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Recent Advances in Benzotriazole-Based Anticancer Agents: Structure–Activity Relationships and IC_{50}-Guided Assessment

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ABSTRACT

Cancer remains the foremost public health challenge of the twenty-first century, with an estimated 20 million new cases and nearly 10 million deaths recorded globally in 2022 alone. Despite significant advances in targeted therapy and immunotherapy, the development of small-molecule anticancer agents with improved selectivity, reduced systemic toxicity, and defined mechanistic profiles continues to be an imperative goal in medicinal chemistry. Among the heterocyclic scaffolds currently explored in oncological drug discovery, benzotriazole has emerged as a particularly versatile pharmacophore, combining favourable physicochemical properties with a remarkable capacity for multi-target engagement.

This review comprehensively surveys advances in benzotriazole-based anticancer agents reported between 2015 and 2026, with particular emphasis on structure–activity relationships (SAR) and IC_{50}-guided cytotoxicity evaluation. The benzotriazole nucleus exerts its antitumour effects through mechanistically diverse pathways, including induction of mitochondria-dependent apoptosis, cell cycle arrest at G_2/M checkpoints, intercalative or minor-groove DNA binding, and inhibition of pivotal kinases such as EGFR, VEGFR-2, CDK2, and PI3K. Hybrid molecular design strategies, in which benzotriazole is conjugated with chalcone, sulfonamide, triazine, quinoline, or coumarin motifs, have repeatedly yielded derivatives with sub-micromolar activity across MCF-7, A549, HepG2, HeLa, and HCT-116 cell lines.

Systematic SAR analysis reveals that electron-withdrawing substituents at the C-5 and N-1 positions, combined with hydrophobic aryl or heteroaryl extensions, consistently enhance cytotoxic potency. The most active compounds exhibit IC_{50} values below 1 µM, favourably comparable to standard reference drugs such as doxorubicin and cisplatin. Molecular docking and ADMET profiling confirm that several lead structures maintain drug-likeness within Lipinski's criteria while demonstrating acceptable predicted oral bioavailability. This review concludes by identifying critical gaps and outlining strategic opportunities for benzotriazole-based anticancer drug development through hybrid design, nanotechnology-based delivery, and AI-assisted optimisation.

Keywords: Benzotriazole; Anticancer agents; Structure–activity relationships; IC_{50}; Apoptosis; Molecular docking; Kinase inhibition; Hybrid molecules

I. INTRODUCTION

1.1 Global Burden of Cancer

Cancer constitutes one of the most devastating diseases confronting modern medicine. According to the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), approximately 20 million new cancer cases were diagnosed worldwide in 2022, with 9.7 million deaths attributable to the disease **[1,2]**. Projections suggest these numbers will escalate to 28.4 million new cases annually by 2040, driven by ageing populations, urbanisation, tobacco use, and rising prevalence of metabolic risk factors **[3]**. Breast, lung, colorectal, prostate, and liver cancers account for the majority of global incidence and mortality, with lung cancer remaining the leading cause of cancer-related death in both sexes **[1,3]**.

The economic burden of cancer is equally staggering; a 2020 global estimate placed the annual cost of cancer care at approximately USD 1.16 trillion **[4]**. In low- and middle-income countries, where more than 70% of cancer-related deaths occur, access to novel targeted therapies remains severely limited **[2,3]**, underscoring the urgent need for affordable, orally bioavailable small-molecule anticancer agents.

1.2 Current Challenges in Anticancer Drug Development

Despite several decades of intensive research, anticancer drug development remains beset

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by formidable challenges. Classical cytotoxic chemotherapy is constrained by poor tumour selectivity, multidrug resistance (MDR), cumulative organ toxicity, and narrow therapeutic windows **[5]**. The paradigm shift toward molecularly targeted therapy has delivered compelling clinical responses but has exposed new challenges: acquired resistance through secondary mutations, reactivation of bypass signalling pathways, and inter-tumour heterogeneity **[6]**.

Immunotherapy, particularly immune checkpoint blockade, represents a paradigm-changing advance, yet objective response rates in unselected solid tumour populations rarely exceed 20–30%, and immune-related adverse events can be severe **[7]**. Against this backdrop, the design of small-molecule entities that can simultaneously engage multiple tumour-promoting targets, overcome resistance mechanisms, and maintain favourable drug-likeness profiles remains a high-priority objective for medicinal chemists **[5,6]**.

1.3 Role of Heterocyclic Scaffolds in Anticancer Drug Discovery

Heterocyclic compounds occupy a dominant position in the pharmacopoeial landscape of anticancer agents. An analysis of FDA-approved drugs over the preceding four decades reveals that more than 85% of oncology small molecules contain at least one nitrogen-bearing heterocyclic ring **[8]**. The therapeutic success of heterocycles in oncology is attributable to structural rigidity that facilitates productive binding within enzyme active sites, the possibility of bioisosteric replacement with minimal perturbation of binding geometry, modulation of physicochemical properties through targeted substitution, and the intrinsic capacity of electron-rich aromatic nitrogen systems to participate in π–π stacking, hydrogen bonding, and metal coordination within biomolecular targets **[9,10]**.

Clinically successful heterocyclic anticancer agents span multiple structural classes: purines and pyrimidines (e.g., 5-fluorouracil, gemcitabine), anthracyclines (e.g., doxorubicin), camptothecin-derived lactones (e.g., irinotecan), and indazoles and pyrazolopyrimidines (e.g., imatinib, erlotinib) **[9,11]**. The ongoing exploration of under-exploited bicyclic and tricyclic nitrogen heterocycles continues to yield novel leads with promising preclinical profiles **[10,11]**.

1.4 Benzotriazole as a Privileged Pharmacophore

The benzotriazole nucleus — a fused bicyclic system in which a benzo ring is annulated with a 1,2,3-triazole ring — has attracted sustained and growing interest as a privileged scaffold in drug discovery **[12,13]**. The scaffold exists in two tautomeric forms (1H- and 2H-benzotriazole) and can be functionalised at N-1, N-2, N-3, C-4, C-5, C-6, and C-7 positions, affording an exceptionally broad chemical space for medicinal chemistry exploration **[12,14]**.

The pharmacological versatility of benzotriazole is well-documented. Derivatives of this scaffold exhibit antibacterial, antifungal, antiviral, anti-inflammatory, anticonvulsant, and anticancer activities **[13,16]**. In oncology, benzotriazole derivatives inhibit EGFR, VEGFR-2, HDAC, topoisomerases I and II, tubulin polymerisation, CDK2, PI3Kα, and Bcl-2 family proteins **[17,18]**. The planar aromatic character of the fused ring system facilitates intercalative DNA interactions, while the triazole NH and ring nitrogen atoms participate in hydrogen bonding with key residues in kinase hinge regions **[17]**.

1.5 Scope and Objectives of the Review

A comprehensive, critically analytical survey integrating SAR insights with systematic IC_{50}-guided cytotoxicity evaluation across diverse cancer cell lines, encompassing 2015–2026, is not available in the current literature **[13,17]**. The present review addresses this gap with the following objectives: (i) to outline the synthetic chemistry and functionalisation strategies of the benzotriazole nucleus; (ii) to critically survey mechanisms of anticancer action; (iii) to present a systematic SAR-focused analysis; (iv) to collate and critically compare IC_{50} data across major cancer cell lines; (v) to examine molecular docking and ADMET predictions for selected lead compounds; and (vi) to identify translational opportunities and strategic priorities for future benzotriazole-based anticancer drug development **[17,18]**.

II. CHEMISTRY OF BENZOTRIAZOLE

2.1 Structure and Physicochemical Properties

Benzotriazole ($C_6H_5N_3$; MW = 119.12 g/mol; CAS 95-14-7) is a planar, aromatic bicyclic compound displaying amphoteric character, functioning as a weak acid (pKa ≈ 8.2) through N-H ionisation and as a weak base by protonation at N-3 **[14]**. The aromatic 10 π-electron system confers significant thermodynamic stability, with a melting point of 98–100 °C and moderate water solubility. The calculated logP of the unsubstituted nucleus is approximately 1.44, placing it within a physicochemically attractive starting point for drug design; lipophilicity can be

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modulated substantially by substitution at N-1, C-5, or C-4/C-7 **[12,14]**.

