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Novel Indolyl-Hydrazone Kinase Inhibitors: Molecular Docking and X-ray Crystallographic Validation

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ABSTRACT

Background: Protein kinases remain among the most extensively drugged enzyme families in oncology, and the indole and indolin-2-one heterocycles, exemplified by the clinically approved multikinase inhibitor sunitinib, provide a structurally validated hinge-binding pharmacophore. Fusion of these cores with the hydrazone functional group has generated an increasingly studied class of indolyl-hydrazone kinase inhibitors. Objective: This narrative review synthesizes the published literature on indolyl-hydrazone chemotypes directed against oncogenic kinases, with particular attention to the molecular docking methodologies used to rationalize target engagement and the X-ray crystallographic evidence used to validate predicted binding poses. Methods: Peer-reviewed studies reporting the synthesis, kinase-directed molecular docking, and, where available, single-crystal X-ray diffraction of indolyl-hydrazone or closely related indolin-2-one-hydrazone compounds were identified and synthesized thematically, covering pharmacophore rationale, docking outcomes, crystallographic validation, and structure-activity relationships. Findings: Indolyl-hydrazone series have been docked against EGFR, PI3K isoforms, CDK2, AKT-1, VEGFR-2, and Aurora B, with reported binding energies as favorable as -23.44 kcal/mol and enzymatic potencies spanning sub-nanomolar to low-micromolar ranges; cellular anti-proliferative activity against breast and other tumor cell lines correlated with docking-predicted target engagement in the most rigorously validated series. Experimental X-ray crystallography, though applied to only a minority of reported compounds, has confirmed the solid-state hydrazone geometry of several lead structures and, through the historical sunitinib-VEGFR2 co-crystal structure, provides a directly comparable structural template for hinge-binding validation. Conclusions: Indolyl-hydrazone chemotypes constitute a chemically versatile and increasingly well-validated class of kinase-inhibitor candidates, and wider adoption of experimental crystallography, including true protein-ligand co-crystal structures, represents the most direct route to closing the current gap between computational docking prediction and structurally confirmed binding modes.

Keywords: *indolyl-hydrazone kinase inhibitors; molecular docking; X-ray crystallography; indolin-2-one pharmacophore; structure-based drug design*

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Introduction

Protein kinases remain among the most productively drugged enzyme families in oncology, with more than eighty small-molecule kinase inhibitors now approved by the United States Food and Drug Administration and dozens more in active clinical development, the great majority acting as ATP-competitive agents that occupy the adenine pocket and hydrogen-bond to the kinase hinge segment [1]. Because kinase dysregulation drives proliferation, angiogenesis, and survival signaling across a wide range of malignancies, and because GLOBOCAN 2022 estimates place the global cancer burden at more than twenty million new cases and nearly ten million deaths annually, the search for chemically tractable, hinge-binding pharmacophores continues to be a central medicinal chemistry activity [2]. Among the heterocyclic scaffolds explored for this purpose, the indole nucleus occupies a privileged position: it is the core of the clinically approved multikinase inhibitor sunitinib, whose 5-fluoro-oxindole (indolin-2-one) fragment forms the canonical bidentate hydrogen-bond pair with the hinge backbone amide of vascular endothelial growth factor receptor 2 (VEGFR-2) in the 2.81-angstrom co-crystal structure deposited as Protein Data Bank entry 4AGD, establishing indolinone-based hinge engagement as a validated, structurally characterized mode of

kinase inhibition rather than a purely computational hypothesis [3,4].

