

**DIDYMIN: CHEMISTRY, PHARMACOKINETICS, DRUG DELIVERY,
AND PHARMACOLOGICAL ACTIVITIES OF A
CITRUS FLAVANONE GLYCOSIDE -
A COMPREHENSIVE REVIEW**

Review Article

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ABSTRACT:

Didymin (neoponcirin; isosakuranetin 7-O-rutinoside) is a citrus flavanone glycoside that has moved, within roughly a decade, from an obscure minor constituent of citrus juice to a compound credited with one of the broadest preclinical pharmacological footprints among dietary flavonoids. Reported activities now span oncology, hepatology, cardiology, nephrology, neurology, metabolic disease, gastroenterology, pain physiology, and reproductive toxicology.

This review brings together, for the first time in a single account, both sides of the didymin literature that have previously been treated separately: the pharmaceutical-sciences foundation of the molecule (its chemistry and stereochemistry, natural distribution and probable biosynthesis, extraction and analytical quantification, intestinal fate, and the delivery strategies available to address its central translational obstacle of poor aqueous solubility) and the full breadth of its reported disease-model pharmacology.

Across eleven distinct activity domains, a recurring mechanistic vocabulary emerges: activation of the Nrf-2/Keap-1 antioxidant axis, suppression of NF- κ B/TLR4-driven inflammation and the NLRP3 inflammasome, modulation of PI3K/Akt and ERK/MAPK signalling, and regulation of the intrinsic Bax/Bcl-2/caspase apoptotic cascade, with Raf-kinase-inhibitor protein (RKIP) emerging as a connecting node across several organ systems.

This review also transparently highlights an important data-integrity concern within the existing literature: a foundational study on the hepatoprotective effects of didymin was formally retracted in 2025 due to concerns regarding image integrity, and its findings have been excluded from the present review.

The review further identifies several key barriers to the clinical translation of didymin, including the absence of a validated stereospecific bioanalytical method, the predominant reliance on rodent, nematode, and in vitro models, the lack of dedicated human pharmacokinetic and safety studies, and the limited investigation of strategies to overcome its poor solubility, with cyclodextrin complexation being the only formulation approach evaluated to date.

Keywords: Didymin; isosakuranetin; flavanone glycoside; citrus flavonoid; bioavailability; pharmacokinetics; drug delivery; pharmacology

1. INTRODUCTION

Citrus fruits are among the richest dietary sources of flavonoids, a class of polyphenolic secondary metabolites with well-documented antioxidant, anti-inflammatory, cardioprotective, and anticancer properties [1][2][3][4]. Within the flavanone subclass, which also contains the far better-studied hesperidin, naringin, and eriocitrin, didymin has historically attracted comparatively little attention despite co-occurring with these relatives in oranges, mandarins, bergamot, and grapefruit. Chemically, didymin is the 7-O-rutinoside of isosakuranetin (4'-methoxy-naringenin), differing from hesperidin only in the absence of a 3'-hydroxyl group on the aglycone, a single substitution that nonetheless appears to translate into a distinctive pharmacological signature.

Interest in didymin has progressively expanded beyond its initial recognition as a naturally occurring dietary flavonoid, particularly following early experimental studies demonstrating its ability to induce apoptosis in cancer cell lines that exhibited resistance to conventional apoptotic stimuli. These findings provided an important basis for investigating didymin as a potential

bioactive and therapeutic compound rather than merely as an incidental constituent of the diet. Subsequently, the growing body of experimental evidence encompassing its potential anticancer and other pharmacological activities was brought together in a comprehensive 2018 survey, which represented an important milestone in consolidating the available therapeutic literature on didymin and in highlighting its emerging potential for further biomedical investigation [5]. In the years since that survey, the literature has expanded substantially, increasingly using multi-omics approaches, transcriptomics, metabolomics, network pharmacology, and molecular docking, to resolve didymin's mechanisms in far greater detail, and extending its evaluated activities into organ systems not previously examined, including the gut microbiome-macrophage axis, the cerebrovascular unit after haemorrhagic and ischaemic stroke, the male reproductive axis, and, most recently, chronic neuropathic pain.

A parallel body of work, largely separate from this disease-model literature, has addressed the pharmaceutical-sciences questions that are routine for any candidate natural product: where exactly the compound occurs and in what quantities, how it is best extracted and measured, what happens to it after oral intake, and how its evident solubility problem might be solved. These questions remain only partially answered for didymin specifically, and are frequently addressed only by extrapolation from better-characterised citrus relatives such as hesperidin and naringenin.

This review is organised to bring both literatures together into a single, self-contained account. It first addresses didymin's chemical identity, natural occurrence, biosynthesis, extraction, and analytical quantification; it then turns to the compound's absorption, distribution, metabolism, and excretion, and to the drug-delivery strategies available to improve its bioavailability; and it finally surveys, activity by activity, the full range of biological effects reported for didymin across preclinical models. Throughout, an explicit effort has been made to distinguish findings that are genuinely compound-specific from mechanistic themes, antioxidant enzyme induction, NF- κ B suppression, PI3K/Akt modulation, that recur across the citrus flavanone class as a whole [1][3]. A further, less common contribution of this review is methodological rather than pharmacological: An important data-integrity issue within the didymin literature should also be acknowledged. A widely cited study investigating the hepatoprotective effects of didymin was formally retracted in November 2025 following concerns regarding the integrity and reliability of

the published images, which were identified through post-publication peer review [6]. In view of this retraction, the findings and conclusions of the affected study have not been considered as supporting evidence in the present review. Nevertheless, the retraction is explicitly documented to ensure transparency and to alert readers who may consult earlier reviews, systematic discussions, or other secondary sources that incorporated the original findings before the retraction occurred. This approach is intended to maintain the evidentiary integrity of the present review and to ensure that conclusions regarding the pharmacological and hepatoprotective potential of didymin are based only on studies that remain part of the available, non-retracted evidence base [6].