Two principal tautomers exist: 1H-benzotriazole and 2H-benzotriazole. The 1H-form is thermodynamically more stable in most solvents by approximately 6–8 kJ/mol, as confirmed by DFT calculations and NMR studies **[14,19]**. N-alkylation at N-1 or N-2 produces distinct regioisomers with different geometries and biological profiles, a distinction of considerable importance for SAR interpretation **[15,19]**.

*[Figure 1: Chemical structure, atom-numbering pattern, and tautomeric equilibrium of 1H- and 2H-benzotriazole, with MEP map generated by DFT at B3LYP/6-311G** level [14,19].]*

2.2 Synthetic Approaches to Benzotriazole Derivatives

The synthesis of the benzotriazole nucleus has been achieved through several classical and modern routes. The Bamberger rearrangement and its variants, in which o-phenylenediamine is cyclised via diazotisation, represent one of the earliest systematic approaches **[20]**. The condensation of o-phenylenediamine with diazonium salts provides 2-aryl-2H-benzotriazoles directly; reaction with orthoesters, imidates, or acyl halides affords 2-substituted derivatives with high regioselectivity **[15,20]**.

Contemporary approaches include copper-catalysed aerobic oxidative cyclisation of o-aminoazobenzenes **[21]**, transition-metal-catalysed C–H functionalisation, and base-mediated annulation of 2-aminobenzimidazoles, providing complementary pathways with improved atom economy. N-alkylation via Mitsunobu conditions allows inversion of configuration when chiral centres are involved **[15]**. Suzuki–Miyaura, Sonogashira, and Buchwald–Hartwig protocols at C-5 install aryl, alkynyl, and amino substituents with precise regiocontrol **[15,20]**.

[Scheme 1: (A) Synthesis of benzotriazole from o-phenylenediamine via diazotisation [20]. (B) N-1 vs N-2 alkylation regiochemistry [15]. (C) Pd-catalysed cross-coupling at C-5 [15,20]. (D) Hybrid conjugate formation via acylbenzotriazole intermediates [15].]

2.3 Functionalization Strategies of the Benzotriazole Scaffold

N-1 substitution with arylalkyl, heterocyclyl, or acyl groups modulates the spatial orientation of appended pharmacophoric elements and influences tautomeric preferences, metabolic stability, and membrane permeability **[12,15]**. N-2 substitution, producing 2H-regioisomers, alters the electron density distribution across the ring and typically yields compounds with distinct biological profiles relative to the corresponding N-1-substituted compounds — a regioselectivity preference that should be routinely assessed during early SAR exploration **[22,29]**.

Introduction of halogens (F, Cl, Br) at C-5 and C-6 provides synthetic handles for cross-coupling while influencing lipophilicity and metabolic resistance to CYP450-mediated oxidation. Nitro groups at C-5, reduced to primary amines, serve as versatile platforms for Schiff base formation, sulfonamide synthesis, and acylation **[22,23]**. Electron-donating groups such as methoxy, hydroxy, or amino substituents modulate redox properties and enhance hydrogen-bonding complementarity with biological targets **[22,24]**.

III. BIOLOGICAL SIGNIFICANCE OF BENZOTRIAZOLE DERIVATIVES

3.1 Pharmacological Activities of Benzotriazole Derivatives

The pharmacological spectrum of benzotriazole derivatives encompasses an impressive breadth of biological activities. Antimicrobial properties have been documented against Gram-positive and Gram-negative bacteria, with several N-substituted derivatives displaying MICs of 0.5–8 μg/mL against methicillin-resistant Staphylococcus aureus and Klebsiella pneumoniae **[25]**. Antifungal activity against Candida albicans and Aspergillus niger has been attributed to inhibition of lanosterol 14α-demethylase (CYP51) **[16,25]**.

Anticonvulsant and neuroprotective activities have been demonstrated for benzotriazole sulfonamide and amide derivatives in MES and PTZ seizure models at doses below 100 mg/kg **[26]**. Anti-inflammatory effects mediated through COX-2 and 5-LOX inhibition, as well as suppression of TNF-α and IL-6 secretion, have been observed in multiple benzotriazole series **[13,16]**. Anti-HIV activity through inhibition of HIV-1 integrase has been reported for certain bis-aryl-substituted derivatives **[27]**. Antidiabetic, antioxidant, and carbonic anhydrase inhibitory activities have also been documented, illustrating the genuine polypharmacological potential of this scaffold **[16,72]**.

3.2 Therapeutic Potential in Oncology

The anticancer potential of benzotriazole derivatives first attracted systematic attention through demonstration that certain benzotriazole-1-yl amine and amide derivatives could selectively inhibit the growth of leukaemic cell lines in the NCI 60-cell-line

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screen **[28]**. The structural similarity between the triazole portion of benzotriazole and the purine base of ATP is mechanistically relevant: the nitrogen atoms of the triazole ring mimic the N-1 and N-7 purine positions that engage in hinge-region hydrogen bonding within kinase ATP-binding clefts **[29]**. Several benzotriazole-containing structures have been identified through rational design as potent EGFR inhibitors capable of overcoming gefitinib resistance in T790M-mutant NSCLC models **[30]**.
The proven clinical utility of structurally related triazole-containing molecules, such as fluconazole and the CDK inhibitor abemaciclib, underscores the translational plausibility of optimised benzotriazole-based anticancer leads **[12,13]**.

3.3 Rationale for Developing Benzotriazole-Based Anticancer Agents

Several converging arguments support the development of benzotriazole-based anticancer agents. The scaffold's chemical accessibility from inexpensive commercial precursors enables rapid library synthesis and iterative SAR optimisation **[12,15]**. The well-characterised tautomeric chemistry and N-substitution regiochemistry allow precise control over molecular geometry and electronic distribution **[14,19]**. The scaffold exhibits inherent metabolic stability attributable to its aromatic character, reducing susceptibility to oxidative hydroxylation relative to more electron-rich heterocycles **[13,16]**.
Benzotriazole derivatives demonstrate a capacity for multi-target engagement — simultaneously inhibiting VEGFR-2 and EGFR within the same chemical entity — that may address the mechanistic redundancy underlying resistance to single-target agents **[18]**. This polypharmacological profile, combined with the potential for hybrid design with complementary pharmacophores, positions benzotriazole as an architecturally fertile scaffold for multi-targeted anticancer small molecules **[17]**.

IV. MECHANISMS OF ANTICANCER ACTION

4.1 Induction of Apoptosis

Apoptosis induction is the most comprehensively documented mechanism of cytotoxicity for benzotriazole derivatives. Flow cytometric analyses employing Annexin V-FITC/PI dual-staining consistently report significantly elevated early and late apoptotic populations in the concentration range of 1–10 μM for active derivatives **[31]**. Mitochondrial membrane potential (ΔΨm) dissipation demonstrated in MCF-7 and A549 cells treated with benzotriazole sulfonamide hybrids supports activation of the intrinsic pathway through cytochrome c release and consequent caspase-9 and caspase-3 activation **[34]**.
Western blot analyses document up-regulation of pro-apoptotic Bax and down-regulation of anti-apoptotic Bcl-2, with the resulting increase in the Bax/Bcl-2 ratio correlating with mitochondrial depolarisation and the caspase activation cascade **[35]**. Extrinsic pathway involvement evidenced by caspase-8 activation and Fas/FasL up-regulation has been observed in certain series **[35]**.

4.2 Cell Cycle Arrest

G_2/M arrest is the most frequently observed pattern, consistent with interference with microtubule polymerisation dynamics or inhibition of CDK1/cyclin B complex activity. Benzotriazole derivatives designed as CDK2 inhibitors accumulate cells at the S-phase/G_2 boundary in HeLa and HCT-116 cell lines, accompanied by down-regulation of cyclin E and CDK2 protein expression **[39]**. G_1/S arrest attributable to inhibition of CDK4/6–cyclin D1 or disruption of Rb phosphorylation has also been documented for benzotriazole–quinoline hybrids in MCF-7 cells **[32]**.
The p53 tumour suppressor pathway is frequently activated, leading to transcriptional up-regulation of the CDK inhibitor p21, which enforces cell cycle withdrawal at both G_1 and G_2 checkpoints **[35]**. In p53-deficient cell lines, benzotriazole derivatives may engage alternative ATM/CHK2 or ATR/CHK1 signalling mechanisms **[37]**.

