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Discovery of potent and selective inhibitors of 11β-HSD1 for the treatment of metabolic syndrome

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Abstract—High throughput screening efforts have identified a novel class of dichloroaniline amide 11β-HSD1 inhibitors. SAR studies initiated from dichloroaniline **4** focused on retaining the potency and selectivity profile of the lead.
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The glucocorticoid hormones, cortisol in human and corticosterone in rodents, play key roles in lipid and glucose metabolism. Patients with glucocorticoid excess (Cushing's disease) are typically obese, insulin resistant, and have high fasting blood glucose levels. However, in diabetic patients, circulating cortisol levels are not elevated.[1] To determine if there is a link between glucocorticoid concentrations and human diabetes, researchers focused on the reductase 11β-hydroxysteroid dehydrogenase (11β-HSD1). This enzyme converts inactive cortisone to cortisol in tissues such as liver and fat, increasing the local concentration of active glucocorticoid. Evidence of the importance of inhibition of 11β-HSD1 came from homozygous 11β-HSD1 knockout mice as well as animals engineered to overexpress 11β-HSD1.[2] The knockout animals showed decreased fasting blood glucose levels and decreased glycogenolysis. Selective over-expression of 11β-HSD1 in mouse adipose tissue increased visceral fat mass, when fed a high fat diet, and the animals were hyperglycemic and insulin resistant.[3]

Carbenoxolone (**1**), a non-selective 11β-HSD1 inhibitor, has been shown to decrease glycogenolysis in diabetic patients (Fig. 1).[4] However, carbenoxolone also caused a rise in blood pressure, likely due to inhibition of 11β-HSD2.[4] Researchers at Biovitrum and Merck have reported the preparation of small molecule inhibitors of 11β-HSD1 (**2** and **3**) that showed efficacy in rodents. The Biovitrum inhibitor, BVT.2733, when dosed via mini-pump, lowered glucose and insulin levels. Additionally, continuous infusion of **2** reduced expression levels of mRNA encoding for PEPCK and G6Pase.[5] The short-acting Merck inhibitor **3** was reported to lower glucose levels, decrease food intake, improve insulin sensitivity, and decrease the rate of body weight gain in STZ treated DIO mice. In ApoE knockout mice, triazole **3** was shown to decrease the rate of atherosclerotic plaque formation.[6] In light of the preliminary validation of 11β-HSD1 as a treatment for the metabolic syndrome, we present a novel series of potent, selective dichloroaniline 11β-HSD1 inhibitors.

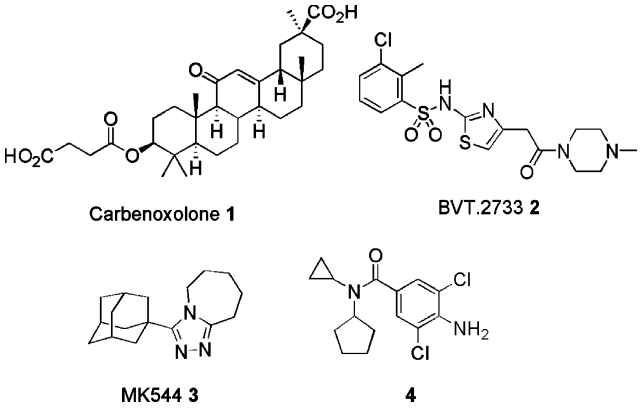

Figure 1. Representative 11β-HSD1 inhibitors.

Keywords: 11β-HSD1 inhibitor; 11β-hydroxysteroid dehydrogenase.
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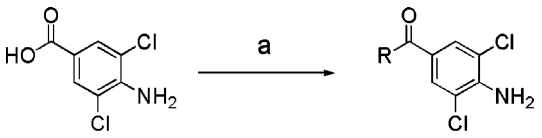


5 6

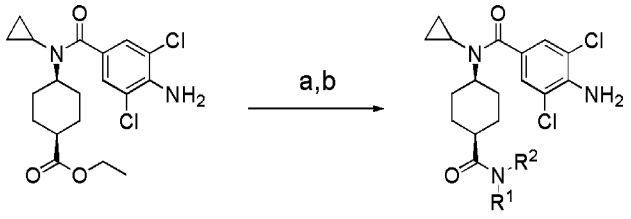
Scheme 1. Reagents and conditions: (a) amines, TBTU, DMF, 100 °C, 12 h, 70%.

Dichloroaniline amides (**6**) were initially prepared as shown in Scheme 1 where 4-amino-3,5-dichlorobenzoic acid was coupled with a variety of secondary amines.

Elaboration of amide **6** began with hydrolysis, which was accomplished under standard conditions, to provide the carboxylic acid (Scheme 2). Coupling of the acid with amines provided a second group of dichloroaniline 11β-HSD1 inhibitors.

