

First-in-Class Dual PDGFR/Carbonic Anhydrase IX/XII Inhibitors: 6,7-Dimethoxyquinoline-Sulfonamides as Promising Antileukemic Agents

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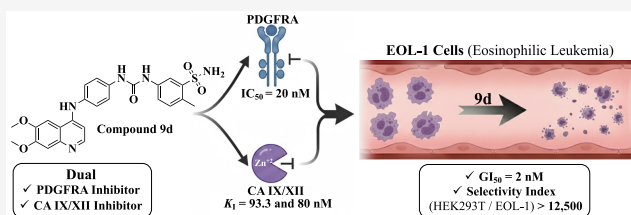


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ABSTRACT: Leukemia remains a challenging hematological malignancy, with limited therapeutic options. To address this unmet need, we report quinoline–sulfonamide hybrids as first-in-class dual inhibitors of platelet-derived growth factor receptor (PDGFR) and carbonic anhydrase (CA) IX/XII. Structure–activity relationship studies identified compound **9d** as a potent lead, exhibiting strong inhibition of PDGFRA ($IC_{50} = 20$ nM) and CA IX/XII ($K_i = 93.3$ and 80.0 nM, respectively), along with exceptional antiproliferative activity in FIP1L1–PDGFRA-driven EOL-1 cells ($GI_{50} = 2$ nM), comparable to clinical agents. Mechanistic analyses revealed that **9d** effectively abrogates PDGFRA signaling, induces G0/G1 cell-cycle arrest, and triggers apoptosis. Molecular docking and 200 ns molecular dynamics simulations supported stable dual binding of **9d** within the ATP-binding pocket of PDGFR and the catalytic cleft of CA IX. By simultaneously targeting oncogenic PDGFRA signaling and hypoxia-driven pH regulation (CA IX/XII), **9d** represents a promising lead for preclinical development in PDGFR/CA IX/XII-driven leukemias.



1. INTRODUCTION

Leukemia, a heterogeneous group of hematological malignancies, is characterized by the uncontrolled proliferation and accumulation of abnormal hematopoietic cells in the bone marrow and peripheral blood.^{1,2} Leukemia remains a major global health burden, with estimated 487,294 new cases and 305,405 deaths reported worldwide in 2022.³ It is the most prevalent malignancy among children, accounting for nearly 30% of pediatric cancers while also contributing substantially to cancer morbidity and mortality across all age groups.^{4,5} Despite advancements in therapeutic strategies, including chemotherapy, radiation, stem cell transplantation, and targeted therapies, leukemia remains a challenging disease to treat, often associated with high relapse rates and significant morbidity and mortality, particularly in aggressive forms such as acute myeloid leukemia (AML).^{6,7} The complexity of leukemia, stemming from its diverse genetic landscape and the dynamic interplay with the bone marrow microenvironment, necessitates the continuous development of novel and more effective therapeutic interventions.⁸

Protein kinases play pivotal roles in regulating fundamental cellular processes, including proliferation, differentiation, and survival. Dysregulation of kinase activity, often through mutations, gene fusions, or overexpression, leads to uncontrolled cell growth and is a hallmark of many cancers, including

leukemia.^{9,10} Oncogenic kinases serve as crucial drivers of leukemia pathogenesis and represent attractive therapeutic targets.¹¹ Among the most well-characterized oncogenic kinases in leukemia are Platelet-Derived Growth Factor Receptors (PDGFRs), Fms-like Tyrosine Kinase 3 (FLT3), and KIT.¹² Activating mutations or fusions involving PDGFR (e.g., FIP1L1–PDGFRA fusion) are implicated in various myeloproliferative neoplasms and hypereosinophilic syndromes, leading to the aberrant activation of downstream signaling pathways, such as PI3K/AKT, JAK/STAT, and MAPK/ERK, which promote uncontrolled cell proliferation and survival.¹³

The advent of tyrosine kinase inhibitors (TKIs) has revolutionized the treatment landscape for several leukemias, most notably with imatinib (Figure 1A) for chronic myeloid leukemia (CML), which targets the BCR-ABL fusion protein.¹⁴ However, despite their clinical success, the therapeutic efficacy of PDGFR-directed inhibitors remains

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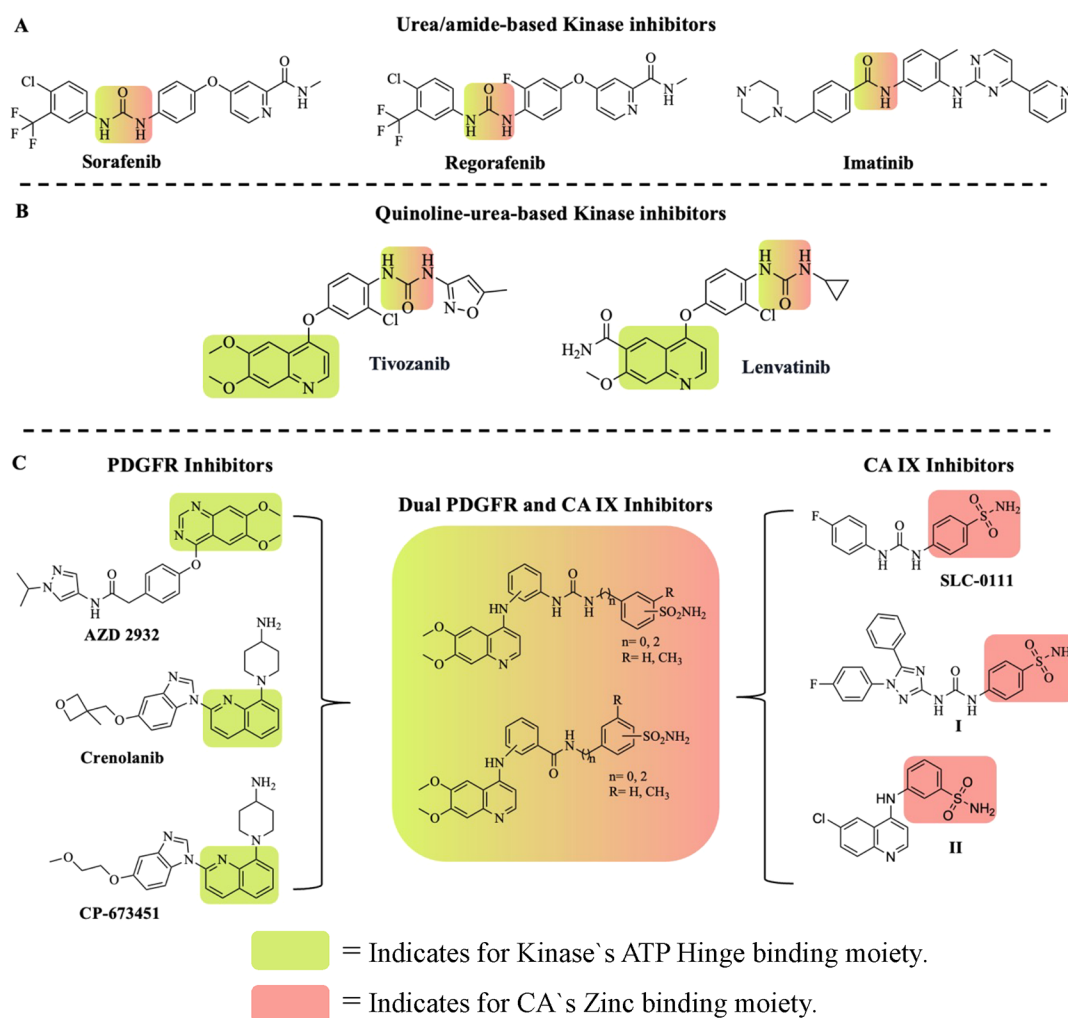


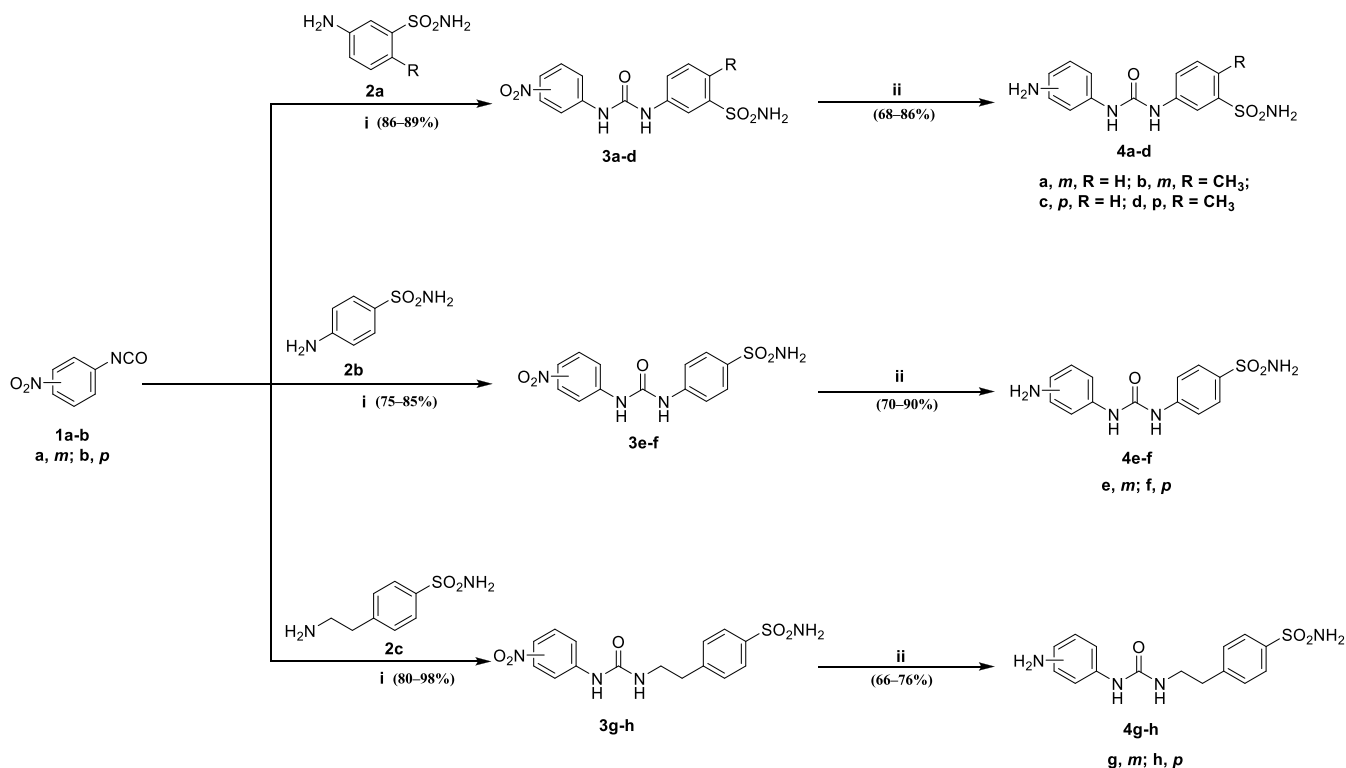
Figure 1. Design strategy for developing dual PDGFR/carbonic anhydrase IX/XII inhibitors. (A) Representative structures of clinically relevant urea- and amide-linker-based kinase inhibitors (sorafenib, regorafenib, and imatinib). (B) Representative structures of clinically relevant quinoline-urea-based kinase inhibitors (tivozanib, lenvatinib). (C) Design rationale for target quinoline-sulfonamide hybrids (**9a–h** and **10a–h**) as dual PDGFR/CA IX inhibitors.

limited by several challenges. Resistance frequently emerges through secondary mutations within the PDGFR kinase domain (on-target resistance) as well as compensatory activation of parallel signaling pathways (off-target resistance).^{15,16} Moreover, many clinically used PDGFR inhibitors exhibit insufficient selectivity, resulting in undesirable off-target toxicities that restrict dose escalation and narrow their therapeutic window.¹⁷ These limitations underscore the urgent need for new strategies that can overcome resistance and enhance therapeutic durability, which has motivated medicinal chemists to develop next-generation inhibitors with improved potency, selectivity, and sustained clinical efficacy, particularly in resistance-prone leukemias.

Beyond direct oncogenic signaling, the tumor microenvironment plays a critical role in cancer progression and therapeutic response. Hypoxia, a common feature of solid tumors, is also prevalent in the bone marrow niche where leukemic cells reside, creating a unique microenvironment that fosters disease progression and drug resistance.¹⁸ In response to hypoxia, cells upregulate hypoxia-inducible factors (HIFs), which in turn activate genes involved in adapting to low-oxygen conditions, including CA IX/XII.¹⁹ CA IX/XII are a transmembrane metalloenzyme that play a crucial role in pH regulation by

catalyzing the reversible hydration of CO₂ to carbonic acid, thereby contributing to extracellular acidification and intracellular pH homeostasis.²⁰ In parallel with these oncological considerations, carbonic anhydrase inhibitors (CAIs) possess a long and well-established clinical history, with their therapeutic effects arising from the inhibition of distinct CA isoforms. Clinically approved CAIs are primarily used for the treatment of glaucoma through inhibition of the cytosolic CA II.^{21,22} CAIs are also employed as antiepileptic agents by targeting neuronal isoforms, including CA VII and CA XII.^{23–25} In addition, increasing evidence supports the use of CAIs as anti-inflammatory agents, an activity frequently associated with inhibition of the ubiquitously expressed CA I, II, IX, and XII isoforms.^{26,27} Collectively, this extensive pharmacological background provides a strong rationale for the development of isoform-selective CA inhibitors, particularly CA IX-targeted agents, for cancer therapy. Notably, CA IX/XII display limited expression in most normal tissues but are markedly overexpressed in various solid and hematological malignancies, including leukemia, especially under hypoxic conditions.^{28,29}

In leukemia, CA IX/XII expression has been documented in acute myeloid leukemia (AML) and adult T-cell leukemia (ATL) cells, where it plays a crucial role in the survival of

Scheme 1. General Synthesis of 4ah Intermediates^a

^aReagents and conditions: (i) Dry acetonitrile, 80 °C, 6 h. (ii) Methanol, 10% Pd/C, H₂, rt, 5 h.

leukemic cells under hypoxic conditions.^{28,29} The acidic extracellular environment generated by CA IX/XII activity promotes tumor cell proliferation, invasion, and metastasis and contributes to drug resistance by impairing the uptake and efficacy of certain chemotherapeutic agents.^{30,31} Accordingly, CA IX/XII represents an attractive therapeutic target, particularly in hypoxia-adapted leukemias.

The strategy of incorporating CA IX/XII inhibition into a polypharmacological framework is well-precedented in medicinal chemistry. Dual-acting agents that combine CA IX inhibition with a blockade of additional oncogenic pathways have demonstrated anticancer activity, including hybrid molecules targeting CA IX in conjunction with vascular endothelial growth factor receptor-2 (VEGFR-2),^{32,33} epidermal growth factor receptor (EGFR),³⁴ or tubulin polymerization.³⁵ These studies validate the inclusion of CA IX/XII inhibition as part of a multitarget approach to disrupt tumor survival and proliferation.

Building on this concept, our work pairs CA IX/XII inhibition with targeting of platelet-derived growth factor receptors (PDGFRs), key oncogenic driver in specific leukemias. In PDGFR-driven diseases, such as FIP1L1–PDGFRA-positive eosinophilic leukemia, pharmacological inhibition of the fusion kinase suppresses the primary proliferative signal but does not fully address the hypoxia-adapted phenotype within the bone marrow niche.^{28,36} Under hypoxic conditions, CA IX/XII maintain intracellular pH homeostasis, support metabolic buffering, and limit intracellular drug accumulation, thereby enabling leukemic cell persistence despite upstream kinase inhibition.²⁸ Consequently, simultaneous inhibition of PDGFR and CA IX/XII is anticipated to produce more durable antileukemic responses than targeting either pathway alone, consistent with the

principles of rational polypharmacology aimed at overcoming resistance and compensatory survival mechanisms.³⁷

Despite this therapeutic promise, the development of dual-targeting agents presents notable medicinal chemistry challenges. Achieving balanced potency against both targets while maintaining a favorable safety profile is inherently demanding as compounds must effectively engage each target at therapeutically relevant concentrations without introducing undue off-target toxicity. Addressing these challenges requires careful scaffold optimization and systematic structure–activity relationship studies.³⁸

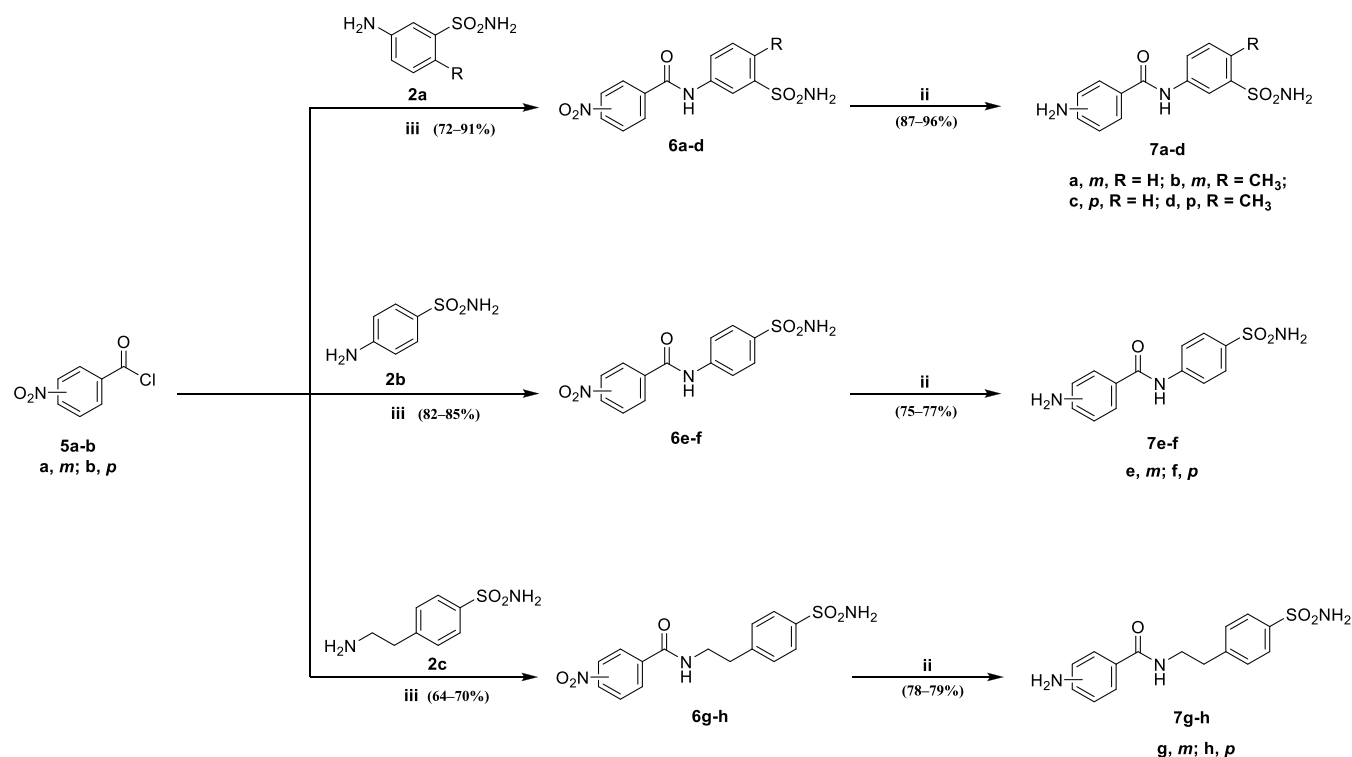
2. RESULTS AND DISCUSSION

2.1. Rational Design of Dual PDGFR and CA IX/XII Inhibitors

We adopted a hybrid pharmacophore strategy that integrates structural motifs from established PDGFR kinase inhibitors with CA IX/XII-targeting sulfonamides.

