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Target Nomination Is Not Target Validation: A Causal Evidence Architecture for Connecting Natural-Product Perturbations, Omics Signatures, Binding Evidence, and Phenotypic Rescue

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ABSTRACT

Natural products can produce rich phenotypic, transcriptional, morphological, and proteomic responses while leaving the molecular cause of those responses uncertain. Contemporary target-discovery approaches increasingly connect compound perturbations with omics signatures, predicted targets, proteome-wide engagement, genetic sensitivity, and pathway effects, but these observations do not carry equivalent causal meaning. This article develops a proposed causal evidence architecture for separating target nomination from progressively stronger target validation. The architecture distinguishes evidence that identifies plausible targets from evidence of physical engagement, functional dependency, phenotype mediation, and counterfactual rescue. It further treats orthogonality as the ability of independent experiments to eliminate different alternative explanations rather than as the simple accumulation of concordant assays. Particular attention is given to natural products, for which polypharmacology, covalent reactivity, probe derivatization, context-dependent exposure, and downstream network responses can make apparently coherent target narratives misleading. The proposed architecture therefore preserves uncertainty, conflicting observations, multiple-target explanations, and assay non-detectability as explicit evidence states rather than forcing every dataset toward a single mechanistic conclusion. Causal confidence is intended to inform what experiment or development decision should follow, not to provide a universal numerical validation score. The framework does not establish prospective predictive performance, therapeutic efficacy, or clinical relevance. Instead, it provides an evidence-bounded structure for asking whether a nominated protein is merely associated with a natural-product response, physically engaged by the compound, functionally necessary for that response, or sufficiently supported to justify stronger mechanistic interpretation.

Keywords: Natural products, Target deconvolution, Target engagement, Chemoproteomics, Phenotypic rescue, Causal inference

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Introduction

Target identification is often narrated as though discovery of a plausible protein completes a mechanistic explanation. In practice, target assessment is multidimensional: biological rationale, compound–target engagement, functional consequences, perturbational specificity, and contextual relevance answer related but different questions [1]. A target can therefore be compelling as a hypothesis while remaining weakly supported as the molecular cause of an observed phenotype. This distinction is consequential because preclinical target-validation failures can arise from non-specific perturbations, inappropriate models, reagent artifacts, compensatory biology, or interpretations that outrun what an experiment actually tests [2]. For natural products,

these problems are intensified by structural complexity, chemically reactive motifs, incomplete exposure information, multiple interacting proteins, and phenotypes emerging from more than one molecular event.

Recent natural-product target-discovery literature encompasses computational inference, affinity-based methods, chemical proteomics, thermal or solubility-based profiling, and functional validation strategies [3, 4]. These technologies substantially enlarge the observable evidence space, but greater observability does not mean that every observation carries the same causal weight. A transcriptomic signature may nominate a pathway; a proteomic shift may support target engagement; a resistance mutation may connect target perturbation to compound response; and rescue may test whether reversing the proposed target perturbation changes the phenotype. Treating all four as interchangeable confirmations obscures the different alternative explanations each can exclude.

This article therefore proposes a causal evidence architecture for natural-product target assessment. Its central distinction is between nomination evidence, which makes a target plausible, and validation evidence, which increasingly constrains alternative explanations linking compound, target, and phenotype. The architecture does not define an automatic ladder in which every project must traverse identical experiments. Instead, evidence is organized by the question it can answer: whether a target is associated with the perturbation, physically engaged, functionally implicated, necessary or sufficient within the tested system, and supported by an intervention capable of altering the predicted compound–phenotype relationship.

The purpose is consequently not to replace existing target-identification technologies with a new assay or numerical score. It is to provide a proposed logic for combining them without collapsing prediction into engagement, engagement into functional mediation, or mechanistic evidence into therapeutic validation. This separation is particularly important when natural products generate multi-target, context-dependent, or apparently contradictory evidence.

