Bridging Type 2 Diabetes and Alzheimer's Disease: Assembling the Puzzle Pieces in the Quest for the Molecules With Therapeutic and Preventive Potential

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Abstract: Type 2 diabetes (T2D) and Alzheimer's disease (AD) are two age-related amyloid diseases that affect millions of people worldwide. Broadly supported by epidemiological data, the higher incidence of AD among type 2 diabetic patients led to the recognition of T2D as a tangible risk factor for the development of AD. Indeed, there is now growing evidence on brain structural and functional abnormalities arising from brain insulin resistance and deficiency, ultimately highlighting the need for new approaches capable of preventing the development of AD in type 2 diabetic patients. This review provides an update on overlapping pathophysiological mechanisms and pathways in T2D and AD, such as amyloidogenic events, oxidative stress, endothelial dysfunction, aberrant enzymatic activity, and even shared genetic background. These events will be presented as puzzle pieces put together, thus establishing potential therapeutic targets for drug discovery and development against T2D and diabetes-induced cognitive decline—a heavyweight contributor to the increasing incidence of dementia in developed countries. Hoping to pave the way in this direction, we will present some of the most promising and well-studied drug leads with potential against both pathologies, including their respective bioactivity reports, mechanisms of action, and structure–activity relationships. Med. Res. Rev., 38, No. 1, 261–324, 2018

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1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia resulting from deficient insulin production, action, or both. According to the International Diabetes Federation, type 2 diabetes (T2D), the so-called noninsulin-dependent diabetes, accounts for at least 90% of all cases of diabetes, affecting approximately 415 million people around the world.[1] Due to increasing rates of obesity, unhealthy diets, physical inactivity, and average life expectancy in developed countries, this number is expected to rise to 642 million in 2040.[1] Untreated or uncontrolled T2D is associated with macrovascular, microvascular, and neuropathic complications, and is the leading cause of cardiovascular disease, kidney failure, blindness, and low limb amputation.[1]

In contrast to type 1 diabetes (T1D), where little or no insulin is produced by the pancreas due to an autoimmune-mediated destruction of β-cells, T2D primarily arises as a result of hyperinsulinemia accompanied by impaired glucose tolerance, which is believed to be an outcome of compensatory insulin signaling dysfunction in peripheral tissues.[2,3] Peripheral insulin resistance is in turn associated with compensatory insulin hypersecretion[4] and decreased hepatic insulin clearance,[5] leading to a pathological vicious cycle of high blood glucose and insulin levels. In advanced disease stages, type 2 diabetic patients also present with reductions in β-cell mass and function as a consequence of massive and unregulated insulin production, eventually leading to insufficient pancreatic insulin secretion that may require the therapeutic use of exogenous insulin or insulin analogues in order to control blood sugar levels of these patients.[6,7]

Even though the mechanistic causes of insulin resistance are not fully established yet, the consequences are even more wide ranging than first predicted by Professor Wilhelm Falta, in 1931.[8] Indeed, insulin resistance in the hippocampus has been more and more supported by evidence as a key mechanistic mediator of cognitive dysfunction and dementia.[9] When compared to healthy subjects, the risk of dementia—including clinical diagnosis of both vascular dementia and Alzheimer's disease (AD)—is actually up to 73% higher in people with T2D,[10] and the process of cognitive decline seems to begin early in prediabetic stages of insulin resistance,[9,11] where fasting glucose levels are abnormally high but below the threshold for diabetes, thus alluding to a mechanistic link between insulin sensitivity and dementia.[12] Moreover, in a study conducted by Rdzak and co-workers at Yale-New Hospital in Connecticut, only one in over 500,000 patients with T1D (who self-administer insulin on a daily basis to control glycemia) was diagnosed with AD, which highlights the importance of insulin brain signaling, compromised in T2D patients, for AD development.[13]

AD is the most common form of dementia, accounting for 60% to 80% of all cases[14] and affecting more than 35 million people worldwide.[15,16] It usually manifests in people older than 65 years of age, with progressive memory loss and gradual decline in cognitive function, although the pathophysiological mechanisms linked to these symptoms are not completely understood to this point.[16] It is clear, however, that accumulation of misfolded proteins in the brain, namely amyloid-β (Aβ) aggregates and neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein, are key triggers of oxidative and inflammatory damage, which then culminate in synaptic dysfunction and neuronal death.[15,17]

In the light of the epidemiological data supporting the link between T2D and AD, the increasing incidence of the latter[18] is possibly a consequence not only of population ageing alone, but also of the diabetes epidemic itself. It is estimated that about 193 million people living with diabetes are undiagnosed and unaware of the long-term damage caused by the disease.[1] If research efforts are assertively directed toward new multitarget therapies that can simultaneously tackle both diseases, then perhaps in the future it will be possible to attenuate or even prevent the product of cumulative neuropathological events that begin to take place in the earliest stages of T2D.

This review starts by providing an updated background on common and interconnected targets for drug discovery and development against both T2D and AD. It covers crucial pathological events involved in the etiology of both type diseases, from metabolic alterations and vascular damage to amyloidogenic processes, together with peripheral and brain insulin resistance. Altered activity of key enzymes such as phosphodiesterase (PDE), glycogen synthase kinase 3β (GSK-3β), and insulin degrading enzyme (IDE) is also a major link between both diseases, and thus will also be explored. Prospective causal links between such events and the onset of AD in diabetic patients will be established and assembled together as puzzle pieces. Moreover, in the light of the demanding therapeutic needs of T2D and AD (which are inherent to their pathophysiological complexity), a comprehensive search for multitarget molecules suitable for therapeutic use or as leads for further development will also be carried out. Given that nature is the richest source of molecular scaffolds with broad bioactivity, we herein provide an overview on promising nature-derived and nature-inspired compounds robustly reported in the literature. Furthermore, the latest and most promising rationally designed molecules with selectivity for some of the described therapeutic targets will also be presented, ultimately focusing on highlighting structural requirements of innovative, potential "two in one" approaches for the treatment of T2D and the prevention of AD.

2. COMORBIDITIES OF T2D: A COMPLEX DISEASE

Potentiated by high-fat hypercaloric diets, lack of physical activity, and/or genetic predisposition, T2D is characterized by a complex set of risk factors that include high blood pressure, hyperglycemia, insulin resistance, dyslipidemia, and abdominal obesity, which increase the possibility of heart disease and coronary events.[19,20] Interestingly, these features have also been reported as indicators of patients with risk of progression from mild cognitive impairment to AD.[21,22] In fact, greater cognitive and affective decline was found to occur in AD patients with this set of risk factors when compared to those without, together with more significant pathological white matter changes.[23,24] Seemingly, these events are not only linked to insulin resistance—a hallmark of T2D—but also to vascular endothelial dysfunction,[23] which is in agreement with the recognized association between pathological alterations in cerebral microvasculature and decreased cognitive function in patients with AD.[25,26] Briefly, this suggests that cerebrovascular damage may arise in type 2 diabetic patients as a cognitive impairment-promoting factor, being supported by reports showing that T2D and AD mouse models present similar vascular, behavioral, and cognitive abnormalities, with congruent underpinning causes.[27–30]

Oxidative stress and neuroinflammation are also coexisting features of AD,[31] and both are taken as key prompting factors of cerebrovascular dysfunction.[32,33] The diabetes-promoted underlying causes for these phenomena are vast. In T2D, peripheral insulin resistance leads to hyperglycemia, which is itself a cause of tissue damage and oxidative stress.[34] First, high plasma glucose levels favor the nonenzymatic glycation of proteins, lipids, and nucleic acids—thus interfering with their physiological functions, with subsequent formation of advanced glycation end-products (AGEs) and development of common diabetic complications such as retinopathy, nephropathy, or neuropathy.[35] These compounds can furthermore interact with receptors for advanced-glycation end-products (RAGEs)—which are overexpressed in the brains of AD patients[36]—to trigger inflammatory response mechanisms and the production of reactive oxygen species (ROS).[37] Moreover, all the unused glucose excess is massively converted into sorbitol by aldol reductase in the polyol pathway, with collateral depletion of cellular nicotinamide adenine dinucleotide phosphate in its reduced form (NADPH),[34] which is a major cofactor of antioxidant enzyme systems. An oxidative environment will induce lipid peroxidation and nitric oxide (NO) inactivation in the endothelium, with consequent endothelial cell damage and

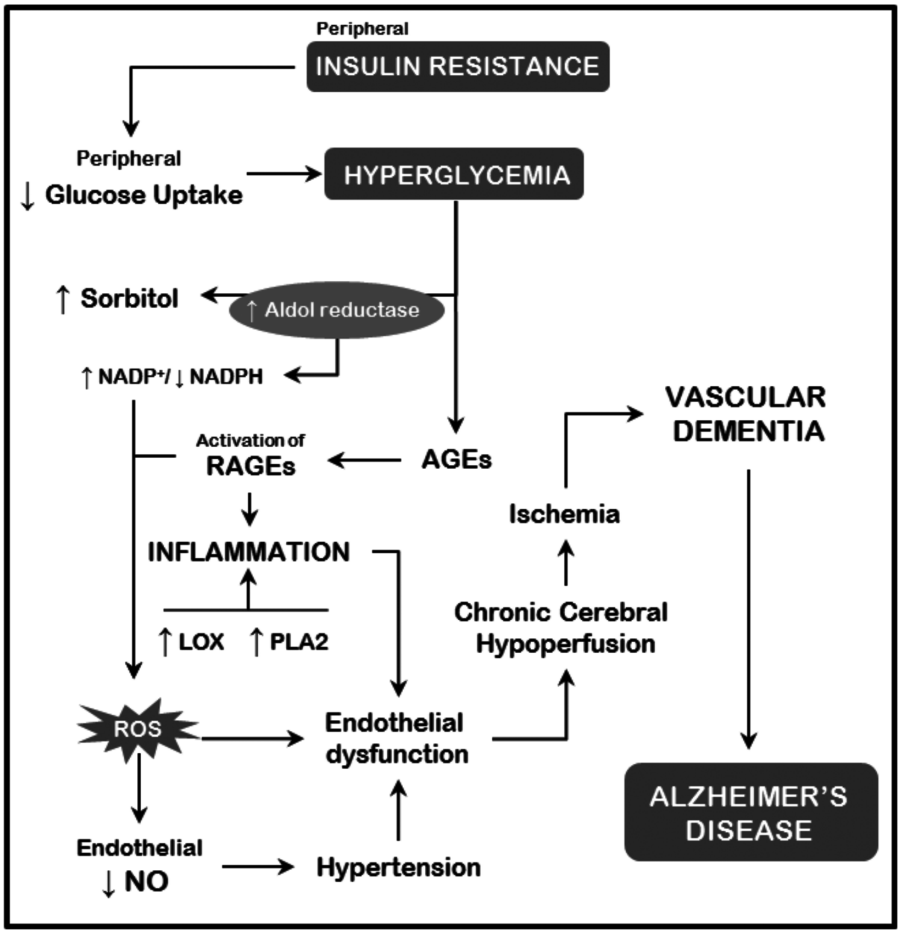


Figure 1. The way from peripheral insulin resistance to endothelial dysfunction, vascular dementia, and AD through oxidative stress and proinflammatory events. $NADP^+$, oxidized form of nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; AGEs, advanced glycation end-products; RAGEs, receptors for advanced glycation end-products; LOX, lipoxygenase; PLA2, phospholipase A2; ROS, reactive oxygen species; NO, nitric oxide.

reduced endothelium-dependent vasodilation, ultimately leading to chronic hypertension.[38–41] Oxidative enzymes involved in lipid metabolism such as phospholipase A2 (PLA2) and lipoxygenase (LOX) also contribute to inflammation and endothelial damage,[42–45] and both have been found to be increased in patients with diabetes and AD.[46]

Shortly, oxidative stress, hypertension, and chronic inflammation are some of the pathophysiological alterations associated with hyperglycemia, dyslipidemia, and insulin resistance,[47–49] typically found in type 2 diabetic patients, and somehow all of them contribute to endothelial dysfunction, which leads to vascular cognitive impairment by blocking or reducing blood flow into the brain (Fig. 1).[50,51] Prolonged hypoperfusion can lead to neuronal and neuroglial ischemic damage and may even accelerate Aβ overproduction and aggregation, eventually triggering the development of AD.[52,53] Mixed dementia—a combination of vascular dementia and AD—is presumably more common with increasing age,[54] thus supporting a cause–effect relationship between these two types of dementia. Yet, AD is often characterized by other significant pathological features that include extracellular amyloid plaques

composed by Aβ and NFTs containing hyperphosphorylated tau that contribute to oxidative and inflammatory damage, cerebrovascular damage, blood–brain barrier (BBB) dysfunction, and neuronal death.[15,55] Interestingly, tau pathology is also triggered by hyperglycemia.[56] In fact, caspase-3-dependent proteolytic cleavage of tau was found to be aberrantly increased in the brains of type 2 diabetic mice, in agreement with in vitro experiments showing that high glucose levels can trigger this process in a similar way to that of Aβ, potentially exerting an additive effect. Aβ may furthermore establish another important connecting point with T2D due to cross-seeding events, which will be explored in the following Section 3.

3. AMYLOIDOGENESIS: A COMMON PROCESS TO BOTH PATHOLOGIES

More than a dozen unrelated human proteins can undergo abnormal assembly in vivo to form cytotoxic amyloid fibrils.[57] Two of these proteins—the proislet amyloid polypeptide (proIAPP) and amyloid β precursor protein (AβPP)—are frontrunners in the misfolding processes taking place in T2D and AD, respectively. IAPP or amylin is the major component of pancreatic amyloid deposits found in most type 2 diabetic patients,[58,59] and arises from posttranslational modifications in its precursor, proIAPP. Physiologically, IAPP exerts an autocrine or paracrine effect on β-cells, acting as a modulator of insulin secretion, being also involved in glycogen synthesis and glucagon secretion, gastric emptying, and calcium metabolism.[60] As previously mentioned, early stages of T2D are characterized by hyperglycemia and compensatory hyperinsulinemia due to peripheral insulin resistance and impaired insulin clearance. Since IAPP is cosecreted with insulin by pancreatic β-cells, insulin hypersecretion collaterally leads to IAPP accumulation in the pancreas with the formation of cytotoxic extracellular amyloid deposits, which ultimately results in β-cell dysfunction and death.[61,62] In fact, incomplete N-terminal enzymatic cleavage of proIAPP into IAPP has been proposed as a triggering factor for IAPP amyloid formation indicating that the first intracellular aggregates partially consist of proIAPP. Briefly, proIAPP acts as a seed by recruiting mature IAPP inside β-cell vesicles, which are eventually released and continue to enlarge outside the pancreas.[60,63,64] The so formed cytotoxic aggregates end up inducing β-cell apoptosis in later stages of T2D, giving rise to hypoinsulinemic states such as those typically found in type 1 diabetic patients. IAPP oligomers also accumulate in the heart and kidneys, which accelerates diabetic heart failure.[65,66]

A. IAPP in the Brain

Since IAPP was first found to cross the BBB,[67] much research has been focused on the effects of IAPP in the brain, as well as possible consequences of its overproduction in early stages of T2D. In vitro experiments have shown that soluble oligomers of human IAPP are cytotoxic in pancreatic and neuronal cell lines by affecting Ca^{2+} homeostasis[68,69] and by causing cell membrane disruption.[70,71] On the other hand, recent in vivo studies suggest that IAPP might exert neuroprotective effects to some extent,[72,73] in addition to its reported physiological actions in the brain, which include relaxation of cerebrovascular structure, reduction of food intake, and regulation of adipose energy reserves.[74–81] One of these studies found that chronic intraperitoneal injections of IAPP reduced both behavioral impairment and brain beta-amyloid pathology in a murine model of AD.[73] Another study found a positive correlation between plasma IAPP levels and cognitive function among elderly subjects; however, this correlation disappeared among those with diabetes.[72] The aggregating environment of T2D may in fact trigger the cytotoxic IAPP oligomer formation, accounting for the greater susceptibility of AD onset in diabetic patients with increased levels of IAPP.[82–84] The described neuroprotective effects are ought to be exclusive to nondiabetic clinical backgrounds, which are associated with merely physiological

IAPP plasma concentrations. In fact, a recent study has shown that brains of type 2 diabetic patients with AD have IAPP deposits inside the neurons promoting the synthesis of proinflammatory cytokine interleukin (IL)-1β and additionally forming adducts with 4-hydroxynoneal (4-HNE), a marker of peroxidative membrane injury. These effects were also observed in mice expressing human IAPP or injected with aggregated human IAPP, thus indicating that elevated circulating levels of IAPP directly correlate with neuronal damage induced by oxidative stress and aberrant inflammatory responses, although the mechanisms underlying intracellular IAPP uptake have not been elucidated to this point.[85]

B. Amyloidogenesis in AD

The amyloid cascade hypothesis of AD posits that gradual and progressive neuronal loss is directly related to the accumulation of Aβ aggregates in the brain parenchyma, which are nowadays accepted as surface catalysts in the formation of diffusible neurotoxic Aβ oligomers.[86,87] The fibrillogenic Aβ peptides are formed after sequential proteolytic cleavage of AβPP—a transmembrane glycoprotein with a large extracellular domain—by β secretase enzyme-1 (BACE-1) and γ secretase.[16] A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$ are the most common Aβ isoforms resulting from this process: while the first is predominant, the last has the higher tendency to aggregate and is found in Aβ deposits of most diseased brains.[16,88] Interestingly, soluble intermediary A$\beta_{1\text{-}42}$ oligomers seem to be more neurotoxic than the actual fibrils,[89,90] accounting for most of the pathological events associated to impaired synaptic plasticity and memory loss.[91,92]

C. What Can We Learn From Genetic Studies?

In the early 1990s, the apolipoprotein E-ε4 (APOE-ε4) allele was identified as the most important genetic risk factor for sporadic AD and, contrasting with the amyloid hypothesis, Aβ deposition in the brain was regarded as an APOE-ε4-related consequence rather than the primary cause of late-onset AD, due to impaired clearance of Aβ often combined with its overproduction.[93–95] The APOE-ε4 genotype is also associated with insulin resistance[96,97] and, interestingly, the association between T2D and AD is particularly significant among APOE-ε4 carriers, who have much higher chances of developing AD when compared to non-diabetic APOE-ε4 carriers.[98] Indeed, studies show that the APOE-ε4 genotype accelerates age-dependent hippocampal atrophy and memory impairment by increasing brain beta-amyloid burden due to dysfunctional Aβ production, aggregation, and clearance.[98–102] In the central nervous system (CNS), apolipoprotein E (APOE) is mainly expressed by astrocytes, being involved in the regulation of lipid transport, glucose metabolism, and neuronal signaling, among others. It can also bind to Aβ, essentially reducing its cytotoxicity by promoting APOE receptor-mediated uptake and degradation.[103] However, effective Aβ binding and uptake is only carried out by APOE-ε2 and APOE-ε3 isoforms: APOE-ε4—which does not interact with Aβ as strongly—acts more as a "pathological chaperone" by catalyzing the formation of brain beta-amyloid deposits to a greater extent.[103–106] In agreement, APOE-ε4 carriers have lower Aβ levels in the cerebrospinal fluid (CSF).[107]

Modified expression or activity of AβPP cleaving enzymes is also believed to be a relevant cause of the beta-amyloid pathology in late-onset AD. BACE-1 expression, for instance, is upregulated in the brain cortex of sporadic AD patients[108,109] and can be triggered by AGE/RAGE interaction, oxidative stress, decreased endothelial NO derived by eNOS, hypoxia, and ischemic injury—a set of characteristic features of T2D.[110–114] Moreover, recent data show that BACE-1 overexpression is extended to the BBB, playing a critical role in the development of cerebral amyloid angiopathy.[115] Interestingly, overproduced Aβ can itself stimulate BACE-1 expression[116]

and, indeed, it is supposed that Aβ accumulation can be both a cause and a consequence of BBB dysfunction—a distinctive trait of AD and other neurodegenerative disorders.[55]

Unlike BACE-1, mutations in presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) proteins—which form the catalytic core of γ secretase as catalytically active subunits—have been linked to the far more uncommon early-onset familial AD.[117] These mutations highly favor γ secretase cleaving activity toward the generation of longer and more aggregation-prone A$\beta_{1\text{-}42}$ peptides.[117,118] Mutations in AβPP itself can also occur, although they only account for about 0.5% of all cases of AD.[117] These are associated to both sporadic and familial disease forms, and while AβPP mutations near the BACE-1 cleaving site increase Aβ production in general, mutations around γ secretase site also contribute to an increased relative ratio of A$\beta_{1\text{-}42}$ to A$\beta_{1\text{-}40}$.[119,120]

D. Cross-Seeding Events Between AChE, IAPP, and Aβ

Apparently, Aβ peptide assembly into amyloid fibrils can be triggered and accelerated by other aggregation-prone molecules acting as seeds in the fibrillization process. Acetylcholinesterase (AChE), for instance—an enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh) in the CNS—is one of those entities,[121,122] and was found to colocalize with Aβ deposits in the hippocampus of AD patients.[123,124] In fact, small peptides resulting from AChE C-terminus cleavage have a substantial sequence homology to the comparable region of Aβ, and have been shown to spontaneously self-assemble into amyloid fibrils.[125,126] AChE-containing Aβ aggregates—which are actually more neurotoxic than Aβ aggregates alone—are believed to be a result of successive incorporation of Aβ monomers onto an initial amyloid fibril enclosing an AChE-Aβ complex, in a classic heterologous amyloid seeding process where AChE acts as a seed for the accumulation of the misfolded protein bulk.[127,128] In T2D, a similar phenomenon may occur between periphery-derived IAPP and hippocampal Aβ. Indeed, these peptides share about 25% of amino acid sequence identity in regions that are essential for self-assembly.[129] It was found that circulating IAPP is able to promote homologous seeding in the cerebrovascular structure and brain parenchyma of type 2 diabetic patients, causing diabetic brain damage and neurodegeneration.[130] Moreover, it prompts heterologous seeding with Aβ to form amyloid deposits in the brain and blood vessel walls of patients with AD,[131] simultaneously inducing failure of Aβ elimination from the brain and further enhancing Aβ-promoted neuronal dysfunction.[130] Together, these data indicate that independent and cross-seeding amyloid events constitute a molecular connection between T2D and AD (Fig. 2), strongly encouraging the development of new multitarget inhibitors of amyloid fibril formation as therapeutic and preventive strategies against both conditions.

4. PERIPHERAL AND CEREBRAL INSULIN RESISTANCE

Due to the epidemiologic link between T2D and AD, dysfunctional insulin activity in the hippocampus is currently receiving considerable attention for its role in cognitive decline.[9] Indeed, postmortem studies of patients with AD provided solid evidence of dysfunctional insulin signaling in the brain, including downregulation of insulin receptors and lack of insulin sensitivity, which can be labeled as cerebral insulin resistance.[132–135] Furthermore, age-related insulin resistance is associated with chronic neuroinflammation,[136] an early pathological feature of the neurodegenerative process[137] that leads to increased BBB permeability.[138] Insulin resistance and aberrant insulin levels usually go hand in hand and, in fact, not only the risk of AD was found to be higher in people with hyperinsulinemia,[139] typical of early stages of T2D, but also higher c-peptide levels, which serve as a measure of insulin secretion rates, seem to be related

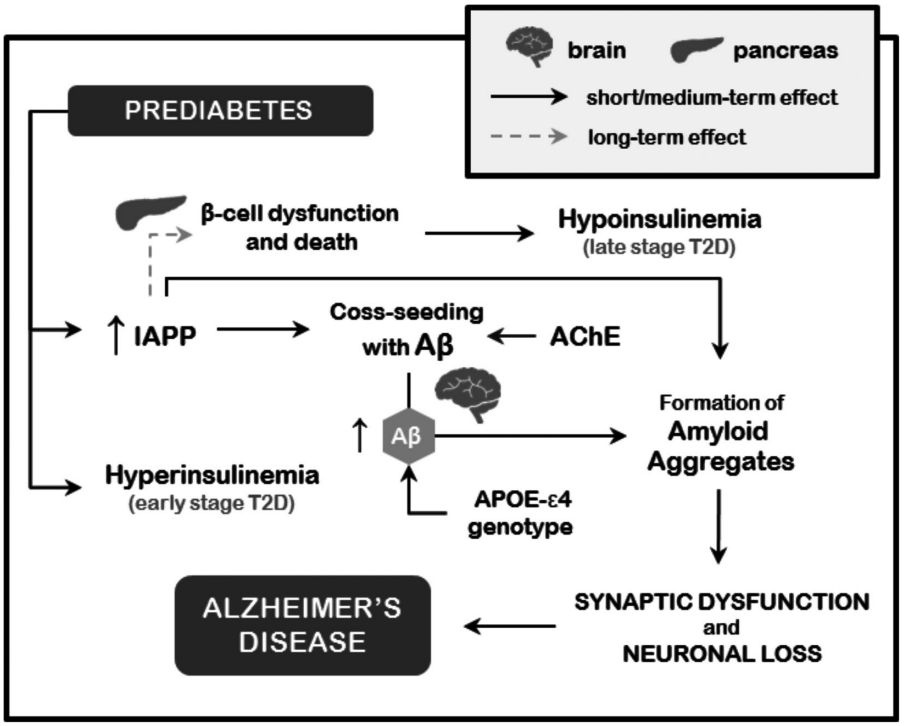


Figure 2. The way from prediabetes to the formation of brain amyloid aggregates through amyloid cross-seeding events. IAPP, islet amyloid polypeptide; Aβ, amyloid β; T2D, type 2 diabetes; AChE, acetylcholinesterase; APOE-ε4, apolipoprotein E-ε4.

to worse cognition even among people without diabetes.[140] Thus, it is crucial to understand if and how peripheral insulin resistance and abnormal insulin levels can extend themselves into the brain—eventually contributing to the onset of AD—and what mechanisms are possibly involved.

A. Insulin in the Brain

Although peripheral insulin action is tightly coupled to glucose homeostasis, in the CNS these two phenomena are thought to be mostly independent.[141,142] Yet, a growing number of non-metabolic actions of insulin in the CNS are being reported, such as neuronal survival under oxygen and glucose deprivation, synaptic maintenance, stimulation of dendritic formation, learning, and memory,[143] thus supporting the importance of brain insulin signaling in the preservation of cognitive function. Indeed, insulin receptors are distributed throughout the brain, including the hippocampus[144] and the effects of their modulation go far beyond the above described neuronal processes, as they have a big impact on the CNS response to environmental and peripheral stimuli, such as the control of food intake, satiety, body weight, and reproductive functions.[145,146] Despite the existence of insulin-secreting neurons,[147] insulin is mainly produced by pancreatic β-cells. Yet, there is significant evidence indicating that sympathetic and parasympathetic nervous systems regulate peripheral insulin secretion through the release of hypothalamic monoamines, and induced hypothalamic lesions have led to peripheral insulin resistance, thus suggesting that it may actually have its origin in the brain.[148]

Peripherally produced insulin can be readily transported through receptor-mediated transcytosis across BBB endothelial cells.[143,149] It is known that this transport system can be regulated, positively or negatively, depending on physiological conditions. Whereas streptozotocin (STZ)-induced diabetic mice—a T1D model—have increased insulin transport through the BBB into the brain and CSF, hyperglycemic states induced by single IP injections of glucose seem to have a pronounced inhibitory effect on that transport.[150] The difference is that, whereas in the first case the production of insulin by the pancreas is abolished, in the second case—a more approximate situation to that observed in prediabetes and early T2D—hyperglycemia is accompanied by elevated levels of plasma insulin. Accordingly, obesity induced by high-fat diets, a well-established risk factor for T2D, also leads to decreased insulin transport across the BBB.[151] The mechanism by which combined elevated glucose and insulin plasma levels lead to reduced insulin uptake into the brain is yet to be elucidated, but it seems that insulin affinity for brain endothelial cells under these physiological conditions is somehow significantly diminished. Nonetheless, the above data suggest that decreased insulin transport into the brain in T2D may be a triggering factor of neurodegeneration and cognitive decline. In fact, there is evidence that AD patients have higher plasma insulin and lower CSF insulin when compared to healthy adults,[152] thus supporting this point.

B. Brain Insulin Deficiency

Deficient brain insulin levels seem to provide the ideal conditions for amyloidogenic events to take place, which directly connect T2D to AD. In fact, the lack of insulin allows constitutive Aβ production by keeping GSK-3 permanently activated.[153,154] This serine/threonine kinase was originally isolated from skeletal muscle[155] and is mostly known for its role in glycogen metabolism and regulation of apoptosis, as a downstream target of insulin-mediated PI3K/Akt signaling, vital to the translocation of glucose transporters to the cell surface in response to the activation of insulin receptors after a meal.[156] It has been demonstrated that overexpression or overactivation of GSK-3β in rodent models of T2D is associated with impaired ability of insulin to activate glucose utilization and glycogen synthesis.[157] Yet, it is widely expressed in the brain[158] and its enduring activation due to brain insulin deficiency is associated not only with Aβ production and induced neuronal damage,[159] but also with long-term synaptic depression processes[160] and tau hyperphosphorylation.[161–163] Moreover, there is evidence that BACE-1 expression is upregulated in the absence of insulin, which boosts the extracellular accumulation of Aβ in the brain.[164] Conversely, Aβ oligomers stimulate neuronal downregulation of insulin receptors, with subsequent dysfunctional brain insulin signaling and insulin resistance,[165,166] thus intensifying the process of cognitive decline. This effect was reversed by insulin itself,[166] which essentially reinforces its neuroprotective effects.