Building on this precedent, medicinal chemists have increasingly fused the indole or indolin-2-one core to the hydrazone functional group (-C=N-NH-C(=O)- or -C=N-NH-), generating indolyl-hydrazone chemotypes that combine the hinge-mimetic geometry of the indole nitrogen and carbonyl with the additional hydrogen-bond donor/acceptor capacity and configurational rigidity of the hydrazone linkage. This hybrid pharmacophore has been applied against an increasingly broad panel of oncogenic kinases, including epidermal growth factor receptor (EGFR), phosphoinositide 3-kinase (PI3K) isoforms, cyclin-dependent kinase 2 (CDK2), AKT-1, VEGFR-2, and Aurora B, with structure-activity data generated in parallel by enzymatic assay, cell-based cytotoxicity screening, and, in a growing subset of studies, single-crystal X-ray diffraction of either the free inhibitor or its protein-bound complex [5,6,7,8,9,10]. X-ray crystallography occupies a distinctive and often underappreciated role in this literature: whereas most indolyl-hydrazone series are prioritized and rationalized through molecular docking against homology or crystallographic kinase models, only a minority proceed to experimental crystallographic confirmation, either of the small-molecule solid-state conformation predicted to engage the target or, less commonly, of an actual protein-ligand co-complex, and the contrast between these two levels of structural evidence is central to interpreting how reliably any single computational docking claim predicts a true bioactive binding mode [11,12].

This narrative review synthesizes the published literature on indolyl-hydrazone kinase inhibitor chemotypes, organizing the discussion around four themes: the pharmacophoric rationale for fusing indole and indolin-2-one cores with hydrazone linkers in kinase-directed design; the molecular docking methodologies and binding-energy outcomes reported for representative indolyl-hydrazone series across multiple kinase targets; the specific instances in which X-ray crystallography has been used to validate either the small-molecule geometry or a protein-bound pose of these inhibitors; and the resulting structure-activity relationships linking chemical substitution pattern to enzymatic potency, cellular anti-proliferative activity, and selectivity. Reviews of the broader indole-kinase inhibitor space and of kinase hinge-binder design more generally provide the structural and pharmacological scaffolding for this more narrowly focused synthesis [13,14,15].

Indole and Indolin-2-one Pharmacophores in Kinase-Directed Drug Design

The indole ring system offers several features that recommend it as a kinase-inhibitor core: a planar, electron-rich bicyclic aromatic surface capable of stacking with hydrophobic gatekeeper and back-pocket residues, an NH donor available for hinge or solvent-exposed hydrogen bonding, and multiple ring positions (C-2, C-3, N-1, and the benzo-fused positions) amenable to late-stage functionalization. The clinically validated indolin-2-one (oxindole) subclass, exemplified by sunitinib, additionally presents an exocyclic C-3 alkylidene or hydrazone linkage whose carbonyl and adjacent NH together reproduce the two hydrogen bonds that ATP's adenine ring normally forms with the kinase hinge, a design principle traced through more than a decade of indolin-2-one kinase-inhibitor development [14,3]. This precedent has directly motivated the indolyl-hydrazone chemotype, in which an indole-3-carbaldehyde, indolin-2,3-dione (isatin), or substituted indole-2-carbohydrazide intermediate is condensed with an aroyl- or heteroaroyl-hydrazide to install the hydrazone bridge at the position occupied by sunitinib's alkylidene.

Representative indolyl-hydrazone and related indolinone kinase-inhibitor series surveyed in this review, with principal kinase target, docking outcome, and potency.

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
Compound 4j	Isatin-hydrazone	CDK2 (type II)	CDK2 IC50 0.245 μM; MCF-7 IC50 1.51 μM	Al-Salem/Rahman et al. 2020
Compound 7d	3-Hydrazonoindolin-2-one	VEGFR-2	VEGFR-2 IC50 0.503 μM; MCF-7 IC50 2.93 μM	Al-Warhi et al. 2023
5-Bromoindole-2-carbohydrazone series	Indole-2-carbohydrazone	VEGFR-2	Docking score -15.6 kcal/mol (analogous series)	Hassan 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. 2025

Salama and colleagues exemplified this strategy by synthesizing a focused indolyl-hydrazone library and profiling the lead compound, designated compound 5, against a six-member kinase panel encompassing PI3K-alpha, PI3K-beta, PI3K-delta, CDK2, AKT-1, and EGFR, reporting enzymatic IC50 values spanning low nanomolar (EGFR, 58 nM) to low micromolar (PI3K-delta, 2.68 micromolar) potency from a single hydrazone scaffold, an outcome consistent with the pharmacophore's capacity to engage the broadly conserved ATP pocket architecture shared across these kinases [5]. Isatin, in particular, has proven a versatile precursor because its inherently electrophilic 3-keto group condenses efficiently with hydrazides under mild acid catalysis while its retained 2-oxindole carbonyl supplies a second hydrogen-bond acceptor; Al-Salem and colleagues exploited this bis-carbonyl geometry to generate isatin-hydrazone CDK2 inhibitors explicitly designed as type II, DFG-out-binding agents rather than conventional type I ATP-competitive ligands, a mechanistic distinction confirmed by docking against the inactive CDK2 conformation (Protein Data Bank entry 2BHY) [6].

