CHAPTER 14

Translational Research in Stress Neuroendocrinology: 11β-Hydroxysteroid Dehydrogenase 1 (11β-HSD1), A Case Study

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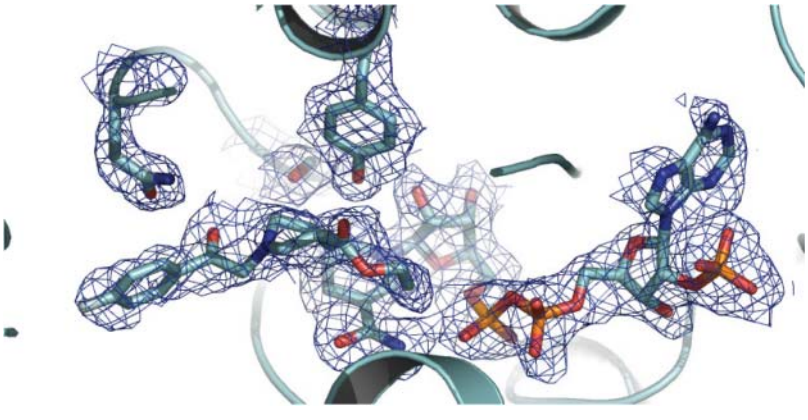

A novel small molecule inhibitor bound in the active site of 11β-HSD1. Courtesy of University of Edinburgh.

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14.1 Introduction

In addition to the metabolic complications of obesity and the metabolic syndrome, the clinical manifestations of both endogenous Cushing's syndrome and iatrogenic glucocorticoid administration include neuropsychiatric problems related to excess cortisol exposure. Emerging studies in humans have shown that subtle activation of the hypothalamic–pituitary–adrenal (HPA) axis, with resultant higher morning cortisol levels, is also associated with poorer cognitive function in older age (MacLullich *et al.*, 2005; Reynolds *et al.*, 2010). Dysregulation of the HPA axis is therefore thought to be a key mechanism underlying cognitive decline and dementia. In our ageing society, dementia is an increasing burden on health care resources and there is a demand to find novel targets for therapeutic manipulation. In this review we discuss the evidence that the HPA axis may be an important therapeutic target to exploit to prevent cognitive decline and discuss the approach to designing drugs to manipulate the HPA axis that are suitable for clinical use.

14.1.1 The hypothalamic–pituitary–adrenal (HPA) axis

The principal biological mechanism underlying adaptation to stress is the HPA axis, which regulates secretion of glucocorticoid hormones (cortisol in humans and other mammals, corticosterone in rodents) to maintain homeostasis. Secretion of glucocorticoids follows a circadian rhythm regulated by the suprachiasmatic nucleus of the hypothalamus, with plasma cortisol levels peaking prior to activity (i.e. in the morning in humans) and in response to 'stress'. Glucocorticoid secretion is autoregulated by a negative feedback loop whereby glucocorticoids regulate secretion of corticotrophin releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) at the level of the hypothalamus and pituitary. The typical circadian pattern is not made up of a simple smooth change in hormone levels over the 24 hours but is the result of changes in the activity of an underlying ultradian rhythm (Walker *et al.*, 2012). Cortisol is released from the adrenal gland in discrete pulses that result in rapidly changing levels of hormone, both in blood and within the tissues. The change in pulse amplitude, and to a lesser extent frequency, makes up the circadian rhythm and the changes in HPA activity that occur in response to altered physiological and pathological conditions. This pulsatility of glucocorticoid secretion is also an important factor in determining the responsivity of the HPA axis to stress and the transcriptional responses of glucocorticoid responsive genes.

Circulating glucocorticoids are predominantly bound to corticosteroid binding globulin (CBG) (70–75%) and albumin (15–20%). Binding proteins are saturated at high physiological levels, allowing greater diurnal fluctuation in free steroid levels. CBG may also deliver glucocorticoids to

target cells. As glucocorticoids are highly lipophilic they readily diffuse across biological membranes into the cytoplasm. Evidence is emerging that glucocorticoid transport in and out of cells is actively controlled by membrane transporters. A large number (~50) of human members of the ATP-binding cassette (ABC) transporter family have been identified that act as importers or exporters in cells. The ABC transporters vary in their tissue distribution and range of substrate specificities and several ABC transporters are known to transport steroids. For example, the Mdr/p-glycoprotein/ABC1 transporter acts particularly at the blood–brain barrier to partially exclude specific corticosteroids from the brain.

Once inside the cell, glucocorticoids exert their actions by binding to intracellular receptors. Glucocorticoids bind with differing affinity to two types of receptors: the low affinity glucocorticoid receptor or Type 2 receptor (GR) is widely distributed in the brain and periphery. In contrast, the mineralocorticoid or Type 1 receptor (MR) has an extremely high affinity for glucocorticoids but is more restricted in anatomical localization, e.g. to distal nephron, colon and sweat glands and hippocampus. GR and MR are activated upon ligand binding and the receptor–ligand complex translocates to the nucleus, binding to glucocorticoid response elements in the promoter regions of target genes to influence gene transcription. Additionally, MR and probably GR mediate rapid non-genomic effects, probably via sites on the cell membrane, but the detailed mechanisms of this are still unknown.

Access to steroid receptors is regulated by local activity of the 11β-hydroxysteroid dehydrogenase enzymes (11β-HSDs) (Chapman *et al.*, 2013). These enzymes catalyze the interconversion of the active steroid cortisol and its inactive metabolite cortisone. 11β-HSD2 in kidney inactivates cortisol and protects MR from cortisol. In contrast 11β-HSD1 reactivates cortisol from inactive cortisone in many sites, thus amplifying local tissue glucocorticoid levels and ensuring adequate activation of GR. Cortisol regenerated by 11β-HSD1 in these tissues is released into the bloodstream so therefore impacts on circulating levels, in addition to tissue concentrations. A further component regulating circulating cortisol concentrations is the rate of clearance or metabolism of cortisol, primarily by the hepatic A ring reductases (5α- and 5β-reductases). Thus the circulating cortisol pool is determined by three sources: adrenal cortisol production, cortisol regeneration by 11β-HSD1 and cortisol clearance predominantly by the hepatic A-ring reductases.

