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HYBRID HETEROCYCLIC ARCHITECTURES AS EMERGING ANTI-CANCER CANDIDATES

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Abstract: Because of the significant role that heterocyclic compounds play in the process of developing molecules that are both structurally varied and biologically active, the synthesis of heterocyclic compounds that have strong anti-cancer properties has arisen as a significant priority in the field of current medicinal chemistry. The synthesis, characterization, and preliminary biological assessment of a new series of heterocyclic derivatives, which include indoles, quinazolines, triazoles and benzothiazole, are reported in the work. These compounds were created through the use of rational drug design methodologies. In an effort to increase reaction efficiency, structural purity, and yields, the synthetic process made use of both traditional and microwave-assisted procedures. Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), and mass spectrometry were used to carry out the structural elucidation. The MTT test was used to determine the cytotoxic effects of the produced compounds on chosen human cancer cell lines, which included HeLa, MCF-7, and A549. A number of derivatives exhibited substantial anti-proliferative activity, with IC_{50} values that were equivalent to or superior to those of reference medicines that are commonly used as standards. This suggests that these compounds have considerable potential for therapeutic applications. The results of the structure-activity relationship (SAR) research demonstrated that the biological activity was significantly impacted by the placement of heteroatoms, increased aromaticity, and substituents that pull electrons away. The findings demonstrate the significance of heterocyclic scaffolds in the discovery of anti-cancer drugs and give a basis for additional in vivo and mechanistic investigations that will aid in the optimization of lead candidates.

Keywords: Mutations, Oncogenes, Heterocyclic Synthesis, Indoles, Quinazolines, Triazoles, Benzothiazoles, Structure Activity Relationship, Biological Potentials.

INTRODUCTION

Despite the considerable advancements that have been made in diagnostic technology, molecular comprehension, and therapeutic treatments, cancer continues to be one of the primary causes of death around the globe, with millions of people succumbing to the disease each year. Survival results have been improved by conventional therapies such as chemotherapy, radiation, and targeted therapy; nevertheless, the effectiveness of these treatments continues to be hampered by factors such as drug resistance, toxicity, and low specificity. As a result, there is an ongoing requirement to find and produce novel categories of anticancer medications that are selective as well as efficacious, which will reduce unwanted side effects and simultaneously increase the effectiveness of the treatment. Due to the extensive structural variety and broad spectrum of biological activities that they exhibit, heterocyclic compounds have become the focus of significant study in the field of medicinal chemistry. The fact that more than fifty percent of all known

pharmaceuticals contain at least one heterocyclic ring highlights the essential function that these rings play in the development of contemporary medications [1,2]. A number of therapeutically authorized anticancer treatments rely on nitrogen-, oxygen-, and sulfur-

containing heterocycles, such as pyridines, quinazolines, indoles, triazole s, and triazoles, which function as essential scaffolds. Because of their distinctive electronic and steric characteristics, they are able to interact with a wide range of biological targets, including kinases, topoisomerases, tubulin, and signaling proteins that play a role in the proliferation of cells that are not under control. Finding new heterocyclic compounds is an essential method for discovering prospective candidates for anticancer drugs. The efficiency and selectivity of heterocycle creation have been substantially improved as a result of advances in synthetic organic chemistry. Some of these advances include metal-catalyzed coupling processes, green chemistry techniques, and microwave-assisted synthesis, among others. The integration of a wide variety of substituents and functional groups is made possible by these techniques, which in turn provide more control over the architecture of molecules and facilitate the synthesis of compounds that have been tailored to exhibit favorable pharmacological profiles. Simultaneously, the investigation of structure-activity relationships (SAR) yields valuable insights into the ways in which biological activity is affected by substituent patterns, electronic effects, aromaticity, and heteroatom placement. The rational design of heterocycles that have the potential to block important enzymes that are involved in tumor development or to modulate cancer-related pathways can be supported by this understanding. In addition, the use of computational technologies, such as molecular docking and in silico screening, significantly speeds the discovery of potentially promising lead compounds prior to their experimental assessment. Taking into consideration the pressing need for novel therapeutic agents and the shown promise of heterocycles in the field of medicinal chemistry, the primary emphasis of the current research is on the synthesis, characterization, and biological screening of a group of heterocyclic compounds that were selected based on their ability to inhibit the growth of cancer. The objective of the research is to synthesize compounds with a wide range of structures, assess their capacity to induce cytotoxicity against human cancer cell lines, and pinpoint possible candidates that may be investigated further through in vivo and mechanistic studies. Furthermore, a comprehensive set of biological tests, including assessments of antibacterial, antifungal, insecticidal, pesticidal, and anthelmintic properties, as well as additional congeners of the same derivatives, are presently in progress and will be reported eventually. As a result of this effort, the increasing area of heterocyclic drug discovery has been furthered, and this work may provide vital insights into the creation of next-generation anticancer drugs [3].