On the PDGFR inhibition side, the 6,7-dimethoxyquinoline scaffold was selected as the core pharmacophore. This privileged motif is known to support ATP-competitive binding within the kinase hinge region and significantly contributes to the compound's potency and selectivity against PDGFR.³⁹ This choice is validated by clinical and preclinical PDGFR inhibitors, including AZD2932, a dual PDGFR/VEGFR inhibitor with nanomolar potency,⁴⁰ crenolanib, a potent PDGFR/FLT3 inhibitor,⁴¹ and CP-673451, a potent and selective PDGFR inhibitor.⁴² (Figure 1).

For the CA IX-directed component, we drew inspiration from classical sulfonamide-based inhibitors such as SLC-0111 (*K_i* = 45.1 nM), which employs a benzenesulfonamide zinc-binding group connected via a flexible ureido linker.⁴³ In such

Scheme 2. General Synthesis of 7a–h Intermediates^a

^aReagents and Conditions: (iii) Dry acetone pyridine (2.0 equiv), rt, 2 h. (ii) Methanol, 10% Pd/C, H₂, rt, 5 h.

scaffolds, the sulfonamide coordinates the catalytic Zn²⁺ ion, while the ureido linker engages in hydrogen bonding and accommodates conformational flexibility. Previous studies have shown that replacement of the SLC-0111 tail with a 1,5-diphenyltriazole moiety enhances CA IX/XII inhibition (e.g., compound I; $K_i = 8.3$ nM).⁴⁴ Moreover, aminoquinoline-bearing analogues (e.g., compound II; $K_i = 8.4$ nM)⁴⁵ have demonstrated improved CA IX activity, suggesting productive synergy between kinase- and CA XI-directed pharmacophores (Figure 1).

To tether 6,7-dimethoxyquinoline (PDGFR motif) with benzenesulfonamide (CA motif), we utilized ureido and amide linkers. This strategy was guided by the established role of these linkers in promoting high-affinity binding for both target classes. The ureido linker, in particular, is a fundamental component of numerous potent and selective inhibitors targeting the tumor-associated isoforms CA IX and XII. Concurrently, amide and urea linkers are a critical motif for many PDGFR inhibitors, as exemplified by sorafenib (a multikinase inhibitor targeting Raf, VEGFR, PDGFR, FLT3, and c-KIT)⁴⁶ and regorafenib (VEGFR1–3, TIE2, PDGFR- β , FGFR, Raf)⁴⁷ and the amide-bearing imatinib, a selective BCR-ABL inhibitor with activity against PDGFR⁴⁸ (Figure 1A). Beyond these, a significant class of quinoline-based kinase inhibitors feature urea linkages, such as tivozanib (a potent and selective VEGFR TKI with some PDGFR activity)⁴⁹ and lenvatinib (a multikinase inhibitor targeting VEGFR, FGFR, PDGFR, KIT, and RET).⁵⁰ These precedents highlight the utility of quinoline-based scaffolds and amide/urea linkers in driving the high-affinity binding to PDGFR (Figure 1B).

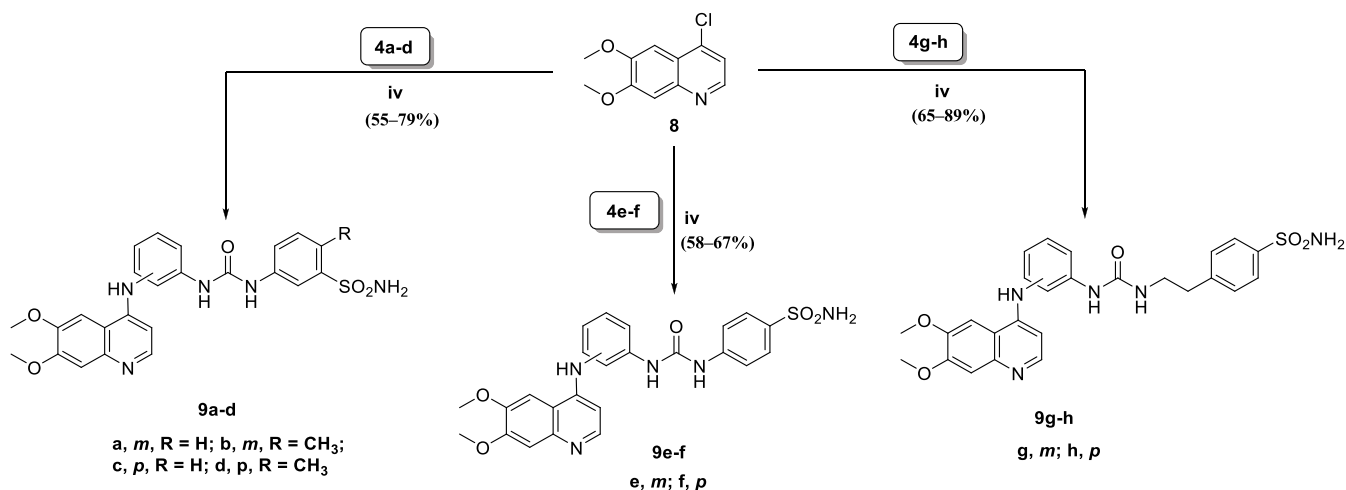
Building on these precedents, to achieve dual-target activity, we designed a library of quinoline-sulfonamide hybrids (9a–h and 10a–h, Figure 1), in which a sulfonamide zinc-

binding group was tethered to a PDGFR-directed quinoline scaffold via tailored linkers, positioning the kinase-binding domain distal to the zinc-binding moiety for bifunctional engagement. Structural diversity was introduced by varying linker type, polarity, rigidity, and quinoline/sulfonamide orientation (meta vs para) to probe structure–activity relationships. The resulting compounds were evaluated for PDGFR inhibition, carbonic anhydrase isoform selectivity, and antiproliferative activity in leukemia models with the goal of identifying a potent antileukemic scaffold.

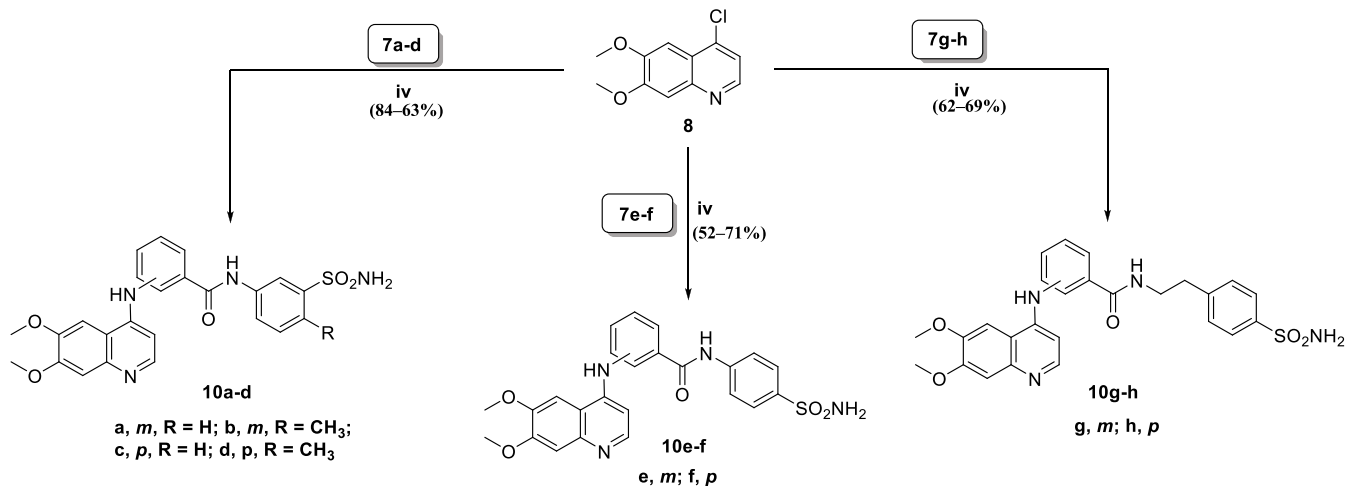
2.2. Synthesis of Quinoline–Sulfonamide Hybrids

Our synthetic strategy was designed to generate a focused library of quinoline–sulfonamide hybrids incorporating either ureido or amide linkers, enabling the exploration of structure–activity relationships (SAR) relevant to dual PDGFR and CA IX/XII inhibition. Four sulfonamide precursors were selected to assess the impact of sulfonamide regioisomerism and linker extension: *meta*-sulfanilamide (**2a**), *para*-methylsubstituted *meta*-sulfanilamide (**2a'**), *para*-sulfanilamide (**2b**), and *para*-aminoethyl-substituted benzenesulfonamide (**2c**).

Two complementary routes were employed to access key nitroaryl sulfonamide intermediates. In the first (Scheme 1), ureido-substituted sulfonamides (**3a–h**) were prepared by reacting the four sulfonamide precursors (**2a–c**) with *meta*- or *para*-substituted nitrophenyl isocyanates in dry acetonitrile. In the second scheme (Scheme 2), amide derivatives (**6a–h**) were obtained via Schotten–Baumann amidation of the same sulfonamide precursors with *meta*- or *para*-nitrobenzoyl chloride in acetone, utilizing pyridine as both a nucleophilic catalyst and a base to neutralize the liberated hydrochloric acid. Catalytic hydrogenation of the resulting nitro intermediates (**3a–h** and **6a–h**) with 10% Pd/C in methanol furnished the corresponding aniline derivatives (**4a–h** and **7a–h**).

Scheme 3. General Synthesis of Ureido-Linked Quinoline–Sulfonamide Hybrids 9a–h^a

^aReagents and conditions: (iv) isopropanol, catalytic 35% HCl (2–6 drops), 100 °C, 48 h.

Scheme 4. General Synthesis of Amide-Linked Quinoline Sulfonamide Hybrids 10a–h^a

^aReagents and conditions: (iv) isopropanol, catalytic 35% HCl, 100 °C, 48 h.

Finally, 4a–h and 7a–h hybridized with 4-chloro-6,7-dimethoxyquinoline (8) via acid-promoted nucleophilic substitution, affording two series of target compounds: quinoline-conjugated ureido benzenesulfonamides (9a–h, Scheme 3) and their amide analogues (10a–h, Scheme 4). This library, comprising regioisomeric sulfonamide and quinoline cores with varied linker chemistries, provides a platform for systematic SAR studies aimed at elucidating structural requirements for selective dual inhibition of PDGFR kinase and cancer-associated CA IX/XII.

2.3. Kinase Inhibitory Profile: Potency and Selectivity

To comprehensively assess the inhibitory potential of the synthesized quinoline-sulfonamides hybrids against key oncogenic kinases relevant to leukemia, a multitiered approach was employed, encompassing initial screening, quantitative IC₅₀ determination, and detailed kinome selectivity profiling.

2.3.1. Initial Kinase Screening. The inhibitory activity of the synthesized sulfonamides was first assessed at 1 μM against a representative leukemia-relevant kinase panel comprising PDGFRA, FLT3-ITD, KIT, and ABL (Table 1). Compounds 9c and 9d emerged as the most potent PDGFRA inhibitors,

Table 1. Residual Kinase Inhibitory Activities against PDGFRA, FLT3-ITD, KIT, and ABL. Assessed at 1 μM

Comp.	Residual Activity (%) at 1 μM			
	PDGFRA	FLT3-ITD	KIT	ABL
9a	45.8	69.7	21.8	102.2
9b	55.9	94.2	21.0	97.1
9c	6.7	34.3	18.9	94.5
9d	6.7	28.2	18.0	79.2
9e	93.2	80.0	46.6	98.9
9f	26.5	54.4	20.8	94.6
9g	99.3	112.0	102.4	98.6
9h	32.6	43.7	36.4	107.6
10a	77.5	87.4	35.8	84.3
10b	66.5	70.4	25.2	89.6
10c	93.5	95.9	87.9	107.7
10d	93.9	90.1	83.8	99.9
10e	106.1	110.9	58.9	108.6
10f	105.9	122.4	97.9	104.1
10g	99.0	90.5	70.2	97.1
10h	93.0	105.3	87.0	86.8

reducing the residual kinase activity to 6.7%. Both also inhibited KIT (residual activity ~18–19%), while **9d** displayed additional activity against FLT3-ITD (28%), compared to 34% for **9c**. In contrast, most other analogues, particularly the **10a–h** series, showed negligible PDGFRA inhibition (>90% residual activity). Activity against ABL was generally weak across both series (>70% residual activity). This initial screening highlighted **9c** and **9d** as primary leads for further investigation of their PDGFR inhibitory capabilities.

2.3.2. PDGFRA IC₅₀. The pronounced PDGFRA inhibition exhibited by **9c** and **9d** prompted us to determine their half-maximal inhibitory concentrations (IC₅₀ values) against this primary target. To quantify the inhibitory potency, IC₅₀ values against PDGFRA were determined for representative compounds (**9c** and **9d**) with sunitinib included as a reference. Compounds **9c** and **9d** exhibited exceptional nanomolar potency (IC₅₀ = 30 and 20 nM, respectively), comparable to that of sunitinib (IC₅₀ = 50 nM). These data establish **9c** and **9d** as lead PDGFRA inhibitors within the series (Table 2).

Table 2. PDGFRA IC₅₀ ± SD for Compounds **9c** and **9d**

Comp.	PDGFRA IC ₅₀ (μM)
9c	0.03 ± 0.02
9d	0.02 ± 0.01
Sunitinib	0.05 ± 0.03

2.4. Receptor Kinase Selectivity Profiling

To delineate selectivity, compound **9d** was profiled at 1 μM against a broad human receptor kinase panel comprising 59 wild-type and 11 mutant enzymes (Figure 2). Potent inhibition (residual activity <25%) was observed across several receptor tyrosine kinase families, including VEGFRs (FLT1, FLT4, KDR), type III RTKs (PDGFRα, FLT3, KIT, CSF1R), MET,

and RET. Moderate inhibition (25–50% residual activity) extended to kinases such as BLK, LYN, TRK family members, FGFR2/3, ALK, and Eph kinases. Weak or negligible activity (>50% residual activity) was observed for the majority of the tyrosine kinase subfamily of the human kinome, including FGFR1, BTK, ROS, and TIE2.

Compounds **9c** and **9d** thus emerge as potent and selective PDGFR inhibitors, with activity comparable to that of the clinically approved multitargeted RTK inhibitor sunitinib.^{51,52} Kinome selectivity profiling revealed that **9d** exerts focused inhibition within specific RTK subfamilies, most notably, PDGFR and closely related type III RTKs, while sparing a large proportion of the kinome. This selectivity profile supports **9d** as a promising candidate for targeted therapy in PDGFR-driven malignancies.

2.5. Carbonic Anhydrase Inhibition

Given the critical role of CA IX/XII in maintaining pH homeostasis within the hypoxic tumor microenvironment, which is also relevant to leukemia, the synthesized compounds were evaluated for their inhibitory activity against hCA isoforms I, II, IX, and XII, with acetazolamide (AAZ) serving as a reference standard.

2.5.1. Inhibition of Physiological Isoforms (CA I/II). Minimizing activity against the widely expressed cytosolic isoforms CA I and II is essential to reduce systemic off-target effects. Most of our compounds exhibited substantially weaker inhibition of CA I compared to AAZ (K_i = 250 nM). For instance, compound **9b** displayed very weak CA I inhibition (K_i = 3054 nM), whereas compound **10h** showed moderate potency (K_i = 125.3 nM). A similar trend was observed for CA II (AAZ K_i = 12.0 nM), with several compounds showing diminished activity. Within the ureido-linked series, **9h** (K_i = 26.9 nM) was the most potent, while **9b** was the weakest (K_i = 259.9 nM). In the amide-linked series, **10g** (K_i = 17.3 nM) emerged as the most active CA II inhibitor. Compounds **9c** and **9d**, while the most potent PDGFR inhibitors, exhibited markedly reduced activity against the physiological CA isoforms compared to AAZ. Specifically, they were ~3- and 11-fold less potent on CA I (K_i = 770 and 2704 nM, respectively) and ~9.5- and 15-fold less potent on CA II (K_i = 115 and 177 nM, respectively). Overall, inhibition of CA I/II remained in the low to moderate nanomolar range, supporting a favorable selectivity profile toward tumor-associated isoforms (Table 3).

2.5.2. Inhibition of Tumor-Associated Isoforms CA IX/XII. The majority of our synthesized compounds demonstrated strong inhibition against hypoxia-induced CA IX/XII isoforms. Among them, compound **9h** emerged as an exceptionally potent CA IX inhibitor (K_i = 6.3 nM), surpassing the value of the reference inhibitor AAZ (K_i = 25.0 nM). Compound **9f** also exhibited high potency (K_i = 8.5 nM), with both compounds sharing a *para*-orientation of the sulfonamide and quinoline moieties, a feature that consistently favored CA IX activity. Within the amide-linked analogues, **10f** (K_i = 14.7 nM) retained strong CA IX inhibition, highlighting the contribution of spatial arrangement to the activity. For CA XII, compound **9e** (K_i = 14.1 nM) was the most potent, followed by compounds **9f** (19.4 nM) and **10f** (K_i = 23.5 nM). The *para*-sulfonamide configuration again proved to be advantageous for dual CA IX/XII inhibition. In contrast, compounds **9c** and **9d**, although primarily being the most potent PDGFR inhibitors of the series, also retained moderate

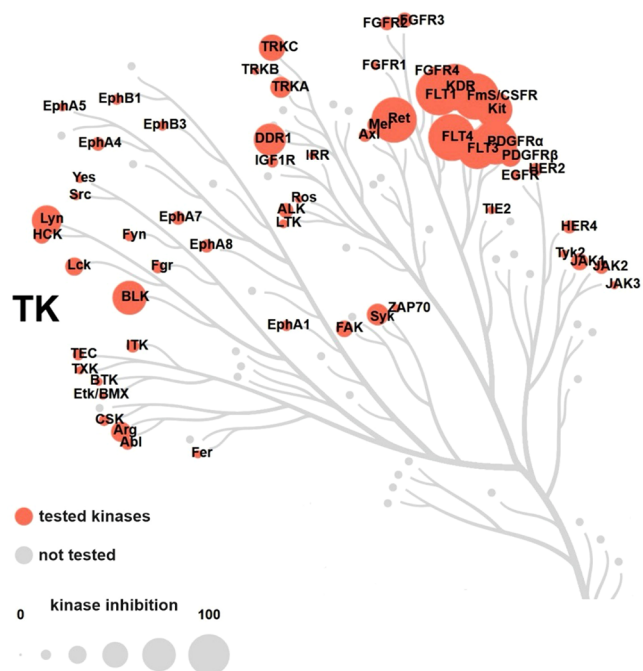


Figure 2. Kinase selectivity profile of compound **9d** determined at 1 μM against 59 selected wild-type human receptor kinases.^{53,54} Complete profiling data are provided in Figure S121 (Supporting Information).

Table 3. Inhibition Data of Human CA Isoforms I, II and Cancer-Associated CA IX and XII Using AAZ as a Reference Drug^a

Compound	K_i (nM) ^a			
	CA I	CA II	CA IX	CA XII
9a	735.2	142.5	38.9	57.5
9b	3054	259.3	84.5	88.0
9c	701.7	115.0	59.7	65.6
9d	2704	177.0	93.3	80.0
9e	333.2	36.1	18.7	14.1
9f	409.9	84.2	8.5	19.4
9g	298.8	50.1	18.5	41.3
9h	265.6	26.9	6.3	50.1
10a	734.4	396.7	50.8	68.2
10b	2947	133.5	108.6	104.0
10c	715.5	322.2	64.0	74.7
10d	1232	187.1	131.3	96.7
10e	521.3	86.5	46.9	39.9
10f	303.0	23.1	14.7	23.5
10g	185.6	17.3	20.3	46.7
10h	125.3	39.8	29.0	33.8
AAZ	250.0	12.0	25.0	5.7

^aMean from 3 different assays, by a stopped-flow technique (errors were in the range of ± 5 –10% of the reported values).

yet meaningful activity against the tumor-associated isoforms, with K_i values of 59.7 and 93.3 nM for CA IX and 65.6 and 80.0 nM for CA XII, respectively.