14.1.2 Dysregulation of the HPA axis and disease

Glucocorticoids play a vital role in adaptation to environmental stressors, including starvation, infection or injury. Release of cortisol in response to these stressors induces a wide range of adaptive responses that may

be homeostatic (adjusting systems to maintain stability despite changing conditions) or allostatic (responding to perturbations in a changing environment). Thus responses include the release of fuel by facilitating gluconeogenesis and lipolysis, maintaining cardiovascular homeostasis by inducing sodium retention and vasoconstriction, altering mood and memory focusing on 'fight or flight' responses, and acting as a brake on the innate immune response. A healthy stress response is typically characterized by a rapid rise in cortisol within minutes, followed by a return to baseline levels within 1–2 hours following the stress exposure. Chronic elevations in cortisol, however, may be maladaptive, as illustrated by the many manifestations of Cushing's syndrome, including obesity, type 2 diabetes, hypertension, dyslipidaemia, depression, memory loss and impaired wound healing. Dysfunctional regulation of the hypothalamic–pituitary–adrenal (HPA) axis has been proposed as an important biological mechanism underlying these traits in the general population. Thus epidemiological data suggest that overexposure to cortisol through subtle activation of the HPA axis may contribute to stress-related diseases including the metabolic syndrome, depression, cognitive decline and accelerated ageing. In addition, a number of psychiatric and metabolic diseases have been associated with changes in cortisol pulsatility. The associations of the HPA axis with the metabolic syndrome have been reviewed in detail elsewhere (Anagnostis *et al.*, 2009); here we focus on evidence that the HPA axis contributes to cognitive decline.

14.1.2.1 Dementia

The number of people with dementia in the United Kingdom is set to rise dramatically over the next 40 years with estimates from around 700,000 to 1.7 million as the population ages (Russ *et al.*, 2012). Age-related cognitive decline has been linked to dysregulation of the HPA axis, with resultant chronically increased exposure of the hippocampus and prefrontal cortex to elevated glucocorticoid levels. These two structures play a key role in long-term memory, declarative and working memory, and are particularly sensitive to the deleterious effects of glucocorticoids. Glucocorticoid receptors are highly expressed in these areas in both rodents and humans, and alterations in hippocampal structure are associated with a number of consequences for memory and behaviour. In rodents, manipulations that reduce plasma glucocorticoid concentrations or their effects on target tissues, such as adrenalectomy with low-dose glucocorticoid replacement in midlife, neonatal handling or antidepressant therapy from mid-life, attenuate cognitive decline with ageing, suggesting a causative role for glucocorticoid excess in the etiology of cognitive decline. In humans, administration of exogenous glucocorticoids, or elevated endogenous glucocorticoids, as occurs in Cushing's syndrome, is associated with affective, cognitive and psychotic disorders. Higher salivary cortisol levels

are also associated with poorer cognitive performance in people with mild cognitive impairment whilst patients with Alzheimer's disease have higher cortisol levels in association with lower hippocampal volumes.

In people without overt cognitive disease, more subtle alterations in HPA axis function have also been linked with cognitive decline. Higher plasma cortisol levels at 09:00 h have been associated with poorer age-related cognitive ability, including poorer performance on declarative memory tests in a small group of elderly, otherwise healthy, male volunteers. Such changes in HPA axis function are also associated with alterations in brain structure in key loci involved in memory, including the prefrontal cortex and left anterior cingulate cortex. We have also shown that alterations in the HPA axis contribute to cognitive decline in people with type 2 diabetes. In a large cohort study of older people with type 2 diabetes, we showed that higher morning fasting cortisol levels were associated with significantly lower general cognitive ability and with poorer performance in two cognitive domains, including working memory and processing speed (Reynolds *et al.*, 2010). The latter is the first cognitive domain to show a decline with ageing and is an early predictor of dementia. There were also trends for poorer cognitive function in other domains including mental flexibility, non-verbal memory, immediate and delayed memory, and general cognitive ability.

14.1.3 Exposure to excess cortisol over the life course

Morning cortisol levels vary widely between individuals, with genetic factors reported to contribute 35–60% of this variation. Another contributing factor is early-life experience. Increasing evidence in animal models and emerging data in humans are supportive of the hypothesis that overexposure of the developing fetus is associated with low birthweight and with lifelong activation of the HPA axis (Reynolds, 2013). In studies of men and women across the life span, high morning cortisol levels have been associated with lower birthweight, with a meta-analysis of 11 studies showing that cortisol concentrations fell on average by 25.3 (95% CI, 5.9–44.8) nmol/l per kilogram increase in birthweight. A blood sample collected for measurement of cortisol in the morning, under fasting conditions, in the setting of a research study in an unfamiliar environment, is considered a robust measure of a 'stressed' cortisol sample. It has therefore been hypothesized that low birthweight is associated with enhanced biological responses to stress secondary to central activation of the HPA axis. This is supported by studies in men and women showing increased plasma cortisol responses to stimulation with SynACTHen (synthetic ACTH) and increased salivary cortisol responses to stress tests, including the Trier Psychosocial Stress Test (TSST) and cold-pressor test. This subtle elevation in cortisol is associated with detrimental effects on cognition. The 1958 birth cohort study showed that higher cortisol levels at 45 years

were associated with poorer verbal memory and fluency at 50 years, with a contribution from childhood cognition to these associations. Likewise, in older people, the risk of memory impairment increases after years of cumulative increases in basal cortisol levels.

14.1.4 Therapeutic manipulations

If higher lifetime levels of cortisol are associated with age-related cognitive impairment, is it possible that therapeutic manipulations to lower cortisol levels may help to improve cognitive function? Potential strategies would be antagonism of the receptor and/or its signalling pathway or reducing ligand availability. For the HPA axis this is challenging as manipulations to generally lower cortisol activity (e.g. administration of the GR antagonist RU38486) would have potentially detrimental effects, resulting in symptoms of glucocorticoid deficiency, as seen in Addison's disease. Lower cortisol also associates with immunological abnormalities and post-traumatic stress disorder. Moreover, long-term therapy with GR antagonists leads to activation of the HPA axis and reversal of the competitive blockade, as well as adrenal hyperplasia. One potential alternative strategy therefore is to antagonize local glucocorticoid action by targeting pre-receptor metabolism of cortisol. This is an attractive approach if any compensatory HPA axis activation is incomplete and adrenal stress responses are maintained. 11β-HSD1 is widely expressed in the brain in rodents, particularly in the hippocampus, cerebellum and neocortex, and has also been demonstrated in the human hippocampus, pre-frontal cortex and cerebellum. In a small, randomized, double-blind, placebo-controlled, crossover study, administration of the 11β-HSD inhibitor, carbenoxolone, improved verbal fluency after four weeks in ten healthy elderly men (aged 55–75 years) and improved verbal memory after six weeks in 12 people with type 2 diabetes (Sandeep *et al.*, 2004). Whether or not this short-term effect on cognitive ability could be reproduced over longer time periods, and therefore help reduce the cognitive decline associated with ageing, is unknown. Nevertheless, this work highlights 11β-HSD1 as a target for the treatment of cognitive impairment, particularly in the context of Alzheimer's dementia (AD).

