



Spatial Pharmacology Reveals Where Natural Products Act Across Tissues, Niches, Microenvironments, Disease Compartments

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ABSTRACT

Natural-product pharmacology is usually interpreted through concentrations, targets, pathways, and phenotypes, yet each of these can vary across physical space. Plasma exposure or whole-tissue abundance may therefore coexist with markedly different concentrations across anatomical compartments, cellular neighborhoods, barrier environments, and metabolically distinct regions. This article develops an original conceptual framework for treating spatial location as an explicit coordinate of pharmacological inference. The framework separates four questions that are frequently conflated: where a compound or locally formed species is detected, whether pharmacologically relevant exposure is achieved at that location, whether a target or susceptible cellular state is available there, and whether a local response is observed. Spatial mass spectrometry imaging, spatial metabolomics, transcriptomics, proteomics, and multiplexed tissue imaging provide complementary evidence for these questions, but no single modality establishes site of action. Natural-product examples further show that formulation, tissue architecture, local metabolism, and disease microenvironment can reshape what reaches a region and what molecular response is subsequently measured. The proposed Location–Exposure–Target–Response framework therefore treats spatial mechanism as a convergence problem rather than a colocalization problem. It also distinguishes accumulation from action and requires uncertainty in molecular identity, quantitation, resolution, and registration to remain visible during interpretation. The framework is intended as an evidence-organizing and hypothesis-generating structure, not a validated predictor of efficacy, toxicity, or clinical tissue selectivity. Prospective testing should determine whether this structured convergence improves pharmacological interpretation or lead prioritization.

Key Words: Spatial pharmacology, Natural products, Mass spectrometry imaging, Spatial omics, Tissue microenvironment, Site of action

eIJPPR 2026; 16(3):166-174

HOW TO CITE THIS ARTICLE: Van der Meer E, Jansen L, de Vries B, Claes S. Spatial Pharmacology Can Reveal Where a Natural Product Actually Acts Across Tissues, Cellular Niches, Microenvironments, and Disease Compartments. Int J Pharm Phytopharmacol Res. 2026;16(3):166-74. <https://doi.org/10.51847/ru6XojoCiR>

INTRODUCTION

Pharmacological reasoning conventionally asks whether a compound reaches sufficient exposure, engages a relevant target, modifies a pathway, and produces a phenotype. Those questions are usually expressed without an explicit spatial coordinate, even though tissues are organized into compartments, cell populations, vascular and stromal

interfaces, and locally distinct biochemical environments. Mass spectrometry imaging has made this omission increasingly visible by allowing drugs, metabolites, and endogenous molecules to be mapped directly within intact tissue architecture [1]. The consequence is not that spatial imaging replaces pharmacokinetics or mechanistic pharmacology. Rather, it shows that “where” can be an

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Received: 21 March 2026; **Revised:** 23 June 2026; **Accepted:** 25 June 2026

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independent source of information about the plausibility of a claimed mechanism.

This distinction matters because a systemic concentration and a whole-organ concentration can conceal different local exposure states. Spatial analyses of absorption, distribution, metabolism, and toxicology demonstrate that drug-related signals can vary across tissue regions, while mechanistic modeling of the human central nervous system and brain tumors shows that penetration and exposure can differ across anatomically constrained compartments [2, 3]. The same problem is especially important for natural products, whose physicochemical heterogeneity, formulation dependence, metabolic conversion, and frequently pleiotropic phenotypes make a single averaged tissue value an uncertain proxy for the molecular species actually encountered by a target-bearing cell.

The central argument developed here is therefore deliberately narrower than a claim that spatial colocalization identifies mechanism. A compound can be present where it does not act, absent from a measured pixel despite biologically relevant exposure below detection, or converted locally into a species with a different distribution. Likewise, a target can be expressed where it is inaccessible, unengaged, or functionally irrelevant. This article proposes that site-of-action reasoning should preserve four analytically separate questions—location, exposure, target state, and response—and should only strengthen a mechanistic interpretation when evidence across these dimensions converges without being contradicted by plausible spatial alternatives.