2. CHEMISTRY AND STRUCTURAL IDENTITY

Didymin (neoponcirin; isosakuranetin 7-O-rutinoside) belongs to the flavanone-glycoside group of citrus flavonoids, sharing the characteristic flavan nucleus, two aromatic rings joined by a dihydropyrone ring, with hesperidin, narirutin, and naringin. Structurally, didymin sits between naringenin (the unmethylated flavanone abundant in grapefruit) and hesperidin (the corresponding rutinoside of hesperetin, which carries an additional 3'-hydroxyl group). The aglycone, isosakuranetin, is linked at the 7-hydroxyl of the A-ring to rutinose, an α -1,6-linked L-rhamnopyranosyl-D-glucopyranoside, the same disaccharide found in hesperidin and narirutin. This linkage confers a solubility profile substantially better than the free aglycone but still poor in absolute terms, a property didymin shares with essentially the entire flavanone-rutinoside family [2][3]. Because the underlying chemistry is shared so closely with hesperidin and naringenin, physicochemical data generated for those better-studied relatives, aqueous solubility as a function of pH and temperature, cyclodextrin complexation behaviour, and photostability, offer a reasonable, if imperfect, first approximation of didymin's own behaviour wherever compound-specific measurements are still lacking.

Like every flavanone, didymin possesses a chiral centre at C-2 of the dihydropyrone ring, yet the overwhelming majority of pharmacological and pharmacokinetic work on citrus flavanones, including, so far as could be determined, every published didymin study, has used racemic or unspecified-configuration material rather than resolved individual enantiomers. This is not a merely academic gap: intravenous administration of racemic homoeriodictyol, isosakuranetin, and taxifolin to rats revealed clear enantiomer-dependent differences in half-life, clearance, and

non-renal elimination fraction, with the (–)-(S)-enantiomer of isosakuranetin, didymin's own aglycone, predominating in lemon, grapefruit, and other citrus matrices [7]. Comparable stereoselective differences have been documented for hesperetin, naringenin, and eriodictyol, in which the (2S)-epimers of the parent glycosides and the S(–)-enantiomers of the aglycones were both the dominant natural forms and pharmacokinetically distinguishable after intravenous dosing [8]. Chiral high-performance liquid chromatographic methods capable of resolving these enantiomers already exist for hesperetin, naringenin, and related flavanones [9], but no equivalent chiral assay has yet been reported for didymin or isosakuranetin specifically. Until one is developed, reported didymin pharmacokinetic and quantification data should be understood as describing an undefined mixture of stereoisomers rather than a single, fully characterised chemical entity.

3. NATURAL OCCURRENCE AND BIOSYNTHESIS

Didymin occurs across numerous Citrus species, sweet orange, mandarin, bergamot, and grapefruit among them, typically as a minor constituent relative to hesperidin and narirutin in the same juice or peel matrix. Quantitative HPLC profiling of bergamot juice placed didymin among the lower-abundance flavanone glycosides detected, with limits of detection and quantitation comparable to those for narirutin [10], while chromatographic analysis of enriched clementine juice reported didymin at roughly 4–6 mg per 100 mL, one to two orders of magnitude below the neohesperidin and hesperidin content of the same samples [11]. This relative scarcity, rather than any particular chemical instability, is the most plausible explanation for why didymin has been isolated and studied far less often than its citrus relatives.

Biosynthetically, didymin is presumed to arise through the canonical flavanone pathway common to all citrus flavanones: chalcone synthase and chalcone isomerase convert p-coumaroyl-CoA and malonyl-CoA to naringenin chalcone and then (2S)-naringenin; a flavonoid-specific O-methyltransferase is inferred to generate isosakuranetin by 4'-O-methylation, analogous to the hydroxylation and methylation steps recently characterised for the flavone branch of citrus flavonoid biosynthesis [12]; and a rhamnosyltransferase, structurally analogous to the substrate-promiscuous 1,6-rhamnosyltransferases already characterised in Citrus and Chrysanthemum for related flavanone and flavone rutinosides, is presumed responsible for the final 7-O-rutinosylation step. Critically, the specific glycosyltransferase(s) that rutinosylate

isosakuranetin to yield didymin have not yet been cloned or biochemically characterised, so this pathway remains an inference from homologous systems rather than a directly demonstrated route.

A more unusual feature of didymin's natural history is its reported presence outside Citrus altogether: in the Mediterranean herb *Origanum vulgare* L., in the traditional Chinese mint *Mentha haplocalyx* Briq. (where it is described as the plant's principal analgesic constituent), and in the Mexican medicinal plant *Clinopodium mexicanum*, where it is more often referred to by its older synonym neoponcirin. Whether this reflects convergent evolution of comparable rhamnosyltransferase activity in unrelated plant families, retention of an ancestral glycosylation pathway, or simply more extensive phytochemical screening of these particular species, is not resolved in the literature and represents a natural question for future biosynthetic and phylogenomic work.

4. EXTRACTION AND ANALYTICAL QUANTIFICATION

Didymin has, to date, essentially always been recovered and quantified as part of a broader citrus flavanone-glycoside profile rather than as a dedicated target analyte. Conventional solvent maceration (methanol or aqueous methanol) remains the most common extraction approach in studies that report didymin content, but ultrasound-assisted extraction has repeatedly been shown, for citrus peel flavanone glycosides generally, to shorten extraction time and improve yield through cavitation-driven disruption of plant cell walls [13][14]. Microwave-assisted extraction offers a further, essentially untested alternative for didymin specifically: applied to hesperidin recovery from Citrus unshiu peel, response-surface-optimised microwave heating combined with low-temperature crystallisation achieved yields comparable to prolonged room-temperature solvent extraction in a fraction of the processing time [15]. No ultrasound- or microwave-optimisation study targeting didymin recovery specifically appears to have been published, which is a straightforward methodological gap given how well established both techniques are for its more abundant relatives. Quantification has relied almost exclusively on reversed-phase HPLC with diode-array or mass-spectrometric detection, using co-injection with authentic didymin standards for peak identification against narirutin, hesperidin, naringin, and other co-eluting flavanones [10][11]. A validated LC-MS/MS bioanalytical method specific to didymin in plasma or other biological matrices, of the kind long established for other poorly

soluble flavonoid glycosides, including validated solid-phase-extraction HPLC assays for hesperetin and naringenin in human plasma and urine [16], has not been published outside the plasma concentration-time data reported alongside a cyclodextrin inclusion-complex formulation study [17]. Any future assay would also need to resolve the stereochemical issue described above: chiral stationary-phase HPLC methods validated for hesperetin and naringenin [9] provide a template not yet adapted for didymin or isosakuranetin. Method development and validation for any such assay would be expected to follow the internationally harmonised ICH Q2(R1) framework for specificity, linearity, accuracy, precision, and quantitation limits [18].