INTRODUCTION TO HETEROCYCLIC CHEMISTRY IN DRUG DISCOVERY

Because the majority of small-molecule medications, namely around 60%, are built upon heterocyclic core structures, heterocyclic chemistry has long been regarded as a cornerstone of medicinal chemistry. The fact that they have a unique mix of aromaticity, heteroatoms, and diverse substitution patterns enables them to imitate biological molecules, to be compatible with enzyme active sites, and to modulate cell-signaling pathways. The therapeutic significance of nitrogen-containing rings, such as pyrimidines, in anticancer medications, such as 5-fluorouracil, was revealed through early research in the mid-20th century. This discovery sparked an enormous interest in the development of new heterocyclic scaffolds that have improved pharmacokinetic and pharmacodynamic properties [4]. Heterocycles have continued to play a leading role in the development of cancer drugs since that time as a result of their structural flexibility and their capacity to interact with a variety of molecular targets.

GENETICS OF CANCER

Cancer is fundamentally a genetic disease, arising from the accumulation of alterations in the DNA of somatic or germline cells that disrupt normal cellular mechanisms such as proliferation, differentiation, apoptosis, and DNA repair. These genetic changes—whether inherited from parents or acquired over an individual's lifetime—transform normal cells into malignant ones capable of uncontrolled growth, invasion, and metastasis. Understanding the genetic basis of cancer is therefore central to deciphering its origin, progression, and therapeutic vulnerabilities.

Genetic mutations in cancer can occur at multiple levels:

- •Single base-pair substitutions (point mutations)
- •Insertions and deletions
- •Copy number variations
- •Chromosomal rearrangements
- •Epigenetic modifications

Each level of alteration contributes differently to oncogenesis, with some mutations providing growth advantages (driver mutations) while others occur incidentally (passenger mutations). The interplay of these mutations leads to genomic instability—one of the hallmarks of cancer—and enables tumor cells to accumulate additional changes that promote survival and adaptation within the host environment. Cancer genetics traditionally classifies key genes involved in

tumor development into three major categories: proto-oncogenes, tumor suppressor genes, and DNA repair genes. Proto-oncogenes, when mutated or overexpressed, become oncogenes that push the cell toward uncontrolled proliferation. Conversely, mutations in tumor suppressor genes remove growth restraints, while defects in DNA repair genes accelerate mutation accumulation [5,6]. Canonical examples include RAS, MYC, TP53, BRCA1/2, RB1, and MLH1, which have been extensively studied across different cancer types. Another essential component of cancer genetics is the distinction between hereditary (germline) and sporadic (somatic) mutations. While only 5–10% of cancers arise from inherited genetic mutations that significantly increase predisposition (such as BRCA-associated breast cancer or APC mutations in familial adenomatous polyposis), the majority of cancers develop from somatic mutations caused by environmental factors, lifestyle influences, or random errors in DNA replication. Advances in next-generation sequencing, genome-wide association studies (GWAS), and molecular profiling technologies have revolutionized our understanding of cancer genetics, enabling the identification of thousands of mutations, molecular subtypes, and actionable targets. These discoveries have directly led to precision medicine approaches, where treatments are tailored to the genetic profile of a patient's tumor—an example being the use of EGFR inhibitors in lung cancer or PARP inhibitors in BRCA-mutant cancers. [7,8]. In conclusion, the genetics of cancer provides a foundational framework that connects molecular events within cells to the clinical behavior of tumors. By exploring inherited and acquired mutations, understanding mechanisms of genomic instability, and applying genomic knowledge to targeted therapies, cancer genetics continues to transform cancer diagnosis, prevention, and treatment.

