

SPECTROSCOPIC, ELECTRONIC PROPERTIES, REACTIVITY ANALYSES AND MOLECULAR DOCKING STUDIES OF THE BIOLOGICAL APPLICATION OF 4-NITROPHENYL CHLOROFORMATE ON LUNG CANCER TARGETS

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ABSTRACT

The 4-Nitrophenyl chloroformate(4NPCM) is a significant activated chloroformate compound with potential applications in synthesis of organic compounds and chemical biology. The present work is a combined experimental and theoretical study of 4-nitrophenyl chloroformate. The potential-energy surface was explored and the most stable molecular conformation was found using the optimized geometry obtained at the B3LYP/6-311++G(d,p) level of density functional theory. The vibrational properties of the compound were studied by experimental Fourier-transform infrared and Fourier-transform Raman spectroscopy. The observed spectral bands were assigned with the help of calculated vibrational frequencies, infrared intensities and Raman activities. Experimental and theoretical studies of the UV-Vis spectra of 4NPCM in different solvent media were carried out to study its electronic absorption properties. The frontier molecular orbital analysis was conducted to calculate the energy gap between the highest occupied and lowest unoccupied molecular orbitals, and to determine the chemical stability and reactivity of the compound. Additionally, the molecular electrostatic potential analysis was carried out to identify the reactive sites involved in possible intermolecular interactions. Moreover, molecular docking studies were performed against the selected lung cancer-related protein targets to study the binding mode and key ligand-protein interactions. The results give a detailed insight into the structure, vibrations, electronic, solvent dependent and molecular interaction properties of 4-nitrophenyl chloroformate, and support its further study in chemical and bio-molecular research.

KEYWORDS: DFT; Spectroscopic; HOMO-LUMO; MEP; Molecular docking; Lung cancer;

1. INTRODUCTION

Chloroformate esters represent an essential class of synthetic intermediates, extensively employed as acylating agents in synthesis of organic compounds and play a key part in the construction of carbamates, carbonates, and peptide-coupling framework architectures. Of these reagents 4-nitrophenyl chloroformate (4NPCM) is of particular importance due to the strongly electron withdrawing para nitro substituent. The strong electronic effect increases the electrophilicity of the carbonyl carbon and makes the 4-nitrophenolate group a good leaving group in nucleophilic acyl substitution reactions. Thus, 4NPCM is widely used to activate hydroxyl and amino functional groups for the preparation of prodrugs, polymer-drug conjugates and biologically active derivatives, and also as a key coupling agent in nucleoside and peptide chemistry.

Although 4-nitrophenyl chloroformate is routinely used as a synthetic reagent, there is no systematic and consolidated study on its structural, electronic and vibrational properties in the literature. A detailed knowledge of these basic properties is essential both to explain its chemical reactivity and to predict its behaviour in different media. In the present study, the FT-IR, FT-Raman and UV-Visible spectroscopic techniques were systematically studied for 4NPCM. Vibrational modes follow complementary selection rules in infrared and Raman scattering and the combination of FT-IR and FT-Raman spectroscopes gives a complete spectral fingerprint. This combination enables unambiguous assignments of important diagnostic modes including carbonyl (C=O), carbon-chlorine (C-Cl), nitro -NO₂, and aromatic ring vibrations, together with quantum chemical modelling [1,2].

In recent years, several computational and experimental investigations have reported the structural, vibrational and electronic properties of related nitrophenyl and carbonyl-substituted derivatives. For example, Szafran et al.[3] performed detailed FT-IR, FT-Raman and DFT investigations on nitrophenyl esters, showing that the

strong electron withdrawing character of the para-nitro group greatly enhances the carbonyl stretching frequency (C=O) and modifies the surface charge topology over the benzene ring. Likewise, Subashchandrabose et al.[4] and Govindasamy et al.[5] investigated different nitro substituted aromatic systems using B3LYP calculations and PED analysis and reported characteristic asymmetric (1520-1540 cm^{-1}) and symmetric (1330-1360 cm^{-1}) nitro stretching modes, which are in close agreement with the vibrational signatures observed in 4-nitrophenyl chloroformate.

Density functional theory (DFT) has become a standard and computationally feasible method to relate experimental spectroscopic observations to theoretical electronic structure models [6, 7]. In this work, the optimised ground-state geometry of 4NPCM was calculated by using DFT calculations and its vibrational spectra were simulated, which helped to assign the normal modes quantitatively by Potential Energy Distribution (PED) analysis. A potential energy surface scan was performed to locate the global minimum conformer and to investigate the rotational barriers about the flexible C–O and C–N torsional coordinates.

To provide insight into the electronic architecture governing molecular reactivity, frontier molecular orbital (FMO) analysis was performed to compute the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), energy band gap (ΔE) and derived conceptual DFT parameters (chemical hardness, softness and global reactivity indices) [1, 6, 8]. The energy gap is a crucial metric of the intramolecular charge transfer (ICT) and kinetic stability. Molecular electrostatic potential (MEP) mapping was performed to determine the localised electrophilic and nucleophilic regions on the surface [6, 9]. Additionally, condensed Fukui functions and local dual descriptors (Δr) were calculated to better identify nucleophilic and electrophilic attack sites in space [1].

Finally, considering the biological importance of nitrophenyl derivatives as enzyme inhibitors, chemical biology and drug delivery platforms [8,10], computational molecular docking simulations were conducted to investigate the binding affinities, active site orientations and non-covalent interaction networks of 4NPCM against selected cancer-related target proteins. The full structural, electronic and computational characterisation of 4-nitrophenyl chloroformate has been carried out by FT-IR, FT-Raman and UV-Vis spectroscopies in combination with DFT-based geometry optimisation, PES analysis, FMO/MEP mapping, dual descriptor calculations, NBO analysis and molecular docking.

2.0 METHODS AND MATERIALS

2.1 Experimental

The title compound, 4-nitrophenyl chloroformate (4NPCM) was commercially available from Avara Synthesis and used directly in the characterisation studies without further purification steps. Vibrational and electronic spectroscopic measurements were collected using standard analytical instrumentation. The Fourier-transform infrared (FT-IR) spectrum was acquired over the region of 4000-400 cm^{-1} at the Government College of Engineering, Bargur. The Fourier-transform Raman (FT-Raman) spectrum was recorded in the range of 4000-50 cm^{-1} at Jamal Mohamed College, Tiruchirappalli. Additionally, the experimental UV-Visible absorption spectrum of 4NPCM was measured in the range of 200-600 nm using chloroform as the solvent at the Government College of Engineering, Bargur.

2.2. Theoretical

All quantum chemical calculations for 4-nitrophenyl chloroformate (4NPCM) were performed with the Gaussian 09W [11] software suite, using GaussView 5.0 [12] for molecular modeling and visualization. A preliminary potential energy surface (PES) scan was executed by systematically varying two flexible dihedrals SC1 (C8–O2–C13–C11) and SC2 (O3–N6–C7–C10) in 36 degree increments to pinpoint the ground-state global minimum conformation. Geometry optimization of the minimum-energy structure was subsequently carried out without symmetry constraints using Density Functional Theory (DFT) with the B3LYP functional and the 6-311++G(d,p) basis set [13, 14]. Harmonic vibrational frequencies were calculated and found to have no imaginary frequencies and were scaled by a factor of 0.961 to take into account anharmonicity. Normal mode assignments were quantitatively assessed by Potential Energy Distribution (PED) analysis using VEDA4 [15]. The like Polarizable Continuum Model (PCM) was used for solvent effects (Chloroform, Water, DMSO and Acetone) [16]. The Time-Dependent DFT with M06-2X functional [17] with the 6-311++G(d,p) basis set was used to simulate the vertical electronic excitation energies, absorption maxima (λ_{max}), oscillator strengths and frontier molecular orbital (FMO) parameters. Reactivity descriptors based on the conceptual DFT, Fukui functions and local dual descriptors, have been obtained from the Mulliken population charges of the neutral and ionised states. Finally, target receptor binding was studied with AutoDock [18] by adding Gasteiger charges and polar hydrogens and performing 100 Lamarckian Genetic Algorithm (LGA) conformational searches to calculate the hydrogen-bonding distances and binding energies against target anticancer proteins 1X2J and 5ZSY.

