



Natural Products Should Become Chemical Probes Before They Become Drug Leads to Separate Target Engagement from Phenotypic Association

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ABSTRACT

Natural products frequently enter lead-optimization programs because they produce compelling phenotypes, yet a phenotype identifies a biological response rather than its molecular cause. This translational perspective argues that, when a discrete or experimentally testable molecular mechanism is proposed, natural products should first be evaluated as fit-for-purpose chemical probes before medicinal chemistry is organized around lead status. The distinction matters because target engagement, selectivity, perturbational specificity, and causal contribution are different evidential questions. The analysis integrates contemporary chemical-probe standards, live-cell target-engagement methods, chemoproteomic selectivity profiling, natural-product target-identification strategies, orthogonal chemistry, and control design. On that basis, a probe-before-lead sequence is proposed in which a phenotypic association becomes progressively more interpretable only after the nominated target is engaged in a relevant biological context, competing target interactions are characterized, and orthogonal perturbations support the proposed molecular explanation. The framework does not treat probe quality as equivalent to drug-likeness, nor does it require every useful natural product to operate through a single target. Polypharmacological and mechanism-agnostic phenotypic programs remain legitimate when their evidential claims are correspondingly bounded. The practical implication is not that mechanistic work should delay all optimization, but that optimization should not harden an uncertain target narrative into a lead-development premise. The proposed sequence remains a conceptual translational strategy rather than a prospectively validated decision rule and requires testing across diverse natural-product classes, targets, assay systems, and disease contexts.

Key Words: Natural products, Chemical probes, Target engagement, Chemoproteomics, Phenotypic screening, Translational pharmacology

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INTRODUCTION

A natural product that suppresses a disease-relevant cellular phenotype has demonstrated biological activity, not a complete mechanism. Phenotypic drug discovery is valuable precisely because it can reveal useful biology without requiring a predefined target, but that advantage

creates a later attribution problem: the observed response may arise from one target, several targets, an indirect pathway effect, or a context-specific perturbation that is not captured by the preferred molecular explanation. Experience with phenotypic discovery therefore treats target deconvolution as a distinct scientific task rather than

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an automatic consequence of obtaining an active phenotype [1].

The same caution applies when chemical and genetic perturbations appear to converge. Their effects are produced through different intervention modes, kinetics, degrees of target suppression, compensatory responses, and off-target liabilities. Agreement can strengthen a hypothesis, but disagreement does not necessarily falsify the underlying biology, and either modality can generate misleading mechanistic confidence when treated as self-interpreting. Recent analysis of phenotypic screening emphasizes these limitations and reinforces the need to distinguish a reproducible phenotype from a securely attributed molecular cause [2].

Direct target-facing measurements change the evidential question. In a recent chemical-tool study, phenotypic screening was integrated with chemical proteomics and NanoBRET target-engagement validation, illustrating how a cellular response can be connected to a physical protein interaction rather than inferred from phenotype alone [3]. Such integration does not make every detected interaction causal. It does, however, establish a more defensible starting point for asking whether the proposed target is occupied at relevant concentrations, whether alternative binders exist, and whether functional perturbation tracks with engagement.

The central argument developed here is therefore deliberately conditional. When a natural product is being advanced under a target-centered therapeutic hypothesis, the molecule should first earn the status of an interpretable chemical probe. Probe before lead is proposed here as an evidential ordering principle, not as a rule that phenotypic discovery must begin with targets. It shifts the critical question from whether a molecule produces an attractive response to whether the molecular explanation used to justify optimization has survived direct and orthogonal testing. This distinction is especially consequential for natural products because chemically unusual structures and biologically rich phenotypes can make mechanistic plausibility appear stronger than the evidence establishing it.

Why natural products are frequently advanced before their mechanism is secure

Natural products occupy an unusually productive but difficult region of drug-discovery space. Their structural diversity, stereochemical complexity, biosynthetic provenance, and history of biological selection can generate pharmacology that is difficult to reproduce with simpler synthetic collections. At the same time, discovery programs may face challenges in supply, dereplication, structural characterization, tractable derivatization, target identification, and translation across biological systems.

Contemporary analysis of natural products in drug discovery therefore supports their continuing value while emphasizing that biological activity does not eliminate the chemical and mechanistic problems attached to the starting material [4].

