



Electrophilic Natural Products as Covalent Drug Leads Through Reactivity Control, Target Selectivity, Proteome Engagement, and Medicinal Chemistry Optimization

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ABSTRACT

Electrophilic natural products are often interpreted through an unhelpful binary: their reactive groups are either treated as liabilities to be removed or as privileged motifs that confer covalent pharmacology. Neither position adequately reflects how covalent drugs are discovered or how natural electrophiles behave in biological systems. This conceptual framework examines electrophilic natural products as candidate covalent leads by separating intrinsic chemical reactivity from molecular recognition, target-site engagement, kinetic behavior, proteome selectivity, functional pharmacology, and developability. Warhead identity alone cannot determine whether covalency will be productive, selective, reversible, durable, or toxic. Natural electrophiles instead occupy a continuum in which scaffold architecture, electronic activation, local protein environment, exposure, and time jointly influence observed reactivity. Building on these distinctions, the article develops a proposed Reactivity–Recognition–Engagement framework for lead selection and positions chemoproteomic evidence as a critical gate between plausible covalent binding and credible proteome-level selectivity. Medicinal chemistry is treated not as a process of eliminating electrophilicity, but of tuning warhead reactivity, recognition geometry, and exposure so that target engagement is favored over uncontrolled chemical attack. The framework does not define validated thresholds, predict clinical safety, or establish universal advancement rules. Its purpose is to organize experimental evidence so that electrophilic natural products are neither rejected because they react nor advanced merely because they covalently bind. Electrophilicity is thereby reframed as a controllable pharmacological variable rather than a categorical lead-selection verdict.

Key Words: *Electrophilic natural products, Covalent drugs, Chemoproteomics, Kinetic selectivity, Medicinal chemistry, Target engagement*

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INTRODUCTION

Covalent pharmacology has moved from a liability-centered view of chemical reactivity toward a design problem in which bond formation can be useful when coupled to molecular recognition, appropriate kinetics, and controllable exposure. Modern covalent drugs demonstrate that irreversible or reversible bond formation can be

deliberately engineered, but their success does not imply that an electrophilic group is intrinsically favorable [1]. For natural products, the interpretive problem is sharper. Electrophilic natural products can function as mechanistic probes, reveal ligandable residues, and provide starting points for pharmacological intervention, yet their biological activities may also involve multiple targets or chemically promiscuous reactions [2].

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The central mistake is therefore to classify electrophilic natural products by warhead presence alone. Contemporary covalent medicinal chemistry encompasses electrophiles with substantially different reaction mechanisms, activation requirements, steric environments, and opportunities for electronic tuning [3]. A chemically reactive motif is only the beginning of the pharmacological question. What matters is whether the parent scaffold directs that reactivity toward a biologically meaningful nucleophile at a rate, concentration, and duration compatible with preferential engagement. The same formal electrophilic functionality may consequently behave very differently when embedded in different molecular architectures or exposed to different protein environments. This article develops that distinction into an original conceptual framework for electrophilic natural-product lead selection. It treats intrinsic reactivity, pre-covalent recognition, proteome engagement, kinetic selectivity, downstream function, exposure, and safety as connected but non-equivalent evidential domains. The proposed framework does not assign universal numerical thresholds, convert a covalent adduct into evidence of efficacy, or treat a restricted chemoproteomic profile as proof of clinical safety. Instead, it organizes the questions that should be answered before electrophilic natural products are either discarded as indiscriminately reactive or advanced because a preferred target can be modified.

Under this view, electrophilicity is a design variable whose value is conditional on biological discrimination. A credible covalent lead is therefore not simply a molecule that reacts, nor even one that reacts with an intended protein. It is a scaffold in which chemical reactivity can be progressively reconciled with recognition, proteome-level engagement, kinetic behavior, functional consequence, exposure, and de-risking.

