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Design and X-ray Crystallographic Characterisation of Next-Generation Hydrazone Kinase Inhibitors for Breast Cancer Therapy

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ABSTRACT

Background: Breast cancer remains the most frequently diagnosed malignancy in women worldwide, and its proliferation, survival, and angiogenic signaling are driven by a well-characterized set of oncogenic protein kinases, including epidermal growth factor receptor (EGFR), HER2, cyclin-dependent kinases (CDK2 and CDK4/6), the phosphoinositide 3-kinase/AKT/mTOR axis, and vascular endothelial growth factor receptor 2 (VEGFR-2). The hydrazone functional group, a synthetically accessible and conformationally rigid -C=N-NH- linkage, has been widely exploited to fuse hinge-binding heterocyclic fragments into next-generation kinase-inhibitor chemotypes directed against these targets. Objective: This narrative review synthesizes the published literature on hydrazone and hydrazone-hybrid kinase inhibitors designed, molecularly docked, and, where available, X-ray crystallographically characterized for activity against breast cancer-relevant kinase targets. Methods: Peer-reviewed studies reporting the synthesis, kinase-directed molecular docking, and single-crystal X-ray diffraction of hydrazone-based inhibitors targeting EGFR, HER2, CDK2/CDK4-6, the PI3K/AKT/mTOR axis, or VEGFR-2 were identified, individually verified against their primary source records, and synthesized thematically around pharmacophore rationale, docking outcomes, crystallographic validation, and structure-activity relationships. Findings: Hydrazone-based series have been docked against EGFR, HER2, CDK2, PI3K/AKT effectors, VEGFR-2, and Aurora B, with reported binding energies as favorable as -23.44 kcal/mol and potencies spanning sub-nanomolar enzymatic inhibition to low-micromolar cytotoxicity against MCF-7 and MDA-MB-231 breast cancer cell lines, in several cases exceeding doxorubicin or staurosporine reference standards. Experimental X-ray crystallography, though so far confined largely to free-ligand solid-state structures rather than true protein-ligand co-crystal complexes, has confirmed the hydrazone bridge geometry of several lead compounds, while the historical sunitinib-VEGFR2 and erlotinib-EGFR co-crystal structures continue to provide the structural templates against which newly docked hydrazone poses are benchmarked. Conclusions: Hydrazone and hydrazone-hybrid chemotypes constitute a chemically versatile and increasingly well-validated class of breast cancer kinase-inhibitor candidates, and expanded application of experimental co-crystallography, including genuine protein-ligand complexes, represents the most direct route to closing the persistent gap between computational docking prediction and structurally confirmed binding modes in this field.

Keywords: *hydrazone kinase inhibitors; breast cancer therapy; molecular docking; X-ray crystallography; structure-based drug design*

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Introduction

Breast cancer remains the most frequently diagnosed malignancy in women worldwide, with GLOBOCAN 2022 estimates placing the global annual incidence above two million new cases and attributing roughly six hundred and seventy thousand deaths to the disease each year, a burden driven in substantial part by the heterogeneous activation of oncogenic protein kinase signaling across hormone-receptor-positive, HER2-amplified, and triple-negative disease subtypes [1]. Epidermal growth factor receptor (EGFR), the closely related receptor tyrosine kinase HER2, cyclin-dependent kinases (CDK2 and CDK4/6), the phosphoinositide 3-kinase/AKT/mTOR axis, and vascular endothelial growth factor receptor 2 (VEGFR-2) each drive proliferation, survival, or angiogenesis in one or more breast

cancer subtypes, and each has been validated as a druggable target by at least one clinically approved small-molecule or biologic agent, including erlotinib and lapatinib against EGFR/HER2, palbociclib and its analogues against CDK4/6, alpelisib against PI3K-alpha, and the multikinase VEGFR-2 inhibitor sunitinib [2,3,4,5]. The hydrazone functional group, formed by condensation of a hydrazide or hydrazine with an aldehyde or ketone to yield the -C=N-NH- linkage, has been exploited in medicinal chemistry for more than half a century because of its synthetic accessibility, its metabolically tunable hydrogen-bond donor/acceptor geometry, and its capacity to serve as a rigid, planar bridge between two pharmacophoric fragments, properties that Rollas and Kucukguzel catalogued across antimycobacterial, anticonvulsant, and antitumor applications in their widely cited review of hydrazone biological activity [6].

