

Synthesis, Electronic Structure Analysis, Pharmacological Evaluation and Molecular Dynamics Investigation of Pyrazole-Triazole Derivative as a Computationally Predicted FGFR-Targeting Candidate Against Glioblastoma

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Glioblastoma remains a highly invasive brain tumour with limited therapeutic options, highlighting the need for novel FGFR-targeted inhibitors. The objective of the work to explore structural characteristics, electronic behaviour and pharmacological potential of 5-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-triazolidin-3-one (5CPTO) as a *in silico*-predicted FGFR-binding candidate. A combined computational and experimental strategy was employed, integrating DFT, spectroscopic analysis and computational docking studies to elucidate the structural, spectral and biological attributes of the compound. The optimized geometry showed a nearly planar molecular configuration with preservation of the aromatic framework. Experimental and theoretical FT-IR and NMR data were in close agreement, supporting the assignment of the principal functional groups. Molecular orbital analysis gave a HOMO–LUMO energy gap of 4.757 eV, consistent with moderate electronic stability and intramolecular charge-transfer characteristics. NBO analysis indicated appreciable π -electron delocalization within the molecular framework. MEP mapping and Fukui function analysis located the principal electrophilic and nucleophilic sites, while topological analysis identified covalent as well as weak non-covalent interactions involved in molecular stabilization. ADMET predictions indicated favourable oral bioavailability, low toxicity, and conformity with Lipinski's drug-likeness criteria. Molecular docking with FGFR4 (PDB ID: 7DTZ) yielded a binding energy of -7.73 kcal/mol, with hydrogen bonds and hydrophobic contacts contributing to ligand recognition at the binding site. During the 100 ns MD simulation, the 5CPTO–FGFR4 complex maintained stable RMSD, RMSF, and radius of gyration profiles. MMPBSA calculations gave favourable binding free-energy values, with van der Waals and hydrophobic interactions making major energetic contributions. These computational findings support the structural stability, electronic characteristics, and FGFR4-binding capability of 5CPTO and provide a basis for its further evaluation through *in vitro* enzymatic and cell-based studies related to glioblastoma.

Keywords: Synthesis, Molecular docking, MD simulation, Spectroscopy, ADMET, DFT.

INTRODUCTION

Heterocyclic compounds constitute a diverse class of organic molecules with a broad range of structural, physico-chemical, and biological properties, with their reactivity and stability influenced by the nature and arrangement of heteroatoms within the ring system [1]. Nitrogen-containing hetero-

cycles have received considerable attention in medicinal chemistry owing to their diverse biological activities and their frequent occurrence in bioactive molecules [2]. Such heterocyclic scaffolds and their derivatives have contributed substantially to the development of therapeutic agents and include several important five-membered nitrogen-containing ring systems [3]. Their structural diversity and ability to partici-

EXPERIMENTAL

pate in specific molecular interactions enable nitrogen heterocycles to function as pharmacophoric components in numerous biologically active compounds. Among these scaffolds, pyrazole is a well-established five-membered nitrogen-containing heterocycle found in a variety of biologically and therapeutically relevant molecules. Pyrazole derivatives have been reported to possess diverse biological activities, including antiviral [4], antitumor [5], anti-inflammatory [6], antibacterial [7], insecticidal and fungicidal [8] and anticancer activities [9]. Modified pyrazole scaffolds are present in several pharmaceutical compounds, reflecting the versatility of this heterocyclic framework in medicinal chemistry. Their biological and chemical properties have led to the application of pyrazole derivatives in agrochemical research and development [1]. The pyrazole ring is further utilized as a synthetic building block in multistep organic transformations [10] and as a ligand framework for coordination with metal centers [11].

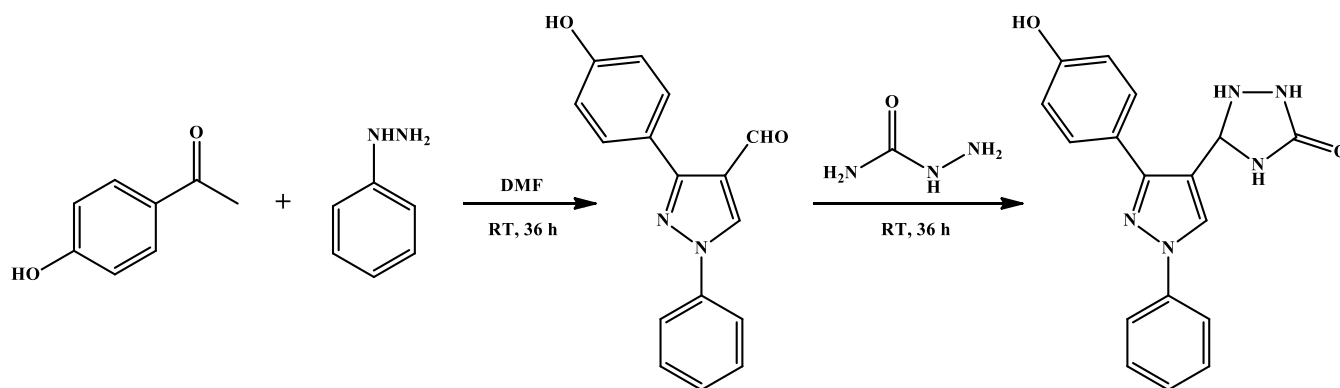
Glioma is a major primary malignancy of the central nervous system and includes localized and diffuse forms [12,13]. According to the WHO classification, gliomas are graded from I to IV, with glioblastoma (GBM) representing the highest-grade and most aggressive form, characterized by frequent recurrence and poor survival [14,15]. Fibroblast growth factor receptor (FGFR) signalling has been implicated in glioma progression, while therapeutic targeting of this pathway remains challenging due to resistance mechanisms and limitations in treatment response. The current use of surgery, radiotherapy, and chemotherapy highlights the need for new therapeutic scaffolds targeting relevant molecular pathways [16].

In the present study, 5-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-triazolidin-3-one ($C_{17}H_{14}N_5OCl$; 5CPTO) was synthesized and characterized using experimental FT-IR and NMR spectroscopy and quantum chemical calculations. DFT, NBO, frontier molecular orbital and molecular electrostatic potential analyses were employed to investigate its structural and electronic properties. ADMET analysis was used to assess its pharmacokinetic and toxicity profiles, while molecular docking and 100 ns molecular dynamics simulations were performed to examine its interaction and stability with FGFR4. These studies provide a computational basis for further *in vitro* evaluation of 5CPTO as a potential antiglioma compound.

All chemicals and solvents were of LR grade and procured from the reputed commercial vendors. Melting points were determined using a melting point apparatus and are reported uncorrected. FT-IR spectra (Perkin-Elmer Spectrum Two FT-IR spectrophotometer) were recorded using an FT-IR spectrophotometer and expressed in cm^{-1} . 1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using a Bruker Avance 400 NMR spectrometer in $DMSO-d_6$ with TMS as the internal standard. Reaction progress was monitored by thin-layer chromatography (TLC) on F₂₅₄ silica gel plates under UV light.

Synthesis: Phosphorus oxychloride ($POCl_3$) and dimethylformamide (DMF) were added to a mixture of 1-(4-chlorophenyl)ethan-1-one (4'-chloroacetophenone) and phenylhydrazine to facilitate Vilsmeier-Haack cyclization. The reaction mixture was stirred at room temperature for 36 h and the progress of the reaction was monitored by TLC on silica gel plates using ethyl acetate/*n*-hexane as the mobile phase. After completion, the reaction mixture was filtered and the crude product was recrystallized from ethanol to afford 3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde in 89% yield. The resulting aldehyde was subsequently treated with semicarbazide in chloroform and stirred at room temperature for 36 h. Reaction progress was monitored by TLC using ethyl acetate/*n*-hexane as the mobile phase. The precipitated solid was collected by filtration and purified by recrystallization from ethanol. The target compound, 5-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-triazolidin-3-one (5CPTO), was obtained as a white crystalline solid in 92% yield (**Scheme-I**).

Quantum computational techniques: The DFT-based quantum chemical calculations performed using B3LYP functional and 6-311++G(d,p) basis set to calculate the electronic properties of molecules [17-21], through Gaussian 16w [22]. Geometry optimization was performed to obtain the minimum-energy molecular structure, total electronic energy and harmonic vibrational frequencies. The optimized structure was visualized using Chemcraft [23]. The calculated vibrational frequencies were scaled by a factor of 0.961 to account for systematic deviations associated with the computational method [24]. Vibrational assignments and potential energy distribution (PED) analysis were performed using VEDA [25]. The electronic structure was examined through



Scheme-I: Synthetic route of the title compound (5CPTO)

natural bond orbital (NBO) analysis at the same level of theory to evaluate second-order donor–acceptor interactions and electron delocalization. Molecular electrostatic potential (MEP) surfaces and frontier molecular orbital (FMO) properties were calculated and visualized using GaussView [26]. Topological analysis of the electron density was carried out using Multiwfn [27]. Drug-likeness was evaluated using SwissADME according to Lipinski's rule, while pharmacokinetic and toxicity-related properties were predicted using pkCSM [28,29]. PDBsum was used to analyze protein structural features, including backbone dihedral angles and the distribution of residues within favoured and allowed conformational regions [30].

Docking studies: The anticancer potential of 5CPTO was investigated through molecular docking against the kinase domain of fibroblast growth factor receptor 4 (FGFR4). The docking calculations were performed using AutoDock 4.2 and AutoDock Tools 1.5.6 [31]. The crystal structure of FGFR4 (PDB ID: 7DTZ), co-crystallized with a covalent 2-amino-pyrimidine-based inhibitor and determined at a resolution of 2.01 Å, was selected as the receptor structure. The structure was retrieved from the Protein Data Bank [32]. Before docking, crystallographic water molecules and the co-crystallized ligand were removed, followed by the addition of polar hydrogen atoms and assignment of atomic charges. Docking was performed using the Lamarckian genetic algorithm (LGA) [33] and the resulting ligand–receptor interactions were analyzed using Discovery Studio Visualizer 4.1 [34]. The docking protocol was validated by re-docking the co-crystallized ligand, which resulted in an RMSD of 2.17 Å between the predicted and experimental binding orientations. The corresponding superimposed structures are presented in Fig. 1.

