

RESEARCH MONOGRAPH . COMPOUND PROFILE

Bryostatin-1

A marine-derived macrolide isolated from the bryozoan Bugula neritina, distinguished from classical phorbol ester PKC activators by isoform-selective modulation producing anti-inflammatory, synaptogenic, and latent HIV-reversing activity at sub-nanomolar concentrations without tumor-promoting effects.

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ABSTRACT

Bryostatin-1, the prototypical member of the bryostatin family of macrocyclic lactones, is a highly oxygenated 26-membered macrolide originally isolated from the marine bryozoan *Bugula neritina* and subsequently attributed to the bacterial endosymbiont *Candidatus Endobugula sertula*. The compound binds the C1 regulatory domain of protein kinase C (PKC) at the conserved diacylglycerol (DAG) binding site with sub-nanomolar affinity, modulating both conventional (α , β I/ β II, γ) and novel (δ , ϵ , η , θ) PKC isoforms. Despite competitive displacement of phorbol esters from the C1 domain, bryostatin-1 produces functionally distinct downstream signaling characterized by initial PKC activation followed by isoform-selective downregulation, a pharmacological profile that distinguishes it from tumor-promoting phorbol esters and underwrites its diverse therapeutic applications across oncology, neurology, immunology, and infectious disease.

The compound entered clinical development through the National Cancer Institute (NCI) in the early 1990s as an antineoplastic agent, advancing through more than 80 Phase 1 and Phase 2 oncology trials enrolling over 1,500 patients across multiple solid tumor and hematological malignancy indications. The principal dose-limiting toxicity in oncology trials was myalgia, observed at doses of 25 to 50 micrograms per square meter administered by intravenous infusion. Single-agent antitumor activity was modest, though combination regimens with cytarabine in acute myeloid leukemia and with vincristine in non-Hodgkin lymphoma produced response rates warranting further investigation. The

oncology program did not yield a registration-quality efficacy signal, and active NCI-sponsored oncology development was substantially curtailed by 2010.

A second clinical trajectory, initiated by Alkon and colleagues at the Blanchette Rockefeller Neurosciences Institute and subsequently advanced by Synaptogenix (formerly Neurotrope), repositioned bryostatin-1 as a cognitive enhancement agent for Alzheimer's disease on the basis of PKC epsilon activation, synaptogenesis induction, and amyloid precursor protein alpha-secretase processing. A Phase 2a trial (Nelson et al. 2017) demonstrated safety at 25 micrograms per square meter and produced signals of PKC epsilon activation and cognitive stabilization. A larger NIH-sponsored Phase 2 trial (NCT04538066) in 122 patients with moderately severe Alzheimer's disease reported that the primary endpoint (change from baseline in Severe Impairment Battery score at week 28) was not met with statistical significance across the full analysis population. However, prespecified secondary analysis of the severe cohort (Mini-Mental State Examination 10 to 14) demonstrated that bryostatin-treated patients showed no significant cognitive decline over 10 months, compared with placebo patients who declined by 12.8 Severe Impairment Battery points, a finding published in the Journal of Alzheimer's Disease in 2023. The compound was well tolerated in the Alzheimer's trials with no drug-related serious adverse events and no cases of the myalgia observed at higher oncology doses.

A third research application addresses HIV latency reversal. Bryostatin-1 reactivates latent HIV-1 provirus through PKC-mediated NF-kappaB activation in both lymphocytic and monocytic cellular reservoirs, including astrocytes, at low

nanomolar concentrations. The Wender laboratory at Stanford developed a scalable 29-step total synthesis (2017) and characterized synthetic bryostatin analogs (bryologs) as latency-reversing agents with an expanded therapeutic window, establishing the foundation for a "kick and kill" eradication strategy in combination with antiretroviral therapy.

Pharmacokinetics following intravenous administration are characterized by a two-compartment disposition model with distribution and elimination half-lives of approximately 1 and 23 hours, respectively. The compound distributes widely to lung, liver, gastrointestinal tract, and adipose tissue, with evidence of enterohepatic circulation. Renal excretion accounts for approximately 23 percent of the administered dose in the first 12 hours; fecal excretion accounts for approximately 40 percent by 72 hours. The compound is not orally bioavailable at therapeutically relevant concentrations and is administered exclusively by intravenous infusion in clinical applications.

This monograph reviews the chemistry, natural source, total synthesis, and supply chain of bryostatin-1; the PKC isoform pharmacology in molecular detail; the comprehensive pharmacokinetic record; the clinical evidence base across oncology, Alzheimer's disease, and HIV latency reversal indications; reconstitution, sourcing, and handling considerations for laboratory work; stack-interaction implications; the adverse-event and safety profile; and a comparative assessment of five PKC-modulating compounds (prostratin, ingenol mebutate, phorbol 12-myristate 13-acetate, SUW133, and TPPB) against bryostatin-1 on five competency standards (novelty, effect size, promising potential, side-effect profile, and overall validation). The compound is not approved by any regulatory authority for

any indication. It is supplied as a research-grade preparation; investigators should obtain analytical confirmation of identity and purity on every lot.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Bryostatin 1 (no INN has been formally assigned; "bryostatin 1" is the conventional pharmacological name used throughout the peer-reviewed literature and in regulatory filings).

Development codes: NSC 339555 (National Cancer Institute Developmental Therapeutics Program). This designation is the canonical identifier in the NCI-sponsored oncology trial literature and in the NCI Natural Products Repository [1].

Chemical Abstracts Service registry number: 83314-01-6.

International Union of Pure and Applied Chemistry name: (1S,3S,5Z,7R,8E,11S,12S,13E,15S,17R,21R,23R,25S)-25-acetyloxy-1,11,21-trihydroxy-17-[(1R)-1-hydroxyethyl]-5,13-bis(2-methoxy-2-oxoethylidene)-10,10,26-trimethyl-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.13,7.111,15]nonacos-8-ene-12-yl (2E,4E)-2,4-octadienoate. The compound is a heavily oxygenated macrolide featuring a 26-membered macrolactone ring incorporating three substituted tetrahydropyran (oxane) subunits designated the A, B, and C rings [2, 3].

1.2 Structural class and chemistry

Bryostatin-1 is a marine macrolide natural product belonging to the bryostatin family, a group of at least 21 structurally related macrocyclic lactones (bryostatins 1 through 21) isolated from or associated with the marine bryozoan *Bugula neritina*. The structural class is characterized by the 26-membered macrolactone ring system, three embedded tetrahydropyran rings connected by a methylene bridge (A to B) and a propenyl chain (B to C), two exocyclic methyl enoate groups appended to the B and C rings, and an (E,E)-octa-2,4-dienoate ester at C-20 that distinguishes bryostatin-1 from other family members (bryostatin-2 carries a myristate ester at this position; other bryostatins carry alternative acyl groups) [2, 3].

Molecular formula: C₄₇H₆₈O₁₇.

Exact mass: 904.4508 g/mol (monoisotopic).

Molecular weight: 905.02 g/mol.

Physical properties: White to off-white amorphous solid. Soluble in dimethyl sulfoxide (DMSO), ethanol, methanol, and chloroform; practically insoluble in water. The compound is light-sensitive and should be stored protected from light at minus 20 degrees Celsius or below. Optical rotation has been reported as $[\alpha]_D$ approximately plus 60 degrees (c 0.1, methanol) [3].

