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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Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/med.21440

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.

Key words: type 2 diabetes; diabetes-induced dementia; Alzheimer’s disease; natural products; drug design
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 \(\beta\)-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 \(\beta\)-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-\(\beta\) (A\(\beta\)) 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.\textsuperscript{19, 20} Interestingly, these features have also been reported as indicators of patients with risk of progression from mild cognitive impairment to AD.\textsuperscript{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.\textsuperscript{23, 24} Seemingly, these events are not only linked to insulin resistance—a hallmark of T2D—but also to vascular endothelial dysfunction,\textsuperscript{23} which is in agreement with the recognized association between pathological alterations in cerebral microvasculature and decreased cognitive function in patients with AD.\textsuperscript{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.\textsuperscript{27–30}

Oxidative stress and neuroinflammation are also coexisting features of AD,\textsuperscript{31} and both are taken as key prompting factors of cerebrovascular dysfunction.\textsuperscript{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.\textsuperscript{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.\textsuperscript{35} These compounds can furthermore interact with receptors for advanced-glycation end-products (RAGEs)—which are overexpressed in the brains of AD patients\textsuperscript{36}—to trigger inflammatory response mechanisms and the production of reactive oxygen species (ROS).\textsuperscript{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),\textsuperscript{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. Oxidative enzymes involved in lipid metabolism such as phospholipase A2 (PLA2) and lipoxygenase (LOX) also contribute to inflammation and endothelial damage, and both have been found to be increased in patients with diabetes and AD.

Shortly, oxidative stress, hypertension, and chronic inflammation are some of the pathophysiological alterations associated with hyperglycemia, dyslipidemia, and insulin resistance, 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). Prolonged hypoperfusion can lead to neuronal and neuroglial ischemic damage and may even accelerate Aβ overproduction and aggregation, eventually triggering the development of AD. Mixed dementia—a combination of vascular dementia and AD—is presumably more common with increasing age, 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.

Medicinal Research Reviews DOI 10.1002/med
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.\textsuperscript{15, 55} Interestingly, tau pathology is also triggered by hyperglycemia.\textsuperscript{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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{65, 66}

A. IAPP in the Brain

Since IAPP was first found to cross the BBB,\textsuperscript{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\textsuperscript{2+} homeostasis\textsuperscript{68, 69} and by causing cell membrane disruption.\textsuperscript{70, 71} On the other hand, recent in vivo studies suggest that IAPP might exert neuroprotective effects to some extent,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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-hydroxynonenal (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 Aβ1-40 and Aβ1-42 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 Aβ1-42 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 resistance96,97 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 patients108,109 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 expression116
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.\textsuperscript{55}

Unlike BACE-1, mutations in presenilin 1 (\textit{PSEN1}) and presenilin 2 (\textit{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.\textsuperscript{117} These mutations highly favor γ secretase cleaving activity toward the generation of longer and more aggregation-prone Aβ\textsubscript{1-42} peptides.\textsuperscript{117, 118} Mutations in AβPP itself can also occur, although they only account for about 0.5% of all cases of AD.\textsuperscript{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 Aβ\textsubscript{1-42} to Aβ\textsubscript{1-40}.\textsuperscript{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 fibrillation process. Acetylcholinesterase (AChE), for instance—an enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine (ACH) in the CNS—is one of those entities,\textsuperscript{121, 122} and was found to colocalize with Aβ deposits in the hippocampus of AD patients.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{130} Moreover, it prompts heterologous seeding with Aβ to form amyloid deposits in the brain and blood vessel walls of patients with AD,\textsuperscript{131} simultaneously inducing failure of Aβ elimination from the brain and further enhancing Aβ-promoted neuronal dysfunction.\textsuperscript{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.\textsuperscript{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.\textsuperscript{132–135} Furthermore, age-related insulin resistance is associated with chronic neuroinflammation,\textsuperscript{136} an early pathological feature of the neurodegenerative process\textsuperscript{137} that leads to increased BBB permeability.\textsuperscript{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,\textsuperscript{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
to worse cognition even among people without diabetes. 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. 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, 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, 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. Despite the existence of insulin-secreting neurons, 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.
Peripherally produced insulin can be readily transported through receptor-mediated transcytosis across BBB endothelial cells.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{152} thus supporting this point.

