Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

VBIT-4

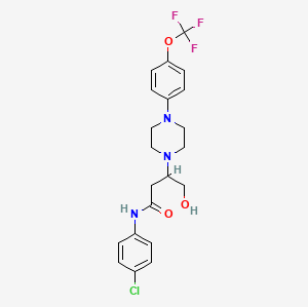
Evidence Summary

VDAC1 is overexpressed in AD, T2DM, myocardial infarction, cancer, and other diseases. VBIT-4 has shown therapeutic benefit in numerous preclinical models, but no studies have tested it in humans.

Neuroprotective Benefit: VDAC1 is overexpressed in the brains of Alzheimer's patients. Preclinical studies showed VBIT-4 preserved cognitive function and decreased neuronal loss, apoptosis, and neuroinflammation. VBIT-4 has not been tested in humans.

Aging and related health concerns: VDAC1 is overexpressed in patients with diabetes, myocardial infarction, cancer, and others. VBIT-4 has shown therapeutic benefit in some rodent models. No data exist in humans.

Safety: VBIT-4 treatment has been well-tolerated in rodents. No studies have tested the safety of VBIT-4 in humans.

Availability: available as research grade	**Dose**: not established	**Chemical formula:** $C_{21}H_{23}ClF_3N_3O_3$ **MW**: 457.9  Source: PubChem
Half-life: not documented in humans	**BBB**: penetrant in mice	
Clinical trials: No clinical trials of VBIT-4 have been registered or completed.	**Observational studies**: N/A	

What is it?

VBIT-4 is a small molecule that inhibits the oligomerization of VDAC1 (voltage-dependent anion channel-1) and prevents apoptosis and mitochondrial dysfunction (Verma et al., 2022). High-throughput screening and medicinal chemistry were employed to develop VBIT-4 and related compounds (VBIT-12, VBIT-3) that directly interacted with VDAC1 and prevented VDAC1 oligomerization, apoptosis, and mitochondrial dysfunction (Ben-Hail et al., 2016).

VDAC1 is a protein expressed primarily in the outer mitochondrial membrane and serves as a mitochondrial gatekeeper for the passages of metabolites (e.g., pyruvate, malate, succinate, NADH/NAD+, and others), ions (e.g., calcium), fatty acids, cholesterol, heme, and nucleotides (reviewed in Shoshan-Barmatz et al., 2018; Shoshan-Barmatz et al., 2020). VDAC1 regulates energy production, metabolism, reactive oxygen species production, lipid oxidation, and mitochondria-mediated apoptosis. VDAC1 represents a hub protein that interacts with over 150 other proteins including Aβ and phosphorylated tau. Under stress or in the presence of apoptosis inducers (including Aβ), VDAC1 is overexpressed and oligomerizes, forming a large channel that enables the release of pro-apoptotic proteins (e.g., cytochrome c, AIF) to the cytosol, and interacts with apoptosis-regulating proteins, Bcl-2, Bcl-xL, and hexokinase. VDAC1 oligomers can also allow the release of mtDNA, which triggers type-I interferon responses (Verma et al., 2022; Shoshan-Barmatz et al., 2020). VDAC1 also plays a role in endoplasmic reticulum (ER)-mitochondria cross-talk, regulation of autophagy, and inflammation (reviewed in Shoshan-Barmatz et al., 2020).

Mitochondrial dysfunction is an early event in many neurodegenerative diseases, as well as in metabolic and inflammatory conditions. VBIT-4 has been tested in preclinical models of Alzheimer's disease (Verma et al., 2022), amyotrophic lateral sclerosis (ALS)(Shteinfer-Kuzmine et al., 2022), diabetes (Zhang et al., 2019), surgical pain (Wei et al., 2023), ulcerative colitis (Verma et al., 2022), systemic lupus erythematosus (Kim et al., 2019), and others.

Neuroprotective Benefit: VDAC1 is overexpressed in the brains of Alzheimer's patients. Preclinical studies showed VBIT-4 preserved cognitive function and decreased neuronal loss, apoptosis, and neuroinflammation. VBIT-4 has not been tested in humans.

Types of evidence:

- 0 clinical trials
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

No studies have tested whether VBIT-4 prevents dementia or age-related cognitive decline in humans.

Human research to suggest benefits to patients with dementia:

No studies have tested VBIT-4 in human patients with dementia.

