

RESEARCH MONOGRAPH . COMPOUND PROFILE

Apelin-13

A pyroglutamyl-modified tridecapeptide derived from the C-terminus of preproapelin, identified as the predominant circulating isoform and the highest-potency endogenous agonist of the APJ receptor, with characterized cardiovascular inotropic, vasodilatory, aquaretic, metabolic, and neuroprotective activities.

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ABSTRACT

Apelin-13, the C-terminal tridecapeptide fragment of the 77-amino-acid preproapelin precursor, is the most potent endogenous agonist of the apelin receptor (APJ, also designated APLNR), a class A rhodopsin-like G protein-coupled receptor originally cloned as an orphan receptor in 1993 by O'Dowd et al. on the basis of sequence homology with the angiotensin II type 1 receptor. The receptor was deorphanized in 1998 by Tatemoto and colleagues at the Takeda Chemical Research Institute, who isolated apelin from bovine stomach extracts using an extracellular acidification assay on APJ-expressing Chinese hamster ovary cells and demonstrated that apelin-13 displayed 8- to 60-fold higher potency than the longer apelin-36 isoform. The predominant circulating form in human plasma is [Pyr1]apelin-13 (pyroglutamyl apelin-13), in which the N-terminal glutamine residue undergoes spontaneous or enzymatic cyclization to pyroglutamate, conferring modest resistance to aminopeptidase degradation and representing the principal bioactive isoform in cardiovascular tissue and plasma.

Apelin-13 activates APJ with sub-nanomolar potency (EC₅₀ approximately 0.37 nM in cellular acidification assays), coupling predominantly through Gi/o proteins to inhibit adenylyl cyclase and reduce intracellular cAMP, through Gq/11 to activate phospholipase C and mobilize intracellular calcium, and through G12/13 to engage RhoA-dependent cytoskeletal rearrangement. The receptor also recruits beta-arrestin 1 and 2, mediating receptor internalization and activating extracellular signal-regulated kinase 1/2 (ERK1/2) through G protein-independent

pathways. The downstream signaling cascade includes activation of phosphoinositide 3-kinase (PI3K)/Akt, endothelial nitric oxide synthase (eNOS), AMP-activated protein kinase (AMPK), and inhibition of reactive oxygen species generation, collectively producing the cardiovascular, metabolic, and cytoprotective effects that define the pharmacological profile.

The cardiovascular pharmacology of apelin-13 is the most extensively characterized domain. In human clinical studies, systemic infusion of [Pyr1]apelin-13 at 30 to 300 nmol/min produces a sustained approximately 10 percent increase in cardiac index, increased ejection fraction, reduced systemic vascular resistance by approximately 12 percent, and reduced mean arterial pressure by approximately 4 percent, effects observed in both healthy volunteers and patients with chronic heart failure and chronic kidney disease. The mechanism involves direct positive inotropic action on cardiomyocytes through APJ-mediated calcium sensitization, nitric oxide-dependent vasodilation in resistance arteries, and counter-regulatory opposition to the renin-angiotensin-aldosterone system. Preclinical models demonstrate cardioprotective effects in myocardial infarction, ischemia-reperfusion injury, pressure-overload hypertrophy, and diabetic cardiomyopathy, with mechanisms including salvage of the peri-infarct border zone, mobilization of endogenous cardiac stem cells, and suppression of pathological fibrosis.

The renal pharmacology is defined by the functional antagonism between apelin and arginine vasopressin (AVP) at the collecting duct. Apelin-13 inhibits vasopressin-induced translocation of aquaporin 2 (AQP2) water channels to the apical membrane of principal cells through Gi-mediated inhibition of cAMP/protein

kinase A signaling, producing a diuretic (aquaretic) effect that opposes AVP-driven water reabsorption. This reciprocal regulation positions the apelin/AVP axis as a physiological rheostat for water homeostasis, with therapeutic implications for hyponatremia and states of AVP excess.

Metabolic pharmacology encompasses insulin-sensitizing and glucoregulatory effects. Apelin-13 administration in diabetic rodent models reduces blood glucose, increases serum insulin, improves pancreatic islet mass, and enhances glucose uptake in skeletal muscle through AMPK-dependent GLUT4 translocation. Neuroprotective activity has been demonstrated in models of ischemic stroke, diabetes-associated cognitive decline, and excitotoxic injury, with mechanisms including antioxidant defense through the SIRT3/FoxO3 pathway, anti-inflammatory cytokine modulation, and direct neuronal survival signaling through PI3K/Akt.

The principal pharmacokinetic limitation of apelin-13 is its extremely short plasma half-life. Native [Pyr1]apelin-13 has a plasma half-life of approximately 21 to 24 minutes in rodents, driven by rapid proteolytic degradation at the Leu5-Ser6 peptide bond by neprilysin, angiotensin-converting enzyme 2 (ACE2), and plasma kallikrein. This has motivated extensive medicinal chemistry efforts to develop stabilized analogues (macrocytic peptides, D-amino acid substitutions, PEGylation) and small-molecule APJ agonists (AMG-986, BMS-986224, azelaprag) for chronic administration.

This monograph reviews the chemistry, identification, and structural biology of apelin-13; the discovery and deorphanization history of the APJ receptor; the molecular pharmacology across Gi, Gq, G12/13, and beta-arrestin pathways; the pharmacokinetic

profile and proteolytic degradation pathways; the preclinical evidence base across cardiovascular, renal, metabolic, and neurological domains; the clinical evidence from human hemodynamic studies; sourcing and quality verification for research-grade material; reconstitution and handling protocols; stack interactions with vasoactive and metabolic agents; the adverse-event and safety profile; and a comparative assessment of five APJ receptor agonist candidates against apelin-13 on five competency standards. The compound is not an approved therapeutic agent in any jurisdiction. It is supplied as a research-grade peptide; investigators should obtain analytical confirmation of identity, purity, and peptide content on every lot.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Systematic peptide name: Apelin-13.

Sequence designation: The C-terminal 13 amino acids of the 77-residue human preproapelin precursor protein. The linear amino acid sequence is Gln-Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Met-Pro-Phe (single-letter code: QRPRLSHKGPMPPF).

Pyroglutamyl form: [Pyr1]apelin-13 (also written [Glp1]apelin-13, pyroglutamyl apelin-13). This is the predominant circulating isoform in human plasma, in which the N-terminal glutamine undergoes spontaneous or enzymatic cyclization to a pyroglutamate residue. The pyroglutamyl form displays modestly enhanced resistance to aminopeptidase degradation relative to the native glutamine form.

Other apelin isoforms: Apelin-13 is one of several bioactive fragments derived from preproapelin. Other characterized isoforms include apelin-36 (the full-length mature peptide), apelin-17, and apelin-12. Apelin-13 and [Pyr1]apelin-13 display the highest receptor binding affinity and biological potency among these isoforms, with 8- to 60-fold greater activity than apelin-36 in cellular acidification assays [1].

Chemical Abstracts Service registry numbers: 217082-58-1 (apelin-13, native glutamine form); 217082-60-5 ([Pyr1]apelin-13, pyroglutamyl form).

PubChem Compound Identifier: CID 25078060 (apelin-13).

UniProt accession: Q9ULZ1 (human preproapelin/APLN gene product). Apelin-13 corresponds to residues 65 to 77 of the preproapelin sequence.

Target receptor: APJ receptor (also designated APLNR, apelin receptor, angiotensin receptor-like 1). IUPHAR receptor nomenclature: apelin receptor. The receptor gene is APLNR (chromosome 11q12.1 in humans).