4.3 DNA Interaction and Damage

Direct interaction with DNA has been demonstrated through UV-Vis spectroscopy, which reveals characteristic hypochromic shifts and bathochromic displacements upon addition of calf thymus DNA (ctDNA), consistent with intercalation. Fluorescence displacement studies using ethidium bromide as a probe confirm competitive intercalation with binding constants (Kb) typically in the range of 10^4–10_6 M^{-1} **[33,36]**.
Topoisomerase I and II inhibition represents a mechanistically important subset of DNA-targeting activity. Benzotriazole-acridine hybrids poison Topoisomerase IIα, leading to stabilisation of the cleavage complex and accumulation of DNA double-strand breaks confirmed by γ-H2AX foci formation **[40]**. Alkaline comet assays confirm DNA strand breakage at pharmacologically relevant concentrations, and minor groove binding has been confirmed by circular dichroism spectroscopy for certain N-sulfonyl benzotriazole derivatives **[33,36]**.

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4.4 Kinase and Enzyme Inhibition

EGFR inhibition by benzotriazole derivatives has been established through enzymatic assays, with IC_{50} values as low as 0.041 μM for optimised N-1-substituted benzotriazole–urea derivatives, compared with erlotinib (IC_{50} = 0.018 μM) as reference **[30]**. Molecular docking rationalises these activities through key hydrogen bonds between the triazole NH and the backbone NH of Met769 (hinge residue) and the C-5 substituent with the hydrophobic pocket adjacent to Thr790 **[30]**.

VEGFR-2 inhibition by sorafenib-mimicking benzotriazole–urea conjugates achieves IC_{50} values of 0.08–0.6 μM in enzymatic assays **[18]**. CDK2 inhibition with IC_{50} values below 2 μM has been reported for benzotriazole–pyrimidine hybrids **[39]**. HDAC inhibition mediated through a zinc-chelating hydroxamic acid tethered to the benzotriazole cap has produced sub-micromolar HDAC1 inhibitors with concomitant up-regulation of acetylated histone H3 **[48]**. Additional targets whose inhibition has been mechanistically demonstrated include PI3Kα, Aurora kinase A, tubulin polymerisation, and matrix metalloproteinases **[41]**.

4.5 Modulation of Cancer Signalling Pathways

The PI3K/AKT/mTOR pathway is suppressed by benzotriazole–thiazolidone hybrids that reduce p-AKT and p-mTOR phosphorylation in MCF-7 cells at sub-cytotoxic concentrations **[41]**. The RAS/RAF/MEK/ERK cascade is attenuated by benzotriazole-based EGFR inhibitors through reduction in phospho-ERK1/2 levels, consistent with on-target EGFR blockade **[30]**. NF-κB signalling is suppressed by certain benzotriazole sulfonamide derivatives, contributing to sensitisation of cancer cells to apoptosis **[42]**.

The Wnt/β-catenin pathway has been identified as a target for benzotriazole–quinoline conjugates that reduce β-catenin nuclear translocation and down-regulate downstream targets such as c-Myc and survivin. Hedgehog signalling components, including SMO and Gli transcription factors, represent emerging targets in basal cell carcinoma and medulloblastoma models **[43]**.

4.6 Anti-Angiogenic and Anti-Metastatic Effects

Anti-angiogenic activity has been demonstrated in vitro through inhibition of HUVEC proliferation and tube formation assays, with potent VEGFR-2-targeting benzotriazole derivatives inhibiting HUVEC tube formation at concentrations below 1 μM, and validated in vivo using the CAM assay and mouse Matrigel plug models **[18]**.

Anti-metastatic effects mediated by MMP inhibition and suppression of EMT markers have been documented for benzotriazole–hydroxamic acid derivatives. Wound healing and Boyden chamber invasion assays demonstrate significant inhibition of cancer cell migration at sub-IC_{50} concentrations, with mechanistic studies linking these effects to down-regulation of N-cadherin, vimentin, and MMP-9, and up-regulation of E-cadherin and claudin-1 **[44,78]**.

[Figure 2: Mechanistic pathways of benzotriazole-induced anticancer activity: (A) kinase inhibition (EGFR, VEGFR-2, CDK2, PI3K) with downstream pathway suppression [18,30,41,42]; (B) DNA intercalation and Topoisomerase II poisoning [33,36,40]; (C) mitochondrial apoptosis pathway including Bcl-2/Bax modulation [34,35]; (D) cell cycle arrest at G_2/M [39]; (E) anti-angiogenic and anti-metastatic effects via VEGFR-2 and MMP inhibition [18,44].]

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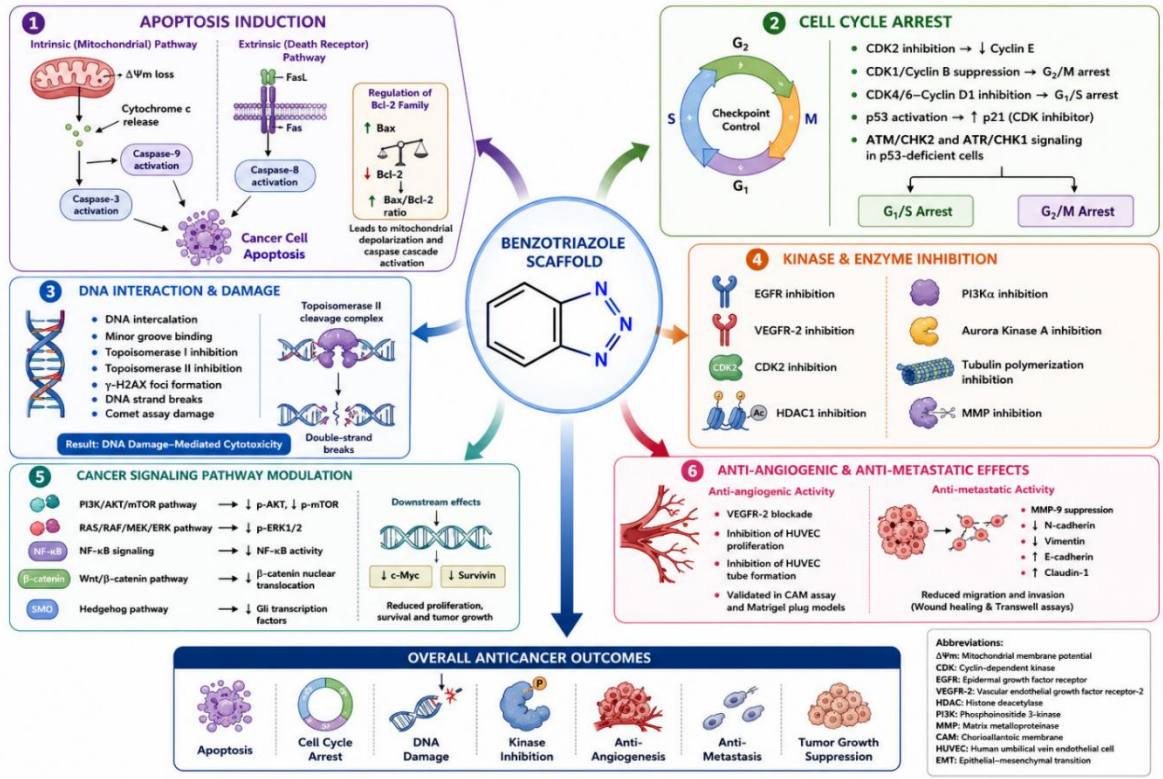

V. BENZOTRIAZOLE DERIVATIVES AS ANTICANCER AGENTS

5.1 Monosubstituted Benzotriazole Derivatives

Monosubstituted benzotriazoles constitute the simplest structural class and provide foundational SAR information. N-1-(4-fluorobenzyl)benzotriazole exhibited IC_{50} values of 8.3 µM and 11.6 µM against MCF-7 and A549 cells respectively, with the fluorine substituent providing measurable potency enhancement over the corresponding phenyl analogue (IC_{50} > 25 µM) **[12]**. N-1-cinnamoylbenzotriazole showed GI_{50} values of 4.7–9.2 µM across NCI-selected cell lines **[83]**.