Natural electrophiles across a continuum of chemical reactivity

Natural electrophiles occupy a chemically and biologically heterogeneous space. Their ability to modify nucleophilic residues can be measured directly in complex proteomes: multiplexed thiol-reactivity profiling has shown that electrophilic natural products can generate distinguishable

cysteine-engagement patterns and nominate candidate protein targets [4]. Such observations argue against treating “electrophilic” as a single mechanistic category. They also show why observed cellular reactivity cannot be reduced to the nominal presence of an enone, epoxide, aldehyde, or another reactive motif.

A useful counterexample to a simple high-reactivity model is the natural-product-inspired latent electrophile examined by Byun and colleagues. A comparatively tempered electrophilic system could still support covalent inhibition when embedded within an appropriate recognition scaffold [5]. Productive covalency can therefore emerge from conditional chemical reactivity and target proximity rather than from maximizing solution-phase electrophilicity. This distinction is particularly important for natural-product medicinal chemistry because reducing intrinsic reactivity need not eliminate useful covalent pharmacology when scaffold architecture preserves productive recognition.

Authentic natural products also demonstrate that covalent engagement is not confined to synthetic warhead design. Withaferin A and withanone can form covalent interactions with the SARS-CoV-2 main protease in a defined biochemical setting [6]. That finding establishes target-level covalent chemistry for those compounds in the studied system; it does not establish organism-level efficacy, proteome selectivity, favorable exposure, or clinical utility. The distinction illustrates why evidence for bond formation and evidence for drug-like behavior must remain separate.

Electrophilic natural products are therefore better considered along a qualitative continuum of effective biological reactivity. Intrinsic electrophilicity, scaffold-controlled approach to the target, local residue environment, competing nucleophiles, concentration, and time can shift where a compound appears on that continuum. Unless these factors are measured under comparable conditions, the continuum should not be converted into an artificial numerical ranking.

The constructs needed to interpret this continuum without collapsing chemical reactivity into pharmacological selectivity are summarized in **Table 1**.

Table 1. Operational distinctions for interpreting electrophilic natural products as covalent lead candidates

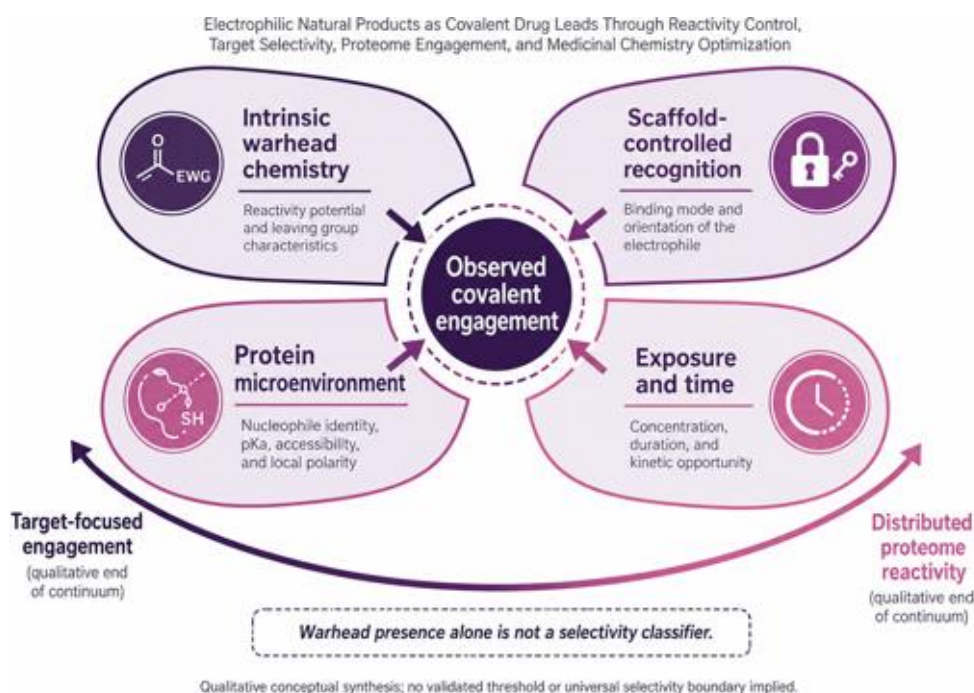
Construct	Operational meaning in this framework	Evidence it can provide	Inference it cannot provide
Electrophilic warhead	Structural functionality capable of reacting with an appropriate biological nucleophile	Plausibility and mechanism of covalent chemistry	Target selectivity, efficacy, or safety
Intrinsic reactivity	Electrophile reaction propensity under defined chemical conditions	Comparative chemical behavior under matched conditions	Cellular target preference or proteome selectivity
Proteome engagement	Experimentally detected modification of protein sites in a biological mixture	Identity and distribution of detectably occupied sites	Functional importance of every occupied site