3.0 RESULTS AND DISCUSSION

3.1. Potential Energy Surface (PES) Scan and Geometrical Analysis

To determine the most stable molecular conformation of 4-nitrophenyl chloroformate (4NPCM), a potential energy surface (PES) scan was carried out in Gaussian 09W by systematically scanning two important dihedral angles, SC1 (C8–O2–C13–C11) and SC2 (O3–N6–C7–C10). The obtained energy surface shows that the global

minimum conformation corresponds to dihedral values $SC1 = 179.99^\circ$ and $SC2 = 0.08^\circ$, with a total optimised energy of -1080.6765 Hartree (Fig. 1). This suggests a near planar arrangement of the phenyl ring, the nitro group and the chloroformate moiety in its ground state.

The optimised structural parameters computed at the DFT/B3LYP/6-311++G(d,p) level and the comparative related experimental single-crystal XRD data[19] are presented in Table 1 and Fig. 2. The calculated distance for the carbonyl bond (C13=O5) is 1.184 Å, which is in good agreement with the experimental value of 1.208 Å. The theoretical values of C13–O2 (1.346 Å) and C8–O2 (1.407 Å) for the ester-linkage framework are consistent with the XRD findings of 1.340 and 1.432 Å, respectively. The bond length between the nitro group and the aromatic ring (N6–C7) is predicted to be 1.481 Å, which is in good agreement with the experimental value of 1.470 Å.

The calculated C8–O2–C13 bond angle of 117.1° is comparable to the crystallographic value of 116.6° in terms of angular geometry. The angle of carbonyl region (O2–C13–O5) is calculated as 127.8° , while it is 123.5° experimentally. In addition, the aromatic carbon backbone exhibits the typical C–C bond lengths of 1.389–1.391 Å theoretically and 1.385–1.398 Å experimentally, indicating that the selected computational level can describe the structural features of 4NPCM accurately.

3.2. Vibrational Spectral Analysis (FT-IR and FT-Raman)

4-Nitrophenyl chloroformate (4NPCM) has been characterized in terms of its structural and functional features by vibrational spectral analysis. The experimental Fourier-transform infrared (FT-IR) and FT-Raman spectra have been interpreted and compared with theoretical harmonic calculations. The vibrational frequencies calculated at B3LYP/6-311+G(d,p) level have been scaled by a factor of 0.961 to take into account the anharmonicity, electron correlation and basis set limitations. Quantitative assignments of normal modes were carried out using Veda4 software on the basis of computed Potential Energy Distribution (PED) percentages. The comparative experimental and calculated FT-IR and FT-Raman spectra are presented in Fig. 3 and Fig. 4, respectively. The detailed experimental frequencies, scaled theoretical values, relative intensities and PED assignments are compiled in Table 2.

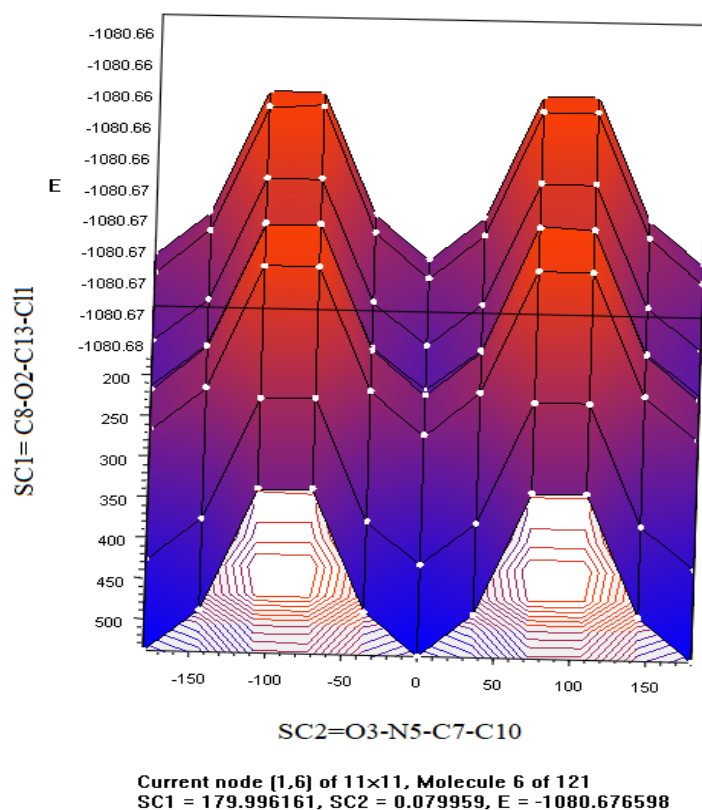


Fig. 1. Potential energy surface (PES) scan of the title molecule as a function of the chosen dihedral angles $SC1 = C8-O2-C13-Cl1$ and $SC2 = O3-N5-C7-C10$.

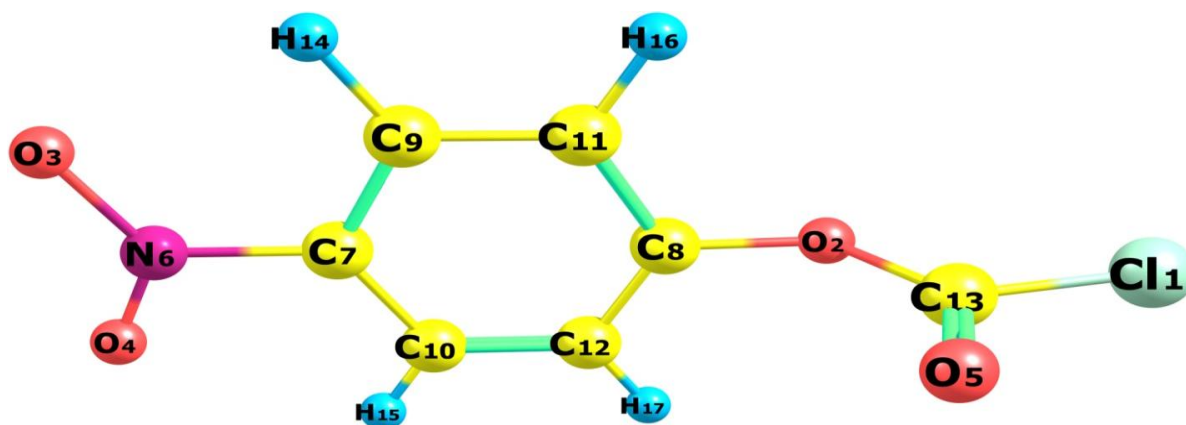


Fig. 2. Optimised molecular arrangement and atom labelling of 4-Nitrophenyl chloroformate (4NPCM) calculated at B3LYP/6-311++G(d,p) level of theory.

Table 1. Geometric parameters of 4NPCM molecule

Bond Length (Å)			Bond angle(degree)		
Bond	Theoretical	XRD ^a	Bond	Theoretical	XRD ^a
C11-C13	1.765	-	C11-C13-O2	108.2	-
O2-C8	1.407	1.432	C11-C13-O5	124.0	-
O2-C13	1.346	1.340	C8-O2-C13	117.1	116.6
O3-N6	1.224	1.226	O2-C8-C11	118.8	120.6
O4-N6	1.224	1.227	O2-C8-C12	118.8	120.3
O5-C13	1.184	1.208	O2-C13-O5	127.8	123.5
N6-C7	1.481	1.470	O3-N6-O4	125.0	123.9
C7-C9	1.391	1.385	O3-N6-C7	117.5	117.5
C7-C10	1.391	1.398	O4-N6-C7	117.5	117.5
C8-C11	1.389	1.385	N6-C7-C9	118.8	118.4
C8-C12	1.389	1.390	N6-C7-C10	118.8	118.54
C9-C11	1.39	1.398	C9-C7-C10	122.3	122.5
C9-H14	1.081	0.950	C7-C9-C11	118.8	118.4
C10-C12	1.39	1.385	C7-C9-H14	119.8	120.0
C10-H15	1.081	0.990	C7-C10-C12	118.8	119.7
C11-H16	1.082	0.990	C7-C10-H15	119.8	121.0
C12-H17	1.082	0.990	C11-C8-C12	122.4	121.0
			C8-C11-C9	118.9	121.0
			C8-C11-H16	120.1	120.0
			C8-C12-C10	118.9	120.0
			C8-C12-H17	120.1	121.0
			C11-C9-H14	121.5	121.0.
			C9-C11-H16	121.0	121.0
			C12-C10-H15	121.5	121.0
			C10-C12-H17	121.0	121.0

a- Reference taken from [19]