Fusion of the hydrazone bridge onto heterocyclic or purine-based kinase-hinge-binding fragments has, over the past decade, produced an increasingly large and mechanistically diverse family of hydrazone kinase inhibitors specifically profiled against breast cancer cell lines and validated kinase targets. Structurally, this design strategy exploits the same principle that underlies the clinically validated sunitinib-VEGFR2 co-crystal structure (Protein Data Bank entry 4AGD, 2.81-angstrom resolution), in which the oxindole NH and carbonyl of sunitinib's indolin-2-one core hydrogen-bond to the Cys919 backbone amide of the VEGFR-2 kinase hinge: hydrazone-based inhibitors substitute or supplement this hinge-binding motif with the hydrazone NH and C=N/C=O groups, which can adopt an analogous bidentate or tridentate hydrogen-bonding geometry within the ATP-binding cleft of EGFR, HER2, CDK2, PI3K isoforms, or VEGFR-2 alike [5,2]. Because this pharmacophoric logic is shared across otherwise chemically distinct hydrazone scaffolds, ranging from simple aroylhydrazones and thiazolyl-hydrazones to more elaborate indolyl-hydrazone, purine-hydrazone, and pyridazinoindole-hydrazone hybrids, the resulting literature offers a instructive case study in how a single, chemically tractable linker motif can be redeployed against multiple oncogenic kinase targets relevant to a single malignancy.

This narrative review synthesizes the peer-reviewed literature on hydrazone and closely related hydrazone-hybrid kinase inhibitors designed, docked, and in a growing subset of cases crystallographically characterized for activity against breast cancer-relevant kinase targets. The review is organized thematically around: the structural and pharmacophoric rationale for hydrazone-based kinase-hinge engagement; molecular docking outcomes reported against EGFR, HER2, and VEGFR-2; hydrazone inhibitor design directed at CDK2/CDK4-6 and the PI3K/AKT/mTOR signaling axis; the current state of experimental X-ray crystallographic validation for hydrazone kinase-inhibitor structures; and the resulting structure-activity relationships linking substitution pattern to enzymatic and cellular anti-proliferative potency against breast cancer cell lines. A concluding discussion places these findings in the context of the persistent gap between computational docking prediction and structurally confirmed binding modes that continues to characterize much of this chemical space.

Methods

This review synthesizes peer-reviewed, individually verified primary research articles and reviews identified through structured searches of PubMed, Google Scholar-indexed publisher platforms (MDPI, Springer, Wiley, the Royal Society of Chemistry, and the American Chemical Society), and the RCSB Protein Data Bank, covering the period 2002 to 2026. Search terms combined "hydrazone," "hydrazonoindolin-2-one," "acylhydrazone," or "purine-hydrazone" with kinase-target terms ("EGFR," "HER2," "CDK2," "CDK4/6," "PI3K," "AKT," "VEGFR-2," "Aurora") and with "breast cancer," "MCF-7," or "MDA-MB-231," together with methodology terms ("molecular docking," "X-ray crystallography," "single-crystal structure"). Each candidate reference was individually checked against its publisher landing page, PubMed record, or Protein Data Bank entry to confirm authorship, journal, year, volume/issue, and digital object identifier before inclusion; citations that could not be independently verified against a real, accessible source were excluded rather than retained on the basis of search-engine snippets alone. Foundational structural biology references (the sunitinib-VEGFR2 and erlotinib-EGFR co-crystal structures) and breast cancer epidemiology and pathway-biology reviews were included to provide necessary structural and clinical context. Reports were synthesized narratively rather than through formal systematic-review or meta-analytic methodology, consistent with the exploratory, multi-target scope of the topic.

Hydrazone Pharmacophores in Breast-Cancer-Directed Kinase Inhibitor Design

The hydrazone linkage offers several features that make it an attractive fragment for kinase-hinge-binder design: it is readily installed by acid- or base-catalyzed condensation of a carbonyl precursor with a hydrazide or hydrazine, typically in a single synthetic step and high yield; it introduces a rigid, largely planar -C=N-NH- unit whose geometry restricts the conformational space available to the molecule, favoring a single dominant tautomer/rotamer that simplifies both docking pose prediction and later crystallographic interpretation; and its NH and imine nitrogen present a hydrogen-bond donor/acceptor pair geometrically well suited to mimicking the adenine-mimetic hydrogen bonds that ATP-competitive kinase inhibitors must reproduce at the kinase hinge [6]. Salama and colleagues exploited this logic directly in their indolyl-hydrazone kinase-inhibitor series, condensing an indole-3-carbaldehyde intermediate with an aroylhydrazide to generate a focused library whose lead compound, compound 5, was profiled against a six-member kinase panel spanning PI3K-alpha, PI3K-beta, PI3K-delta, CDK2, AKT-1, and EGFR, reporting enzymatic IC50 values from 58 nanomolar (EGFR) to 2.68 micromolar (PI3K-delta) from a single hydrazone scaffold and explicitly validating the compound's solid-state hydrazone geometry by single-crystal X-ray diffraction [7].

