**Friend or enemy? Review of 17β-HSD10 and its role in human health or disease**

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**ABSTRACT**

17β-hydroxysteroid dehydrogenase (17β-HSD10) is a multifunctional human enzyme with important roles both as a structural component and also as a catalyst of many metabolic pathways. This mitochondrial enzyme has important functions in the metabolism, development and aging of the neural system, where it...
is involved in the homeostasis of neurosteroids, especially in regard to estradiol, changes in which make it an essential part of neurodegenerative pathology. These roles therefore, indicate that 17β-HSD10 may be a possible druggable target for neurodegenerative diseases including Alzheimer’s disease (AD), and in hormone dependent cancer. The objective of this review is to provide a summary about physiological functions and pathological roles of 17β-HSD10 and the modulators of its activity.

KEYWORDS

17β-hydroxysteroid dehydrogenase type 10; HSD10; neurosteroid; estradiol; Alzheimer’s disease; cancer
List of abbreviations

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<th>Abbreviation</th>
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<tr>
<td>AB</td>
<td>amyloid beta</td>
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<td>ABAD-DP</td>
<td>amyloid beta-binding alcohol dehydrogenase decoy peptide</td>
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<td>AD</td>
<td>Alzheimer's disease</td>
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<td>APP</td>
<td>amyloid precursor protein</td>
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<td>ATP</td>
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<td>CoA</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>ER</td>
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<td>E\textsubscript{R\alpha}</td>
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<td>ERAB</td>
<td>ER-associated amyloid beta-peptide-binding protein</td>
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<td>FDA</td>
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<td>GABA\textsubscript{A}</td>
<td>gamma-aminobutyric acid receptor type A</td>
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<td>HADH II</td>
<td>L-3-hydroxyacyl-CoA dehydrogenase type II</td>
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<td>4-HNE</td>
<td>4-hydroxy-2-nonenal</td>
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<td>17β-HSD10</td>
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<td>IC\textsubscript{50}</td>
<td>half-maximal inhibitory concentration</td>
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<td>IDE</td>
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<td>LPS</td>
<td>lipopolysaccharide</td>
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<td>MHBD</td>
<td>2-methyl-3-hydroxybutyryl-CoA dehydrogenase</td>
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<td>mPTP</td>
<td>mitochondrial permeability transition pore</td>
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<td>MRPP</td>
<td>mitochondrial ribonuclease P protein</td>
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<td>MRXS 10</td>
<td>X-linked mental retardation</td>
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<td>mt</td>
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<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
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<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>PD</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<td>SAAC</td>
<td>acetoacetyl coenzyme A</td>
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<td>SAR</td>
<td>structure-activity relationship</td>
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<td>SDR</td>
<td>short-chain dehydrogenase/reductase</td>
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<td>SCHAD</td>
<td>short-chain L-3-hydroxyacyl coenzyme A dehydrogenase</td>
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**Introduction**

17β-HSD10 is a mitochondrial enzyme involved in the metabolism of a wide range of substrates, including neurosteroids (He et al. 2019) and sex steroids, maintaining their physiological level (Shafqat et al. 2003). However, it also plays an important role in tRNA processing as a structural component of RNase P (Holzmann et al. 2008). Its abnormal function including inherited mutations leads to disruption in mitochondrial physiology and is thought to be one of the underlying pathological causes for diseases such as Alzheimer disease (He et al. 2018) and some forms of cancer (Yang et al. 2005). This paper discusses the role of 17β-HSD10 in physiology, as well as its connections to various diseases. The first section gives an overview of 17β-HSD10 structure, localization and physiological functions. In the second section, the role of 17β-HSD10 in disease, specifically in neurodegenerative disorders and cancer are discussed, which together are attracting more and broader interest in this enzyme. For these reasons, in the last ten years, several research groups have produced novel compounds with the potential to modulate/inhibit its enzymatic activity or protein-protein interaction with the toxic peptide amyloid beta (Aβ). The available modulators/inhibitors of 17β-HSD10 are discussed in the third main part of this review.

**The 17β-HSD10 and its uniqueness within the short-chain dehydrogenase/reductase family**

17β-hydroxysteroid dehydrogenase type 10 (EC 1.1.1.3; OMIM 300256) is a mitochondrial protein involved in many physiological pathways. It belongs to a family of 17β-hydroxysteroid dehydrogenases, oxidoreductase enzymes that play an essential role in steroid metabolism. Except for 17β-HSD5, such enzymes belong to the short-chain dehydrogenase/reductase (SDR) family (Shafqat et al. 2003; Jörnvall et al. 1995). To date, 14 different mammalian isoenzymes, which differ in substrate specificity, their preferred reaction in vivo, or subcellular and tissue distribution, have been identified. Only 17β-HSD10 and 17β-HSD8 are localized in mitochondria, others can be found in peroxisomes (17β-HSD4), in the cytoplasm (17β-HSD1, 17β-HSD5, 17β-HSD14), are secreted (17β-HSD11, 17β-HSD13) or localized in the endoplasmic reticulum (ER) (17β-HSD2, 17β-HSD3, 17β-HSD6, 17β-HSD7, 17β-HSD9, and 17β-HSD12) (Marchais-Oberwinkler et al. 2011; Lukacik et al. 2006). Within this enzyme family, 17β-HSD10 is the only one, which is able to bind amyloid-beta (Aβ), the peptide involved in the onset of Alzheimer’s disease. This feature is given by an unique amino acid region called loop D (Ld) which acts as an Aβ-binding site (Lustbader et al. 2004), which is absent in other SDR enzymes.
Gene and protein structure

17β-HSD10 enzyme is encoded by *HSD17B10* gene located on chromosome Xp11.2 (He et al. 1998; Korman and Yang 2007). The chromosome domain comprising *HSD17B10* gene (Xp11.21-p11.22) presumably can escape chromosome X inactivation (Miller and Willard 1998; He et al. 2011), leading to two active genes in females. The gene is divided into 6 exons and 5 introns, with an overall size of about 4 kilobases (kb). 17β-HSD10 mRNA is provided maternally (Rauschenberger et al. 2010) and studies of molecular evolution indicate that *HSD17B10* is an ancient housekeeping gene (Marques et al. 2006). The NAD-binding (nicotinamide adenine dinucleotide) region located on the protein’s N-terminus (res. 17-23) is encoded by exons 1-3, whilst the other three exons encode catalytic residues of the active site, which are located on the C-terminal substrate-binding region. Exon 5 is an alternative exon and can be spliced to produce several different transcripts creating several isoforms of the enzyme (Yang et al. 2007a).

Considering the possible X chromosome inactivation escape, there could be *HSD17B10* expression levels with regard to gender (Yang and He 2001).

Protein expression is tissue-specific and depends on the physiological conditions. In adult and fetal brains, the expression is most abundant in neuronal cells of the hippocampus and amygdala (He et al. 2005). Other tissues with high expression include gonads, liver, and heart (Yan et al. 1997; Yan et al. 2007). Historically, this protein was considered to be present in the ER (Yan et al. 1997) and initially named ERAB (ER-associated amyloid beta-peptide-binding protein), but further studies confirmed its presence to be only in mitochondria (He et al. 2001; Oppermann et al. 1999). Later, the N-terminal mitochondria-targeting sequence was identified and this sequence was found to form a helical structure, homologous to other mitochondrial matrix proteins (He et al. 2002; Shafqat et al. 2003; He et al. 2001). Mitochondrial localization was further confirmed using various techniques such as subcellular fractionation of rat liver (Luo et al. 1995), immunohistological analysis of brains from both Alzheimer’s disease and healthy patients (Frackowiak et al. 2001) or confocal microscopy of neuroblastoma cells (He et al. 1999).