1.2 Structural class and chemistry

Apelin-13 is a linear tridecapeptide belonging to the class of endogenous bioactive peptides that serve as ligands for G protein-coupled receptors. It is classified within the apelin peptide family, which is defined by derivation from a single gene product (APLN) and convergent binding to the APJ receptor.

Molecular formula: C₆₉H₁₁₁N₂₃O₁₆S (apelin-13, native form); C₆₉H₁₀₈N₂₂O₁₆S ([Pyr1]apelin-13, pyroglutamyl form).

Molecular weight: 1550.83 Da (apelin-13, native form); 1533.85 Da ([Pyr1]apelin-13, pyroglutamyl form).

Physical properties: White to off-white lyophilized powder. Freely soluble in water, dilute aqueous buffers, and dimethyl sulfoxide. Sparingly soluble in organic solvents. The peptide is hygroscopic and should be stored desiccated.

Structural features: The N-terminal RPRL motif (residues 2 through 5) forms a characteristic beta-turn that is critical for APJ receptor recognition and binding. The C-terminal phenylalanine (Phe13) penetrates deep into the transmembrane binding pocket of the receptor and is essential for receptor activation. Crystallographic studies at 2.6 angstrom resolution have demonstrated a two-site binding mode in which the peptide adopts a curved, extended conformation across the extracellular loops and transmembrane helices of the APJ receptor [2, 3].

Chirality: All amino acid residues in native apelin-13 are L-configured. The compound contains one sulfur-bearing residue (Met11) that is susceptible to oxidation under harsh conditions.

1.3 Synthesis

Apelin-13 is produced by solid-phase peptide synthesis (SPPS) using standard Fmoc (9-fluorenylmethyloxycarbonyl) chemistry. The synthesis proceeds from the C-terminal phenylalanine anchored to a Wang or Rink amide resin through sequential coupling of protected amino acids using activating reagents such as HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate) or HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) in the presence of N,N-diisopropylethylamine. Global deprotection

and cleavage from the resin are performed with trifluoroacetic acid cocktails containing scavengers (triisopropylsilane, water, ethanedithiol) to prevent side reactions on the methionine thioether and arginine guanidinium groups.

For the pyroglutamyl form, the N-terminal glutamine is cyclized to pyroglutamate either by spontaneous cyclization during cleavage and purification (which occurs readily under acidic conditions at elevated temperature) or by enzymatic conversion using glutaminyl cyclase. Alternatively, pyroglutamic acid can be directly coupled as the final residue during SPPS.

Purification is by reversed-phase high-performance liquid chromatography (RP-HPLC) on C18 columns with acetonitrile/water/trifluoroacetic acid gradient elution. The product is characterized by HPLC purity assessment, electrospray ionization mass spectrometry (ESI-MS) for molecular weight confirmation, and amino acid analysis. Research-grade material is typically supplied at greater than 95 percent purity by HPLC; higher purity (greater than 98 percent) is available from specialized peptide suppliers.

2. Abstract

Apelin-13 is the most potent endogenous peptide agonist of the apelin receptor (APJ/APLNR), a class A G protein-coupled receptor that has emerged as a therapeutic target of substantial interest in cardiovascular medicine, renal physiology, metabolic disease, and neuroscience [1, 4]. The peptide was isolated in 1998 by Tatemoto and colleagues from bovine stomach extracts as the endogenous ligand for the previously orphan APJ receptor, which had been cloned in 1993 by O'Dowd et al. on the basis of its sequence homology with the angiotensin II type 1 receptor [5, 6]. Among the several bioactive isoforms derived from the preproapelin precursor (apelin-36, apelin-17, apelin-13, apelin-12), apelin-13 and its pyroglutamyl derivative [Pyr1]apelin-13 display the highest receptor affinity and biological potency, and [Pyr1]apelin-13 has been identified as the predominant circulating apelin isoform in human plasma [7].

The cardiovascular pharmacology of apelin-13 is defined by three principal effects: positive inotropy (increased cardiac contractility without increased heart rate), peripheral vasodilation (reduced systemic vascular resistance through endothelial nitric oxide release), and counter-regulation of the renin-angiotensin-aldosterone system [8, 9]. In human clinical hemodynamic studies conducted by Japp et al. (2010) and subsequently by Gala-Peralta et al. (2024), systemic infusion of [Pyr1]apelin-13 at 30 to 300 nmol/min produced a sustained increase in cardiac index of approximately 10 percent, reduced systemic vascular resistance by approximately 12 percent, and reduced mean arterial pressure by approximately 4 percent, with effects observed in healthy volunteers, patients with chronic heart failure, and patients with chronic kidney disease [8, 10]. These hemodynamic effects were achieved without significant tachycardia, arrhythmia, or other clinically significant adverse events, establishing a favorable acute safety profile.

Preclinical pharmacology extends across multiple organ systems. In the heart, apelin-13 is cardioprotective in models of myocardial infarction, ischemia-reperfusion injury, and pressure-overload hypertrophy through mechanisms including peri-infarct border zone salvage, endogenous stem cell mobilization, anti-fibrotic signaling, and suppression of pathological hypertrophy [11, 12]. In the kidney, apelin-13 acts as a physiological antagonist of arginine vasopressin (AVP) at the collecting duct, inhibiting vasopressin-induced aquaporin 2 (AQP2) translocation and

producing a diuretic (aquaretic) effect that positions the apelin/AVP axis as a homeostatic regulator of water balance [13, 14]. In metabolic disease, apelin-13 improves insulin sensitivity, reduces blood glucose, enhances pancreatic islet mass, and promotes GLUT4 translocation in skeletal muscle through AMP-activated protein kinase (AMPK)-dependent mechanisms [15, 16]. In the central nervous system, apelin-13 is neuroprotective in models of ischemic stroke, diabetes-associated cognitive decline, and excitotoxic injury through antioxidant, anti-inflammatory, and PI3K/Akt survival signaling pathways [17, 18].

The principal pharmacological limitation is the extremely short plasma half-life. Native [Pyr1]apelin-13 has a half-life of approximately 21 to 24 minutes in rodent plasma, driven by rapid proteolytic cleavage at the Leu5-Ser6 bond by neprilysin and at the C-terminal penultimate proline by angiotensin-converting enzyme 2 (ACE2) [19, 20]. This pharmacokinetic profile has motivated the development of stabilized peptide analogues (macrocytic variants with half-lives exceeding 3 to 24 hours, D-amino acid-substituted analogues) and orally bioavailable small-molecule APJ agonists (AMG-986, BMS-986224, azelaprag) for chronic therapeutic applications [21, 22, 23].

Apelin-13 is not an approved therapeutic agent in any jurisdiction. It is supplied as a research-grade synthetic peptide for *in vitro* and *in vivo* investigation. This monograph documents the compound identification, discovery history, molecular pharmacology, pharmacokinetics, preclinical and clinical evidence, sourcing, handling, stack interactions, safety profile, and a comparative assessment against five APJ receptor agonist candidates.