Why whole-tissue measurements blur spatial mechanism

Whole-tissue measurements are powerful for quantifying average abundance, but averaging also removes information. Spatially resolved metabolomics shows that metabolite distributions can vary substantially within tissue and that spatial coordinates can reveal biochemical organization that bulk measurements merge [4]. For pharmacology, the implication is not that every local metabolic difference is functionally meaningful; it is that a single homogenized value cannot identify which anatomical or cellular region generated that value. A treatment-associated change measured in an organ homogenate can therefore reflect a broad shift, a highly localized response, altered cellular composition, or a mixture of these possibilities.

The same limitation applies to target-related biology. Spatial transcriptomics maps disease-associated expression states within tissue context and demonstrates that molecular programs relevant to drug discovery are not uniformly distributed [5]. Yet transcript abundance is not equivalent to protein abundance, target accessibility, engagement, or causal dependence. Spatial information narrows where a mechanism could plausibly operate; it does not, by itself, complete the mechanistic chain.

Cellular composition adds another level of averaging. Multiplexed single-cell pathology in breast cancer revealed highly structured cellular phenotypes and neighborhoods within tumors [6]. Although that evidence is disease-specific, it establishes a general interpretive problem: whole-tissue measurements can combine pharmacologically different cell populations into one apparent molecular state. **Table 1** organizes the distinct spatial constructs that such averaging can collapse while preserving the inferential boundary associated with each.

Table 1. Spatial constructs concealed by whole-tissue pharmacological averaging

Construct	Meaning in spatial pharmacology	What whole-tissue averaging can obscure	Interpretation boundary
Compartment	Anatomically delimited region with distinct access conditions	Unequal local distribution or retention	Regional abundance does not establish bioavailable exposure
Cellular niche	Local organization of cell types and states	Minority responsive or resistant populations	Cell presence does not establish target dependence
Microenvironment	Local physicochemical and biological conditions	Barrier, stromal, vascular, or matrix effects	Context may modify exposure without being the target
Molecular species	Parent compound, metabolite, conjugate, or other locally present form	Different species occupying different regions	Detection does not establish pharmacological activity
Target state	Local expression or pathway availability	Spatially restricted therapeutic opportunity	Abundance does not equal engagement
Response state	Local molecular or phenotypic change	Regional effects diluted in organ averages	Response does not retrospectively prove upstream causality

note. These distinctions are synthesized for the proposed framework and organize spatial variables that should remain analytically separate during site-of-action interpretation.

Figure 1 extends this problem beyond tissue averaging by showing how distribution, biological context, local metabolism, measurement, and response must remain

distinct until convergent evidence supports a spatial mechanism.



