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Computational evaluation and biological assessment of nitrogen-containing heterocyclic derivatives as potential anticancer agents

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Abstract

Cancer persists as one of the most catastrophic illnesses worldwide, requiring ongoing investigation for more effective and safer treatment medicines. Nitrogen-containing heterocyclic compounds have arisen as attractive options owing to their varied chemical structures, advantageous pharmacokinetic profiles, and significant biological actions against several cancer types. This work included the synthesis and evaluation of a number of nitrogen-containing heterocyclic derivatives, including quinazoline, indole, flavonoid, indazole, and coumarin scaffolds, for their anticancer activities. Various computational methods, including as molecular docking, density functional theory (DFT) calculations, ADMET predictions, and 3D-QSAR modeling, were used to forecast the binding affinities, electronic characteristics, and pharmacokinetic profiles of the synthesized drugs. Biological assessments using MTT assays and flow cytometry on diverse cancer cell lines (A-549, MDA-MB-231, MCF-7, SiHa) revealed substantial anticancer efficacy in certain compounds. Molecular docking experiments demonstrated robust binding interactions of several drugs with thymidylate synthase (PDB ID: 1JU6), suggesting their potential as effective anticancer medicines. The combined experimental and computational results underscore the therapeutic potential of nitrogen-containing heterocyclic compounds in cancer medication development.

Keywords: Nitrogen-containing heterocycles, Anticancer agents, Quinazoline derivatives, Indole derivatives, Indazole derivatives, Molecular docking

Introduction

Cancer continues to be a significant and intricate problem for contemporary medicine, marked by unregulated cellular growth, genetic instability, and the capacity of malignant cells to infiltrate adjacent tissues and metastasis to other organs. Notwithstanding substantial progress in traditional and focused treatment approaches, such as chemotherapy, radiation, immunotherapy, hormone therapy, and molecularly targeted medicines, the worldwide incidence of cancer persists in escalating. A significant drawback of several contemporary anticancer medicines is their accompanying adverse effects, emergence of drug resistance, inadequate selectivity, and diminished effectiveness in certain cancer subtypes. Therefore, there is a pressing need to create innovative, more selective, and safer therapeutic agents that may address these issues while reducing damage to healthy cells. In the continuous quest for efficacious anticancer medicines, heterocyclic compounds have surfaced as one of the most promising and thoroughly investigated groups of molecules in medicinal chemistry. Nitrogen-containing heterocycles have garnered significant interest owing to their remarkable structural variety, advantageous pharmacokinetic characteristics, and extensive array of biological functions. The incorporation of nitrogen atoms into their cyclic structures improves their capacity to engage in various interactions-such as hydrogen bonding, π-π stacking, metal coordination, and electrostatic interactions-with a range of molecular targets implicated in cancer development. The molecular targets include enzymes, receptors, DNA, and signaling proteins that govern essential cellular processes, including cell cycle progression, apoptosis, angiogenesis, and metastasis.

A diverse array of nitrogen-containing heterocyclic frameworks, such as quinazolines, indoles, pyridines, imidazoles, triazoles, and coumarins, is already present among FDA-approved anticancer pharmaceuticals and

experimental compounds. Their extensive application and biological significance arise from the adaptability of their fundamental structures, which may be altered to improve selectivity, potency, and pharmacological safety. The capacity to refine electronic, steric, and hydrophobic characteristics via structural alterations offers medicinal chemists a robust foundation for systematic drug creation. This work focuses on the design, synthesis, computational analysis, and biological evaluation of new nitrogen-containing heterocyclic derivatives as prospective anticancer medicines. The research incorporates many computational methodologies, including as molecular docking, density functional theory (DFT) calculations, pharmacokinetic ADMET predictions, and 3D-QSAR modeling, in conjunction with experimental biological assessments on a range of human cancer cell lines. This collaborative project seeks to uncover promising heterocyclic lead compounds with substantial anticancer activity, excellent safety profiles, and significant potential for future development as targeted therapeutic medicines.

Related Studies

Dudhe, Anshu *et al*. (2023) [3]. Approximately 10 million lives are lost annually due to cancer. Some of the most common forms of cancer are those of the breast, lungs, colon, rectum, and prostate. Smoking, being overweight, drinking too much alcohol, not getting enough fruits and vegetables, and not moving around enough are shocking contributors to around a third of cancer fatalities. Heteroatoms and heterocyclic compounds play an essential role in medicines and are common structural components of many active natural products. According to statistics, most physiologically active chemicals are heterocycles or include a heterocyclic element; among these complex structures, nitrogen-containing heterocycles are the most common structural framework. These results highlight the important and dynamic function of heterocycles in modern drug designs and drug sighting methodology. The primary objective of this literature study was to examine the anti-cancer effects of heterocyclic compounds containing nitrogen that have been described in recent scientific studies.