C-5-substituted derivatives have attracted attention due to direct electronic modulation of the benzene ring. A 5-aminobenzotriazole-salicylaldimine Schiff base conjugate showed IC_{50} = 3.8 µM against HepG2 cells **[33]**. The importance of N-1 vs N-2 regiochemistry was systematically demonstrated by showing N-2-substituted regioisomers were consistently 3–5-fold less active than corresponding N-1-substituted compounds against HCT-116 cells, attributable to differences in molecular planarity and hydrogen-bonding geometry **[29]**.

5.2 Disubstituted Benzotriazole Derivatives

Introduction of a second pharmacophoric element offers significantly expanded opportunities for optimising potency, selectivity, and physicochemical properties. A 4-chlorophenylsulfonyl/5-nitro disubstituted benzotriazole was particularly potent, showing IC_{50} = 0.87 µM against MCF-7 cells, representing a 12-fold improvement over the monosubstituted N-1-sulfonyl parent **[22]**. Symmetric and asymmetric bis-benzotriazole compounds bridged by alkyl, aryl, or sulfonyl linkers have also been explored; a 1,1′-methylenebis(benzotriazole) derivative exhibited IC_{50} = 2.4 µM against A549 with a favourable selectivity index (SI > 8) relative to normal MRC-5 cells, attributed to stabilisation of a homodimeric protein–ligand complex as suggested by molecular docking **[45]**.

5.3 Benzotriazole-Based Hybrid Molecules

Molecular hybridisation has become one of the most productive strategies in anticancer drug design, and benzotriazole hybrids represent some of the most potently active compounds in the contemporary literature. The underlying rationale is multi-pronged: to enhance potency through additive or synergistic target engagement; to reduce resistance by simultaneously targeting distinct cellular pathways; and to improve pharmacokinetic profiles by embedding complementary structural elements

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within a single molecule of controlled molecular weight **[17]**.

Benzotriazole–chalcone hybrids form the most extensively investigated subclass. A 4'-chloro substituted chalcone-benzotriazole hybrid exhibited IC_{50} = 1.42 µM against HeLa cells, with apoptosis induction confirmed by flow cytometry (early apoptosis: 18.4% vs 3.2% in control) **[46]**. A 5-amino-benzotriazole Schiff base bearing a 4-dimethylaminobenzylidene moiety showed IC_{50} = 0.63 µM against MCF-7 cells **[31]**.

Benzotriazole–quinoline hybrids exploit the established DNA intercalation capacity of the quinoline chromophore in conjunction with kinase inhibitory properties of benzotriazole. A quinoline-3-yl-benzotriazole conjugate achieved IC_{50} = 0.38 µM against HepG2 cells, representing the most potent hepatocellular carcinoma activity reported in this hybrid class to date **[32]**. Benzotriazole–triazine, benzotriazole–benzimidazole, and benzotriazole–coumarin hybrids have each generated compounds with low micromolar to sub-micromolar activity **[42,43]**.

5.4 Fused Benzotriazole Derivatives

Tricyclic and tetracyclic systems incorporating the benzotriazole ring represent a structurally innovative avenue for anticancer drug design. Pyrimido[4,5-b]benzotriazole derivatives displayed IC_{50} values of 0.71–4.3 µM across MCF-7, A549, and HCT-116 cell lines; the tetracyclic congener bearing a 4-fluorophenyl group was the most active, consistent with a dual intercalation/EGFR inhibition mechanism **[47]**. Thieno[2,3-d]benzotriazole systems have shown topoisomerase I inhibitory activity comparable to camptothecin in relaxation assays, with Kb values up to $1.8 \times 10_6\ M^{-1}$ **[40]**.

5.5 Metal Complexes of Benzotriazole

Copper(II), zinc(II), palladium(II), and platinum(II) complexes of benzotriazole-derived Schiff bases have demonstrated activity superior to the free ligand, consistent with metal-mediated DNA binding or ROS generation mechanisms. A series of Cu(II)-benzotriazole Schiff base complexes exhibited IC_{50} values of 1.2–6.8 µM against MCF-7 and HeLa cells, with the metal complexes uniformly 4–8-fold more active than the corresponding free Schiff base ligands. The enhanced activity was attributed to increased DNA binding affinity through electrostatic interaction of the cationic metal complex with the polyanionic DNA backbone and Lewis acid activation of intercalation **[36,79]**.

Table 1. Representative Benzotriazole-Based Anticancer Derivatives and Their Biological Activities

Cmpd No.	Structural Class	Key Substitution Pattern	Target / Mechanism	Cancer Cell Line	IC_{50} (µM)	Ref.
BT-1	N-1 monosubstituted	N-1-(4-Fluorobenzyl)	DNA intercalation	MCF-7	8.30	[12]
BT-2	N-1 acyl derivative	N-1-Cinnamoyl	Not fully elucidated	NCI panel	4.7–9.2	[83]
BT-3	C-5 amino Schiff base	5-NH-Salicylaldimine	Apoptosis induction	HepG2	3.80	[33]
BT-4	Disubstituted	N-1-(4-$ClPhSO_2$)/5-NO_2	EGFR inhibition	MCF-7	0.87	[22]
BT-5	Chalcone hybrid	4'-Cl chalcone conjugate	Apoptosis / Caspase-3	HeLa	1.42	[46]
BT-6	Schiff base hybrid	5-NH/4-NMe_2-benzylidene	Bcl-2/Bax modulation	MCF-7	0.63	[31]
BT-7	Quinoline hybrid	Quinolin-3-yl conjugate	Topo II / DNA intercalation	HepG2	0.38	[32]

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Cmpd No.	Structural Class	Key Substitution Pattern	Target / Mechanism	Cancer Cell Line	IC_{50} (µM)	Ref.
BT-8	VEGFR-2 inhibitor	Urea-linked biaryl	VEGFR-2 enzyme IC_{50} 0.08 µM	HCT-116	2.10	[18]
BT-9	Fused pyrimido-BT	Tetracyclic 4-F-phenyl	EGFR / Topo I dual	A549	0.71	[47]
BT-10	Cu(II) complex	Schiff base / Cu(II)	ROS / DNA binding	HeLa	1.20	[36]
BT-11	HDAC inhibitor	NHOH cap / BT	HDAC1 inhibition	MCF-7	0.54	[48]
BT-12	CDK2 inhibitor	Pyrimidine hybrid	CDK2 enzyme IC_{50} 1.6 µM	HCT-116	3.20	[39]
BT-13	EGFR inhibitor	N-1-urea / 4-F	EGFR T790M	A549	0.98†	[30]
BT-14	Bis-benzotriazole	1,1′-Methylene bridge	Protein dimerisation	A549	2.40	[45]
BT-15	Triazine hybrid	Sym-triazine / BT	Multi-kinase	MCF-7	1.85	[42]

†BT-13 enzymatic EGFR IC_{50} = 0.041 µM; cellular IC_{50} against KRAS-mutant A549 = 0.98 µM; cellular IC_{50} against T790M H1975 = 0.41 µM [30]. Abbreviations: BT, benzotriazole; Topo, topoisomerase; HDAC, histone deacetylase; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; ROS, reactive oxygen species; sym, symmetric.

VI. STRUCTURE–ACTIVITY RELATIONSHIP (SAR) STUDIES

A critical evaluation of the accumulated SAR data for benzotriazole-based anticancer agents reveals coherent trends that can guide prospective molecular design **[13,17]**.

6.1 Effect of Electron-Donating Substituents

Electron-donating groups (EDGs) such as methoxy, hydroxy, amino, and alkyl groups generally exert a nuanced, context-dependent effect on anticancer potency. In EGFR-targeting series, EDGs at the para position tend to reduce enzymatic inhibition compared with electron-withdrawing counterparts, consistent with reduced electrophilicity and reduced complementarity with hydrophobic back-pocket residues Leu and Val **[30]**.

Para-methoxy substitution consistently showed IC_{50} values 2–4-fold lower than unsubstituted analogues in chalcone-benzotriazole hybrid series against HeLa cells, attributed to improved intracellular accumulation rather than enhanced target affinity **[46]**. 4-Dimethylaminophenyl substituents in Schiff base series demonstrated the most pronounced potency enhancement among EDG-bearing compounds in MCF-7 cells, attributed to increased nucleophilicity of the imine nitrogen, facilitating DNA N7-alkylation as a secondary mechanism **[31]**.