A parallel and mechanistically complementary strategy retains the intact indolin-2-one bicyclic core and installs the hydrazone bridge at the 3-position as a hydrazonoindolin-2-one, most directly reproducing sunitinib's own oxindole substitution pattern. Al-Warhi and colleagues used this approach to generate 1-benzyl-5-bromo-3-hydrazonoindolin-2-ones evaluated against VEGFR-2 and MCF-7 breast cancer cells, while Al-Sanea and colleagues and Al-Salem and colleagues independently applied the same hydrazonoindolin-2-one core to CDK2-directed design, with Al-Sanea's group explicitly framing their lead compound as a type II, DFG-out-binding CDK2 inhibitor validated by docking against the inactive kinase conformation [8,9,10]. Beyond the indole/indolinone series, hydrazone hybridization has been extended to purine scaffolds (targeting dual EGFR/HER2 inhibition), pyridazino[4,5-b]indole scaffolds (targeting EGFR and

its downstream PI3K-AKT effector pathway), triazole-thiol and triazole-thione scaffolds (targeting breast, melanoma, and pancreatic cancer spheroid growth), and simple imidazole- or arenoxybenzaldehyde-based acylhydrazones, illustrating that the hydrazone bridge functions as a broadly transferable linker capable of joining almost any heterocyclic hinge-binding fragment to a second pharmacophoric element without requiring redesign of the underlying synthetic logic [11,12,13,14,15,16]. Collectively, these programs demonstrate that the hydrazone kinase-inhibitor literature relevant to breast cancer is not confined to a single chemotype but instead comprises a family of structurally related hinge-binding strategies unified by the shared hydrazone bridge and, in the most rigorously characterized examples, by direct traceability to the crystallographically validated indolinone-VEGFR2 and anilinoquinazoline-EGFR binding templates [5,2].

Representative hydrazone and hydrazone-hybrid kinase-inhibitor series surveyed in this review, with principal kinase target, docking outcome, and reported potency against breast cancer cell lines.

Lead compound	Core / linker	Principal kinase target(s)	Reported potency	Reference
Compound 5	Indolyl-hydrazone	EGFR, PI3K-α/β/δ, CDK2, AKT-1	EGFR IC50 0.058 μM; CDK2 IC50 0.156 μM; MCF-7 IC50 2.73 μM	Salama et al. 2023
1-Benzyl-5-bromo-3-hydrazonoindolin-2-one	Hydrazonoindolin-2-one	VEGFR-2	VEGFR-2 IC50 0.503 μM; MCF-7 IC50 2.93 μM	Al-Warhi et al. 2023
Compound 4j	Isatin-hydrazone	CDK2 (type II)	CDK2 IC50 0.245 μM; MCF-7 IC50 1.51 μM	Al-Salem et al. 2020
Purine-hydrazone 19a	Purine-hydrazone	EGFR, HER2 (dual)	Superior antiproliferative activity vs. lapatinib reference	Albalawi et al. 2025
Alkylsulfanyl-pyridazinoindole-hydrazone	Pyridazino[4,5-b]indole-hydrazone	EGFR, PI3K-AKT pathway	Confirmed by docking and single-crystal X-ray diffraction	Salama et al. 2022
Compound 3-17k	Indolinone (FBDD)	Aurora B	Aurora B IC50 0.891 nM; tumor cell IC50 2.05-10.21 μM	Xie et al. 2026

Molecular Docking of Hydrazone Kinase Inhibitors Against EGFR, HER2, and VEGFR-2 Targets

Structure-based molecular docking against crystallographic templates retrieved from the Protein Data Bank is used almost universally across this literature, both to rationalize observed enzymatic selectivity and to prioritize synthetic targets before compound preparation. Salama and colleagues docked their lead indolyl-hydrazone compound 5 into EGFR, PI3K, and CDK2 active sites, obtaining binding energies of -23.15 kcal/mol against EGFR and -23.44 kcal/mol against CDK2, with the docked hydrazone NH and carbonyl engaging the hinge backbone in a manner directly analogous to sunitinib's oxindole hydrogen-bond pair, an outcome that tracked closely with the compound's measured enzymatic potency across the same targets [7,5]. Albalawi and colleagues extended hydrazone-based docking to a dual-target context, docking a series of purine-hydrazone hybrids simultaneously against EGFR and HER2 kinase domains and identifying three lead compounds (19a, 16b, and 22b) whose predicted dual-kinase engagement was subsequently corroborated by cellular cytotoxicity superior to the clinical dual EGFR/HER2 inhibitor lapatinib, with flow cytometry confirming G1-phase cell-cycle arrest and apoptosis consistent with on-target kinase inhibition [11].