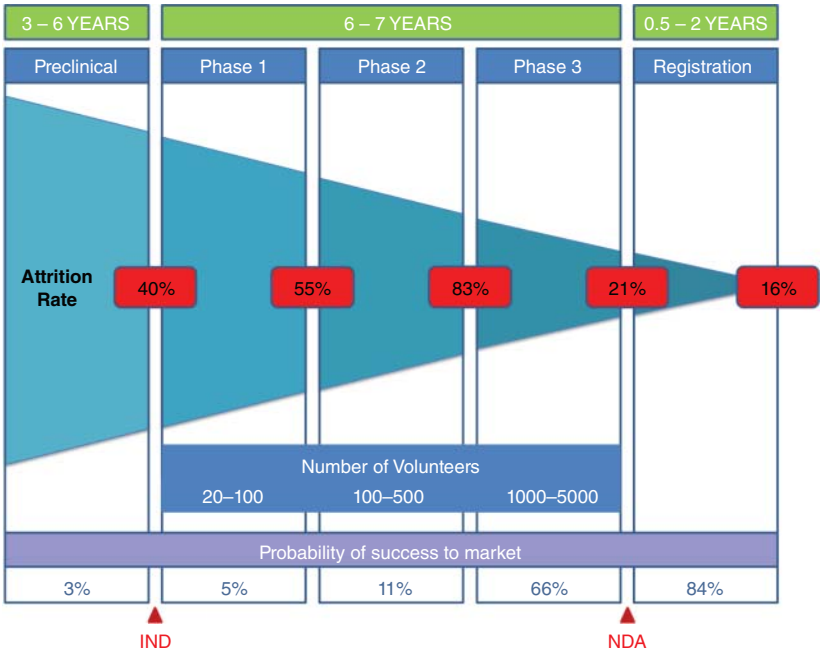
14.2 Small molecule drug discovery and development

14.2.1 Introduction

It has been estimated that approximately 40% of the 2-year increase in life expectancy from 1986- to 2000 can be attributed to the introduction and use of new drugs and it reasonable to conclude that new medicines will be

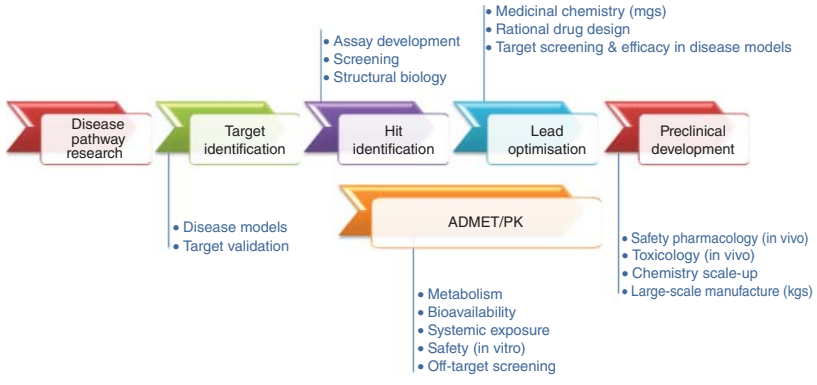
required to maintain current life expectancy levels (Lichtenberg, 2005). However, the process of translating academic and clinical research into marketed therapies remains challenging, time-consuming (10–15 years to market) and inherently risky, with further demands placed on the pharmaceutical industry from ever more stringent regulatory conditions. Less than 0.1% of compounds that enter preclinical testing are estimated to be advanced to first time in human studies, and only 5% of compounds entering initial clinical studies are likely to reach the market. For CNS-targeted drugs the landscape is even more difficult, with new molecular entities entering clinical development having a far lower probability of making it to market than the industry average across other therapeutic areas.

Drug discovery and development consists of a series of stages, which are summarized in Figure 14.1. A preclinical phase, which includes disease pathway research and target discovery, is followed by a drug discovery phase, which generally takes 3–6 years and involves a range of activities to identify and optimize chemical compounds able to specifically modulate the target of interest (Figure 14.2). This phase is discussed in detail in the following sections. If successful, this discovery phase will yield one or more development candidate compounds that undergo scale-up chemistry



Key: IND – investigational new drug, NDA – new drug application.

Figure 14.1 Drug discovery and development process. Data obtained from Arrowsmith *et al.*, 2012.



ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity

PK: Pharmacokinetics

Figure 14.2 Preclinical drug discovery process.

to identify a route of manufacture for bulk production before being profiled *in vivo* for safety, genotoxicity and toxicity (in two species; rodent and non-rodent). If a clear preclinical safety and toxicology profile with an adequate projected safety margin is achieved an investigational new drug (IND in the USA) application (or clinical trials authorization (CTA) in the UK) is submitted to allow first time in human clinical studies (Phase 1). Phase 1 studies are typically carried out in 20–100 healthy volunteers and are aimed at establishing the safety and tolerability of the drug. **Pharmacokinetic** and **pharmacodynamic** parameters are also gathered such that the safe dosing regimen for further clinical studies can be determined. Phase 2 trials are carried out in patients with disease and are aimed at establishing efficacy, identifying the optimal dose and establishing any short-term side effects. These studies are typically carried out in 100–500 patients across several test sites. The greatest attrition occurs during this phase, with approximately 50% of failures occurring due to lack of efficacy, 30% due to strategic decisions and 20% due to safety concerns (Arrowsmith, 2012). If a drug is shown to be sufficiently safe and efficacious during Phase 2, subsequent Phase 3 studies are carried out in a larger, more diverse patient population (1000–5000 patients). These studies, which aim to establish statistically significant evidence of safety and effectiveness, are the longest and most expensive part of the clinical development process since they require multicentre trials across the world. If the data successfully demonstrate efficacy and safety a new drug application (NDA), containing all the scientific information on the drug, is submitted. If this is approved by the regulatory authorities the drug becomes available for prescription, although certain drugs may require additional Phase 4 trials to evaluate long-term effects.

