ABAD: A Potential Therapeutic Target for Aβ-Induced Mitochondrial Dysfunction in Alzheimer's Disease

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Abstract: Amyloid-beta-peptide (Aβ) binding to mitochondrial Aβ–binding alcohol dehydrogenase (ABAD) enzyme triggers a series of events leading to mitochondrial dysfunction characteristic of Alzheimer's disease (AD). Thus this interaction may represent a novel target for treatment strategy against AD. In this review we summarize current findings regarding the ABAD-Aβ interaction, namely structural and biophysical data, available inhibitors and more recent data from proteomic studies.

Key Words: ABAD, Alzheimer's disease, amyloid-β, mitochondrial dysfunction, neuronal cell death, oxidative stress, frentizole, AG18051 inhibitor.

INTRODUCTION

Alzheimer's disease (AD) is the main cause of dementia in the elderly and is characterized by progressive cognitive and memory impairment. Despite the intense research on AD, there is not yet an effective treatment for this disease, and this is because the pathogenic mechanisms underlying the early stages of the disease are not completely understood. The major pathological hallmarks of AD brains are the presence of extracellular senile plaques of amyloid-beta peptides (Aβ), neurofibrilary tangles and loss of neuronal cells, particularly of cholinergic neurons [1, 2]. AD is also associated with loss of synapses, mitochondrial abnormalities and inflammatory responses [3-7].

Although the etiology of AD is not fully understood, the build-up of Aβ is considered to play a central role in the pathogenesis of the disease. Aβ is generated by proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases, resulting predominantly in peptides of 40 {Aβ (1–40)} and 42 {Aβ (1–42)} amino acids in length [8, 9]. According with the original amyloid cascade hypothesis the accumulation of Aβ in extracellular compartment is considered the earliest event in the cascade leading to AD neurodegeneration [10]. Recent data, however, indicated that the soluble oligomeric forms of Aβ are the most toxic species and correlate best with the severity of the disease [11]. Furthermore, it has been shown that the accumulation of intracellular Aβ is neurotoxic and precedes the formation of extracellular plaques [12, 13]. This led to a modification of the amyloid cascade hypothesis which now takes into account that soluble oligomeric forms of Aβ and its intraneuronal accumulation play a key role in the pathogenesis of the disease [13, reviewed in 14]. Therefore, several studies are now focusing on the mechanisms by which Aβ accumulates intracellularly and mediates neurotoxicity.

Increasing evidence suggests that mitochondria may be an important target for intracellular Aβ to exert its neurotoxic effects. Mitochondrial dysfunction and oxidative stress have long been implicated in the AD pathogenesis and they have been found to occur early in the course of the disease. Several mitochondrial alterations have been reported in AD brains and these include reduction in the number of mitochondria [15], decreased brain glucose metabolism [16], reduction in the activities of both tricarboxylic acid cycle enzymes [17] and cytochrome c oxidase (COX) [18], increased oxidative stress [19], Ca^{2+} deregulation [20] and changes in mtDNA [21]. The association between Aβ and mitochondrial dysfunction in AD is supported by the demonstration that Aβ accumulates within mitochondria both of AD transgenic mice and human AD brains [22, 23] and leads to impairment of mitochondrial functions. *In vitro* studies with isolated mitochondria exposed to Aβ suggested that Aβ inhibit COX activity, decrease mitochondrial membrane potential and respiration rates, induce cytochrome c release, induce the permeability transition pore to open, and increase mitochondrial ROS generation [24-27]. Compelling evidence suggests that an important mechanism by which Aβ might induce mitochondrial dysfunction is by interacting with Aβ-binding alcohol dehydrogenase (ABAD), a matrix mitochondrial enzyme with multiple functional roles. In this review, we start by summarizing the physiological functions of ABAD and then current findings showing an important role of the ABAD-Aβ interaction in the pathogenesis of AD.