C. Oxidative Stress, Mitochondrial Dysfunction and Metabolic Brain Ischemia

In addition to insulin deficiency, reduced ATP synthesis, raised levels of ROS, and lipid and protein peroxidation were observed in the brains of Zucker diabetic fatty (ZDF) rats—a genetic model of T2D characterized by obesity and peripheral insulin resistance.[167] Furthermore, evidence shows that high-fat diets, which are similarly associated with obesity and peripheral insulin resistance, promote hippocampal oxidative stress and mitochondrial homeostasis deficiencies, with concomitant cognitive impairment and memory loss.[168–170] This suggests that mitochondrial dysfunction and oxidative stress might play a key role in the development of cerebral insulin resistance. However, although this hypothesis is reinforced by diminished insulin-dependent stimulation of insulin signaling pathways caused by oxidative stress in

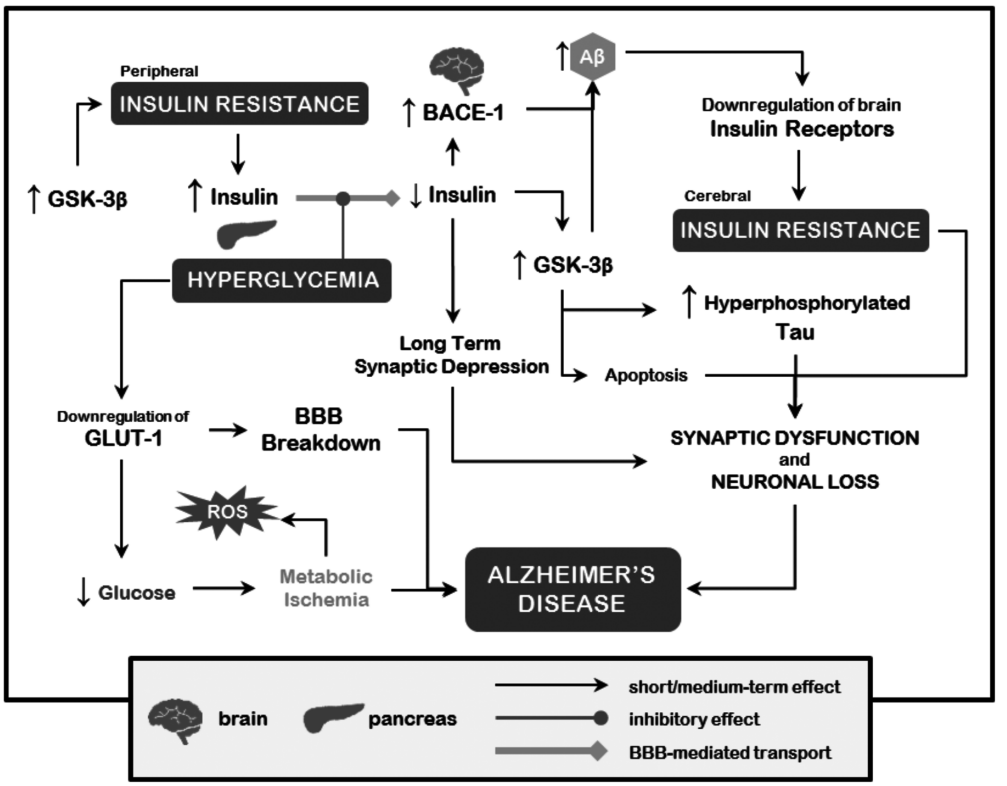


Figure 3. The way from peripheral insulin resistance and hyperglycemia to cerebral insulin resistance through GSK-3β and BACE-1 overactivation. GSK-3β, glycogen synthase kinase 3β; GLUT-1, glucose transporter 1; BACE-1, beta-secretase; ROS, reactive oxygen species; BBB, blood–brain barrier; CNS, central nervous system; Aβ, amyloid β.

peripheral tissues,[171,172] it is thought that insulin resistance might also be a cause for oxidative stress,[173] therefore establishing a two-way cause–effect relationship between both events.

Altered glucose metabolism, by its turn, is known to play a critical role in the physiopathology of AD by triggering oxidative stress and mitochondrial dysfunction.[174] A recent study has shown that peripheral insulin resistance is associated with significantly lower regional cerebral glucose metabolism and worse performance on both immediate and delayed memory.[175] Indeed, glucose is the primary source of energy in the brain and it is now clear that it has memory-enhancing effects.[176] Downregulation of the glucose transporter 1 (GLUT-1) at the BBB occurs spontaneously as a consequence of hyperglycemia,[177] and is also observed in AD patients,[178,179] not only leading to BBB breakdown and early cerebral microvascular degeneration,[180] but also to impaired glucose transport into the brain, which compromises ATP generation and stimulates the production of ROS.[181] Furthermore, BACE-1 activity—also enhanced by insulin deficiency, as mentioned above—impairs neuronal glucose oxidation and reduces the basal oxygen consumption rate,[182] therefore intensifying the hazard of metabolic brain ischemia and consequent cognitive dysfunction. In the light of the data presented and summarized in Figure 3, specific or combined inhibition of both GSK-3β and BACE-1 enzymes comes across as a very reasonable therapeutic approach against T2D and AD. The accuracy of this strategy will be discussed in the final section of this review based on the most recent advances in medicinal chemistry regarding new compounds targeting one or both enzymes.

5. IMPAIRED cGMP AND cAMP-MEDIATED SIGNALING PATHWAYS

The activity of multiple cyclic nucleotide PDE families is enhanced in the subcutaneous adipose tissue of type 2 diabetic patients,[183] and there are studies indicating that this is likely to have implications in diabetes-associated cardiovascular disease.[184,185] PDEs catalyze phosphodiester bond hydrolysis in cyclic guanosine monophosphate (cGMP) and adenosine monophosphate (cAMP), two central second messengers involved in cellular signal transduction pathways that are vital in a vast number physiological processes.[186] cGMP, in particular, plays a major role in NO-promoted smooth muscle relaxation—and, therefore, in blood pressure control—and has been shown to ameliorate vascular inflammation and insulin resistance induced by high-fat feeding.[187,188] Moreover, NO/cGMP signaling pathway in the hippocampus seems to be crucially implicated in synaptic transmission, neuronal plasticity, learning, and memory.[189–191] This signaling cascade is impaired by the action of Aβ[192] and, indeed, reduced levels of cGMP in the CSF have been recently associated with cognitive decline and amyloidogenesis in AD patients.[193]

cAMP, on the other hand, was found to stimulate the postprandial secretion of glucagon-like peptide 1 (GLP-1), an intestinal hormone that potentiates glucose-stimulated insulin secretion by pancreatic β-cells, while inhibiting glucagon secretion by α cells.[194,195] This is particularly relevant in advanced stages of T2D, when β-cells become dysfunctional and lose their ability to produce insulin. As a result, anomalous intraislet signaling fails to inhibit glucagon secretion by α cells even when plasma glucose levels are considerably high, which contributes to exacerbated hyperglycemia in diabetic patients.[196,197] Furthermore, there is strong evidence indicating that cGMP-mediated memory consolidation in the hippocampus requires postsynaptic cAMP signaling,[198,199] and that cAMP itself can inhibit PLA2 and protect neurons against synaptic damage.[200] Yet, cAMP levels were actually found to be increased in the CSF and cerebral microvessels in AD patients.[201,202] It is not clear whether cAMP levels rise by means of a compensatory mechanism or not, but hippocampal cAMP downstream effectors—namely cAMP-dependent protein kinase (PKA) and cAMP response element-binding protein (CREB), the final long-term memory promoters in the pathway[203]—are in fact inactivated or downregulated in the presence of Aβ.[204,205] Downregulated cAMP/PKA/CREB pathway is re-established by PDE inhibitors,[204] thus suggesting that intracellular hippocampal cAMP levels in AD patients may not directly correlate with those in the CSF. In fact, studies using postmortem brain samples of AD patients have shown that while the activity of adenylate cyclase—the enzyme that catalyzes cAMP formation—is decreased in the hippocampus,[206] the expression of several cAMP-specific PDE isoforms is significantly upregulated,[207] which corroborates this point. The trail from PDE increased activity or upregulation to cognitive decline and memory loss is briefly illustrated in Figure 4.

The impact of PDE inhibition on the reversal of main disease traits that tie T2D and AD together has been getting growing attention. From glucose-induced insulin secretion potentiation and insulin sensitivity enhancement, to decreased brain beta-amyloid pathology and neuronal death, PDE inhibitors have been able to deliver extremely promising results[208–214] that accredit PDE as biomolecular link between both pathologies and, at the same time, as a very attractive therapeutic target for their treatment and prevention.

6. IDE: A CRUCIAL PIECE IN THE PUZZLE

Insulin-degrading enzyme (IDE) is a widely expressed thiol zinc-metalloendopeptidase able to degrade insulin and a variety of other substrates such as Aβ, IAPP, and glucagon.[2] It has been pointed out as a possible link between T2D and AD,[215,216] although some results are

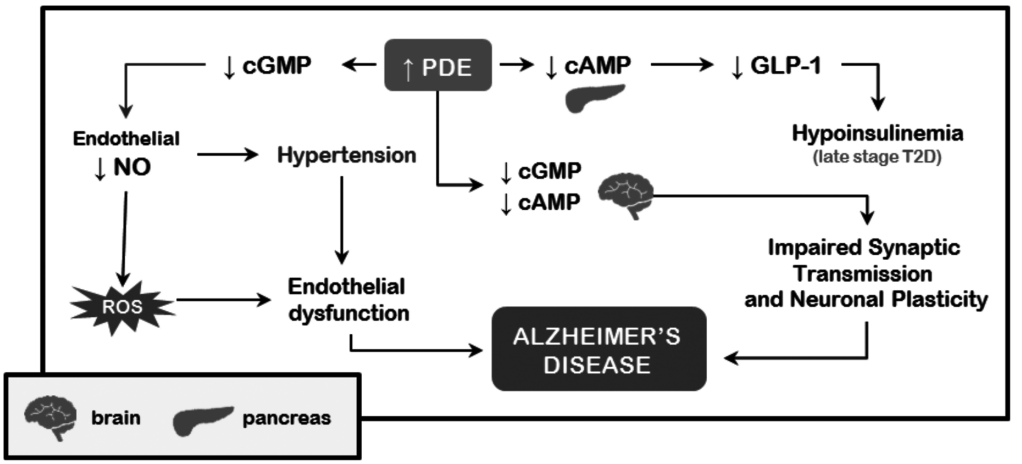


Figure 4. The way from PDE overexpression/overactivation to impaired synaptic transmission and neuronal plasticity through the depletion of cGMP and cAMP levels. NO, nitric oxide; ROS, reactive oxygen species; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase; GLP-1, glucagon-like peptide 1; T2D, type 2 diabetes.

apparently controversial and incoherent. To begin with, in the prediabetes state it is thought that insulin may compete for IDE and thus contribute to decreased Aβ degradation, with subsequent accumulation in the brain.[215] Although AD is associated with cerebral insulin deficiency,[152] peripherally produced and circulating Aβ can still cross the BBB and indeed contribute to AD brain lesions, which supports this hypothesis.[217,218] Moreover, some authors claim that inactivating IDE gene mutations may result in altered IDE expression or activity and, consequently, to low insulin and Aβ clearance rates[215,219]—in fact, some genetic IDE polymorphisms have actually been associated with T2D[220–222] and AD,[223–226] pointing toward IDE function as plausible connecting factor between both diseases. Furthermore, it has been shown that IDE expression and activity are negatively regulated by a number of physiological factors and endogenous molecules,[227] including NO[228,229] and long-chain fatty acids.[230] Basal plasma NO levels were found to be increased in type 2 diabetic patients[40,231,232]—possibly contributing to IDE inactivation—while increased levels of long-chain free fatty acids might be an important connecting point between high-fat hypercaloric diets and impaired IDE-mediated insulin metabolism in T2D.[233]

To corroborate this point, loss of IDE activity owing to IDE knockout (KO) in mice led to glucose intolerance with increased levels of insulin and IAPP in pancreatic β-cells, and concomitant Aβ accumulation in primary neuronal cultures and brain membrane fractions.[2] Similar results were obtained when using the Goto-Kakizaki (GK) nonobese rat model for T2D with decreased IDE activity caused by two missense mutations in the IDE allele.[212] However, other reports suggest that there are inactivating IDE gene mutations leading to impaired insulin secretion[234–236]—a different scenario that rather resembles later stages of T2D, when peripheral insulin resistance is accompanied by pancreatic β-cell dysfunction and insulin deficiency.[237] Indeed, IDE KO mice exposed to a high caloric diet show a bad prognostic for glucose tolerance in comparison to their control linear mates exposed to the same diet (Borges and Macedo, unpublished observations). In agreement, another study carried out using IDE KO mice led to low glucose-stimulated insulin secretion associated with reduced autophagy and an increase in pancreatic α-synuclein.[238] In type 2 diabetic patients, the same inverse correlation between pancreatic IDE and α-synuclein levels was observed.[238] Besides regulating presynaptic vesicle release in the CNS,[239] α-synuclein is able to interact with insulin

secretory granules in the pancreas and inhibit glucose-stimulated insulin secretion.[240] Regardless of being unable to degrade α-synuclein, IDE can actually form stable and irreversible complexes with monomers of aggregating-prone proteins such as this one, thus inhibiting their fibrillization in a nonproteolytical way.[241] Hence, low IDE levels may not only promote α-synuclein-induced inhibition of insulin secretion, but also enhance the amyloid burden in the pancreas.

The above data are apparently contradictory: if on the one hand null IDE expression led to hyperinsulinemia, on the other hand, it appears that also promotes some reduction of glucose-stimulated insulin secretion. In the first case plasma insulin was measured after an overnight fast, while in the second IDE KO mice were subjected to an intraperitoneal (i.p.) glucose injection to address glucose-induced insulin secretion. However, the most evident phenotypic event relates to compromised IDE-mediated insulin clearance in the liver leading to hyperinsulinemia.[229]

In prediabetes or early type 2 diabetes the hyperinsulinemic phenotype is, indeed, prevalent; yet, since mouse IAPP does not form amyloid fibrils,[242] the observed consequences of IDE KO in mice fell short of the real extent of long-term IDE deficiency in humans. With reduced autophagy and IDE-promoted clearance, increased α-synuclein and IAPP levels will enhance the odds of amyloid formation in the pancreas, ultimately leading to β-cell damage and β-cell failure observed in late stages of T2D.[238,243]

Brain insulin deficiency or altered levels of other IDE substrates under pathological conditions may eventually lead to downregulation of brain IDE expression as suggested by significantly reduced IDE levels in insulin-deficient mice.[244] In fact, low hippocampal IDE levels have been found in late-onset AD patients with impaired insulin metabolism associated with the APOE-ε4 allele.[245] Similarly to what happens in the pancreas, IDE acts as a dual protease-chaperone function toward Aβ, forming non-neurotoxic aggregates on top of its degrading activity.[246] Hence, low IDE levels accelerate the formation of Aβ aggregates in the brain,[247,248] which intensifies synaptic dysfunction and cerebrovascular amyloidosis,[249] both hallmarks of AD.

Figure 5 puts together most of the earlier presented pathophysiological features of T2D that converge into the development of AD, fitting them together like puzzle pieces. In this puzzle, IDE inhibition or downregulation—caused either by genetic (IDE polymorphisms) or environmental factors (high-fat hypercaloric diets)—is portrayed, both directly and indirectly, as a brain Aβ enhancing factor, and thus as a front-line player in the neurodegenerative process in cooperation with APOE-ε4 or upregulated PDE and GSK-3β enzymes. Since acute IDE inhibition was shown to improve glucose tolerance by mimicking postprandial insulin pancreatic release,[250,251] the idea of using IDE inhibitors as a valid therapeutic strategy against T2D has been considered and is currently a topic of much debate with unclear long-term effects. Nevertheless, a more recent report presented evidence pointing in the opposite direction, as acute IDE inhibition actually induced glucose intolerance in mice and was therefore shown not to be a viable option in the treatment of T2D.[252] As shown in Figure 5, decreased IDE activity modulates the levels of IAPP and glucagon in vivo in addition to those of insulin, having a significant impact on postprandial gastric emptying and blood glucose levels.[251] Concretely, the loss of IDE activity under these conditions results in lower glucagon-processing and, consequently, enhanced glucagon-stimulated gluconeogenesis, which is considered to be a major pathophysiologic process and therapeutic target of T2D.[253] This fact alone might raise concerns about the potential use of IDE inhibitors in the clinic; yet, it is just as important to consider that, with prolonged administration, such molecules are, in theory, likely to further increase the amyloid burden to which diabetic patients are primarily more susceptible, not only by raising IAPP levels, but also by stimulating α-synuclein and Aβ accumulation, and perhaps inducing long-term amyloid-derived toxicity.[254] Scrupulous investigation is required in order

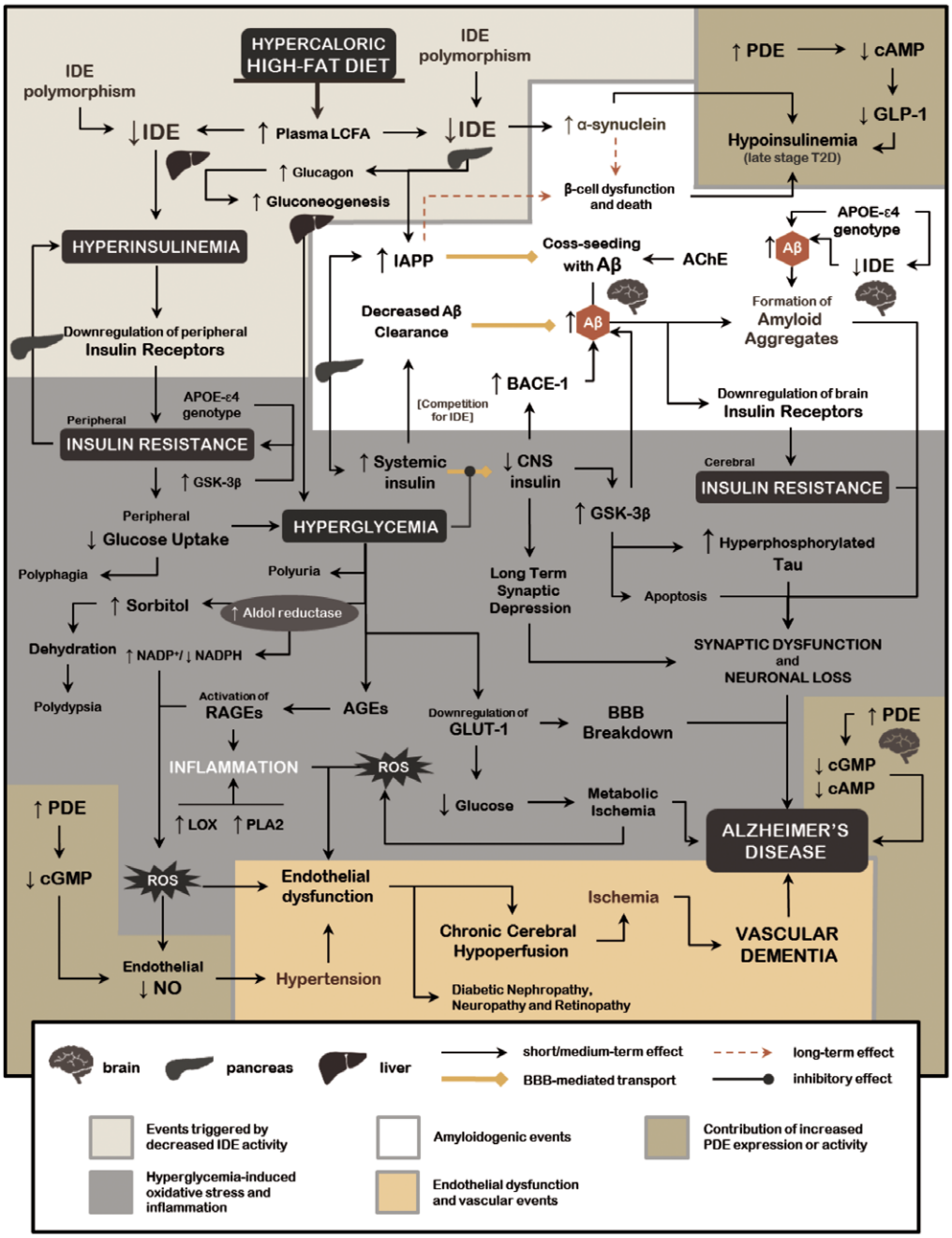


Figure 5. Pathophysiological links bridging type 2 diabetes and Alzheimer's disease: the puzzle pieces put together. IDE, insulin degrading enzyme; $NADP^+$, oxidized form of nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; AGEs, advanced glycation end-products; RAGEs, receptors for advanced glycation end-products; LOX, lipoxygenase; PLA2, phospholipase A2; ROS, reactive oxygen species; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase; GLP-1, glucagon-like peptide 1; T2D, type 2 diabetes; GSK-3β, glycogen synthase kinase 3β; GLUT-1, glucose transporter 1; BACE-1, beta-secretase; BBB, blood–brain barrier; CNS, central nervous system; Aβ, amyloid β; IAPP, islet amyloid polypeptide; AChE, acetylcholinesterase; APOE-ε4, apolipoprotein E-ε4.

to determine the actual applicability of IDE inhibitors in the treatment of T2D, particularly focusing on potential amyloidogenic effects that would trigger the development of AD in such an aggregating-prone pathological environment.

7. TUNING APPROACHES FOR TREATMENT AND PREVENTION

From the classic biguanide metformin (**1**), to the recently Food and Drug Administration (FDA)-approved sodium-glucose cotransporter-2 (SGLT-2) inhibitor empaglifozin (**6**), orally available small-molecule antidiabetic agents are daily used worldwide for the control of T2D[255]—some of which are represented in Table I. GLP-1 analogues such as liraglutide, albiglutide, or dulaglutide are injectable alternatives,[256,257] along with long-acting insulin analogues such as insulin degludec or insulin detemir used in more advanced disease stages.[258]

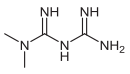
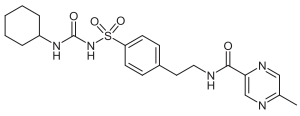
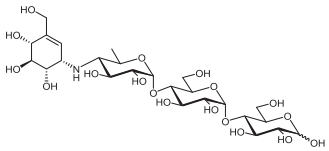
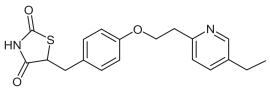
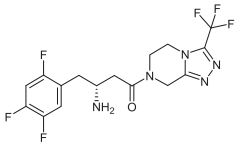
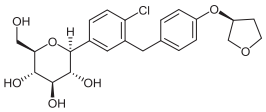
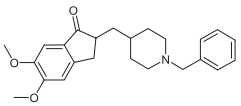
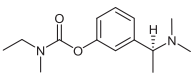
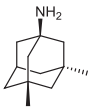
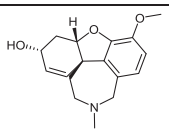
The complex etiology of T2D and the clinical diversity among patients have led to the use of combinations of some of these drugs, together with lifestyle changes regarding diet and exercise.[277–281] However, many of the most commonly used drugs have several side effects.[282] Despite the seemingly good results in clinical trials, approved dipeptidyl peptidase 4 (DPP4) inhibitors have been reported to cause severe joint pain after 1 day to one or more years taking the drug.[283] Ketoacidosis and urinary tract infections have been associated with the use of SGLT-2 inhibitors,[284] while cases of pancreatitis were detected among patients treated with liraglutide, a GLP-1 analogue.[285]

Currently prescribed drugs for the management of AD mostly consist of AChE or both AChE and butyrylcholinesterase (BChE) inhibitors—including donepezil (**7**) and rivastigmine (**8**)—that cause an increase in ACh brain levels, but allow only temporary symptomatic relief[16] (Table I). The glutaminergic *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine (**9**, Table I) is prescribed in latter stages of AD, but similarly to cholinesterase inhibitors, there is no robust evidence of disease-modifying effects.[286] Yet, the very recent discovery that aducanumab significantly reduces brain Aβ plaques in patients with prodromal or mild AD in a dose- and time-dependent manner suggests that new and more effective therapies may be available in the near future.[287] This human monoclonal antibody is currently being tested in phase III clinical trials and, indeed, early diagnosis and therapy might be key when trying to implement disease-modifying therapies for AD.

Based on the shared pathological background of T2D and AD, much effort is also being put into uncovering potential benefits of well-known and commonly used antidiabetic drugs in the treatment and prevention of the latter. On the one hand, metformin (**1**) is associated with increased risk of cognitive decline,[288,289] possibly through oxidative stress-induced increase in AβPP expression and processing. Yet, this effect was reversed by the action of insulin and antioxidant compounds such as curcumin,[290] thus encouraging the concomitant use of such molecules together with prescribed metformin therapy. On the other hand, it has recently been shown that metformin exerts neuroprotective effects, being able to prevent scopolamine- and cisplatin-induced cognitive impairment and brain damage in mice, while reducing BACE-1 activity.[291–294]

Although a pilot trial conducted in patients with amnestic mild cognitive impairment (AMCI) did not reach solid conclusions on whether metformin might be used to effectively control the progression of prodromal AD, by running a larger trial the real effects of long-term metformin treatment in type 2 diabetic patients may be clarified.[295] Thiazolidinediones such as pioglitazone (**4**) might also have intrinsic neuroprotective and anti-inflammatory effects, and the ability to increase brain insulin sensitivity.[296–298] Randomized and open-controlled trials conducted so far reported cognitive and functional improvements led by pioglitazone in type 2 diabetic patients with mild cognitive impairment or AD,[299,300] thus validating this parallel

Table I. Representative Examples of FDA-Approved Small-Molecule Drugs Differing in Terms of Structural Classes/Mechanism of Action for the Treatment of Type 2 Diabetes or AD

Drug	Class	Structure	Effect	References
TYPE 2 DIABETES				
Metformin (1)	biguanide		Suppressed gluconeogenesis, increased peripheral insulin sensitivity, increased peripheral glucose uptake	259 - 261
Glipizide (2)	sulfonylurea		Increased glucose-stimulated insulin secretion, increased peripheral insulin sensitivity, suppressed gluconeogenesis	262, 263
Acarbose (3)	α-glucosidase inhibitor		Decreased intestinal glucose absorption, increased peripheral insulin sensitivity	264, 265
Pioglitazone (4)	thiazolidine-dione		Enhanced insulin signalling, increased peripheral glucose uptake, improved lipid metabolism, suppressed gluconeogenesis	266, 267
Sitagliptin (5)	DPP-4 inhibitor		Increased glucose-stimulated insulin secretion, suppressed gluconeogenesis	268, 269
Empagliflozin (6)	SGLT-2 inhibitor		Reduced renal glucose reabsorption	270
ALZHEIMER'S DISEASE				
Donepezil (7)	Selective AChE inhibitor		Enhanced acetylcholine brain levels	271
Rivastigmine (8)	AChE and BChE inhibitor		Enhanced acetylcholine brain levels	272, 273
Memantine (9)	NMDA receptor antagonist		Reduced NMDA receptor-mediated excitotoxicity by inhibiting prolonged Ca^{2+} neuronal influx	274
Galantamine (10)	Alkaloid		Enhanced acetylcholine brain levels, potentiation of nicotinic acetylcholine activity, attenuation of Aβ deposit formation and neuroinflammation	275, 276

therapeutic and preventive strategy. Long-acting intranasal insulin detemir has also improved cognition in APOE-ε4 carriers with mild cognitive impairment or early AD stage, but the molecular mechanism by which the APOE-ε4 modulates the effect of the insulin analogue is still to be elucidated.[301] Similarly, liraglutide was recently found to prevent chronic inflammation, pathology-specific tau phosphorylation, and the formation of beta-amyloid deposits in the brain, while improving memory impairment in mice.[302–304] A controlled, randomized double-blinded trial to investigate the effect of liraglutide in patients with AD is currently ongoing.[305]

Conversely, the natural compound galantamine (**10**)—another FDA-approved drug for AD (Table I)—has just been pointed out as a potential antidiabetic agent. Indeed, besides the well-known cholinesterase inhibitory activity, galantamine was found to decrease plasma glucose and insulin levels, while improving insulin signaling pathways, lipid metabolism, and β-cell function.[306] These data, on top of its antioxidant, anti-inflammatory and antiapoptotic activity, makes galantamine an excellent example of how nature can be explored toward the discovery of multitarget drug-like molecules. This is particularly relevant in the case of T2D and AD therapeutics due to their complex and multifactorial etiology.

In spite of the drugs in use, an enormous effort has been made by the scientific community aiming at the discovery of highly efficient new molecular entities devoid of major side effects. In the next section, robustly studied lead molecules from natural origin are presented and their mechanisms of action discussed.

