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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

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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

Aβ	amyloid beta
ABAD-DP	amyloid beta-binding alcohol dehydrogenase decoy peptide
AD	Alzheimer´s disease
APP	amyloid precursor protein
ATP	adenosine triphosphate
CoA	coenzyme A
CypD	cyclophilin D
DHT	dihydrotestosterone
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
ER_{α}	estrogen receptor α
ERAB	ER-associated amyloid beta-peptide-binding protein
FDA	Food and drug administration
$GABA_A$	gamma-aminobutyric acid receptor type A
HADH II	L-3-hydroxyacyl-CoA dehydrogenase type II
4-HNE	4-hydroxy-2-nonenal
17β-HSD10	17β-hydroxysteroid dehydrogenase type 10
IC_{50}	half-maximal inhibitory concentration
IDE	insulin-degrading enzyme
kb	kilobase
L_D	loop D
LPS	lipopolysaccharide
MB	methylene blue
MHBD	2-methyl-3-hydroxybutyryl-CoA dehydrogenase
mPTP	mitochondrial permeability transition pore
MRPP	mitochondrial ribonuclease P protein
MRXS 10	X-linked mental retardation
mt	mitochondrial
NAD	nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
PD	Parkinson´s disease

ROS	reactive oxygen species
SAAC	acetoacetyl coenzyme A
SAR	structure-activity relationship
SDR	short-chain dehydrogenase/reductase
SCHAD	short-chain L-3-hydroxyacyl coenzyme A dehydrogenase

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 (L_D) 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 (Ser_{155}, Tyr_{168}, Lys_{172}) 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.* 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-dihydroxypregnan-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 C_{21} 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 (Mg^{2+}-dependent endoribonuclease). MRPP1 and MRPP2 create a stable subcomplex whereas the interaction between this subcomplex and MRPP3 is weak (Holzmann *et al.* 2008; Vilardo and Rossmanith 2015; Vilardo 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 unstability (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 $GABA_A$ 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

2010; Hansson Petersen *et al.* 2008; Audano *et al.* 2018). 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 α_D 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 L_D 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 (K_D 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 IC_{50} (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

(Thr_{108}, $His_{109,}$ and Thr_{110}) 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 IC_{50} 4.4 μM and 9.6 μM, using previously published compounds by Xie (**5** and **6**) (Xie *et al.* 2006) (IC_{50} 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.

Compound	17β-HSD10-Aβ IC_{50} (μM)	Reference
1	1.70	(Lustbader *et al.* 2004)
2	4.40	(Viswanath *et al.* 2017)
3	9.60	(Viswanath *et al.* 2017)
4	200.00	(Xie *et al.* 2006)
5	6.46	(Xie *et al.* 2006)
6	6.56	(Xie *et al.* 2006)

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 (IC_{50} 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 IC_{50} 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_{50} 341.9 μM and 52.7 μM (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_{50} determination.

Further testing of other benzothiazolyl urea analogs showed a compound (**11**) as a potential 17β-HSD10 inhibitor (IC_{50} 3.06 μM) (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_{50} 6.34 μM), 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_{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 $IC_{50} \leq 100$ 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 17β-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 17β-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 17β-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).

Tab. 2. Inhibitors of 17β-HSD10 enzymatic activity (Substrates: Acetoacetyl-CoA (SAAC), radiolabeled 17β-estradiol ([^{14}C]-17β-E2)).

Compound	17β-HSD10 IC_{50} (μM)	Assay substrate	Reference
7	341.9	SAAC	(Valasani *et al.* 2013)
8	52.7	SAAC	(Valasani *et al.* 2013)
11	3.06	SAAC	(Benek *et al.* 2017)
12	1.89	SAAC	(Benek *et al.* 2018)
13	1.67	SAAC	(Benek *et al.* 2018)

14	1.20	SAAC	(Schmidt *et al.* 2020)
15	0.092	SAAC	(Kissinger *et al.* 2004)
16	0.55	[^{14}C]-17β-E2	(Ayan *et al.* 2012)
17	610	[^{14}C]-17β-E2	(Boutin *et al.* 2018)
18	-	-	(Dilly *et al.* 2017a)

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 conection 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 IC_{50} 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.