3. Discovery and Development History

3.1 Identification of the APJ orphan receptor

The apelin receptor (APJ) was first identified in 1993 by O'Dowd and colleagues at the University of Toronto through a degenerate polymerase chain reaction strategy targeting novel G protein-coupled receptors with homology to the angiotensin II receptor family [5]. The cloned receptor shared 31 percent overall amino acid identity with the human angiotensin II type 1 (AT1) receptor and 54 percent identity in the hydrophobic transmembrane domains, leading to its provisional designation as "angiotensin receptor-like 1" (AGTRL1, subsequently renamed APLNR). Despite the sequence homology, the receptor did not bind angiotensin II or any other known angiotensin peptide, and it remained an orphan receptor for five years. During this period, several groups characterized its tissue distribution (brain, heart, lung, kidney, adipose tissue, endothelium) and its signaling properties in heterologous expression systems, establishing that it coupled to pertussis toxin-sensitive Gi/o proteins [5, 6].

3.2 Deorphanization by Tatemoto and isolation of apelin

In 1998, Kazuhiko Tatemoto and colleagues at the Takeda Chemical Research Institute in Osaka screened bovine stomach tissue extracts for ligands capable of activating APJ-expressing Chinese hamster ovary cells in an extracellular acidification assay [1]. The screen identified a 36-amino-acid peptide (apelin-36) derived from a 77-residue precursor protein (preproapelin), which they named "apelin" (APJ Endogenous LIgaNd). Tatemoto et al. further demonstrated that the C-terminal fragments apelin-13 and apelin-17 were substantially more potent than the full-length apelin-36, with apelin-13 showing 8- to 60-fold higher activity in the acidification assay. This finding established apelin-13 as the principal high-potency isoform and the focus of subsequent pharmacological characterization [1].

3.3 Identification of [Pyr1]apelin-13 as the major circulating isoform

In 2013, Zhen et al. developed a specific immunoassay for [Pyr1]apelin-13 and demonstrated that the pyroglutamyl form is the predominant apelin isoform in human plasma, with circulating concentrations in the low picomolar range in healthy subjects [7]. The finding that the pyroglutamyl modification occurs in vivo (through spontaneous cyclization or enzymatic conversion by glutaminy cyclase) established [Pyr1]apelin-13 as the physiologically relevant circulating form and the appropriate isoform for pharmacological and clinical investigation.

3.4 Structural biology of the APJ receptor

The structural basis for apelin-receptor interaction was elucidated through a series of crystallographic and cryo-electron microscopy studies. Ma et al. (2017) reported the crystal structure of the human APJ receptor in complex with an apelin mimetic peptide at 2.6 angstrom resolution, revealing a two-site binding mode in which the peptide engages both the extracellular vestibule and the orthosteric transmembrane pocket [2]. Subsequent studies by Yue et al. (2024) and Cao et al. (2024) resolved the structures of APJ in both monomeric and homodimeric states, demonstrating that apelin-13 binds exclusively to one protomer of the homodimer coupled to Gi protein, with the unliganded protomer adopting a distinct conformation [3, 24]. These structural insights have guided the design of stabilized peptide analogues and small-molecule agonists.

3.5 Identification of Elabela as a second endogenous APJ ligand

In 2013 to 2014, two independent groups identified a second endogenous peptide ligand for the APJ receptor, designated Elabela (also called APELA or Toddler) [25]. Elabela is a 54-amino-acid prepropeptide processed to active fragments of 11, 16, and 32 residues. Elabela and apelin share no sequence homology but bind overlapping sites on the APJ receptor with comparable or greater potency than apelin-13 in some assays. The identification of Elabela demonstrated that the APJ receptor is a dual-ligand system, with implications for the interpretation of apelin-13 pharmacology in the context of endogenous Elabela tone.

3.6 Development of synthetic APJ agonists

The short plasma half-life of native apelin-13 drove medicinal chemistry programs at multiple pharmaceutical companies. Amgen disclosed AMG-986, an orally bioavailable small-molecule APJ agonist, which entered Phase 1 clinical trials in healthy volunteers and heart failure patients and was reported as safe and well tolerated [22]. Bristol-Myers Squibb developed BMS-986224, a potent (K_d 0.3 nM) and selective small-molecule APJ agonist that produced approximately 10 to 15 percent increases in cardiac output in preclinical models and advanced to clinical evaluation [23]. Macrocyclic peptide analogues (including MM07, a biased agonist with approximately 7-fold greater half-life than [Pyr1]apelin-13 ex vivo) were developed at the University of Edinburgh and evaluated in human hemodynamic studies [26]. These programs are ongoing as of the monograph revision date, with no APJ agonist having achieved regulatory approval for any indication.

4. Mechanism of Action

Apelin-13 is a pleiotropic peptide agonist whose pharmacological effects are mediated through multi-pathway activation of the APJ receptor, a class A G protein-coupled receptor expressed in cardiomyocytes, vascular endothelium, vascular smooth muscle, renal collecting duct epithelium, hypothalamic neurons, pancreatic islets, adipocytes, and skeletal muscle. The receptor couples to multiple heterotrimeric G protein families and to beta-arrestin scaffolding proteins, producing tissue-specific downstream signaling cascades.

4.1 G protein-coupled signaling pathways

Gi/o coupling: The predominant signaling pathway in most tissues. APJ activation by apelin-13 inhibits adenylyl cyclase through the Gi alpha subunit, reducing intracellular cyclic AMP (cAMP) concentrations. The Gi beta-gamma subunit activates phosphoinositide 3-kinase (PI3K), leading to Akt phosphorylation and downstream activation of endothelial nitric oxide synthase (eNOS), AMP-activated protein kinase (AMPK), and anti-apoptotic signaling through BAD phosphorylation and inhibition of caspase cascades [4, 27]. In the kidney, Gi-mediated inhibition of cAMP/protein kinase A (PKA) signaling in collecting duct principal cells prevents vasopressin-stimulated aquaporin 2 trafficking, producing the aquaretic effect [13, 14].

Gq/11 coupling: In cardiomyocytes and vascular smooth muscle cells, apelin-13 activates phospholipase C beta (PLCbeta) through Gq/11, generating inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3-mediated calcium release from the sarcoplasmic reticulum contributes to the positive inotropic effect. DAG activates protein kinase C (PKC) isoforms that participate in cardioprotective signaling through ERK1/2 activation [4, 27].

G12/13 coupling: Apelin-13 activates RhoA and Rho-associated protein kinase (ROCK) through G12/13 in certain cell types, contributing to cytoskeletal rearrangement, cell migration, and angiogenic responses in endothelial cells [4].

4.2 Beta-arrestin recruitment and internalization

APJ recruits both beta-arrestin 1 and beta-arrestin 2 upon apelin-13 binding. Beta-arrestin recruitment serves dual functions: it mediates clathrin-dependent receptor internalization (desensitization) and it scaffolds G protein-independent signaling through ERK1/2 mitogen-activated protein kinase [4, 28]. The relative balance between G protein and beta-arrestin signaling (referred to as signaling bias) varies among apelin peptide isoforms and synthetic analogues. C-terminal modifications of apelin-13 that introduce positive charge produce partial agonist behavior disfavoring beta-arrestin 2 recruitment while retaining G_i activation, a finding that has informed the design of biased agonists such as MM07 [26, 29].

4.3 Cardiovascular mechanism

The cardiovascular effects of apelin-13 reflect the composite of three signaling outputs. The positive inotropic effect (increased cardiac contractility) is mediated by $G_q/11$ -dependent calcium sensitization and sodium-hydrogen exchanger activation in cardiomyocytes, producing increased force of contraction without the increased myocardial oxygen demand associated with beta-adrenergic stimulation [8, 9]. The vasodilatory effect is mediated by G_i -dependent PI3K/Akt/eNOS activation in vascular endothelium, producing nitric oxide release and relaxation of underlying smooth muscle [8]. The counter-regulatory effect on the renin-angiotensin-aldosterone system reflects the evolutionary opposition between the APJ and AT1 receptor pathways at the level of vascular tone, cardiac remodeling, and sodium/water handling [4, 9].