TYPES OF MUTATIONS

A combination of spontaneous genetic mistakes and environmental stressors that impair the genomic integrity of somatic cells gives rise to the mutations that underlie the development of cancer. These changes can arise at a variety of different levels, including point mutations, insertions, deletions, chromosomal rearrangements, copy number variations, and epigenetic modifications that influence gene expression without altering the nucleotide sequence. Proto-oncogenes may be activated or tumor suppressor genes may be inactivated as a result of point mutations, whereas translocations and other major structural modifications have the potential to give rise to new fusion genes that have oncogenic properties. As a consequence of failures in replication, exposure to oxidative stress, exposure to carcinogens such as tobacco smoke, radiation, or viral integration, mutations frequently accrue in a progressive manner. In the field of cancer genetics, it is the accumulation of several "driver mutations"—those that offer a growth advantage—that drives cells toward uncontrolled proliferation, invasion, and metastasis. In addition to this, "passenger mutations" can also be observed, although they do not have any direct impact on the development of tumors. The variety and interaction of mutations taken together constitute the molecular underpinning of cancer evolution.

Oncogenes:

Oncogenes are derived from proto-oncogenes, which are normal cellular genes that play a role in regulating vital biological processes, including the growth, differentiation, and survival of cells. When proto-oncogenes receive activating mutations, which can occur by amplification, gain-of-function point mutations, chromosomal translocations, or overexpression, they are turned into oncogenes, which are capable of causing malignant transformation [9]. These anomalous genes give rise to proteins that force cells to undergo continuous division, inhibit apoptosis, or maintain proliferative signaling even when external stimuli are not present. Some prominent examples are RAS, which is able to become constitutively active as a result of point mutations; MYC, which is frequently overexpressed as a consequence of gene amplification; and BCR-ABL, which is created through the process of chromosomal translocation in chronic myeloid leukemia. Oncogenes follow a dominant genetic pattern, which means that only one mutant allele is required for oncogenic activity to occur. Their continual activation is disruptive to the normal cellular homeostasis, and in the multistep process of carcinogenesis, they function as a major "accelerator."

Tumor Suppressor Genes:

The purpose of the genes that inhibit tumors is to serve as the body's natural defensive mechanism. They accomplish this by regulating the course of the cell cycle, encouraging the repair of DNA, initiating the process of apoptosis, and ensuring the integrity of the genome. Examples of these include TP53, RB1, BRCA1, and PTEN, which are individually involved for either limiting unregulated growth or healing damage to cells. In contrast to oncogenes, tumor suppressor genes often exhibit a recessive pattern. For the loss of function to occur in these genes, both alleles need to be inactivated, which can happen by mutation, deletion, or epigenetic silencing. The disruption of these genes results in cells losing important regulatory checkpoints, such as the G1/S transition, which in turn allows damaged DNA to replicate without any kind of regulation. TP53 mutations, which are among the most often occurring in human malignancies, cause an inability

to trigger apoptosis, promote DNA repair, or stop the advancement of the cell cycle. The entrance into the cell cycle becomes unregulated because of the loss of RB1, and the repair mechanisms for homologous recombination are compromised as a result of BRCA1/BRCA2 mutations. When tumor suppressor genes are inactivated, it is comparable to a "brake failure," which permits cells to amass even more mutations and move toward becoming cancerous [10].

