Development of 11β-HSD1 Inhibitors for the Treatment of Type 2 Diabetes

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Abstract: Glucocorticoid action is linked to the development of obesity and insulin resistance. Inhibition of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been proposed as a strategy to suppress glucocorticoid action in a tissue-specific manner. A large variety of 11β-HSD1 inhibitor classes are under investigation by the pharmaceutical industry to treat type 2 diabetes and obesity.

Key Words: 11β-hydroxysteroid dehydrogenase, glucocorticoid receptor, cortisol, ex *vivo* activity, insulin resistance, Cushing's syndrome.

INTRODUCTION

Insulin resistance is often associated with obesity and other disorders such as dyslipidemia, hyperglycemia and hypertension. This group of disorders is collectively referred to as the metabolic syndrome [1]. The development of the metabolic syndrome is complex and many distinct underlying molecular mechanisms have been proposed, among which is the hypothesis that glucocorticoid action stimulates obesity and insulin resistance [2-5]. This notion is supported by clinical findings with Cushing's syndrome, a condition where patients have increased glucocorticoid exposure and develop symptoms similar to the metabolic syndrome [6]. Mechanistically, numerous studies have implicated glucocorticoid activity in the regulation of hepatic gluconeogenesis and lipogenesis, adipose glucose and amino acid utilization, glucose uptake and lipid oxidation in skeletal muscle, and the production of angiotensinogen [2]. Antagonism of the glucocorticoid action is therefore an attractive strategy to treat the disorders of the metabolic syndrome. In fact, treatment of Cushing's syndrome with a synthetic glucocorticoid receptor antagonist, RU 486, resulted in the improvement of metabolic parameters [7, 8].

In humans, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive cortisone to active cortisol and plays a critical role in glucocorticoid action (Fig. (**1**)) [2]. 11β-HSD2 catalyzes the reverse reaction and is responsible for the endogenous production of inactive cortisone (Fig. (**1**)) [2]. 11β-HSD1 is mainly expressed in liver, adipose and brain while 11β-HSD2 is primarily expressed in kidney. In obese subjects, the expression of 11β-HSD1 in adipose tissue is increased when compared with lean controls [9-11], suggesting that elevated 11β-HSD1 activity may contribute to local glucocorticoid excess and insulin resistance. A similar finding was made when comparing the adipose 11β-HSD1 expression between lean and obese monozygotic twins [12], demonstrating that the association of adipose 11β-HSD1 overexpression with obesity is not genetically based. This premise was recapitulated in mice by overexpressing 11β-HSD1 in adipose tissue. These animals developed phenotypes similar to the features of the metabolic syndrome [13, 14]. These data suggest that the increased adipose expression of 11β-HSD1 in obese subjects could lead to the development of the metabolic syndrome through amplification of glucocorticoid action. To determine if suppression of the adipose glucocorticoid action can reduce obesity and mitigate insulin resistance, 11β-HSD2 was overexpressed in the adipose tissue of mice [15]. These animals were protected against diet-induced obesity and insulin resistance [15]. Taken together, these data suggest that 11β-HSD1 inhibitors are potential therapeutics for obesity and type 2 diabetes.

Although both reductase and dehydrogenase activities are associated with the enzyme, 11β-HSD1 is a reductase in intact cells and tissues [16, 17]. Therefore, in *in vitro* assays to assess 11β-HSD1 inhibitors, the reductase assay is more relevant, and compounds should be assessed for their ability to inhibit the reductase activity in structure-activity relationship (SAR) efforts.