Stereochemistry: Bryostatin-1 contains 11 defined stereocenters across the macrolide scaffold and the octa-2,4-dienoate side chain. The absolute configuration was established by X-ray crystallographic analysis of bryostatin-1 and confirmed by total synthesis. All commercially supplied research-grade material and all clinical-trial material is the single natural stereoisomer. Investigators should confirm identity and stereochemical integrity by comparison of optical rotation and chromatographic retention time against authenticated reference standards.

1.3 Synthesis and supply

The natural source of bryostatin-1 is the marine bryozoan *Bugula neritina*, a cosmopolitan fouling organism found in temperate and subtropical coastal waters. The compound is produced not by the bryozoan itself but by the gamma-proteobacterial endosymbiont *Candidatus Endobugula sertula*, which harbors a modular polyketide synthase gene cluster responsible for bryostatin biosynthesis [4]. Natural extraction yields are extremely low, on the order of 1 to 18 micrograms per gram of wet animal tissue, and the NCI program historically required collection of approximately 10,000 to 14,000 gallons of *Bugula neritina* to obtain 18 grams of bryostatin-1, a supply-chain limitation that substantially constrained clinical development [5].

Three landmark total syntheses have addressed the supply problem. The Keck laboratory reported the first total synthesis of bryostatin-1 in 2011, proceeding through approximately 57 total steps with a longest linear sequence of 31 steps [6]. The Wender laboratory at Stanford reported a substantially more efficient synthesis in 2017, proceeding in 29 total steps (19 in the longest linear sequence) with greater than 80 percent average yield per step and 4.8 percent overall yield, establishing the first practical access to gram quantities of synthetic bryostatin-1 and enabling the synthesis of structurally simplified analogs (bryologs) for structure-activity investigation [7]. The Trost laboratory reported the total synthesis of bryostatin 16 (a related family member) in 42 steps [8]. The Wender synthesis uses a convergent

strategy in which preformed and heavily functionalized pyran rings A and C are united by pyran annulation to construct the B ring, followed by macrolactonization and selective functional group manipulation.

An alternative supply approach pursued by Aphios Corporation uses supercritical fluid extraction of cultured *Bugula neritina* colonies to produce research-grade and GMP-grade bryostatin-1 without total synthesis [9]. This material was used in the Synaptogenix-sponsored Alzheimer's disease clinical trials.

2. Abstract

Bryostatin-1 (NSC 339555) is a marine macrolide natural product that modulates protein kinase C (PKC) through high-affinity binding to the C1 regulatory domain at the diacylglycerol (DAG) binding site, producing initial activation followed by isoform-selective downregulation of PKC isozymes [1, 2]. The compound was first isolated from *Bugula neritina* by Pettit and colleagues in 1982 and was advanced into clinical development by the NCI as an antineoplastic agent beginning in 1991. More than 80 Phase 1 and Phase 2 oncology trials enrolling over 1,500 patients established the human safety profile, defined myalgia as the principal dose-limiting toxicity at doses of 25 to 50 micrograms per square meter, and demonstrated modest single-agent antitumor activity insufficient for registration as monotherapy [10, 11].

The compound was repositioned for Alzheimer's disease on the basis of the Alkon laboratory's demonstration that bryostatin-1 activates PKC epsilon in neuronal systems, promotes synaptogenesis through brain-derived neurotrophic factor (BDNF) induction, enhances alpha-secretase processing of amyloid precursor protein (APP), and improves spatial learning and memory in transgenic Alzheimer's disease mouse models at doses well below the oncology dose-limiting threshold [12, 13, 14]. A Phase 2a trial demonstrated safety and PKC epsilon activation at 25 micrograms per square meter [15]. A larger NIH-sponsored Phase 2 trial in 122 patients with moderately severe Alzheimer's disease did not meet its primary endpoint across the full analysis population but demonstrated significant cognitive stabilization in the prespecified severe subgroup over 10 months of treatment [16].

A third application, HIV latency reversal, leverages the PKC-mediated NF-kappaB activation pathway to reactivate latent HIV-1 provirus from cellular reservoirs as part of a "kick and kill" eradication strategy. Bryostatin-1 reactivates latent HIV-1 in both lymphocytic (CD4+ T cell) and monocytic reservoirs at low nanomolar concentrations, and synthetic bryostatin analogs (bryologs) developed by the Wender laboratory have demonstrated in vivo latency reversal with an expanded therapeutic window in humanized mouse models [7, 17, 18].

Pharmacokinetics are characterized by a two-compartment intravenous disposition model with half-lives of approximately 1 hour (distribution) and 23 hours (elimination), wide tissue distribution to lung, liver, gastrointestinal tract, and adi-

pose tissue, and dual renal and fecal excretion with evidence of enterohepatic circulation [19]. The compound is not orally bioavailable and is administered exclusively by intravenous infusion. The principal adverse events at clinical doses are myalgia (dose-limiting at oncology doses), nausea, headache, and fatigue; at the lower Alzheimer's disease doses (25 micrograms per square meter), the compound has been well tolerated with no drug-related serious adverse events [15, 16].

This monograph documents the chemistry, natural source, total synthesis, PKC isoform pharmacology, pharmacokinetics, clinical evidence across oncology, Alzheimer's disease, and HIV applications, sourcing and quality verification, reconstitution and handling, stack interactions, adverse events, and a comparative assessment of five PKC-modulating compounds against bryostatin-1.

3. Discovery and Development History

3.1 Origin and isolation

Bryostatin-1 was first isolated and structurally characterized by George R. Pettit and colleagues at Arizona State University in 1982 from an ethanol extract of the marine bryozoan *Bugula neritina* collected in the Gulf of California [2]. The compound was identified through NCI-sponsored bioassay-guided fractionation as part of a systematic screen of marine invertebrate extracts for antineoplastic activity. The structure was determined by X-ray crystallographic analysis and confirmed by extensive nuclear magnetic resonance spectroscopy and mass spectrometry. The name "bryostatin" derives from Bryozoa, the phylum to which *Bugula neritina* belongs, and "statin," from the Greek "to halt," reflecting the compound's antiproliferative activity in the original screen. Pettit subsequently isolated and characterized bryostatins 2 through 21 from various *Bugula neritina* collections and from related marine organisms [3].

3.2 Identification of PKC as the molecular target

The molecular target of bryostatin-1 was identified as protein kinase C by Kraft and colleagues in 1986, who demonstrated that bryostatin-1 competed with phorbol 12,13-dibutyrate for binding to the PKC C1 domain with sub-nanomolar affinity (K_i approximately 1.35 nM) and produced initial activation followed by downregulation of PKC enzymatic activity in intact cells [20]. This binding profile was paradoxical: phorbol esters that bind the same site are potent tumor promoters, yet bryostatin-1 showed antitumor activity and subsequently was demonstrated to antagonize phorbol ester-induced tumor promotion in mouse skin models. The resolution of this paradox, elaborated over the subsequent two decades, lies in the structurally distinct interaction of bryostatin-1 with the C1 domain, which produces a different membrane-binding geometry, different isoform selectivity of activation and downregulation, and consequently different downstream transcriptional and signaling outputs compared to phorbol esters and DAG [21, 22].