14.2.2 Target discovery

Varying approaches to drug discovery can be taken, but a general paradigm for the small molecule drug discovery process is shown in Figure 14.2. The essential initial steps in the drug discovery process involve painstaking basic and clinical research over a number of years to identify pathways important in disease states and to identify potential disease-modifying drug targets. This research, predominantly carried out in the academic environment, involves the use of both molecular and systems approaches to identify new targets whose modulation is likely to inhibit or reverse disease progression. Since it is critical that the functional role of a putative target can be assessed, the use of transgenic mouse models, where the target gene can be either overexpressed or knocked out, is of particular importance in the validation of new therapeutic targets. Such studies usually require the generation of tissue-specific lines to identify whether modulation of the target ameliorates the disease phenotype. The importance of mouse knock-outs in target discovery and validation has been highlighted in a retrospective evaluation of knock-out phenotypes of the top 100 selling drugs, which demonstrated that knock-out phenotypes correlated with known drug efficacy (Zambrowicz and Sands, 2003).

14.2.2.1 Target selection

Most drugs target proteins. Enzymes and receptors, where there is a defined small molecule binding site, account for approximately 45 and 30% of therapeutic targets respectively. However, not all protein targets are tractable to small molecule modulation. This is particularly true for protein–protein interactions where there are no pre-existing small molecule binding sites and the interface between each protein may present several potentially tractable small molecule binding sites over a large surface area. Therefore, the nature of the target has a major impact on the likelihood of successfully identifying potent modulators for further optimization. Prior to embarking on a drug discovery campaign it is thus important to carry out an assessment of the **druggability** of the target to determine whether the protein is likely to be able to accommodate drug-like compounds at target binding sites.

14.2.2.2 Assay development

Prior to initiating a drug discovery programme it is necessary to design and implement a series of assays to fully assess the quality of potential candidate compounds. This screening cascade typically consists of a primary assay to determine potency at the target and secondary assays to assess selectivity at closely related targets. These assays are usually configured in high throughput format (384- or 1536-well microplate format) to allow rapid

assessment of compounds. Cell-based and functional assays are also generally required, since it is desirable to assess potency in a more biological context. As optimization proceeds a series of additional *in vitro* assays are included to determine physicochemical attributes and to forecast behaviour *in vivo*. These tests are covered in greater detail in subsequent sections.

14.2.3 Hit identification

The key next step in small molecule drug discovery, known as **hit** identification, is the discovery of chemical matter capable of modulating the function of the therapeutic target (Bleicher *et al.*, 2003). The main objective of hit identification is to identify chemical starting points with characteristics, such as low molecular weight and adequate potency at the target, that are amenable to further medicinal chemistry optimization. Strategies to identify chemical starting points can be divided into approaches such as high throughput screening (HTS), which require no information about the target or its ligand(s), and rational approaches that require detailed structural information on the target or its ligands. HTS is the most commonly used method for identifying chemical starting points for further drug discovery and development, with many marketed drugs resulting from hits generated through HTS campaigns. In large pharmaceutical companies HTS campaigns are routinely performed on compound libraries consisting of $>10^6$ compounds. However, not all chemical matter arising from such screens is suitable for onward optimization and, therefore, strategies and appropriate filters are employed to identify compounds with favoured hit-like properties.

14.2.3.1 *In silico* screening

If detailed structural information on the target or ligands is available then focused screening of subsets of compounds may be employed as a cheaper and more rapid alternative to HTS, since this strategy may be more likely to identify hits with desirable properties for optimization. Certain biological targets such as G-protein coupled receptors (GPCRs) or steroidal receptors bind molecules with common features such that it is possible to identify privileged structural motifs that are likely to confer activity towards a particular protein family. It is thus possible to incorporate these privileged motifs into a search of a large compound library to sift and select compounds likely to bind to the target of interest. Similarly, computational modelling may also be employed to build a ligand-based pharmacophore, representing the average shape of ligands known to bind a particular target. Such pharmacophores can then be used to interpolate *in silico* (or virtual) libraries of molecules to search chemical space and pre-select compound libraries for *in vitro* screening. However, it should be noted that ligand-based pharmacophores tend to provide a less accurate representation of the target

binding site since the influence of the protein on the conformation of each ligand is unknown. If structural information on the target protein is available, this enables the generation of a pharmacophore, which is likely to more accurately represent the features of the target site required for molecular binding. This information may be combined with ligand information to prepare a composite pharmacophore for *in silico* screening.

14.2.3.2 Fragment screening

A further application of structural biology for hit identification and the rational optimization of hits is the use of a fragment screening approach combined with structure-based drug design. Fragment screening is based on identifying small molecule fragments, which bind weakly to the biological target but which can be combined or modified to produce highly specific and high affinity compounds. In contrast to HTS libraries, fragment libraries consist of small molecular weight compounds (<300 Da) with low lipophilicities that are more soluble and can thus be readily incubated with proteins at a high concentration. Hits from fragment screens demonstrate lower affinities (mM) than hits from conventional screens (nM–μM) and the approach is highly dependent on the availability of biophysical methods such as surface plasmon resonance able to identify molecules with low affinities. Biological targets that are amenable to rapid iterations of structural biology (X-ray crystallography or nuclear magnetic resonance (NMR)) are also required since a detailed understanding of the molecular interactions of the fragment with the target protein is required to rationally link or modify fragments using the appropriate chemical groups.

Screening campaigns typically yield a variety of potential chemical starting points and it is usually necessary to carry out some preliminary medicinal chemistry to rank hit series ahead of a **lead** optimization campaign. This hit-to-lead chemistry is aimed at establishing preliminary structure-activity relationships (SAR) to build confidence that a particular chemical scaffold is likely to be amenable to further optimization.