Stability during extraction, processing, and storage is a further practical consideration inherited from the wider flavanone-glycoside literature. Flavanones as a class are more photostable during storage than flavones or flavonols, though degradation still accelerates with light exposure, elevated oxygen levels, and alkaline pH [19]. Applied to didymin, this suggests that extraction and storage protocols already developed for hesperidin and naringin, protection from light, moderate temperature, avoidance of strongly alkaline conditions, are a reasonable working default until compound-specific stability data become available. Bioaccessibility studies of citrus flavanones and flavones in food matrices more broadly indicate that the food matrix itself, digestion conditions, and co-ingested macronutrients can each materially alter the fraction of ingested flavonoid glycoside that becomes available for absorption [20], a variable not yet examined for didymin specifically but directly relevant to interpreting any future human feeding study; *in vitro* Caco-2 permeability data on a bioaccessible *Citrus sinensis* polyphenolic fraction, in which only around 8% of ingested flavonoid content permeated the intestinal monolayer after simulated digestion, illustrate how substantial this attenuation can be for the citrus flavanone class as a whole [21].

5. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Direct pharmacokinetic characterisation of didymin remains almost entirely absent from the literature. The clearest available evidence for oral bioavailability in even a qualitative sense comes indirectly, from the demonstration that orally administered didymin is active *in vivo* against neuroblastoma xenografts, which by necessity establishes that meaningful systemic or tumour-site exposure occurs after oral dosing [22]. Beyond this, didymin's intestinal fate can reasonably be inferred from the well-characterised metabolism of structurally related flavonoid

rutinosides. As with hesperidin and narirutin, didymin's rutinose moiety is not a substrate for the small-intestinal sodium-glucose transporter and must first be cleaved before significant absorption occurs, via two enzymatic routes: intestinal brush-border and cytosolic β -glucosidases acting on the terminal glucose [23][24], and microbial α -L-rhamnosidases acting on the rhamnose-glucose linkage further down the gastrointestinal tract. A recent biochemical survey of gut-bacterial GH78 α -L-rhamnosidases specifically characterised their activity against citrus flavanone diglycosides bearing the same α -1,6-rhamnosyl-glucosyl (rutinose) linkage found in didymin, confirming that rhamnose removal from the 7-OH position of flavanone rutinosides is an efficient, well-precedented microbial transformation [25]; a related study specifically cloned and characterised the α -L-rhamnosidase responsible for metabolising the structurally analogous rutinoside-linked flavonoid poncirin in the human gut microbiota, providing a directly relevant enzymatic template for didymin's own likely microbial fate [26]. More broadly, the bidirectional relationship between dietary flavonoids and the gut microbiome, in which microbial deglycosylation determines flavonoid bioavailability while the resulting metabolites in turn reshape microbial community structure, has been reviewed extensively for the flavonoid class as a whole [27].

Once cleaved, the resulting aglycone, isosakuranetin, in didymin's case, is markedly more lipophilic than the parent glycoside and is presumed to cross the enterocyte membrane by passive diffusion, consistent with the general principle that deglycosylation is typically a prerequisite for meaningful absorption of dietary flavonoid glycosides [23]. Direct evidence for this specific transformation comes from work on poncirin, the corresponding neohesperidoside of isosakuranetin: oral administration to mice altered gut microbial diversity and produced isosakuranetin as a detectable urinary metabolite, together with shifts in amino-acid and short-chain-fatty-acid metabolism, the clearest available evidence that isosakuranetin-based flavanone glycosides undergo gut-microbial deglycosylation and systemic aglycone exposure *in vivo*, even though the study used poncirin rather than didymin itself [28]. Whether didymin undergoes the same phase II conjugation (glucuronidation, sulfation, further methylation) documented for other flavonoid aglycones after intestinal or hepatic absorption has not been directly examined.

A further layer of pharmacokinetic complexity specific to didymin is its documented inhibition of the intestinal and hepatic organic anion-transporting polypeptide OATP2B1, alongside

hesperidin and narirutin, at low-micromolar concentrations [29]. Because OATP2B1 handles the intestinal uptake of a range of co-administered drugs, this raises a genuine, if still untested, food-drug interaction concern for patients consuming didymin-rich citrus products alongside OATP2B1-substrate medications, a consideration that sits alongside, rather than resolves, the more fundamental question of how much didymin itself reaches the systemic circulation intact or as its aglycone.

5.1 HUMAN PHARMACOKINETIC DATA FOR STRUCTURALLY RELATED FLAVANONES

In the complete absence of a human pharmacokinetic study of didymin itself, the closest available proxies come from its citrus relatives naringenin, hesperetin, and eriocitrin, all extensively studied after oral administration to healthy volunteers. Single-dose administration of pure hesperetin and naringenin as solid-dispersion capsules produced rapid absorption, plasma concentrations detectable within 20 minutes, peaking at 3.5–4.0 hours, but low absolute bioavailability, with only 3–6% of the administered dose recovered in urine, attributed to extensive first-pass metabolism and microbial C-ring cleavage [30]. Ingestion of orange and grapefruit juice similarly produced modest and highly variable plasma and urinary recovery of naringenin and hesperetin [31], while enzymatic modification of hesperidin to improve its solubility measurably increased bioavailability relative to the native glycoside in a randomised, double-blind, crossover human trial [32], direct clinical evidence that solubility is rate-limiting for this class of compound, consistent with the formulation rationale developed in the next section. A comparative human pharmacokinetic study of eriocitrin and hesperidin found that the more water-soluble eriocitrin produced higher plasma and urinary concentrations of shared downstream metabolites and identified seventeen distinct phase-II flavanone-derived metabolites, further underscoring how strongly aqueous solubility constrains flavanone-glycoside bioavailability in humans [33]. Broader reviews of flavanone dietary sources, bioactivities, and bioavailability reach the same general conclusion across the class [34].

The metabolic fate of these aglycones after absorption has also been characterised in some detail: naringenin undergoes extensive site-dependent intestinal and hepatic glucuronidation, with efflux transporters pumping the resulting glucuronides back into the intestinal lumen and materially limiting net systemic exposure [35], and comparable phase II glucuronidation and sulfation

pathways, together with the efflux transporters handling their conjugates, have been documented for naringin, hesperidin, and their aglycones more broadly [36]. Because didymin's aglycone isosakuranetin is structurally intermediate between naringenin and hesperetin, qualitatively similar glucuronidation and efflux-mediated bioavailability limitations would be a reasonable expectation, though this remains an inference rather than a demonstrated finding specific to didymin. Foundational work establishing that dietary polyphenols in general are subject to this kind of extensive, bioavailability-limiting first-pass metabolism [37] provides the broader conceptual framework within which didymin's own presumed disposition should be interpreted.

