Structural, Spectroscopic Investigation and Binding Mechanism of 2,5-dibromotoluene With Human Serum Albumin

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Abstract

The optimized structure, comparative theoretical and experimental vibrational assignments of 2,5-dibromotoluene (DBT) have been evaluated by density functional theory (DFT) with higher basis set calculations. The global reactivity determination such as energy gap, dipole moment has been explored. The locale reactive sites of the molecule are described by applying the electrostatic potential. The interactions between the bonds are assessed by the natural bond orbital (NBO) investigation. The resonance quality ^1H and ^{13}C (NMR) shifts of the molecule calculated by GIAO method. Optical transparency of the molecule has been analyzed by theoretical UV-Visible spectra. The binding of toluene with serum albumin protein utilizing *in silico* has been validated and subsequently, the present work clears the method for scheming the drugs in the dealing of serum albumin.

Introduction

Toluene's are the class of aromatic hydrocarbon consists of a methyl group attached to a phenyl group. They are made basically from unrefined oil in the petrochemical manufacturing and acts as a solvent for paint thinners, paints, painting ink, rubber, lacquers, glues, many disinfectants, leather tanners, many chemical reactants. In biochemistry research, they can be utilized to separate hemoglobin from red blood cells [1]. Also, the neurotoxin possessions of toluene are expected to be the most wellbeing dangers and therefore inhalation of the vapor may results in tiredness, tipsiness, and cerebral pains. The Government Republic of Germany has created organic resilience ethics for a choice of solvents including toluene. A few organic tests have been arranged for assessing toluene demonstration; these involved the estimation of toluene in breath, blood and urine, and hip uric acid and o-cresol in urine [2].

Albumin is a protein which helps retain fluid in our blood stream. It carries numerous substances including fatty acids, hormones, vitamins, and enzymes. It is capable of 70% to 80% of the osmotic weight of typical plasma, controlling the volume of circulating blood. Commercially accessible albumin is fractionated from blood or plasma from donors. A lower albumin level indicates liver and kidneys diseases. Human Serum Albumin (HSA), the foremost plentiful plasma, could be a multi-field molecule. It shows a surprising ligand-binding capacity, carrier for endless endo-exogenous compounds. HSA is a precious biomarker of numerous diseases such as cancer, post-menopausal weight and extreme graft-versus-host disease [3]. In this study, the binding mechanism of 2,5-dibromotoluene (DBT) with human serum albumin has been studied accompanied by spectroscopic and density functional theory (DFT) computation. For that, the molecular docking examination is performed to recognize the inhibitory nature of the 2,5-dibromotoluene with human serum albumin protein. From the DFT calculations, the vibrational frequencies, molecular orbital energies, Mullikan charge, molecular electrostatic potential, natural bond orbital (NBO) and other molecular properties of DBT have been investigated. The vibrational wave numbers of the molecule have been estimated from potential energy dispersion (PED) by *MOLVIB* program [4].

Methodology

Experimental characterizations

The DBT test with 99% pureness has been purchased from Sigma Aldrich, USA. Perkin Elmer FT-IR spectrometer was recorded by employing a KBr pellet strategy at room temperature with a 1.0 cm^{-1} resolution. Stand-alone Fourier transform-Raman spectrum was taken by applying BRUKER RFS 27 model spectrometer at room temperature with a resolution of 2 cm^{-1} . The FT-IR and FT-Raman spectra have been established in the wavenumber range $4000\text{--}400\text{ cm}^{-1}$ and $4000\text{--}50\text{ cm}^{-1}$, respectively.

Quantum chemical calculations

The *GAUSSIAN 09W* program [5] exploiting Becke-3-Lee-Yang-Parr (B3LYP) hybrid functional [6, 7] with the standard 6-311++G (d,p) [8] basis set have been utilized for DFT calculations of DBT, at the ground state level. Initially, the optimized

structure of DBT is estimated by the DFT/B3LYP strategy with a 6-311++G (d,p) then the vibrational wave-numbers and intensities are calculated. The scaled quantum mechanical (SQM) [9] strategy ensures between the experiment and DFT computed results. The frontier molecular orbital's (FMOs) of DBT has been visualized using *Gaussview 05* visualization program [10]. The UV-Vis range of DBT have been computed (without any solvation) by time-dependent (TD)-DFT/B3LYP strategy related with the polarizable continuum model (PCM). The ^{13}C and ^1H NMR shielding was recreated using the Gauge-Invariant-atomic orbital (GIAO) method.