8. UNVEILING NATURAL LEADS

A. Resveratrol

This stilbenoid (**11**, Fig. 6) is abundantly present in grapes, blueberries, raspberries, and mulberries, and has been extensively studied regarding its exceptional cardioprotective, anti-inflammatory, and antioxidant activities.[307] In the past decade, resveratrol was found to remodel Aβ soluble oligomers and fibrils into nontoxic aggregates, and to stimulate proteasome-mediated Aβ clearance.[308, 309] It is believed that resveratrol can also exert neuroprotective effects in vascular dementia, having the ability to relieve oxidative stress-induced ischemia in rat hippocampus, thus protecting the integrity of the BBB.[310–312] Additionally, this natural product is capable of preventing pyramidal cell death in the hippocampus, while restoring both functional and structural synaptic deficits caused by chronic cerebral hypoperfusion in rats.[313–315] Similarly to Aβ, resveratrol can also remodel IAPP cytotoxic aggregates into off-pathway species.[316] Recent data resulting from a molecular dynamics study suggests that this compound is inserted in the hydrophobic pocket resulting from the interaction between two IAPP molecules, and also prevents IAPP interaction with negatively charged membranes by forming complexes with specific regions of the amyloid polypeptide.[317] Another interesting study involving mutational analysis established the importance of His18 and both Phe15 and Tyr37 aromatic amino acid residues—which are part of the core structure of the IAPP fibril—as crucial for the described interaction.[318] This is in agreement with the generally accepted hypothesis that aromatic–aromatic interactions are on the basis of the amyloid self-assembly process,[318–320] and that π–π stacking between polyphenolic aromatic rings and aromatic side chain amino acids of amyloid proteins may be of great importance in the disruption of their characteristic lamellar cross-β-sheet structures.[57, 321] In addition, the phenolic hydroxy groups of resveratrol may further enhance the stability of the inhibitor-protein complex through the establishment of hydrogen bonds.[321] Since the type of interactions that promote the formation of cross-β-sheet quaternary

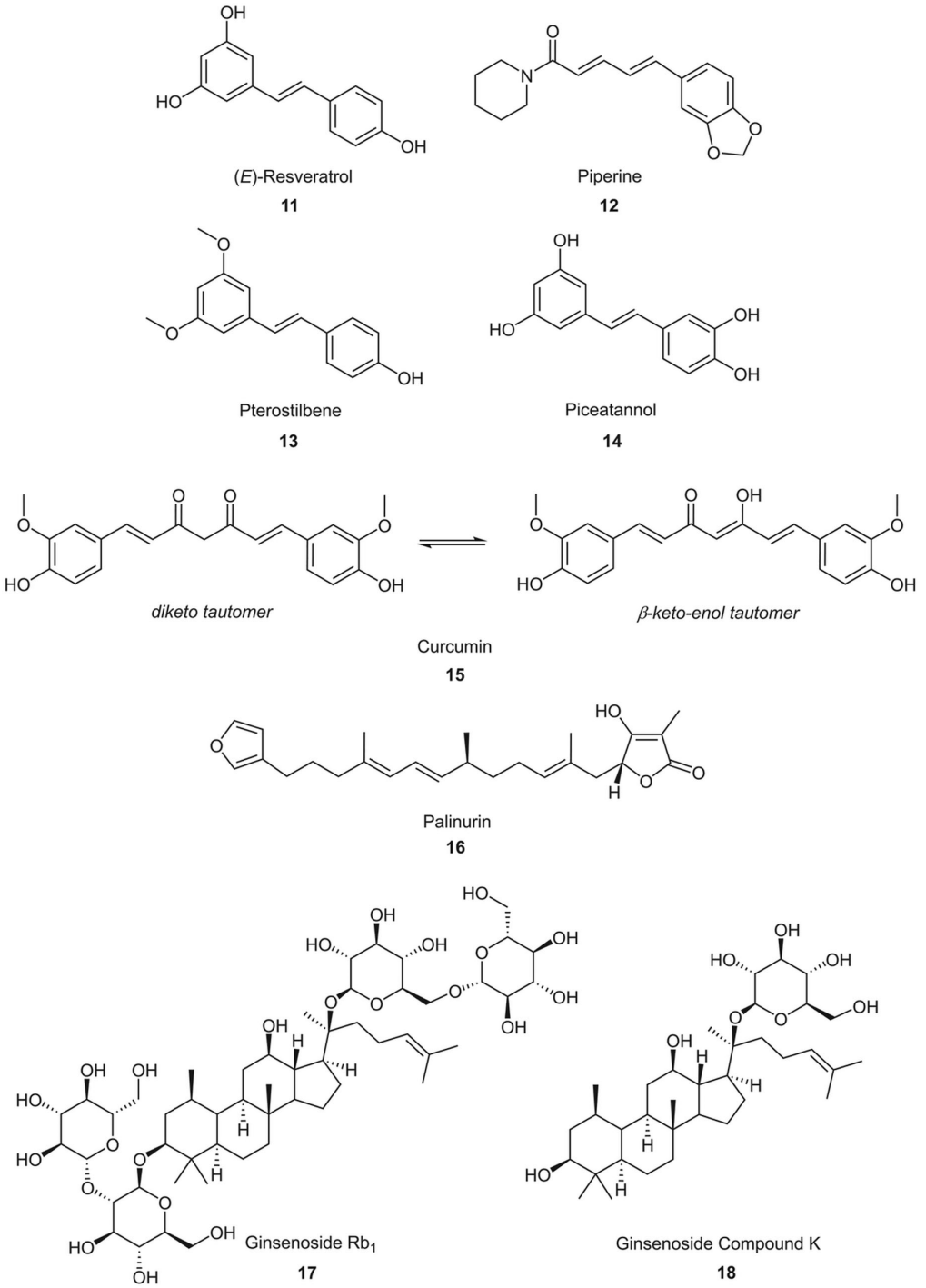


Figure 6. Chemical structure of natural compounds **11–18** with potential as therapeutic leads against diabetes-induced cognitive decline.

structures throughout the misfolding process seems to be common to all amyloid polypeptides, these structural requirements may apply to the inhibition of both Aβ and IAPP.[321,322]

Inhibition of IAPP fibrillization is most likely just one of the antidiabetic mechanisms by which resveratrol acts in humans. Indeed, when tested in diabetic rat models, resveratrol was able to reduce hepatic RAGE expression and plasma glucose levels,[323] at the same time enhancing peripheral insulin sensitivity.[324–326] In clinical trials, short-term resveratrol supplementation (1 g/day for 45 days) significantly decreased systolic blood pressure, fasting plasma glucose levels, glycated hemoglobin (A1c), insulin, and insulin resistance in type 2 diabetic patients already receiving antidiabetic medication when compared to the placebo group.[327] A similar study reporting the effects of long-term supplementation with resveratrol (250 mg daily for 3 months) in combination with metformin was concordant, thus supporting the potential of resveratrol as adjuvant in antidiabetic therapies.[328] Remarkably, a dosage of only 5 mg twice a day for 4 weeks improved insulin sensitivity in type 2 diabetic patients; however, it had no significant effects in β-cell function,[329] even though the ability of resveratrol to enhance β-cell function by decreasing the expression of several PDE isoforms has recently been confirmed.[330] Furthermore, 50 mg of resveratrol twice a day for 60 days reduced the size of foot ulcers[331]—a severe complication of diabetes that, when poorly managed, can eventually lead to lower limb amputation. Alternatively, a recent paper reported the lack of success to improve glycemic control in patients with T2D managed by diet and receiving 500 mg of resveratrol twice daily over 5 weeks, with no significant effects in GLP-1 secretion, gastric emptying, or energy intake.[332] However, the study only included nonobese patients, and thus might not reflect the tangible antidiabetic effects of resveratrol in obese patients with poorly controlled T2D.

Resveratrol is currently being tested in a number of phase II and III clinical trials for T2D, which aim to evaluate the impact of both short- and long-term supplementation on glycemic control, inflammation, endothelial and mitochondrial function, intrahepatic and cardiac lipid content, among other key metabolic parameters.[333] A few other ongoing trials—including a phase IV trial—are focusing on the effects of resveratrol on mild to moderate AD. Results of a completed randomized, double-blind controlled phase II trial suggest that 500 mg of resveratrol once daily (with dose escalation over 13 weeks to 2 g/day) may indeed revert some pathological features of AD, such as low CSF and plasma $A\beta_{1\text{-}40}$ levels, although no significant changes in $A\beta_{1\text{-}42}$ or hyperphosphorylated tau were observed, neither insulin metabolism nor hippocampal volume were altered.[334]

Despite being able to permeate the BBB,[335] the rather low bioavailability of resveratrol due to rapid intestine and liver metabolism is pointed out as a thoughtful activity limiting issue in vivo.[336,337] Indeed, this could justify the above described inability of resveratrol to revert major hallmarks of AD. The 3-*O*-glucuronide and 3-*O*-sulfate conjugates were identified as the most abundant resveratrol metabolites, while free *trans*-resveratrol virtually undetectable in rodent urine and plasma samples.[338] Even though it has been suggested that in vivo β-glucuronidase and sulfatase enzymes might increase the intracellular concentration of resveratrol at specific target sites,[337,339] they may not compensate for the loss of effect at a systemic level.

To overcome the bioavailability issue, the combined administration of resveratrol with piperine (**12**, Fig. 6) was tested in rats and caused a 15-fold increase in maximum plasma concentrations, possibly by inhibiting its glucuronidation in the gastrointestinal tract,[340] which strongly encourages further investigation on the therapeutic outcome of this strategy. The synthesis of bioavailable resveratrol analogues—not as susceptible to conjugation but capable of retaining the desired activity—also comes across as an attractive approach for taking advantage of the full therapeutic and preventive potential of the natural lead structure. Interestingly, two natural resveratrol analogues also found in grapes and berries, pterostilbene (**13**) and piceatannol (**14**) also depicted in Figure 6, display strong antidiabetic and

neuroprotective activities as well,[341–347] and thus may lead the way into the synthesis of optimized
resveratrol-based bioactive compounds. In particular, pterostilbene was considered an even more potent modulator of cognitive function and cellular stress than resveratrol itself, and demonstrated improved bioavailability when compared to resveratrol, associated with higher plasma and brain concentrations.[348] Both methoxy groups in positions 3 and 5 enhance the lipophilicity of pterostilbene but make it less susceptible to glucuronidation, although it is still converted into the 4′-*O*-sulfate conjugate to a major extent.[349] Notwithstanding, these data provide important clues for structural optimization of resveratrol, which shall be explored in this review.

B. Curcumin

Curcumin (**15**, Fig. 6), a natural diphenylheptanoid occurring as an interconvertible mixture of diketo and β-keto-enol tautomers, is the major curcuminoid found in *Curcuma longa* L. rhizomes, commonly known as turmeric, and is responsible for its characteristic yellow color.[350] Turmeric has been used in Ayurveda, Unani, and Siddha medicine in the treatment of hepatic and biliary disorders, diabetic wounds, among others maladies.[351] Based on the traditional applications, curcumin has been extensively studied in the past few decades, reported as an extremely potent antioxidant[352] with the capacity of preventing inflammation and insulin resistance.[353] Indeed, it is seemingly able to protect pancreatic β-cells from apoptosis and to enhance glucose-induced insulin secretion.[354] In STZ-induced diabetic rats, curcumin caused a significant decrease in fasting plasma glucose, while increasing insulin levels, and induced a significant upregulation of pancreatic CREB and phospholipase C, at the same time maintaining normal cAMP and cGMP levels in the pancreas.[355] This can be explained by its PDE inhibitory activity.[330]

Similarly to resveratrol, curcumin—a brain permeable compound[356]—is able to reorganize Aβ aggregates into nontoxic off-pathway species,[357] while significantly reducing the formation of human IAPP amyloid fibrils.[358] Furthermore, it was found to attenuate diabetic-induced apoptosis in retinal neurons[359] and, accordingly, it was also able to prevent the activation of proapoptotic signaling pathways in primary hippocampal neuron cultures treated with endothelin-1 (ET-1)—a vasoconstrictor peptide produced by endothelial cells that is increased in the brain of AD.[360] In a recent study, curcumin exhibited neuroprotective effects in an AD mouse model that manifested in improved active avoidance and locomotor activity.[361] Moreover, it is a powerful GSK-3β inhibitor, with an IC_{50} of 66.3 nM.[362] The previously mentioned role of GSK-3β as a key player in a variety of processes such as insulin action, cell division, apoptosis, and tau phosphorylation turns this enzyme into a suitable molecular target for T2D and associated neurodegenerative disorders, in particular AD.[363] This is supported by tests in mice with high-fat diet-induced cognitive impairment, showing that GSK-3β inhibition improves insulin brain levels and increases brain-derived neurotrophic factor (BDNF), which may be on the basis of enhanced synaptic plasticity and the observed improvements in memory consolidation.[364]

At least two clinical trials envisaged to evaluate the benefits of curcumin administration to type 2 diabetic patients are currently in progress.[365] To this point, significant improvements in β-cell function and peripheral insulin sensitivity were found in prediabetic patients when 750 mg of curcumin were administered twice a day, for 9 months. Moreover, while 16.4% of the placebo group developed T2D, none of the patients supplemented with curcumin were diagnosed with this condition by the end of the 9 month period,[366] thus supporting the use of this compound in the clinic. Nevertheless, poor plasma solubility with consequent low

bioavailability has been pointed out as a plausible explanation for limited efficacy of curcumin in clinical trials for AD.[367–369] In order to address this issue, several water-soluble curcumin analogues with improved pharmacokinetic properties have been developed, and some of the most promising ones will be presented in the next section.

C. Palinurin

Marine organisms are rich sources of lead scaffolds with a wide range of interesting bioactivities.[370] Palinurin is one of such scaffolds: this linear furansesquiterpene (**16**, Fig. 6) was first isolated from the Mediterranean marine sponge *Ircinia variabilis* in 1979,[371] and was recently found to be an allosteric GSK-3β inhibitor.[370]

On the basis of preliminary studies suggesting a non-ATP-competitive inhibition mechanism, palinurin was further studied in 2013 to explore the type of interaction established with the target enzyme.[370] Indeed, with an IC_{50} of 1.9 μM, it binds to an allosteric site located at the N-terminal lobe of GSK-3β—known as the binding pocket no. 5—and creates structural constraints that condition the accessibility of the ATP γ-phosphate to the substrate-binding site. The tetronic unit of palinurin was found to be much more specifically bound to the enzyme than the hydrophobic tail, with interactions between the carbonyl oxygen and Tyr56, and between the deprotonated hydroxy group and Lys86. This peculiar binding mode is seemingly responsible for the selectivity of the compound towards other kinases such as casein kinase 2 (CK2) or cyclic-dependent kinase 7 (CDK5) with $IC_{50} > 100$ μM or > 25 μM, respectively, since in these cases one of the key amino acid residues is mutated, thus compromising the described specificity toward the tetronic unit.

Due to its high affinity toward the allosteric site of GSK-3β, palinurin comes across a very promising starting point for future development. Together, the above data provide important hints for rationally planned modifications in the original scaffold with the purpose of increasing its potency against the therapeutic target, which ought to focus on affinity enhancement between the hydrophobic chain and the allosteric binding site.

D. Ginsenosides

Ginsenosides are a class of dammarane-type triterpene glycosides differing in both sugar composition and placement that are found in *Panax ginseng*, broadly used in Chinese traditional medicine for its nootropic and antiageing effects.[372] Although the neuroprotective activities of ginsenosides have been widely studied, it was not until the past few years that these compounds started to get attention for their antidiabetic potential. In fact, among the most promising molecules, ginsenoside Rb1 (**17**, Fig. 6) was found to significantly reduce fasting plasma glucose levels, while decreasing hepatic fat accumulation and insulin resistance markers in obese rodents.[373,374] This compound promotes GLP-1 release, thus enhancing glucose-stimulated insulin secretion by β-cells through the activation of cAMP/PKA/CREB signaling in vitro, which has been pointed out as a key mechanism involved in its antidiabetic activity.[375,376] Moreover, it significantly attenuates neuroinflammation,[377,378] Aβ-induced neurotoxicity, and tau hyperphosphorylation in cortical neurons.[379,380]

Interestingly, although ginsenoside Rb1 is believed to have increased oral exposure in diabetic mice via enhanced intestinal permeability and decreased deglycosylation by the intestinal microflora,[381] ginsenoside compound K (**18**, Fig. 6) is also perceived as a very promising lead for the treatment of diabetes-associated cognitive decline. Indeed, it exerts significant antihyperglycemic effects, which are believed to be related to an ability to restore insulin signaling through the upregulation of insulin receptors and its downstream effectors PI3K and Akt, with consequent induced expression of GLUT-4 glucose transporters.[382] In addition, it is able to

upregulate the expression of the low-affinity glucose transporter GLUT-2 in β-cells, which is known to be directly involved in the glucose-stimulated insulin secretion process due to its role as a "glucose sensor."[383,384] Ginsenoside compound K was also found to protect pancreatic β-cells against apoptosis[385] and to stimulate the intestinal release of GLP-1 in vitro,[386] while inhibiting the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase)—two important hepatic enzymes involved in gluconeogenesis.[387]

Ginsenoside compound K can also promote Aβ clearance in vitro,[388] and was shown to exert anti-inflammatory and neuroprotective effects in a rodent model of cerebral ischemia.[389] Even though it has not been as extensively studied as ginsenoside Rb1, these reports demonstrate that in vivo enzymatic transformation of ginsenoside Rb1 into compound K, the major metabolite of all known ginsenosides able to reach the systemic circulation,[390] may not necessarily be a disadvantage.

E. O- and C-Glycosyl Flavonoids

Although natural flavonoids are commonly known for their antioxidant properties, they have also been associated to major cardioprotective, antidiabetic, antiamyloidogenic, anti-inflammatory, and AChE inhibitory effects,[16,391–394] thus being acknowledged for their utility against diabetes and related neurodegenerative disorders. They can occur as aglycones or as the corresponding glycosylated forms—either as *O*-glycosides or *C*-glycosyl derivatives; yet, the relevance of the sugar moiety in the amyloid fibril disruption process makes glycosylated flavonoids preferable when considering amyloid polypeptides such as Aβ or IAPP as appropriate therapeutic targets.[391] Moreover, sugar derivatives exhibit often desirable pharmacokinetic properties associated with enhanced water solubility. Although several *O*-glycosylflavonoids such as (-)-3-*O*-(β-D-allosyl)epicatechin (**19**), 7-*O*-(β-D-glucosyl)apigenin (**20**), or 3-*O*-(β-D-glucosyl)cyanidin (**21**) (Fig. 7) have been associated to major antidiabetic,[395] antiamyloidogenic,[391] and neuroprotective effects,[396,397] *C*-glycosyl derivatives have received growing attention for their insusceptibility to in vivo hydrolysis by glycosidases. Moreover, they generally exhibit stronger antioxidant and antidiabetic activities than the corresponding *O*-glycosides and aglycones, making them more appealing scaffolds for further development.[398]

Vitexin (**22**) and isovitexin (**23**) (Fig. 7)—the *C*-glucosyl derivatives of apigenin in positions 8 and 6 of the flavone scaffold, respectively—are naturally occurring compounds in edible plants such as Mung beans (*Vigna radiata*) or bamboo leaves (*Phyllostachys nigra*).[399,400] The methanol extract of *Ficus deltoidea* leaves, which is also enriched in both compounds, displayed major antidiabetic effects when administered to SZT-induced diabetic rats, including plasma glucose reduction, increased insulin secretion, and reduced gluconeogenesis through the inhibition of PEPCK and G6Pase hepatic enzymes. Furthermore, it enhanced the expression of GLUT-4 glucose transporters in skeletal muscles.[401] However, the potency of these compounds is divergent and seemingly dependent upon the glycosylation pattern: in fact, when tested separately in sucrose-loaded induced diabetic mice, isovitexin significantly reduced postprandial glucose levels when administered orally at 20 mg/kg, while vitexin required a higher dose (50 mg/kg) to achieve a similar effect. They were both inhibitors of α-glucosidase with IC_{50} values of 4.1 and 6.7 μg/mL, respectively[402]—which is correspondingly equivalent to 9.48 and 15.50 μM—and they were both inhibitors of the formation of AGEs in vitro.[403]

So far, it is not known whether vitexin and isovitexin exert antiamyloidogenic effects toward Aβ, although both aglycone apigenin and 7-*O*-(β-D-glucosyl)apigenin do exhibit those properties.[391] Nevertheless, they are active against AChE and BChE—with IC_{50} values ranging from 6.24 μM to 12.16 μM—and vitexin, in particular, is also able to inhibit BACE-1, with an IC_{50} of 51.07 μM. Notably, the presence of the sugar moiety makes the difference in these activities, since apigenin was a far worse AChE and BChE inhibitor (IC_{50} = 34.43 and 29.11 μM,

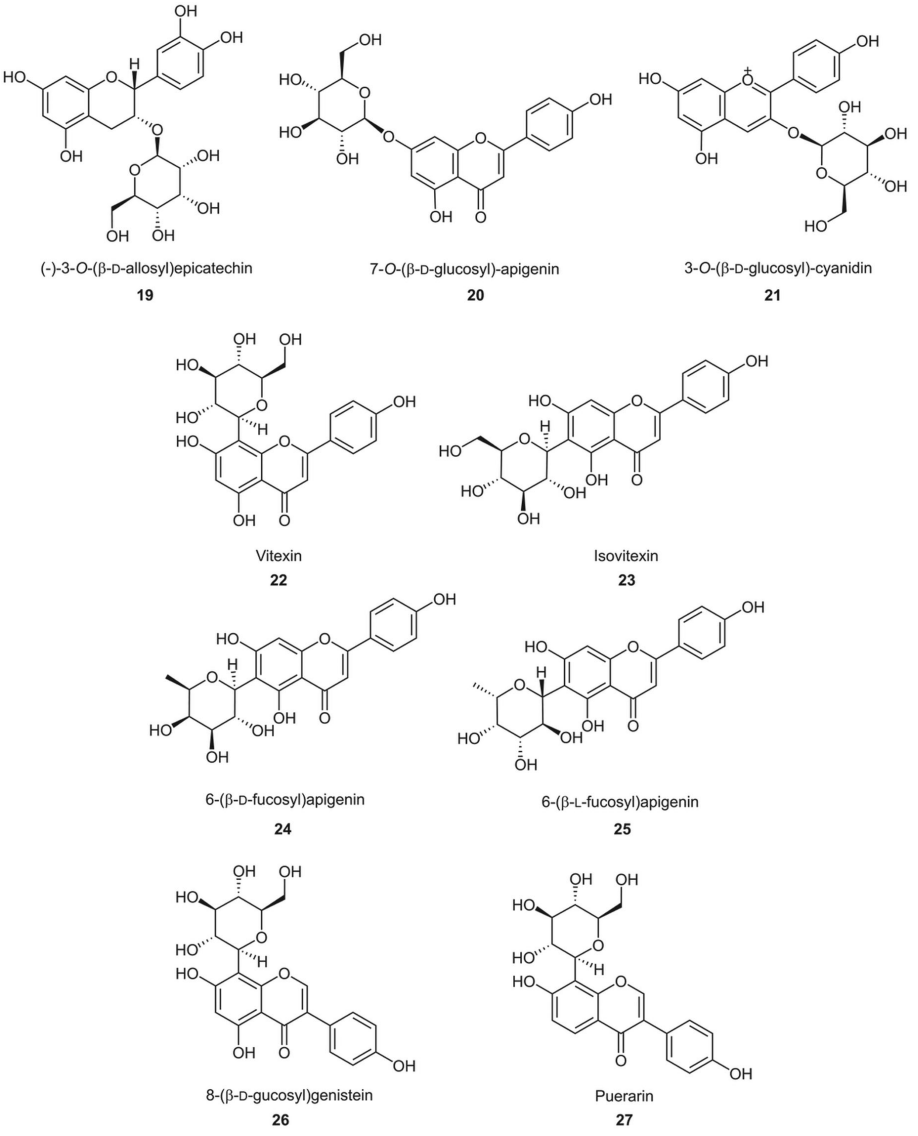


Figure 7. Chemical structure of glycosylflavonoids **19–27** with antidiabetic, antiamyloidogenic, and/or neuroprotective effects.

respectively) and was not able to inhibit BACE-1 to a significant extent ($IC_{50} > 100\ \mu M$).[404] Again, in PC12 cells, the protective effects of vitexin against Aβ-induced toxicity were more significant than those of isovitexin.[405] It would be interesting to assess if these *C*-glucosyl derivatives exert synergistic effects, which—if so—would support the development of an optimized combination of both compounds for further evaluation.

Another pair of *C*-glycosyl apigenin derivatives, 6-(β-D-fucosyl)apigenin (**24**) and 6-(β-L-fucosyl)apigenin (**25**) (Fig. 7) isolated from *Averrhoa carambola*, also showed antidiabetic potential. When administered to hyperglycemic Wistar rats (50 mg/kg), both flavonoids significantly reduced plasma glucose levels.[406,407] The D-fucosyl derivative promoted glucose uptake in soleus muscle, and led to a substantial increase in glycogen levels in muscle and liver tissues by enhancing cellular insulin signaling pathways. This suggested that this compound is somehow

able to mimic insulin actions.[406] The enantiomer 6-(β-L-fucosyl)apigenin was found to induce muscular glycogen synthesis in normoglycemic rats, involving the inactivation of GSK-3. Moreover, it enhanced glucose-induced insulin secretion.[407] Whether both enantiomers exert similar or complementary antidiabetic effects still needs to be clarified. This information would provide new clues about the importance of sugar configuration in the bioactivity of both apigenin *C*-fucosyl derivatives.

The 4′,5,7-trihydroxyisoflavone analogue to apigenin is genistein, which *C*-glucosyl derivative in position 8, 8-(β-D-glucosyl)genistein (**26**, Fig. 7), is the major component of the Portuguese plant *Genista tenera* ethyl acetate extract, recently revealed to be an extremely potent antidiabetic agent.[408] The extract did not show toxicity toward human lymphocytes when tested at 2 mg/mL. Moreover, in addition to its antioxidant effect, it was found to inhibit G6Pase and α-glucosidase to a higher extent than acarbose (**3**, Table I), while reducing AChE activity to 19% at 2.31 μM, a tenfold lower concentration than that needed for rivastigmine to inhibit the enzyme in the same assay.[409,410]

8-(β-D-glucosyl)genistein was able to normalize plasma glucose levels of STZ-induced diabetic Wistar rats within 7 days of treatment (4 mg/kg/day). Moreover, it increased insulin circulation and potentiated glucose-induced insulin secretion, which is suggestive of β-cell regenerating effects. Remarkably, the compound was able to inhibit human IAPP fibrillization, virtually preventing its aggregation into toxic amyloid oligomers—even prior to insoluble fibril formation. Additionally, nuclear magnetic resonance (NMR) studies pointed toward the same binding epitope in the presence of IAPP and A$\beta_{1\text{-}42}$ polypeptides, suggesting potential neuroprotective effects as well.[408] The results confirmed the already expected key role of both aromatic rings in the resulting interaction, and reinforced the importance of the sugar moiety in the antiamyloidogenic activity of this compound.

Puerarin (**27**, Fig. 7) is a direct analogue of 8-(β-D-glucosyl)genistein, only lacking a hydroxy group at position 5. It is abundantly found in *Pueraria lobata* and has also been found to exert major antidiabetic effects in STZ-induced mice, with the ability to upregulate insulin expression and stimulate β-cell survival.[411–413] It furthermore improved the learning and memory functions of these animals by inhibiting AChE activity, while reducing inflammatory cytokines and oxidative markers in the hippocampus,[414] which is indicative that the compound is able to cross the BBB and act in the CNS. The only published report comparing the bioactivity of 8-(β-D-glucosyl)genistein and puerarin indicates that the latter is more effective at enhancing glucose uptake into 3T3-L1 adipocytes,[415] even though this ought to be one among many other mechanisms by which both of these multitarget compounds exert their therapeutic effect.

It is clear that nature is definitely a vast and valuable source of lead compounds displaying exceptional therapeutic profiles against T2D and AD. The nutraceutical and pharmaceutical development of such structures should be highly encouraged, which may involve chemical modification in order to achieve better pharmacokinetic profiles and higher selectivity toward desired molecular targets, as above highlighted.

9. STRUCTURE–ACTIVITY RELATIONSHIPS AND STRUCTURAL OPTIMIZATION OF NATURAL LEADS

A. Resveratrol Analogues

In order to assess the importance of hydroxylation pattern in the protective effects of resveratrol against obesity and T2D, a small library of resveratrol analogues was developed in 2013, including monohydroxy, dihydroxy, and trihydroxy derivatives of the stilbene skeleton.[416] 3-*trans*-hydroxystilbene **28** (Fig. 8) stood out from the group for its ability to reduce adipocyte

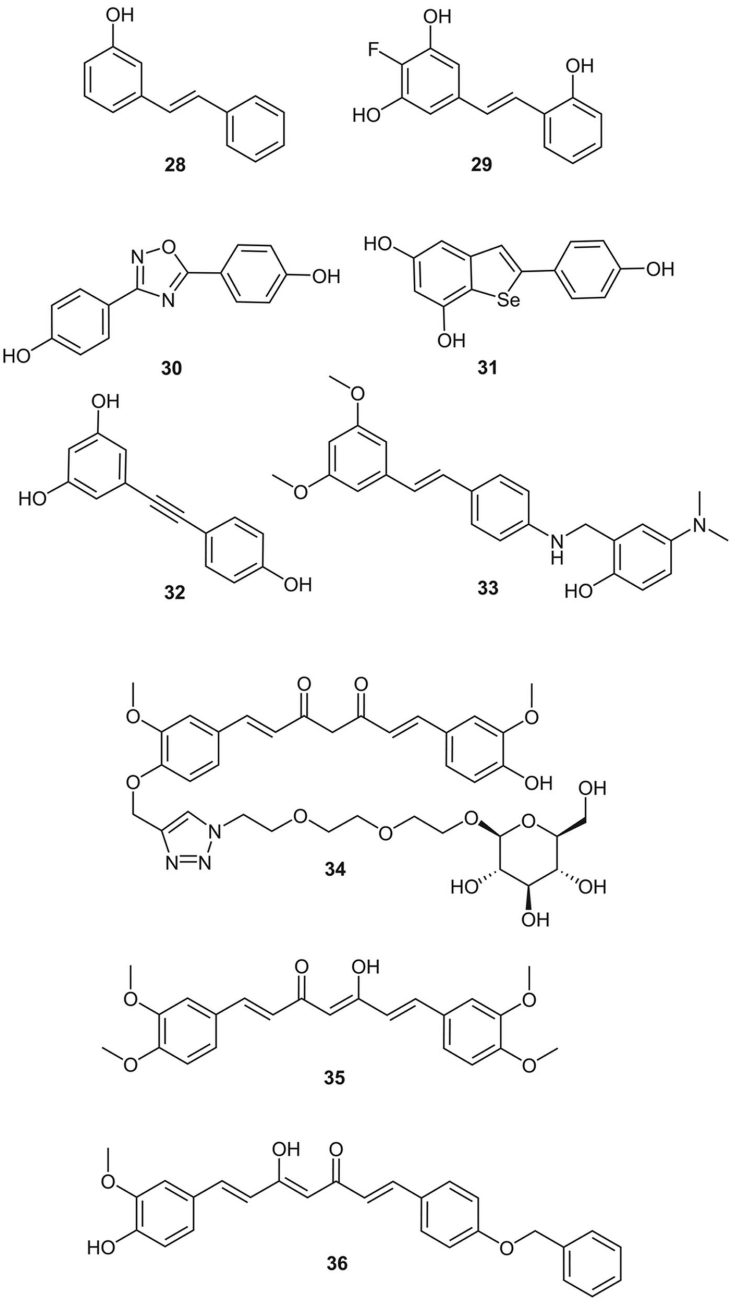


Figure 8. Chemical structure of resveratrol analogues **28–33** and curcumin analogues **34–36**.

differentiation, while increasing glucose uptake in insulin-resistant C2C12 skeletal muscle cells in the presence of insulin, resulting in enhanced insulin sensitivity, glucose tolerance, and reduction of obesity in vivo. Like resveratrol, compound **28** activates 5′-AMP-activated protein kinase (AMPK), which is involved in GLUT-4 translocation to the cell surface,[417,418] but whereas resveratrol was associated to genotoxic effects in mammalian cells, compound **28** was not.[419] Together, these data are indicative of the importance of the hydroxy group in

position 3 for the antidiabetic activity of resveratrol, but not in position 4′, which is related to its antioxidant effects due to the olefinic double bond contribution to resonance stabilization of the phenoxyl radical. This suggests that the therapeutic potential of resveratrol is much more related to its chemical structure than to its antioxidant activity alone, for which it is commonly known. Another study reported the synthesis of several resveratrol analogues as AChE and BChE inhibitors with potential to alleviate the symptoms of AD.[420] Compound **29** (Fig. 8) carrying an additional fluorine atom in position 4 was interestingly found to inhibit BChE in a selective way ($IC_{50} = 0.01$ mM vs. $IC_{50} = 0.94$ mM for AChE), which was attributed to the hydroxy group in position 2′, responsible for the affinity toward BChE. According to the previous study, since compound **29** maintains the hydroxy group at position 3, it would be interesting to assess whether it can exert antidiabetic effects similar to those found for compound **28**.

