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Design, Synthesis, and Anticancer Evaluation of Pyrimidine-based Derivatives

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ABSTRACT

Cancer remains a leading global health challenge, driving an urgent need for novel therapeutic agents that offer greater efficacy and safety. The pyrimidine nucleus, a foundational component of DNA, RNA, and key cofactors, stands as a privileged scaffold in anticancer drug discovery. This systematic review comprehensively analyzes the design, synthesis, and biological evaluation of new pyrimidine-based derivatives reported over the past decade. We detail modern design strategies, including molecular hybridization with heterocycles and natural products, scaffold morphing via ring fusion, and rational targeting of critical pathways such as kinase signaling (EGFR, VEGFR, CDKs) and nucleotide metabolism (TS, DHFR). The synthesis of these libraries leverages both classical cyclocondensation reactions and advanced, efficient methodologies. A critical analysis of *In vitro* data across diverse cancer cell lineages reveals potent, often nanomolar, cytotoxicity and elucidates established mechanisms of action, including apoptosis induction, cell cycle arrest, and enzyme inhibition. A comprehensive Structure-Activity Relationship discussion identifies key pharmacophoric features and substituent effects governing potency and selectivity. Despite promising results, significant challenges remain, particularly the frequent lack of in vivo validation and detailed mechanistic studies. Future progress hinges on integrating computational chemistry for rational design and ADMET prediction, exploring emerging targets, and rigorously pursuing the translational pathway to clinically viable, multi-targeted anticancer agents.

Keywords: Design, Synthesis, Pyrimidine-Based Derivatives

INTRODUCTION

Cancer remains one of the most formidable global health challenges of our time, constituting a leading cause of morbidity and mortality worldwide[1]. According to the World Health Organization's International Agency for Research on Cancer, the global burden is projected to escalate dramatically, with new cancer cases expected to rise to over 35 million by 2050-a 77% increase from 2024 data. This surge is driven by complex factors including aging populations, lifestyle changes, environmental carcinogens, and persistent health disparities that limit access to early detection and preventative care[2,3]. The human cost is immense, with millions of lives lost annually, while the socioeconomic impact strains healthcare systems, reduces workforce productivity, and imposes catastrophic financial burdens on families. Despite significant advances in early diagnosis and multimodal treatment regimens comprising surgery, radiotherapy, and chemotherapy, the therapeutic landscape is fraught with limitations. Conventional cytotoxic agents, the long-standing cornerstone of pharmacotherapy, often suffer from severe, dose-limiting toxicities, narrow therapeutic indices, and the inevitable development of multidrug resistance, leading to therapeutic failure and disease relapse. Furthermore, many treatments lack specificity, causing widespread damage to healthy proliferating cells and severely compromising patients' quality of life[4,5]. These stark realities underscore an urgent and perpetual need for the discovery and development of novel, more effective, and safer anticancer agents. The paradigm is shifting from broadly cytotoxic drugs to targeted, mechanism-based therapies designed to interfere with specific molecular pathways fundamental to oncogenesis, metastasis, and survival. Innovation in drug design focuses on improving selectivity for malignant cells, overcoming resistance mechanisms, and minimizing adverse effects, thereby offering hope for more durable responses and improved survival outcomes. The pursuit of novel chemotherapeutic scaffolds is, therefore, not merely an academic endeavor but a critical imperative in the ongoing global fight against cancer, aiming to transform it from a often-lethal diagnosis to a manageable chronic condition[6].

The term "privileged scaffold" in drug discovery refers to a core molecular structure capable of yielding potent and selective ligands for diverse biological targets through systematic modification. The pyrimidine ring, a six-membered heterocycle featuring two nitrogen atoms at the 1 and 3 positions, epitomizes this concept. Its profound significance in medicinal chemistry, particularly in oncology, is inextricably linked to its fundamental role in the chemistry of life itself, providing an unparalleled platform for designing biomimetic agents that can seamlessly integrate into and disrupt critical cellular processes[7].

Biological Significance of Pyrimidine in Nucleotides and Co-factors

The pyrimidine nucleus is a foundational building block of life, forming the core of three key nucleobases: cytosine, thymine, and uracil. Cytosine and thymine are essential components of deoxyribonucleic acid (DNA), while cytosine and uracil are integral to ribonucleic acid (RNA). This central role in genetic material means that any synthetic analogue of a pyrimidine base is inherently poised to interfere with the replication, transcription, and translation processes that are hyperactive in rapidly proliferating cancer cells[8]. Beyond nucleic acids, pyrimidine derivatives are crucial co-factors in cellular metabolism and biochemistry. For instance, thiamine (vitamin B1), which contains a pyrimidine moiety, is a vital coenzyme in carbohydrate metabolism. Similarly, the pyrimidine ring is present in essential molecules like folic acid, a key cofactor in one-carbon transfer reactions necessary for the de novo synthesis of purines and thymidylate[9].

Historic and Clinically Approved Pyrimidine-Based Anticancer Drugs

The translation of pyrimidine's biological role into therapeutic triumph has a rich and successful history, solidifying its status as a cornerstone of cancer chemotherapy. The prototype and perhaps most iconic agent is 5-Fluorouracil (5-FU), a uracil analogue first synthesized in 1957[10]. Its mechanism is a masterclass in antimetabolite action: following intracellular activation, it forms a covalent complex with thymidylate synthase (TS), irreversibly inhibiting the synthesis of thymidine monophosphate (dTMP), a crucial precursor for DNA replication. Furthermore, its metabolites can be misincorporated into RNA and DNA, leading to catastrophic cellular dysfunction[11]. The clinical success of 5-FU, despite its toxicity, validated the pyrimidine-antimetabolite strategy and spurred the development of prodrugs like Capecitabine, an orally administered agent that is enzymatically converted to 5-FU preferentially in tumor tissue, offering improved convenience and a better safety profile. Expanding on this theme,