Kumar, Adarsh *et al*. (2023) [5]. Worldwide, cancer remains a significant obstacle to healthcare. Although there are a number of anticancer medications on the market, many of them are ineffective, have serious side effects, are not safe, or are resistant to other forms of the disease. Therefore, the development of safer and more targeted anticancer medications is of the utmost importance. There is at least one heteroatom in more than 85 percent of medications that have a physiological effect. The most prevalent heterocyclic framework consists of nitrogen heterocycles. We have assembled the pharmacological features and list of FDA-approved heterocyclic medicines containing nitrogen atoms in this research. In addition, our research has revealed nitrogen-containing heterocycles such as pyrimidine, quinolone, carbazole, pyridine, imidazole, benzimidazole, triazole, β-lactam, indole, pyrazole, quinazoline, quinoxaline, satin, pyrrole-benzodiazepines, and pyrido [2,3-d] pyrimidines. These compounds are utilized in the treatment of various cancer types, and we have detailed their biochemical mechanisms of action and cellular targets.

Kumar, Naresh *et al*. (2022) [1]. Uncontrolled development and aberrant cell division define cancer, a major public health concern worldwide. With 9.6 million fatalities in 2018, or 1 in 6 deaths worldwide, cancer is the second most deadly disease in the world, according to the World Health Organization. The National Cancer Registry Programmed Report 2020, published by the ICMR-India, predicts that 13,90,000 cases of cancer would be reported in India in 2020, with the likelihood of this figure increasing to 15,70,000 by 2025. Cancer, particularly in its advanced stages, remains incurable despite the development of several anticancer medications, none of which are without adverse effects. Nearly everyone these days is ill with some form of illness. The development of new, highly effective bioactive compounds is, hence, an urgent matter. In an effort to improve human health, several scientists are focusing on discovering new biological targets or creating novel lead compounds. Nevertheless, new lead compounds are continually being discovered using heterocycles. Heterocyclic cores are present in many pharmaceutically authorized medications due to their exciting pharmacological qualities; for example, doxorubicin, methortrexate, vinblastine, vincristine, daunorubicin, 5-fluorouracil, etc. As a result, the study of heterocyclic chemicals offers an intriguing opportunity to create new anticancer medications. In this study, we zeroed in on heterocyclic chemicals with anticancer properties, both naturally occurring and manufactured. In addition, research into the action mechanism of several heterocyclic anticancer drugs has been ongoing.

Amewu, Richard *et al*. (2021) [2]. Cancer is a complicated disease category characterized by uncontrolled growth of cells that may metastasize to other organs. Recent advances in genomics, bioinformatics, and cheminformatics have led to the identification of drugs that inhibit various putative targets. These targets include, but are not limited to, members of the vascular endothelial growth factor (VEFG) family, fibroblast growth factors (FGF), platelet derived growth factors (PDGF), epidermal growth factor (EGF), thymidine phosphorylase (TP), and neuropeptide Y4 (NY4). Many cancer chemotherapy therapies have serious side effects, including drug resistance, systemic toxicity, and medication ineffectiveness. As a result, state-of-the-art cancer therapies are being considered that effectively target many potential targets in separate cancer cells. However, there is a lack of knowledge about heterocyclic compounds and their multimodal anticancer capabilities, despite the fact that these compounds have made significant contributions to chemotherapeutics for the treatment of numerous illnesses. Thus, this study provides a comprehensive database of heterocyclic multitarget anticancer agents derived from both natural and synthetic sources, together with their IC50 values and the biological targets they inhibit.

Avula, Satya *et al*. (2021) [4]. One of the top killers on a global scale is cancer. To combat this fatal illness, effective pharmaceuticals are necessary. Natural products have produced several "lead compounds" that might be used in the creation of new drugs, and they have long been an important source of anticancer medicines. Recently, thanks to developments in chemical synthesis and bio evaluation methods, these naturally occurring lead compounds have been used to make effective anticancer medications. Several

O-heterocycles have been studied as promising cancer treatments among the bioactive chemicals found in nature. In most cases, these chemicals have completely new structures and action mechanisms. A few chosen O-heterocycles have been briefly covered in this review article as potential anticancer drugs. Several compounds have been detailed, including their bioactivities, chemical compositions, and natural sources. Proper research on the structure-activity connection and the mechanism of actions, as well as the efficient bio evaluation of these compounds and their synthetic alterations, have been highlighted as means to enhance their analogues. This article has shown how naturally occurring O-heterocyclic chemicals are becoming more important in the fight against cancer.