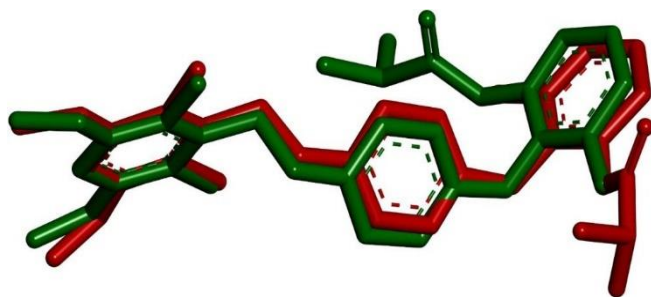


Fig. 1. Docking protocol validation by superimposition of native and redocked ligand conformations (RMSD = 2.17 Å)

The biological relevance of FGFR4 as a glioma target requires careful interpretation. FGFR1 and FGFR3 are more frequently reported in glioma and glioblastoma, whereas FGFR4 has been studied more extensively in other malignancies including hepatocellular carcinoma. The present docking analysis was consequently used as a structural assessment of 5CPTO binding within the FGFR4 kinase domain rather than as evidence of FGFR4-specific antiglioma activity. Further validation using an FGFR1 structure, such as PDB ID 7WCL, together with experimental studies, would help establish the relevance of FGFR-directed activity to glioma.

Methodology for MD simulation: Molecular dynamics simulations (GROMACS 2022.4) [35] were employed to elucidate the protein-ligand interactions. The protein topology

was generated using amber99sb-ildn [36]. The system was solvated with TIP3P water in a cubic box and neutralized with Na⁺ counterions. Topologies of the ligands with their force field parameters were derived by SwissParam web server [37]. Minimization was carried out and equilibration (NVT/NPT, 300 K), 100 ns production runs were performed. Binding energetics were assessed via MMPBSA (gmx MMPBSA module) [38], utilizing 1000 frames from the last 10 ns to evaluate stability and interactions under simulated physiological conditions.

RESULTS AND DISCUSSION

Spectroscopic analysis

Vibrational analysis: Theoretical analysis indicates that 5CPTO possesses C₁ point group symmetry and consists of 38 atoms, giving rise to 108 normal vibrational modes. All computed modes are both IR and Raman active, as summarized in Table-1. In heterocyclic systems, N-H stretching vibrations are commonly observed within the 3500-3000 cm⁻¹ region [39]. For 5CPTO, calculated N-H stretching bands appear at 3471, 3449 and 3331 cm⁻¹, which are in match with 3470 cm⁻¹ which is experimentally recorded (Fig. 2).

The C-H stretching vibrations generally fall within 3200-3000 cm⁻¹ range [40], reflecting the minimal substituent effects. The experimental IR spectrum of 5CPTO shows C-H stretching peaks in 3146 and 3049 cm⁻¹, while theoretical calculations predict corresponding vibrations between 2870 and 3149 cm⁻¹. Carbonyl (C=O) stretching is typically found in the 1850-1550 cm⁻¹ region [41]. The experimental FTIR at 1746 cm⁻¹ corresponds well with the computed value of 1699 cm⁻¹, exhibiting a PED contribution of 82%.

The C-C stretching vibrations normally occur from 1650 to 1100 cm⁻¹ [42]. For 5CPTO, the theoretical results reveal prominent C-C vibrations at 1570 cm⁻¹ (66%), 1425 cm⁻¹ (35%), 1257 cm⁻¹ (80%) and 1217 cm⁻¹ (55%), which agree closely with experimental bands observed at 1406 and 1217 cm⁻¹. The vibrations involving ring-halogen interactions are particularly important due to symmetry reduction and heavy atom effects [43]. According to Mooney [44,45], C-X halogen substituted carbons stretching typically appears between 1129 and 480 cm⁻¹. The FT-IR band observed at 488 cm⁻¹ in 5CPTO can be assigned to C–Cl stretching, which falls within the expected spectral range. The close match between the experimental and calculated frequencies supports this assignment.

NMR analysis: The ¹H and ¹³C NMR spectra of 5CPTO were recorded in DMSO-d₆ and are shown in Fig. 3. The theoretical chemical shifts were calculated using the GIAO method with the IEFPCM solvation model [46,47]. The experimental and calculated ¹H and ¹³C chemical shifts are compared in Table-2, which confirmed the good agreement and supporting the proposed molecular structure of 5CPTO.

Carbonyl carbons in amides and carboxylic acids generally resonate in the 160-220 ppm region of the ¹³C NMR spectrum due to deshielding by the electronegative oxygen atom [48]. In 5CPTO, the C22 carbonyl carbon exhibited a calculated chemical shift of 172.923 ppm. Aromatic carbons typically appear between 100 and 175 ppm, with their chemi-

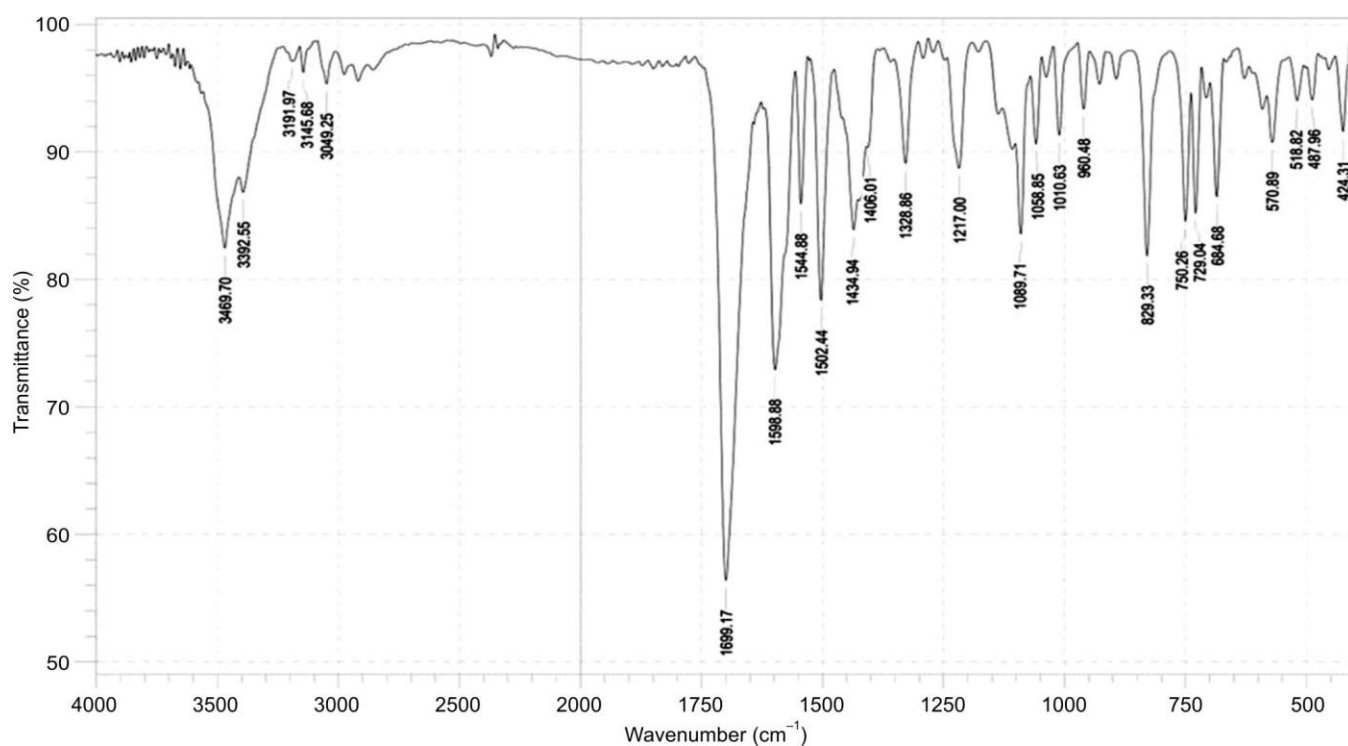
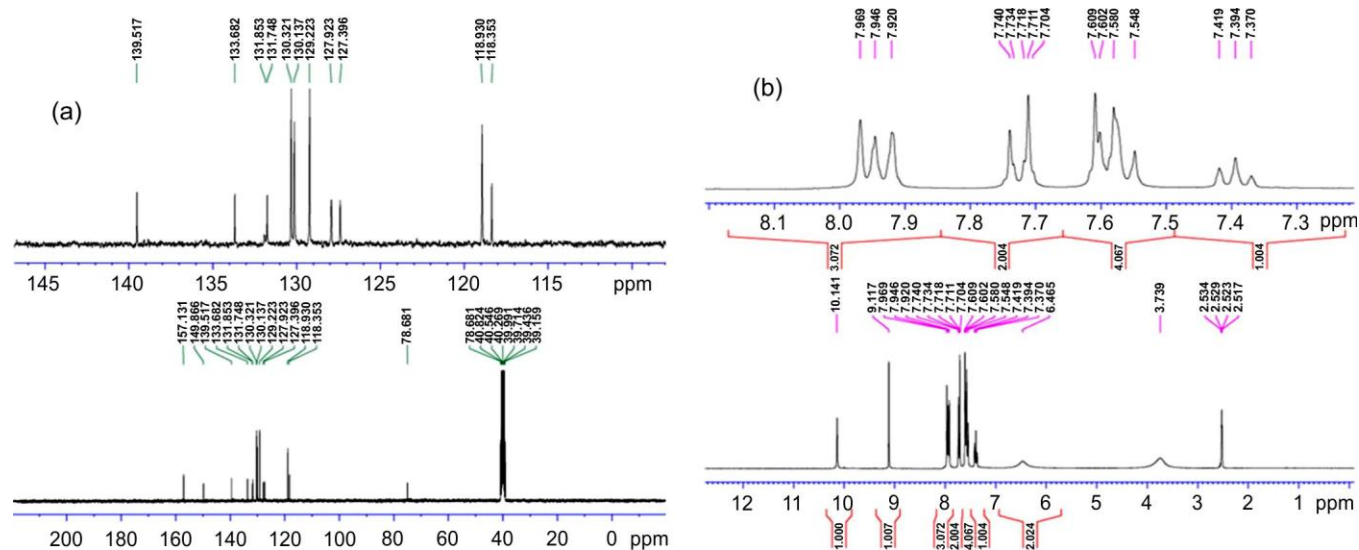
TABLE-1
 EXPERIMENTAL–THEORETICAL VIBRATIONAL DATA AND PED ASSIGNMENTS OF 5CPTO

