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Design, ADME, Molecular Docking, And Molecular Dynamics Simulation Study Of Tyrosine Derivatives Of Isatin – Isatin-Para-Aminobenzoic Acid Conjugates.

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ABSTRACT

Twenty eight novel isatin-Para-Aminobenzoic Acid Schiff bases have been screened for their potential usefulness against several propitious cancer targets. Molecular docking was implemented using the crystalline structure of three possible target receptors, wherein the binding affinity of the compounds were established by docking with the target binding site. Computer predictions were made using MOE 2024.06, and all ligands were drawn using ChemDraw version 22.0.0. In silico ADME prediction studies unveiled the remarkable prospects for receptor interaction, and the drug-likeness properties were estimated utilizing the Swiss ADME website. Furthermore, Molecular Dynamics simulations of ligand 5 docked at the active site of 6QNG (Carbonic anhydrase 12) were executed using Desmond-v7.9 tool of the Schrodinger Suite for 50 ns, computing RMSD, RMSF, Ligand-Protein Contacts, and Ligand Torsion Profile results. Findings indicate the most effective binding energy within the receptor pocket that demonstrates potential activity against the Carbonic anhydrase 12 receptor. Compound 11 gives the highest binding affinity of – 10.94 kcal/mol with Carbonic anhydrase 12 and good scores of -9.45 and -8.57 with Carbonic anhydrase and Dihydrofolate reductase, respectively. The majority of compounds were found to obey Lipinski's rule of five, with superior absorption from the alimentary tract, two-thirds of the compounds are not substrates for P-glycoprotein, and all ligands don't penetrate the blood-brain barrier. Molecular dynamics simulations indicate a Mean Protein RMSD of 1.6 Å, a ligand RMSD of 3.5 Å, and the RMSF analysis shows that the protein residues that interact with the ligand stay within a distance of under 1 Å.
Keywords: isatin, tyrosine derivatives, ADME, molecular docking.

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INTRODUCTION

From a Global standpoint, cancer accounts for the second-major cause of mortality behind cardiovascular diseases. Malignancy-linked fatalities have risen sharply in the last decade. In the dearth of efficacious management, tumor-linked deaths would double in the following few years. [1] Cancer is an ailment characterized by the atypical growth of cells that can disseminate to or attack encircling tissues and organs. Despite the considerable number of subsisting cancer remedies, chemotherapy stay the principal avenue to cancer care. Nevertheless, chemotherapy has a number of drawbacks, such as inadequate effectiveness, a lack of selectivity, high costs, Opportunities for genotoxicity, and the evolution of drug resistance. Additionally, it provokes copious side effects due to destruction to normal body cells and organ toxicity, which in turn diminishes the wellness for patients with cancer. [2,3]

Schiff bases are a significant category of organic compounds which are discerned for their assorted pharmacological properties, including antibacterial, anti-inflammatory, antipyretic, antifungal, and antimalarial effects, along with their utilizations in dyes, pigments, precursor materials, intermediates in organic synthesis, catalysts, and stabilizers for polymers. The biological activities ascribed to Schiff bases are largely due to the imine group, which can be regulated by amending the functional groups in the molecules. Moreover, Schiff bases are considered as splendid ligands since the imine groups make chelated complexes with metal ions, demonstrating a firm affinity for transition metal ions.[4-6]

Isatin (1H-indole-2,3-dione). is a bicyclic aromatic heterocyclic compound. Isatin and its derivatives are a valuable modern category of heterocyclic molecules with many substantial activities and are well-endured in humans. Isatin has been stated to have various biological activities. Many isatin-scaffold based compounds have been synthesized and shown to own distinctive pharmacological effects, like anti-HIV, antimicrobial, anti-tubercular, antiviral, antitumor, antioxidant, anticonvulsant, anti-inflammatory, Analgesic, Anti-fungal and CNS depressant activities. [7-9]

There has been a growing need for the progress and discovery of novel antineoplastic substances with unique and new targets and a new molecular structure that may help to mitigate the persistently increased cases of cancer-mediated death. [10]

Computational Method

The structures of proposed Schiff base derivatives are illustrated in the table. 1. To assess the cytotoxic effect of these compounds, an in silico molecular docking (MOE) was conducted to evaluate their interaction with three proteins (5FL5, 6QNG, 5SDB). The crystal structures of macromolecular proteins were protonated using the Amber99 force field. ADME (absorption, distribution, metabolism, and excretion) properties were predicted employing the Swiss ADME tool of Swiss Drug Design Server to ensure drug-likeness and pharmacokinetic compatibility of the compounds. Desmond-v7.9 was utilized to implement Molecular Dynamics simulations to get insights into their molecular behavior. Simulations were run for 50 ns under physiological conditions, during which binding mechanisms and conformational changes in the ligand-protein complexes could be analyzed. Computations were based on the structure of the three proteins (5FL5, 6QNG, 5SDB). It includes the discovery and optimization of potent and selective compounds for pharmaceutical use based on the three-dimensional structure of biological target molecules using docking algorithms. (11, 12).