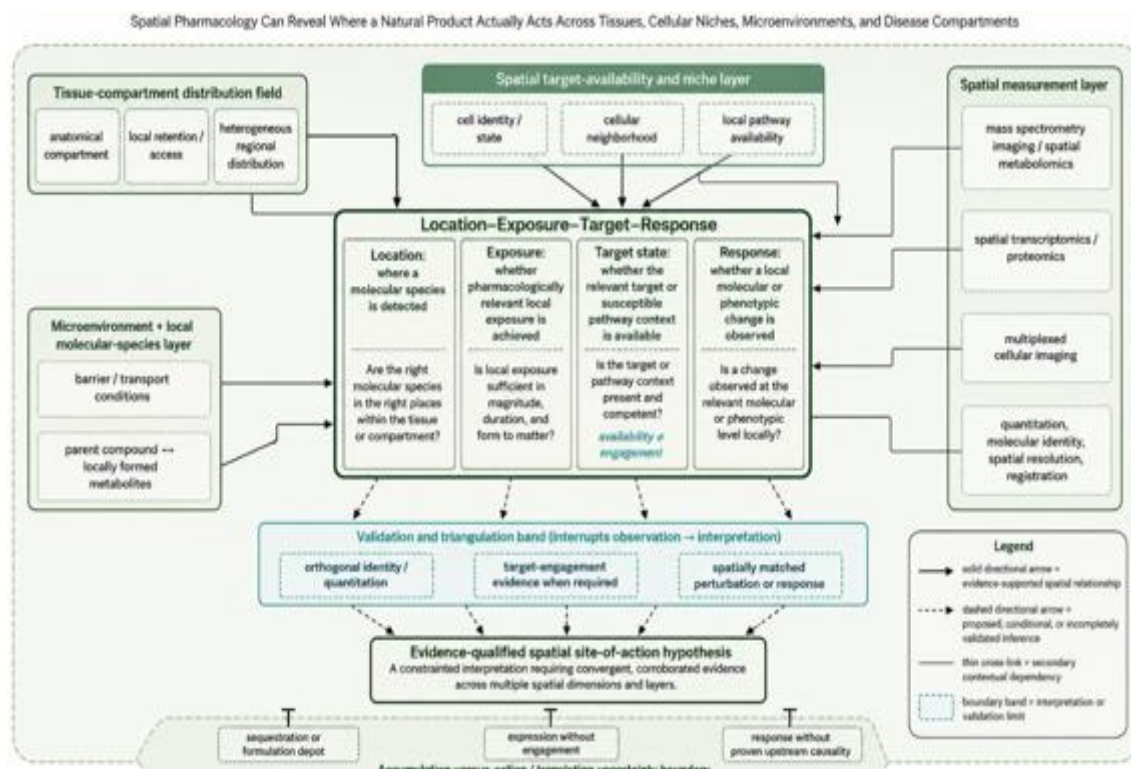


Figure 1. Spatial pharmacology as a multilevel constraint on site-of-action inference

Compound distribution across tissue compartments

Spatial heterogeneity begins with the compound itself. In a transdermal delivery model, mass spectrometry imaging visualized the distribution of berberine delivered from chitosan microneedles, demonstrating that a natural-product molecule can be mapped in relation to formulation and tissue penetration [7]. The relevant inference is limited but important: formulation and route help determine where a compound is detected. A strong local signal may still represent a depot, extracellular retention, binding to tissue components, or total rather than unbound drug. Consequently, distribution should be treated as a spatial exposure precursor, not as a synonym for site of action. Natural-product toxicology provides a second example. Dual mass spectrometry imaging and spatial metabolomics have been used to examine nitidine chloride disposition together with nephrotoxicity-associated metabolic changes [8]. Such co-mapping can reveal whether exposure and biological perturbation occupy overlapping or discordant regions. It cannot establish that a particular metabolic alteration is a direct mediator of toxicity without additional mechanistic evidence. This distinction is central to a spatial framework because a local response may lie downstream of tissue injury, compensatory biology, or a neighboring-cell effect rather than direct compound action. Distribution is also conditioned by tissue context. Multimodal molecular imaging has shown that nanomedicine distribution can correlate with features of the tumor microenvironment [9]. Although nanomedicine

transport cannot be generalized directly to free natural-product molecules, the study demonstrates the broader point that tissue architecture can shape where therapeutic material accumulates. A spatial pharmacological description should therefore identify the compartment, route and formulation, and should state whether the mapped signal represents parent compound, derived species, or an analytically unresolved feature.

Spatial variation in target availability

A compound can reach a region that lacks the biological state required for its intended effect. Spatial proteomics provides direct evidence that pathway-relevant protein states are geographically organized within disease tissue; in a severe skin disease, spatial proteomic analysis identified JAK-related biology that was subsequently interrogated therapeutically [10]. This type of evidence strengthens local mechanistic plausibility because it connects tissue geography with pathway context. It still does not justify treating protein abundance as direct evidence of binding, occupancy, or target engagement. Target availability is also a property of cellular organization, not merely molecular abundance. Spatial mapping of rheumatoid arthritis synovium identified a LYVE1-positive macrophage network associated with response to therapy [11]. The appropriate interpretation is associative: a cellular niche can mark a local biological context relevant to treatment response without establishing

the local drug concentration or proving that the niche itself causes the response.

The proposed framework therefore treats target state as a spatially resolved eligibility condition. Depending on the mechanism under consideration, eligibility may involve expression of a molecular target, presence of a responsive cell type, pathway competence, or a disease-specific cellular state. These forms of availability should not be collapsed into a single binary “target present” label. Where a manuscript or computational model moves from availability to engagement, that transition requires its own evidence.

Cellular niches as distinct pharmacological environments

Single cells do not experience pharmacology in isolation. Spatial multi-omics of tumors has demonstrated that resistance-associated cellular states and microenvironmental vulnerabilities can be organized across local neighborhoods, while three-dimensional imaging mass cytometry shows that highly multiplexed cellular and molecular architecture can be reconstructed through tissue volume [12, 13]. Together, these studies support treating a niche as a spatially organized biological context rather than simply a list of cell types.