NON-SELECTIVE 11β-HSD1 INHIBITORS

There are several non-selective 11β-HSD1 inhibitors. The licorice derivatives carbenoxolone (CBX) and glycyrrhetinic acid (GA) inhibit both 11β-HSD1 and 11β-HSD2 [18, 19]. CBX is a hemisuccinate ester of GA (Fig. (**2**)). CBX is a potent 11β-HSD1 inhibitor with an IC_{50} of 17 and 30 nM, respectively, in two independent reports [18, 19]. It is several-fold more potent against 11β-HSD2 in both reductase and dehydrogenase assays [19]. However, the potency of GA for 11β-HSD1 is inconsistent in two different reports [18, 19]. In contrast to a potency value similar to that of CBX reported by Diederich *et al*. [19], Hult and co-workers reported an IC_{50} with GA for 11β-HSD1 in the μM range [18]. Human 11β-HSD1 was used in both studies but they were from different sources [18, 19]. The enzyme source is unlikely the cause of the discrepancy because both studies

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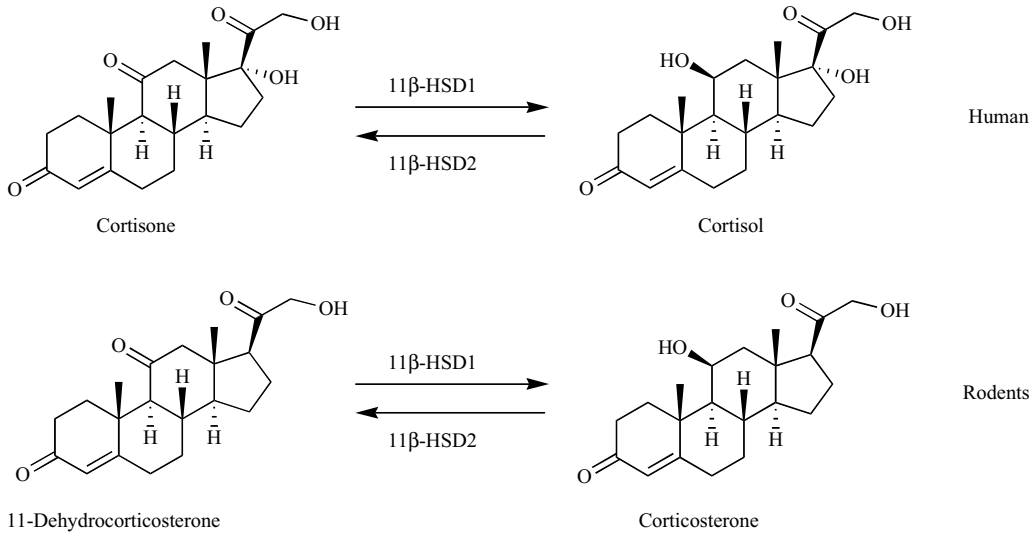


Fig. (1). The reactions catalyzed by 11β-HSDs.

found similar potency values with CBX [18, 19]. The other difference is that Hult *et al*. used cortisone as substrate in their assay, while Diederich *et al*. used 11-dehydrodexamethasone [18, 19]. The discrepancy between the reported GA potency values could be due to its slow binding kinetics. We used cortisone as substrate and recombinant human 11β-HSD1 expressed in *E. coli* in our study, and obtained an IC_{50} value with GA similar to that reported by Diederich *et al*. [20, 21]. Our data as well as those by Diederich suggest that CBX and GA have similar potency values for human 11β-HSD1. Several bile acids are also 11β-HSD1 inhibitors but they are not as potent as the licorice derivatives [19]. Metyrapone is a drug that decreases cortisol levels in man and animals (Fig. (**2**)) [22]. It is an inhibitor of 11β-hydroxylase, a key enzyme involved in the final step of cortisol biosynthesis [22]. Metyrapone is also a weak inhibitor of 11β-HSD1, but the potency is in the sub to single mM range [19, 22], not comparable to those of the bile acids or licorice derivatives. The SAR of GA derivatives was briefly explored. Introduction of an 11α-methyl-11β-hydroxyl group to GA significantly reduced the activity for 11β-HSD2 while maintaining the activity for 11β-HSD1 [23].