Preparation Of Ligands

The molecular structures were depicted using ChemDraw Ultra 22.0.0, then all ligands were saved as SMILES files to be opened in MOE for structure preparation, where these were protonated 3D at a temperature of 300 ˚C and pH 7 and energy minimized with MOE, applying default parameters. The MMFF94x force field was applied with no periodicity, and the constraints were kept at the rigid water molecule level. [13-15]

Preparation Of Proteins

The proteins crystal structures were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) at http://www.rcsb.org/pdb/home/home.do. The crystallographic water molecules, except those involved in ligand-protein interactions as a water bridge

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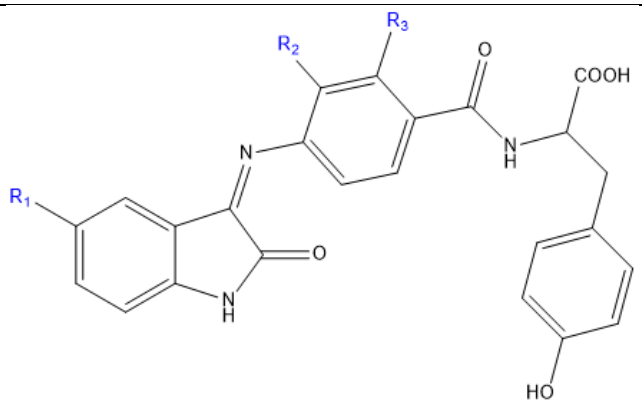
were removed. The protein structure was 3D protonated, and then energy minimization was performed utilizing the MOE software with default parameters. In the molecular docking procedure, receptors and solvent atoms were subjected, and polar hydrogens were added. During the docking process, the ligand atom was selected, rescoring1 was set at London dG and rescoring2 at GBVI/WSA dG, running to record the ligand interaction with the protein. [13-15]

Table 1: Chemical structure of the designed compounds



Ligand ID	R1	R2	R3
1	H	H	H
2	H	H	Me
3	H	H	OMe
4	H	Cl	H
5	Me	H	H
6	Me	H	Me
7	Me	H	OMe
8	Me	Cl	H
9	OMe	H	H
10	OMe	H	Me
11	OMe	H	OMe
12	OMe	Cl	H
13	NO_2	H	H
14	NO_2	H	Me
15	NO_2	H	OMe
16	NO_2	Cl	H
17	F	H	H
18	F	H	Me
19	F	H	OMe
20	F	Cl	H
21	Cl	H	H
22	Cl	H	Me
23	Cl	H	OMe
24	Cl	Cl	H
25	Br	H	H
26	Br	H	Me
27	Br	H	OMe
28	Br	Cl	H

In silico drug-likeness and ADMET prediction

The physicochemical properties and drug-likeness of target ligands were estimated utilizing the SwissADME website http://www.swissadme.ch/index.php (16). While the drug-likeness scores were calculated using the Molsoft LLC website https://www.molsoft.com/mprop/ (17).

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Molecular Dynamics Simulation

Molecular dynamics (MD) simulations, an improved method for studying macromolecular ligand-receptor interactions, provide dynamic insight beyond the static nature of molecular docking. Ligand 5 was selected for MD simulations based on docking results. The system was carried out using the SPC liquid sample in a 10 Å box with an OPLS4 charge neutralized with 0.15 M NaCl at neutral PH. Simulations were run for 50 nanoseconds at 300 kV to investigate bond dynamics and stability.

RESULTS AND DISCUSSION

The goal is to create effective substances that demonstrate selectivity and possess optimal ADME (absorption, distribution, metabolism, and excretion) characteristics.