Modes	Experimental frequency	Theoretical frequency		IR intensity		PED assignment (%)
	IR	Unscaled	Scaled	Relative	Absolute	
108	3470	3611	3471	35	4	ν NH100
107		3589	3449	41	5	ν NH99
106		3466	3331	4	0	ν NH100
105	3146	3276	3149	1	0	ν CH99
104		3220	3094	1	0	ν CH96
103		3206	3081	2	0	ν CH98
102		3200	3075	2	0	ν CH91
101		3198	3074	6	1	ν CH92
100		3192	3067	1	0	ν CH100
99		3191	3066	20	3	ν CH95
98		3179	3055	8	1	ν CH91
97		3175	3052	4	1	ν CH93
96	3049	3171	3047	2	0	ν CH94
95		2986	2870	35	4	ν CH100
94	1699	1817	1746	792	100	ν OC82
93	1599	1641	1577	65	8	β HCC18 + ν CC55
92		1636	1573	16	2	β HCC34 + ν CC27
91		1634	1570	3	0	ν CC66
90	1545	1610	1547	20	3	ν CC55 + β HCC11
89	1502	1580	1518	69	9	β HCC10 + ν CC45
88		1541	1481	6	1	β HCC29 + ν CC27
87		1531	1471	153	19	β HCC49 + ν CC13
86		1499	1440	52	7	β HCC74
85	1435	1496	1437	3	0	ν CC40 + β HCC36
84	1406	1483	1425	74	9	ν CC35
83		1440	1384	35	4	β HCC24 + ν CC26
82		1435	1379	22	3	β HCN13 + ν NN22 + ν CC18
81		1409	1355	12	2	β HCC40
80		1403	1349	43	5	β HNC55
79	1329	1378	1324	54	7	β HNC43 + β HCC11 + ν NN11
78		1366	1313	44	6	β HCC16 + ν CC30
77		1357	1304	16	2	β HCC75
76		1348	1296	36	5	β HCC37
75		1343	1290	5	1	ν CC77 + β HCC10
74		1331	1279	3	0	β HCC70
73		1308	1257	5	1	ν CC80
72		1270	1221	81	10	ν NN21 + β HCN14
71	1217	1266	1217	26	3	ν CC55
70		1240	1192	71	9	β HCC20 + ν CC23
69		1226	1178	13	2	ν CC14 + β HCC30 + β HCN13
68		1209	1162	1	0	β HCC55 + ν CC16
67		1200	1153	0	0	β HCC70 + ν CC19
66		1183	1137	0	0	ν CC11 + β HCC76
65		1148	1104	32	4	β HCC46 + ν NN21
64	10090	1135	1091	4	1	β HCC38 + ν CC37
63		1104	1061	29	4	ν CC33 + β HCC29
62	1059	1102	1059	45	6	ν CC36 + ν ClC12
61		1089	1046	10	1	ν NC55
60		1080	1038	60	8	β HCC13 + ν NN41
59	1011	1058	1017	6	1	β HCC10 + ν CC42
58		1045	1004	3	0	β HCC24 + ν CC52
57		1031	991	21	3	β HCC49 + ν NN16
56		1023	983	77	10	β HCC16 + ν NN29

55		1016	976	1	0	ν CC29 + β HCC55
54		1003	964	38	5	ν NN53
53	960	1002	963	0	0	τ HCCH95
52		997	958	12	2	τ HCCC75
51		984	946	2	0	τ HCCN90
50		978	940	23	3	β HCN13 + ν CC20 + τ HCCC25
49		977	939	16	2	τ HCCC60
48		923	887	5	1	τ HCCN89
47		911	875	34	4	ν NN46 + β HCC12
46	829	858	824	32	4	τ HCCC130
45		854	821	25	3	ν CC12 + τ HCCC14
44		845	812	0	0	τ HCCC82
43		843	810	13	2	τ HCCC80
42		831	799	28	4	τ HCCC55
41	750	793	762	58	7	τ HNCO36 + β CCN11
40		771	741	30	4	τ HCCN72
39		770	740	25	3	τ HNCO23 + τ HCCN13
38		755	726	12	2	τ HCCC24
37		740	711	8	1	β HCC25 + ν CIC11
36		721	693	5	1	τ HCCC71
35	685	711	683	26	3	β HCC35
34		702	674	26	3	τ HCCC91
33		692	665	12	2	β HCC40
32		653	627	0	0	τ HCCC60
31		645	620	1	0	β HCC78
30		630	606	0	0	β HCC78
29	571	593	570	44	6	β HNC36 + τ HNCN11
28		569	547	87	11	τ HNCN57
27	519	542	521	40	5	β CCN11 + β HNC39
26		524	503	52	7	β HNC14 + ν CIC12 + τ HCCC112
25		520	500	31	4	τ HCCC60
24	488	508	488	15	2	ν CIC20
23		499	480	63	8	β CCN12 + τ HCNC29
22		447	430	4	0	β CCC17
21	424	424	407	1	0	τ HCCC86
20		416	400	0	0	τ HCCC89
19		412	396	5	1	β CCC16 + τ HCCC114
18		378	363	1	0	τ HCCN18
17		330	317	0	0	β CCC33
16		315	303	1	0	β CCC15 + τ HCCC131
15		271	260	1	0	τ HCNC45
14		263	252	2	0	τ HNNC52
13		241	231	3	0	τ HCCH15
12		217	208	2	0	β HCC27
11		207	199	0	0	τ HCCC24
10		170	163	1	0	τ HCCC150
9		141	136	3	0	β HCC12 + τ HCCC42
8		120	115	3	0	β CCN15 + τ HCCC47
7		92	89	1	0	τ HCCC165
6		70	68	1	0	τ HCCC64
5		53	51	1	0	β HCC49
4		52	50	0	0	τ HCCC115
3		40	38	0	0	τ HCCC67
2		33	32	1	0	τ HCCC47
1		27	26	0	0	τ CNCC77

^aScaling factor: 0.961 for B3LYP/6-311++G(d,p); ^bRelative IR absorption normality intensities with highest peak absorption equal to 100;

^c ν -stretching, β -Bending, τ -Torsion

Fig. 2. Experimental IR spectrum (4000-400 cm^{-1}) of 5CPTOFig. 3. Experimental (a) ^{13}C NMR and (b) ^1H NMR spectra of 5CPTO in DMSO solventTABLE-2
COMPARISON OF EXPERIMENTAL AND THEORETICAL CHEMICAL SHIFTS OF 5CPTO

Atom	Theoretical	Experimental	Atom	Theoretical	Experimental	Atom	Theoretical	Experimental
C1	147.639	149.866	C14	134.740	—	H29	8.528	7.969
C2	133.674	130.321	C15	131.886	127.923	H30	8.387	7.946
C3	133.724	131.748	C16	134.569	133.682	H31	7.856	7.734
C4	137.966	139.517	C17	121.583	118.353	H32	7.689	7.548
C5	134.430	131.853	C18	75.147	78.681	H33	7.689	7.704
C6	133.197	130.137	C22	172.923	—	H34	7.689	7.609
C7	157.511	157.131	H25	7.689	7.580	H35	5.886	7.394
C8	125.197	127.396	H26	8.270	7.740	H36	3.972	3.739
C10	133.020	129.223	H27	8.270	7.920	H37	4.237	6.465
C12	146.151	—	H28	7.689	7.602	H38	5.243	7.370
C13	124.016	118.93	—	—	—	—	—	—

cal shifts influenced by the electronic nature of the attached substituents [49]. The twelve aromatic carbons (C1–C6 and C12–C17) of 5CPTO showed the experimental signals in the range of 118.353–149.866 ppm, while the calculated values ranged from 121.583–147.639 ppm. The pyrazole ring carbons C7, C8 and C10 showed calculated chemical shifts of 157.511, 125.197 and 133.020 ppm, respectively, compared with experimental values of 157.131, 127.396, and 129.223 ppm. For the triazolidine carbon C18, the experimental and calculated chemical shifts were 75.17 and 78.68 ppm, respectively, giving a difference of approximately 3.5 ppm.

Aromatic protons generally resonate in the 6.5–8.0 ppm range [50,51]. In 5CPTO, the aromatic protons H25–H28 and H30–H34 exhibited experimental chemical shifts of 7.689–8.387 ppm, compared with calculated values of 7.548–7.946 ppm. The protons H36, H37 and H38 showed experimental shifts of 3.739, 6.465 and 7.370 ppm, respectively, whereas the corresponding calculated values were 3.972, 4.237 and 5.243 ppm. The pyrazole proton H29 appeared at 7.969 ppm experimentally and 8.528 ppm theoretically. The signals observed at 2.50–2.52 ppm were attributed to residual solvent/water signals associated with DMSO- d_6 .

Structural optimization and geometry: The optimized geometry of 5CPTO, along with the corresponding atomic numbering scheme, was generated using Gaussian16W and visualized through GaussView, as illustrated in Fig. 4. The calculated geometric parameters for the lowest-energy configuration are tabulated in Table-3. Key bond-length and bond-angle values used in the discussion were extracted from the computed output. The C-H bond lengths in the aromatic parts of the molecule are essentially uniform and were calculated to fall within the narrow range of 1.081 to 1.084 Å for the ring

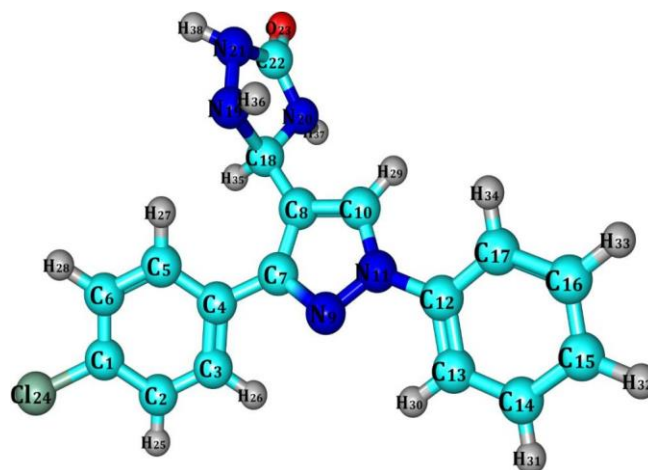


Fig. 4. Molecular optimized structure of the compound 5CPTO

C-H bonds (C13–H30 = 1.081 Å, C3–H26 = 1.083 Å, C5–H27 = 1.084 Å), consistent with typical aromatic C-H distances [52]. Furthermore, X-H distances associated with amine/heteroatom-bound hydrogens are shorter (N19–H36 = 1.018 Å, N20–H37 = 1.011 Å, N21–H38 = 1.012 Å), reflecting the different bonding nature of N-H compared to aromatic C-H. One slightly longer C-H distance (C18–H35 = 1.099 Å) is observed for the exocyclic CH and may be affected by the local bonding environment around C18. The C-C bond lengths within the two aromatic rings are in a tight range typical for delocalized benzene systems (approximately 1.389 to 1.403 Å for C1–C2 = 1.393 Å, C3–C4 = 1.403 Å, C12–C13 = 1.396 Å). Exocyclic single C-C bonds and connections to heteroatom containing fragments are predictably longer (for example, C8–C18 = 1.494 Å). In contrast, more localized C-N/C-O and