Target-directed covalency	Bond formation favored by scaffold recognition and productive target geometry	Mechanistically plausible preferential engagement	Universal selectivity across concentrations or proteomes
Defined natural-product target adduct	Direct covalent modification of a specified protein by a natural product	Target-level covalent interaction	In vivo therapeutic effectiveness or acceptable safety

Note: These definitions are operational components of the proposed framework rather than validated kinetic, regulatory, or safety classifications.

Figure 1 integrates the section's central inference: electrophilic functionality acquires pharmacological meaning only after the chemical scaffold is interpreted

within the molecular and biological conditions governing observed covalent engagement.



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Figure 1. Electrophilicity becomes pharmacologically meaningful only through context

Warhead identity, intrinsic reactivity, and biological environment

Warhead identity matters because electrophiles engage nucleophiles through different chemical mechanisms and display different dependencies on substitution, activation, steric access, and local environment. Medicinal-chemistry analyses of targeted covalent inhibitors show that warhead classes can be tuned across broad chemical space rather than treated as interchangeable reactive handles [7]. For electrophilic natural products, however, the reactive group is embedded within a larger scaffold whose conformation, polarity, substituent pattern, and noncovalent contacts can change the conditions under which bond formation becomes favorable.

This distinction becomes especially important when intrinsic reactivity is estimated using simplified chemical surrogates. N-Acryloylindole-based chemoproteomic probes illustrate that electrophile design can change which cysteine and oxidized-thiol environments are detectably engaged in cells [8]. Such experiments are informative

about chemical behavior and accessible proteome space, but their results remain conditional on probe structure, concentration, uptake, accessibility, sample preparation, and analytical detection. Probe reactivity should therefore not be converted directly into a general ranking of natural-product selectivity.

Proteome-wide comparison of structurally diverse electrophiles provides a stronger reason to reject that simplification. Different electrophilic chemotypes produce distinct residue- and site-engagement patterns, demonstrating that proteome selectivity emerges from an interaction between electrophile chemistry and protein context rather than from warhead identity alone [9]. A warhead that appears moderate in a simplified nucleophile assay may react efficiently within a protein environment that supplies favorable positioning, electrostatics, or residue accessibility; conversely, substantial intrinsic electrophilicity need not guarantee access to a particular target nucleophile.

The practical consequence is that intrinsic reactivity should be measured and optimized without being promoted to a surrogate for biological selectivity. A useful electrophile is not merely one that reacts slowly enough to appear safe or rapidly enough to secure occupancy. Its reactivity must be compatible with the recognition, localization, and exposure conditions that discriminate the intended site from competing nucleophiles.

Proximity-driven covalency versus uncontrolled chemical attack

The mechanistic value of covalency depends critically on what happens before bond formation. In proximity-driven covalency, noncovalent recognition first positions an electrophile close to a geometrically and chemically compatible nucleophile, increasing the probability that reaction occurs within the intended complex. Precision-guided reversible covalent JNK inhibitors demonstrate that scaffold architecture can direct a Michael-acceptor warhead toward a selected cysteine while retaining reversible covalent behavior as an explicit design feature [10]. This establishes the feasibility of recognition-enabled covalency in a defined kinase system; it does not establish a universal property of Michael acceptors.