3.3 NCI oncology development

The NCI selected bryostatin-1 for preclinical development in the late 1980s on the basis of its novel mechanism of action and broad-spectrum activity in the NCI 60-cell-line screen. The Investigational New Drug application was filed in 1991, and Phase 1 dose-escalation studies began the same year. The Phase 1 program established the maximum tolerated dose at approximately 25 to 50 micrograms per square meter administered as a 1-hour or 24-hour intravenous infusion, with myalgia as the dose-limiting toxicity [10, 11]. Over the subsequent 15 years, more than 80 Phase 1 and Phase 2 trials were conducted across melanoma, renal cell carcinoma, non-small cell lung cancer, colorectal cancer, non-Hodgkin lymphoma, chronic lymphocytic leukemia, multiple myeloma, and acute myeloid leukemia indications. Single-agent response rates were generally low (less than 10 percent partial response rates in most solid tumor trials). Combination regimens with cytarabine in relapsed and refractory acute myeloid leukemia produced complete remission rates of 25 to 35 percent in some studies, and combination with vincristine in aggressive non-Hodgkin lymphoma produced objective response rates warranting further investigation [23, 24].

The NCI oncology program was substantially curtailed by approximately 2010, reflecting the convergence of several factors: modest single-agent activity, the extremely limited natural supply (which constrained trial enrollment and dosing flexibility), the dose-limiting myalgia that restricted dose escalation, and the emergence of more effective targeted therapies in most of the studied indications. The compound did not receive regulatory approval for any oncology indication.

3.4 Repositioning for Alzheimer's disease

Beginning in the early 2000s, the Alkon laboratory at the Blanchette Rockefeller Neurosciences Institute (subsequently the West Virginia University Rockefeller Neuroscience Institute) characterized bryostatin-1 as a cognitive enhancement agent on the basis of several converging preclinical observations: PKC epsilon activation in hippocampal neurons promotes synaptogenesis and dendritic spine remodeling through BDNF and insulin-like growth factor pathways [12]; PKC alpha activation promotes non-amyloidogenic alpha-secretase processing of APP, increasing production of the neurotrophic soluble APP alpha (sAPPalpha) fragment and reducing amyloidogenic beta-secretase processing [13]; and bryostatin-1 at doses substantially below the oncology maximum tolerated dose (5 to 30 micrograms per

square meter equivalents) improved spatial learning and memory in both aged rats and in transgenic Tg2576 Alzheimer's disease mice [14]. The cognitive enhancement was demonstrated to persist for weeks after the treatment course, consistent with a synaptic remodeling mechanism rather than acute pharmacological effect.

Neurotrope (subsequently renamed Synaptogenix) was founded to advance this application. A Phase 2a open-label trial (Nelson et al. 2017) in 9 patients with moderately severe Alzheimer's disease demonstrated safety at 25 micrograms per square meter, confirmed PKC epsilon activation in blood mononuclear cells as a pharmacodynamic biomarker, and produced signals of cognitive stabilization on the Severe Impairment Battery (SIB) [15]. A larger NIH-sponsored, randomized, double-blind, placebo-controlled Phase 2 trial (NCT04538066) enrolled 122 patients with moderately severe Alzheimer's disease across multiple U.S. sites. The primary endpoint (change from baseline in SIB total score at week 28) was not met with statistical significance across the full analysis population. In the prespecified severe subgroup analysis (baseline MMSE 10 to 14), bryostatin-treated patients showed no significant cognitive decline throughout the 10-month trial period, while placebo patients declined by 12.8 SIB points, a clinically meaningful difference published in the Journal of Alzheimer's Disease in 2023 [16]. Synaptogenix subsequently initiated a Phase 2b trial and completed enrollment.

3.5 HIV latency reversal

The recognition that PKC activation drives NF-kappaB-mediated reactivation of latent HIV-1 provirus led to investigation of bryostatin-1 as a latency-reversing agent (LRA) beginning approximately 2009. Mehla et al. (2010) demonstrated that bryostatin-1 reactivated latent HIV-1 in both Jurkat-derived latency cell line models and in primary CD4+ T cells from aviremic patients on antiretroviral therapy [17]. Subsequent work by Gutierrez et al. (2016) showed that bryostatin-1 reactivated latent HIV-1 in astrocytes through a PKC-alpha and PKC-delta dependent mechanism, extending the latency-reversal activity to central nervous system reservoirs [18]. The Wender laboratory's scalable synthesis enabled production of synthetic bryostatin analogs (bryologs) optimized for HIV latency reversal with reduced toxicity, and the bryolog SUW133 demonstrated in vivo latency reversal in a humanized mouse model of HIV infection [7, 25].

4. Mechanism of Action

Bryostatin-1 is a modulator of protein kinase C (PKC), a family of serine/threonine kinases that transduce lipid second-messenger signals and regulate cell proliferation, differentiation, apoptosis, immune function, synaptic plasticity, and gene expression. The PKC family comprises three subfamilies: conventional (cPKC: alpha, betaI, betaII, gamma), which require both DAG and calcium for activation; novel (nPKC: delta, epsilon, eta, theta), which require DAG but not calcium; and atypical (aPKC: zeta, iota/lambda), which require neither DAG nor phorbol ester binding for activation. Bryostatin-1 binds the C1 regulatory domain present in conventional and novel PKC isoforms; it does not bind atypical PKC isoforms, which lack a functional C1 domain [20, 21].

4.1 C1 domain binding and PKC activation

The C1 domain of PKC contains the conserved binding site for the endogenous activator DAG and for pharmacological modulators including phorbol esters and bryostatins. Bryostatin-1 binds this site with sub-nanomolar affinity (K_i approximately 1.35 nM against [3H]phorbol 12,13-dibutyrate in mixed rat brain PKC preparations), displacing phorbol esters competitively [20]. The binding event promotes PKC translocation from the cytosol to the plasma membrane, conformational opening of the pseudosubstrate domain, and catalytic activation. At short time scales (minutes to hours), bryostatin-1 is a potent PKC activator, producing phosphorylation of PKC substrates including myristoylated alanine-rich C-kinase substrate (MARCKS), extracellular signal-regulated kinase (ERK), and various transcription factors [21].

4.2 Isoform-selective downregulation

The distinguishing pharmacological feature of bryostatin-1 relative to phorbol esters is the pattern of subsequent PKC downregulation. Prolonged exposure to bryostatin-1 (hours to days) produces isoform-selective downregulation of certain PKC isoforms through ubiquitin-proteasome-mediated degradation, while other isoforms are relatively spared or show biphasic kinetics. The pattern is cell-type dependent but consistently differs from the pattern produced by phorbol esters such as phorbol 12-myristate 13-acetate (PMA). In many cell types, bryostatin-1 produces

sustained activation of PKC epsilon while producing downregulation of PKC alpha and PKC delta, whereas PMA downregulates all activated isoforms with relatively uniform kinetics [22, 26]. This isoform selectivity is the molecular basis for the divergent biological effects of bryostatin-1 and phorbol esters: bryostatin-1 is anti-neoplastic and non-tumor-promoting, while phorbol esters are tumor-promoting.

Molecular dynamics simulations have demonstrated that the bryostatin-1-PKC complex adopts a membrane-binding geometry that is structurally distinct from the phorbol ester-PKC and DAG-PKC complexes, producing different exposure of the C1 domain hydrophobic surface to the lipid bilayer and consequently different membrane residence time, membrane microdomain partitioning, and access to membrane-proximal substrates [22]. These structural differences at the C1-membrane interface propagate to different downstream signaling outputs despite competitive binding at the same site.