TABLE-3
LIST OF OPTIMIZED BOND LENGTHS (Å) AND BOND ANGLES (°) PARAMETERS OF 5CPTO

Bond length (Å)	Values	Bond length (Å)	Values	Bond angle (°)	Values	Bond angle (°)	Values	Bond angle (°)	Values
C1-C2	1.393	C12-C17	1.397	C2-C1-C6	121.0	C7-N9-N11	106.0	C14-C15-H32	120.3
C1-C6	1.390	C13-C14	1.391	C2-C1-C124	119.5	C10-C8-C18	126.1	C16-C15-H32	120.2
C1-C124	1.758	C13-H30	1.081	C1-C2-C3	119.2	C8-C10-N11	107.2	C15-C16-C17	120.5
C2-C3	1.389	C14-C15	1.394	C1-C2-H25	120.1	C8-C10-H29	130.1	C15-C16-H33	120.2
C2-H25	1.083	C14-H31	1.084	C6-C1-C124	119.6	C8-C18-N19	112.6	C17-C16-H33	119.3
C3-C4	1.403	C15-C16	1.393	C1-C6-C5	119.4	C8-C18-N20	113.9	C16-C17-H34	119.8
C3-H26	1.083	C15-H32	1.084	C1-C6-H28	120.1	C8-C18-H35	109.7	N19-C18-N20	101.7
C4-C5	1.402	C16-C17	1.392	C3-C2-H25	120.7	N9-N11-C10	111.7	N19-C18-H35	108.0
C4-C7	1.473	C16-H33	1.084	C2-C3-C4	121.1	N9-N11-C12	120.5	C18-N19-N21	104.9
C5-C6	1.393	C17-H34	1.083	C2-C3-H26	119.7	N11-C10-H29	122.6	C18-N19-H36	107.3
C5-H27	1.084	C18-N19	1.488	C4-C3-H26	119.2	C10-N11-C12	127.8	N20-C18-H35	110.5
C6-H28	1.083	C18-N20	1.470	C3-C4-C5	118.5	N11-C12-C13	119.5	C18-N20-C22	108.3
C7-C8	1.429	C18-H35	1.099	C3-C4-C7	119.8	N11-C12-C17	120.2	C18-N20-H37	119.5
C7-N9	1.333	N19-N21	1.432	C5-C4-C7	121.7	C13-C12-C17	120.3	N21-N19-H36	106.2
C8-C10	1.379	N19-H36	1.018	C4-C5-C6	120.9	C12-C13-C14	119.4	N19-N21-C22	110.4
C8-C18	1.494	N20-C22	1.393	C4-C5-H27	120.3	C12-C13-H30	119.3	N19-N21-H38	114.8
N9-N11	1.352	N20-H37	1.011	C4-C7-C8	129.5	C12-C17-C16	119.6	C22-N20-H37	115.1
C10-N11	1.361	N21-C22	1.401	C4-C7-N9	120.0	C12-C17-H34	120.6	N20-C22-N21	106.2
C10-H29	1.076	N21-H38	1.012	C6-C5-H27	118.9	C14-C13-H30	121.3	N20-C22-O23	127.6
N11-C12	1.423	C22-O23	1.209	C5-C6-H28	120.5	C13-C14-C15	120.7	C22-N21-H38	115.6
C12-C13	1.396			C8-C7-N9	110.4	C13-C14-H31	119.3	N21-C22-O23	126.3
				C7-C8-C10	104.7	C15-C14-H31	120.1		
				C7-C8-C18	129.1	C14-C15-C16	119.5		

C-Cl bonds have values consistent with their bond orders (C7-N9 = 1.333 Å, N11-C12 = 1.423 Å, C22-O23 = 1.209 Å for the carbonyl and C1-Cl24 = 1.758 Å). Based on the literature, the C-Cl bond length in aromatic systems typically ranges from 1.74 to 1.78 Å, which aligns well with DFT studies [53].

The optimized geometry indicates that the phenyl rings largely retain their aromatic character, with small changes in bond lengths and angles at substituted and bridging positions [54]. The endocyclic and exocyclic bond angles remain close to the ideal value of 120° for an aromatic ring, although local deviations occur near the substituted sites. For example, the C2-C1-C6 and C2-C3-C4 angles are 121.0° and 121.1°, respectively, whereas C3-C4-C5 is reduced to 118.5°. Larger deviations occur at the ring-bridge junctions, with C4-C7-C8 and C7-C8-C18 angles of 129.5° and 129.1°, respectively, reflecting geometric adjustment around the connecting groups.

The carbonyl C22-O23 bond length of 1.209 Å is consistent with a C=O double bond. The C18-N19 (1.488 Å), N19-N21 (1.432 Å), and N9-N11 (1.352 Å) distances reflect the different bonding environments within the heteroatom containing framework, with shorter N-N distances consistent with increased bond order or conjugative effects. The angles N19-C18-N20 (101.7°), C18-N19-N21 (104.9°) and N20-C22-O23 (127.6°) are consistent with the influence of hybridization and lone-pair interactions on the local geometry. Thus, the aromatic rings remain close to planar, while substitution and bridging groups introduce localized structural distortions that may influence the electronic distribution and molecular interactions of 5CPTO.

Biological interaction studies

Drug likeness and ADMET: The physico-chemical and pharmacological parameters of 5CPTO are summarized in Table-4. The compound has a molecular weight of 339.78 g/mol and a topological polar surface area (TPSA) of 70.98 Å², which falls within the range generally considered compatible with good membrane permeability [55]. It contains three

hydrogen-bond donors and three hydrogen-bond acceptors, satisfying the corresponding criteria of Lipinski's rule of five and supporting its drug-like physico-chemical profile.

5CPTO has a molar refractivity of 102.41 and a fraction of *sp*³-hybridized carbon atoms of 0.06, consistent with a predominantly aromatic and relatively rigid molecular framework. Three rotatable bonds provide a limited degree of conformational flexibility. The compound passed the Lipinski, Ghose, Veber, Egan and Muegge drug-likeness filters [56-60]. Its predicted bioavailability score of 0.55 further supports its suitability for continued pharmacological investigation. These computational descriptors place 5CPTO within commonly accepted drug-like chemical space and support its further experimental evaluation.

ADMET analysis: *In silico* ADMET analysis was performed using the pkCSM platform to assess the predicted pharmacokinetic and toxicity characteristics of 5CPTO (Table-5). The predicted water solubility was -3.547 log mol/L, while the Caco-2 permeability and intestinal absorption values were 0.825 log P_{app} (10⁻⁶ cm/s) and 93.6%, respectively. These values support the possibility of good gastrointestinal absorption. The predicted skin permeability of -2.735 log K_p suggests limited transdermal absorption. 5CPTO was predicted to be a P-glycoprotein substrate, indicating that transporter-mediated efflux could influence its cellular disposition.

The predicted VD_{ss} was -0.007 log L/kg, with plasma protein binding of 0.034. The predicted BBB permeability (log BB = -1.117) and CNS permeability (log PS = -2.22) point to limited passive CNS penetration. This property is an important consideration for a compound proposed for glioblastoma therapy. Although disruption of the blood-brain barrier can occur within glioblastoma-associated tumour regions, the extent to which this would increase 5CPTO exposure cannot be established from the present computational data. The predicted CNS distribution should, therefore, be considered a limitation requiring experimental investigation.

The metabolic predictions identified 5CPTO as a potential substrate of CYP2D6 and CYP3A4 and as a predicted inhibitor of CYP1A2, CYP2C19, CYP2C9, and CYP3A4, with no predicted inhibition of CYP2D6 [61,62]. These findings raise the possibility of CYP-mediated drug interactions that should be examined experimentally. The predicted total clearance was -0.036 log mL/min/kg, while 5CPTO was not predicted to be a substrate of the renal OCT2 transporter [63].

The predicted toxicity profile was favourable for several endpoints. 5CPTO was predicted to be negative in the Ames test and non-hepatotoxic, whereas hERG II inhibition was predicted and represents a potential cardiac-safety concern requiring experimental assessment. The predicted oral rat LD₅₀ was 2.421 mol/kg, the maximum tolerated clinical dose was 0.714 log mg/kg/day, and the chronic LOAEL was 2.558 log mg/kg/day. These predictions provide an initial assessment of the safety profile, but they cannot substitute for experimental toxicological studies [64,65]. The ADMET results support further investigation of 5CPTO, while its limited predicted CNS penetration and potential hERG liability remain important issues for lead optimization. Experimental studies are required to establish its pharmacokinetic behaviour, brain exposure, drug-interaction profile and safety.

TABLE-4
PHYSICO-CHEMICAL AND
DRUG-LIKE PROPERTIES OF 5CPTO

Physico-chemical properties	Values
Molecular weight	339.78 g/mol
Num. heavy atoms	24
Num. arom. heavy atoms	17
Fraction Csp3	0.06
Num. rotatable bonds	3
Num. H-bond acceptors	3
Num. H-bond donors	3
Molar Refractivity	102.41
TPSA	70.98 Å ²
Consensus Log P _{ow}	2.39
Log S (ESOL)	-4.03
Drug likeness	
Lipinski	Yes
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55

TABLE-5
ADMET PROFILE OF 5CPTO COVERING KEY PHARMACOKINETIC AND TOXICITY PARAMETERS

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-3.547	Numeric (log mol/L)
Absorption	Caco2 permeability	0.825	Numeric (log Papp in 10 ⁻⁶ cm/s)
Absorption	Intestinal absorption (human)	93.618	Numeric (% Absorbed)
Absorption	Skin permeability	-2.735	Numeric (log Kp)
Absorption	P-glycoprotein substrate	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution	VDss (human)	-0.007	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0.034	Numeric (Fu)
Distribution	BBB permeability	-1.117	Numeric (log BB)
Distribution	CNS permeability	-2.22	Numeric (log PS)
Metabolism	CYP2D6 substrate	Yes	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	Yes	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitor	Yes	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitor	Yes	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitor	Yes	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitor	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitor	Yes	Categorical (Yes/No)
Excretion	Total clearance	-0.036	Numeric (log mL/min/kg)
Excretion	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.714	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	Yes	Categorical (Yes/No)
Toxicity	Oral rat acute toxicity (LD ₅₀)	2.421	Numeric (mol/kg)
Toxicity	Oral rat chronic toxicity (LOAEL)	2.558	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)
Toxicity	Skin sensitization	No	Categorical (Yes/No)
Toxicity	<i>T. pyriformis</i> toxicity	0.35	Numeric (log µg/L)
Toxicity	Minnow toxicity	1.031	Numeric (log mM)

Molecular docking: According to the literature, pyrazole-based molecules are effective in treating cancer. Furthermore, the PASS prediction also confirms that the 5CPTO molecule exhibits antineoplastic (brain cancer) properties, as depicted in Table-6. Fibroblast growth factor receptors (FGFRs) play a crucial role in brain cancer, particularly in aggressive tumours like glioblastoma multiforme (GBM). Normally, FGFRs help regulate essential cellular processes, such as cell growth, development and blood vessel formation. However, when genetic mutations cause FGFR signalling to malfunction, it can drive tumour growth, disease progression and drug resistance in GBM. Excessive FGFR activity fuels tumour cell proliferation and survival, making FGFRs a promising

target for therapy. By inhibiting FGFRs, it's possible to reduce tumour growth and overcome drug resistance. As FGFRs are key players in critical signalling pathways, targeting them could be a game-changer in treating brain cancers, such as GBM. This highlights the need for therapeutic efforts focused on FGFRs to develop effective treatments [66,67]. The Ramachandran plot was used to evaluate the quality of these proteins [68,69], confirming their excellent stereochemical properties. Protein 7DTZ has 92.8% of its residues in favoured regions ϕ/ψ con-formational regions and 7.2% in generally allowed regions as shown in Fig. 5.