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Acknowledgements and conflict of interest disclosure

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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).

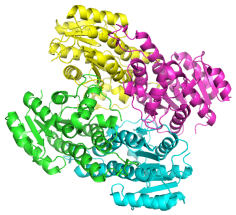
Fig. 6. Structure of fused pyrazole compound AG18051 (**15**) (Kissinger *et al.* 2004).

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)



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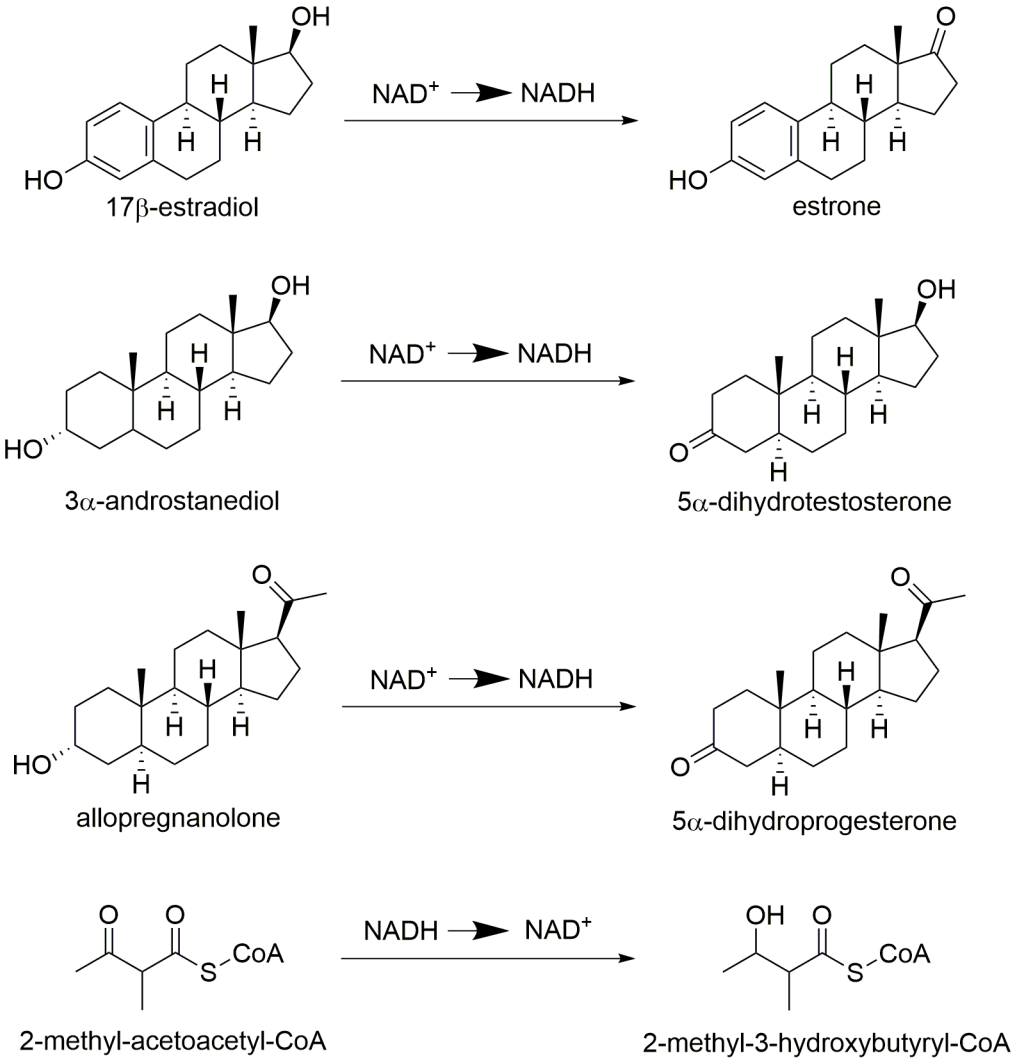