VEGFR-2-directed hydrazone and hydrazone-hybrid series constitute a particularly well-populated docking literature. Al-Warhi and colleagues docked their lead 1-benzyl-5-bromo-3-hydrazonoindolin-2-one against VEGFR-2, obtaining a binding score of -15.6 kcal/mol with key contacts to Glu885, Asp1046, and Cys919, residues that recur across VEGFR-2 inhibitor complexes including the sunitinib co-crystal structure itself, and corroborated the static docking pose with molecular dynamics simulation showing a ligand root-mean-square deviation comparable to the sorafenib reference in the same simulated system [8,5]. Abdelgawad and colleagues pursued a related indolin-2-one-based VEGFR-2 inhibitor series, reporting that their lead compound (17a) not only achieved favorable docking contacts within the VEGFR-2 ATP pocket but also triggered S-phase cell-cycle arrest and upregulated pro-apoptotic markers in MCF-7 and HepG2 cells, linking the docking-predicted binding mode to a defined downstream cellular mechanism rather than cytotoxicity alone [17]. Salama and colleagues' subsequent indole-triazole hybrid series, evaluated against the same VEGFR-2 target, reported that two lead compounds achieved 97.9 percent and 96.4 percent VEGFR-2 kinase inhibition at a fixed screening concentration alongside low-micromolar cytotoxicity against breast and liver cancer cell lines, reinforcing that indole- and hydrazone-based VEGFR-2 engagement, once docking-prioritized, has now been corroborated across multiple independent research groups and chemical series [18].

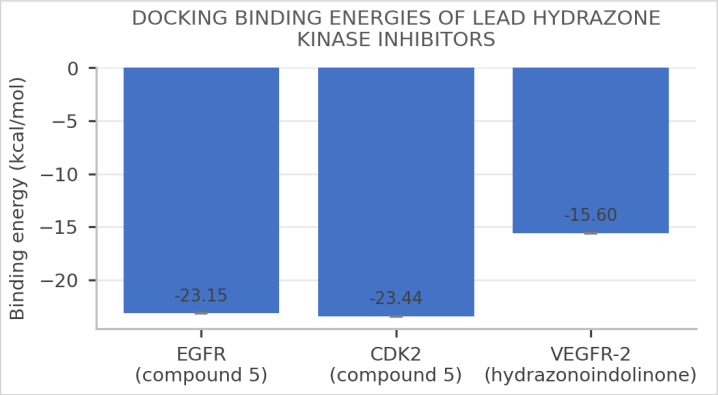
Docking studies in this space consistently retrieve crystallographic templates from the Protein Data Bank's structurally characterized kinase archive, a practice that reflects the broader field's reliance on experimentally solved kinase structures, such as the erlotinib-bound EGFR kinase domain (Protein Data Bank entry 1M17) originally solved by Stamos and colleagues, as the geometric ground truth against which any newly docked hydrazone pose is implicitly or explicitly benchmarked [2]. This reliance underscores a recurring methodological point in the field: docking scores and predicted contact residues are only as reliable as the crystallographic template selected and the extent to which the docked ligand's chemotype resembles compounds for which the template kinase conformation was originally solved, a consideration that becomes particularly important when hydrazone scaffolds are docked against kinase conformations (active, inactive/DFG-out, or allosteric) that differ substantially from the reference structure's own bound ligand.