This distinction becomes particularly important when the same molecule is treated simultaneously as a biological tool and a therapeutic lead. Natural products can serve as resources for chemical probes because their bioactivity can reveal protein function and pathway organization; however, a molecule used to interrogate biology is judged by whether it generates an interpretable perturbation, whereas a therapeutic lead must eventually satisfy exposure, safety, formulation, efficacy, and developability requirements that are only partly overlapping [5]. Collapsing these roles creates a translational shortcut: once a plausible target has been nominated, medicinal chemistry may begin optimizing potency or drug-like properties before the target explanation has been adequately challenged.

The difficulty is not an absence of target-identification technology. Contemporary natural-product workflows encompass affinity-based capture, photoaffinity strategies, stability-based approaches, proteome-wide mass-spectrometric methods, and other label-free technologies [6]. These strategies do not interrogate exactly the same molecular event and are vulnerable to different sources of bias. Label-based methods can alter the parent scaffold or depend on linker position and probe retention of activity; label-free methods can depend on protein abundance, detectability, thermal behavior, chemical stability, or other context-specific properties.

Recent comparisons of labeled and label-free natural-product target-identification strategies accordingly support complementarity rather than interchangeability [7]. A target recovered by one approach may be a credible mechanistic candidate, but the experiment that produced the candidate also determines what can legitimately be inferred from it. A capture experiment may establish enrichment under defined conditions; a stability shift may indicate ligand-dependent protein behavior; a phenotypic correlation may prioritize a pathway. None should silently be converted into the stronger claim that the nominated protein is the direct and phenotype-driving target.

For this reason, “mechanism secure” should not mean simply that a single target name has been produced by one platform. It should mean that the target claim has reached an evidential state appropriate to the therapeutic decisions being made. The distinctions developed to this point are summarized in **Table 1** so that bioactivity, candidate identification, probe use, and mechanistic qualification are not treated as interchangeable milestones.

Table 1. Distinct evidential states before a natural product is treated as a mechanistically qualified lead

Construct	What is established	What remains unresolved	Appropriate interpretation
Phenotypic association	Compound exposure is associated with a defined biological response.	Direct molecular cause, relevant binder, and contribution of alternative targets.	Bioactive starting point; not a mechanism.
Candidate target	A target is prioritized through an identification strategy.	Direct engagement, selectivity, and functional contribution to the phenotype.	Mechanistic hypothesis requiring further testing.
Probe-oriented natural product	The molecule is used to interrogate biological function or target relationships.	Whether tool modifications preserve pharmacology relevant to the parent structure.	Research-tool role; not automatically therapeutic-lead status.
Target-identification strategy	Labeled and label-free approaches provide distinct routes to candidate-target evidence.	Method-specific false positives, false negatives, and transferability across systems.	Complementary evidence requiring method-aware interpretation.

What makes a molecule a chemical probe?

A chemical probe is not defined merely by affinity for a protein or by the ability to change a phenotype. It is a fit-for-purpose experimental reagent whose chemical identity, potency, selectivity, cellular activity or engagement, concentration of use, and control strategy are sufficiently characterized to make the resulting perturbation interpretable. Current probe-selection guidance converges on this multidimensional view while emphasizing that criteria must be adapted to probe modality, including covalent probes and targeted degraders [8–10].

That definition creates an important asymmetry between a probe and a lead. For a probe, the primary question is epistemic: can the molecule generate a perturbation from which a defensible conclusion about target function can be drawn? For a lead, the eventual question is therapeutic: can an optimized molecule deliver a favorable intervention in an organism at an acceptable exposure, safety, and pharmaceutical profile? These questions overlap, but they are not equivalent. A natural product may be valuable as a mechanistic probe yet be unsuitable as a drug starting point because of instability, difficult synthesis, limited exposure, reactive chemistry, or other developability constraints. Conversely, a chemically attractive development candidate can remain a poor probe if the molecular basis of its phenotype is ambiguous.

The proposed perspective therefore uses probe quality as a gate on interpretation, not as a synonym for universal molecular excellence. A probe can be adequate for one target, concentration range, cell system, or mechanistic question and inadequate for another. Probe quality is consequently relational to the inference being attempted. Selectivity against irrelevant proteins does not compensate for failure to engage the nominated target in cells, and high biochemical potency does not compensate for an uncontrolled phenotype at the concentrations required experimentally.

This principle is particularly important for natural products because mechanistic interrogation frequently requires modification of structures whose stereochemistry, functional groups, conformational preferences, or intracellular distribution contribute to activity. Probe construction can therefore improve observability while simultaneously changing the object being observed. A natural-product derivative bearing a linker, reporter, reactive group, or affinity handle cannot simply inherit the mechanistic claims attached to the parent compound. Before a target-centered lead program is justified, the probe system should support experiments whose positive and negative outcomes can both be interpreted without assuming the conclusion that the experiments were designed to test.