Molecular Docking

Molecular docking is a computational simulation approach that scrutinizes the optimal binding pose of a ligand within the target's binding site. Negative values of binding affinities indicate strong binding, with more negative magnitudes representing stronger binding and more favorable conformation of the complex formed. Furthermore, the molecular docking simulation was conducted to affirm the anticancer effectiveness of the designed isatin-para aminobenzoic acid derivatives by exploring binding means along with the situation of ligands in the binding pocket of the screened targets. The obtained poses were sorted as reported by their score excellence, and Table 2 reveals binding affinities of the superior pose of each one of the 28 designed ligands with the carbonic anhydrase 9 (5FL5), carbonic anhydrase 12 (6QNG), and human dihydrofolate reductase (5SDB). [18] The molecular docking outcomes table (2) highlights that compounds 3, 7, 11, 13, 15, 18, 22, and 25 show good binding affinities across multiple target proteins, suggesting these ligands are promising candidates for further experimental validation. Among the three target proteins tested, the best binding scores were recorded for 6QNG with seven ligand scores more than -10 kcal/mol, of which ligand 11 gives the highest score of -10.94 kcal/mol. The lowest binding affinities gained were for 5SDB, with the majority of ligands scoring less than -9.00 kcal/mol, of which ligand 2 displays the lowest score of -8.12.

Table 2: The binding scores (kcal/mol) of the 28 ligands with carbonic anhydrase 9 (5FL5), carbonic anhydrase 12 (6QNG), and human dihydrofolate reductase (5SDB).

ligands	proteins				ligands	proteins		
	5FL5	6QNG	5SDB			5FL5	6QNG	5SDB
1	-9.65	-9.13	-8.45		**15**	-9.76	-9.90	-8.92
2	-9.37	-9.86	-8.12		**16**	-9.99	-9.74	-9.16
3	-9.72	-10.11	-8.82		**17**	-9.65	-9.86	-8.86
4	-9.89	-9.31	-8.37		**18**	-9.60	-10.23	-8.20
5	-9.78	-9.58	-8.20		**19**	-8.61	-8.69	-8.65
6	-9.19	-9.50	-8.60		**20**	-9.57	-9.49	-8.47
7	-9.91	-10.42	-9.12		**21**	-9.75	-8.87	-8.48
8	-9.76	-9.54	-8.48		**22**	-9.05	-10.29	-8.65
9	-9.44	-9.32	-8.81		**23**	-10.07	-9.76	-8.52
10	-9.44	-9.42	-8.62		**24**	-9.99	-9.83	-8.83
11	-9.45	-10.94	-8.57		**25**	-9.78	-10.06	-8.77
12	-9.84	-9.42	-8.50		**26**	-9.23	-9.09	-8.58
13	-9.95	-10.24	-8.73		**27**	-9.64	-9.24	-8.44
14	-9.09	-9.72	-8.97		**28**	-8.81	-9.61	-8.66

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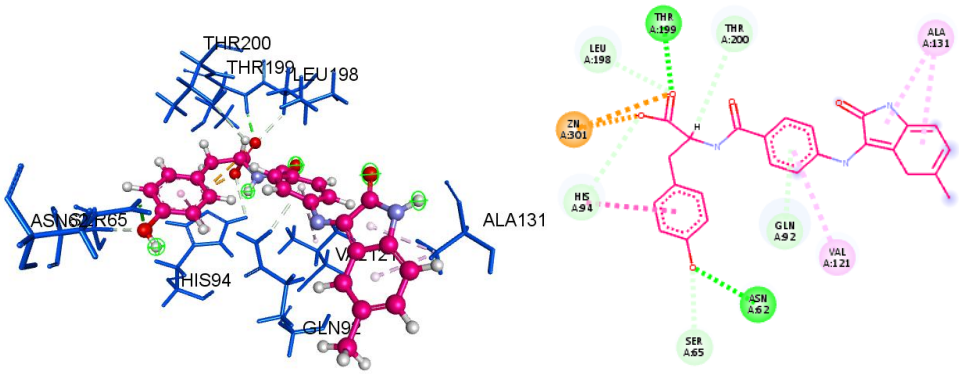

Fig. 1: 3D (A) and 2D of ligand 11 pose with carbonic anhydrase 12

ADME Prediction

The choice of modern drugs and designing their proper formulation type are principally conditional on the physicochemical properties, which encompass the hydrogen-bonding capability, solubility, molecular weight (MW), topological polar surface area (TPSA), and molar refractivity (MR); hence, the physicochemical properties (Table 3) are the crucial aspects for obtaining modern drug candidates and must align with the drug-likeness rules like Lipinski's rule, Muegge's rules, Ghose's filter, Veber's filter, Pfizer rule for CNS activity, Egan's filter and lead-like rule which are critical during the early stages of drug discovery process enabling the assessment whether the compound under study is acceptable as drug-like molecule. According to these guidelines, we determined that the majority of Schiff base ligands comply with the four drug-likeness principles, and these derivatives qualify as potential new drug candidates (19).