4.4 Renal mechanism

In the kidney, apelin-13 opposes arginine vasopressin (AVP) at the collecting duct through a well-characterized molecular pathway. AVP binds the V2 vasopressin receptor on basolateral membranes of principal cells, activating G_s -coupled adenylyl cyclase and increasing cAMP, which through PKA phosphorylates aquaporin 2 (AQP2) and promotes its exocytic insertion into the apical membrane to permit water reabsorption. Apelin-13 binds APJ on the same cells and activates G_i , which inhibits adenylyl cyclase and reduces cAMP, thereby preventing AQP2 phosphorylation and apical insertion [13, 14]. The net effect is increased free water clearance (aquaresis). Apelin-13 additionally reduces epithelial sodium channel (ENaC) activity in collecting duct cells, contributing to natriuresis [30].

4.5 Metabolic mechanism

In skeletal muscle, apelin-13 promotes glucose uptake through AMPK-dependent translocation of GLUT4 glucose transporters to the plasma membrane, an insulin-independent pathway that is preserved in insulin-resistant states [15, 16]. In adipose tissue, apelin modulates lipolysis and adipogenesis through AMPK and ERK1/2 signaling. In pancreatic beta cells, apelin-13 has been reported to enhance insulin secretion and to promote beta cell survival through anti-apoptotic PI3K/Akt signaling [15, 16]. The composite metabolic effect profile positions apelin-13 as an insulin-sensitizing and glucoregulatory peptide with therapeutic potential in type 2 diabetes and metabolic syndrome.

4.6 Neuroprotective mechanism

Apelin-13 exerts neuroprotective effects through multiple pathways. In ischemic stroke models, apelin-13 reduces infarct volume and improves neurological outcomes through PI3K/Akt-mediated neuronal survival signaling, suppression of inflammatory cytokines (TNF-alpha, IL-1beta, IL-6), and reduction of oxidative stress through superoxide dismutase and catalase activation [17, 18]. In diabetes-associated cognitive decline models, apelin-13 improves cognitive performance (Y-maze, Morris water maze) through the SIRT3/FoxO3 pathway, reducing mitochondrial dysfunction and oxidative damage [18]. The receptor is expressed at moderate density in hypothalamus, hippocampus, and cortex, consistent with central nervous system bioactivity.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and bioavailability

Apelin-13 is a 1.5 kDa peptide with no oral bioavailability in its native form. The peptide is rapidly degraded by gastrointestinal proteases and has negligible absorption across the intestinal epithelium. Administration in research and clinical settings is therefore parenteral: intravenous infusion (the standard clinical route for hemodynamic studies), subcutaneous injection (research applications), intraperitoneal injection (rodent studies), and intranasal delivery (experimental, used in stroke models at 100 micrograms in mice) [8, 10, 31].

5.2 Distribution

The volume of distribution has not been precisely characterized in humans. In rodent pharmacokinetic studies, [Pyr1]apelin-13 distributes rapidly to cardiovascular tissue, kidney, lung, and brain. The peptide crosses the blood-brain barrier to some degree, consistent with the expression of APJ in hypothalamic and hippocampal neurons and the observed central effects of systemically administered apelin-13 [4, 19]. Plasma protein binding has not been formally characterized but is expected to be low for a small hydrophilic peptide.

5.3 Metabolism and degradation

The pharmacokinetics of apelin-13 are dominated by rapid proteolytic degradation. The primary cleavage site is the Leu5-Ser6 peptide bond, which is the first cleavage observed in plasma across multiple species (human, rat, mouse) [19, 20]. The principal proteases responsible for apelin-13 degradation are:

Neprilysin (NEP, CD10): A zinc-dependent metalloendopeptidase that cleaves apelin-13 at the Leu5-Ser6 bond and is the only protease demonstrated to fully inactivate the peptide by abolishing receptor binding of the resulting fragments [19].

Angiotensin-converting enzyme 2 (ACE2): Removes the C-terminal phenylalanine (Phe13) from apelin-13, producing apelin-13(1-12), which retains partial receptor binding activity but has substantially reduced potency [20, 32]. The ACE2 cleavage is physiologically significant because ACE2 is co-expressed with APJ in cardiovascular tissue and modulates local apelin bioactivity.

Plasma kallikrein: Cleaves apelin-13 at internal sites, contributing to overall degradation. Furin also cleaves apelin peptides but has been demonstrated principally with the longer apelin-36 isoform [19].

5.4 Plasma half-life

In rat plasma, [Pyr1]apelin-13 has a half-life of approximately 24 minutes *ex vivo*. In *vivo* pharmacokinetic studies in rats following intravenous bolus administration report a clearance of 0.049 L/h/kg and a half-life of approximately 0.36 hours (approximately 21 minutes) [19, 33]. The half-life in human plasma has not been formally determined through pharmacokinetic studies, but the rapid clearance observed during clinical infusion studies (requiring continuous infusion to maintain hemodynamic effects) is consistent with a similarly short half-life [8, 10].

5.5 Stabilized analogues

The short half-life has driven development of stabilized apelin-13 analogues. Macrocyclic analogues bearing lactam bridges or disulfide constraints show plasma half-lives of greater than 3 hours (versus 24 minutes for native [Pyr1]apelin-13), with some D-amino acid-substituted macrocycles achieving half-lives of 6.8 to greater than 24 hours [21, 34]. These analogues retain sub-nanomolar binding affinity (K_i as low as 0.15 nM for macrocyclic analogue 15) and produce prolonged cardiovascular effects *in vivo* [21, 34]. MM07, a cyclic apelin analogue developed at the University of Edinburgh, achieves approximately 7-fold greater half-life than [Pyr1]apelin-13 *ex vivo* and has been evaluated in human hemodynamic studies as a biased agonist favoring G protein over beta-arrestin signaling [26].

5.6 Routes of administration in research

Intravenous infusion: The standard route for clinical hemodynamic studies. Doses of 30 to 300 nmol/min of [Pyr1]apelin-13 have been used in human studies, with infusion durations of 6 to 120 minutes [8, 10].

Intraperitoneal injection: The standard route for rodent pharmacology studies. Doses of 10 to 50 nmol/kg/day have been used in cardiovascular, metabolic, and neuroprotection studies [11, 12, 15].

Subcutaneous injection: Used in chronic rodent dosing studies, typically at 20 nmol/kg/day via osmotic minipump or repeated injection [11].

Intrabrachial infusion: Used for assessment of local forearm vascular effects in human volunteers. Doses of 0.3, 1, and 3 nmol/min have been infused into the brachial artery with measurement of forearm blood flow [8].

Intranasal delivery: Experimental route used in mouse stroke models at 100 micrograms per administration. Provides rapid access to the central nervous system through the olfactory epithelium [31].

6. Preclinical Pharmacology

6.1 Cardiac contractility and inotropy

Apelin-13 increases cardiac contractility in isolated cardiomyocyte preparations and in intact hearts through Gq/11-dependent calcium sensitization. In isolated perfused rat hearts, apelin-13 at nanomolar concentrations increases developed pressure and the rate of pressure development (dP/dt) without increasing heart rate [9, 11]. The positive inotropic effect is nitric oxide-independent in the myocardium (distinguishing it from the vascular mechanism) and is abolished by Gq/11 inhibition or by phospholipase C blockade. Importantly, apelin-13 does not increase myocardial oxygen consumption to the same degree as beta-adrenergic agonists at equivalent increases in contractility, a finding that supports its investigation as a heart failure therapeutic [9].

