

RESEARCH MONOGRAPH . COMPOUND PROFILE

Carnosic-Acid

A catechol-type abietane diterpene isolated from Rosmarinus officinalis and Salvia officinalis, distinguished by a pro-electrophilic mechanism of Nrf2 activation through oxidative conversion to a quinone intermediate that S-alkylates Keap1 cysteine residues, conferring context-dependent neuroprotective, anti-inflammatory, and cytoprotective activity selectively in tissues under oxidative stress.

DOCUMENT	KDC-MN-1524
ISSUED	2026-05-15
REVISION	1.0
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COMPOUND CLASS	Phenolic abietane diterpene with pro-electrophilic Nrf2/Keap1 pathway activation and pleiotropic antioxidant, anti-inflammatory, and neuroprotective activity

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ABSTRACT

Carnosic acid is the principal lipophilic phenolic diterpene of rosemary (*Salvia rosmarinus*, formerly *Rosmarinus officinalis*) and common sage (*Salvia officinalis*), constituting approximately 1.5 to 5 percent of dried leaf mass and serving as the primary bioactive antioxidant constituent of commercial rosemary extracts designated E392 in European food-additive regulations. The compound belongs to the abietane diterpenoid structural class and is characterized by a catechol (ortho-dihydroxybenzene) moiety on ring C of the phenanthrene skeleton that confers both direct radical-scavenging activity and, more distinctively, pro-electrophilic activation of the Keap1/Nrf2/ARE cytoprotective transcriptional pathway. Under conditions of oxidative stress, the catechol ring of carnosic acid undergoes two-electron oxidation to an ortho-quinone electrophile that forms covalent thioether adducts with reactive cysteine residues (principally Cys151, Cys273, and Cys288) on the Keap1 sensor protein, releasing the transcription factor Nrf2 from proteasomal degradation and permitting its nuclear translocation and transactivation of antioxidant response element (ARE)-driven phase 2 genes including heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase catalytic subunit (GCLC), and thioredoxin reductase 1. This pro-electrophilic mechanism is pharmacologically significant because it renders the compound conditionally active: carnosic acid is converted to its electrophilic effector form preferentially in tissues experiencing elevated reactive oxygen species flux, thereby concentrating cytoprotective gene induction at sites of pathological oxidative damage rather than systemically. The

neuroprotective activity of carnosic acid has been characterized in multiple rodent models of neurodegeneration and neuroinflammation, including 6-hydroxydopamine and MPTP models of Parkinson's disease, middle cerebral artery occlusion models of ischemic stroke, controlled cortical impact models of traumatic brain injury, and transgenic mouse models of Alzheimer's disease (5xFAD). In the 5xFAD model, a diacetyl pro-drug derivative (diAcCA) with improved oral stability and approximately 20 percent greater bioavailability than the parent compound reduced amyloid plaque burden, suppressed microglial and astrocytic inflammatory gene expression, and normalized spatial and working memory at oral doses translating to feasible human-equivalent exposures. Beyond neuroprotection, carnosic acid has demonstrated preclinical anti-inflammatory activity through suppression of NF-kappaB signaling and reduction of pro-inflammatory cytokines (TNF-alpha, IL-1beta, IL-6) in models of colitis, hepatotoxicity, periodontitis, and high-fat-diet-induced neuroinflammation; antiproliferative and pro-apoptotic activity against multiple cancer cell lines (melanoma, glioma, breast, prostate, colon, renal, esophageal) through modulation of PI3K/AKT/mTOR, MAPK, and STAT3 signaling pathways; hepatoprotective activity against acetaminophen-induced and carbon-tetrachloride-induced liver injury; and antiadipogenic activity through PPARgamma modulation. Pharmacokinetically, carnosic acid is orally bioavailable (approximately 40 to 65 percent in rodent models), undergoes extensive hepatic phase II glucuronidation, and achieves measurable plasma concentrations in humans after oral administration of standardized rosemary extract. The oral median lethal dose in mice exceeds 7100 mg/kg, and 30-day repeated-dose toxicity studies at 150 mg/kg/day in rats produced no significant histopathological changes. The Joint

FAO/WHO Expert Committee on Food Additives has established an acceptable daily intake of 0 to 0.6 mg/kg body weight per day for carnosic acid and carnosol combined. Human clinical data remain limited; no registration trials have been completed for any therapeutic indication, and the compound is not approved as a medicine by any regulatory authority. This monograph reviews the chemistry, natural occurrence, and isolation of carnosic acid; the pro-electrophilic Nrf2/Keap1 mechanism in molecular detail; the pharmacokinetic record including absorption, distribution, metabolism, and elimination; the preclinical pharmacology across neuroprotective, anti-inflammatory, anticancer, and hepatoprotective domains; the limited clinical evidence base; sourcing and quality verification; reconstitution and handling; stack interactions; adverse-event and safety signal; and a comparative assessment of five alternative Nrf2-activating or rosemary-derived compounds against carnosic acid on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Carnosic acid.

Marketed extract designations: E392 (European Union food additive designation for rosemary extracts standardized to carnosic acid and carnosol content). The E392 designation covers the extract rather than the isolated compound; carnosic acid is the principal active constituent [1].

Chemical Abstracts Service registry number: 3650-09-7.

PubChem Compound Identifier: CID 65126.

International Union of Pure and Applied Chemistry name: (4aR,10aS)-5,6-dihydroxy-1,1-dimethyl-7-(propan-2-yl)-2,3,4,9,10,10a-hexahydro-1H-phenanthrene-4a(4bH)-carboxylic acid. The systematic name reflects the trans-fused decalin system bearing an isopropyl-substituted catechol ring and a bridgehead carboxylic acid [2].

Synonyms in the pharmacological literature: Salvin (historical); the compound is occasionally designated "rosemary phenolic diterpene" or "CA" in abbreviated usage. It should not be confused with carnosine (beta-alanyl-L-histidine), a dipeptide with an unrelated structure and pharmacology.

1.2 Structural class and chemistry

Carnosic acid is a phenolic abietane diterpene. The abietane skeleton is a tricyclic C₂₀ diterpenoid framework common to a large family of plant secondary metabolites, including abietic acid (the resin acid prototype), tanshinones (from *Salvia miltiorrhiza*), and ferruginol. Carnosic acid is distinguished within this class by the ortho-dihydroxyl (catechol) substitution at C-11 and C-12 on the aromatic C-ring and by the carboxylic acid at the C-20 bridgehead position [3].

Molecular formula: C₂₀H₂₈O₄.

Exact monoisotopic mass: 332.1988 g/mol.

Molecular weight: 332.43 g/mol.

Physical properties: White to pale yellow crystalline powder. Soluble in methanol, ethanol, dimethyl sulfoxide, and lipid matrices; sparingly soluble in water (aqueous solubility approximately 0.1 mg/mL at 25 degrees Celsius and neutral pH). Melting point approximately 190 to 195 degrees Celsius. The compound is sensitive to oxidation in air and light; degradation products include carnosol (the gamma-lactone formed by intramolecular lactonization of the oxidized quinone), rosmanol, epirosmanol, and 7-methylepirosmanol [4].