14.2.4 Lead optimization

The goal of drug discovery is to develop a therapy that is beneficial over current therapies and it is essential that the requirements of a new drug are defined clearly at the inception of the drug discovery process. To aid this, detailed target product and development **candidate** profiles are generated, which define the required endpoints of the candidate compound across a range of properties. **Lead optimization** is the process by which lead compounds are chemically modified to improve their characteristics such that they closely match those required for a development candidate and subsequent drug (the target product). Potency and selectivity are closely monitored throughout lead optimization with

structure–activity relationships used to guide chemical modifications such that the optimal potency is achieved. If available, structural information from protein–ligand complexes may also be used to rationally design modifications of lead molecules by optimizing specific interactions with the protein target. However, lead optimization is a multiparameter process and improvements in *in vitro* potency and selectivity must also be aligned with the appropriate levels of metabolic stability, systemic exposure and toxicity (Figure 14.3). It is often the case that modifications that improve one parameter, such as target binding affinity, have a detrimental effect on another parameter, such as metabolic stability. Lead optimization is thus a constant balancing act that requires multiple iterations of medicinal chemistry and biological testing to attain the full range of properties required for a particular development candidate.

14.2.4.1 Physicochemical properties

During lead optimization a detailed understanding of the physicochemical properties of each molecule is required since properties such as molecular weight (MW), **lipophilicity** (logP), polar surface area (PSA) and hydrogen-bonding capacity are closely linked to the metabolic, pharmacokinetic and safety properties of compounds. Alignment of each of these parameters is fundamental for the success of any drug discovery project. For orally targeted drugs, these parameters are encapsulated in the Lipinski Rule of 5, which states that a compound is likely to be orally active and drug-like if it has a mass <500 Da, a logP <5, <5 hydrogen bond

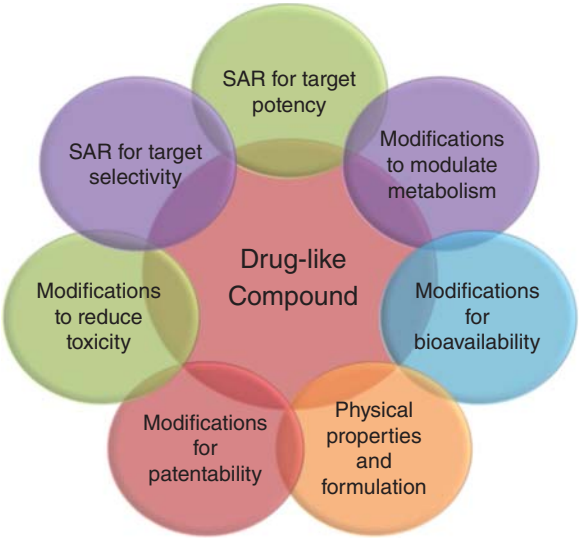


Figure 14.3 Lead optimization process. SAR: structure-activity relationships.

donors and <10 hydrogen bond acceptors. These rules have been further refined to include parameters such as polar surface area and rotatable bonds, such that compounds with polar surfaces areas <140 Å^2 and <10 rotatable bonds have been shown to have a high probability of being orally bioavailable.

14.2.4.2 Drug absorption and distribution

The developability of a lead compound is dependent on the ability to achieve sufficient *in vivo* drug concentrations to produce a therapeutic effect, while maintaining an adequate margin of safety. To achieve this, the absorption, distribution, metabolism, excretion, toxicity (ADMET) and pharmacokinetic (PK) profiles of lead compounds are closely monitored through a variety of *in vitro* and *in vivo* assays. The various barriers to absorption are illustrated in Figure 14.4. For orally administered therapies a drug must be able to access the systemic circulation via intestinal absorption. To achieve this, the drug must move from the intestinal lumen, through an unstirred water layer and mucus coat next to the epithelial cell surface. Transport across the epithelial layers may take place by either transcellular or paracellular flux, where the drug solute then meets a basement membrane, interstitial space, the mesenteric capillary wall and finally the mesenteric circulation. The entire absorption process thus consists of a number of potential resistances, with molecular properties having a major influence on a drug's absorption. Plasma membrane partitioning is

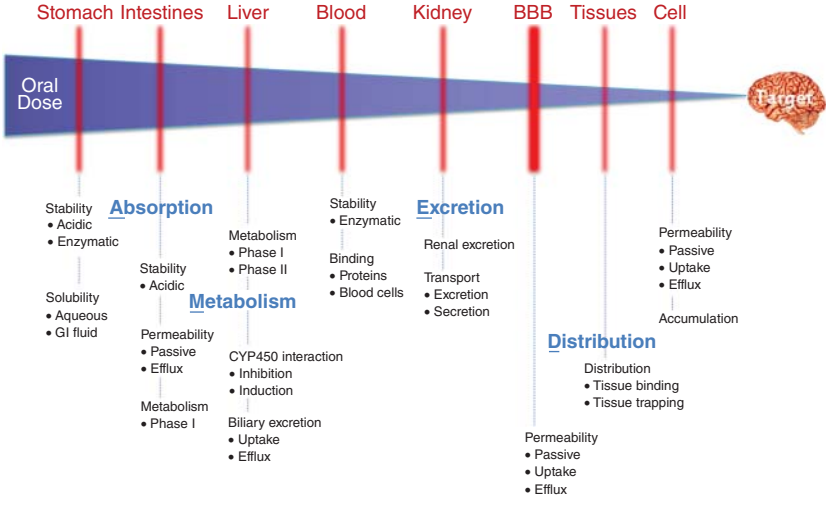


Figure 14.4 Pharmacokinetic barriers between an oral dose and a CNS drug target. Note that the same barriers exist for a peripheral drug target, but without the blood-brain-barrier (BBB). Diagram adapted from Reichel *et al.*, 2009.

governed by molecular surface area, lipophilicity and polarity, with polar H-bonding disfavouring partitioning. For ionizable drugs, permeability is also pH-dependent: neutral species are able to undergo transcellular passive diffusion, whereas charged species are restricted to paracellular transport.

Systemic absorption may also be affected by transporters such as P-glycoprotein (P-gp), which is found in many cells including those lining the intestine and blood–brain barrier (BBB). This transporter (or efflux pump) can limit the oral or brain exposure of compounds that serve as substrates; therefore, *in vitro* screens for P-gp activity are routinely included during lead optimization to highlight any potential liabilities that may reduce the proportion of a drug able to reach the systemic circulation.