Over the past few years, other resveratrol analogues with more extensive structural modifications have been synthesized and evaluated. For instance, the 1,2,4-oxadiazole derivative **30** depicted in Figure 8 exhibited higher antioxidant and anti-inflammatory activities than resveratrol, being able to prevent the interaction of nuclear factor κB (NF-κB) with DNA through the formation of a hydrogen bond and cation–π interactions between the central ring and Arg56 of p50, a DNA-binding residue.[421] Moreover, while compound **31** exhibited enhanced antioxidant activity attributable to selenium-induced reduction of O–H bond dissociation of hydroxy groups in positions 3 and 5,[422] analogue **32** displayed higher anti-inflammatory activity but lower scavenging capacity due to the presence of the central triple bond.[423] Another analogue, compound **33**, resulted from the combination of pterostilbene (**13**) and the pharmacophore moiety of clioquinol, a well-known metal chelator that improved cognition in patients with AD in a phase II clinical trial.[424,425] This nontoxic and centrally penetrant compound was conceived based on studies reporting the role of Fe, Cu, and Zn ions in the promotion of oxidative stress and Aβ aggregation,[426–429] and was indeed able to significantly inhibit self and Cu(II)-induced $A\beta_{1\text{-}42}$ fibrillization.[424] To this point, none of these four compounds have been tested for antidiabetic effects, notwithstanding their potential as multitarget compounds against diabetes-induced cognitive decline.

B. Curcumin Analogues

Aiming at addressing bioavailability issues, the synthetic curcumin derivative **34** (Fig. 8) was developed and succeeded at amplifying the already powerful activity of the original scaffold,[430] previously explored in this review. This water-soluble sugar conjugate is anticipated to reach the CNS via GLUT-1 transporters located at the BBB, and is 1000 times more potent against Aβ and tau aggregation, being active at 8 nM and 0.1 nM, respectively. Moreover, it is an even more powerful antioxidant than curcumin and, in fact, based on the antidiabetic effects exerted by the natural molecule, compound **34** is likely to have a strong therapeutic impact not only against AD, but against T2D as well.

A more recent study featuring several other curcumin synthetic analogues allowed the establishment of structure–activity relationships on the original scaffold based on their antidiabetic activity. Among all synthesized analogues resulting from modifications in the length of the linker segment, cyclization of the latter and changes hydroxyl or methyl substitution patterns, compound **35** (Fig. 8) was the most potent antihyperglycemic agent (100 μg/kg) in alloxan-induced diabetic Wistar rats.[431] According to this report, methylation of all phenolic groups does not prejudice the antidiabetic effects of the natural lead, but analogues lacking the original methoxy groups or only displaying free hydroxy groups are not as active in vitro, and are not active in vivo at all. Moreover, variations in the linker pattern of curcumin were not well tolerated: a 7-carbon linker bearing two oxo groups was found to be a better molecular pattern

than 5- or 9-cabon linkers bearing just one oxo group or a central aromatic ring, respectively. More flexible linkers were generally associated with enhanced glucose-reducing ability.

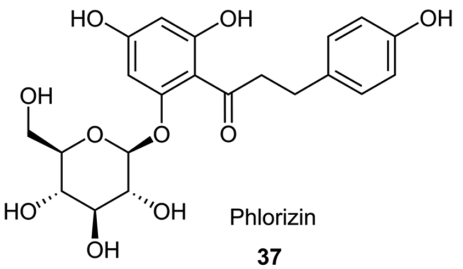
Another recent report presented a new curcumin-based potent BACE-1 and GSK-3β dual inhibitor **36** (Fig. 8).[432] With IC_{50} values of 0.97 μM and 0.90 μM, respectively, the compound was envisaged based on the potential of the β-keto-enol tautomer of curcumin to modulate both enzymes, namely by interacting with BACE-1 catalytic dyad composed by Asp32 and Asp228, and through the formation of a Michael adduct with GSK-3β, resulting from a Michael addition of Cys199 to the unsaturated ketone. Different moieties were introduced in the aryl side rings, but whereas bis-*para*-benzyloxyphenyl or *para*-methoxy substituted symmetric analogues were the most potent BACE-1 and GSK-3β inhibitors, respectively, compound **36** displayed a more balanced dual low-micromolar inhibition profile. This analogue could permeate the BBB, did not show significant neurotoxicity effects, and displayed moderate antioxidant activity. Similarly to compound **34**, its antidiabetic activity was not evaluated to this point, but the confirmed ability to inhibit GSK-3β along with the reminiscent curcumin scaffold is strongly indicative of the possibility of such effects, which should definitely be explored.

C. Dihydrochalcone Analogues

Selective inhibition of the high-capacity and low-affinity SGLT-2 transporter—which is responsible for 80–90% of renal glucose reabsorption from urine into the blood—has emerged as a promising therapeutic strategy against T2D.[433,434] Phlorizin (**37**, Fig. 9), a naturally occurring dihydrochalcone flavonoid found in apples, was first isolated in 1835 and has been extensively studied owing to its ability to competitively inhibit both SGLT-1 and SGLT-2.[435,436] Despite being a potent antidiabetic agent, this *O*-glycoside was found to be unsuitable for clinical use due to its low bioavailability and susceptibility to in vivo degradation, along with undesired gastrointestinal side effects related to lack of selectivity toward SGLT-2.[433,436,437] Notwithstanding, it has served as the basis for new antidiabetic therapies such as the early mentioned FDA-approved empagliflozin (**6**, Table I), along with other antihyperglycemic gliflozins selective for SGLT-2 with improved pharmacokinetic profile,[438] including dapagliflozin (**38**, Fig. 9) and canagliflozin (**39**, Fig. 9), also used in the clinic. However, these molecules are still associated with severe side effects, as recently announced by FDA,[284] thus encouraging further investigation on structural optimization of the lead scaffold.

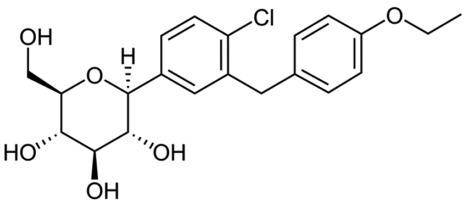
The novel ertugliflozin (**40**, Fig. 9)—a dapagliflozin (**38**) analogue—is currently being evaluated in phase III clinical trials in type 2 diabetic patients.[439] With an IC_{50} of 0.877 nM toward human SGLT-2 (vs. 1960 nM against SGLT-1), this compound (**40**) led to significant improvement in regard to glycemic control, blood pressure lowering, and body weight reduction.[440] In contrast with structural changes in the aglycone moiety applied in the generation of previous compounds that did not allow the maintenance of optimal drug-like physicochemical properties, the investigation behind the discovery of ertugliflozin **40** uncovered the tolerability of the sugar unit to modifications in position 5. Moreover, the dioxa-bicyclo[3.2.1] octane system enhanced the rigidity of the molecule, and had a strong impact in the potency and selectivity toward SGLT-2, at the same time allowing optimal safety and pharmacokinetic profile associated with low hepatic clearance.[440,441]

After the capacity of the dihydrochalcone scaffold to reduce Aβ-induced cytotoxicity has been disclosed,[442] further nootropic, neuroprotective, and neurotrophic effects were attributed to the natural lead,[443,444] thus highlighting its multitarget mode of action and ability to serve as a model for the treatment of diabetes-induced cognitive decline. Interestingly, in silico studies have suggested a potential role of gliflozins in the symptomatic relief of AD by inhibiting AChE.[445,446] Moreover, in addition to its known antihyperglycemic activity, empagliflozin (**6**) was found to ameliorate vascular injury and cognitive function of obese type 2 diabetic mice,



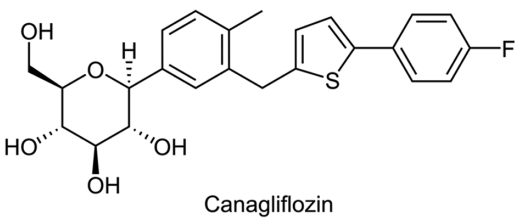
Phlorizin

37



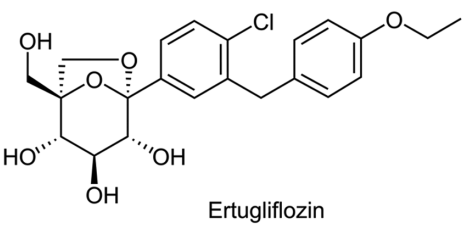
Dapagliflozin

38



Canagliflozin

39



Ertugliflozin

40

Figure 9. Chemical structure of phlorizin **37** and phlorizin-inspired *C*-glycosyl selective SGLT-2 inhibitors with antihyperglycemic activity: dapagliflozin **38**, canagliflozin **39**, and ertugliflozin **40**.

as a result of attenuated cerebral oxidative stress and increased BDNF.[447] Together, these data prospect the use of SGLT-2 inhibitors in the prevention of AD in type 2 diabetic patients, and reinforce the utility of natural compounds as leads for multitarget drug discovery and development.

Yet, the generation of potent and selective approaches is also possible through rational design based on structural information collected about molecular targets common to T2D and AD, and despite the multifactorial nature of both diseases—which seem to require wide-range acting therapeutic strategies—some of the most interesting small-molecule PDE and GSK-3β inhibitors under development will be presented in the next section.

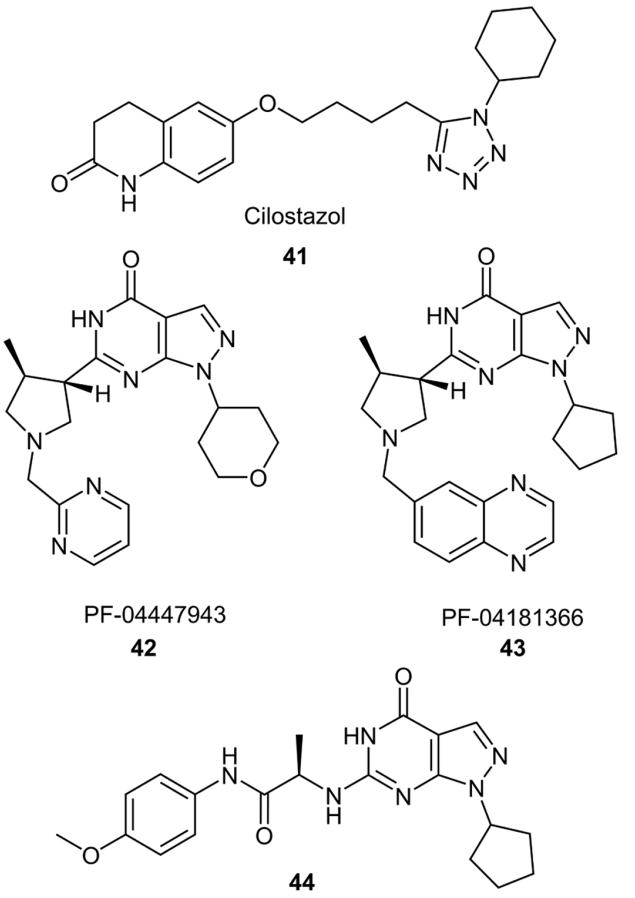


Figure 10. Chemical structure of PDE inhibitors **41–44** with potential against diabetes-induced cognitive decline.

10. RATIONAL DESIGN AND SYNTHESIS OF NOVEL MOLECULAR ENTITIES TACKLING COMMON THERAPEUTIC TARGETS OF T2D AND AD

A. PDE Inhibitors

Over the years, selective PDE inhibitors have been studied and developed for their potential as pharmaceutical agents. More than 20 human PDE genes encode for over a hundred PDE isoforms and, for instance, while PDE5, PDE6, and PDE9 specifically recognize cGMP, PDE4, PDE7, and PDE8 isoforms direct their hydrolytic capacity toward cAMP.[448] Moreover, whereas PDE3 seems to be involved in processes such as platelet aggregation and regulation of the antilipolytic effect of insulin,[449,450] PDE5 inhibition improves muscle microvascular blood flow and insulin-stimulated glucose uptake.[451,452] In addition, PDE9 inhibitors have shown to be have potential for the treatment of metabolic syndrome, with reported activities against insulin resistance, hyperglycemia, dyslipidemia, cardiovascular disease and obesity.[453–456]

Cilostazol (**41**, Fig. 10)—a quinolinone derivative—is a known PDE3 inhibitor approved by the FDA in 1999 for the reduction of symptoms of intermittent claudication,[457] and is here presented as a great example of how old and commonly used drugs could be a source of new therapeutic solutions. Indeed, cilostazol has recently been rediscovered as a promising agent against vascular dementia and even AD: it was found to prevent memory impairment

and cognitive deficits in mice by APOE-mediated Aβ accumulation, tau phosphorylation, and oxidative stress levels.[458,459] The compound was also regarded as a BBB-protective drug against cerebral ischemic injury[460] and, in rat models of chronic cerebral hypoperfusion, it was able to prevent white matter disintegration[461] and to improve diabetes-associated cognitive impairment.[209]

A pilot study published in 2013 confirmed the ability of this PDE inhibitor to prevent cognitive decline in patients with AD and, more recently, a retrospective analysis validated its beneficial effects in patients with mild dementia already receiving donepezil treatment.[462,463] With such positive results, cilostazol is currently being tested in a phase II clinical trial in patients with mild cognitive impairment, and in a phase III trial in type 2 diabetic patients, as encouraged by several other preclinical reports showing antihyperglycemic, insulin-sensitizing, anti-inflammatory, and endothelial protective effects associated with this compound.[464–469] The latest publications showing attenuating effects on the severity of lower limb diabetic ulceration and peripheral arterial occlusive disease in patients with T2D are additionally encouraging[470,471] and, together, these data support the use of cilostazol as a basis for new preventive and therapeutic approaches against diabetes-induced cognitive impairment through the selective inhibition of PDE3.

A more recently developed pyrazolopyrimidinone derivative—PF-04447943 (**42**, Fig. 10)—was conceived to selectively inhibit PDE9A for the treatment of cognitive disorders associated with impaired cGMP signaling.[472] On the basis of a screening hit from the Pfizer neuroscience PDE platform,[473] the previously reported PDE9A inhibitor PF-04181366 (**43**, Fig. 10) was rationally improved to overcome selectivity issues over PDE1C—a PDE isoform expressed in the cardiac tissue, as well as high human microsomal clearance that would compromise optimal half-life and dose projection in humans. First, after simplifying the quinoxaline to a phenyl ring, the replacement of the lipophilic cyclopentyl group by the more polar 4-tetrahydropiranyl moiety could significantly enhance in the selectivity over PDE1C, which is favored by the interaction between the pyran oxygen and Tyr424 residue present at the PDE9A binding site, and absent in that of PDE1C. Later, the incorporation of two nitrogen atoms in the benzene ring could finally deliver the desired pharmacokinetic properties, at the same time optimizing the interactions with the target by stacking with Phe441—which is an Ile in PDE1C, on top of two hydrogen bonds established by the pyrazolopyrimidinone moiety with Gln453, which are typically found in PDE inhibitors.[474]

Ultimately, this highly potent (IC_{50} = 8 nM) selective PDE9A inhibitor was found to be centrally penetrant, while displaying an excellent tolerability and metabolic profile in humans.[475] It is interesting to note that, despite being a substantially polar molecule, PF-04447943 can still cross the BBB due to its limited number of hydrogen donors, low basic pK_a, and molecular weight.[472] In fact, it revealed a good impact on hippocampal synaptic plasticity and improved cognitive function in rodents, while elevating cGMP levels in multiple brain regions and in CSF.[476,477] Hence, the compound moved on to clinical trials. Unfortunately, the encouraging preclinical data have not translated to major clinical benefits, as the recently published results of a phase II trial in patients with AD did not state any significant differences between a 12 week treatment with 25 mg of PF-04447943 twice a day and the placebo, both in terms of cognition and behavior.[478] Apparently, the induced cGMP enhancing effect is not be enough to create a strong impact on the cognitive performance of these patients—at least not enough to compensate for the damage caused by several other pathological features associated with AD.

Nevertheless, even though PF-04447943 has only been explored regarding its therapeutic utility against neurodegenerative disorders, the above cited potential of PDE9 inhibition for the treatment of metabolic syndrome suggests a possible application of this type of compounds in T2D as well. In fact, another compound bearing the pyrazolopyrimidinone core

(**44**, Fig. 10) was developed in 2014 to selectively inhibit PDE9A, but instead, the authors focused on its potential antihyperglycemic activity.[479] With an IC_{50} of 0.60 nM and a predicted K_i of 0.50 nM, it was shown to interact with Met365 and Tyr424 residues through the cyclopentyl ring, and with Gln452 and Phe456 residues though the central pyrazolopyrimidinone moiety by means of two hydrogen bonds and π–π interactions, respectively. Importantly, the D-Ala unit strengthens the affinity of this compound with PDE9A by establishing an additional hydrogen bond with Ala452. It was well tolerated in mice and soluble in gastric and intestinal fluids, but quite unstable in mouse liver microsomes, with a half-life of only 23 min. Nevertheless, compound **44** significantly reduced the 8-Br-cAMP dexamethasone induced expression of PEPCK and G6Pase—indeed suggesting a potential antihyperglycemic effect.

B. GSK-3β Inhibitors

In 2007, the thiadiazolidinone NP031112 (**45**, Fig. 11) was found to possess outstanding anti-inflammatory and neuroprotective effects, and has received much attention since then for its potential therapeutic role against brain disorders.[480] It resulted from the improvement of NP00111 (**46**), and NP01138 (**47**) (Fig. 11), two thiadiazolidinones that had been previously reported by the same research team.[481] This compound, later named tideglusib, is an irreversible, non-ATP-competitive GSK-3β inhibitor with IC_{50} values in the nanomolar range, and was also found to protect neural stem cells against the overactivation of NMDA receptors that lead to neuronal excitotoxicity.[482,483] In transgenic mice overexpressing human mutant AβPP and tau protein, tideglusib was able to prevent hippocampal neuronal cell death and associated memory loss, while promoting a decrease in the levels of phosphorylated tau and a reduction in brain beta-amyloid burden.[484,485] Tideglusib is generally well tolerated,[486] thus indicating that GSK-3β can be safely targeted. Although recent results from a completed phase II trial show that the compound does not show significant clinical efficacy in patients with mild to moderate AD, the authors highlight the need for dosage and disease stage adjustments, as mild patients and low dose tests consistently produced better responses.[487]

Tideglusib is also an agonist of the peroxisome proliferator-activated receptor gamma (PPARγ), which is involved in regulating fatty acid storage and glucose metabolism.[480] The ability to interact with this receptor is pointed out as another mechanism by which the compound exerts its activity and is likely related to the thiadiazolidinone moiety. In fact, thiazolidinediones such as the previously presented FDA-approved pioglitazone (**4**, Table I) are PPARγ agonists with antidiabetic activity. This suggests that, in addition to the known potential against neurodegenerative processes, tideglusib-like compounds may display antidiabetic properties to some extent, which—if so—would make them suitable candidates for the prevention of AD in T2D.

The optimization of imidazopyridine derivatives led to another promising GSK-3β inhibitor, conceived as a potential antidiabetic candidate.[488] Biological evaluation of several synthesized analogue structures allowed the accomplishment of a robust structure–activity relationship study, focusing not only on enzymatic activity and cellular potency, but also on their metabolic stabilities and physicochemical properties. The most promising compound (**48**, Fig. 11) exhibited an outstanding 8 nM IC_{50} against GSK-3β, and an 80 nM EC_{50} on Rd cells, together with acceptable metabolic stability on mouse and human microsomes and optimal calculated log *P* and polar surface area (PSA) parameters. Although lower IC_{50} and EC_{50} values were obtained for other compounds—for instance bearing 4-methoxypyridinyl (**49**, Fig. 11) or 4-ethoxypyridinyl (**50**, Fig. 11) moieties instead of the 6-methylpyridinyl group in the first case—they were excluded due to insufficient oral exposure in mice or low metabolic stability, respectively.

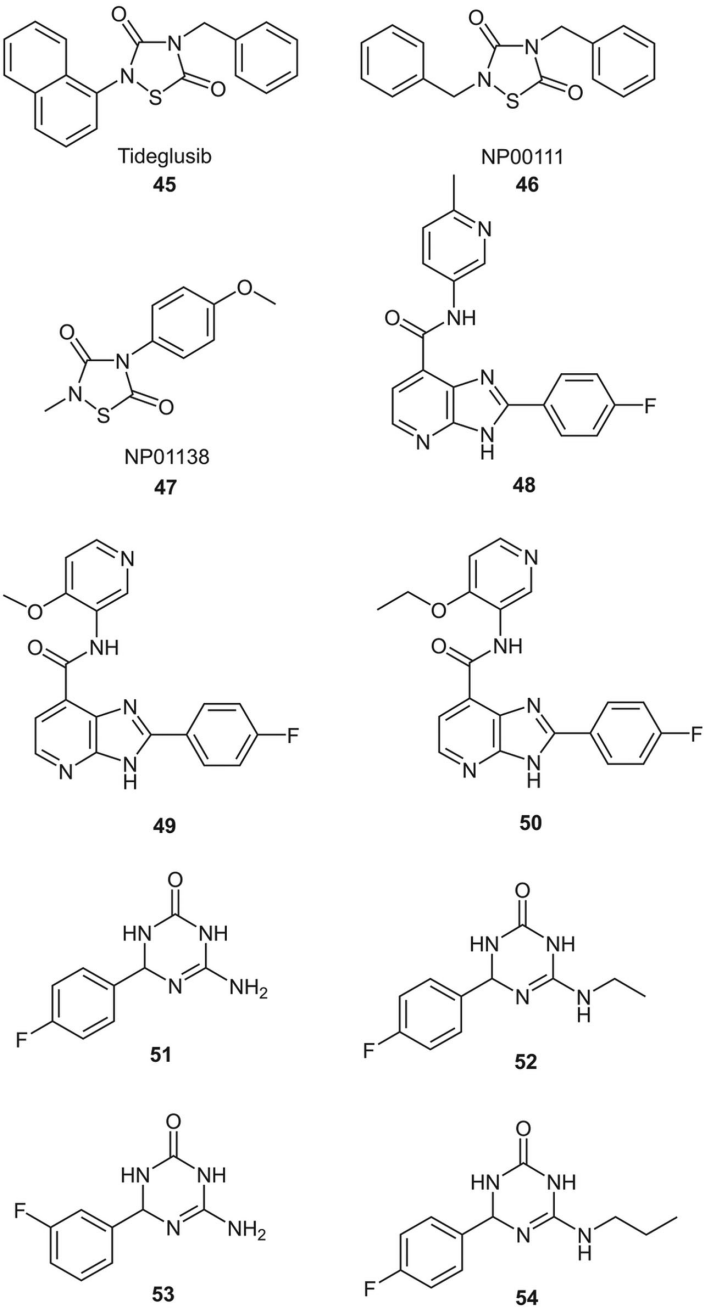


Figure 11. Chemical structure of thiadiazolidinones **45–47** and imidazopyridines **48–50** with GSK-3β inhibitor activity, together with triazone derivatives **51–54** with dual BACE-1 and GSK-3β inhibitor activity.

Unlike tideglusib, these compounds seem to compete with ATP for the catalytic pocket of GSK-3β, as revealed by the X-ray crystallographic analysis of a representative analogue synthesized in this study. Interestingly, the imidazopyridine core serves simultaneously as a hydrogen donor and acceptor moiety, being able to interact with both the amino and carbonyl

groups of Val135. On the other hand, the amide carbonyl moiety was found to interact with Asp200 through a water hydrogen binding network.

Compound **48** was then evaluated in an oral glucose tolerance test (OGTT) in mice. At only 10 mg/kg, this imidazopyridine derivative could effectively induce a significant decrease in plasma glucose levels after 30 min of oral administration, thus supporting its therapeutic potential against T2D. Although other unexplored mechanisms of action might also be involved, this study suggests a possible relationship between GSK-3β inhibition and systemic glucose lowering effects, thus encouraging the development of similar antidiabetic approaches for the prevention of cognitive decline in diabetic patients.

C. A Novel Triazinone-Based Dual BACE-1 and GSK-3β Inhibitor

Given the complex and multifactorial nature of AD, a set of triazinone derivatives was designed and synthesized aiming at the development of the first class of dual BACE-1/ GSK-3β inhibitors.[489] Using a fragment-based strategy, the goal was to combine a guanidine moiety and a cyclic amide group in the same molecule: whereas the first fragment would be responsible for the binding to the catalytic site of BACE-1 as reported for several BACE-1 inhibitors,[490,491] the second one would simultaneously serve as a hydrogen donor and acceptor, thus establishing key hydrogen bond interactions with the ATP-binding site of GSK-3β.

Molecular modeling studies identified the 6-amino-4-phenyl triazinone scaffold as the starting point in this investigation. Preliminary structure–activity relationship studies initially led to the identification of two reference compounds, **51** (BACE-1 $IC_{50} = 18.03\ \mu M$; GSK-3β $IC_{50} = 14.67\ \mu M$) and **52** (BACE-1 $IC_{50} = 16.05\ \mu M$; GSK-3β $IC_{50} = 7.11\ \mu M$), also presented in Figure 11. Then, by changing the substitution pattern of the aromatic ring with both electronwithdrawing or electron donating groups and by exploring other *N*-alkyl or *N*-aryl groups on the exocyclic amino moiety at position 6, the original structures were successively and rationally changed, and their activity evaluated.

Firstly, while the 3-fluoro-phenyl derivative **53** (Fig. 11) was found to be the most potent BACE-1 inhibitor ($IC_{50} = 10.18\ \mu M$), it had lower activity against GSK-3β when compared to both reference molecules. Moreover, the 4-fluoro-phenyl derivative with an *N*-propyl moiety (**54**, Fig. 11) was the best GSK-3β inhibitor among all synthesized analogues ($IC_{50} = 4.34\ \mu M$), but could not maintain the BACE-1 inhibitor potency of the previous compounds. Indeed, although alkylation or arylation of 6-amino group could generally improve the anti-GSK-3β effects of these triazinone derivatives, such alterations conversely spoiled BACE-1 inhibitor activity, probably due to steric or electronic effects hampering the recognition by the catalytic site of the enzyme.

Despite the low neurotoxicity of these four compounds, their ability to reduce cellular $A\beta_{1\text{-}40}$ levels was only moderate (**51**, **52**, and **53**) or negligible (**54**). Nevertheless, compound **52** could still produce a statistically significant reduction of cellular $A\beta_{1\text{-}40}$ levels in about 15%, which has been reported as being enough to induce a significant reduction in brain beta-amyloid burden at old age.[492] Furthermore, both compounds **52** and **54**—the best GSK-3β inhibitors—exhibited significant neuroprotective effects in primary cultures of astrocytes and microglia, by preventing lipopolysaccharide (LPS)-induced inflammation and nitrite formation. These two analogues also displayed neurogenic effects in neurosphere cultures of primary rat stem cells, thus suggesting an ability to induce the differentiation of immature neurones and potentiate endogenous brain regeneration. In addition, compound **52** could successfully cross the BBB in mice.

By targeting two major players of the beta-amyloid and tau pathological network, this structurally simple and low molecular weight compound **52** may definitively serve as the basis for the development of very promising therapeutic strategies against AD, with the potential

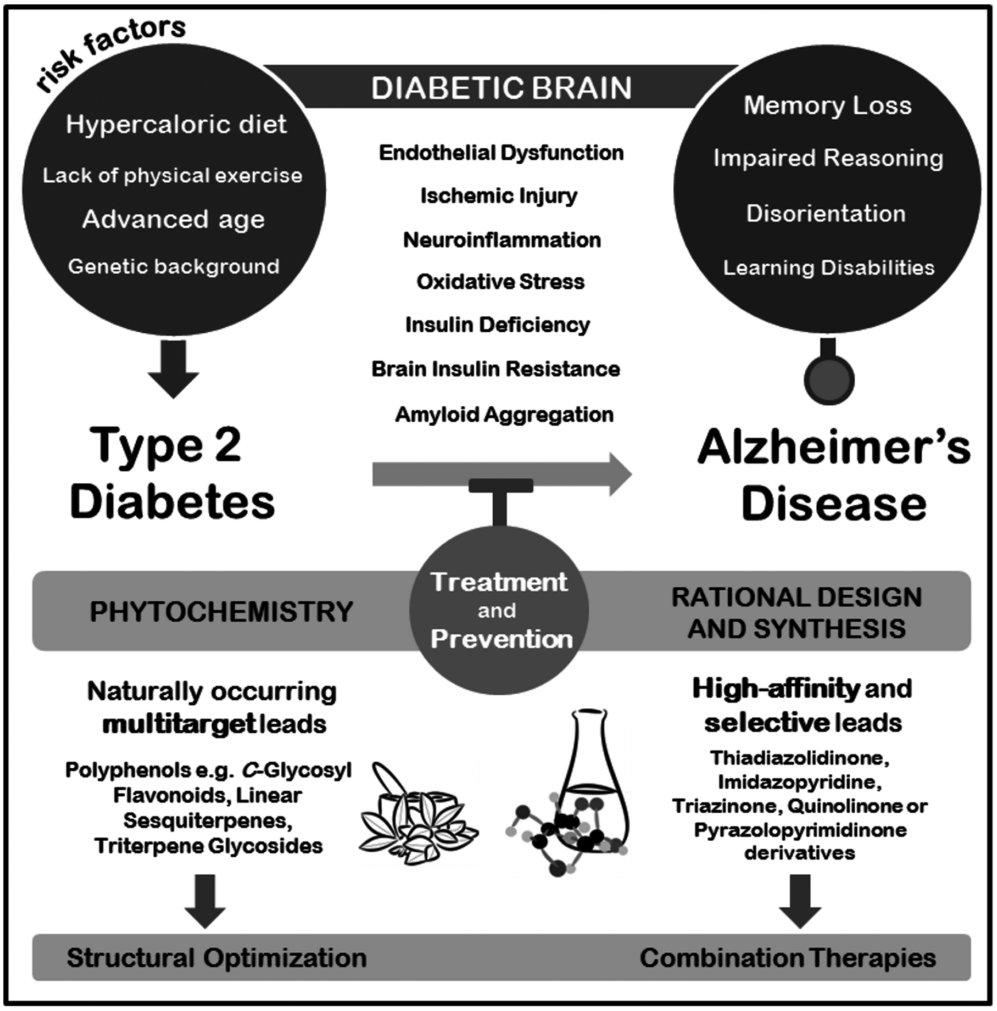


Figure 12. Summary of diabetic brain characteristic features bridging type 2 diabetes and Alzheimer's disease, and approaches for treatment and prevention: the potential of naturally occurring multitarget leads versus high-affinity, selective leads generated through rational design and synthesis.

to create greater disease-modifying effects than plain single-targeted approaches. Moreover, being a GSK-3β inhibitor, it could also lead to an innovative way of controlling T2D, while preventing the development of commonly associated neurodegenerative events.