Protein and ligand search

The 3D precious structure proteins such as human serum albumin (PDB ID: 1A06) and plasma-derived human serum albumin (PDB ID: 5Z0B) were accomplished from the Proteins Information Bank (<http://www.rcsb.org/pdb/>) [11]. These structures can be utilized for docking studies. The protein structure and amino corrosive position, the docking handle were utilized by Disclosure Studio (Adaptation: 2017 R2 client) [12]. Target ligand DBT and its structure were initiated from exposed ligand databases: PubChem (<http://pubchem.ncbi.nlm.nih.gov>).

Molecular docking

The docking of protein-ligand is accomplished by Auto Dock Vina (Version: 4.2.1) [13]. For docking, the ligand and all water elements are evacuated to get ready for the protein structures, whereas cofactors are kept as a portion of the authoritative compact. Computational docking could be an open strategy utilized to recognize the ligand-proteins binding interactions. To understand the degree of docking energy affinities (Kcal/Mol), the receptor and ligand structures are arranged in pdbqt format. For each ligand, Auto Dock Vina produced energy affinity values for ten distinctive docking postures. In our method, we isolated the protein particles to discover receptors with a great binding affinity to DBT.

Results And Discussion

Molecular geometry analysis and symmetry

The structure of DBT is optimized using the DFT/B3LYP level of theory employing a 6-311++G (d, p) ground set. The corresponding structure of DBT is appeared in Fig. 1. The least energy has been calculated as $-5418.72330173\text{a.u.}$ The obtained negative energy value affirmed that the DBT could be the stable structure on the potential energy surface [14]. In addition, the geometrical parameters such as bond lengths and bond angles are recorded in Table 1 with the relevant XRD information of the title molecule [15]. It seems that the optimized parameters with higher basis set calculations concur well with the XRD data. The effect of the ring framework can be well fixed by the rise in bond length of C2-Br7 and C5-Br10 (1.921 and 1.916 by B3LYP and 1.86 Å by XRD). The other bond lengths C1-C2, C1-C6, C1-C12, C2-C3, C3-C4, C4-C5, and C5-C6 which are calculated as 1.401, 1.401, 1.505, 1.391, 1.392, 1.389 and 1.389 Å, respectively (1.42, 1.45, 1.46, 1.37, 1.43, 1.34 and 1.38 Å by experimental). From the DFT calculations, the C2-C1-C6, C1-C2-C3, C1-C2-Br7, C3-C2-Br7, C2-C3-C4, C3-C4-C5, C4-C5-C6 and C1-C6-C5 bond lengths are computed as 117.04° , 122.06° , 120.15° , 117.77° , 120.02° , 118.64° , 121.21° and 121.00° , correspondingly, (experimental values: 118° , 121° , 119° , 123° , 121° , 122° , 123° , 119°). Moreover, these asymmetry deviations may be due to the methyl and bromine groups interlinked with molecule. Besides, the geometry of DBT has C_1 point group symmetry. The thermodynamics parameters of DBT are counted in Table 2. In our study, the calculated dipole moment and total energy of DBT are assessed as 0.6392 Debye and $72.883\text{ kcal mol}^{-1}$, respectively, and the immaterial zero-point vibration energy ($67.28997\text{ kcal mol}^{-1}$) is acquired. These thermodynamics constraints can be utilized in the evaluation of chemical reactions and to discover the extra thermodynamic energies of DBT.

Vibrational spectral analysis

DBT molecule comprises 15 atoms and thus its 39 vibrations are present in both vibrational spectra (IR and Raman). Figs. 2 and 3 expose the experimental and computed FTIR and FT-Raman spectra. The vibrational intensities and the assignment of DBT have appeared in Table 3. Irregularities among the calculated and observed vibrational frequencies, since theoretical

values are carried out on free molecule, but experiments are done on liquid sample. Therefore, computed wave numbers have been scaled, utilizing the scale factor 0.9613 and for the B3LYP strategy [16].