Objectives

- To synthesize a library of nitrogen-containing heterocyclic derivatives, including quinazoline, indole, indazole, flavonoid, and coumarin-based scaffolds.
- To evaluate the *in-vitro* anticancer activity of the synthesized compounds against multiple human cancer cell lines, including A-549, MDA-MB-231, MCF-7, and SiHa.
- To investigate the molecular interactions of the synthesized compounds with the target enzyme thymidylate synthase (PDB ID: 1JU6) using molecular docking studies.

Materials and Methods

In this study, a combination of experimental and computational methods was employed to explore the anticancer potential of nitrogen-containing heterocyclic compounds. All computational analyses were conducted using a Windows 10 system equipped with Intel® CoreTM i3-4005U CPU, employing the Schrödinger 2020-1 drug discovery suite. Green chemistry protocols were followed to ensure environmentally sustainable synthesis procedures. Density Functional Theory (DFT) calculations for compounds 1b-6b were carried out using Gaussian 16 software at the B3LYP-D3BJ functional level with the 6-311++G(d,p) basis set, and visualized via GaussView 6.0 to determine electronic parameters such as HOMO-LUMO energies. For 3D-QSAR modeling, molecular alignment and CoMFA/CoMSIA models were developed using SYBYL-X.2.1, applying Tripos force fields, Gasteiger-Hückel charges, and Powell's gradient optimization.

Chemicals for synthesis were sourced from several commercial vendors and used without further purification. Reactions were carried out under a nitrogen atmosphere and monitored by TLC using silica gel plates, with purification achieved by flash or column chromatography. The synthesized compounds were characterized by melting point determination, FTIR, HPLC, mass spectrometry, and NMR spectroscopy.

Biological evaluation involved cell cycle distribution analysis using flow cytometry (FACS Aria systems), where A-549 lung cancer cells were treated with the target compounds, followed by staining with RNase and propidium iodide for DNA content measurement. Molecular docking studies utilized the Cryo-EM structure (PDB ID: 7CKR) of the MCT1/Basigin-2 complex with preparation performed via Schrödinger's Prime module. Molecular dynamics (MD) simulations were conducted for 100 ns using Schrödinger's Desmond software, with systems solvated in a 10Å SPC water box, neutralized by Na^+ and Cl^- ions, and equilibrated through steepest descent and LBFGS algorithms to ensure stability.

Results

Molecular dynamics (MD) simulation

Figure 1 shows the results of a long-range MD simulation research that investigated the binding and structural stability of compound 187 in human MCT1. Various metrics were examined to study the stability and dynamic behavior of the ligand-protein complex during the simulation, including the root mean square deviation (RMSD) of the Cα atoms in the protein and the ligand, the protein's root mean square fluctuation (RMSF), and the mapping of the protein-ligand contact.

The RMSD of each frame collected from the complete trajectory may be used to evaluate the structural conformation of the protein Cα atoms throughout the MD simulation. The simulation's small deviation and constant fluctuation of the RMSD showed that the protein-ligand combination was stable. Figure 1 shows the calculated root-mean-squared deviation (RMSD) of carbon atoms in each frame as a function of simulation time.

(b). After the protein Cα atoms were bound to the ligand, the RMSD was shown to be almost balanced, with small fluctuations between 1.3 Å and 2.40 Å. In addition to the RMSD of the protein Cα atoms, the ligand's RMSD was also assessed and shown to be very low. An average of 3.6 Å was maintained for the ligand RMSD after the first variation caused by equilibration. Throughout the simulation run, the compound 187 bound protein complex showed no structural or conformational changes, as demonstrated by small RMSD fluctuations and a convergent pattern. To study how individual amino acids affect the stability of protein-ligand interactions, the RMSF value is essential.

The orientation hanges of each amino acid Cα atom throughout the simulation as opposed to its initial orientation in the native state is what it is. The effect of compound 187 on MCT1's flexibility was investigated by calculating RMSF values. During the simulation, compound 187 interacted with 21 amino acids of the MCT1 protein, as shown in figure 1 (c). These amino acids were TYR 34, LYS 38, SER 62, LEU 66, ALA 67, MET 69, TYR 70, SER 154, PRO 155, LEU 158, PHE 274, PHE 278, LEU 281, VAL 282, ARG 313, SER 371; PHE 367; LEU 374; VAL 402; LEU 403; PRO 407. Lipid interaction in two dimensions.

In the 100 ns simulation, at 64% of the time, the oxygen atom of the indazole-3-carboxamide group formed a water-mediated hydrogen bond with the negatively charged residue AR 313. Based on the ligand interaction histogram, it can be seen that the compound was positioned in the active pocket through hydrophobic interactions with various residues, including the hinge region, "gatekeeper" residues (TYR 34, LYS 38, LEU 66, TYR 70, SER 154, LEU 158, PHE 274, PHE 278, LEU 281, VAL 282, ARG 313, PHE 367, SER 371, LEU 374, VAL 402), water bridges between TYR 34, SER 154, ARG 313 and SER 371, and direct hydrogen bonding with ARG 313 (figure 1 d).