STRUCTURAL FEATURES OF 11β-HSD1

The crystal structures of 11β-HSD1 enzymes from guinea pig, human, and mice have been resolved. In all cases, the structures were resolved using truncated enzymes lacking the N-terminal membrane attachment domain expressed in *E. coli* [24-26]. The enzyme is a homodimer of two subunits

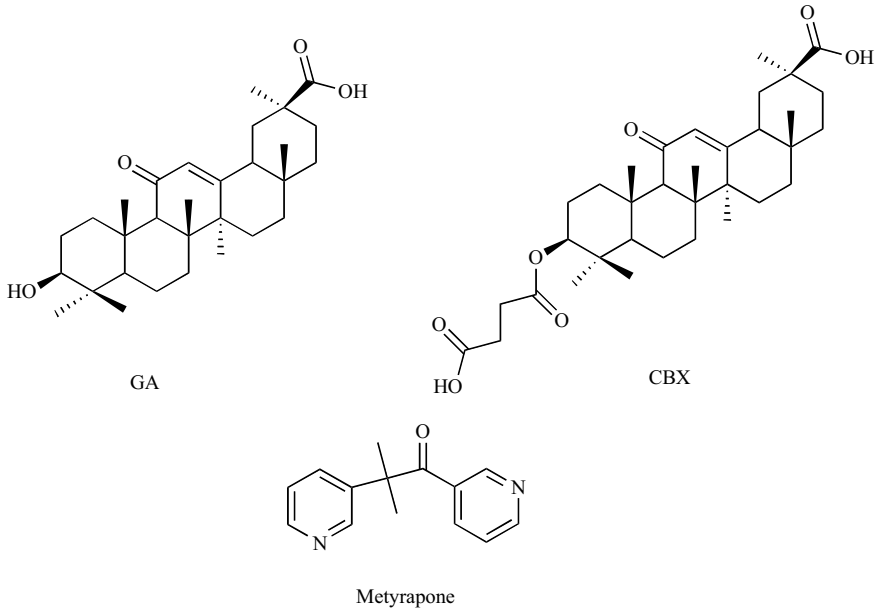


Fig. (2). Structures of non-selective 11β-HSD1 inhibitors: GA, CBX, and metyrapone.

with the C-terminus of one subunit interacting with the active site of the other [24-26]. The key residues in substrate binding include the signature motif YSASK at the catalytic center, with Y183 (human) being the key residue [24-26]. The serine residues determine the rate of catalysis but are not involved in substrate binding [27]. Based on the crystal structure with CHAPS, cortisol was docked into the active site and the A ring is predicted to be directed towards the distal end of the binding pocket, with its C-3 keto group pointing to solvent [25]. This model predicts that S170, L171, A172, Y177 and Y183 are involved in the interactions with cortisol [25]. In addition, through mutagenesis studies and structural modeling, we identified the roles of Y177 and Y280 in substrate and inhibitor binding [20, 21]. In contrast to the implication from the crystal structure [24], Y177 is not a hydrogen bond donor to the C-3 keto group of cortisol [20]. Instead, it is involved in physical interaction with the substrate [20]. Y280 at the C-terminus caps the active site of the dimer partner [24], and is not critical in substrate or CBX binding [21]. However, it is important in the binding of GA [21].