\section*{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 A\textbeta production by keeping GSK-3 permanently activated.\textsuperscript{153,154} This serine/threonine kinase was originally isolated from skeletal muscle\textsuperscript{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.\textsuperscript{156} It has been demonstrated that overexpression or overactivation of GSK-3\textbeta in rodent models of T2D is associated with impaired ability of insulin to activate glucose utilization and glycogen synthesis.\textsuperscript{157} Yet, it is widely expressed in the brain\textsuperscript{158} and its enduring activation due to brain insulin deficiency is associated not only with A\textbeta production and induced neuronal damage,\textsuperscript{159} but also with long-term synaptic depression processes\textsuperscript{160} and tau hyperphosphorylation.\textsuperscript{161–163} Moreover, there is evidence that BACE-1 expression is upregulated in the absence of insulin, which boosts the extracellular accumulation of A\textbeta in the brain.\textsuperscript{164} Conversely, A\textbeta oligomers stimulate neuronal downregulation of insulin receptors, with subsequent dysfunctional brain insulin signaling and insulin resistance,\textsuperscript{165,166} thus intensifying the process of cognitive decline. This effect was reversed by insulin itself,\textsuperscript{166} which essentially reinforces its neuroprotective effects.

\section*{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.\textsuperscript{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.\textsuperscript{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...
peripheral tissues,\textsuperscript{171,172} it is thought that insulin resistance might also be a cause for oxidative stress,\textsuperscript{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.\textsuperscript{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.\textsuperscript{175} Indeed, glucose is the primary source of energy in the brain and it is now clear that it has memory-enhancing effects.\textsuperscript{176} Downregulation of the glucose transporter 1 (GLUT-1) at the BBB occurs spontaneously as a consequence of hyperglycemia,\textsuperscript{177} and is also observed in AD patients,\textsuperscript{178,179} not only leading to BBB breakdown and early cerebral microvascular degeneration,\textsuperscript{180} but also to impaired glucose transport into the brain, which compromises ATP generation and stimulates the production of ROS.\textsuperscript{181} Furthermore, BACE-1 activity—also enhanced by insulin deficiency, as mentioned above—imparts neuronal glucose oxidation and reduces the basal oxygen consumption rate,\textsuperscript{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-3\(\beta\) 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.

\textit{Medicinal Research Reviews} DOI 10.1002/med
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, and there are studies indicating that this is likely to have implications in diabetes-associated cardiovascular disease. 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 of physiological processes. 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. Moreover, NO/cGMP signaling pathway in the hippocampus seems to be crucially implicated in synaptic transmission, neuronal plasticity, learning, and memory. This signaling cascade is impaired by the action of Aβ and, indeed, reduced levels of cGMP in the CSF have been recently associated with cognitive decline and amyloidogenesis in AD patients. 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. 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. Furthermore, there is strong evidence indicating that cGMP-mediated memory consolidation in the hippocampus requires postsynaptic cAMP signaling, and that cAMP itself can inhibit PLA2 and protect neurons against synaptic damage. Yet, cAMP levels were actually found to be increased in the CSF and cerebral microvessels in AD patients. 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—are in fact inactivated or downregulated in the presence of Aβ. Downregulated cAMP/PKA/CREB pathway is re-established by PDE inhibitors, 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, the expression of several cAMP-specific PDE isoforms is significantly upregulated, 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 that accredit PDE as a 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. It has been pointed out as a possible link between T2D and AD, although some results are
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. Although AD is associated with cerebral insulin deficiency, peripherally produced and circulating Aβ can still cross the BBB and indeed contribute to AD brain lesions, which supports this hypothesis. 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—in fact, some genetic IDE polymorphisms have actually been associated with T2D and AD, 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, including NO and long-chain fatty acids. Basal plasma NO levels were found to be increased in type 2 diabetic patients—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.

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. 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. However, other reports suggest that there are inactivating IDE gene mutations leading to impaired insulin secretion—a different scenario that rather resembles later stages of T2D, when peripheral insulin resistance is accompanied by pancreatic β-cell dysfunction and insulin deficiency. 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. In type 2 diabetic patients, the same inverse correlation between pancreatic IDE and α-synuclein levels was observed. Besides regulating presynaptic vesicle release in the CNS, α-synuclein is able to interact with insulin.