Accordingly, the ureido-linked derivatives, particularly those incorporating *para*-quinoline and *para*-sulfonamide motifs, displayed superior activity against tumor-associated CA IX/XII while maintaining reduced inhibition of CA I/II. These findings support their potential as selective CA IX/XII anticancer agents tailored to the hypoxic microenvironment of leukemia.

2.6. Structure–Activity Relationship (SAR) Analysis

The combined kinase and carbonic anhydrase inhibition data for compounds **9a–h** and **10a–h** reveal distinct SAR trends that underpin their biological activities and inform further optimization.

2.6.1. PDGFR SAR. Analysis of the kinase inhibition data revealed clear structural determinants of potency and selectivity toward PDGFR. The ureido-linked series (**9a–h**) consistently outperformed the corresponding amide-linked analogues (**10a–h**), particularly against PDGFRA and KIT. Within this series, the orientation of 6,7-dimethoxyquinoline proved critical: *para*-substituted derivatives (e.g., **9c**, **9d**) exhibited potent PDGFR inhibition, whereas the corresponding *meta*-substituted analogues were markedly less active, underscoring strict spatial requirements for optimal binding. Conversely, *meta*-substituted sulfonamides showed potent PDGFRA inhibition compared to their *para*-substituted counterparts. Introduction of a *para*-methyl substituent ($R_1 = \text{CH}_3$) in **9d** broadened the inhibitory profile by slightly enhancing FLT3-ITD (28.2%) and ABL (79.2%) inhibition relative to **9c** ($R_1 = \text{H}$; 34.3% and 94.5%, respectively) while maintaining comparable potency against PDGFRA and KIT.

In contrast, several derivatives (e.g., **9e**, **9g**, **10c–h**) showed weak activity across the kinase panel, likely due to steric or electronic factors that impair favorable engagement. To sum up, the SAR highlights the ureido linker, *para*-substituted dimethoxyquinoline, and *meta*-sulfonamide configuration as key drivers of PDGFR inhibition, while *para*-methylation enhances activity against FLT3-ITD and ABL, which are therapeutically relevant targets in acute myeloid leukemia^{55,56} (Figure 3).

2.6.2. Carbonic Anhydrase SAR. CA inhibition data revealed three main determinants of tumor-associated CA IX/XII potency and selectivity: the linker, the sulfonamide orientation, and the quinoline substitution pattern. As observed for PDGFR, ureido-linked derivatives (**9a–h**) consistently showed greater potency and selectivity toward CA IX than their amide-linked counterparts (**10a–h**). For example, **9h** (CA IX $K_i = 6.3$ nM) was substantially more potent than **10h** (CA IX $K_i = 29.0$ nM), indicating that the urea functionality provides an optimal geometry and hydrogen-bonding profile for productive anchoring within the CA IX active site. The orientation of the sulfonamide group also played a decisive role in CA inhibition. *Para*-substituted sulfonamides outperformed their *meta* counterparts, as seen with **9f** and **9h** versus **9b** and **9d**, consistent with the notion that the *para*-orientation better aligns the sulfonamide for zinc

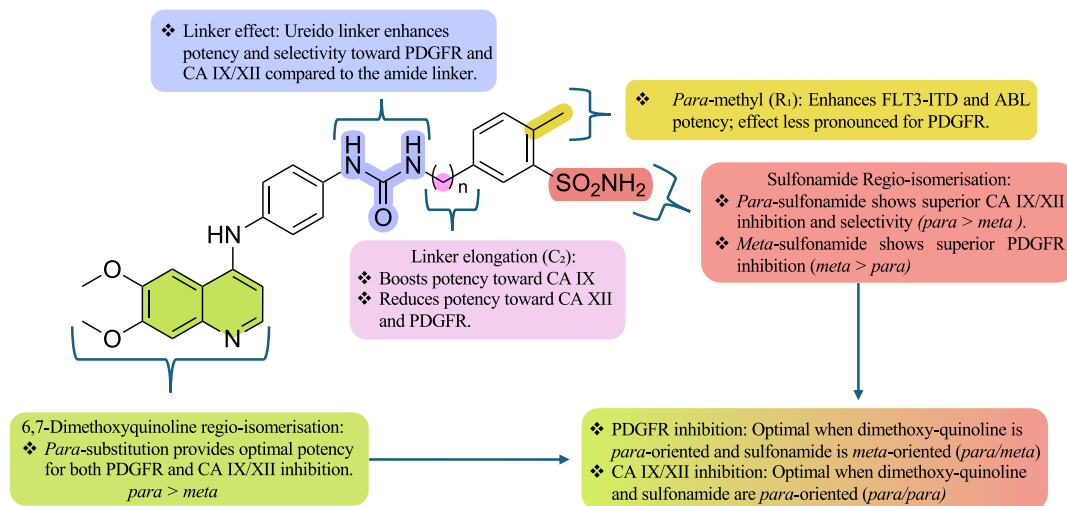


Figure 3. Key structure–activity relationships for PDGFR and CA IX/XII dual inhibition.

coordination within the active site. In addition, the 6,7-dimethoxyquinoline tail in the *para*-position (e.g., **9f**, **9h**, **10f**) enhanced CA IX/XII inhibition, likely due to favorable π - π stacking and hydrophobic interactions with the enzyme pocket. Elongation of the linker by two carbons further improved CA IX activity, as observed for **9h** (CA IX K_i = 6.3 nM) compared to **9f** (K_i = 8.5 nM), but this modification reduced CA XII potency, indicating a trade-off between potency and isoform selectivity (Figure 3).

Among evaluated compounds, **9f** displayed the most favorable CA selectivity profiles, with selectivity indices (Table S1, Supporting Information) of 48.2 (CA I/IX) and 9.9 (CA II/IX), followed by **9h** (Table S1, Supporting Information; CA I/IX = 42.1; SI CA II/IX = 4.3). **9d** exhibited favorable selectivity toward tumor-associated isoforms, showing one of the highest SI CA I/XII (33.8), along with CA I/IX = 29.0, CA II/IX = 1.9, and CA II/XII = 2.2 (Table S1, Supporting Information). CA SAR trends highlight a consistent structural motif for tumor-selective CA inhibition: a ureido linker, *para*-sulfonamide substitution, and a *para*-conjugated 6,7-dimethoxyquinoline tail. Compounds incorporating these features represent promising scaffolds for the selective inhibition of CA IX/XII with minimal activity against CA I/II.

Taken together, the SAR analysis defines a unified pharmacophoric framework underlying the dual PDGFR and CA IX/XII inhibitory activity. For both targets, high potency is driven by a ureido linker in combination with a *para*-substituted 6,7-dimethoxyquinoline core, establishing a common structural requirement for the dual activity. Fine-tuning of target preference is primarily governed by the orientation of the sulfonamide group: a *meta*-oriented sulfonamide preferentially enhances PDGFR inhibition, whereas a *para*-oriented sulfonamide favors CA IX/XII inhibition. Importantly, modulation of the sulfonamide orientation allows biasing of PDGFRA kinase versus carbonic anhydrase activity while retaining nanomolar potency against both targets, highlighting the tunability of this scaffold and its suitability for balanced or target-weighted dual inhibition.

2.7. Antiproliferative Activity

To evaluate cellular efficacy and selectivity, the synthesized compounds were tested in a leukemia-focused panel designed to probe PDGFR dependence and hypoxia-inducible CA IX expression.⁵⁷ EOL-1 cells, harboring the FIP1L1-PDGFR fusion, served as a direct model of PDGFR-driven eosinophilic leukemia,⁵⁸ while MV4-11 cells represented a model for FLT3-ITD-driven AML.⁵⁹ Additional leukemia lines (THP-1, RS4-11, HL60, K562, and Kasumi-1) captured diverse subtypes with varying kinase dependencies, thereby reflecting clinically relevant heterogeneity.⁶⁰ Notably, several of these leukemia cell lines, including EOL-1 and MV4-11, are known to express CA IX, particularly under hypoxic conditions prevalent in the bone marrow microenvironment, thus providing a relevant model for assessing the contribution of CA IX inhibition to the overall antiproliferative effect.

To experimentally validate hypoxia-induced CA IX expression in these cellular models, MV4-11 and EOL-1 cells were treated with cobalt chloride (CoCl_2 ; 100 and 200 μM) for 24 and 48 h, and the expression of hypoxia-associated markers was analyzed by immunoblotting (Figure 4). Under normoxic conditions, HIF-1 α could barely be detected in both cell lines. In contrast, CoCl_2 treatment resulted in the robust

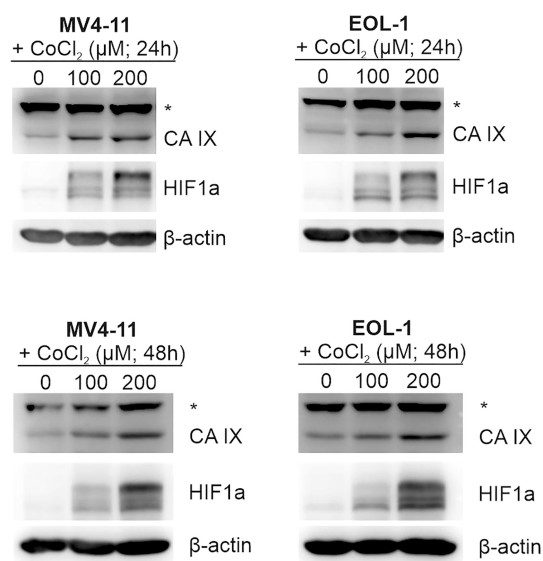


Figure 4. CoCl_2 -induced hypoxia upregulates CAIX expression in MV4-11 and EOL-1 Cells. MV4-11 and EOL-1 cells were treated with cobalt chloride (CoCl_2 ; 0, 100, or 200 μM) for 24 or 48 h to induce hypoxia. Protein expression levels of CAIX and hypoxia-inducible factor-1 α (HIF-1 α) were analyzed by Western blotting. CoCl_2 treatment resulted in dose- and time-dependent stabilization of HIF-1 α and a corresponding increase in CAIX expression in both cell lines. β -actin was used as a loading control. The asterisk (*) indicates an uncharacterized band observed during CAIX detection, as reported in the antibody datasheet.

stabilization of HIF-1 α in a concentration- and time-dependent manner, with a more pronounced induction observed at 200 μM and after 48 h. Consistent with HIF-1 α activation, CA IX expression was markedly upregulated in both MV4-11 and EOL-1 cells following CoCl_2 exposure. CA IX levels increased progressively with higher CoCl_2 concentrations and prolonged incubation, confirming the effective activation of hypoxia-responsive signaling pathways. β -actin levels remained constant across all conditions, verifying equal protein loading. Collectively, these results confirm that MV4-11 and EOL-1 cells mount a robust hypoxic response and upregulate CA IX under hypoxia-mimicking conditions, thereby validating their suitability as cellular models to explore the contribution of CA IX inhibition to the antiproliferative activity of dual PDGFR/CA IX inhibitors.

Beyond leukemia, HEK293T cells were utilized as a non-malignant control for evaluating general cytotoxicity and selectivity against non-malignant cells.⁶¹ In EOL-1 cells, the ureido-linked derivatives (**9a–h**) showed the highest cytotoxicity, with **9d** emerging as the most potent analogue, exhibiting a GI_{50} of 2 nM. This cellular potency is in excellent agreement with its biochemical PDGFR α inhibition (IC_{50} = 20 nM), providing strong evidence for an on-target activity (Table 4). Compound **9d** also demonstrated pronounced cytotoxicity against MV4-11 (GI_{50} = 0.263 μM), possibly due to its residual activity against FLT3-ITD. On Kasumi-1 and RS4-11, compound **9d** exhibited weak cytotoxicity, with GI_{50} values of 2.081 and 4.599 μM , respectively. Compound **9c** displayed a similar selectivity profile (EOL-1, GI_{50} = 12 nM; MV4-11, 1.304 μM ; Kasumi-1, 2.671 μM).

In contrast, cell lines lacking strong PDGFR, FLT3, or KIT dependence (THP-1, HL60) were largely insensitive (GI_{50} > 25 μM), and the amide-linked series (**10a–h**) was broadly

Table 4. Antiproliferative Activity (GI_{50} , μM) of Compounds 9a–h and 10a–h and Reference Kinase Inhibitors against a Panel of Leukemia and Non-malignant Cell Lines

Comp.	$GI_{50} \pm SD$ (μM)							
	EOL-1	MV4-11	THP-1	RS4-11	HL60	K562	Kasumi-1	HEK293T
9a	21.655 $\pm$ 1.902	>25	>25	>25	>25	>25	>25	>25
9b	1.056 $\pm$ 0.060	>25	>25	>25	>25	>25	>25	>25
9c	0.012 $\pm$ 0.004	1.304 $\pm$ 0.310	>25	21.759 $\pm$ 1.345	>25	>25	2.671 $\pm$ 0.478	>25
9d	0.002 $\pm$ 0.002	0.263 $\pm$ 0.114	24.912 $\pm$ 0.125	4.599 $\pm$ 0.150	19.435 $\pm$ 2.513	19.653 $\pm$ 1.238	2.081 $\pm$ 0.219	>25
9e	>25	>25	>25	>25	>25	>25	>25	>25
9f	1.188 $\pm$ 0.017	>25	>25	>25	>25	>25	>25	>25
9g	>25	>25	>25	>25	>25	>25	>25	>25
9h	0.501 $\pm$ 0.080	20.640 $\pm$ 2.401	>25	>25	>25	>25	>25	>25
10a	>25	>25	>25	>25	>25	>25	>25	>25
10b	22.079 $\pm$ 4.132	>25	>25	>25	>25	>25	>25	>25
10c	>25	>25	>25	>25	>25	>25	>25	>25
10d	23.700 $\pm$ 1.838	>25	>25	>25	>25	>25	>25	22.777 $\pm$ 0.358
10e	>25	>25	>25	>25	>25	>25	>25	>25
10f	>25	>25	>25	>25	>25	>25	>25	>25
10g	>25	>25	>25	>25	>25	>25	>25	>25
10h	>25	>25	>25	>25	>25	>25	>25	>25
Cabozantinib	0.001 $\pm$ 0.000	0.024 $\pm$ 0.010	>25	3.652 $\pm$ 0.372	21.356 $\pm$ 3.624	2.858 $\pm$ 0.297	0.055 $\pm$ 0.010	18.238 $\pm$ 4.211
Sorafenib	<0.0001	0.016 $\pm$ 0.000	8.357 $\pm$ 0.471	6.316 $\pm$ 1.179	5.398 $\pm$ 0.347	6.544 $\pm$ 1.823	0.081 $\pm$ 0.006	12.576 $\pm$ 0.179
Imatinib	<0.0001	>25	>25	>25	>25	0.293 $\pm$ 0.074	0.938 $\pm$ 0.044	>25

inactive. In K562, compound **9d** showed weak inhibition (GI_{50} = 19.653 μM), consistent with its limited ABL activity in the kinase panel (Table 4). Importantly, no significant cytotoxicity was detected in the normal cell line (HEK293T) for the ureido analogues (GI_{50} > 25 μM). Compounds **9c** and **9d** exhibited wide therapeutic windows for eosinophilic leukemia (HEK293T/EOL-1) with about >2000-fold selectivity for **9c** and about >12,500-fold selectivity for **9d**, compared to about >18,000-fold selectivity for cabozantinib. This favorable therapeutic window underscores the on-target nature of the observed effects rather than nonspecific toxicity. Moreover, activity in models that upregulate CA IX under hypoxia, such as EOL-1 and MV4–11, is consistent with the proposed dual PDGFR/CA IX inhibition profile of these compounds.

Notably, the potency of **9d** in EOL-1 cells approached that of reference multikinase inhibitors cabozantinib (GI_{50} = 1 nM

in EOL-1) while retaining superior selectivity over non-malignant cells, further strengthening its therapeutic potential. Taken together, these findings highlight the potential of **9d** as a highly potent and selective inhibitor for PDGFR-driven leukemias, with activity comparable to that of established clinical agents such as cabozantinib and sorafenib, underscoring its promise as a first-in-class dual PDGFR/CA IX/XII-targeted antileukemic agent.

2.8. Cellular Mechanistic Studies

To elucidate the cellular mechanisms underlying the antiproliferative activity of the synthesized compounds, particularly the lead compound **9d**, a series of in-depth cellular studies were conducted. These investigations focused on the inhibition of key signaling pathways, induction of apoptosis, and effects on the cell cycle progression in leukemia models.

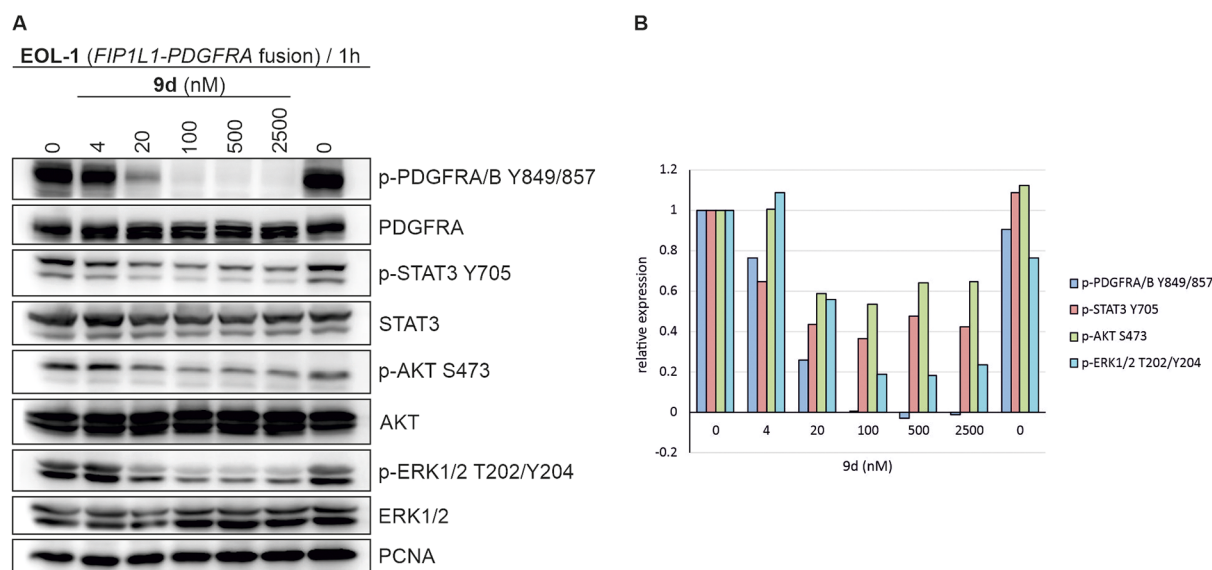


Figure 5. Inhibition of PDGFRA signaling pathways by compound 9d in EOL-1 cells. EOL-1 cells expressing the FIP1L1–PDGFRA fusion kinase were treated with compound 9d (0–2500 nM) for 1 h. (A) Representative Western blots showing the phosphorylation status of PDGFRA α/β (Y849/857) and downstream signaling proteins STAT3 (Y705), AKT (S473), and ERK1/2 (T202/Y204), together with the corresponding total protein levels. (B) Densitometric quantification of phosphorylated proteins, normalized to their respective total protein levels and expressed relative to untreated control cells. PCNA was used as a loading control.