The 17β-HSD10 protein creates a homotetramer with a total molecular mass of 108 kDa (Fig. 1), each subunit is made up of 261 amino acids (He et al. 1998; Powell et al. 2000) and is in contact with other three subunits. All four active sites are located on the surface of the tetramer with a distance of about 30 Å between the reactive C4N atoms in the NAD+ cofactor (Kissinger et al. 2004). Each monomer comprises of the active site including the triad of amino acid residues (Ser155, Tyr168, Lys172) and Rossmann fold dinucleotide-binding motif, typical for SDR enzymes (Kissinger et al. 2004; Marques et al. 2008). The Rossmann fold consists of a central β-sheet of seven parallel strands, bordered by six α-helices, three on
Each side (Powell et al. 2000). Nicotinamide cofactor binds into the conserved sequence “GGXXGXG” (G is glycine; X is other amino acid residue).

17β-HSD10 function

Importance of 17β-HSD10

17β-HSD10 seems to be involved in many physiological functions. It is considered to be essential for normal neuronal development and functioning of mitochondria (Shafqat et al. 2003; Yang et al. 2009; Vilardo and Rossmanith 2015), while any defects in expression or function lead to disruptions in homeostasis. Complete loss of 17β-HSD10 is probably lethal, causing death in early embryogenesis as was shown in studies using mice and Xenopus embryos. The HSD17B10 gene knock-out in mice embryos result in apoptosis and death in the early stages of embryogenesis caused by mitochondrial dysfunction. On the other hand, the pathological aftermath in HSD17B10 knock-out Xenopus embryos and conditional knock-out mice-derived cells can be successfully rescued by wild-type (non-mutated) 17β-HSD10 DNA constructs microinjected into cells (Rauschenberger et al. 2010; Carlson et al. 2015; Zschocke 2012). Drosophila expresses the protein Scully which has a high homology with human 17β-HSD10. Mutation in this protein leads to a lethal phenotype, so it is assumed to be important in germline formation and essential for cell survival. Scully mutants have reduced number of mitochondria, which are aberrant in shape and show accumulation of lipid material from membranes, which is typical for human β-oxidation diseases (Torroja et al. 1998). In Caenorhabditis elegans, it was found, that reduction in activity of SDR enzymes is connected with a shorter lifespan (Murphy et al. 2003).

Though a 17β-HSD10 knock-out is lethal (Zschocke 2012), a knock-down or loss of catalytic function leads to pathological phenotype (though still with significantly reduced cell proliferation and viability) and this holds true for gene mutations and duplications (Froyen et al. 2008). This implicates that 17β-HSD10 has an essential role in cell survival which is independent on its catalytic activity (Deutschmann et al. 2014). Probably only its presence, providing some residual structural function and structural integrity, and its import into mitochondria, is essential for embryonic survival. The same finding was found in humans, where a mutation in the HSD17B10 gene results in disease with various clinical manifestations ranging from mild to progressive neurodegeneration (Zschocke 2012; Ofman et al. 2003; Vilardo and Rossmanith 2015).


**Enzymatic function**

The substrate-binding site of the enzyme is formed by the C-terminal end (residues 203-220) and is located close to the catalytic triad to form a wide and mainly hydrophobic cleft together with the coenzyme-binding site at the bottom of the gorge. Binding of the substrate follows the opening of the cleft which exposes the catalytic triad, which promotes hydride transfer between the cofactor and a substrate. Substrate is bound into the catalytic site via hydrogen bonds between the oxygen of Tyr\(_{168}\) and hydrogen of Ser\(_{155}\) (Nordling et al. 2001; Kissinger et al. 2004), while the NAD\(^+\) cofactor interacts with the enzyme via hydrogen bonds with the entire catalytic triad (Ser\(_{155}\), Lys\(_{172}\), and Tyr\(_{168}\)) (Yan et al. 2007).

The active site of the enzyme is very flexible, allowing the processing of a wide range of various substrates (Yang et al. 2011; He et al. 2018). Over time, various reactions catalyzed by 17β-HSD10 have been discovered and so many alternative names for the enzyme have occurred such as 2-methyl-3-hydroxybutyryl-coenzyme A dehydrogenase (2-methyl, 3-hydroxybutyryl-CoA, MHBD) (Ofman et al. 2003), human brain short chain L-3-hydroxyacyl coenzyme A dehydrogenase (SCHAD) (He et al. 1999; Shafqat et al. 2003), ABAD (amyloid-binding alcohol dehydrogenase) (Yan et al. 1999) or L-3-hydroxyacyl-CoA dehydrogenase type II (HADH II) (Furuta et al. 1997; He et al. 1998).

Concerning the potential physiological substrates, 17β-HSD10 can catalyze dehydrogenation of 17β-OH of estrogens, 3α-OH dehydrogenation of androgens and exhibits NADH-dependent L-3-hydroxyacyl-CoA dehydrogenase activity in the oxidation of the hydroxyacyl-CoA of fatty acids and branched-chain amino acid isoleucine (Fig. 2). Oxidation of simple alcohols has been reported as well (Yan et al. 1999; He et al. 2002), but the overall alcohol dehydrogenase activity was found to be negligible (Yang et al. 2014).

**Steroid metabolism**

The major and most important substrates processed by 17β-HSD10 are estrogens and androgens. 17β-HSD10 can catalyze the turnover of the most potent neuroactive steroids in the brain (He et al. 2019), specifically oxidation of allopregnanolone to 5α-dihydroprogesterone and 3α,5α-3,21-dihydroxypregn-20-one to 5α-dihydrodeoxycorticosterone (Belelli and Lambert 2005). These steroids are positive allosteric modulators of GABA\(_A\) receptors (gamma-aminobutyric acid receptor type A) and are essential for normal GABAergic neuron function (He et al. 2000a; He et al. 2005; Yang et al. 2007a). The enzyme further catalyzes the formation of the androgen 5-dihydrotestosterone from 3α-androstanediol, maintaining its intracellular level, and also the oxidation of 17β-estradiol, a potent female sex steroid, to its less potent product estrone (Shafqat et al. 2003; Lim et al. 2011; Yan et al. 1999; He et al. 2000b).
Participation in the conversion of these important steroids suggests that 17β-HSD10 plays a fundamental role in the metabolism of neuroactive compounds and the physiology of steroid hormones (Yang et al. 2014). Oxidative activity of 17β-HSD10 towards 20β-OH and 21-OH groups in C21 steroids suggests that it is also involved in the metabolism of gestagens and glucocorticoids and that it can participate in the isomerization of bile acids (Shafqat et al. 2003) and the metabolism of phytanic acid (Kobayashi et al. 1996).

Estradiol, the major human estrogen plays a pivotal role in female reproductive physiology, but it does also have a role in male reproduction (Nelson and Bulun 2001), as well as in bone (Väänänen and Härkönen 1996) and lipid (Palmisano et al. 2017) metabolism. Moreover, it can stimulate the neuronal system as a potent neuroactive steroid (Maggi et al. 2004). In addition, estradiol acts via binding to the estrogen receptor α (ERα) and β (ERβ) (Greene et al. 1986; Kuiper et al. 1996), which dimerizes after binding and translocates to the nucleus, where it can modulate transcription of various genes (Marino et al. 2006). Estradiol has a trophic effect on growth, development and survival of cholinergic neurons (Newhouse and Dumas 2015) and its levels are crucial in many brain diseases, such as neurotransmitter impairments (schizophrenia) (Kulkarni et al. 2012), ischemic damage (van der Spuy and Pretorius 2012), immune system dysfunction (multiple sclerosis) (Collongues et al. 2018) or neurodegenerative diseases (AD) (Pozzi et al. 2006; Xu et al. 1998), as well as in breast cancer (Chang 2011; Kota et al.). An important role for estradiol is thought to be in the normal aging process (Lejri et al. 2018; Grimm and Eckert 2017), where it is able to regulate mitochondrial homeostasis, preserving the structure of mitochondria by reducing oxidative stress and preventing cytochrome c release, and apoptosis (Morkuniene et al. 2002).