4.3 PKC epsilon activation and synaptogenesis

PKC epsilon is a novel PKC isoform highly expressed in hippocampal and cortical neurons. Activation of PKC epsilon by bryostatin-1 promotes synaptogenesis through several downstream pathways: induction of brain-derived neurotrophic factor (BDNF) expression, activation of the Ras-ERK-CREB signaling cascade, and stimulation of dendritic spine formation and maturation [12, 14]. In cultured cortical neurons, bryostatin-1 increased the number of presynaptic boutons (synaptophysin-positive puncta) through a PKC-dependent mechanism while simultaneously reducing the density of immature dendritic spines, suggesting a shift from immature to mature synaptic contacts [27]. In aged rats and transgenic Alzheimer's disease mice, bryostatin-1 administered at sub-oncological doses increased hippocampal mushroom spine density, increased synaptophysin and spinophilin immunoreactivity, and improved performance on spatial learning and memory tasks including the Morris water maze and radial arm water maze [14].

4.4 Alpha-secretase processing of APP

PKC alpha activation by bryostatin-1 promotes non-amyloidogenic processing of amyloid precursor protein (APP) by enhancing alpha-secretase (ADAM10/ADAM17) activity at the plasma membrane. Alpha-secretase cleaves APP within the amyloid-beta domain, producing the neurotrophic soluble APP alpha (sAPPalpha) fragment and precluding generation of the amyloidogenic amyloid-beta peptide that forms

the characteristic plaques of Alzheimer's disease. In Tg2576 transgenic mice expressing human APP with the Swedish mutation, bryostatin-1 treatment reduced brain amyloid-beta 1-40 and 1-42 levels and increased sAPPalpha, consistent with enhanced alpha-secretase processing [13]. The dual PKC epsilon (synaptogenic) and PKC alpha (anti-amyloidogenic) mechanisms provide a mechanistic rationale for the cognitive benefits observed in both preclinical and clinical Alzheimer's disease studies.

4.5 Immune modulation and anti-inflammatory effects

Bryostatin-1 modulates innate immune function through PKC-dependent pathways in dendritic cells, macrophages, and microglia. In bone-marrow-derived dendritic cells and macrophages, bryostatin-1 reciprocally inhibited production of pro-inflammatory cytokines (interleukin-6, interleukin-12) while promoting an anti-inflammatory phenotype characterized by interleukin-10 and arginase-1 expression [28]. In the central nervous system, bryostatin-1 attenuated neuroinflammation in experimental autoimmune encephalomyelitis (EAE) and cuprizone-induced demyelination models, reduced microglial activation, and promoted oligodendrocyte differentiation and remyelination through PKC-dependent modulation of innate immune signaling [28]. These anti-inflammatory effects contribute to the neuroprotective profile and may be relevant to the Alzheimer's disease application, where neuroinflammation is a recognized disease driver.

4.6 HIV latency reversal

Latent HIV-1 provirus integrated into the genome of resting CD4+ T cells and other cellular reservoirs is transcriptionally silent and invisible to the immune system and to antiretroviral therapy. Reactivation of latent provirus ("kick") to enable immune-mediated or viral cytopathic clearance ("kill") is a leading strategy for HIV eradication. Bryostatin-1 reactivates latent HIV-1 through PKC-mediated activation of NF-kappaB, which binds the HIV-1 long terminal repeat (LTR) promoter and drives proviral transcription. The reactivation has been demonstrated in Jurkat-derived latency cell lines (J-Lat), in primary resting CD4+ T cells from aviremic patients, and in astrocytes, extending the latency-reversal activity to the central nervous system reservoir [17, 18, 25]. The compound is among the most potent latency-reversing agents identified in ex vivo patient cell assays, active at low nanomolar concentrations.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and administration

Bryostatin-1 is not orally bioavailable at therapeutically relevant concentrations, reflecting its high molecular weight (905 Da), extensive first-pass hepatic metabolism, and poor aqueous solubility. All clinical applications use intravenous infusion. In oncology trials, the compound was administered as a 1-hour or 24-hour intravenous infusion at doses of 25 to 50 micrograms per square meter of body surface area [10, 11]. In Alzheimer's disease trials, a single dose of 25 micrograms per square meter was administered as a 1-hour intravenous infusion [15, 16].

5.2 Distribution

Preclinical pharmacokinetic studies in mice using radiolabeled bryostatin-1 demonstrated wide tissue distribution following intravenous administration, with highest concentrations in lung, liver, gastrointestinal tract, and adipose tissue [19]. The high gastrointestinal tract concentration, combined with substantial fecal excretion, suggests enterohepatic circulation. The compound crosses the blood-brain barrier, a property confirmed by the pharmacodynamic effects observed in central nervous system models (synaptogenesis, microglial modulation, HIV latency reversal in astrocytes) and by direct measurement of brain PKC epsilon activation following systemic administration in rodent models [14, 19].

Plasma disposition following intravenous administration is characterized by a two-compartment model. In preclinical studies, the distribution half-life was approximately 1.05 hours and the elimination half-life was approximately 22.97 hours [19]. In human oncology trials at doses of 25 to 50 micrograms per square meter, peak plasma concentrations were achieved at 1 to 2 hours after the start of infusion, and the compound was detectable in plasma for at least 24 hours after dosing [10, 15].

5.3 Metabolism

The metabolic pathways of bryostatin-1 have not been fully characterized at the enzymatic level. The majority of circulating radioactivity in preclinical studies was associated with intact drug for up to 24 hours after dosing, suggesting relatively slow metabolic transformation [19]. Hepatic metabolism is presumed to involve oxidative and conjugative pathways, but the specific cytochrome P450 isoforms responsible have not been definitively identified. The absence of defined CYP involvement limits prediction of drug-drug interactions through metabolic pathways, though the hepatic extraction and wide tissue distribution suggest vulnerability to inhibitors of hepatic uptake and efflux transporters.

5.4 Elimination

Dual renal and fecal elimination has been characterized in preclinical studies. Urinary excretion accounted for approximately 23 percent of the administered dose within the first 12 hours following intravenous administration. By 72 hours, approximately equal amounts of radioactivity (approximately 40 percent each) were recovered in urine and feces, with the fecal component consistent with biliary excretion and possible enterohepatic circulation [19]. Total recovery accounted for approximately 80 percent of the administered dose by 72 hours. Renal impairment and hepatic impairment effects on bryostatin-1 pharmacokinetics have not been formally characterized in dedicated studies.

5.5 Special populations

Pharmacokinetic data in special populations (pediatric, geriatric, renal impairment, hepatic impairment) are limited. The Alzheimer's disease clinical population consisted primarily of elderly patients (mean age approximately 70 to 80 years), and the compound was well tolerated in this population without dose adjustment [15, 16]. Pediatric pharmacokinetics have not been characterized. Pregnancy and lactation data are absent; the compound is a PKC modulator with potential effects on cell proliferation and differentiation, and use in pregnancy is contraindicated in the absence of safety data.

6. Clinical Evidence Base

6.1 Oncology

The oncology clinical program for bryostatin-1 is the largest and longest-established evidence base, comprising more than 80 NCI-sponsored Phase 1 and Phase 2 trials across approximately 15 years of active enrollment. The program established the human safety profile, defined the dose-limiting toxicity (myalgia), characterized the pharmacokinetics, and evaluated antitumor activity across multiple solid tumor and hematological malignancy indications [10, 11].

Phase 1 trials established the maximum tolerated dose at approximately 25 to 50 micrograms per square meter administered as a 1-hour intravenous infusion, with myalgia as the dose-limiting toxicity at higher doses. The myalgia was cumulative, dose-dependent, and in some cases severe enough to require dose reduction or treatment discontinuation [10].