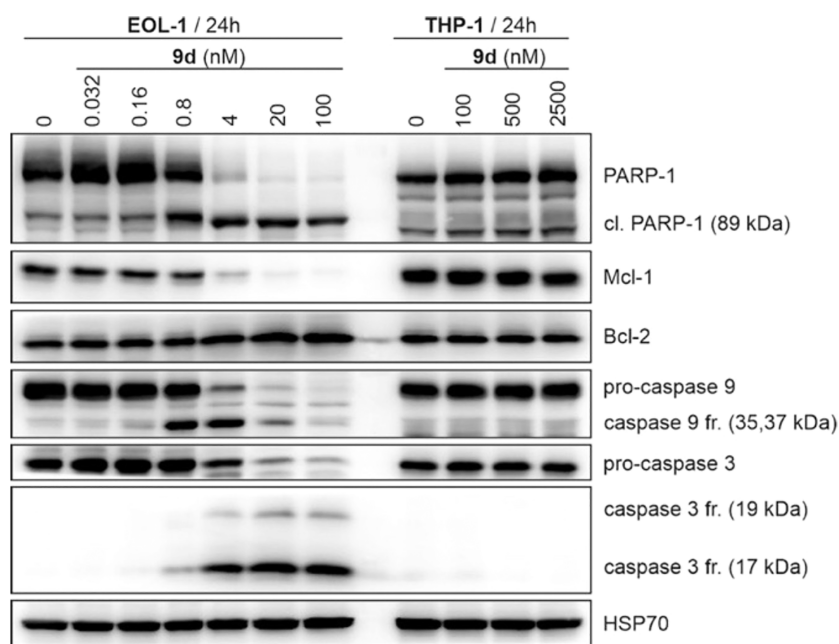


Figure 6. Western blot analysis of apoptotic markers in EOL-1 and THP-1 cells treated with the indicated concentrations of 9d for 24 h. HSP70 served as the loading control. Densitometric quantification of protein levels is provided in the [Supporting Information](#) (Figure S122).

2.8.1. PDGFRA Signaling Pathway Inhibition. To investigate the cellular basis of the antiproliferative activity of the lead compound 9d, we examined its effects on FIP1L1–PDGFRA signaling in EOL-1 cells, which constitutively express this oncogenic fusion kinase. Cells were treated with increasing concentrations of 9d (0, 4, 20, 100, 500, and 2500 nM) for 1 h, and protein phosphorylation was assessed by Western blot (Figure 5A). Compound 9d induced a clear, dose-dependent inhibition of FIP1L1–PDGFRA phosphorylation (p-PDGFRA/B Y849/857), with substantial reduction detected from 20 nM and near-complete dephosphorylation at 100 nM. At the same time, total PDGFRA protein levels were

unchanged across all concentrations, indicating that 9d primarily inhibits the kinase activity rather than affecting protein stability. Consistent with the inhibition of upstream PDGFRA phosphorylation, the phosphorylation of key downstream signaling molecules was also abrogated in a dose-dependent manner. Phosphorylation of STAT3 (p-STAT3 Y705), a crucial transcription factor involved in cell proliferation and survival, was markedly reduced at concentrations of 9d from 20 nM upward. Similarly, the phosphorylation of AKT (p-AKT S473), a central component of the PI3K/AKT pathway, exhibited a clear dose-dependent decrease, with substantial inhibition observed at concentrations

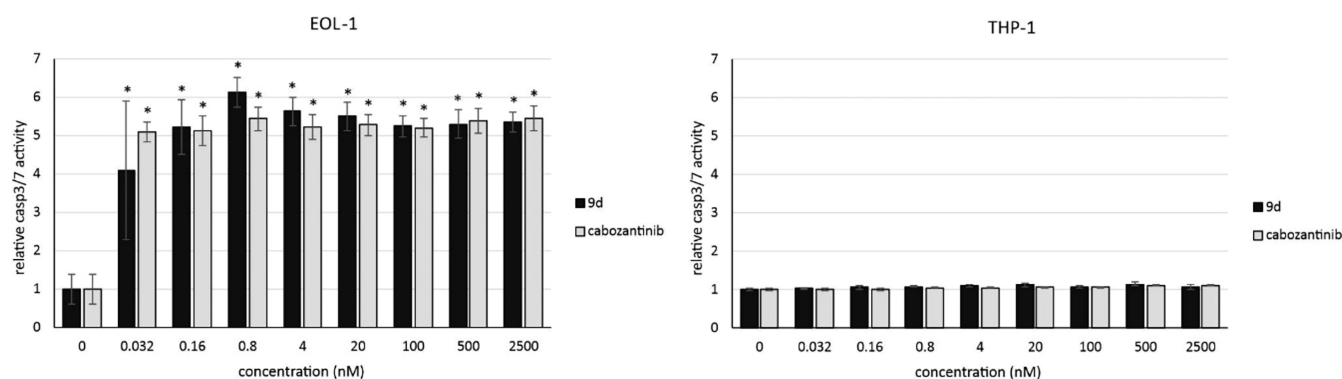


Figure 7. Caspase3/7 activation in EOL-1 and THP-1 cells following treatment with the indicated concentrations of **9d** or cabozantinib for 24 h. Statistical significance was determined by one-way ANOVA, followed by Dunnett's multiple-comparison test versus the untreated control ($*p < 0.01$).

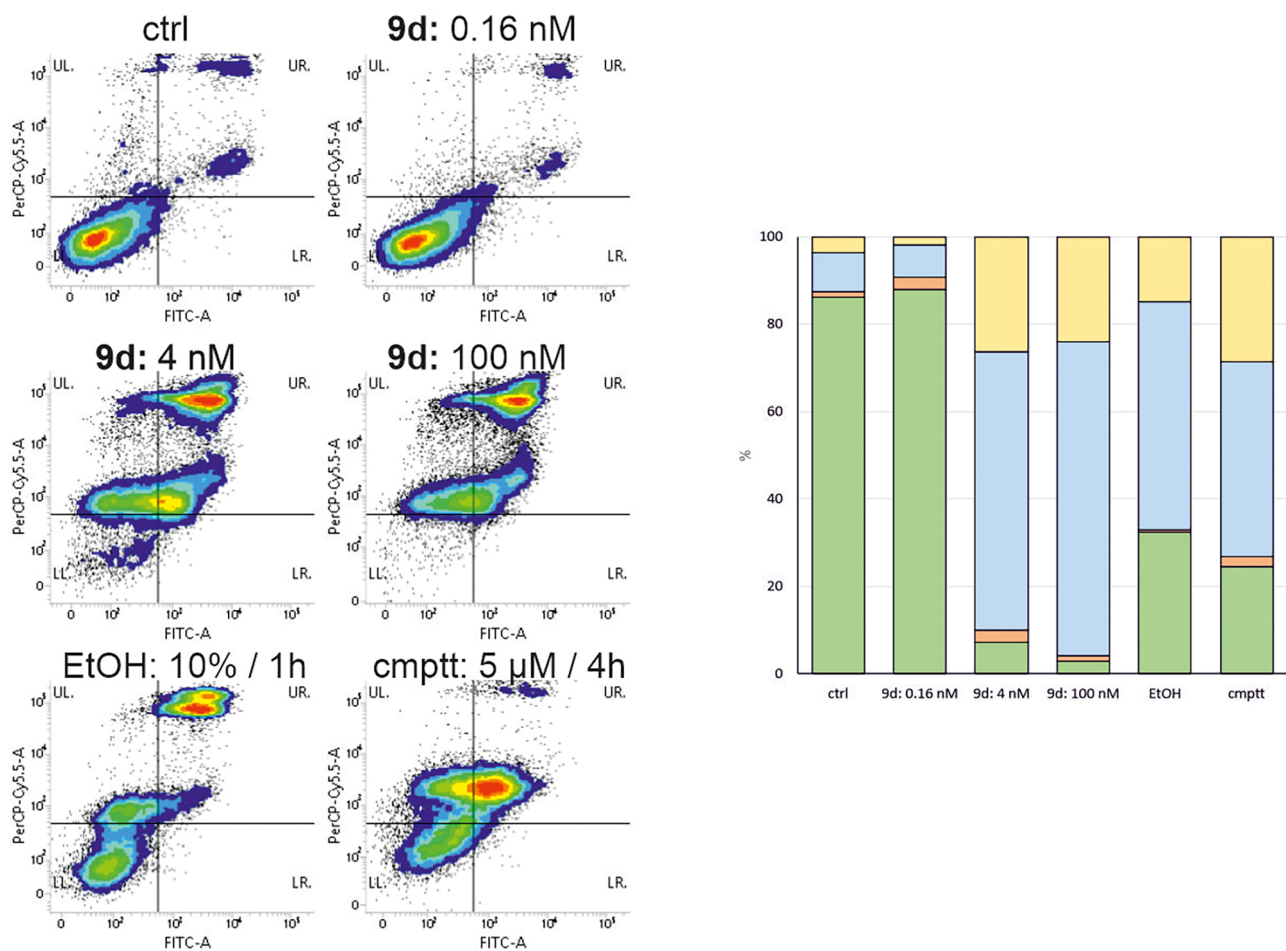


Figure 8. Annexin V/propidium iodide flow cytometric analysis of apoptosis induced by compound **9d**. Representative Annexin V-FITC/propidium iodide density plots (left) and quantitative distribution of cell populations among individual quadrants (right) are shown. EOL-1 cells were treated with increasing concentrations of compound **9d** for 24 h prior to staining and flow cytometric analysis. Cells treated with 10% ethanol (1 h) or camptothecin (**cmptt**) (5 μ M, 4 h) were included as necrotic and apoptotic controls, respectively.

of 100 nM and above. Furthermore, the phosphorylation of ERK1/2 (p -ERK1/2 T202/Y204), a critical effector in the MAPK pathway, was also significantly diminished by **9d** treatment, with strong inhibition evident from 100 nM. These effects were confirmed by densitometric quantification normalized to the respective total protein levels (Figure 5B). Notably, the total protein levels of STAT3, AKT, and ERK1/2 remained unchanged, confirming that **9d** selectively interferes

with kinase activity and signaling rather than altering protein expression or stability (Figure 5).

Together, these findings provide compelling cellular evidence that **9d** directly inhibits the FIP1L1-PDGFR α fusion kinase and its downstream pathways. The rapid and dose-dependent blockade of PDGFR α -driven JAK/STAT, PI3K/AKT, and MAPK/ERK signaling, which play a pivotal role in cell proliferation, survival, differentiation, and angiogenesis, is

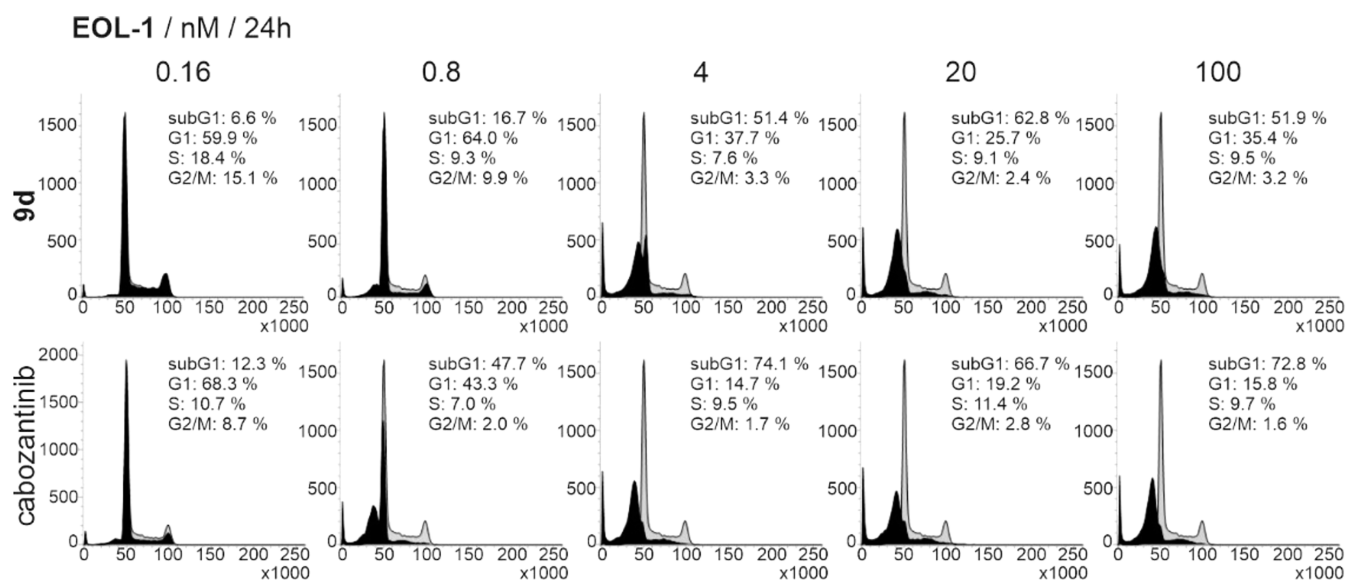


Figure 9. Cell cycle analysis of EOL-1 cells treated with **9d** and cabozantinib for 24 h. The cells were treated with the indicated concentrations of the compounds (black histograms), and the results were compared with those of the untreated control sample (gray histograms; sub-G1: 6.2%; G1: 60.1%; S: 19.8%; G2/M: 14.0%).

consistent with the potent antiproliferative effect of **9d** in EOL-1 cells ($GI_{50} = 2$ nM) and with its low nanomolar potency against PDGFRA ($IC_{50} = 20$ nM). By simultaneously disabling multiple prosurvival and proliferative cascades, **9d** effectively disrupts the oncogenic signaling that drives EOL-1 cell growth. These results validate PDGFRA as a key cellular target of **9d** and support its therapeutic potential in malignancies characterized by aberrant PDGFRA signaling.

2.8.2. Induction of Apoptosis. To investigate whether compound **9d** induces apoptosis, EOL-1 and THP-1 leukemia cells were exposed to increasing concentrations of **9d** for 24 h, and protein expression levels of key apoptotic and anti-apoptotic regulators were evaluated by Western blot using HSP70 as a loading control (Figure 6). In EOL-1 cells, compound **9d** induced apoptosis in a clearly concentration-dependent manner. Cleavage of PARP-1 (poly ADP-ribose) polymerase 1, a hallmark of apoptosis, was detectable at concentrations as low as 0.8 nM, with progressive accumulation of the 89 kDa cleaved PARP-1 fragment at 4 nM and higher. This effect was accompanied by a marked decrease in the expression of the antiapoptotic protein Mcl-1 (myeloid cell leukemia 1), showing substantial reduction at 4 nM and almost complete depletion at 20 nM. In contrast, Bcl-2 (B-cell lymphoma 2) levels remained largely unchanged across all tested concentrations.

Caspase activation was also evident in EOL-1 cells: pro-caspase-9 decreased concomitantly with the appearance of 35- and 37 kDa cleaved fragments, while pro-caspase-3 was processed to its 19- and 17 kDa active forms, both of which were clearly detectable at 4 nM and higher, indicating activation of the intrinsic apoptotic pathway (Figure 6). These effects were supported by densitometric quantification of apoptotic markers normalized to the loading control HSP70 (Figure S122).

Consistent with these findings, quantitative analysis of effector caspases 3/7 revealed a robust, dose-dependent increase in enzymatic activity in EOL-1 cells treated with **9d**, with significant activation already apparent at 0.032 nM and reaching a maximum of ~7-fold increase at 0.8 nM (Figure 7).

The magnitude and kinetics of caspase 3/7 activation closely paralleled those observed with cabozantinib, underscoring the potent proapoptotic activity of **9d** in this cellular context.

By contrast, THP-1 cells, which display lower sensitivity to **9d** in proliferation assays, showed no significant signs of apoptosis even at substantially higher concentrations. This was consistent with the results of the caspase3/7 assay, which showed that no activation occurred following treatment with **9d** or cabozantinib (Figure 7).

Taken together, these findings demonstrate that compound **9d** triggers apoptosis in EOL-1 cells through activation of the intrinsic apoptotic pathway, as evidenced by caspase9 and caspase3 cleavage, caspase 3/7 activation, and PARP-1 fragmentation and Mcl-1 downregulation. The stable expression of Bcl-2 suggests that Mcl-1 degradation constitutes a primary apoptotic driver in this context. Notably, the induction of apoptosis at subnanomolar concentrations in EOL-1 cells (PARP-1 cleavage at 0.8 nM) is in excellent agreement with the antiproliferative potency ($GI_{50} = 2$ nM) of **9d** and its potent inhibition of the PDGFRA fusion kinase. In contrast, the weaker apoptotic response observed in THP-1 cells is consistent with the lack of a PDGFR dependency. Together, the mechanistic and cellular findings establish a direct link between PDGFR inhibition and activation of the mitochondrial apoptotic cascade, highlighting the therapeutic potential of compound **9d** in PDGFRA-driven leukemias.

To further confirm apoptosis induction at the single-cell level, EOL-1 cells were treated with increasing concentrations of compound **9d** for 24 h and analyzed by Annexin V–FITC/propidium iodide staining, followed by flow cytometry (Figure 8). Treatment with **9d** resulted in a concentration-dependent decrease in the viable cell population (Annexin V[−]/PI[−]), accompanied by a corresponding increase in both early apoptotic (Annexin V⁺/PI[−]) and late apoptotic (Annexin V⁺/PI⁺) fractions. At higher concentrations, the resulting apoptotic profile closely resembled that observed with the positive control camptothecin, whereas ethanol-treated cells predominantly accumulated in the Annexin V[−]/PI⁺ quadrant, indicative of necrotic cell death. These data provide

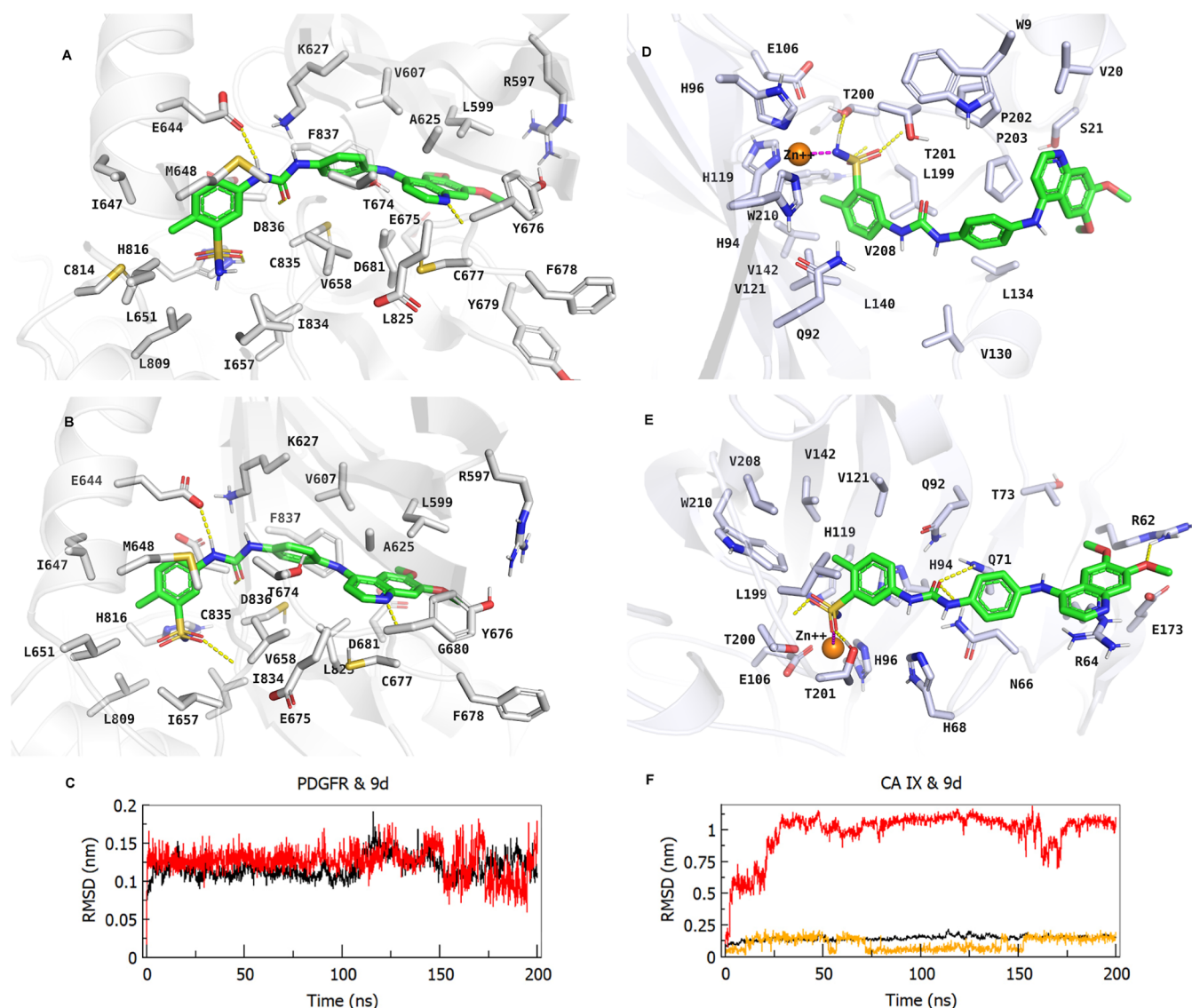


Figure 10. Molecular interaction profile of compound **9d** with PDGFRA and CA IX based on Glide docking and 200 ns molecular dynamics (MD) simulations. (A) Docking pose of **9d** in the ATP-binding cleft of PDGFRA, showing hinge binding with Cys677 and π - π stacking with Phe837. (B) Final MD-refined binding pose after 200 ns, confirming the persistence of key hydrogen bonds and hydrophobic contacts. (C) RMSD plot of the PDGFRA-**9d** complex, with the protein backbone in black and the ligand in red, indicating stable binding. (D) Docking pose of **9d** in the CA IX catalytic site, highlighting Zn^{2+} coordination (orange sphere), Thr200/201 hydrogen bonds, and π -stacking with His94. (E) Final pose after 200 ns MD, retaining metal chelation and new adaptive H-bonds with Gln71, Asn66, and Arg62. (F) RMSD plot of the CA IX-**9d** complex, with the protein backbone in black, Zn^{2+} in orange, and the ligand in red, confirming long-term conformational stability.

independent single-cell evidence that compound **9d** induces programmed cell death rather than nonspecific cytotoxicity, fully supporting activation of the intrinsic apoptotic pathway.