Rodent studies extend this picture further upstream. Oral administration of naringenin and its glycosides to rats showed that the aglycone is absorbed considerably faster and more completely than the glycosylated forms, reinforcing the deglycosylation-first principle already discussed for didymin's own rutinose moiety [38]. Comparable rat plasma work identified the specific glucuronide and sulfate conjugates of orally administered hesperidin, providing a template metabolite-identification strategy not yet applied to didymin [39]. In humans, the well-documented capacity of grapefruit-derived naringin to precipitate clinically significant drug interactions, originally attributed to furanocoumarin-mediated CYP3A4 inhibition but subsequently linked more broadly to citrus flavonoid pharmacokinetics, illustrates that even modest systemic exposure to a citrus flavanone glycoside can carry pharmacological consequences beyond the compound's intended activity [40], directly relevant to the OATP2B1 interaction already noted for didymin. Earlier quantitative surveys of narirutin, naringin, hesperidin, and neohesperidin content across citrus varieties [41], and of flavanone absorption following citrus juice or purified naringin and hesperidin administration [42], together with more recent Caco-2-based work on naringenin intestinal permeability under simulated antibiotic-modified gut conditions [43], form much of the empirical foundation from which didymin's own likely absorption behaviour must, for now, be extrapolated. Validated stereospecific HPLC assays for hesperetin and naringenin in serum and urine [44][45] illustrate the level of bioanalytical rigour a dedicated didymin assay would ultimately need to match.

6. DRUG DELIVERY AND BIOAVAILABILITY ENHANCEMENT STRATEGIES

Poor aqueous solubility is the central pharmaceutical obstacle standing between didymin's extensive preclinical pharmacology and any translational application, and at present cyclodextrin inclusion complexation is the only enhancement strategy tested against didymin directly. β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin inclusion complexes, characterised by FT-IR, powder X-ray diffraction, differential scanning calorimetry, and scanning electron microscopy, improved didymin's aqueous solubility and oral bioavailability in rodent studies while preserving its chemosensitising activity in tumour models [17], consistent with cyclodextrins' long-established, broadly applicable role as pharmaceutical solubilising agents for poorly soluble small molecules [46]. The closely related flavanone glycoside hesperidin indicates how much further this single approach can be pushed: molecular-level amorphous inclusion complexes of hesperidin and hesperetin with 2-hydroxypropyl- β -cyclodextrin have been characterised down to the specific intermolecular interactions responsible for improved apparent solubility and accompanying biological activity [47], a depth of physicochemical characterisation not yet attempted for didymin's own cyclodextrin complexes. A recent mini-review of hesperidin and hesperetin bioavailability strategies more broadly underscores just how central solubility-enhancing formulation has become to translating this entire flavanone family into clinically meaningful exposure [48].

Beyond cyclodextrins, a substantial body of formulation science developed for structurally related citrus flavanones has not yet been applied to didymin. Self-nanoemulsifying drug delivery systems (SNEDDS), isotropic oil/surfactant/co-surfactant mixtures that spontaneously form nanoscale emulsion droplets on dilution in gastrointestinal fluid, have substantially improved the oral bioavailability of naringenin, the unmethylated flavanone aglycone most structurally similar to didymin's own aglycone isosakuranetin, producing markedly greater plasma exposure than the free flavanone in rats [49]. A comparable bioactive SNEDDS approach improved apigenin's drug loading roughly 500-fold relative to the free flavone and demonstrated favourable in vivo bioavailability and stability outcomes [50]. Solid dispersion technology offers a technically simpler alternative pursued extensively for hesperidin: kneading and solvent-evaporation solid dispersions with mannitol or polyvinylpyrrolidone carriers increased hesperidin's aqueous solubility several-fold and improved dissolution rate [51], amorphous solid dispersions with polymeric excipients such as Soluplus® and hydroxypropylmethylcellulose achieved comparable apparent-solubility gains through full amorphisation of the crystalline

flavanone [52], and a ball-milled solid dispersion of hesperidin with polyvinylpyrrolidone measurably increased both peak plasma concentration and total systemic exposure relative to the unformulated compound in a rat colitis model, alongside improved therapeutic efficacy [53]. Phospholipid-complex (phytosome) technology represents a further, chemically distinct option: complexing polyphenolic phytoconstituents with phosphatidylcholine-based phospholipids has been shown across numerous plant-derived actives to improve membrane permeation and oral bioavailability without altering the parent molecule's structure [54]. More broadly, nanoparticle-mediated flavonoid delivery has been shown across a range of systems to enhance not only bioavailability but also anti-inflammatory efficacy, by sustaining flavonoid exposure and modulating proinflammatory cytokine production more effectively than free compound [55], and a dedicated meta-analysis of citrus flavonoid nanocarrier formulations specifically has confirmed the superiority of nanocarrier-based delivery over conventional formulations across the citrus flavonoid class [56]. None of SNEDDS, solid dispersion, phospholipid complexation, or nanoparticle encapsulation has yet been reported for didymin specifically, but the close structural relationship between didymin's aglycone and naringenin or hesperetin makes each a reasonable, and on present evidence likely productive, next step for improving didymin's oral exposure beyond what cyclodextrin complexation alone has achieved. A comprehensive review of the biological fate and delivery-system landscape for citrus flavonoids as a class reaches the same conclusion: increased bioavailability through appropriate delivery systems consistently translates into enhanced bioefficacy in animal models [57].

At the commercialisation end of the spectrum, a multi-flavonoid citrus formulation containing didymin at approximately 1% of total flavonoid content, alongside eriocitrin, hesperidin, and naringin, has already been evaluated in a randomised, placebo-controlled human trial for prediabetes management [58]. While this cannot be interpreted as evidence for didymin's own pharmacokinetics or clinical efficacy in isolation, it demonstrates that citrus flavanone-glycoside blends containing didymin are technically formulable, stable, and acceptable for human oral administration, a necessary, if not sufficient, precondition for any future didymin-specific clinical development.