Target engagement as the first mechanistic threshold

Once a target has been nominated, the first mechanistic question should be whether the natural product actually engages that target in the biological context in which the relevant phenotype is observed. Target-engagement assays are designed to distinguish physical interaction or occupancy from biochemical potency, pathway modulation, and downstream phenotypic response [11]. This distinction matters because potency measured against an isolated protein does not establish intracellular access, an adequate unbound concentration, correct subcellular localization, or occupancy of that target in an intact biological system.

The available experimental repertoire is broad. Fluorescent tracer systems can support BRET-, FRET-, and fluorescence-polarization-based measurements, while curated tracer resources can facilitate target-engagement assay development by identifying experimentally characterized starting reagents [12]. Other approaches exploit ligand-induced changes in thermal stability, proteolytic sensitivity, chemical reactivity, competitive binding, affinity enrichment, or direct binding-site capture.

Each format turns a particular physical consequence of ligand–protein interaction into an observable readout; consequently, the evidential meaning of “engagement” remains assay dependent.

Live-cell measurement illustrates why this layer cannot be replaced by isolated biochemical profiling. Quantitative analysis of cyclin-dependent kinase inhibitors using live-cell engagement assays demonstrated that cellular potency and selectivity relationships can differ from those inferred exclusively from biochemical measurements [13]. The implication for natural products is not that one assay platform should become mandatory. Rather, engagement should be established at a biological level appropriate to the mechanistic claim. If the claimed mechanism is intracellular, evidence confined to an isolated purified protein leaves a substantial inferential gap.

Accordingly, this article proposes target engagement as the first mechanistic threshold after phenotype-to-target nomination. Passing that threshold means that the nominated target is demonstrably contacted under conditions relevant to the experiment. It does not establish that target engagement caused the phenotype, that the interaction is selective, or that the engaged target will be therapeutically useful. Those stronger claims require additional evidence.

This ordering also changes how an apparently negative result is interpreted. Failure to reproduce a phenotype after medicinal-chemistry modification may reflect loss of target engagement rather than invalid biology; conversely, preserved phenotype despite loss of nominated-target engagement should trigger reconsideration of the mechanistic hypothesis. Engagement therefore functions not only as confirmation but as a way to make discordance experimentally informative before a lead program becomes anchored to an uncertain molecular explanation.

Selectivity as a requirement for interpretation

Target engagement becomes mechanistically useful only when interpreted against the rest of the molecule’s interaction landscape. A natural product that occupies the nominated target while simultaneously engaging other proteins capable of producing the same phenotype cannot be treated as target-specific evidence. Large-scale chemical-proteomic analyses of kinase inhibitors and cysteine-reactive chemotypes demonstrate that compounds classified by a preferred target can possess broader and chemotype-dependent interaction landscapes than would be inferred from a single biochemical assay or nominal target designation [14–16].

Selectivity should therefore be treated as a relational property rather than a permanent label attached to a structure. It depends on which proteins are interrogated, compound concentration, exposure duration, cellular

permeability, subcellular localization, target abundance, competing proteins, assay format, and the biological state in which the molecule is tested. A compound may appear selective in a biochemical panel yet become less discriminating in cells because intracellular exposure or protein abundance changes the set of meaningfully occupied targets. Conversely, a nominal biochemical off-target may contribute little in a cellular context if it is inaccessible or insufficiently occupied.

This has two consequences for probe-before-lead reasoning. First, selectivity profiling should be designed around the inference required from the probe rather than around an abstract demand for absolute single-target action. A probe can remain informative despite documented secondary interactions when those interactions are experimentally characterized and cannot plausibly account for the measured phenotype under the conditions used. The important requirement is not chemical exclusivity but sufficient discrimination among competing mechanistic explanations.

Second, occupancy and selectivity must be interpreted together. A weak interaction can become experimentally important when the working concentration produces substantial occupancy, whereas an apparently potent biochemical interaction may have little functional relevance if the corresponding protein is not meaningfully reached in the relevant compartment. The concentration used to generate the phenotype therefore becomes part of the mechanistic claim: increasing exposure to obtain a larger response can also move the experiment into a different target-interaction regime.