C-H vibrations

The aromatic C-H stretching assemblies are generally found in the wave number interval $3100\text{--}3000\text{ cm}^{-1}$ [17, 18]. In this study, theoretically scaled C-H vibrations of DBT has been found at $3153, 3110$ and 3113 cm^{-1} (**These are established by their TED values and** nearly 95%). The C-H experimental FT-IR band recognized at $3183, 3112\text{ cm}^{-1}$ and FT-Raman at $3111, 3092\text{ cm}^{-1}$ also agrees with the calculated results. In-plane C-H vibrations are coupled with C-C stretching vibrations and are identified in the $1300\text{--}1100\text{ cm}^{-1}$ [19, 20]. The strong C-H in-plane stretching vibrations of DBT has been computed at $1281, 1236, 1191\text{ cm}^{-1}$, the corresponding FT-IR and FT-Raman bands found at bands assigned at $1272, 1244, 1189, 1283, 1210$ and 1194 cm^{-1} . The absorption groups stemming from C-H out of plane is occurred in $950\text{--}800\text{ cm}^{-1}$ region [21, 22]. These C-H out of plane vibrations from DFT are obtained as $933, 865, 682\text{ cm}^{-1}$ and the equivalent experimental peaks found at $952, 893, 888$ and 686 cm^{-1} .

C-Br vibrations

C-Br stretching as well as C-Br deformation is noted in the vibrational range $650\text{--}485\text{ cm}^{-1}$ and $300\text{--}140\text{ cm}^{-1}$, [23,24] respectively. The stretching C-Br vibrations of DBT are identified at 874 and 752 cm^{-1} in FT-IR. The C-Br in-plane deformation vibrational bands found at $430, 427$ and 331 cm^{-1} in the experimental spectra (nearly 70% TED). The equivalent computational bands have been seen at $434, 369\text{ cm}^{-1}$. The C-Br out-of-plane vibrations are moreover recognized and reinforced by implies of the literature results [25] and values are noted in Table 3.

Methyl group vibrations

Normally, the CH_3 in-plane and CH_3 out plane stretching vibrations occur in the $2975\text{--}2840\text{ cm}^{-1}$ region [26, 27]. In our investigation, computational frequencies at $3062, 3033$ and 2982 which fits with the experimental frequencies $3098, 3053, 2997, 2993\text{ cm}^{-1}$ are assigned for methyl in-plane, out-of-plane and asymmetric vibrations (nearly 92% TED). Usually, CH_3 group distortions are found in between $1450\text{--}1400\text{ cm}^{-1}$ [28]. For DBT, the CH_3 in-plane, out-of-plane and symmetric distortions are found at $1421, 1395$ and 1381 cm^{-1} from DFT calculations, which are coincide with observed results. The other methyl vibrations are well assigned and are given in Table 3.

Electronic properties

The frequency of oscillation (f), excitation energies (E), electronic transition, UV-vis spectral studies of DBT are computed by TD-DFT method [29]. For DBT, a solid peak has been observed at 246 nm with oscillator quality $f = 0.0003$ and energy = 5.0337 eV as exposed in Fig. 4. For this strong peak, the transition of charges from HOMO to LUMO describes $\pi \rightarrow \pi^*$ transition by 50% contribution. The HOMO is covering by π holding type orbitals on bromine atoms and phenyl group. LUMO is localized on methyl and benzene ring system by π anti-bonding type orbital's. The other energizing state of DBT is computed at 233 nm with $E = 5.3178\text{ eV}$ and oscillator frequency of 0.0674 . For that, the $\pi \rightarrow \pi^*$ transition is calculated from HOMO to L+3 (66%). HOMO is contained mainly over bromine atoms by π type orbitals. LUMO+3 is confined by π^* orbitals on methyl group and ring system. Another energize state has been computed at 254 nm with frequency $f = 0.0209$ and energy = 4.8657 eV . This has the most elevated major contributions (96%) from HOMO to LUMO+2 relates $\pi \rightarrow \pi^*$ exchange transition as given in Table 4. Hence, the DBT has been unsaturated due to the $\pi \rightarrow \pi^*$ type transition arises with substitutions in the aromatic ring of the molecule. These properties of the DBT reflect the eigen values of HOMO and LUMO [30]. The HOMO-LUMO gap of DBT is found as 5.6628 eV . The most notable ($E_{\text{HOMO}-2} = -8.1454\text{ eV}$) energy permits to be the excellent electron giver and the LUMO ($E_{\text{LUMO}+2} = -0.7940\text{ eV}$) implies the electron leading acceptor. The corresponding energy

gaps obtained as 7.3514 eV. The various frontier orbitals of DBT are plotted in Fig. 5. Further molecular properties such as hardness, softness and electron affinity are calculated by using Koopmans' theorem [31] and are illustrated in Table 5.