other nucleoside analogues emerged. Gemcitabine (2',2'-difluorodeoxycytidine), a deoxycytidine analogue, represents a more sophisticated evolution. It is phosphorylated intracellularly to its active diphosphate and triphosphate forms. The diphosphate inhibits ribonucleotidereductase, starving the cell of deoxyribonucleotides, while the triphosphate is incorporated into elongating DNA strands[12]. However, after incorporation, it allows for one additional nucleotide to be added before causing "masked chain termination," making the damage irreparable and triggering apoptosis. Cytarabine (Ara-C), another nucleoside analogue, follows a similar activation pathway and primarily causes chain termination during DNA synthesis. These agents have become mainstays in treating a range of cancers, including pancreatic (gemcitabine), leukemias (cytarabine), and breast and colorectal cancers (5-FU/capecitabine). Their enduring clinical utility, spanning decades, serves as powerful testament to the enduring potency and adaptability of the pyrimidine scaffold. They provide both the inspiration and the pharmacophoric blueprint for the ongoing design of new generations of targeted pyrimidine-based therapeutics aimed at overcoming the limitations of their predecessors[13].

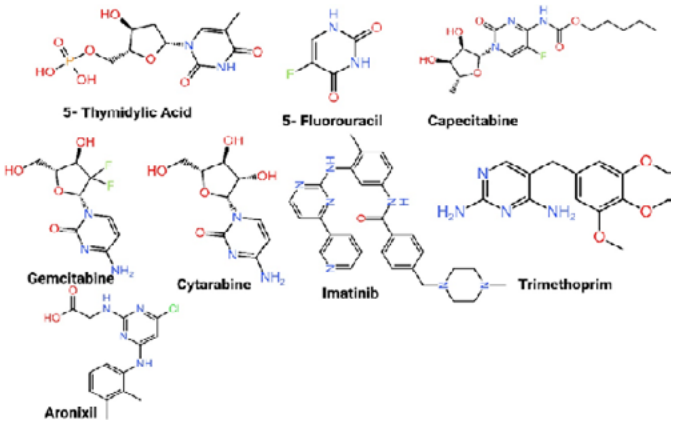
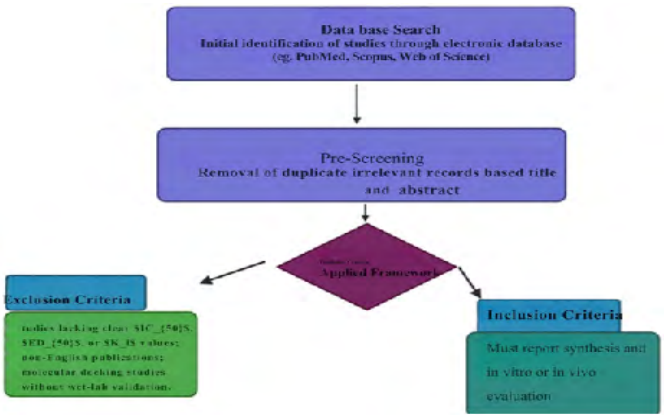


Fig. 1. Marketed drugs containing the pyrimidine scaffold

Methodology Systematic Review Protocol

To ensure reproducibility and minimize bias, a rigorous systematic methodology was employed. A comprehensive literature search was conducted across major electronic databases—PubMed, Scopus, Web of Science, and SciFinder—using tailored Boolean operators combining keywords related to "pyrimidine," "synthesis," and "anticancer activity." The search was limited to studies published between 2013 and 2025 to capture the most recent decade of innovation. Pre-defined eligibility criteria focused on primary research articles describing novel synthetic pyrimidine derivatives evaluated for cytotoxicity in specific cancer cell lines *(In vitro)* and/or animal models *(In vivo)*, with mechanistic studies prioritized. The study selection process followed the PRISMA guidelines, detailed in a flow diagram, to document the screening and exclusion of records. Data from included studies were extracted into a standardized table, and their methodological quality was assessed using established tools (e.g., ToxRTool for *In vitro* studies).



Design Strategies for Novel Pyrimidine-Based Anticancer Agents

Molecular Hybridization Approaches

Molecular hybridization stands as one of the most prolific and successful strategies in modern anticancer drug design, particularly when applied to a privileged scaffold like pyrimidine. This approach involves the covalent fusion of two or more distinct pharmacophoric units—each with known biological activity—into a single new chemical entity[14]. The primary rationale is to create a hybrid molecule that can either interact with multiple biological targets simultaneously (a multi-target-directed ligand, MTDL) or exhibit a synergistic effect by enhancing affinity for a single target, thereby overcoming drug resistance, improving efficacy, and potentially reducing side effects through a more refined pharmacological profile[15]. For pyrimidine derivatives, this has unlocked a vast chemical space, leading to compounds with remarkable potency and novel mechanisms.