In postmortem frontal cortex specimens of people who had Alzheimer's disease, VDAC1 protein levels (measured by immunoblotting and quantitative densitometry) were significantly increased compared to specimens from controls (Manczak et al., 2012). Five samples each were available from Alzheimer's disease Braak stages I/II, Braak stages III/IV, Braak stages V/VI, and age-matched controls. VDAC1 levels appear to progressively increase as Alzheimer's disease progresses. Based on co-immunoprecipitation and immunofluorescence studies, VDAC1 interacted with Aβ and phosphorylated tau in the frontal cortex of Alzheimer's patients.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Mitochondrial dysfunction is an early event in many neurodegenerative diseases and results in reduced metabolism, disruption of calcium homeostasis, increased oxidative reactive oxygen species, increased lipid peroxidation, and apoptosis (reviewed in Shoshan-Barmatz et al., 2018).

In a mouse model of Alzheimer's disease (5xFAD mice, 3 months old with amyloid pathology but before cognitive impairment), treatment with VBIT-4 (20 mg/kg, twice a week in drinking water) for 5 months prevented the impairment in radial arm water maze performance (measured by number of errors and time to reach platform) seen in 5xFAD mice treated with vehicle (Verma et al., 2022). The VBIT-4-treated 5xFAD mice performed comparably to wild-type mice in both error numbers and time to reach the platform. In the open field habituation test, the 5xFAD mice treated with VBIT-4 for 5 months performed similarly to the wild-type mice. In the T-maze test, which assesses spatial long-term memory, 5xFAD mice treated with VBIT-4 for 5 months performed similarly to wild-type mice, while 5xFAD mice treated with vehicle made significantly fewer entries to the correct arm. Similarly, 5xFAD mice treated with VBIT-4 for 5 months had preserved performance on the Y-maze test comparable to that of wild-type mice, while 5xFAD mice treated with vehicle had significantly fewer correct triads.

In 5xFAD mouse brain, VDAC1 was overexpressed in neurons surrounding Aβ plaques, and this was associated with neuronal cell death (Verma et al., 2022). VBIT-4 treatment protected against neuronal loss (measured by class III beta-tubulin staining) and reduced the area occupied by Aβ plaques by 20% in 5xFAD mice compared to vehicle-treated controls. However, VBIT-4 treatment did not have any effect on p-tau levels in 5xFAD mice. In 5xFAD mice, expression of synaptic proteins (synaptophysin and PSD-95) in the cortex and hippocampus was reduced by 60-70%, but VBIT-4 treatment completely prevented this decrease such that expression was comparable to wild-type mice.

In 5xFAD mice, apoptotic cells (measured by TUNEL-stained cells) were increased by 3-fold compared to wild-type mice, but VBIT-4 treatment for 5 months significantly reduced apoptosis (Verma et al., 2022). Also in 5xFAD mice, activated caspase-3 levels (apoptotic marker) were increased by 2.5-fold in both the cortex and hippocampus, but levels were greatly reduced in VBIT-4-treated 5xFAD mice. Levels of p53, which regulates apoptosis and senescence, was higher in the cortex and hippocampus of 5xFAD mice compared to wild-type mice, but VBIT-4 treatment in 5xFAD mice significantly reduced p53 levels to those comparable to wild-type mice.

In 5xFAD mice, astrocyte morphology is altered, while VBIT-4 treatment increased processes with greater surface area, more branches, and more branching points in astrocytes (Verma et al., 2022). Also in 5×FAD mice, microglia had short and thick processes with an amoeboid-shape, whereas VBIT-4 treatment resulted in significantly larger microglia with more and longer processes.

In 5xFAD mice, a marker of neuroinflammation, p-NFκB-p65, was significantly increased compared to wild-type mice (Verma et al., 2022). Its mRNA level was dramatically increased by ~150-fold in 5xFAD mouse brain, but VBIT-4 treatment prevented this increase in both the cortex and hippocampus to a level comparable to that in wild-type mice. The levels of cytokines IL-1β and TNF-α, regulated by NFκB, were also significantly increased in 5xFAD mice, while VBIT-4 treatment prevented this increase. IL-1β mRNA expression in the 5×FAD mouse cortex was about 70-fold higher than that in wild-type mice, and VBIT-4 treatment greatly reduced this level. The NLRP3 inflammasome is critical for the innate immune system and is associated with neuroinflammation in Alzheimer's disease. In the cortex and hippocampus of 5xFAD mice, NLRP3 was highly expressed, while VBIT-4 treatment prevented the increase. Caspase-1 is activated in the NLRP3 inflammasome and turns proinflammatory cytokines into active forms. Caspase-1 expression was increased in the cortex and hippocampus of 5xFAD mice, but not in VBIT-4-treated mice.