NMR spectral analysis

The optimized DBT has been utilized in the calculation of ^{13}C and ^1H NMR spectra using DFT/ B3LYP 6-311++G (d, p) method employing the GIAO strategy. It is the effective way to interpret the structure of huge biomolecules. The computational ^{13}C isotropic shift values of the DBT with tetramethyl silane (TMS) as a reference is recorded in Table 6. The calculated ^{13}C spectra have appeared in Fig. 6. In common, the chemical shift range of aromatic carbon molecules lies from 100 to 200 ppm [32]. In this case, the computational ^{13}C NMR shift values of the aromatic ring carbons are gotten in the range 135.92 to 147.07 ppm. The high electronegative properties of the bromine atoms deliver positive charges to the carbon atoms. The highest shift of aromatic carbons C1, C2 and C5 are found as 147.07, 146.64 and 147.15 ppm, which are due to the attachment of bromine atoms and methyl group. The methyl carbon C12 gives the lowest shift at 22.39 ppm, since it is coupled to the three H atoms. The 15H protons linked with methyl group exhibits the lowest shift at 1.37 ppm. Hydrogens connected straightforwardly, their protecting diminishes shielding, and the resonance leads to higher wavenumber. Hydrogens put closer to electron donor, the resonance moved to lower wavenumber. The computed chemical shifts of H8, H9 and H11 attached directly to carbon atoms have the most extreme of 7.56, 7.44 and 7.64 ppm and are given in Fig. 7 and Table 7.

Molecular electrostatic potential surface analysis

Molecular electrostatic potential (MEP) surface can give the responsive locales of electrophilic, nucleophilic, molecular shape as well as hydrogen holding reactions [33]. This MEP surface makes a difference to find the electron - deficient, slightly deficient, rich, slightly rich by understanding its color codes as blue color, light blue color, red, and yellow, respectively. The MEP surface of DBT has been portrayed in Fig. 8. The negative potential of DBT is found over the bromine atoms Br7 and Br10, which are due to the lone pair of bromine atoms. The atom C6 is also electronegative since it is prepared to be held adjacent to bromine. The positive locales are nucleophilic and are found in the hydrogens of methyl group (H13, H14 and H15). The MEP of DBT explains that the methyl group and bromine atoms are probably outbreak of the reactive sites.

Natural bond orbital analysis

The interaction between the donor and acceptor molecular bonds gives a helpful basis set for exploring the charge exchange interaction in the molecule frameworks [34]. NBO investigation of DBT is performed at the DFT/B3LYP/6-311++G (d, p) level of basis set and the calculated values are recorded in Table 8. In common, higher the esteem of stabilization energy $E(2)$ in NBO will lead to more giving tendency from electron donors to electron acceptors and causes more prominent degree of conjugation in any system. As recorded in Table 8, the solid interaction ($E(2) = 10.69$ kcal/mol) is gotten between the π (C1-C2) orbital and π^* (C3 - C4) orbital, and another stabilization of 10.48 kcal/mol is observed between π (C5- C6) orbital and π^* (C1 - C2) orbital, which are the characteristic highlights of bioactivity of DBT [35].

Mulliken atomic charges

Mulliken charge distribution gives a vital part in scheming the electro negativity, electrostatic potential, dipole moment, polarizability and electronic structure of the molecule. These properties are well studied by the atomic charge influence [36]. The Mulliken population of DBT is examined with B3LYP/6-311++G (d, p) method and are noted in Table 9. In DBT, the positive values (0.209, 0.213, 0.204, 0.173, 0.173 and 0.145) of hydrogen atoms H8, H9, H11, H13, H14, and H15 represents that DBT is more acidic. Both negative and positive values of carbon atoms C1, C2, C3, C4, C5, C6 and C12 are highly influenced by their substituents. In this study, the delocalization of charges occurring through (C1) carbon atom, it holds the highest positive charges (0.494). Further, the two electronegative bromine atoms (Br7 and Br10) dominate the largest negative charge of DBT (-0.182 and -0.184). The graphical representation of charges in DBT has been exhausted in Fig. 9.

