-C6 (π-π^*, 19.47 kcal/mol), C1-C2 to C5-C6 (π-π^*, 19.22 kcal/mol), and C1-C2 to C3-C4 (π-π^*, 17.88 kcal/mol). The electronic transition due to the N atom in the molecule is N15 to O17-H22 (n-σ *, 11.56 kcal/mol), this is n-σ *transitions with very less E^2 value hence this transition is relatively less favored among the top ten transitions in the molecule. All these transitions though theoretically favorable, only a few transitions will be allowed by the selection rules, which can be identified by the oscillator strength and HOMO- LUMO contribution, as is done in the following section.

Table: 5

Second-order perturbation theory of Fock matrix in NBO basis of P-Bromo-DL-phenylalanine

Donor	Type of bond	Occupancy	Acceptor	Type of bond	Occupancy	Energye(2) Kcal/mol	E(j)-e(i)	F(i,j)
O 17	n	1.79042	C 14 - O 16	π^*	0.20907	41.43	0.38	0.112
O 16	n	1.84883	C 14 - O 17	σ^*	0.08933	30.98	0.64	0.128
C 3 - C 4	π	1.66517	C 1 - C 2	π^*	0.38307	21.59	0.27	0.069
C 5 - C 6	π	1.65731	C 1 - C 2	π^*	0.38307	21.21	0.27	0.068

C 5 - C 6	π	1.65731	C 3 - C 4	π*	0.35621	21.04	0.28	0.069
O 16	n	1.84883	C 13 - C 14	σ*	0.09351	19.81	0.6	0.098
C 3 - C 4	π	1.66517	C 5 - C 6	π*	0.30586	19.47	0.29	0.067
C 1 - C 2	π	1.68434	C 5 - C 6	π*	0.30586	19.22	0.3	0.068
C 1 - C 2	π	1.68434	C 3 - C 4	π*	0.35621	17.88	0.3	0.066
N 15	n	1.92455	O 17 - H 22	σ*	0.03821	11.56	0.73	0.083
Br 11	n	1.93771	C 1 - C 2	π*	0.38307	9.72	0.3	0.053
O 17 - H 22	σ	1.98318	C 14 - O 16	σ*	0.03242	6.25	1.33	0.082
N 15	n	1.92455	C 12 - C 13	σ*	0.03305	6.05	0.7	0.059
C 2 - C 3	σ	1.96832	C 1 -Br 11	σ*	0.03611	5.56	0.8	0.059
C 5 - C 6	σ	1.96827	C 1 -Br 11	σ*	0.03611	5.56	0.79	0.059
O 17	n	1.97491	C 13 - C 14	σ*	0.09351	4.91	0.95	0.062
C 12 - H 18	σ	1.96977	C 13 - N 15	σ*	0.01978	4.83	0.82	0.056
N 15	n	1.92455	C 13 - C 14	σ*	0.09351	4.71	0.68	0.051
C 13 - H 23	σ	1.9567	C 14 - O 16	σ*	0.20907	4.68	0.58	0.048
C 5 - H 9	σ	1.97797	C 3 - C 4	σ*	0.02404	4.61	1.08	0.063
C 3 - H 8	σ	1.9787	C 4 - C 5	σ*	0.02476	4.54	1.09	0.063
C 6 - H 10	σ	1.97834	C 1 - C 2	σ*	0.02626	4.39	1.09	0.062
C 2 - H 7	σ	1.97841	C 1 - C 6	σ*	0.0263	4.34	1.09	0.062
C 12 - H 19	σ	1.97776	C 4 - C 5	σ*	0.02476	4.17	1.07	0.06
C 2 - C 3	σ	1.96832	C 1 - C 2	σ*	0.02626	3.94	1.27	0.063
C 5 - C 6	σ	1.96827	C 1 - C 6	σ*	0.0263	3.86	1.27	0.062
C 6 - H 10	σ	1.97834	C 4 - C 5	σ*	0.02476	3.71	1.09	0.057
C 2 - H 7	σ	1.97841	C 3 - C 4	σ*	0.02404	3.68	1.09	0.057
C 5 - H 9	σ	1.97797	C 1 - C 6	σ*	0.0263	3.56	1.08	0.055
C 5 - C 6	σ	1.96827	C 4 - C 12	σ*	0.02256	3.53	1.11	0.056