Stereochemistry: Carnosic acid contains two stereocenters at C-4a (the bridgehead carbon bearing the carboxylic acid) and C-10a (the ring-junction carbon). The natural product is the (4aR,10aS) enantiomer. Synthetic preparations and isolated material should be confirmed for enantiomeric identity by optical rotation or chiral chromatography [2].

Oxidative degradation cascade: Under aerobic conditions, particularly in the presence of light or elevated temperature, the catechol ring of carnosic acid undergoes sequential oxidation. The initial two-electron oxidation produces the ortho-quinone, which can either (a) react with biological nucleophiles (the pharmacologically productive Keap1 S-alkylation pathway) or (b) undergo intramolecular lactonization to yield carnosol, a structurally related but pharmacologically distinct compound with an intact gamma-lactone ring replacing the catechol. Further oxidation of carnosol produces rosmanol and related products. This cascade is the primary stability concern for the isolated compound and for rosemary extracts standardized to carnosic acid content [4, 5].

1.3 Natural occurrence and isolation

Carnosic acid is biosynthesized in the glandular trichomes of rosemary (*Salvia rosmarinus*, syn. *Rosmarinus officinalis*) and sage (*Salvia officinalis*) leaves through the methylerythritol phosphate (MEP) terpenoid pathway. The biosynthetic route proceeds from geranylgeranyl diphosphate through copalyl diphosphate (catalyzed by copalyl diphosphate synthase) to miltiradiene (catalyzed by miltiradiene synthase), followed by cytochrome P450-mediated oxidative aromatization of ring C and sequential hydroxylation to yield ferruginol, 11-hydroxyferruginol, and finally carnosic acid [6].

Typical concentrations in dried rosemary leaves range from 1.5 to 5 percent by dry weight, making carnosic acid the most abundant phenolic diterpene in the plant. Concentrations vary with cultivar, geographic origin, harvest season, and post-harvest drying conditions. Supercritical carbon dioxide extraction, pressurized liquid extraction, and conventional solvent extraction (ethanol, methanol, or acetone) are the principal industrial isolation methods. Commercial rosemary extracts for food-additive use (E392) are typically standardized to contain 10 to 25 percent carnosic acid by HPLC [1, 7].

1.4 Synthesis

Total chemical synthesis of carnosic acid has been achieved through multiple routes, though the complexity of the trans-fused abietane skeleton with the labile catechol functionality makes practical synthesis challenging. The Breskorn and Fuchs (1962) structural elucidation and the subsequent Matsumoto et al. (2002) and Simmons et al. (2008) total syntheses proceed through construction of the trans-decalin core by Robinson annulation or Diels-Alder strategies, followed by aromatization of ring C and regioselective dihydroxylation [8]. Industrial supply is exclusively by extraction from plant material rather than by chemical synthesis, owing to the cost advantage of rosemary cultivation and extraction. The pro-drug derivative di-AcCA (diacetylcarnosic acid) has been synthesized by acetylation of both catechol hydroxyl groups, conferring improved stability to oxidative degradation while maintaining complete deacetylation to carnosic acid in gastric conditions [9].

2. Abstract

Carnosic acid (CA) is the dominant lipophilic phenolic diterpene of rosemary and sage, with a well-characterized pro-electrophilic mechanism of Nrf2/Keap1 pathway activation that distinguishes it from conventional direct antioxidants and from electrophilic Nrf2 activators that act constitutively regardless of cellular redox state. The compound is converted to its pharmacologically active ortho-quinone form under conditions of oxidative stress, and this conditional activation confines the cytoprotective transcriptional response to tissues experiencing pathological oxidant flux [10, 11].

The Nrf2-activating mechanism has been validated across multiple disease-relevant in vitro and in vivo systems. In neurodegeneration models, carnosic acid protects dopaminergic neurons against 6-hydroxydopamine toxicity, reduces infarct volume in middle cerebral artery occlusion ischemia, attenuates cortical contusion injury and mitochondrial dysfunction after traumatic brain injury, and normalizes cognitive deficits and reduces amyloid plaque burden in the 5xFAD transgenic mouse model of Alzheimer's disease [9, 12, 13, 14]. In inflammation models, carnosic acid suppresses NF-kappaB-driven cytokine production in lipopolysaccharide-stimulated macrophages and microglial cells and reduces disease severity in models of colitis, hepatotoxicity, and periodontitis [15, 16, 17]. In cancer models, carnosic acid induces apoptosis and cell-cycle arrest in melanoma, glioma, breast, prostate, colon, renal, and esophageal cancer cell lines through modulation of PI3K/AKT/mTOR, MAPK/ERK, and STAT3 signaling, with synergistic effects when combined with temozolomide, carmustine, and lomustine in glioma and melanoma [18, 19, 20, 21].

Pharmacokinetically, carnosic acid is orally bioavailable in rodents (approximately 40 to 65 percent), undergoes hepatic glucuronidation as the principal phase II metabolic transformation, and achieves measurable plasma concentrations in humans after oral administration of rosemary extract [22, 23]. The compound has low acute toxicity (mouse oral LD50 greater than 7100 mg/kg) and no significant histopathological toxicity at 150 mg/kg/day for 30 days in rats [24]. The JECFA acceptable daily intake for combined carnosic acid and carnosol is 0 to 0.6 mg/kg body weight per day [25].

No human registration trials have been completed for any therapeutic indication. The compound is available as a food-grade antioxidant additive (E392) and as a research-grade chemical from multiple suppliers. Investigators should confirm identity, purity, and enantiomeric configuration on every lot, given the known oxidative instability and the potential for degradation to carnosol during storage and handling.

3. Discovery and Development History

3.1 Identification and structural elucidation

Carnosic acid was first isolated from rosemary leaves by Wenkert et al. (1965) and structurally elucidated by Breskorn and Fuchs (1962), who established the abietane diterpene skeleton and the catechol substitution pattern through chemical degradation and spectroscopic analysis [8, 26]. The compound was recognized early as the principal antioxidant constituent of rosemary, a property that had been exploited empirically in Mediterranean cuisine and food preservation for centuries. The structural relationship to carnosol (the oxidation-derived gamma-lactone) was established in the same period.

3.2 Food-additive development

The commercial development of carnosic acid has been principally in the food-additive and preservative domain rather than as a pharmaceutical. Rosemary extracts standardized to carnosic acid and carnosol content were evaluated by the European Food Safety Authority (EFSA) and received the E392 food-additive designation for use in oils, fats, sauces, bakery products, meat products, and fish products [1]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated rosemary extract and established the acceptable daily intake at its 82nd meeting [25]. The food-grade application remains the dominant commercial use of carnosic acid.

3.3 Identification of Nrf2/Keap1 mechanism

The pharmacological significance of carnosic acid expanded substantially with the characterization of the Nrf2/Keap1 activation mechanism by Satoh et al. (2008) at the Burnham Institute for Medical Research (now Sanford Burnham Prebys Medical Discovery Institute). The Satoh laboratory demonstrated that carnosic acid activates Nrf2 through a pro-electrophilic mechanism: under oxidative conditions, the compound is converted to its quinone form, which S-alkylates critical cysteine residues on Keap1, releasing Nrf2 for nuclear translocation [10]. This work estab-

lished the conceptual framework for carnosic acid as a conditionally active cytoprotectant and provided the rationale for its investigation in neurodegenerative and neuroinflammatory disease models.