THE DEVELOPMENT OF SELECTIVE INHIBITORS OF 11β-HSD1

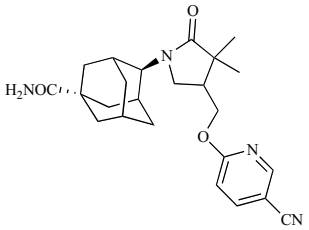
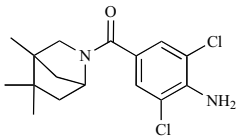
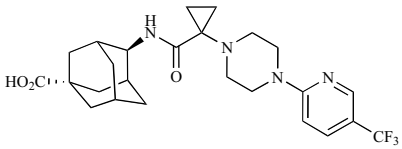
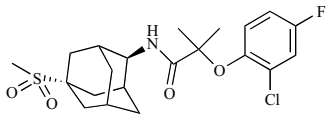
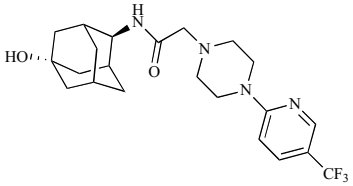
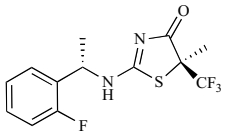
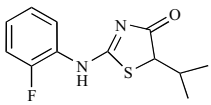
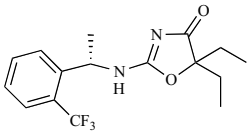
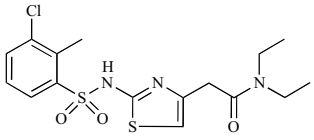
11β-HSD1 is primarily expressed in liver, adipose, and brain [2]. Based on the elevated 11β-HSD1 expression in the adipose of obese human subjects [9-12] as well as mouse genetic studies [13-15], adipose is the primary target tissue of 11β-HSD1 inhibitors to treat obesity and insulin resistance [2]. In addition, hepatic overexpression of 11β-HSD1 only led to mild insulin resistance and dyslipidemia [28], suggesting that the hepatic 11β-HSD1 activity may play a smaller metabolic role compared with the adipose enzyme. However, given the role of glucocorticoid action in hepatic lipid metabolism [2], inhibition of the hepatic 11β-HSD1 activity may help improve lipid profiles. Taken together, we propose that the primary target tissue is adipose and the secondary target tissue is liver. To inhibit the adipose 11β-HSD1, an optimal distribution of the inhibitor to fat tissue is essential. The hepatic distribution is unlikely an issue since orally available compounds have first-pass and subsequent circulation-based hepatic distribution. For adipose penetration, compound lipophilicity is an important property. However, lipophilicity could affect the solubility and bioavailability. Therefore, an optimal profile includes good solubility and bioavailability and a certain degree of lipophilicity for adipose penetration.

A variety of 11β-HSD1 inhibitors have been disclosed in the patent literature [29]. This review will focus on the compounds reported in recent publications. The various chemical compound series and their activities have been under investigation by multiple pharmaceutical companies and research institutions. One interesting observation is the variation of compound potency across species (see below and Table **1**). There are significant differences between the binding pockets of human and mouse enzymes [25, 26], which provides a structural basis for the cross-species potency shifts. Since most pharmacodynamic (PD) and diabetes models are rodents, acceptable rodent (especially mouse) potency makes it convenient to evaluate the PD effect and efficacy of a given compound. For example, Abbott Laboratories have reported several chemical series of 11β-HSD1 inhibitors, among which are lactams [30], dichloroaniline amides [31], admantane aminoamides [32], admantane sulfones and sulfonamides [33], and E-5-hydroxy-2-admantamines [34]. One of the most potent compounds from each series is shown in Table **1** (Compounds 1-5). All these compounds have single nanomolar potency for the human enzyme (Table **1**). However, the admantane aminoamide (compound 3) has far lower mouse potency than human potency. The butyrolactam (compound 1) and the admantane sulfones (compound 4) are both equipotent against the human and mouse enzymes (Table **1**). The admantamine (compound 5) is several-fold less potent against mouse 11β-HSD1 than the human enzyme. Two chemical series of 11β-HSD1 inhibitors with a thiazolone core from Amgen have smaller molecular weights [35, 36]. Compounds 6 and 7 are examples from these series and have lower double digit nanomolar potency against human 11β-HSD1 (Table **1**). Compound 6 has lower albeit acceptable mouse potency (Table **1**). The co-crystal structure of compound 7 with human 11β-HSD1 revealed several key interactions between the inhibitor and the binding pocket [36]. The *exo* tautomer of compound 7 forms hydrogen bonding with S170 and Y183 [36]. In addition, the inhibitor forms a hydrophobic, edge-to-face stacking interaction with Y177 [36]. This finding is consistent with our earlier results that Y177 provides physical interactions for substrate and inhibitor binding [20]. Compounds 8-10 are examples of the oxazolones [37], arylsulfonamidothiazoles [38], and piperdine amides [39], respectively, reported by Biovitrum. The most potent is compound 8. Modifications of the triazole series by Merck led to several variable structural classes [40-42]. All (compounds 11-13) have activities against the human and mouse enzymes with compound 11 being the most potent in either case. In addition, several novel series have been reported by other institutions (compounds 14-16) but they are not as potent as the compounds discussed above.