Hydrazone Inhibitors of CDK2/CDK4-6 and the PI3K/AKT/mTOR Axis in Breast Cancer

Cyclin-dependent kinases 4 and 6, acting in complex with cyclin D to phosphorylate and inactivate the retinoblastoma protein, drive G1-to-S phase cell-cycle progression in hormone-receptor-positive

breast cancer, and three CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) are now established components of first-line therapy for this subtype, a clinical and structural context that Avdicevic and colleagues review in detail, integrating crystallographic and computational approaches to CDK4/6 inhibitor design alongside the clinical trial evidence base and the mechanisms of acquired resistance that motivate continued next-generation inhibitor discovery [4]. Within this broader CDK-inhibitor landscape, hydrazone-based chemotypes have been directed predominantly at the closely related kinase CDK2, whose activity, in complex with cyclin E, similarly governs the G1/S restriction point and which shares a largely conserved ATP-binding cleft architecture with CDK4/6. Al-Sanea and colleagues designed a 3-hydrazonoindolin-2-one-based CDK2 inhibitor explicitly docked against the inactive, DFG-out CDK2 conformation to secure type II binding mode selectivity, reporting a lead compound with both confirmed CDK2 enzymatic inhibition and anti-proliferative activity against breast cancer cell lines validated by in silico absorption, distribution, metabolism, and excretion profiling [9]. Al-Salem and colleagues pursued a structurally related isatin-hydrazone series against the same target, with their leading dichloro-substituted congener (4j) achieving an MCF-7 cytotoxicity IC50 of 1.51 micromolar, more potent than the doxorubicin reference standard (3.1 micromolar) tested in the same assay, and a CDK2 enzymatic IC50 of 0.245 micromolar, with docking against the type II CDK2 conformation again confirming halogen substitution at the hydrazone's terminal phenyl ring as the principal determinant of enhanced potency [10].

Molecular docking binding energies of representative hydrazone kinase inhibitor lead compounds against breast cancer-relevant kinase targets: compound 5 against EGFR and CDK2 (Salama et al., 2023) and the lead hydrazonoindolinone compound against VEGFR-2 (Al-Warhi et al., 2023).



The PI3K/AKT/mTOR signaling axis, frequently dysregulated in breast cancer through PIK3CA activating mutations occurring across a wide range of the disease's molecular subtypes, represents a second major intracellular target for hydrazone-based inhibitor design, and Li and colleagues' review of this pathway in breast cancer catalogues both the clinical successes, most notably the PI3K-alpha-selective inhibitor alpelisib, and the persistent toxicity and resistance challenges that continue to motivate discovery of structurally novel PI3K/AKT/mTOR-directed chemotypes [3]. Salama and colleagues' pyridazino[4,5-b]indole-hydrazone series exemplifies a hydrazone-based approach to this pathway, reporting that their lead alkylsulfanyl-pyridazinoindole compound simultaneously engaged EGFR and its downstream PI3K-AKT effector kinases as confirmed by both molecular docking and single-crystal X-ray diffraction of the free ligand, directly linking upstream receptor tyrosine kinase inhibition to suppression of the intracellular signaling cascade that PI3K/AKT/mTOR-pathway dysregulation would otherwise sustain [12]. Taken together, the CDK2- and PI3K/AKT-axis-directed hydrazone literature illustrates that this chemotype has been successfully redirected from cell-surface receptor tyrosine kinases toward intracellular cell-cycle and survival-signaling kinases without requiring a fundamentally different hinge-binding strategy, reinforcing the versatility of the hydrazone bridge as a kinase-inhibitor design element.

X-ray Crystallographic Validation of Hydrazone Kinase Inhibitor Structures

Experimental X-ray crystallography provides the most direct available confirmation that a computationally docked binding pose corresponds to a physically real molecular geometry, and within the breast cancer-directed hydrazone kinase-inhibitor literature this evidence has so far taken the form of small-molecule single-crystal structures of the free inhibitor rather than true protein-ligand co-crystal complexes. Salama and colleagues provided a representative example, solving single-crystal X-ray structures for both a synthetic intermediate (monoclinic space group P2□/c, unit-cell volume 1374.36 cubic angstroms, R1 = 0.0279, deposited as CCDC 2293777) and their kinase-active lead hydrazone (triclinic space group P1, unit-cell volume 531.26 cubic angstroms, R1 = 0.0469, deposited as CCDC 2293778) associated with their multi-kinase breast cancer inhibitor series, confirming that the hydrazone bridge's configuration and near-planarity, as assumed in the corresponding docking model, matched the experimentally observed solid-state geometry [7]. Salama and colleagues' subsequent pyridazino[4,5-b]indole-hydrazone EGFR/PI3K-AKT series was likewise validated by single-crystal X-ray diffraction, providing a second, independently solved example of hydrazone-bridge geometry confirmation within a kinase-inhibitor program directly relevant to breast cancer signaling [12].