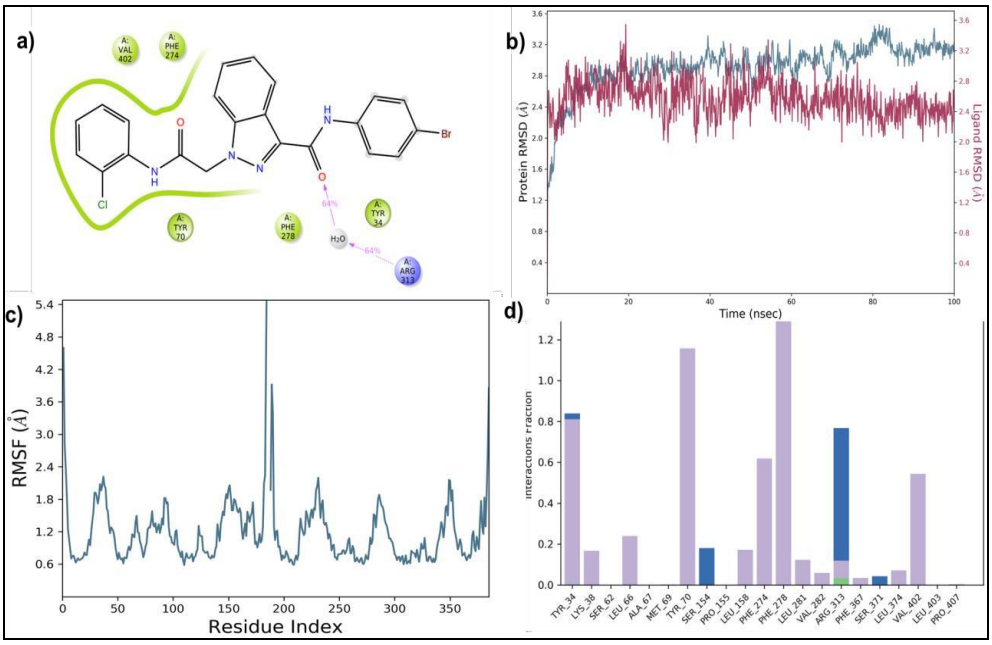

Fig 1: MD simulation analysis of compound 187 in human MCT1 (PDB ID: 7CKR) (a) 2D interaction diagram (b) RMSD (Protein RMSD is shown in grey while RMSD of compound are shown in red) (c) protein RMSF (d) Protein–ligand contact analysis of MD

Biological evaluation

To evaluate the efficacy of the produced compounds, this research used a panel of several human cancer cell lines. The National Centre for Cell Science (NCCS) in Pune provided the cell lines A-549 (lung cancer), MDA-MB-231 (triple negative breast cancer), MCF-7 (breast cancer), SiHa (cervical cancer). The inhibitory impact of the produced compounds was studied by selecting these cell lines based on the expression levels of MCT1 and MCT4, or both. In contrast to MCF-7, which only expresses MCT1, MDA-MB-231, SiHa, and A-549 cells mostly express MCT4.

***In-vitro* Anticancer Activity**

Examination of the first group of chemicals

Screening in MTT assay

The first step of synthesis included the synthesis of sixty-eight target compounds, including quinazoline, N-substituted benzylated indole, flavonoids, indazole cyanoacrylate derivatives, and coumarin. The invitro cytotoxic effects of a series of all produced compounds were assessed using the MTT test against MCT1 and MCT4 overexpressing cell lines (SiHa, MDA-MB-231, MCF-7, and A-549 cell lines). At first, two concentrations of each chemical were tested: 10 µM and 25 µM. The positive control used the reference inhibitor INDCHC, whereas the negative control utilized the vehicle DMSO.

We tested the coumarin compounds in a first series against MCT1 overexpressing SiHa cells in low glucose medium under normoxic circumstances. As shown in figure 2, chemicals 56, 57, and 58 had a positive impact on cell viability. Like the well-known MCT1 inhibitor INDCHC, this has similar effects. We further tested the same set of synthetic series against MDA-MB 231 cells (breast cancer cells), which are known to express MCT4 and, to a lesser degree, MCT1, in our screening process. Screening findings showed that under standard incubation and medium conditions, the majority of chemicals did not significantly impact cell viability (figure 3). Numerous chemicals failed to affect cell survival when tested against SiHa cells in normoxic settings utilizing high glucose medium (figure 4). Table 1 summarizes the results of the MTT test screening of the produced compounds.