Estradiol seems to be one of the crucial factors in AD by regulating neuroinflammation (Pozzi et al. 2006) because ERα and ERβ are present on microglia and monocytes. Other neuroprotective effects (Manthey and Behl 2006) are controlled by the regulation of APP trafficking and metabolism, thus reducing the formation of pathological forms of aggregated Aβ (Xu et al. 1998; Gandy and Petanceska 2001). It also modulates the activity of IDE (Amtul et al. 2010) and other factors involved in Aβ degradation (Jayaraman et al. 2012).

Further, it was found that 17β-HSD10 can degrade aldehydes, such as 4-hydroxy-2-nonenal (4-HNE), which is a product of lipid peroxide metabolism and is highly toxic and capable of promoting neuronal death (Kruman and Mattson 1999). For this reason, it was thought that 17β-HSD10 contributes to the metabolic stress-protective response by utilization of ketone bodies, especially β-hydroxybutyrate for energy production in an energy-deficient environment (He et al. 2000a). However, the catalytic efficiency of 17β-HSD10 for β-hydroxybutyrate is low, and so this enzyme probably plays a limited role in energy
production from ketone bodies (Du Yan et al. 2000), but its involvement in steroid metabolism seems to be essential for physiological functions.

**Natural binding partners**

17β-HSD10 can bind to other proteins, such as ERα, cyclophilin D (CypD), and tRNA methyltransferase (Carlson et al. 2015; Pagani and Eckert 2011; Jazbutyte et al. 2009; Holzmann et al. 2008). Interaction of 17β-HSD10 with ERα regulates intracellular estrogen levels because the binding of ERα to 17β-HSD10 inhibits its enzymatic function and blocks estradiol degradation. High levels of estradiol disrupt this interaction, while low levels promote the complex formation (Jazbutyte et al. 2009). Estradiol can also reduce oxidative stress by inhibiting cytochrome c release and apoptosis, thus protecting mitochondrial structure and function (Morkuniene et al. 2002; Morkuniene et al. 2006). Because 17β-HSD10 metabolizes estradiol to its less potent metabolite estrone, the interaction of ERα with 17β-HSD10 might be a natural method to prevent the decrease of intracellular estradiol levels (Jazbutyte et al. 2009; Morkuniene et al. 2002), enabling its cytoprotective function.

Binding of 17β-HSD10 to CypD was found to occur in PC12 (pheochromocytoma) cancer cells (Carlson et al. 2015). CypD is peptidyl-prolyl cis-trans isomerase acting as a part of the mitochondrial permeability transition pore (mPTP) playing an important role in cell death (Giorgio et al. 2010). 17β-HSD10-CypD binding prevents CypD translocation to the inner mitochondrial membrane and mPTP formation. However, in the presence of elevated levels of the Aβ peptide, the 17β-HSD10-CypD complex is disrupted and CypD can form the mPTP pore, which subsequently leads to the cell death (Carlson et al. 2015; Kristofikova et al. 2018). It might be that in cancer, cells might also use overexpression of 17β-HSD10 as a tool to prevent mPTP opening, thus preventing themselves from cell death (Carlson et al. 2015).

In addition to the enzymatic activity, 17β-HSD10 has a pivotal role as a structural component of the mitochondrial RNase P complex (EC 3.1.26.5) (Holzmann et al. 2008). Human mitochondrial RNase P is a unique enzyme composed of three proteins and does not need a trans-acting RNA for catalysis of RNA and tRNA cleavage. This enzyme complex is composed of MRPP1 (mitochondrial ribonuclease P protein 1, tRNA methyltransferase), MRPP2 (17β-HSD10) and MRPP3 (Mg²⁺-dependent endoribonuclease). MRPP1 and MRPP2 create a stable subcomplex whereas the interaction between this subcomplex and MRPP3 is weak (Holzmann et al. 2008; Vlardo and Rossmanith 2015; Vlardo and Rossmanith 2013; Oerum et al. 2018). 17β-HSD10 is essential for stable MRPP1 protein expression and mutations of this enzyme result in reduced activity of the RNase P components and defective RNA processing causing the disordered function of mitochondria in HSD10 mediated diseases (Deutschmann et al. 2014). Knock-down of
17β-HSD10 in human cells suppress mitochondrial tRNA processing plus methylation, and causes accumulation of unprocessed tRNA precursors necessary for mitochondrial protein translation, leading to decreased protein synthesis, insufficient energy production, and mitochondrial disruption (Holzmann et al. 2008; Vilardo et al. 2012; Chatfield et al. 2015; Vilardo and Rossmanith 2015).

**17β-HSD10 related diseases**

**HSD10 disease**

HSD10 mitochondrial disease (OMIM 300438), also called 17β-hydroxysteroid dehydrogenase 10 deficiency, MHBD deficiency, 3-hydroxyacyl-CoA dehydrogenase II deficiency or X-linked mental retardation (MRXS10) (Lenski et al. 2007) is an inborn error and a multifactorial disorder caused by mutation of the *HSD17B10* gene resulting in the disturbance of many metabolic pathways catalyzed by 17β-HSD10 (Yang et al. 2007a). Typical symptoms include X-linked mental retardation, choreoathetosis, cardiomyopathy, neurodegeneration, and abnormal behavior. The clinical phenotype of patients with the HSD10 disease is variable (Ensenauer et al. 2002; Lenski et al. 2007) and depends on the exact position of the mutation in a gene. Some mutations thus affect only enzymatic activity, whilst some cause the instability of the protein structure, with both resulting in a wide spectrum of symptoms from mild to severe (Ofman et al. 2003; Vilardo and Rossmanith 2015). As the *HSD17B10* gene is located on the X chromosome, males with HSD10 disease usually have a more severe disease presentation (Ofman et al. 2003). Patients with HSD10 disease are commonly divided into 4 groups: neonatal form, infantile form, juvenile form, and atypical presentation (Zschocke 2012).

There are several reported missense mutations in the *HSD17B10* gene. Most prevalent (about 50% of cases) is the mutation R130C in exon 4, which causes a 90% decrease in catalytic activity, decreased protein levels and its instability (Yang et al. 2009). Such a high occurrence of this mutation is caused by the presence of a highly methylated cytosine at position 130 which can be easily deaminated to form thymine, thus generating the substitution of arginine to cysteine which ultimately leads to disruption of the protein structure and enzymatic activity. Patients with this mutation have a more serious clinical phenotype compared with patients carrying other mutations (Yang et al. 2013). 17β-HSD10 also plays a role in isoleucine degradation (Ofman et al. 2003) and its malfunction leads to accumulation of degradation metabolites such as 2-methyl-3-hydroxybutyrate and tiglylglycine (Korman 2006). Moreover, stable concentration and homeostasis of neurosteroids are critical for proper cognitive development. Blockade or depletion of 17β-HSD10 in patients can cause imbalance in neurosteroid metabolism and metabolism of GABA<sub>A</sub> modulators, which therefore could be the cause of the neurological dysfunction,
damage of cognitive process and psychomotor retardation (Yang et al. 2009; He et al. 2005; Porcu et al. 2016). To date, there is no effective treatment for this disease (Zschocke 2012).