Molecular docking of 5CPTO with FGFR4 (PDB ID: 7DTZ) gave a calculated binding energy of -7.73 kcal/mol

TABLE-6
BIOLOGICAL ACTIVITY PREDICTED DATA OF 5CPTO USING PASS ONLINE TOOL

Pa	Pi	Activity
0.545	0.066	5-O-(4-Coumaroyl)-D-quinic acid 3'-monooxygenase inhibitor
0.529	0.028	CYP2A8 substrate
0.525	0.089	Glycosylphosphatidylinositol phospholipase D inhibitor
0.512	0.005	Antineoplastic (brain cancer)
0.453	0.039	HMGCS2 expression enhancer
0.433	0.185	Nootropic
0.421	0.115	Phthalate 4,5-dioxygenase inhibitor
0.417	0.074	Pterin deaminase inhibitor
0.403	0.100	Chloride peroxidase inhibitor

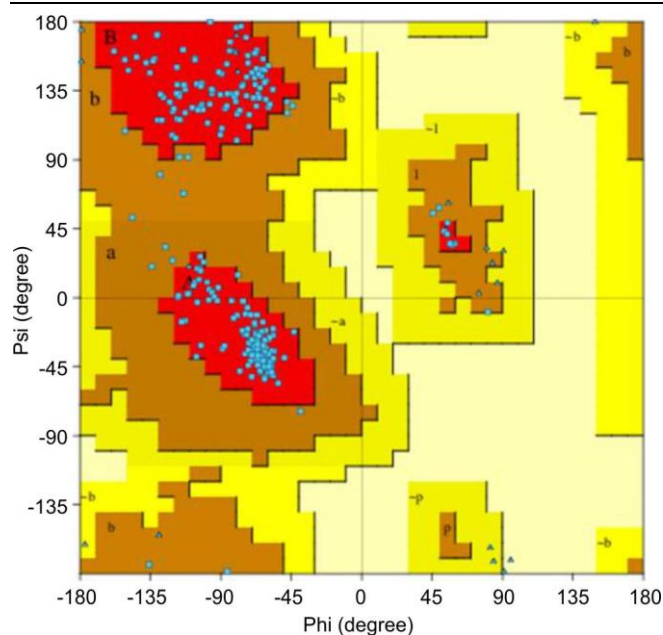


Fig. 5. Ramachandran plot of selected FGFR protein (7DTZ)

and an estimated inhibition constant (K_i) of 2.15 μM . The 3D and 2D interaction maps (Fig. 6) indicate that 5CPTO occupies the receptor binding cavity and is stabilized by hydrogen-bonding and π -type interactions (Table-7). Four hydrogen bonds were identified with GLU520 (2.36 Å), MET524 (2.54 Å), CYS552 (3.44 Å) and ASP630 (2.92 Å). A π -cation interaction with LYS503 and a π - σ interaction with PHE478 were observed, while alkyl and π -alkyl contacts involving ALA501, VAL481 and LEU619 provided additional non-covalent interactions. These interactions support the ability of 5CPTO to accommodate the FGFR4 binding site. The docking results provide a structural basis for further assessment of 5CPTO–FGFR4 interactions, which was subsequently examined through molecular dynamics simulations.

MD simulation: Molecular dynamics (MD) simulations were performed to examine the conformational stability and behaviour of the docked 5CPTO–FGFR4 complex, accounting for protein flexibility that is not fully represented by conventional docking using a static receptor structure. The best-ranked docked complex was subjected to a 100 ns simulation and root mean square deviation (RMSD), root mean

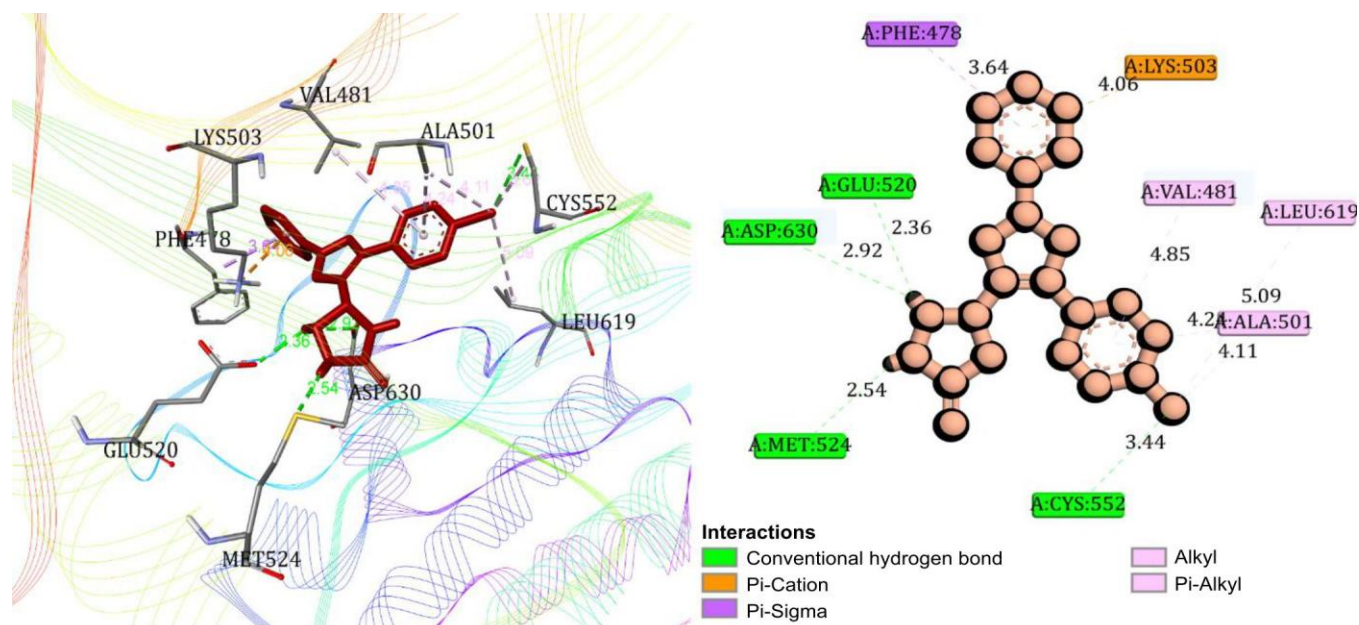


Fig. 6. 3D and 2D representations of the molecular docking interactions of the protein–ligand complex

TABLE-7
PROTEIN-LIGAND MOLECULAR DOCKING INTERACTION DETAILS WITH
BINDING ENERGY, INHIBITION CONSTANT AND INTERMOLECULAR ENERGY

Protein (PDB)	Binding energy (kcal/mol)	Inter molecular energy (kcal/mol)	Inhibition constant (μM)	Protein residues	Ligand groups	Type	Distance (Å)
7DTZ	-7.73	-8.63	2.15	ASP630	NH	Conventional hydrogen bond	2.92
				GLU520	NH	Conventional hydrogen bond	2.36
				MET524	NH	Conventional hydrogen bond	2.54
				CYS552	CCl	Conventional hydrogen bond	3.44
				LEU619	CCl	Alkyl	5.09
				ALA501	CCl	Alkyl	4.11
				ALA501	Ring	Pi-alkyl	4.23
				VAL481	Ring	Pi-alkyl	4.85
				LYS503	Ring	Pi-cation	4.06
				PHE478	Ring	Pi-sigma	3.64

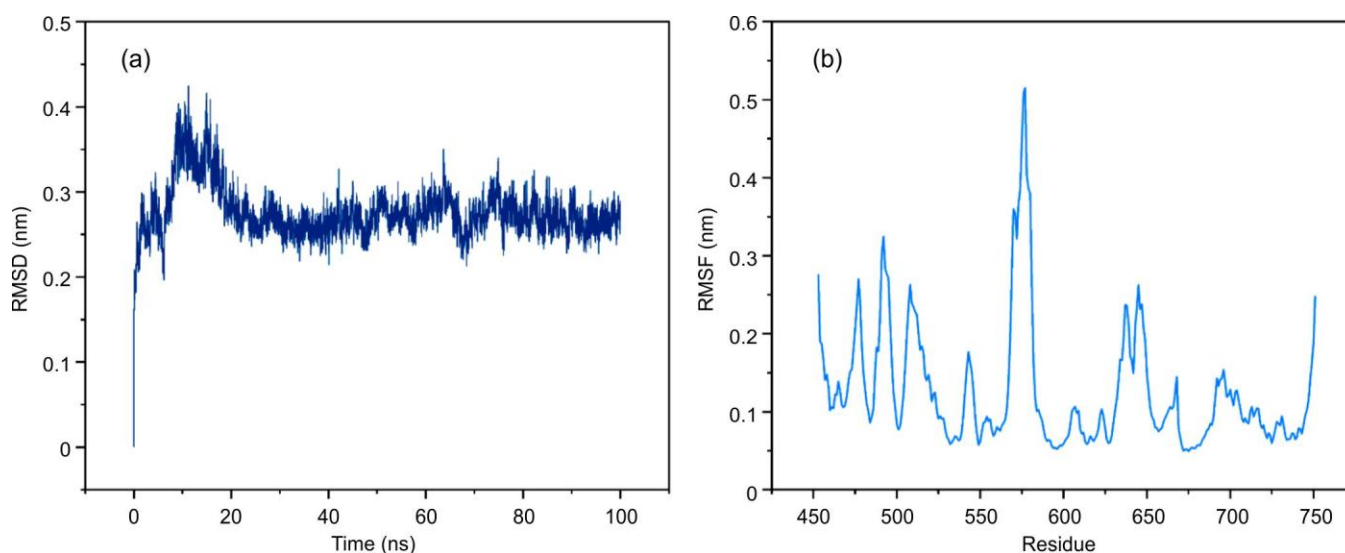


Fig. 7. (a) RMSD and (b) RMSF profiles of the protein–ligand complex

square fluctuation (RMSF) and radius of gyration (Rg) were analyzed to characterize the trajectory [70–72].