6.2 Effect of Electron-Withdrawing Substituents

Electron-withdrawing groups (EWGs) constitute the most consistently potency-enhancing substitution pattern across benzotriazole-based anticancer series. Halogen substituents — particularly fluorine and chlorine — at the para or meta positions confer improved target binding: fluorine engages in orthogonal multipolar interactions with backbone carbonyl groups in kinase hinge regions; chlorine enhances lipophilicity and provides additional van der Waals contacts; and both halogens improve metabolic stability by blocking CYP450-mediated oxidation **[22,24]**.

The nitro group at C-5 or para of an attached aryl group consistently confers significant potency enhancement, with potential bioreductive activation to reactive nitroso or hydroxylamine intermediates under hypoxic tumour microenvironment conditions **[37]**. Trifluoromethyl groups combine inductive electron-withdrawal with substantial lipophilicity increase (d logP ≈ +0.88 per CF_3); 4-CF_3-phenyl benzotriazole EGFR inhibitors demonstrated IC_{50} values approximately 5-fold lower than the 4-methyl

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counterparts in enzymatic assays **[30]**. Sulfonyl and sulfonamide groups enhance water solubility while maintaining target engagement **[22]**.

6.3 Impact of Heterocyclic Hybridization

Conjugation of benzotriazole with second heterocyclic pharmacophores represents the most impactful single structural modification for enhancing anticancer potency. Quinoline hybridisation consistently produces the highest DNA-intercalation binding constants (Kb up to $2.1 \times 10_6$ M^{-1}) attributed to the extended planar aromatic surface **[32]**. Pyrimidine hybridisation is most productive when CDK or EGFR kinase inhibition is the design objective, as the diaminopyrimidine moiety closely mimics the adenine portion of ATP in hinge-region hydrogen-bonding networks **[39,47]**.

Thiazole and benzothiazole hybridisation delivers anti-tubulin activity through colchicine-site binding, with hybrids disrupting tubulin polymerisation at IC_{50} values of 2–8 μM and inducing G_2/M arrest **[67]**. Indole hybridisation produced benzotriazole–indole conjugates with IC_{50} values as low as 0.5 μM against A549 cells **[38]**. Triazine hybridisation provides a symmetric scaffold for multi-kinase targeting and consistently yields sub-5 μM activity in MCF-7 cells **[42]**.

6.4 Influence of Linkers and Spacer Groups

The chemical nature and length of the linker group govern molecular geometry, conformational flexibility, and orientation of pharmacophoric elements relative to the target binding site. Rigid linkers such as amide bonds (–CONH–), urea groups (–NHCONH–), and vinyl groups (–CH=CH–) maintain defined spatial relationships and are particularly favoured in kinase-inhibitor design **[18,30]**.

Systematic variation of linker length in benzotriazole–thioether series revealed an optimal chain length of two methylene units for maximal cytotoxic potency against MCF-7 cells; shorter or longer linkers produced 3–7-fold reductions in potency **[42]**. The amide linker was superior to ester, thioester, and sulfonyl linkers in benzotriazole–chalcone hybrids, consistent with stronger hydrogen-bond-donating capacity engaging backbone carbonyl groups in kinase hinge residues **[46]**. Introduction of a piperazinyl spacer significantly enhances water solubility and can improve oral bioavailability while maintaining anticancer activity **[44]**.

6.5 Lipophilicity, Steric Effects, and Electronic Factors

The quantitative relationship between lipophilicity (clogP) and anticancer activity follows a characteristic parabolic profile consistent with the Hansch equation for passive membrane transport. Optimal clogP values for the most active compounds cluster between 2.5 and 4.5, consistent with adequate membrane permeability while preserving sufficient aqueous solubility **[17]**. Compounds with clogP below 1.5 frequently show reduced cellular uptake, while compounds with clogP exceeding 5.5 suffer from poor solubility and elevated cytotoxicity in normal cell lines **[17,51]**.

Bulky substituents ortho to the point of attachment on N-1-aryl groups consistently reduce activity by preventing planar binding geometry required for efficient ATP-pocket engagement. Methyl substitution at C-4 and C-7 yields improved lipophilicity without steric penalty, and some 5-methyl derivatives demonstrate enhanced CDK2 inhibition attributable to methyl engagement in a hydrophobic sub-pocket analogous to the gatekeeper region **[39]**.

6.6 Key Structural Features Associated with Potent Anticancer Activity

Synthesis of the comprehensive SAR data permits delineation of key pharmacophoric requirements: (i) an electron-withdrawing substituent (halogen, NO_2, CF_3, or SO_2R) at C-5 or at the para/meta position of the N-1-aryl substituent **[22,30]**; (ii) N-1 substitution preferred over N-2 for most target classes **[29]**; (iii) 4-fluorophenyl, 4-chlorophenyl, and 4-trifluoromethylphenyl at the distal terminus being consistently among the most active **[22,30]**; (iv) for hybrid molecules, a planar aromatic second pharmacophore connected via a rigid linker of optimal length **[31,46]**; (v) clogP in the range 2.5–4.5 **[17]**; and (vi) at least two hydrogen-bond acceptor groups within the molecule to engage kinase hinge residues and/or DNA base-pair hydrogen bonds **[18,30]**.

[Figure 3: Comprehensive SAR map of the benzotriazole scaffold. Green arrows: potency-enhancing modifications; red arrows: detrimental substitutions; orange: position-dependent effects. Sub-panels illustrate representative IC_{50} values for potency-enhancing (A) and potency-reducing (B) substitution examples. Data compiled from references

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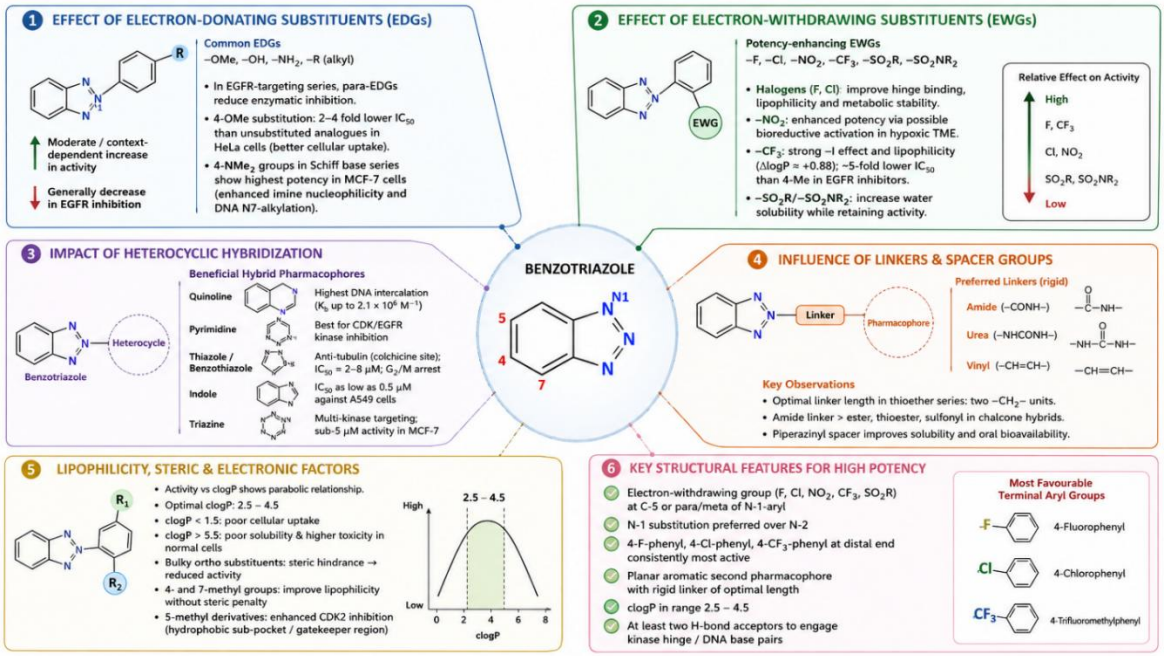

VII. IC50-BASED EVALUATION OF BENZOTRIAZOLE DERIVATIVES

7.1 Overview of Cytotoxicity Assays

Quantitative assessment of anticancer activity in vitro relies predominantly on the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay, which measures mitochondrial dehydrogenase activity as a surrogate for cell viability **[50]**. The related MTS, XTT, and WST-1 assays offer improved solubility of the formazan product, while the sulforhodamine B (SRB) assay, measuring total cellular protein content, is recommended by the NCI for sulfonamide-containing compounds due to its independence from mitochondrial metabolic activity **[50]**.