Phase 2 monotherapy trials in melanoma, renal cell carcinoma, non-small cell lung cancer, colorectal cancer, head and neck squamous cell carcinoma, and ovarian cancer generally reported objective response rates below 10 percent, insufficient for registration [11, 23]. A Phase 2 trial in renal cell carcinoma (Pagliaro et al. 2000) at 35 to 40 micrograms per square meter on days 1, 8, and 15 of a 4-week cycle reported no objective responses in 25 evaluable patients [29]. A Phase 2 trial in metastatic melanoma reported a partial response rate of approximately 5 percent [11].

Combination regimens produced more encouraging signals. Bryostatin-1 in combination with cytarabine in relapsed and refractory acute myeloid leukemia produced complete remission rates of 25 to 35 percent in some studies, exploiting the ability of bryostatin-1 to prime leukemia cells for cytarabine-induced apoptosis through PKC-mediated modulation of cell cycle checkpoints [24]. Bryostatin-1 in combination with vincristine in aggressive non-Hodgkin lymphoma produced objective responses in a subset of patients [23]. These combination findings, while not of registration quality, contributed to the understanding of bryostatin-1 as a sensitizer or modulator rather than a direct cytotoxic agent.

The oncology program was substantially curtailed by approximately 2010 owing to the convergence of modest single-agent activity, supply limitations, the dose-limiting myalgia, and the emergence of more effective targeted therapies.

6.2 Alzheimer's disease

The Alzheimer's disease clinical program for bryostatin-1 was initiated on the basis of the preclinical cognitive enhancement data generated by the Alkon laboratory and has been advanced by Synaptogenix.

Phase 2a trial (Nelson et al. 2017): An open-label study in 9 patients with moderately severe Alzheimer's disease (MMSE 10 to 18) receiving bryostatin-1 at 25 micrograms per square meter as a single 1-hour intravenous infusion demonstrated safety and tolerability. PKC epsilon levels in peripheral blood mononuclear cells increased significantly at 1 and 24 hours post-infusion, confirming target engagement. The SIB total score showed a non-significant trend toward improvement at week 13 in the expanded access cohort [15].

Phase 2 trial (NCT02431468): A randomized, double-blind, placebo-controlled trial in approximately 150 patients with moderately severe to severe Alzheimer's disease evaluated multiple doses and schedules of bryostatin-1. This trial produced preliminary safety and dosing information that informed the design of the subsequent NIH-sponsored trial.

NIH-sponsored Phase 2 trial (NCT04538066): This randomized, double-blind, placebo-controlled trial enrolled 122 patients with moderately severe Alzheimer's disease across multiple U.S. sites. The trial was supported by the National Institute on Aging and the National Cancer Institute. Patients received bryostatin-1 at 25 micrograms per square meter or placebo administered intravenously over a treatment course spanning multiple infusions. The primary endpoint was the change from baseline in SIB total score at week 28 (after completion of the second course of treatment). The primary endpoint was not met with statistical significance across the full analysis population: the mean SIB change was plus 1.4 points in the bryostatin group and plus 0.6 points in the placebo group [16].

However, prespecified secondary analysis of the severe cohort (baseline MMSE 10 to 14) demonstrated a clinically and statistically significant treatment effect. Bryostatin-treated patients in this subgroup showed no significant cognitive decline over the entire 10-month trial period, whereas placebo patients declined by

12.8 SIB points, a magnitude of decline consistent with the expected natural history of moderately severe Alzheimer's disease over this time frame. No drug-related serious adverse events were reported, and no cases of myalgia were observed at the 25 microgram per square meter dose, consistent with the myalgia being a dose-dependent effect at the higher oncology doses [16].

Synaptogenix subsequently initiated a Phase 2b trial to further evaluate bryostatin-1 in the severe Alzheimer's disease population identified by the secondary analysis, and enrollment was completed.

6.3 HIV latency reversal

The HIV latency reversal application of bryostatin-1 is principally preclinical and early translational. Ex vivo studies using resting CD4+ T cells from aviremic HIV-positive patients on suppressive antiretroviral therapy have demonstrated that bryostatin-1 at low nanomolar concentrations is among the most potent latency-reversing agents identified, comparable to or exceeding the potency of other PKC agonists (prostratin, ingenol-3-angelate) and substantially more potent than HDAC inhibitors (vorinostat, romidepsin) in standard latency reversal assays [17, 25].

The Wender laboratory's synthesis of bryostatin analogs (bryologs) has produced compounds with enhanced latency-reversal activity and reduced toxicity. The bryolog SUW133 demonstrated in vivo HIV latency reversal in a humanized BLT mouse model, with measurable increases in HIV RNA in plasma of mice receiving SUW133 in combination with antiretroviral therapy, representing one of the first demonstrations of in vivo latency reversal by a PKC modulator [25]. The bryolog prodrug approach developed by Wender et al. further expanded the therapeutic window by providing sustained release of the active PKC modulator from an inactive precursor [30].

A single-dose clinical pharmacokinetic and pharmacodynamic study of bryostatin-1 as a latency-reversing agent in HIV-positive individuals has been reported, establishing feasibility of the clinical approach [31]. Larger clinical trials of bryostatin-1 or bryologs as HIV latency-reversing agents have not been reported.

6.4 Other investigated indications

Bryostatin-1 has been investigated in preclinical models for multiple sclerosis (based on the anti-inflammatory and remyelination-promoting effects in EAE and cuprizone models) [28], traumatic brain injury, stroke (based on the neuroprotective and synaptogenic mechanisms), learning and memory enhancement in non-disease models, and immune modulation for cancer immunotherapy (based on the effects on dendritic cell maturation and antigen presentation). None of these indications has advanced to registration-quality clinical trials.

7. Reported Research Dose Ranges

7.1 Oncology doses

Phase 1 and Phase 2 oncology trials used doses of 25 to 50 micrograms per square meter of body surface area, administered as a 1-hour or 24-hour intravenous infusion. Dosing schedules varied across trials: weekly infusions for 3 of 4 weeks, bi-weekly infusions, and continuous 24-hour infusion schedules were all evaluated. The maximum tolerated dose was defined at approximately 25 to 40 micrograms per square meter for the 1-hour infusion schedule, with myalgia as the dose-limiting toxicity [10, 11].

7.2 Alzheimer's disease doses

The Alzheimer's disease clinical program used a single dose level of 25 micrograms per square meter administered as a 1-hour intravenous infusion. This dose was selected on the basis of preclinical dose-response data demonstrating cognitive enhancement at sub-oncological doses and on the Phase 2a safety and PKC epsilon activation data. The lower dose was well tolerated without the myalgia observed at higher oncology doses [15, 16].

7.3 Preclinical research doses

In rodent models, bryostatin-1 is administered intraperitoneally or intravenously at doses of 5 to 30 micrograms per square meter (allometrically scaled) or 10 to 40 micrograms per kilogram body weight. Cognitive enhancement in aged rats and transgenic Alzheimer's disease mice has been demonstrated at doses as low as 10 micrograms per kilogram administered twice weekly for 4 to 12 weeks [14]. In vitro PKC activation and HIV latency reversal are observed at concentrations of 1 to 100 nanomolar [17, 20].

8. Sourcing and Quality Verification

Bryostatin-1 is available from multiple research-grade suppliers, though supply has historically been constrained by the difficulty of obtaining the compound from natural sources and the complexity of total synthesis.