3.4 Expansion to neuroprotection research

The Bhatt, Bhatt, Lipton, and Bhatt laboratories at Scripps Research Institute and collaborating institutions extended the Nrf2 characterization to multiple in vivo disease models between 2010 and 2025. Key demonstrations include neuroprotection in MPTP and 6-OHDA Parkinson's disease models [12], reduction of infarct volume and neurological deficit in ischemic stroke [13], attenuation of mitochondrial dysfunction and neuronal cytoskeletal degradation in traumatic brain injury [14], and, most recently, efficacy of the diacetyl pro-drug diAcCA in the 5xFAD transgenic mouse model of Alzheimer's disease with transcriptomic evidence of inflammatory gene downregulation and neuroprotective gene upregulation [9]. A 2025 Scripps Research press release described diAcCA as a potential anti-inflammatory drug candidate for Alzheimer's disease, with ongoing preparation for clinical translation [27].

3.5 Current development state

No investigational new drug (IND) application has been filed for carnosic acid or diAcCA in any jurisdiction as of the monograph preparation date. The compound is not in active commercial pharmaceutical development. The current research state is one of strong preclinical validation across multiple disease models, established food-safety data, and absent clinical-stage development. The diAcCA pro-drug represents the most advanced pharmaceutical formulation effort and the most plausible path to clinical translation.

4. Mechanism of Action

Carnosic acid is a multi-pathway compound. Its principal molecular activities, characterized in cell-free, cell-based, and in vivo systems, are pro-electrophilic activation of the Keap1/Nrf2/ARE antioxidant transcriptional pathway; suppression of NF-kappaB-driven inflammatory signaling; modulation of PI3K/AKT/mTOR and MAPK proliferative and survival signaling; and direct radical-scavenging antioxidant activity. These activities operate at overlapping concentration ranges and contribute differentially to the observed neuroprotective, anti-inflammatory, anti-cancer, and hepatoprotective effects.

4.1 Keap1/Nrf2/ARE pathway activation

The Keap1/Nrf2/ARE pathway is the principal inducible cytoprotective transcriptional system in mammalian cells. Under basal conditions, the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) is sequestered in the cytoplasm by its repressor protein Keap1 (Kelch-like ECH-associated protein 1), which functions as a substrate adaptor for the Cullin 3-RING-box 1 (Cul3-Rbx1) E3 ubiquitin ligase complex. Keap1-mediated polyubiquitination targets Nrf2 for proteasomal degradation, maintaining low basal Nrf2 levels and correspondingly low expression of ARE-driven phase 2 genes [28].

Carnosic acid activates this pathway through a pro-electrophilic mechanism that is distinct from most characterized Nrf2 inducers. The catechol ring on the C-ring of the abietane skeleton (the 11,12-dihydroxy substitution) is oxidized under conditions of elevated reactive oxygen species to an ortho-quinone. The C-14 position of the quinone becomes a Michael acceptor electrophile that reacts with the thiolate anions of reactive cysteine residues on Keap1, principally Cys151 (in the BTB domain), Cys273, and Cys288 (in the intervening region). The resulting thioether (S-alkylated) adducts conformationally disrupt the Keap1-Nrf2 interaction, preventing ubiquitination and stabilizing Nrf2 for nuclear translocation [10, 11].

The pharmacological significance of this mechanism is the conditional activation: the oxidation of catechol to quinone requires an oxidative environment. In healthy cells with low reactive oxygen species flux, carnosic acid remains predominantly in its reduced catechol form and produces minimal Keap1 modification. In

cells under oxidative stress (as in neurodegenerative lesions, ischemic tissue, or sites of active inflammation), the elevated oxidant burden drives quinone formation and Keap1 S-alkylation, concentrating the Nrf2-mediated cytoprotective response at the site of pathology. Satoh et al. (2008) demonstrated this conditional activation in mixed cortical cultures and in the MCAO stroke model, showing that carnosic acid activated Nrf2-dependent gene expression preferentially in the ischemic penumbra (high oxidative stress) rather than in the contralateral hemisphere (low oxidative stress) [10].

The downstream transcriptional consequences of Nrf2 nuclear translocation include upregulation of heme oxygenase-1 (HMOX1/HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase catalytic and modifier subunits (GCLC, GCLM), glutathione S-transferases (GSTA1, GSTM1, GSTP1), thioredoxin reductase 1 (TXNRD1), and ferritin light and heavy chains (FTL, FTH1). The composite effect is a coordinated increase in cellular glutathione synthesis, quinone detoxification, iron sequestration, and biliverdin/bilirubin-mediated antioxidant capacity [28].

4.2 NF-kappaB suppression

Carnosic acid suppresses the NF-kappaB inflammatory signaling pathway through multiple characterized mechanisms. In lipopolysaccharide-stimulated macrophage and microglial cell models, carnosic acid inhibits IkappaB kinase (IKK) phosphorylation, stabilizes IkappaBalpha, and reduces nuclear translocation of NF-kappaB p65/RelA. The downstream consequence is reduced transcription of NF-kappaB-dependent pro-inflammatory mediators including tumor necrosis factor alpha (TNF-alpha), interleukin-1 beta (IL-1beta), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) [15, 16].

A crosstalk between the Nrf2/HO-1 axis and NF-kappaB suppression has been characterized in SH-SY5Y neuroblastoma cells exposed to paraquat. Carnosic acid simultaneously induced Nrf2/HO-1 expression and suppressed NF-kappaB activation, with pharmacological inhibition of HO-1 partially reversing the NF-kappaB suppression, indicating that the anti-inflammatory effect is partially downstream of Nrf2-mediated HO-1 induction [16]. A 2022 study in APP/PS1 transgenic mice demonstrated that nanocarrier-packaged carnosic acid inhibited the CEBPbeta-NF-kappaB interaction in microglia and astrocytes, reducing mature IL-1beta, TNF-alpha, and IL-6 production in brain tissue and ameliorating cognitive deficits [29].

4.3 PI3K/AKT/mTOR and MAPK pathway modulation

In cancer cell lines, carnosic acid modulates the PI3K/AKT/mTOR survival and proliferative signaling axis. Inhibition of AKT phosphorylation and downstream mTOR signaling has been demonstrated in breast cancer (MCF-7, MDA-MB-231), prostate cancer (PC-3, LNCaP), colon cancer (HCT116), and renal carcinoma (Caki) cell lines [18, 20]. The MAPK/ERK pathway is activated by carnosic acid in some cell types (contributing to pro-apoptotic signaling through sustained ERK activation) and inhibited in others, reflecting the context dependence of MAPK signaling in proliferation versus stress response [19]. STAT3 inactivation through Src kinase inhibition has been characterized in Caki renal carcinoma cells, producing apoptosis through caspase-3 activation and PARP cleavage [21].

The dual action on PTEN (a negative regulator of PI3K/AKT) has been characterized in the context of heterotopic ossification, where carnosic acid functions as both an Nrf2 activator and a PTEN/AKT suppressor, linking the antioxidant and antiproliferative mechanisms [30].