Natural-product-inspired chemistry provides a complementary example. Discovery of a drug-like DCAF11 ligand chemotype based on an arylidene-indolinone scaffold shows that a natural-product-like electrophilic architecture can combine molecular recognition with covalent engagement in a tractable ligand [11]. Together with latent-electrophile design [5], this evidence supports a central medicinal-chemistry principle: maximal bulk electrophilicity is unnecessary when recognition and geometry increase the effective probability of reaction at the intended site.

The opposite state—uncontrolled chemical attack—should likewise not be inferred merely from the presence of a reactive motif. In the framework proposed here, it denotes a pattern in which covalent reaction becomes insufficiently discriminating across competing biological nucleophiles relative to the intended target. Proteome-wide electrophile comparisons show why that interpretation must remain context dependent [9]. Broad engagement may change with concentration, exposure duration, cellular environment, or scaffold modification; therefore, no fixed structural boundary can separate productive from uncontrolled covalency.

Proximity-driven covalency and uncontrolled attack are consequently mechanistic interpretations requiring comparative evidence, not labels assigned by inspection. A solution-phase reactivity measurement can characterize one chemical dimension, a target adduct can establish bond formation, and a potency assay can establish an

experimental response, but none alone demonstrates that recognition governs reaction in a cellular proteome. The distinction ultimately requires convergence among chemical reactivity, target recognition, kinetic behavior, and proteome engagement.

Target selectivity must be defined at the proteome level

A covalent lead cannot be called selective merely because it modifies an intended purified target. Site-resolved chemoproteomics can identify occupied residues across complex protein mixtures, moving attribution beyond potency or enrichment alone [12]. Activity-guided mapping in primary human T cells further shows that cysteine ligandability and functional consequence are heterogeneous in cells [13], while CysDB demonstrates that available quantitative cysteine evidence is distributed unevenly across proteins and datasets [14]. Failure to detect a site is therefore not proof that it is inaccessible.

Chemoproteomic selectivity also has measurement boundaries. Structural analysis has identified warhead- and coverage-specific biases in cysteine-focused datasets [15]. Proteome-wide mapping of cysteine-reactive drugs likewise shows that compounds can produce comparatively focused or widely distributed engagement patterns [16]. For electrophilic natural products, selectivity should therefore mean preferential intended-site engagement relative to measurable competitors at specified concentration and time, with negative findings qualified by coverage, abundance, accessibility, and analytical detectability. Detection establishes engagement; it does not establish the functional importance of every occupied site. Selectivity must also be interpreted relative to exposure. A compound that gives a narrow map at a low concentration but acquires numerous additional sites near the concentration required for function presents a different development problem from a compound whose target advantage remains stable.

Reversible and irreversible covalent engagement as different pharmacological strategies

Reversible and irreversible covalency are different pharmacological strategies, not a simple safety ranking. Reversible covalent inhibition depends on mass action, noncovalent affinity, and adduct stability [17]. Dynamic cysteine-reactive thiomethyltetrazines further demonstrate that covalent reversibility can be engineered and observed in live-cell experiments [18]. These findings establish design possibilities, not a general advantage for reversible warheads.

Irreversible engagement can instead provide durable occupancy when recognition is favorable and exposure is controlled. Its behavior nevertheless depends on target turnover and accumulation of off-target modification. The

relevant comparison is therefore whether either modality maintains useful separation between intended and unintended engagement over the biological time window of interest. Target turnover adds another layer: irreversible occupancy may outlast free drug when protein resynthesis is slow, whereas rapid turnover can shorten the functional consequence of an irreversible adduct. Reversible engagement may require sustained exposure to maintain occupancy. These are pharmacological consequences of the complete target–compound system rather than properties inferable from the word “reversible” or “irreversible” alone.