The RMSD profile (Fig. 7a) showed a rapid increase during the initial equilibration period, reaching approximately 0.25 nm within the first few nanoseconds and a maximum of 0.42 nm at around 11 ns. The RMSD subsequently decreased to approximately 0.27–0.30 nm and remained within this range from about 20 ns to the end of the simulation, with only minor fluctuations between 60 and 80 ns. The average RMSD of approximately 0.28–0.30 nm during the later phase suggests that the protein backbone reached a relatively stable conformational state after equilibration.

RMSF analysis (Fig. 7b) showed that most residues fluctuated within 0.05–0.20 nm, while higher mobility was observed in residues 575–577, where the RMSF reached approximately 0.44–0.51 nm. The residues involved in ligand interactions generally exhibited lower fluctuations, approximately 0.06–0.24 nm, consistent with comparatively restricted local mobility within the binding region. The higher fluctuations observed in selected loop regions are consistent with the higher flexibility commonly associated with such structural segments.

The radius of gyration (Rg) was used to monitor changes in the compactness of the protein during the simulation (Fig. 8). Rg values remained between approximately 2.06 and 2.10 nm during the first 15 ns, followed by a decrease to approximately 2.01–2.03 nm at 20–30 ns. From approximately 30 to 70 ns, the values remained relatively stable at 2.01–2.04 nm, with a brief decrease to around 2.00 nm near 60 ns. During the final 30 ns, Rg fluctuated between approximately 2.03 and 2.06 nm. The absence of sustained increases in Rg suggests that no major loss of compactness or unfolding occurred during

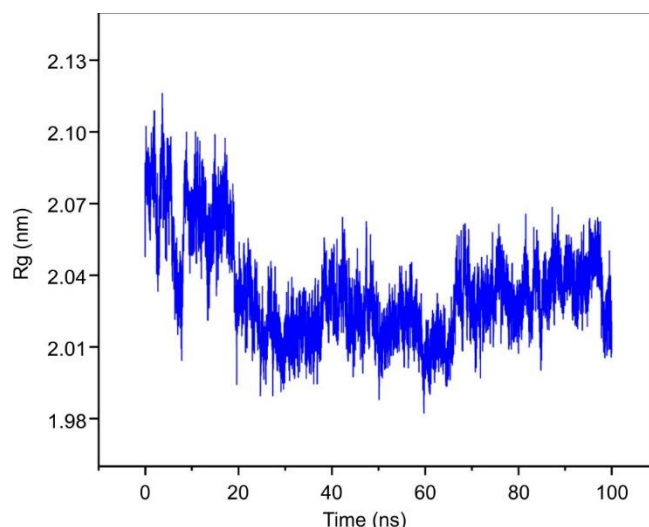


Fig. 8. Radius of gyration (Rg) profile of the protein–ligand complex

the 100 ns trajectory. The combined RMSD, RMSF and Rg profiles support the maintenance of a relatively stable and compact conformational state of the 5CPTO–FGFR4 complex throughout the latter part of the simulation.

MM-PBSA: The molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) method was used to estimate the binding free energy of the 5CPTO–FGFR4 complex by considering the major energetic contributions from van der Waals, electrostatic and solvation terms [73]. The calculated total binding free energy was –28.239 kcal/mol (Table-8), supporting a thermodynamically favourable interaction between 5CPTO and FGFR4 within the limitations of the MM-PBSA approach.

TABLE-8
MM-PBSA BINDING FREE-ENERGY COMPONENTS OF THE PROTEIN–LIGAND COMPLEX

Van der Waals interaction energy ($\Delta E_{VDWAALS}$) (kcal/mol)	Electrostatic interaction energy (ΔE_{EEL}) (kcal/mol)	Polar solvation energy (ΔE_{GB}) (kcal/mol)	Non-polar solvation energy (ΔE_{SURF}) (kcal/mol)	Gas-phase energy (ΔG_{GAS}) (kcal/mol)	Solvation energy (ΔG_{SOLV}) (kcal/mol)	Total binding free energy (ΔG_{TOTAL}) (kcal/mol)
-34.398	-9.17	19.823	-4.494	-43.568	15.328	-28.239

Van der Waals interactions provided the largest favourable contribution (-34.398 kcal/mol), while the electrostatic contribution was -9.17 kcal/mol. The non-polar solvation term contributed -4.494 kcal/mol to the calculated binding energy. The gas-phase energy was -43.568 kcal/mol, whereas the solvation contribution was +15.328 kcal/mol, partially offsetting the favourable gas-phase interactions. The resulting negative binding free energy suggests that van der Waals and electrostatic interactions, together with favourable non-polar contributions, contribute to the calculated stability of the 5CPTO-FGFR4 complex.

Electronic and reactivity studies

FMO analysis: The electronic behaviour of the 5CPTO molecule was analyzed using FMO concept. The computed HOMO and LUMO energies were -6.451 eV and -1.694 eV, respectively, giving an energy gap (ΔE) of 4.757 eV (Table-9). This moderate gap suggests that the compound maintains a suitable balance between chemical stability and reactivity, which indicate its potential role in charge-transfer interactions [74].

TABLE-9 HOMO-LUMO ENERGY VALUES AND GLOBAL DESCRIPTOR PARAMETER VALUES OF 5CPTO	
Parameters	Values
HOMO (eV)	-6.451
LUMO (eV)	-1.694
Ionization potential	6.451
Electron affinity	1.694
Energy gap (eV)	4.757
Electronegativity	4.072
Chemical potential	-4.072
Chemical hardness	2.379
Chemical softness	0.210
Electrophilicity index	3.486
Electronic charge	1.712
Electron donating capability (ω^-)	5.819
Electron accepting capability (ω^+)	1.747

The HOMO of 5CPTO is predominantly localized over the aromatic and heteroatom-containing regions, while the LUMO extends across the conjugated framework (Fig. 9). This distribution suggests the presence of electron-donating and

electron-accepting regions within the molecule and supports intramolecular charge-transfer characteristics. The calculated ionization potential (6.451 eV) and electron affinity (1.694 eV) are consistent with its electron-donating and electron-accepting abilities. The electronegativity (4.072 eV), chemical hardness (2.379 eV) and electrophilicity index (3.486 eV) indicate moderate resistance to electronic perturbation and a measurable tendency toward electron acceptance [75]. The calculated softness of 0.634 provides further information on the electronic reactivity of 5CPTO but should not be used alone to predict toxicity [76]. The electron-donating power ($\omega^- = 5.819$ eV) and electron-accepting power ($\omega^+ = 1.747$ eV) indicate a greater tendency toward electron donation. These FMO-derived descriptors provide useful information on the electronic characteristics and potential reactivity of 5CPTO.

MEP analysis: The molecular electrostatic potential (MEP) map of 5CPTO provides information on the distribution of electrostatic potential and the regions that may participate in intermolecular interactions (Fig. 10) [77,78]. The surface ranges from red, representing regions of relatively negative potential, to blue, representing relatively positive potential. The most pronounced negative potential is located around the carbonyl oxygen (O23), identifying this region as a likely site for electrophilic interactions. Positive potential is mainly distributed around selected nitrogen-bound and aromatic hydrogen regions, which may favour interactions with electron-rich sites. The chlorine atom (Cl24) exhibits comparatively weak electrostatic character.

The negative-potential regions around the carbonyl and heteroatom-containing groups are consistent with their ability to participate in hydrogen-bonding and electrostatic interactions. The relatively positive regions around aromatic hydrogens may contribute to π -associated and hydrophobic contacts during protein binding. Halogen-containing hydrophobic contacts are frequently observed in protein-ligand complexes and can contribute to ligand recognition and binding [79,80]. The MEP profile therefore provides a useful description of the potential interaction sites of 5CPTO and complements the docking results.

Fukui: Fukui functions were calculated to characterize the local reactivity of 5CPTO and to identify sites with higher susceptibility toward electrophilic and nucleophilic inter-

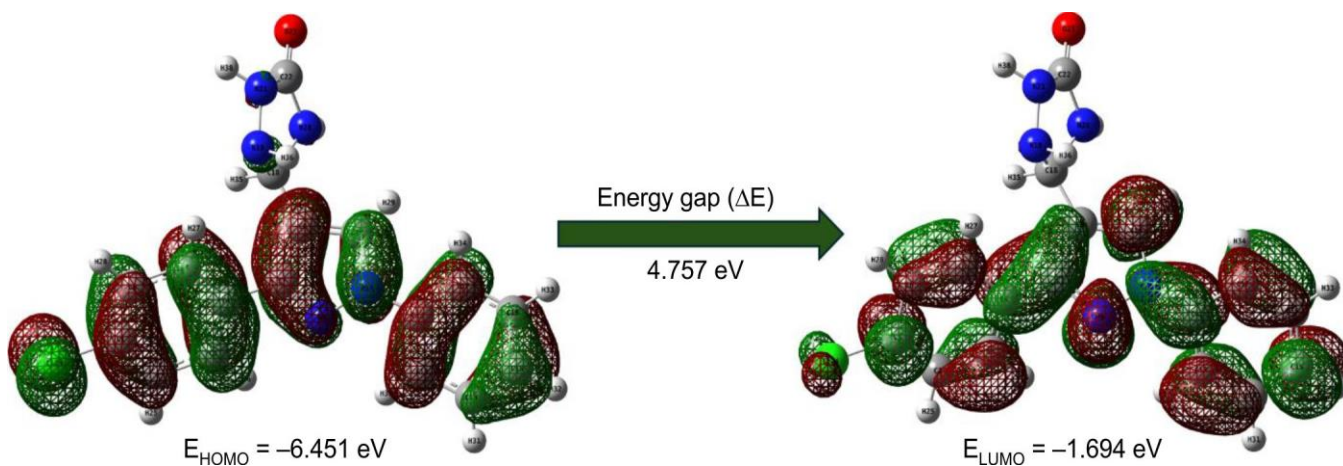


Fig. 9. HOMO-LUMO energy level diagram of 5CPTO

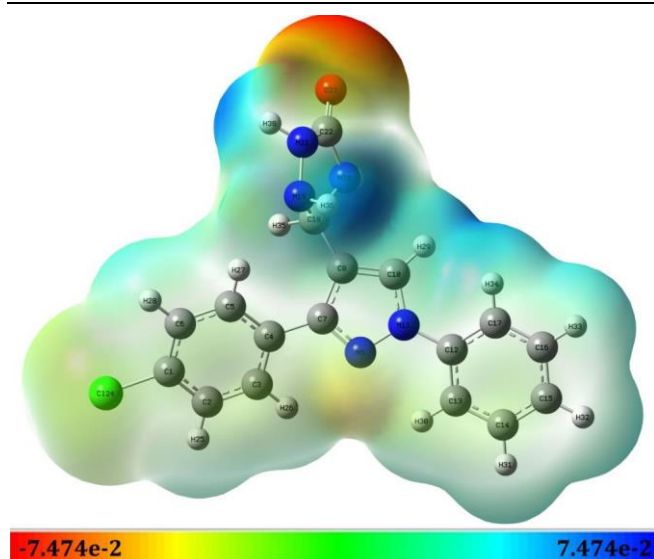


Fig. 10. Molecular electrostatic potential (MEP) map of 5CPTO

actions [81,82]. Unlike MEP analysis, Fukui functions provide a quantitative descriptor of site-specific chemical reactivity. Based on the calculated $\Delta f(r)$ values (Table-10), C22, C1, N19, C18, C5, C2, N20, O23, N21, C4, C8, N11 and Cl24 were identified as sites with greater electrophilic character, whereas N9, C10, C17, C12, C15, C14, C13, C16, C7, C6 and C3 exhibited higher nucleophilic character.