Hybrids with Other Heterocycles leverage the complementary pharmacological properties of different ring systems. The fusion creates novel architectures that can interact with diverse binding sites. A prominent example is the integration of the 1,2,3-triazole ring, often installed via click chemistry[16,17]. The triazole moiety is more than just a bioisostere for an amide bond or a metabolically stable linker; it participates in key dipole interactions, hydrogen bonding, and π-stacking within enzyme active sites. Pyrimidine-triazole hybrids have demonstrated potent inhibition of kinases like EGFR and BRAF, as well as tubulin polymerization, leading to dual antiproliferative and anti-migratory effects. Similarly, quinazoline (a fused pyrimidine-benzene system) is itself a renowned anticancer scaffold (e.g., gefitinib, erlotinib). Further hybridization of a pyrimidine with a quinazoline or other heterocycles like indole (a component of many natural tubulin inhibitors) creates complex polyheterocyclic systems. For instance, pyrimidine-indole hybrids have shown exceptional ability to disrupt microtubule dynamics and induce mitochondrial-mediated apoptosis, often exhibiting nanomolar cytotoxicity against multidrug-resistant cell lines. Other hybrids incorporate imidazole, thiazole, or oxadiazole rings, each contributing specific electronic and steric properties that modulate solubility, target affinity, and cellular uptake[18,19]. Conjugates with Natural Products represent a powerful subset of hybridization, marrying the robust, target-focused pyrimidine core with the inherent bioactivity, structural diversity, and often favorable safety profiles of natural product pharmacophores. This strategy aims to capitalize on the unique mechanisms of natural compounds while improving their drug-like properties through synthetic modification. Chalcone-pyrimidine conjugates are a major area of research. Chalcones, with their α,β-unsaturated ketone system, are known inducers of oxidative stress and apoptosis. Attaching them to a pyrimidine ring often results in compounds with enhanced pro-apoptotic activity, potent inhibition of VEGF-induced angiogenesis, and significant tubulin destabilization, outperforming the parent fragments. Coumarin-pyrimidine hybrids are another successful class. Coumarins possess intrinsic anticancer activity through topoisomerase inhibition, cell cycle arrest, and angiogenesis suppression. Their fusion with pyrimidine, frequently at the 6 or 7 position of the coumarin, yields molecules that are potent multi-kinase inhibitors or topoisomerase II poisons, with improved lipophilicity and cellular permeability. Similarly, conjugation with flavonoid scaffolds (e.g., flavone, chromone) merges the pyrimidine's antimetabolite potential with the flavonoid's antioxidant, anti-inflammatory, and kinase-inhibitory properties. These hybrids frequently demonstrate dual inhibition of key pathways, such as simultaneously targeting thymidylate synthase and the PI3K/Akt/mTOR survival cascade, leading to profound cytotoxic synergy. Beyond these, hybrids with ursolic acid, artemisinin, or curcumin motifs are being explored to confer additional capabilities like hedgehog pathway inhibition or ferroptosis induction.

The limitations of traditional, single-target cancer drugs—often overcome by complex disease pathways and drug resistance—have spurred innovation in drug design. While combining separate drugs is one approach, it introduces challenges like unpredictable interactions and added patient burden. A more elegant solution lies in hybrid molecules: single chemical entities crafted to engage multiple cancer targets simultaneously[20,21]. The pyrimidine scaffold, due to its fundamental role in cell biology, has become a central hub for constructing these multi-tasking agents.

Several promising hybrids illustrate this strategy. For instance, researchers have fused pyrimidine with a coumarin natural product to create

a compound that selectively kills HER2-positive breast cancer cells. This hybrid works by triggering reactive oxygen species (ROS) and activating JNK signaling, leading to DNA damage and apoptosis. In another approach, linking pyrimidine to a 1,2,3-triazole ring yielded compounds with potency surpassing the standard drug 5-Fluorouracil in liver and cervical cancer cells. One such hybrid achieved its effects by inhibiting the Wee1 kinase and disrupting sphingolipid signaling, halting cell proliferation. The fusion of pyrimidine with an indole moiety has produced agents that attack cancer's structural framework. These hybrids destabilize microtubules—the cell's internal scaffolding—and inhibit the Hedgehog signaling pathway, a key driver in many cancers, as demonstrated in both cellular and zebrafish models. This dual action suppresses tumor cell migration and invasion[22].

Computational design is also paving the way. One in silico-designed triazole-pyrimidine hybrid showed strong potential to inhibit a key factor in gastric cancer, though its real-world efficacy awaits validation. Other successful hybrids incorporate diverse fragments Thiazolidinone-pyrimidine hybrids with halogen substitutions showed potent activity against lung cancer cells, likely by inhibiting the ROS1 kinase[23,24]. Acridone-pyrimidine hybrids exhibited a triple threat: they intercalate into DNA, inhibit the pro-survival Akt kinase, and combat multidrug resistance. A dual-pyrimidine-quinazolinone hybrid inhibited a metabolic enzyme (DPP-4) and arrested the cell cycle in colorectal cancer cells. Despite these successes, the path is not without hurdles. Some hybrids, like certain pyrimidine-glycosides, have shown unacceptable toxicity to healthy human cells in early tests, halting their development.

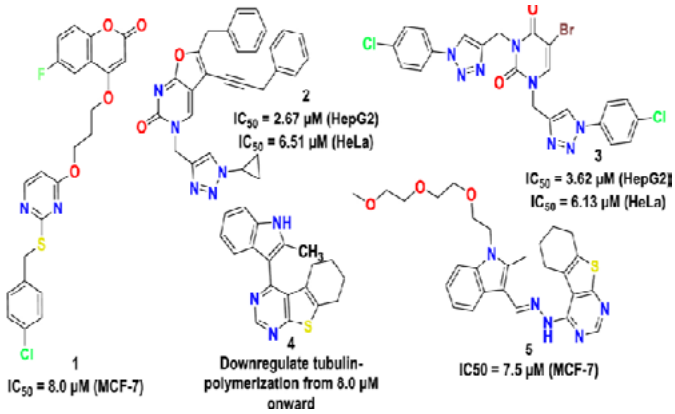


Fig. 2. Pyrimidine-based hybrid molecules as anticancer agent

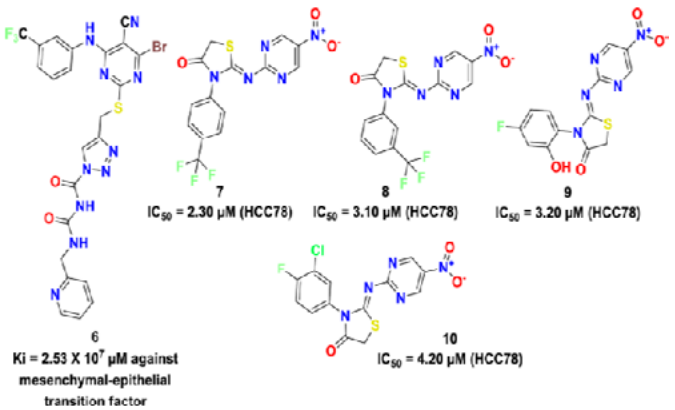


Fig. 3. Pyrimidine-derived hybrid molecules developed as anticancer agents

Scaffold Morphing and Ring Fusion/Expansion

Beyond linking discrete units, a powerful strategy for diversifying the pyrimidine core involves scaffold morphing, where the fundamental heterocyclic structure itself is altered through ring fusion or expansion[25,26]. This transforms the simple, monocyclic pyrimidine into more complex, rigid polycyclic systems, which can dramatically enhance binding affinity, selectivity, and metabolic stability by presenting a larger, more complementary surface area to biological