Why target discovery produces hypotheses before mechanisms

Perturbational data are powerful because they permit a compound-induced state to be compared with large reference spaces. The Connectivity Map established this logic at scale by organizing gene-expression responses to chemical and genetic perturbations, enabling similarity or reversal patterns to generate mechanism hypotheses [5]. Such patterns can identify biologically meaningful neighborhoods without demonstrating that the matched gene or pathway is the directly engaged molecular target.

Image-based phenotypic profiling extends the same principle into cell morphology. Large matched chemical and genetic perturbation resources allow compounds and gene perturbations to be compared across high-dimensional cellular features [6]. Perturbation-specific transcriptional mapping similarly can connect antimicrobial responses with target-related perturbations and thereby prioritize plausible mechanisms [7]. These approaches become stronger when chemical and genetic perturbations converge, but convergence may still reflect a shared downstream process, stress response, or pathway state rather than identical molecular engagement.

Machine-learning models can further compress transcriptional signatures into representations useful for target prediction [8]. Their output nevertheless remains a prediction conditioned by training labels, perturbational context, representation quality, and the biological states present in the underlying data. High predictive confidence cannot itself demonstrate binding.

The same caution applies when genetic and small-molecule screens appear to agree. Chemical perturbations may have off-target activities or concentration-dependent effects, whereas genetic perturbations can produce compensation, dosage artifacts, or biology distinct from acute pharmacological modulation [9]. Accordingly, target discovery usually begins by narrowing explanatory possibilities. A mechanistic claim requires experiments designed not merely to reproduce the same association, but to distinguish the proposed target from credible alternatives.

Sources of target-nomination evidence

Chemical proteomics can move target discovery closer to molecular interaction by identifying proteins captured, modified, stabilized, destabilized, or otherwise altered in the presence of a small molecule [10]. Yet "chemoproteomic evidence" is not a single evidential category. Affinity capture, covalent labeling, stability profiling, and site-level proteolytic approaches interrogate different physical consequences of compound–protein interaction and have different susceptibility to indirect effects.

For natural products, cellular thermal shift assay and thermal proteome profiling can reveal ligand-associated changes in protein thermal stability [11]. Tissue-based thermal profiling additionally shows why context matters: engagement detectability can depend on compound exposure, protein abundance, tissue composition, and the thermal behavior of the protein itself [12]. A thermal shift therefore supports an engagement-compatible relationship but does not establish that the shifted protein mediates the phenotype.

Limited-proteolysis chemoproteomics can detect interaction-associated conformational changes and provide information about candidate binding regions [13]. Solvent-induced proteome stability profiling supplies a distinct physicochemical readout of target engagement [14]. Agreement between these assays can strengthen an interaction hypothesis, but their outputs should not be counted as independent causal confirmations unless the relevant sources of error are genuinely different.

Proteome integral solubility alteration provides another proteome-wide route to target deconvolution [15]. Solubility shifts can nevertheless arise from complexes, pathway remodeling, or indirect protein-state changes. Genetic selection adds a different evidential dimension: compounds can be linked to genes whose alteration changes susceptibility [16]. CRISPR-based mutagenesis scanning can go further by identifying resistance-conferring variants in candidate targets [17]. Such evidence is functionally informative, although resistance can also arise through bypass pathways or altered fitness.

These distinctions are summarized in **Table 1**, which classifies common nomination evidence by the question each modality can legitimately answer.