11. CONCLUDING REMARKS

Promoted by the ingestion of hypercaloric diets and lack of physical exercise, T2D is mostly developed in older subjects with a propitious genetic background, and leads to a complex set of interrelated pathophysiological features such as hyperglycemia, insulin resistance, pancreatic dysfunction, vascular damage, ischemic injury, inflammation and oxidative stress (Fig. 12). Over time, these events potentiate the onset of AD, which is a multifactorial disorder itself. The development of new antidiabetic strategies targeting common pathological processes comes across not only as a very appealing therapeutic strategy, but also as a public health emerging need.

Although rational design and synthesis has led to highly potent and selective drug candidates with optimal pharmacokinetic profiles such as the thiadiazolidinone or pyrazolopyrimidinone derivatives herein disclosed, these approaches frequently fail to deliver good clinical results, particularly when tested for their ability to reverse neurodegeneration and cognitive impairment in patients with AD.[493,494] Apparently, the inhibition of one therapeutic target alone does not compensate for the damage caused by several other players in the pathological process, which may be the main underlying cause for such disappointing outcomes. The complex nature of this neurodegenerative disorder may in fact require the concomitant use of more than one bioactive compound, or the development of innovative molecules able to tackle multiple targets at once. Nevertheless, when looking for a prophylactic strategy against diabetes-induced cognitive decline, it would still be important to evaluate its long-term effects in early stage type 2 diabetic patients with no clinical signs of dementia: indeed, a preventive approach does not necessarily reverse disease once it has been settled, but may well prevent it from settling at all. Therefore, the therapeutic approach should take into account both the stage of diabetes and/or AD. Whether there is a subgroup of patients with diabetes due to its genotypic and phenotypic characteristics could profit from an early therapeutic approach is still to unveil.

Compounds from natural sources such as the polyphenols and their glycosyl derivatives here described offer an alternative in the search for multitarget scaffolds with such capability. In fact, most of them simultaneously display antioxidant, anti-inflammatory and antihyperglycemic activities, and some are even able to interfere with the activity of specific enzymes involved in the etiology of both T2D and AD, such as PDE and GSK-3β. Moreover, they may also modulate protein–protein interactions on the basis of toxic oligomer and fibril formation, or even inhibit secretase and cholinesterase enzymes, thus increasing the chances of achieving the desired preventive effects. Even though compounds such as resveratrol or curcumin are associated with relevant bioavailability issues, they still come across as promising multitarget starting points for structural optimization, namely through the introduction of water-soluble sugar moieties—which have been considered important for enhanced bioactivity as well.

This review aims at encouraging the scientific community to develop multitarget approaches against both T2D and AD. By covering pathophysiological pathways underlying both diseases, as well as mechanisms of action and structure–activity relationships on the presented set of molecules, this work contributes to promote the progress of antidiabetic drug discovery and development, and innovation toward prevention of diabetes-induced AD.

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REFERENCES

1. International Diabetes Federation, IDF Diabetes Atlas, 7th ed. International Diabetes Federation, 2015.
2. Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guenette S. Insulin-degrading enzyme regulates the levels of insulin, amyloid

beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci USA 2003;100(7):4162–4167.

3. Abdul-Hay SO, Kang D, McBride M, Li L, Zhao J, Leissring MA. Deletion of insulin-degrading enzyme elicits antipodal, age-dependent effects on glucose and insulin tolerance. PLoS One 2011;6(6):e20818.
4. Polonsky KS, Sturis J, Bell GI. Seminars in medicine of the Beth Israel Hospital, Boston. Non-insulin-dependent diabetes mellitus—A genetically programmed failure of the beta cell to compensate for insulin resistance. N Engl J Med 1996;334(12):777–783.
5. Kotronen A, Juurinen L, Tiikkainen M, Vahkavaara S, Yki-Järvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology 2008;135(1):122–130.
6. Del Prato S, Marchetti P. Beta and alpha-cell dysfunction in type 2 diabetes. Horm Metab Res 2004;36(11–12):775–781.
7. Mayfield JA, White RD. Insulin therapy for type 2 diabetes: Rescue, augmentation and replacement of beta-cell function. Am Fam Physician 2004;70(3):489–500.
8. Falta W, Boller R. Insularer und Insulinresistenter Diabetes. Kkinische Wochenschrift 1931;10:438–443.
9. Biessels GJ, Reagan LP. Hippocampal insulin resistance and cognitive dysfunction. Nat Rev Neurosci 2015;16(11):660–671.
10. Koekkoek PS, Kappelle LJ, Berg E, Rutten GE, Biessels GJ. Cognitive function in patients with diabetes mellitus: Guidance for daily care. Lancet Neurol 2015;14(3):329–340.
11. Xu W, Caracciolo B, Wang HX, Winblad B, Bäckman L, Qiu C, Fratiglioni L. Accelerated progression from mild cognitive impairment to dementia in people with diabetes. Diabetes 2010;59(11):2928–2935.
12. Williamson R, McNeilly A, Sutherland C. Insulin resistance in the brain: An old-age or new-age problem? Biochem Pharmacol 2012;84(6):737–745.
13. Rdzak GM, Abdelghany O. Does insulin therapy for type 1 diabetes mellitus protect against Alzheimer's disease? Pharmacotherapy 2014;34(12):1317–1323.
14. Alzheimer's Association. 2014. Alzheimer's disease facts and figures. Alzheimer's Dement 2014;10(2):e47–e49.
15. Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med 2010;362(4):329–344.
16. Dias C, Rauter AP. In: Jimenéz-Barbero J, Cañada FJ, Martín-Santamaría S, Eds. Carbohydrates in Drug Design and Discovery (RSC Drug Discovery Series No. 43). Cambridge: The Royal Society of Chemistry; 2015. p 180–204.
17. Selkoe DJ. Alzheimer's disease: Genes, proteins, and therapy. Physiol Rev 2001;81(2):741–766.
18. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: A systematic review and metaanalysis. Alzheimer's Dement 2013;9(1):63–75.
19. Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. Circulation 2003;108(4):414–419.
20. Ballantyne CM, Hoogeveen RC, McNeill AM, Heiss G, Schmidt MI, Duncan BB, Pankow JS. Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study. Int J Obes (Lond) 2008;32(2):21–24.
21. Solfrizzi V, Scafato E, Capurso C, D'Introno A, Colacicco AM, Frisardi V, Vendemiale G, Baldereschi M, Crepaldi G, Di Carlo A, Galluzzo L, Gandin C, Inzitari D, Maggi S, Capurso A, Panza F. Italian Longitudinal Study on Ageing Working Group. Metabolic syndrome, mild cognitive impairment, and progression to dementia. The Italian Longitudinal Study on Aging. Neurobiol Aging 2011;32(11):1932–1941.

22. Viticchi G, Falsetti L, Buratti L, Luzzi S, Bartolini M, Acciarri MC, Provinciali L, Silvestrini M. Metabolic syndrome and cerebrovascular impairment in Alzheimer's disease. Int J Geriatr Psychiatry 2015;30(12):1164–1170.
23. Hishikawa N, Yamashita T, Deguchi K, Wada J, Shikata K, Makino H, Abe K. Cognitive and affective functions in diabetic patients associated with diabetes-related factors, white matter abnormality and aging. Eur J Neurol 2015;22(2):313–321.
24. Hishikawa N, Fukui Y, Sato K, Kono S, Yamashita T, Ohta Y, Deguchi K, Abe K. Cognitive and affective functions in Alzheimer's disease patients with metabolic syndrome. Eur J Neurol 2016;23(2):339–345.
25. Silvestrini M, Pasqualetti P, Baruffaldi R, Bartolini M, Handouk Y, Matteis M, Moffa F, Provinciali L, Vernieri F. Cerebrovascular reactivity and cognitive decline in patients with Alzheimer's disease. Stroke 2006;37(4):1010–1015.
26. Viticchi G, Falsetti L, Vernieri F, Altamura C, Bartolini M, Luzzi S, Provinciali L, Silvestrini M. Vascular predictors of cognitive decline in patients with mild cognitive impairment. Neurobiol Aging 2012;33(6):1127.e1–9.
27. Humpel C, Marksteiner J. Cerebrovascular damage as a cause for Alzheimer's disease. Curr Neurovasc Res 2005;2(4):341–347.
28. Sena CM, Pereira AM, Carvalho C, Fernandes R, Seiça RM, Oliveira CR, Moreira PI. Type 2 diabetes aggravates Alzheimer's disease-associated vascular alterations in the aorta of mice. J Alzheimers Dis 2015;45(1):127–138.
29. Carvalho C, Machado N, Mota PC, Correia SC, Cardoso S, Santos RX, Santos MS, Oliveira CR, Moreira PI. Type 2 diabetic and Alzheimer's disease mice present similar behavioral, cognitive, and vascular anomalies. J Alzheimers Dis 2013;35(3):623–635.
30. Carvalho C, Cardoso S, Correia SC, Santos RX, Santos MS, Baldeiras I, Oliveira CR, Moreira PI. Metabolic alterations induced by sucrose intake and Alzheimer's disease promote similar brain mitochondrial abnormalities. Diabetes 2012;61(5):1234–1242.
31. Yao Y, Chinnici C, Tang H, Trokanowski JQ, Lee VM, Praticò D. Brain inflammation and oxidative stress in a transgenic mouse model of Alzheimer-like brain amyloidosis. J Neuroinflammation 2004;1(1):21.
32. Di Napoli M, Shah IM. Neuroinflammation and cerebrovascular disease in old age: A translational medicine perspective. J Aging Res 2011;2011:857484.
33. Tripathy D, Yin X, Sanchez A, Luo J, Martinez J, Grammas P. Cerebrovascular expression of proteins related to inflammation, oxidative stress and neurotoxicity is altered with aging. J Neuroinflammation 2010;7:63.
34. Chung SS, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. J Am Soc Nephrol 2003;14(8 Suppl 3):S233–S236.
35. Singh VP, Bali A, Singh N, Jagg AS. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol 2014;18(1):1–14.
36. Yan SD, Chen X, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature 1996;382(6593):685–691.
37. Stirban A, Gawlowski T, Roden M. Vascular effects of advances glycation end products: Clinical effects and molecular mechanisms. Mol Metab 2014;3(2):94–108.
38. Kesavulu MM, Giri R, Kameswara RB, Apparao C. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetes with microvascular complications. Diabetes Metab 2000;26(5):387–392.
39. Singhania N, Puri D, Madhu SV, Sharma SB. Assessment of oxidative stress and endothelial dysfunction in Asian Indians with type 2 diabetes mellitus with and without macroangiopathy. QJM 2008;101(6):449–455.
40. Maejima K, Nakano S, Himeno M, Tsuda S, Makiishi H, Ito T, Nakagawa A, Kigoshi T, Ishibashi T, Nishio M, Uchida K. Increased basal levels of plasma nitric oxide in type 2 diabetic subjects. Relationship to microvascular complications. J Diabetes Complications 2001;15(3):135–143.

41. Ozden S, Tatlipinar S, Biçer N, Yaylali V, Yildirim C, Ozbay D, Güner G. Basal serum nitric oxide levels in patients with type 2 diabetes mellitus and different stages of retinopathy. Can J Ophthalmol 2003;38(5):393–396.
42. Zalewski A, Macphee C, Nelson JJ. Lipoprotein-associated phospholipase A2: A potential therapeutic target for atherosclerosis. Curr Drug Targets Cardiovasc Haematol Disord 2005;5(6):527–532.
43. Tsimikas S, Tsironis LD, Tselepis AD. New insights into the role of lipoprotein(a)-associated phospholipase A2 in atherosclerosis and cardiovascular disease. Arterioscler Thromb Vasc Biol 2007;27(10):2094–2099.
44. Wilensky RL, Shi Y, Mohler ER, Hamamdzic D, Burgert ME, Li J, Postle A, Fenning RS, Bollinger JG, Hoffman BE, Pelchovitz DJ, Yang J, Mirabile RC, Webb CL, Zhang L, Zhang P, Gelb MH, Walker MC, Zalewski A, Macphee CH. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. Nat Med 2008;14(10):1059–1066.
45. Hennig B, Chow CK. Lipid peroxidation and endothelial cell injury: Implications in atherosclerosis. Free Radic Biol Med 1988;4(2):99–106.
46. Sun GY, Horrocks LA, Farooqui AA. The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory responses in neurodegenerative diseases. J Neurochem 2007;103(1):1–16.
47. Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. Life Sci 2009;84(21–22):705–712.
48. Tsiomalos K, Athyros VG, Karagiannis A, Mikhailidis DP. Endothelial dysfunction in metabolic syndrome: Prevalence, pathogenesis and management. Nutr Metab Cardiovasc Dis 2010;20(2):140–146.
49. Monteiro R., Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm 2010;2010:289645.
50. Raffaitin C, Gin H, Empana JP, Helmer C, Berr C, Tzourio C, Portet F, Dartigues JF, Alpérovitch A, Barberger-Gateau P. Metabolic syndrome and risk for incident Alzheimer's disease or vascular dementia: The Three-City Study. Diabetes Care 2009;32(1):169–174.
51. Solfrizzi V, Scafato E, Capurso C, D'Introno A, Colacicco AM, Frisardi V, Vendemiale G, Baldereschi M, Crepaldi G, Di Carlo A, Galluzzo L, Gandin C, Inzitari D, Maggi S, Capurso A, Panza F. Italian Longitudinal Study on Ageing Working Group. Metabolic syndrome and the risk of vascular dementia: The Italian Longitudinal Study on Ageing. J Neurol Neurosurg Psychiatry 2010;81(4):433–440.
52. De La Torre JC. Impaired cerebromicrovascular perfusion. Summary of evidence in support of its causality in Alzheimer's disease. Ann N Y Acad Sci 2000;924:136–152.
53. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Baterman RJ. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science 2010;330(6012):1774.
54. Selnes OA, Vinters HV. Vascular cognitive impairment. Nat Clin Pract Neurol 2006;2(10):538–547.
55. Erickson MA, Banks WA. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. J Cereb Blood Flow Metab 2013;33(10):1500–1513.
56. Kim B, Backus C, Oh S, Feldman EL. Hyperglycemia-induced tau cleavage in vitro and in vivo: A possible link between diabetes and Alzheimer's disease. J Alzheimers Dis 2013;34(3):727–739.
57. Sacchettini JC, Kelly JW. Therapeutic strategies for human amyloid diseases. Nat Rev Drug Discov 2002;1(4):267–275.
58. Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB. Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci USA 1987;84(23):8628–8632.
59. Westermark P, Wilander E, Westermark GT, Johnson KH. Islet amyloid polypeptide-like immunoreactivity in the islet B cells of type 2 (non-insulin-dependent) diabetic and non-diabetic individuals. Diabetologia 1987;30(11):887–892.

60. Westermark P, Andersson A, Westermark GT. Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol Rev 2011;91(3):795–826.
61. Huang CJ, Haataja L, Gurlo T, Butler AE, Wu X, Soeller WC, Butler PC. Induction of endoplasmic reticulum stress-induced beta-cell apoptosis and accumulation of polyubiquitinated proteins by human islet amyloid polypeptide. Am J Physiol Endocrinol Metab 2007;293(6):E1656–E1662.
62. Hull RL, Zraika S, Udayasankar J, Aston-Mourney K, Subramanian SL, Kahn SE. Amyloid formation in human IAPP transgenic mouse islets and pancreas, and human pancreas, is not associated with endoplasmic reticulum stress. Diabetologia 2009;52(6):1102–1111.
63. Paulsson JF, Andersson A, Westermark P, Westermark GT. Intracellular amyloid-like deposits contain unprocessed pro-islet amyloid polypeptide (proIAPP) in beta cells of transgenic mice overexpressing the gene for human IAPP and transplanted human islets. Diabetologia 2006;49(6):1237–1246.
64. Marzban L, Rhodes CJ, Steiner DF, Haataja L, Halban PA, Verchere CB. Impaired NH2-terminal processing of human proislet amyloid polypeptide by the prohormone convertase PC2 leads to amyloid formation and cell death. Diabetes 2006;55(8):2192–2201.
65. Gong W, Liu ZH, Zeng CH, Peng A, Chen HP, Zhou H, Li LS. Amylin deposition in the kidney of patients with diabetic nephropathy. Kidney Int 2007;72(2):213–218.
66. Despa S, Margulies KB, Chen L, Knowlton AA, Havel PJ, Taegtmeyer H, Bers DM, Despa F. Hyperamylinemia contributes to cardiac dysfunction in obesity and diabetes: A study in humans and rats. Circ Res 2012;110(4):598–608.
67. Banks WA, Kastin AJ, Maness LM, Huang W, Jaspan JB. Permeability of the blood-brain barrier to amylin. Life Sci 1995;57(22):1993–2001.
68. Kawahara M, Kuroda Y, Arispe N, Rojas E. Alzheimer's beta-amyloid, human islet amylin, and prion protein fragment evoke intracellular free calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell line. J Biol Chem 2000;275(19):14077–14083.
69. Casas S, Novials A, Reimann F, Gomis R, Gribble FM. Calcium elevation in mouse pancreatic beta cells evoked by extracellular human islet amyloid polypeptide involves activation of the mechanosensitive ion channel TRPV4. Diabetologia 2008;51(12):2252–2262.
70. Janson J, Ashley RH, Harrison D, McIntyre S, Butler PC. The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. Diabetes 1999;48(3):491–498.
71. Anguiano M, Nowak RJ, Lansbury PT, Jr. Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. Biochemistry 2002;41(38):11338–11343.
72. Qiu WQ, Au R, Zhu H, Wallack M, Liebson E, Li H, Rosenzweig J, Mwamburi M, Stern RA. Positive association between plasma amylin and cognition in a homebound elderly population. J Alzheimers Dis 2014;42(2):555–563.
73. Zhu H, Wang X, Wallack M, Li H, Carreras I, Dedeoglu A, Hur JY, Zheng H, Li H, Fine R, Mwamburi M, Sun X, Kowall N, Stern RA, Qiu WQ. Intraperitoneal injection of the pancreatic peptide amylin potently reduces behavioural impairment and brain amyloid pathology in murine models of Alzheimer's disease. Mol Psychiatry 2015;20(2):252–262.
74. Westfall TC, Curfman-Falvey M. Amylin-induced relaxation of the perfused mesenteric arterial bed: Meditation by calcitonin gene-related peptide receptors. J Cardiovasc Pharmacol 1995;26(6):932–936.
75. Edvinsson L, Goadsby PJ, Uddman R. Amylin: Localization, effects on cerebral arteries and on local cerebral blood flow in the cat. Scientific World J 2001;1:168–180.
76. Arnelo U, Reidelberger R, Adrian TE, Larsson J, Permert J. Sufficiency of postprandial plasma levels of islet amyloid polypeptide for suppression of feeding in rats. Am J Physiol 1998;275(5 Pt 2): R1537–R1542.

77. Clementi G, Caruso A, Cutuli VM, de Bernardis E, Prato A, Amico-Roxas M. Amylin given by central or peripheral routes decreases gastric emptying and intestinal transit in the rat. Experientia 1996;52(7):677–679.
78. Isaksson B, Wang F, Permert J, Olsson M, Fruin B, Herrington MK, Enochsson L, Erlanson-Albertsson C, Arnelo U. Chronically administered islet amyloid polypeptide in rats serves as an adiposity inhibitor and regulates energy homeostasis. Pancreatology 2005;5(1):29–36.
79. Lutz TA. Pancreatic amylin as a centrally acting satiating hormone. Curr Drug Targets 2005;6(2):181–189.
80. Rushing PA, Hagan MM, Seeley RJ, Lutz TA, Woods SC. Amylin: A novel action in the brain to reduce body weight. Endocrinology 2000;141(2):850–853.
81. Olsson M, Herrington MK, Reidelberger RD, Permert J, Arnelo U. Comparison of the effects of chronic central administration and chronic peripheral administration of islet amyloid polypeptide on food intake and meal pattern in the rat. Peptides 2007;28(7):1416–1423.
82. Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ. Risk of dementia among persons with diabetes mellitus: A population-based cohort study. Am J Epidemiol 1997;145(4):301–308.
83. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology 1999;53(9):1937–1942.
84. Wang KC, Woung LC, Tsai MT, Liu CC, Su YH, Li CY. Risk of Alzheimer's disease in relation to diabetes: A population-based cohort study. Neuroepidemiology 2012;38(4):237–244.
85. Verma N, Ly H, Liu M, Chen J, Zhu H, Chow M, Hersh LB, Despa F. Intraneuronal amylin deposition, peroxidative membrane injury and increased IL-1β synthesis in brains of Alzheimer's disease patients with type-2 diabetes and in diabetic HIP rats. J Alzheimers Dis 2016;53(1):259–272.
86. Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: An appraisal for the development of therapeutics. Nat Rev Drug Discov 2011;10(9):698–712.
87. Cohen SIA, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, Otzen DE, Vendruscolo M, Dobson CM, Knowles TPJ. Proliferation of amyloid-β42 aggregates occurs through a secondary nucleation mechanism. Proc Natl Acad Sci USA 2013;110(24):9758–9763.
88. Welander H, Frånberg J, Graff C, Sundström E, Winblad B, Tjernberg LO. Abeta43 is more frequent than Abeta40 in amyloid plaque cores from Alzheimer's disease brains. J Neurochem 2009;110(2):697–706.
89. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol 1999;46(6):860–866.
90. Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliott JI, Van Nostrand WE, Smith SO. Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. Nat Struct Mol Biol 2010;17(5):561–567.
91. Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, Krafft GA, Klein WL. Alzheimer's disease-affected brain: presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proc Natl Acad Sci USA 2003;100(18):10417–10422.
92. Shankar GM, Li S, Mehta TH, Garcia-Muñoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid-beta protein dimers isolated from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008;14(8):837–842.
93. Corder EH, Woodbury MA. Genetic heterogeneity in Alzheimer's disease: A grade of membership analysis. Genet Epidemiol 1993;10(6):495–499.
94. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90(20):9649–9653.

95. Morris GP, Clark IA, Vissel B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol Commun 2014;2:135.
96. VanFossen BT, Watson GS, Baker LC, Rhoads KW, Cholerton BA, Reger MA, Plymate SR, Schellenberg G, Craft S. Statin users without an APOE-epsilon4 allele have increased insulin resistance. J Alzheimers Dis 2010;19(4):1149–1153.
97. Ong QR, Chan ES, Lim ML, Cole GM, Wong BS. Reduced phosphorylation of brain insulin receptor substrate and Akt proteins in apolipoprotein-E4 targeted replacement mice. Sci Rep 2014;4:3754.
98. Peila R, Rodriguez BL, Launer LJ. Honolulu-Asia Aging Study. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. Diabetes 2002;51(4):1256–1262.
99. Yin J, Turner GH, Coons SW, Maalouf M, Reiman EM, Shi J. Association of amyloid burden, brain atrophy and memory deficits in aged apolipoprotein ε4 mice. Curr Alzheimer Res 2014;11(3):283–290.
100. Ye S, Huang Y, Müllendorff K, Dong L, Giedt G, Meng EC, Cohen FE, Kuntz ID, Weisgraber KH, Mahley RW. Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. Proc Natl Acad Sci USA 2005;102(51):18700–18705.
101. Hass S, Weidemann A, Utermann G, Baier G. Intracellular apolipoprotein E affects amyloid precursor protein processing and amyloid Abeta production in COS-1 cells. Mol Genet Genomics 2001;265(5):791–800.
102. Verghese PB, Castellano JM, Garai K, Wang Y, Jiang H, Shah A, Bu G, Frieden C, Holtzman DM. ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions. Proc Natl Acad Sci USA 2013;110(19):E1807–E1816.
103. Jordán J, Galindo MF, Miller RJ, Reardon CA, Getz GS, LaDu MJ. Isoform-specific effect of apolipoprotein E on cell survival and beta-amyloid-induced toxicity in rat hippocampal pyramidal neuronal cultures. J Neurosci 1998;18(1):195–204.
104. Wisniewski T, Frangione B. Apolipoprotein E: A pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 1992;135(2):235–238.
105. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer's disease. Proc Natl Acad Sci USA 1993;90(20):9649–9653.
106. Gearing M, Mori H, Mirra SS. Abeta-peptide length and apolipoprotein E genotype in Alzheimer's disease. Ann Neurol 1996;39(3):395–399.
107. Tapiola T, Pirttilä T, Mehta PD, Alafuzofff I, Lehtovirta M, Soininen H. Relationship between apoE genotype and CSF beta-amyloid (1-42) and tau in patients with probable and definite Alzheimer's disease. Neurobiol Aging 2000;21(5):735–740.
108. Fukumoto H, Cheung BS, Hyman BT, Irizarry MC. Beta-secretase protein and activity are increased in the neocortex in Alzheimer's disease. Arch Neurol 2002;59(9):1381–1389.
109. Holsinger RM, McLean CA, Beyreuther K, Masters CL, Evin G. Increased expression of the amyloid precursor beta-secretase in Alzheimer's disease. Ann Neurol 2002;51(6):783–786.
110. Guglielmotto M, Aragno M, Tamagno E, Vercellinatto I, Visentin S, Medana C, Catalano MG, Smith MA, Perry G, Danni O, Boccuzzi G, Tabaton M. AGEs/RAGE complex upregulates BACE1 via NF-κB pathway activation. Neurobiol Aging 2012;33(1):196.e13–27.
111. Guglielmotto M, Aragano M, Autelli R, Giliberto L, Novo E, Colombatto S, Danni O, Parola M, Smith MA, Perry G, Tamagno E, Tabaton M. The up-regulation of BACE1 mediated by hypoxia and ischemic injury: Role of oxidative stress and HIF1alpha. J Neurochem 2009;108(4):1045–1056.
112. Tong Y, Zhou W, Fung V, Christensen MA, Qing H, Sun X, Song W. Oxidative stress potentiates BACE1 gene expression and Abeta generation. J Neural Transm (Vienna) 2005;112(3):455–469.
113. Austin SA, Santhanam AV, Hinton DJ, Choi DS, Katusic ZS. Endothelial nitric oxide deficiency promotes Alzheimer's disease pathology. J Neurochem 2013;127(5):691–700.

114. Ko SY, Ko HA, Chu KH, Shieh TM, Chi TC, Chen HI, Chang WC, Chang SS. The possible mechanism of advanced glycation end products (AGEs) for Alzheimer's disease. PLoS One 2015;10(11):e0143345.
115. Devraj K, Poznanovic S, Spahn C, Schwall G, Harter PN, Mittelbronn M, Antoniello K, Paganetti P, Muhs A, Heilemann M, Hawkins RA, Schrattenholz A, Liebner S. BACE-1 is expressed in the blood-brain barrier endothelium and is upregulated in a murine model of Alzheimer's disease. J Cereb Blood Flow Metab 2016;36(7):1281–1294.
116. Mamada N, Tanokashira D, Hosaka A, Kametani F, Tamaoka A, Araki W. Amyloid β-protein oligomers upregulate the β-secretase, BACE1, through a post-translational mechanism involving its altered subcellular distribution in neurons. Mol Brain 2015;8(1):73.
117. Tang YP, Gershon ES. Genetic studies in Alzheimer's disease. Dialogues Clin Neurosci 2003;5(1):17–26.
118. Szaruga M, Veugelen S, Benurwar M, Lismont S, Sepulveda-Falla D, Lleo A, Ryan NS, Lashley T, Fox NC, Murayama S, Gijsen H, De Strooper B, Chávez-Gutiérrez L. Qualitative changes in human γ-secretase underlie familial Alzheimer's disease. J Exp Med 2015;212(12):2003–2013.
119. Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, Vigo-Pelfrey C, Lieberburg I, Selkoe DJ. Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. Nature 1992;360(6405):672–674.
120. De Jonghe C, Esselens C, Kumar-Singh S, Craessaerts K, Serneels S, Checler F, Annaert W, Van Broeckhoven C, De Strooper B. Pathogenic APP mutations near the gamma-secretase cleavage site differentially affect Abeta secretion and C-terminal fragment stability. Hum Mol Genet 2001;10(16):1665–1671.
121. Alvarez A, Bronfman F, Pérez CA, Vicente M, Garrido J, Inestrosa NC. Acetylcholinesterase, a senile plaque component, affects the fibrillogenesis of amyloid-beta-peptides. Neurosci Lett 1995;201(1):49–52.
122. Inestrosa NC, Alvarez A, Calderón F. Acetylcholinesterase is a senile plaque component that promotes assembly of amyloid beta-peptide into Alzheimer's filaments. Mol Psychiatry 1996;1(5):359–361.
123. Morán MA, Mufson EJ, Gómez-Ramos P. Colocalization of cholinesterases with beta amyloid protein in aged and Alzheimer's brains. Acta Neuropathol 1993;85(4):362–369.
124. Ulrich J, Meier-Ruge W, Probst A, Meier E, Ipsen S. Senile plaques: Staining for acetylcholinesterase and A4 protein: A comparative study in the hippocampus and entorhinal cortex. Acta Neuropathol 1990;80(6):624–628.
125. Greenfield SA, Zimmermann M, Bond CE. Non-hydrolytic functions of acetylcholinesterase. The significance of C-terminal peptides. FEBS J 2008;275(4):604–611.
126. Cottingham MG, Hollinshead MS, Vaux DJ. Amyloid fibril formation by a synthetic peptide from a region of human acetylcholinesterase that is homologous to the Alzheimer's amyloid-beta peptide. Biochemistry 2002;41(46):13539–13547.
127. Alvarez A, Alarcón R, Opazo C, Campos EO, Muñoz FJ, Calderón FH, Dajas F, Gentry MK, Doctor BP, De Mello FG, Inestrosa NC. Stable complexes involving acetylcholinesterase and amyloid-beta peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils. J Neurosci 1998;18(9):3213–3223.
128. Dinamarca MC, Sagal JP, Quintanilla RA, Godoy JA, Arrázola MS, Inestrosa NC. Amyloid-beta-acetylcholinesterase complexes potentiate neurodegenerative changes induced by the Abeta peptide. Implications for the pathogenesis of Alzheimer's disease. Mol Neurodegener 2010; 5:4.
129. O'Nuallain B, Williams AD, Westermark P, Wetzel R. Seeding specificity in amyloid growth induced by heterologous fibrils. J Biol Chem 2004;279(17):17490–17499.
130. Jackson K, Barisone GA, Diaz E, Jin LW, DeCarli C, Despa F. Amylin deposition in the brain: A second amyloid in Alzheimer's disease? Ann Neurol 2013;74(4):517–526.