targets. Fused Bicyclic Systems represent the most direct evolution. The quinazoline scaffold (benzene fused to pyrimidine) is a preeminent success story, forming the core of blockbuster kinase inhibitors like erlotinib and gefitinib. This fusion increases planarity and lipophilicity, optimizing the molecule for deep insertion into the ATP-binding pockets of tyrosine kinases. Similarly, pyrimido[4,5-d]pyrimidines (a pyrimidine fused with another pyrimidine) create a dense, nitrogen-rich scaffold that is a potent inhibitor of key enzymes like dihydrofolatereductase and thymidylate synthase, often with greater potency than their monocyclic predecessors. Other fused systems, such as pteridines or purine analogues (imidazole-fused pyrimidines), mimic essential biological cofactors, allowing them to effectively disrupt folate metabolism and nucleotide biosynthesis pathways[27,28]. Tricyclic and Polycyclic Analogues push this concept further, constructing elaborate frameworks that can engage in complex interactions with diverse targets. Examples include angular systems like pyrimido[4,5-b]quinolines or linear fusions such as acridopyrimidines. These extended planar structures are particularly adept at DNA intercalation, leading to direct genetic disruption and topoisomerase inhibition. Their increased rigidity often translates to improved pharmacokinetic profiles by reducing conformational flexibility and susceptibility to enzymatic degradation. Furthermore, the specific arrangement of rings and substituents in these polycyclic systems can be finely tuned to selectively inhibit a specific kinase isoform or to simultaneously occupy adjacent pockets in a protein target, fostering the development of highly specific and potent multi-target agents. This structural sophistication, born from simple scaffold morphing, continues to yield novel chemical entities that probe new biological space in anticancer drug discovery[29,30].

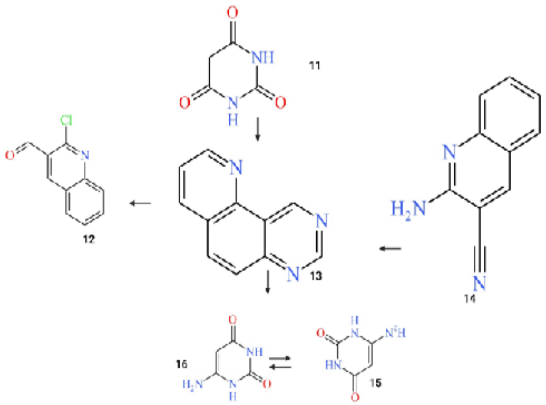


Fig. 4. The main building cycles for pyrimido[4,5-b]quinolines

Targeted Drug Design

Kinase Inhibitors (EGFR, VEGFR, BRAF, CDKs)

A predominant and highly successful application of modern pyrimidine chemistry is the rational design of kinase inhibitors. Kinases are enzymes that transfer phosphate groups, acting as critical "on/off" switches in cellular signaling pathways that govern proliferation, survival, and angiogenesis[31]. Their dysregulation is a hallmark of cancer, making them prime therapeutic targets. The pyrimidine scaffold is exceptionally well-suited for this role, as its planar structure and hydrogen-bonding capabilities allow it to compete with ATP for binding in the kinase's catalytic pocket[32].

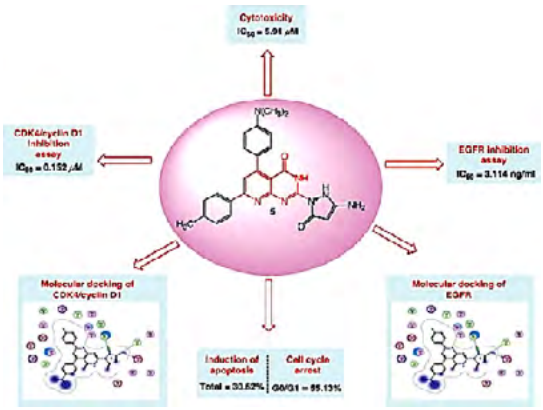


Fig. 5. Pyrimidine derivatives as dual inhibitors of EGFR and CDK4/cyclin D1 represents a cutting-edge strategy in targeted cancer therapy

EGFR Inhibitors The Epidermal Growth Factor Receptor is a key driver in cancers like non-small cell lung cancer (NSCLC). First-generation drugs like erlotinib and gefitinib, based on a quinazoline core (a fused pyrimidine), revolutionized treatment for patients with activating EGFR mutations. Newer pyrimidine-based derivatives aim to overcome resistance mutations (e.g., T790M, C797S) and improve brain penetration. VEGFR Inhibitors Targeting the Vascular Endothelial Growth Factor Receptor disrupts tumor angiogenesis, starving tumors of nutrients. Pyrimidine-based multi-kinase inhibitors like sorafenib (a diarylurea containing a pyrimidine core) inhibit VEGFR-2/3, among other targets, and are used in renal cell and hepatocellular carcinoma. BRAF Inhibitors The BRAF kinase, particularly the V600E mutant, constitutively activates the MAPK pathway in melanomas and other cancers. Vemurafenib, a pyrimidine-sulfonamide hybrid, is a potent and selective inhibitor of mutant BRAF, demonstrating how subtle modifications to the pyrimidine ring can confer

exquisite selectivity for a single mutant kinase over the wild-type form. CDK Inhibitors Cyclin-Dependent Kinases regulate the cell cycle. Dysregulated CDK activity leads to uncontrolled proliferation. Pyrimidine forms the core of several CDK4/6 inhibitors, such as palbociclib and abemaciclib, which are now standard-of-care in hormone receptor-positive breast cancer. These drugs typically feature a pyrimidine ring substituted with an aminopyridine or similar group to achieve selective, reversible inhibition, inducing G1 cell cycle arrest.