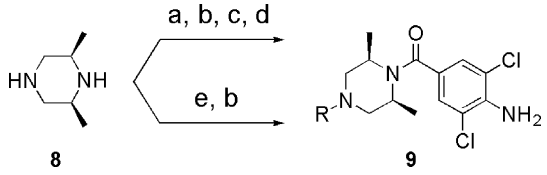
Another series of compounds was prepared by the route outlined in Scheme 3. The least hindered amine in *cis*-2,6-dimethyl piperazine was selectively masked as a *tert*-butoxy carbamate.[7] Coupling with 4-amino-3,5-dichlorobenzoic acid furnished the desired amide product. Cleavage of the protecting group under acidic conditions provided a crystalline amine hydrochloride core. Analogs were prepared by standard nucleophilic substitution reactions. Alternatively, palladium catalyzed cross-coupling of 2,6-dimethyl piperazine with aryl halides provided substituted piperazines that were, in turn, coupled with 4-amino-3,5-dichlorobenzoic acid.[8]

To explore the steric environment of the 11β-HSD1 enzyme, we investigated 2,6-dimethyl-4-amino piperidines.



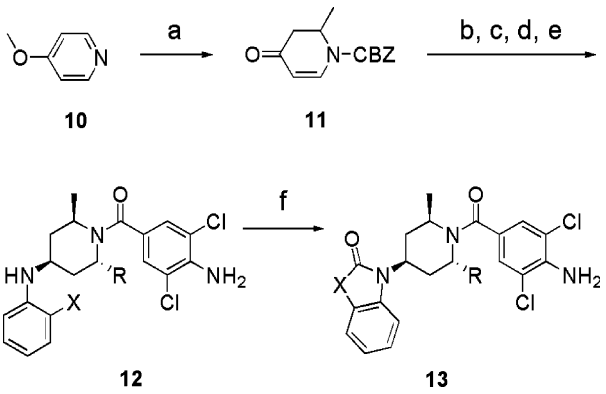
6g 7

Scheme 2. Reagents and conditions: (a) LiOH, THF/H_2O, 80 °C, 12 h, 90%; (b) R_1R_2NH, TBTU, DMF, 100 °C, 12 h, 80%.



8 9

Scheme 3. Reagents and conditions: (a) $(Boc)_2O$, THF, rt, 12 h, 80%; (b) TBTU, DMF, 4-amino-3,5-dichlorobenzoic acid, 100 °C, 12 h, 40%; (c) 4 M HCl in dioxane, THF, rt, 3 h, 90%; (d) RX, DMF, Cs_2CO_3, 180 °C, 60 min, 10–50%; (e) $Pd_2(dba)_3$, ArBr, NaOtBu, BINAP, toluene, 150 °C, 15 min, 30–50%.



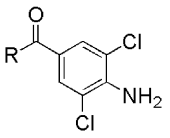
Scheme 4. Reagents and conditions: (a) benzyl chloroformate, MeMgBr, THF, −25 °C, 2 h, 1 M HCl, rt, 1 h, 50%; (b) Zn, AcOH, 80 °C, 12 h, 80% or MeMgBr, THF, CuBr DMS, $BF_3{\cdot}OEt_2$, −78 °C, 2.5 h, 60%; (c) H_2, Pd/C, MeOH, 1 atm, 12 h; (d) TBTU, DMF, 4-amino-3,5-dichlorobenzoic acid, 100 °C, 12 h, 40%; (e) anilines, $Na(OAc)_3BH$, AcOH, DCE, rt, 12 h, 20–50%; (f) triphosgene, Et_3N, CH_2Cl_2, 0 °C, 2 h, 10–30%.

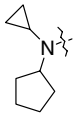
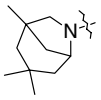
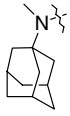
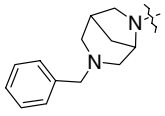
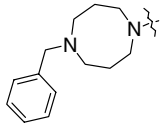
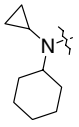
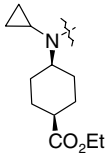
Such compounds were prepared as shown in Scheme 4 using chemistry pioneered by the Comins group.[9] By sequential addition of benzyl chloroformate, MeMgBr and aqueous acid to 4-methoxypyridine **10**, enone **11** was efficiently produced. Enone **11** was then processed in two different ways. Reduction with zinc was followed by hydrogenolysis of the amine protecting group.[10] The resulting amine was coupled with 4-amino-3,5-dichloro benzoic acid. Reductive amination with 2-aminophenol or 1,2-diaminobenzene provided monomethyl piperidines **12** (R = H). Imine reduction favored the syn isomer with a 10:1 selectivity ratio. Subsequent reaction with triphosgene afforded either the cyclic carbamate or urea (**13**, R = H). Alternatively, enone **11** was treated with methyl cuprate. The 2,6-dimethyl ketone was formed in a 3:1 *trans*/*cis* ratio. Subsequent transformations to analogs of **13** (R = Me) proceeded as described above with an additional reductive amination carried out using 1,2-diamino benzene.