Table 1. Evidence classes in natural-product target nomination and the inferential boundary attached to each

Evidence class	Primary question answered	Strongest defensible interpretation	Important competing explanation	Proper role in assessment
Perturbational transcriptomics	Does the compound produce a state resembling a known perturbation?	Target or pathway hypothesis	Shared downstream response	Nomination
Morphological profiling	Does cellular phenotype resemble a chemical or genetic reference?	Phenotypic-mechanism proximity	Many mechanisms yield similar morphology	Nomination
Predictive modeling	Which targets best fit learned perturbational patterns?	Ranked target hypothesis	Training-domain or label bias	Nomination
Affinity/chemical proteomics	Which proteins are captured, modified, or enriched?	Candidate physical interaction	Probe bias or indirect capture	Nomination to engagement
Thermal/stability profiling	Which proteins change physicochemical stability?	Engagement-compatible target	Indirect stabilization or detectability failure	Engagement support
Proteolysis/solvent/solubility profiling	Which proteins show interaction-associated structural or solubility changes?	Orthogonal engagement evidence	Complex remodeling or protein-state effects	Engagement support
Genetic sensitivity/resistance	Does changing a gene alter compound response?	Functional target or pathway dependency	Bypass pathways or fitness effects	Functional validation

Note: Categories describe evidential functions rather than a universal rank. A negative result may be uninformative when the assay cannot detect the relevant interaction.

Evidence that moves a target toward causal validation

Evidence becomes more causally discriminating when an intervention tests what should happen if the nominated target truly mediates the compound phenotype. Genetic rescue is especially informative because restoration or alteration of the proposed target can test whether the expected biological response is reversed, attenuated, or otherwise modified [18]. Rescue is not infallible—construct dosage, incomplete restoration, localization, and context can alter the result—but it asks a counterfactual question that observational concordance does not.

Chemical and genetic rescue designs can also identify mechanism-specific resistance and pathway dependence. Studies of targeted protein degradation illustrate how rescue and resistance experiments can separate intended target loss from alternative degradation-related effects [19]. The transferable principle is evidential rather than

modality-specific: changing the proposed causal component should alter the response in the direction predicted by the mechanism.

Recent natural-product studies demonstrate the value of combining engagement with orthogonal functional evidence rather than ending at target capture or profiling. Examples involving orpinolide, naamidine J, and quercetin integrate target-oriented proteomic or engagement evidence with additional biochemical, genetic, pathway, or phenotypic experiments [20–22]. Their validation depth and biological contexts differ, so they should not be treated as interchangeable demonstrations of a universal hierarchy. Collectively, however, they illustrate why a target claim becomes more persuasive when independent experiments address distinct alternatives.

Covalent interactions require similar discipline. Adenanthin has been linked to covalent targeting of the NF-κB p65 subunit together with functional pathway effects [23]. Covalency can strengthen evidence of physical interaction, but electrophilic reactivity may also broaden off-target chemistry. Specificity and phenotype mediation therefore remain separate questions.

The proposed architecture treats causal validation as progressive elimination of plausible alternative explanations, not accumulation of assay counts. **Table 2** sets out the principal discriminating tests and the causal question each contributes.

Table 2. Experimental evidence that can move an engaged target toward causal phenotype attribution

Validation evidence	Causal question tested	What a supportive result strengthens	Residual uncertainty
Orthogonal engagement assay	Is the same target detected by a different physical principle?	Confidence in compound–target interaction	Both assays may still report indirect state changes
Resistance-conferring target alteration	Does changing the target alter compound susceptibility?	Functional target linkage	Resistance may involve fitness or pathway compensation
Target depletion or perturbation	Does reducing target function phenocopy or modify the compound response?	Functional dependency	Genetic and pharmacological perturbations may differ
Genetic rescue	Does restoration or resistant target expression reverse the predicted phenotype?	Counterfactual causal attribution	Rescue completeness and expression context matter
Biochemical/pathway linkage	Does target modulation connect to the predicted downstream mechanism?	Mechanistic coherence across levels	The pathway may also be reached through other targets
Probe/parent-compound concordance	Does target evidence survive changes in target-detection strategy?	Fidelity of probe-based assignment	Derivatization or exposure differences can remain
Specificity challenge	Does the mechanism survive testing against plausible competing targets or reactivity?	Exclusion of alternative explanations	Unmeasured targets may still contribute

The relationships developed through these sections are integrated in **Figure 1**. The figure makes explicit that nomination, engagement, functional mediation, rescue, conflict, and development interpretation are different evidential states rather than interchangeable confirmations.