Thymidylate Synthase Inhibitors & Dihydrofolate Reductase (DHFR) Inhibitors

The enzymes Thymidylate Synthase and Dihydrofolate Reductase are pivotal, sequential nodes in the de novo pathway for synthesizing thymidine monophosphate (dTMP), a critical building block for DNA replication. Their inhibition starves rapidly dividing cancer cells of this essential nucleotide, leading to "thymineless death" and making them two of the most historically validated targets in cancer chemotherapy. Pyrimidine-based compounds are uniquely suited to inhibit these enzymes due to their structural mimicry of the natural pyrimidine substrates, with design strategies evolving from classic antimetabolites to sophisticated multi-target agents.

Thymidylate Synthase Inhibitors catalyze the reductive methylation of deoxyuridine monophosphate (dUMP) to dTMP, using 5,10-methylenetetrahydrofolate (mTHF) as a cofactor. The prototypical pyrimidine-based TS inhibitor is 5-Fluorouracil (5-FU) and its prodrugs. Its active metabolite, 5-fluoro-dUMP (FdUMP), forms a stable covalent ternary complex with TS and mTHF, leading to irreversible enzyme inhibition. The quest for newer pyrimidine TS inhibitors focuses on overcoming the resistance mechanisms that limit 5-FU's efficacy, such as increased TS expression or reduced activation. This has led to the development of novel benzylpyrimidine and quinazoline analogues that act as direct, non-covalent TS inhibitors with different binding modes. Furthermore, the pyrimidine core has been integrated into dual-targeting hybrids—for instance, molecules that combine a TS-inhibiting pyrimidine pharmacophore with a folate moiety or a topoisomerase inhibitor. These hybrids can simultaneously disrupt dTMP synthesis and cause additional DNA damage, exhibiting synergistic effects and activity against 5-FU-resistant cell lines. The core pyrimidine-dione or -diamine structure remains central, with strategic substitutions (e.g., at the N1, C5, and C6 positions) modulating lipophilicity, cellular uptake, and binding affinity to the TS active site.

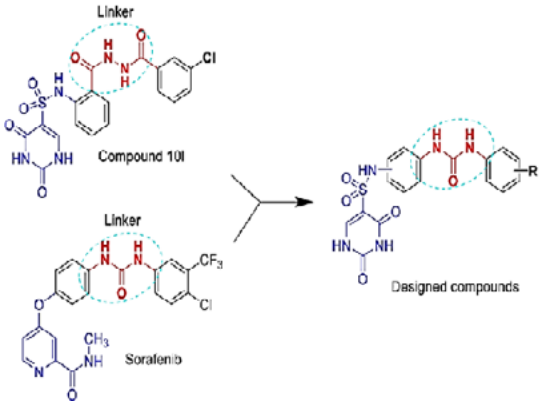


Fig. 6. N-phenyl-(2,4-dihydroxypyrimidine-5-sulfonamido) phenylurea hybrid as a dual-targeting antitumor agent

Dihydrofolate Reductase Inhibitors play an equally crucial role by maintaining the cellular pool of tetrahydrofolate (THF), the essential folate cofactor required for the TS reaction and purine synthesis. While the classic antifolate methotrexate is a pteridine-based structure, pyrimidine-based DHFR inhibitors represent a significant and potent class. These compounds, such as the antimalarial drug pyrimethamine and its anticancer analogues, function by competitively and tightly binding to the DHFR active site, mimicking the dihydrofolate substrate[34,35]. Modern design leverages the pyrimidine ring as a scaffold to anchor key pharmacophoric elements: a 2,4-diaminopyrimidine motif is nearly universal, with the 4-amino group forming critical hydrogen bonds with conserved aspartate residues in the binding pocket. The C5 position of the pyrimidine ring is a key site for modification; adding lipophilic aryl or aryloxy groups (e.g., as in trimethoprim-inspired molecules) enhances binding affinity and can be tuned to selectively inhibit human versus bacterial DHFR, or to overcome resistance via mutations. Recent advances involve creating DHFR-TS dual inhibitors—single molecules featuring a 2,4-diaminopyrimidine head (for DHFR binding) connected via a suitable linker to a dUMP-mimicking tail (for TS binding). These ambitious hybrids aim to cause a complete blockade of the dTMP synthesis pathway. Additionally, non-classical lipophilic antifolates with bulky pyrimidine substituents are designed to enter cells via passive diffusion rather

than the diminished folate carrier, bypassing a common transport-mediated resistance mechanism seen with methotrexate.