THE PHYSIOLOGICAL FUNCTIONS OF ABAD

ABAD is a NAD-dependent oxidoreductase, member of the short chain dehydrogenase reductase (SDR) family. Human ABAD was identified in a yeast two-hybrid system as a binding partner of Aβ [28], and subsequently, found to have an identical cDNA sequence to L-3-hydroxyacyl-CoA dehydrogenase [29, 30]. ABAD forms a homotetramer and each

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monomer contains a Rossman fold dinucleotide-binding motif and the Ser/Lys/Tyr catalytic triad characteristic of the SDR enzymes [31, 32]. This enzyme differs from other members of SDR family by virtue of its mitochondrial localization, its capacity to bind Aβ and its ability to use a broad array of substrates [reviewed in 33]. In view of this multiple substrate specificities, ABAD is able to participate in several metabolic pathways and accordingly, has been referred by other names such as, 3-hydroxyacyl-coA dehydrogenase type 2 (HADH2) [29], 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) [34] and 2-methyl-3-hydroxybutyryl-coA dehydrogenase (MHBD) [35]. The 3-hydroxyacyl-coA dehydrogenase activity of ABAD enables it to participate in the third step of the mitochondrial beta-oxidation, oxidising 3-hydroxyacyl-coAs, with a preference for short chain substrates [30]. The enzyme is required in the isoleucine catabolism, where it catalyses the conversion of 2-methyl-3-hydroxybutyryl-coA (MHB) to 2-methyl-acetoacetyl-coA [35]. The ability of the enzyme to utilise the ketone body β-hydroxybutyrate, a major energetic substrate during neuronal deprivation, has raised a question about its role in enhancing the cellular response to metabolic stress [36]. A role in sex steroid and neurosteroid metabolism was also attributed to the enzyme due to its 3α- and 17β-hydroxysteroid dehydrogenase activities [37, 38]. It catalyzes the oxidative inactivation of 17β-estradiol in estrogen metabolism, and the generation of 5α-dihydrotestosterone from 3α-adiol in androgen metabolism.

Accumulating evidence suggests that ABAD might play important functions in the brain. Recently, ABAD was shown to be the enzyme that effectively catalyzes the oxidation of the neurosteroids allopregnanolone and allotetrahydrodeoxycorticosterone, which are positive allosteric modulators of $GABA_A$ receptors in brain [39, 40]. These neurosteroids bind to $GABA_A$ receptors and, through their 3α-hydroxyl groups, increase receptor opening frequency and duration of opening [41]. Therefore ABAD appears to play an essential role in the maintenance of GABAergic neuronal function and thus, in the regulation of neuronal excitability. More recently, ABAD was reported to be one of the proteins of the postsynaptic density (PSD) [42], which is a complex of proteins with important roles in maintaining the structural integrity of the synapses and in mediating the responses to neurotransmitter released from presynaptic axon terminals [43].

Mutations in the gene encoding ABAD (HSD17B10 gene) have been associated with a progressive neurodegenerative disease [35] and a new syndromic form X-linked mental retardation (XLMR) [44]. HSD17B10 gene is organized into six exons and five introns and maps to chromosome Xp11.2 [30]. Three missense mutations (R130C, L122V, and N247S) in the ABAD coding region [35, 45] were reported to cause MHBD deficiency, an inborn error of the isoleucine catabolism, where this pathway is impaired due to deficiency of MHB activity of the enzyme. Patients with MHBD deficiency show accumulation and increased excretion of the isoleucine pathway metabolites tiglylglycine and MHB, and severe neurological symptoms, including progressive loss of metal and motor skills, mental retardation and epilepsy [35, 45, 46]. The severe neurological abnormalities that characterize this disease indicate that, besides the function in the isoleucine catabolism, important functions of ABAD in the nervous system are also affected. A silent mutation at exon 5, which affects alternative splicing of this exon, has been recently associated with a new syndromic form of XLMR with a unique clinical presentation characterized by mild mental retardation, choreoathetosis and abnormal behaviour [44]. The reduction in the amount of ABAD, rather than the aberrant alternative splicing, was suggested to underlie the pathogenic mechanism of the disease.

There is also evidence supporting a role of the enzyme in embryonic development both in vertebrates and invertebrates. Mutational inactivation of the Drosophila counterpart of ABAD, termed scully, resulted in a lethal phenotype during embryonic and pupal development [47]. Mutants displayed non-functional gonads, lipid accumulation and aberrant mitochondria. The testes displayed deficiency of 3-hydroxyacyl-coA dehydrogenase activity, indicating that ABAD is the principal enzyme with such activity in this tissue. More recently, the expression pattern of ABAD gene was analysed during amphioxus and zebrafish early development [48], with the results of this study suggesting an important role of the enzyme during embryogenesis of these fishes.