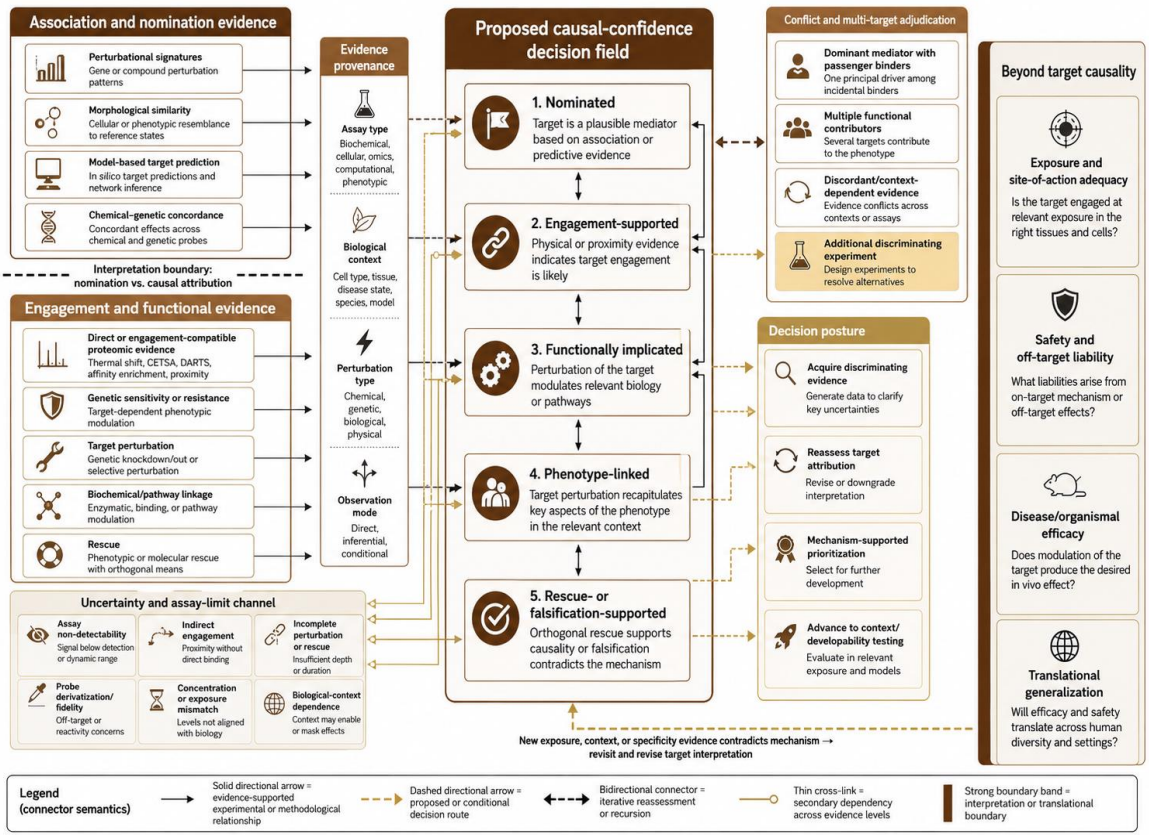

Figure 1. Proposed multilevel causal evidence architecture separating target nomination, target engagement, and phenotype mediation in natural-product research

Triangulation across orthogonal evidence

Orthogonality should describe independence of evidential logic, not merely the use of different laboratory platforms. Thermostability-assisted limited proteolysis, organ-resolved PISA, and time-induced PISA illustrate how proteome-wide target evidence can depend on different physicochemical readouts, biological contexts, and temporal windows [24–26]. Concordance across such assays is useful because each can expose limitations invisible to another, yet correlated stability changes can still arise from shared downstream remodeling. The relevant question is therefore not how many assays are positive, but which alternative explanation each assay is capable of challenging. Two assays that both respond to the same protein-complex rearrangement may add less causal information than one engagement assay paired with a genetic intervention.