Table (3) The physicochemical properties, drug-likeness, and drug-likeness scores of the designed compounds

ID	GI	BBB	Pgp	BS	MWT	nHBA	nHBD	TPSA(Å)	iLOGP	WLOGP	nLV
1	High	No	No	0.56	429.42	6	4	128.09	1.53	2.32	0
2	High	No	Yes	0.56	443.45	6	4	128.09	1.99	2.63	0
3	High	No	Yes	0.56	459.45	7	4	137.32	1.73	2.33	0
4	High	No	No	0.56	463.87	6	4	128.09	2.15	2.97	0
5	High	No	Yes	0.56	443.45	6	4	128.09	2.02	2.63	0
6	High	No	Yes	0.56	457.48	6	4	128.09	1.98	2.94	0
7	High	No	Yes	0.56	473.48	7	4	137.32	1.86	2.64	0
8	High	No	No	0.56	477.90	6	4	128.09	1.68	3.28	0
9	High	No	Yes	0.56	459.45	7	4	137.32	2.08	2.33	0
10	High	No	Yes	0.56	473.48	7	4	137.32	2.23	2.64	0
11	Low	No	Yes	0.56	489.48	8	4	146.55	1.90	2.34	0
12	Low	No	No	0.56	493.90	7	4	137.32	2.21	2.98	0
13	Low	No	No	0.11	474.42	8	4	173.91	1.50	2.23	0
14	Low	No	No	0.11	488.45	8	4	173.91	1.51	2.54	1
15	Low	No	No	0.11	504.45	9	4	183.14	1.29	2.24	1
16	Low	No	No	0.11	508.87	8	4	173.91	1.74	2.88	1
17	High	No	No	0.56	447.42	7	4	128.09	1.59	2.88	0
18	High	No	No	0.56	461.44	7	4	128.09	1.95	3.19	0
19	Low	No	Yes	0.56	477.44	8	4	137.32	1.66	2.89	0
20	High	No	No	0.56	481.86	7	4	128.09	1.55	3.53	0
21	High	No	No	0.56	463.87	6	4	128.09	2.09	2.97	0
22	High	No	No	0.56	477.90	6	4	128.09	2.11	3.28	0
23	Low	No	No	0.56	493.90	7	4	137.32	1.91	2.98	0
24	High	No	No	0.56	498.31	6	4	128.09	1.89	3.63	0
25	High	No	No	0.56	508.32	6	4	128.09	1.99	3.08	1
26	High	No	No	0.56	522.35	6	4	128.09	2.01	3.39	1

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27	Low	No	No	0.56	538.35	7	4	137.32	2.26	3.09	1
28	High	No	No	0.56	542.77	6	4	128.09	2.15	3.74	1

Molecular Dynamics Simulation

The 6QNG (Carbonic anhydrase 12) protein, selected depending upon the results of docking at the same time, is a key mediator in the pH adjustment of the cancer microenvironment, and its elevated expression has been found in several human cancers. It represents a key enzymatic protein worried in biological pathways, making it a prime target for designing inhibitors (20). Molecular dynamics (MD) simulation turned into accomplished to evaluate the binding balance and interaction profile of the compound 5 with the 6QNG protein. Simulation parameters blanketed an NPT ensemble at 300 K for 50 ns.

The RMSD

The Root Mean Square Deviation (RMSD) of the protein-ligand complex turned into calculated to evaluate the overall system balance. The RMSD values of the ligand and protein stabilized after the preliminary equilibration phase (~10 ns), suggesting that the device reached a regular state. This displays minimal conformational changes inside the protein-ligand complex at some stage in the simulation, indicative of stable binding.

Protein RMSD

The protein's RMSD values remained in the suitable range (below 2 Å), confirming the structural stability of the protein throughout the MD simulation. These values advise that the protein's conformational flexibility did not exceed standard thermal fluctuations, ensuring the binding site maintained its geometry fig (2).