4.4 Direct antioxidant activity

Independent of the Nrf2-mediated transcriptional response, carnosic acid possesses direct radical-scavenging activity attributable to the catechol ring. The ortho-dihydroxyl system can donate hydrogen atoms to lipid peroxyl radicals and other reactive oxygen and nitrogen species, functioning as a chain-breaking antioxidant in lipid-phase oxidation. This direct antioxidant activity is the basis for the food-preservative application and contributes to the cytoprotective activity in acute oxidative insults before the slower transcriptional Nrf2 response can take effect [5, 31].

4.5 Additional characterized targets

HSF-1 (heat shock factor 1) activation by carnosic acid has been reported, leading to upregulation of heat shock protein expression (HSP70, HSP27) and contributing to chaperone-mediated proteostasis under stress conditions [27]. PPARgamma activation has been characterized in adipocyte models, contributing to antiadipogenic and insulin-sensitizing effects at concentrations in the low-micromolar range [32].

Ferroptosis inhibition through Nrf2/GPX4 pathway activation has been demonstrated in periodontal disease models, extending the cytoprotective mechanism to iron-dependent cell death pathways [17].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Carnosic acid is absorbed from the gastrointestinal tract after oral administration. Deng et al. (2009) characterized the pharmacokinetics in rats after intravenous (10 mg/kg) and oral (90 mg/kg) administration, reporting oral bioavailability of approximately 40 percent after 6 hours [22]. A separate study reported absolute oral bioavailability of 65 percent, with the higher value likely reflecting more complete recovery of glucuronidated metabolites [23]. Time to peak plasma concentration after oral gavage in rats is approximately 30 to 60 minutes. Peak plasma concentration after 90 mg/kg oral administration in rats was approximately 3 to 5 micrograms per milliliter [22].

Carnosic acid is lipophilic (log P approximately 4.0) and is absorbed more efficiently in the presence of dietary fat or lipid vehicles. Lecithin-based nanoemulsion systems have been developed to improve oral bioavailability further, with reported improvements in area under the curve (AUC) of 2- to 3-fold compared to suspension formulations [33]. The pro-drug diAcCA demonstrates approximately 20 percent improvement in bioavailability over unmodified carnosic acid, attributable to protection of the catechol ring from pre-systemic oxidation during gastrointestinal transit, with complete deacetylation occurring in gastric acid conditions [9].

A human pharmacokinetic study (Meoni et al., 2023) developed and validated an HPLC-UV method for determination of carnosic acid in human plasma and applied it to a prototype pharmacokinetic study in a healthy volunteer receiving oral rosemary extract. Carnosic acid was detectable in plasma, confirming human oral absorption, though comprehensive pharmacokinetic parameters (C_{max}, T_{max}, AUC, half-life) from adequately powered human studies remain unpublished [34].

5.2 Distribution

Volume of distribution data in rats indicate moderate tissue distribution. The compound is lipophilic and partitions into lipid-rich tissues. Brain penetration has been inferred from the demonstrated central nervous system pharmacological activity in rodent models of neurodegeneration and traumatic brain injury, though direct measurement of brain-to-plasma ratios by mass spectrometry imaging or microdialysis has not been reported in comprehensive form. Plasma protein binding has not been formally characterized in published studies but is expected to be substantial given the lipophilicity [22, 23].

5.3 Metabolism

Carnosic acid undergoes extensive hepatic phase II metabolism, principally glucuronidation. Large amounts of glucuronidated metabolites were detected in plasma and urine in the Deng et al. (2009) rat pharmacokinetic study, and the glucuronide conjugates were responsible for the relatively high calculated bioavailability despite poor metabolic stability of the parent compound [22]. Cytochrome P450-mediated oxidative metabolism has been characterized in vitro using human hepatocytes. Carnosic acid inhibits CYP2C9- and CYP3A4-catalyzed reactions and induces CYP2B6 and CYP3A4 mRNA and enzyme activity in a dose-dependent manner, indicating both inhibitory and inductive interactions with the CYP enzyme system [35]. The oxidative degradation of carnosic acid to carnosol, rosmanol, and related abietane quinones also occurs in vivo and constitutes a non-enzymatic elimination pathway [4].

5.4 Elimination

Renal excretion of glucuronidated metabolites is the principal elimination pathway. Biliary excretion contributes to fecal elimination. The plasma elimination half-life in rats after intravenous administration is approximately 65 to 90 minutes, reflecting rapid hepatic conjugation and renal clearance [22]. The relatively short half-life in rodents suggests that sustained pharmacological effect at Nrf2-relevant concentrations may require multiple daily dosing or sustained-release formulation in any clinical application.

5.5 Routes of administration

Oral administration is the principal route studied in preclinical models, using gavage of carnosic acid in suspension (typically in 0.5 percent carboxymethylcellulose or corn oil vehicle) or in standardized rosemary extract. Intraperitoneal administration has been used in acute neuroprotection studies. Topical application has been studied in dermatological and wound-healing models. Intravenous administration has been characterized pharmacokinetically but is not a practical research route owing to the poor aqueous solubility of the compound. The diAcCA pro-drug is formulated for oral administration [9, 22].

5.6 Special populations

No human special-population pharmacokinetic data exist. Carnosic acid is present in rosemary, a widely consumed culinary herb, and dietary exposure across populations (including children, the elderly, and pregnant women) is long-standing at food-additive levels. The JECFA acceptable daily intake of 0 to 0.6 mg/kg body weight per day applies to the general population including children [25].

6. Preclinical Pharmacology

6.1 Neuroprotection

The neuroprotective pharmacology of carnosic acid is the most extensively characterized preclinical domain and spans multiple disease models.

Parkinson's disease models: Carnosic acid (1 to 10 mg/kg intraperitoneal) protects dopaminergic neurons in the substantia nigra pars compacta against 6-hydroxydopamine toxicity in rats and against MPTP toxicity in mice. Protection is associated with increased striatal Nrf2 nuclear translocation, elevated HO-1 and NQO1 expression, and preserved tyrosine hydroxylase immunoreactivity [12]. The effect is abolished by pharmacological or genetic inhibition of Nrf2, confirming pathway dependence.

Ischemic stroke models: In the middle cerebral artery occlusion (MCAO) model in mice, carnosic acid administered at the time of reperfusion reduced infarct volume by approximately 35 to 50 percent and improved neurological deficit scores. Nrf2 activation was localized to the ischemic penumbra, consistent with the pro-electrophilic conditional activation mechanism [10, 13].

Traumatic brain injury models: Miller et al. (2015) characterized carnosic acid in the controlled cortical impact model in mice, reporting that administration (1 mg/kg intraperitoneal) decreased mitochondrial dysfunction, reduced oxidative damage markers (4-hydroxynonenal, protein carbonyls), and attenuated neuronal cytoskeletal degradation (spectrin breakdown products) through Nrf2/ARE-dependent mechanisms [14]. A sex-dependent modulation of the Nrf2 antioxidant response was observed, with male mice showing greater Nrf2 activation than female mice at equivalent doses [36].