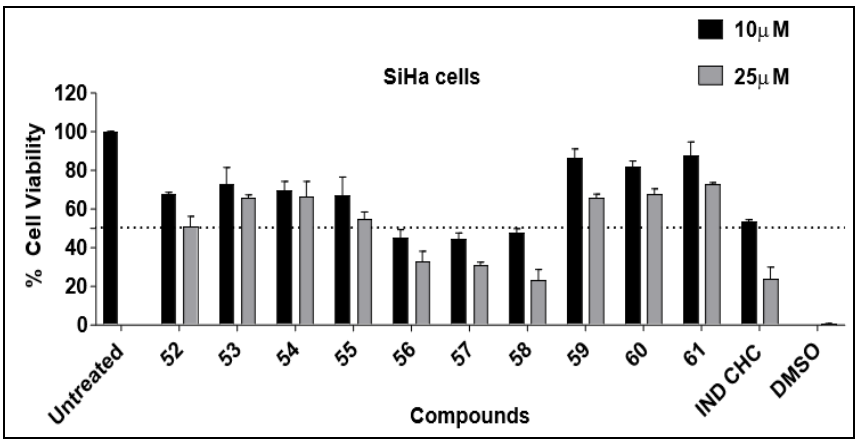

Fig 2: Screening of coumarin derivatives against SiHa cells in low glucose media in MTT assay

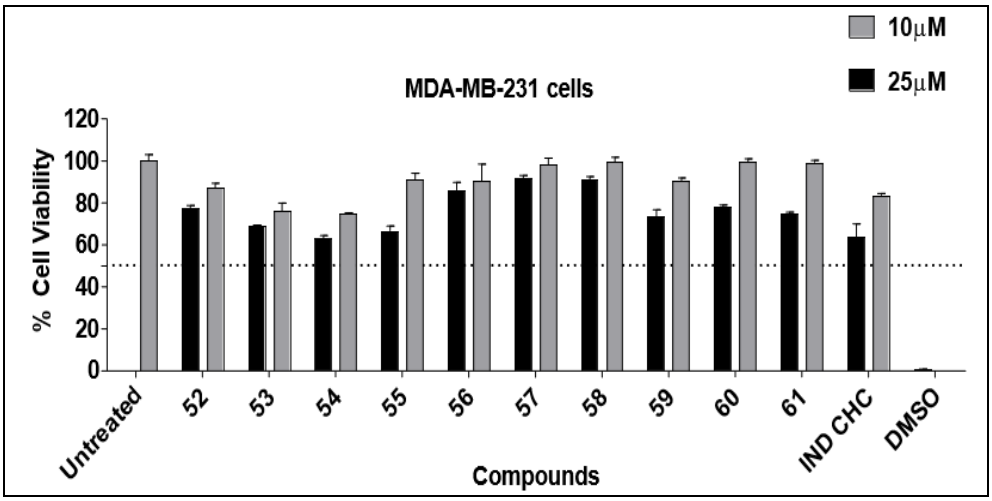

Fig 3: Screening of coumarin derivatives against MDA-MB-231 cells in low glucose media in MTT assay

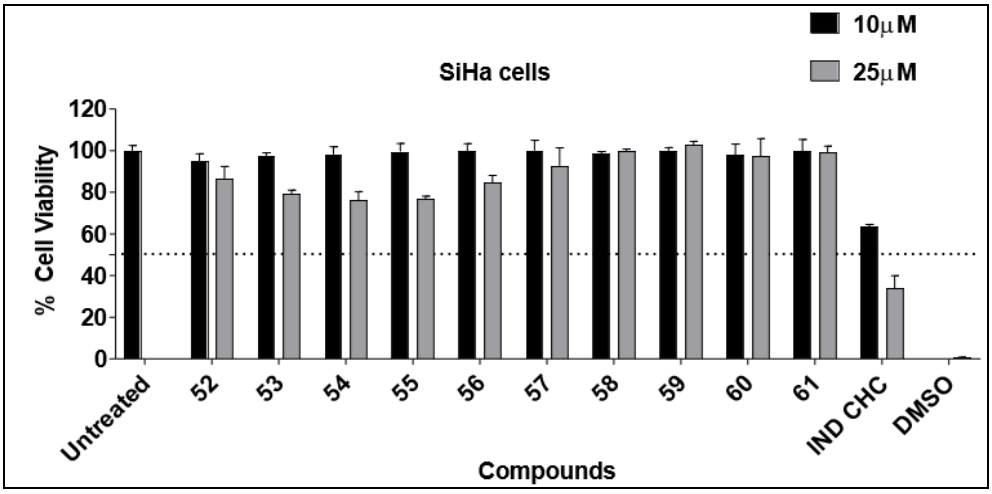

Fig 4: Screening of coumarin derivatives against SiHa cells in high glucose hypoxia condition in MTT assay

Separately, we tested quinazoline- and flavonoid-based derivatives against SiHa and MDA-MB-231 cell lines, which overexpress MCT1 and MCT4, respectively. Even at high concentrations, none of the compounds showed any inhibition of MCT1 and MCT4 in the first screening at a dose of 25 μM, as compared to normal IND CHC. The scaffold was not further investigated for screening purposes since it did not block MCT.