Kinetic selectivity and the time dimension of covalent binding

Covalent pharmacology is time dependent. Endpoint potency can misrepresent potency or selectivity when reaction continues during an assay, as shown for covalent EGFR inhibitors [19]. A single IC₅₀ can therefore confound recognition, reaction rate, and incubation time. Dose- and time-response analysis can estimate (K_I), providing a more mechanistically informative comparator

for covalent fragments and leads [20]. COOKIE-Pro extends this logic to the proteome by estimating (k_{inact}) and (K_I), for intended and competing proteins [21]. These parameters do not predict efficacy or safety, but they help reveal whether selectivity arises mainly from recognition, covalent rate, or both. This distinction matters for medicinal chemistry because two analogues with similar endpoint inhibition can embody different optimization problems. One may possess strong pre-covalent recognition but a poorly positioned or weakly reactive electrophile; another may compensate for weak recognition through faster covalent chemistry. Their apparent potencies can converge even though the second profile is more vulnerable to off-target reaction as exposure increases.

In the proposed framework, kinetic selectivity is the time-resolved separation between intended and competing engagement under specified conditions. **Table 2** distinguishes the principal kinetic quantities; **Figure 2** shows how prolonged exposure can preserve or erode an

Table 2. Mechanistic distinctions needed to interpret time-dependent covalent engagement

Dimension	Interpretive value	Boundary
Endpoint potency	Activity under one assay time	Can shift as reaction proceeds
(K_I)	Affinity-related pre-covalent component	Context and model dependent
(k_{inact})	Covalent inactivation-rate component	Does not describe exposure
(k_{inact}/K_I)	Matched-condition covalent efficiency	Not equivalent to cellular selectivity
Reversible covalency	Dynamic adduct occupancy	Reversibility does not ensure selectivity
Proteome kinetic selectivity	On/off-target kinetic separation	Coverage remains incomplete

Note: No universal kinetic threshold for advancement is proposed.

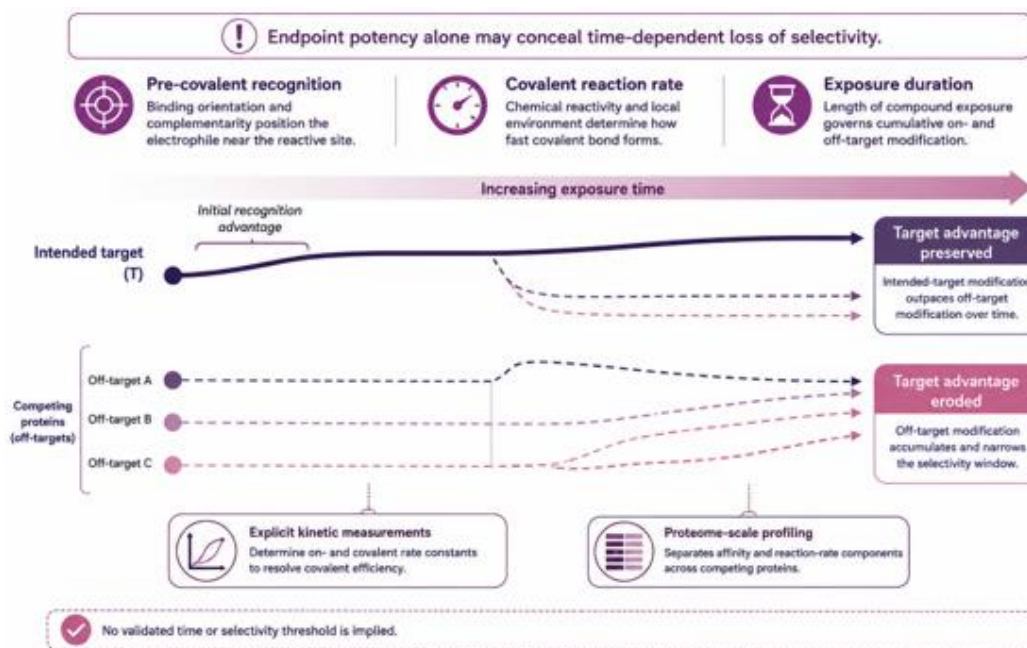


Figure 2. Recognition advantage can persist or erode as covalent exposure proceeds

A reactivity–recognition–engagement framework for lead selection

The evidence supports separating three questions before integrating them. Reactivity asks what electrophilic chemistry the scaffold can express under defined conditions. Recognition asks whether noncovalent interactions and geometry preferentially position that chemistry near an intended nucleophile. Engagement asks what the compound actually occupies across the measurable proteome at relevant concentration and time. The Reactivity–Recognition–Engagement model is proposed here as an organizing framework, not a validated score.