As mentioned, mutation of HSD17B10 causes a reduced RNase P activity and its consequences. This was identified by analyzing the tissue of HSD10 disease patients, where the reduced assembly and activity of respiratory chain complexes (except complex II) were found (Chatfield et al. 2015). Similarly, patients with a defect in RNase Z, which cleaves the 3´end of tRNA, have nearly identical clinical symptoms as patients with HSD10 disease, suggesting that the accumulation of precursor transcripts may also interfere with ribosomes and block their function in the translation of mitochondrial proteins (Haack et al. 2013). It is proved that the severity of certain clinical symptoms in patients with HSD10 mediated disease is not consistent with the level of remaining enzymatic activity of the mutated enzyme, and mitochondrial integrity and functionality is dependent only on the presence of the 17β-HSD10 protein (Rauschenberger et al. 2010; Deutschmann et al. 2014). To date, several case studies of HSD10 disease patients with variable phenotypes and clinical manifestation have been described (Zschocke et al. 2000; Ensenauer et al. 2002; Olpin et al. 2002; Sutton et al. 2003; Poll-The et al. 2004; Sass et al. 2004; Perez-Cerda et al. 2005; Cazorla et al. 2007; Lenski et al. 2007; García-Villoria et al. 2010; Rauschenberger et al. 2010; Seaver et al. 2011; Zschocke 2012; Fukao et al. 2014; Falk et al. 2016; Richardson et al. 2017; Waters et al. 2019; Akagawa et al. 2017). However, the number of affected individuals is relatively low, usually with siblings or relatives with the same mutation pattern in one family. For this reason, research and development of possible treatment strategy is very difficult and is hard to hypothesize the future trends in this field.

Cancer

Due to the important role of 17β-HSDs in steroid metabolism, several 17β-HSD family members are implicated in some cancer types (Nordling et al. 2001), specifically breast (Jansson et al. 2006; Wang et al. 2008; Zhang et al. 2012), endometrial (Cornel et al. 2012), colorectal (Rawłuszko et al. 2011) and prostate (He and Yang 2006). Overexpression of 17β-HSD10 under nutrient-limiting conditions in cancer cells can help the cells keep cellular homeostasis by providing additional steroid metabolites that can promote survival under unfavorable conditions and so protect the cells against cell death. The overexpression of 17β-HSD10 in some prostate and bone cancer types (He et al. 2003), and especially in the pheochromocytoma cells, leads to increased cell growth both in vitro and in vivo. Higher expression of 17β-HSD10 influences activity of respiratory chain and generation of energy, thus supporting cell growth and making the cells more resistant to oxidative stress, which ultimately results in promoting tumorigenesis and aggressiveness of the cancer cells. 17β-HSD10 higher level is connected with bad prognosis of these patients (Carlson et al. 2015). In prostate cancer, 17β-HSD10 enables steroid synthesis,
catalyzing the alternative synthesis pathway of androgen by generating dihydrotestosterone (DHT) in the absence of testosterone (He and Yang 2006; Ayan et al. 2012). In osteosarcoma patients, the expression level of 17β-HSD10 was shown to be a possible predictive marker for chemotherapy response (Salas et al. 2009). Similarly in colorectal cancer, high 17β-HSD10 expression is associated with better overall survival (Amberger et al. 2016). Based on these findings, human 17β-HSD10 is considered to be a potential drug target for certain cancer types (He et al. 2001; Ayan et al. 2012). However, there are no new findings in this area in recent years, except of profiling of risperidone (discussed further in section of inhibitors) as an anti-cancer drug in clinical trials (Dilly et al. 2019). The role of 17β-HSD10 in cancer is not thoroughly determined and further studies have to be performed to better understand its involvement.

**Neurodegenerative disorders**

17β-HSD10 has the ability to promote neurodegenerative processes and apoptosis of neurons thereby playing an important role in neurodegenerative disorders (Oppermann et al. 1999; Yan et al. 1999). A role for 17β-HSD10 is confirmed in Parkinson’s disease (PD) (Tieu et al. 2004), which is a neurodegenerative disease manifested by the reduction of dopaminergic neurons in the substantia nigra resulting in motor symptoms as shaking, rigidity and postural instability, and walking problems (Opara et al. 2017). Patients with PD have significantly downregulated the expression of 17β-HSD10, resulting in reduced levels of the enzyme in dopaminergic neurons. Thus, it implied that an elevated level of 17β-HSD10 might protect against PD hallmarks (Froemming and Sames 2007; Tieu et al. 2004).

**Alzheimer’s disease and the role of amyloid β**

Alzheimer’s disease (AD) is the most common cause of dementia with a significant impact on individuals and society, affecting over 50 million people worldwide (Sahyouni et al. 2016; Wolfe 2017; Vos et al. 2016). The progress of the disease is connected with neuropathological changes caused by the presence of extracellular plaques of Aβ and neurofibrillary tangles of hyperphosphorylated tau-protein, resulting in loss of neuronal cells and decline of cognitive function. The disease mostly occurs sporadically at age around 65, but some cases (5%) are caused by inherited mutations in genes for enzymes responsible for Aβ turnover resulting in an earlier age of onset (Burns and Iliffe 2009; Feldman 2007). Available pharmacotherapy is based only on treating the disease symptoms by improving the cognitive function of AD patients with cholinesterase inhibitors (donepezil, galantamine, rivastigmine) or N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) (Noetzli and Eap 2013; Wang et al. 1999). There is a need to find other therapeutic approaches, especially to prevent and retard the disease progression in affected
individuals. The potential targets and approaches in AD therapy evolution are extensively reviewed elsewhere (Van Bulck et al. 2019; Anand et al. 2017). Despite the intensive research in this field and many clinical trials realized to date (Huang et al. 2020), a therapy is not yet available for clinical use.

AD is connected with the changes or disruption of many physiological pathways. Levels of toxic products of lipid peroxidation such as 4-HNE are elevated in brains of AD patients (Sayre et al. 1997), while levels of neuroprotective neurosteroids estradiol and allopregnanolone are significantly decreased (Marx et al. 2006). Levels of these neurosteroids are strongly linked to the disease state. In addition, lower expression and activity of enzymes required for energy production in mitochondria (both in the electron transport chain and ATP synthase) are detected in AD patients (Blass et al. 2000; Parker et al. 1994). Neuronal cells in AD have lower utilization of energy through glycolysis and utilize ketone bodies as an alternative fuel (Ishii et al. 1997), so they are highly dependent on mitochondrial energy production and susceptible to mitochondrial dysfunction (Moreira et al. 2009).

Mitochondria as a major energy producer, play a central role in the pathology of AD and are involved in Aβ-induced neuronal impairment (Chen and Yan 2010). Significant mitochondrial dysfunction in brains from 3×Tg-AD mice (transgenic mice harboring three mutations associated with a familial form of Alzheimer’s disease) was detected (Yao et al. 2007), including defective electron transport chain enzymatic activity, accumulating ROS (reactive oxygen species), and impaired energy metabolism which are all early features of AD pathology (Chen and Yan 2010; Lustbader et al. 2004; Borger et al. 2011). Chronic oxidative stress in AD causes mitochondrial abnormalities and a significant elevation of mtDNA and cytochrome c oxidase levels, whereas the number of mitochondria is significantly reduced (Hirai et al. 2001).

The main fibril protein forming senile plaques is the Aβ peptide, a major biomarker of the AD. Aβ can accumulate in mitochondria, bind to mitochondrial proteins, leading to mitochondria-mediated cellular toxicity and neuronal death (Glenner and Wong 1984; Burns and Iliffe 2009; Chen and Yan 2010; Pagani and Eckert 2011; Borger et al. 2011). Aβ protein is formed from an amyloid precursor protein (APP) after cleavage by a β-secretase and γ-secretase complex, which leads to the production of Aβ(1-40), or the more toxic Aβ(1-42) peptide. These peptides are degraded by an insulin-degrading enzyme (IDE) and neprilysin (Evin and Weidemann 2002) (Fig. 3). Aβ was found to be associated with mitochondria of both transgenic mice expressing mutant APP, and AD patients, providing a direct link between Aβ and the dysfunction of mitochondria in AD. Aβ is transported into mitochondria by various mechanisms (receptor-dependent pathway and translocase of the inner/outer membrane machinery) or directly from the Golgi apparatus/endoplasmic reticulum membrane contact. Any imbalance or defect in these pathways may cause pathological accumulation of Aβ in mitochondria (Anandatheerthavarada et al. 2003; Chen and Yan
Once inside the mitochondria, Aβ can affect transport of molecules through mitochondrial membranes, respiratory chain, and interact with several matrix proteins (Caspersen et al. 2005; Benek et al. 2015; Lustbader et al. 2004).