Molecular docking and interactions analysis

After thoroughly examining the six compounds' inherent properties and predicting their possible ADMET patterns, our next goal is to study how they could interact with certain proteins. To accomplish this, molecular docking methods will be used, which will allow for the approximate determination of their binding affinities with different proteins. A three-dimensional model of the protein's binding site is first generated using this approach, and then a database of small molecules is searched for those that can fit into the model and form strong interactions. In the end, the specific interactions between our compounds and the target protein will be better understood thanks to this iterative method that allows us to predict an empirical binding mechanism and determine the affinities of the compounds.

The target protein for our inquiry has been determined to be thymidylate synthase, which was found in PDB (1JU6). The findings may be seen in Table of the supporting information. It is clear from the low and negative binding energies that all the compounds that were tested had a strong affinity for the target proteins. Particularly encouraging for anticancer action, these binding energies were lower than those linked with 5-FU, the reference medicine. In addition, the research examined the interactions between the target protein (1JU6) and six compounds (1b-6b), as well as the reference ligands 5-FU. All of these compounds showed a strong affinity for 1JU6 and showed considerable anticancer activity *in vitro*.

The compounds' interactions with the active site were shown in two- and three-dimensional models, with the hydrogen bond surface included in the latter. Out of the six compounds, compound 3b stands out with the best docking score (−8.50 kcal/mol) in the ligand-protein interactions shown in Figures 5 and 6. The other compounds were 4b, 2b, 1b, 5b, and 6b, with binding energies of -6.90 kcal/mol and -6.0 kcal/mol, respectively, in decreasing order. After complexing with 1JU6, compound 1b interacted with nine residues: two carbon-hydrogen bonds with GLU87 and GLY222, two conventional hydrogen bonds with TRP109 and ASN226, and four hydrophobic contacts with ILE108, LEU221, PHE225, and MET311.

Compound 3b formed traditional hydrogen bonds with ASN226 and ASN112, carbon-hydrogen bonds with GLY222 and TRP109, and five hydrophobic contacts with ILE108, LEU221, PHE225, and MET311. One of these interactions was a π-π T-shaped bond. Research has already identified the active site of thymidylate synthase (TS), which includes amino acid residues like ILE108, CYS195, ASP218, GLN214, LEU221, and PHE225. Our research shows that 5-FU's action mechanism involves interactions with three important amino acid residues in this active site: CYS195, GLN214, and ASP218.