Alzheimer's disease models: The 2025 diAcCA study in 5xFAD transgenic mice demonstrated that oral administration of the pro-drug reduced amyloid plaque burden in hippocampus and cortex, downregulated inflammatory gene expression (including microglial activation markers and astrocytic GFAP), upregulated neuroprotective genes enriched in axonogenesis and synaptic plasticity pathways, and normalized spatial and working memory as assessed by Morris water maze and novel

object recognition [9, 27]. The therapeutic advantage of the pro-drug approach was attributed to improved oral stability and bioavailability, delivering higher sustained carnosic acid concentrations to brain tissue.

Depression models: Carnosic acid (10 to 40 mg/kg oral) alleviated depression-like behavior in ovariectomized mice through Nrf2/HO-1 pathway activation, providing evidence for activity in neuropsychiatric models beyond classical neurodegeneration [37].

6.2 Anti-inflammatory pharmacology

Carnosic acid suppresses inflammatory responses in multiple organ systems. In lipopolysaccharide-stimulated RAW 264.7 macrophages, carnosic acid (5 to 20 micromolar) dose-dependently reduces TNF-alpha, IL-1beta, IL-6, and PGE2 production through IKK/NF-kappaB pathway inhibition [15]. In colitis models (DSS-induced murine colitis), oral carnosic acid reduces disease activity index scores, colonic shortening, and histological damage [38]. In high-fat-diet-induced neuroinflammation, carnosic acid alleviates brain injury through NF-kappaB-regulated inflammation and caspase-3-associated apoptosis [39]. In periodontitis models, carnosic acid inhibits ferroptosis through Nrf2/GPX4 pathway activation, reducing alveolar bone loss and gingival inflammation [17].

6.3 Anticancer pharmacology

Carnosic acid has demonstrated antiproliferative and pro-apoptotic activity in a broad panel of cancer cell lines.

Melanoma: In B16F10 melanoma cells, carnosic acid inhibited proliferation (IC50 approximately 20 to 40 micromolar), induced G0/G1 cell-cycle arrest, and enhanced the anticancer effects of carmustine (BCNU) and lomustine (CCNU). In vivo, carnosic acid at 100 mg/kg oral produced significant tumor growth inhibition without hepatotoxicity (normal AST and ALT) [19].

Glioma: Carnosic acid potentiated the anticancer effect of temozolomide in U87 and U251 glioma cell lines by inducing apoptosis (increased cleaved caspase-3 and PARP) and autophagy (increased LC3-II and beclin-1). The combination index analysis indicated synergism at clinically relevant temozolomide concentrations [20].

Breast cancer: Antiproliferative activity has been characterized in MCF-7 (ER-positive) and MDA-MB-231 (triple-negative) breast cancer cell lines, with inhibition of PI3K/AKT/mTOR signaling and induction of caspase-dependent apoptosis [18].

Prostate cancer: Carnosic acid inhibited proliferation and induced apoptosis in PC-3 and LNCaP prostate cancer cell lines. Rosmarinic acid and carnosic acid from rosemary extract have been evaluated together for prostate cancer chemoprevention, with the lipophilic diterpene fraction (carnosic acid and carnosol) showing greater antiproliferative potency than the hydrophilic fraction (rosmarinic acid) [40].

Esophageal cancer: Carnosic acid induced antiproliferation and anti-metastatic properties in esophageal squamous cell carcinoma through MAPK signaling pathway activation, producing G2/M cell-cycle arrest and DNA damage [41].

Renal carcinoma: In Caki cells, carnosic acid induced apoptosis through Src/STAT3 signaling pathway inactivation [21].

6.4 Hepatoprotective pharmacology

Carnosic acid protects against hepatotoxicity through Nrf2-mediated upregulation of hepatic antioxidant defenses. In acetaminophen-induced liver injury in mice, carnosic acid pretreatment (30 to 100 mg/kg oral) potentiated Nrf2-mediated antioxidant capacity, reduced hepatic glutathione depletion, lowered serum transaminase (ALT, AST) levels, and decreased centrilobular necrosis [42]. In carbon tetrachloride-induced hepatotoxicity models, similar protective effects have been demonstrated [15].

6.5 Other preclinical activities

Additional characterized preclinical activities include antiadipogenic effects through PPARgamma modulation in 3T3-L1 adipocytes [32]; antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and moderate activity against Gram-negative organisms [7]; and cardioprotective effects against ischemia-reperfusion injury in isolated heart preparations through Nrf2-dependent mechanisms [43].

7. Clinical Evidence Base

7.1 Human pharmacokinetic studies

A prototype pharmacokinetic study (Meoni et al., 2023) validated an HPLC-UV analytical method for carnosic acid determination in human plasma and applied it after oral administration of standardized rosemary extract to a healthy volunteer. The study confirmed that carnosic acid is absorbed into human plasma after oral rosemary extract administration and that the analytical method is suitable for use in Phase 1 clinical studies [34]. Comprehensive human pharmacokinetic parameters from adequately powered crossover or parallel-group studies have not been published.

7.2 Rosemary extract clinical studies

Human clinical investigations of carnosic acid have been conducted principally in the context of rosemary extract supplementation rather than as isolated compound administration. Rosemary extract standardized to carnosic acid content has been evaluated in small human studies for cognitive function, mood, and metabolic endpoints.

Rosemary extract at doses of 100 to 1000 mg per day has been used in clinical studies to reduce symptoms of common mental health disorders, with significant improvements noted at higher doses. A randomized controlled trial evaluating rosemary extract for cognitive function in healthy adults reported modest improvements in processing speed and attention following acute supplementation, though attribution of these effects specifically to carnosic acid (versus rosmarinic acid or other rosemary constituents) was not possible from the study design [44].

7.3 Absence of registration trials

No Phase 2 or Phase 3 registration trials have been completed for carnosic acid or its pro-drug derivative diAcCA for any therapeutic indication. The compound is not approved as a medicine by any regulatory authority. The preparation of diAcCA for

clinical translation, as described by the Lipton laboratory at Scripps Research in 2025, represents the most advanced clinical-development effort, but IND filing has not been publicly confirmed [9, 27].

7.4 Food safety evaluations

The JECFA evaluation of rosemary extract (expressed as carnosic acid and carnosol) established an ADI of 0 to 0.6 mg/kg body weight per day based on the 30-day oral toxicity study NOAEL of 60 mg/kg/day in rats with a 100-fold safety factor [25]. The EFSA evaluation of rosemary extracts as food additives (E392) similarly concluded that use at specified maximum levels in food categories was not of safety concern [1].

8. Sourcing and Quality Verification

Research-grade carnosic acid is supplied by multiple chemical and biochemical suppliers (Sigma-Aldrich/MilliporeSigma, Cayman Chemical, Santa Cruz Biotechnology, Selleck Chemicals, MedChemExpress, StressMarq Biosciences, Toronto Research Chemicals) at greater than 95 percent purity by HPLC. Supply is in milligram to gram quantities as the free acid. Pricing is moderate, typically in the range of \$30 to \$150 per 100 milligrams of research-grade material.

Food-grade rosemary extract standardized to carnosic acid content (10 to 25 percent by HPLC) is available from ingredient suppliers (Naturex/Givaudan, Kemin Industries, Vitiva, Frutarom) in kilogram quantities for food-additive and nutraceutical applications.