C 2 - C 3	σ	1.96832	C 4 - C 12	σ*	0.02256	3.51	1.12	0.056
C 3 - H 8	σ	1.9787	C 1 - C 2	σ*	0.02626	3.49	1.09	0.055
C 3 - C 4	π	1.66517	C 12 - C 13	σ*	0.03305	3.44	0.61	0.044
C 2 - C 3	σ	1.96832	C 3 - C 4	σ*	0.02404	3.32	1.27	0.058
C 4 - C 5	σ	1.97273	C 3 - C 4	σ*	0.02404	3.3	1.27	0.058
C 3 - C 4	σ	1.97422	C 4 - C 5	σ*	0.02476	3.27	1.27	0.058
Br 11	n	1.97636	C 1 - C 6	σ*	0.0263	3.27	0.86	0.047
Br 11	n	1.97636	C 1 - C 2	σ*	0.02626	3.26	0.86	0.047
C 5 - C 6	σ	1.96827	C 4 - C 5	σ*	0.02476	3.19	1.27	0.057
C 1 - C 6	σ	1.98022	C 1 - C 2	σ*	0.02626	3.18	1.3	0.057
C 1 - C 2	σ	1.97981	C 2 - C 3	σ*	0.01727	3.16	1.3	0.057
C 1 - C 2	σ	1.97981	C 1 - C 6	σ*	0.0263	3.15	1.3	0.057
C 12 - H 18	σ	1.96977	C 3 - C 4	σ*	0.02404	3.14	1.07	0.052
C 3 - C 4	σ	1.97422	C 2 - C 3	σ*	0.01727	3.12	1.27	0.056
C 13 - H 23	σ	1.9567	C 4 - C 12	σ*	0.02256	3.12	0.93	0.048
C 1 - C 6	σ	1.98022	C 5 - C 6	σ*	0.01679	3.03	1.31	0.056
C 4 - C 5	σ	1.97273	C 5 - C 6	σ*	0.01679	2.99	1.27	0.055
C 1 -Br 11	σ	1.9842	C 2 - C 3	σ*	0.01727	2.96	1.21	0.054
C 13 - H 23	σ	1.9567	N 15 - H 21	σ*	0.01054	2.85	0.93	0.046
C 1 -Br 11	σ	1.9842	C 5 - C 6	σ*	0.01679	2.84	1.22	0.053
C 12 - H 19	σ	1.97776	C 13 - C 14	σ*	0.09351	2.71	0.84	0.043
C 4 - C 5	σ	1.97273	C 3 - H 8	σ*	0.01433	2.69	1.13	0.049
C 3 - C 4	σ	1.97422	C 5 - H 9	σ*	0.01401	2.47	1.16	0.048
C 4 - C 12	σ	1.97638	C 2 - C 3	σ*	0.01727	2.46	1.2	0.049

UV-VISIBLE ANALYSIS of P-Bromo-DL-phenylalanine :

The theoretical UV-Visible electronic transitions are determined for the molecule P-Bromo-DL-phenylalanine through B3LYP/6-33++G (d,p) method and basis set along with TD-SCF functional. The experimental spectrum of the compound is recorded by solving in DMSO solvent. The calculated and experimental excitation energies, absorption wavelength, oscillator strength, and HOMO-LUMO contributions are presented in Table 6. The UV theoretical and experimental spectra are presented in Figure 6. The top ten most probable transitions which are discussed in the NBO analysis, at DMSO phase are found to have energy gaps 5.0368, 5.1852, 5.3167, 5.4165, 5.5266, 5.5808, 5.7823, 5.821, 5.9218, 6.0654 eV and their absorption wavelengths are 246.16, 239.11, 233.2, 228.9, 224.34, 222.16, 214.42, 213, 209.37, 204.41 nm and corresponding oscillator strengths are 0.0122, 0.0012, 0.015, 0.0471, 0.0496, 0.1517, 0.0043, 0.0107, 0.0035, 0.0016 respectively.

The oscillator strength values are the indicator of the absorption coefficient or the intensity of these transitions, observation of the oscillator strength for different transitions, in this molecule indicates that the first n- π^* at 246 nm and the three π-π^* transition at wavelengths 224, 222, 214 nm will have the appreciable intensity in both theoretical and experimental Uv-Vis spectra. These observations coincide with the conclusions drawn in the NBO analysis based on the E^2 values. All the n- σ^* & π -σ^* transitions are not favored as expected.