Cross-level triangulation becomes stronger when molecular engagement is connected to system response. Integration of multi-omics with PISA has been used to narrow antibiotic mode-of-action hypotheses by bringing proteome-state evidence into contact with broader cellular responses [27]. Such convergence does not automatically identify the direct causal target: several downstream pathways may converge on a similar omics state. It instead reduces the space of plausible mechanisms and identifies where a discriminating intervention is needed. The proposed framework therefore records whether evidence concerns molecular interaction, pathway response, or phenotype, rather than allowing one level to substitute for another.

Natural-product studies can similarly combine network or omics signals with biochemical and biological validation. Work on nicotiflorin illustrates a multilayer strategy in which computational or systems-level inference is followed by targeted experimental interrogation [28]. The evidential classes should remain visible rather than being fused into a single “validated” label. A predicted network neighborhood and a measured target-engagement event may agree, for example, while still leaving open whether that engaged target is necessary for the phenotype. Accordingly, the proposed architecture treats orthogonality as falsification-oriented. Independent observations add most value when they test different links in the compound–target–phenotype chain. Convergence that cannot eliminate a credible competing explanation increases coherence but not necessarily causal confidence, whereas a strategically chosen discordant or rescue experiment may be more informative than several concordant

measurements [1]. This interpretation also gives negative findings a role: a technically informative negative result can reduce causal confidence, while a negative result from an assay incapable of observing the relevant interaction should remain classified as uninformative.

A causal architecture for natural-product target assessment

Natural products make single-target narratives particularly vulnerable because one compound can engage multiple proteins through distinct covalent and non-covalent interactions. Proteome-wide mapping of withaferin A exemplifies a broad target landscape in which detection of multiple interactors does not reveal which, if any, are necessary for the observed phenotype [29]. The proposed architecture therefore begins by preserving target multiplicity rather than collapsing all evidence toward one preferred protein. A nominated target remains one candidate explanation among alternatives until functional evidence narrows that set.

A second checkpoint concerns measurement fidelity. Chemical probes can increase sensitivity and enable target capture, but derivatization can modify permeability, localization, reactivity, or binding. Probe-based mapping of honokiol illustrates why captured proteins should be interpreted alongside evidence that the probe remains pharmacologically representative of the parent compound [30]. Probe fidelity is therefore treated as a boundary on the interpretation of engagement evidence. When fidelity is uncertain, the target assignment may still be useful for hypothesis generation, but it should not inherit the full evidential meaning of the unmodified natural product.

Recent syntheses of natural-product target-identification strategies emphasize the growing complementarity of computational prediction, chemical proteomics, and functional validation [31]. Proteomic strategies in traditional Chinese medicine further show that coverage, detectability, abundance, and workflow-specific biases remain important constraints [32]. Current natural-product target-discovery technologies consequently justify integration but do not establish a universally validated causal hierarchy [33]. This distinction is important because technological sophistication can increase confidence in measurement without automatically increasing confidence in causal interpretation.

The architecture proposed here organizes evidence into five qualitative states: nominated, engagement-supported, functionally implicated, phenotype-linked, and rescue- or falsification-supported. Movement between states is conditional, not automatic. A target may remain at one state when the next test is technically unavailable, fail because an assay is uninformative, or branch into several target explanations. Evidence can also move backward when a specificity challenge contradicts the working mechanism. The architecture additionally retains provenance: the reader should be able to identify which evidence class produced each state, whether the relationship is direct or inferential, and which alternative explanations remain open.

This structure is intentionally not a numerical score. A single well-designed rescue experiment may be more discriminating than several correlated omics measurements, while a broad engagement landscape may require parallel testing of several candidates. **Table 3** translates the proposed states into an experiment-selection workflow in which the next action is determined by the unresolved causal question rather than by a fixed assay sequence.

