ChatGPT

Comprehensive summary of PubMed-indexed studies involving the AMPK activator R419

R419 is a synthetic small-molecule that indirectly activates AMP-activated protein kinase (AMPK) by inhibiting mitochondrial complex I. The compound was originally identified through structure–activity optimization of pyridine dicarboxamides and has been evaluated in multiple biological contexts ranging from metabolic regulation to cancer and pain. The summaries below cover all PubMed-indexed articles and reviews that are dedicated to or make explicit mention of R419. Citations point to lines in the referenced articles.

Key studies where R419 is the primary experimental compound

2013 – AMPK activation through mitochondrial regulation results in increased substrate oxidation and improved metabolic parameters in models of diabetes (PLOS One)

- **Objective** – To determine whether inhibiting mitochondrial complex I with R419 can activate AMPK and improve metabolic parameters in vitro and in diabetic mice.
- **Methods** – R419's potency at complex I inhibition and AMPK activation was compared with metformin. The researchers tested glucose uptake in C2C12 myotubes, hepatic glucose production, fatty-acid oxidation and lipogenesis in hepatocytes, and metabolic fluxes in db/db mice using tracer analysis.
- **Key findings**:
- R419 inhibited complex I with high potency (IC50 ≈ 100 nM) and activated AMPK at lower concentrations than metformin [1].
- In cultured myocytes, R419 increased glucose uptake through an AMPK-dependent mechanism [1].
- In hepatocytes, R419 suppressed glucose production and increased fatty-acid oxidation; these effects were partly AMPK-independent [1].
- Treatment of db/db mice upregulated genes involved in fatty-acid oxidation and branched-chain-amino-acid catabolism; carbon-13 tracer analysis indicated increased oxidation of glucose and palmitate [1].
- **Significance** – This study introduced R419 as a potent complex I inhibitor that activates AMPK and modulates nutrient metabolism. It provided proof-of-concept that mitochondrial complex I inhibition can improve metabolic parameters in diabetic models.

2015 – The AMPK activator R419 improves exercise capacity and skeletal-muscle insulin sensitivity in obese mice (Molecular Metabolism)

- **Objective** – To evaluate the effects of R419 on whole-body exercise capacity and insulin sensitivity in obese, insulin-resistant mice.

- **Methods** – Obese mice with diet-induced insulin resistance were treated orally with R419. Insulin tolerance tests, treadmill running tests, and biochemical analyses of skeletal muscle were performed. Wild-type and muscle-specific AMPK knockout (AMPK-MKO) mice were compared [2].
- **Key findings**:
- R419 improved insulin tolerance, increased glucose disposal and GLUT4 content in skeletal muscle without altering body mass [2].
- The compound enhanced treadmill running capacity and increased mitochondrial electron-transport chain content in wild-type mice, but not in AMPK-MKO mice [2].
- Improvement in insulin sensitivity occurred independently of skeletal-muscle AMPK, whereas improved exercise capacity required muscle AMPK [2].
- **Significance** – R419 was shown to improve skeletal-muscle insulin sensitivity and exercise performance. The study demonstrated a differential role for muscle AMPK in the metabolic versus exercise effects of R419 and highlighted the therapeutic potential of indirect AMPK activators.

2016 – The potent indirect AMPK activator R419 attenuates mitogen-activated protein kinase signalling, inhibits nociceptor excitability and reduces pain hypersensitivity in mice (Pain Reports)

- **Objective** – To test whether R419 can activate AMPK in sensory neurons and attenuate pain signalling.
- **Methods** – Dorsal-root-ganglion (DRG) neurons were treated with R419. The researchers measured AMPK activation, mitogen-activated protein kinase (MAPK) signalling, protein synthesis and neuronal excitability. In vivo, the compound was administered locally in mouse models of nerve-growth-factor-induced pain and postoperative pain [3].
- **Key findings**:
- R419 activated AMPK in sensory neurons, reduced phosphorylation of MAPKs (ERK, p38 and JNK), inhibited nascent protein synthesis and decreased DRG neuron excitability [3].
- The compound suppressed nerve-growth-factor-induced increases in neuronal excitability and mechanical pain amplification [3].
- Local application of R419 prevented postsurgical pain and blocked hyperalgesic priming, indicating long-lasting analgesic effects [3].
- **Significance** – These results identified R419 as a potent analgesic candidate that suppresses MAPK signalling and nociceptor excitability via AMPK activation. The study broadened the therapeutic scope of AMPK activators into pain management.