The proposed threshold is consequently perturbational specificity rather than purity of action. The question is whether the observed biological effect can reasonably be attributed to the nominated target once competing engagements have been measured and constrained. This formulation deliberately preserves space for multi-target pharmacology while preventing a target-centered lead narrative from resting on engagement data that were never interpreted against realistic alternatives. Selectivity is thus not the end of mechanistic validation; it is the condition that makes subsequent causal tests interpretable.

Cellular perturbation without target attribution

A cellular response remains mechanistically ambiguous even when it is accompanied by extensive molecular change. Proteomic, transcriptomic, imaging, or pathway-level perturbations may reveal what happens after compound exposure without identifying which molecular interaction initiated the response. Cell-compatible photoaffinity chemoproteomics can narrow this gap by mapping small-molecule binding sites directly in cellular

systems, yet a detected binder still requires functional interpretation [17].

Activity-based protein profiling extends target discovery into native proteomes and is particularly useful when ligandable residues can be interrogated chemically, but its observable proteome remains constrained by probe chemistry and analytical coverage [18]. More broadly, mass-spectrometry-based proteomics can support target identification, engagement analysis, mechanism-of-action studies, and pharmacodynamic characterization, but these outputs occupy different evidential levels [19].

The practical distinction is therefore between molecular change and molecular cause. A protein whose abundance, phosphorylation state, localization, or thermal behavior changes after treatment may sit downstream of the relevant interaction. For a target-centered natural-product hypothesis, such perturbational evidence should prioritize mechanistic experiments rather than substitute for them. The proposed probe-before-lead sequence therefore requires direct target-facing evidence before downstream cellular consequences are interpreted as target attribution.

Orthogonal chemistry for testing causality

No single target-identification method eliminates the possibility of method-specific artefact. Chemoproteomic approaches differ in capture chemistry, competition format, proteome coverage, dependence on ligand derivatization, and sensitivity to protein abundance or

accessibility. Their value therefore increases when mechanistically different methods converge on the same target hypothesis [20].

Reversible-ligand chemical proteomics can identify binding sites in native systems and thereby strengthen direct-interaction evidence, but binding-site detection does not by itself establish that the interaction drives the phenotype [21]. Natural-product probe construction adds another complication: installing a photoaffinity group, reporter, linker, or enrichment handle can change affinity, localization, permeability, or the off-target profile. Work with honokiol-derived probes illustrates the utility of purpose-built natural-product probes while also reinforcing the need to establish that the modified probe retains pharmacology relevant to the parent structure [22]. Orthogonality should therefore mean more than repeating a result with a second version of the same assay. The strongest mechanistic case combines distinct questions: does the molecule engage the proposed target, does an independent chemistry reproduce that interaction, do competing interactions remain plausible, and does functional perturbation track with the target hypothesis? Convergence raises mechanistic confidence; discordance should redirect investigation rather than be suppressed to preserve a lead narrative.

Table 2 distinguishes the evidential questions that orthogonal experiments are intended to separate.

Table 2. Experimental distinctions required to move from molecular interaction toward causal interpretation

Evidence type	Primary question	Positive result supports	Does not establish
Direct engagement	Is the nominated target contacted in the relevant system?	Physical interaction or occupancy.	Phenotype causality.
Binding-site mapping	Where does the molecule interact with the protein?	Site-level interaction evidence.	Functional consequence.
Orthogonal chemistry	Does independent chemistry recover the same target relationship?	Reduced dependence on one capture strategy.	Complete selectivity.
Parent–probe concordance	Does the derivatized probe retain relevant parent behavior?	Interpretability of probe-based target identification.	Identical pharmacology in every context.
Functional perturbation	Does changing target function alter the compound response?	Contribution of the target hypothesis.	Therapeutic validity by itself.

The completed argument is synthesized in **Figure 1** as a proposed hierarchy in which phenotype remains the starting observation, whereas lead-level mechanistic

confidence depends on progressively constrained interpretation.