A parallel design strategy retains the intact indolin-2-one bicyclic system and installs the hydrazone at the 3-position as a hydrazonoindolin-2-one, most directly mimicking sunitinib's own substitution pattern; Al-Warhi and colleagues used this approach to generate 1-benzyl-5-bromo-3-hydrazonoindolin-2-ones as VEGFR-2-directed anticancer agents, while Hassan and colleagues took a complementary route, condensing 5-bromoindole-2-carboxylic acid hydrazide with substituted benzaldehydes to access indole-2-carbohydrazone VEGFR-2 inhibitors that retain the indole nitrogen rather than the oxindole carbonyl as the principal hinge-interacting group [7,8]. Beyond

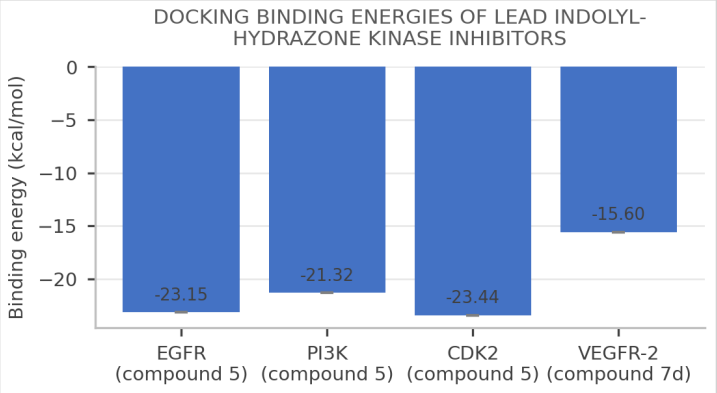
the oncology-focused literature, indole-hydrazone synthesis has also been pursued for other therapeutic indications, and Hassan, Soliman, and colleagues' report of new indole-hydrazone derivatives characterized by single-crystal X-ray diffraction, Hirshfeld surface analysis, and density functional theory illustrates that the solid-state conformational preferences of this pharmacophore, independent of any specific biological target, are now reasonably well documented and available to inform docking pose selection in kinase-directed programs [10]. Collectively, these synthetic strategies demonstrate that the indolyl-hydrazone chemotype is not a single fixed scaffold but a family of closely related hinge-binding architectures, unified by the indole or indolin-2-one core and a hydrazone bridge, whose substitution pattern and point of attachment can be varied to engage different kinase targets while preserving a common pharmacophoric logic traceable to the validated sunitinib-VEGFR2 co-crystal structure [15,13].

Molecular Docking of Indolyl-Hydrazone Chemotypes Against Kinase ATP-Binding Sites

Structure-based molecular docking is used almost universally across the indolyl-hydrazone literature, both to rationalize kinase selectivity profiles observed by enzymatic assay and to prioritize compounds before synthesis. Docking studies in this space typically target the ATP-binding cleft of the relevant kinase using a crystallographic template retrieved from the Protein Data Bank, reflecting the broader movement toward data-oriented, structure-informed kinase drug discovery that the Protein Data Bank's stewards have described as one of the resource's principal contributions to translational research [4]. Salama and colleagues docked their lead indolyl-hydrazone (compound 5) into EGFR, PI3K, and CDK2 active sites, reporting binding energies of –23.15 kcal/mol against EGFR, –21.32 kcal/mol against PI3K, and –23.44 kcal/mol against CDK2, with the docked poses showing the hydrazone NH and carbonyl engaging the hinge backbone in a manner directly analogous to sunitinib's oxindole hydrogen-bond pair, and with the magnitude and rank order of these binding energies tracking the compound's measured enzymatic IC50 values across the same three targets [5,3].