A further, largely unexplored avenue relevant to didymin's supply chain is enzymatic or biocatalytic production, as opposed to extraction from citrus biomass. An immobilised-

rhamnosidase reaction platform has been used to construct a novel, high-efficiency biosynthetic route to the related flavanone glycoside hesperetin-7-O-glucoside, in which the enzyme is reused across repeated reaction cycles to build glycosidic bonds under controlled conditions [59]. Given that essentially the same class of rhamnosyltransferase and rhamnosidase enzymes discussed above is implicated in both the natural biosynthesis and the microbial degradation of didymin's rutinose linkage, an analogous immobilised-enzyme or whole-cell biocatalytic strategy could, in principle, offer a scalable, extraction-independent route to producing didymin or its aglycone isosakuranetin at higher purity than is currently achievable from citrus by-products, an approach that has not yet been attempted for this compound.

7. PHARMACOLOGICAL ACTIVITIES OF DIDYMIN

The following sections survey didymin's reported biological activities in turn. Across all eleven domains, a comparatively small and recurring set of molecular mechanisms appears repeatedly, and is highlighted explicitly at the end of each subsection where relevant, before being drawn together in the summary table that follows.

7.1 *Anticancer activity*

The earliest and still most extensively cited pharmacological property of didymin is its anticancer activity. In human neuroblastoma cells, orally administered didymin suppressed tumour growth by inducing apoptosis through the intrinsic, mitochondrial Bcl-2-family-regulated pathway [60], the caspase-dependent execution phase of which has been characterised in molecular detail elsewhere [61] and by inhibiting angiogenic signalling, among the first findings to establish didymin as an orally active, non-toxic candidate for targeting paediatric solid tumours [22]. In non-small-cell lung cancer cells, didymin activates a Fas-mediated apoptotic pathway both in vitro and in xenograft models, with concurrent inhibition of proliferative signalling [62]. Beyond direct cytotoxicity, didymin has also demonstrated the ability to chemosensitise multidrug-resistant cancer cells to conventional chemotherapeutic agents, an effect substantially amplified when the compound's bioavailability is improved through cyclodextrin complexation [17]. Collectively, these studies position didymin as a dietary phytochemical with adjuvant potential in oncology, though, as with most flavonoid anticancer data, clinical confirmation is entirely lacking.

7.2 Antioxidant activity and cellular stress resistance

Antioxidant activity underlies almost every other biological effect attributed to didymin and is the most consistently reproduced finding across the literature, consistent with the broader recognition that oxidative stress, an imbalance between reactive oxygen species and endogenous antioxidant defences, is a common upstream driver of tissue injury across organ systems [63]. In a *Caenorhabditis elegans* model of UV-irradiation stress, didymin reduced reactive oxygen species accumulation, increased superoxide dismutase activity, and promoted nuclear translocation of the transcription factor DAF-16, acting at least in part through the insulin/IGF-1-like (*daf-2*) signalling pathway to upregulate the stress-response genes *sod-3* and *hsp-16.2* [64]. This mechanistic insight, that didymin's antioxidant action is not purely chemical radical scavenging but also transcriptional, engaging conserved longevity and stress-resistance pathways, recurs throughout the organ-specific toxicity studies discussed below, most of which report restoration of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activities alongside reduced malondialdehyde and reactive oxygen species levels following didymin treatment. At the transcriptional level, this antioxidant activity is very frequently attributed to activation of the Keap1-Nrf2-antioxidant response element axis, the master regulatory system governing inducible cellular antioxidant gene expression [65].

7.3 Anti-inflammatory activity

Suppression of inflammatory signalling is the second recurring mechanistic thread in the didymin literature, frequently converging on the PI3K/Akt survival pathway that governs cell-fate decisions across almost all of the organ systems discussed in this section [66]. Across hepatic, cardiac, cerebral, and intestinal injury models, didymin consistently reduces expression or activation of nuclear factor kappa-B (NF- κ B) and toll-like receptor 4 (TLR4), a pattern-recognition receptor whose downstream NF- κ B signalling is a well-established target for anti-inflammatory phytochemicals more broadly [67], and of downstream pro-inflammatory cytokines including tumour necrosis factor- α , interleukin-1 β , and interleukin-6. In models of myocardial infarction and intracerebral haemorrhage specifically, didymin also inhibits the NLRP3 (or, via the adaptor Asc, the caspase-1/GSDMD) inflammasome pathway [68], thereby limiting pyroptotic cell death in addition to classical apoptosis. This dual antioxidant/anti-

inflammatory character is likely the mechanistic common denominator that allows a single compound to show benefit across such structurally unrelated organ injuries.

7.4 Hepatoprotective activity

The liver is, by volume of published work, the organ system in which didymin's protective activity has been most thoroughly characterised, although one widely cited early study in this area requires an important caveat. Didymin was originally reported as the hepatoprotective constituent of *Origanum vulgare* L., ameliorating carbon-tetrachloride-induced hepatic injury via upregulation of Raf-kinase-inhibitor protein (RKIP), a small, evolutionarily conserved regulator best known for suppressing Raf/MEK/ERK and NF- κ B signalling in other physiological and oncological contexts [69][70]. That original 2017 report was formally retracted by the publisher in November 2025 after image-duplication and manipulation concerns raised through post-publication peer review [6], and it is therefore excluded here as a source of evidence; readers who encounter it cited elsewhere in the didymin literature should treat its specific claims with caution pending any independent replication. A related paper by an overlapping author group, reporting that didymin alleviates CCl₄-induced hepatic fibrosis via RKIP-dependent inhibition of ERK and PI3K/Akt signalling [71], has not itself been retracted, but shares authorship with the retracted study and is flagged here for the same reason; it should be weighted cautiously until independently confirmed rather than treated as settled evidence.

Independently sourced hepatoprotective evidence for didymin is nonetheless substantial. Transcriptomic and metabolomic profiling of a chronic CCl₄ fibrosis model showed that didymin alleviates endoplasmic-reticulum stress, through the ATF6, IRE1 α , and PERK arms of the unfolded-protein response, a signalling network increasingly implicated in liver disease more broadly [72], and normalises glycerophospholipid metabolism [73]. A parallel multi-omics study of lipopolysaccharide/D-galactosamine-induced acute liver injury reached a consistent conclusion, again implicating glycerophospholipid metabolism and the PI3K/Akt and TLR4/NF- κ B pathways [74]. Didymin's hepatoprotective activity extends to metabolic liver disease: in dexamethasone- and high-fat-diet-induced non-alcoholic fatty liver disease, didymin decreased hepatocyte apoptosis and lipid accumulation by inhibiting both the TLR4/NF- κ B and PI3K/Akt pathways [75], while in diet-induced metabolic dysfunction-associated fatty liver disease, didymin was shown to bind directly to and activate Sirt1, stimulating mitochondrial biogenesis

and lipophagy while reducing apoptosis, an effect abolished by pharmacological Sirt1 inhibition and thereby confirming Sirt1 as the proximate target [76]. Most recently, didymin has been shown to protect against polystyrene-nanoplastic-induced hepatic damage, restoring Nrf-2/Keap-1 signalling, normalising antioxidant enzyme activities and transaminase levels, and reducing hepatic inflammatory and apoptotic markers [77], evidence that didymin's hepatoprotective spectrum extends to emerging environmental-pollutant toxicities and not solely to classical chemical or dietary insults.