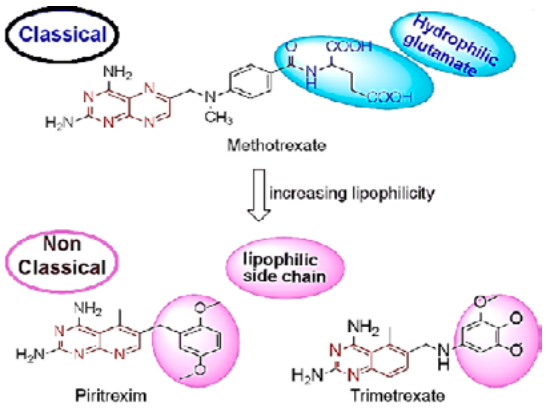


Fig. 7. Dihydrofolatereductase inhibition effect of 5-substituted pyrimidine

In vitro Anticancer Evaluation: Mechanisms and Potency

The curated panel of cancer cell lines presented here represents the cornerstone of modern in vitro anticancer drug discovery, most notably exemplified by the US National Cancer Institute's NCI-60 screening panel[37]. This diverse collection is strategically designed to encompass the pathological heterogeneity of human cancers, providing a crucial first-line biological filter for evaluating the potency and potential spectrum of activity of novel therapeutic agents, such as pyrimidine derivatives. The inclusion of cancer types—Leukemia, Non-Small Cell Lung Cancer, Colon Cancer, Central Nervous System (CNS)[38] Cancer, Melanoma, Ovarian Cancer, Renal Cancer, Prostate Cancer, and Breast Cancer—ensures that compounds are tested against a wide array of genetic backgrounds, histologies, and inherent resistance mechanisms. Each lineage serves as a specific model[38] Leukemia cell lines (CCRF-CEM, HL-60, K-562, etc.) represent liquid tumors and are critical for assessing activity against hematological malignancies, often revealing agents that interfere with rapid cell division and apoptotic pathways. Solid tumors are extensively covered, with the lung cancer subset (A549, NCI-H460, etc.) including adenocarcinomas and large cell carcinomas, models essential for testing agents targeting EGFR, KRAS, or ALK pathways[39]. The colon cancer panel (HCT-116, HT29, etc.) is vital for compounds aimed at pathways like Wnt/β-catenin or mismatch repair deficiency, while the CNS lines (SF-268, U251, etc.) present the challenge of the blood-brain barrier and the aggressive nature of gliomas[40,41].

Table 1: Cell line of *in vitro* anti-cancer

Cancer Type	Cell Line	Key Characteristics & Common MOA
Leukemia	CCRF-CEM	T-cell lymphoblastic leukemia. Used for DNA damaging agents (alkylators, topo inhibitors), antimetabolites, and apoptosis inducers
	HL-60(TB)	Promyelocytic leukemia. Classic model for differentiation therapy (e.g., ATRA-induced differentiation). Also for apoptosis
	K-562	CML-derived; expresses Bcr-Abloncoprotein. Model for tyrosine kinase inhibitors (e.g., Imatinib) and immunotherapies (NK cell cytotoxicity)
	MOLT-4	T-cell acute lymphoblastic leukemia. Similar use to CCRF-CEM for cytotoxic agents
	RPMI-8226	Multiple myeloma model. Used for proteasome inhibitors (e.g., Bortezomib), immunomodulatory drugs
	SR	Drug-sensitive leukemic line, often used in comparative studies with resistant lines
Non-Small Cell	A549/ATCC	Adenocarcinoma with KRAS mutation. Common workhorse for cytotoxicity, apoptosis, and studies on RAS pathway inhibitors
Lung Cancer	NCI-H460	Large cell lung cancer. Often highly proliferative and sensitive; used for initial screening
	NCI-H226	Squamous cell carcinoma. Used for studies targeting squamous cell markers/pathways
	NCI-H23	Adenocarcinoma with KRAS mutation.
	EKVX, HOP-62, HOP-92, NCI-H322M, NCI-H522	Part of NCI-60 diversity panel. Used to assess drug selectivity across different lung cancer genotypes
Colon Cancer	HCT-116	Common, microsatellite unstable, p53 wild-type. Benchmark for DNA damage and chemotherapy response
	HT29	p53 mutant, mucin-producing. Often used in combination therapy and drug resistance studies
	SW-620	Derived from a lymph node metastasis. Key model for invasion, migration, and metastasis-inhibition MOAs
	COLO 205, HCC-2998, HCT-15, KM12	NCI-60 panel lines providing genetic diversity (e.g., different MSI status, mutations in APC, KRAS)

CNS Cancer	U251	Glioblastoma. Primary model for glioma biology, invasiveness, and crossing the blood-brain barrier
	SF-268, SF-295, SF-539	Glioblastoma/astrocytoma lines. Used for alkylating agents (e.g., Temozolomide) and PI3K/AKT pathway inhibitors
	SNB-19, SNB-75	CNS carcinoma/melanoma. Used in panel for CNS-active compounds.
Melanoma	MDA-MB-435	Note: Historically used as melanoma but now believed to be a misidentified breast cancer line. Use with caution
	SK-MEL-28	Harbors a BRAF V600E mutation. Primary model for BRAF inhibitors (e.g., Vemurafenib) and resistance mechanisms
	LOX IMVI	Highly metastatic melanoma. Used for invasion inhibition and cytotoxic screening.
	M14, UACC-257, UACC-62, SK-MEL-2, SK-MEL-5, MALME-3M	NCI-60 panel providing diversity in BRAF/NRAS mutation status for targeted therapy studies
Ovarian Cancer	OVCAR-3	High resistance to multiple drugs. Model for multidrug resistance (MDR1/P-gp) and overcoming chemoresistance
	SK-OV-3	Lack of functional BRCA1, HER2 amplified. Used for PARP inhibitors and targeted therapies
	NCI/ADR-RES	A derivative of OVCAR-8 selected for doxorubicin resistance. The classic model for P-glycoprotein-mediated MDR
	IGROV1, OVCAR-4, OVCAR-5, OVCAR-8	Panel representing different sensitivities to platins and taxanes
Renal Cancer	786-0	Clear cell renal carcinoma (CCRCC) with VHL mutation. Model for angiogenesis inhibitors (e.g., anti-VEGF)
	ACHN	Papillary renal cell carcinoma. Used in broader panels for renal cancer drug screening
	CAKI-1	CCRCC, relatively chemosensitive. Used for immune-mediated killing assays as well
	A498, RXF 393, SN12C, TK-10, UO-31	NCI-60 panel lines representing renal cancer heterogeneity
Prostate Cancer	PC-3	Androgen receptor negative, highly metastatic to bone. Model for androgen-independent disease and bone metastasis
	DU-145	Androgen receptor negative, less aggressive than PC-3. Used for cytotoxicity and cell cycle arrest studies
Breast Cancer	MCF7	Luminal A type, ER+/PR+, HER2-. The classic model for estrogen-dependent growth and endocrine therapy (e.g., Tamoxifen)
	MDA-MB-231	Triple-negative breast cancer (TNBC), highly aggressive/invasive. Key model for metastasis, invasion, and targeted TNBC therapies
	T-47D	Luminal, ER+/PR+. Used alongside MCF7 for endocrine agent studies.
	BT-549	TNBC, metaplastic. Used for studies on EMT (Epithelial-Mesenchymal Transition) and invasion
	MDA-MB-468	TNBC, expresses EGFR. Model for EGFR-targeted therapies in TNBC
	HS 578T	TNBC, derived from a carcinosarcoma. Used in diverse breast cancer panels