In a different mouse model of Alzheimer's disease (APP/PS1, 8 months old), VDAC1 was significantly upregulated, but treatment with VBIT-4 (25 mg/kg, i.p.) for 4 weeks resulted in decreased VDAC1 expression (Zhou et al., 2023). APP/PS1 mice exhibited impaired performance on the Morris water maze (measured by longer latency to find the platform), but VBIT-4 treatment significantly reduced the latency to find the platform.

Amyotrophic lateral sclerosis (ALS) can be caused by mutations in the superoxide dismutase (SOD1). SOD1 mutants can interact with VDAC1 and affect its normal function (Shteinfer-Kuzmine et al., 2022). In rodent models of ALS (SOD1G93A mice and rats), VDAC1 oligomerization is increased in the spinal cord mitochondria. In SOD1G93A mice, treatment with a VDAC1 oligomerization inhibitor, VBIT-12 (20 mg/kg/day in drinking water), showed some benefit on muscle endurance (measured by grip strength in the hindlimbs), even though it did not significantly delay disease onset or improve survival.

In SOD1G93A mice treated with VBIT-12 administered intraperitoneally starting at 60 days and continued every other day, there was a trend for a delay in disease onset and longer survival, though this was not statistically significant (Shteinfer-Kuzmine et al., 2022). In these same mice, VBIT-12 treatment (i.p.) preserved their limb muscle strength for a significantly longer period of time. VBIT-12-

treated SOD1G93A mice showed a significantly greater pulling strength from their forelimbs and hindlimbs during disease progression compared to the vehicle-treated group.

In motor-neuron-like NSC-34 cells transfected with mutant SOD1 (SOD1G93A), cell survival was reduced by 25-30% compared to the survival of cells transfected with normal SOD1, but incubation with VBIT-12 partially prevented the cell death (Shteinfer-Kuzmine et al., 2022).

In iPSC-derived motor neurons from ALS patients carrying mutations in TDP-43, VBIT-4 administration prevented the cytosolic accumulation of mtDNA and inflammation (Yu et al., 2020).

APOE4 interactions: Unknown

Aging and related health concerns: VDAC1 is overexpressed in patients with diabetes, myocardial infarction, cancer, and others. VBIT-4 has shown therapeutic benefit in some rodent models. No data exist in humans.

Types of evidence:

- 0 clinical trials
- Numerous laboratory studies

Type 2 diabetes: POTENTIAL BENEFIT BASED ON PRECLINICAL STUDIES

In type 2 diabetes, VDAC1 is overexpressed and is mistargeted to the plasma membrane in human insulin-secreting islet β-cells (Zhang et al., 2019; Shoshan-Barmatz et al., 2020).

In pooled islets from 5 type 2 diabetes donors and one with impaired glucose tolerance, administration of VBIT-4 for 1 hour increased islet ATP content by 5-fold in the presence of 1 and 16.7 mM glucose (Zhang et al., 2019). The islets from type 2 diabetes patients show severe blunting of glucose-stimulated insulin secretion, but this is increased nearly 4-fold in parallel with improved ATP generation with the administration of VBIT-4 (or anti-VDAC antibodies or metformin).

In a mouse model of diabetes (db/db mice), VBIT-4 treatment (25 mg/kg/day, i.p.) started at 42 days-old and continued for 5 weeks prevented hyperglycemia and maintained normal glucose tolerance and physiological regulation of insulin secretion (Zhang et al., 2019). Insulin sensitivity was also improved in

the VBIT-4-treated db/db mice, as measured by HOMA-IR. Upon VBIT-4 cessation, blood glucose concentrations increased gradually, reaching those of vehicle-treated mice. VBIT-4 treatment did not affect body weight. In older db/db mice (14-week-old) with established hyperglycemia, VBIT-4 treatment only marginally lowered blood glucose.