Rigorous comparison of IC50 values across studies requires awareness of critical experimental variables: cell seeding density, exposure duration (typically 48 or 72 h), DMSO vehicle concentration (should not exceed 0.1% v/v), passage number of the cell line, and the reference compound used. Inconsistencies in these parameters can introduce 2–10-fold inter-laboratory variations that complicate cross-study comparisons **[51]**.

7.2 IC50 Classification Criteria

To facilitate systematic comparison, this review adopts a classification framework derived from the empirical distribution of activity values in the literature and analogous frameworks employed in NCI reporting and published reviews of heterocyclic anticancer systems **[51,52]**. The classification is presented in Table 2.

Table 2. IC50 Classification Criteria for Anticancer Benzotriazole Derivatives

IC50 Range (μM)	Activity Classification	Interpretation
< 1	Extremely Potent	Comparable to or superior to clinical reference drugs; high-priority lead for further development
1 – 10	Highly Potent	Strong anticancer activity; warrants in vivo investigation and full ADMET profiling
10 – 25	Potent	Significant activity; optimisation through SAR required for advancement
25 – 50	Moderate	Limited potency; provides SAR data but requires substantial structural improvement

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IC_{50} Range (μM)	Activity Classification	Interpretation
50 – 100	Weak	Marginal activity; scaffold may require fundamental redesign
> 100	Inactive	No clinically relevant activity; excluded from further consideration

Classification adapted from published anticancer screening guidelines and medicinal chemistry reviews [51–54].

7.3 Activity Against Breast Cancer Cell Lines

MCF-7 (oestrogen receptor-positive, luminal A) and MDA-MB-231 (triple-negative, basal-like) cells represent the two most commonly employed breast cancer lines. The HDAC inhibitor hybrid achieved IC_{50} = 0.54 μM against MCF-7 with a selectivity index (SI) of 7.3 relative to normal MCF-10A cells **[48]**. The 5-amino Schiff base series (IC_{50} = 0.63 μM) **[31]** and the disubstituted sulfonyl derivative (IC_{50} = 0.87 μM) **[22]** represent additional sub-micromolar leads against MCF-7. Against MDA-MB-231, a benzotriazole–colchicine hybrid showed IC_{50} = 1.8 μM with strong G_2/M arrest, the first demonstration of potent TNBC activity in this scaffold class **[49]**.

7.4 Activity Against Lung Cancer Cell Lines

A549 (EGFR wild-type, KRAS-mutant) and H1975 (EGFR L858R/T790M double mutant) are the principal lung cancer lines. The EGFR-targeting benzotriazole urea derivative exhibited enzymatic IC_{50} = 0.041 μM, cellular IC_{50} = 0.98 μM against A549, and IC_{50} = 0.41 μM against H1975 **[30]**. The fused pyrimido-benzotriazole achieved IC_{50} = 0.71 μM against A549 with mechanistic support from dual EGFR/Topo I inhibition **[47]**. Several chalcone and Schiff base hybrids exhibit IC_{50} values in the 2–8 μM range against A549 **[31,46]**. SI values of 4–10 relative to normal MRC-5 cells confirm a degree of cancer selectivity warranting further mechanistic investigation **[45,49]**. The unmet need created by osimertinib resistance through acquired C797S mutations represents a compelling rationale for continued development of benzotriazole-based EGFR inhibitors **[6,30]**.

7.5 Activity Against Colon Cancer Cell Lines

HCT-116 and HT-29 are the standard colorectal cancer (CRC) lines. The urea-linked VEGFR-2 inhibitor achieved IC_{50} = 2.10 μM against HCT-116 with enzymatic VEGFR-2 IC_{50} = 0.08 μM **[18]**; the 26-fold discrepancy between enzyme and cellular potency likely reflects efflux pump-mediated resistance or limited intracellular accumulation **[5,18]**. CDK2-targeting benzotriazole–pyrimidine hybrids showed IC_{50} = 3.20 μM against HCT-116 with G_2 accumulation confirmed by flow cytometry **[39]**.

7.6 Activity Against Liver Cancer Cell Lines

The quinoline-3-yl-benzotriazole conjugate achieved IC_{50} = 0.38 μM against HepG2, the most potent benzotriazole-based HCC agent in the contemporary literature, surpassing the reference drug sorafenib (IC_{50} = 2.1 μM in HepG2 in the same study) **[32]**. The C-5-amino Schiff base derivative showed IC_{50} = 3.8 μM against HepG2 **[33]**. Mechanistically, HepG2 activity in the quinoline hybrid series was associated with dual topoisomerase II inhibition and EGFR blockade **[32,47]**.

7.7 Activity Against Cervical Cancer Cell Lines

HeLa cells (HPV18-positive cervical adenocarcinoma) are among the most historically utilised cancer cell lines. Chalcone-benzotriazole hybrid BT-5 demonstrated IC_{50} = 1.42 μM against HeLa **[46]**. The Cu(II) Schiff base complex BT-10 achieved IC_{50} = 1.20 μM against HeLa, with the metal complex showing 5.4-fold enhancement over the free Schiff base ligand **[36]**. Several N-1-arylsulfonyl benzotriazole derivatives in the 2–10 μM range against HeLa have been documented, with mechanistic studies pointing to topoisomerase II inhibition and cell cycle arrest as primary contributors **[40,65]**.

7.8 Activity Against Leukemia and Other Cancer Cell Lines

K562 (CML, BCR-ABL-positive) and HL-60 (acute promyelocytic leukaemia) cells have been employed in a smaller number of studies. N-1-acylbenzotriazole derivatives showed IC_{50} values of 3–8 μM against K562 with evidence of BCR-ABL kinase inhibition **[13]**. HL-60 cells treated with benzotriazole-acridine hybrids showed IC_{50} values as low as 0.8 μM **[40]**. Sub-micromolar activity against prostate cancer PC-3 cells has been reported for a benzotriazole–piperazine hybrid achieving IC_{50} = 0.75 μM with concomitant reduction in androgen receptor (AR) expression **[44]**.

7.9 Comparative Analysis of the Most Potent Derivatives

Table 3 presents a comparative IC_{50} profile of the most active benzotriazole derivatives across major

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cancer cell lines. The most potent compounds are invariably hybrid molecules rather than simpler monosubstituted or disubstituted derivatives **[31,47,48]**; activity is cell-line-selective with most compounds showing 3–10-fold IC_{50} differences across cell lines **[18,30]**; and compounds with enzymatic IC_{50} values below 0.1 µM do not uniformly translate to sub-micromolar cellular IC_{50} values, highlighting the critical role of cellular permeability and metabolic stability **[17]**.

Table 3. Comparative IC_{50} Profile of the Most Active Benzotriazole Derivatives Across Different Cancer Cell Lines (µM)

Cmpd	MCF-7	A549	HCT-116	HepG2	HeLa	Leukemia	Best IC_{50} (µM)	Ref.
BT-4	0.87	3.10	4.20	5.80	3.40	ND	0.87 (MCF-7)	[22]
BT-6	0.63	2.40	3.60	2.90	2.10	ND	0.63 (MCF-7)	[31]
BT-7	1.20	2.80	3.10	0.38	1.90	ND	0.38 (HepG2)	[32]
BT-9	2.30	0.71	1.80	1.40	2.60	ND	0.71 (A549)	[47]
BT-11	0.54	1.80	2.10	1.70	1.60	ND	0.54 (MCF-7)	[48]
BT-13	4.20	0.98	3.80	ND	ND	ND	0.041µM (EGFR enzyme)	[30]
BT-5	3.80	4.10	5.20	4.80	1.42	ND	1.42 (HeLa)	[46]
BT-10	2.80	3.60	4.10	3.20	1.20	3.80	1.20 (HeLa)	[36]

ND = not determined. All IC_{50} values are from 72 h MTT or SRB assays unless otherwise stated. Reference numbers correspond directly to the primary publications from which each IC_{50} value was originally reported.