Taken together, these data indicate that ABAD play a variety of functions, namely, in energy metabolism, isoleucine catabolism and steroid metabolism. Importantly, the involvement of ABAD in maintenance of GABAergic and synaptic functions together with the neurological symptoms associated with clinical cases of deficiency of this enzyme, suggested that it might have essential roles in brain.

EVIDENCE OF ABAD-Aβ INTERACTION AND ITS ROLE IN THE PATHOGENESIS OF AD

ABAD was identified to bind Aβ in a yeast-two hybrid screening [28]. The expression levels of this enzyme were found to be increased in brains of AD patients when compared to non-demented age matched controls [23, 28]. Evidence that ABAD-Aβ interaction occurs in AD brains came from confocal and electron microscopy studies, which demonstrated that ABAD colocalizes with Aβ in mitochondria of brains both of patients with AD and of transgenic mice overexpressing ABAD and mutant APP (Tg mAPP/ABAD) [23]. These double transgenic mice, which were developed to study the effects of neuronal ABAD in an Aβ-rich environment, displayed exaggerated oxidative stress, increased generation of ROS, and severe impairment in spatial learning and memory [23]. Neurons from these animals showed generation of ROS, DNA fragmentation, lactate dehydrogenase release and cytochrome c release. In another study, cultured neurons from Tg mAPP/ABAD mice also displayed decreases activity of COX, spontaneous release of ROS, loss of mitochondrial membrane potential, decreased ATP and release of cytochrome c from mitochondria with subsequent induction of caspase-3-like activity followed by apoptotic cell death [49]. Taken together, these studies provided evidence that ABAD-Aβ interaction might trigger a series of events leading to mitochondrial dysfunction, namely oxidative stress generation and neuronal apoptosis.

The exact molecular mechanisms through which ABAD-Aβ interaction might induce mitochondrial dysfunction are not yet completely clear. Both *in vitro* [50] and structural studies [23, 51] indicated that Aβ inhibits the enzymatic activity of ABAD, suggesting that a direct consequence of ABAD-Aβ interaction is an impairment of ABAD metabolic functions in mitochondria from neurons. Furthermore, inhibition of ABAD enzymatic activity in the several metabolic pathways that it participate, possibly might also result in the accumulation of upstream metabolites, which in turn might exert adverse effects on neurons [52]. Moreover, ABAD-Aβ interaction might activate, directly or indirectly, other signalling cascades within neurons. In this respect, a recent proteomic study [53, 54] revealed that ABAD-Aβ interaction up-regulates the levels of some proteins in neuronal cells. These structural and proteomic studies of ABAD-Aβ interaction are discussed in more detail in the next two sections.

STRUCTURAL DETAILS OF ABAD-Aβ INTERACTION

In vitro binding studies demonstrated that Aβ peptides (1–42, 1–40, and 1–20) bind to ABAD in a dose-dependent manner while the C-terminal portion of Aβ(25–35) does not bind to ABAD [23]. This suggests that Aβ might bind to ABAD *via* its N-terminus. To determine structural details of the ABAD-Aβ interaction, crystallographic studies were performed [23]. The crystal structure of ABAD-Aβ complex revealed the structural basis for the inhibition of the ABAD enzymatic activity by Aβ, previously reported by *in vitro* studies [50]. In the ABAD-Aβ crystal structure, the ABAD structure shows substantial distortion of the NAD and substrate binding cavities. The L_E loop containing the catalytic triad is highly deformed and most residues of L_D and L_F loops are disordered Fig. (**1**), suggesting that Aβ binding might induce a conformational change in the ABAD structure. This conformational change likely prevents NAD binding to the enzyme, as no electron density for NAD was observed in the ABAD-Aβ complex. By preventing NAD binding to ABAD, Aβ inhibits the ABAD activity. No electron density was observed for Aβ. However, the observation that in the rat ABAD structure in the presence of NAD and absence of the substrate, the L_F loop is also disordered while the L_D loop is well ordered, suggested that Aβ binding might have influenced the dynamics and conformation of L_D loop, and hence, that this could be the binding site of Aβ. Subsequent site directed mutagenesis studies confirmed that L_D loop plays a critical role in Aβ binding [23]. Moreover, a synthesized peptide encompassing the residues from L_D loop (residues 92 to 120) of human ABAD [termed ABAD decoy peptide (ABAD-DP)] inhibited ABAD-Aβ interaction, confirming that this region is sufficient to mediate Aβ binding to ABAD [23]. Unfortunately, the molecular details of the interface between ABAD and Aβ could not be revealed due to the lack of electron density of Aβ and of L_D loop.