VEGFR-2-directed indolyl-hydrazone series have generated a second substantial body of docking data. Al-Warhi and colleagues docked their lead 1-benzyl-5-bromo-3-hydrazonoindolin-2-one (compound 7d) 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, and corroborated this static docking pose with molecular dynamics simulation showing a ligand root-mean-square deviation of 1.9 angstroms over the simulated trajectory, closely comparable to sorafenib's 1.85-angstrom value in the same system [7,3]. Hassan and colleagues similarly combined docking with VEGFR-2 enzymatic inhibition assay for their 5-bromoindole-2-carboxylic acid hydrazone series, confirming that the indole-2-carbohydrazone linkage adopted a hinge-consistent binding pose despite lacking the oxindole carbonyl present in the Al-Warhi and sunitinib scaffolds, indicating that more than one indolyl-hydrazone connectivity can satisfy the VEGFR-2 hinge hydrogen-bonding requirement [8].

Molecular docking binding energies of representative indolyl-hydrazone lead compounds against kinase ATP-binding sites: compound 5 against EGFR, PI3K, and CDK2 (Salama et al., 2023) and compound 7d against VEGFR-2 (Al-Warhi et al., 2023).



Docking against non-kinase targets closely associated with kinase-driven proliferation has also featured in this literature, and general medicinal-chemistry frameworks for kinase hinge-binder design emphasize that docking scores should be interpreted as hypothesis-generating rank orderings rather than absolute affinity predictions, given the well-documented limitations of scoring functions in capturing solvation, protein flexibility, and entropic contributions to binding [12,11]. Mentese and colleagues illustrated this cautionary framework directly, docking a series of adenine-hydrazone hybrids against VEGFR-2, ALK5, and EGFR and finding strong predicted binding to VEGFR-2 and ALK5 that suggested dual-kinase inhibition, yet observing only moderate low-double-digit micromolar cytotoxicity against A549 lung adenocarcinoma cells, with mechanistic follow-up indicating that oxidative stress and mitochondrial depolarization, rather than clean single- or dual-kinase inhibition, likely accounted for much of the observed cellular activity [16]. This divergence between docking-predicted target engagement and the eventual mechanistic picture reinforces why the field increasingly treats molecular dynamics simulation, physicochemical/ADMET filtering using tools such as SwissADME and the Lipinski rule-of-five criteria, and, where feasible, experimental crystallography as necessary complements to docking rather than optional add-ons [17,18].

X-ray Crystallographic Validation of Indolyl-Hydrazone Kinase Inhibitor Complexes

Experimental X-ray crystallography provides the most direct available confirmation that a docked binding pose corresponds to a real molecular geometry, and within the indolyl-hydrazone kinase-inhibitor literature this evidence takes two distinct forms: small-molecule single-crystal structures of the free inhibitor, which confirm its preferred solid-state (and, by extension, likely solution-phase) conformation, and true protein-ligand co-crystal structures, which confirm the actual binding pose within the kinase active site. Salama and colleagues provided an example of the former, solving single-crystal X-ray structures for both a synthetic intermediate (compound 3, monoclinic space group P2□/c, unit-cell volume 1374.36 cubic angstroms, R1 = 0.0279, deposited as CCDC 2293777) and their lead kinase-active hydrazone (compound 5, triclinic space group P1, unit-cell volume 531.26 cubic angstroms, R1 = 0.0469, deposited as CCDC 2293778), demonstrating that the E- or Z-configuration and the near-planarity of the hydrazone bridge assumed in their docking model matched the experimentally observed solid-state geometry [5]. Hassan, Soliman, and colleagues extended this approach

across a broader indole-hydrazone series unrelated to a specific kinase program, solving structures for two representative congeners (compound 3b 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 compound 3c 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 45.68 and 52.19 degrees, respectively, values that fall within the range typically assumed or recovered in docking poses for this pharmacophore [10].