For natural products, this distinction is potentially consequential because the same nominal tissue exposure may encounter different cell states, neighboring-cell signals, stromal structures, or pathway configurations in different regions. The proposition that these differences alter natural-product action is a framework-level hypothesis unless exposure and response are measured together. Spatial cellular data alone do not report the amount of compound reaching the niche, and drug localization alone does not specify which cells are pharmacologically competent.

Accordingly, the framework separates three questions within niche analysis: which cells occupy the region, what local signaling or pathway state characterizes them, and whether the relevant molecular species can access that region at pharmacologically meaningful exposure. Maintaining these questions as separate evidence layers avoids converting descriptive tissue heterogeneity into an unsupported mechanism.

Microenvironmental control of local drug response

Local pharmacology can also be modified by physical barriers and extracellular conditions. Spatial pharmacokinetic/pharmacodynamic modeling in airway mucus demonstrates how a barrier environment can be represented explicitly when estimating local drug behavior [14]. The importance of such modeling is conceptual as much as quantitative: nominal dose or systemic exposure cannot be assumed to translate uniformly into

concentration at the biological interface. Model output, however, remains conditional on its geometry, transport assumptions, and parameterization and should not be reported as measured local exposure.

Spatially organized response states provide a complementary biological layer. Single-cell and spatial-omics studies of cancer therapy show that cellular hallmarks associated with response and resistance can vary with local state and microenvironment [15]. This evidence supports interpreting response as context dependent, but it does not establish a universal rule for natural products or prove that every resistant niche arises from inadequate drug access.

The framework therefore treats microenvironment as a modifier that can act on more than one part of the pharmacological chain. A barrier can modify exposure; neighboring cells can modify pathway state; local chemistry can alter molecular stability or conversion; and these effects may converge on response without sharing the same causal route. Spatial pharmacology is most informative when those alternatives remain visible rather than being compressed into a single “microenvironment effect.”

Spatial metabolism and local formation of active species

Spatial exposure is not limited to the administered molecule. Metabolism can generate molecular species with different persistence, localization, permeability, and pharmacological relevance. After inhaled ciclesonide administration, desorption electrospray ionization mass spectrometry imaging separately visualized the parent drug and its metabolites within rat lung tissue [16]. Likewise, MALDI imaging showed tissue distributions of duloxetine and major metabolites across mouse brain, liver, kidney, and spleen [17]. These studies establish that parent-compound location cannot automatically represent the spatial disposition of derived species.

For natural products, this distinction may be especially important when extensive biotransformation produces metabolites or conjugates whose target-facing distribution differs from that of the administered structure. The proposed framework therefore treats **molecular-species identity** as part of spatial exposure rather than an analytical detail added after localization. A parent-compound hotspot may be pharmacologically less informative than a lower-abundance active species elsewhere, whereas locally accumulated metabolites may also be inactive products of clearance. Spatial coexistence of a compound and metabolite is consequently insufficient to rank their mechanistic importance without orthogonal evidence.

Table 2 separates the spatial observations that can otherwise be conflated when parent compound, metabolite,

exposure, target state, and response are interpreted together.

Table 2. Distinguishing spatial presence from pharmacologically interpretable local action

Spatial observation	What it establishes	Competing interpretation	Evidence needed for stronger inference
Parent compound detected	Molecular species is locally measurable	Retention, binding, depot, extracellular signal	Quantitative or accessibility-sensitive exposure evidence
Metabolite detected	A derived species reaches or forms in the region	Inactive clearance product	Identity confirmation and functional relevance
Target-rich region	Relevant molecular or cellular context is present	Target inaccessible or nonessential	Engagement or pathway-dependence evidence
Local molecular response	Regional biology changes with treatment	Secondary injury or indirect signaling	Temporally and spatially matched perturbation
Spatial overlap	Two signals occupy compatible coordinates	Coincidence or shared tissue determinant	Orthogonal convergence across exposure, target, and response

note. The distinctions are proposed interpretive categories. No spatial observation alone is treated as sufficient evidence of site of action.

Figure 2 shows why the spatial trajectory from administered molecule to observed response can branch through barriers and local metabolism rather than following a single monotonic path.