131. Oskarsson ME, Paulsson JF, Schultz SW, Ingelsson M, Westermark P, Westermark GT. In vivo seeding and cross-seeding of localized amyloidosis: A molecular link between type 2 diabetes and Alzheimer's disease. Am J Pathol 2015;185(3):834–846.
132. Convit A, Wolf OT, Tarshish C, de Leon MJ. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. Proc Natl Acad Sci USA 2003;100(4):2019–2022.
133. Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R, O'Neill C. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. Neurobiol Aging 2010;31(2):224–243.
134. Yarchoan M, Toledo JB, Lee EB, Arvanitakis Z, Kazi H, Han LY, Louneva N, Lee VM, Kim SF, Trojanowski JQ, Arnold SE. Abnormal serine phosphorylation of insulin receptor substrate 1 is associated with tau pathology in Alzheimer's disease and tauopathies. Acta Neuropathol 2014;128(5):679–689.
135. De Felice FG, Lourenco MV, Ferreira ST. How does brain insulin resistance develop in Alzheimer's disease? Alzheimers Dement 2014;10(1 Suppl):S26–S32.
136. Adzovic L, Lynn AE, D'Angelo HM, Crockett AM, Kaercher RM, Royer SE, Hopp SC, Wenk GL. Insulin improves memory and reduces chronic neuroinflammation in the hippocampus of young but not aged brains. J Neuroinflammation 2015;12:63.
137. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. Nat Rev Immunol 2014;14(7):463–477.
138. Weiss N, Miller F, Cazaubon S, Couraud PO. The blood-brain barrier in brain homeostasis and neurological diseases. Biochim Biophys Acta 2009;1788(4):842–857.
139. Luchsinger JA, Tang MX, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer's disease. Neurology 2004;63(7):1187–1192.
140. Okereke O, Hankinson SE, Hu FB, Grodstein F. Plasma C peptide level and cognitive function among older women without diabetes mellitus. Arch Intern Med 2005;165(14):1651–1656.
141. Hom FG, Goodner CJ, Berrie MA. A [3H]2-deoxyglucose method for comparing rates of glucose metabolism and insulin responses among rat tissues in vivo. Validation of the model and the absence of an insulin effect on the brain. Diabetes 1984;33(2):141–152.
142. Hasselbalch SG, Knudsen GM, Videbaek C, Pinborg LH, Schmidt JF, Holm S, Paulson OB. No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. Diabetes 1999;48(10):1915–1921.
143. Banks WA, Owen JB, Erickson MA. Insulin in the brain: There and back again. Pharmacol Ther 2012;136(1):82–93.
144. Doré S, Kar S, Rowe W, Quirion R. Distribution and levels of [125I]IGF-1, [125I]-IGFII and [125I]insulin receptor binding sites in the hippocampus of aged memory-unimpaired and -impaired rats. Neuroscience 1997;80(4):1033–1040.
145. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. Endocrinol Metab Clin North Am 2008;37(4):811–823.
146. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. Science 2000;289(5487):2122–2125.
147. Devaskar SU, Giddings SJ, Rajakumar PA, Carnaghi LR, Menon RK, Zahm DS. Insulin gene expression and insulin synthesis in mammalian neuronal cells. J Biol Chem 1994;269(11):8445–8454.
148. Das UN, Repossi G, Dain A, Eynard AR. Is insulin resistance a disorder of the brain? Front Biosci (Landmark Ed) 2011;16:1–12.
149. Duffy KR, Pardridge WM. Blood-brain barrier transcytosis of insulin in developing rabbits. Brain Res 1987;420(1):32–38.
150. Banks WA, Jaspan JB, Kastin AJ. Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. Peptides 1997;18(10):1577–1584.

151. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes 2000;49(9):1525–1533.
152. Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D, Jr. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: Relationship to severity of dementia and apolipoprotein E genotype. Neurology 1998;50(1):164–168.
153. Cross DA, Alessi DR, Vandenheede JR, McDowell HE, Hundal HS, Cohen P. The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletal muscle cell line L6 is blocked by wortmannin, but not by rapamycin: Evidence that wortmannin blocks activation of the mitogen-activated protein kinase pathway in L6 cells between Ras and Raf. Biochem J 1994;303(Pt 1):21–26.
154. Phiel CJ, Wilson CA, Lee VM, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature 2003;423(6938):435–439.
155. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. Eur J Biochem 1980;107(2):519–527.
156. Pap M, Cooper GM. Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-kinase/Akt cell survival pathway. J Biol Chem 1998;273(32):19929–19932.
157. Henriksen EJ, Dokken BB. Role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. Curr Drug Targets 2006;7(11):1435–1441.
158. Woodgett JR. Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J 1990;9(8):2431–2438.
159. DaRocha-Souto B, Coma M, Pérez-Nievas BG, Scotton TC, Siao M, Sánchez-Ferrer P, Hashimoto T, Fan Z, Hudry E, Barroeta I, Serenó L, Rodríguez M, Sánchez MB, Hyman BT, Gómez-Isla T. Activation of glycogen synthase kinase-3 beta mediates β-amyloid induced neuritic damage in Alzheimer's disease. Neurobiol Dis 2012;45(1):425–437.
160. Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J, Lo E, Wu D, Saule E, Bouschet T, Matthews P, Isaac JT, Bortolotto ZA, Wang YT, Collingridge GL. LTP inhibits LTD in the hippocampus via regulation of GSK3beta. Neuron 2007;53(5):703–717.
161. Li X, Lu F, Tian Q, Yang Y, Wang Q, Wang JZ. Activation of glycogen synthase kinase-3 induces Alzheimer-like tau hyperphosphorylation in rat hippocampus slices in culture. J Neural Transm (Vienna) 2006;113(1):93–102.
162. Koh SH, Noh MY, Kim SH. Amyloid-beta-induced neurotoxicity is reduced by inhibition of glycogen synthase kinase-3. Brain Res 2008;1188:254–262.
163. Špolcová A, Mikulášková B, Kršková K, Gajdošechová L, Zórad Š, Olszanecki R, Suski M, Bujak-Giż ycka B, Železná B, Maletínská L. Deficient hippocampal insulin signalling and augmented tau phosphorylation is related to obesity- and age-induced peripheral insulin resistance: A study in Zucker rats. BMC Neurosci 2014;15:111.
164. Devi L, Alldred MJ, Ginsberg SD, Ohno M. Mechanisms underlying insulin deficiency-induced acceleration of β-amyloidosis in a mouse model of Alzheimer's disease. PLoS One 2012;7(3):e32792.
165. Zhao WQ, De Felice FG, Fernandez S, Chen H, Lambert MP, Quon MJ, Krafft GA, Klein WL. Amyloid beta oligomers induce impairment of neuronal insulin receptors. FASEB J 2008;22(1):246–260.
166. Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H, Silverman MA, Kazi H, Melo HM, McClean PL, Holscher C, Arnold SE, Talbot K, Klein WL, Muñoz DP, Ferreira ST, De Felice FG. An anti-diabetes agent protects the mouse brain from defective insulin signalling caused by Alzheimer's disease-associated Aβ oligomers. J Clin Invest 2012;122(4):1339–1353.
167. Raza H, John A, Howarth FC. Increased oxidative stress and mitochondrial dysfunction in Zucker diabetic rat liver and brain. Cell Physiol Biochem 2015;35(3):1241–1251.
168. Morrison CD, Pistell PJ, Ingram DK, Johnson WD, Liu Y, Fernandez-Kim SO, White CL, Purpera MN, Uranga RM, Bruce-Keller AJ, Keller JN. High fat increases hippocampal oxidative stress

and cognitive impairment in aged mice: Implications for decreased Nrf2 signalling. J Neurochem 2010;114(6):1581–1589.

169. Treviño S, Aguilar-Alonso P, Flores Hernandez JA, Brambila E, Guevara J, Flores G, Lopez-Lopez H, Muñoz-Arenas G, Morales-Medina JC, Toxqui V, Venegas B, Diaz A. A high calorie diet causes memory loss, metabolic syndrome and oxidative stress into hippocampus and temporal cortex of rats. Synapse 2015;69(9):421–433.
170. Petrov D, Pedrós I, Artiach G, Sureda FX, Barroso E, Pallàs M, Casadesús G, Beas-Zarate C, Carro E, Ferrer I, Vazquez-Carrera M, Folch J, Camins A. High-fat diet-induced deregulation of hippocampal insulin signalling and mitochondrial homeostasis deficiencies contribute to Alzheimer's disease pathology in rodents. Biochim Biophys Acta 2015;1852(9):1687–1699.
171. Wei Y, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, Manrique C, Strump CS. Angiotensin II-induced NADPH oxidase activation impairs insulin signalling in skeletal muscle cells. J Biol Chem 2006;281(46):35137–35146.
172. Rudich A, Kozlovsky N, Potashnik R, Bashan N. Oxidant stress reduces insulin responsiveness in 3T3-L1 adipocytes. Am J Physiol 1997;272(5 Pt 1):E935–E940.
173. De la Monte SM. Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's disease. Drugs 2012;72(1):49–66.
174. Chen Z, Zhong C. Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: Implications for diagnostic and therapeutic strategies. Prog Neurobiol 2013;108:21–43.
175. Willette AA, Bendlin BB, Starks EJ, Birdsill AC, Johnson SC, Christian BT, Okonkwo OC, La Rue A, Hermann BP, Koscik RL, Jonaitis EM, Sager MA, Asthana S. Association of insulin resistance with cerebral glucose uptake in late middle-aged adults at risk for Alzheimer's disease. JAMA Neurol 2015;72(9):1013–1020.
176. Messier C, Gagnon M. Glucose regulation and cognitive functions: Relation to Alzheimer's disease and diabetes. Behav Brain Res 1996;75(1–2):1–11.
177. Cornford EM, Hyman S, Cornford ME, Clare-Salzler M. Down-regulation of blood-brain glucose transport in the hyperglycemic nonobese diabetic mouse. Neurochem Res 1995;20(7):869–873.
178. Mooradian AD, Chung HC, Shah GN. GLUT-1 expression in the cerebra of patients with Alzheimer's disease. Neurobiol Aging 1997;18(5):469–474.
179. Kalaria RN, Harik SI. Reduced glucose transporter at the blood-brain barrier and in cerebral cortex in Alzheimer's disease. J Neurochem 1989;53(4):1083–1088.
180. Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, Sengillo JD, Hillman S, Kong P, Nelson AR, Sullivan JS, Zhao Z, Meiselman HJ, Wenby RB, Soto J, Abel ED, Makshanoff J, Zuniga E, De Vivo DC, Zlokovic BV. GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. Nat Neurosci 2015;18(4):521–530.
181. Liu Y, Song XD, Liu W, Zhang TY, Zuo J. Glucose deprivation induces mitochondrial dysfunction and oxidative stress in PC12 cell line. J Cell Mol Med 2003;7(1):49–56.
182. Findlay JA, Hamilton DL, Ashford ML. BACE1 activity impairs neuronal glucose oxidation: Rescue by beta-hydroxybutyrate and lipoic acid. Front Cell Neurosci 2015;9:382.
183. Omar B, Banke E, Ekelund M, Frederiksen S, Degerman E. Alterations in cyclic nucleotide phosphodiesterase activities in omental and subcutaneous adipose tissues in human obesity. Nutr Diabetes 2011;1:e13.
184. Netherton SJ, Jimmo SL, Palmer D, Tilley DG, Dunkerley HA, Raymond DR, Russell JC, Absher PM, Sage EH, Vernon RB, Maurice DH. Altered phosphodiesterase 3-mediated cAMP hydrolysis contributes to a hypermotile phenotype in obese JCR:LA-cp rat aortic vascular smooth muscle cells: Implications for diabetes-associated cardiovascular disease. Diabetes 2002;51(4):1194–1200.
185. Giannetta E, Isidori AM, Galea N, Carbone I, Mandosi E, Vizza CD, Naro F, Morano S, Fedele F, Lenzi A. Chronic inhibition of cGMP phosphodiesterase 5A improves diabetic cardiomyopathy: A randomized, controlled clinical trial using magnetic resonance imaging with myocardial tagging. Circulation 2012;125(19):2323–2333.

186. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: Essential components in cyclic nucleotide signalling. Annu Rev Biochem 2007;76:481–511.
187. Rizzo NO, Maloney E, Pham M, Luttrell I, Wessells H, Tateya S, Daum G, Handa P, Schwartz MW, Kim F. Reduced NO-cGMP signalling contributes to vascular inflammation and insulin resistance induced by high-fat feeding. Arterioscler Thromb Vasc Biol 2010;30(4):758–765.
188. Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, Daum G, Clowes AW, Morton GJ, Schwartz MW, Kim F. Endothelial NO/cGMP/VASP signalling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. Diabetes 2011;60(11):2792–2801.
189. Prickaerts J, de Vente J, Honig W, Steinbusch HW, Blokland A. cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. Eur J Pharmacol 2002;436(1–2):83-87.
190. Bon CL, Garthwaite J. On the role of nitric oxide in hippocampal long-term potentiation. J Neurosci 2003;23(5):1941–1948.
191. Domek-Ł opaciń ska KU, Strosznajder JB. Cyclic GMP and nitric oxide synthase in aging and Alzheimer's disease. Mol Neurobiol 2010;41(2–3):129–137.
192. Puzzo D, Vitolo O, Trinchese F, Jacob JP, Palmeri A, Arancio O. Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity. J Neurosci 2005;25(29):6887–6897.
193. Ugarte A, Gil-Bea F, García-Barroso C, Cedazo-Minguez Á, Ramírez MJ, Franco R, García-Osta A, Oyarzabal J, Cuadrate-Tejedor M. Decreased levels of guanosine 3', 5'-monophosphate (cGMP) in cerebrospinal fluid (CSF) are associated with cognitive decline and amyloid pathology in Alzheimer's disease. Neuropathol Appl Neurobiol 2015;41(4):471–482.
194. Friedlander RS, Moss CE, Mace J, Parker HE, Tolhurst G, Habib AM, Wachten S, Cooper DM, Gribble FM, Reimann F. Role of phosphodiesterase and adenylate cyclase isozymes in murine colonic glucagon-like peptide 1 secreting cells. Br J Pharmacol 2011;163(2):261–271.
195. Ong WK, Gribble FM, Reimann F, Lynch MJ, Houslay MD, Baillie GS, Furman BL, Pyne NJ. The role of the PDE4D cAMP phosphodiesterase in the regulation of glucagon-like peptide-1 release. Br J Pharmacol 2009;157(4):633–644.
196. Moon JS, Won KC. Pancreatic α-cell dysfunction in type 2 diabetes: Old kids on the block. Diabetes Metab J 2015;39(1):1–9.
197. Campbell JE, Drucker DJ. Islet α cells and glucagon: Critical regulators of energy homeostasis. Nat Rev Endocrinol 2015;11(6):329–338.
198. Bollen E, Puzzo D, Rutten K, Privitera L, De Vry J, Vanmierlo T, Kenis G, Palmeri A, D'Hooge R, Balschun D, Steinbusch HM, Blokland A, Prickaerts J. Improved long-term memory via enhancing cGMP-PKG signalling requires cAMP-PKA signalling. Neuropsychopharmacology 2014;39(11):2497–2505.
199. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. Neuron 2002;35(4):605–623.
200. Bate C, Williams A. cAMP-inhibits cytoplasmic phospholipase A2 and protects neurons against amyloid-β-induced synapse damage. Biology (Basel) 2015;4(3):591–606.
201. Martínez M, Fernández E, Frank A, Guaza C, de la Fuente M, Hernanz A. Increased cerebrospinal fluid cAMP levels in Alzheimer's disease. Brain Res 1999;846(2):265–267.
202. Grammas P, Roher AE, Ball MJ. Increased accumulation of cAMP in cerebral microvessels in Alzheimer's disease. Neurobiol Aging 1994;15(1):113–116.
203. Kandel ER. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, BREB-2, and CPEB. Mol Brain 2012;5:14.
204. Vitolo OV, Sant'Angelo A, Constanzo V, Battaglia F, Arancio O, Shelanski M. Amyloid beta-peptide inhibition of the PKA/CREB pathway and long-term potentiation: Reversibility by drugs that enhance cAMP signalling. Proc Natl Acad Sci USA 2002;99(20):13217–13221.

205. Pugazhenthi S, Wang M, Pham S, Sze CI, Eckman CB. Downregulation of CREB expression in Alzheimer's brain and in Aβ-treated rat hippocampal neurons. Mol Neurodegener 2011;6:60.

206. Schnecko A, Witte K, Bohl J, Ohm T, Lemmer B. Adenylyl cyclase activity in Alzheimer's brain: Stimulatory and inhibitory signal transduction pathways are differently affected. Brain Res 1994;644(2):291–296.

207. Pérez-Torres S, Cortés R, Tolnay M, Probst A, Palacios JM, Mengod G. Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. Exp Neurol 2003;182(2):322–334.

208. Boland BB, Alarcón C, Ali A, Rhodes CJ. Monomethylated-adenines potentiate glucose-induced insulin production and secretion via inhibition of phosphodiesterase activity in rat pancreatic islets. Islets 2015;7(2):e1073435.

209. Kwon KJ, Lee EJ, Kim MK, Kim SY, Kim JN, Kim JO, Kim HJ, Kim HY, Han JS, Shin CY, Han SH. Diabetes augments cognitive dysfunction in chronic cerebral hypoperfusion by increasing neuronal death: Implication of cilostazol for diabetes mellitus-induced dementia. Neurobiol Dis 2015;73:12–23.

210. Ramirez CE, Nian H, Yu C, Gamboa JL, Luther JM, Brown NJ, Shibao CA. Treatment with sildenafil improves insulin sensitivity in prediabetes: A randomized, controlled trial. J Clin Endocrinol Metab 2015;100(12):4533–4540.

211. Nawrocki AR, Rodriguez CG, Toolan DM, Price O, Henry M, Forrest G, Szeto D, Keohane CA, Pan Y, Smith KM, Raheem IT, Cox CD, Hwa J, Renger JJ, Smith SM. Genetic deletion and pharmacological inhibition of phosphodiesterase 10A protects mice from diet-induced obesity and insulin resistance. Diabetes 2014;63(1):300–311.

212. Cuadrado-Tejedor M, Garcia-Barroso C, Sanzhez-Arias J, Mederos S, Rabal O, Ugarte A, Franco R, Pascual-Lucas M, Segura V, Perea G, Oyarzabal J, Garcia-Osta A. Concomitant histone deacetylase and phosphodiesterase 5 inhibition synergistically prevents disruption in synaptic plasticity and it reverses cognitive impairment in a mouse model of Alzheimer's disease. Clin Epigenetics 2015;7:108.

213. McGirr A, Lipina TV, Mun HS, Georgiou J, Al-Amri AH, Ng E, Zhai D, Elliott C, Cameron RT, Mullins JG, Liu F, Baillie GS, Clapcote SJ, Roder JC. Specific inhibition of phosphodiesterase-4B results in anxiolysis and facilitates memory acquisition. Neuropsychopharmacology 2016;41(4):1080–1092.

214. Lueptow LM, Zhan CG, O'Donnell JM. Cyclic GMP-mediated memory enhancement in the object recognition test by inhibitors of phosphodiesterase-2 in mice. Psychopharmacology (Berl) 2016;233(3):447–456.

215. Qiu WQ, Folstein MF. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: Review and hypothesis. Neurobiol Aging 2006;27(2):190–198.

216. Akter K, Lanza EA, Martin SA, Myronyuk N, Rua M, Raffa RB. Diabetes mellitus and Alzheimer's disease: Shared pathology and treatment. Br J Clin Pharmacol 2011;71(3):365–376.

217. Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B, Zlokovic BV. Circulating amyloid-beta peptide crosses the blood-brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. Vascul Pharmacol 2002;38(6):303–313.

218. Roher AE, Esh CL, Kokjohn TA, Castaño EM, Van Vickle GD, Kalback WM, Patton RL, Luehrs DC, Daugs ID, Kuo YM, Emmerling MR, Soares H, Wuinn JF, Kaye J, Connor DJ, Silverberg NB, Adler CH, Seward JD, Beach TG, Sabbagh MN. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. Alzheimers Dement 2009;5(1):18–29.

219. Farris W, Mansourian S, Leissring MA, Eckman EA, Bertram L, Eckman CB, Tanzi RE, Selkoe DJ. Partial loss-of-function mutations in insulin-degrading enzyme that induce diabetes also impair degradation of amyloid beta-protein. Am J Pathol 2004;164(4):1425–1432.

220. Gu F, Efendic S, Nordman S, Ostenson CG, Brismar K, Brookes AJ, Prince JA. Quantitative trait loci near the insulin-degrading enzyme (IDE) gene contribute to variation in plasma insulin levels. Diabetes 2004;53(8):2137–2142.

221. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle AV, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445(7130):881–885.

222. Zhao J, Deliard S, Aziz AR, Grant SF. Expression analyses of the genes harboured by the type 2 diabetes and pediatric BMI associated locus on 10q23. BMC Med Genet 2012;13:89.

223. Gutiérrex-Hermosillo H, Díaz De León-González E, Palacios-Corona R, Cedillo-Rodríguez JA, Camacho-Luis A, Reyes-Romero MA, Medina-Chávez JH, Blandón PA. C allele of the rs2209972 single nucleotide polymorphism of the insulin degrading enzyme gene and Alzheimer's disease in type 2 diabetes, a case control study. Med Clin (Barc) 2015;144(4):151–155.

224. Zhang Y, Wang B, Wan H, Zhou Q, Li T. Meta-analysis of the insulin degrading enzyme polymorphism and susceptibility to Alzheimer's disease. Neurosci Lett 2013;541:132–137.

225. Zuo X, Jia J. Promoter polymorphisms which modulate insulin degrading enzyme expression may increase susceptibility to Alzheimer's disease. Brain Res 2009;1249:1–8.

226. Kim M, Hersh LB, Leissring MA, Ingelsson M, Matsui T, Farris W, Lu A, Hyman BT, Selkoe DJ, Bertram L, Tanzi RE. Decreased catalytic activity of the insulin-degrading enzyme in chromosome 10-linked Alzheimer disease families. J Biol Chem 2007;282(11):7825–7832.

227. Hulse RE, Ralat LA, Wei-Jen T. Structure, function and regulation of insulin-degrading enzyme. Vitam Horm 2009;80:635–648.

228. Cordes CM, Bennett RG, Siford GL, Hamel FG. Nitric oxide inhibits insulin-degrading enzyme activity and function through S-nitrosylation. Biochem Pharmacol 2009;77(6):1064–1073.

229. Natali A, Ribeiro R, Baldi S, Tulipani A, Rossi M, Venturi E, Mari A, Macedo MP, Ferrannini E. Systemic inhibition of nitric oxide synthesis in non-diabetic individuals produces a significant deterioration in glucose tolerance by increasing insulin clearance and inhibiting insulin secretion. Diabetologia 2013;56(5):1183–1191.

230. Hamel FG, Upward JL, Bennett RG. In vitro inhibition of insulin-degrading enzyme by long-chain fatty acids and their coenzyme A thioesters. Endocrinology 2003;114(6):2404–2408.

231. Chien WY, Yang KD, Eng HL, Hu YH, Lee PY, Wang ST, Wang PW. Increased plasma concentration of nitric oxide in type 2 diabetes but not in nondiabetic individuals with insulin resistance. Diabetes Metab 2005;31(1):63–68.

232. Ozden S, Tatlipinar S, Biçer N, Yaylali V, Yildirim C, Ozbay D, Güner G. Basal serum nitric oxide levels in patients with type 2 diabetes mellitus and different stages of retinopathy. Can J Ophthalmol 2003;38(5):393–396.

233. Afonso RA, Lautt WW, Ribeiro RT, Legare DJ, Macedo MP. Insulin resistance in two animal models of obesity: A comparison of HISS-dependent and HISS-independent insulin action in high-fat diet-fed and Zucker rats. Proc West Pharmacol Soc 2007;50:110–114.

234. Grarup N, Rose CS, Andersson EA, Andersen G, Nielsen AL, Albrechtsen A, Clausen JO, Rasmussen SS, Jørgensen T, Sandbaek A, Lauritzen T, Schmitz O, Hansen T, Pedersen O. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: Validation and extension of genome-wide association studies. Diabetes 2007;56(12):3105–3111.

235. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M, RISC Consortium, UK type 2 Diabetes Genetics Consortium. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. Diabetes 2007;56(12):3101–3104.

236. Rudovich N, Pivovarova O, Fisher E, Fischer-Rosinsky A, Spranger J, Möhlig M, Schulze MB, Boeing H, Pfeiffer AF. Polymorphisms within insulin-degrading enzyme (IDE) gene determine insulin metabolism and risk of type 2 diabetes. J Mol Med (Berl) 2009;87(11):1145–1151.

237. Rolla A. The pathophysiological basis for intensive insulin replacement. Int J Obes Relat Metab Disord 2004;28(Suppl 2):S3–S7.

238. Steneberg P, Bernardo L, Edfalk S, Lundberg L, Backlund F, Ostenson CG, Edlund H. The type 2 diabetes-associated gene ide is required for insulin secretion and suppression of α-synuclein levels in β-cells. Diabetes 2013;62(6):2004–2014.

239. Bellani S, Sousa VL, Ronzitti G, Valtorta F, Meldolesi J, Chieregatti E. The regulation of synaptic function by alpha-synuclein. Commun Integr Biol 2010;3(2):106–109.

240. Geng X, Lou H, Wang J, Li L, Swanson AL, Sun M, Beers-Stolz D, Watkins S, Perez RG, Drain P. α-Synuclein binds the K(ATP) channel at insulin-secretory granules and inhibits insulin secretion. Am J Physiol Endocrinol Metab 2011;300(2):E276–E286.

241. Sharma SK, Chorell E, Steneberg P, Vernersson-Lindahl E, Edlund H, Wittung-Stafshede P. Insulin-degrading enzyme prevents α-synuclein fibril formation in a nonproteolytical manner. Sci Rep 2015;5:12531.

242. Westermark P, Engström U, Johnson KH, Westermark GT, Betsholtz C. Islet amyloid polypeptide: Pinpointing amino acid residues linked to amyloid fibril formation. Proc Natl Acad Sci USA 1990;87(13):5036–5040.

243. Bennett RG, Hamel FG, Duckworth WC. An insulin-degrading enzyme inhibitor decreases amylin degradation, increases amylin-induced cytotoxicity, and increases amyloid formation in insulinoma cell cultures. Diabetes 2003;52(9):2315–2320.

244. Jolivalt CG, Lee CA, Beiswenger KK, Smith JL, Orlov M, Torrance MA, Masliah E. Defective insulin signalling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: Parallels with Alzheimer's disease and correction by insulin. J Neurosci Res 2008;86(15):3265–3274.

245. Cook DG, Leverenz JB, McMillan PJ, Kulstad JJ, Ericksen S, Roth RA, Schellenberg GD, Jin LW, Kovacina KS, Craft S. Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. Am J Pathol 2003;162(1):313–319.

246. De Tullio MB, Castelletto V, Hamley IW, Martino Adami PV, Morelli L, Castaño EM. Proteolytically inactive insulin-degrading enzyme inhibits amyloid formation yielding non-neurotoxic aβ peptide aggregates. PLoS One 2013;8(4):e59113.

247. Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, Rosner MR, Safavi A, Hersh LB, Selkoe DJ. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. J Biol Chem 1998;273(49):32730–32738.

248. Vekrellis K, Ye Z, Qiu WQ, Walsh D, Hartley D, Chesneau V, Rosner MR, Selkoe DJ. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. J Neurosci 2000;20(5):1657–1665.

249. Lifshitz V, Benromano T, Weiss R, Blanga-Kanfi S, Frenkel D. Insulin-degrading enzyme deficiency accelerates cerebrovascular amyloidosis in an animal model. Brain Behav Immun 2013;30:143–149.

250. Leissring MA, Malito E, Hedouin S, Reinstatler L, Sahara T, Abdul-Hay SO, Choudhry LC, Manolopoulou M, Tang WJ, Stein RL, Cuny CG, Selkoe DJ. Designed inhibitors of insulin-degrading enzyme regulate the catabolism and activity of insulin. PLoS One 2010;5(5):e10504.

251. Maianti JP, McFedries A, Foda ZH, Kleiner RE, Du XQ, Leissring MA, Tang WJ, Charron MH, Seeliger MA, Saghatelian A, Liu DR. Anti-diabetic activity of insulin-degrading enzyme inhibitors mediated by multiple hormones. Nature 2014;511(7507):94–98.

252. Deprez-Poulain R, Hennuyer N, Bosc D, Liang WG, Enée E, Marechal X, Charton J, Totobenazara J, Berte G, Jahklal J, Verdelet T, Dumont J, Dassonneville S, Woitrain E, Gauriot M, Paquet C, Duplan I, Hermant P, Cantrelle FX, Sevin E, Culot M, Landry C, Herledan A, Piveteau C, Lippens G, Leroux F, Tang WJ, van Endert P, Staels B, Deprez B. Catalytic site inhibition of insulin-degrading enzyme by a small molecule induces glucose intolerance in mice. Nat Commun 2015;6:8250.

253. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: A pathophysiologic and therapeutic makeover. J Clin Invest 2012;122(1):4–12.

254. Costes S, Butler PC. Insulin-degrading enzyme inhibition, a novel therapy for type 2 diabetes? Cell Metab 2014;20(2):201–203.