2018 – Synthesis and biological evaluation of complex I inhibitor R419 and its derivatives as anticancer agents in HepG2 cells (Bioorganic & Medicinal Chemistry Letters)

- **Objective** – To synthesise R419 derivatives and evaluate their anticancer activity against human hepatocellular carcinoma (HepG2) cells.
- **Methods** – A series of derivatives were prepared by modifying the piperazine and pyridine rings of R419. Cytotoxicity (IC50 values), AMPK activation, ATP depletion and apoptosis indicators were assessed [4].
- **Key findings**:
- R419 itself exhibited significant anticancer activity against HepG2 cells (IC50 ≈ 5.2 ± 0.9 μM) [4].

- Nine derivatives retained anticancer activity; derivative H20 was most potent and induced marked ATP depletion, AMPK activation, decreased Bcl-2/Bax ratio and necrotic cell death [4].
- H20 also exhibited strong complex-I inhibitory activity [4].
- **Significance** – This study demonstrated that R419 and analogues have anticancer potential via complex-I inhibition and AMPK activation. It also provided insights into structure–activity relationships for improving potency and reducing off-target effects.

2021 – AMPK mediates energetic stress-induced liver GDF15 (FASEB Journal)

- **Objective** – To investigate whether energetic stress and AMPK activation induce expression of growth-differentiation factor 15 (GDF15), a stress response cytokine implicated in appetite regulation.
- **Methods** – Wild-type and AMPKβ1-null mice were treated with R419, the AMP mimetic AICAR, or the direct AMPK activator A769662. Gene expression, endoplasmic-reticulum (ER) stress markers and food intake were assessed [5].
- **Key findings**:
- R419 and AICAR induced ER and energetic stress in liver tissue, increased GDF15 mRNA and protein levels and suppressed food intake [5].
- The direct AMPK activator A769662 increased GDF15 independently of ER stress, highlighting differences between direct and indirect activators [5].
- In AMPKβ1-null mice, R419's induction of GDF15 was attenuated, indicating AMPKβ1 involvement [5].
- **Significance** – The study linked energetic stress and AMPK activation to hepatic GDF15 induction and appetite suppression. It showed that R419's metabolic effects extend to endocrine regulation of feeding.

2022 – Structure–activity relationships leading to the identification of the indirect activator of AMPK, R419 (Bioorganic & Medicinal Chemistry)

- **Objective** – To detail the medicinal-chemistry programme that led to R419 and to optimise its pharmacokinetic and safety profile.
- **Methods** – Researchers synthesized and evaluated a series of pyridine dicarboxamide analogues. Microsomal stability, AMPK activity, hERG channel inhibition and in vivo efficacy were assessed [6].
- **Key findings**:
- Substitutions on the piperazine and pyridine rings improved microsomal stability and reduced hERG inhibition while maintaining AMPK activation [6].
- The optimized compound R419 retained potent on-target activity, demonstrated oral bioavailability and activated AMPK in vivo, improving metabolic parameters in db/db and diet-induced obesity mouse models [6].
- **Significance** – This medicinal chemistry paper clarified how R419 was discovered and optimized. It emphasized balancing potency with safety and pharmacokinetic properties to produce a viable preclinical candidate.

2021 – Brain-permeable AMPK activator R481 raises glycaemia by autonomic nervous-system activation and amplifies the counter-regulatory response to hypoglycaemia in rats (Frontiers in Endocrinology)

- **Objective** – To compare the systemic effects of a brain-permeable AMPK activator (R481) with those of R419, which is largely excluded from the brain.
- **Key findings**:
- In vitro, both R481 and R419 increased AMPK activity in neuronal cell lines [7].
- In rats, R481 crossed the blood–brain barrier, raised blood glucose and amplified counter-regulatory responses to hypoglycaemia, whereas R419 did not alter glucose excursion [7].
- **Significance** – This study highlighted that brain permeability is critical for certain systemic effects of AMPK activators. R419 served as a negative control, underscoring that its metabolic actions occur largely outside the central nervous system.

Additional scientific articles that reference R419

These works are not dedicated studies of R419 but cite it as an example of an AMPK activator or complex I inhibitor.

2019–2024 reviews and studies on AMPK or mitochondrial inhibitors

- **Natural dihydrophenanthrene plant compounds study (2022 J Biol Chem)** – This article characterizes plant-derived compounds that activate AMPK through the allosteric drug-and-metabolite binding site. In its introduction, the authors cite R419 as an AMPK activator known to improve exercise capacity and insulin sensitivity in obese mice [8]. There is no experimental use of R419 in the paper.
- **Comprehensive review of benzoylpiperidine fragments (2024 Molecules)** – This medicinal-chemistry review lists R419 as the indirect AMPK activator resulting from optimisation of pyridine diamides and cites the 2018 HepG2 anticancer study and the 2022 medicinal-chemistry optimisation paper. It does not present new data on R419.
- **Optimization of pharmacokinetic and in vitro safety profile of pyridine diamide indirect AMPK activators (2023 J Med Chem)** – This article, which is pay-walled, reports further modifications of the R419 scaffold to improve pharmacokinetic properties and reduce off-target effects. It cites the 2022 SAR paper as precedent and is expected to describe analogues of R419; however, specific experimental details were unavailable at the time of writing.
- **Novel benzimidazole and indole AMPK activators (2022–2023 Bioorg Med Chem Lett)** – These studies report new direct AMPK activators and cite R419 as a potent indirect activator for comparison. They do not perform experiments with R419.
- **Natural phenanthrene compounds, exercise reviews and other AMPK-focused articles (2022–2024)** – Multiple reviews on AMPK function, exercise physiology, or metabolic regulation cite the 2015 R419 study when listing small-molecule activators. These references highlight R419 as an example of a mitochondria-targeting AMPK agonist but do not provide new experimental data.