Melanoma lines (SK-MEL series, LOX IMVI) are characterized by frequent BRAF mutations, serving as key models for targeted inhibitor development. Ovarian and renal cancer cell lines (OVCAR series, A498, UO-31) often exhibit distinct drug resistance profiles and are used to identify compounds that can overcome multidrug resistance or target clear cell or papillary histologies[42]. The prostate (PC-3, DU-145) and breast cancer panels (MCF7, MDA-MB-231, etc.) are further stratified by hormone receptor status (ER/PR, HER2), enabling the identification of targeted agents for specific clinical subtypes, such as CDK4/6 inhibitors for ER+ breast cancer or novel therapies for triple-negative disease[43]. The power of this panel lies not just in its diversity but in the deep molecular characterization of each line-their known oncogenic driver mutations, gene expression profiles, and proteomic signatures. This allows researchers to move beyond simple cytotoxicity (measured as % Growth Inhibition or GI50) and perform mechanistic correlations, identifying whether a new pyrimidine compound's activity is associated with specific molecular features, such as EGFR amplification, p53 mutation status, or BRCAness. Screening across this panel helps prioritize lead compounds with broad-spectrum efficacy or, conversely, exquisite selectivity for a particular cancer type, guiding subsequent mechanistic studies and *in vivo* model selection[44]. Ultimately, this systematic approach using a standardized cell line panel is indispensable for translating chemical synthesis efforts into biologically relevant anticancer candidates, providing the foundational data that bridges medicinal chemistry and preclinical oncology[45].

Critical Analysis and Structure-Activity Relationship (SAR)

The systematic analysis of the literature reveals clear and impactful Structure-Activity Relationship (SAR) trends for pyrimidine-based anticancer agents, delineating how specific molecular modifications dictate potency, selectivity, and mechanism of action[46]. A comprehensive SAR summary begins with the impact of substituents at core positions. The C2 position is exceptionally versatile; small amino groups (NH_2) are crucial for dihydrofolatereductase (DHFR) inhibition, forming vital hydrogen bonds, while bulkier aryl or heteroaryl groups at C2 are often hallmarks of kinase inhibitors (e.g., anilines in EGFR inhibitors), providing necessary hydrophobic interactions in the ATP-binding pocket. Substitution at C4 typically involves amino, oxo, or thioxogroups[47,48]. A 4-amino group is essential for classical antimetabolites like cytarabine and for many DHFR inhibitors, whereas a 4-oxo or 4-thioxo moiety is common in uracil/thymine analogues (e.g., 5-FU) and dihydropyrimidinones from Biginelli reactions, with thioxo often enhancing tubulin-binding affinity. The C5 position serves as a primary handle for modulating lipophilicity, electronic character, and steric bulk. Introducing hydrophobic aryl or heteroaryl rings at C5 frequently boosts potency against kinases and tubulin, as these groups occupy adjacent hydrophobic regions in the target proteins. Electron-withdrawing groups like halogens (e.g., fluorine in 5-FU) are critical for irreversible enzyme inhibition in antimetabolites[49]. Finally, the C6 position is similarly sensitive; small alkyl groups can be tolerated, but larger substitutions, especially fused ring systems as in quinazolines, dramatically enhance planar surface area for π-stacking interactions in kinase domains, directly correlating with increased binding affinity and selectivity, as evidenced by the evolution from simple pyrimidines to gefitinib[50].

Beyond the core, the role of linkers and hybrid fragments is paramount in dictating the pharmacological profile of modern multi-target agents[51,52]. The nature of the linker—whether a simple methylene chain, a piperazine, or a 1,2,3-triazole ring—profoundly influences molecular flexibility, conformation, and bioavailability. Flexible alkyl chains can allow a hybrid molecule to adapt to distinct binding sites, but may reduce affinity. Rigid or semi-rigid linkers like triazoles or amides lock the pharmacophores in a favorable orientation, potentially enhancing dual-target engagement; the triazole, in particular, adds dipole interactions and metabolic stability. The hybrid fragment itself dictates the secondary mechanism: conjugation with a chalcone moiety introduces pro-apoptotic and anti-angiogenic properties; fusion with a coumarin imparts topoisomerase inhibition; and attachment of an indole fragment directs the molecule toward tubulin polymerization disruption. The SAR indicates that successful hybridization is not merely additive; it often creates synergistic effects where the new molecule's potency exceeds that of either parent fragment, primarily by enabling simultaneous disruption of complementary pathways within the cancer cell[53,54].

Furthermore, the influence of stereochemistry and conformation is critical, especially in saturated or partially saturated derivatives like dihydropyrimidinones (DHPMs). For these scaffolds, the stereochemistry at chiral centers (often at C4 of the DHPM ring) can lead to significant differences in biological activity. One enantiomer or diastereomer may fit precisely into an asymmetric enzymatic pocket, such as tubulin's colchicine site, while its mirror image shows markedly reduced affinity[55]. Molecular conformation, governed by ring fusion and substituent sterics, also determines target selection. The planar, rigid conformation of fused polycyclic systems (e.g., acridopyrimidines) is ideal for DNA intercalation, while a slightly puckered conformation might be optimal for fitting into the allosteric pocket of a specific kinase isoform[55,56].