The carbonyl oxygen (O23) and nitrogen containing regions identified by the Fukui analysis are consistent with the hydrogen-bonding interactions observed in the docking study. The reactive aromatic carbon sites may contribute to π -associated and dispersion interactions within the binding

pocket. The Cl24 site may participate in non-covalent contacts, although its specific contribution to binding cannot be established from Fukui analysis alone. The Fukui results provide complementary information on the local electronic reactivity of 5CPTO and help rationalize some of the interaction patterns observed in the docking analysis.

Topological analyses

ELF and LOL: Topological analysis using the electron localization function (ELF) and localized orbital locator (LOL) was performed to examine electron localization and bonding characteristics in 5CPTO [83,84]. The ELF and LOL maps generated using Multiwfn are shown in Fig. 11. High ELF values are mainly observed around the C–C and C–N bonds, consistent with localized electron density associated with covalent bonding [85]. The N11 atom of the pyrazole ring exhibits localized electron density that may be associated with its lone-pair character. Slightly lower ELF values around the C1–Cl24 and C–H bonds indicate comparatively lower electron localization in these regions. The LOL distribution is consistent with the ELF analysis, with pronounced localization along the C–C, C–N and C–O bonding regions. The combined ELF and LOL profiles support the presence of localized covalent bonds together with electron delocalization across the conjugated framework. These results provide complementary information on the electronic structure of 5CPTO and its distribution of localized and delocalized electron density.

RDG: The combined view of the RDG isosurface and scatter plot (Fig. 12) shows a balanced presence of hydrogen bonds, van der Waals forces and steric interactions, which confirmed the structural stability and flexible binding abilities of molecule. The isosurface colour coding, blue, green

TABLE-10
HIRSHFELD CHARGES AND FUKUI DUAL-DESCRIPTOR PARAMETERS OF 5CPTO

Atoms	(0,1)	(1,2)	(-1,2)	f0	f+	f–	$\Delta f(r)$
N9	-0.151	-0.127	-0.206	-0.039	-0.023	-0.055	0.032
C10	0.059	0.107	-0.016	-0.061	-0.048	-0.075	0.027
C17	-0.002	0.044	-0.062	-0.053	-0.047	-0.060	0.013
C12	0.054	0.076	0.021	-0.028	-0.022	-0.033	0.011
C15	0.006	0.093	-0.091	-0.092	-0.087	-0.097	0.010
C14	0.016	0.066	-0.044	-0.055	-0.050	-0.060	0.010
C13	0.009	0.053	-0.045	-0.049	-0.044	-0.054	0.010
C16	0.013	0.066	-0.049	-0.057	-0.053	-0.062	0.009
C7	0.038	0.077	-0.003	-0.040	-0.040	-0.041	0.001
C6	0.010	0.065	-0.046	-0.056	-0.055	-0.056	0.001
C3	0.017	0.066	-0.031	-0.049	-0.049	-0.048	0.000
C22	0.207	0.216	0.199	-0.008	-0.009	-0.008	-0.001
C1	0.034	0.082	-0.012	-0.047	-0.048	-0.046	-0.002
N19	-0.022	-0.011	-0.031	-0.010	-0.011	-0.009	-0.002
C18	0.117	0.128	0.109	-0.010	-0.011	-0.009	-0.002
C5	0.000	0.046	-0.043	-0.045	-0.046	-0.044	-0.002
C2	0.011	0.063	-0.039	-0.051	-0.052	-0.050	-0.002
N20	-0.008	0.001	-0.012	-0.007	-0.009	-0.004	-0.005
O23	-0.334	-0.301	-0.361	-0.030	-0.033	-0.027	-0.005
N21	0.033	0.061	0.012	-0.025	-0.028	-0.021	-0.007
C4	-0.005	0.029	-0.031	-0.030	-0.034	-0.026	-0.008
C8	-0.044	0.001	-0.067	-0.034	-0.045	-0.023	-0.022
N11	0.023	0.069	0.003	-0.033	-0.046	-0.021	-0.026
Cl24	-0.081	0.031	-0.153	-0.092	-0.112	-0.072	-0.039

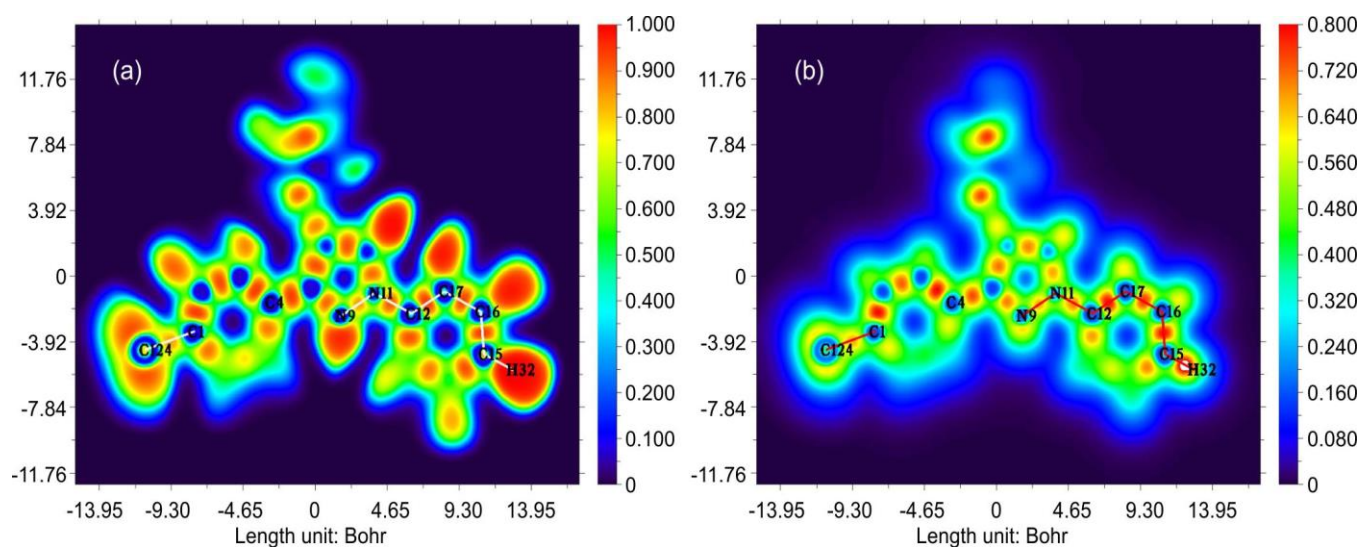


Fig. 11. (a) Electron localization function and (b) localized orbital locator maps of 5CPTO

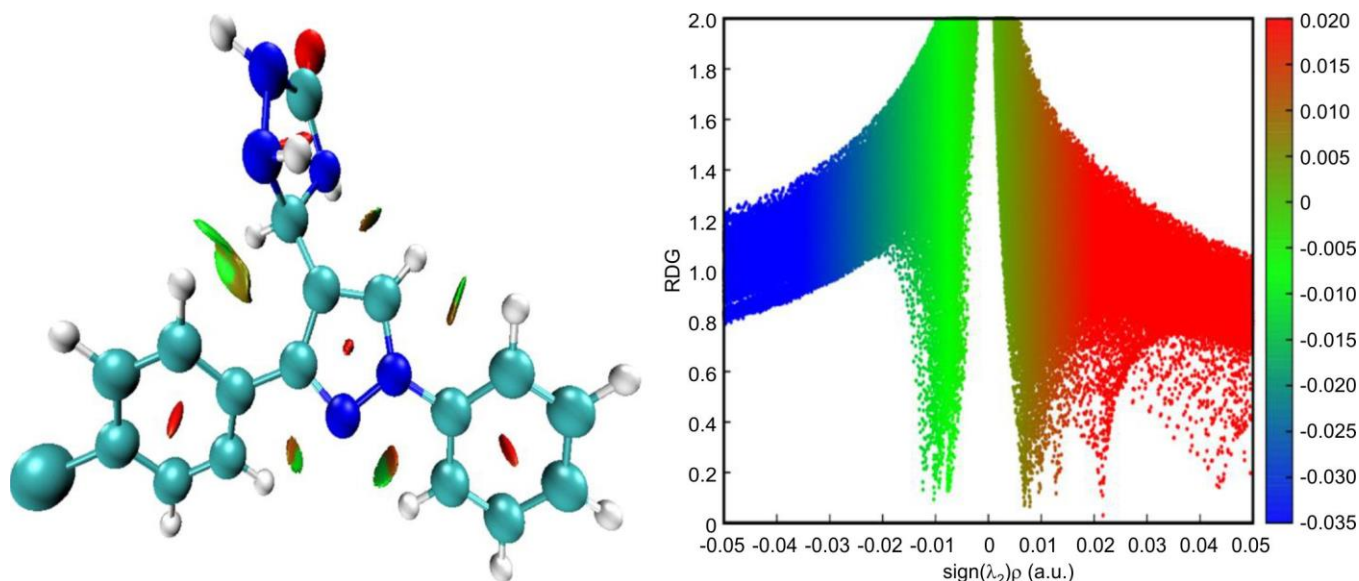


Fig. 12. Reduced density gradient of 5CPTO showing the non-covalent interaction surface (left) and corresponding 2D scatter plot (right)

and red, represents different interaction strengths [86]. Blue areas $(\lambda_2)\rho < 0$ indicate attractive hydrogen bonds and electrostatic forces, mainly around nitrogen and hydrogen atoms, confirming strong intramolecular H-bonding that stabilises the heterocyclic core. Green regions $(\lambda_2)\rho$ nearly 0 suggest weak van der Waals forces and π - π stacking, spread over aromatic and heteroaromatic rings, which are important for molecular planarity and π -conjugation. Red zones $(\lambda_2)\rho > 0$ reveal steric repulsion, primarily in the fused ring system, highlighting localized electron density crowding in π -electron rich areas [87]. The scatter plot supports these observations, with sharp red spikes (0.01 to 0.025 a.u.) indicating steric effects, green spikes (0.005 to 0.02 a.u.) representing weak dispersive interactions and broad blue regions showing attractive hydrogen bonds. The observed attractive, dispersive and repulsive interactions provide a balanced description of the non-covalent interaction profile of 5CPTO and contribute to its molecular stabilization.