14.2.4.3 Drug metabolism

The blood supply to the gastrointestinal tract is drained through the hepatic portal vein; therefore, for an orally absorbed drug the entire absorbed dose will pass through the liver where it undergoes first-pass metabolism. The fraction of drug reaching the systemic circulation can thus be markedly reduced if its metabolism in the liver is high. Drug metabolism in the liver may be separated into phase I and phase II processes whereby a drug may first be oxidatively metabolized to produce a more polar metabolite, which is subsequently conjugated before being excreted. Many enzymes are able to contribute to phase I metabolism, but the most important metabolic enzymes in the liver are the CYP450 enzymes, which are able to catalyse a range of oxidative reactions and accept a wide spectrum of small molecule substrates. The five main forms of CYP450 that are involved in the metabolism of drugs in man are CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, with each enzyme isoform displaying a preference for chemotypes with particular molecular properties. Although the interactions of compounds with particular CYP450s are complex, it is generally accepted that increases in lipophilicity favour increased hepatic clearance, particularly by CYP3A4, although this effect may be offset by the ionization state of a particular molecule. During lead optimization predicted human hepatic clearance is monitored by testing compounds in human liver microsomes (phase I metabolism) or primary hepatocytes (phase I and II metabolism) since these assays are amenable to relatively high throughput analysis and avoid time-consuming and costly *in vivo* studies.

Since most drugs are metabolized by CYP450 enzymes it is also important to identify whether a compound inhibits a particular CYP450 isozyme as this will highlight any potential drug–drug interactions, which could lead to adverse toxicity if the drug is co-administered with another drug. To assess this, *in vitro* assays for each of the main CYP450 enzymes are routinely conducted during lead optimization, with potent inhibitors generally rejected in favour of compounds with low CYP450 inhibition.

The expression of CYP450 enzymes may be induced by stimulation of specific receptors and it is important to determine whether a lead compound has any impact on this induction of CYP450 expression. Of particular importance is the pregnane X receptor (PXR), which mainly induces expression of CYP3A4, but also CYPs 2C8, 2C9 and 2C19. Since CYP3A4 metabolizes >50% of prescription drugs it is important to include assays of PXR in the lead optimization screening cascade. CYP induction may also be assessed in hepatocyte assays; however, these studies are expensive and time-consuming and are usually limited to later stages of lead optimization.

14.2.4.4 Plasma protein binding

In the blood, drugs bind to plasma proteins and this binding has implications for clearance, volume of distribution and efficacy since it affects the amount of free drug in the circulation. It is widely accepted that only free drug is available to interact with target tissues, where it may elicit a pharmacological response; therefore, it is critical to establish the level of plasma protein binding so that robust pharmacokinetic/pharmacodynamics (PK/PD) predictions can be constructed. Free drug levels are of particular importance for CNS-active compounds where only free drug is able to cross the blood–brain barrier. Plasma protein-binding is highly associated with the ionization state, with acids generally showing greater binding than neutrals, followed by zwitterions and bases. Plasma protein binding also rises with increases in lipophilicity; therefore, control of physicochemical parameters is essential to maintain an adequate level of free drug.

14.2.4.5 Cardiac safety

During lead optimization it is usually necessary to include a variety of screens to assess any potential toxicity or safety issues that may be encountered when a drug is administered *in vivo*. Of particular note is cardiac safety and early recognition of potential liabilities associated with blockade of the hERG channel is now an essential part of any drug discovery programme. hERG (the human ether-a-go-go related gene) codes for the α-subunit of the IKr potassium channel and its blockade leads to prolongation of the QT interval, leading to Torsades de Pointes and sudden cardiac death. Moderately high throughput automated patch clamp assays are routinely included during lead optimization. However, late-stage compounds are profiled in more time-consuming and accurate manual patch clamps studies of hERG and other important ion channels. Compounds displaying significant hERG inhibition are unlikely to be progressed to candidate selection.

14.2.4.6 Pharmacodynamics

As lead optimization progresses towards candidate selection it is desirable to understand the relationship between *in vivo* exposure (pharmacokinetics,

or PK) and target engagement (pharmacodynamics, or PD) since it is essential to be able to determine the required level of target engagement to achieve efficacy. PK studies form a critical part of any drug discovery campaign, with studies routinely carried out in rodents, where drug levels may be measured in blood over a long time period using mass spectrometric analysis. Pharmacokinetics/pharmacodynamics (PK/PD) relationships may be established if the drug levels can be related to an appropriate biomarker, from which target engagement can be inferred. These studies are typically performed before *in vivo* studies in disease models to ensure that appropriate drug levels and target engagement can be achieved.

14.2.4.7 Targeting the CNS

For drugs that are targeted towards the brain, several other criteria must also be considered to ensure that a compound is able to cross the blood–brain barrier (BBB). The BBB controls the selective transport of materials into the brain and consists of tight junctions of endothelial cells that contain both uptake and efflux transporters capable of restricting drug permeation. Drug delivery to the brain is thus governed by a compound's ability to passively transport through the endothelial cell layer, access specific uptake mechanisms or act as a substrate for particular efflux pumps such as P-gp (Figure 14.4). The transport of compounds across the BBB is also dependent on plasma protein binding, ionization state, plasma concentrations over time and cerebral blood flow. Many of these properties can be altered by making specific modifications to the chemical structure of a lead compound.

General rules to increase the probability of designing compounds that are able to access the CNS have been proposed by various research groups. It is widely accepted that the logP, partition coefficient (logD), hydrogen-boding potential, PSA, MW, charge and P-gp substrate status of a compound have a significant impact on its ability to cross the blood–brain barrier. General rules, similar to those of Lipinski, have been proposed to help select CNS-penetrant compounds since CNS-active drugs tend to be more lipophilic, be more rigid, have fewer hydrogen-bond donors and lower PSA when compared to non-CNS drugs. More recently these general rules have been further refined to incorporate ADME parameters, binding efficiencies and safety parameters such that optimal values for each parameter have been defined (Wager *et al.*, 2010). A holistic approach has subsequently been proposed for the multiparameter optimization (MPO) of parameters to ensure CNS penetration. In this approach an MPO desirability score is produced by combining data for logP, logD, MW, PSA, number of hydrogen bond donors (HBD) and pK_a (pK_a is the acid dissociation constant; it gives a measure of the charge on a molecule at a given pH). This scoring system allows for a suboptimal property of one

parameter to be offset against an optimal property of another parameter such that the overall quality of a molecule can be assessed. Using this system, compounds yield an MPO score between 0 and 6, with MPO scores >4 considered likely to yield CNS-penetrant compounds (74% of marketed CNS drugs possess an MPO score >4).