Medicinal Research Reviews DOI 10.1002/med
secretory granules in the pancreas and inhibit glucose-stimulated insulin secretion. 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. 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.

In prediabetes or early type 2 diabetes the hyperinsulinemic phenotype is, indeed, prevalent; yet, since mouse IAPP does not form amyloid fibrils, 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.

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. In fact, low hippocampal IDE levels have been found in late-onset AD patients with impaired insulin metabolism associated with the APOE-ɛ4 allele. 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. Hence, low IDE levels accelerate the formation of Aβ aggregates in the brain, which intensifies synaptic dysfunction and cerebrovascular amyloidosis, 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-ɛ4 or upregulated PDE and GSK-3β enzymes. Since acute IDE inhibition was shown to improve glucose tolerance by mimicking postprandial insulin pancreatic release, 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. 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. 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. 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. 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 empagliflozin (6), orally available small-molecule antidiabetic agents are daily used worldwide for the control of T2D—some of which are represented in Table I. GLP-1 analogues such as liraglutide, albiglutide, or dulaglutide are injectable alternatives, along with long-acting insulin analogues such as insulin degludec or insulin detemir used in more advanced disease stages.

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. However, many of the most commonly used drugs have several side effects. 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. Ketoacidosis and urinary tract infections have been associated with the use of SGLT-2 inhibitors, while cases of pancreatitis were detected among patients treated with liraglutide, a GLP-1 analogue.

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 (Table I). The glutamnergic 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. 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. 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, 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, 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.

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. Thiazolidinediones such as pioglitazone (4) might also have intrinsic neuroprotective and anti-inflammatory effects, and the ability to increase brain insulin sensitivity. 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, thus validating this parallel...
<table>
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<tr>
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<th>Structure</th>
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<td>Glipizide</td>
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<td>Acarbose</td>
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<td>Enhanced acetylcholine brain levels, potentiation of nicotinic acetylcholine activity, attenuation of Aβ deposit formation and neuroinflammation</td>
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*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
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.\textsuperscript{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.\textsuperscript{302–304} A controlled, randomized double-blinded trial to investigate the effect of liraglutide in patients with AD is currently ongoing.\textsuperscript{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 \(\beta\)-cell function.\textsuperscript{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.\textsuperscript{307} In the past decade, resveratrol was found to remodel A\(\beta\) soluble oligomers and fibrils into nontoxic aggregates, and to stimulate proteasome-mediated A\(\beta\) clearance.\textsuperscript{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.\textsuperscript{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.\textsuperscript{313–315} Similarly to A\(\beta\), resveratrol can also remodel IAPP cytotoxic aggregates into off-pathway species.\textsuperscript{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.\textsuperscript{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.\textsuperscript{318} This is in agreement with the generally accepted hypothesis that aromatic–aromatic interactions are on the basis of the amyloid self-assembly process,\textsuperscript{318–320} and that \(\pi–\pi\) 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-\(\beta\)-sheet structures.\textsuperscript{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.\textsuperscript{321} Since the type of interactions that promote the formation of cross-\(\beta\)-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. 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, at the same time enhancing peripheral insulin sensitivity. 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. 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. 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, even though the ability of resveratrol to enhance β-cell function by decreasing the expression of several PDE isoforms has recently been confirmed. Furthermore, 50 mg of resveratrol twice a day for 60 days reduced the size of foot ulcers—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. 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. 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 Aβ1-40 levels, although no significant changes in Aβ1-42 or hyperphosphorylated tau were observed, neither insulin metabolism nor hippocampal volume were altered.

Despite being able to permeate the BBB, the rather low bioavailability of resveratrol due to rapid intestine and liver metabolism is pointed out as a thoughtful activity limiting issue in vivo. 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. 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, 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 (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, 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 and piceatannol also depicted in Figure 6, display strong antidiabetic and
neuroprotective activities as well,\textsuperscript{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.\textsuperscript{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.\textsuperscript{349} Notwithstanding, these data provide important clues for structural optimization of resveratrol, which shall be explored in this review.