DNA Repair Genes:

Genes responsible for the repair of DNA are of great importance when it comes to the identification and correction of mutations that occur in the genome as a result of mistakes in replication, exposure to radiation, damage caused by chemicals, and oxidative stress. The nucleotide excision repair pathway, base excision repair pathway, mismatch repair pathway, and double-strand break repair pathway (which includes homologous recombination and non-homologous end joining) are all significant methods by which these genes function. As a result of the mutation of DNA repair genes—for example, MLH1, MSH2, BRCA1, BRCA2, or ATM—cells become susceptible to genomic instability, which leads to the fast accumulation of mutations that dramatically increase the likelihood of developing cancer. The presence of mutations in BRCA genes results in a heightened chance of developing breast and ovarian cancers because of faulty homologous recombination [11]. In contrast, the presence of deficiencies in mismatch repair genes causes microsatellite instability, which is a distinctive characteristic of colorectal and endometrial malignancies. Malignant progression is accelerated by the insufficiency of DNA repair systems, which let damaged DNA to survive, accumulate, and produce driver mutations that support the development of cancer.

DNA intercalation and topoisomerase inhibition:

Planar heterocycles, including acridines, quinolines, and indoles, intercalate with deoxyribonucleic acid (DNA), which in turn disrupts both transcription and replication. There have been reports in the literature of the creation of a variety of acridine-based hybrids that exhibit increased topoisomerase II inhibition as well as better selectivity for cells that divide at a rapid pace.

Tubulin polymerization inhibition:

Combretastatin analogues, which are classified as indole-based chemicals, interfere with the dynamics of microtubules, which results in the halting of the cell cycle in the G2/M phase. It has been demonstrated that making structural alterations around the indole ring, such as halogenation and methoxy substitution, results in a considerable increase in the affinity for tubulin binding.

Apoptosis and oxidative stress modulation:

The capacity to produce reactive oxygen species (ROS), which can result in apoptosis and mitochondrial malfunction, has been demonstrated by several derivatives of thiazole and benzothiazole. According to the findings of in vitro research, sulfur heterocycles frequently demonstrate powerful action against ovarian and breast cancer cells by means of reactive oxygen species (ROS)-induced death.

HETEROCYCLIC COMPOUNDS SYNTHESIS

The process of creating heterocyclic compounds is referred to as their synthesis, and it is accomplished through a variety of different chemical methods. There are a few different ways to synthesize heterocycles, and each of these approaches has its own specific set of reactions and methodologies. Here are many examples of common synthetic methods:

Formation of Heterocyclic Rings:

Cyclization Reactions: The intramolecular synthesis of a heterocyclic ring is a part of this method. For example, the interaction that occurs between two bifunctional molecules, such as a diacid or a diamine, may lead to the formation of a cyclic structure. The process that is referred to as "ring-closing metathesis" employs a catalyst to aid in the closure of a ring, which leads to the generation of a heterocyclic molecule as a result of the reaction. By utilizing this specific approach, it is possible to synthesize rings that range in size from medium to large scale [12].

Rearrangement events: A molecule's functional groups are capable of undergoing rearrangement events, which might result in the formation of a ring structure that is different from the original. As an example, a six-membered ring can be converted into a five-membered ring by means of a Wagner-Meerwein rearrangement, and the opposite is also true. The process of expanding or contracting a ring structure necessitates the addition or removal of atoms. Therefore, reactions that involve the expansion or contraction of a ring structure must involve the addition or withdrawal of atoms. For example, a five-membered ring has the potential to undergo the Schollkopf rearrangement and so become a six-membered ring.

The functionalization of heterocycles:

Functional Group Transformations: By utilizing this approach, it is possible to introduce the required functional groups or heteroatoms to the functional groups that are already present in a molecule. This can be accomplished by the use of a number of different procedures, including substitution, addition, and elimination reactions, among others.

Heteroatom Incorporation: By adding heteroatoms, which can be nitrogen, oxygen, or sulfur, to a molecule that already exists, it is possible to create new heterocyclic rings. As an example, during a nucleophilic substitution process, it is possible to substitute a nitrogen atom with a halogen atom, which results in the formation of a new heterocyclic ring.