The effects of ABAD-DP were then tested in cell culture, which first involved the generation of a permeable form of ABAD-DP, Tat-ABAD-DP, through addition of the cell-membrane transduction domain of the human immunodeficiency virus-1 (HIV-1) Tat protein. When Tat-ABAD-DP inhibitor was added to cultured neurons of transgenic mice overexpressing ABAD and Aβ, oxidative stress and cytochrome c release were suppressed [23]. This result supports the hypothesis that the ABAD-Aβ interaction is not only a key factor in the potentiation of Aβ-induced cytotoxicity, but also may be an effective drug target for therapeutic intervention in AD.

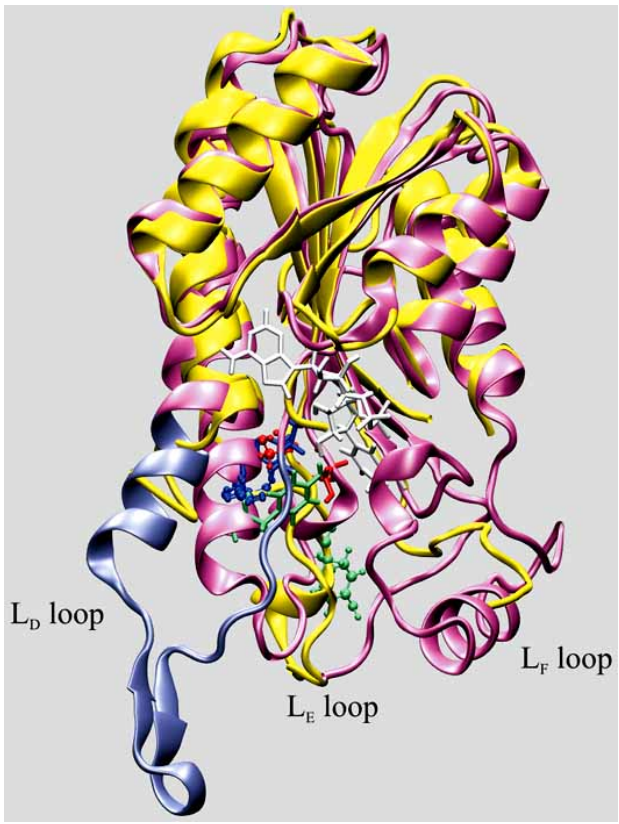


Fig. (1). Superposition of a monomer of the structure of human ABAD in complex with NAD (pdb: 1u7t), shown in pink, with the structure of human ABAD in complex with Aβ (pdb: 1so8), shown in yellow. NAD is colored white and residues from the catalytic triad are colored green (Tyr168), blue (Lys172) and red (Ser155) and shown in CPK representation for 1so8 structure and in licorice representation for 1u7t structure. The L_D, L_E and L_F loops which undergo large changes upon Aβ binding are indicated and the L_D loop is colored blue in the 1u7t structure.

Further details of the ABAD-Aβ interaction were obtained from surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) studies [51]. These studies showed that ABAD binds to Aβ oligomers, the most toxic forms of Aβ, and not to monomers. This is interesting as both oligomeric and monomeric forms of Aβ have been detected in mitochondria [55]. However the precise oligomeric state of Aβ that binds ABAD remains to be determined. These studies also confirm the findings from crystallographic studies by showing that Aβ binding to ABAD inhibits NAD binding and is associated with conformational changes in the ABAD-Aβ complex. Binding of Aβ and NAD to ABAD were shown to be mutually exclusive as NAD was shown to inhibit ABAD-Aβ interaction. The mechanism of this inhibition is however, not clear. SPR studies also provided insights

into the driving forces of the ABAD-Aβ interaction. This interaction is driven by a favourable entropic change, which overcomes the unfavourable enthalpy change, indicating that the hydrophobic interactions and the increase in protein dynamics of ABAD are the dominant driving forces of the ABAD-Aβ interaction. The increased ABAD dynamics is likely due to an increase in the flexibility of the L_D, L_E and L_F loops, deformed in the ABAD-Aβ crystal structure, upon Aβ binding [51].