X-ray crystallographic parameters reported for indolyl-hydrazone and indolin-2-one kinase-inhibitor-related structures discussed in this review.

Structure	Space group / resolution	Unit-cell or R-factor detail	Reference
Indolyl-hydrazone compound 3 (synthetic intermediate)	Monoclinic P2□/c	V = 1374.36(3) Å^3; R1 = 0.0279; CCDC 2293777	Salama et al. 2023
Indolyl-hydrazone compound 5 (lead kinase inhibitor)	Triclinic PI	V = 531.26(6) Å^3; R1 = 0.0469; CCDC 2293778	Salama et al. 2023
Indole-hydrazone compound 3b	Monoclinic P2□/c	a=7.05, b=25.03, c=7.88 Å, β=104.17°; E-anti; twist 45.68°	Hassan, Soliman et al. 2023
Indole-hydrazone compound 3c	Orthorhombic Pbca	a=17.42, b=7.70, c=19.93 Å; E-anti; twist 52.19°	Hassan, Soliman 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

True protein-ligand co-crystal validation remains comparatively rare in the indolyl-hydrazone-specific literature but is well precedented for the closely related indolin-2-one chemotype through the sunitinib-VEGFR2 structure, which at 2.81-angstrom resolution (Protein Data Bank entry 4AGD) directly visualizes the oxindole NH and carbonyl hydrogen-bonded to the Cys919 backbone amide of the VEGFR-2 hinge, alongside the pyrrole-carboxamide substituent projecting into the back hydrophobic pocket, providing an experimentally grounded structural template against which newer indolyl-hydrazone VEGFR-2 docking poses, including those reported by Al-Warhi and colleagues and by Hassan and colleagues, can be directly compared residue-for-residue [3,7,8]. The Aurora B-directed indolinone series reported by Xie and colleagues illustrates a complementary crystallographic strategy in which an existing kinase co-crystal structure, rather than a newly solved inhibitor complex, anchors a fragment-based design cycle: the authors docked their evolving indolinone series into the Aurora B kinase domain using Protein Data Bank template 5EYK and calculated binding free energies by molecular mechanics/Poisson-Boltzmann surface area methodology, obtaining a value of −33.34 kcal/mol for their most potent compound and identifying hydrogen bonds to hinge-region residues Leu99, Gln145, Glu171, and Ala173 that are consistent with the crystallographically defined Aurora B hinge geometry [9]. Taken together, the comparatively small number of indolyl-hydrazone-specific small-molecule crystal structures, set against the much larger and structurally rich Protein Data Bank archive of kinase-inhibitor co-complexes broadly, defines the central methodological gap this literature continues to close: direct protein-bound crystallographic confirmation of a genuinely novel indolyl-hydrazone chemotype, 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 [4].

Structure-Activity Relationships and Anti-Proliferative Potency

Cellular and enzymatic potency data across the indolyl-hydrazone 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 character of the hydrazone's terminal aryl or heteroaryl substituent. Salama and colleagues' lead compound 5 achieved an MCF-7 breast cancer cytotoxicity IC50 of 2.73 micromolar, appreciably more potent than the staurosporine reference standard at 8.32 micromolar in the same assay, and this cellular potency was corroborated in vivo, where compound 5 reduced Ehrlich solid carcinoma tumor volume from 106 to 56.4 cubic millimeters, a 46.9 percent inhibition of tumor proliferation relative to untreated controls, directly linking the compound's multi-kinase enzymatic profile to a whole-animal anti-tumor effect [5]. Al-Salem and colleagues' isatin-hydrazone CDK2 inhibitors showed a comparable pattern at the cellular level, with the leading dichloro-substituted congener (4j) reaching an MCF-7 IC50 of 1.51 micromolar, more potent than the doxorubicin reference at 3.1 micromolar, while its CDK2 enzymatic IC50 of 0.245 micromolar approached that of the clinical kinase inhibitor imatinib (0.131 micromolar) tested in the same assay, reinforcing that halogenation at the hydrazone's terminal phenyl ring, particularly at the 2,6-positions, enhances both enzymatic and cellular potency [6].