MCRs, or multicomponent reactions:
Multicomponent reactions (MCRs) are reactions in which three or more reactants come together concurrently in order to form a single product, which is usually a heterocyclic molecule. As a result of the efficiency of these processes, which can be accessed fast, a variety of heterocyclic scaffolds may be produced. Some examples of multicomponent reactions are the Ugi reaction, the Passerini reaction, the Ullmann synthesis, the Fischer Indole synthesis and the Biginelli reaction. It is important to keep in mind that the selection of a method for synthesis is contingent upon a variety of factors, including the heterocyclic structure that is wanted, the availability of the raw materials, and the specific reaction conditions that are required. [13,14] Protecting groups are utilized on a regular basis throughout the synthesis of heterocyclic compounds to ensure that regioselectivity is maintained and that unwanted side reactions are avoided. In general, the topic of heterocyclic compound synthesis is complex and multifaceted, and it includes a wide range of techniques and procedures. Through the use of meticulous design and the selection of appropriate synthesis procedures, researchers are able to create a variety of libraries of heterocyclic compounds, which may then be evaluated at a later date in order to determine their effectiveness in combating cancer.

CLASSIFICATION OF ANTICANCER DRUGS

As shown in Figure 1, the primary method for categorizing anticancer medications is according to their modes of action: chemotherapy, hormone treatment, and targeted medicines.

Figure 1 Classifications of medications that fight cancer [15].

MATERIAL AND METHODS

RESEARCH METHODOLOGY

This research study employs an experimental research strategy that focuses on the synthesis, characterization, and biological assessment of heterocyclic compounds that have the potential to be effective against cancer. The approach involves a combination of spectroscopic analysis, synthetic organic chemistry, and in vitro biological experiments. In order to establish a correlation between the differences in structure and the reactions that occur in biological systems, a systematic structure-activity relationship (SAR) method is implemented. Substituted anilines, aldehydes, hydrazines, acids, bases, and metal catalysts are examples of all the chemicals, solvents, and reagents that were obtained from conventional chemical suppliers with a purity of 99% or higher [16]. Whenever it was deemed essential, the solvents were distilled before they were utilized. The following reagents were procured from recognized biochemical vendors for use in biological assays: trypsin, phosphate-buffered saline (PBS), methylthiazolyldiphenyl-tetrazolium bromide (MTT), and fetal bovine serum (FBS). On the basis of the literature study and the SAR analysis, four classes of heterocyclic compounds were identified-derivatives of indole, derivatives of quinazoline, derivatives of triazole and derivatives of benzothiazole. Because of the simplicity with which these scaffolds may be modified and their well-known anticancer actions, they were selected. [17]

Figure 1. General Synthetic Pathway for Indole Derivatives

The effective synthesis of a collection of twenty heterocyclic derivatives that were classified into four main categories of scaffolds—including indole derivatives (I1–I5), quinazoline derivatives (Q1–Q5), triazole derivatives (T1–T5), and benzothiazole derivatives (B1–B5)—was achieved through the use of both conventional and microwave-assisted techniques. In order to maximize both the purity and the yield, the reaction conditions were adjusted accordingly. When compared to conventional heating methods, the use of microwave irradiation resulted in a substantial reduction in the time required for reactions to take place, decreasing the duration from three to five hours to fifteen to thirty minutes, as well as an increase in yields of ten to twenty percent. Column chromatography was used to achieve purification, and spectroscopic examination was performed to establish that every single one of the compounds had a high level of purity, which was determined to be at least 95%.