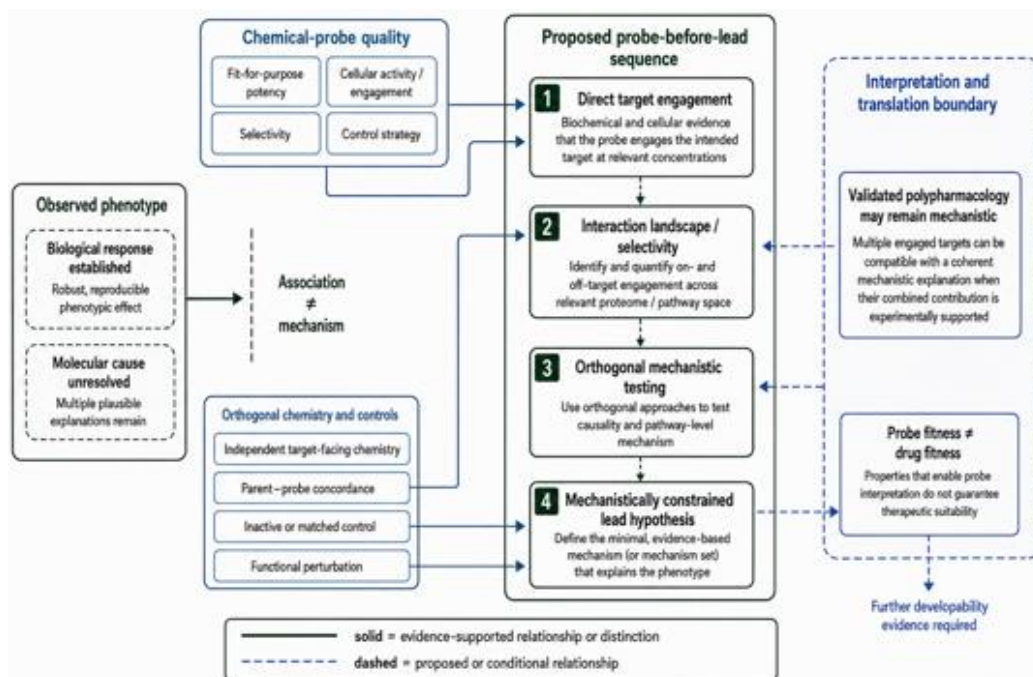


Figure 1. From phenotypic association to a mechanistically constrained lead hypothesis: a proposed probe-before-lead framework

Probe-quality analogs and inactive controls

Controls determine whether an apparently specific probe result can support a target-level inference. A systematic assessment of cell-based biomedical research found substantial shortcomings in chemical-probe use, including inappropriate probe selection, concentrations, and control practices [23]. For natural products, this problem is amplified when structural modification changes physicochemical behavior or when the parent molecule already engages several proteins.

An inactive analogue is useful only if its loss of activity is mechanistically interpretable. Comparative analysis of chemical-probe negative controls shows that an analogue can lose off-target interactions together with intended-target activity, making an active-versus-inactive comparison deceptively clean [24]. A stronger design therefore combines, where feasible, a matched inactive control with an independently structured active probe and direct engagement measurements.

The purpose is not to manufacture perfect controls. It is to expose alternative explanations. If two distinct active chemotypes engage the same target and reproduce the phenotype while a well-characterized inactive analogue does neither, confidence increases. If the controls disagree, the disagreement is mechanistically informative and should precede further lead optimization.

A probe-before-lead development sequence

Natural-product target identification now includes affinity-based, stability-based, proteomic, and phenotype-linked approaches that can progressively refine candidate

mechanisms [25]. The perspective proposed here organizes these capabilities into a sequence: establish the phenotype, nominate plausible targets, demonstrate relevant engagement, characterize competing interactions, test the mechanism with orthogonal chemistry and controls, and only then use the resulting evidence to justify target-centered lead optimization.

Computational or integrated phenotype–interaction approaches can be valuable earlier in this sequence because they prioritize hypotheses efficiently. A recent natural-product workflow combining virtual interaction and phenotypic target characterization illustrates this hypothesis-generating role [26]. Such convergence should not, however, be relabelled as direct engagement when physical interaction has not been measured.

The sequence is therefore a decision architecture rather than a mandatory experimental checklist. Different targets and compound classes will require different assays, and some steps can proceed iteratively. Its purpose is to prevent increasingly expensive optimization from depending on a mechanistic assertion whose evidential status has never been made explicit.

When probe optimization and drug optimization diverge

Natural-product probe development frequently modifies a scaffold to create observability: a handle may permit enrichment, photocrosslinking, fluorescence, or quantitative engagement analysis. Reviews of natural-product-derived probes illustrate how such modifications can illuminate drug–target relationships while introducing

design requirements that are specific to mechanistic interrogation [27].

Drug optimization asks different questions. Exposure, stability, metabolic liability, formulation, safety margins, synthetic accessibility, and efficacy may favor structural changes that are irrelevant—or even undesirable—for a research probe. Conversely, a highly informative probe may contain a reporter or reactive element that would never be retained in a therapeutic candidate.