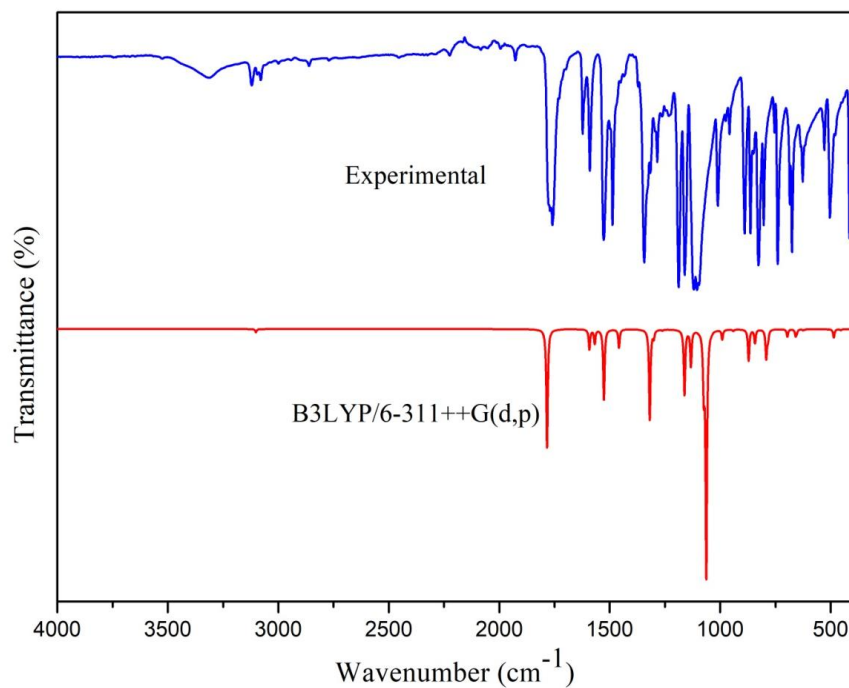


Fig. 3. Comparison of the experimental FT-IR spectrum with the theoretical FT-IR spectrum calculated at the B3LYP/6-311++G(d,p) level.

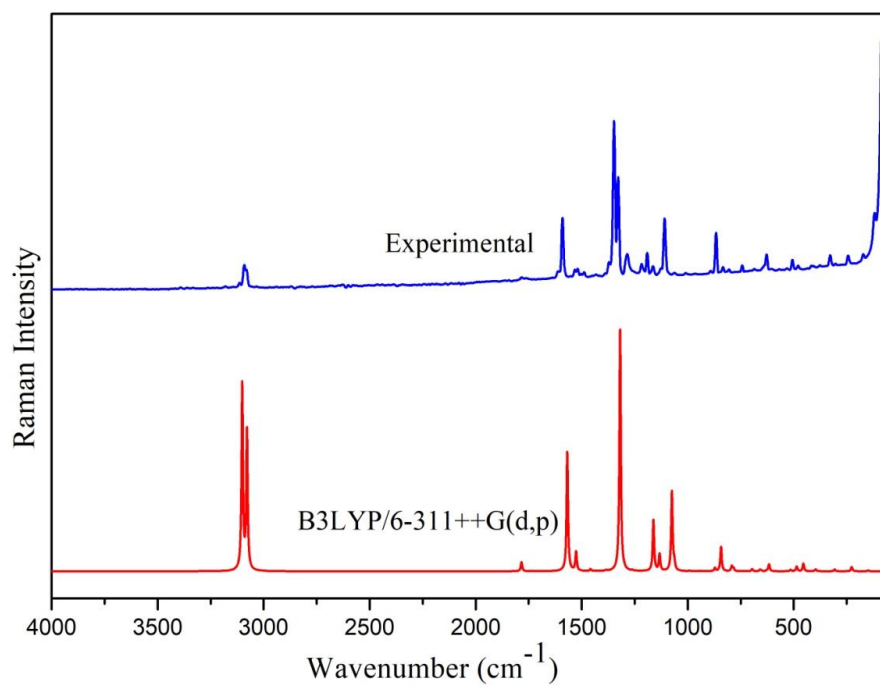


Fig. 4. Comparison of experimental FT-Raman spectrum with theoretical FT-Raman spectrum calculated at B3LYP/6-311++G(d,p) level.

Table 2. FT-IR, FT-Raman and PED analyses of 4NPCM molecule

Mod e No	Experimental Wavenumber (cm ⁻¹)		Theoretical Wavenumber (cm ⁻¹)		I _{IR} ^c	I _{RAMAN} ^d	Assignments (PED) ^a
	FTIR	FT-Raman	Unscaled	Scaled ^b			
45	3125	3113(w)	3227	3101	1	71	αCH(92)
44	3097		3227	3101	1	6	αCH(94)
43	3983	3092(m)	3204	3079	0	27	αCH(94)
42	-	3082(m)	3203	3078	0	31	αCH(52)
41	1773	1783(w)	1856	1784	49	4	αOC(94)
40	1618(vs)	1592(s)	1657	1592	8	0	αCC(50)
39	1589(vs)	1587(s)	1632	1568	6	49	αCC(54)
38	1526(vs)	1532(m)	1588	1527	29	8	αON(78)
37	1491(vs)	1521(m)	1517	1458	8	1	β HCC(66)
36	1342(w)	1370(w)	1442	1385	0	0	αCC(34)
35	1342(vs)	1347(vs)	1373	1319	37	100	αON(82)+β ONO(12)
34	1318(m)	1327(vs)	1353	1301	3	0	αCC(84)
33	1282(m)	1682(m)	1313	1262	0	0	β HCC(84)
32	1155(vs)	1163(w)	1209	1162	27	21	αOC(38)+β HCC(20)+αCC(26)
31	1120(vs)	1111(s)	1179	1133	15	7	β HCC(48)
30	1109(vs)	1060(w)	1121	1077	1	0	β HCC(71)+αCC(26)
29	1092(vs)		1118	1075	22	33	αNC(20)+β CCC(26)
28	1014(m)		1106	1063	100	3	αOC(66)+β OCCl(13)
27	979(m)	1010(w)	1031	991	4	0	β CCC(87)
26	961(m)		994	955	0	0	tHCCC(88)
25	-		979	941	1	0	tCCCC(16)+tHCCC(68)
24	981(s)	867(s)	907	871	13	1	β COC(13)+OUTOCCC(17)
23	862(s)	832(m)	877	843	6	10	β ONO(38)+β CCC(12)
22	803(s)	804(m)	836	803	0	0	tHCCC(100)
21	-		824	792	12	2	β OCCl(10)+tHCCC(30)
20	742(vs)	741(m)	816	784	4	1	αCC(25)+αOC(-19)+β ONO(24)
19	682(s)	685(w)	724	696	3	1	OUTOCON(68)
18	-		685	659	3	1	tCCCC(54)+OUTOCON(12)
17	-		680	654	1	0	OUTOOCIC(94)
16	623(m)	628(s)	651	625	0	0	αNC(14)+β ONO(12)+β CCC(25)
15	622(w)		641	616	0	3	β CCC(63)
14	527(m)	507(m)	536	515	0	1	β ONC(57)+β NCC(13)
13	499(S)	478(m)	506	486	4	2	αClC(16)+tCCCC(31)+OUTOCC(22)
12	-		473	454	0	3	αClC(40)+β OCCl(41)+tCCCC(92)
11	410(s)		423	407	0	0	tCCCC(92)
10	-		414	398	1	0	β ClCO(13)+αNC(32)+β OCCl(15)+β CCC(11)
9	-		411	395	0	1	β OCC(58)+β ONC(15)
8	-	328(m)	319	307	0	1	OUTNCCC(38)+β COC(12)+β ClCO(-10)+tCCCC(13)
7	-	243(m)	236	227	0	2	αOC(17)+β ClCO(36)
6	-		231	222	0	0	β NCC(63)+β ONC(-18)
5	-	117(m)	155	149	1	0	OUTNCCC(-23)+β

							COC(34)+tCCCC(-14)
4	-	85(s)	56	53	0	0	OUTNCCC(45)+ β COC(22)+tCCCC(18)
3	-	63(s)	53	51	0	0	tClCOC(84)+ β OCC(10)
2	-	-	50	48	0	0	tONCC(95)
1	-	-	26	25	0	2	tCOCC(91)

^a α -stretching, α_a -Symmetrical stretching, α_a -asymmetrical stretching, β -bending, T-torsion, vs-very strong, s-strong, m-medium, w-weak, vw-very weak.

^bscaling factor : 0.961 for B3LYP/6-311+G(d,p)

^cRelative absorption intensities normalized with highest peak absorption equal to 100.