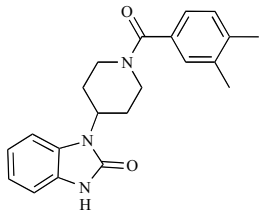
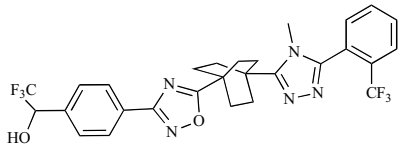
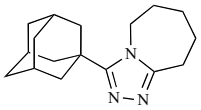
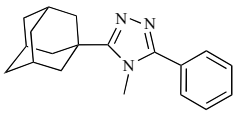
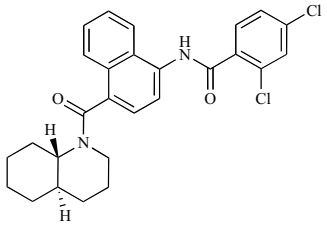
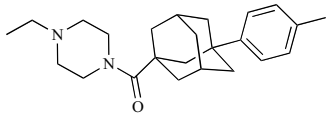
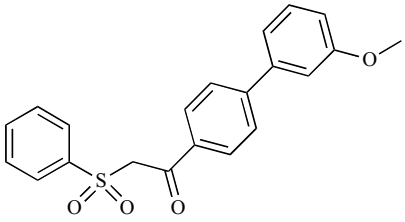
In addition to potency, pharmacokinetic (PK) properties such as good *in vivo* metabolic stability and long plasma half-life are important parameters in selecting compounds. Since adipose is the primary target tissue for 11β-HSD1 inhibitors, a PD effect consistent with the PK data and *in vitro* potency is critical in lead optimization. Key compounds with PK, PD or efficacy data are summarized in Table **2** [30, 32, 33-38, 40-42, 44, 46-48]. A typical PD study with 11β-HSD1 inhibitors is an *ex vivo* assay in a mouse model where tissues are harvested post-dosing with a given compound. The harvested tissue pieces, containing the compound *via in vivo* distribution, are incubated with a substrate *in vitro* to monitor substrate-to-product conversion catalyzed by 11β-HSD1 within the tissues [41, 47]. Compared with vehicle control, a reduction in the total activity after compound dosing indicates the inhibitory effect of the compound. Similarly, an *in vivo* PD assay is conducted by injecting a radiolabeled substrate to mice following compound dosing to monitor the generation of radiolabeled product in the circulation [41]. A number of 11β-HSD1 inhibitors that have been evaluated in PK and/or PD studies are summarized in Table **2**.

Several compounds in Table **2** were evaluated in diabetes models. Abbott tested a butyrolactam in a diet-induced obesity (DIO) mouse model [30]. After 14-day repeated dosing,

Table 1. Summary of Different Chemical Series of 11β-HSD1 Inhibitors

Chemical Series	Structure	Company	h-HSD1 K_i or IC_{50} (nM)*	m-HSD1 K_i or IC_{50} (nM)*	Ref.
1		Abbott	3 (IC_{50})	2 (IC_{50})	[30]
2		Abbott	4	NA (rat K_i=8)	[31]
3		Abbott	7	500	[32]
4		Abbott	7	4	[33]
5		Abbott	8	34	[34]
6		Amgen	22	130	[35]
7		Amgen	17	NA	[36]
8		Biovitrum	19	NA	[37]
9		Biovitrum	52 (IC_{50})	284 (IC_{50})	[38]

(Table 1. Contd….)

Chemical Series	Structure	Company	h-HSD1 K_i or IC_{50} (nM)*	m-HSD1 K_i or IC_{50} (nM)*	Ref.
10		Biovitrum	100	NA	[39]
11		Merck	1.4 (IC_{50})	<1 (IC_{50})	[40]
12		Merck	7.5 (IC_{50})	97 (IC_{50})	[41]
13		Merck	37 (IC_{50})	109 (IC_{50})	[42]
14		Novartis	100 (IC_{50})	NA	[43]
15		Univ. of Edinburgh	82 (IC_{50})	81 (IC_{50})	[44]
16		Wyeth	60 (IC_{50})	NA	[45]

Note: NA, not available; one example for each chemical series is included in the table.