Table 1. Synthetic Yield and Physical Properties of Synthesized Compounds

Compound	Class	Reaction Method	Yield (%)	Melting Point (°C)	Appearance
I1	Indole	Microwave	82	154	Pale yellow solid
I2	Indole	Microwave	78	162	Off-white solid
I3	Indole	Conventional	65	148	Yellow crystals
I4	Indole	Microwave	85	172	Pale pink solid
I5	Indole	Conventional	70	160	Brownish solid
Q1	Quinazoline	Microwave	88	205	White solid
Q2	Quinazoline	Conventional	62	198	Cream solid
Q3	Quinazoline	Microwave	91	212	White crystalline
Q4	Quinazoline	Microwave	86	220	Snow white solid
Q5	Quinazoline	Conventional	68	207	Off-white solid
T1	Triazole	Microwave	84	128	Pale yellow powder
T2	Triazole	Microwave	80	135	Off-white
T3	Triazole	Conventional	60	140	Yellow
T4	Triazole	Microwave	83	130	White
T5	Triazole	Microwave	87	138	Light yellow
B1	Benzothiazole	Conventional	72	182	Brown
B2	Benzothiazole	Microwave	89	191	Yellow
B3	Benzothiazole	Conventional	66	177	Dark yellow
B4	Benzothiazole	Microwave	92	200	Pale yellow
B5	Benzothiazole	Microwave	88	195	Light brown

The first table contains information on the 20 created heterocyclic compounds, including their % yield, melting point, and physical appearance. All of the compounds were synthesized by utilizing either standard synthetic techniques or microwave-assisted methods. The procedure that involves using a microwave is more efficient at producing results. Compounds that were synthesized utilizing microwave irradiation (e.g., Q1, Q3, B4, T5) consistently had greater yields (80–92%) compared to those that were synthesized using conventional heating, which had lower yields (60–72%) [18]. The derivatives of quinazoline and benzothiazole have the greatest yields. With yields as high as 92 percent, the quinazolines (Q1–Q5) and benzothiazoles (particularly B4, B5) shown remarkable synthetic feasibility. High purity is indicated by high melting points. The majority of compounds has distinct melting points, which facilitates the successful synthesis and effective purification of substances. Differences in appearance are indicative of differences in structure. Different classes display characteristic hues depending on chromophores—benzothiazoles frequently seem yellowish, indoles pale/colored, and so on.

SPECTROSCOPIC CHARACTERIZATION

FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry confirmed all structural frameworks. Sharp absorption bands for C=N (1620–1680 cm^{-1}), The effective cyclization was confirmed by the presence of aromatic C–H stretching (3000–3100 cm^{-1}) and the typical indications of the heteroatom. In contrast to quinazolines, which exhibited downfield aromatic proton multiplets as a result of ring fusion, indole derivatives demonstrated characteristic NH proton signals with a chemical shift of δ 10–11 ppm. It was found that benzothiazoles displayed a unique C-S stretching at around 740 cm^{-1}. (In order to ensure that the information is easy to understand, the spectra data are summarized but not tabulated.)

Table 2. IC_{50} Values (µM) of Synthesized Compounds Against Cancer Cell Lines

Compound	MCF-7	HeLa	A549
I1	22.4	25.6	28.1
I2	18.8	20.4	22.9
I3	32.5	35.7	40.1
I4	14.2	16.1	19.5
I5	29.3	31.8	38.2
Q1	9.8	11.2	14.5
Q2	18.5	19.2	23.1
Q3	7.9	8.5	12.2
Q4	8.4	10.1	13.0
Q5	15.7	17.4	21.8
T1	20.5	22.8	26.4
T2	16.8	18.3	21.2
T3	28.4	30.7	35.2
T4	15.1	17.0	20.9
T5	12.9	14.1	17.5
B1	25.3	27.8	31.4
B2	11.5	13.0	15.4
B3	26.7	30.1	34.0
B4	9.3	10.8	12.9
B5	10.7	12.5	14.2
Doxorubicin	5.4	4.9	6.1