The same ten transitions in gas phase have energy gaps 4.9817, 5.1003, 5.1978, 5.3897, 5.4589, 5.5214, 5.6252, 5.7957, 5.8904, 6.0392 ev and their absorption wavelengths are 248.48, 243.09, 238.53, 230.04, 227.12, 224.55, 220.41, 213.93, 210.49, 205.3 nm and corresponding oscillator strengths are 0.0094, 0.0029, 0.0038, 0.0372, 0.102, 0.0063, 0.0614, 0.005, 0.0064, 0.0026 respectively. This UV - Visible analysis in different phases shows that there is a variation in the wavelengths as well as the intensity of absorption of different peaks which indicates the solvent effect on these electronic transitions. The n- π^* transition is predicted to be of weak intensity, only one π - π^* transition at 224 nm is predicted with very high intensity, hence in the theoretical spectrum, only this peak is visible.
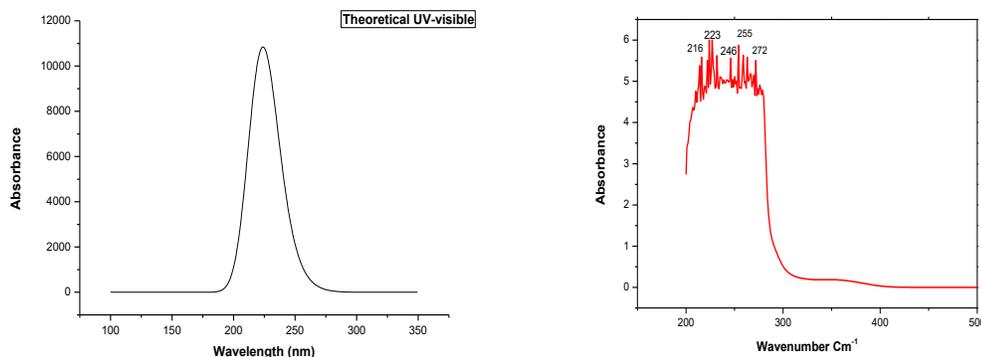
Fig. 5 The Theoretical and Experimental UV-Visible spectra of P-Bromo-DL-Phenyl Alanine

Table: 6

Theoretical electronic absorption spectra of P-Bromo-DL-phenylalanine 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				
4.9817		248.88	0.0094	HOMO->LUMO (43%)
5.1003		243.09	0.0029	HOMO->L+1 (36%)
5.1978		238.53	0.0038	H-1->LUMO (50%)
5.3897		230.04	0.0372	HOMO->L+3 (33%)
5.4589		227.12	0.102	HOMO->L+2 (69%)
5.5214		224.55	0.0063	H-1->L+1 (83%)
5.6252		220.41	0.0614	H-1->L+2 (85%)
5.7957		213.93	0.005	H-1->L+4 (52%)
5.8904		210.49	0.0064	H-2->LUMO (50%)
6.0392		205.3	0.0026	HOMO->L+4 (78%)
Ethanol				
5.0368	246	246.16	0.0122	HOMO->LUMO (66%)
5.1852		239.11	0.0012	HOMO->L+2 (70%)
5.3167		233.2	0.015	H-1->LUMO (37%)
5.4165		228.9	0.0471	HOMO->L+1 (51%)
5.5266		224.34	0.0496	H-1->LUMO (23%)

5.5808	223	222.16	0.1517	H-1->L+1 (60%)
5.7823	216	214.42	0.0043	HOMO->L+3 (36%)
5.821		213	0.0107	H-1->L+2 (29%)
5.9219		209.37	0.0035	H-2->L+2 (51%)
6.0654		204.41	0.0016	H-1->L+3 (31%)

Homo – Lumo Analysis:

The HOMO represents the orbitals that have electrons with high occupancy and tends to give them to orbitals with less occupancy called LUMO and these orbitals are together called frontier Molecular orbitals (FMO). The energy gap between them is related to the molecular properties of the molecule [15-17]. The HOMO – LUMO orbital for the p-Bromo-dl-phenylalanine molecule is shown in Fig. 6, while the parameter calculated are shown in Table 7. In this molecule, the prominent interaction between frontier orbitals is found to be n → π* transition. The monitoring result, HOMO→LUMO orbital transitions implied an electron density between alanine groups. The title compound has an energy gap is found at (ΔE) = -0.267eV.