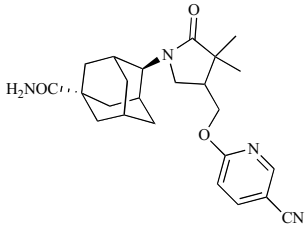
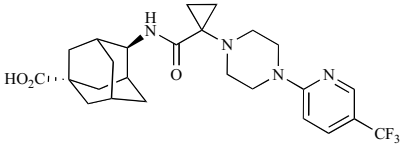
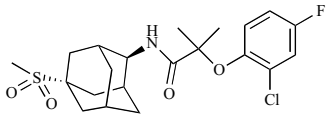
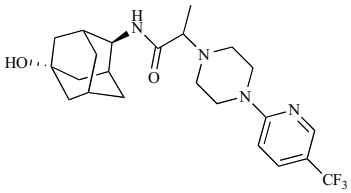
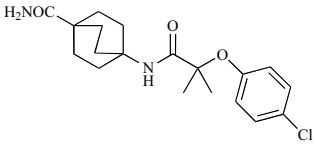
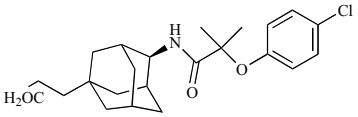
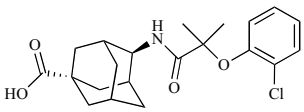
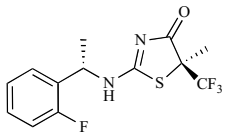
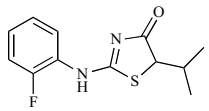
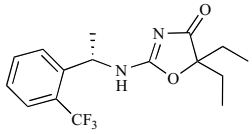
h-HSD1, human 11β-HSD1; m-HSD1, mouse 11β-HSD1.

* Values represent K_i unless indicated as IC_{50}.

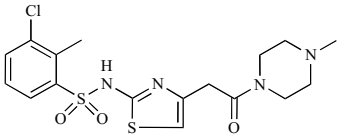
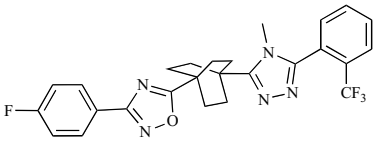
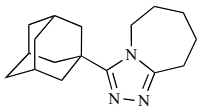
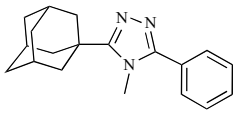
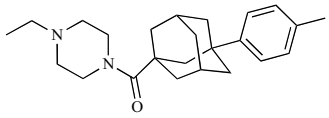
plasma insulin and triglyceride levels decreased [30]. Biovitrum tested an arylsulfonamidothiazole compound in KKA^y mice for up to 11 days and observed a glucose-lowering effect [38]. Merck conducted a thorough *in vivo* study with a triazole compound [41]. This compound lowered fasting glucose and improved other metabolic parameters in two mouse models of type 2 diabetes [41]. It also decreased aortic lesions in a mouse atherosclerosis model [41].

Although CBX is a non-selective 11β-HSD1 inhibitor, a clinical study with CBX in type 2 diabetic patients suggests that 11β-HSD1 inhibition has potential metabolic benefits [49]. CBX did not affect the glucose disposal rate during hyperinsulinemia but reduced glucose production rate during hyperglucagonemia in lean male patients with type 2 diabetes [49], and this is likely driven by reduced glycogenolysis [49]. In healthy volunteers, CBX decreased lipolysis [50],