In cells grown in high-glucose media (30 mM) for 36 hours, VDAC1 mRNA increased in primary lung endothelial cells and decreased in human skin fibroblasts (Belosludtsev et al., 2023). In lung endothelial cells grown in high-glucose media, administration of VBIT-4 (5 µM) countered the increase in reactive oxygen species (H2O2) and mitochondrial permeability transition pore opening. In endothelial cells, a hyperglycemic condition resulted in a significant decrease in the mitochondrial membrane potential. In normoglycemic conditions, VBIT-4 (5 µM) administration alone reduced mitochondrial membrane potential in endothelial cells. However, VBIT-4 (5 µM) administration did not affect mitochondrial membrane potential under hyperglycemic conditions.

Cardiovascular disease: MIXED BENEFIT IN RAT MODEL OF MYOCARDIAL INFARCTION

In an immunohistochemical analysis, human cardiac tissues from post-myocardial infarction overexpress VDAC1 compared to tissue from non-infarcted individuals (Klapper-Goldstein et al., 2020). Tissues from short-term and long-term myocardial infarction showed a 7-fold and 25-fold higher VDAC1 expression compared to non-infarcted tissue. VDAC1 overexpression is also seen in heart tissue from patients with chronic ventricular dilation/dysfunction.

In a rat model of myocardial infarction (induced by excessive aldosterone), VDAC1 expression was markedly increased in both ventricular and atrial tissues, while VBIT-4 treatment (25-30 mg/kg/day in drinking water) attenuated the atrial fibrotic load (Klapper-Goldstein et al., 2020). However, VBIT-4 treatment did not have a significant effect on the susceptibility to atrial fibrillation episodes induced by burst pacing, or the expression levels of VDAC1 or cytochrome c. A numeric tendency of reduction in apoptotic staining was also observed but the results were not statistically significant.

Cancer: POTENTIAL BENEFIT BASED ON IN VITRO STUDIES

VDAC1 is overexpressed in various tumors obtained from patients as well as in animal models of cancer (reviewed in Shoshan-Barmatz et al., 2020). The excess VDAC1 contributes to cancer cell metabolism by facilitating the passage of essential metabolites and ATP.

In breast adenocarcinoma (MCF-7) culture, a very high concentration (30 µM) of VBIT-4 for 48 hours led to a decrease in mitochondrial membrane potential, an increase in reactive oxygen species (ROS) production (e.g., H202), an increase in ROS-positive cells, and an increase in cell death (Belosludtsev et al., 2024). A lower concentration of VBIT-4 (5 µM) did not affect mitochondrial membrane potential or the number of ROS-positive cells.

Pain: POTENTIAL BENEFIT IN A MOUSE MODEL OF POST-SURGICAL PAIN

In a mouse model of chronic pain (induced by 6 hours of sleep deprivation before and after skin/muscle surgery), VBIT-4 treatment (50 µg/10µL injected intrathecally) reduced microglial activation and duration of mechanical pain, measured by ipsilateral paw withdrawal threshold and latency test, compared to vehicle-treated controls (Wei et al., 2023). There were no effects of VBIT-4 treatment on contralateral mechanical pain. During the pain maintenance phase, VDAC1 protein expression and oligomerization were upregulated in the spinal cord of these mice, which promoted ATP release and expression of microglial activation-related inflammatory factors (IL-1β and CCL2). In an *in vitro* study, LPS (which triggers innate immunity and inflammation) induced microglial activation, VDAC1 expression and oligomerization, ATP release, and IL-1β and CCL2 expression, but administration of VBIT-4 partly reversed microglial ATP release and IL-1β and CCL2 expression.

Inflammatory diseases: POTENTIAL BENEFIT IN MOUSE MODELS OF ULCERATIVE COLITIS

Mitochondrial dysfunction has been implicated as a trigger of inflammatory bowel diseases including Crohn's disease and ulcerative colitis. VDAC1 is overexpressed by 6- to 8-fold in the colon of people with Crohn's disease, ulcerative colitis, and chronic colitis, compared to healthy donors (Verma et al., 2022).