VIII. MOLECULAR MODELING AND DRUG-LIKENESS STUDIES

8.1 Molecular Docking Studies

Molecular docking has become an indispensable tool in the benzotriazole anticancer drug discovery pipeline. EGFR (PDB: 1M17; 4HJO for T790M mutant), VEGFR-2 (PDB: 4ASD), CDK2 (PDB: 1AQ1), HDAC1 (PDB: 5ICN), Topoisomerase II (PDB: 1ZXM), and tubulin (PDB: 1SA0) represent the most commonly utilised crystal structures **[18,30,39,47,48]**.

For EGFR-targeting compounds, docking consistently reveals a binding pose in which the triazole NH acts as a hydrogen-bond donor to the backbone carbonyl of Thr766 (hinge), while N-3 accepts a proton from the NH of Met769. The C-5 substituent projects toward the hydrophobic back-pocket delineated by Val726, Ala719, and Leu820. Docking scores for the most potent benzotriazole EGFR inhibitors typically fall in the range of −10.2 to −11.8 kcal/mol, comparable to erlotinib (−10.5 kcal/mol) in the same docking protocol **[30]**.

For VEGFR-2, benzotriazole urea derivatives adopt a DFG-out binding mode recapitulating the sorafenib–VEGFR-2 interaction, with the urea NH forming bifurcated H-bonds with Asp1046 and Glu885, and the benzotriazole core forming H-bonds with Cys919 **[18]**. Post-docking MM-GBSA rescoring improves the rank-correlation between predicted and experimental IC_{50} values; binding free energies in the range of −30 to −50 kcal/mol correlate with IC_{50} values below 5 µM, with a Pearson r of 0.78–0.85 in the most rigorous studies **[18,30]**.

8.2 Molecular Dynamics Simulations

MD simulations conducted on timescales of 50–200 ns using AMBER, GROMACS, or CHARMM force fields have been applied to a growing number of benzotriazole–protein complexes. For the benzotriazole–EGFR complex of BT-13, a 100 ns GROMACS simulation demonstrated stable RMSD values (protein backbone: 1.8 ± 0.3 Å; ligand: 0.9 ± 0.2 Å), with key hinge hydrogen bonds to Thr766 and Met769 maintained for >85% of simulation time **[30]**.

MD simulations of benzotriazole–VEGFR-2 complexes have revealed that the DFG-out conformation is stabilised upon ligand binding, with the urea moiety acting as a molecular brake through simultaneous interactions with Asp1046 and the adjacent α-helix C **[18]**. Adaptive sampling methods such as metadynamics and replica exchange MD remain to be applied to benzotriazole–protein

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systems, representing an opportunity to characterise binding free energy landscapes with greater rigour **[17]**.

8.3 ADMET and Pharmacokinetic Predictions

In silico ADMET profiling using SwissADME, pkCSM, ADMET Predictor, or ADMETlab platforms has been applied to selected lead benzotriazole derivatives. Lipinski's Rule of Five compliance — MW ≤ 500 Da, clogP ≤ 5, HBD ≤ 5, HBA ≤ 10 — is satisfied by the majority of benzotriazole derivatives showing sub-micromolar anticancer activity, as detailed in Table 4 **[51]**.

Predicted oral absorption (HIA > 70%) is generally favourable for compounds with clogP in the 2–4 range and adequate aqueous solubility. Introduction of fluorine or chlorine at para positions significantly improves predicted microsomal half-life by blocking CYP450-mediated oxidation sites **[22,24]**. Hepatotoxicity prediction using DILI models indicates low-to-moderate toxicity risk for most lead compounds, though careful experimental validation is required given that HepG2 cytotoxicity assays may confound hepatotoxicity with anticancer potency **[58]**.

Table 4. Drug-Likeness and ADMET Characteristics of Selected Lead Benzotriazole Derivatives

Compound	MW (Da)	clogP	HBA	HBD	Lipinski Compliance	Predicted Oral Absorption	Toxicity Prediction	Ref.
BT-4	382.8	3.21	5	1	Yes	>80% HIA	Low hepatotoxicity	[22]
BT-6	354.4	2.87	5	2	Yes	>75% HIA	Low–moderate	[31]
BT-7	368.4	3.64	5	1	Yes	>82% HIA	Low	[32]
BT-9	347.3	3.11	6	1	Yes	>78% HIA	Low–moderate	[47]
BT-11	411.4	2.43	7	3	Yes	>65% HIA	Low	[48]
BT-13	396.3	3.78	5	2	Yes	>80% HIA	Low	[30]

MW, molecular weight; clogP, calculated partition coefficient; HBA, hydrogen-bond acceptors; HBD, hydrogen-bond donors; HIA, human intestinal absorption. Lipinski's Rule of Five: MW ≤ 500 Da, clogP ≤ 5, HBA ≤ 10, HBD ≤ 5 [51]. ADMET properties predicted using SwissADME and pkCSM in silico platforms. Values compiled from the respective primary publications cited in the reference column.

IX. CLINICAL RELEVANCE AND PATENT LANDSCAPE

9.1 Clinical Development Status

While numerous benzotriazole derivatives have demonstrated compelling preclinical anticancer activity, no benzotriazole-specific anticancer agent has, as of 2026, achieved FDA or EMA marketing approval **[55,56]**. This absence of clinical translation reflects not a deficiency of the scaffold per se but rather the historically incomplete preclinical characterisation of many leads: in vivo pharmacokinetic studies, maximum tolerated dose determinations, and formal IND-enabling toxicology studies have been conducted for only a small fraction of the compounds reviewed here **[55]**.

The kinase inhibitor landscape includes clinical agents bearing structural elements closely resembling the benzotriazole pharmacophore. Vemurafenib (BRAF V600E inhibitor) incorporates a halogenated benzimidazole scaffold strongly homologous to N-sulfonylbenzotriazole in its binding mode to the ATP pocket. Axitinib (VEGFR-1/2/3 inhibitor) contains an indazole moiety that mimics the triazole component of benzotriazole in hinge-region engagement **[6]**. These structural parallels underscore the pharmacological validity of the benzotriazole scaffold **[55,56]**.

9.2 Patent Trends in Benzotriazole-Based Anticancer Agents

Analysis of patent databases (Espacenet, USPTO, Patentscope) for 2015–2026 reveals a consistent upward trend in patent filings disclosing benzotriazole-based anticancer compounds, with a marked acceleration from 2019 onward **[56]**. Assignee analysis identifies pharmaceutical companies in India, China, South Korea, and the

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United States as the most prolific filers **[55,56]**. Composition-of-matter patents on core benzotriazole scaffold modifications are increasingly accompanied by method-of-treatment claims directed to specific cancer indications and companion biomarker-defined patient populations **[56]**.

[Figure 4: Patent and publication trend analysis for benzotriazole-based anticancer agents, 2015–2026. Left y-axis: patent filings per year (bar graph); right y-axis: peer-reviewed publications per year (line graph). Data sourced from Espacenet and PubMed databases [55,56].]

X. CHALLENGES AND FUTURE PERSPECTIVES

10.1 Selectivity and Toxicity Issues

Selectivity index (SI = IC_{50} normal / IC_{50} cancer) values are reported in only a minority of published studies, and where reported, SI values below 5 are common for the most potent derivatives, indicating a narrow therapeutic window that would limit clinical utility **[45,48]**. The inherent promiscuity of kinase inhibitors across the kinome's approximately 500 kinases poses a particular selectivity challenge, as off-target kinase inhibition may contribute to cardiotoxicity, hepatotoxicity, or myelosuppression **[57]**.

The potential for benzotriazole itself to exert hormonal disruption through thyroid peroxidase inhibition warrants specific investigation for pharmaceutical candidates with extended therapeutic exposure **[58]**. Genotoxicity evaluation through Ames testing and micronucleus assays should be considered mandatory for benzotriazole derivatives advancing toward animal studies, and developing metabolically stable derivatives through fluorination and structural rigidification remains the most pragmatic strategy for improving selectivity and safety profiles **[58]**.