2022 Frontiers in Oncology article on NDUFC1 knockdown in hepatocellular carcinoma

- This study investigated the role of NDUFC1, a complex I subunit, in hepatocellular carcinoma. In the discussion, the authors referenced the 2018 Bioorg Med Chem Lett paper on R419 and its derivatives

as part of the literature on complex I inhibitors [9]. R419 was not studied experimentally in this work.

2019–2023 conference abstracts and posters on pain

- Conference abstracts in *The Journal of Pain* (e.g., "The mitochondrial complex-I inhibitor R419 potently stimulates AMPK in dorsal root ganglion neurons and reduces incision-evoked pain in vivo") echo findings from the 2016 Pain Reports study but do not report additional data. These abstracts confirm and publicize the analgesic potential of R419.

Additional contexts where the term R419 is used

- **VHH R419 antibody** – Outside the AMPK field, "R419" refers to a camelid single-domain antibody whose crystal structure is deposited in the RCSB PDB. This antibody is unrelated to the small-molecule AMPK activator and should not be confused with it.
- **Sample identifiers and page numbers** – Some articles and research protocols include "R419" as part of a figure label, page number or sample identifier. These instances have no connection to the AMPK activator and are not summarized here.

Summary and outlook

R419 emerged from structure–activity optimisation of pyridine dicarboxamides as a potent mitochondrial complex I inhibitor and indirect AMPK activator. Preclinical studies demonstrate that R419 improves insulin sensitivity, enhances exercise capacity via muscle AMPK, stimulates substrate oxidation, suppresses hepatic glucose production and reduces pain hypersensitivity. Derivatives such as H20 exhibit anticancer activity via energy depletion and AMPK activation. R419 also induces GDF15 expression under energetic stress and has been used as a negative control for brain-permeable AMPK activators. Medicinal chemistry efforts have refined R419's pharmacokinetic and safety profiles, making it a promising preclinical tool for investigating metabolic diseases, cancer and pain. Ongoing research into analogues and related scaffolds continues to build upon the foundational insights provided by these studies.

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[1] AMPK activation through mitochondrial regulation results in increased substrate oxidation and improved metabolic parameters in models of diabetes - PubMed
https://pubmed.ncbi.nlm.nih.gov/24339975/

[2] The AMPK activator R419 improves exercise capacity and skeletal muscle insulin sensitivity in obese mice - PMC
https://pmc.ncbi.nlm.nih.gov/articles/PMC4563030/

[3] The potent, indirect adenosine monophosphate-activated protein kinase activator R419 attenuates mitogen-activated protein kinase signaling, inhibits nociceptor excitability, and reduces pain hypersensitivity in mice - PMC
https://pmc.ncbi.nlm.nih.gov/articles/PMC5034875/

[4] Synthesis and biological evaluation of Complex I inhibitor R419 and its derivatives as anticancer agents in HepG2 cells - PubMed
https://pubmed.ncbi.nlm.nih.gov/30001917/

5 AMPK mediates energetic stress-induced liver GDF15 - PubMed
https://pubmed.ncbi.nlm.nih.gov/33337559/

6 Structure activity relationships leading to the identification of the indirect activator of AMPK, R419 - PubMed
https://pubmed.ncbi.nlm.nih.gov/35973281/

7 Brain Permeable AMP-Activated Protein Kinase Activator R481 Raises Glycaemia by Autonomic Nervous System Activation and Amplifies the Counterregulatory Response to Hypoglycaemia in Rats - PubMed
https://pubmed.ncbi.nlm.nih.gov/34975743/

8 Natural (dihydro)phenanthrene plant compounds are direct activators of AMPK through its allosteric drug and metabolite–binding site - PMC
https://pmc.ncbi.nlm.nih.gov/articles/PMC9108889/

9 Knockdown of NDUFC1 inhibits cell proliferation, migration, and invasion of hepatocellular carcinoma - PMC
https://pmc.ncbi.nlm.nih.gov/articles/PMC9479186/