\textbf{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 \textit{Curcuma longa} L. rhizomes, commonly known as turmeric, and is responsible for its characteristic yellow color.\textsuperscript{350} Turmeric has been used in Ayurveda, Unani, and Siddha medicine in the treatment of hepatic and biliary disorders, diabetic wounds, among others maladies.\textsuperscript{351} Based on the traditional applications, curcumin has been extensively studied in the past few decades, reported as an extremely potent antioxidant with the capacity of preventing inflammation and insulin resistance.\textsuperscript{352} Indeed, it is seemingly able to protect pancreatic β-cells from apoptosis and to enhance glucose-induced insulin secretion.\textsuperscript{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.\textsuperscript{355} This can be explained by its PDE inhibitory activity.\textsuperscript{330}

Similarly to resveratrol, curcumin—a brain permeable compound\textsuperscript{356}—is able to reorganize Aβ aggregates into nontoxic off-pathway species,\textsuperscript{357} while significantly reducing the formation of human IAPP amyloid fibrils.\textsuperscript{358} Furthermore, it was found to attenuate diabetic-induced apoptosis in retinal neurons\textsuperscript{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.\textsuperscript{360} In a recent study, curcumin exhibited neuroprotective effects in an AD mouse model that manifested in improved active avoidance and locomotor activity.\textsuperscript{361} Moreover, it is a powerful GSK-3β inhibitor, with an IC\textsubscript{50} of 66.3 nM.\textsuperscript{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.\textsuperscript{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.\textsuperscript{364}

At least two clinical trials envisaged to evaluate the benefits of curcumin administration to type 2 diabetic patients are currently in progress.\textsuperscript{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,\textsuperscript{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. 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. Palinurin is one of such scaffolds: this linear furansesquiterpene (16, Fig. 6) was first isolated from the Mediterranean marine sponge *Ircinia variabilis* in 1979, and was recently found to be an allosteric GSK-3β inhibitor.

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. 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. 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. 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. Moreover, it significantly attenuates neuroinflammation, Aβ-induced neurotoxicity, and tau hyperphosphorylation in cortical neurons.

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, 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. 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."\textsuperscript{383, 384} Ginsenoside compound K was also found to protect pancreatic β-cells against apoptosis\textsuperscript{385} and to stimulate the intestinal release of GLP-1 in vitro,\textsuperscript{386} while inhibiting the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase)—two important hepatic enzymes involved in gluconeogenesis.\textsuperscript{387}

Ginsenoside compound K can also promote Aβ clearance in vitro,\textsuperscript{388} and was shown to exert anti-inflammatory and neuroprotective effects in a rodent model of cerebral ischemia.\textsuperscript{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,\textsuperscript{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,\textsuperscript{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.\textsuperscript{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,\textsuperscript{395} antiamyloidogenic,\textsuperscript{391} and neuroprotective effects,\textsuperscript{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.\textsuperscript{398}

Vitexin (22) and isovitexin (23) (Fig. 7)—the C-glycosyl 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).\textsuperscript{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.\textsuperscript{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\textsubscript{50} values of 4.1 and 6.7 μg/mL, respectively,\textsuperscript{402}—which is correspondingly equivalent to 9.48 and 15.50 μM—and they were both inhibitors of the formation of AGEs in vitro.\textsuperscript{403}

So far, it is not known whether vitexin and isovitexin exert antiamyloidogenie effects toward Aβ, although both aglycone apigenin and 7-O-(β-D-glucosyl)apigenin do exhibit those properties.\textsuperscript{391} Nevertheless, they are active against AChE and BChE—with IC\textsubscript{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\textsubscript{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\textsubscript{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 μM)\(^4\). Again, in PC12 cells, the protective effects of vitexin against Aβ-induced toxicity were more significant than those of isovitexin.\(^5\) 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.\(^6\)\(^,\(^7\) 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. 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. 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. 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. 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. 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.