Table 3. Proposed evidence-state workflow for natural-product target assessment

Current evidence state	Main unresolved question	Preferred next discriminating action	Interpretation if unresolved
Nominated	Is the protein physically engaged?	Orthogonal engagement test	Retain as hypothesis
Engagement-supported	Does engagement affect compound response?	Genetic or functional perturbation	Binding may be non-mediating
Functionally implicated	Does the target mediate the phenotype?	Rescue or resistance design	Pathway association remains possible
Phenotype-linked	Is attribution target-specific?	Competing-target and specificity challenge	Multi-target explanation remains open
Rescue/falsification-supported	Does the mechanism persist across context?	Exposure/context replication	Causality remains system-bounded

Conflicting and multi-target evidence

Conflict is not evidence failure; it is information about which mechanistic explanation remains underdetermined. Chemical-probe practice demonstrates why apparently coherent results can be misleading when concentrations,

inactive controls, selectivity, or orthogonal probes are inadequate [34]. In the proposed architecture, evidence generated with a poorly characterized intervention is downgraded at its source rather than allowed to accumulate downstream as if it were independent confirmation. This prevents a technically weak perturbation from becoming persuasive merely because several downstream readouts respond consistently.

Multi-target binding creates a different problem. Large-scale kinase-inhibitor chemoproteomics has shown that compounds nominally associated with one target can exhibit broad and variable interaction landscapes [35]. A proteomic list therefore cannot by itself distinguish a dominant causal target, passenger binders, or a phenotype produced by several functionally contributing targets. Withaferin A provides an analogous natural-product reason to preserve broad target landscapes until functional adjudication is performed [29]. Ranking the most strongly detected protein may be operationally convenient, but signal magnitude is not a validated surrogate for phenotypic mediation.

The architecture consequently separates three explanations for multi-target evidence: one principal mediator with non-mediating binders, several targets that contribute jointly, and unresolved discordance caused by context or measurement. These explanations should not be averaged into a single confidence score. A multi-target mechanism becomes stronger only when perturbing more than one engaged target changes the phenotype in a manner consistent with their proposed contributions. The intervention logic of rescue remains useful here because target alteration can test whether the observed phenotype depends on the proposed component rather than merely accompanies it [18].

Discordance should likewise be localized. Agreement in engagement but disagreement in phenotype points to a different problem from disagreement between two engagement assays or between genetic and chemical perturbation. The proposed approach therefore asks which link has failed before deciding whether the mechanism should be weakened, revised, or split into context-specific alternatives. **Table 4** summarizes the principal boundaries that should trigger adjudication rather than automatic escalation of causal language.

Table 4. Boundary conditions requiring target-evidence adjudication

Boundary	Misleading interpretation to avoid	Required adjudication
Weak probe fidelity	Captured protein equals parent-compound target	Verify probe/parent concordance
Broad engagement	Most enriched protein is causal	Test functional mediation
Discordant assays	One assay must be wrong	Examine assay principle and context
Multiple functional hits	Single-target mechanism	Test joint versus dominant contribution
Negative engagement test	Target is absent	Assess assay detectability and exposure
Context shift	Mechanism is universal	Re-test in relevant biological setting

Using causal confidence in drug-discovery decisions

Causal confidence is useful only if it changes what is done next. Human-genetic support provides a useful analogy: across drug-development portfolios, genetic support is associated with altered probabilities of success, but it does not guarantee the outcome of an individual target program [36]. Likewise, stronger compound–target causality should change the posture of a natural-product program without being converted into a deterministic success label. The relevant decision may be to invest in deeper mechanism work, test exposure in a more relevant system, compare analogues, or pause a target claim that remains underdetermined.