Hassan, Soliman, and colleagues extended crystallographic characterization of the indole-hydrazone pharmacophore beyond a specific kinase program, solving structures for two representative indole-hydrazone congeners (one in monoclinic space group P2□/c with unit-cell parameters a = 7.05, b = 25.03, c = 7.88 angstroms and beta = 104.17 degrees, and a second in orthorhombic space group Pbca with a = 17.42, b = 7.70, c = 19.93 angstroms), both confirming the E-anti hydrazone configuration in the solid state and quantifying the indole-hydrazone dihedral twist angle at approximately 46 and 52 degrees, respectively, alongside Hirshfeld surface analysis and density functional theory computations that further characterize the intermolecular packing forces governing this pharmacophore's crystal geometry [19]. Although this study was not itself directed at a breast cancer kinase target, its crystallographic and computational characterization of the indole-hydrazone scaffold's preferred solid-state conformation is directly transferable to docking pose selection and validation for the breast cancer-relevant indolyl-hydrazone and hydrazonoindolinone series discussed above, since all these compounds share the same core hydrazone dihedral geometry.

X-ray crystallographic parameters reported for hydrazone kinase-inhibitor-related structures and their historical structural templates discussed in this review.

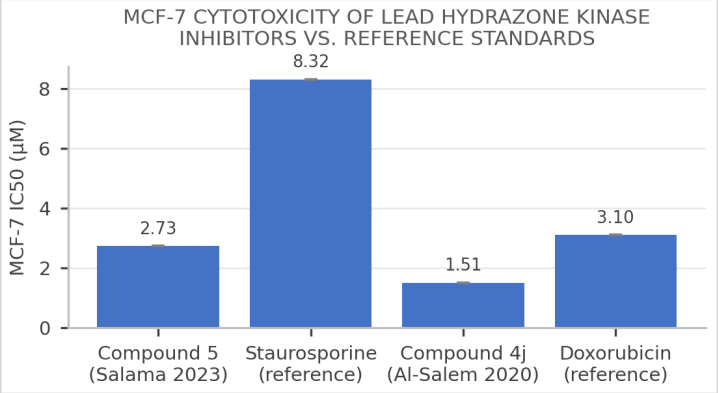
Structure	Space group / resolution	Unit-cell or R-factor detail	Reference
Indolyl-hydrazone compound 3 (synthetic intermediate)	Monoclinic P2₁/c	V = 1374.36(3) Å³; R1 = 0.0279; CCDC 2293777	Salama et al. 2023
Indolyl-hydrazone compound 5 (lead kinase inhibitor)	Triclinic P1	V = 531.26(6) Å³; R1 = 0.0469; CCDC 2293778	Salama et al. 2023
Indole-hydrazone compound 3b	Monoclinic P2₁/c	a=7.05, b=25.03, c=7.88 Å, β=104.17°; E-anti; twist ~46°	Hassan et al. 2023
Indole-hydrazone compound 3c	Orthorhombic Pbca	a=17.42, b=7.70, c=19.93 Å; E-anti; twist ~52°	Hassan et al. 2023
Sunitinib-VEGFR2 kinase domain co-crystal (PDB 4AGD)	Resolution 2.81 Å	Oxindole NH/carbonyl hinge hydrogen bonds to Cys919 backbone	McTigue et al. 2012
Erlotinib-EGFR kinase domain co-crystal (PDB 1M17)	Anilinoquinazoline complex	4-anilinoquinazoline hinge-region binding geometry	Stamos et al. 2002

True protein-ligand co-crystal validation remains, at the time of this review, largely absent from the hydrazone-specific breast cancer kinase-inhibitor literature, but is well precedented for the structurally related indolinone and anilinoquinazoline chemotypes through the sunitinib-VEGFR2 structure (Protein Data Bank entry 4AGD, 2.81-angstrom resolution) and the erlotinib-EGFR kinase domain structure (Protein Data Bank entry 1M17), both of which directly visualize the hinge hydrogen-bonding geometry that newly reported hydrazone docking poses are implicitly designed to reproduce [5,2]. The Aurora B-directed indolinone series reported by Xie and colleagues illustrates a complementary fragment-based crystallographic strategy in which an existing kinase co-crystal structure, rather than a newly solved inhibitor complex, anchors iterative design: the authors docked their evolving indolinone series into the Aurora B kinase domain using an established crystallographic template and calculated binding free energies by molecular mechanics/Poisson-Boltzmann surface area methodology, obtaining a favorable value of -33.34 kcal/mol for their most potent compound (3-17k, Aurora B IC50 = 0.891 nanomolar) and identifying hinge-region hydrogen-bonding contacts consistent with the crystallographically defined Aurora B active site [20]. Taken together, the comparatively small number of hydrazone-specific small-molecule crystal structures solved to date, set against the much larger archive of structurally characterized kinase-inhibitor co-complexes available in the Protein Data Bank more broadly, defines the central methodological gap that this literature continues to close: direct protein-bound crystallographic confirmation of a genuinely novel hydrazone-kinase complex, as opposed to confirmation of the free-ligand conformation or reliance on structurally related historical co-crystal templates, remains the exception rather than the rule.