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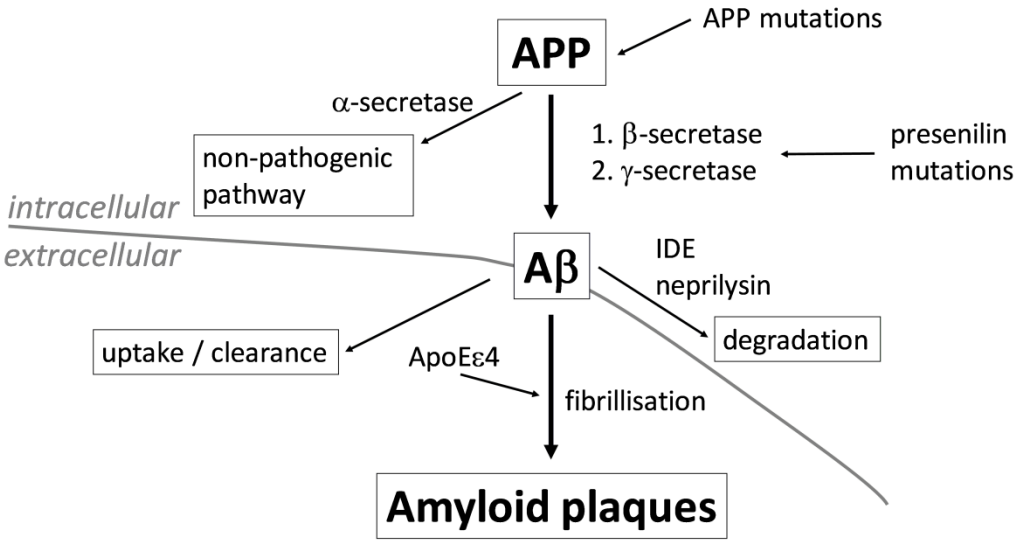

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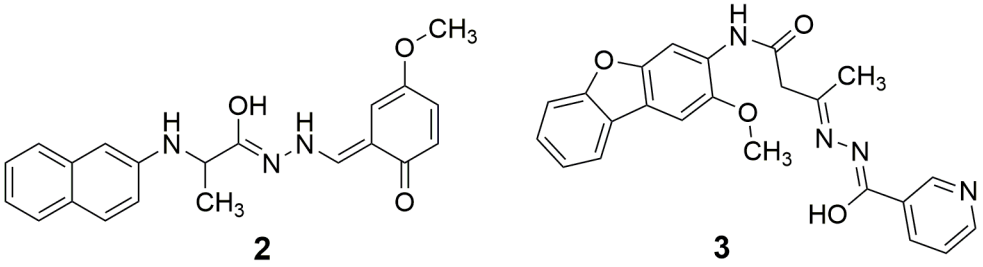

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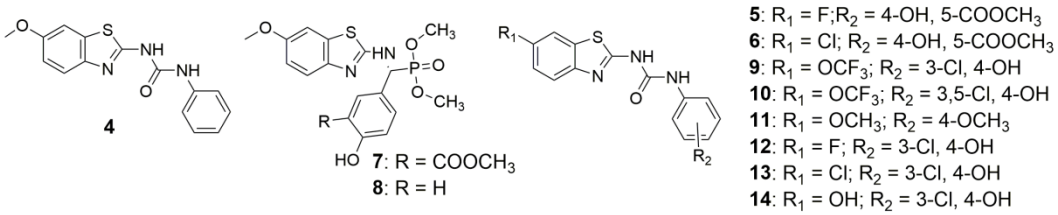