The pro-drug derivative diAcCA is not yet commercially available as of the monograph preparation date; it is a research compound synthesized by the Lipton laboratory [9].

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 95 percent (greater than 98 percent for mechanism-of-action studies where carnosol contamination would confound results)
- Mass spectrometry confirmation of molecular weight (333.2066 for the protonated species $[M+H]^+$, 332.1988 monoisotopic)
- Assessment of carnosol content (the principal degradation product), which should be below 2 percent in fresh high-purity lots
- Optical rotation measurement or chiral HPLC to confirm the natural (4aR,10aS) configuration
- Karl Fischer or loss-on-drying analysis for residual water content
- Storage under inert atmosphere (nitrogen or argon) at minus 20 degrees Celsius, protected from light, to minimize oxidative degradation to carnosol

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. Given the known oxidative instability of carnosic acid, on-receipt and pre-experiment analytical confirmation is strongly recommended for any investigation where dose-response precision is critical.

9. Reconstitution and Handling

Carnosic acid is supplied as a crystalline or amorphous powder. The compound is poorly soluble in water and is typically reconstituted in organic solvents for in vitro work.

Stock solution preparation for in vitro studies: Dissolve in dimethyl sulfoxide (DMSO) at 10 to 50 mM to prepare concentrated stock solutions. Store aliquots at minus 20 degrees Celsius under nitrogen. Working dilutions should be prepared immediately before use in culture medium, with final DMSO concentration not exceeding 0.1 percent (v/v) to avoid solvent cytotoxicity. Ethanol and methanol are alternative solvents; aqueous dilution from ethanol stocks should be performed rapidly to avoid precipitation.

Formulation for oral gavage in rodent studies: Suspend in 0.5 percent carboxymethylcellulose sodium, in corn oil, or in polyethylene glycol 400 at concentrations of 1 to 20 mg/mL. Corn oil vehicles improve absorption owing to the lipophilicity of the compound. Formulations should be prepared fresh daily or stored at 2 to 8 degrees Celsius for no more than 48 hours, protected from light and air.

Stability considerations: Carnosic acid is sensitive to oxidation, particularly in solution, at elevated temperature, and in the presence of light or transition metal ions. The catechol ring undergoes oxidation to the ortho-quinone, followed by lactonization to carnosol. Solutions in DMSO are more stable than aqueous solutions; storage under inert atmosphere (nitrogen or argon) extends stability substantially. Repeated freeze-thaw cycles should be avoided; aliquoting into single-use volumes is recommended. Lyophilized or solid material stored under inert atmosphere at minus 20 degrees Celsius is stable for at least 24 months.

Light sensitivity: The compound is moderately photosensitive. Storage containers should be amber glass or foil-wrapped, and bench-time exposure to ambient light during experimental preparation should be minimized.

10. Stack Interactions and Combinations

Carnosic acid interacts with several drug classes through its CYP enzyme effects and through its multi-pathway pharmacology. Investigators conducting research with carnosic acid in combination with other research compounds should consider:

CYP enzyme interactions: Carnosic acid inhibits CYP2C9 and CYP3A4 and induces CYP2B6 and CYP3A4 at higher concentrations [35]. Concurrent use with drugs metabolized by CYP2C9 (warfarin, phenytoin, NSAIDs) or CYP3A4 (cyclosporine, statins, protease inhibitors, many benzodiazepines) may alter the exposure of the co-administered drug. The clinical significance of these interactions at dietary or supplemental carnosic acid exposures is uncertain but should be considered in high-dose research applications.

Other Nrf2 activators: Concurrent use with other Nrf2-activating compounds (sulforaphane, dimethyl fumarate, bardoxolone methyl, curcumin, CDDO-Me) may produce additive or synergistic Nrf2 pathway activation. The combination of carnosic acid with sulforaphane has been studied in vitro and produces additive HO-1 induction; whether this translates to therapeutic advantage or to excessive Nrf2 activation (which has been associated with pro-tumorigenic effects in some contexts) has not been systematically evaluated [28].

Antioxidants and radical scavengers: Concurrent N-acetylcysteine (NAC) may attenuate the pro-electrophilic activation of carnosic acid by scavenging the reactive oxygen species required for catechol-to-quinone conversion. NAC pretreatment has been shown to abrogate carnosic acid-induced apoptosis in cancer cell lines, confirming the ROS-dependence of the quinone-mediated mechanism [18]. Investigators studying the Nrf2-mediated effects should avoid concurrent NAC or other potent radical scavengers.

Chemotherapeutic agents: Carnosic acid has demonstrated synergistic anti-cancer effects with temozolomide (glioma), carmustine and lomustine (melanoma), and potentially other alkylating agents [19, 20]. The mechanism is plausibly related to carnosic acid-induced ROS generation and Nrf2-mediated modulation of drug re-

sistance pathways. However, Nrf2 activation has also been associated with chemoresistance in some tumor types through upregulation of drug efflux transporters and glutathione conjugation enzymes; context-dependent evaluation is essential.

Anti-inflammatory agents: Concurrent use with conventional NSAIDs, corticosteroids, or biological anti-inflammatory agents (anti-TNF, anti-IL-6) may produce additive anti-inflammatory effects through complementary pathway suppression (NF-kappaB suppression by carnosic acid, COX inhibition by NSAIDs, glucocorticoid receptor activation by steroids).

Cholinesterase inhibitors and memantine: The Alzheimer's disease research application of carnosic acid may involve concurrent use with donepezil, rivastigmine, galantamine, or memantine. No direct pharmacokinetic interactions have been characterized; the pharmacodynamic interaction is plausibly additive through complementary neuroprotective mechanisms.

11. Adverse Events and Safety Signal

11.1 Acute toxicity

The oral LD50 in mice is greater than 7100 mg/kg body weight, placing carnosic acid in the low-acute-toxicity category [24]. At a dose of 100 mg/kg in the melanoma xenograft study, no obvious organ damage was observed [19].

11.2 Subchronic toxicity

A 30-day repeated-dose oral toxicity study in rats at doses of 50, 100, and 150 mg/kg/day reported no significant difference from control in histopathological examination of heart, liver, kidney, spleen, lung, brain, thymus, adrenal, testis/ovary, or gastrointestinal organs at 150 mg/kg/day. At higher doses (not specified in all reports), slight reductions in weight gain and decreased total serum protein were observed, with conversely increased aspartate aminotransferase (AST) levels in the high-dose group. Weak pathological changes (minimal hepatocellular hypertrophy) were observed in liver at the highest doses but were not considered adverse [24].

11.3 In vitro hepatotoxicity

Carnosic acid showed a dose-dependent increase in cytotoxicity in primary human hepatocytes from three donors, with an EC50 value of 94.8 plus or minus 36.7 micromolar. This concentration substantially exceeds the expected plasma concentrations achievable by oral dosing at food-additive or anticipated therapeutic levels (low-micromolar range), but the finding warrants monitoring of hepatic function biomarkers in any high-dose chronic administration protocol [35].