6.2 Myocardial infarction and ischemia-reperfusion injury

In rodent models of permanent coronary artery ligation, apelin-13 infusion (beginning at the time of ligation or at 24 hours post-infarction) salvages the peri-infarct border zone, reduces infarct size by 20 to 35 percent, and preserves left ventricular ejection fraction at 4 weeks compared to vehicle [11, 12]. The mechanism involves mobilization of endogenous c-kit-positive cardiac stem cells to the border zone, suppression of cardiomyocyte apoptosis through PI3K/Akt/Bad signaling, reduction of inflammatory macrophage infiltration, and attenuation of collagen deposition (anti-fibrotic effect) [11, 12].

In ischemia-reperfusion models (transient coronary occlusion followed by reperfusion), apelin-13 pretreatment reduces reperfusion injury through activation of the reperfusion injury salvage kinase (RISK) pathway (PI3K/Akt and ERK1/2) and through reduction of reactive oxygen species generation [11].

6.3 Cardiac hypertrophy and heart failure

In pressure-overload models of cardiac hypertrophy (transverse aortic constriction in mice), chronic apelin-13 administration attenuates pathological hypertrophy and preserves systolic function. The anti-hypertrophic mechanism involves AMPK activation, which inhibits the calcineurin/NFAT pathway responsible for the hypertrophic transcriptional program [11, 35]. In models of established heart failure, apelin-13 improves cardiac output and reduces pulmonary congestion, consistent with the human hemodynamic data [9, 11].

6.4 Vascular biology

Apelin-13 produces nitric oxide-dependent vasodilation in resistance arteries through endothelial APJ activation and PI3K/Akt/eNOS phosphorylation. In endothelium-denuded preparations, apelin-13 produces vasoconstriction through smooth muscle APJ/Gq signaling, indicating that the net vascular effect depends on endothelial integrity [8, 36]. This observation has been confirmed in vivo: in animals with intact endothelium, apelin-13 reduces blood pressure; in models of endothelial dysfunction (asymmetric dimethylarginine exposure, advanced atherosclerosis), apelin-13 may paradoxically increase vascular resistance [36]. The clinical relevance of this dual vascular mechanism is under investigation.

Apelin-13 also promotes angiogenesis in developmental and post-ischemic models through APJ-mediated endothelial cell proliferation, migration, and tube formation, with signaling through VEGF-dependent and VEGF-independent pathways [4].

6.5 Renal pharmacology

In rodent models, apelin-13 produces dose-dependent diuresis (aquaresis) through inhibition of AQP2 apical insertion in collecting duct principal cells. The mechanism involves Gi-mediated reduction of cAMP and PKA activity, opposing the V2 receptor-mediated antidiuretic signaling of AVP [13, 14]. In water-deprived rats, endogenous apelin is suppressed (allowing maximal AVP-driven water reabsorption), while AVP is elevated; upon rehydration, apelin rises and AVP falls, demonstrating the reciprocal physiological regulation [13]. Apelin-13 additionally reduces ENaC-mediated sodium reabsorption in the collecting duct, contributing to natriuresis

[30]. In chronic kidney disease models, apelin-13 infusion preserves glomerular filtration rate and reduces proteinuria through anti-inflammatory and anti-fibrotic mechanisms [10, 37].

6.6 Metabolic pharmacology

In db/db diabetic mice and high-fat diet-induced obese mice, chronic apelin-13 administration (intraperitoneal, 2 to 4 weeks) reduces fasting blood glucose by 20 to 40 percent, increases serum insulin levels, improves glucose tolerance, and enhances insulin sensitivity as measured by homeostatic model assessment [15, 16]. The glucoregulatory mechanism involves AMPK-dependent GLUT4 translocation in skeletal muscle (an insulin-independent glucose uptake pathway), enhanced glucose-stimulated insulin secretion from pancreatic beta cells, and increased pancreatic islet mass through anti-apoptotic signaling [15, 16]. In adipose tissue, apelin-13 enhances fatty acid oxidation through AMPK activation and modulates adipokine secretion [15].

6.7 Neuroprotection

In middle cerebral artery occlusion (MCAO) models of ischemic stroke, apelin-13 (administered intravenously or intranasally) reduces infarct volume by 25 to 45 percent and improves neurological deficit scores [17, 31]. The neuroprotective mechanism involves PI3K/Akt activation, reduction of inflammatory cytokine expression (TNF-alpha, IL-1beta), preservation of blood-brain barrier integrity, and suppression of neuronal apoptosis [17].

In streptozotocin-induced diabetic rats, apelin-13 improves cognitive performance in Y-maze and Morris water maze testing by reducing hippocampal oxidative stress through the SIRT3/FoxO3 pathway, enhancing superoxide dismutase and catalase activity, and reducing malondialdehyde and 4-hydroxynonenal levels [18]. The compound also demonstrates anti-inflammatory activity in the central nervous system through suppression of microglial activation and reduction of NF-kappaB-dependent cytokine production [17, 18].

6.8 Anti-inflammatory and cytoprotective effects

Apelin-13 has demonstrated cytoprotective activity in models of cisplatin-induced nephrotoxicity (reduction of renal tubular apoptosis and oxidative stress), septic shock (hemodynamic stabilization and reduction of catecholamine requirements in cecal ligation and puncture models), and ischemia-reperfusion injury of erythrocytes in diabetic rats [37, 38, 39]. The anti-inflammatory mechanism is broadly attributable to PI3K/Akt-mediated suppression of NF-kappaB and to reduction of reactive oxygen species generation through AMPK activation.

7. Clinical Evidence Base

7.1 Cardiovascular hemodynamics in healthy volunteers

The first systematic clinical evaluation of apelin-13 hemodynamics was reported by Japp et al. (2010) in *Circulation* [8]. In a randomized, double-blind, placebo-controlled, crossover study, [Pyr1]apelin-13 was infused systemically at 30 and 300 nmol/min in 12 healthy male volunteers. At 300 nmol/min, apelin-13 produced a significant increase in cardiac output, increased forearm blood flow, and reduced systemic vascular resistance and mean arterial pressure. Intrabrachial infusion at 0.3 to 3 nmol/min confirmed dose-dependent forearm vasodilation. All infusions were well tolerated with no serious adverse events, no clinically significant electrocardiographic changes, and no tachycardia [8].

7.2 Heart failure

In the same Japp et al. (2010) publication, [Pyr1]apelin-13 infusion (30 to 300 nmol/min) was administered to patients with chronic heart failure (NYHA class II to III). The compound produced an approximately 10 percent sustained increase in cardiac index with increased ejection fraction on echocardiography and reduced peripheral vascular resistance, demonstrating that the hemodynamic benefit is preserved in the failing heart [8]. The absence of reflex tachycardia distinguished apelin-13 from catecholaminergic inotropes and supported the hypothesis of a distinct, potentially safer inotropic mechanism.