7.5 Cardioprotective activity

Three independent cardiotoxicity models converge on a consistent cardioprotective profile for didymin. Against doxorubicin-induced cardiotoxicity, didymin pretreatment suppressed reactive-oxygen-species generation, preserved mitochondrial membrane potential, and reduced cardiomyocyte apoptosis via activation of the PI3K/Akt/Nrf2 axis [78]. Against paraquat-induced cardiac damage, didymin restored antioxidant enzyme activity, reduced cardiac injury biomarkers (creatinine kinase-MB, troponin, lactate dehydrogenase), suppressed inflammatory cytokines, and normalised the Bax/Bcl-2/caspase apoptotic balance, with corresponding improvement in cardiac histoarchitecture [79]. In a model of ischaemia-reperfusion myocardial infarction, didymin reduced myocardial fibrosis and apoptosis and inhibited the NLRP3/ASC/caspase-1 inflammasome both in vivo and in H9C2 cardiomyocytes, at doses as low as 1–4 mg/kg [80].

7.6 Nephroprotective activity

Although evidence supporting a direct renoprotective effect of didymin remains relatively limited compared with the more extensive literature on its hepatoprotective and cardioprotective properties, the available findings are consistent with its broader antioxidant and anti-inflammatory pharmacological profile. In an experimental rat model of cadmium-induced renal injury, administration of didymin was associated with restoration of the Nrf2/Keap1 signalling pathway, improvement in the activity of endogenous renal antioxidant enzymes, and attenuation of inflammatory and apoptotic responses. These biochemical and molecular changes were accompanied by improvements in renal histopathological features, suggesting that didymin may exert protective effects against chemically induced renal oxidative and inflammatory damage

[81]. However, the potential renoprotective effects of didymin against other emerging environmental nephrotoxics remain poorly investigated. In particular, as of the time of writing, no peer-reviewed study has specifically evaluated the protective effects of didymin against nanoplastic-induced renal injury. This represents an important gap in the current literature and warrants further investigation, particularly in view of the reported protective effects of didymin against nanoplastic-associated hepatic injury [77]. Future studies should therefore determine whether the antioxidant, anti-inflammatory, and anti-apoptotic mechanisms attributed to didymin in other organ systems can also provide meaningful protection against nanoplastic-induced renal toxicity.

7.7 Neuroprotective activity

Didymin's neuroprotective activity has been demonstrated across three distinct models of acute cerebrovascular injury, extending a broader precedent for neuroprotection among citrus-derived flavonoids more generally [82]. Following experimental intracerebral haemorrhage, didymin improved neurobehavioural performance and reduced blood-brain-barrier disruption by upregulating RKIP, which in turn suppressed Asc/Caspase-1/GSDMD-mediated microglial pyroptosis and downstream release of interleukin-1 β , tumour necrosis factor- α , and myeloperoxidase [83]. In a model of cerebral ischaemia-reperfusion injury, didymin pretreatment reduced neuronal apoptosis and inflammatory cytokine levels in both rat brain tissue and oxygen-glucose-deprived PC12 cells by activating the PPAR- γ /RXRA signalling pathway, a receptor with an independently documented anti-inflammatory and neuroprotective role in ischaemic brain injury [84], and pharmacological inhibition of PPAR- γ abolished the protective effect, confirming its mechanistic necessity [85]. Most recently, in a rat model of subarachnoid haemorrhage, didymin improved neurological outcome scores, reduced microglial activation and brain oedema, and preserved blood-brain-barrier integrity by downregulating matrix metalloproteinase-9, a protease independently linked to blood-brain-barrier breakdown after ischaemic and haemorrhagic stroke [86], while preserving the tight-junction proteins ZO-1 and occludin in haemoglobin-stimulated brain microvascular endothelial cells [87].

7.8 Antidiabetic and metabolic activity

Didymin's antidiabetic potential was first established through a battery of enzyme-inhibition assays showing strong inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), a well-validated antidiabetic and anti-obesity drug target in its own right [88], α -glucosidase, aldose reductase, the rate-limiting enzyme of the polyol pathway implicated in diabetic neuropathy, retinopathy, and nephropathy [89], and advanced glycation end-product formation, a further pathway well recognised for driving diabetic vascular and renal complications [90], with molecular docking confirming high-affinity binding within the active sites of these targets [91]. In insulin-resistant HepG2 cells, didymin increased glucose uptake and activated the IRS-1/PI3K/Akt/GSK-3 axis while suppressing hepatic gluconeogenic enzymes [91]. Complementary work in hyperglycaemia-exposed human umbilical vein endothelial cells showed that didymin prevents glucose-induced endothelial dysfunction and death by limiting reactive-oxygen-species generation, restoring the eNOS/iNOS balance, and reducing monocyte adhesion and NF- κ B activation, mechanisms directly relevant to the vascular complications of diabetes [92]. Most recently, in high-fat-diet-induced impaired glucose tolerance, didymin was shown to protect pancreatic β -cells by enhancing mitochondrial biogenesis and function, improving insulin secretion, and reducing β -cell apoptosis; these benefits were abolished by a mitochondrial electron-transport inhibitor, confirming mitochondrial support as the operative mechanism [93]. This body of work sits within a wider, well-established literature on citrus flavonoids and diabetic complications more generally [94][95][96].