The framework should not be cysteine-exclusive because covalent ligand discovery increasingly addresses other nucleophilic residues, although evidence depth remains uneven across residue classes [22]. Candidates can fail at any dimension: excessive reactivity can overwhelm recognition; strong recognition can coexist with inefficient covalency; favorable target kinetics can deteriorate at higher exposure; and selective occupancy can remain pharmacologically irrelevant.

Advancement should therefore favor controlled convergence rather than maximal electrophilicity: sufficient chemical reactivity, plausible recognition advantage, and preferential biological engagement. The framework deliberately avoids assigning weights to these dimensions. A strong result in one dimension cannot automatically compensate for a failure in another; exceptionally selective target binding, for example, does not rescue a scaffold whose metabolite reacts broadly, while low solution reactivity does not rescue a candidate that requires excessive exposure for occupancy.

The dimensions are also experimentally iterative. Medicinal-chemistry changes intended to reduce reactivity can alter conformation, permeability, or residence at the target, so the recognition and engagement layers must be re-measured rather than assumed to remain constant. Conversely, a change introduced to improve affinity may reposition the warhead and alter covalent kinetics. Functional consequence, exposure, metabolism, and safety therefore qualify whether controlled convergence survives optimization rather than merely whether it was observed in the starting natural product.

Chemoproteomic evidence as a gatekeeper for advancement

If proteome selectivity is central to an electrophilic lead, chemoproteomics should act as an advancement gate rather than a late confirmation. Label-free workflows can profile cysteine-reactive fragments at substantial depth and support earlier elimination of compounds whose apparent

target preference collapses in complex mixtures [23]. Coverage, however, remains incomplete.

Engagement becomes more interpretable when paired with independent biology. Integrated phenotypic and chemoproteomic analysis of dietary electrophiles in platelets demonstrates the complementarity of functional and target-engagement evidence [24]. Integrated chemical genomics and chemical proteomics can likewise strengthen target prioritization through orthogonal information [25]. Neither approach makes concordance equivalent to causal proof.

The proposed gate is therefore evidential, not numerical: advancement requires increasing concordance among direct site engagement, proteome distribution, concentration and time dependence, and orthogonal function. The most informative chemoproteomic experiment is consequently not always the one with the largest number of quantified sites, but the one that tests the relevant competing explanation: whether target engagement disappears when the recognition element is weakened, whether off-target occupancy expands with exposure, or whether a site-specific perturbation changes the phenotype. A restricted target map is favorable evidence, but it does not replace mechanism, pharmacology, exposure, or safety.

Medicinal chemistry strategies for tuning electrophilic natural product scaffolds

Natural-product scaffolds can intertwine recognition and electrophilicity, so optimization should not treat the warhead as an isolated substituent. Nimbolide illustrates this principle: chemoproteomics identified covalent engagement of RNF114 and enabled exploitation of that interaction in targeted protein degradation [26]. Subsequent work translated RNF114-directed covalent chemistry into simpler synthetic ligands and degraders that mimic a key component of the natural-product mechanism [27].

The broader medicinal-chemistry task is to preserve useful recognition while tuning electronics, steric access, scaffold geometry, permeability, and exposure. Natural-product complexity makes these changes especially interdependent: deleting a reactive substituent can remove the covalent mechanism, but modifying a remote stereochemical or lipophilic feature may also change pre-covalent orientation or cellular access. Matched analogue series should therefore measure chemical reactivity and biological engagement in parallel rather than assuming that structural changes act through one property only. Lower intrinsic reactivity is valuable only when sufficient target engagement remains; increasing reactivity is defensible only when recognition and exposure preserve discrimination. Optimization should seek the widest

practical separation between intended engagement and competing proteome reaction, rather than the weakest possible electrophile.