Structure-Activity Relationships and Anti-Proliferative Potency Against Breast Cancer Cell Lines

Cellular and enzymatic potency data across the surveyed hydrazone kinase-inhibitor literature span more than three orders of magnitude, from sub-nanomolar Aurora B enzymatic inhibition to double-digit micromolar cytotoxicity, and the pattern of variation tracks both the specific kinase target engaged and the electronic and steric character of the hydrazone's terminal aryl or heteroaryl substituent. Salama and colleagues' lead indolyl-hydrazone compound 5 achieved an MCF-7 breast cancer cytotoxicity IC50 of 2.73 micromolar, appreciably more potent than the staurosporine reference standard (8.32 micromolar) in the same assay, and this cellular potency translated into a whole-animal anti-tumor effect, with compound 5 reducing Ehrlich solid carcinoma tumor volume by 46.9 percent relative to untreated controls, directly linking the compound's multi-kinase enzymatic profile to in vivo efficacy [7]. Among the VEGFR-2-directed hydrazonoindolinone series, Al-Warhi and colleagues reported that their lead chlorophenyl-substituted compound achieved a VEGFR-2 enzymatic IC50 of 0.503 micromolar and an MCF-7 cytotoxicity IC50 of 2.93 micromolar, modestly more potent than the corresponding fluorophenyl analog and comparable in cellular potency to the doxorubicin reference standard, indicating that halogen identity at this position measurably modulates both target engagement and cellular activity [8].

MCF-7 breast cancer cell cytotoxicity (IC50, μM) of representative hydrazone kinase inhibitor lead compounds relative to the doxorubicin or staurosporine reference standard used in each respective study.



Thiazole-, triazole-, and imidazole-based acylhydrazones directed more broadly at breast cancer cell lines corroborate this substituent-dependent pattern. Sermuksnyte and colleagues' triazole-thiol hydrazone series suppressed migration and spheroid growth of MDA-MB-231 breast cancer cells in a substituent-dependent manner, and their subsequent pyridine-triazole-thione-hydrazone series, evaluated for combined antimicrobial and antitumor activity, likewise showed cytotoxic potency that varied systematically with the electron-withdrawing or electron-donating character of the hydrazone's terminal aromatic ring [13,14]. Dilek's imidazole-based hydrazone series achieved cytotoxic activity against MCF-7 cells with IC50 values below 9.3 micromolar for all five synthesized congeners, outperforming the cisplatin reference standard, with the most potent compound additionally showing favorable binding stability in molecular dynamics simulation against its docked target, while Aydin and colleagues' N-acylhydrazone and 1,3,4-oxadiazole series, evaluated against MDA-MB-231 alongside lung and prostate cancer cell lines, found the hydrazone congeners generally more potent than their oxadiazole cyclization products, indicating that ring closure of the open hydrazone to the oxadiazole heterocycle is not automatically potency-enhancing in this chemotype [15,16].

The Aurora B-targeted indolinone series from Xie and colleagues occupies the most potent end of the spectrum surveyed here, with

the lead compound achieving a sub-nanomolar Aurora B enzymatic IC50 of 0.891 nanomolar, essentially matching the reference inhibitor CCT241736 (2.137 nanomolar), while retaining low-micromolar cytotoxicity across a multi-cell-line tumor panel alongside markedly weaker activity against non-transformed epithelial lines, yielding selectivity indices considerably more favorable than those typically reported elsewhere in this chemical space [20]. Not every hydrazone kinase-inhibitor series achieves comparably strong target-cytotoxicity concordance, and comparison across the surveyed studies underscores a recurring theme: docking-predicted kinase engagement is a necessary but insufficient condition for potent, mechanistically confirmed anti-proliferative activity, and studies that combine docking, enzymatic kinase assay, cellular cytotoxicity against breast cancer cell lines, and, in the strongest cases, X-ray crystallographic structure determination provide substantially more convincing evidence of a genuine kinase-inhibitor mechanism than docking or cytotoxicity data considered in isolation.