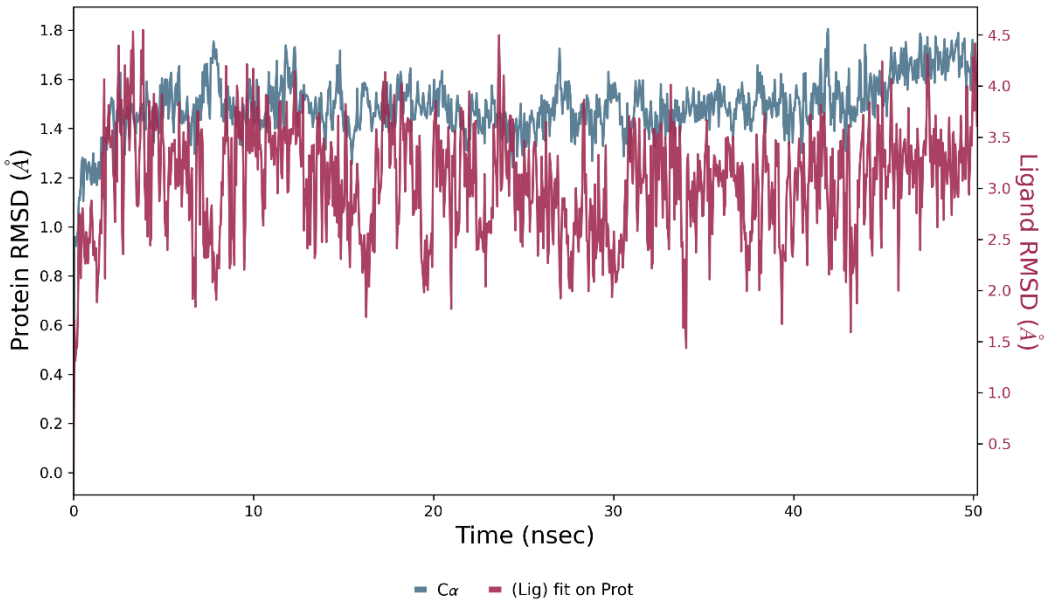

Figure 2: protein and ligand RMSD

Ligand RMSD

The ligand RMSD, measured relative to the protein backbone, stabilized at 3.5 Å, demonstrating its relatively constant positioning inside the binding pocket. This implies that the ligand 5 did no longer showed off vast glide and maintained favorable interactions with the 6QNG protein fig (2).

Protein RMSF

The Root Mean Square Fluctuation (RMSF) of protein residues indicated better flexibility in loop areas and terminal ends, as predicted. However, residues worried in ligand binding showed minimum fluctuations, confirming their role in strong interactions with the 4b compound fig (3).

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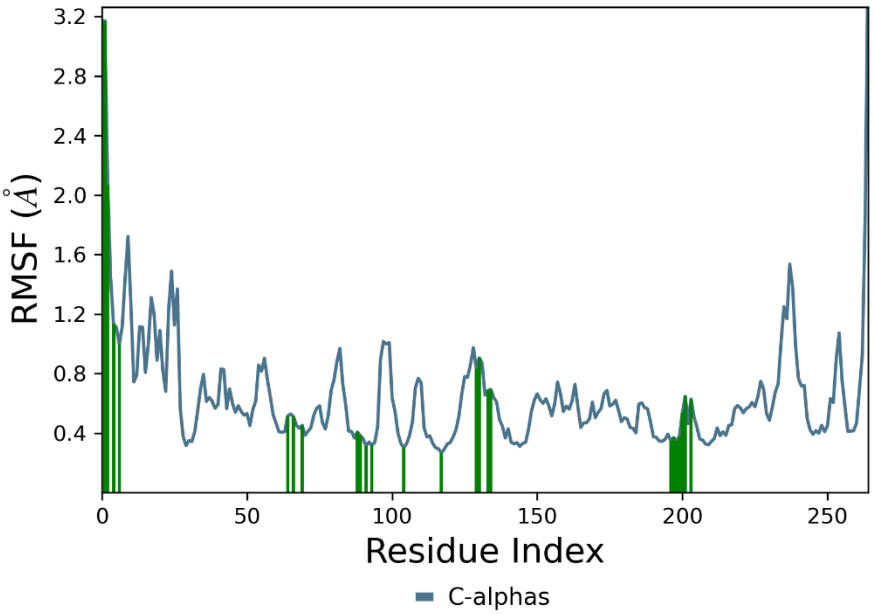

Figure (3); RMSF of protein

Ligand RMSF

The RMSF values for compound 5's atoms remained low in the course of the simulation, suggesting a strong binding conformation within the protein's active site. This stability is indicative of strong protein-ligand interactions, which might be critical for the compound's inhibitory activity fig (4).