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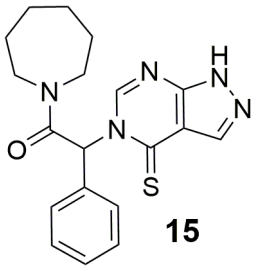

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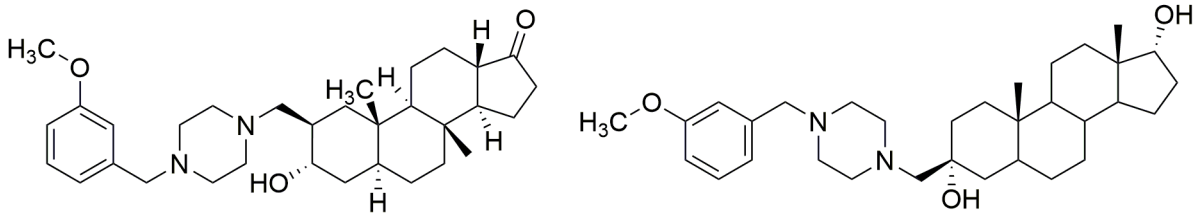

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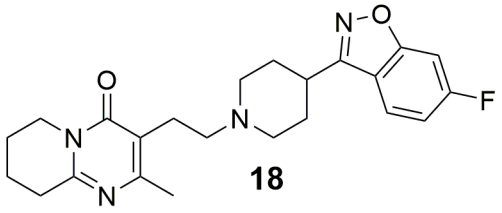

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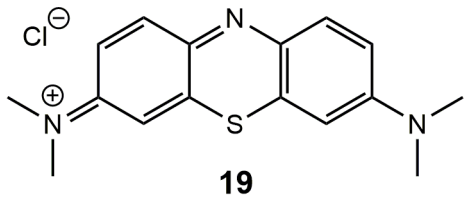

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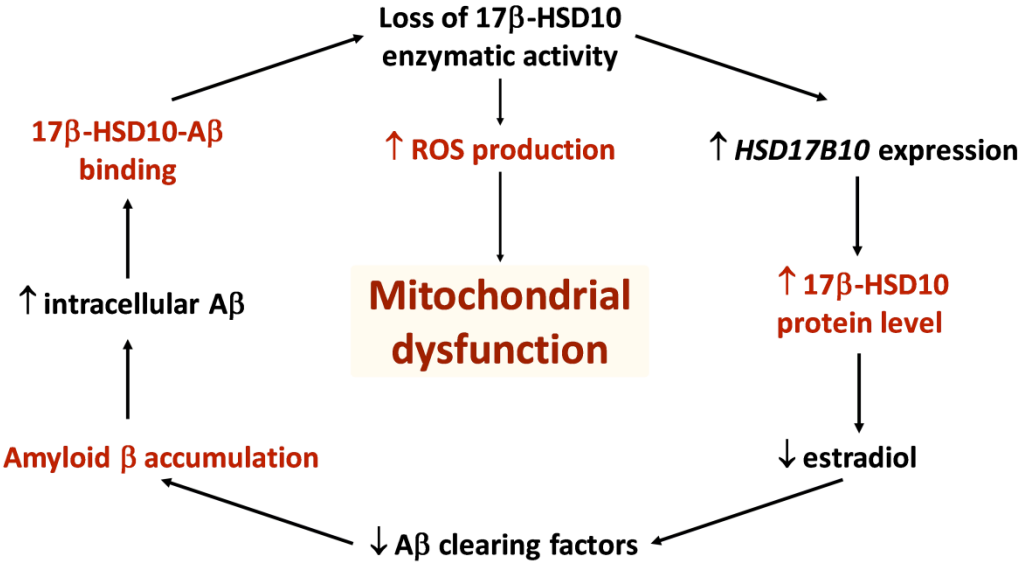

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