^dRelative Raman intensities normalized to 100.

(a) Aromatic C–H Vibrations

Aromatic ring C–H stretching modes typically appear in the region 3000–3150 cm⁻¹ [20]. For 4NPCM, the computed C–H stretching modes (Modes 42–45) span 3078–3101 cm⁻¹ with PED contributions exceeding 90%. Experimentally, these modes register as a weak band at 3125 cm⁻¹ in FT-IR and distinct peaks at 3113, 3092, and 3082 cm⁻¹ in the FT-Raman spectrum. The in-plane C-H bending vibrations (β HCC) are assigned in the 1077–1458 cm⁻¹ range (Modes 30–33 and 37) to the sharp FT-IR bands at 1109, 1120, 1155, 1282, 1492 cm⁻¹ and the Raman bands at 1521 cm⁻¹ - 1060 cm⁻¹. The low wavenumber region (792–955 cm⁻¹ (Modes 21–26) is mainly occupied by out-of-plane C–H deformations, which are well correlated with experimental bands around 803–961 cm⁻¹.

(b) Carbonyl (C=O) Group Vibrations

The carbonyl stretching vibration (α OC) is an important diagnostic band, because it is highly infrared active due to the large dipole moment change during vibration [20]. In 4NPCM, Mode 41 is pure carbonyl stretching (94% PED). The theoretically scaled frequency at 1784 cm⁻¹ is in good agreement with the strong band observed at 1773 cm⁻¹ in FT-IR and 1783 cm⁻¹ in FT-Raman. The increase in frequency compared to normal aliphatic ketones is due to the electronegative chlorine atom and the oxygen atom of the chloroformate group, which withdraw electron density and strengthen the carbonyl bond.

(c) Nitro (-NO₂) Group Modes

Nitro group vibrations have strong signatures in FT-IR and FT-Raman spectra due to strong resonance and inductive interactions with the aromatic ring [20–22]. The asymmetric nitro stretching vibration (α ON) is assigned to Mode 38 (78% PED), predicted at 1527 cm⁻¹ and observed at 1526 cm⁻¹ in FT-IR and 1532 cm⁻¹ in Raman. The symmetric stretching mode (α ON) appears as a very strong band at 1342 cm⁻¹ in FT-IR and 1347 cm⁻¹ in FT-Raman, matching the theoretical mode at 1319 cm⁻¹ (Mode 35, 82% PED). The nitro group bending (β ONO) and deformation modes contribute to lower-frequency vibrations around 832–862 cm⁻¹ (Mode 23, 38% PED).

(d) Aromatic Ring C=C and Linkage Vibrations

Aromatic ring skeletal stretching modes (α CC) are identified at 1618 cm⁻¹ and 1589 cm⁻¹ in FT-IR, and at 1592 cm⁻¹ and 1587 cm⁻¹ in FT-Raman. These observed bands correspond closely to calculated frequencies at 1592 cm⁻¹ (Mode 40, 50% PED) and 1568 cm⁻¹ (Mode 39, 54% PED). The C–O ester bond stretching mode (α OC) contributes heavily to Mode 28 (66% PED), calculated at 1063 cm⁻¹ and observed as a strong FT-IR peak at 1014 cm⁻¹. Lastly, C–Cl stretching vibrations (α ClC) appear at lower frequencies (Modes 12 and 13), corresponding to experimental FT-IR bands at 499 cm⁻¹ and 410 cm⁻¹.

3.3. UV-Visible Spectral and Frontier Molecular Orbital Analysis

The electronic absorption characteristics and solvatochromic behavior of 4-nitrophenyl chloroformate (4NPCM) were investigated experimentally in chloroform and simulated computationally across four media of varying polarity (Chloroform, Water, DMSO, and Acetone) using time-dependent density functional theory (TD-DFT) at the M062X/6-311++G(d,p) level (Fig. 5). In chloroform, the experimental spectrum displays a dominant absorption peak at 263 nm (4.7267 eV) alongside a secondary shoulder at 306 nm (4.0625 eV), which closely matches the calculated transitions at 271 nm ($f = 0.0280$, 92% HOMO→LUMO and 297 nm ($f = 0.0004$, 89% H-5→LUMO), respectively (Table 3). The change to polar solvents (Water, DMSO and Acetone) leads to a small bathochromic (red) shift of the main HOMO→LUMO excitation to 275–276 nm ($f = 0.0270 - 0.0285$) with excitation energies of 4.5041–4.5205 eV and confirms the stabilisation of the excited state in polar media.

The analysis of the frontier molecular orbitals (FMO) shows the HOMO probability density on the central aromatic ring and the LUMO density on the whole conjugated framework between the nitro (-NO₂) and chloroformate (OCOCl) groups (Fig. 6). The estimated energy band gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$) is decreased from 4.6145 eV in chloroform ($E_{\text{HOMO}} = -8.0989$ eV, $E_{\text{LUMO}} = -3.4844$ eV) to 4.5101–4.5307 eV in polar solvents which confirms the improved intramolecular charge transfer (ICT) and increased electronic reactivity in dielectric media and data are compiled in Table 4. The derived conceptual DFT parameters reveal a high chemical hardness ($\eta = 2.2551$ – 2.3073 eV) with a strong electrophilicity index ($\omega = 7.2691$ – 7.3774 and electron accepting capability ($\omega^+ = 4.6617$ – 4.7751) (Table 4). This strong electrophilic character is caused by

synergistic electron-withdrawing effects of the substituents which promote active-site receptor binding in molecular docking.

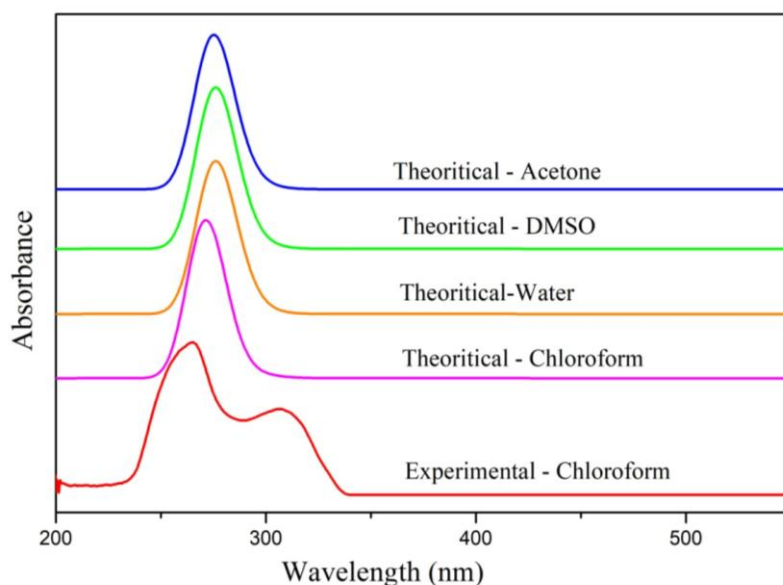


Fig. 5. Experimental UV–Vis absorption spectrum in chloroform compared with theoretical spectra calculated in different solvent media (chloroform, water, DMSO and acetone) using the TD-DFT/B3LYP/6-311++G(d,p) method.

Table 3. UV-Visible analyses of title molecule using different solvent effects.