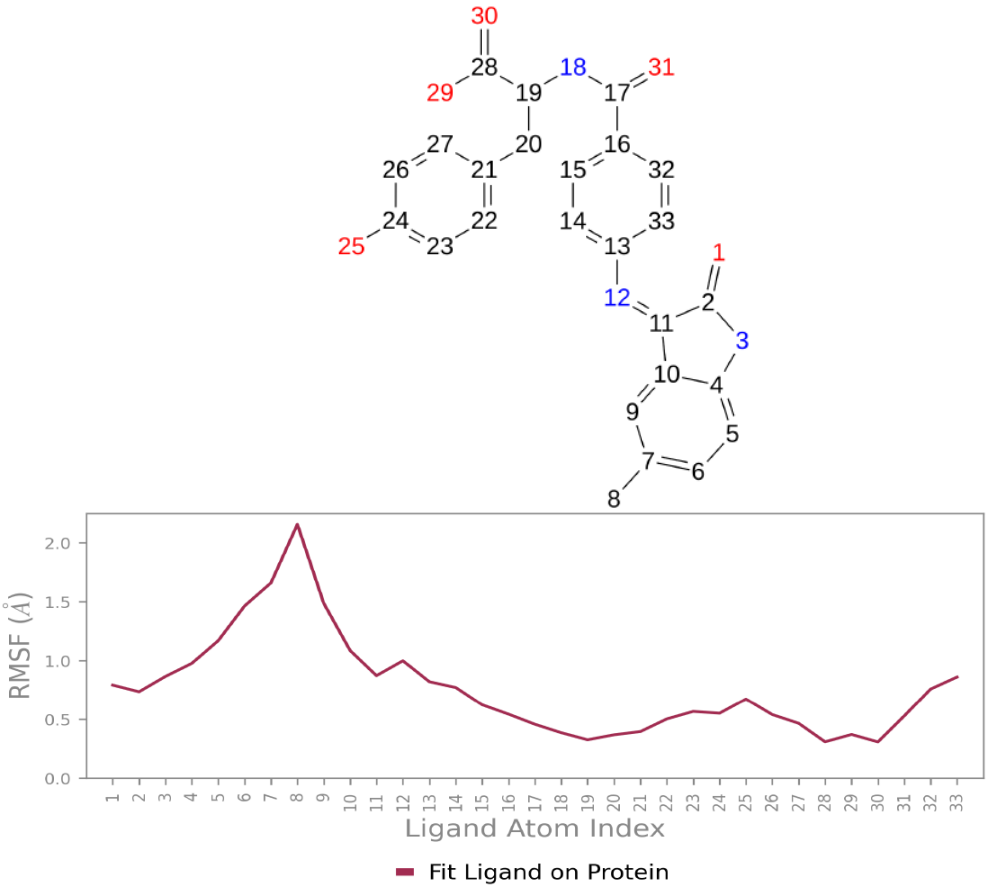

Fig (4): ligand RMSF

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Ligand-Protein Contacts

Ligand RMSF analysis discovered constrained atomic fluctuations, mainly for atoms engaged in essential hydrogen bonding and hydrophobic interactions. This shows that the ligand continues an inflexible conformation whilst bound, reducing entropic consequences all through binding.

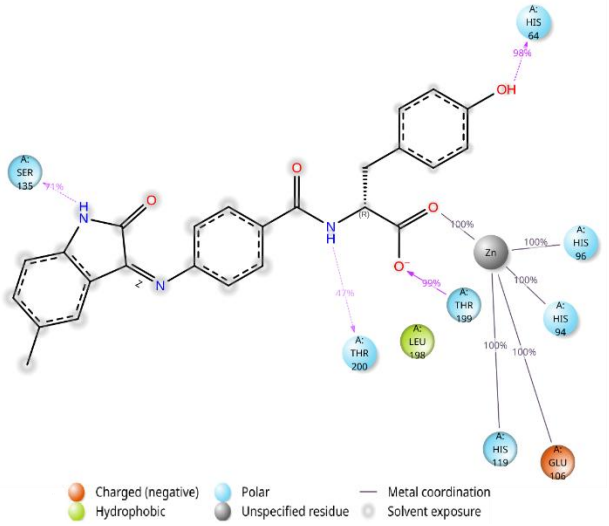

Figure (5); Ligand- Protein Contacts

Protein-Ligand Contacts (cont.)