Translating covalent engagement into functional pharmacology

Target engagement is a mechanistic intermediate rather than a pharmacological endpoint. Nimbolide provides an example in which RNF114 engagement was connected to PARP1 trapping and context-dependent synthetic lethality in BRCA-mutated cancer models [28]. This supports a defined mechanism in the studied setting, not generalized anticancer efficacy.

Chemoproteomic and phosphoproteomic profiling of salvianolic acid A likewise connected covalent target evidence with mTORC1-associated signaling changes [29]. Such convergence strengthens functional interpretation while remaining compatible with polypharmacology.

The proposed framework therefore places functional pharmacology downstream of engagement. A credible mechanism should show more than co-occurrence between occupancy and response: perturbing the engaged target or residue should, where technically feasible, alter the response in the predicted direction, and functional effects should occur within an exposure range compatible with measured target engagement. Failure of these relationships does not prove that the compound lacks pharmacology; it indicates that the proposed target mechanism is incomplete or that polypharmacology remains plausible.

Safety liabilities that cannot be inferred from warhead presence alone

Covalent chemistry can produce persistent off-target adducts, reactive metabolites, and immune or organ-specific liabilities, but serious adverse-reaction risk cannot be predicted from electrophile presence alone [30]. Exposure, metabolic activation, tissue distribution, target turnover, proteome breadth, adduct persistence, and host response may contribute separately.

A selective chemoproteomic profile also does not establish safety. Proteome engagement can reveal broad reaction under tested conditions [16], while proteome-scale kinetics can show accumulation of off-target modification [21], but neither reproduces an organism. The appropriate safety question is whether the parent compound and relevant chemical species generated in vivo produce tolerable intended and unintended reactivity. Metabolic transformation may also unmask stronger electrophiles or change tissue distribution. Electrophilicity therefore requires empirical de-risking rather than structural exoneration or condemnation.

Development boundaries and experimental requirements

Negative chemoproteomic findings are provisional because peptide detectability, abundance, structure, preparation, and warhead-specific biases constrain coverage [15]. Advancement should therefore combine concentration- and time-resolved engagement with direct site assignment and orthogonal functional validation. Proteome-scale kinetics demonstrate that affinity-related and reaction-rate components can be separated across intended and competing proteins [21], while scalable chemoproteomics makes broader early triage increasingly practical [23].

Where metabolism can alter the reactive species, profiling the parent compound alone is insufficient. Development studies should also test whether the engagement pattern is reproducible across relevant cell states or tissues rather than assuming that one proteome represents the organism. Clinical safety remains outside the inferential reach of chemistry and proteomics; exposure, metabolites, tissue specificity, immune consequences, and conventional toxicology require dedicated assessment [30].

CONCLUSION

Control reactivity rather than eliminating it

Electrophilic natural products should neither be rejected because they contain reactive functionality nor advanced because they form a preferred covalent adduct. Their value depends on whether reactivity can be subordinated to recognition, maintained as preferential engagement across competing proteins, and translated into meaningful pharmacology at an exposure compatible with development.

The proposed Reactivity–Recognition–Engagement framework keeps intrinsic chemistry, pre-covalent recognition, proteome occupancy, kinetic discrimination, functional consequence, and developability distinct while showing how they must converge. Chemoproteomics and kinetic profiling reveal where apparent selectivity becomes distributed or time dependent; medicinal chemistry can then rebalance scaffold recognition, warhead behavior, and exposure.

The framework is conceptual, not prospectively validated. It supplies no universal thresholds and does not predict clinical safety. Its practical proposition is narrower: electrophilic-natural-product optimization should seek to control covalency well enough that reactivity becomes discriminating pharmacology rather than uncontrolled chemical attack. That reframing preserves useful electrophilic chemistry while demanding evidence that the biological system, not structural intuition alone, supports the claim of control.

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