7.9 Gastrointestinal activity

Two independent lines of evidence now support a role for didymin in intestinal inflammation, extending what was, until recently, a single-study finding. An early mechanistic study demonstrated that didymin reprogrammes pro-inflammatory M1-like macrophages toward an anti-inflammatory M2-like phenotype in a dextran-sulfate-sodium model of colitis, and traced this effect to enhanced fatty acid oxidation driven by induction of *Hadhb* expression, with in vivo confirmation that promoting *Hadhb*-dependent fatty acid oxidation was sufficient to drive the M1-to-M2 shift and alleviate colitis [97]. A more recent, mechanistically distinct study in the same DSS-colitis model found that didymin reduced colonic expression of interleukin-6, interleukin-1 β , and tumour necrosis factor- α , restored the tight-junction proteins claudin-1 and zona occludens-1, and favourably shifted gut microbiota composition, increasing Firmicutes and

Verrucomicrobiota while decreasing Bacteroidota and Proteobacteria; network pharmacology and molecular docking further implicated the microbiota-derived metabolites L-ornithine and saccharin as mediators that modulate STAT3 and NF- κ B signalling downstream of didymin treatment [98]. Taken together with the broader literature on gut dysbiosis in inflammatory bowel disease [99], this gut-microbiome axis is one of the more novel mechanistic threads in the recent didymin literature and suggests that at least part of the compound's systemic anti-inflammatory activity may be indirect, mediated through effects on the intestinal microbial community rather than acting solely at the tissue level.

7.10 Analgesic and CNS-modulatory effects

Two lines of evidence, separated by more than a decade, indicate that didymin also has direct effects on the central nervous system beyond its role in acute cerebrovascular injury. Isolated from the Mexican medicinal plant *Clinopodium mexicanum*, didymin (reported under its synonym 2(S)-neoponcirin) produced anxiolytic-like, sedative, and antinociceptive effects in mice comparable in some respects to diazepam and ibuprofen, with the anxiolytic action shown to depend on the GABAergic system through co-administration experiments with muscimol and picrotoxin [100]. Far more recently, in a rat model of chronic constriction injury, a standard model of neuropathic pain, didymin, identified as the principal analgesic constituent of the traditional Chinese herb *Mentha haplocalyx*, significantly alleviated mechanical allodynia, restored expression of the mitophagy markers LC3 and TOM20, and suppressed the NLRP3/ASC/GSDMD pyroptotic cascade and NF- κ B phosphorylation in the spinal cord, an effect shown by molecular docking and pharmacological RKIP inhibition to depend on didymin's binding to and upregulation of RKIP [101]. Taken together with the RKIP-dependent anti-pyroptotic mechanism already described for intracerebral haemorrhage, this recent work suggests that RKIP upregulation may be a more general mechanistic thread linking didymin's actions across several distinct neurological conditions, and identifies neuropathic pain as a newly opened and still very preliminary area of investigation.

7.11 Gonadoprotective and reproductive activity

Reproductive toxicology is the most recently opened area of didymin research. In male rats exposed to the persistent environmental toxicant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD),

co-administration of didymin restored the activity of testicular antioxidant enzymes, normalised the expression of steroidogenic enzymes (3β - and 17β -hydroxysteroid dehydrogenase, steroidogenic acute regulatory protein, CYP11A1, and CYP17A1), restored circulating follicle-stimulating hormone, luteinising hormone, and testosterone, improved sperm count, viability, and motility, reduced sperm morphological anomalies, and normalised the Bax/caspase-3/Bcl-2 apoptotic balance in testicular tissue, with corresponding improvement in histopathology [102]. At present, this study represents the only published evidence supporting a potential gonadoprotective effect of didymin. Notably, the protective activity was demonstrated in the context of exposure to an endocrine-disrupting environmental toxicant, rather than in response to a conventional chemotherapeutic agent or metabolic challenge. This finding broadens the potential biological relevance of didymin beyond its more commonly investigated antioxidant, anti-inflammatory, and cytoprotective effects. Furthermore, the observation is consistent with emerging evidence suggesting that didymin may exert protective effects against a range of environmentally relevant toxicants, including newer classes of pollutants such as nanoplastics. Collectively, these findings indicate that the potential of didymin to mitigate environmentally induced reproductive and systemic toxicity warrants further investigation in well-characterised preclinical models.

Table 1. Summary of biological activities, mechanisms, and representative evidence for Didymin.

Biological activity	Reported mechanism(s)	Representative model(s) / reference(s)
Anticancer	Fas-mediated and mitochondrial (Bax/Bcl-2) apoptosis; chemosensitisation of drug-resistant tumour cells	Neuroblastoma, NSCLC, MDR cancer models [22][62][17]
Antioxidant / stress resistance	ROS/RNS scavenging; SOD, catalase, GPx, GSR upregulation; Nrf2/DAF-16 nuclear translocation via insulin/IGF-1-like (daf-2) pathway	<i>C. elegans</i> UV-irradiation model [64]; recurrent finding across organ-toxicity studies
Anti-inflammatory	Suppression of NF- κ B/TLR4 signalling; reduced TNF- α , IL-1 β , IL-6; NLRP3	Hepatic, cardiac, cerebral, colonic injury models

	inflammasome inhibition	[75][80][83][98]
Hepatoprotective	ER-stress alleviation and glycerophospholipid normalisation; TLR4/NF- κ B and PI3K/Akt inhibition; Sirt1-driven mitochondrial biogenesis and lipophagy; Nrf-2/Keap-1 modulation	CCl4 fibrosis, LPS/D-galactosamine acute injury, dexamethasone- and diet-induced NAFLD/MAFLD, polystyrene-nanoplastic hepatotoxicity [73][74][76][77]
Cardioprotective	PI3K/Akt/Nrf2 activation; NLRP3 inflammasome and apoptosis suppression; restoration of antioxidant enzyme activity	Doxorubicin- and paraquat-induced cardiotoxicity; ischaemia-reperfusion myocardial infarction [78][79][80]
Nephroprotective	Restoration of Nrf-2/Keap-1 signalling; reduced renal inflammatory and apoptotic markers	Cadmium-induced renal injury [81]
Neuroprotective	Asc/Caspase-1/GSDMD pyroptosis inhibition (RKIP-dependent); PPAR- γ /RXRA activation; MMP-9 suppression preserving blood-brain-barrier integrity	Intracerebral haemorrhage, cerebral ischaemia-reperfusion, subarachnoid haemorrhage [83][85][87]
Antidiabetic / metabolic	Inhibition of PTP1B, α -glucosidase, aldose reductase, and AGE formation; IRS-1/PI3K/Akt/GSK-3 activation; mitochondrial support of pancreatic β -cells	Insulin-resistant HepG2 cells, hyperglycaemic HUVECs, high-fat-diet impaired glucose tolerance [91][92][93]
Gastrointestinal	Hadhb-driven fatty acid oxidation promoting M1-to-M2 macrophage repolarisation; restoration of gut-barrier proteins claudin-1/ZO-1; gut microbiota and amino-acid metabolism modulation	DSS-induced ulcerative colitis [97][98]
Analgesic / CNS-modulatory	GABAergic potentiation (anxiolytic/sedative/antinociceptive);	Anxiolytic-antinociceptive mouse models [100]; chronic