The major role of 17β-HSD10 in AD pathology is its interaction with Aβ resulting in mitochondrial dysfunction and neuronal impairment (Lustbader et al. 2004). 17β-HSD10 was first identified as a binding partner of Aβ in 1997 from a yeast two-hybrid screen against HeLa cDNA and was reported to be an intracellular endoplasmic reticulum-associated amyloid beta binding protein, which could mediate the cellular toxicity of Aβ (Yan et al. 1997). As indicated above later, its localization was found to be actually within mitochondria, and so the abbreviation ABAD (Yan et al. 1999) began to be used, though given its substrates, today the term 17β-HSD10 is more suitable.

Significantly, elevated expression of 17β-HSD10 protein was found in the brains of AD patients, including astrocytes (He et al. 2005; Borger et al. 2011), where it forms a 17β-HSD10-Aβ complex contributing to Aβ-induced toxicity by promoting mitochondrial dysfunction (Yan et al. 1999; Benek et al. 2015; Lustbader et al. 2004). It is known that Aβ binds to 17β-HSD10 via a region called loop D, containing residues 92-120, a short β-hairpin structure on the beginning of αD helix. This sequence is unique within the SDR enzyme family making 17β-HSD10 the only SDR enzyme able to bind Aβ. The 17β-HSD10-Aβ complex formation has been confirmed by various experiments, but Aβ and L0 could not be seen in crystallographic studies due to poor electron density in this region, suggesting a disordered conformation (Lustbader et al. 2004).

Interaction with Aβ is highly specific and occurs at nanomolar concentrations (Kd of 88 nM (Yan et al. 1997)), while micromolar levels of Aβ are required for inhibition of 17β-HSD10 enzymatic activity (Yan et al. 1999; Oppermann et al. 1999). It is predicted that only aggregated oligomeric Aβ is able to inhibit 17β-HSD10 enzymatic activity (Yan et al. 2007). Binding between Aβ and 17β-HSD10 is mainly through hydrophobic interactions. The generation of this interaction changes protein conformation, which was proven by using surface plasmon resonance and studies of co-crystal structures of 17β-HSD10 in complex with Aβ (Lustbader et al. 2004; Yan et al. 2007). After binding of Aβ, distortion of the NAD-binding pocket prevents binding of the NAD cofactor to the enzyme, leading to loss of the enzymatic activity, and thereby resulting in an elevation of mitochondrial stress and loss of neuron viability (Aitken et al. 2016; Lustbader et al. 2004).

17β-HSD10 is able to degrade toxic aldehydes (Murakami et al. 2009), but when binding Aβ it potentially changes its substrate specificity (Yan et al. 1999). Thus aldehydes created from brain lipids cannot be detoxified therefore damaging/killing neurons (Takuma et al. 2005; Lustbader et al. 2004; Du Yan et al. 2000; Murakami et al. 2009). Williams et al. (Williams et al. 2006) proved elevated levels of toxic...
aldehydes in some brain regions of patients with early AD and mild cognitive impairment. Moreover, binding of Aβ disables binding of 17β-HSD10 to MRPP1 and the formation of mtRNase P, resulting in loss of 17β-HSD10-associated tRNA:m1R9 methyltransferase activity in vitro as well (Vilardo and Rossmanith 2013). Additionally, the interaction of 17β-HSD10 with Aβ results in altered expression levels of other proteins such as the elevation of peroxiredoxin II and endophilin AI. Both these proteins can serve as indicators for 17β-HSD10-Aβ interaction as their levels return to physiological levels after perturbation of this toxic interaction (Yao et al. 2007; Ren et al. 2008). Peroxiredoxin II is an antioxidant enzyme controlling peroxide levels in cells and protects neurons from toxic levels of Aβ (Yao et al. 2007). Its elevated level in AD is probably a compensatory mechanism of neurons attempting to protect themselves from the toxic effects of Aβ. However, peroxiredoxin II is deactivated by cyclin-dependent kinase 5, which is hyperactivated in AD, leading to accumulation of toxic peroxides (Liu et al. 2016). Endophilin AI is a protein with a role in vesicle formation localized in cytoplasm, which is present in presynaptic nerve termini. Elevated levels of endophilin I lead to stimulation of the c-Jun N-terminal kinase pathway leading to disrupted synaptic activity, elevated ROS production and activation of p38 MAP kinase, contributing to mitochondrial dysfunction and decline of cognitive function (Reutens and Begley 2002; Ren et al. 2008; Yu et al. 2018).

Role of neurosteroids in neurodegenerative disorders

Neuroactive steroids (e.g. estrogens, pregnanes, and androstanes) are molecules produced endogenously in the body (and mainly in the brain) and exhibit many physiological functions. Many of these molecules are able to modulate the functions of the brain and the nervous system through interaction with GABA_A receptors, and can exhibit neuroprotective and anti-apoptotic effects. Levels of neuroactive steroids change during our lifetime and in many physiological conditions including the ovarian cycle, pregnancy, and stress, but their homeostasis can be disturbed in many neurodegenerative disorders (Porcu et al. 2016). Thus, maintaining their levels and modulation of their metabolism could be a possible therapeutic target in some steroid-dependent diseases.

As mentioned above, estrogens are steroid hormones acting through the estrogen receptors (α and β), expressed in various parts of the body, but mainly in the central nervous system. These hormones display various neuroprotective activities and restoration or maintaining of their levels could serve as a potential preventive and therapeutic strategy in many neurodegenerative diseases (Manthey and Behl 2006).

The main human estrogen, 17β-estradiol, is a hormone with a highly important role in reproductive physiology of both men and women, metabolism of lipids, and in brain functions such as memory.
mechanisms and mood control. This neurosteroid has a strong neuroprotective ability with potential anti-inflammatory and anti-apoptotic capabilities. Estradiol controls the inflammatory response by blocking gene expression of pro-inflammatory genes via the estrogen receptors on the brain’s inflammatory cells (Pozzi et al. 2006) and show also direct antioxidant activity, which is receptor independent (Manthey and Behl 2006; Porcu et al. 2016). It has a neuroprotective effect by regulating APP trafficking, metabolism and it regulates the accumulation of Aβ by influencing the expression of IDE. Treatment with estradiol reduces the formation of hyper-aggregated Aβ(1-42) and hyperphosphorylation of tau-protein in AD models both in in vitro and in vivo experiments (Xu et al. 1998; Jayaraman et al. 2012; Alvarez-De-La-Rosa et al. 2005). It may explain why women are more susceptible to AD (Yang et al. 2011; He et al. 1999), especially after menopause where the level of estradiol is significantly reduced (Jayaraman et al. 2012).

17β-HSD10 metabolizes estradiol (Yan et al. 1999; He et al. 2000b) and decreases its levels, therefore providing a direct link between the physiological role of this enzyme and AD pathogenesis. Based on these estradiol findings, hormone replacement therapy for AD prevention has been studied. However, at present, no clinical trial using hormone therapy has provided any protection against AD or cognitive improvement. Moreover, some studies showed a potential increase risk of dementia (Henderson 2014; Savolainen-Peltonen et al. 2019; Shumaker 2004). Hypothetically, the partial inhibition of 17β-HSD10 enzymatic activity and thus maintaining physiological levels of steroids might be way forward.