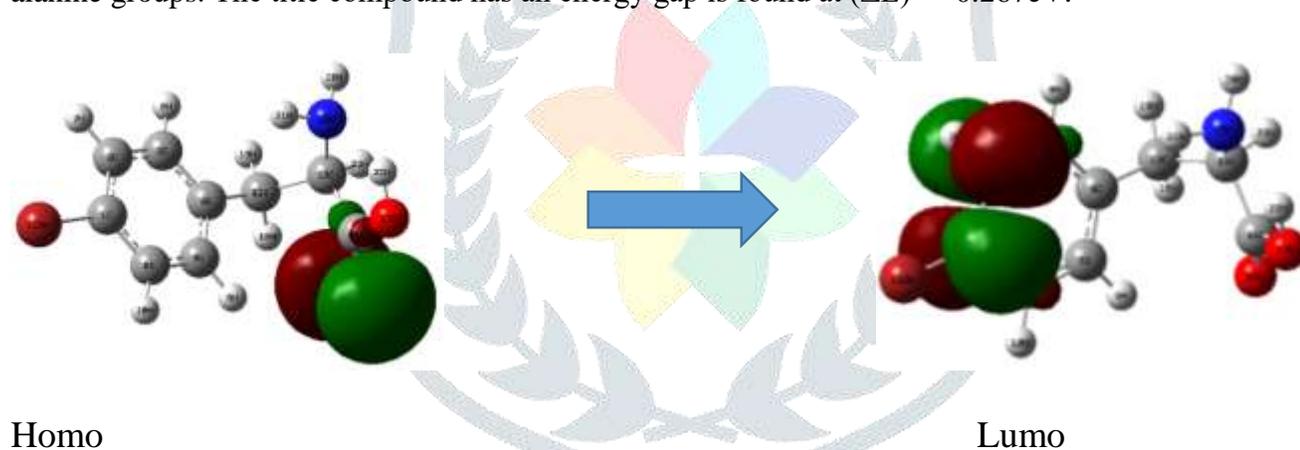Homo   Lumo

Fig. 6 Homo – Lumo of p-Bromo-dl-phenylalanine

The figure clearly shows the prominent HOMO orbitals are lying around the COOH group in alanine whereas the LUMO orbitals lie only in the phenyl ring, adjacent to Br substitution.

Table.7

HOMO, LUMO, electronegativity, global hardness and softness, electrophilicity index of P-Bromo-DL-phenylalanine

Parameter	Gas
E_{HOMO}(ev)	-0.26789
E_{LUMO}(ev)	-0.00024

$\Delta E_{\text{HOMO-LUMOGAS}}$ (ev)	-0.26765
Electronegativity (χ) (ev)	0.134065
Global hardness(η) (ev)	0.133825
Global softness (s) (ev)	0.5353
Electrophilicity index (ω) (ev)	0.06715

MEP:

In the present study, a 3D plot of molecular electrostatic potential (MEP) map of p-Bromo-dl-phenylalanine is illustrated in Fig.7. The MEP reveals the electron density distribution over the molecule which constitutes electrostatic potential. The MEP is a useful property to study reactivity, where an electrophilic reaction is attracted towards the negative regions (where the electron distribution effect is dominant) [18-19], which are shown in red color. While the maximum positive region which preferred site for a nucleophilic attack is shown as blue color. The importance of MEP lies in the fact that it simultaneously displays molecular size, shape as well as positive, negative, and neutral electrostatic potential regions in terms of color grading. The regions above the alanine group are found to be more negative potentials and over the hydrogen atoms, particularly on amino group shows more positive region. The different values of the electrostatic potential at the surface are represented by different colors. Potential increases in the order red < orange < yellow < green < blue. As can be seen from the MEP map of the title molecule, while regions having the negative potential are over the chain carbon and oxygen atoms, the region having the positive potential are overall hydrogen atoms.
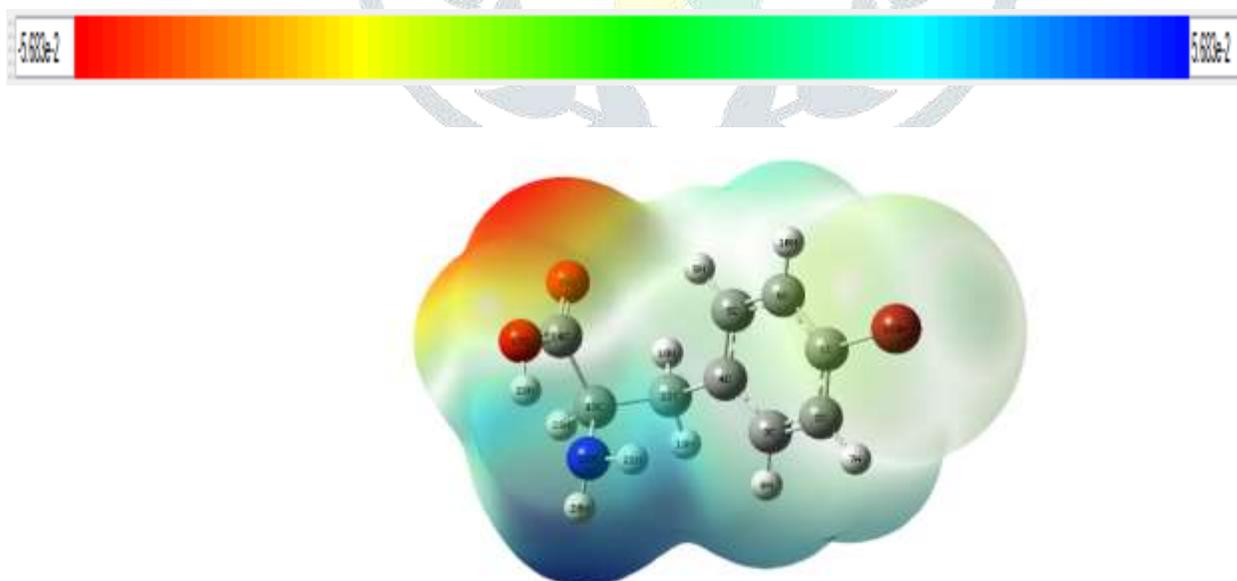