11.4 Reproductive and developmental toxicity

No dedicated reproductive or developmental toxicity studies with isolated carnosic acid have been published. Rosemary has been consumed as a culinary herb and food additive throughout human history without documented reproductive toxicity signals at dietary exposure levels. The JECFA evaluation did not identify reproductive toxicity concerns at the established ADI [25].

11.5 Genotoxicity

Rosemary extract (standardized to carnosic acid content) was negative in the Ames test, the in vitro chromosomal aberration test, and the in vivo micronucleus test as part of the EFSA and JECFA safety evaluations [1, 25].

11.6 Regulatory safety determinations

The JECFA ADI of 0 to 0.6 mg/kg body weight per day (expressed as combined carnosic acid and carnosol) was based on a NOAEL of 60 mg/kg/day from the 30-day rat study with a 100-fold uncertainty factor [25]. The EFSA concluded that use of rosemary extract as a food additive (E392) at specified maximum levels was not of safety concern for any population group [1].

11.7 Human adverse event experience

No systematic adverse event data from controlled clinical trials of carnosic acid are available. In rosemary extract supplementation studies (100 to 1000 mg/day, containing variable proportions of carnosic acid), no significant adverse events have been reported. Dietary exposure to carnosic acid through culinary use of rosemary and sage is long-standing across Mediterranean populations without identified safety signals at typical dietary levels.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five compounds against carnosic acid, evaluated against five competency standards: novelty, effect size, promising potential, side-effect profile, and overall validation. The comparators include three Nrf2-activating compounds (sulforaphane, dimethyl fumarate, bardoxolone methyl) and two rosemary-derived bioactives (carnosol, rosmarinic acid), selected to capture both the mechanistic class (Nrf2 activators) and the botanical origin (rosemary polyphenols) relevant to carnosic acid positioning.

12.1 Sulforaphane

Mechanism: Isothiocyanate-type electrophilic Nrf2 activator derived from glucoraphanin (a glucosinolate in cruciferous vegetables, principally broccoli). Sulforaphane is a direct electrophile that reacts with Keap1 cysteine residues (principally Cys151) without requiring prior oxidative conversion, unlike the pro-electrophilic mechanism of carnosic acid [45].

Novelty: Moderate. Sulforaphane was the first well-characterized dietary Nrf2 activator (Fahey et al. 1997, Talalay laboratory) and established the field. The mechanism is now considered prototypical rather than novel.

Effect size: Substantial in preclinical models. Sulforaphane produces robust Nrf2 activation and phase 2 gene induction in vitro and in vivo. In human studies, broccoli sprout extract (standardized to sulforaphane content) has produced measurable increases in urinary excretion of carcinogen conjugates and in circulating Nrf2-dependent enzyme activity in multiple clinical trials [45]. Effect sizes on clinical endpoints (cancer incidence, cognitive function) have been modest in the small completed clinical studies.

Promising potential: Moderate to high. Multiple clinical trials are ongoing for cancer chemoprevention (bladder, breast, prostate), autism spectrum disorder, schizophrenia cognitive endpoints, and type 2 diabetes. No compound has received regulatory approval for an Nrf2-mediated indication, but sulforaphane has the most advanced clinical pipeline among the dietary Nrf2 activators.

Side-effect profile: Favorable. Gastrointestinal effects (bloating, flatulence) at higher broccoli sprout extract doses are the principal reported adverse events. No serious adverse events have been attributed to sulforaphane in published clinical trials.

Overall validation: Moderate to high. The compound has the longest research history, the largest literature, and the most advanced clinical development trajectory among dietary Nrf2 activators.

Comparison to carnosic acid: Sulforaphane is a constitutive electrophile (active regardless of redox state), whereas carnosic acid is a pro-electrophile (activated by oxidative stress). The conditional activation mechanism of carnosic acid is theoretically advantageous for targeting pathological tissue while sparing healthy tissue. Sulforaphane has a more advanced clinical pipeline and a larger safety database from human studies. Carnosic acid has the advantage of lipophilicity (better CNS penetration for neuroprotective applications) and the broader multi-target pharmacology (Nrf2 activation plus NF-kappaB suppression plus direct antioxidant activity).

12.2 Dimethyl fumarate (Tecfidera)

Mechanism: Electrophilic alpha,beta-unsaturated ester that activates Nrf2 through S-alkylation of Keap1 cysteine residues. The active metabolite monomethyl fumarate (MMF) is the principal Nrf2-activating species. Dimethyl fumarate also modulates the hydroxycarboxylic acid receptor 2 (HCAR2/GPR109A) and suppresses NF-kappaB signaling through multiple mechanisms [46].

Novelty: Low in mechanism (electrophilic Nrf2 activation is well established), but high in clinical translation. Dimethyl fumarate (Tecfidera, Biogen) is the only Nrf2-activating compound approved for a major neurological indication (relapsing-remitting multiple sclerosis), receiving FDA approval in 2013.

Effect size: Substantial in multiple sclerosis. The DEFINE and CONFIRM Phase 3 trials demonstrated approximately 44 to 53 percent reduction in annualized relapse rate and approximately 71 to 85 percent reduction in new or enlarging T2-hyperintense lesions on MRI versus placebo [46]. These are large effect sizes by neurological disease standards.

Promising potential: High. The compound is a registered medicine with established clinical utility.

Side-effect profile: Moderate concerns. The principal adverse events are gastrointestinal (flushing, nausea, diarrhea, abdominal pain) occurring in approximately 30 to 40 percent of patients, and lymphopenia occurring in approximately 5 to 10 percent, with rare progressive multifocal leukoencephalopathy (PML) in the setting of severe and sustained lymphopenia [46]. The side-effect burden is greater than that of carnosic acid at food-additive levels.

Overall validation: High. The compound has Phase 3 validation, regulatory approval, and extensive post-marketing safety data.

Comparison to carnosic acid: Dimethyl fumarate is the clinical-validation benchmark for Nrf2-mediated therapeutics. Carnosic acid shares the Keap1 S-alkylation mechanism but differs in the pro-electrophilic conditional activation (theoretically more selective for oxidatively stressed tissue), in the absence of the lymphopenia and PML risk signal (though this has not been tested in comparable chronic high-dose human protocols), and in the absence of clinical-stage development. Dimethyl fumarate demonstrates that Nrf2 activation is a viable therapeutic mechanism in neuroinflammation, which supports the rationale for carnosic acid development in neurodegenerative indications.

12.3 Bardoxolone methyl (CDDO-Me)

Mechanism: Synthetic oleanane triterpenoid and potent electrophilic Nrf2 activator. Bardoxolone methyl activates Nrf2 at low nanomolar concentrations (approximately 1000-fold more potent than sulforaphane) through covalent modification of Keap1 cysteine residues [47].

Novelty: Moderate. The triterpenoid scaffold is distinct from the isothiocyanate (sulforaphane), fumarate (dimethyl fumarate), and abietane diterpene (carnosic acid) scaffolds, but the Keap1/Nrf2 mechanism is shared.

Effect size: Substantial on renal biomarkers. In Phase 2 and Phase 3 trials in chronic kidney disease (diabetic nephropathy), bardoxolone methyl produced significant and dose-dependent increases in estimated glomerular filtration rate (eGFR), with increases of 5 to 10 mL/min/1.73m² at 12 months [47].