Solvents	Experimental		Theoretical				Major contributions
	λ_{max} (nm)	Band gap(eV)	Wavelength(λ_{max}) (nm)	Band gap(eV)	Osc. Strength(f)	Energy (cm^{-1})	
Chloroform	-	-	362	3.4340	0	27638	H-3->LUMO (88%)
	306	4.0625	297	4.1856	0.0004	33669	H-5->LUMO (89%)
	263	4.7267	271	4.5872	0.0280	36895	HOMO->LUMO (92%)
Water			358	3.4724	0	27971	H-3->LUMO (87%)
			294	4.2283	0.0004	34011	H-6->LUMO (90%)
			276	4.5041	0.0270	36266	HOMO->LUMO (93%)
DMSO			358	3.4724	0	27952	H-3->LUMO (87%)
			294	4.2283	0.0004	33989	H-6->LUMO (89%)
			276	4.5041	0.0285	36270	HOMO->LUMO (94%)
Acetone			358	3.4724	0	27904	H-3->LUMO (87%)
			295	4.2140	0.0004	33942	H-5->LUMO (88%)
			275	4.5205	0.0273	36390	HOMO->LUMO (93%)

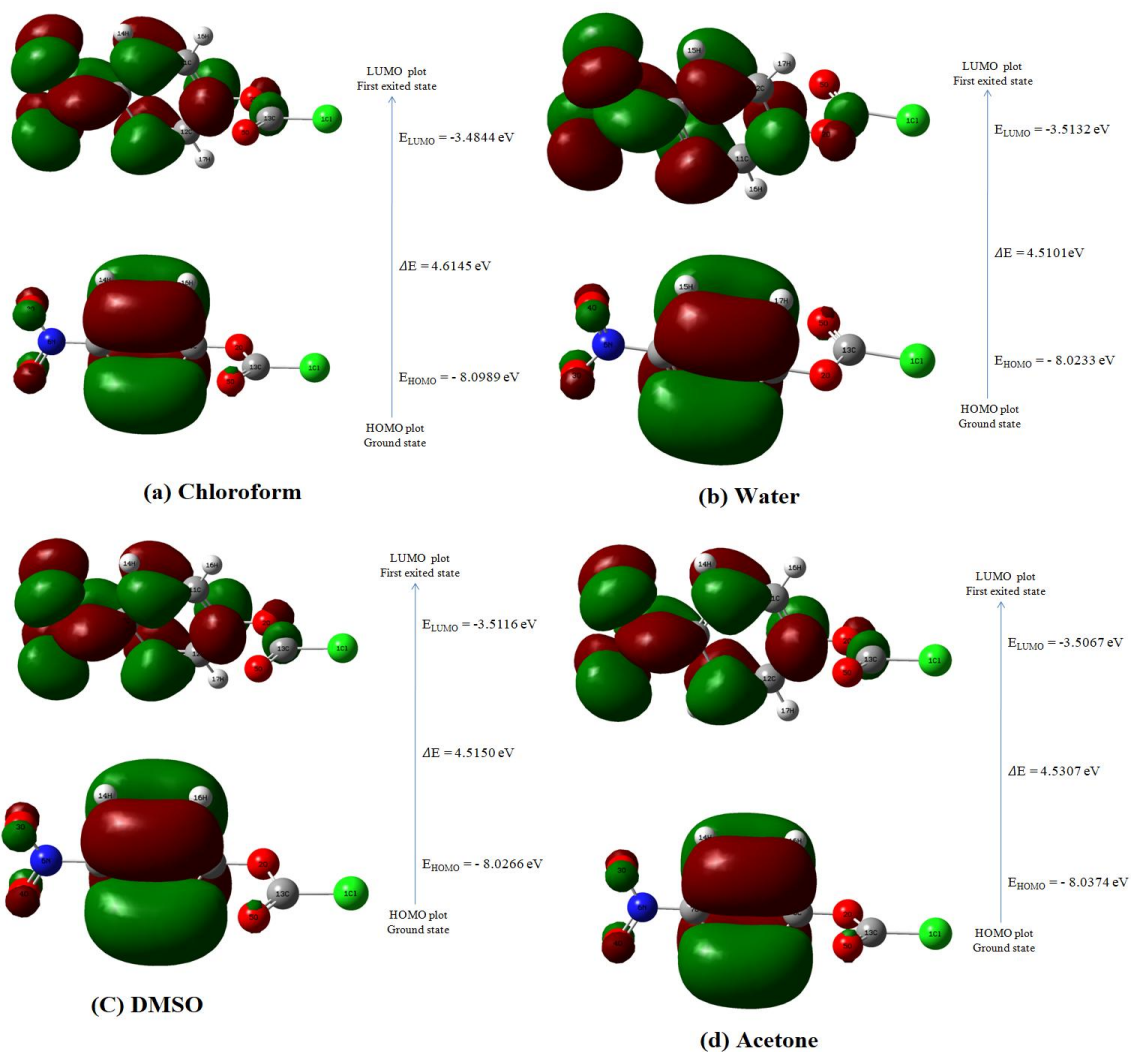


Fig. 6. Isosurfaces of the frontier molecular orbitals (HOMO–LUMO) and the corresponding values of energy gap calculated in different solvent media: (a) Chloroform, (b) Water, (c) DMSO and (d) Acetone.

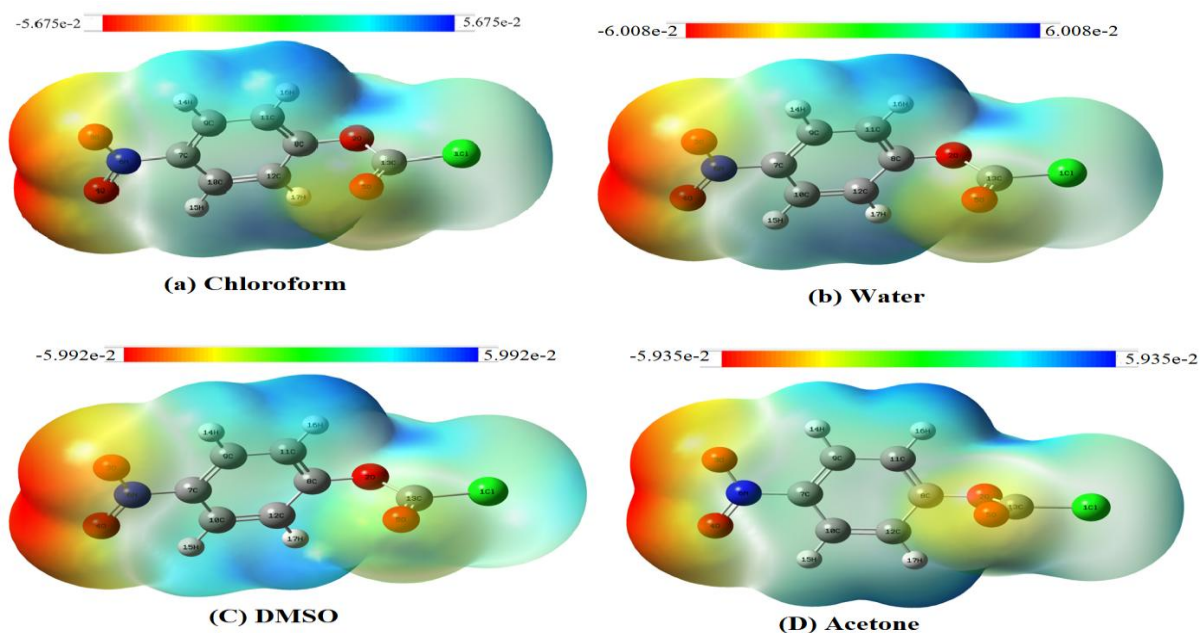


Fig. 7. Molecular electrostatic potential (MEP) surfaces mapped for the title compound in solvent media: (a) chloroform, (b) water, (c) DMSO and (d) acetone.

Table 4. Chemical parameters of 4NPCM using different solvent media.

Parameters	B3LYP/6-311++G(d,p)			
	Chloroform	Water	DMSO	Acetone
E _{Homo} (eV)	-8.0989	-8.0233	-8.0266	-8.0374
E _{Lumo} (eV)	-3.4844	-3.5132	-3.5116	-3.5067
Ionization potential(eV)	8.0989	8.0233	8.0266	8.0374
Electron affinity(eV)	3.4844	3.5132	3.5116	3.5067
Energy gap(eV)	4.6145	4.5101	4.5150	4.5307
Electronegativity(eV)	5.7917	5.7683	5.7691	5.7721
Chemical potential(eV)	-5.7917	-5.7683	-5.7691	-5.7721
Chemical hardness(eV)	2.3073	2.2551	2.2575	2.2654
Chemical softness(eV ⁻¹)	0.2167	0.2217	0.2215	0.2207
Electrophilicity index(eV)	7.2691	7.3774	7.3715	7.3535
Electronic charge	2.5102	2.5579	2.5555	2.5480
Electron donating capability (w-)	10.4533	10.5434	10.5383	10.5227
Electron accepting capability (w+)	4.6617	4.7751	4.7692	4.7507

3.4 MEP analyses

The Molecular Electrostatic Potential (MEP) mapping of 4-nitrophenyl chloroformate (4NPCM) was carried out at B3LYP/6-311++G(d,p) level in four solvent environments (Chloroform, Water, DMSO and Acetone) to locate the active electronic regions and to interpret its non-covalent binding behaviour in the biological systems [23, 24] (Fig. 7). The resulting electrostatic surface shows clearly defined electronegative regions (coloured in red and orange) around the nitro group oxygen atoms (O3, O4) and the carbonyl oxygen (O5). The electron-rich pockets are the preferred binding sites for electrophilic species and act as the main hydrogen-bond acceptor upon target binding. Electropositive regions (blue–cyan shading) are prominent in the aromatic ring hydrogens (H14–H17) and electron-deficient carbonyl carbon (C13). The positive potential is due to the strong electron-withdrawing nature of the nitro and chloroformate groups, making these sites perfect targets for the incoming nucleophiles.