Electronic population and bonding studies

NBO analysis: The bonding structure, electronic stability, charge distribution and conjugative interactions in the compound were studied using natural bond orbital (NBO) analysis [88]. $E(2)$ is a measure of conjugation; the higher the value, the stronger the electronic interactions [89]. This energy is manifested in the form of a ratio $E(2) = q_i [F_{(i,j)}^2 / (E_i - E_j)]$ and measures the degree of delocalization between donor (i) and acceptor (j) electron density. The equation involves the occupation of the orbital of the donors (q_i), orbital energies (E_i and E_j) and the elements of the Fock matrix ($F_{i,j}$). As a result, the $E(2)$ values allow the explanation of the phenomenon of hydrogen-bonding of molecules with each other and as well as an electron flow [90]. The NBO calculations are outlined in Table-11. The π - π^* delocalization between neighbouring aromatic and heteroaromatic rings contributes substantially to the stability of molecule, as evident from the

TABLE-11
NBO PARAMETERS SHOWING DONOR–ACCEPTOR INTERACTIONS, ORBITAL
OCCUPANCY AND STABILIZATION ENERGIES CALCULATED FOR 5CPTO

Donor	Type	Ed/e	Acceptor	Type	Ed/e	E(2) ^a (kcal/mol)	E(j)-E(i) ^b (a.u.)	F(i,j) ^c (a.u.)
C1-C6	π^*	0.389	C2-C3	π^*	0.303	174.960	0.020	0.080
C7-N9	π^*	0.410	C8-C10	π^*	0.352	173.760	0.010	0.070
N21-H38	σ	1.977	N21-C22	σ^*	0.089	131.960	3.830	0.646
N21-H38	σ	1.977	N21-H38	σ^*	0.018	70.400	3.540	0.446
N21-C22	σ	1.991	N21-C22	σ^*	0.089	67.000	4.010	0.472
C7-N9	π^*	0.410	C4-C5	π^*	0.368	59.370	0.030	0.063
N20	LP(1)	1.799	C22	LP*(1)	0.806	56.840	0.190	0.111
N19-N21	σ	1.987	N21-H38	σ^*	0.018	53.480	3.710	0.398
N21-H38	σ	1.977	C10-H29	σ^*	0.014	48.530	0.890	0.186
N21	LP(1)	1.824	C22	LP*(1)	0.806	46.890	0.200	0.104
N21-C22	σ	1.991	C10-H29	σ^*	0.014	43.640	1.060	0.192
N19-N21	σ	1.987	C10-H29	σ^*	0.014	41.620	1.060	0.187
N11	LP(1)	1.516	C8-C10	π^*	0.352	38.020	0.290	0.097
N21-H38	σ	1.977	C17-H34	σ^*	0.013	37.570	0.930	0.168
N19-N21	σ	1.987	N21-C22	σ^*	0.089	35.120	4.000	0.341
N21-C22	σ	1.991	C17-H34	σ^*	0.013	34.290	1.110	0.174
N19-N21	σ	1.987	C17-H34	σ^*	0.013	29.770	1.100	0.162
N11	LP(1)	1.516	C7-N9	π^*	0.410	28.910	0.280	0.082
N21-C22	σ	1.991	C22-O23	σ^*	0.012	28.640	3.260	0.273
C8-C10	π	1.792	C7-N9	π^*	0.410	27.940	0.280	0.083
N21	LP(1)	1.824	N21-C22	σ^*	0.089	27.700	3.530	0.286

^aE(2) Energy of Hyper-conjugative interaction; ^bEnergy difference between donor and acceptor I and j NBO orbitals; ^cF(i,j) Fock matrix element between I and j NBO orbitals.

high stabilization energies of 174.96 and 173.76 kJ/mol for interactions such as $\pi^*(\text{C1-C6})$ to $\pi^*(\text{C2-C3})$ and $\pi^*(\text{C7-N9})$ to $\pi^*(\text{C8-C10})$, respectively. The $\sigma(\text{N21-H38})$ to $\sigma^*(\text{N21-C22})$ transition, with a stabilization energy of 131.96 kJ/mol, reflects strong hyperconjugation at the triazolidinone segment.

Lone pair interactions also play a significant role in enhancing molecular stability, as exemplified by LP(N20) to LP*(C22) and LP(N21) to LP*(C22) with stabilization energies of 56.84 kJ/mol and 46.89 kJ/mol, respectively. Furthermore, the interaction $\sigma(\text{N21-H38})$ to $\sigma^*(\text{C10-H29})$ (48.53 kJ/mol) highlights the importance of N-H hyperconjugation in electron delocalization. The LP(N11) to $\pi^*(\text{C8-C10})$ transition (38.02 kJ/mol) demonstrates intramolecular charge transfer from pyrazole nitrogen to the conjugated π -system, facilitating electron density redistribution across the heterocyclic core. The $\sigma^*(\text{C22-O23})$ interaction, involving the carbonyl oxygen, with a stabilization energy of 28.64 kJ/mol, confirms conjugative effects that further stabilise the triazolidinone ring through delocalized charge transfer. The bonding orbital occupancies are approximately 1.97-1.99 Ed/e for σ - and π -bonds, while nitrogen lone pairs show slightly lower values (1.52 to 1.82), indicating active electron donation to anti-bonding orbitals. However, the stability of 5CPTO is primarily

governed by robust π - π^* delocalization across the aromatic and heteroaromatic rings, complemented by LP*- σ^* and LP- π^* hyperconjugative interactions mainly involving the N21-C22-O23 segment. These donor–acceptor interactions contribute to electron delocalization within 5CPTO and provide insight into its electronic structure and reactivity.

Charge transfer study: The electron–hole analysis was performed to examine the changes in electron and hole distributions following electronic excitation and the resulting charge redistribution in 5CPTO [91]. The parameters of the first three excited states are listed in Table-12 and the corresponding hole (blue) and electron (green) distributions are shown in Fig. 13. For the first excited state, the excitation energy was 4.289 eV, with a D index of 1.302 Å and a Δr value of 1.302 Å, which indicate a moderate separation between the electron and hole distributions. The electron and hole densities were mainly localized over the pyrazole and aromatic regions, suggesting localized intramolecular charge transfer with some delocalization across the heterocyclic framework.

The second excited state had an excitation energy of 4.731 eV, with a D index of 2.884 Å and a Δr value of 1.969 Å. The larger D index indicates higher spatial separation between the electron and hole centroids in this state. The third

TABLE-12
CHARGE TRANSFER (ELECTRON-HOLE) VALUES OF THE FIRST THREE EXCITED STATES USING TD-DFT METHOD

Parameters	First excited state	Second excited state	Third excited state
Excitation energy E (eV)	4.289	4.731	4.747
Charge transfer length D index (Å)	1.302	2.884	0.68
Δr index (Å)	1.302	1.969	2.378
t index (Å)	-1.959	-0.918	-2.372

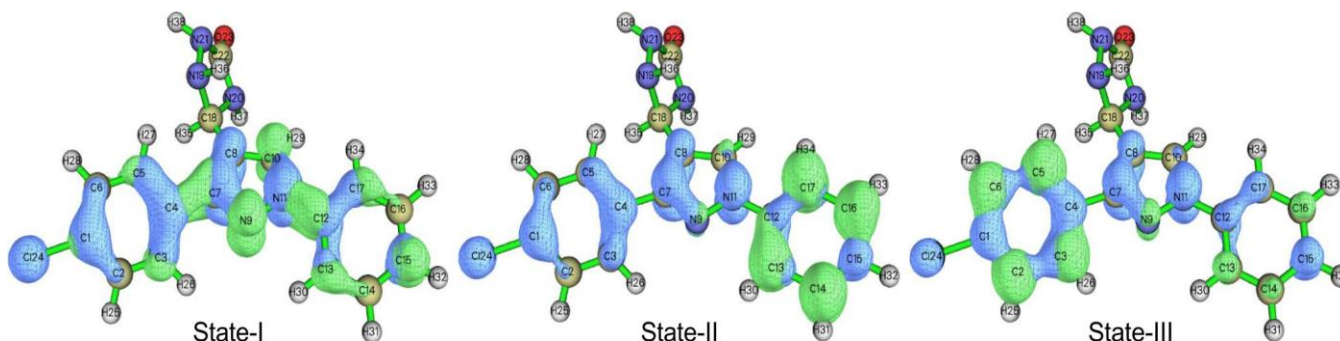


Fig. 13. TD-DFT-based charge-transfer distribution of 5CPTO across the first three excited states

excited state, with an excitation energy of 4.747 eV, showed a D index of 0.680 Å and a Δr value of 2.378 Å, with the electron and hole densities mainly distributed over the aromatic and pyrazole regions. The differences in D and Δr values among the three excited states indicate variations in the extent of charge redistribution following excitation. These results provide insight into the excited-state electronic behaviour and intramolecular charge-transfer characteristics of 5CPTO.

Conclusion

The molecular structure, electronic, spectroscopic, physico-chemical, and biological properties of 5-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-triazolidin-3-one (5CPTO) were investigated using DFT and complementary computational methods. The optimized geometry and the close agreement between calculated and experimental FT-IR and NMR data support the structural assignment and the reliability of the computational analysis. FMO, NBO, MEP, Fukui function and topological analyses provided information on electron delocalization, electronic reactivity and potential interaction sites within the molecule. The predicted pharmacokinetic and ADMET profiles were consistent with drug-like properties, good intestinal absorption, non-mutagenic behaviour and low predicted hepatotoxicity. Molecular docking with FGFR4 (PDB ID: 7DTZ) gave a favourable calculated binding interaction involving hydrogen bonds, π -cation, hydrophobic and other non-covalent contacts. The 100 ns molecular dynamics simulation maintained a relatively stable 5CPTO–FGFR4 complex, while MM-PBSA calculations yielded a favourable estimated binding free energy. The computational results provide a basis for further investigation of 5CPTO as an FGFR-interacting compound with possible relevance to anti-glioblastoma research and nonlinear optical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no

data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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