Research-grade suppliers: Sigma-Aldrich (catalog B7431, greater than or equal to 99 percent purity by HPLC), Cayman Chemical, MedChemExpress, TargetMol, Tocris, and BOC Sciences supply bryostatin-1 at greater than 98 percent purity by HPLC. The compound is supplied as a solid (amorphous powder) in quantities of 10 micrograms to 1 milligram at the research scale. Pricing is substantially higher than most small-molecule research compounds, reflecting the difficulty of synthesis: typical pricing is \$200 to \$500 per 50 micrograms for high-purity material at the time of monograph preparation [9].

GMP-grade supply: Aphios Corporation (Woburn, Massachusetts) produces GMP-grade bryostatin-1 using supercritical fluid extraction from cultured *Bugula neritina* colonies. This material was used in the Synaptogenix Alzheimer's disease clinical trials and is the principal current source for clinical-grade material [9].

Synthetic supply: The Wender laboratory synthesis provides a route to synthetic bryostatin-1 and bryostatin analogs at the gram scale. Synthetic material is available through research collaborations and through commercial suppliers (Sigma-Aldrich, MedChemExpress) who source from synthetic routes [7].

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

- High-performance liquid chromatography area-percent purity, with target greater than 98 percent
- High-resolution mass spectrometry confirmation of molecular weight (expected $[M+Na]^+$ at m/z 927.4399; $[M+H]^+$ at m/z 905.4579)
- Optical rotation measurement ($[\alpha]_D$ approximately plus 60 degrees, c 0.1, methanol) to confirm stereochemical integrity
- Proton nuclear magnetic resonance spectroscopy (500 MHz, $CDCl_3$) for structural confirmation against published spectral data

- Karl Fischer or thermogravimetric analysis for residual water content

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. Given the high cost and limited supply of bryostatin-1, lot-to-lot variability should be assessed, and on-receipt analytical confirmation is strongly recommended for any investigation where dose-response precision is important.

9. Reconstitution and Handling

Bryostatin-1 is supplied as a white to off-white amorphous solid. The compound is practically insoluble in water and must be reconstituted in organic solvent for research applications.

Stock solution preparation: Dissolve in DMSO to prepare stock solutions at concentrations of 0.1 to 10 mM (approximately 0.09 to 9.05 mg/mL). The compound is freely soluble in DMSO, ethanol, and methanol. For in vivo administration, stock solutions in DMSO can be further diluted into formulation vehicles; one validated formulation for preclinical studies is 10 percent DMSO, 40 percent polyethylene glycol 300, 5 percent Tween 80, and 45 percent saline [32]. The final DMSO concentration in the injection vehicle should be minimized (target less than 10 percent by volume) to reduce solvent-related toxicity.

Clinical formulation: For clinical administration, bryostatin-1 has been formulated in polyethylene glycol 400 and ethanol for dilution into 5 percent dextrose in water or 0.9 percent sodium chloride for intravenous infusion. The clinical formulation used in the Synaptogenix trials was prepared from GMP-grade material by Aphios Corporation [9].

Storage: Store the solid compound at minus 20 degrees Celsius or below, protected from light, in a tightly sealed container under inert atmosphere (nitrogen or argon) if possible. The compound is sensitive to light, oxygen, and moisture. Stock solutions in DMSO are stable for up to 3 months when stored at minus 20 degrees Celsius in aliquots. Repeated freeze-thaw cycles should be avoided; aliquot stock solutions into single-use volumes at the time of preparation [32].

Handling precautions: Bryostatin-1 is a potent PKC modulator active at nanomolar concentrations. Standard laboratory precautions for handling potent bioactive compounds should be observed: use gloves, avoid inhalation of powder, and handle in a ventilated area. The compound is not classified as a known human carcinogen; however, its activity as a PKC modulator and its effects on cell proliferation and differentiation warrant careful handling. Avoid contact with skin and eyes.

Stability: Reconstituted solutions should be used within the stability window (3 months at minus 20 degrees Celsius for DMSO stock solutions). Working dilutions in aqueous buffers should be prepared fresh and used within 24 hours. The compound is susceptible to hydrolysis of the ester linkages (particularly the C-20 octa-2,4-dienoate ester) under acidic or basic conditions; maintain solutions at neutral pH.

10. Stack Interaction Considerations

Bryostatin-1 interacts with several drug classes through its PKC-modulating mechanism and through potential pharmacokinetic interactions. Investigators conducting research with bryostatin-1, especially in combination regimens, should consider:

Other PKC modulators: Concurrent use with phorbol esters (PMA/TPA, prostatin), ingenol derivatives (ingenol mebutate, ingenol-3-angelate), or other C1 domain ligands produces competitive binding at the PKC C1 domain. Depending on relative concentrations and binding kinetics, the combination may produce additive, synergistic, or antagonistic effects on PKC isoform activation and downregulation. Bryostatin-1 has been demonstrated to antagonize PMA-induced tumor promotion in mouse skin models, and concurrent exposure may produce unpredictable downstream signaling outcomes [20, 21].

Cytotoxic chemotherapy: Bryostatin-1 has been combined clinically with cytarabine, vincristine, fludarabine, and other cytotoxic agents [23, 24]. The combination with cytarabine in acute myeloid leukemia exploits PKC-mediated priming of leukemia cells for ara-C-induced apoptosis. Timing of administration is critical: bryostatin-1 is typically administered 24 hours before cytarabine to allow PKC-mediated sensitization before cytotoxic exposure. Concurrent administration may produce different effects than sequential administration.

Immunosuppressants: PKC is a critical node in T cell receptor signaling; bryostatin-1 modulation of PKC in T cells may interfere with the mechanism of calcineurin inhibitors (cyclosporine, tacrolimus) and mTOR inhibitors (sirolimus, everolimus). Concurrent use in transplant or autoimmune settings has not been characterized and should be approached with caution.

Antiretroviral therapy: In the HIV latency-reversal context, bryostatin-1 is administered in combination with suppressive antiretroviral therapy (ART). No pharmacokinetic interactions between bryostatin-1 and standard ART regimens (integrase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors) have been formally characterized. Protease inhibitors that are strong CYP3A4 inhibitors (ritonavir, cobicistat) may theoretically affect bryostatin-1 metabolism, though the specific CYP involvement in bryostatin-1 metabolism has not been defined.

QT-prolonging agents: Bryostatin-1 has not been associated with QT interval prolongation in clinical trials. However, PKC modulation can affect cardiac ion channel function, and concurrent use with known QT-prolonging agents should be monitored.

Cholinesterase inhibitors and memantine: The Alzheimer's disease clinical trials specifically excluded concurrent memantine use, as preclinical data suggested that memantine may interfere with the PKC-mediated cognitive enhancement mechanism. Concurrent cholinesterase inhibitors (donepezil, rivastigmine, galantamine) were permitted in some trials and did not produce identified safety concerns [15, 16].

Nonsteroidal anti-inflammatory drugs: The myalgia that is the dose-limiting toxicity at oncology doses has been managed with NSAIDs (ibuprofen, naproxen) in clinical trials without identified pharmacokinetic or pharmacodynamic interactions [10].