The dichloroaniline amides were evaluated in 11β-HSD1 binding assays using truncated human and rat enzymes. The cellular assay for 11β-HSD1 activity was performed in HEK293 cells transfected with full length h-HSD1 cDNA.[11]

High throughput screening efforts led to the identification of dichloroaniline amide **4** that was a potent inhibitor of both the human and rat isoforms of 11β-HSD1 and was highly selective against 11β-HSD2 (Table 1).[12] In rat, dichloroaniline **4** was shown to be a short acting inhibitor of 11β-HSD1. When compound **4** was tested for metabolic stability in rat microsomes, it was rapidly metabolized. Subsequent metabolite identification experiments demonstrated that the major mode of metabolism was hydroxylation of the cyclopentyl ring. No aniline oxidation was observed. Lead **4** also had poor water solubility. Thus, we began a research effort to identify additional potent, selective dichloroaniline inhibitors of 11β-HSD1.

Table 1. First generation dichloroaniline 11βHSD1 inhibitors (**6**)



Compound	R	h-HSD1 K_i[a] (nM)	r-HSD1 K_i[a] (nM)	HEK HSD1 IC_{50}[a] (nM)
4		8	16	140
6a		4	8	17
6b		10	6	100
6c		4	4	41
6d		16	2700	280
6e		53	210	960
6f		14	5.3	130
6g		28	4	900

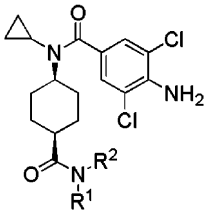
[a] Values are geometric means of two experiments.

Polycyclic amides **6a**–**c** were potent against human and rat 11β-HSD1. They are also selective against both human and rat 11β-HSD2. Cellular potency of compounds **6a** and **6c** was significantly improved over the lead compound.[13]

In an effort to lower the lipophilicity of this series, we attempted to introduce polar functionality. Compounds **6d** and **6e** indicated human 11β-HSD1 could tolerate a basic nitrogen. Unfortunately, the amine had a deleterious effect on cellular activity. The cyclopropyl cyclohexyl amide **6f** was as potent as lead compound **4**. Functionalization of the cyclohexyl ring at the 4 position with a carboethoxy group led to compound **6g**. This ester retained potency against rat 11β-HSD1 while suffering a drop in cellular potency. Analogs of **6g** were pursued in hopes of recovering cellular activity.

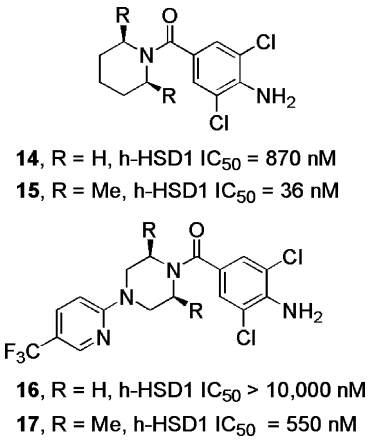
In general, lipophilic secondary amides were preferred by rat 11β-HSD1 (Table 2). Compounds **7a** and **7b** were representative examples of a group of compounds that contained small, non-polar groups. These potent com-

Table 2. Dichloroaniline amides 11βHSD1 inhibitor **7**



Compound	R^1	R^2	h-HSD1 K_i[a] (nM)	r-HSD1 K_i[a] (nM)	HEK HSD1 IC_{50}[a] (nM)
7a		H	17	39	640
7b		H	20	68	330
7c		Me	60	14	5700
7d		H	700	20	>30,000
7e		=R^1	130	5.2	6400
7f		=R^1	560	9.0	>30,000
7g		H	1100	17	>30,000

[a] Values are geometric means of two experiments.



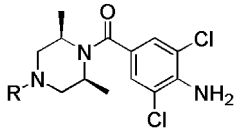
Scheme 5. Steric bulk around amide increases h-HSD1 potency.

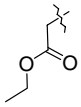
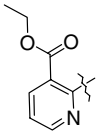
pounds did not show improved cellular potency. The move to small, polar substituents, such as ester **7c**, also did not improve cellular potency. Similarly, tertiary amide **7e** was also significantly less active in the HEK assay. The incorporation of acidic (**7d**) or basic (**7f** and **7g**) substituents at this position also decreased the human 11β-HSD1 potency and led to a complete loss of cellular activity. However, these compounds were potent against r-HSD1, indicating that charged substituents were preferred at this position in the rat isoform.