Taken together, these structural studies have demonstrated that Aβ inhibits the ABAD enzymatic activity by inducing a conformational change in ABAD structure which inhibits binding of NAD to the enzyme. Clearly, the investigation related with the ABAD-Aβ interaction would benefit from the existence of a complete model of this complex showing the interface between ABAD and Aβ. However the lack of three-dimensional structures of Aβ oligomers makes difficult to predict the full Aβ-ABAD complex through molecular modelling.

PROTEOMIC STUDIES: IDENTIFICATION OF SIGNALLING CASCADES ACTIVATED BY ABAD-Aβ INTERACTION

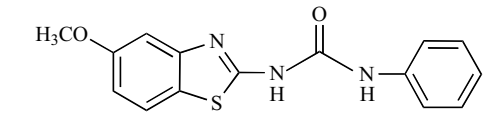
Recently, the possible signalling events affected by ABAD-Aβ interaction were investigated by analysing protein expression differences between brains of transgenic mice overexpressing ABAD and Aβ, solely ABAD or Aβ and non-transgenic mice. These proteomic studies identified two proteins, endophilin I [53] and peroxiredoxin II [54], whose expression was increased in brains from transgenic animals overexpressing ABAD and Aβ. Furthermore, increased expression of these two proteins was also detected in brains of AD patients, suggesting their potential involvement in AD pathogenesis. Endophilin I is a protein involved in synaptic vesicle endocytosis [56] and has been reported to regulate c-Jun N-terminal kinase (JNK) activation [57]. Activation of JNK pathway has been implicated in AD [58] and Aβ has been reported to enhance activation of JNK in cortical neurons, which in turn lead to neuronal apoptosis [59-63]. The demonstration that endophilin I can significantly increase the JNK activation in cortical neurons exposed to Aβ, with subsequent death of neurons, suggest a potential new signalling pathway contributing to the activation of JNK in AD [53]. Peroxiredoxin II has been previously reported to be increased in several neurodegenerative diseases [64, 65]. This protein is a member of the peroxiredoxin family that is involved in antioxidant protection and redox signalling [66]. Therefore, the increased expression of peroxiredoxin II in AD diseased brain is indicative of the neurons attempting to protect themselves from increased oxidative stress induced by Aβ toxicity [54]. Importantly, the inhibition of the ABAD-Aβ interaction with the Tat-ABAD-DP inhibitor led the expression levels, both of peroxiredoxin II and endophilin I, to return to normal, confirming that the increase in expression of these two proteins is caused by Aβ binding to ABAD [53, 54]. This also suggests that the expression levels of these two proteins are indicators of the ABAD-Aβ interaction [53, 54].

Taken together, these data indicate that ABAD-Aβ interaction activates two distinct pathways, one involving peroredoxin II to protect neurons from increased oxidative stress and the other involving endophilin I which contribute to neuronal death *via* activation of JNK pathway. Given that peroxiredoxin II and endophilin I are localized in cytoplasm, these studies also suggest that the ABAD-Aβ interaction activate signal transduction pathways in the cytosol that leads to increase of these two proteins. Further work is needed to unravel the mechanisms by which ABAD-Aβ interaction results in the increase of peroxiredoxin II and endophilin I.

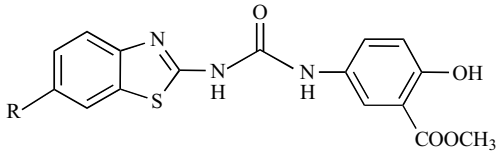
INHIBITORS OF ABAD-Aβ INTERACTION AND OF ABAD

Tat-ABAD-DP was the first inhibitor identified for the ABAD-Aβ interaction and since then, it has been widely used both in *in vitro* and *in vivo* studies aimed to investigate the roles of this interaction in AD.

More recently, frentizole, a benzothiazole urea approved by FDA as an immunosuppressive drug, was identified to inhibit the ABAD-Aβ interaction in ELISA-based screening assay [67]. A library of 45 frentizole analogs was then constructed, and the structure-activity relationship (SAR) study indicated that: a) the urea moiety was required for inhibitory activity, b) small electron-withdrawing groups were preferred at the benzothiazole ring and c) compounds with a hydroxyl group at the para position of the phenylurea were more potent. The combination of these features allowed the identification of compounds 1 and 2 shown in Fig. (**2**) as the two most potent inhibitors of the ABAD-Aβ interaction. However, the specificity of these compounds to ABAD-Aβ interaction and its ability to decrease the citotoxicity of Aβ remains to be demonstrated.