Genetics-guided prioritization demonstrates how heterogeneous evidence can be formalized while preserving indication specificity [37]. The present architecture does not import those scoring systems or propose universal numerical thresholds. Instead, it uses qualitative causal states to distinguish decisions such as further target deconvolution, discriminating rescue, context replication, exposure testing, or mechanism-supported prioritization. A lower-confidence target is not necessarily discarded; it is routed toward the experiment most capable of resolving the remaining uncertainty. Conversely, a higher-confidence target is not automatically advanced if the underlying phenotype lacks disease relevance or feasible exposure.

Evidence-integration platforms also show the value of retaining provenance across heterogeneous target–disease evidence [38]. Provenance is essential because several weak or correlated sources should not appear equivalent to one strong intervention. Where sources disagree, the disagreement should remain inspectable. This principle is particularly useful for natural products because computational predictions, proteomic hits, pathway

measurements, and phenotypic observations can be generated at different concentrations, times, or biological levels.

Limitations and validation boundaries

Chemical tools remain only as informative as their potency, selectivity, target engagement, controls, and biological context permit [39]. The proposed architecture cannot repair a weak perturbation merely by organizing its outputs more carefully. It also cannot define a universal minimum number of assays, because causal informativeness depends on the target, compound, model, and alternative explanations under consideration. Some targets may be inaccessible to one engagement method, while some phenotypes cannot be cleanly rescued without perturbing the wider system.

Negative evidence requires particular caution. Failure to observe a thermal or stability shift may reflect assay blind spots rather than absence of interaction [11]. Conversely, tissue-level engagement can be real while remaining insufficient to establish exposure at the relevant site of action, organismal efficacy, safety, or clinical benefit [12]. The architecture therefore includes an "uninformative test" possibility rather than forcing every negative result into falsification. This category is important because absence of evidence and evidence of absence have different causal implications.

The framework is also not prospectively validated. Its qualitative states and transition logic should be tested against blinded target-identification problems that include true negatives, ambiguous mechanisms, polypharmacology, probe failures, and context-dependent effects. Rigorous controls are particularly important because systematic examination of chemical-probe use has documented how inadequate selectivity assessment and controls can compromise mechanistic interpretation [34]. Prospective testing should therefore evaluate whether the framework improves experiment selection and error detection, not merely whether retrospective examples can be fitted to its categories.

Finally, target causality is only one layer of drug discovery. Even a well-supported molecular mechanism remains bounded by pharmacokinetics, tissue exposure, toxicity, disease relevance, reproducibility, and translational generalization. Those questions require their own evidence and should not be represented as automatic consequences of mechanistic confidence.

Conclusion

Target nomination and target validation should be treated as different scientific tasks. Omics signatures, morphological similarity, predictive models, chemical proteomics, and stability profiling can identify plausible targets or support physical engagement, but none automatically demonstrates that an engaged protein mediates a natural-product phenotype. Stronger causal interpretation emerges when evidence addresses different links in the compound–target–phenotype chain and, crucially, when interventions such as resistance, perturbation, rescue, or specificity challenges eliminate plausible alternatives.

The proposed causal evidence architecture preserves this non-equivalence. It separates nomination, engagement, functional implication, phenotype linkage, and rescue- or falsification-supported interpretation while retaining assay failure, probe distortion, context dependence, conflicting evidence, and polypharmacology as explicit possibilities. Orthogonality is defined by independence of the question tested rather than by assay count, and multi-target evidence is adjudicated rather than compressed into a single preferred explanation. Evidence can therefore strengthen, stall, branch, or retreat as new experiments constrain the working mechanism.

Causal confidence is a guide to the next experiment or decision, not a certificate of therapeutic validity. A mechanism-supported target may justify stronger prioritization or transition to exposure and context testing, but target causality does not establish pharmacokinetic adequacy, organismal efficacy, safety, clinical benefit, or implementation readiness. The value of the architecture lies in making those boundaries visible and in preserving the provenance of each inference. Its next test is prospective: whether directing experiments toward unresolved alternatives improves the reliability, interpretability, and efficiency of natural-product target assessment across chemically and biologically diverse systems.

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