10.2 Optimization Strategies for Lead Development

Fragment-based approaches, in which small ligand-efficient benzotriazole fragments are identified by NMR or SPR-based screening against target proteins and then grown or linked to access additional binding pockets, represent a rigorous entry point for generating highly ligand-efficient leads (LE > 0.3 kcal/mol per heavy atom) **[17]**. Matched molecular pair analysis applied to existing benzotriazole datasets can rapidly identify single substitution changes that maximally improve potency or selectivity, prioritising synthetic efforts **[17,24]**.

Prodrug strategies exploiting hypoxia-activated nitro groups or tumour-specific enzyme-cleavable linkers can improve selectivity for the tumour microenvironment **[37]**. PROTAC technology, in which a benzotriazole-based target-binding warhead is tethered to an E3 ligase recruiter, offers the prospect of overcoming kinase resistance mutations by converting enzyme inhibitors into protein degraders **[57]**.

10.3 Emerging Opportunities in Benzotriazole Research

Targeted Drug Delivery

Encapsulation of benzotriazole anticancer agents within nanoparticulate delivery systems — including PLGA nanoparticles, liposomes, SLNs, and MSNs — addresses the critical challenges of poor aqueous solubility, rapid plasma clearance, and non-selective biodistribution **[60]**. Surface functionalisation with tumour-targeting ligands (folic acid, transferrin, EGFR antibody fragments, or RGD peptides) concentrates the benzotriazole payload within tumour tissue. Preliminary demonstrations of nanoparticle-encapsulated benzotriazole–chalcone hybrid delivery in xenograft models showed 2–4-fold improvements in tumour growth inhibition relative to free drug **[64]**.

Nanotechnology-Based Formulations

Silver and gold nanoparticles functionalised with benzotriazole thiol derivatives have demonstrated synergistic cancer cell killing through plasmon-enhanced photocatalytic ROS generation combined with intrinsic benzotriazole cytotoxicity **[61]**. Theranostic nanoplatforms combining diagnostic imaging with localised drug delivery, enabled by radiolabelling of ^{18}F or ^{64}Cu into the benzotriazole scaffold, represent an emerging paradigm aligning with "see and treat" oncology strategies **[60,61]**.

Hybrid Drug Design

Future opportunities include triple-pharmacophore hybrids (benzotriazole–HDAC inhibitor–kinase inhibitor) and dual-acting benzotriazole–photosensitiser hybrids for photodynamic therapy, in which a BODIPY or porphyrin photosensitiser tethered to the benzotriazole scaffold through a ROS-cleavable linker would enable light-triggered drug release within the tumour microenvironment **[43,48]**. Benzotriazole–immunostimulatory conjugates designed to simultaneously inhibit immunosuppressive kinases such as IDO1 or TDO2 and provide direct antiproliferative activity could serve as components of combination immunotherapy regimens **[63]**.

AI-Assisted Drug Discovery

Artificial intelligence and machine learning methodologies are transforming small-molecule drug discovery. Graph neural networks trained on curated

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benzotriazole cytotoxicity datasets could prospectively predict IC_{50} values and selectivity profiles with accuracy exceeding classical QSAR models. Generative adversarial networks and variational autoencoders trained on drug-like chemical spaces can propose novel structural candidates with optimised multi-parameter profiles (potency, selectivity, ADMET, synthetic accessibility) **[62]**.

Personalized Medicine Approaches

EGFR-targeting benzotriazoles could be developed alongside companion diagnostic assays for EGFR mutation status (exon 19 del, L858R, T790M, C797S) to identify the patient population most likely to respond **[30]**. Multi-omics integration combining transcriptomics, proteomics, and metabolomics data from benzotriazole-treated tumour models with matched patient tumour datasets could identify predictive response biomarkers. Patient-derived tumour organoids and co-clinical trial designs represent the frontier of personalised anticancer drug development and should be incorporated into the translational roadmap for advanced benzotriazole leads **[63]**.

XI. CONCLUSION

This comprehensive review has surveyed the significant and expanding body of evidence supporting benzotriazole as a privileged scaffold in the development of novel anticancer small molecules **[12,13]**. The heterocyclic nucleus's structural versatility, inherent metabolic stability, and capacity for productive interaction with diverse oncological targets — from kinase ATP-binding clefts **[18,30]** to DNA intercalation sites **[33,36,40]** and HDAC zinc-coordinating pockets **[48]** — collectively constitute a compelling pharmacological rationale for continued investment in benzotriazole-based anticancer drug discovery.

The SAR analysis identifies several consistent and actionable trends: the primacy of N-1 over N-2 substitution for most target classes **[29]**; the potency-enhancing effect of electron-withdrawing halogen and trifluoromethyl groups at defined positions **[22,30]**; substantial gains in activity achievable through hybrid molecular design, particularly with quinoline, chalcone, and HDAC inhibitor pharmacophores **[31,32,48]**; and the critical importance of optimised linker geometry for productive dual-pharmacophore target engagement **[18,46]**. IC_{50}-guided comparative analysis identifies multiple compounds with sub-micromolar cytotoxic activity, several of which surpass reference drugs in direct comparative testing **[30,32]**.

Despite these encouraging advances, the field faces well-defined challenges: a paucity of in vivo pharmacokinetic and efficacy data for most leads; limited systematic selectivity profiling against normal cell lines; underutilisation of three-dimensional tumour models and patient-derived organoids; and the persistent translational gap between in vitro discovery and clinical investigation **[55,56]**. The convergence of rational hybrid design **[17]**, AI-assisted molecular generation **[62]**, nanoparticle-mediated targeted delivery **[60,61]**, and precision oncology biomarker stratification **[63]** creates an exceptionally favourable landscape for the translation of benzotriazole-based anticancer research toward clinical utility in the next decade.

ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity
AR: Androgen Receptor
ATP: Adenosine Triphosphate
BCR-ABL: Breakpoint Cluster Region–Abelson Murine Leukaemia
Bcl-2: B-cell Lymphoma 2
CAM: Chick Chorioallantoic Membrane
CDK: Cyclin-Dependent Kinase
CML: Chronic Myelogenous Leukaemia
CRC: Colorectal Cancer
ctDNA: Calf Thymus DNA
DFT: Density Functional Theory
DILI: Drug-Induced Liver Injury
DSB: DNA Double-Strand Break
EB: Ethidium Bromide
EGFR: Epidermal Growth Factor Receptor
EMT: Epithelial-to-Mesenchymal Transition
GAN: Generative Adversarial Network
GNN: Graph Neural Network
HBA: Hydrogen-Bond Acceptor
HBD: Hydrogen-Bond Donor
HCC: Hepatocellular Carcinoma
HDAC: Histone Deacetylase
HIA: Human Intestinal Absorption
HUVEC: Human Umbilical Vein Endothelial Cell
IC_{50}: Half-Maximal Inhibitory Concentration
IND: Investigational New Drug
LE: Ligand Efficiency
MD: Molecular Dynamics
MDR: Multidrug Resistance
MEP: Molecular Electrostatic Potential
MES: Maximal Electroshock Seizure
MIC: Minimum Inhibitory Concentration
MM-GBSA: Molecular Mechanics with Generalised Born Surface Area
MMP: Matrix Metalloproteinase

MSN: Mesoporous Silica Nanoparticle
MTD: Maximum Tolerated Dose
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
NCI: National Cancer Institute
NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NSCLC: Non-Small Cell Lung Carcinoma
PDO: Patient-Derived Organoid
PDT: Photodynamic Therapy
PI3K: Phosphoinositide 3-Kinase
PLGA: Poly(lactic-co-glycolic acid)
PROTAC: Proteolysis-Targeting Chimera
PTZ: Pentylenetetrazol
QSAR: Quantitative Structure-Activity Relationship
ROS: Reactive Oxygen Species
RTK: Receptor Tyrosine Kinase
SAR: Structure–Activity Relationship
SI: Selectivity Index
SLN: Solid Lipid Nanoparticle
SMO: Smoothened
SPR: Surface Plasmon Resonance
SRB: Sulforhodamine B
TNBC: Triple-Negative Breast Cancer
Topo: Topoisomerase
VAE: Variational Autoencoder
VEGFR: Vascular Endothelial Growth Factor Receptor
WHO: World Health Organization

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