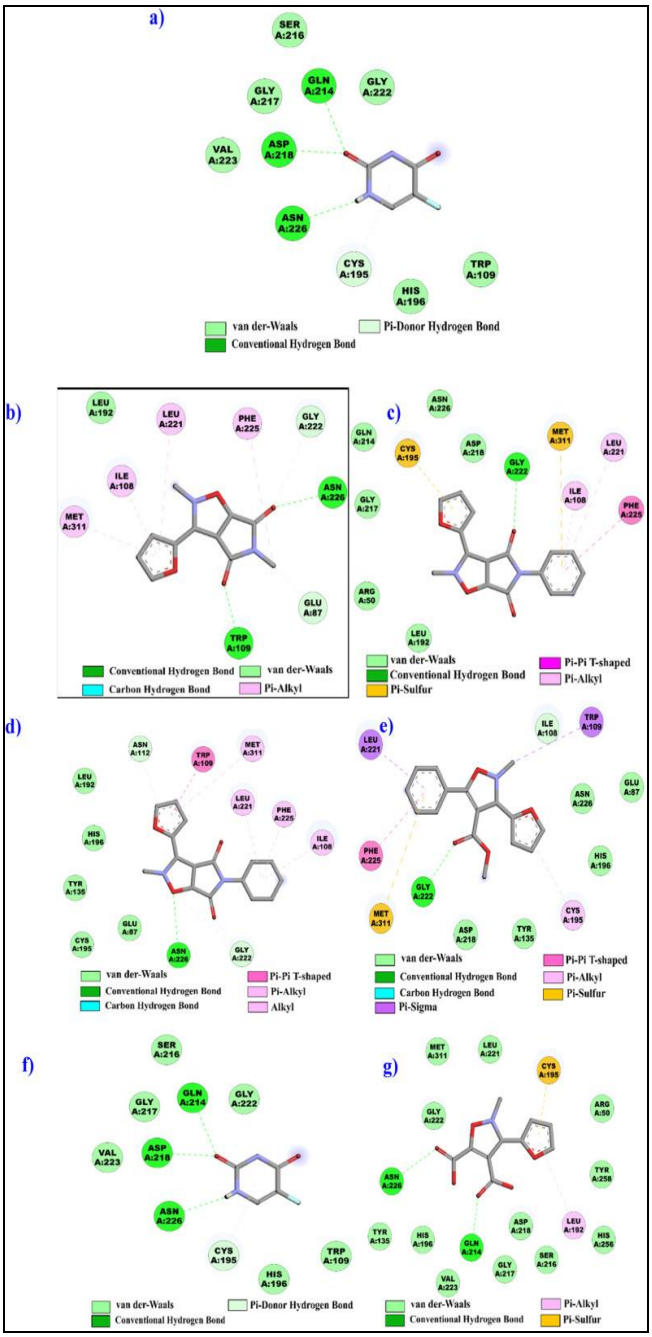

Fig 5: Docked poses of the six designed compounds (1b-6b) and 5-FU with 1JU6.

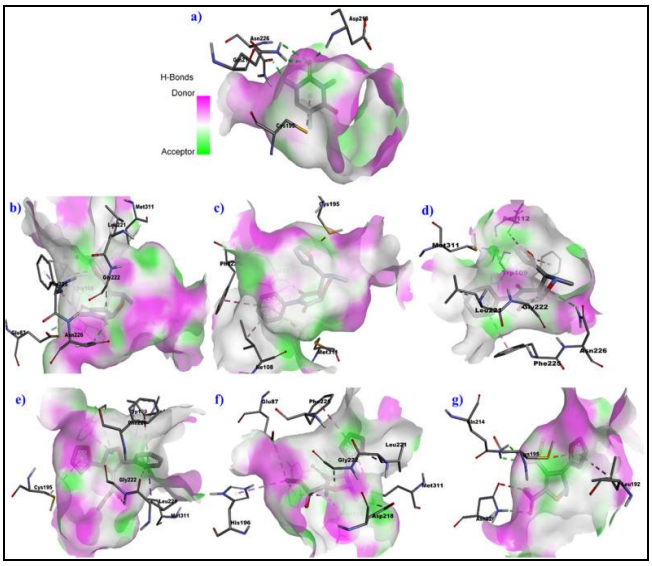

Fig 6: Hydrogen bond surface mapped the locations of hydrogen bonds in of ligand-protein

Density functionals theory calculations

Density functional theory (DFT) computations were performed in their entirety for compounds 1b-6b. The B3LYP-D3BJ functional, which incorporates Grimme's third dispersion function enhanced with Becke-Johnson's damping function, was used to do these calculations at the 6-311++G(d,p) theoretical level, with the use of the Gaussian 16 software package. The results were examined using the Gauss View 6.0 program, which also included the energies of the Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO), among other relevant electronic properties.

A Density of States (DOS) graph was created using the Gauss Sum 2.2 application. We optimized the shape of the target compound without imposing any geometric limitations. The electrostatic potential and DOS were also computed using this improved shape. We used the B3LYP approach in the context of Time-Dependent DFT (TD-DFT) with the 6-311++G(d,p) basis set to analyze the UV-visible spectra and excited state dipole moments.

The molecular reactivity indices were computed to evaluate the drug-targeting capabilities of these compounds. To start, we checked that the fully optimized geometric structure did not include any imaginary vibration frequencies. Then, we analyzed the EHOMO, ELUMO, and energy gap ΔE.

ΔE=ELUMO−EHOMO

The data from the frontier molecular energy were used to produce parameters like electronegativity (χ), chemical potential (μ), chemical hardness (η), and softness (S).

$$\mu = -\chi = \frac{1}{2}(E_{LUMO} + E_{HOMO})$$

$$\eta = \frac{1}{2}(E_{LUMO} - E_{HOMO})$$

$$S = \frac{1}{2\eta}$$

We also evaluated the global electrophilicity ω to clarify the relationship between energy fluctuation and maximal electron transfer.

$\omega=\mu 22\eta$

ADMET prediction (absorption, distribution, metabolism, excretion, and toxicity)

Analyzing toxicity and other pharmacokinetic data is essential for determining how a medicine acts in the body. These parameters include ADME (absorption, distribution, metabolism, and excretion) factors. The AdmetSAR server and other online tools play a crucial role in this evaluation. Data files including the drug's molecular structure and SMILES strings are required to carry out these calculations. In a transformation procedure, they are used to translate this data into valuable information about pharmacokinetics. Better judgments on the drug's possible use in treating different medical diseases are made possible by this method's thorough grasp of the drug's behavior.

Molecular structure alignment

In order to establish a trustworthy 3D-QSAR model, the data set needed to be aligned and contour maps needed for the CoMSIA and CoMFA experiments were generated using molecule 14, as seen in Figure 7.