Fig. 7 MEP of p-Bromo-dl-phenylalanine

Molecular Docking Study

Docking is an essential tool used for drug discovery and delivery nowadays. Many programs for performing molecular docking have been developed which makes it a notable tool in the pharmaceutical research field [20]. The approach due to docking is used to model the interaction between a protein and the small molecule at the atomic level that allows to characterize the behaviorisms of small molecules at the binding place of target proteins and to evaluate basic biochemical processes. The two basic works in the docking process are the prediction of the ligand conformation and its binding site and orientation and the assessment of binding affinity. These two are related to the binding pose of the molecule at the active protein site ranking of the conformations through scoring function.

In this study, to know binding sites Cavity detection program PASS has been utilized. This study reveals that derivatives consisting of strong electron-withdrawing groups such as NH_2 and OH have better binding potential on the active site of the enzyme.

A docking study carried out on the molecule P-Bromo-DL-phenylalanine showed that the molecule dock with the protein 3HMC, which is an endolysin, which means a protein used as bacteria Phage. Their development as novel antibacterial agents offered many potential advantages over conventional antibiotics. The computed results of the docking are shown in Table 8 and   Fig. 8.

The predicted binding conformation reveals that the electron-withdrawing substituent NH_2 & COOH and their position on the phenylalanine group have affected the binding orientation and pattern of the molecule on the binding pocket of the enzyme. Here there are four hydrogen bonds altogether formed with the target protein, at mainly two cites in the molecule, two bonds are formed at NH_2 group and two at COOH group. Among them, three bonds are formed with the same bonding length 2.0 Å, which means the binding energy is the same for all these bonds. The fourth one is formed at the double-bonded O in the ester group, with a bond length of 1.9 Å. Any bond with length 2.0 Å does not show the Hydrogen bond characteristics fully, hence this binding is weak when compared to the hydrogen bond binding. Hence the present molecule P-Bromo-DL-phenylalanine can be used for antibacterial drugs.

Fig.8 Molecular Docking of P-Bromo-DL-Phenyl Alanine

Table.8

The Docking binding pose for P-Bromo-DL-Phenyl Alanine

Protein (PDB ID)	Bonded Residues	Bond Distance (Å)
3HMC	ASP 175	2.0
	TYR 148	2.0
	GLU 164	2.0
	TYR 148	1.9

Conclusion:

The molecule p-Bromo-dl-phenylalanine was subjected to thorough structural, vibrational, NBO, UV, NMR, and docking analyses, and the following observations are made. The structural analysis exposed that there is no change in the structural parameters of the phenyl ring except at C1 & C4 where the substitutional group is attached. The CC and CH bond length values in the alanine group are found to be enhanced due to the presence of double O and N atoms in the group. The CC and CH aromatic stretching

vibrations are found to be enhanced to the usual values. But these vibrations along with CO modes are unusually found enhanced in the alanine groups due to the presence of N and O atoms. The NMR and charge analysis show that the C14 has the highest value 167 ppm due to the double-bonded atom. The shift of 4C (149 ppm) shows that the alanine group as such is highly electron-withdrawing in nature.

According to the perturbation theory, the transition between O17 to C14-O16 (n- π^*, 41.45 kcal/mol) is the most probable transition which happens in the ester (COOH) inside the alanine group. The other probable π-π^* transitions are taking place within the benzene ring. This prediction was confirmed by the Uv-Vis analysis, both theoretically and experimentally,   which indicates that only the first n- π^* and the four π-π^* transitions will have the appreciable intensity in both theoretical and experimental Uv-Vis spectra.

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