14.3 11β-HSD1 inhibitors

11β-HSD1 is predominantly found in metabolic tissues such as liver and adipose and, as noted above in Section 14.1, there is comprehensive evidence from studies in rodents and in humans that regulation of cortisol levels via inhibition of 11β-HSD1 provides a therapeutic strategy to treat cardiometabolic conditions in humans. Based on this evidence many pharmaceutical companies have embarked on drug discovery programmes focused on metabolic disease.

14.3.1 Target and drug properties

11β-HSD1 is an NADPH-dependent ketoreductase enzyme, which interconverts cortisone (inactive) and cortisol in humans. *In vivo*, the enzyme acts predominantly as a reductase since it is co-localized in the endoplasmic reticulum with the enzyme hexose-6-phosphate dehydrogenase, which is thought to provide the NADPH cofactor required to drive unidirectional ketoreductase activity. Thus, any *in vitro* drug screening assay should be designed with this in mind as inhibitors that specifically inhibit the 11β-HSD enzyme dehydrogenase reaction are unlikely to be efficacious. Despite containing a large, hydrophobic active site, which is required to accommodate the lipophilic steroid substrate, 11β-HSD1 has proven to be a highly tractable target for the development of potent and selective inhibitors with drug-like characteristics. A wide range of chemotypes has been shown to potently inhibit the enzyme and the details of many medicinal chemistry optimization programmes have been disclosed in the scientific and patent literature (Sun *et al.*, 2011). A typical **pharmacophore** for an 11β-HSD1 inhibitor can be described since certain common features predominate across the various medicinal chemistry programmes. The majority of inhibitors reported contain a central group, able to form hydrogen bonding interactions, such as a carbamate (e.g. BI-35585), sulphonamide (e.g. PF-915275), lactam (e.g. AMG-221) or heterocycle (e.g. MK-0916) (Figure 14.5). This group is usually flanked by lipophilic groups that form hydrophobic interactions within the steroid binding site of the enzyme. Several X-ray structures of inhibitors bound to human 11β-HSD1 have been disclosed in the scientific literature and it is thus possible to build an average pharmacophore by overlaying these

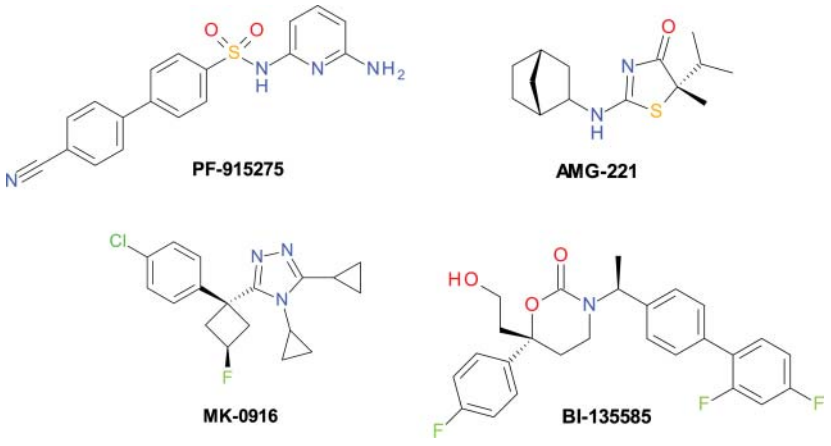


Figure 14.5 Chemical structure of selected 11β-HSD1 inhibitors.

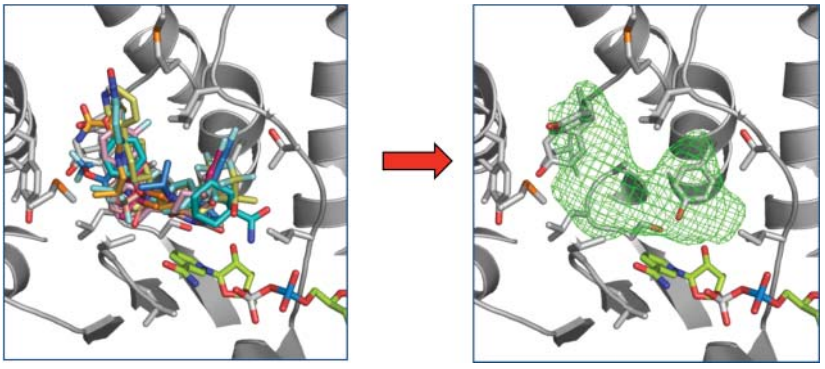


Figure 14.6 11β-HSD1 pharmacophore.

structures. Figure 14.6 shows that, despite containing diverse chemical groups, a typical 11β-HSD1 inhibitor adopts a V-shaped conformation in the active site of the enzyme. This pharmacophore may be employed to computationally dock compounds into the active site of the enzyme such that rational modifications may be made to a molecule that are likely to generate specific interactions with active site residues, thus improving potency and selectivity.

The prevalence of lipophilic groups in most inhibitors can present potential problems with solubility and metabolic stability; however, it has been shown that these issues can be overcome by attaching polar groups at key positions without a reduction in target potency or selectivity. Many compounds have been shown to possess suitable oral pharmacokinetic and pharmacodynamic profiles in target tissues (liver and adipose) for further development and several compounds have entered clinical

studies in humans for the treatment of metabolic disease. These compounds include PF-915275 (Pfizer), HSD-016 (Wyeth/Pfizer), AMG-221 (Amgen), MK-0916 and MK-0736 (Merck), AZD-4017 and AZD-8329 (AstraZeneca), RO-5093151 (Roche), BI-135585 (Boehringer Ingleheim), BMS-816336 and BMS-770767 (Bristol Myers Squibb), LY-2523199 (Lilly) and INCB-13739 (Incyte) (Figure 14.5). Of the published studies, only INCB-13739 has been shown to modestly improve metabolic parameters with reductions in glycated hemoglobin (HbA1c), fasting plasma glucose, plasma lipids and body weight observed in a Phase 2b study of metformin-resistant type 2 diabetics. However, it remains to be seen whether selective 11β-HSD1 inhibitors will be progressed to market for metabolic indications since these improvements in metabolic parameters may not be of sufficient magnitude to compete with those elicited by current therapies, such as metformin and the, more recently developed, gliptins.