In a mouse model of ulcerative colitis (induced by DSS), treatment with a VDAC1 oligomerization inhibitor, VBIT-12 (in drinking water), suppressed weight loss, diarrhea, rectal bleeding, pro-inflammatory cytokine production, crypt and epithelial cell damage, and focal inflammation (Verma et al., 2022). VBIT-12 administration also inhibited the infiltration of inflammatory cells, apoptosis, mtDNA release, and activation of caspase-1 and NLRP3 inflammasome. DSS-induced reduction in colon length was by 45%, but with VBIT-12 treatment (started on day 2), the reduction in colon length was 22%. DSS-induced body weight reduction was by 32% on day 10, but with VBIT-12 treatment, the reduction was 10%. In this mouse model, VBIT-12 was more effective than VBIT-4 in protecting against DSS-induced colon pathology. In a different mouse model of ulcerative colitis (induced by TNBS), VBIT-12 treatment (started on day 1 or 2 post-TNBS treatment) also prevented body weight loss. In control mice not

exposed to DSS or TNBS, VBIT-12 administration for 7 days had no effect on colon length, body weight, or colon morphology.

Autoimmune diseases: POTENTIAL BENEFIT IN A MOUSE MODEL OF SLE

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by β cell hyperactivity, abnormally activated T cells, and defects in the clearance of apoptotic cells. In a mouse model of SLE (MpJ-Faslpr mice), VBIT-4 treatment blocked the development of skin lesions and the thickening of the epidermis that accompanies leukocyte infiltration, suppressed facial and dorsal alopecia, and decreased spleen and lymph node weights, without affecting mortality or body weight (Kim et al., 2019). VBIT-4 treatment also decreased cell-free mtDNA, type-I interferon signaling, and neutrophil extracellular traps.

Safety: VBIT-4 treatment has been well-tolerated in rodents. No studies have tested the safety of VBIT-4 in humans.

Types of evidence:

- 0 clinical trials
- Several laboratory studies

In mice that were exposed to VBIT-4 for 4 months, there were no signs of toxicity, reflected by body weight, behavior, organ histochemistry, and others (Ben-Hail et al., 2016). Detailed results were not shown in the publication. The authors also noted that VBIT-4 had no effect on cell growth and viability, though the precise conditions and doses were not discussed in detail.

In mice, VBIT-4 showed an elimination half-life of 7.6 hours (Verma et al., 2022). In rats, a single-dose toxicity study for VBIT-4 showed no treatment-related mortality or clinical signs, and no significant changes in hematology or in serum chemistry parameters.

In a rat model of myocardial infarction (induced by excessive aldosterone), VBIT-4 treatment (25-30 mg/kg/day in drinking water) was well-tolerated, with the final body weight being similar to the control group (Klapper-Goldstein et al., 2020). VBIT-4 treatment did not significantly affect cardiac echocardiographic parameters.

A cell culture study examined the effect of VBIT-4 on healthy cells (Belosludtsev et al., 2024). Very high concentrations of VBIT-4 (15-30 µM) suppressed mitochondrial respiration in state 3 and 3UDNP, increased H2O2 production in mitochondria, and decreased the Ca2+ retention capacity, but increased the time of Ca2+-dependent mitochondrial swelling. In rat liver mitochondria, the highest VBIT-4 concentration (30 µM) significantly reduced complex I activity by 25 %, complex III activity by 50%, and complex IV activity by 29%, but there was no change in complex II activity. VBIT-4 also induced depolarization of organelles fueled by substrates of complex I (but not complex II) of the respiratory chain. In a molecular docking study, VBIT-4 interacted with the rotenone-binding site on complex I with similar affinity. It is worth noting that at a lower concentration (5 µM), VBIT-4 does not inhibit mitochondrial respiration and stimulates respiration in state 3 upon oxidation of complex I substrates.

Drug interactions: Drug interactions with VBIT-4 have not been documented. Theoretically, VBIT-4 may interact with other drugs that target VDAC1, such as metformin.

Sources and dosing:

VBIT-4 has been studied in various rodent models. No studies have been carried out in humans, so there is no dosage information for humans. In a mouse model of Alzheimer's disease (5xFAD mice), the dose of VBIT-4 that prevented cognitive impairment was 20 mg/kg, twice a week in drinking water (Verma et al., 2022).

Research underway:

Based on ClinicalTrials.gov, there are no ongoing clinical trials testing VBIT-4.

Search terms:

Pubmed, Google: VBIT-4

Websites visited for VBIT-4:

- Clinicaltrials.gov (0)
- NIH RePORTER (0)
- DrugAge (0)
- Geroprotectors (0)
- PubChem

- DrugBank.ca (0)
- Cafepharma (0)
- Pharmapro.com (0)

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