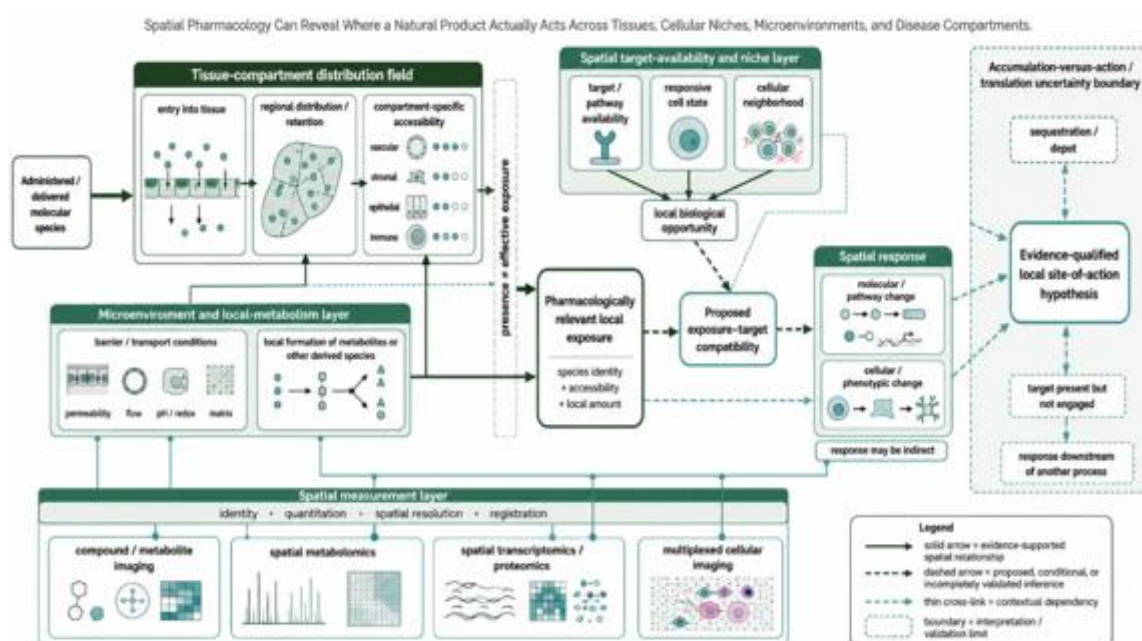


Figure 2. From distributed molecular species to an evidence-qualified local pharmacological response

Measuring natural product localization with imaging and spatial omics

Natural-product spatial pharmacology requires measurement systems capable of distinguishing administered molecules from local biological response. In prostate-cancer xenografts, multimode MALDI mass spectrometry imaging was used to examine matriline-associated spatial metabolic reprogramming [18]. The experiment supports spatial profiling of treatment-associated biochemical change, but it does not make each altered metabolite a direct mechanistic mediator or demonstrate target engagement. More broadly, an updated synthesis of herbal-medicine applications documents expanding use of mass spectrometry imaging for

constituent localization and pharmacological or toxicological investigation [19].

Measurement confidence nevertheless determines how far an image can support pharmacological interpretation. Quantitative MSI requires calibration and normalization strategies; raw ion intensity should not automatically be read as local concentration [20]. Low-molecular-weight compounds also pose analytical challenges involving ionization efficiency, chemical background, sensitivity, and other platform-dependent factors [21]. Consequently, absence of a detectable signal may indicate insufficient analytical sensitivity rather than biological absence.

Molecular identification creates a second boundary. Small-molecule MSI annotation can be complicated by isomers, isobars, adducts, incomplete fragmentation, and database

dependence, so a spatial feature does not become a securely identified compound merely because its measured mass is plausible [22]. Quantitative performance also varies with platform and workflow, and multicenter work demonstrates the importance of explicitly evaluating reproducibility rather than assuming transportability from one implementation [23, 24].

Spatial resolution provides a third constraint. Atmospheric-pressure MALDI has demonstrated lateral resolution approaching cellular dimensions [25], while tissue-expansion mass-spectrometry imaging further extends single-cell-scale molecular profiling across multiple biomolecular classes [26]. These capabilities should not be projected onto datasets acquired at coarser resolution. Finally, computational pipelines such as MSIpixel can automate components of MSI annotation and quantitation [27], but automation remains an analytical layer whose output depends on input quality, molecular identification, calibration, and validation.