Molecular docking analysis

Serum albumin frequently implied as blood albumin, which is found in vertebrate blood. Human serum albumin (HSA) is the maximum rich protein in human blood plasma and used to treat diseases due to hypo albuminemia (low albumin) and hyper albuminemia (high albumin). The general structure of albumin is branded by many long α helices possessing large shape, which is essential for coordinating blood weight. Albumin comprises eleven official spaces for hydrophobic compounds [37]. Serum plays a central part in toxicology and medicates advancement to diverse tissues. The official liking of serum is causally related to natural forms and toxic impacts [38]. HSA comprises of three helical spaces with eight sets of twofold disulphide bridges. Each space of HSA is separated into two subdomains. From the past report, the two-protein crystal structure of ligand-free HSA (ID: 1AO6) and plasma – derived human serum albumin (ID: 5Z0B) are served as guides for analysts to explore in the biomedical properties and restorative applications. These proteins are demonstrated to be voiced in several tissues and cells and thus binding with ligand will lead to get very efficient therapeutic drugs and proves little renal clearance [39].

The targeted proteins have been composed from the protein data bank (PDB). At first, the receptor and ligand DBT molecule have been displaced utilizing Auto Dock graphical Devices. At that point, the polar H bond and Kollman charges have been involved to focus on selected proteins. Then, the Lamarckian Hereditary Calculation is utilized for calculations within the Auto Dock program [40]. Moreover, the DBT molecule (ligand) is docked well with all focused proteins utilizing Auto Dock program. The docking parameters such as binding energy, ligand efficiency and interacted residues of DBT with proteins are calculated.

Our *in-silico* investigations revealed that the DBT molecule interacts with the serum human ligand-free protein HSA (ID: 1AO6) and plasma – derived human serum albumin (ID: 5Z0B) and are represented in Figs. 10 and 11, and their official binding energy are detailed in Table 10. The present work reflects that DBT binds with 1AO6 protein through the binding free energy (ΔG°) of -5.00 KJmol^{-1} . The highest binding energy (ΔG°) is found to be -5.4 KJmol^{-1} for DBT with 5Z0B. According to our docking inquiries, the inhibition activity of two serum human proteins is impaired by DBT. As a result, it is sensible to expect that DBT has potent serum albumin efficacy.

Conclusions

The optimized molecular parameters of 2,5-dibromotoluene is performed by the DFT/B3LYP using 6-311++(d,p) basis set and correlated well with the XRD data. MEP investigation appears the electrophilic and nucleophilic responsive locales of the title molecule. The Mulliken charge distribution and molecular orbital analysis confirm the bioactivity nature of the molecule. The computed chemical shifts of ^{13}C and ^1H NMR reflects the structural information of the molecule. The NBO indicates the intramolecular charge exchange and stability of the molecule. The docking results evident that the inhibition activity is negatively affected by the title molecule. Among the selected proteins, the plasma – derived human serum albumin (5Z0B) has the good binding energies with the target. Therefore, it is sensible that the title molecule might have potent serum albumin efficacy.

Declarations

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Author contribution Conceptualization: SJ and MR; methodology: SJ and MR; formal analysis and investigation: PM and SJ; writing—original draft preparation: PM and MR; writing—review and editing: SJ and MR; supervision: SJ and MR

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Code availability Not applicable.

Competing interests The authors declare no competing interests

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Tables

Table 1 Optimized geometrical parameters of 2,5-dibromotoluene

Parameters	Method/Basis set	Experimental ²¹
Bond length(Å)		
C1-C2	1.401	1.42
C1-C6	1.401	1.45
C1-C12	1.505	1.46
C2-C3	1.391	1.37
C2-Br7	1.921	1.41
C3-C4	1.392	1.43
C4-C5	1.389	1.34
C5-Br10	1.916	1.86
C5-C6	1.389	1.38
Bond angle(°)		
C2-C1-C6	117.04	118
C1-C2-C3	122.06	121
C1-C2-Br7	120.15	119
C3-C2-Br7	117.77	123
C2-C3-C4	120.02	121
C3-C4-C5	118.64	122
C3-C4-H9	120.37	119
C5-C4-H9	120.98	123
C4-C5-C6	121.21	123
C1-C6-C5	121.00	119