2.8.3. Cell Cycle Analysis. To investigate the mechanism by which compound **9d** inhibits cell proliferation, its effect on cell cycle progression was analyzed in EOL-1 cells, which are highly sensitive to **9d** due to expression of the FIP1L1-PDGFR fusion kinase. Cells were treated with increasing concentrations of **9d** (0.16, 0.8, 4, 20, and 100 nM) or cabozantinib for 24 h, and DNA content was assessed by flow cytometry (Figure 9). Treatment with **9d** caused a dose-dependent accumulation of cells in the G0/G1 phase, accompanied by a decrease in the S and G2/M populations. A modest effect was observed at 0.16 nM, whereas a significant G0/G1 arrest emerged at 0.8 nM and was sustained at higher concentrations. From 4 nM onward, a sub-G1 population

appeared, which expanded markedly at 20 and 100 nM, indicating induction of apoptosis following prolonged arrest. Consistent with Annexin V/PI apoptosis analysis (Figure 8), the emergence of the sub-G1 population from 4 nM onward reflects apoptotic cell death. Cabozantinib produced a comparable pattern of G0/G1 accumulation and sub-G1 emergence, consistent with its known PDGFR-inhibitory activity. The observed G0/G1 arrest is consistent with the blockade of PDGFRA-driven signaling pathways as **9d** strongly inhibited the phosphorylation of PDGFRA and its downstream effectors, STAT3, AKT, and ERK1/2. By preventing transmission of mitogenic signals, **9d** halted cell cycle progression at the G1 checkpoint, which in turn triggered apoptotic cell death, as confirmed by PARP-1 cleavage and caspase activation. Thus, **9d** exerts a dual effect in FIP1L1-PDGFR-driven cells: it induces potent growth arrest at low

nanomolar concentrations, followed by apoptosis at higher doses or prolonged exposure. The parallel effects of cabozantinib further validate the on-target mechanism of **9d** and highlight its promise as a selective therapeutic agent against PDGFRA-driven leukemias.

Compound **9d** emerged as a potent and selective PDGFR inhibitor, directly targeting the oncogenic driver of certain leukemias. The functional assays demonstrated robust suppression of FIP1L1–PDGFRA phosphorylation and downstream signaling (STAT3, AKT, and ERK1/2). Biochemically, **9d** inhibited PDGFRA with an IC_{50} of 20 nM, comparable to the clinically used multikinase inhibitor sunitinib (IC_{50} = 50 nM). These effects translated into pronounced antiproliferative activity in EOL-1 cells (GI_{50} = 2 nM), accompanied by G_0/G_1 arrest and apoptosis, consistent with an on-target mechanism and benchmarked closely to that of cabozantinib (GI_{50} = 1 nM). Importantly, **9d** displayed a wide therapeutic window (>12,500-fold; GI_{50} > 25,000 nM in HEK293T), comparable to that of cabozantinib (~18,000-fold), thereby excluding nonspecific cytotoxicity.

Beyond the PDGFR blockade, **9d** also inhibited CA IX/XII, hypoxia-associated isoforms frequently upregulated in the bone marrow niche. Although secondary to its kinase activity, this effect may mitigate microenvironment-driven resistance, further enhancing therapeutic potential. Collectively, these findings position **9d** as a promising candidate for PDGFR-driven leukemias, combining direct suppression of the oncogenic pathway with the added ability to counteract hypoxia-mediated survival mechanisms.

2.9. Molecular Modeling

To gain molecular-level insights into the binding characteristics of compound **9d**, *in silico* docking and molecular dynamics (MD) simulations were performed against two therapeutically relevant targets: PDGFRA (PDB ID: 6JOL) and CA IX (PDB ID: 5FL4) (Figure 10). The docking studies indicated that **9d** binds in a manner compatible with the DFG-out conformation of PDGFRA. A critical hydrogen bond was observed between the nitrogen atom of the 6,7-dimethoxyquinolin-4-yl ring and the backbone amide of Cys677 (2.01 Å), anchoring the ligand in the hinge region. The ureido carbonyl further formed bidentate interactions with Asp836 (1.84 Å) and His816 (2.80 Å), both located near the DFG loop. Additional stabilizing contacts were detected with Glu675 (2.27 Å) and Cys835 (2.77 Å) and again with Cys677 (2.71 Å). A secondary polar interaction with His816 (2.96 Å) supported this binding pattern. The central phenyl linker established a T-shaped π – π stacking interaction with Phe837 (5.47 Å), a conserved feature among type-II inhibitors, which is known to stabilize the inactive DFG-out conformation of kinases by engaging both the ATP site and adjacent hydrophobic pockets. Moreover, the ligand was enveloped by hydrophobic residues—Leu599, Ile647, Leu651, Cys814, Leu825, and Met648—which facilitated van der Waals and alkyl– π interactions, enhancing the overall binding affinity and pocket complementarity (Figure 10A). In comparison, the reference ligand sunitinib displayed a similar hinge interaction with Cys677 (2.49 Å) and formed an electrostatic contact with Lys627 (1.92 Å). Sunitinib also engaged Asp836 (2.70 Å) and showed π – π stacking with Phe837 (5.06 Å). Despite its rigid linker and slightly shallower burial in the hydrophobic pocket, sunitinib achieved a Glide docking score of –8.208 kcal/mol. Interestingly, **9d** exhibited a substantially more favorable

score of –12.155 kcal/mol, suggesting superior binding energetics attributed to an extended interaction footprint and optimal spatial accommodation.

Following the docking step, a 200 ns MD simulation was performed to evaluate the dynamic stability of the PDGFRA–**9d** complex (Video S1). The simulation trajectory confirmed the persistence of key pharmacophoric interactions. The hydrogen bond with Cys677 remained stable (2.16 Å), as did polar interactions with Asp836 (1.93 Å) and Glu675 (2.49 Å). New hydrogen bonds formed during the trajectory, notably with Val658 (2.58 Å) and Cys835 (2.70 Å), indicating the evolution of an adaptive hydrogen-bonding network. The π – π stacking with Phe837 was preserved with an average interplanar distance of 4.98 Å. Hydrophobic interactions with Leu599, Leu825, and Met648 were consistently retained throughout the trajectory (Figure 10B). RMSD analysis showed that the protein backbone remained stable, with fluctuations below 0.20 nm. At the same time, the ligand rmsd stabilized after approximately 25 ns, confirming the robustness of the binding pose under dynamic conditions (Figure 10C).

In the CA IX binding site, compound **9d** adopted a conserved sulfonamide-based binding mode characteristic of carbonic anhydrase inhibition. The deprotonated sulfonamide nitrogen chelated the catalytic Zn^{2+} ion at a distance of 2.04 Å, closely mimicking the metal coordination of established inhibitors. This sulfonamide interaction was further stabilized by two hydrogen bonds with Thr200 (1.76 Å) and Thr201 (1.93 Å). π – π stacking between the 6,7-dimethoxyquinolin-4-yl ring and His94 (4.91 Å), along with a hydrogen bond between ureido and Gln92 (2.00 Å), contributed to the optimized binding orientation. Additional van der Waals contacts were established with Val121, Val142, Leu199, and Trp210 (Figure 10D). The reference ligand SLC-0111 exhibited a comparable binding pattern, coordinating Zn^{2+} at 2.18 Å and forming hydrogen bonds with Thr200 (1.96 Å) and Gln92 (1.98 Å). It also engaged in π – π stacking with His94 and hydrophobic interactions with Val121, Val130, and Leu199. While SLC-0111 achieved a slightly better docking score (–6.548 kcal/mol) than **9d** (–5.634 kcal/mol), the latter successfully recapitulated all key interaction motifs required for potent CA IX inhibition.

To assess the stability of the complex in a dynamic environment, a 200 ns MD simulation was performed for the CA IX–**9d** system (Video S2). The sulfonamide– Zn^{2+} coordination remained intact (2.03 Å), and the hydrogen bonds with Thr200 and Thr201 were preserved. Additional hydrogen bonds developed during the simulation, including interactions between the urea carbonyl group and Gln71 (2.56 Å) and Asn66 (2.74 Å), as well as a hydrogen bond between the quinoline methoxy oxygen and Arg62 (2.61 Å). These interactions reflect a dynamically expanded hydrogen bonding network, contributing to the stabilization of the ligand within the binding pocket. A π – π stacking interaction between the benzenesulfonamide ring and His94 was sustained throughout the simulation, with a reduced average distance of 4.33 Å. Additionally, hydrophobic interactions with Arg64, Val121, and Trp210 remained consistent, contributing to the stability of the ligand–protein complex (Figure 10E). The RMSD profile confirmed structural stability, with the protein backbone remaining below 0.25 nm and the ligand rmsd plateauing after 30 ns, affirming the conformational integrity of **9d** within the catalytic pocket (Figure 10F). These computational analyses support that compound **9d** possesses a dual inhibitory profile

against both PDGFRA and CA IX, characterized by conserved interaction motifs, dynamic stability, and favorable docking energetics, highlighting its potential as a multitarget anticancer scaffold. The 2D ligand–protein interaction diagrams for compound **9d** and the reference inhibitors SLC-0111 (CA IX) and sunitinib (PDGFRA), corresponding to both initial docking poses and MD-refined final poses, are provided in the Supporting Information (Figures S1–S6).

3. CONCLUSIONS

In summary, we report the successful design and biological validation of quinoline–sulfonamide hybrids as the first dual inhibitors of PDGFR and CA IX/XII. Among them, compound **9d** emerged as the most potent analogue, showing strong inhibition of PDGFRA ($IC_{50} = 20$ nM) and CA IX/XII ($K_i = 93.3$ and 80.0 nM), along with exceptional antiproliferative activity in FIP1L1–PDGFRA-driven EOL-1 eosinophilic leukemia cells ($GI_{50} = 2$ nM). **9d**'s potency surpassed that of sunitinib and was comparable to that of cabozantinib, while it showed minimal cytotoxicity toward non-malignant and other cancer cell lines. Mechanistic studies have demonstrated that **9d** abrogates PDGFRA signaling, induces G_0/G_1 cell-cycle arrest, and promotes apoptosis, with efficacy comparable to that of the clinically established multikinase inhibitor cabozantinib. Molecular docking and 200 ns molecular dynamics simulations confirmed the stable binding of **9d** within the ATP-binding pocket of PDGFRA and the catalytic cleft of CA IX, highlighting persistent interactions with key residues and Zn metal coordination, which reinforces the proposed dual-inhibitory mechanism at the atomic level.

To the best of our knowledge, although multitargeted agents addressing either PDGFR or CA IX/XII have been reported, quinoline–sulfonamide hybrids represent the first class of compounds rationally designed to simultaneously inhibit both targets, supporting a “first-in-class” designation. These findings provide a strong foundation for further preclinical evaluation, including in vivo validation in PDGFR/CA IX/XII-driven leukemia models alongside optimization of physicochemical and pharmacokinetic properties and exploration of targeted delivery or prodrug strategies to enhance therapeutic potential.

4. EXPERIMENTAL PART

4.1. General Information

All commercially available chemical reagents were purchased from TCI, Wako, or Sigma-Aldrich and used directly without further purification. NMR spectra were recorded on Bruker Ascend 400 (1H NMR: 400 MHz, ^{13}C NMR: 100 MHz) spectrometers at 298 K. Coupling constants (J) are denoted in Hz, and chemical shifts (δ) are in ppm. The abbreviations s, d, t, q, dd, and ddd denote the resonance multiplicities singlet, doublet, triplet, quartet, doublet of doublets, and doublet of doublets, respectively. Mass spectrometric data were obtained using a Thermo Fisher Scientific LTQ Orbitrap XL. All compounds were confirmed to be greater than 95% pure by analytical HPLC. The most potent compounds, **9c** and **9d**, exhibited purities greater than 97%.

4.2. General Synthetic Procedures for Ureido-Substituted Sulfonamides (3a–h)

A dried round-bottom flask was charged with the appropriate sulfanilamide (1.0 equiv) and purged with nitrogen to establish an inert atmosphere. Dry acetonitrile was injected into the reaction flask under a nitrogen atmosphere. Separately, 4-

nitroaryl isocyanate (1.0 equiv), predissolved in dry acetonitrile, was added dropwise to the sulfonamide solution at room temperature under a nitrogen atmosphere. The reaction mixture was stirred for 10 min at ambient temperature and then heated to reflux (82 °C) and stirred for 6 h. After the mixture was cooled to room temperature, the precipitate was collected by vacuum filtration and washed sequentially with cold acetonitrile and a nonpolar solvent (hexane or diethyl ether).

4.2.1. 3-(3-(3-Nitrophenyl)ureido)-benzenesulfonamide (3a). Yellow powder. Yield: 87%. 1H NMR (400 MHz, DMSO- d_6): δ 9.27 (s, 1H), 9.18 (s, 1H), 8.58 (t, $J = 2.2$ Hz, 1H), 8.11 (d, $J = 1.7$ Hz, 1H), 7.84 (ddd, $J = 8.2, 2.3, 0.8$ Hz, 1H), 7.73 (ddd, $J = 8.2, 2.1, 0.8$ Hz, 1H), 7.62–7.54 (m, 2H), 7.52–7.42 (m, 2H), 7.37 (s, 2H).

4.2.2. 2-Methyl-5-(3-(3-nitrophenyl)ureido)-benzenesulfonamide (3b). Yellow powder. Yield: 86%. 1H NMR (400 MHz, DMSO- d_6): δ 9.21 (s, 1H), 9.09 (s, 1H), 8.56 (t, $J = 2.0$ Hz, 1H), 8.06 (d, $J = 2.2$ Hz, 1H), 7.83 (dd, $J = 8.1, 2.1$ Hz, 1H), 7.71 (dd, $J = 8.0, 1.6$ Hz, 1H), 7.61–7.49 (m, 2H), 7.38 (s, 2H), 7.28 (d, $J = 8.3$ Hz, 1H), 2.51 (s, 3H).

4.2.3. 3-(3-(4-Nitrophenyl)ureido)-benzenesulfonamide (3c). Yellow powder. Yield: 89%. 1H NMR (400 MHz, DMSO- d_6): δ 9.47 (s, 1H), 9.25 (s, 1H), 8.27–8.14 (m, 2H), 8.09 (s, 1H), 7.80–7.64 (m, 2H), 7.62–7.55 (m, 1H), 7.53–7.43 (m, 2H), 7.36 (s, 2H).

4.2.4. 2-Methyl-5-(3-(4-nitrophenyl)ureido)-benzenesulfonamide (3d). Yellow powder. Yield: 86%. 1H NMR (400 MHz, DMSO- d_6): δ 9.47 (s, 1H), 9.20 (s, 1H), 8.19 (d, $J = 9.1$ Hz, 2H), 8.07 (d, $J = 2.0$ Hz, 1H), 7.70 (d, $J = 9.1$ Hz, 2H), 7.55 (dd, $J = 8.1, 2.0$ Hz, 1H), 7.38 (s, 2H), 7.29 (d, $J = 8.3$ Hz, 1H), 2.52 (s, 3H).

4.2.5. 4-(3-(3-Nitrophenyl)ureido)-benzenesulfonamide (3e). White powder. Yield: 75%. 1H NMR (400 MHz, DMSO- d_6): δ 9.32 (s, 1H), 9.23 (s, 1H), 8.55 (t, $J = 2.2$ Hz, 1H), 7.85 (ddd, $J = 8.2, 2.3, 0.9$ Hz, 1H), 7.78–7.69 (m, 3H), 7.66–7.61 (m, 2H), 7.58 (t, $J = 8.2$ Hz, 1H), 7.24 (s, 2H).

4.2.6. 4-(3-(4-Nitrophenyl)ureido)-benzenesulfonamide (3f). White powder. Yield: 85%. 1H NMR (400 MHz, DMSO- d_6): δ 9.55 (s, 1H), 9.31 (s, 1H), 8.20 (d, $J = 9.1$ Hz, 2H), 7.75 (d, $J = 8.7$ Hz, 2H), 7.70 (d, $J = 9.1$ Hz, 2H), 7.63 (d, $J = 8.7$ Hz, 2H), 7.25 (s, 2H).

4.2.7. 4-(2-(3-(3-Nitrophenyl)ureido)ethyl)-benzenesulfonamide (3g). White powder. Yield: 80%. 1H NMR (400 MHz, DMSO- d_6): δ 9.04 (s, 1H), 8.51 (t, $J = 2.2$ Hz, 1H), 7.80–7.70 (m, 3H), 7.62 (dd, $J = 8.1, 1.3$ Hz, 1H), 7.49 (t, $J = 8.2$ Hz, 1H), 7.44 (d, $J = 8.3$ Hz, 2H), 7.28 (s, 2H), 6.34 (t, $J = 5.6$ Hz, 1H), 3.44–3.35 (m, 2H), 2.85 (t, $J = 7.1$ Hz, 2H).

4.2.8. 4-(2-(3-(4-Nitrophenyl)ureido)ethyl)-benzenesulfonamide (3h). Yellow powder. Yield: 98%. 1H NMR (400 MHz, DMSO- d_6): δ 9.30 (s, 1H), 8.12 (d, $J = 9.2$ Hz, 2H), 7.75 (d, $J = 8.2$ Hz, 2H), 7.60 (d, $J = 9.2$ Hz, 2H), 7.43 (d, $J = 8.2$ Hz, 2H), 7.31 (s, 2H), 6.47 (t, $J = 5.6$ Hz, 1H), 3.44–3.34 (m, 2H), 2.84 (t, $J = 7.0$ Hz, 2H).