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 Aβ1-42 polypeptides, suggesting potential neuroprotective effects as well. 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 diabetic mice, with the ability to upregulate insulin expression and stimulate β-cell survival. 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, 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, 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. 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, but whereas resveratrol was associated to genotoxic effects in mammalian cells, compound 28 was not. 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 phenoxy 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.\textsuperscript{420} Compound 29 (Fig. 8) carrying an additional fluorine atom in position 4 was interestingly found to inhibit BChE in a selective way (IC\textsubscript{50} = 0.01 mM vs. IC\textsubscript{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.\textsuperscript{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,\textsuperscript{422} analogue 32 displayed higher anti-inflammatory activity but lower scavenging capacity due to the presence of the central triple bond.\textsuperscript{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.\textsuperscript{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,\textsuperscript{426–429} and was indeed able to significantly inhibit self and Cu(II)-induced Aβ<sub>1-42</sub> fibrillization.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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). 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 tautomeric 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-benzyloxymethyl 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. 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. 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. 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 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, 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. 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. 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 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.

After the capacity of the dihydrochalcone scaffold to reduce Aβ-induced cytotoxicity has been disclosed, further nootropic, neuroprotective, and neurotrophic effects were attributed to the natural lead, 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. 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.
as a result of attenuated cerebral oxidative stress and increased BDNF. 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.

*Medicinal Research Reviews* DOI 10.1002/med
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 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.\textsuperscript{458, 459} The compound was also regarded as a BBB-protective drug against cerebral ischemic injury\textsuperscript{460} and, in rat models of chronic cerebral hypoperfusion, it was able to prevent white matter disintegration\textsuperscript{461} and to improve diabetes-associated cognitive impairment.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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.\textsuperscript{472} On the basis of a screening hit from the Pfizer neuroscience PDE platform,\textsuperscript{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.\textsuperscript{474}

Ultimately, this highly potent (IC\textsubscript{50} = 8 nM) selective PDE9A inhibitor was found to be centrally penetrant, while displaying an excellent tolerability and metabolic profile in humans.\textsuperscript{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\textsubscript{a}, and molecular weight.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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

\textit{Medicinal Research Reviews} DOI 10.1002/med
(44, Fig. 10) was developed in 2014 to selectively inhibit PDE9A, but instead, the authors focused on its potential antihyperglycemic activity. 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 $\pi-\pi$ 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-3$\beta$ 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. 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. This compound, later named tideglusib, is an irreversible, non-ATP-competitive GSK-3$\beta$ 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. In transgenic mice overexpressing human mutant A$\beta$PP 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. Tideglusib is generally well tolerated, thus indicating that GSK-3$\beta$ 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.

Tideglusib is also an agonist of the peroxisome proliferator-activated receptor gamma (PPAR$\gamma$), which is involved in regulating fatty acid storage and glucose metabolism. 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 PPAR$\gamma$ 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-3$\beta$ inhibitor, conceived as a potential antidiabetic candidate. 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-3$\beta$, 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.
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.
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. 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, 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 IC₅₀ = 18.03 μM; GSK-3β IC₅₀ = 14.67 μM) and 52 (BACE-1 IC₅₀ = 16.05 μM; GSK-3β IC₅₀ = 7.11 μM), also presented in Figure 11. Then, by changing the substitution pattern of the aromatic ring with both electron-withdrawing 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 (IC₅₀ = 10.18 μM), 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 (IC₅₀ = 4.34 μM), 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 Aβ₁₋₄₀ levels was only moderate (51, 52, and 53) or negligible (54). Nevertheless, compound 52 could still produce a statistically significant reduction of cellular Aβ₁₋₄₀ levels in about 15%, which has been reported as being enough to induce a significant reduction in brain beta-amyloid burden at old age. 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
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.

11. CONCLUDING REMARKS

Medicinal Research Reviews DOI 10.1002/med
Although rational design and synthesis has led to highly potent and selective drug candidates with optimal pharmacokinetic profiles such as the thiazolidinone 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.\textsuperscript{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-3\(\beta\). 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.

\textbf{ACKNOWLEDGMENTS}

EU is gratefully acknowledged for the support of the projects entitled “Diagnostic and Drug Discovery Initiative for Alzheimer’s Disease” (D3i4AD), FP7-PEOPLE-2013-IAPP, GA 612347, and “PERsonalised ICT supported Service for Independent Living and Active Ageing” (PERSSILAA), FP7-ICT-2013-10, GA 610359. Fundação para a Ciência e a Tecnologia is also acknowledged for the support of the project MULTI/UID 0612/2013, PTDC/BIM-MET/2115/2014, and for the Ph.D. grant awarded to A.M.M. (SFRH/BD/93170/2013).

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