	RKIP upregulation suppressing spinal NLRP3/ASC/GSDMD pyroptosis and NF- κ B activation	constriction injury neuropathic pain model [101]
Gonadoprotective	Restoration of steroidogenic enzymes (3 β -/17 β -HSD, StAR, CYP11A1, CYP17A1) and gonadotrophins; antiapoptotic support of spermatogenesis	TCDD-induced testicular toxicity [102]

8. Safety, Toxicity, and Antimicrobial Considerations

No study reviewed here reported adverse effects attributable to didymin itself in control or vehicle-treated arms, and in each disease model the compound was used to ameliorate an experimentally induced injury rather than to produce toxicity, broadly reassuring, but falling well short of a formal toxicological characterisation. Systematic acute- and repeat-dose toxicology, genotoxicity, and reproductive / developmental toxicity studies dedicated specifically to didymin, as opposed to citrus extracts in general, do not appear to have been published. Likewise, unlike several other citrus flavonoids, direct evidence of antimicrobial or antibacterial activity specific to didymin was not identified; the antimicrobial properties often generalised to 'citrus flavonoids' as a class have, for the most part, been demonstrated for other family members such as naringenin, hesperetin, and nobiletin rather than for didymin itself [58].

9. Knowledge Gaps and Future Directions

Several concrete gaps emerge from this review, spanning both the pharmaceutical-sciences and pharmacological literatures.

First, no validated bioanalytical method, LC-MS/MS, chiral HPLC, or otherwise, for didymin in plasma, urine, or tissue has been published outside a single cyclodextrin-formulation pharmacokinetic study; developing and validating such a method, ideally with the stereospecificity and sensitivity now routine for other flavonoid glycosides, is a prerequisite for any further in vivo pharmacokinetic work.

Second, didymin's own absorption, plasma protein binding, tissue distribution, metabolite profile, and excretion route remain uncharacterised in unformulated form; the existing gut-metabolism evidence is drawn almost entirely from didymin's structural relatives and from the closely related glycoside poncirin rather than from didymin itself, and no human feeding or supplementation study of didymin in isolation has been conducted.

Third, extraction and analytical methods optimised specifically for didymin recovery, rather than as an incidental co-analyte in broader citrus flavonoid profiling, have not been developed, despite well-established ultrasound- and microwave-assisted extraction protocols for the citrus flavanone class as a whole.

Fourth, no nanocarrier, self-emulsifying, solid-dispersion, or phospholipid-complex formulation has been tested against didymin, despite consistent bioavailability gains reported for exactly these classes of intervention with structurally related flavanones, and no enzymatic or biocatalytic production route has been explored as an alternative to citrus extraction.

Fifth, didymin's C-2 stereochemistry has been entirely overlooked; every study reviewed here appears to have used unresolved or presumed-racemic material, despite clear precedent from other citrus flavanones that enantiomers of the same molecule can differ meaningfully in pharmacokinetic behaviour.

Sixth, the biosynthetic pathway responsible for didymin's characteristic 7-O-rutinosylation remains inferred rather than demonstrated, and cloning the responsible glycosyltransferase(s) would both clarify didymin's unusual distribution across non-citrus taxa and potentially open a biotechnological route to production independent of citrus extraction.

On the pharmacological side, the evidence base remains almost entirely preclinical: rodent, nematode, and cell-culture studies dominate the literature, and no controlled human trial of isolated didymin has been identified, the closest analogue being its presence as a minor constituent of a multi-flavonoid citrus blend tested in a small human prediabetes trial. The food-drug interaction potential arising from didymin's inhibition of intestinal OATP2B1 has received little attention and could be clinically relevant for patients consuming citrus products alongside OATP2B1-substrate medications. Dedicated studies evaluating the formal toxicological profile, genotoxic potential, and antimicrobial activity of didymin remain limited or unpublished,

representing important gaps in the current evidence base. In addition, several molecular pathways—including Nrf2/Keap1, NF- κ B/TLR4, PI3K/Akt, NLRP3, and the Bax/Bcl-2/caspase and RKIP signalling axes—are repeatedly reported across studies involving different organ systems. Although the recurrence of these pathways may indicate a degree of convergent pharmacological activity, it is also possible that their frequent identification reflects the relatively narrow range of molecular targets and signalling pathways routinely investigated in the existing didymin literature. Consequently, it remains difficult to determine whether these pathways represent genuinely central mechanisms of action or are partly a consequence of methodological and hypothesis-selection biases. Future research would benefit from hypothesis-driven yet pathway-independent approaches, including unbiased proteomic, transcriptomic, and multi-omics analyses, to identify additional molecular targets and establish the broader mechanistic landscape of didymin. Furthermore, in view of the formal retraction of a previously cited hepatoprotective study in 2025, researchers should exercise particular caution when using older references and verify the current publication and retraction status of relevant studies before incorporating their findings into new research or reviews.

10. CONCLUSION

Didymin has evolved, in under two decades, from a structurally minor citrus flavanone glycoside into a compound with one of the broadest documented preclinical pharmacological profiles among dietary flavonoids, with credible evidence of anticancer, antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, nephroprotective, neuroprotective, antidiabetic, gastrointestinal, analgesic, and gonadoprotective activity.

This breadth is underpinned by a comparatively small number of recurring molecular mechanisms, chiefly centred on the Nrf-2/Keap-1 antioxidant axis, NF- κ B/TLR4- and NLRP3-driven inflammatory signalling, PI3K/Akt-dependent cell survival, RKIP-mediated regulation of kinase and inflammasome signalling, and control of the intrinsic apoptotic cascade.

Set against this pharmacological breadth, however, is a pharmaceutical-sciences foundation that has not kept pace: didymin's chemistry and natural distribution are reasonably well mapped, but its biosynthesis, analytical quantification, absorption, and metabolic fate are understood mainly by extrapolation from better-studied citrus relatives rather than through compound-specific data,

and only cyclodextrin complexation has been tested against its defining solubility problem. Realising didymin's therapeutic potential will require sustained investment in solubility-enhancing formulation science, a validated bioanalytical method, systematic toxicological characterisation, and, ultimately, controlled clinical evaluation, alongside careful, retraction-aware use of the existing literature, without which its considerable preclinical promise will remain, as it has for most of its history, confined to the laboratory bench.

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