Frentizole IC_{50}=200



1 R=Cl IC_{50}=6.46
2 R=F IC_{50}=6.56

Fig. (2). Structure of frentizole and of two frentizole analogs shown to inhibit the ABAD-Aβ interaction.

Although inhibitors of ABAD may not have therapeutic applicability in AD since Aβ inhibits the ABAD enzymatic activity, they may be valuable for dissecting ABAD's roles in the pathogenesis of AD, specifically those that concern the role that the enzyme activity plays in this process. Recently, AG18051 (1-azepan-1-yl-2-phenyl-2-(4-thioxo-1,4-dihydropyrazolo[3,4-d]pyrimidin-5-yl)-ethanone; Fig. (**3**)) was identified as a potent inhibitor of ABAD (IC_{50} of 92 nM) [32]. The inhibitor, which has no close structural similarity to known ABAD substrates, binds in the active-site cavity of the enzyme and reacts with the NAD^+ cofactor to form a covalent adduct. Given the multiple metabolic functions of ABAD, difficulties may arise when trying to design specific

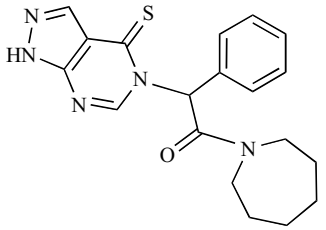


Fig. (3). Structure of the AG18051 (1-azepan-1-yl-2-phenyl-2-(4-thioxo-1,4-dihydro-pyrazolo[3,4-d]pyrimidin-5-yl)-ethanone) inhibitor.

inhibitors that target this enzyme [31]. Nevertheless, the AG18051 inhibitor appears to be specific for ABAD as it showed no detectable inhibition of two closely related members of the SDR family (*E. coli* 7α-hydroxysteroid dehydrogenase and *Streptomyces hydrogenans* 3α-20β-hydroxysteroid dehydrogenase) [32].

CONCLUSION

Increasing evidence suggest that Aβ-induced mitochondrial dysfunction play a central role in the pathogenesis of AD and point toward the involvement of ABAD in this process. ABAD, a multifunctional enzyme, has been found to be upregulated in brains of patients with AD and to interact with Aβ in these brains. Interaction between Aβ and ABAD contributes to mitochondrial dysfunction by inducing oxidative stress and apoptosis in neurons. Furthermore, by interacting with ABAD, Aβ inhibits the normal enzyme activity, possibly impairing the important functions of ABAD in brain, and activates signalling cascades that lead to increase in expression of two proteins with differing actions, peroxiredoxin II is an antioxidant and endophilin I is involved in activation of neuronal cell death. Importantly, blocking of ABAD-Aβ interaction reverse the increase of these two proteins and protects against Aβ-induced cytotoxicity, thus suggesting that this interaction is a potential drug target for treatment of AD. In this regard, the search for small molecule inhibitors of the ABAD-Aβ interaction has already started and two frentizole analogs have been recently identified. A better knowledge of which functions of ABAD are impaired due to its interaction with Aβ, of the signalling cascades activated by this interaction, and of the molecular details of the ABAD-Aβ complex may, however, be required to effectively devise therapeutic approaches for intervention in AD based on the ABAD-Aβ interaction.

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ABBREVIATIONS

ABAD = Amyloid-beta binding alcohol dehydrogenase

ABAD-DP = ABAD decoy peptide

Aβ = Amyloid-beta peptide

AD = Alzheimer's disease

AG18051 = 1-azepan-1-yl-2-phenyl-2-(4-thioxo-1,4-dihydro-pyrazolo[3,4-d]pyrimidin-5-yl)-ethanone

APP = Amyloid precursor protein

COX = Cytochrome c oxidase

ERAB = Endoplasmic reticulum-associated Aβ-binding

HADH2 = 3-hydroxyacyl-coA dehydrogenase type 2

HIV-1 = Human immunodeficiency virus-1

17β-HSD10 = 17β-hydroxysteroid dehydrogenase type 10

MHB = 2-methyl-3-hydroxybutyryl-coA

MHBD = 2-methyl-3-hydroxybutyryl-coA dehydrogenase

NMR = Nuclear magnetic resonance

PSD = Postsynaptic density

ROS = Reactive oxigen species

SDR = Short chain dehydrogenase reductase

SPR = Surface plasmon resonance

XLMR = X-linked mental retardation

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