**Inhibition of 17β-HSD10-Aβ interaction or enzymatic activity as a therapeutic target**

The ability of 17β-HSD10 to metabolize a wide range of chemically different substrates and participate in many physiological pathways makes it a very intricate target for disease therapy (Yang et al. 2014; Powell et al. 2000). Due to the important role of 17β-HSD10 in the metabolism of active neurosteroids, modulation of its enzymatic activity could restore the neurosteroid homeostasis and neuroprotection (Boutin et al. 2018; Marques et al. 2009) and thus it could be a valuable approach for AD and cancer treatment (Carlson et al. 2015; Ayan et al. 2012). In addition, prevention of 17β-HSD10 binding to Aβ or disrupting this interaction have a significant positive effect on mitochondria and Aβ-induced cellular stress (Borger et al. 2013; Lustbader et al. 2004; Marques et al. 2008) both in cell lines and mouse models (Yao et al. 2011). Finding novel and promising inhibitors is a challenging procedure. To date, several compounds targeting 17β-HSD10-Aβ interaction or directly 17β-HSD10 enzymatic activity have been developed. According to their nature and structure, such inhibitors can be divided into five groups:
mimetics of loop D (1-3), benzothiazolyl ureas (e.g. 4-13), fused pyrazole compounds (e.g. 14), steroidal inhibitors (e.g. 15-16), atypical antipsychotics and other compounds (17-18).

The fused pyrazoles were the first produced and are very potent inhibitors of 17β-HSD10, but were not further developed in the last decade. The most abundant and published inhibitors belong to benzothiazolyl ureas and steroidal compounds. This approach is most probably related to the possibilities of easier molecular design of such inhibitors as they have to be druglike, membrane penetrable, which is especially important for CNS delivery purposes. Mimetics of loop D and atypical antipsychotics are being the further directions for AD or anti-cancer use, but there is limited knowledge on their behavior in vivo.

**Mimetics of loop D**

As mentioned above, loop D is a short region unique for 17β-HSD10 which is responsible for Aβ binding (Lustbader et al. 2004). Based on its structure sequence Lustbader et al. synthesized a peptide comprising residues 92-120, called ABAD decoy peptide (ABAD-DP, 1), which was able to inhibit the interaction of 17β-HSD10 both with Aβ(1-40) and Aβ(1-42) having IC₅₀ (half-maximal inhibitory concentration) 4.9 μM and 1.7 μM in vitro (Tab. 1). As a control, a reverse sequence peptide (ABAD-RP) was made and confirmed to be inactive (Lustbader et al. 2004). For the study of 17β-HSD10-Aβ inhibition in vivo, ABAD-DP was fused with the cell-membrane transduction domain from the HIV-1 virus (ABAD-DP-Tat) enabling the peptide to cross biological membranes. This improved structure was able to prevent cytochrome c release, suppress ROS production and decrease toxicity induced by Aβ in cultured neurons from Tg ABAD mice, while ABAD-RP-Tat had no effect. However, the use of this structure as a therapeutic seems to be complicated due to its peptidyl character, which can be degraded by various peptidases. For stabilization of the decoy peptide, fusion with thioredoxin-1 was made (Yang et al. 2007b) and this fusion peptide decreased Aβ-induced toxicity in PC12 cell culture and to recover disturbed Aβ-induced redox homeostasis. In vivo studies of decoy peptide consisting from 17β-HSD10 residues 93-116 containing mitochondrial matrix targeting sequence and Tat fragment (Tat-mito-ABAD-DP) (Ren et al. 2008; Yao et al. 2007) which was administered intraperitoneally to mice revealed disruption of 17β-HSD10-Aβ interaction and decreased the production of AD markers, peroxiredoxin II and endophilin I, leading to improvement in cognitive functions of transgenic animals (Yao et al. 2011).

Since peptides mimicking the binding part of the enzyme have limitations as drug candidates, there was an effort to find non-peptidyl inhibitors targeting “hot spots” of loop D with the potential to inhibit the 17β-HSD10-Aβ binding (Viswanath et al. 2017). The most important residues responsible for Aβ-binding
(Thr₁₀⁸, His₁₀⁹, and Thr₁₁⁰) were used for the virtual screening of various chemical databases and for in silico design. The analysis of best hits using an ELISA (enzyme-linked immunosorbent assay) assay revealed 2 promising lead candidates, 2 and 3 (Fig. 4) inhibiting the 17β-HSD10-Aβ interaction with IC₅₀ 4.4 μM and 9.6 μM, using previously published compounds by Xie (5 and 6) (Xie et al. 2006) (IC₅₀ 6.46 μM and 6.56 μM ) as a standard (Tab. 1, Fig. 4). Treatment of HT22 cells (a mouse hippocampal neuronal cell line) with 5 μM of compounds 2 and 3 was shown to improve mitochondrial dysfunction and reduce Aβ-induced cell toxicity (Viswanath et al. 2017).

Tab. 1. Inhibitors of 17β-HSD10-Aβ interaction.

<table>
<thead>
<tr>
<th>Compound</th>
<th>17β-HSD10-Aβ IC₅₀ (μM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.70</td>
<td>(Lustbader et al. 2004)</td>
</tr>
<tr>
<td>2</td>
<td>4.40</td>
<td>(Viswanath et al. 2017)</td>
</tr>
<tr>
<td>3</td>
<td>9.60</td>
<td>(Viswanath et al. 2017)</td>
</tr>
<tr>
<td>4</td>
<td>200.00</td>
<td>(Xie et al. 2006)</td>
</tr>
<tr>
<td>5</td>
<td>6.46</td>
<td>(Xie et al. 2006)</td>
</tr>
<tr>
<td>6</td>
<td>6.56</td>
<td>(Xie et al. 2006)</td>
</tr>
</tbody>
</table>

Benzothiazolyl ureas

In an ELISA screening of potential compounds interacting with 17β-HSD10 and/or Aβ, frentizole (4) (Fig. 5), a commonly used FDA-approved (food and drug administration) drug for rheumatoid arthritis and systemic lupus, was revealed as an inhibitor of the Aβ-17β-HDS10 interaction (IC₅₀ 200 μM, Tab. 1) (Xie et al. 2006). This parent structure subsequently served as a scaffold for the preparation of several analogs (Fig. 5). SAR (structure-activity relationship) analysis revealed the urea is an important component for the inhibitory activity, whereas amide compounds showed significantly lower inhibition. Various substitutions of benzothiazole and phenylurea can affect the inhibitory ability and showed a preference for small electron-withdrawing groups on benzothiazole moiety and a 4-OH substitution of the phenylurea moiety, resulting in two potent inhibitors (5, 6) of 17β-HSD10-Aβ binding with IC₅₀ below 10 μM (Tab. 1) (Xie et al. 2006).

Further modification of known structures based on in silico models revealed a novel class of benzothiazole amino phosphonate analogues (Valasani et al. 2013). Benzothiazole phosphonates should have improved
properties for crossing the biological membranes, blood-brain barrier and entering target cells. Two best compounds were selected (7, 8, Fig. 5) and inhibitory ability was determined (Vangavaragu et al. 2014). These compounds inhibit the 17β-HSD10 enzymatic activity with an IC\textsubscript{50} 341.9 \textmu M and 52.7 \textmu M (Tab. 2) and have the ability to rescue mitochondrial function, adenosine triphosphate (ATP) and cytochrome c oxidase activity levels with no adverse toxic effects on cells (Valasani et al. 2013; Valaasani et al. 2014).

Hroch et al. prepared novel riluzole-based benzothiazolyl ureas (Hroch et al. 2016) with two promising compounds (9, 10). The most effective structural pattern was a combination of 4-hydroxy and 3-chlorine or 3,5-dichlorine substitution of the phenyl ring. However, these compounds were only poorly soluble, disallowing IC\textsubscript{50} determination.

Further testing of other benzothiazolyl urea analogs showed a compound (11) as a potential 17β-HSD10 inhibitor (IC\textsubscript{50} 3.06 \textmu M) (Benek et al. 2017). This structure served as a structural basis for the development of a new generation of 17β-HSD10 inhibitors (Hroch et al. 2016; Benek et al. 2017; Aitken et al. 2019; Schmidt et al. 2020) (Tab. 2). Some of the previously published benzothiazolylphenylureas were screened as potential inhibitors of CK1 (casein kinase), other enzyme connected to AD pathology (Benek et al. 2018). Two more compounds (12, 13) being low micromolar inhibitors of both 17β-HSD10 and CK1, were identified.