255. U.S. Food and Drug Administration. http://www.fda.gov/ForPatients/Illness/Diabetes/ucm408682.htm (accessed September 2016).
256. Gelhorn HL, Bacci ED, Poon JL, Boye KS, Suzuki S, Babineaux SM. Evaluating preferences for profiles of glucagon-like peptide-1 receptor agonists among injection-naïve type 2 diabetes patients in Japan. Patient Prefer Adherence 2016;10:1337–1348.
257. Zaccardi F, Htike ZZ, Webb DR, Khunti K, Davies MJ. Benefits and harms of once-weekly glucagon-like peptide-1 receptor agonist treatments: A systematic review and network meta-analysis. Ann Intern Med 2016;164(2):102–113.
258. Atkin S, Javed Z, Fulcher G. Insulin degludec and insulin apart: Novel insulins for the management of diabetes mellitus. Ther Adv Chronic Dis 2015;6(6):375–388.
259. Sirtori CR, Pasik C. Re-evaluation of a biguanide, metformin: Mechanism of action and tolerability. Pharmacol Res 1994;30(3):187–228.
260. Hundal RS, Krssak M, Durfour S, Laurent D, Lebon V, Chandramouli V, Inzucchi SE, Schumann WC, Petersen KF, Landau BR, Shulman GI. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes 2000;49(12):2063–2069.
261. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: An update. Ann Intern Med 2002;137(1):25–33.
262. Lebovitz HE, Feinglos MN. Mechanism of action of the second-generation sulfonylurea glipizide. Am J Med 1983;75(5B):46–54.
263. Prendergast BD. Glyburide and glipizide, second-generation oral sulfonylurea hypoglycemic agents. Clin Pharm 1984;3(5):473–485.
264. Clissold SP, Edwards C. Acarbose. A preliminary review of its pharmacodynamics and pharmacokinetic properties, and therapeutic potential. Drugs 1988;35(3):214–243.
265. Chiasson JL, Josse RG, Leiter LA, Mihic M, Nathan DM, Palmason C, Cohen RM, Wolever TM. The effect of acarbose on insulin sensitivity in subjects with impaired glucose tolerance. Diabetes Care 1996;19(11):1190–1193.
266. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: A 6-month randomized placebo-controlled dose-response study. The Pioglitazone 001 Study Group. Diabetes Care 2000;23(11):1605–1611.
267. Smith U. Pioglitazone: Mechanism of action. Int J Clin Pract Suppl 2001;121:13–18.
268. Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, Wang AQ, Zeng W, Musson D, Winchell G, Davies MJ, Ramael S, Gottesdiener KM, Wagner JA. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: Results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 2005;78(6):675–688.
269. Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE. Sitagliptin Study 021 Group. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes Care 2006;29(12):2632–2637.
270. Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P. Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: Characterization and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab 2012;14(1):83–90.
271. Sugimoto H, Ogura H, Arai Y, Limura Y, Yamanishi Y. Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. Jpn J Pharmacol 2002;89(1):7–20.
272. Bullock R. The clinical benefits of rivastigmine may reflect its dual inhibitory mode of action: An hypothesis. Int J Clin Pract 2002;56(3):206–214.
273. Giacobini E, Spiegel R, Enz A, Veroff AE, Cutler NR. Inhibition of acetyl- and butyrylcholinesterase in the cerebrospinal fluid of patients with Alzheimer's disease by rivastigmine: Correlation with cognitive benefit. J Neural Transm (Vienna) 2002;109(7–8):1053–1065.

274. Cosman KM, Boyle LL, Porteinsson AP. Memantine in the treatment of mild-to-moderate Alzheimer's disease. Expert Opin Pharmacother 2007;8(2):203–214.

275. Dajas-Bailador FA, Heimala K, Wonnacott S. The allosteric potentiation of nicotinic acetylcholine receptors by galantamine is transduced into cellular responses in neurons: Ca^{2+} signals and neurotransmitter release. Mol Pharmacol 2003;64(5):1217–1226.

276. Wu Z, Zhao L, Chen X, Cheng X, Zhang Y. Galantamine attenuates amyloid-β deposition and astrocyte activation in APP/PS1 transgenic mice. Exp Gerontol 2015;72:244–250.

277. Veleba J, Kopeckv J, Jr., Janovska P, Kuda O, Horakova O, Malinska H, Kazdova L, Oliyarnyk O, Skop V, Trnovska J, Hajek M, Skoch A, Flachs P, Bardova K, Rossmeisl M, Olza J, de Castro GS, Calder PC, Gardlo A, Fiserova E, Jensen J, Bryhn M, Kopecky J, Sr., Pelikanova T. Combined intervention with pioglitazone and n-3 fatty acids in metformin-treated type 2 diabetic patients: Improvement of lipid metabolism. Nutr Metab (Lond) 2015;12:52.

278. Ahmann A. Combination therapy in type 2 diabetes mellitus: Adding empagliflozin to basal insulin. Drugs Context 2015;4:212288.

279. Moses RG, Round E, Shentu Y, Golm GT, O'Neill EA, Gantz I, Engel SS, Kaufman KD, Goldstein BJ. A randomized clinical trial evaluating the safety and efficacy of sitagliptin added to the combination of sulfonylurea and metformin in patients with type 2 diabetes mellitus and inadequate glycemic control. J Diabetes 2016;8(5):701–711.

280. Tang YZ, Wang G, Jiang ZH, Yan TT, Chen YJ, Yang M, Meng LL, Zhu YJ, Li CG, Li Z, Yu P, Ni CL. Efficacy and safety of vidagliptin, sitagliptin, and linagliptin as add-on therapy in Chinese patients with T2DM inadequately controlled with dual combination of insulin and traditional oral hypoglycemic agent. Diabetol Metab Syndr 2015;7:91.

281. Saboo B, Reddy GC, Juneja S, Kedia AK, Manjrekar P, Rathod R; behalf of GLOBE Investigators. Effectiveness and safety of fixed dose combination of acarbose/metformin in Indian Type 2 diabetes patients: Results from observational GLOBE Study. Indian J Endocrinol Metab 2015;19(1):129–135.

282. Bennett WL, Maruthur NM, Sigh S, Segal JB, Wilson LM, Chatterjee R, Marinopoulos SS, Puhan MA, Ranasinghe P, Block L, Nicholson WK, Hutfless S, Bass EB, Bolen S. Comparative effectiveness and safety of medications for type 2 diabetes: An update including new drugs and 2-drug combinations. Ann Intern Med 2011;154(9):602–613.

283. U.S. Food and Drug Administration. http://www.fda.gov/Drugs/DrugSafety/ucm459579.htm (accessed September 2016).

284. U.S. Food and Drug Administration. http://www.fda.gov/Drugs/DrugSafety/ucm475463.htm (accessed September 2016).

285. U.S. Food and Drug Administration. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm198543.htm (accessed November 2016).

286. Tabaton M, Perry G, Zhu X, Lee H, Casadesus G, Smith MA. Memantine: "Hypothesis testing" not "disease modifying" in Alzheimer's disease. Am J Pathol 2010;176(2):540–541.

287. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J, Qian F, Arastu M, Li M, Chollate S, Brennan MS, Quintero-Monzon O, Scannevin RH, Moore Arnold H, Engber T, Rhodes K, Ferrero J, Hang Y, Mikulskis A, Grimm J, Hock C, Nitsch RM, Sandrock A. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature 2016;537(7618):50–56.

288. DiTacchio KA, Heinemann SF, Dziewczapolski D. Metformin treatment alters memory function in a mouse model of Alzheimer's disease. J Alzheimers Dis 2015;44(1):43–48.

289. Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H, Woodward M, Boundy K, Ellis KA, Bush AI, Faux NG, Martins R, Szoeke C, Rowe C, Watters DA, AIBL Investigators. Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care 2013;36(10):2981–2987.

290. Picone P, Nuzzo D, Caruana L, Messina E, Barera A, Vasto S, Di Carlo M. Metformin increases APP expression and processing via oxidative stress, mitochondrial dysfunction and NF-κB activation: Use of insulin to attenuate metformin's effect. Biochim Biophys Acta 2015;1853(5):1046–1059.
291. Mostafa DK, Ismail CA, Ghareeb DA. Differential metformin dose-dependent effects on cognition in rats: Role of Akt. Psychopharmacology (Berl) 2016;233(13):2513–2524.
292. Allard JS, Perez EJ, Fukui K, Carpenter P, Ingram DK, de Cabo R. Prolonged metformin treatment leads to reduced transcription of Nrf2 and neurotrophic factors without cognitive impairment in older C57BL/6J mice. Behav Brain Res 2016;301:1–9.
293. Zhou W, Kavelaars A, Heijnen CJ. Metformin prevents cisplatin-induced cognitive impairment and brain damage in mice. PLoS One 2016;11(3):e0151890.
294. Hettich MM, Matthes F, Ryan DP, Griesche N, Schröder S, Dorn S, Krauβ S, Ehninger D. The anti-diabetic drug metformin reduces BACE1 protein level by interfering with the MID1complex. PLoS One 2014;9(7):e102420.
295. Luchsinger JA, Perez T, Chang H, Mehta P, Steffener J, Pradabhan G, Ichise M, Manly J, Devanand DP, Bagiella E. Metformin in amnestic mild cognitive impairment: results of a pilot randomized placebo controlled clinical trial. J Alzheimers Dis 2016;51(2):501–514.
296. Yin QQ, Pei JJ, Xu S, Luo DZ, Dong SQ, Sun MH, You L, Sun ZJ, Liu XP. Pioglitazone improves cognitive function via increasing insulin sensitivity and strengthening antioxidant defence system in fructose-drinking insulin resistance rats. PLoS One 2013;8(3):e59313.
297. Crenshaw DG, Asin K, Gottschalk WK, Liang Z, Zhang N, Roses AD. Effects of low doses of pioglitazone on resting-state functional connectivity in conscious rat brain. PLoS One 2015;10(2):e0117973.
298. Prakash A, Kumar A, Ming LC, Mani V, Majeed AB. Modulation of the nitrergic pathway via activation of PPAR-γ contributes to the neuroprotective effect of pioglitazone against streptozotocin-induced memory dysfunction. J Mol Neurosci 2015;56(3):739–750.
299. Hanyu H, Sato T, Kiuchi A, Sakurai H, Iwamoto T. Pioglitazone improved cognition in a pilot study on patients with Alzheimer's disease and mild cognitive impairment with diabetes mellitus. J Am Geriatr Soc 2009;57(1):177–179.
300. Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer's disease. Neurobiol Aging 2011;32(9):1626–1633.
301. Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, Callaghan M, Arbuckle M, Behl C, Craft S. Long acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. J Alzheimers Dis 2015;45(4):1269–1270.
302. McClean PL, Jalewa J, Hölscher C. Prophylactic liraglutide treatment prevents amyloid plaque deposition, chronic inflammation and memory impairment in APP/PS1 mice. Behav Brain Res 2015;293:96–106.
303. Hansen HH, Barkholt P, Fabricius K, Jelsing J, Terwel D, Pyke C, Knudsen LB, Vrang N. The GLP-1 receptor agonist liraglutide reduces pathology-specific tau phosphorylation and improves motor function in a transgenic hTauP301L mouse model of tauopathy. Brain Res 2016;1634:158–170.
304. Hansen HH, Fabricius K, Barkholt P, Niehoff ML, Morley JE, Jelsing J, Pyke C, Knudsen LB, Farr SA, Vrang N. The GLP-1 receptor agonist liraglutide improves memory function and increases hippocampal CA1 neuronal numbers in a senescence-accelerated mouse model of Alzheimer's disease. J Alzheimers Dis 2015;46(4):877–888.
305. ClinicalTrials. gov. https://clinicaltrials.gov/ct2/show/NCT01843075 (accessed September 2016).
306. Ali MA, El-Abhar HS, Kamel MA, Attia AS. Antidiabetic effect of galantamine: Novel effect for a known centrally acting drug. PLoS One 2015;10(8):e0134648.
307. Kuršvietienė L, Stanevičienė I, Mongirdienė A, Bernatonienė J. Multiplicity of effects and health benefits of resveratrol. Medicina (Kaunas) 2016;52(3):148–155.

308. Ladiwala AR, Lin JC, Bale SS, Marcelino-Cruz AM, Bhattacharya M, Dordick JS, Tessier PM. Resveratrol selectively remodels soluble oligomers and fibrils of amyloid Abeta into off-pathways conformers. J Biol Chem 2010;285(31):24228–24237.

309. Marambaud P, Zhao H, Davies P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. J Biol Chem 2005;280(45):37377–37382.

310. Meng Z, Li J, Zhao H, Liu H, Zhang G, Wang L, Hu HE, Li DI, Liu M, Bi F, Wang X, Tian G, Liu Q, Buren B. Resveratrol relieves ischemia-induced oxidative stress in the hippocampus by activating SIRT1. Exp Ther Med 2015;10(2):525–530.

311. Li W, Tan C, Liu X, Wang X, Gui Y, Qin L, Deng F, Yu Z, Hu C, Chen L. Resveratrol ameliorates oxidative stress and inhibits aquaporin 4 expression following rat cerebral ischemia-reperfusion injury. Mol Med Rep 2015;12(5):7756–7762.

312. Zhao HF, Li N, Wang Q, Cheng XJ, Li ZM, Liu TT. Resveratrol decreases the insoluble Aβ1-42 level in hippocampus and protects the integrity of the blood-brain barrier. Neuroscience 2015;310:641–649.

313. Anastácio JR, Netto CA, Castro CC, Sanches EF, Ferreira DC, Noschang C, Krolow R, Dalmaz C, Pagnussat A. Resveratrol treatment has neuroprotective effects and prevents cognitive impairment after chronic cerebral hypoperfusion. Neurol Res 2014;36(7):627–633.

314. Hong JH, Lee H, Lee SR. Protective effect of resveratrol against neuronal damage following transient global cerebral ischemia in mice. J Nutr Biochem 2016;27:146–152.

315. Li H, Wang J, Wang P, Rao Y, Chen L. Resveratrol reverses the synaptic plasticity deficits in a chronic cerebral hypoperfusion rat model. J Stroke Cerebrovasc Dis 2016;25(1):122–128.

316. Mishra R, Sellin D, Radovan D, Gohlke A, Winter R. Inhibiting islet amyloid polypeptide fibril formation by the red wine compound resveratrol. Chembiochem 2009;10(3):445–449.

317. Lolicato F, Raudino A, Milardi D, La Rosa C. Resveratrol interferes with the aggregation of membrane-bound human-IAPP: A molecular dynamics study. Eur J Med Chem 2015;92:876–881.

318. Tu LH, Young LM, Wong AG, Ashcroft AE, Radford SE, Raleigh DP. Mutational analysis of the ability of resveratrol to inhibit amyloid formation by islet amyloid polypeptide: Critical evaluation of the importance of aromatic-inhibitor and histidine-inhibitor interactions. Biochemistry 2015;54(3):666–676.

319. Marek P, Abedini A, Song B, Kanungo M, Johnson ME, Gupta R, Zaman W, Wong SS, Raleigh DP. Aromatic interactions are not required for amyloid fibril formation by islet amyloid polypeptide but do influence the rate of fibril formation and fibril morphology. Biochemistry 2007;46(11):3255–3261.

320. Tracz SM, Abedini A, Driscoll M, Raleigh DP. Role of aromatic interactions in amyloid formation by peptides derived from human amylin. Biochemistry 2004;43(50):15901–15908.

321. Porat Y, Abramowitz A, Gazit E. Inhibition of amyloid fibril formation by polyphenols: Structural similarity and aromatic interactions as a common inhibition mechanism. Chem Biol Drug Des 2006;67(1):27–37.

322. Rambaran RN, Serpell LC. Amyloid fibrils: Abnormal protein assembly. Prion 2008;2(3):112–117.

323. Khazaei M, Karimi J, Sheikh N, Goodarzi MT, Saidijam M, Khodadadi I, Moridi H. Effects of resveratrol on receptor for advanced glycation end products (RAGE) expression and oxidative stress in the liver of rats with type 2 diabetes. Phytother Res 2016;30(1):66–71.

324. González-Rodríguez Á, Santamaría B, Mas-Gutierrez JA, Rada P, Fernández-Millán E, Pardo V, Álvarez C, Cuadrado A, Ros M, Serrano M, Valverde ÁM. Resveratrol treatment restores peripheral insulin sensitivity in diabetic mice in a sirt1-independent manner. Mol Nutr Food Res 2015;59(8):1431–1442.

325. Côté CD, Rasmussen BA, Duca FA, Zadeh-Tahmasebi M, Baur JA, Daljeet M, Breen DM, Filippi BM, Lam TK. Resveratrol activates duodenal Sirt1 to reverse insulin resistance in rats through a neuronal network. Nat Med 2015;21(5):498–505.

326. Sadi G, Pektas MB, Koca HB, Tosun M, Koca T. Resveratrol improves hepatic insulin signalling and reduces the inflammatory response in streptozotocin-induced diabetes. Gene 2015;570(2):213–220.

327. Movahed A, Nabipour I, Louis XL, Thandapilly SJ, Yu L, Kalantarhormozi M, Rekabpour SJ, Netticadan T. Antihyperglycemic effects of short term resveratrol supplementation in type 2 diabetic patients. Evid Based Complement Alternat Med 2013;2013:851267.

328. Bhatt JK, Thomas S, Nanjan MJ. Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. Nutr Res 2012;32(7):537–541.

329. Brasnyó P, Molnár GA, Mohás M, Markó L, Laczy B, Cseh J, Mikolás E, Szijártó IA, Mérei A, Halmai R, Mészáros LG, Sümegi B, Wittmann I. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. Br J Nutr 2011;106(3):383–389.

330. Rouse M, Younès A, Egan JM. Resveratrol and curcumin enhance pancreatic β-cell function by inhibiting phosphodiesterase activity. J Endocrinol 2014;223(2):107–117.

331. Bashmakov YK, Assaad-Khalil SH, Abou Seif M, Udumyan R, Megallaa M, Rohoma KH, Zeitoun M, Petyaev IM. Resveratrol promotes foot ulcer size reduction in type 2 diabetes patients. ISRN Endocrinol 2014;2014:816307.

332. Thazhath SS, Wu T, Bound MJ, Checklin HL, Standfield S, Jones KL, Horowitz M, Rayner CK. Administration of resveratrol for 5 wk has no effect on glucagon-like peptide 1 secretion, gastric emptying, or glycemic control in type 2 diabetes: A randomized controlled trial. Am J Clin Nutr 2016;103(1):66–70.

333. ClinicalTrials. gov. https://clinicaltrials.gov/ct2/results?term=resveratrol+type+2+diabetes&Search=Search (accessed September2016).

334. Turner RS, Thomas RG, Craft S, van Dyck CH, Mintzer J, Reynolds BA, Brewer JB, Rissman RA, Raman R, Aisen PS, Alzheimer's Disease Cooperative Study. A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer's disease. Neurology 2015;85(16):1383–1391.

335. Wang Q, Xu J, Rottinghaus GE, Simonyi A, Lubahn D, Sun GY, Sun AY. Resveratrol protects against global cerebral ischemic injury in gerbils. Brain Res 2002;958(2):439–447.

336. Walle T, Hsieh F, DeLegge MH, Oatis JE, Jr., Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos 2004;32(12):1377–1382.

337. Walle T. Bioavailability of resveratrol. Ann N Y Acad Sci 2011;1215:9–15.

338. Yu C, Shin YG, Chow A, Li Y, Kosmeder JW, Lee YS, Hirschelman WH, Pezzuto JM, Mehta RG, van Breemen RB. Human, rat, and mouse metabolism of resveratrol. Pharm Res 2002;19(12):1907–1914.

339. Patel KR, Andreadi C, Britton RG, Horner-Glister E, Karmokar A, Sale S, Brown VA, Brenner DE, Singh R, Steward WP, Gescher AJ, Brown K. Sulfate metabolites provide an intracellular pool for resveratrol generation and induce autophagy with senescence. Sci Transl Med 2013;5(205):205ra133.

340. Johnson JJ, Nihal M, Siddiqui IA, Scarlett CO, Bailey HH, Mukhtar H, Ahmad N. Enhancing the bioavailability of resveratrol by combining it with piperine. Mol Nutr Food Res 2011;55(8):1169–1176.

341. Elango B, Dornadula S, Paulmurugan R, Ramkumar KM. Pterostilbene ameliorates streptozotocin-induced diabetes through enhancing antioxidant signalling pathways mediated by Nrf2. Chem Res Toxicol 2016;29(1):47–57.

342. Oritani Y, Okitsu T, Nishimura E, Sai M, Ito T, Takeuchi S. Enhanced glucose tolerance by intravascularly administered piceatannol in freely moving healthy rats. Biochem Biophys Res Commun 2016;470(3):753–758.

343. Llarena M, Andrade F, Hasnaoui M, Portillo MP, Pérez-Matute P, Arbones-Mainar JM, Hijona E, Villanueva-Millán MJ, Aguirre L, Carpéné C, Aldámiz-Echevarría L. Potential renoprotective effects of piceatannol in ameliorating the early-stage nephropathy associated with obesity in obese Zucker rats. J Physiol Biochem 2016;72(3):555–566.

344. Minakawa M, Miura Y, Yagasaki K. Piceatannol, a resveratrol derivative, promotes glucose uptake through glucose transporter 4 translocation to plasma membrane in L6 myocytes and suppresses blood glucose levels in type 2 diabetic model db/db mice. Biochem Biophys Res Commun 2012;422(3):469–475.

345. Fu Z, Yang J, Wei Y, Li J. Effects of piceatannol and pterostilbene against β-amyloid-induced apoptosis on the PI3K/Akt/Bad signalling pathway in PC12 cells. Food Funct 2016;7(2):1014–1023.

346. Zhou Y, Zhang XM, Ma A, Zhang YL, Chen YY, Zhou H, Li WJ, Jin X. Orally administered pterostilbene attenuates acute cerebral ischemia-reperfusion injury in a dose- and time-dependent manner in mice. Pharmacol Biochem Behav 2015;135:199–209.

347. Kim HJ, Lee KW, Lee HJ. Protective effects of piceatannol against beta-amyloid-induced neuronal cell death. Ann N Y Acad Sci 2007;1095:473–482.

348. Chang J, Rimando A, Pallas M, Camins A, Porquet D, Reeves J, Shukitt-Hale B, Smith MA, Joseph JA, Casadesus G. Low-dose pterostilbene, but not resveratrol, is a potent neuromodulator in aging and Alzheimer's disease. Neurobiol Aging 2012;33(9):2062–2071.

349. Azzolini M, La Spina M, Mattarei A, Paradisi C, Zoratti M, Biasutto L. Pharmacokinetics and tissue distribution of pterostilbene in the rat. Mol Nutr Food Res 2014;58(11):2122–2132.

350. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: A short review. Life Sci 2006;78(18):2081–2087.

351. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological action and medicinal applications. Curr Sci 2004;87(1):44–53.

352. Zhao BL, Li XJ, He RG, Cheng SJ, Xin WJ. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. Cell Biophys 1989;14(2):175–185.

353. Maithilikarpagaselvi N, Sridhar MG, Swaminathan RP, Zachariah B. Curcumin prevents inflammatory response, oxidative stress and insulin resistance in high fructose fed male Wistar rats: potential role of serine kinases. Chem Biol Interact 2016;244:187–194.

354. Hao F, Kang J, Cao Y, Fan S, Yang H, An Y, Pan Y, Tie L, Li X. Curcumin attenuates palmitate-induced apoptosis in MIN6 pancreatic β-cells through PI3K/Akt/FoxO1 and mitochondrial survival pathways. Apoptosis 2015;20(11):1420–1432.

355. Naijil G, Anju TR, Jayanarayanan S, Paulose CS. Curcumin pretreatment mediates antidiabetogenesis via functional regulation of adrenergic receptor subtypes in the pancreas of multiple low-dose streptozotocin-induced diabetic rats. Nutr Res 2015;35(9):823–833.

356. Garcia-Alloza M, Borrelli LA, Rozkalne A, Hyman BT, Bacskai BJ. Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer's mouse model. J Neurochem 2007;102(4):1095–1104.

357. Thapa A, Jett SD, Chi EY. Curcumin attenuates amyloid-β aggregate toxicity and modulates amyloid-β aggregation pathway. ACS Chem Neurosci 2016;7(1):56–68.

358. Daval M, Bedrood S, Gurlo T, Huang CJ, Costes S, Butler PC, Langen R. The effect of curcumin on human islet amyloid polypeptide misfolding and toxicity. Amyloid 2010;17(3–4):118–128.

359. Li J, Wang P, Zhu Y, Chen Z, Shi T, Lei W, Yu S. Curcumin inhibits neuronal loss in the retina and elevates Ca^{2+}/calmodulin-dependent protein kinase II activity in diabetic rats. J Ocul Pharmacol Ther 2015;31(9):555–562.

360. Stankowska DL, Krishnamoorthy VR, Ellis DZ, Krishnamoorthy RR. Neuroprotective effects of curcumin on endothelin-1 mediated cell death in hippocampal neurons. Nutr Neurosci 2015. doi: 10.1080/1028415X.2015.1119377.

361. Huang HC, Zheng BW, Guo Y, Zhao J, Zhao JY, Ma XW, Jiang ZF. Antioxidative and neuroprotective effects of curcumin in an Alzheimer's disease rat model co-treated with intracerebroventricular streptozotocin and subcutaneous D-galactose. J Alzheimers Dis 2016;52(3):899–911.

362. Bustanji Y, Taha MO, Almasri IM, Al-Ghussein MA, Mohammad MK, Alkhatib HS. Inhibition of glycogen synthase kinase by curcumin: Investigation by simulated molecular docking and subsequent in vitro/in vivo evaluation. J Enzyme Inhib Med Chem 2009;24(3):771–778.

363. Maqbool M, Mobashir M, Hoda N. Pivotal role of glycogen synthase kinase-3: A therapeutic target for Alzheimer's disease. Eur J Med Chem 2016;107:63–81.

364. Sharma S, Taliyan R. Neuroprotective role of indirubin-3'-monoxime, a GSKβ inhibitor in high fat diet induced cognitive impairment in mice. Biochem Biophys Res Commun 2014;452(4):1009–1015.

365. ClinicalTrials. gov. https://clinicaltrials.gov/ct2/results?term=curcumin+type+2+diabetes&Search=Search (accessed September2016).
366. Chuengsamarn S, Rattanamongkolgul S, Luechapudiporn R, Phisalaphong C, Jirawatnotai S. Curcumin extract for prevention of type 2 diabetes. Diabetes Care 2012;35(11):2121–2127.
367. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. Mol Pharm 2007;4(6):807–818.
368. Ringman JM, Frautschy SA, Teng E, Begum AN, Bardens J, Beigi M, Gylys KH, Badmaev V, Heath DD, Apostolova LG, Porter V, Vanek Z, Marshall GA, Hellemann G, Sugar C, Masterman DL, Montine TJ, Cummings JL, Cole GM. Oral curcumin for Alzheimer's disease: Tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. Alzheimers Res Ther 2012;4(5):43.
369. Gooze KG, Shah TM, Sohrabi HR, Rainey-Smith SR, Brown B, Verdile G, Martins RN. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. Br J Nutr 2016;115(3):449–465.
370. Bidon-Chanal A, Fuertes A, Alonso D, Pérez DI, Martínez A, Luque FJ, Medina M. Evidence for a new binding mode to GSK-3: Allosteric regulation by the marine compound palinurin. Eur J Med Chem 2013;60:479–489.
371. Alfano G, Cimino G, De Stefano S. Palinurin, a new linear sesterterpene from a marine sponge. Experientia 1979:35(9):1136–1137.
372. Shen LH, Zhang JT. Ginsenoside Rg1 promotes proliferation of hippocampal progenitor cells. Neurol Res 2004;26(4):422–428.
373. Xiong Y, Shen L, Liu KJ, Tso P, Xiong Y, Wang G, Woods SC, Liu M. Antiobesity and antihyperglycemic effects of ginsenoside Rb1 in rats. Diabetes 2010;59(20):2505–2512.
374. Yu X, Ye L, Zhang H, Zhao J, Wang G, Guo C, Shang W. Ginsenoside Rb1 ameliorates liver fat accumulation by upregulating perilipin expression in adipose tissue of db/db obese mice. J Ginseng Res 2015;39(3):199–205.
375. Liu C, Zhang M, Hu MY, Guo HF, Li J, Yu YL, Jin S, Wang XT, Liu L, Liu XD. Increased glucagon-like peptide-1 secretion may be involved in antidiabetic effects of ginsenosides. J Endocrinol 2013;217(2):185–196.
376. Park SM, Hong AM, Sung SR, Lee JE, Kwon DY. Extracts of *Rehmanniae radix*, *Ginseng radix* and *Scutellariae radix* improve glucose-stimulated insulin secretion and β-cell proliferation through IRS2 induction. Genes Nutr 2008;2(4):347–351.
377. Lee JS, Song JH, Sohn NW, Shin JW. Inhibitory effects of ginsenoside Rb1 on neuroinflammation following systemic lipopolysaccharide treatment in mice. Phytother Res 2013;27(9):1270–1276.
378. Jang M, Lee MJ, Choi JH, Kim EJ, Nah SY, Kim HJ, Lee S, Lee SW, Kim YO, Cho IH. Ginsenoside Rb1 attenuates acute inflammatory nociception by inhibition of neuronal ERK phosphorylation by regulation of the Nrf2 and NF-κB pathways. J Pain 2016;17(3):282–297.
379. Qian YH, Han H, Hu XD, Shi LL. Protective effect of ginsenoside Rb1 on beta-amyloid protein (1-42)-induced neurotoxicity in cortical neurons. Neurol Res 2009;31(7):663–667.
380. Chen X, Huang T, Zhang J, Song J, Chen L, Zhu Y. Involvement of calpain and p25 of CDK5 pathway in ginsenoside Rb1's attenuation of beta-amyloid peptide25-35-induced tau hyperphosphorylation in cortical neurons. Brain Res 2008;1200:99–106.
381. Liu C, Hu M, Guo H, Zhang M, Zhang J, Li F, Zhong Z, Chen Y, Li Y, Xu P, Li J, Liu L, Liu X. Combined contribution of increased intestinal permeability and inhibited deglycosylation of ginsenoside Rb1 in the intestinal tract to the enhancement of ginsenoside Rb1 exposure in diabetic rats after oral administration. Drug Metab Dispos 2015;43(11):1702–1710.
382. Jiang S, Ren D, Li J, Yuan G, Li H, Xu G, Han X, Du P, An L. Effects of compound K on hyperglycemia and insulin resistance in rats with type 2 diabetes mellitus. Fitoterapia 2014; 95:58–64.

383. Gu J, Li W, Xiao D, Wei S, Cui W, Chen W, Hu Y, Bi X, Kim Y, Li J, Du H, Zhang M, Chen L. Compound K, a final intestinal metabolite of ginsenosides, enhances insulin secretion in MIN6 pancreatic β-cells by upregulation of GLUT2. Fitoterapia 2013;87:84–88.

384. Thorens B. GLUT2 in pancreatic and extra-pancreatic gluco-detection (review). Mol Membr Biol 2001;18(4):265–273.

385. Guan FY, Gu J, Li W, Zhang M, Ji Y, Li J, Chen L, Hatch GM. Compound K protects pancreatic islet cells against apoptosis through inhibition of the AMPK/JNK pathway in type 2 diabetic mice and in MIN6 β-cells. Life Sci 2014;107(1–2):42–49.