11. Adverse Events and Safety

11.1 Common adverse events (oncology doses)

The adverse-event profile of bryostatin-1 is well characterized from the extensive oncology clinical program enrolling over 1,500 patients [10, 11, 23]:

- Myalgia (dose-limiting toxicity; 30 to 60 percent incidence at doses of 25 to 50 micrograms per square meter; dose-dependent, cumulative, can be severe and treatment-limiting)
- Nausea (10 to 20 percent)
- Vomiting (5 to 15 percent)
- Fatigue (10 to 20 percent)
- Headache (5 to 10 percent)
- Phlebitis at the infusion site (5 to 10 percent with peripheral intravenous access)
- Diarrhea (5 to 10 percent)

The myalgia is the principal safety signal and the most clinically significant adverse event. The mechanism is incompletely understood but is hypothesized to involve PKC-mediated modulation of calcium handling in skeletal muscle or PKC-dependent activation of nociceptive pathways. The myalgia typically develops within 24 to 48 hours of infusion, peaks at 48 to 72 hours, and resolves over 5 to 14 days. It is dose-dependent and cumulative across treatment cycles [10].

11.2 Adverse events at Alzheimer's disease doses

At the lower doses used in Alzheimer's disease trials (25 micrograms per square meter), bryostatin-1 was well tolerated. The NIH-sponsored Phase 2 trial reported no drug-related serious adverse events and no cases of myalgia. Common adverse events at Alzheimer's disease doses included diarrhea, headache, fatigue, and weight loss, with incidence rates comparable to placebo. No fatal adverse events were attributed to treatment [15, 16].

11.3 Rare and serious adverse events

Across the entire clinical experience (oncology and Alzheimer's disease), the following rare or serious events have been reported:

- Severe myalgia requiring hospitalization (rare at oncology doses; not observed at Alzheimer's disease doses)
- Hypotension during infusion (rare)
- Thrombocytopenia (rare, generally mild and reversible)
- Hepatic enzyme elevation (rare, generally transient and clinically insignificant)
- Hypersensitivity reactions (very rare)

No drug-related deaths have been attributed to bryostatin-1 across the entire clinical program.

11.4 Safety in context

The safety profile of bryostatin-1 at the Alzheimer's disease dose (25 micrograms per square meter) is substantially more favorable than the oncology safety profile, reflecting the steep dose-response relationship for myalgia. The extensive human safety database from the oncology program (over 1,500 patients) provides high confidence in the characterization of the adverse-event spectrum. The compound does not produce the tumor-promoting activity associated with phorbol esters, has not been associated with secondary malignancy in clinical follow-up, and has not produced organ toxicity (cardiac, renal, hepatic, pulmonary) at studied doses [16].

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five PKC-modulating compounds against bryostatin-1, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five comparators were selected as the principal PKC C1 domain ligands with substantive research or clinical pharmacology programs across the therapeutic areas relevant to bryostatin-1 (oncology, neurodegeneration, HIV latency reversal).

12.1 Prostratin (12-deoxyphorbol 13-acetate)

Mechanism: Non-tumor-promoting phorbol ester; binds the PKC C1 domain and activates conventional and novel PKC isoforms. Like bryostatin-1, prostratin does not promote tumor formation despite C1 domain binding, distinguishing it from tumor-promoting phorbol esters. Principal application is HIV latency reversal [33].

Development trajectory: Prostratin was identified as a traditional medicine from the Samoan tree *Homalanthus nutans* and was characterized as a PKC activator and HIV latency-reversing agent by the AIDS Research Alliance and Stanford University. The compound has been evaluated in ex vivo patient cell latency reversal assays and in preclinical models. A first-in-human clinical trial for HIV latency reversal has been discussed but not reported in published form as of the most recent monograph revision.

Novelty: Low to moderate. The phorbol ester scaffold is well established. The non-tumor-promoting property is shared with bryostatin-1 but is unusual within the phorbol ester class.

Effect size: Moderate in ex vivo HIV latency reversal assays. Prostratin is less potent than bryostatin-1 in most comparative latency reversal assays, typically requiring micromolar concentrations versus low nanomolar for bryostatin-1 [17, 25].

Promising potential: Low to moderate. The compound has not advanced to clinical trials for any indication. Research-grade material is readily available from chemical suppliers. The natural source (*Homalanthus nutans* bark) is more accessible than *Bugula neritina* but provides modest yields.

Side-effect profile: Preclinical only. The non-tumor-promoting property is favorable. Systemic toxicity at effective HIV latency-reversal doses has not been characterized in humans.

Overall validation: Low to moderate. The compound has a moderate preclinical literature, principally in HIV latency reversal, but no clinical validation. It serves as a reference non-tumor-promoting PKC activator.

Comparison to bryostatin-1: Bryostatin-1 is substantially more potent for HIV latency reversal (nanomolar versus micromolar), has a vastly larger clinical safety database, and has validated clinical applications beyond HIV. Prostratin has the advantages of simpler structure, easier synthesis, and potentially lower production cost.

12.2 Ingenol mebutate (ingenol-3-angelate, PEP005)

Mechanism: Diterpene ester PKC activator; binds the PKC C1 domain with nanomolar affinity and activates conventional and novel PKC isoforms. Produces distinct isoform-selectivity pattern compared to bryostatin-1 and phorbol esters. The compound was approved (as Picato) for topical treatment of actinic keratosis on the basis of PKC-mediated keratinocyte cell death and immune cell recruitment [34].

Development trajectory: Ingenol mebutate was developed by LEO Pharma and received FDA approval in 2012 for topical treatment of actinic keratosis. In 2020, the European Medicines Agency recommended suspension of the marketing authorization following increased incidence of skin cancer in the treated areas in a long-term safety study, and LEO Pharma withdrew the product from U.S. and European markets in 2020 [34]. As an HIV latency-reversing agent, ingenol-3-angelate has been evaluated in ex vivo assays and in preclinical models, with potency intermediate between prostratin and bryostatin-1 [25].

Novelty: Low. The diterpene ester scaffold is well characterized, and the PKC C1 domain binding mechanism is shared with other comparators.

Effect size: Moderate in actinic keratosis (approximately 40 to 55 percent complete clearance in Phase 3 trials). Moderate in HIV latency reversal assays.

Promising potential: Low. The topical actinic keratosis indication has been withdrawn owing to safety concerns. The HIV latency-reversal application has not advanced to clinical trials. The compound serves as a reference PKC activator for mechanistic research.

Side-effect profile: The topical application produced local skin reactions (erythema, flaking, crusting, swelling) as expected pharmacological effects. The long-term safety concern (increased skin cancer incidence in treated areas) led to market withdrawal, raising a systemic safety concern for PKC activators [34]. This concern is specific to the topical keratinocyte application and has not been observed with systemically administered bryostatin-1 at clinical doses.

Overall validation: Moderate. The compound achieved regulatory approval (subsequently withdrawn) and has a substantial clinical dataset in the dermatology indication. The safety signal has reduced enthusiasm for PKC activators in general.

Comparison to bryostatin-1: Ingenol mebutate achieved regulatory approval (unlike bryostatin-1) but was subsequently withdrawn for safety concerns that have not been observed with bryostatin-1. Bryostatin-1 has a larger systemic safety database and a more diverse therapeutic portfolio. The ingenol mebutate withdrawal has complicated the regulatory narrative for PKC modulators, though the safety concern appears specific to chronic topical PKC activation in keratinocytes rather than to intermittent systemic administration.

12.3 Phorbol 12-myristate 13-acetate (PMA, TPA)

Mechanism: The prototypical PKC activator and tumor promoter. Binds the C1 domain with low nanomolar affinity and produces activation followed by downregulation of all conventional and novel PKC isoforms. PMA is the reference standard PKC activator in pharmacological research and the compound against which bryostatin-1's distinctive pharmacology was first defined [20, 21].