3.5 Fukui functions and dual descriptors (Δr) analyses

The Fukui functions and dual descriptors (Δr) provide clear information about the site selective chemical reactivity of 4NPCM. The local dual descriptor parameter (Δr) is a powerful tool to identify the electropositive centers prone to nucleophilic attack and the electron-rich regions that are favoured by the incoming electrophiles [25-26].

As shown in Table 5, the most significant nucleophilic attack sites ($\Delta r > 0$) are the oxygen atoms O2 ($\Delta r = +0.6090$), O5 ($\Delta r = +0.5405$), O4 ($\Delta r = +0.4425$), and O3 ($\Delta r = +0.4420$), as well as the aromatic carbons C11/C12 ($\Delta r = +0.2610$) and C7 ($\Delta r = +0.2574$). High positive values of the dual descriptor on oxygen atoms show their ability to interact nucleophilically and serve as main hydrogen bond acceptors during target receptor binding.

Conversely, the highest reactive sites for electrophilic attack ($\Delta r < 0$) are at the carbonyl carbon C13 ($\Delta r = -0.7059$), the nitro nitrogen N6 ($\Delta r = -0.4416$), and the ring protons H14/H15 ($\Delta r = -0.2398$), and H16/H17 ($\Delta r = -0.2252$). The strong negative dual descriptor at C13 indicates that the carbonyl carbon of the chloroformate moiety is highly electrophilic, which is due to the combined electron-withdrawing effect of the attached oxygen and chlorine atoms. These numerical trends are directly correlated with the surface charge distribution as presented in the MEP maps and explain the main non-covalent interactions observed in molecular docking.

Table 5. Fukui functions and Dual descriptors of 4NPCM molecule

Atoms	Mulliken atomic charges			Fukui functions			dela r
	0, 1 (N)	N +1(-1, 2)	N-1(1,2)	fr+	fr ⁻	fr ⁰	
C11	0.0534	-0.0032	0.0741	-0.0565	-0.0208	-0.0386	-0.0357
O2	-0.5474	-0.2736	-0.2122	0.2738	-0.3352	-0.0307	0.6090
O3	-0.3780	-0.1925	-0.1215	0.1855	-0.2565	-0.0355	0.4420
O4	-0.3779	-0.1925	-0.1209	0.1855	-0.2570	-0.0358	0.4425
O5	-0.5307	-0.2760	-0.2449	0.2547	-0.2858	-0.0155	0.5405
N6	0.4603	0.2873	0.1917	-0.1730	0.2686	0.0478	-0.4416
C7	0.0601	0.0524	0.3252	-0.0077	-0.2651	-0.1364	0.2574
C8	0.3216	0.1905	0.4180	-0.1311	-0.0964	-0.1137	-0.0347
C9	-0.1508	-0.0557	-0.0787	0.0951	-0.0721	0.0115	0.1672

C10	-0.1508	-0.0557	-0.0793	0.0951	-0.0715	0.0118	0.1666
C11	-0.2134	-0.1383	-0.0274	0.0750	-0.1860	-0.0555	0.2610
C12	-0.2133	-0.1383	-0.0266	0.0750	-0.1867	-0.0559	0.2617
C13	0.7169	0.3740	0.3539	-0.3430	0.3630	0.0100	-0.7059
H14	0.2475	0.1125	0.1428	-0.1350	0.1048	-0.0151	-0.2398
H15	0.2475	0.1125	0.1428	-0.1350	0.1047	-0.0152	-0.2397
H16	0.2275	0.0983	0.1316	-0.1292	0.0959	-0.0166	-0.2252
H17	0.2275	0.0983	0.1315	-0.1292	0.0960	-0.0166	-0.2252

3.6. Molecular docking

Molecular docking simulations were performed using AutoDock for 100 search runs to investigate the binding modes and inhibitory activity of 4-nitrophenyl chloroformate (4NPCM) against two macromolecular targets (1X2J [27] and 5ZSY [28]) associated with lung cancer. The study of compound interaction on these protein structures justifies the structural basis of its possible anticancer activity in a lung malignancy model. The thermodynamic docking parameters and calculated inhibition constant (K_i) indicate the favourable binding of 4NPCM in the active-site pockets of both the receptors.

Comparing the binding profiles, 1X2J has a greater thermodynamic preference than 5ZSY. The highest ranking pose for 1X2J yielded a lower binding free energy of -6.49 kcal/mol (intermolecular energy of -7.38 kcal/mol). The highest complex for 5ZSY scored -5.59 kcal/mol (intermolecular energy of -6.49 kcal/mol). This difference in binding is stabilized by a more extensive hydrogen bonding network in the 1X2J active site. Specifically, Conformation 1 (Run 67) for 1X2J exhibits an inhibition constant of 17.59 μm and forms four strong hydrogen bonds involving residues ARG 483 (1.8 Å), SER 508 (1.9 Å), ARG 415 (2.1 Å), and SER 555 (2.1 Å). Secondary poses for 1X2J also display multiple polar contacts: Conformation 13 (Run 25; $K_i = 93.45\mu\text{m}$) engages VAL 514 (2.1 Å), VAL 467 (2.4 Å), and VAL 324 (2.1 Å) and 1.9 Å), while Conformation 27 (Run 29; $K_i = 139.61\mu\text{m}$) anchors via ARG 415 (2.1 Å and 2.5 Å) and VAL 465 (2.3 Å).

In contrast, docking complexes with the 5ZSY target exhibit fewer stabilizing hydrogen-bonding contacts. Conformation 1 (Run 6; $K_i = 79.71\mu\text{m}$) interacts via a single hydrogen bond with LYS 39 (1.9 Å). Similarly, Conformation 44 (Run 23; $K_i = 493.32\mu\text{m}$) forms a sole hydrogen bond with LEU 73 (2.0 Å), whereas Conformation 45 (Run 29; $K_i = 505.98\mu\text{m}$) establishes three polar contacts with LYS 39 (2.1 Å), ARG 36 (2.4 Å), and VAL 37 (2.2 Å). Table 6 summarizes the binding energies, distances of the residues in the active site, and the inhibition constants, while the 2D, 3D, and surface molecular docking orientations are presented in Fig. 8 (for 1X2J) and Fig. 9 (for 5ZSY). The better hydrogen-bonding network and pocket fit are consistent with higher stability of the complex of 4NPCM bound to 1X2J receptor.