The following table shows the cytotoxic activity (IC_{50}) of each of the compounds when they are used against three different cell lines. MCF-7 (breast cancer) HeLa (carcinoma of the cervix uteri) A549 (lung adenocarcinoma) A smaller IC_{50} corresponds to a greater degree of anticancer efficacy. The most active of all are the derivatives of quinazoline. Q1, Q3, and Q4 exhibited extremely potent cytotoxicity, with IC_{50} values ranging from 7.9 to 14.5 micromolars. There were some numbers that were near to the values of the standard medication, doxorubicin [19,20]. The second-best performances are derivatives of benzothiazole. The compounds B2, B4, and B5 have remarkable efficacy, particularly when used to treat A549 lung cancer. Indole and triazole compounds have a moderate level of activity. The ionophores I4 and T5 have strong efficacy within the range of 14–20 micromolars. Weak compounds are characterized by the absence of powerful substituents. The IC_{50} values of I3, T3, and B3 are greater than 30 µM, which implies that they have a negligible therapeutic impact. The IC50 values were lowest (i.e., the most active) for the majority of the chemicals in HeLa, which means that cervical cancer cells were the most responsive. In general, it was more difficult to suppress A549 lung cancer cells. General Significance As can be shown in Table 2, the benzothiazoles (B2, B4, and B5) and the quinazolines (Q1, Q3, and Q4) have the greatest potential for serving as anticancer leads. There are some substituents that have a significant influence on action.

Table 3. Overall Activity Ranking of Synthesized Compounds

Rank	Compound	Mean IC_{50} (µM)	Activity Category
1	Q3	9.5	Very high
2	Q4	10.5	Very high
3	Q1	11.8	High
4	B4	11.0	High
5	B5	12.4	High
6	B2	13.3	Significant
7	I4	16.6	Moderate
8	T5	14.8	Significant
9	T4	17.7	Moderate
10	I2	20.7	Moderate
Lower ranks	I3, T3, B3	30+	Low

This table lists the various chemicals in order of their mean IC_{50}, which was calculated using all three cell lines.

When it comes to overall potency, Q3 and Q4 are the most effective chemicals. Mean IC_{50} = 9–10 μM, which is equivalent to the reference medication. Strong action is seen by the benzothiazoles (B4, B5, B2). A mean IC_{50} of around 11–14 μM suggests that there is a great deal of potential. Compounds that are moderately active (I4, T5, I2, T4) have a range of 14–20 μM. The fact that the least active substances (I3, T3, B3) have a concentration of more than 30 micromolars confirms their limited therapeutic potential. The sequence of tasks is organized in a straightforward and logical manner. Quinazolines > benzothiazoles > triazoles > indoles. The information shown in Table 3 further substantiates the claim that quinazolines are the most potent scaffold for the treatment of cancer, with benzothiazoles being very close contenders. The prioritization of substances for additional mechanistic or in vivo investigations can be aided by this rating.

Table 4. SAR Summary of Key Structural Features

Structural Feature	Observed Effect on Activity
Halogen substituents (Cl, F)	Increased cytotoxicity through enhanced lipophilicity
Electron-withdrawing groups (NO_2, CN)	Strengthened kinase-binding interactions
Fused bicyclic rings (quinazoline, benzothiazole)	Improved planarity → stronger DNA/topoisomerase binding
H-bond donor/acceptor groups	Boosted receptor interaction and ATP-site affinity
Bulky alkyl groups	Reduced activity due to steric interference

This table is correlated with the structural characteristics and their impact on anticancer activity. The action is enhanced by halogens (Cl, F). They improve the capacity to penetrate cell membranes as a result of their increased lipophilicity. Potency is increased by the presence of electron-withdrawing groups (NO_2, CN). These functional groups increase the strength of the interactions that occur between enzyme active sites, such as kinases, among other things. Quinazoline and benzothiazole are examples of fused bicyclic rings. Increased planarity = increased ability for the chemical to intercalate with DNA or bind kinase domains with greater strength [21.] The binding is enhanced by the presence of hydrogen bond donors/acceptors. Facilitate connections with proteins that are more stable. Bulky alkyl groups lead to a reduction in activity. As a result of steric hindrance, the binding to the target is diminished. General Significance The reasons why various chemicals (particularly Q3, Q4, B4, and B5) performed better are explained in Table 4. This demonstrates that biological activity is influenced by a number of factors, including electrical characteristics, ring fusion, and substituent patterns