Development trajectory: PMA is not a drug candidate; it is a research tool compound. Its tumor-promoting activity precludes clinical development for any indication. The compound has been used extensively as a reference PKC activator in ex vivo HIV latency reversal assays, where it is among the most potent LRAs identified [17].

Novelty: None for therapeutic applications. The compound defined the field of PKC pharmacology.

Effect size: Maximal PKC activation in vitro. Very high potency in HIV latency reversal assays. Produces robust tumor promotion in mouse skin models.

Promising potential: None as a therapeutic agent. Continues to serve as the reference PKC activator for pharmacological research.

Side-effect profile: Tumor-promoting activity precludes clinical use. Produces inflammation, hyperplasia, and tumor promotion in chronic exposure models.

Overall validation: Maximal as a research tool. Zero as a therapeutic agent.

Comparison to bryostatin-1: PMA and bryostatin-1 bind the same site on PKC but produce fundamentally different biological outcomes: PMA promotes tumors, bryostatin-1 is antineoplastic. This divergence is the central pharmacological observation that defines bryostatin-1's therapeutic potential and that demonstrates the importance of C1 domain binding geometry and isoform-selective downregulation kinetics in determining downstream outcomes [21, 22]. Bryostatin-1 is the clinically translatable PKC modulator; PMA is the research reference standard.

12.4 SUW133 (Wender bryolog)

Mechanism: Designed synthetic analog of bryostatin-1 (bryolog) that retains the critical C1 domain pharmacophore elements while simplifying the macrolide scaffold. Binds PKC C1 domain with comparable affinity to bryostatin-1 (low nanomolar Ki). Produces PKC activation, NF-kappaB-mediated HIV latency reversal, and PKC epsilon-dependent synaptogenic activity similar to bryostatin-1 in cellular models [7, 25].

Development trajectory: Developed by the Wender laboratory at Stanford as part of the function-oriented synthesis program that produced scalable access to bryostatin-1 and structurally simplified analogs. SUW133 demonstrated in vivo HIV latency reversal in a humanized BLT mouse model, representing one of the first demonstrations of in vivo latency reversal by a PKC modulator [25]. Prodrug versions of SUW133 have been developed to expand the therapeutic window [30]. The compound has not entered human clinical trials.

Novelty: High. The function-oriented synthesis approach that produced SUW133 is a methodological innovation in natural product chemistry. The compound demonstrates that the full structural complexity of bryostatin-1 is not required for therapeutic activity.

Effect size: Comparable to bryostatin-1 in HIV latency reversal assays and in pre-clinical PKC activation and synaptogenesis models [25]. In vivo latency reversal was demonstrated in the humanized mouse model.

Promising potential: Moderate to high. The compound represents the leading next-generation candidate in the bryostatin class, with a simplified and scalable synthetic route, demonstrated in vivo activity, and an emerging prodrug strategy for therapeutic window expansion. Clinical development has not been initiated.

Side-effect profile: Preclinical only. In humanized mice, SUW133 was tolerated at doses producing measurable latency reversal. The simplified structure may produce a different toxicity profile from bryostatin-1, but this has not been characterized in formal toxicology studies.

Overall validation: Low to moderate. Strong preclinical data with in vivo proof of concept in a disease-relevant model, but no human safety or efficacy data.

Comparison to bryostatin-1: SUW133 shares the PKC-modulating mechanism of bryostatin-1 with comparable in vitro potency and demonstrated in vivo activity. Bryostatin-1 has the advantage of a comprehensive human safety database (over 1,500 patients), clinical trial data in Alzheimer's disease, and GMP-grade supply. SUW133 has the advantage of a more practical synthesis (fewer steps, higher yield, more scalable), potentially enabling broader access and lower cost. The two compounds are complementary: bryostatin-1 as the clinically validated prototype and SUW133 as the synthetically optimized next-generation candidate.

12.5 TPPB (1-(4-{[1-(4-fluorophenyl)-1H-indol-3-yl]carbonyl}piperidin-1-yl)-2-phenylethan-1-one)

Mechanism: Small-molecule PKC activator; binds the C1 domain and activates conventional and novel PKC isoforms. TPPB has been characterized as an activator of PKC alpha and PKC epsilon in neuronal cell lines and has been compared to bryostatin-1 for effects on APP processing (alpha-secretase activation and sAPPalpha production) in SH-SY5Y neuroblastoma cells [35].

Development trajectory: TPPB is principally a research tool compound used for mechanistic studies of PKC isoform activation in neuronal systems. The compound has not entered clinical development for any indication. It has been used as a comparator in studies of bryostatin-1-mediated APP processing and PKC isoform activation.

Novelty: Low. The indolyl-carbonyl piperidine scaffold is not structurally related to bryostatins or phorbol esters but targets the same C1 domain binding site. The mechanism is non-novel.

Effect size: In SH-SY5Y cells, TPPB produced dose-dependent activation of PKC alpha and PKC delta and enhanced sAPPalpha production, with effects generally comparable to bryostatin-1 at micromolar concentrations [35]. TPPB required higher concentrations than bryostatin-1 to achieve comparable effects, reflecting lower C1 domain binding affinity.

Promising potential: Low. The compound is a research tool without clinical development trajectory.

Side-effect profile: Preclinical only. No human safety data. In vitro cytotoxicity at high concentrations has been reported.

Overall validation: Low. Limited preclinical literature. The compound serves as a reference small-molecule PKC activator for mechanistic comparison with bryostatin-1 in neuronal models.

Comparison to bryostatin-1: TPPB is a lower-potency PKC activator used as a comparator in mechanistic studies. Bryostatin-1 has vastly greater pharmacological characterization, clinical data, and therapeutic development. TPPB's principal value is as a tool compound for dissecting the contributions of individual PKC isoforms to the neuroprotective and anti-amyloidogenic effects attributed to bryostatin-1.

12.6 Comparator summary

The five comparators occupy distinct positions in the PKC modulator landscape. **Prostratin** is a non-tumor-promoting phorbol ester with moderate HIV latency-reversal activity but substantially lower potency than bryostatin-1 and no clinical development. **Ingenol mebutate** achieved and subsequently lost regulatory approval for topical actinic keratosis, with the withdrawal creating a cautionary precedent

for PKC activators though the safety concern is application-specific. **PMA** is the reference PKC activator and the compound against which bryostatin-1's unique pharmacology is defined; its tumor-promoting activity precludes clinical translation. **SUW133** is the most promising next-generation candidate, sharing bryostatin-1's mechanism with a more practical synthetic route and demonstrated in vivo activity, but lacking human clinical data. **TPPB** is a research tool compound useful for mechanistic studies but without therapeutic development potential.

Bryostatin-1 occupies a unique position as the only compound in the panel with extensive human clinical experience (over 1,500 patients), the only compound with clinical efficacy data in Alzheimer's disease, and the only compound with a GMP-grade supply chain supporting ongoing clinical trials. Its principal limitations are the difficulty and cost of synthesis (partially addressed by the Wender route), the dose-limiting myalgia at oncology doses (not observed at Alzheimer's disease doses), and the absence of oral bioavailability requiring intravenous administration for all clinical applications. The PKC epsilon selectivity, the absence of tumor promotion, and the multi-indication therapeutic portfolio (oncology, neurodegeneration, HIV, immune modulation) distinguish bryostatin-1 from all comparators and support its continued investigation as a first-in-class therapeutic PKC modulator.

13. References

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