The protein-ligand touch evaluation discovered multiple hydrogen bonds, hydrophobic interactions, and water bridges, with a few interactions persisting for over 70% of the simulation time. Key residues, including [HIS64, HIS94, HIS96, GLU106, SER135, THR199], played pivotal roles in anchoring the ligand in the binding web page.

Protein-Ligand Contacts

Hydrogen bonds, ionic bonds, metal coordination, water bridges and hydrophobic interactions contributed notably to the ligand's binding affinity. The staying power of these interactions over the simulation underscores the ligand's potential as a robust inhibitor.

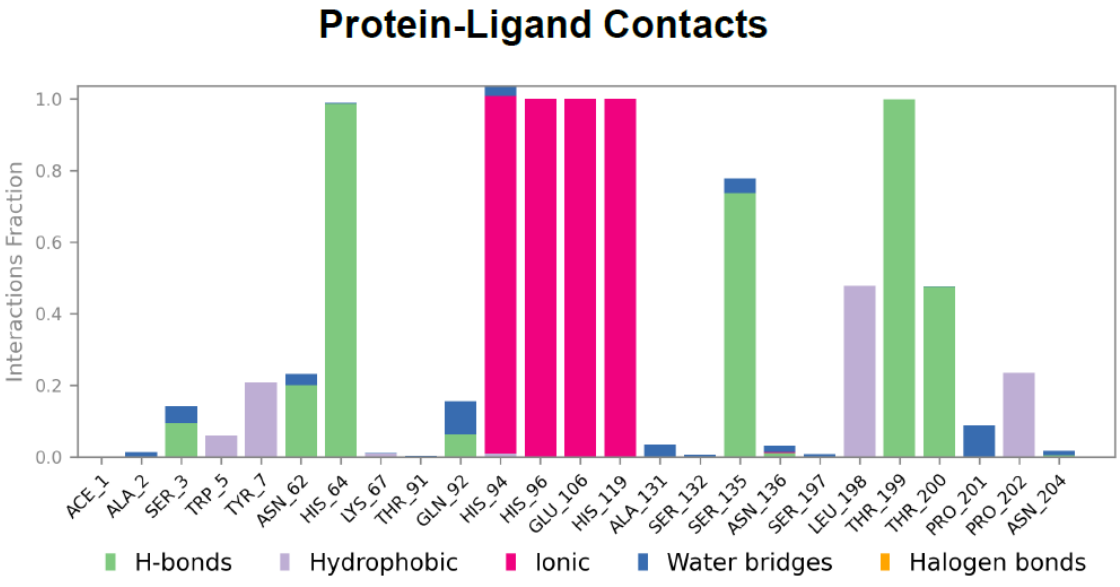

Figure (6); Protein-ligand contact histogram

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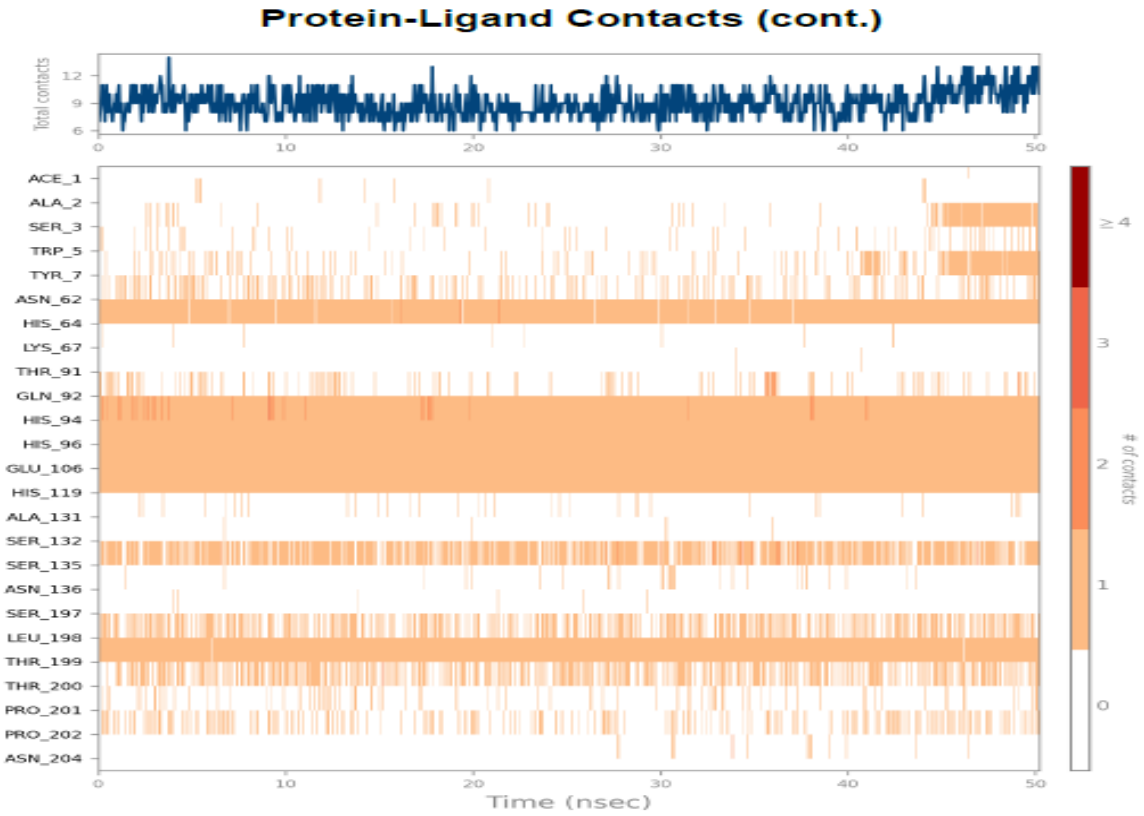