The benzothiazole urea scaffold was combined with other structural patterns valuable for inhibition of other enzymes relevant to AD pathophysiology to possibly obtain a dual effect. A combination of frentizole and rasagiline scaffolds for inhibiting both 17β-HSD10 and MAO (monoamine oxidase) revealed one compound exhibiting good potency for MAO (IC\textsubscript{50} 6.34 \textmu M), but no activity towards 17β-HSD10 (Hroch et al. 2017). Searching for novel lead structures for 17β-HSD10 inhibitors using high-throughput screening of more than 6500 compounds from different collections and libraries (Aitken et al. 2017) revealed 16 hits. These inhibitors were identified to be in the micromolar range and might be capable to cross the blood-brain barrier. One of the most promising hits was raloxifene, a selective estrogen receptor modulator, used as a drug for osteoporosis treatment in postmenopausal women (Barrett-Connor 2006). This compound or its analogs could be helpful in restoring estrogen homeostasis important for neuroprotection.

**Fused pyrazole compounds**

Small molecule inhibitors based on fused pyrazoles were found to have a good inhibitory activity towards 17β-HSD10 (Abreo et al. 2005)). The most potent inhibitor to date is AG18051 (15, IC\textsubscript{50} 92 nM) (Kissinger et al. 2004) (Tab. 2, Fig. 6), a pyrazolopyrimidine based compound able to bind into the substrate-binding
site of the 17β-HSD10 enzyme. This structure has no structural similarity with any known 17β-HSD10 substrates but is sterically and electrostatically complement with its active site (Marques et al. 2008b). Crystallization of the enzyme in the presence of AG18051 and NAD+ cofactor showed cooperative binding of inhibitor and cofactor forming a covalent adduct inside the enzyme active site. Binding of the inhibitor in the absence of cofactor is very weak (Kissinger et al. 2004). The structural analysis of this inhibitor binding revealed stabilization of the enzyme structure after cofactor and inhibitor binding (Marques et al. 2008b), mainly in substrate-binding loop, which is very flexible in the absence of ligand. In vitro, AG18051 was able to reduce ROS formation and cellular toxicity induced by Aβ in SH-SY5Y (human neuroblastoma cell line) cells and restore a normal level of estradiol (Lim et al. 2011). The structure of this inhibitor was further studied in silico to find chemical modifications in the benzene and azepane ring to improve its binding ability (Marques et al. 2008a). A SAR study of AG18051 modifications identified a group of 18 inhibitors with IC_{50} in the nanomolar range (7 compounds with IC_{50} ≤ 100 nM) (Abreo et al. 2005).

**Steroidal inhibitors**

As the 17β-HSD10 is a steroid-processing enzyme, steroid analogs could be potentially inhibitors. A number of steroidal compounds, which are known inhibitors of other steroidogenic enzymes, were tested to reveal their inhibitory potency to 17β-HSD10 (Ayan et al. 2012). The most potent structures were further modified to reveal the DHT-based reversible inhibitor (16) with an IC_{50} 0.55 μM (Tab. 2, Fig. 7). However, this compound is not selective and inhibits 17β-HSD3, which is (together with 17β-HSD10) overexpressed in prostate cancer forms.

Structure of compound 16 was further used for SAR studies and several analogs were prepared (Boutin et al. 2018) followed by inhibitory ability testing using radiolabeled allopregnanolone and estradiol as the enzyme substrates. The most potent inhibitor was found to be a 17α-OH alcohol derivative of androsterone (17) with an estimated IC_{50} 235 μM towards allopregnanolone and 610 μM towards estradiol (Tab. 2), which could aid crossing the blood-brain barrier. The calculated IC_{50} for 16 using allopregnanolone and the estradiol assay was 985 μM and 710 μM, while previously it was measured as 0.55 μM in HEK293 cell line (Ayan et al. 2012). Apparently, different methods used for inhibition determination can lead to completely different results. Inhibition properties between steroidal (Ayan et al. 2012) and non-steroidal (Vangavaragu et al. 2014) 17β-HSD10 inhibitors were compared using recombinant enzyme and cellular model system (Boutin and Poirier 2018). Using purified recombinant enzyme, the non-steroidal compound (7) was found to be a stronger inhibitor than a steroidal one (16). However, using HEK293 cells overexpressing 17β-HSD10, the non-steroidal inhibitor did not affect
enzymatic activity, whereas steroidal inhibitor inhibited the enzymatic reaction. The ability of the inhibitor to enter the cell was not determined, thus it is possible, that the non-steroidal inhibitor could not inhibit the enzyme inside the cell. Alternatively, it is possible that the enzyme works slightly differently in the isolated recombinant form compared to situation in the cell line. Still, these approaches are just experimental models and so can behave quite differently compared to a complex system of a human cell let alone the human body.

Atypical antipsychotics and other compounds
A new group of $17\beta$-HSD10 inhibitors are compounds, which are routinely used in clinical practice as drugs for psychiatric disorders. Some atypical antipsychotics were found to have additional effects as anti-cancer drugs (Chen et al. 2018). The most promising compound is risperidone (18) (Fig. 8), an FDA-approved drug for treating schizophrenia (Marder and Meibach 1994), which was found to interact with $17\beta$-HSD10 in a drug screen against the whole genome model of Drosophila melanogaster T7 bacteriophage library (Dilly et al. 2017a). In silico docking of risperidone into the crystal structure of $17\beta$-HSD10 predicted a competitive binding mode within the cofactor binding site, similar to that found for steroidal inhibitors (Ayan et al. 2012). In vitro, risperidone is able to suppress proliferation and invasion and to induce apoptosis in HCC (human hepatocarcinoma cell line) cells (Chen et al. 2018). The novel combination of risperidone with rumenic acid, called VAL401, was tested and shown a significant slowdown of PC3 cell (prostate cancer cell line) proliferation in vitro and PC3-induced tumor growth xenografts in vivo in mice (Dilly et al. 2017a; Dilly et al. 2017b). Based on these data, VAL401 was assumed to be a repurposed drug for adenocarcinomas or prostate cancer and there has been a completed clinical trial stage II for lung cancer treatment using it in 2018 (Dilly et al. 2019).

<table>
<thead>
<tr>
<th>Compound</th>
<th>$17\beta$-HSD10 IC$_{50}$ ($\mu$M)</th>
<th>Assay substrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>341.9</td>
<td>SAAC</td>
<td>(Valasani et al. 2013)</td>
</tr>
<tr>
<td>8</td>
<td>52.7</td>
<td>SAAC</td>
<td>(Valasani et al. 2013)</td>
</tr>
<tr>
<td>11</td>
<td>3.06</td>
<td>SAAC</td>
<td>(Benek et al. 2017)</td>
</tr>
<tr>
<td>12</td>
<td>1.89</td>
<td>SAAC</td>
<td>(Benek et al. 2018)</td>
</tr>
<tr>
<td>13</td>
<td>1.67</td>
<td>SAAC</td>
<td>(Benek et al. 2018)</td>
</tr>
<tr>
<td>14</td>
<td>1.20</td>
<td>SAAC</td>
<td>(Schmidt et al. 2020)</td>
</tr>
<tr>
<td>15</td>
<td>0.092</td>
<td>SAAC</td>
<td>(Kissingier et al. 2004)</td>
</tr>
<tr>
<td>16</td>
<td>0.55</td>
<td>[14C]-17β-E2</td>
<td>(Ayan et al. 2012)</td>
</tr>
<tr>
<td>17</td>
<td>610</td>
<td>[14C]-17β-E2</td>
<td>(Boutin et al. 2018)</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>-</td>
<td>(Dilly et al. 2017a)</td>
</tr>
</tbody>
</table>