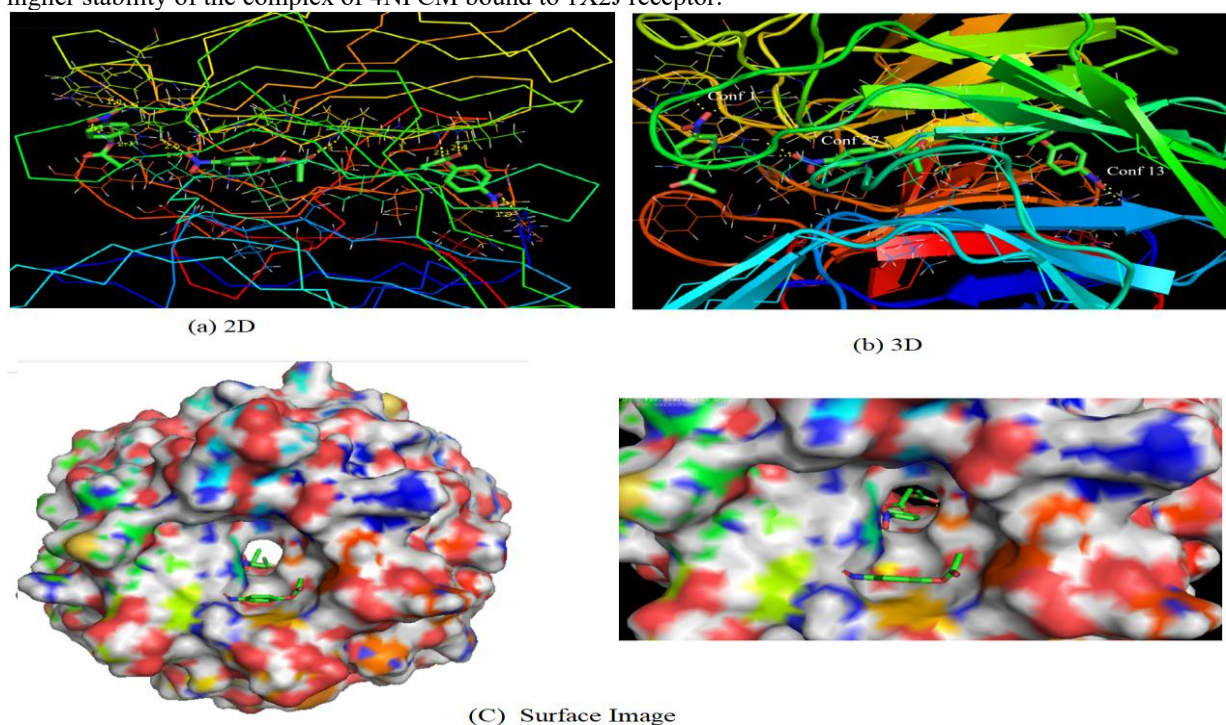


Fig. 8. Molecular docking interaction views of (a) 2D interaction network, (b) 3D ribbon representation, and (c) surface binding cavity layout for the 4NPCM ligand binding to the 1X2J target protein in different conformations (1, 13 and 27).

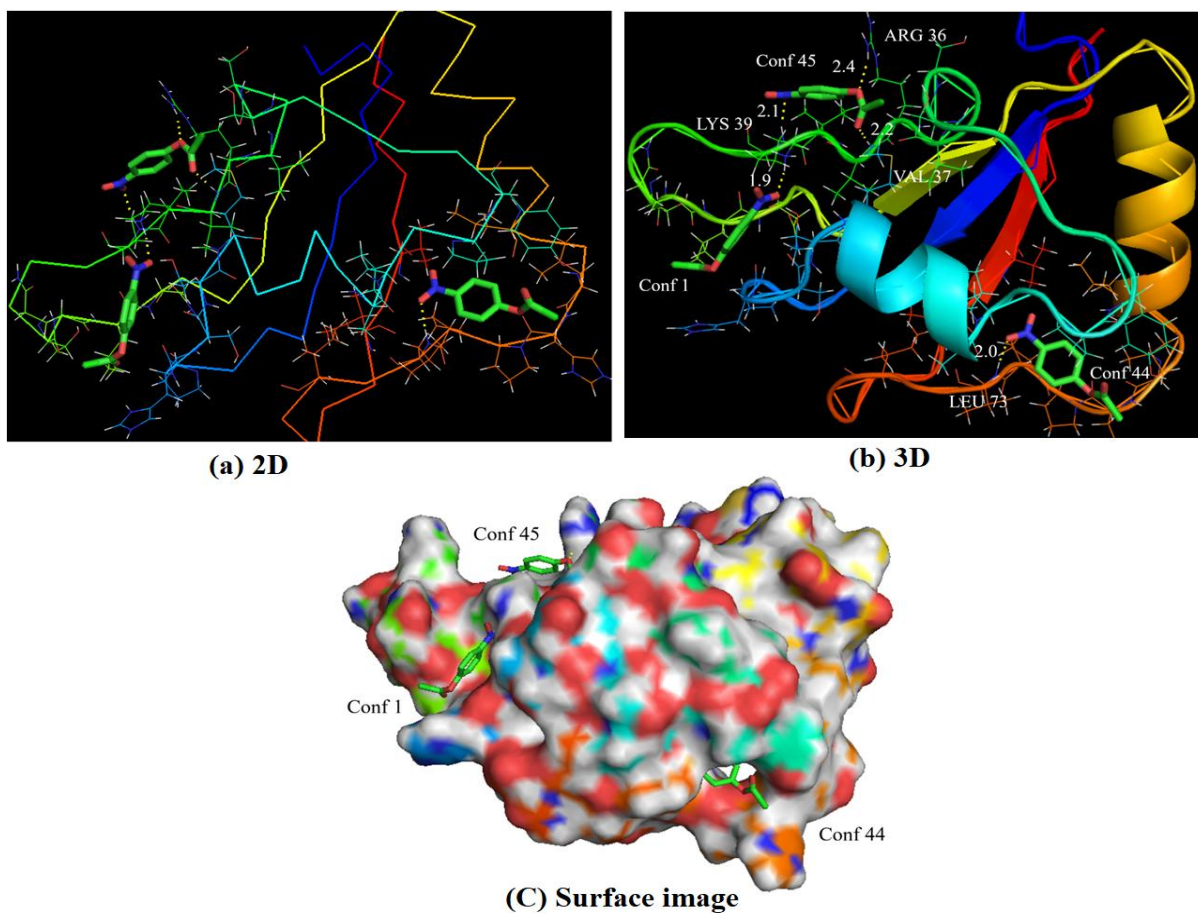


Fig. 9. Molecular docking interaction views of (a) 2D interaction network, (b) 3D ribbon representation, and (c) surface binding cavity layout for the 4NPCM ligand binding to the 5ZSY target protein in different conformations (1, 44 and 45).

Table 6. Anticancer investigations of 4NPCM compound.

PDB ID	Conformation	Rank= cluster rank (sub rank) (Out of 100)	Binding energy(ΔG) kcal/mol	Inhibition Constant Ki(micromolar)	Hydrogen bonds	Bond residue(bond distance Å)	Intermolecular Energy kcal/mol	Reference RMSD
1X2J	1(Run 67)	1(1)=1	-6.49	17.59	(O...HN)	ARG 483(1.8)	-7.38	68.16
					(O...HO)	SER 508(1.9)		
					(O...HN)	ARG 415(2.1)		
					(O...HO)	SER 555(2.1)		
	13 (RUN 25)	2(7)=27	-5.50	93.45	(O...HN)	VAL 514(2.1)	-6.39	74.67
					(O...HN)	VAL 467(2.4)		
					(O...HN)	VAL 324(2.1)		
					(O...HN)	VAL 324(1.9)		
	27(Run 29)	4(3)= 36	-5.26	139.61	(O...HN)	ARG 415(2.1)	-6.15	72.98
5ZSY					(O...HN)	ARG 415(2.5)		
					(O...HN)	VAL 465(2.3)		
	1(Run 6)	1(1)=1	-5.59	79.71	(O...HN)	LYS 39(1.9)	-6.49	14.27
	44(Run 23)	4(4)=47	-4.51	493.32	(O...HN)	LEU 73(2.0)	-5.41	11.69
	45(Run 29)	6(1)=50	-4.5	505.98	(O...HN)	LYS 39(2.1)	-5.39	11.72
					(O...HN)	ARG 36(2.4)		
					(O...HN)	VAL 37(2.2)		

4. CONCLUSION

The present work reports a comprehensive study of 4-nitrophenyl chloroformate (4NPCM) by means of experimental FT-IR, FT-Raman and UV-Vis spectroscopy and quantum chemical DFT, TD-DFT and molecular docking simulations. The potential energy surface (PES) scan showed the global minimum conformation at dihedral angles SC1 = 179.99° and SC2 = 0.08° (E = -1080.676598 a.u.) in good structural agreement with the

experimental XRD data. The scaled vibrational frequencies (0.961 factor) and Potential Energy Distribution (PED) assignments confirmed the key diagnostic bands such as the carbonyl stretch at 1773 cm⁻¹ (FT-IR) and 1783 cm⁻¹ (FT-Raman) and asymmetric and symmetric nitro stretching modes at 1526 cm⁻¹ and 1342 cm⁻¹. Solvent-dependent TD-DFT calculations accurately reproduced the main absorption band at 263 nm (HOMO→LUMO transition) in chloroform and frontier orbital analysis showed a narrowing band gap (from 4.6145 eV in chloroform to 4.5101 eV in water) and a high electrophilicity index ($\omega = 7.2691-7.3774$ eV) due to the strong electron-withdrawing effects of the nitro and chloroformate groups. Surface charge topology and Fukui dual descriptors (Δr) indicated that the oxygen atoms (O2–O5) are the principal hydrogen-bond acceptors ($\Delta r > 0$) and the carbonyl carbon C13 is a major electrophilic site ($\Delta r = -0.7059$). Finally, AutoDock simulations of 100 runs showed a high thermodynamic preference and complex stability to lung cancer target protein 1X2J ($\Delta G = -6.49$ kcal/mol, $K_i = 17.59$ μ M) over 5ZSY ($\Delta G = -5.59$ kcal/mol, $K_i = 79.71$ μ M) with a multi-point hydrogen-bonding network with the ARG 483, SER 508, ARG 415 and SER 555 residues.