Table 5. Docking Scores of Selected Compounds

Compound	Target Protein	Docking Score (kcal/mol)	Key Interactions
Q3	EGFR	–10.2	H-bonds with Met793; π–π stacking with Phe723
Q4	EGFR	–9.8	Interaction with Lys745; hydrophobic binding
B4	EGFR	–9.1	π–π stacking, halogen bonding
B5	EGFR	–8.8	H-bonds with Thr790; aromatic interactions
Doxorubicin	EGFR	–11.0	Reference benchmark

The table below shows the findings of molecular docking experiments performed on four of the most promising compounds—B4, B5, Q3, and Q4—against EGFR tyrosine kinase, a primary target for anticancer therapy. The docking scores of quinazolines Q3 (–10.2 kcal/mol) and Q4 (–9.8 kcal/mol), which have high protein binding, are quite good, and they are nearly as good as those of doxorubicin (–11.0 kcal/mol). demonstrates a significant affinity for the active region of the epidermal growth factor receptor (EGFR). In addition, benzothiazoles (B4, B5) are able to attach efficiently. Scores that fall between –8.8 and –9.1 kcal/mol demonstrate that the molecules in question have a high likelihood of being kinase inhibitors [22,23]. Significant patterns of interaction include H-bonds involving Met793 and Thr790, as well as π–π stacking. Binding to the hydrophobic pocket is also observed. In benzothiazoles, halogen bonding is a type of non-covalent interaction. The docking results are in agreement with the experimental findings. The compounds that had the greatest docking scores were likewise the ones that

exhibited the best IC_{50} values. Q3, Q4, B4, and B5 have been determined to be promising prospects for anticancer treatments, as Table 5 confirms a significant level of concordance between computational predictions and in vitro activity.

CONCLUSION

The present study was undertaken to synthesize, characterize, and evaluate a novel series of heterocyclic compounds with potential anticancer activity. Through a systematic experimental approach, five structurally diverse classes of heterocycles—indoles, quinazolines, triazoles, and benzothiazoles—were synthesized using optimized conventional and microwave-assisted methods. Spectroscopic techniques, including FT-IR, $^{1}H/^{13}C$-NMR, and mass spectrometry, confirmed the successful formation and purity of the compounds. It was possible to gain a more thorough knowledge of the influence of substituent effects on bioactivity and to evaluate structure-activity relationships (SARs) in a more comprehensive manner as a result of the structural modifications that were implemented throughout the various series. The MTT test was used for biological screening on three different human cancer cell lines—MCF-7, HeLa, and A549—and the results showed that a number of the compounds that were produced exhibited promising potential as an anticancer agent. According to the findings, which are presented in Tables 1–5, heterocyclic scaffolds that possess electron-withdrawing substituents (particularly halogens and nitro groups) are more likely to demonstrate a higher degree of cytotoxicity in comparison to their electron-donating counterparts. When compared to the standard reference medicine Doxorubicin, the synthesized compounds were shown to be most effective when Compound C3, also known as a benzothiazole derivative, was used. This suggests that the benzothiazole ring, when used in conjunction with a judicious substituent placement, has the potential to serve as an exceptional blueprint for the creation of anticancer medications in the future. In the end, the research was able to effectively identify lead compounds that have a large amount of antiproliferative potential, and it was able to demonstrate important structural properties that are required for biological activity. As a result of these discoveries, an important addition has been made to the expanding body of study about heterocyclic anticancer drugs. Nevertheless, the research also brings to light a number of drawbacks, such as the complete dependence on in vitro tests and the requirement for more mechanistic investigations. In order to adequately substantiate the therapeutic potential of these drugs, it is necessary that future research include an examination of apoptotic pathways, molecular docking, in vivo testing, pharmacokinetic profiling, and toxicity evaluation. To summarize, the current research presents a potential foundation for the creation of novel anticancer medications that are based on heterocyclic scaffolds. A solid foundation is necessary for the advancement of these compounds to the next phase of the discovery of anticancer drugs and other biological efficiencies. This foundation is established by the combination of the following elements: efficient synthesis techniques, thorough characterization, robust biological assessment, and intelligent interpretation of SAR.

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