Discussion

Three cross-cutting observations emerge from this synthesis. First, the hydrazone bridge has proven to be a chemically versatile and synthetically economical linker capable of joining a wide variety of heterocyclic hinge-binding fragments, indole, indolin-2-one, purine, pyridazinoindole, imidazole, thiazole, and triazole among them, to generate inhibitors directed at essentially every major kinase target implicated in breast cancer pathogenesis, from cell-surface receptor tyrosine kinases (EGFR, HER2, VEGFR-2) through intracellular cell-cycle regulators (CDK2, and by extension the clinically validated CDK4/6 axis) to survival-signaling kinases within the PI3K/AKT/mTOR cascade. This breadth of applicability, evident across the studies surveyed here, indicates that the hydrazone motif functions less as a target-specific pharmacophore and more as a generalizable hinge-engagement strategy whose ultimate kinase selectivity is determined largely by the identity of the heterocyclic fragment to which it is attached. Second, molecular docking against Protein Data Bank-derived crystallographic templates is now near-universal practice across this literature, and in the best-documented cases (the indolyl-hydrazone, hydrazonoindolinone, and pyridazinoindole-hydrazone series discussed above) is corroborated by molecular dynamics simulation, in vitro enzymatic kinase inhibition assay, and cellular cytotoxicity testing against breast cancer cell lines, a multi-layered validation strategy that substantially strengthens confidence in the proposed binding mode relative to docking alone. Third, and most consequentially for the field's future direction, experimental X-ray crystallographic confirmation remains disproportionately confined to free-ligand solid-state structures rather than true protein-ligand co-crystal complexes: even the most thoroughly characterized hydrazone series surveyed here rely on structurally related but chemically distinct historical co-crystal templates, principally the sunitinib-VEGFR2 and erlotinib-EGFR structures, to argue by analogy that a docked hydrazone pose is plausible, rather than presenting direct crystallographic visualization of the hydrazone inhibitor bound within its own target kinase's active site.

This validation gap has practical consequences for structure-based optimization. Analogy to a related co-crystal template can reliably establish that a docked pose is geometrically plausible and consistent with known hinge-binding chemistry, but it cannot substitute for direct visualization of ligand-specific contacts, induced-fit conformational changes in the kinase activation loop or gatekeeper residue, or ordered water molecules that a genuine co-crystal structure would reveal and that often prove decisive for structure-guided potency and selectivity optimization in subsequent medicinal chemistry cycles. The comparatively low throughput and technical demands of kinase-domain protein crystallization and diffraction relative to small-molecule crystallization likely explain much of this disparity, and cryo-electron microscopy, now capable in favorable cases of resolving kinase-inhibitor complexes at near-atomic resolution, represents a plausible complementary route to closing this gap for hydrazone kinase inhibitors going forward, even though no cryo-electron microscopy structure of a hydrazone-class breast cancer kinase inhibitor complex was identified among the sources reviewed here.

Conclusion

Hydrazone and hydrazone-hybrid kinase inhibitors constitute a chemically versatile and increasingly well-populated class of breast cancer-directed anticancer agents, with reported series engaging EGFR, HER2, CDK2, the PI3K/AKT/mTOR axis, VEGFR-2, and Aurora B through a hinge-binding logic traceable to experimentally solved co-crystal structures of sunitinib and erlotinib bound to their respective kinase targets. Molecular docking, applied almost universally across this literature and increasingly supplemented by molecular dynamics simulation, enzymatic kinase inhibition assay, and cellular cytotoxicity testing against breast cancer cell lines, has proven effective for rank-ordering candidate compounds and rationalizing observed kinase-selectivity profiles. Reported potencies span from sub-nanomolar enzymatic inhibition to double-digit micromolar cytotoxicity, with the most convincingly validated compounds, those combining docking, enzymatic assay, cellular testing, and single-crystal X-ray diffraction in a single study, consistently ranking among the most potent and mechanistically well-supported agents surveyed. The persistent scarcity of true protein-ligand co-crystal structures for hydrazone-class kinase inhibitors, as distinct from free-ligand solid-state structures or reliance on structurally related historical templates, represents the most significant remaining gap between computational prediction and direct structural confirmation in this field, and continued investment in experimental co-crystallography, potentially supplemented by cryo-electron microscopy for less readily crystallized kinase targets, represents the most direct route to closing it and to advancing next-generation hydrazone kinase inhibitors toward more definitive preclinical development for breast cancer therapy.

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