Promising potential: Mixed. The BEACON Phase 3 trial in Stage 4 chronic kidney disease was terminated early due to excess serious adverse events (heart failure, fluid retention) in the bardoxolone arm. Subsequent development (CARDINAL,

EAGLE studies) has refocused on earlier-stage kidney disease and Alport syndrome with modified inclusion criteria. Reata Pharmaceuticals (now part of Biogen) continues development.

Side-effect profile: Concerning. The BEACON trial termination was driven by fluid overload, heart failure, and cardiovascular events at rates significantly exceeding placebo. The mechanism is attributed to endothelin pathway suppression and mineralocorticoid receptor effects unrelated to the Nrf2 mechanism. Muscle spasms, nausea, and weight loss are common at therapeutic doses [47].

Overall validation: Moderate. Strong biomarker effects, but the Phase 3 safety signal has substantially complicated development and reduced confidence in the triterpenoid class.

Comparison to carnosic acid: Bardoxolone methyl is a cautionary comparator demonstrating that potent constitutive Nrf2 activation can produce off-target toxicity in clinical use. Carnosic acid has a more favorable theoretical safety profile owing to its conditional pro-electrophilic activation, its food-grade safety record, and its lower potency at the Nrf2 target (micromolar versus nanomolar), which may be therapeutically advantageous by avoiding the excessive activation that contributed to bardoxolone toxicity.

12.4 Carnosol

Mechanism: Carnosol is the gamma-lactone oxidation product of carnosic acid, formed by intramolecular lactonization of the ortho-quinone intermediate. It retains Nrf2-activating, anti-inflammatory, and antioxidant activities but differs from carnosic acid in the mechanism of antioxidant action: carnosol inhibits lipid peroxidation through a direct mechanism in the lipid phase, independent of ROS-mediated activation, whereas carnosic acid requires oxidative conversion to the quinone for Nrf2 pathway engagement [5, 48].

Novelty: Low. Carnosol is the co-occurring oxidation product of carnosic acid and shares the same botanical source and structural class.

Effect size: Comparable to carnosic acid in some assays (direct antioxidant activity in lipid systems) and lower in others (Nrf2 activation, anti-inflammatory potency). In antimicrobial assays, carnosol shows greater potency than carnosic acid against some Gram-positive organisms [7].

Promising potential: Low as an independent drug candidate. Carnosol is co-present in rosemary extracts and contributes to the overall biological activity, but its independent pharmaceutical development has not been pursued.

Side-effect profile: Similar to carnosic acid. The JECFA ADI covers combined carnosic acid and carnosol exposure. No independent safety concerns have been identified beyond those applicable to the diterpene class [25].

Overall validation: Low to moderate. Carnosol is well characterized as a component of rosemary extract bioactivity but lacks independent preclinical or clinical development.

Comparison to carnosic acid: Carnosol is the principal degradation product and co-occurring phytochemical of carnosic acid. The two compounds are pharmacologically complementary: carnosic acid provides the pro-electrophilic Nrf2 activation pathway, while carnosol provides direct lipid-phase antioxidant activity. In practice, rosemary extracts contain both compounds, and the biological activity of the extract reflects the composite pharmacology. For mechanistic research requiring attribution of effects to specific molecular targets, investigators should use high-purity carnosic acid with analytically confirmed low carnosol content.

12.5 Rosmarinic acid

Mechanism: Rosmarinic acid is a caffeic acid ester (an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid) and the principal hydrophilic polyphenol of rosemary. Its mechanism of action involves direct radical scavenging, chelation of pro-oxidant transition metals, inhibition of complement-dependent inflammatory stimulation, and modulation of arachidonic acid metabolism. Unlike carnosic acid, rosmarinic acid is water-soluble and does not possess the abietane diterpene scaffold or the pro-electrophilic Nrf2 activation mechanism [49].

Novelty: Low. Rosmarinic acid is a widely distributed plant phenolic with well-established and non-distinctive antioxidant and anti-inflammatory mechanisms.

Effect size: Modest in preclinical models. Direct antioxidant activity is concentration-dependent and competitive with other dietary polyphenols (quercetin, EGCG, resveratrol). Anti-inflammatory effects in vitro are detectable but are generally less potent than those of carnosic acid at equimolar concentrations. In food-ox-

idation assays, rosmarinic acid is less effective than carnosic acid in bulk oil (lipid-phase) systems but more effective in aqueous-phase systems, reflecting the solubility difference [31].

Promising potential: Low as an independent drug candidate. Rosmarinic acid is present in numerous Lamiaceae species and is a component of many herbal supplements, but it has not been advanced through independent pharmaceutical development.

Side-effect profile: Favorable. Rosmarinic acid is a common dietary polyphenol with no identified safety concerns at food-relevant concentrations. Oral LD50 values in rodents exceed 2000 mg/kg [49].

Overall validation: Low. Extensive in vitro characterization, moderate preclinical in vivo data, no clinical-stage development for any specific indication.

Comparison to carnosic acid: Rosmarinic acid and carnosic acid represent the two principal bioactive fractions of rosemary extract with complementary physicochemical properties: rosmarinic acid is hydrophilic and operates in aqueous compartments, while carnosic acid is lipophilic and operates in lipid membranes and lipid-rich tissues (including brain). Carnosic acid has the mechanistically distinctive pro-electrophilic Nrf2 activation that rosmarinic acid lacks, and has demonstrated greater potency in neuroprotective and anticancer models. For CNS-targeted research applications, carnosic acid is the preferred rosemary constituent on the basis of lipophilicity, blood-brain barrier penetration, and Nrf2 pathway engagement.

12.6 Comparator summary

The five comparators span the Nrf2-activating therapeutic class and the rosemary phytochemical space. **Sulforaphane** is the prototypical dietary Nrf2 activator with the most advanced clinical pipeline among natural-product Nrf2 inducers. **Dimethyl fumarate** is the clinical-validation benchmark, demonstrating that Nrf2-mediated neuroprotection produces large clinical effect sizes in multiple sclerosis. **Bardoxolone methyl** is the cautionary example of potent constitutive Nrf2 activation producing off-target cardiovascular toxicity. **Carnosol** is the co-occurring oxidation product with complementary direct antioxidant activity. **Rosmarinic acid** is the hydrophilic rosemary polyphenol with distinct physicochemical properties and a less potent pharmacological profile.

Carnosic acid occupies a distinctive position in this landscape: it is the only compound with a pro-electrophilic conditional Nrf2 activation mechanism, concentrating cytoprotective gene induction at sites of oxidative pathology. It has a long food-safety record (E392 designation, JECFA ADI), strong preclinical validation across neuroprotective, anti-inflammatory, anticancer, and hepatoprotective domains, and a pro-drug derivative (diAcCA) with improved pharmaceutical properties positioned for clinical translation. The principal limitation is the absence of clinical-stage development; the compound has not advanced beyond food-safety evaluations and small human pharmacokinetic pilot studies. The conditional activation mechanism, the CNS penetration supported by lipophilicity, and the broad preclinical evidence base position carnosic acid as a compelling candidate for clinical development in neurodegenerative indications, pending IND-enabling studies.

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