From this SAR analysis, key promising pharmacophoric features can be identified. A recurrent high-value pharmacophore is the 2,4-diaminopyrimidine motif, a near-universal signature for potent DHFR inhibition and a component of many kinase-directed scaffolds. The 4-anilinoquinazoline system remains a gold-standard pharmacophore for EGFR inhibition[57,58]. For microtubule disruption, a 5-aryl-3,4-dihydropyrimidin-2(1H)-one core with specific substitutions at the aryl ring and N1 position emerges as a highly potent template. Additionally, the 1,2,3-triazole-linked hybrid system has solidified itself not just as a linker but as an integral pharmacophore enhancing dipole interactions and bioavailability across multiple target classes. A comparison of

potency and selectivity across different structural classes reveals distinct hierarchies and therapeutic niches[59]. Classical antimetabolites (e.g., 5-FU, cytarabine analogues) often exhibit high potency (nanomolar to low micromolar IC_{50}) but can suffer from broader toxicity due to their mechanism, impacting all rapidly dividing cells. Their selectivity is often pharmacokinetic (targeting cells with high nucleotide demand) rather than truly target-based. In contrast, kinase inhibitor-based pyrimidines (quinazolines, pyrimido-pyrimidines) can achieve extraordinary selectivity for mutant over wild-type kinases (e.g., vemurafenib for BRAF V600E) and low nanomolar potency, translating to a high therapeutic index in genetically defined cancers. The tubulin-targeting dihydropyrimidinones frequently show impressive nanomolar cytotoxicity, often superior to doxorubicin or 5-FU in screens, and can retain activity in multidrug-resistant cell lines, though their *in vivo* selectivity requires careful optimization to mitigate neurotoxicity risks. Multi-target hybrid molecules represent an intriguing middle ground; their individual potency against a single target might be slightly lower (micromolar range) than a highly optimized single-target inhibitor, but their ability to concurrently hit two or more pathways often results in superior overall efficacy, synergy, and a higher barrier to resistance in cellular models. However, this polypharmacology can complicate selectivity and toxicity predictions, underscoring the need for rigorous *in vivo* validation. In summary, the evolution from simple antimetabolites to targeted kinase inhibitors and sophisticated hybrids reflects a broader trend in oncology drug design: a relentless pursuit of agents that marry the profound cell-killing potency of cytotoxic chemotherapies with the precise, tumor-specific targeting of modern molecular therapeutics, with the pyrimidine scaffold proving endlessly adaptable to this challenge[60].

Current Challenges and Future Perspectives

Despite significant progress, the development of pyrimidine-based anticancer agents faces persistent challenges that define the critical path forward. A major limitation in current literature is the preponderance of promising *in vitro* data that lacks subsequent validation in robust *in vivo* models, coupled with often superficial or entirely unexplored mechanisms of action (MoA). Addressing these gaps is paramount. Here, computational chemistry offers transformative tools; molecular docking and dynamics simulations enable rational, target-guided design and MoA elucidation, while in silico ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) profiling allows for the early prioritization of leads with favorable drug-like properties, streamlining optimization. Future discovery must also look beyond established targets. Emerging opportunities include modulating protein-protein interactions, targeting cancer stem cells, and exploiting vulnerabilities like ferroptosis or specific metabolic dependencies. Furthermore, the inherent multi-target potential of many pyrimidine hybrids positions them ideally for rational combination therapies with existing standards of care or for drug repurposing initiatives, potentially overcoming resistance and improving outcomes. The ultimate pathway to clinical translation requires a disciplined, integrated approach. It necessitates moving from cell panels to patient-derived xenografts and genetically engineered mouse models, conducting rigorous pharmacokinetic/pharmacodynamic (PK/PD) and toxicology studies, and designing compounds with clear intellectual property and clinical development strategies. Closing these gaps between benchside innovation and bedside application is the essential next chapter for pyrimidine-based anticancer research.

CONCLUSION

The pyrimidine ring system has proven to be an exceptionally versatile and resilient cornerstone in the ongoing quest for novel anticancer therapeutics. As this systematic review illustrates, its inherent biological significance provides a unique platform for designing biomimetic agents capable of disrupting the fundamental processes of cancer cell proliferation and survival. The evolution from classic antimetabolites like 5-fluorouracil to sophisticated, targeted kinase inhibitors and multi-target hybrid molecules underscores the scaffold's remarkable adaptability. Strategies such as molecular hybridization and scaffold morphing have exponentially expanded the chemical space, yielding compounds with enhanced potency, the ability to overcome drug resistance, and novel mechanisms of action.The critical analysis of structure-activity relationships provides a valuable roadmap for future design, clarifying the distinct roles of substituents at each core position and the impact of linkers and hybrid fragments. While

in vitro screening across comprehensive cell line panels provides essential proof-of-concept and helps prioritize leads, the transition to meaningful clinical candidates requires overcoming persistent gaps. The foremost challenges include advancing promising compounds into rigorous *in vivo* efficacy and toxicology studies, deepening mechanistic elucidation beyond initial cytotoxicity, and employing computational tools early in the design process to optimize drug-like properties.Looking forward, the future of pyrimidine-based anticancer agents lies in the intelligent integration of polypharmacology, aiming for selective multi-target inhibition to improve efficacy and durability of response. Embracing emerging biological targets and strategies like drug repurposing will further unlock the potential of this privileged scaffold. Ultimately, by bridging innovative synthetic chemistry with robust translational biology, the next generation of pyrimidine derivatives holds significant promise for delivering the more effective, targeted, and tolerable therapies urgently needed in the global fight against cancer.

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Conflict of interest

The author declare that we have no conflict of interest.

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