Declaration of competing interest

The authors declare no conflict of interest

CRediT authorship contribution statement

K. Theepanji: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **R. Raj Muhamed:** Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **M. Habib Rahuman:** Writing – original draft, Investigation, Formal analysis. **Gopathy Ramalingam:** Writing – original draft, Investigation, Conceptualization. **A. Thamarai:** Writing – original draft, Methodology, , Data curation, Conceptualization. **K. Karthika:** Writing – review & editing, Methodology, Investigation. **M. Raja:** Writing – review & editing, Methodology, Conceptualization.

Data availability

Data will be made available on request.

Supplementary materials

Nil

REFERENCES

1. N.G. Haress, N.A. Al-Sagheer, M.S. Abdel-Aziz, A.A. El-Emam, Spectroscopic investigation (FT-IR and FT-Raman), vibrational assignments, HOMO-LUMO analysis and molecular docking study of 2-(adamantan-1-yl)-5-(4-nitrophenyl)-1,3,4-oxadiazole, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 138 (2015) 876–885. <https://doi.org/10.1016/j.saa.2014.11.109>
2. E. Fazal, C.Y. Panicker, H.T. Varghese, S. Nagarajan, B.S. Sudha, J.A. War, S.K. Srivastava, B. Harikumar, P.L. Anto, Spectroscopic investigation (FT-IR, FT-Raman), HOMO–LUMO, NBO analysis and molecular docking study of 4-chlorophenyl quinoline-2-carboxylate, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 145 (2015) 260–269. <https://doi.org/10.1016/j.saa.2015.01.089>
3. M. Szafran, A. Komasa, E. Bartoszak-Adamska, Vibrational spectra and DFT calculations of 3-carboxy-1-methylpyridinium chloride (trigonelline hydrochloride), *J. Raman Spectrosc.* 38 (9) (2007) 1175–1184. <https://doi.org/10.1002/jrs.1752>
4. S. Subashchandrabose, H. Saleem, V. Thanikachalam, G. Manikandan, FT-IR, FT-Raman spectra, HOMO-LUMO, NBO and MEP analysis of 1-(2-chloro-4-nitrophenyl)piperazine by DFT method, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 77 (4) (2010) 829–837. <https://doi.org/10.1016/j.saa.2010.08.016>
5. P. Govindasamy, M. Gunasekaran, M. Srinivasan, S. Periandy, Spectroscopic (FT-IR, FT-Raman), NBO, HOMO–LUMO, UV–Vis and molecular docking study of 2-amino-4-chloro-6-methoxypyrimidine, *J. Mol. Struct.* 1074 (2014) 705–718. <https://doi.org/10.1016/j.molstruc.2014.06.052>
6. A. Suvitha, Geometry optimization, HOMO and LUMO energy, molecular electrostatic potential, NMR, FT-IR and FT-Raman analyses on 4-nitrophenol, *Eur. Phys. J. Appl. Phys.* 70 (3) (2015) 30401. <https://doi.org/10.1051/epjap/2015140510>
7. Y. Erdogdu, T. Yurdakul, Molecular docking and comparative vibrational spectroscopic analysis, HOMO-LUMO, polarizabilities, and hyperpolarizabilities of N-(4-bromophenyl)-4-nitrobenzamide by different DFT (B3LYP, B3PW91, and MPW1PW91) methods, *Polycycl. Aromat. Compd.* 41 (2) (2021) 332–347. <https://doi.org/10.1080/10406638.2019.1599354>
8. A. Tawfiq, A.H. Hattab, Studies on the DFT calculations and molecular docking of versatile molecular sensor 1-(6-aminopyridin-2-yl)-3-(4-nitrophenyl) urea, *Results Chem.* 4 (2022) 100346. <https://doi.org/10.1016/j.rechem.2022.100346>
9. A. Tawfiq, Infrared spectrum, NBO, HOMO–LUMO, MEP and molecular docking studies of (2E)-3-(3-nitrophenyl)-1-[4-piperidin-1-yl]prop-2-en-1-one, *J. Mol. Struct.* 1249 (2022) 131580. <https://doi.org/10.1016/j.molstruc.2021.131580>
10. A. Tawfiq, O.J. Mahdi, A.H. Hattab, Syntheses, characterization, DFT and molecular docking of N-(4-chlorophenyl)-1-(4-nitrophenyl)methanimine and derivatives, *Al-Nahrain J. Sci.* 29 (1) (2026) 15–24.
11. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T.

- Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford, CT, 2009.
12. R. Dennington, T.A. Keith, J.M. Millam, GaussView, Version 5, Semichem Inc., Shawnee Mission, KS, 2009.
13. A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, *J. Chem. Phys.* 98 (1993) 5648–5652. <https://doi.org/10.1063/1.464913>
14. R.G. Parr, W. Yang, Density-Functional Theory of Atoms and Molecules, Oxford University Press, New York, 1989.
15. M.H. Jamróz, Vibrational energy distribution analysis (VEDA): Scopes and limitations, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 114 (2013) 220–230. <https://doi.org/10.1016/j.saa.2013.05.096>
16. J. Tomasi, B. Mennucci, R. Cammi, Quantum mechanical continuum solvation models, *Chem. Rev.* 105 (2005) 2999–3094. <https://doi.org/10.1021/cr9904009>
17. Y. Zhao, D.G. Truhlar, The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements, *Theor. Chem. Acc.* 120 (2008) 215–241. <https://doi.org/10.1007/s00214-007-0310-x>
18. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J. Comput. Chem.* 30 (2009) 2785–2791. <https://doi.org/10.1002/jcc.21256>
19. S.N. Sheshadri, C.S. Chidan Kumar, S. Naveen, M.K. Veeraiah, K. Raghava Reddy, I. Warad, Crystal structure and Hirshfeld surface analysis of 2-(4-nitrophenyl)-2-oxoethyl benzoate, *Acta Crystallogr. Sect. E Crystallogr. Commun.* 75 (10) (2019) 1572–1576. <https://doi.org/10.1107/S2056989019013069>
20. R.M. Silverstein, F.X. Webster, D.J. Kiemle, Spectrometric Identification of Organic Compounds, 7th ed., John Wiley & Sons, New York, 2005.
21. G. Socrates, Infrared and Raman Characteristic Group Frequencies: Tables and Charts, 3rd ed., John Wiley & Sons, Chichester, 2001.
22. G. Varsányi, Assignments for Vibrational Spectra of Seven Hundred Benzene Derivatives, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 30 (1974) 220–225.
23. P. Politzer, J.S. Murray, The electrostatic potential as a descriptor of molecular properties, *Theor. Chem. Acc.* 108 (2002) 134–142. <https://doi.org/10.1007/s00214-002-0363-9>
24. R. Nithya Balaji, A. Celine Kavida, J. Joy Priscilla, T. Alamelu, Drakshayani Sriramsetti, J. Satheesh Kumar, P. Vinayagam, M. Raja, Synthesis, Characterization, Chemical Reactivity Analyses and Biological Investigations of (E)-1-(5-Chloro-2-Hydroxy Benzylidene)Semicarbazide, *International Journal of Drug Delivery Technology*, 16(84s) (2026) 1776–1791; DOI: 10.25258/ijddt.16.84s.181
25. C. Morell, A. Grand, A. Toro-Labbé, Theoretical support for using an operational dual descriptor for chemical reactivity, *Chem. Phys. Lett.* 425 (2006) 355–360. <https://doi.org/10.1016/j.cplett.2006.05.003>
26. C. Morell, A. Grand, A. Toro-Labbé, New dual descriptor for chemical reactivity, *J. Phys. Chem. A* 109 (2005) 205–212. <https://doi.org/10.1021/jp046577a>
27. B. Padmanabhan, K.I. Tong, T. Ohta, Y. Nakamura, M. Scharlock, M. Ohtsuji, M.I. Kang, A. Kobayashi, S. Yokoyama, M. Yamamoto, Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer, *Mol. Cell* 21 (5) (2006) 689–700. <https://doi.org/10.1016/j.molcel.2006.01.013>
28. H. Li, B. Zhao, X. Wang, Y. Zhang, Structural basis of the human RBM10 RRM2 domain and its lung cancer-related mutants, *RCSB Protein Data Bank* (2018). PDB ID: 5ZSY. <https://doi.org/10.2210/pdb5ZSY/pdb>