Table 2. Summary of 11β-HSD1 Inhibitors with Reported PK/PD Data

Chemical Series	Company	PK Data	PD Data	Efficacy Studies	Ref.
	Abbott	MLM Mouse iv and po PK	Mouse *ex vivo* Liver/Fat 1 h/7 h/16 h	DIO mice	[30]
	Abbott	MLM Mouse iv and po PK	NA	NA	[32]
	Abbott	MLM/HLM Mouse and monkey iv and po PK	Mouse *ex vivo* Liver/Fat/Brain 1 h/7 h/16 h	NA	[33]
	Abbott	MLM/HLM Mouse iv and po PK	Mouse *ex vivo* Liver/Fat/Brain 1 h/7 h	NA	[34]
	Abbott	MLM	Mouse *ex vivo* Liver/Fat/Brain 7 h/16 h	NA	[46]
	Abbott	MLM Mouse iv and po PK	Mouse *ex vivo* Liver/Fat/Brain 7 h/16 h	NA	[47]
	Abbott	MLM Mouse iv and po PK	Mouse *ex vivo* Liver/Fat/Brain 1 h/7 h/16 h	NA	[48]
	Amgen	Rat iv and po PK	Mouse *ex vivo* Fat 2 h/6 h	NA	[35]
	Amgen	Rat iv and po PK	NA	NA	[36]
	Biovitrum	HLM/RLM	NA	NA	[37]

(Table 2. Contd….)

Chemical Series	Company	PK Data	PD Data	Efficacy Studies	Ref.
	Biovitrum	NA	NA	KKA[y]	[38]
	Merck	Mouse, rat, and dog iv and po PK	Mouse *in vivo* 4 h/16 h	NA	[40]
	Merck	NA	Mouse *in vivo* up to 25 h Mouse *ex vivo* Liver/Fat/Brain 1 h/4 h/6 h	DIO mice HF/STZ mice apoE KO	[41]
	Merck	NA	Mouse *in vivo* 1 h/ 4 h	NA	[42]
	Univ. of Edinburgh	NA	Mouse *ex vivo* Liver/Fat/Brain 1 h	NA	[44]

Note: NA, not available; for PD data, assay formats (*ex vivo* or *in vivo*), tissue types and time points post-dosing are summarized.

HLM, human liver microsomal stability; MLM, mouse liver microsomal stability; RLM, rat liver microsomal stability; iv, intravenous injection; po, oral administration.

reinforcing the notion of beneficial effects with 11β-HSD1 inhibition. Recently, Incyte Corporation reported interim data from their Phase IIa study with an 11β-HSD1 inhibitor in type 2 diabetic patients [51]. The analysis showed preliminary evidence of a beneficial effect on glycemic control and the compound appears to reduce LDL, total cholesterol and triglycerides [51].

THERAPEUTIC ISSUES AND FUTURE PROSPECTS

It has been demonstrated both genetically and pharmacologically in animal models that inhibition of 11β-HSD1 is a potential strategy to improve insulin sensitivity and other metabolic parameters [15, 41]. Further, preliminary clinical data with non-selective and selective 11β-HSD1 inhibitors suggest that these compounds have beneficial metabolic effects [49-51]. However, hypothalamic-pituitary-adrenal (HPA) axis activation remains a concern for this pathway. For example, 11β-HSD1 knockout mice exhibited HPA axis activation [52]. Further, mild HPA axis activation was also observed in 11β-HSD1 deficient humans [53].

Repeated oral administration of an 11β-HSD1 inhibitor resulted in the reduction of atherosclerotic lesions in apoE knockout mice on Western diet [41]. This observation is interesting in that the lipid effects in the same animals were marginal [41], which may not fully explain the plaque reduction. This finding suggests that the inhibitor may have a direct effect on the vascular wall. 11β-HSD1 has been shown to be expressed in aortic endothelial cells [54], and its activity may be involved in endothelial dysfunction and the formation of atherosclerotic plaques. The potential use of an 11β-HSD1 inhibitor to treat cardiovascular disease is an interesting addition to the beneficial effects of inhibiting this target. Moreover, a recent study with CBX suggests that 11β-HSD1 inhibition could improve cognitive function [55]. These findings open the possibility of expanding the clinical use of 11β-HSD1 inhibitors.

CONCLUSIONS

Glucocorticoid action has been linked to the development of multiple metabolic disorders including obesity and insulin resistance. Evidence of tissue-specific glucocorticoid excess has been observed in obese humans where adipose 11β-HSD1 expression is increased. These data along with genetic and pharmacologic studies in animal models suggest that 11β-HSD1 inhibitors are potential treatments for type 2 diabetes and other metabolic disorders. A large variety of chemical series of 11β-HSD1 inhibitors have been reported. The diversity of these compounds holds promise of clinical success in developing this class of anti-diabetic drugs.

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