A location–exposure–target–response framework

The evidence considered above supports a distinction among measurements that are often compressed into one mechanistic narrative. Spatial pharmacology can establish where a compound or metabolite is detectable [1], but the proposed framework asks three additional questions before treating that location as a candidate site of action.

Location identifies the anatomical, histological, cellular, or subcellular coordinate associated with a measured molecular species. Exposure asks whether the relevant species is present there in a pharmacologically meaningful and accessible form rather than merely retained or analytically visible. Target asks whether the molecular target, susceptible cell state, or required pathway context is locally available and, where the proposed mechanism requires it, whether engagement has been demonstrated. Response asks whether a spatially compatible molecular, cellular, or phenotypic change occurs.

These dimensions are proposed as a convergence framework, not as a validated scoring system. The value lies precisely in permitting discordance. Nitidine chloride imaging, for example, shows that compound localization and regional metabolic perturbation can be examined together without making their overlap mechanistically self-explanatory [8]. Spatial proteomics likewise shows that local pathway information can strengthen therapeutic interpretation, but abundance and pathway state remain distinct from direct engagement [10].

The strongest spatial mechanism hypothesis would therefore arise when molecular identity, local exposure, target-compatible biology, and response converge at commensurate spatial and temporal resolution while credible competing explanations are weakened. Partial

convergence should produce correspondingly weaker language. A localization map can support a distribution claim; localization plus target availability can support mechanistic plausibility; adding appropriately matched response can strengthen that plausibility. None of these combinations should be converted into causal certainty simply by adding more spatial modalities.

Distinguishing where a compound accumulates from where it acts

Accumulation is an observation; action is an interpretation requiring additional evidence. The berberine delivery study demonstrates how tissue localization can be resolved after a defined formulation and route [7]. Yet a locally intense signal could reflect a formulation depot, extracellular trapping, membrane association, high total concentration with low unbound fraction, or slow clearance. The image identifies a place where material is detected, not automatically the biological interface at which pharmacological action occurs.

Metabolites further complicate this distinction. Parent and metabolite distributions can diverge across tissues [17], so assigning activity to the administered structure solely because it dominates bulk exposure may overlook the species facing a local target. The converse error is also possible: a metabolite may accumulate strongly while being pharmacologically inactive.

Target-state evidence does not eliminate the problem. Spatial proteomics can identify pathway-relevant local biology [10], but colocalization of compound and target still does not prove occupancy or functional dependence. The proposed framework therefore treats accumulation-to-action as an interpretation boundary. Crossing it requires evidence appropriate to the claimed mechanism rather than visual overlap alone.

Integrating spatial evidence into computational pharmacology

Computational pharmacology commonly represents exposure with compartments, concentrations, or averaged tissue properties. Spatial PK modeling of CNS and brain-tumor penetration shows that anatomically resolved exposure can instead be treated explicitly [3]. The proposed extension is to represent location not as a descriptive annotation but as a state variable linked to molecular species, accessibility, target context, and measurement resolution.

Barrier conditions can also enter mechanistic models as local modifiers. Spatial PK/PD modeling in airway mucus illustrates how transport through a structured local environment can influence predicted pharmacological behavior [14]. Such representations remain model

dependent and should retain parameter uncertainty rather than being interpreted as measured concentration fields.

A spatial computational framework should also preserve provenance. Automated MSI processing can facilitate compound annotation and quantitation [27], but computational convenience should not collapse molecular identity, calibration confidence, spatial resolution, target evidence, and response into an undifferentiated score. A useful model would instead carry these dimensions separately, permit missing or discordant evidence, and expose the assumptions required to connect one level to the next. Whether such a representation improves prediction or lead ranking remains an empirical question.

Translational consequences for tissue-selective therapeutics

Spatial pharmacology changes what “selectivity” can mean. A compound may be chemically selective for a target yet pharmacologically nonselective across tissues if exposure reaches target-bearing compartments associated with toxicity. Conversely, apparently modest whole-organ exposure could conceal high concentration in a therapeutically relevant niche. Spatial-omics evidence that response and resistance are conditioned by local cellular states supports treating tissue context as a component of therapeutic interpretation [15].