4.3. General Synthetic Procedures for Amide-Substituted Sulfonamides (6a–h)

A dried round-bottom flask was charged with the appropriate sulfonamide (1.0 equiv) and flushed with nitrogen to establish an inert atmosphere. The compound was dissolved in a minimal volume of dry acetone under a nitrogen atmosphere,

followed by the addition of pyridine (2.0 equiv) as a base. Separately, 4-nitrobenzoyl chloride (1.1 equiv), predissolved in dry acetone, was added dropwise to the reaction mixture at 0 °C over 10–15 min. The reaction mixture was stirred for an additional 30 min at 0 °C and then allowed to warm to room temperature, where it was stirred for 2 h. After completion, the resulting solid was collected by vacuum filtration and washed sequentially with cold acetone, a nonpolar solvent (hexane or diethyl ether), and distilled water (to remove pyridine HCl salt).

4.3.1. 3-Nitro-*N*-(3-sulfamoylphenyl)benzamide (6a). White powder. Yield: 72%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.87 (s, 1H), 8.83 (t, *J* = 1.9 Hz, 1H), 8.51–8.39 (m, 2H), 8.35 (d, *J* = 1.5 Hz, 1H), 8.00 (dt, *J* = 6.7, 2.1 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.65–7.54 (m, 2H), 7.42 (s, 2H).

4.3.2. *N*-(4-Methyl-3-sulfamoylphenyl)-3-nitrobenzamide (6b). White powder. Yield: 78%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.78 (s, 1H), 8.80 (t, *J* = 1.7 Hz, 1H), 8.51–8.37 (m, 2H), 8.35 (d, *J* = 2.2 Hz, 1H), 7.92 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.39 (s, 2H), 7.37 (s, 1H), 2.56 (s, 3H).

4.3.3. 4-Nitro-*N*-(3-sulfamoylphenyl)benzamide (6c). White powder. Yield: 91%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.85 (s, 1H), 8.38 (t, *J* = 8.6 Hz, 3H), 8.21 (d, *J* = 8.8 Hz, 2H), 7.97 (dt, *J* = 6.5, 2.2 Hz, 1H), 7.65–7.53 (m, 2H), 7.41 (s, 2H).

4.3.4. *N*-(4-Methyl-3-sulfamoylphenyl)-4-nitrobenzamide (6d). White powder. Yield: 88%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.77 (s, 1H), 8.39 (s, 1H), 8.36 (d, *J* = 2.1 Hz, 2H), 8.20 (d, *J* = 8.7 Hz, 2H), 7.92 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.40 (s, 2H), 7.37 (d, *J* = 8.4 Hz, 1H), 2.56 (s, 3H).

4.3.5. 3-Nitro-*N*-(4-sulfamoylphenyl)benzamide (6e). White powder. Yield: 82%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.87 (s, 1H), 8.81 (s, 1H), 8.44 (dd, *J* = 18.0, 7.6 Hz, 2H), 7.96 (d, *J* = 8.6 Hz, 2H), 7.86 (dd, *J* = 14.1, 8.3 Hz, 3H), 7.30 (s, 2H).

4.3.6. 4-Nitro-*N*-(4-sulfamoylphenyl)benzamide (6f). White powder. Yield: 85%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.87 (s, 1H), 8.39 (d, *J* = 8.8 Hz, 2H), 8.20 (d, *J* = 8.9 Hz, 2H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.31 (s, 2H).

4.3.7. 3-Nitro-*N*-(4-sulfamoylphenethyl)benzamide (6g). White powder. Yield: 70%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.07–8.90 (m, 1H), 8.66 (s, 1H), 8.38 (d, *J* = 7.2 Hz, 1H), 8.27 (d, *J* = 7.1 Hz, 1H), 7.78 (dd, *J* = 14.7, 7.6 Hz, 3H), 7.45 (d, *J* = 7.4 Hz, 2H), 7.30 (s, 2H), 3.55 (t, *J* = 13.1 Hz, 2H), 3.02–2.88 (t, *J* = 13.1 Hz, 2H).

4.4. 4-Nitro-*N*-(4-sulfamoylphenethyl)benzamide (6h). White powder. Yield: 64%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.93 (t, *J* = 5.4 Hz, 1H), 8.31 (d, *J* = 8.7 Hz, 2H), 8.03 (d, *J* = 8.7 Hz, 2H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.30 (s, 2H), 3.55 (dd, *J* = 12.9, 6.8 Hz, 2H), 2.94 (t, *J* = 7.1 Hz, 2H).

4.4.1. General Procedure for Catalytic Hydrogenation of Nitro-Functionalized Intermediates

Catalytic hydrogenation of nitrophenyl sulfonamide intermediates (3a-h and 6a-h) was performed using 10% palladium on carbon (Pd/C) as the catalyst. To a nitrogen-purged reaction flask, the nitro-substituted precursor (1.0 mmol) and 10% Pd/C (120 mg) were suspended in methanol. The reaction mixture was stirred at room temperature under an atmosphere of hydrogen for 5 h. Upon completion, the mixture

was filtered through a short pad of Celite, using methanol as the eluent. In cases requiring an increased elution strength, a methanol–DMSO mixture was employed. The filtrate was concentrated under reduced pressure. When DMSO was used, the crude product was precipitated by the addition to ice-cold water, followed by vacuum filtration. The resulting aniline-functionalized sulfonamides (4a–h and 7a–h) were dried under vacuum and used directly in subsequent reactions without further purification.

4.4.2. 3-(3-(3-Aminophenyl)ureido)-benzenesulfonamide (4a). Pale yellow powder. Yield: 77%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.87 (s, 1H), 8.41 (s, 1H), 8.06 (t, *J* = 1.8 Hz, 1H), 7.51 (ddd, *J* = 8.0, 2.0, 1.4 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.40 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.34 (s, 2H), 6.90 (t, *J* = 7.9 Hz, 1H), 6.78 (t, *J* = 2.0 Hz, 1H), 6.55 (ddd, *J* = 8.0, 2.0, 0.8 Hz, 1H), 6.20 (ddd, *J* = 8.0, 2.1, 0.9 Hz, 1H), 5.02 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.73, 149.55, 145.09, 140.75, 140.37, 129.87, 129.55, 121.37, 119.15, 115.39, 108.92, 106.83, 104.52. HRMS-ESI calcd for C₁₃H₁₅N₄O₃S [M + H]⁺, 307.08594; found, 307.08606.

4.4.3. 5-(3-(3-Aminophenyl)ureido)-2-methylbenzenesulfonamide (4b). Off-white powder. Yield: 86%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.77 (s, 1H), 8.33 (s, 1H), 8.03 (d, *J* = 1.0 Hz, 1H), 7.50 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.35 (s, 2H), 7.24 (d, *J* = 8.3 Hz, 1H), 6.89 (t, *J* = 7.9 Hz, 1H), 6.79 (s, 1H), 6.54 (d, *J* = 7.7 Hz, 1H), 6.19 (d, *J* = 7.6 Hz, 1H), 5.02 (s, 2H), 2.53 (s, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.36, 149.18, 142.29, 140.11, 137.88, 132.60, 129.13, 128.41, 120.94, 116.85, 108.32, 106.23, 103.93, 40.44, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.14. HRMS-ESI calcd for C₁₄H₁₇N₄O₃S [M + H]⁺, 321.10159; found, 321.10162.

4.4.4. 3-(3-(4-Aminophenyl)ureido)-benzenesulfonamide (4c). White powder. Yield: 68%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.82 (s, 1H), 8.19 (s, 1H), 8.04 (d, *J* = 1.3 Hz, 1H), 7.53 (d, *J* = 6.8 Hz, 1H), 7.41 (dt, *J* = 16.6, 6.5 Hz, 2H), 7.32 (s, 2H), 7.09 (d, *J* = 6.9 Hz, 2H), 6.52 (d, *J* = 6.9 Hz, 2H), 4.80 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.81, 144.66, 144.34, 140.68, 129.39, 128.23, 121.06, 120.82, 118.42, 114.85, 114.19, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for C₁₃H₁₅N₄O₃S [M + H]⁺, 307.08594; found, 307.08591.

4.4.5. 5-(3-(4-Aminophenyl)ureido)-2-methylbenzenesulfonamide (4d). White powder. Yield: 79%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.71 (s, 1H), 8.11 (s, 1H), 7.99 (d, *J* = 2.2 Hz, 1H), 7.52 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.33 (s, 2H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 2H), 6.52 (d, *J* = 8.6 Hz, 2H), 4.78 (s, 2H), 2.50 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.85, 144.22, 142.20, 138.17, 132.51, 128.35, 128.04, 120.92, 120.84, 116.74, 114.15, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.10. HRMS-ESI calcd for C₁₄H₁₇N₄O₃S [M + H]⁺, 321.10159; found, 321.10153.

4.4.6. 4-(3-(3-Aminophenyl)ureido)-benzenesulfonamide (4e). Off-white powder. Yield: 70%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.94 (s, 1H), 8.49 (s, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.20 (s, 2H), 6.91 (t, *J* = 7.9 Hz, 1H), 6.77 (s, 1H), 6.57 (d, *J* = 7.7 Hz, 1H), 6.21 (d, *J* = 7.6 Hz, 1H), 5.04 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.15, 149.21, 143.06, 139.89, 136.66, 129.17, 126.88, 117.31, 108.53, 106.33, 104.02, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for C₁₃H₁₅N₄O₃S [M + H]⁺, 307.08594; found, 307.08591.

4.4.7. 4-(3-(4-Aminophenyl)ureido)-benzenesulfonamide (4f). Yellow powder. Yield: 90%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.98 (s, 1H), 8.35 (s, 1H), 7.70 (d, $J = 8.7$ Hz, 2H), 7.58 (d, $J = 8.6$ Hz, 2H), 7.18 (s, 2H), 7.09 (d, $J = 8.5$ Hz, 2H), 6.52 (d, $J = 8.5$ Hz, 2H), 4.80 (s, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 152.62, 144.38, 143.40, 136.29, 128.13, 126.81, 120.98, 117.11, 114.13, 40.14, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $\text{C}_{13}\text{H}_{15}\text{N}_4\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$, 307.08594; found, 307.08585.

4.4.8. 4-(2-(3-(3-Aminophenyl)ureido)ethyl)-benzenesulfonamide (4g). White powder. 66%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.15 (s, 1H), 7.76 (d, $J = 8.1$ Hz, 2H), 7.42 (d, $J = 8.1$ Hz, 2H), 7.31 (s, 2H), 6.83 (t, $J = 7.9$ Hz, 1H), 6.69 (s, 1H), 6.49 (d, $J = 7.8$ Hz, 1H), 6.12 (d, $J = 7.7$ Hz, 1H), 6.03 (t, $J = 5.3$ Hz, 1H), 4.92 (s, 2H), 3.36–3.28 (m, 2H), 2.81 (t, $J = 6.7$ Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 155.16, 149.00, 143.96, 142.06, 141.02, 129.21, 128.95, 125.77, 107.55, 105.88, 103.50, 40.25, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.90, 35.66. HRMS-ESI calcd for $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$, 335.11724; found, 335.11731.

4.4.9. 4-(2-(3-(4-Aminophenyl)ureido)ethyl)-benzenesulfonamide (4h). Pale yellow powder. Yield: 76%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.92 (s, 1H), 7.76 (d, $J = 8.3$ Hz, 2H), 7.41 (d, $J = 8.3$ Hz, 2H), 7.31 (s, 2H), 6.99 (d, $J = 8.7$ Hz, 2H), 6.46 (d, $J = 8.7$ Hz, 2H), 5.91 (t, $J = 5.7$ Hz, 1H), 4.67 (s, 2H), 3.36–3.27 (m, 2H), 2.80 (t, $J = 7.0$ Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 155.71, 144.03, 143.48, 142.02, 129.53, 129.21, 125.75, 120.39, 114.21, 40.39, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 35.79. HRMS-ESI calcd for $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$, 335.11724; found, 335.11731.

4.4.10. 3-Amino-N-(3-sulfamoylphenyl)benzamide (7a). White powder. Yield: 87%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.39 (s, 1H), 8.40 (s, 1H), 7.94 (d, $J = 6.4$ Hz, 1H), 7.54 (d, $J = 7.5$ Hz, 2H), 7.39 (s, 2H), 7.22–7.02 (m, 3H), 6.78 (d, $J = 6.9$ Hz, 1H), 5.34 (s, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 166.69, 148.86, 144.54, 139.81, 135.50, 129.29, 128.91, 123.14, 120.56, 117.26, 117.14, 114.89, 113.05, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 314.05698; found, 314.05692.

4.4.11. 3-Amino-N-(4-methyl-3-sulfamoylphenyl)benzamide (7b). Pale yellow powder. Yield: 93%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.28 (s, 1H), 8.38 (d, $J = 1.7$ Hz, 1H), 7.86 (dd, $J = 8.2, 1.8$ Hz, 1H), 7.32 (d, $J = 8.3$ Hz, 2H), 7.12 (dt, $J = 11.7, 8.9$ Hz, 3H), 6.75 (d, $J = 7.3$ Hz, 1H), 5.32 (s, 2H), 2.54 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 166.47, 148.82, 142.10, 137.37, 135.58, 132.36, 130.43, 128.85, 123.22, 119.20, 117.02, 114.85, 113.05, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.32. HRMS-ESI calcd for $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 328.07263; found, 328.07248.

4.4.12. 4-Amino-N-(3-sulfamoylphenyl)benzamide (7c). Off-white powder. Yield: 92%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.06 (s, 1H), 8.35 (s, 1H), 8.01–7.86 (m, 1H), 7.75 (d, $J = 8.5$ Hz, 2H), 7.50 (d, $J = 4.8$ Hz, 2H), 7.35 (s, 2H), 6.61 (d, $J = 8.5$ Hz, 2H), 5.81 (s, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 165.52, 152.44, 144.42, 140.23, 129.54, 129.14, 122.93, 120.55, 119.96, 117.10, 112.60, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 314.05698; found, 314.05698.

4.4.13. 4-Amino-N-(4-methyl-3-sulfamoylphenyl)benzamide (7d). White powder. Yield: 96%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.98 (s, 1H), 8.36 (s, 1H), 7.88 (d, J

$= 7.1$ Hz, 1H), 7.74 (d, $J = 8.2$ Hz, 2H), 7.33 (s, 2H), 7.29 (d, $J = 8.3$ Hz, 1H), 6.60 (d, $J = 8.3$ Hz, 2H), 5.78 (s, 2H), 2.54 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 165.39, 152.32, 141.99, 137.81, 132.25, 129.77, 129.48, 123.06, 120.72, 119.05, 112.60, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.27. HRMS-ESI calcd for $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 328.07263; found, 328.07260.

4.4.14. 3-Amino-N-(4-sulfamoylphenyl)benzamide (7e). Pale yellow powder. Yield: 77%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.40 (s, 1H), 7.93 (d, $J = 8.7$ Hz, 2H), 7.78 (d, $J = 8.7$ Hz, 2H), 7.27 (s, 2H), 7.12 (dt, $J = 30.8, 7.6$ Hz, 3H), 6.77 (d, $J = 7.3$ Hz, 1H), 5.35 (s, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 166.81, 148.86, 142.41, 138.48, 135.46, 128.88, 126.50, 119.69, 117.15, 114.89, 113.02, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.90. HRMS-ESI calcd for $\text{C}_{13}\text{H}_{14}\text{N}_3\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$, 292.07504; found, 292.07498.

4.4.15. 4-Amino-N-(4-sulfamoylphenyl)benzamide (7f). White powder. Yield: 75%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.06 (s, 1H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.80–7.69 (m, 4H), 7.23 (s, 2H), 6.61 (d, $J = 8.6$ Hz, 2H), 5.83 (s, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 165.59, 152.52, 142.87, 137.86, 129.60, 126.42, 120.44, 119.42, 112.57, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.90. HRMS-ESI calcd for $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 314.05698; found, 314.05692.

4.4.16. 3-Amino-N-(4-sulfamoylphenethyl)benzamide (7g). White powder. Yield: 78%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.33 (t, $J = 5.2$ Hz, 1H), 7.74 (d, $J = 8.1$ Hz, 2H), 7.42 (d, $J = 8.1$ Hz, 2H), 7.28 (s, 2H), 7.05 (t, $J = 7.7$ Hz, 1H), 6.99 (s, 1H), 6.90 (d, $J = 7.5$ Hz, 1H), 6.67 (d, $J = 7.7$ Hz, 1H), 5.21 (s, 2H), 3.55–3.40 (m, 2H), 2.90 (t, $J = 7.0$ Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 167.11, 148.68, 143.94, 142.02, 135.55, 129.18, 128.68, 125.74, 116.44, 114.28, 112.81, 40.35, 40.14, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 34.83. HRMS-ESI calcd for $\text{C}_{15}\text{H}_{17}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 342.08288; found, 342.08813.

4.4.17. 4-Amino-N-(4-sulfamoylphenethyl)benzamide (7h). White powder. Yield: 79%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.11 (s, 1H), 7.74 (d, $J = 7.8$ Hz, 2H), 7.54 (d, $J = 8.1$ Hz, 2H), 7.41 (d, $J = 7.8$ Hz, 2H), 7.29 (s, 2H), 6.52 (d, $J = 8.1$ Hz, 2H), 5.59 (s, 2H), 3.45 (d, $J = 5.8$ Hz, 2H), 2.88 (t, $J = 6.6$ Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 166.33, 151.59, 144.06, 141.97, 129.13, 128.67, 125.71, 121.24, 112.57, 40.30, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 35.08. HRMS-ESI calcd for $\text{C}_{15}\text{H}_{17}\text{N}_3\text{O}_3\text{NaS}$ $[\text{M} + \text{Na}]^+$, 342.08288; found, 342.08823.

4.5. General Procedure for *N*-Arylation of Aniline-Functionalized Sulfonamides (9a–h and 10a–h)

A dried round-bottom flask was charged with the appropriate aniline-functionalized sulfonamide (**4a–h** or **7a–h**, 1.0 equiv) and 4-chloro-6,7-dimethoxyquinoline (**8**, 1.0 equiv). The mixture was suspended in isopropanol, and a catalytic amount of 35% concentrated HCl (2–6 drops) was added dropwise. The reaction was carried out under a nitrogen atmosphere and heated at 100 °C for 48 h. After completion, the hot mixture was filtered, and the resulting solid was washed sequentially with methanol and/or acetone and hexane. The crude product was stirred in 10% aqueous Na_2CO_3 to neutralize acidic byproducts and then filtered and washed with distilled water to afford **9a–h** and **10a–h**.

4.5.1. 3-(3-(3-((6,7-Dimethoxyquinolin-4-yl)amino)-phenyl)ureido)benzenesulfonamide (9a). Yellow powder.

Yield: 70%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.31 (s, 1H), 9.20 (s, 1H), 9.05 (s, 1H), 8.33 (d, J = 5.9 Hz, 1H), 8.07 (t, J = 1.7 Hz, 1H), 7.80 (s, 1H), 7.70 (t, J = 1.9 Hz, 1H), 7.58–7.52 (m, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.44–7.40 (m, 1H), 7.39–7.34 (m, 1H), 7.34 (s, 2H), 7.29 (s, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.00 (dd, J = 7.9, 1.2 Hz, 1H), 6.88 (d, J = 5.9 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 153.27, 152.97, 149.35, 149.15, 145.47, 145.19, 142.25, 141.21, 140.60, 140.54, 130.24, 129.88, 121.41, 119.34, 117.11, 115.43, 114.93, 113.60, 113.22, 105.46, 102.04, 101.02, 56.61, 56.22, 40.60, 40.39, 40.19, 39.98, 39.77, 39.56, 39.35. HRMS-ESI calcd for $\text{C}_{24}\text{H}_{24}\text{N}_5\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 494.14927; found, 494.14917.