Another compound which is potentially connected with 17β-HSD10 modulation is methylene blue (MB, methylthionium chloride, 19, Fig. 9) (Zakaria et al. 2016). MB is an FDA approved drug for methemoglobinemia therapy (Sikka et al. 2011) but recently came into interest as a possible therapy for Alzheimer’s disease. Methylene blue is reported to enhance cell viability, reduce Aβ oligomerization, and both 17β-HSD10 function and protein expression level in LPS (lipopolysaccharide) mouse model (Zakaria et al. 2016). However, the connection of MB with 17β-HSD10 is questionable because it is supported only by one publication (Zakaria et al. 2016) and the current interest with MB is its potential role in protein aggregation related tau pathology. Methylene blue, under the trade name TRx-0014, has been reported to pass phase I and II of clinical trials as a compound which is able to prevent tau aggregation and dissolve tau aggregates in AD (Wischik et al. 2015). Improvements in cognitive function of AD patients after long-term administration of TRx-0014, and the next generation compound Trx-0237 with better pharmacokinetic properties have been reported and now is being under investigation in a phase III clinical trials for the treatment of AD. However, these studies have been criticized because of uncertainties in methodology, and thus remain slightly controversial (Medina 2018).

**CONCLUSION**

Increasing life expectancy and aging of the population is leading to an increase in the incidence of aging-associated diseases like Alzheimer’s disease (AD) and cancer. Both diseases affect globally millions of people and the number of cases is rising. Current treatments are not quite effective and new therapeutic approaches are being extensively studied. A possible strategy for Alzheimer’s disease could be the modulation of enzymes involved in its progression, such as 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10), which had been found to be overexpressed in AD (Yang and He 2001) and is a binding partner of amyloid-β peptide (Yan et al. 1997). Formation of the 17β-HSD10-Aβ interaction leads to an imbalance in neurosteroid levels, mitochondrial dysfunction and neuronal death (Lustbader et al. 2004).
Involvement of 17β-HSD10 in the metabolism of neuroprotective steroidal hormone 17β-estradiol provides a possible link between the physiological roles of 17β-HSD10 and pathology of AD (Yang et al. 2014). In cancer, this enzyme appears to help cancer cells survive by generating specific androgens during hormone ablation therapy (Ayan et al. 2012). For these reasons, 17β-HSD10 is studied as potential target for AD and cancer treatment.

Overexpression of 17β-HSD10 in brains of AD patients (He et al. 2003; He et al. 2005) provides a connection between mitochondrial dysfunction and the interaction with Aβ, which affects both its enzymatic and structural functions, leading to mitochondrial dysfunction and neuronal death (Yan et al. 1997; Lustbader et al. 2004). Elevated level of 17β-HSD10 in AD may be a natural mechanism to restore the level of this enzyme’s activity reduced by its binding to intracellular Aβ (Yang et al. 2011). The role of 17β-HSD10 in AD seems to be a chain reaction. Intracellular Aβ binds 17β-HSD10 enzyme and blocks its enzymatic function, which leads to compensation by elevated expression of the enzyme. The overexpressed enzyme contributes to the lowering of neuroprotective estradiol levels leading to insufficient expression of Aβ-clearance factors controlled by estradiol. Lack of effective clearance of intracellular Aβ then causes its increased levels and increased binding to 17β-HSD10 enzyme and the process continues (Fig. 10). Thus, disruption of 17β-HSD10-Aβ binding and restoring its physiological level, or inhibition of the overexpressed enzyme could lead to the resurrection of estradiol homeostasis. Inhibitors (both of 17β-HSD10-Aβ interaction or direct inhibitors of enzymatic activity) that have been discovered so far were shown to have beneficial effect both in in vitro and in vivo models of AD. The most potent inhibitors are able to inhibit 17β-HSD10 activity with an IC₅₀ ranging from low micromolar to nanomolar level (Kissinger et al. 2004; Abreo et al. 2005; Lustbader et al. 2004; Xie et al. 2006; Viswanath et al. 2017; Valasani et al. 2013; Benek et al. 2017; Benek et al. 2018; Ayan et al. 2012; Boutin et al. 2018). These structures should serve as a starting point for further development of novel and more effective compounds.

The second major pathological condition, which is connected to 17β-HSD10, is cancer, where the overexpression of this enzyme was found to stimulate growth and invasiveness of cancer cells (He and Yang 2006; Carlson et al. 2015). This finding implicates that 17β-HSD10 could be a potential drug target not only for AD but also for several hormone dependent cancer types (He et al. 2001). In searching for anti-cancer drugs targeting 17β-HSD10, an FDA approved atypical antipsychotic risperidone was identified. It is now being evaluated in clinical trials to treat adenocarcinoma and prostate cancer (Dilly et al. 2019).
To conclude, 17β-HSD10 is an important mitochondrial protein for cell homeostasis and survival (a friend). However, its role might change under pathological conditions (its overexpression or/and increased Aβ levels) when it can be turned into an enemy. For this reason, the treatment strategy using 17β-HSD10 inhibitors or inhibitors of Aβ and 17β-HSD10 protein-protein interaction might be the option of choice to reduce 17β-HSD10 related diseases.

---Human subjects---
Involves human subjects:
If yes: Informed consent & ethics approval achieved:
=> if yes, please ensure that the info "Informed consent was achieved for all subjects, and the experiments were approved by the local ethics committee." is included in the Methods.

ARRIVE guidelines have been followed:
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Acknowledgements and conflict of interest disclosure

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(HSDs) reveal multiple specificities in bile acid and steroid hormone metabolism: characterization of multifunctional 3alpha/7alpha/7beta/17beta/20beta/21-HSD. Biochem. J. 376, 49–60.


Figure legends

Fig. 1. Crystal structure of 17β-HSD10 homotetramer (subunits are depicted in the different colors, adopted from (Kissinger et al. 2004)(PDB: 1U7T).

Fig. 2. Several reactions catalyzed by 17β-HSD10.

Fig. 3. Formation and metabolism of Aβ. Aβ is produced from amyloid precursor protein via proteolytic cleavage by β-secretase and γ-secretase. Mutations in APP, secretases or their components affect the APP cleavage and lead to increased generation of Aβ, followed by its aggregation and formation of oligomers, fibrils, and amyloid plaques. Non-aggregated soluble forms of Aβ can be degraded by neprilysin and insulin-degrading enzyme or can be cleared by uptake mechanism involving apolipoprotein E. Figure modified from (Evin and Weidemann 2002).

Fig. 4. 17β-HSD10 inhibitors targeting interaction with Aβ (Viswanath et al. 2017).

Fig. 5. Frentizole (4) and its benzothiazolyl ureas-based analogues (7-14) (Xie et al. 2006; Valasani et al. 2013; Hroch et al. 2016; Benek et al. 2017; Benek et al. 2018; Schmidt et al. 2020).

Fig. 6. Structure of fused pyrazole compound AG18051 (15) (Kissinger et al. 2004).

Fig. 7. Steroidal inhibitors (16-17) of 17β-HSD10 (Ayan et al. 2012; Boutin et al. 2018).

Fig. 8. Structure of risperidone (18) (Dilly et al. 2017a).

Fig. 9. Structure of methylene blue (19).

Fig. 10. 17β-HSD10-Aβ pathology cascade. Aβ binds intracellularly to 17β-HSD10, thus it is inhibiting its enzymatic activity. Loss of enzymatic active protein leads to natural compensatory mechanism by increasing expression of 17BHSD10 gene and increased 17β-HSD10 protein level. Higher level of enzyme causes increased degradation of estradiol, which modulates expression of Aβ clearing factors (e.g. IDE), resulting in accumulation of Aβ. Further, amyloid can enter into mitochondria, binds 17β-HSD10 and the cascade is repeated. (↑ denotes increase, ↓ denotes decrease)