14.3.2 Targeting the brain

The main focus of most pharmaceutical companies has been on inhibiting 11β-HSD1 in peripheral tissues, with many programmes actively aimed at minimizing CNS penetration. In the brain, 11β-HSD1 is highly expressed in regions such as the hippocampus, frontal cortex and cerebellum. These regions are important for cognition and the hippocampus, in particular, has high expression of glucocorticoid receptors. The amplification of intracellular glucocorticoids by 11β-HSD1 appears to have a particular impact with ageing since aged 11β-HSD1 knock-out mice have improved spatial memory and better maintenance of hippocampal long-term potentiation than age-matched controls, while increased glucocorticoid activity is associated with greater hippocampal atrophy and memory impairment in elderly humans. Elevated levels of glucocorticoids also correlate with memory impairment in patients with Alzheimer's disease (AD). Reducing glucocorticoid action in the CNS has therefore emerged as an important therapeutic goal in the treatment of age-associated cognitive impairment and Alzheimer's disease, with 11β-HSD1 becoming a potential target for the symptomatic treatment of Alzheimer's disease.

14.3.2.1 Drugs active on the brain

As noted above, most published 11β-HSD1 medicinal chemistry programmes have had a specific focus on metabolic disease with numerous chemotypes disclosed. However, relatively little information on CNS-orientated programmes has been published. Both AbbVie and the University of Edinburgh have disclosed the details of compounds with CNS inhibition. Figure 14.7 shows the structures of two compounds from each programme that display CNS inhibition. Strikingly each of the

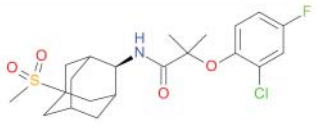
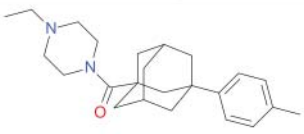
	
Abbott Laboratories	**University of Edinburgh**
hHSD1 (HEK cells): IC_{50} = 98nM	hHSD1 (HEK cells): IC_{50} = 82nM
hHSD1: K_i = 7nM	mHSD1 (CHO cells): IC_{50} = 81nM
mHSD1: K_i = 4nM	
Brain inhibition: 90% (1h), 77% (16h)	Brain inhibition: 39% (1h)
Reference: Sorensen *et al*. (2007).	Reference: Webster *et al*. (2007).

Figure 14.7 Compounds shown to inhibit 11β-HSD1 in the brain.

compounds contains lipophilic adamantyl groups and amide motifs that are able to form hydrogen-bonding interactions, although the arrangement of the amide adjacent to the adamantyl group is different. The X-ray structure of the AbbVie compound in complex with human 11β-HSD1 has been disclosed (Protein Data Bank code (pdb): 2ILT) and shows that the inhibitor adopts a V-shaped conformation within the steroid binding pocket of the enzyme, with the carbonyl moiety of the amide forming hydrogen-bonding interactions with the residues responsible for substrate ketone reduction.

14.3.2.2 Studies on cognition in rodents

Two independent studies of the short-term effects of selective 11β-HSD1 antagonists in rodents have been published by the University of Edinburgh and AbbVie. In the first of these studies, conducted in a cohort of cognitively impaired aged mice, short-term administration (10 days) of the selective inhibitor UE1961 (structure not disclosed) was shown to lead to an improvement in spatial memory as assessed by performance in the Y-maze (Sooy *et al.*, 2010). Strikingly, and in alignment with data from aged *hsd11b1*-/+ mice with only 50% enzyme function, the study demonstrated that a submaximal level of 11β-HSD1 inhibition in the brain was sufficient to reverse cognitive deficits. The mechanism by which the inhibitor is able to elicit this dramatic effect on cognition was not explored within the published study. However, previous work on *hsd11b1*-/- mice demonstrated that aged mice with a lifelong lack of 11β-HSD1 activity display increased long-term potentiation in hippocampal neurons when compared

to aged-matched controls (Yau *et al.*, 2007). Short-term administration of two AbbVie 11β-HSD1 inhibitors of unknown structure (A-801195 and A-918446) to both mice and rats also led to improvements in a range of cognitive tasks, further supporting the use of selective 11β-HSD1 inhibitors for the treatment of age-related disorders (Mohler *et al.*, 2011). In this study a significant increase in cAMP response element-binding protein (CREB) phosphorylation within the mouse cingulate cortex was noted, but there were no apparent changes in acetylcholine release in the medial prefrontal cortex or hippocampus. It would thus appear that the memory-improving effects of these inhibitors may be exerted via glucocorticoid-mediated increased CREB activation/phosphorylation and not modulation of cholinergic activity. Although these studies provide compelling evidence for potential symptomatic improvement in memory, the effects of 11β-HSD1 inhibition on memory and disease pathology in rodent models of Alzheimer's disease have not been disclosed.

14.3.2.3 Clinical studies

Based on these positive preclinical data in rodents both AbbVie (ABT-384) and the University of Edinburgh (UE2343) have progressed compounds into clinical studies in humans. The structural details of ABT-384 have recently been disclosed, showing it to contain elements of the compound shown in Figure 14.7 (Katz *et al.*, 2013) The University of Edinburgh compound is likely to be based around a thiophene amide scaffold. Peer-reviewed data from the ABT-384 clinical programme have been published. A pharmacodynamic study of ABT-384 using a stable isotope d4-cortisol tracer has been reported, but it remains uncertain whether ABT-384 inhibited 11β-HSD1 adequately in the brain at the selected doses since data were presented for only two control subjects without administration of ABT-384 and there is some doubt that the tracer technology used in the study differentiated sufficiently between systemic and central contributions to enzyme inhibition (Katz *et* al., 2013). In a subsequent Phase 2a comparative study with the acetylcholinesterase inhibitor donepezil, ABT-384 did not affect the primary endpoint of the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog) score in patients with baseline Mini Mental State Examination (MMSE) of 10–24 (max. score is 30), diagnosed with Alzheimer's dementia for 1 year (Marek *et al.*, 2014). Although the data from this Phase 2a study show no cognitive improvement with ABT-384 administration using the ADAS-Cog cognitive test as an endpoint, there is considerable scope for further investigation of the clinical effects of 11β-HSD1 inhibition using more sensitive cognitive measures at doses known to inhibit 11β-HSD1 in the brain. Moreover, recent US Food & Drug Administration (FDA) guidance suggests that studies should be performed in patient cohorts

with early/mild disease where the effects of glucocorticoid-dependent symptoms may be more apparent. 11β-HSD1 thus remains a promising target for the potential treatment of cognitive impairment in dementia.

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