The decision to pursue substituted piperazine and amino piperidine dichloroaniline analogs was made based on the compounds shown in Scheme 5. Cyclohexyl amide **14** was found to be a weak inhibitor of human 11β-HSD1. However, when methyl groups were added as steric bulk around the amide carbonyl, the resulting amide **15** was greater than 20-fold more potent. Similarly, 2,6-dimethyl aryl piperazine **17** was marginally active while the non-methylated analog **16** was completely inactive. Thus, a goal became to exploit these observations to make polar analogs that maintained cellular potency.

Incorporation of the *cis*-2,6-dimethyl piperazine moiety to the dichloroaniline scaffold was generally tolerated by rat 11β-HSD1. The potency depended on the substituents appended to the 4 position of the piperazine (Table 3). Pyridyl piperazine **9a**, while potent against r-HSD1, was inactive in cells. Alkyl and aryl piperazines **9b** and **9c** provided the first indication that this scaffold could possess cellular potency. Introduction of both a nitrogen and a second substituent at the ortho positions of the aryl piperazine core also furnished active analogs. Meta- and para-substituted aryl piperazines were inactive or less active than their ortho-substituted comparators. Compounds **9d** and **9e** were examples that displayed polar functional groups and still had cellular

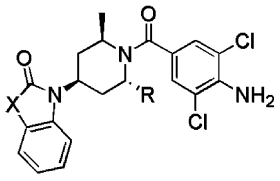
Table 3. 2,6-Dimethyl piperazine dichloroaniline analogs with general structure **9**



Compound	R	h-HSD1 K_i[a] (nM)	r-HSD1 K_i[a] (nM)	HEK HSD1 IC_{50}[a] (nM)
9a		100	6	>10,000
9b		13	14	490
9c		10	18	190
9d		19	23	360
9e		15	5	370

[a] Values are geometric means of two experiments.

Table 4. 4-Aminopiperidine dichloroaniline SAR



Compound	R	X	h-HSD1 K_i[a] (nM)	r-HSD1 K_i[a] (nM)	HEK HSD1 IC_{50}[a] (nM)
13a	H	O	13	140	380
13b	Me	O	12	160	72
13c	Me	N	22	39	76

[a] Values are geometric means of two experiments.

activity. Additionally, **9e** was found to be more than 10-fold more soluble than lead compound **4**.

The highly substituted *trans* piperidine amides were also well tolerated by h-HSD1 (Table 4). Dimethyl urethane **13b** was significantly more active against 11β-HSD1 in cells than the monomethyl variant (**13a**). Urea **13c** proved to be a dual potent human/rat 11β-HSD1 inhibitor that maintained cellular potency.

In summary, we have described SAR for a novel series of dichloroaniline amide 11β-HSD1 inhibitors and identified several dual human/rat potent compounds. The rat isoform tended to accommodate polar analogs better than the human enzyme. While the SAR for cellular potency was less well defined, we were able to discover several dual potent inhibitors, **6a**, **6c**, and **13c**, which were also potent against 11β-HSD1 in cells. All of the compounds identified were selective against 11β-HSD2. The discovery of compounds with this desirable biochemical profile is a key step forward in the identification of new small molecule inhibitors of 11β-HSD1 with therapeutic potential.

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11. The human and mouse HSD1 assays were performed in a SPA format using truncated enzyme, expressed in *Escherichia coli*. The HSD2 SPA assay used full-length human and mouse enzyme expressed in baculovirus. The test compounds were incubated with enzyme and ^{3}H-cortisone substrate for 30 min at room temperature. The radioactive cortisol produced was captured and quantified. The cellular assay for 11β-HSD1 activity was performed in HEK293 cells transfected with full length h-HSD1 cDNA. Test compounds were pre-incubated for 30 min with the cells before introduction of the cortisone substrate. After 2 h incubation, the cortisol concentration was determined by fluorescence polarization immuno-assay (FPIA). For more details, see Rohde, J. J.; Pliushchev, M. A.; Sorensen, B. K.; Wodka, D.; Shuai,Q.; Wang, J.; Fung, S.; Monzon, K. M.; Chiou, W. J.; Pan, L.; Deng, X.; Chovan, L. E.; Ramaiya, A.; Mullally, M.; Henry, R. F.; Stolarik, D. F.; Imade, H. M.; Marsh, K. C.; Beno, D.; Fey, T. A.; Droz, B. A.; McDowell, C.; Brune, M. E.; Camp, H. S.; Sham, H. L.; Frevert, E. U.; Jacobson, P. B.; Link, J. T. *J. Med. Chem.*, submitted for publication.
12. All of the compounds described in this paper were assayed against both human and rat 11β-HSD2 and found to be inactive (IC_{50} > 10 μM).
13. A strategic decision was made to move away from amides such as **6a** due to the fact compounds containing the same lipophilic amine substituent began appearing in the patent literature. See Kampen, Gita, Camilla, Tejlgaard, Andersen, Henrik, Sune, WO 2004/089415, 2004.