Figure (7); Protein-ligand interactions during Time

The Torsion Profile

The evaluation highlighted the stability of rotatable bonds inside the ligand. The major torsions exhibited restrained conformational pressure, suggesting the ligand adopted an energetically favorable orientation within the active site.

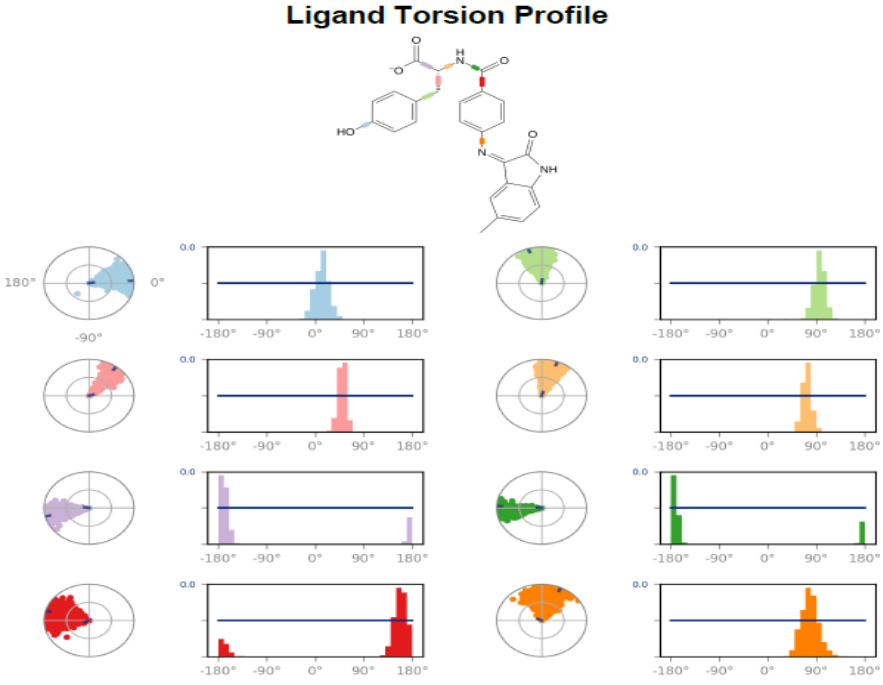

Figure (8); ligand Torsion Profile.

CONCLUSION

The computational study reveals exciting opportunities for developing new anticancer agents by focusing on several crucial targets. Sophisticated techniques such as molecular docking, ADME evaluation, and molecular dynamics simulations were employed to assess the candidate compounds. The docking analysis indicated robust binding of most of compounds to the target human proteins, with a notable preference for compounds 3, 7, 11, 13, 15, 18, 22, and 25 across the targets, suggesting their potential efficacy in anticancer therapy. The ADME evaluation illustrated favorable pharmacokinetic characteristics and limited penetration of the blood-brain barrier. The molecular dynamics simulation demonstrates the compound's stable and specific binding to the 6QNG protein, with robust interactions that are critical for anticancer activity. The choice of 6QNG as a target aligns with its essential biological role, making it a valid model for studying potential anticancer agents. Further experimental validation and optimization of the compounds are warranted based on these computational insights.

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