4.5.2. 5-(3-(3-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)-2methylbenzenesulfonamide (9b). Yellow powder. Yield: 55%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.35 (s, 1H), 9.21 (s, 1H), 9.13 (s, 1H), 8.31 (d, J = 5.9 Hz, 1H), 8.06 (d, J = 2.1 Hz, 1H), 7.81 (s, 1H), 7.68 (s, 1H), 7.51 (dd, J = 8.2, 2.1 Hz, 1H), 7.40–7.32 (m, 3H), 7.31–7.24 (m, 2H), 7.19 (d, J = 8.1 Hz, 1H), 7.00 (d, J = 7.9 Hz, 1H), 6.87 (d, J = 5.9 Hz, 1H), 3.95 (d, J = 10.2 Hz, 6H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 152.82, 152.72, 148.90, 148.74, 145.25, 142.30, 140.97, 140.23, 137.76, 132.62, 129.79, 128.68, 121.15, 117.01, 116.58, 114.41, 113.31, 112.76, 105.25, 101.71, 100.68, 56.25, 55.83, 19.19.

4.5.3. 3-(3-(4-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)benzenesulfonamide (9c). Yellow powder. Yield: 74%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.29 (s, 1H), 9.21 (s, 1H), 9.10 (s, 1H), 8.27 (d, J = 5.9 Hz, 1H), 8.09 (t, J = 1.8 Hz, 1H), 7.80 (s, 1H), 7.59 (d, J = 8.9 Hz, 3H), 7.48 (t, J = 7.8 Hz, 1H), 7.45–7.40 (m, 1H), 7.37 (s, 2H), 7.31 (d, J = 8.8 Hz, 2H), 7.27 (s, 1H), 6.66 (d, J = 5.9 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 152.75, 152.67, 149.66, 148.64, 145.21, 142.30, 141.84, 137.78, 136.99, 133.33, 132.62, 128.65, 124.90, 121.18, 119.38, 117.06, 112.76, 105.18, 101.54, 99.67, 56.21, 55.80, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.16. HRMS-ESI calcd for $\text{C}_{24}\text{H}_{24}\text{N}_5\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 494.14927; found, 494.14920.

4.5.4. 5-(3-(4-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)-2methylbenzenesulfonamide (9d). Yellow powder. Yield: 79%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.32 (s, 1H), 9.22 (s, 1H), 9.10 (s, 1H), 8.26 (d, J = 5.5 Hz, 1H), 8.06 (d, J = 2.2 Hz, 1H), 7.82 (s, 1H), 7.58 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 2.2 Hz, 1H), 7.35 (s, 2H), 7.30 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 6.65 (d, J = 5.9 Hz, 1H), 5.75 (s, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 152.75, 152.67, 149.66, 148.64, 145.21, 142.30, 141.84, 137.78, 136.99, 133.33, 132.62, 128.65, 124.90, 121.18, 119.38, 117.06, 112.76, 105.18, 101.54, 99.67, 56.21, 55.80, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.16. HRMS-ESI calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 508.16492; found, 508.16492.

4.5.5. 4-(3-(3-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)benzenesulfonamide (9e). Yellow powder. Yield: 58%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.09 (s, 1H), 8.90 (s, 1H), 8.76 (s, 1H), 8.29 (d, J = 5.4 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.67 (s, 1H), 7.64–7.57 (m, 3H), 7.32 (t, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.21 (s, 2H), 7.11 (dd, J = 8.1, 1.1 Hz, 1H), 6.97 (dd, J = 7.9, 1.2 Hz, 1H), 6.90 (d, J = 5.4 Hz, 1H), 3.92 (d, J = 12.1 Hz, 6H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 152.34, 151.82, 148.34, 148.08, 146.53, 145.71, 142.86, 141.49, 140.35, 136.97, 129.70, 126.90, 117.62, 115.97, 114.18, 113.52, 111.93, 108.06, 101.49, 101.18, 55.99, 55.60.

4.5.6. 4-(3-(4-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)benzenesulfonamide (9f). Yellow powder. Yield: 67%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.10 (s, 1H), 8.85 (s, 1H), 8.61 (s, 1H), 8.23 (d, J = 5.3 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.67 (s, 1H), 7.62 (d, J = 8.9 Hz, 2H), 7.53 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 7.23 (s, 1H), 7.21 (s, 2H), 6.66 (d, J = 5.3 Hz, 1H), 3.93 (s, 3H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 152.33, 151.52, 148.23, 148.06, 147.14, 145.77, 142.93, 136.78, 135.45, 134.98, 126.82, 123.88, 119.54, 117.42, 113.55, 108.24, 100.98, 100.05, 55.90, 55.45, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $\text{C}_{24}\text{H}_{24}\text{N}_5\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 494.14927; found, 494.14917.

4.5.7. 4-(2-(3-(3-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)ethyl)benzenesulfonamide (9g). White powder. Yield: 65%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.00 (s, 1H), 8.64 (s, 1H), 8.29 (d, J = 5.7 Hz, 1H), 7.75 (d, J = 8.3 Hz, 2H), 7.73 (s, 1H), 7.60 (t, J = 1.9 Hz, 1H), 7.42 (d, J = 8.3 Hz, 2H), 7.27 (dd, J = 16.9, 7.8 Hz, 4H), 7.04 (dd, J = 8.2, 1.1 Hz, 1H), 6.88 (dd, J = 7.9, 1.3 Hz, 1H), 6.84 (d, J = 5.6 Hz, 1H), 6.21 (t, J = 5.7 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 2.83 (t, J = 7.0 Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 155.15, 152.25, 148.45, 147.76, 146.57, 143.83, 143.75, 142.07, 141.58, 140.57, 129.51, 129.18, 125.76, 115.41, 113.56, 113.40, 111.82, 106.57, 101.35, 100.87, 56.04, 55.65, 40.26, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 35.55. HRMS-ESI calcd for $\text{C}_{26}\text{H}_{28}\text{N}_5\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 522.18057; found, 522.18036.

4.5.8. 4-(2-(3-(4-((6,7-Dimethoxyquinolin-4-yl)amino)phenyl)ureido)ethyl)benzenesulfonamide (9h). White powder. Yield: 80%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.59 (s, 1H), 8.54 (s, 1H), 8.20 (d, J = 5.3 Hz, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.66 (s, 1H), 7.44 (d, J = 8.59 Hz, 4H), 7.21 (s, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.58 (d, J = 5.3 Hz, 1H), 6.23 (t, J = 5.5 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.46 (s, 2H), 2.84 (t, J = 7.0 Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 155.36, 151.55, 148.28, 148.07, 147.56, 145.72, 143.90, 142.17, 137.04, 133.76, 129.22, 125.79, 124.27, 118.72, 113.46, 108.22, 101.02, 99.83, 55.95, 55.50, 40.35, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 35.65. HRMS-ESI calcd for $\text{C}_{26}\text{H}_{28}\text{N}_5\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 522.18057; found, 522.18054.

4.5.9. 3-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(3-sulfamoylphenyl)benzamide (10a). White powder. Yield: 77%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.57 (s, 1H), 8.90 (s, 1H), 8.37 (s, 1H), 8.34 (d, J = 5.1 Hz, 1H), 8.03–7.87 (m, 2H), 7.71 (t, J = 5.4 Hz, 1H), 7.68 (s, 1H), 7.56 (t, J = 5.9 Hz, 4H), 7.38 (s, 2H), 7.28 (s, 1H), 6.95 (d, J = 5.1 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 165.53, 151.72, 148.35, 148.29, 146.01, 145.77, 144.57, 141.46, 139.48, 135.67, 129.46, 129.32, 124.61, 123.26, 122.08, 120.80, 120.73, 117.39, 114.27, 108.29, 101.50, 100.95, 55.89, 55.52, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4\text{O}_5\text{S}$ [$\text{M} + \text{H}$] $^+$, 479.13837; found, 479.13788.

4.5.10. 3-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(4-methyl-3-sulfamoylphenyl)benzamide (10b). Brown Powder. Yield: 84%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.50 (s, 1H), 9.07 (s, 1H), 8.38 (d, J = 2.1 Hz, 1H), 8.34 (d, J = 5.4 Hz, 1H), 7.95 (s, 1H), 7.91 (dd, J = 8.2, 2.2 Hz, 1H), 7.77–7.73 (m, 1H), 7.72 (s, 1H), 7.61–7.54 (m, 2H), 7.38 (s, 2H), 7.34 (d, J = 8.5 Hz, 1H), 7.29 (s, 1H), 6.93 (d, J = 5.4 Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 165.28, 152.05, 148.49, 147.50,

146.55, 144.95, 142.14, 141.08, 137.05, 135.81, 132.41, 130.79, 129.50, 124.96, 123.38, 122.49, 121.12, 119.35, 114.03, 107.47, 101.28, 101.11, 55.98, 55.60, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.28. HRMS-ESI calcd for $C_{25}H_{25}N_4O_5S$ $[M + H]^+$, 493.15402; found, 493.15393.

4.5.11. 4-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(3-sulfamoylphenyl)benzamide (10c). White powder. Yield: 63%. 1H NMR (400 MHz, DMSO- d_6): δ 10.42 (s, 1H), 9.03 (s, 1H), 8.41 (d, J = 5.2 Hz, 1H), 8.38 (t, J = 4.8 Hz, 1H), 8.04 (d, J = 8.7 Hz, 2H), 8.01–7.94 (m, 1H), 7.64 (s, 1H), 7.59–7.50 (m, 2H), 7.44 (d, J = 8.7 Hz, 2H), 7.38 (s, 2H), 7.31 (s, 1H), 7.11 (d, J = 5.2 Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 165.11, 151.87, 148.52, 148.24, 146.18, 145.16, 144.75, 144.53, 139.77, 129.31, 129.28, 127.35, 123.14, 120.50, 119.12, 117.30, 115.00, 108.30, 103.50, 101.05, 55.90, 55.57, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $C_{24}H_{23}N_4O_5S$ $[M + H]^+$, 479.13837; found, 479.13828.

4.5.12. 4-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(4-methyl-3-sulfamoylphenyl)benzamide (10d). Pale yellow powder. Yield: 83%. 1H NMR (400 MHz, DMSO- d_6): δ 10.34 (s, 1H), 9.00 (s, 1H), 8.39 (dd, J = 6.1, 3.7 Hz, 2H), 8.03 (d, J = 8.7 Hz, 2H), 7.92 (dd, J = 8.2, 2.2 Hz, 1H), 7.63 (s, 1H), 7.43 (d, J = 8.7 Hz, 2H), 7.38–7.31 (m, 3H), 7.30 (s, 1H), 7.09 (d, J = 5.2 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 2.56 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 164.98, 151.90, 148.55, 148.27, 146.18, 145.03, 144.85, 142.11, 137.38, 132.40, 130.45, 129.29, 127.56, 123.29, 119.26, 119.21, 115.00, 108.29, 103.43, 101.06, 55.93, 55.60, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 19.30. HRMS-ESI calcd for $C_{25}H_{25}N_4O_5S$ $[M + H]^+$, 493.15402; found, 493.15402.

4.5.13. 3-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(4-sulfamoylphenyl)benzamide (10e). Pale yellow powder. Yield: 52%. 1H NMR (400 MHz, DMSO- d_6): δ 10.59 (s, 1H), 8.90 (s, 1H), 8.34 (d, J = 5.0 Hz, 1H), 8.00–7.89 (m, 3H), 7.81 (d, J = 8.6 Hz, 2H), 7.73–7.65 (m, 2H), 7.61–7.53 (m, 2H), 7.28 (s, 3H), 6.95 (d, J = 5.2 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 165.74, 151.76, 148.39, 148.32, 146.02, 145.78, 142.10, 141.50, 138.83, 135.72, 129.52, 126.56, 124.65, 122.14, 120.77, 119.95, 114.30, 108.30, 101.55, 100.94, 55.91, 55.55, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $C_{24}H_{23}N_4O_5S$ $[M + H]^+$, 479.13837; found, 479.13815.

4.5.14. 4-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(4-sulfamoylphenyl)benzamide (10f). Yellow Powder. Yield: 71%. 1H NMR (400 MHz, DMSO- d_6): δ 10.47 (s, 1H), 9.17 (s, 1H), 8.40 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 8.7 Hz, 2H), 7.96 (d, J = 8.9 Hz, 2H), 7.86–7.73 (m, 2H), 7.65 (s, 1H), 7.45 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 1.5 Hz, 3H), 7.10 (d, J = 5.4 Hz, 1H), 3.94 (d, J = 8.5 Hz, 6H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 165.20, 152.00, 148.57, 147.81, 145.66, 145.08, 145.05, 142.39, 138.48, 129.40, 127.52, 126.50, 119.75, 119.33, 114.87, 107.89, 103.34, 101.12, 55.92, 55.58, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89. HRMS-ESI calcd for $C_{24}H_{23}N_4O_5S$ $[M + H]^+$, 479.13837; found, 479.13824.

4.5.15. 3-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(4-sulfamoylphenethyl)benzamide (10g). White powder. Yield: 62%. 1H NMR (400 MHz, DMSO- d_6): δ 8.84 (s, 1H), 8.62 (t, J = 5.1 Hz, 1H), 8.31 (d, J = 5.3 Hz, 1H), 7.79 (s, 1H), 7.73 (d, J = 8.3 Hz, 2H), 7.66 (s, 1H), 7.56–7.50 (m, 1H), 7.47 (d, J = 5.2 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.26 (s, 1H), 6.87 (d, J = 5.3 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.56–3.50 (m, 2H), 2.92 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100

MHz, DMSO- d_6): δ 166.01, 151.71, 148.32, 148.27, 145.98, 145.93, 143.77, 142.11, 141.27, 135.74, 129.29, 129.15, 125.71, 124.17, 121.56, 120.56, 114.20, 108.27, 101.32, 100.96, 55.89, 55.51, 40.47, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 34.75. HRMS-ESI calcd for $C_{26}H_{27}N_4O_5S$ $[M + H]^+$, 507.16967; found, 507.16943.

4.5.16. 4-((6,7-Dimethoxyquinolin-4-yl)amino)-N-(4-sulfamoylphenethyl)benzamide (10h). White powder. Yield: 69%. 1H NMR (400 MHz, DMSO- d_6): δ 8.91 (s, 1H), 8.49 (t, J = 5.4 Hz, 1H), 8.36 (d, J = 5.2 Hz, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.62 (s, 1H), 7.44 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H), 7.29 (s, 2H), 7.28 (s, 1H), 7.03 (d, J = 5.2 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.52 (dd, J = 12.7, 6.8 Hz, 2H), 2.94 (t, J = 7.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 165.86, 151.87, 148.50, 148.28, 146.11, 145.11, 144.28, 143.93, 142.06, 129.21, 128.60, 128.03, 125.76, 119.55, 114.82, 108.28, 102.98, 101.04, 55.93, 55.60, 40.46, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 34.93. HRMS-ESI calcd for $C_{26}H_{27}N_4O_5S$ $[M + H]^+$, 507.16967; found, 507.16931.

4.6. Kinase Assays

PDGFRA, FLT3-ITD, and KIT were purchased from ProQinase; Abl was produced in Sf9 insect cells and purified on a NiNTA column (Qiagen). PDGFRA, FLT3-ITD, and KIT were assayed with peptide substrate (AGLT (poly(Ala, Glu, Lys, Tyr) (6:2:5:1) hydrobromide for PDGFRA and FLT3-ITD or GGMEDIYFEFMGGKKK for KIT) in the presence of 1 μ M ATP, 0.05 μ Ci $[\gamma^{33}P]$ ATP, and the test compound in a final volume of 10 μ L, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μ M Na-orthovanadate, 1.2 mM DTT, 2.5 μ g/50 μ L PEG20,000). Abl was assayed with 500 μ M concentration of a synthetic peptide (GGEAIYAAPFKK), 10 μ M $[\gamma^{33}P]$ ATP, and the appropriate quantity of the test compound in a final volume of 10 μ L, all in a reaction buffer (25 mM Tris, pH 7.5, 5 mM $MgCl_2$, 0.5 mM EGTA, 1 mM DTT, 0.01% Brij35). The reactions were stopped by adding 5 μ L of 3% aq. H_3PO_4 . Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3 $\times$ with 0.5% aq. H_3PO_4 , and finally air-dried. Kinase inhibition was quantified using an FLA-7000 digital image analyzer.

4.7. Kinome Profiling

Protein kinase selectivity of **9d** was evaluated at a single concentration (1 μ M) by screening against 70 human receptor tyrosine kinases (59 wild-type and 11 mutant variants) at Eurofins Discovery.

4.8. Carbonic Anhydrase Inhibition Assay

The experimental procedures for the conducted carbonic anhydrase stopped-flow assay were executed as previously described.^{62–64}

4.9. $CoCl_2$ -Induced Hypoxia and Analysis of CA IX Expression

MV4–11 and EOL-1 cells were treated with cobalt chloride ($CoCl_2$) to induce hypoxia-mimicking conditions. Cells were exposed to $CoCl_2$ for 24 or 48 h, after which protein expression levels were analyzed by immunoblotting. Carbonic anhydrase IX (CA IX) was detected using an anti-CA IX antibody (clone HL1410, Invitrogen), and hypoxia-inducible factor 1 α (HIF-1 α) was detected using an anti-HIF-1 α antibody (clone S4, BD Transduction Laboratories). β -Actin was detected using an anti-actin antibody (clone C4, Santa

Cruz Biotechnology) and served as a loading control. During CA IX detection, an additional uncharacterized band was observed, consistent with information provided in the antibody datasheet.

4.10. Cell Cultures and Antiproliferative Assay

Human cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (EOL-1, THP-1, RS4-11, Kasumi-1), the Cell Lines Service (MV4-11), and the European Collection of Authenticated Cell Cultures (HL60, K562, HEK293T) and were cultivated according to the providers' instructions. For antiproliferative assays, cells were seeded at a density of 20,000 cells per well (except for K562 and HEK293, which were seeded at a density of 5,000 cells per well) and the next day were treated with 6 concentrations of test compounds. After the 72 h incubation period, 20 μ L of resazurin (Merck; final concentration 10 μ g/mL) solution was added for 4 h. Fluorescence of resorufin corresponding to live cells was measured at 560/590 nm (excitation/emission) using Infinite F Plex (Tecan).

4.11. Immunoblotting

Cell lysates were separated on SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. After 1 h blocking in 4% BSA in TBS with Tween 20, overnight incubation with specific primary antibodies, and 1 h incubation with peroxidase-conjugated secondary antibodies, peroxidase activity was detected with Super Signal West Pico reagents (Thermo Scientific) using a CCD camera LAS-4000 (Fujifilm). The following specific antibodies were purchased from Cell Signaling: anti-PDGFR α (D1E1E), antiphospho-PDGFR α / β Y849/857 (C43E9), anti-STAT3 (79D7), antiphospho-STAT3 Y705 (D3A7), anti-Akt (C67E7), antiphospho-Akt S473 (D9E), anti-ERK1/2, anti-phospho-ERK1/2 T202/Y204, anti-PARP-1 (46D11), anti-Mcl-1 (D3SA5), anti-caspase-9, and anti-HSP70. Anti-Bcl-2 was purchased from Sigma-Aldrich, anti-caspase-3 (31A1067) was purchased from Santa Cruz Biotechnology, and anti-PCNA (clone PC-10) was generously gifted by Dr. B. Vojtěšek (Masaryk Memorial Cancer Institute, Brno).