386. Kim K, Park M, Lee YM, Rhyu MR, Kim HY. Ginsenoside metabolite compound K stimulates glucagon-like peptide-1 secretion in NCI-H716 cells via bile acid receptor activation. Arch Pharm Res 2014;37(9):1193–1200.

387. Wei S, Li W, Yu Y, Yao F, A L, Lan X, Guan F, Zhang M, Chen L. Ginsenoside compound K suppresses the hepatic gluconeogenesis via activating adenosine-5′monopjosphate kinase: A study in vitro and in vivo. Life Sci 2015;139:8–15.

388. Guo J, Chang L, Zhang X, Pei S, Yu M, Gao J. Ginsenoside compound K promotes β-amyloid peptide clearance in primary astrocytes via autophagy enhancement. Exp Ther Med 2014;8(4):1271–1274.

389. Park JS, Shin JA, Jung JS, Hyun JW, Van Le TK, Kim DH, Park EM, Kim HS. Anti-inflammatory mechanism of compound K in activated microglia and its neuroprotective effect on experimental stroke in mice. J Pharmacol Exp Ther 2012;341(1):59–67.

390. Yang XD, Yang YY, Ouyang DS, Yang GP. A review of biotransformation and pharmacology of ginsenoside compound K. Fitoterapia 2015;100:208–220.

391. Ladiwala AR, Mora-Pale M, Lin JC, Bale SS, Fishman ZS, Dordick JS, Tessier PM. Polyphenolic glycosides and aglycones utilize opposing pathways to selectively remodel and inactivate toxic oligomers of amyloid β. Chembiochem 2011;12(11):1749–1758.

392. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Scientific World J 2013;2013:162750.

393. Uriarte-Pueyo I, Calvo MI. Flavonoids as acetylcholinesterase inhibitors. Curr Med Chem 2011;18(34):5289–5302.

394. Rauter AP, Martins A, Borges C, Mota-Filipe H, Pinto R, Sepodes B, Justino J. Antihyperglycemic and protective effects of flavonoids on streptozotocin-induced diabetic rats. Phytother Res 2010;24(Suppl2):S133–S138.

395. Shih CC, Wu JB, Jian JY, Lin CH, Ho HY. (-)-Epicatechin-3-O-β-D-allopyranoside from *Davallia formosana*, prevents diabetes and hyperlipidemia by regulation of glucose transporter 4 and AMP-activated protein kinase phosphorylation in high-fat-fed mice. Int J Mol Sci 2015;16(10):24983–25001.

396. Tarozzi A, Merlicco A, Morroni F, Franco F, Cantelli-Forti G, Teti G, Falconi M, Hrelia P. Cyanidin 3-O-glucopyranoside protects and rescues SH-SY5Y cells against amyloid-beta peptide-induced toxicity. Neuroreport 2008;19(15):1483–1486.

397. Qin L, Zhang J, Qin M. Protective effect of cyanidin 3-O-glucoside on beta-amyloid peptide-induced cognitive impairment in rats. Neurosci Lett 2013;534:285–288.

398. Xiao J, Capanoglu E, Jassbi AR, Miron A. Advance on the flavonoid C-glycosides and health benefits. Crit Rev Food Sci Nutr 2016;56(Suppl 1):S29–S45.

399. Tang D, Dong Y, Guo N, Li L, Ren H. Metabolomic analysis of the polyphenols in germinating mung beans (*Vigna radiata*) seeds and sprouts. J Sci Food Agric 2014;94(8):1639–1647.

400. Zhang Y, Jiao J, Liu C, Wu X, Zhang Y. Isolation and purification of four flavone C-glycosides from antioxidant of bamboo leaves and preparative high-performance liquid chromatography. Food Chem 2008;107(3):1326–1336.

401. Farsi E, Ahmad M, Hor SY, Ahamed MB, Yam MF, Asmawi MZ. Standardized extract of *Ficus deltoidea* stimulates insulin secretion and blocks hepatic glucose production by regulating the ex-

pression of glucose-metabolic genes in streptozotocin-induced diabetes. BMC Complement Altern Med 2014;14:220.

402. Choo CY, Sulong NY, Man F, Wong TW. Vitexin and isovitexin from the leaves of *Ficus deltoidea* with in-vivo α-glucosidase inhibition. J Ethnopharmacol 2012;142(3):776–781.
403. Peng X, Zheng Z, Cheng K, Shan F, Ren G, Chen F, Wang M. Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. Food Chem 2008;106(2):475–481.
404. Choi JS, Islam MN, Ali MY, Kim EJ, Kim YM, Jung HA. Effects of C-glycosylation on anti-diabetic, anti-Alzheimer's disease and anti-inflammatory potential of apigenin. Food Chem Toxicol 2014;64:27–33.
405. Guimarães CC, Oliveira DD, Valdevite M, Saltoratto AL, Pereira SI, França SC, Pereira AM, Pereira PS. The glycosylated flavonoids vitexin, isovitexin, and quercetrin isolated from *Serjania erecta* Radlk (Sapindaceae) leaves protect PC12 cells against amyloid-β25-35 peptide-induced toxicity. Food Chem Toxicol 2015;86:88–94.
406. Cazarolli LH, Kappel VD, Pereira DF, Moresco HH, Brighente IM, Pizzolatti MG, Silva FR. Anti-hyperglycemic action of apigenin-6-C-β-fucopyranoside from *Averrhoa carambola*. Fitoterapia 2012;83(7):1176–1183.
407. Cazarolli LH, Folador P, Moresco HH, Brighente IM, Pizzolatti MG, Silva FR. Stimulatory effect of apigenin-6-C-β-L-fucopyranoside on insulin secretion and glycogen synthesis. Eur J Med Chem 2009;44(11):4668–4673.
408. Jesus AR, Dias C, Matos AM, de Almeida RF, Viana AS, Marcelo F, Ribeiro RT, Macedo MP, Airoldi C, Nicotra F, Martins A, Cabrita EJ, Jiménez-Barbero J, Rauter AP. Exploiting the therapeutic potential of 8-β-D-glucopyranosylgenistein: Synthesis, antidiabetic activity, and molecular interaction with islet amyloid polypeptide and amyloid β-peptide (1-42). J Med Chem 2014;57(22):9463–9472.
409. Rauter AP, Martins A, Lopes R, Ferreira J, Serralheiro LM, Araújo ME, Borges C, Justino J, Silva FV, Goulart M, Thomas-Oates J, Rodrigues JA, Edwards E, Noronha JP, Pinto R, Mota-Filipe H. Bioactivity studies and chemical profile of the antidiabetic plant *Genista tenera*. J Ethnopharmacol 2009;122(2):384–393.
410. Rauter AP, Jesus A, Martins A, Dias C, Ribeiro R, Macedo MP, Justino J, Mota-Filipe H, Pinto R, Sepodes B, Medeiros M, Jiménez-Barbero J, Airoldi C, Nicotra F. New C-glycosylpolyphenol antidiabetic agents, effect on glucose tolerance and interaction with beta-amyloid. Therapeutic applications of the synthesized agent(s) and of *Genista tenera* ethyl acetate extracts containing some of those agents. WO 2013132470 A2, 2013. App. filled Mar9, 2012; pub. Sep12, 2013.
411. Hsu FL, Liu IM, Kuo DH, Chen WC, Su HC, Cheng JT. Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. J Nat Prod 2003;66(6):788–792.
412. Wu K, Liang T, Duan X, Xu L, Zhang K, Li R. Anti-diabetic effects of puerarin, isolated from *Pueraria lobata* (Willd.), on streptozotocin-diabetogenic mice through promoting insulin expression and ameliorating metabolic function. Food Chem Toxicol 2013;60:341–347.
413. Li Z, Shangguan Z, Liu Y, Wang J, Li X, Yang S, Liu S. Puerarin protects pancreatic β-cell survival via PI3K/Akt signalling pathway. J Mol Endocrinol 2014;53(1):71–79.
414. Liu X, Mo Y, Gong J, Li Z, Peng H, Chen J, Wang Q, Ke Z, Xie J. Puerarin ameliorates cognitive deficits in streptozotocin-induced diabetic rats. Metab Brain Dis 2016;31(2):417–423.
415. Kato E, Kawabata J. Glucose uptake enhancing activity of puerarin and the role of C-glucoside suggested from activity of related compounds. Bioorg Med Chem Lett 2010;20(15):4333–4336.
416. Ito-Nagahata T, Kurihara C, Hasebe M, Ishii A, Yamashita K, Iwabuchi M, Sonoda M, Fukuhara K, Sawada R, Matsuoka A, Fujiwara Y. Stilbene analogs of resveratrol improve insulin resistance through activation of AMPK. Biosci Biotechnol Biochem 2013;77(6):1229–1235.

417. Yamaguchi S, Katahira H, Ozawa S, Nakamichi Y, Tanaka T, Shimoyama T, Takahashi K, Yoshimoto K, Imaizumi MO, Nagamatsu S, Ishida H. Activation of AMP-activated protein kinase enhance GLUT4 translocation and its glucose transport activity in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 2005;289(4):E643–E649.

418. Winder WW. Energy-sensing and signalling by AMP-activated protein kinase in skeletal muscle. J Appl Physiol 2001;91(3):1017–1028.

419. Matsuoka A, Furuta A, Ozaki M, Fukuhara K, Miyata N. Resveratrol, a naturally occurring polyphenol, induces sister chromatid exchanges in a Chinese hamster lung (CHL) cell line. Mutat Res 2001;494(1–2):107–113.

420. Csuk R, Albert S, Kluge R, Ströl D. Resveratrol derived butyrylcholinesterase inhibitors. Arch Pharm (Weinheim) 2013;346(7):499–503.

421. Gobec M, Tomašič T, Markovič T, Mlinarič -Rašč an I, Dolenc MS, Jakopin Ż. Antioxidant and anti-inflammatory properties of 1,2,4-oxadiazole analogs of resveratrol. Chem Biol Interact 2015;240:200–207.

422. Tanini D, Panzella L, Amorati R, Capperucci A, Pizzo E, Napolitano A, Menichetti S, d'Ischia M. Resveratrol-based benzoselenophenes with an enhanced antioxidant and chain breaking capacity. Org Biomol Chem 2015;13(20):5757–5764.

423. Antus C, Radnai B, Dombovari P, Fonai F, Avar P, Matyus P, Racz B, Sumegi B, Veres B. Anti-inflammatory effects of a triple-bond resveratrol analog: structure and function relationship. Eur J Pharmacol 2015;748:61–67.

424. Lu C, Guo Y, Yan J, Luo Z, Luo HB, Yan M, Huang L, Li X. Design, synthesis, and evaluation of multitarget-directed resveratrol derivatives for the treatment of Alzheimer's disease. J Med Chem 2013;56(14):5843–5859.

425. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Macfarlane S, Mastwyk M, MacGregor L, Kiers L, Cherny R, Li QX, Tammer A, Carrington D, Mavros C, Volitakis I, Xilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, Masters CL. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer's disease: A pilot phase 2 clinical trial. Arch Neurol 2003;60(12):1685–1691.

426. Huang X, Atwood CS, Moir RD, Hartshorn MA, Tanzi RE, Bush AI. Trace metal contamination initiates the apparent auto-aggregation amyloidosis, and oligomerization of Alzheimer's Abeta peptides. J Biol Inorg Chem 2004;9(8):954–960.

427. Curtain CC, Ali F, Volitakis I, Cherny RA, Norton RS, Beyreuther K, Barrow CJ, Masters CL, Bush AI, Barnham KJ. Alzheimer's disease amyloid-beta binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. J Biol Chem 2001;276(23):20466–20473.

428. Atwood CS, Scarpa RC, Huang X, Moir RD, Jones WD, Fairlie DP, Tanzi RE, Bush AI. Characterization of copper interactions with Alzheimer's amyloid beta peptides: Identification of an attomolar-affinity copper binding site on amyloid beta1-42. J Neurochem 2000;75(3):1219–1233.

429. Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE, Bush AI. Dramatic aggregation of Alzheimer's abeta by Cu(II) is induced by conditions representing physiological acidosis. J Biol Chem 1998;273(21):12817–12826.

430. Dolai S, Shi W, Corbo C, Sun C, Averick S, Obeysekera D, Farid M, Alonso A, Banerjee P, Raja K. "Clicked" sugar-curcumin conjugate: Modulator of amyloid-β and tau peptide aggregation at ultralow concentrations. ACS Chem Neurosci 2011;2(12):694–699.

431. Das KK, Razzaghi-Asl N, Tikare SN, Di Santo R, Costi R, Messore A, Pescatori L, Crucitti GC, Jargar JG, Dhundasi SA, Saso L. Hypoglycemic activity of curcumin synthetic analogues in alloxan-induced diabetic rats. J Enzyme Inhib Med Chem 2016;31(1):99–105.

432. Di Martino RM, De Simone A, Andrisano V, Bisignano P, Bisi A, Gobbi D, Rampa A, Fato R, Bergamini C, Perez DI, Martinez A, Bottegoni G, Cavalli A, Belluti F. Versatility of the curcumin scaffold: Discovery of potent and balanced dual BACE-1 and GSK-3β inhibitors. J Med Chem 2016;59(2):531–544.

433. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev 2011;91(2):733–794.

434. Singh AK, Singh R. Sodium-glucose co-traansport-2 inhibits as add-on therapy to insulin: Rationale and evidences. Expert Rev Clin Pharmacol 2016;9(3):409–418.

435. Gosch C, Halbwirth H, Stich K. Phloridzin: Biosynthesis, distribution and physiological relevance in plants. Phytochemistry 2010;71(8–9):838–843.

436. White JR, Jr. Apple trees to sodium glucose co-transporter inhibitors: A review of SGLT2 inhibition. Clin Diabetes 2010;28(1):5–10.

437. Malathi P, Crane RK. Phlorizin hydrolase: A beta-glucosidase of hamster intestinal brush border membrane. Biochim Biophys Acta 1969;173(2):245–256.

438. Scheen AJ. Pharmacokinetic and pharmacodynamics profile of empagliflozin, a sodium glucose co-transporter 2 inhibitor. Clin Pharmacokinet 2014;53(3):213–225.

439. ClinicalTrials. gov. https://clinicaltrials.gov/ct2/results?term=ertugliflozin+type+2+diabetes&Search=Search (accessed September2016).

440. Mascitti V, Thuma BA, Smith AC, Robinson RP, Brandt T, Kalgutkar AM, Maurer TS, Samas B, Sharma R. On the importance of synthetic organic chemistry in drug discovery: Reflections on the discovery of antidiabetic agent ertugliflozin. MedChemComm 2013;4:101–111.

441. Miao Z, Nucci G, Amin N, Sharma R, Mascitti V, Tugnait M, Vaz AD, Callegari E, Kalgutkar AS. Pharmacokinetics, metabolism, and excretion of the antidiabetic agent ertugliflozin (PF-04971729) in healthy male subjects. Drug Metab Dispos 2013;41(2):445–456.

442. Viet MH, Chen CY, Hu CK, Chen YR, Li MS. Discovery of dihydrochalcone as potential lead for Alzheimer's disease: In silico and in vitro study. PLoS One 2013;8(11):e179151.

443. Ghumatkar PJ, Patil SP, Jain PD, Tambe RM, Sathaye S. Nootropic, neuroprotective and neurotrophic effects of phloretin in scopolamine induced amnesia in mice. Pharmacol Biochem Behav 2015;135:182–191.

444. Liu Y, Zhang L, Liang J. Activation of the Nrf2 defence pathway contributes to neuroprotective effects of phloretin on oxidative stress injury after cerebral ischemia/reperfusion in rats. J Neurol Sci 2015;351(1–2):88–92.

445. Shaikh S, Verma A, Siddiqui S, Ahmad SS, Rizvi SM, Shakil S, Biswas D, Singh D, Siddiqui MH, Shakil S, Tabrez S, Kamal MA. Current acetylcholinesterase-inhibitors: A neuroinformatics perspective. CNS Neurol Disord Drug Targets 2014;13(3):391–401.

446. Rizvi SM, Shakil S, Biswas D, Shakil S, Shaikh S, Bagga P, Kamal MA. Invokana (Canagliflozin) as a dual inhibitor os acetylcholinesterase and sodium glucose co-transporter 2: Advancement in Alzheimer's disease-diabetes type 2 linkage via an enzoinformatics study. CNS Neurol Disord Drug Targets 2014;13(3):447–451.

447. Lin B, Koibuchi N, Hasegawa Y, Sueta D, Toyama K, Uekawa K, Ma M, Nakagawa T, Kusaka H, Kim-Mitsuyama S. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. Cardiovasc Diabetol 2014;13:148.

448. García-Osta A, Cuadrado-Tejedor M, Carcía-Barroso C, Oyarzábal J, Franco R. Phosphodiesterases as therapeutic targets for Alzheimer's disease. ACS Chem Neurosci 2012;3(11):832–844.

449. Degerman E, Belfrage P, Manganiello VC. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). J Biol Chem 1997;272(11):6823–6826.

450. Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: Phosphodiesterase inhibitors. Br J Clin Pharmacol 2011;72(4):634–646.

451. Ayala JE, Bracy BP, Julien BM, Rottman JN, Fueger PT, Wasserman DH. Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice. Diabetes 2007;56(4):1025–1033.

452. Genders AJ, Bradley EA, Rattigan S, Richards SM. cGMP phosphodiesterase inhibition improves the vascular and metabolic actions of insulin in skeletal muscle. Am J Physiol Endocrinol Metab 2011;301(2):E342–E350.

453. Fryburg DA, Gibbs EM. Treatment of insulin resistance syndrome and type 2 diabetes with PDE9 inhibitors. WO 2003037432 A1, 2003. App. filled Sep12, 2002; pub. May8, 2003.

454. Deninno MP, Hughes B, Kemp MI, Palmer MJ, Wood A. PDE9 inhibitors for treating cardiovascular disorders. WO 2003037899 A1, 2003. App. filled Oct22, 2002; pub. May8, 2003.

455. Bell AS, Deninno MP, Palmer MJ, Visser MS. PDE9 inhibitors for treating type 2 diabetes, metabolic syndrome, and cardiovascular disease. WO 2004096811, 2004. App. filled Apr21, 2004; pub. Nov11, 2004.

456. Deninno MP, Andrews M, Bell AS, Chen Y, Eller-Zarbo B, Eshelby N, Etienne JB, Moore DE, Palmer MJ, Visser MS, Yu LJ, Zavadoski WJ, Gibbs EM. The discovery of potent, selective, and orally bioavailable PDE9 inhibitors as potent hypoglycemic agents. Bioorg Med Chem Lett 2009;19(9):2537–2541.

457. Kumar M, Bhattacharya V. Cilostazol: A new drug in the treatment intermittent claudication. Recent Pat Cardiovasc Drug Discov 2007;2(3):181–185.

458. Hiramatsu M, Takiguchi O, Nishiyama A, Mori H. Cilostazol prevents amyloid β peptide (25-35)-induced memory impairment and oxidative stress in mice. Br J Pharmacol 2010;161(8):1899–1912.

459. Park SH, Kim JH, Bae SS, Hong KW, Lee DS, Leem JY, Choi BT, Shin HK. Protective effect of the phosphodiesterase III inhibitor cilostazol on amyloid β-induced cognitive deficits associated with decreased amyloid β accumulation. Biochem Biophys Res Commun 2011;408(4):602–608.

460. Horai S, Nakagawa S, Tanaka K, Morofuji Y, Couraud PO, Deli MA, Ozawa M, Niwa M. Cilostazol strengthens barrier integrity in brain endothelial cells. Cell Mol Neurobiol 2013;33(2):291–307.

461. Choi BR, Kim DH, Back DB, Kang CH, Moon WJ, Han JS, Choi DH, Kwon KJ, Shin CY, Kim BR, Lee J, Han SH, Kim HY. Characterization of white matter injury in a rat model of chronic cerebral hypoperfusion. Stroke 2016;47(2):542–547.

462. Sakurai H, Hanyu H, Sato T, Kume K, Hirao K, Kanetaka H, Iwamoto T. Effects of cilostazol on cognition and regional cerebral blood flow in patients with Alzheimer's disease and cerebrovascular disease: A pilot study. Geriatr Gerontol Int 2013;13(1):90–97.

463. Ihara M, Nishino M, Taguchi A, Yamamoto Y, Hattori Y, Saito S, Takahashi Y, Tsuji M, Kasahara Y, Takata Y, Okada M. Cilostazol add-on therapy in patients with mild dementia receiving donepezil: a retrospective study. PLoS One 2014;9(2):e89516.

464. ClinicalTrials. gov. https://clinicaltrials.gov/ct2/results?term=cilostazol&pg=2 (accessed September 2016).

465. Matsumoto T, Noguchi E, Ishida K, Nakayama N, Kobayashi T, Kamata K. Cilostazol improves endothelial dysfunction by increasing endothelium-derived hyperpolarizing factor response in mesenteric arteries from type 2 diabetics rats. Eur J Pharmacol 2008;599(1–3):102–109.

466. Suzuki K, Uchida K, Nakanishi N, Hattori Y. Cilostazol activates AMP-activated protein kinase and restores endothelial function in diabetes. Am J Hypertens 2008;21(4):451–457.

467. Park SY, Shin HK, Lee JH, Kim CD, Lee WS, Rhim BY, Hong KW. Cilostazol ameliorates metabolic abnormalities with suppression of proinflammatory makers in a db/db mouse model of type 2 diabetes via activation of peroxisome proliferator-activated receptor gamma transcription. J Pharmacol Exp Ther 2009;329(2):571–579.

468. Wada T, Onogi Y, Kimura Y, Nakano T, Fusanobori H, Ishii Y, Sasahara M, Tsuneki H, Sasaoka T. Cilostazol ameliorates systemic insulin resistance in diabetic db/db mice by suppressing chronic inflammation in adipose tissue via modulation of both adipocyte and macrophage functions. Eur J Pharmacol 2013;707(1–3):120–129.

469. Tseng SY, Chao TH, Li YH, Liu PY, Lee CH, Cho CL, Wu HL, Chen JH. Cilostazol improves high glucose-induced impaired angiogenesis in human endothelial progenitor cells and vascular endothelial cells as well as enhances vasculoangiogenesis in hyperglycemic mice mediated by the adenosine monophosphate-activated protein kinase pathway. J Vasc Surg 2016;63(4):1051–1062.e3.

470. Neel JD, Kruse RL, Dombrovskiy VY, Vogel TR. Cilostazol and freedom from amputation after lower extremity revascularization. J Vasc Surg 2015;61(4):960–964.

471. Liu JS, Chuang TJ, Chen JH, Lee CH, Hsieh CH, Lin TK, Hsiao FC, Hung YJ. Cilostazol attenuates the severity of peripheral arterial occlusive disease in patients with type 2 diabetes: The role of plasma soluble receptor for advanced glycation end-products. Endocrine 2015;49(3):703–710.

472. Verhoest PR, Fonseca KR, Hou X, Proulx-Lafrance C, Corman M, Helal CJ, Claffey MM, Tuttl JB, Coffman KJ, Liu S, Nelson F, Kleiman RJ, Menniti FS, Schmidt CJ, Vanase-Frawley M, Liras S. Design and discovery of 6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (PF-04447943), a selective brain penetrant PDE9A inhibitor for the treatment of cognitive disorders. J Med Chem 2012;55(21):9045–9054.

473. Verhoest PR, Proulx-Lafrance C, Corman M, Chenard L, Helal CJ, Hou X, Kleiman R, Liu S, Marr E, Menniti FS, Schmidt CJ, Vanase-Frawley M, Schmidt AW, Williams RD, Nelson FR, Fonseca KR, Liras S. Identification of a brain penetrant PDE9A inhibitor utilizing prospective design and chemical enablement as a rapid lead optimization strategy. J Med Chem 2009;52(24):7946–7949.

474. Ke H, Wang H. Crystal structures of phosphodiesterases and implications on substrate specificity and inhibitor selectivity. Curr Top Med Chem 2007;7(4):391–403.

475. Evans RM, Nicholas T, Le Vu, Qiu R, Martin W, Martin D, Styren S, Fullerton T, Schwam E. Safety and pharmacokinetics of PF-04447943, a PDEA inhibitor, in single and multiple dose Phase 1 studies in healthy volunteers. Alzheimers Dement 2010;6(4):S135.

476. Hutson PH, Finger EN, Magliaro BC, Smith SM, Converso A, Sanderson PE, Mullins D, Hyde LA, Eschle BK, Turnbull Z, Sloan H, Guzzi M, Zhang X, Wang A, Rindgen D, Mazzola R, Vivian JA, Eddins D, Uslaner JM, Bednar R, Gambone C, Le-Mair W, Marino MJ, Sachs N, Xu G, Parmentier-Batteur S. The selective phosphodiesterase 9 (PDE9) inhibitor PF-04447943 (6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) enhances synaptic plasticity and cognitive function in rodents. Neuropharmacology 2011;61(4):665–676.

477. Kleiman RJ, Chapin DS, Christoffersen C, Freeman J, Fonseca KR, Geoghegan KF, Grimwood S, Guanowsky V, Hajós M, Harms JF, Helal CJ, Hoffmann WE, Kocan GP, Majchrzak MJ, McGinnis D, McLean S, Menniti FS, Nelson F, Roof R, Schmidt AW, Seymour PA, Stephenson DT, Tingley FD, Vanase-Frawley M, Verhoest PR, Schmidt CJ. Phosphodiesterase 9A regulates central cGMP and modulates responses to cholinergic and monoaminergic perturbation in vivo. J Pharmacol Exp Ther 2012;341(2):396–409.

478. Schwam EM, Nicholas T, Chew R, Billing CB, Davidson W, Ambrose D, Altstiel LD. A multicentre, double-blind, placebo-controlled trial of the PDE9A inhibitor, PF-04447943, in Alzheimer's disease. Curr Alzheimer Res 2014;11(5):413–421.

479. Shao YX, Huang M, Cui W, Feng LJ, Wu Y, Cai Y, Li Z, Zhu X, Liu P, Wan Y, Ke H, Luo HB. Discovery of a phosphodiesterase 9A inhibitor as a potential hypoglycemic agent. J Med Chem 2014;57(24):103040–10313.

480. Luna-Medina R, Cortes-Canteli M, Sanchez-Galiano S, Morales-Garcia JA, Martinez A, Santos A, Perez-Castillo A. NP031112, a thiadiazolidinone compound, prevents inflammation and neurodegeneration under excitotoxic conditions: Potential therapeutic role in brain disorders. J Neurosci 2007;27(21):5766–5776.

481. Luna-Medina R, Cortes-Canteli M, Alonso M, Santos A, Martínez A, Perez-Castillo A. Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor gamma activation. J Biol Chem 2005;280(22):21453–21462.

482. Domínguez JM, Fuertes A, Orozco L, del Monte-Millán M, Delgado E, Medina M. Evidence for irreversible inhibition of glycogen synthase kinase-3β by tideglusib. J Biol Chem 2012;287(2):893–904.

483. Armagan G, Keser A, Atalayin Ç, Dagci T. Tideglusib protects neural stem cells against NMDA receptor overactivation. Pharmacol Rep 2015;67(5):823–831.

484. Serenó L, Coma M, Rodríguez M, Sánchez-Ferrer P, Sánchez MB, Gich I, Agulló JM, Pérez M, Avila J, Guardia-Laguarta C, Clarimón J, Lleó A, Gómez-Isla T. A novel GSK-3beta inhibitor reduces Alzheimer's pathology and rescues neuronal loss in vivo. Neurobiol Dis 2009;35(3):359–367.
485. DaRocha-Souto B, Coma M, Pérez-Nievaz BG, Scotton TC, Siao M, Sánchez-Ferrer P, Hashimoto T, Fan Z, Hudry E, Barroeta I, Serenó L, Rodríguez M, Sánchez MB, Hyman BT, Gómez-Isla T. Activation of glycogen synthase kinase-3 beta mediates β-amyloid induced neuritic damage in Alzheimer's disease. Neurobiol Dis 2012;45(1):425–437.
486. del Ser T, Steinwachs KC, Gertz HJ, Andrés MV, Gómez-Carillo B, Medina M, Vericat JA, Redondo P, Fleet D, León T. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: A pilot study. J Alzheimers Dis 2013;33(1):205–215.
487. Lovestone S, Boada M, Dubois B, Hüll M, Rinne JO, Huppertz HJ, Calero M, Andrés MV, Gómez-Carrillo B, León T, del Ser T. ARGO Investigators. A phase II trial of tideglusib in Alzheimer's disease. J Alzheimers Dis 2015;45(1):75–88.
488. Lee SC, Kim HT, Park CH, Lee DY, Chang HJ, Park S, Cho JM, Ro S, Suh YG. Design, synthesis and biological evaluation of novel imidazopyridines as potential antidiabetic GSK3β inhibitors. Bioorg Med Chem Lett 2012;22(13):4221–4224.
489. Prati F, De Simone A, Armirotti A, Summa M, Pizzirani D, Scarpelli R, Bertozzi SM, Perez DI, Andrisano V, Perez-Castillo A, Monti B, Massenzio F, Polito L, Racchi M, Sabatino P, Bottegoni G, Martinez A, Cavalli A, Bolognesi ML. 3,4-Dihydro-1,3,5-triazin-2(1H)-ones as the first dual BACE-1/GSK-3β fragment hits against Alzheimer's disease. ACS Chem Neurosci 2015;6(10):1665–1682.
490. Yuan J, Venkatraman S, Zheng Y, McKeever BM, Dillard LW, Singh SB. Structure-based design of β-site APP cleaving enzyme 1 (BACE1) inhibitors for the treatment of Alzheimer's disease. J Med Chem 2013;56(11):4156–4180.
491. Ghosh AK, Brindisi M, Yen YC, Xu X, Huang X, Devasamudram T, Bilcer G, Lei H, Koelsch G, Mesecar AD, Tang J. Structure-based design, synthesis and biological evaluation of novel β-secretase inhibitors containing a pyrazole or thiazole moiety as the P3 ligand. Bioorg Med Chem Lett 2015;25(3):668–672.
492. McConlogue L, Buttini M, Anderson JP, Brigham EF, Chen KS, Freedman SB, Games D, Johnson-Wood K, Lee M, Zeller M, Liu W, Motter R, Sinha S. Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP transgenic mice. J Biol Chem 2007;282(36):26326–26334.
493. Berk C, Sabbagh MN. Successes and failures for drugs in late-stage development for Alzheimer's disease. Drugs Aging 2013;30(10):783–792.
494. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: Clinical trials and drug development. Lancet Neurol 2010;9(7):702–716.

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