Accordingly, successful probe development should generate a lead-optimization hypothesis, not automatically a lead molecule. The mechanistic information can identify which structural features preserve target engagement, which interactions should be avoided, and which pharmacological assumptions deserve further testing. Probe quality and drug quality can converge, but neither should be used as a surrogate for the other.

Using probe evidence to rescue or reject therapeutic hypotheses

Probe evidence is valuable not only when it confirms a preferred mechanism. Its greater translational value may be its capacity to reveal that an attractive phenotype has been assigned to the wrong target. Natural-product chemoproteomics can connect target identification with downstream functional validation, as illustrated by work linking Naamidine J to CSE1L in an acute-lung-injury model [28]. That study supports the feasibility of moving from target discovery toward functional interrogation in a defined biological setting, not a general claim of clinical validity.

Within the proposed framework, a therapeutic hypothesis is rescued when direct engagement, selectivity, orthogonal chemistry, and functional evidence converge sufficiently to make the target explanation more credible. It is redirected when another interaction better explains the phenotype, and rejected when the nominated target cannot account for the response under conditions in which the compound remains active.

This framing makes negative mechanistic evidence productive. Abandoning an incorrect target narrative can preserve a useful phenotype while preventing optimization around the wrong molecular premise.

Exceptions for polypharmacological and phenotypic mechanisms

A probe-before-lead strategy should not turn single-target pharmacology into an ideological requirement. Structurally related multi-kinase inhibitors can produce divergent cellular activities through cumulative effects across individual targets, demonstrating that a biologically meaningful phenotype may be distributed across several molecular interactions [29].

The appropriate exception is therefore validated polypharmacology, not mechanism-free attribution. When multiple targets jointly generate the response, mechanistic work should determine which interactions occur at relevant exposures and whether their combined perturbation reproduces the phenotype. The experimental unit of explanation becomes a target combination rather than one protein.

Some discovery programs may also rationally remain phenotype-led because the therapeutic value of a reproducible systems-level response is clearer than any single molecular explanation. In those cases, the claim should remain phenotype-centered. The framework objects only when a target-specific development narrative is adopted without target-specific evidence.

Translational boundaries

The proposed sequence has several boundaries. First, chemical-probe quality does not establish pharmacokinetic adequacy, safety, formulation feasibility, in vivo efficacy, or clinical benefit. Probe assessment answers whether a mechanistic experiment is interpretable, not whether a medicine will succeed.

Second, engagement and selectivity are conditional on biological context. A target profile measured at one concentration, exposure duration, cell state, or protein-abundance regime may change elsewhere. Target-engagement evidence must therefore be matched to the mechanistic claim rather than treated as a permanent property of the molecule [11].

Third, target-identification technologies remain incomplete. Proteomic methods can miss low-abundance, analytically inaccessible, transient, or chemically incompatible interactions [19]. Negative controls can also fail when structural changes alter off-target pharmacology [24]. Absence of detection is consequently not identical to absence of interaction.

Finally, polypharmacology complicates binary decisions about “the” target. Multi-target explanations remain compatible with a probe-first philosophy when the relevant engagements and their functional contributions are experimentally constrained [29]. The framework should therefore be tested prospectively across chemically and biologically diverse natural-product programs before any claim is made that it improves attrition, efficiency, or therapeutic success.

CONCLUSION

Natural products can generate compelling phenotypes long before their molecular causes are secure. That asymmetry is scientifically productive during discovery but becomes risky when a provisional target explanation begins

directing medicinal chemistry, pharmacology, and translational investment.

This perspective proposes that a natural product advanced under a target-centered therapeutic hypothesis should first become an interpretable chemical probe. The sequence separates phenotype from engagement, engagement from selectivity, selectivity from causal contribution, and mechanistic confidence from drug developability. It also treats orthogonal chemistry, negative controls, and discordant results as instruments for challenging a mechanism rather than ceremonial confirmation steps.

The proposal is intentionally conditional. It does not require every useful phenotype to collapse into a single target, and it allows experimentally supported polypharmacological mechanisms. Nor does probe quality guarantee exposure, safety, efficacy, or clinical value. Its purpose is narrower: to ensure that lead optimization is built on a molecular explanation proportionate to the evidence supporting it.

For natural-product discovery, the most valuable probe may therefore be the one that changes the development decision. It may strengthen a target hypothesis, redirect it toward another interaction, or show that the phenotype should remain mechanistically unresolved. Each outcome is preferable to optimizing a molecule around a mechanism that was never adequately tested.

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