7.3 Chronic kidney disease

Gala-Peralta et al. (2024) reported a randomized, double-blind, placebo-controlled, crossover study of [Pyr1]apelin-13 in patients with chronic kidney disease (stages 3 to 4) and matched healthy controls, published in *Nature Communications* [10]. Systemic infusion at 30 nmol/min reduced mean arterial pressure by approximately 4 percent, systemic vascular resistance by approximately 12 percent, and increased cardiac index by approximately 10 percent compared to placebo, with effects of similar magnitude in both chronic kidney disease patients and healthy controls. The compound was well tolerated with no serious adverse events or electro-

cardiographic changes. Additionally, apelin-13 infusion produced a trend toward increased renal plasma flow and reduced renal vascular resistance in chronic kidney disease patients, suggesting potential renal hemodynamic benefit [10].

7.4 Limitations of the clinical evidence base

The clinical evidence for apelin-13 is limited to acute hemodynamic infusion studies in small cohorts (10 to 30 participants per study). No dose-ranging studies, chronic administration trials, or outcome-driven clinical trials have been completed with native apelin-13. The pharmacokinetic limitation (requiring continuous infusion) precludes practical chronic use of the native peptide. Clinical development of the apelin pathway for chronic indications (heart failure, chronic kidney disease, diabetes) is proceeding through stabilized peptide analogues and small-molecule APJ agonists rather than through native apelin-13.

8. Sourcing and Quality Verification

Research-grade apelin-13 and [Pyr1]apelin-13 are supplied by multiple peptide synthesis companies and biochemical reagent suppliers (Tocris Bioscience, Sigma-Aldrich/Merck, Cayman Chemical, Phoenix Pharmaceuticals, Bachem, AnaSpec, APExBIO, MedChemExpress, Selleckchem, GenScript, and others). The compound is supplied as a lyophilized powder, typically as the trifluoroacetate (TFA) or acetate salt, in quantities ranging from 0.1 mg to 50 mg per vial. Pricing is moderate for a synthetic peptide: approximately \$100 to \$400 per milligram at research scale, with volume discounts available at gram scale from custom synthesis suppliers.

Investigators should confirm compound identity and quality on every lot through:

- Reversed-phase HPLC purity assessment, with acceptance criterion of greater than 95 percent (preferably greater than 98 percent for dose-response studies)
- Electrospray ionization mass spectrometry (ESI-MS) confirming molecular weight: expected $[M+H]^+$ of 1551.83 for apelin-13 or 1534.85 for [Pyr1]apelin-13
- Amino acid analysis confirming composition and stoichiometry
- Peptide content determination (net peptide content is typically 60 to 85 percent of the gross weight for lyophilized TFA salt preparations, with the remainder being water, counterion, and residual salts)
- Endotoxin testing (limulus amebocyte lysate assay) for preparations intended for in vivo administration, with acceptance criterion of less than 0.25 EU/mL at the intended working concentration

The distinction between apelin-13 (native glutamine N-terminus) and [Pyr1]apelin-13 (pyroglutamyl N-terminus) is important. The two forms have different molecular weights, different HPLC retention times, and modestly different pharmacological profiles (the pyroglutamyl form has greater resistance to aminopeptidase degradation). Investigators should specify which form is required for their application and confirm identity by mass spectrometry. The pyroglutamyl form is the physiologically relevant circulating isoform and is preferred for most pharmacological investigations.

Counterfeit or misidentified peptide material is uncommon in the apelin-13 market, but lot-to-lot variability in peptide content and purity is observed across suppliers. On-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

9. Reconstitution and Handling

Apelin-13 and [Pyr1]apelin-13 are supplied as lyophilized powders and require reconstitution prior to use. The peptide is freely soluble in water and aqueous buffers at the concentrations used in research.

Stock solution preparation: Reconstitute the lyophilized peptide in sterile deionized water, phosphate-buffered saline (PBS, pH 7.4), or normal saline (0.9 percent sodium chloride) to a concentration of 1 to 10 mg/mL. For in vitro work, dimethyl sulfoxide (DMSO) can be used as a co-solvent, but aqueous reconstitution is preferred given the peptide's excellent water solubility. Vortex or pipette gently to dissolve; do not sonicate or agitate vigorously, as this may promote aggregation or surface adsorption.

Aliquoting: Prepare single-use aliquots of the stock solution in polypropylene microcentrifuge tubes to avoid repeated freeze-thaw cycles. The peptide may adsorb to glass surfaces; polypropylene or siliconized glass vials are preferred. For low-concentration solutions (less than 10 micromolar), addition of 0.1 percent bovine serum albumin (BSA) or 0.01 percent polysorbate 20 as a carrier protein or surfactant can reduce surface adsorption losses.

Storage: Store lyophilized peptide at minus 20 degrees Celsius, desiccated, protected from light. Reconstituted stock solutions should be stored at minus 20 degrees Celsius (for long-term storage, up to 3 months) or at minus 80 degrees Celsius (for extended storage, up to 6 months). Aqueous solutions stored at 2 to 8 degrees Celsius should be used within 7 days. Avoid repeated freeze-thaw cycles; the peptide tolerates up to 3 freeze-thaw cycles without significant loss of bioactivity in most preparations, but single-use aliquoting is strongly recommended.

Methionine oxidation: The Met11 residue is susceptible to oxidation under exposure to atmospheric oxygen, peroxides, or metal ions. Oxidized apelin-13 (methionine sulfoxide form) retains partial receptor binding activity but has reduced potency. Minimize exposure of solutions to air; work under inert gas (nitrogen or argon) for extended handling. If methionine oxidation is a concern, the stability of each lot should be assessed by HPLC after storage.

Working solutions: Dilute stock solutions to working concentrations in the appropriate vehicle immediately before use. For in vivo administration, use sterile, endotoxin-free saline or PBS. For cell-based assays, dilute in serum-free or complete culture medium as appropriate. The peptide is stable in physiological buffers at room temperature for at least 4 hours.

10. Stack Interaction Considerations

Apelin-13 interacts with several drug and compound classes through its receptor-mediated cardiovascular, renal, and metabolic effects. Investigators conducting research with apelin-13, particularly in combination with other vasoactive or metabolic agents, should consider the following interactions.

Renin-angiotensin-aldosterone system (RAAS) modulators: Apelin-13 and angiotensin II exert opposing effects on vascular tone, cardiac remodeling, and sodium/water balance. Concurrent administration of apelin-13 with ACE inhibitors (enalapril, ramipril), angiotensin receptor blockers (losartan, valsartan), or mineralocorticoid receptor antagonists (spironolactone, eplerenone) may produce additive hypotensive effects and synergistic cardioprotective or renoprotective effects. The combination is mechanistically rational for heart failure and hypertension research but requires monitoring for excessive hypotension [4, 9].

Vasopressin and V2 receptor agonists: Apelin-13 functionally opposes vasopressin at the collecting duct. Concurrent desmopressin (DDAVP) or vasopressin administration may attenuate the aquaretic effect of apelin-13. Conversely, concurrent V2 receptor antagonists (tolvaptan, conivaptan) and apelin-13 may produce additive aquaretic effects [13, 14].

Neprilysin inhibitors: Sacubitril (the neprilysin inhibitor component of sacubitril/valsartan, marketed as Entresto) inhibits the principal protease responsible for apelin-13 degradation. Concurrent sacubitril administration would be expected to prolong apelin-13 half-life and increase bioavailability. This interaction is of clinical relevance because sacubitril/valsartan is a first-line therapy for heart failure with reduced ejection fraction, and the elevation of endogenous apelin levels may contribute to the therapeutic mechanism of sacubitril [19, 40].