The same logic applies to toxicity. Spatial analysis of nitidine chloride demonstrates the possibility of comparing local compound disposition with regional metabolic perturbation in a target organ [8]. Such evidence may help distinguish widespread exposure from compartment-focused injury, although preclinical spatial association cannot itself establish human toxicological risk or a specific causal pathway.

For lead ranking, the proposed implication is therefore conditional rather than prescriptive. A candidate supported by convergent local exposure, relevant target state, and spatially compatible response may deserve different interpretation from one supported only by whole-tissue abundance. Before quantitative spatial evidence influences high-stakes ranking, reproducibility also matters: multicenter validation of quantitative imaging mass spectrometry demonstrates why single-site analytical performance should not be assumed to generalize automatically [24]. No universal spatial threshold or ranking score is proposed here.

Resolution limits and spatial uncertainty

A spatial claim is only as precise as its effective resolution. Micrometer-scale MALDI imaging demonstrates what specialized instrumentation can achieve [25], whereas tissue-expansion MSI shows how newer architectures can move molecular imaging toward single-cell spatial

profiling [26]. These achievements do not make all spatial datasets cellular. A pixel containing multiple cell types cannot legitimately support a cell-specific pharmacological conclusion simply because it has spatial coordinates.

Quantitative and chemical uncertainty are equally important. Quantitative MSI requires explicit calibration and attention to matrix effects [20]. Molecular identification has separate confidence constraints arising from annotation and structural ambiguity [22]. These uncertainties should remain visible independently because a precisely localized but incorrectly identified ion and a correctly identified but poorly quantified compound fail for different reasons.

Platform dependence further limits transferability. Comparative quantitative MSI demonstrates that analytical performance can vary across instrumental approaches [23], while multicenter validation shows that reproducibility itself must be measured [24]. Spatial uncertainty therefore includes more than physical resolution: it encompasses molecular identity, abundance, registration, sampling, temporal mismatch, and the correspondence between analytical pixels and biological units.

Limitations

The proposed framework is conceptual and nonempirical. Its components are grounded in evidence from spatial pharmacology, drug distribution, spatial omics, imaging, PK/PD modeling, and natural-product applications, but the complete Location–Exposure–Target–Response structure has not been prospectively tested as an integrated decision system.

The supporting literature also spans different compounds, diseases, organisms, platforms, and spatial scales. Evidence from oncology, airway pharmacology, neurological compartments, inflammatory disease, conventional pharmaceuticals, and nanomedicine informs transferable principles but cannot establish that identical spatial determinants govern every natural product. Natural products themselves differ substantially in metabolism, formulation, target multiplicity, and analytical detectability.

A further limitation is temporal. Most spatial measurements are snapshots, whereas exposure, metabolism, engagement, and response evolve at different rates. Apparent spatial concordance may therefore disappear or reverse across time. Future evaluation should combine matched dose–time designs, orthogonal molecular identification and quantitation, local target-engagement assays when required, spatially resolved perturbation, appropriate negative controls, and external validation. Such studies could refine, support, or reject components of the proposed framework.

CONCLUSION

Pharmacological mechanism has a spatial dimension because compounds, metabolites, targets, cellular states, barriers, and responses are not uniformly distributed within living tissue. Spatial methods make these differences observable, but observation alone does not solve the inferential problem.

The principal contribution of this article is a proposed framework that keeps Location, Exposure, Target, and Response analytically separate until evidence justifies connecting them. This separation prevents tissue accumulation from being mistaken for active exposure, target abundance from being mistaken for engagement, and local response from being treated as retrospective proof of causality. It also accommodates metabolites, cellular niches, barriers, heterogeneous disease compartments, and measurement uncertainty without forcing them into a single linear mechanism.

Spatial pharmacology should therefore be understood as a strategy for constraining explanations rather than automatically confirming them. Its strongest use in natural-product research may be to identify where mechanistic hypotheses become more plausible, where competing explanations remain viable, and where additional measurements are needed. Whether this framework ultimately improves efficacy interpretation, toxicity assessment, or lead ranking requires prospective testing across compounds, tissues, analytical platforms, and disease contexts.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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