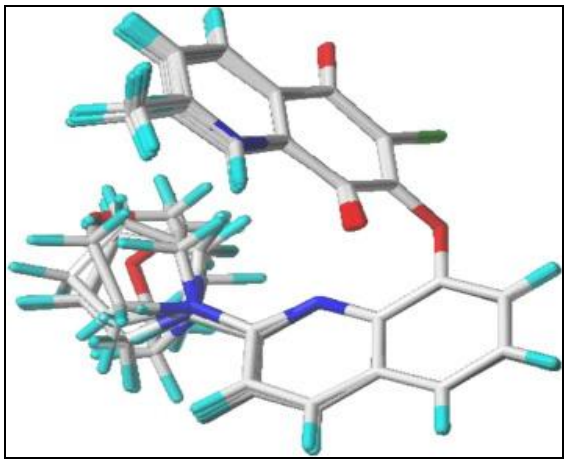

Fig 7: The aligned compounds using molecule 14 as a template.

3D-QSAR models generation

The study's CoMFA and CoMSIA models were built using ten distinct field combinations. The statistical findings that were obtained from these models are shown in Table 1. The best model to use is the one with the lowest number of principal components (N), most significant F-test value, and the highest values of the coefficient of determination of non-cross validation (R2) and cross validation (Q2), as well as the lowest number of the standard error of estimate (SEE).

Table 1: Models' statistical parameters

Generated model	Fields' description	Q2	N	SEE	R2	F
CoMFA / SE	Steric (S)- Electrostatic (E)	0 0.471	6	0.178	0.938	22.500
CoMSIA / SEH	Steric (S)-Hydrophobic (H)-Electrostatic (E)	0.417	4	0.207	0.897	23.870
CoMSIA / SED	Steric (S)- Electrostatic(E)- Donnor of hydrogen bond (D)	0.481	5	0.203	0.910	20.216
CoMSIA / SEA	Steric (S)-Electrostatic (E) - Acceptor of hydrogen bond (A)	0.527	3	0.195	0.900	36.080
CoMSIA / EHA	Electrostatic (E)- Hydrophobic (H)- Acceptor of hydrogen bond (A)	0.441	6	0.203	0.919	16.988
CoMSIA / SEHD	Steric (S)- Electrostatic (E)-Hydrophobic -Donnor of hydrogen bond (D)	0.417	4	0.207	0.897	23.870
CoMSIA / SEHA	Steric (S)-Electrostatic (E)- Hydrophobic (H) – Acceptor of hydrogen bond (A)	0.432	5	0.196	0.915	21.616
CoMSIA / SEDA	Steric (S)- Electrostatic (E)- Donnor (D) and Acceptor (A) of hydrogen bond	0.527	3	0.195	0.900	36.080
CoMSIA / EHDA	Electrostatic (E)-Hydrophobic (H)-Donnor(D) and Acceptor (A) of hydrogen bond	0.441	6	0.203	0.919	16.988
CoMSIA/ SEHDA	Steric (S)- Electrostatic-Hydrophobic (H)- Donor(D) and Acceptor(A) of hydrogen bond	0.432	5	0.196	0.915	21.616

The best-fitting and most optimum model was determined by analyzing the statistical results in Table 1. The model with the greatest Q2(0.527) and R2(0.900), lowest SEE value (0.195), and most significant F-value (36.080) included three main components. With the highest prediction coefficient of determination (Rpred2= 0.899), this model used SEA fields, which stand for steric, electrostatic, and hydrogen bond acceptors. The non-significant value of the internal validation parameter (Q2< 0.5) led to the exclusion of the CoMFA model. Furthermore, the CoMFA model is more strongly rejected due to the high number of main components (N = 6) that are derived via PLS analysis.

Conclusion

The current work effectively synthesized and assessed several nitrogen-containing heterocyclic compounds for their potential anticancer properties. The amalgamation of computational and experimental methodologies for a thorough assessment of the synthesized drugs' molecular characteristics, pharmacokinetics, and biological effectiveness. Molecular docking experiments indicated that many drugs had robust binding affinities for thymidylate synthase, corroborated by advantageous interaction patterns. DFT studies yielded significant insights into electronic structures, while ADMET analysis validated satisfactory pharmacokinetic and toxicity characteristics for the majority of drugs. Biological assessments using MTT tests on diverse cancer cell lines further corroborated the promising anticancer efficacy of chosen derivatives, especially coumarin-based compounds, which exhibited substantial lethal effects. The 3D-QSAR models developed in this research provide a dependable prediction framework for further alterations and optimization of the synthesized scaffolds. Studies indicate that nitrogen-containing heterocyclic molecules are promising scaffolds for the creation of innovative, effective, and safer anticancer medicines.

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