ACE2 modulators: ACE2 cleaves the C-terminal phenylalanine of apelin-13, reducing its potency. Conditions or agents that increase ACE2 activity may reduce apelin-13 bioactivity. ACE2 inhibitors (MLN-4760, in research) would be expected to prolong apelin-13 activity [20, 32].

Insulin and insulin sensitizers: Apelin-13 enhances insulin sensitivity through AMPK-dependent mechanisms. Concurrent administration with metformin (also an AMPK activator) may produce additive glucose-lowering effects. Concurrent insulin administration with apelin-13 has theoretical additive hypoglycemic risk, though this has not been characterized in formal combination studies [15, 16].

Nitric oxide donors: Apelin-13 produces vasodilation through eNOS-dependent nitric oxide release. Concurrent nitric oxide donors (sodium nitroprusside, nitroglycerin, isosorbide dinitrate) may produce additive hypotension. Monitoring for excessive blood pressure reduction is warranted in combination research [8].

Inotropic agents: Concurrent use with catecholaminergic inotropes (dobutamine, milrinone) produces additive increases in cardiac output through different mechanisms. The apelin-13 inotropic mechanism (calcium sensitization) is mechanistically distinct from the beta-adrenergic/cAMP-dependent mechanism of dobutamine and the phosphodiesterase III inhibition mechanism of milrinone. The combination may be investigated in acute heart failure models but has not been characterized for safety in human studies [9].

Diuretics: Concurrent loop diuretics (furosemide), thiazides, or potassium-sparing diuretics and apelin-13 may produce additive volume depletion. Apelin-13 produces aquaresis (free water clearance) rather than natriuresis as a primary mechanism; the combination with natriuretic agents alters the electrolyte composition of the diuretic response [13].

11. Adverse Events and Safety

11.1 Clinical safety data

The clinical safety data for apelin-13 are derived from acute infusion studies in small cohorts. In the Japp et al. (2010) and Gala-Peralta et al. (2024) hemodynamic studies, [Pyr1]apelin-13 infusion at 30 to 300 nmol/min was well tolerated in healthy volunteers, heart failure patients, and chronic kidney disease patients [8, 10]. No serious adverse events were reported. No clinically significant electrocardiographic changes (QT prolongation, arrhythmia, ST-segment changes) were observed during or after infusion. No tachycardia was observed, distinguishing apelin-13 from catecholaminergic inotropes. Mild transient hypotension (consistent with the vasodilatory mechanism) was observed in some subjects at higher infusion rates but did not require intervention [8, 10].

11.2 Preclinical safety observations

In rodent studies, apelin-13 at doses of 10 to 50 nmol/kg/day administered for 2 to 4 weeks has not produced organ toxicity, behavioral abnormalities, or mortality [11, 12, 15]. The compound has no known genotoxic, mutagenic, or teratogenic activity based on available literature (formal regulatory toxicology studies have not been conducted, as the compound has not entered clinical development as a drug candidate).

11.3 Potential safety concerns

Hypotension: The vasodilatory mechanism of apelin-13 carries inherent risk of excessive hypotension, particularly in volume-depleted subjects, in combination with other antihypertensive agents, or at higher infusion rates. Blood pressure monitoring is essential during administration [8].

Endothelial dysfunction context: In models of severe endothelial dysfunction or damage (asymmetric dimethylarginine exposure), apelin-13 may paradoxically increase vascular resistance through unopposed smooth muscle APJ/Gq-mediated

vasoconstriction, potentially exacerbating hypertension [36]. This observation is relevant for research in advanced vascular disease models.

Tumor biology considerations: The APJ receptor is expressed in several tumor types, and the apelin/APJ axis has been implicated in tumor angiogenesis and in some contexts tumor progression. Chronic apelin-13 administration in tumor-bearing models has not been systematically characterized for safety. Investigators working with apelin-13 in oncology contexts should consider the potential for pro-angiogenic effects [4].

Fluid and electrolyte effects: The aquaretic effect of apelin-13 could produce clinically significant free water loss in chronic administration, particularly in combination with other diuretics. Monitoring of fluid balance and serum sodium is warranted in chronic dosing studies [13].

11.4 Absence of formal toxicology

Apelin-13 has not undergone formal preclinical toxicology evaluation (single-dose toxicity, repeat-dose toxicity, reproductive toxicity, carcinogenicity) as required for investigational new drug applications. The available safety data are from pharmacology studies that included tolerability endpoints but were not designed as toxicology studies. Investigators planning in vivo studies should design protocols with appropriate safety monitoring and should not extrapolate the favorable acute safety profile to chronic dosing without additional data.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five APJ receptor agonist candidates against native apelin-13, evaluated on five competency standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile, and overall validation. The five candidates were selected as the principal APJ receptor ligands with substantive preclinical or clinical pharmacology programs, with priority given to compounds that have produced clinical data or that represent mechanistically distinct approaches to APJ activation.

12.1 [Pyr1]apelin-13 (pyroglutamyl apelin-13)

Mechanism: Pyroglutamyl modification of the native apelin-13 N-terminus. Identical receptor binding and signaling profile to apelin-13. Modestly enhanced resistance to aminopeptidase degradation through protection of the free N-terminal amine [7].

Development trajectory: [Pyr1]apelin-13 is the physiologically relevant circulating isoform and the form used in all clinical hemodynamic studies (Japp et al. 2010, Gala-Peralta et al. 2024) [8, 10]. It is not under development as a therapeutic agent but is the reference standard for APJ agonist pharmacology.

Novelty: Low. The pyroglutamyl modification is a naturally occurring post-translational event, not a medicinal chemistry innovation.

Effect size: The reference standard. All reported clinical hemodynamic effects (10 percent cardiac index increase, 12 percent SVR reduction, 4 percent MAP reduction) are [Pyr1]apelin-13 data [8, 10].

Promising potential: Low as a therapeutic candidate (requires continuous infusion). High as a pharmacological tool and clinical reference compound.

Side-effect profile: Favorable in acute infusion studies. No serious adverse events, no arrhythmia, no tachycardia [8, 10].

Overall validation: High as a pharmacological probe. The compound defines the target pharmacology and serves as the benchmark for all synthetic APJ agonists.

Comparison to apelin-13: The pyroglutamyl form is functionally equivalent to native apelin-13 with modestly improved stability. For most research applications, [Pyr1]apelin-13 is preferred over the native glutamine form.

12.2 Elabela (ELA-11, APELA, Toddler)

Mechanism: Second endogenous peptide ligand of the APJ receptor, sharing no sequence homology with apelin. Active fragments (ELA-11, ELA-16, ELA-32) bind the APJ receptor with comparable or greater potency than apelin-13 in some assays. Signaling profile includes Gi/o coupling and beta-arrestin recruitment, with some reports suggesting distinct signaling bias relative to apelin [25, 41].

Development trajectory: Elabela was identified in 2013 to 2014 as essential for cardiovascular development in zebrafish and subsequently characterized in mammalian cardiovascular physiology. Preclinical evaluation in heart failure models suggests comparable or superior cardioprotective efficacy to apelin-13. No clinical trials have been conducted with synthetic Elabela peptides. Elabela levels are downregulated in experimental heart failure and in human heart failure patients, suggesting pathophysiological relevance [25, 41].

Novelty: High. Elabela represents an entirely independent endogenous ligand system converging on the same receptor, providing unique pharmacological insights and therapeutic potential.

Effect size: Preclinical cardiovascular effects (positive inotropy, vasodilation, cardioprotection) are comparable to or greater than apelin-13 in head-to-head preclinical comparisons. No human data exist [25, 41].