4.12. Caspase Assay

Caspase3/7 activity was measured according to a previously published procedure.⁶⁵ Cells (50,000 cells per well of EOL-1 and 20,000 cells per well of THP-1) were cultivated in a 96-well plate and treated with compounds for 24 h (total volume 100 μ L per well). After incubation, 50 μ L of caspase3/7 assay buffer (150 mM HEPES pH 7.4, 450 mM NaCl, 150 mM KCl, 30 mM MgCl₂, 1.2 mM EGTA, 1.5% Nonidet P40, 0.3% CHAPS, 30% sucrose, 30 mM DTT, 3 mM PMSF) containing 150 μ M peptide substrate Ac-DEVD-AMC (Enzo Life Sciences) was added. The caspase3/7 activity was measured after 4 h using Infinite F Plex (Tecan) at 380 nm/430 nm (ex/em). Caspase activity assays were performed in two independent biological experiments, each performed in duplicate. Data are presented as mean $\pm$ SD. Statistical significance was assessed using one-way ANOVA, followed by Dunnett's multiple-comparison test, comparing each concentration to the untreated control.

4.13. EOL-1 Cells Annexin V/Propidium Iodide Staining

EOL-1 cells were seeded at a density of 4.0×10^5 cells/mL and treated with increasing concentrations of compound **9d** for 24 h. As controls, cells were treated with 10% ethanol for 1 h or camptothecin (5 μ M) for 4 h. Following treatment, 1.0×10^6

cells were collected, washed twice with ice-cold phosphate-buffered saline (PBS), and resuspended in 100 μ L of binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂). Propidium iodide (10 μ g/mL; Sigma-Aldrich) and Annexin V (BD Biosciences) were added according to the manufacturer's instructions. Samples were incubated for 30 min in the dark and subsequently analyzed by flow cytometry using a BD FACSVerse instrument with BD FACSuite software (version 1.0.6).

4.14. Cell Cycle Analysis

EOL-1 cells were seeded at a density of 0.4 /mL. After a preincubation period, the cells were treated with the tested compounds for 24 h. After staining with propidium iodide (final concentration of 10 μ g/mL), DNA content was analyzed by flow cytometry (BD FACSVerse with BD FACSuite software, version 1.0.6).

4.15. Molecular Modeling

To explore the molecular binding characteristics of compound **9d**, an integrated in silico protocol combining molecular docking and molecular dynamics (MD) simulations was implemented. Docking studies were performed using the Schrödinger Small Molecule Drug Discovery Suite (v2024-2), while the MD simulations were conducted with GROMACS⁶⁶ 2023.3. For docking studies, high-resolution crystal structures of PDGFRA (PDB ID: 6JOL) and carbonic anhydrase IX (CA IX, PDB ID: 5FL4) were retrieved from the Protein Data Bank. Proteins were prepared by using the Protein Preparation Wizard module in Maestro, including hydrogen addition, bond order assignment, protonation state optimization, and restrained energy minimization with the OPLS4 force field. Ligands were generated using LigPrep at physiological pH (7.0 ± 0.5), and docking was performed using the Glide SP mode.⁶⁷ The best-ranked poses were selected based on docking score and interaction pattern consistency. The accuracy of the docking protocol was evaluated by redocking the cocrystallized reference ligands into their respective binding pockets. The RMSD between docked and crystallographic poses was found to be 0.59 Å for PDGFRA (6JOL) and 0.22 Å for CA IX (5FL4), indicating excellent agreement with experimental geometries and validating the reliability of the docking approach.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jmedchem.5c03037>.

2D docking and molecular dynamics simulation results, NMR spectra, ESI-MS data, and HPLC purity chromatographs (PDF)

Molecular formula strings (CSV)

Molecular simulation video (MP4)

Molecular simulation video (MP4)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PDGFRA, platelet-derived growth factor receptor alpha; CA, carbonic anhydrase; CA I/II/IX/XII, carbonic anhydrase isoforms I, II, IX, and XII; AAZ, acetazolamide; AKT, protein kinase B; DFG, Asp–Phe–Gly motif; ERK, extracellular signal-regulated kinase; FLT3, Fms-like tyrosine kinase 3; FLT3-ITD, Fms-like tyrosine kinase 3 internal tandem duplication; GI₅₀, concentration required to inhibit cell growth by 50%; HIF-1 α , hypoxia-inducible factor-1 alpha; IC₅₀, half-maximal inhibitory concentration; K_i, inhibition constant; KIT, stem cell factor receptor; MAPK, mitogen-activated protein kinase; MD, molecular dynamics; PARP-1, poly(ADP-ribose) polymerase-1; PI3K, phosphoinositide 3-kinase; rmsd, root-mean-square deviation; SAR, structure–activity relationship; STAT3, signal transducer and activator of transcription 3

REFERENCES

- (1) Deschler, B.; Lübbert, M. Acute myeloid leukemia: Epidemiology and etiology. *Cancer* **2006**, *107*, 2099–2107.
- (2) Chen, J.; Pang, W.; Deng, M.; Zheng, R.; Chen, Y.; Zhang, Z.; Tan, Z.; Bai, Z. Global, regional, and national burden of leukemia, 1990–2021: a systematic analysis of the global burden of disease in 2021. *Front Med.* **2025**, *12*, 1542317.
- (3) International Agency for Research on Cancer (IARC). Global Cancer Observatory: Cancer Today, 2022; <https://gco.iarc.fr/today/>. (accessed 20 Jan, 2026)
- (4) Daltveit, D. S.; et al. Global patterns of leukemia by subtype, age, and sex in 185 countries in 2022. *Leukemia* **2025**, *39*, 412–419.
- (5) Steliarova-Foucher, E.; et al. International incidence of childhood cancer, 2001–10: a population-based registry study. *Lancet Oncol.* **2017**, *18*, 719–731.
- (6) Gómez-De León, A.; Demichelis-Gómez, R.; da Costa-Neto, A.; Gómez-Almaguer, D.; Rego, E. M. Acute myeloid leukemia: challenges for diagnosis and treatment in Latin America. *Hematology* **2023**, *28*, 2158015.
- (7) Short, N. J.; et al. Advances in the Treatment of Acute Myeloid Leukemia: New Drugs and New Challenges. *Cancer Discov* **2020**, *10*, S06–S25.
- (8) Ossenkoppele, G.; Montesinos, P. Challenges in the diagnosis and treatment of secondary acute myeloid leukemia. *Crit Rev. Oncol Hematol* **2019**, *138*, 6–13.
- (9) Zhang, J.; Yang, P. L.; Gray, N. S. Targeting cancer with small molecule kinase inhibitors. *Nat. Rev. Cancer* **2009**, *9*, 28–39.
- (10) Fleuren, E. D. G.; Zhang, L.; Wu, J.; Daly, R. J. The kinome ‘at large’ in cancer. *Nat. Rev. Cancer* **2016**, *16*, 83–98.
- (11) Bhanumathy, K.; Balagopal, A.; Vizeacoumar, F. S.; Vizeacoumar, F. J.; Freywald, A.; Giambra, V. Protein Tyrosine Kinases: Their Roles and Their Targeting in Leukemia. *Cancers* **2021**, *13*, 184.
- (12) Masson, K.; Rönnstrand, L. Oncogenic signaling from the hematopoietic growth factor receptors c-Kit and Flt3. *Cell Signal* **2009**, *21*, 1717–1726.
- (13) Verhoeft, K.; Cools, J. The unexpected and unresolved roles of PDGFRA and PDGFRB in T-cell acute lymphoblastic leukemia. *Haematologica* **2020**, *109*, 3461–3462.
- (14) Cruz-Rodriguez, N.; Deininger, M. W. Novel treatment strategies for chronic myeloid leukemia. *Blood* **2025**, *145*, 931–943.

- (15) Alves, R.; et al. Resistance to Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia—From Molecular Mechanisms to Clinical Relevance. *Cancers* **2021**, *13*, 4820.
- (16) Bixby, D.; Talpaz, M. Mechanisms of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategies to overcome resistance. *Hematology* **2009**, *2009*, 461–476.
- (17) Bavaro, L.; Martelli, M.; Cavo, M.; Soverini, S. Mechanisms of Disease Progression and Resistance to Tyrosine Kinase Inhibitor Therapy in Chronic Myeloid Leukemia: An Update. *Int. J. Mol. Sci.* **2019**, *20*, 6141.
- (18) Li, Y.; Zhao, L.; Li, X.-F. Hypoxia and the Tumor Microenvironment. *Technol. Cancer Res. Treat.* **2021**, *20*, 15330338211036304.
- (19) Sakitani, M. Induction of Cancer Stem Cells in Tumor Microenvironment and Involvement of HIFs And Carbonic Anhydrase IX in Oncogenesis of Solid Tumors and Hematological Malignancies Including Adult T-Cell Leukemia/Lymphoma (ATL). *Int. J. Res. Med. Clin. Sci.* **2024**, *2*, 52–76.
- (20) Pastorekova, S.; Gillies, R. J. The role of carbonic anhydrase IX in cancer development: links to hypoxia, acidosis, and beyond. *Cancer Metastasis Rev.* **2019**, *38*, 65–77.
- (21) Fares, M.; et al. Discovery of Potent Dual-Tailed Benzenesulfonamide Inhibitors of Human Carbonic Anhydrases Implicated in Glaucoma and in Vivo Profiling of Their Intraocular Pressure-Lowering Action. *J. Med. Chem.* **2020**, *63*, 3317–3326.
- (22) Tawfik, H. O.; et al. Discovery of Novel Pyridazine-Tethered Sulfonamides as Carbonic Anhydrase II Inhibitors for the Management of Glaucoma. *J. Med. Chem.* **2024**, *67*, 1611–1623.
- (23) Zeidan, M. A.; et al. Shooting an Arrow against Convulsion: Novel Triazole-Grafted Benzenesulfonamide Derivatives as Carbonic Anhydrase II and VII Inhibitors. *J. Med. Chem.* **2025**, *68*, 8873–8893.
- (24) Fadaly, W. A. A.; et al. Novel benzenesulfonamide derivatives linked to diaryl pyrazole tail as potential carbonic anhydrase II/VII inhibitors with anti-epileptic activity. *Eur. J. Med. Chem.* **2025**, *291*, 117619.
- (25) Eldehna, W. M.; et al. Development of isatin-functionalized benzenesulfonamides as novel carbonic anhydrase II and VII inhibitors with antiepileptic potential. *Eur. J. Med. Chem.* **2025**, *292*, 117706.
- (26) Badawi, W. A.; Rashed, M.; Nocentini, A.; Bonardi, A.; Abd-Alhaseeb, M. M.; Al-Rashood, S. T.; Veerakanellore, G. B.; Majrashi, T. A.; Elkaeed, E. B.; Elgendy, B.; et al. Identification of new 4-(6-oxopyridazin-1-yl)benzenesulfonamides as multi-target anti-inflammatory agents targeting carbonic anhydrase, COX-2 and 5-LOX enzymes: synthesis, biological evaluations and modelling insights. *J. Enzyme Inhib. Med. Chem.* **2023**, *38*, 2201407.
- (27) Ragab, M. A.; et al. 4-(5-Amino-pyrazol-1-yl)-benzenesulfonamide derivatives as novel multi-target anti-inflammatory agents endowed with inhibitory activity against COX-2, 5-LOX and carbonic anhydrase: Design, synthesis, and biological assessments. *Eur. J. Med. Chem.* **2023**, *250*, 115180.
- (28) Chen, F.; et al. Pharmacological inhibition of Carbonic Anhydrase IX and XII to enhance targeting of acute myeloid leukaemia cells under hypoxic conditions. *J. Cell. Mol. Med.* **2021**, *25*, 11039–11052.
- (29) Nasu, K.; Yamaguchi, K.; Takanashi, Y.; Sugamura, K.; Harigae, H. Carbonic Anhydrase IX Promotes the Tumorigenicity of Adult T-Cell Leukemia/Lymphoma-Derived Cells in NOG Mice. *Blood* **2015**, *126*, 4822.
- (30) Kalinin, S.; et al. Carbonic Anhydrase IX Inhibitors as Candidates for Combination Therapy of Solid Tumors. *Int. J. Mol. Sci.* **2021**, *22*, 13405.
- (31) Zheng, G.; et al. Identification of carbonic anhydrase 9 as a contributor to pingyangmycin-induced drug resistance in human tongue cancer cells. *FEBS J.* **2010**, *277*, 4506–4518.
- (32) El-Attar, M. A. Z.; et al. Click-modifiable isatin hydrazones as COX-2, VEGFR-2, and carbonic anhydrase inhibitors: A multi-target approach to cancer therapy. *Eur. J. Med. Chem.* **2026**, *302*, 118304.
- (33) Hefny, S. M.; et al. Discovery and Mechanistic Studies of Dual-Target Hits for Carbonic Anhydrase IX and VEGFR-2 as Potential Agents for Solid Tumors: X-ray, *In Vitro*, *In Vivo*, and *In Silico* Investigations of Coumarin-Based Thiazoles. *J. Med. Chem.* **2024**, *67*, 7406–7430.
- (34) Eldehna, W. M.; et al. Discovery of new sulfonamide-tethered 2-aryl-4-anilinoquinazolines as the first-in-class dual carbonic anhydrase and EGFR inhibitors. *Int. J. Biol. Macromol.* **2024**, *279*, 135010.
- (35) Elkotamy, M. S.; et al. Development of pyrazolo[1,5-a]pyrimidine-grafted coumarins as selective carbonic anhydrase inhibitors and tubulin polymerization inhibitors with potent anticancer activity. *Int. J. Biol. Macromol.* **2025**, *303*, 140462.
- (36) Farooqi, A. A.; Siddik, Z. H. Platelet-derived growth factor (PDGF) signalling in cancer: rapidly emerging signalling landscape. *Cell Biochem. Funct.* **2015**, *33*, 257–265.
- (37) Kabir, A.; Muth, A. Polypharmacology: The science of multi-targeting molecules. *Pharmacol. Res.* **2022**, *176*, 106055.
- (38) Ravikumar, B.; Aittokallio, T. Improving the efficacy-safety balance of polypharmacology in multi-target drug discovery. *Expert Opin. Drug Discov.* **2018**, *13*, 179–192.
- (39) Plé, P. A.; et al. Discovery of new quinoline ether inhibitors with high affinity and selectivity for PDGFR tyrosine kinases. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3050–3055.
- (40) Plé, P. A.; et al. Discovery of AZD2932, a new Quinazoline Ether Inhibitor with high affinity for VEGFR-2 and PDGFR tyrosine kinases. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 262–266.
- (41) Mathias, T. J.; et al. The FLT3 and PDGFR inhibitor crenolanib is a substrate of the multidrug resistance protein ABCB1 but does not inhibit transport function at pharmacologically relevant concentrations. *Invest. New Drugs* **2015**, *33*, 300–309.
- (42) Roberts, W. G.; et al. Antiangiogenic and Antitumor Activity of a Selective PDGFR Tyrosine Kinase Inhibitor, CP-673,451. *Cancer Res.* **2005**, *65*, 957–966.
- (43) Bozdag, M.; et al. Discovery of 4-Hydroxy-3-(3-(phenylureido)benzenesulfonamides as SLC-0111 Analogues for the Treatment of Hypoxic Tumors Overexpressing Carbonic Anhydrase IX. *J. Med. Chem.* **2018**, *61*, 6328–6338.
- (44) Elswawi, A. E.; et al. 1,5-Diaryl-1,2,4-triazole Ureas as New SLC-0111 Analogues Endowed with Dual Carbonic Anhydrase and VEGFR-2 Inhibitory Activities. *J. Med. Chem.* **2023**, *66*, 10558–10578.
- (45) Shaldam, M.; et al. Development of Novel Quinoline-Based Sulfonamides as Selective Cancer-Associated Carbonic Anhydrase Isoform IX Inhibitors. *Int. J. Mol. Sci.* **2021**, *22*, 11119.
- (46) Chen, K.-F.; et al. Sorafenib derivatives induce apoptosis through inhibition of STAT3 independent of Raf. *Eur. J. Med. Chem.* **2011**, *46*, 2845–2851.
- (47) Wilhelm, S. M.; et al. Regorafenib (BAY 73–4506): A new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int. J. Cancer* **2011**, *129*, 245–255.
- (48) Manley, P. W.; et al. Imatinib: a selective tyrosine kinase inhibitor. *Eur. J. Cancer* **2002**, *38*, S19–S27.
- (49) De Luca, A.; Normanno, N. Tivozanib, a pan-VEGFR tyrosine kinase inhibitor for the potential treatment of solid tumors. *IDrugs* **2010**, *13*, 636.
- (50) Yamamoto, Y.; et al. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. *Vasc. Cell* **2014**, *6*, 18.
- (51) Faivre, S.; Demetri, G.; Sargent, W.; Raymond, E. Molecular basis for sunitinib efficacy and future clinical development. *Nat. Rev. Drug Discov* **2007**, *6*, 734–745.
- (52) Papaetis, G. S.; Syrigos, K. N. Sunitinib. *BioDrugs* **2009**, *23*, 377–389.
- (53) Metz, K. S.; et al. Coral: Clear and Customizable Visualization of Human Kinome Data. *Cell Syst.* **2018**, *7*, 347–350.

- (54) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The Protein Kinase Complement of the Human Genome. *Science* **2002**, *298*, 1912–1934.
- (55) Takeuchi, A.; et al. Successful treatment with ABL tyrosine kinase inhibitor for patients with acute myeloid leukemia with BCR-ABL1. *Leuk. Res. Rep.* **2021**, *15*, 100233.
- (56) Borogovac, A.; Sahu, K. K.; Viswanathan, G. K.; Miron, P. M.; Cerny, J. A Case of Acute Myeloid Leukemia Harboring a Rare Three-Way Translocation t(5;7;7) Involving the PDGFRB Gene and Successfully Treated with Imatinib. *Cancer Manage. Res.* **2021**, *13*, 8841–8847.
- (57) Skopek, R.; et al. Choosing the Right Cell Line for Acute Myeloid Leukemia (AML) Research. *Int. J. Mol. Sci.* **2023**, *24*, 5377.
- (58) Cools, J.; et al. The EOL-1 cell line as an in vitro model for the study of FIP1L1-PDGFR α -positive chronic eosinophilic leukemia. *Blood* **2004**, *103*, 2802–2805.
- (59) Quentmeier, H.; Reinhardt, J.; Zaborski, M.; Drexler, H. G. FLT3 mutations in acute myeloid leukemia cell lines. *Leukemia* **2003**, *17*, 120–124.
- (60) Campbell, J.; et al. Large-Scale Profiling of Kinase Dependencies in Cancer Cell Lines. *Cell Rep.* **2016**, *14*, 2490–2501.
- (61) Tan, E.; Chin, C. S. H.; Lim, Z. F. S.; Ng, S. K. HEK293 Cell Line as a Platform to Produce Recombinant Proteins and Viral Vectors. *Front. Bioeng. Biotechnol.* **2021**, *9*, 796991.
- (62) Eldehna, W. M.; et al. Development of isatin-functionalized benzenesulfonamides as novel carbonic anhydrase II and VII inhibitors with antiepileptic potential. *Eur. J. Med. Chem.* **2025**, *292*, 117706.
- (63) Eldehna, W. M.; et al. 4-(Pirazolyl)benzenesulfonamide Ureas as Carbonic Anhydrases Inhibitors and Hypoxia-Mediated Chemo-Sensitizing Agents in Colorectal Cancer Cells. *J. Med. Chem.* **2024**, *67*, 20438–20454.
- (64) Saied, S.; et al. Discovery of indolinone-bearing benzenesulfonamides as new dual carbonic anhydrase and VEGFR-2 inhibitors possessing anticancer and pro-apoptotic properties. *Eur. J. Med. Chem.* **2023**, *259*, 115707.
- (65) Carrasco, R. A.; Stamm, N. B.; Patel, B. K. R. One-Step Cellular Caspase-3/7 Assay. *BioTechniques* **2003**, *34*, 1064–1067.
- (66) Abraham, M. J.; et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, *1–2*, 19–25.
- (67) Friesner, R. A.; et al. Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem.* **2004**, *47*, 1739–1749.