Table 2 The thermodynamic parameters of 2,5-dibromotoluene

Parameters	Method/Basis set
	B3LYP/6-311++G(d,p)
Optimized global minimum Energy (Hartrees)	-5418.723 22.656
Total energy (thermal), E_{total} (kcal mol ⁻¹)	72.883
Heat capacity, C_v (cal mol ⁻¹ k ⁻¹)	31.813
Entropy, S (cal mol ⁻¹ k ⁻¹)	96.882
Total	
Translational	42.424
Rotational	31.801
Vibrational	22.656
Vibrational energy, E_{vib} (kcal mol ⁻¹)	71.106
Zero point vibrational energy, (kcal mol ⁻¹)	67.28997
Rotational constants (GHz)	
A	2.72488
B	0.27727
C	0.25205
Dipole moment (Debye)	0.6392

Table 3 The vibrational frequencies, IR intensity, Raman Activity, force constant and reduced mass and assignment for 2,5-dibromotoluene

S.No	Observed wave number		Wave number		IR Intensity (Km mol ⁻¹)	Raman activity (Å ⁴ amu ⁻¹)	Reduced mass (amu)	Force constant (m dyne Å ⁻¹)	Assignment with PED (%)
	(cm ⁻¹)		(cm ⁻¹)						
	FT-IR	FT-Raman	Calculated	Scaled					
1	3183(ms)	-	3208	3153	0.5252	37.0500	1.0956	6.6467	v C-H (98)
2	-	3111(ms)	3194	3113	0.0641	69.5812	1.0889	6.5480	v C-H (96)
3	3112(w)	3092(w)	3191	3110	1.3207	179.9428	1.0912	6.5479	v C-H (94)
4	3098(w)	-	3115	3062	15.3875	159.3771	1.1016	6.3008	CH ₃ ips (92)
5	3053(w)	-	3086	3033	8.2471	64.7405	1.1002	6.1752	CH ₃ ops(91)
6	2997(ms)	2993(ms)	3034	2982	13.5247	38.4785	1.0364	5.6227	CH ₃ ass (92)
7	1598(ms)	1595(w)	1615	1587	8.2123	37.1757	7.1283	10.9667	v C-C (89)
8	1536(vw)	1549(w)	1587	1560	3.9110	7.7095	5.5815	8.2882	v C-C (87)
9	1488(w)	-	1499	1436	60.4355	1.0223	1.3622	1.8053	v C-C(85)
10	1496(ms)	1437(vw)	1480	1422	10.5739	7.9994	1.0427	1.3463	v C-C (82)
11	1412(ms)	1411(w)	1479	1421	24.7711	3.5069	1.7603	2.2713	CH ₃ ipb(81)
12	1381(m)	1383(vw)	1419	1395	2.3901	1.2917	1.2400	1.4727	CH ₃ opb (78)
13	1298(m)	1308(ms)	1405	1381	11.1889	3.2568	2.8658	3.3357	CH ₃ sb (79)
14	1272(m)	1283(w)	1303	1281	1.5994	1.4907	7.3055	7.3097	bC-H (75)
15	1244(s)	1240(w)	1290	1236	1.8445	1.0237	1.3574	1.3322	bC-H (72)
16	1189(vs)	1194(ms)	1222	1191	5.5414	16.3584	2.4717	2.1775	bC-H(74)
17	1126(vs)	1127(ms)	1158	1138	2.3943	0.9338	1.4429	1.1412	CH ₃ opr (75)
18	1052(vs)	-	1103	1086	31.1088	16.7348	2.1688	1.5569	v C-C (72)
19	1033(ms)	1032(vw)	1060	1042	2.1047	23.3628	1.5075	0.9981	v C-C (73)
20	1021(vw)	1019(vw)	1034	1016	117.4771	0.0402	6.1104	3.8500	v C-C (76)
21	979(w)	-	1015	998	3.4135	0.4945	1.5216	0.9253	CH ₃ ipr (72)
22	952(vs)	-	957	933	0.0872	0.3109	1.3165	0.7115	ω C-H (69)
23	893(s)	888(w)	880	865	10.1312	0.1162	1.3276	0.6066	ω C-H (65)
24	874(vs)	-	856	841	19.5831	0.0485	5.8617	2.5324	v C-Br (71)
25	752(w)	-	817	785	28.4755	0.4842	1.3231	0.5206	v C-Br (70)
26	686(vw)	-	694	682	0.6660	14.2217	4.0550	1.1498	ω C-H (66)
27	698(vs)	692(vs)	693	681	0.3562	2.5365	6.9420	1.9674	b C-C (70)
28	536(s)	-	551	528	3.2828	6.1927	5.6504	1.0111	Rtrigd (70)
29	511(vs)	517(ms)	542	521	3.3350	2.5677	3.9275	0.6798	Rasymd (72)