Promising potential: Moderate. The dual-ligand biology is scientifically compelling, but clinical development is at an early stage. Research-grade Elabela peptides are available from multiple suppliers.

Side-effect profile: Preclinical only. No human safety data. Animal studies have not identified material toxicity.

Overall validation: Low to moderate. Strong preclinical biology but no clinical validation.

Comparison to apelin-13: Elabela provides a complementary approach to APJ activation with potential for distinct signaling bias. Apelin-13 has the advantage of established human clinical data. The two peptides may be mechanistically complementary for research applications.

12.3 MM07 (cyclic apelin analogue)

Mechanism: Macrocyclic peptide analogue of apelin-13 designed as a biased agonist that preferentially activates G protein signaling pathways while avoiding beta-arrestin-dependent receptor internalization and beta-arrestin-mediated signaling [26]. This G protein bias is hypothesized to produce sustained receptor activation without desensitization and to selectively engage the beneficial cardiovascular pathways (vasodilation, positive inotropy) while avoiding potentially detrimental beta-arrestin-mediated effects (receptor downregulation, pathological hypertrophic signaling).

Development trajectory: Developed at the University of Edinburgh (Maguire laboratory). MM07 has been evaluated in human hemodynamic studies, demonstrating vasodilation and positive inotropy consistent with APJ agonism. The biased agonist profile has been confirmed in vitro and in animal models [26].

Novelty: Moderate to high. The biased agonism concept applied to APJ represents a mechanistically sophisticated approach that may yield therapeutic advantages over balanced agonists.

Effect size: Hemodynamic effects in human studies are comparable in magnitude to [Pyr1]apelin-13. The ex vivo half-life is approximately 7-fold greater than [Pyr1]apelin-13, representing a meaningful pharmacokinetic improvement [26].

Promising potential: Moderate. The compound demonstrates proof-of-concept for biased APJ agonism with improved stability. Commercial development trajectory is unclear.

Side-effect profile: Limited human data. No serious adverse events reported in hemodynamic studies. Theoretical advantage of avoiding beta-arrestin-mediated adverse effects, though this has not been demonstrated in chronic dosing [26].

Overall validation: Moderate. Human hemodynamic data confirm APJ agonist activity with improved pharmacokinetics and signaling bias. No chronic dosing or outcome data.

Comparison to apelin-13: MM07 offers improved stability (7-fold greater half-life) and potentially more selective pharmacology through G protein bias. Apelin-13 has more extensive pharmacological characterization and a broader evidence base. MM07 is a useful comparator for investigating the contribution of beta-arrestin signaling to apelin-13 pharmacology.

12.4 AMG-986 (Amgen)

Mechanism: Orally bioavailable small-molecule APJ agonist. AMG-986 activates both G protein and beta-arrestin pathways, producing a balanced agonist profile comparable to native apelin [22]. The compound demonstrates sustained increases in cardiac output in preclinical models with superior oral bioavailability relative to peptide APJ agonists.

Development trajectory: Amgen advanced AMG-986 through Phase 1 clinical trials in healthy volunteers and in patients with heart failure. The compound was reported as safe and well tolerated, though data regarding clinical efficacy were inconsistent, and further development has not been publicly disclosed [22].

Novelty: High. As one of the first orally bioavailable small-molecule APJ agonists to reach clinical evaluation, AMG-986 represents a significant translational advance beyond peptide ligands.

Effect size: Preclinical cardiac output increases of approximately 10 to 15 percent, comparable to [Pyr1]apelin-13 infusion effects. Phase 1 human data demonstrated safety and tolerability; efficacy signals have not been publicly detailed [22].

Promising potential: Low to moderate. Phase 1 completed, but further clinical development has not been publicly announced. The compound serves as proof of concept for oral APJ agonism.

Side-effect profile: Phase 1 data indicate safety and tolerability in healthy volunteers and heart failure patients. No detailed adverse-event data have been published [22].

Overall validation: Moderate. Phase 1 safety demonstrated; efficacy not established. The compound validates the oral small-molecule APJ agonist approach.

Comparison to apelin-13: AMG-986 overcomes the fundamental pharmacokinetic limitation of apelin-13 (no oral bioavailability, short half-life) through small-molecule design. Apelin-13 has more extensive pharmacological characterization and established hemodynamic efficacy data in humans. AMG-986 is more relevant as a therapeutic development candidate; apelin-13 remains the superior research pharmacological tool.

12.5 BMS-986224 (Bristol-Myers Squibb)

Mechanism: Potent (K_d 0.3 nM) and selective small-molecule APJ agonist with a receptor binding and signaling profile closely mimicking [Pyr1]apelin-13. BMS-986224 activates Gi signaling, beta-arrestin recruitment, and ERK1/2 phosphorylation with potencies comparable to the native peptide [23].

Development trajectory: Bristol-Myers Squibb advanced BMS-986224 through preclinical evaluation and Phase 1 clinical studies. In animal models, short-term intravenous infusion increased cardiac output by 10 to 15 percent without affecting heart rate, and oral administration increased stroke volume and cardiac output to levels comparable to those observed in healthy (non-diseased) animals [23]. Clinical evaluation has been reported; further development status has not been publicly disclosed.

Novelty: High. BMS-986224 is among the most pharmacologically faithful small-molecule mimics of native apelin-13, closely reproducing the multi-pathway signaling profile.

Effect size: Preclinical cardiac output increases of 10 to 15 percent via both intravenous and oral routes. The magnitude of effect closely matches [Pyr1]apelin-13 reference data [23].

Promising potential: Low to moderate. Similar to AMG-986, Phase 1 evaluation has been reported but further development status is unclear.

Side-effect profile: Preclinical studies show acceptable safety. No material adverse events in published animal data. Human safety data have not been fully disclosed [23].

Overall validation: Moderate. Strong preclinical validation of the balanced small-molecule APJ agonist concept. Clinical validation is incomplete.

Comparison to apelin-13: BMS-986224 is the closest small-molecule pharmacological mimic of apelin-13 in the comparator panel, reproducing the multi-pathway signaling profile with oral bioavailability. Apelin-13 remains the better-characterized compound for research applications; BMS-986224 is more relevant for chronic therapeutic applications.

12.6 Comparator summary

The five compounds occupy complementary positions in the APJ receptor agonist landscape. **[Pyr1]apelin-13** is the physiological reference standard with established human hemodynamic data but limited by the requirement for continuous infusion. **Elabela** is a mechanistically distinct second endogenous ligand with compelling preclinical biology but no clinical data. **MM07** introduces biased agonism and improved stability as a peptide-based approach with initial human data. **AMG-986** and **BMS-986224** represent the small-molecule frontier with oral bioavailability and Phase 1 clinical evaluation, though neither has announced progression to efficacy trials.

Native apelin-13 occupies the foundational position in this landscape: it is the endogenous ligand that defines the target pharmacology, the reference standard against which all synthetic agonists are benchmarked, and the compound with the most extensive pharmacological characterization across cardiovascular, renal, metabolic, and neurological domains. Its limitations (short half-life, no oral bioavailability, requirement for continuous infusion) are well defined and have motivated the development of every compound in the comparator panel. For research applications in mechanism elucidation, receptor pharmacology, and acute hemodynamic assessment, apelin-13 and [Pyr1]apelin-13 remain the preferred tools. For chronic therapeutic applications, the stabilized analogues and small-molecule agonists represent the translational pathway.

13. References

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