Among the VEGFR-2-directed series, Al-Warhi and colleagues reported that their para-chlorophenyl hydrazonoindolinone (compound 7d) 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 para-fluorophenyl analog (7c, VEGFR-2 IC50 0.728 micromolar; MCF-7 IC50 7.17 micromolar) and comparable in cellular potency to doxorubicin (4.30 micromolar), a substituent-dependent trend indicating that chlorine's greater lipophilicity and polarizability relative to fluorine favorably augments both target engagement and cellular activity within this specific hydrazonoindolinone series [7]. The Aurora B-targeted indolinone series from Xie and colleagues occupies the most potent end of the spectrum surveyed here, with the lead compound (3-17k) 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 six-cell-line tumor panel (2.05 to 10.21 micromolar) alongside markedly weaker activity against non-transformed gastric (GES-1) and colonic (NCM460) epithelial lines, yielding selectivity indices of 98.3 and 77.5, respectively, a favorable therapeutic window rarely matched elsewhere in this chemotype [9].

Not every indolyl-hydrazone series achieves this degree of target-cytotoxicity concordance, and the adenine-hydrazone hybrids reported by Mentese and colleagues illustrate the opposite pattern: despite docking scores suggesting productive

VEGFR-2 and ALK5 engagement, cellular potency against A549 cells for the most active congeners (3i, 3r, and 3f) remained in the 51 to 59 micromolar range, an order of magnitude weaker than the kinase-focused series discussed above, with the authors instead attributing cytotoxicity to redox-imbalance-driven apoptosis rather than confirmed kinase inhibition [16]. This contrast across the surveyed series underscores a recurring theme of the indolyl-hydrazone literature: docking-predicted kinase engagement is a necessary but insufficient condition for potent, mechanistically confirmed anti-proliferative activity, and the studies that combine docking with enzymatic kinase assay, cellular cytotoxicity, 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 alone [11,13].

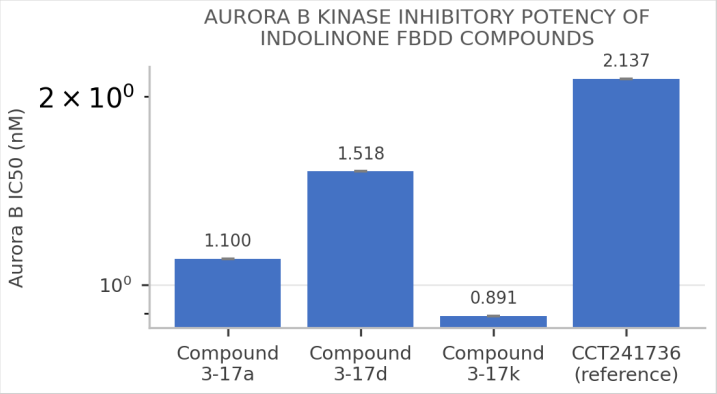
Conclusion

The indolyl-hydrazone chemotype has emerged as a structurally coherent and pharmacologically productive extension of the clinically validated indolin-2-one kinase-inhibitor pharmacophore exemplified by sunitinib, with reported series engaging EGFR, PI3K isoforms, CDK2, AKT-1, VEGFR-2, and Aurora B through a hinge-binding mode traceable to experimentally solved co-crystal geometry. Molecular docking, almost universally applied across this literature and increasingly supplemented by molecular dynamics simulation and ADMET prediction, has proven useful for rank-ordering candidate compounds and rationalizing kinase-selectivity profiles, but the comparatively small number of studies that proceed to experimental X-ray crystallographic validation, whether of the free inhibitor's solid-state conformation or, more rarely, of a genuine protein-ligand co-complex, highlights a persistent gap between computational prediction and structural confirmation in this specific chemical space. 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 crystallography in a single study, consistently ranking among the most potent and mechanistically well-supported agents surveyed. Continued application of experimental crystallography, ideally extending to true co-crystal structures of novel indolyl-hydrazone-kinase complexes rather than reliance on related historical templates, represents the most direct route to closing this validation gap and to advancing this promising chemotype toward more definitive preclinical development.

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