30	498(vw)	492(w)	462	454	4.3721	0.3134	3.7999	0.4780	Rsymd(71)
31	430(m)	427(vw)	442	434	5.9056	0.2950	2.9494	0.3402	b C-Br (69)
32	-	331(vw)	385	369	9.5558	0.3277	5.7633	0.5055	b C-Br (68)
33	-	291(w)	279	274	0.8007	1.1805	5.8751	0.2696	ω C-Br (64)
34	-	226(ms)	227	217	0.7845	0.4481	3.6913	0.1122	ω C-Br (62)
35	-	213(vs)	211	203	0.0148	7.4834	57.8216	1.5213	tRsymd (64)
36	-	194(w)	189	186	1.4578	1.1368	3.0352	0.0644	tRtrigd (63)
37	-	188(vw)	162	156	0.2437	0.1285	10.1951	0.1593	tRasymd (61)
38	-	136(vw)	135	132	0.4449	0.1957	1.0549	0.0114	ω C-C (60)
39	-	62(ms)	72	69	0.1859	0.9325	9.1894	0.0284	CH ₃ twist (58)

Table 4 Molecular orbital contributions of 2,5-dibromotoluene

TDDFT/ B3LYP/6-311++G(d,p) Method				
Energy(eV)	Oscillator strength	Wavelength (nm)	Major contributions	Assignment
4.8657	0.0209	254.81	H $\rightarrow$ L+2 (96%)	$\pi \rightarrow \pi^*$
5.3178	0.0674	233.15	H $\rightarrow$ L+3(66%)	$\pi \rightarrow \pi^*$
5.0337	0.0003	246.31	H $\rightarrow$ L (50%)	$\pi \rightarrow \pi^*$

Table 5 HOMO – LUMO and Global reactivity descriptors for 2,5-dibromotoluene

Molecular Properties	B3LYP/6-311++G(d,p)
	eV
HOMO	-6.786
LUMO	-1.124
$\Delta E(E_{\text{HOMO}} - E_{\text{LUMO}})$ a.u	5.662
Ionization potential (I)	6.786
Electron affinity (A)	1.124
Global hardness(η)	2.831
Global softness (s)	261.57
Electro negativity (χ)	3.955
Chemical potential (μ)	-3.955
Global electrophilicity (w)	2.762

Table 6 The ¹³C NMR chemical shifts with respect to TMS for 2,5-dibromotoluene

Atom	Theoretical values (ppm)	
	Chemical shielding	Chemical shift
1-C	35.39	147.07
5-C	35.41	147.15
2-C	35.79	146.64
3-C	43.69	138.77
6-C	44.55	137.91
4-C	46.53	135.92
12-C	160.07	22.39

Table 7 The ^1H chemical shifts with respect to TMS for 2,5-dibromotoluene

Atom	Theoretical values (ppm)	
	Chemical shielding	Chemical shift
11-H	24.23	7.64
8-H	24.31	7.56
9-H	24.43	7.44
13-H	29.62	2.25
14-H	30.16	1.71
15-H	30.50	1.37

Table 8 Natural bond orbital analyses for 2,5-dibromotoluene

Donor(i)	ED(i) (e)	Acceptor (j)	ED (j) (e)	Stabilization energy E(2) (kJ mol ⁻¹)	Energy difference E(j) – E(i) (a.u.)	Fock matrix element F (I,j) (a.u.)
π (C1-C2)	0.83259	π^* (C3-C4)	0.16176	10.69	0.29	0.071
π (C1-C2)	0.83259	π^* (C5-C6)	0.19484	9.27	0.28	0.065
π (C3-C4)	0.83527	π^* (C1-C2)	0.19791	9.03	0.28	0.064
π (C3-C4)	0.83527	π^* (C5-C6)	0.19484	10.23	0.27	0.067
π (C5-C6)	0.84199	π^* (C1-C2)	0.19791	10.48	0.29	0.071
π (C5-C6)	0.84199	π^* (C3-C4)	0.16176	9.69	0.29	0.067
σ (C1-C6)	0.97925	σ^* (C1-C2)	0.01698	2.29	1.27	0.068
σ (C1-C6)	0.97925	σ^* (C2-Br7)	0.01771	2.61	0.8	0.058
σ (C1-C6)	0.97925	σ^* (C5-C6)	0.01301	2.04	1.26	0.064
σ (C1-C6)	0.97925	σ^* (C5-Br10)	0.01667	2.56	0.8	0.057
σ (C1-C12)	0.98896	σ^* (C2-C3)	0.01239	1.68	1.16	0.056
σ (C1-C12)	0.98896	σ^* (C5-C6)	0.01301	1.39	1.16	0.051
σ (C2-C3)	0.98956	σ^* (C1-C2)	0.01698	1.92	1.3	0.063
σ (C2-C3)	0.98956	σ^* (C1-C12)	0.00825	1.8	1.11	0.056
σ (C3-C4)	0.98074	σ^* (C2-C3)	0.01239	1.84	1.27	0.061
σ (C3-C4)	0.98074	σ^* (C4-C5)	0.01305	1.84	1.27	0.061
σ (C3-H8)	0.98907	σ^* (C1-C2)	0.01698	2.00	1.09	0.059
σ (C4-H9)	0.98880	σ^* (C5-C6)	0.01301	2.14	1.07	0.061
σ (C5-C6)	0.98931	σ^* (C1-C6)	0.01241	1.8	1.3	0.061
σ (C5-C6)	0.98931	σ^* (C1-C12)	0.00825	1.55	1.11	0.052
σ (C5-C6)	0.98931	σ^* (C4-C5)	0.01305	1.55	1.29	0.056
σ (C5-	0.99260	σ^* (C1-	0.01241	1.3	1.22	0.051

Br10)		C6)				
σ (C5-Br10)	0.99260	σ^* (C3-C4)	0.00914	1.38	1.22	0.052
σ (C6-H11)	0.98858	σ^* (C1-C2)	0.01698	2.13	1.08	0.061
σ (C6-H11)	0.98858	σ^* (C4-C5)	0.01305	2.06	1.08	0.06
σ (C12-H13)	0.99261	σ^* (C1-C6)	0.01241	4.85	1.08	0.053
σ (C12-H14)	0.98825	π^* (C1-C2)	0.19791	2.08	0.53	0.046

Table 9 Mulliken's charge of 2,5-dibromotoluene

Atom	Mulliken's atomic charges
	B3LYP/6-311++G(d,p)
C1	0.494
C2	0.074
C3	-0.281
C4	0.041
C5	0.021
C6	-0.654
Br7	-0.182
H8	0.209
H9	0.213
Br10	-0.184
H11	0.204
C12	-0.448
H13	0.173
H14	0.173
H15	0.145

Table 10 Docking calculation showing interacting residues, binding residues involved in H-bonding to reported active sites

S.NO	Protein ID	Binding energy	Ligand efficiency	Inhibit constant	Internal efficiency	Interacted Residues	Ligand and Protein atom involved in H-bonding
1	1A06	-5.0	-0.39	25.89	-5.5	HIS 288,LEU 203, LYS 281, VAL 433,LEU 284,ASN429,LEU 430	LEU 155,LEU 154,GLU 17
2	5Z0B	-5.4	-0.41	29.75	-5.9	SER, THR, TYR, LYS, ARG, TRP, HIS	SER, THR, TYR

Figures

Figure 1

Optimized structure of 2,5-dibromotoluene

Figure 2

FT-IR plot for 2,5-dibromotoluene

Figure 3

FT-Raman plot for 2,5-dibromotoluene

Figure 4

UV plot for 2,5-dibromotoluene

Figure 5

Frontier molecular orbitals of 2,5-dibromotoluene

Figure 6

^{13}C NMR plot for 2,5-dibromotoluene

Figure 7

^1H NMR for 2,5-dibromotoluene

Figure 8

MEP plot for 2,5-dibromotoluene

Figure 9

Mullikan charges for 2,5-dibromotoluene

Figure 10

Protein 1A06 interactions with ligand DBT

Figure 11

Protein 5Z0B interactions with ligand DBT