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Discovery and Initial SAR of Arylsulfonylpiperazine Inhibitors of 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1)

Daqing Sun *, Zhulun Wang, Yongmei Di, Juan C. Jaen †, Marc Labelle ‡, Ji Ma, Shichang Miao †, Athena Sudom, Liang Tang, Craig S. Tomooka, Hua Tu, Stefania Ursu, Nigel Walker, Xuelei Yan, Qiuping Ye, Jay P. Powers *,†

Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, USA

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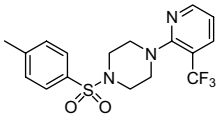
ABSTRACT

High-throughput screening of a small-molecule compound library resulted in the identification of a series of arylsulfonylpiperazines that are potent and selective inhibitors of human 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1). Optimization of the initial lead resulted in the discovery of compound (*R*)-**45** (11β-HSD1 IC_{50} = 3 nM).

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a key enzyme that acts as an NADPH-dependent reductase capable of converting the inactive 11β glucocorticoids such as cortisone into their active form, (e.g., cortisol) in specific tissues, such as liver, adipose, and brain tissues. Therefore, 11β-HSD1 regulates tissue-specific glucocorticoid levels.[1–4] Conversely, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a structurally related isoenzyme of 11β-HSD1, catalyzes the conversion of cortisol to cortisone utilizing NAD as a cofactor. 11β-HSD2 is expressed in cells that contain the mineralocorticoid receptor (MR) and protects the MR by converting cortisol to the inactive form, cortisone.[5]

Aberrant glucocorticoid action in the liver and adipose tissue has been linked to insulin resistance and dyslipidemia. Therefore, selective inhibition of 11β-HSD1 over 11β-HSD2 is a promising strategy to improve insulin sensitivity and treat type 2 diabetes, and has attracted significant attention from the pharmaceutical research community.[6–12]

We identified arylsulfonylpiperazine **1** (Fig. 1, human 11β-HSD1 IC_{50} = 16 nM) as a potent inhibitor of 11β-HSD1 by high-throughput screening of a small-molecule compound library utilizing a purified recombinant human enzyme. Compound **1** did not significantly inhibit 11β-HSD2 under similar conditions (11β-HSD2 IC_{50} > 10 uM).



1

Figure 1. Initial 11β-HSD1 inhibitor hit.

Analogs of **1** were synthesizd via the routes outlined in Schemes 1–3.[13] Compounds **2–22** and **38–44** were prepared as outlined in Scheme 1. Treatment of 2-chloro-3-trifloromethylpyridine with excess piperazine produced pyridylpiperazine **47**, which was then coupled with benzoic acid in the presence of HBTU/HOBt to yield amide **3**. Reaction between **47** and phenyl isocyanate gave urea **4**, while treatment of **47** with the appropriate benzenesulfonyl chlorides in dichloromethane gave arylsulfonylpiperazines **2, 6–22**, and **38–44**. Reductive amination of **47** with benzaldehyde afforded the benzylpiperazine **5**.

Compounds **23–27** were prepared by treatment of 4- methylbenenesulfonyl chloride with pyridylpiperazines **48**, which could be synthesized by nucleophilic displacement of the chlorine in substituted 2-chloropyridines with piperazine (Scheme 2).

* Corresponding authors. Tel.: +1 650 244 2195 (D.S.); +1 650 210 2949 (J.P.P.).
E-mail addresses: daqings@amgen.com (D. Sun), jpowers@chemocentryx.com (J.P. Powers).
† Currently at ChemoCentryx, Inc., Mountain View, CA 94043.
‡ Currently at Lundbeck Research USA, Inc., 215 College Road, Paramus, New Jersey 07652.

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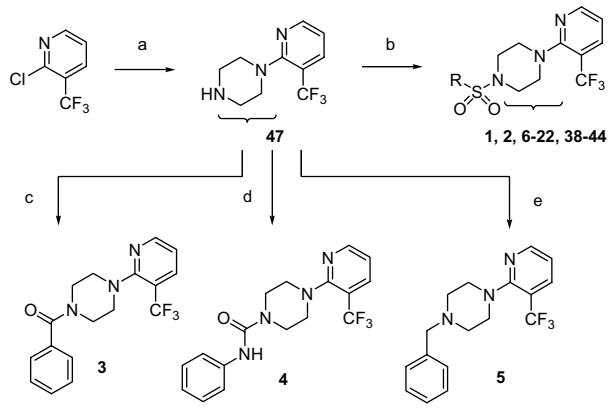
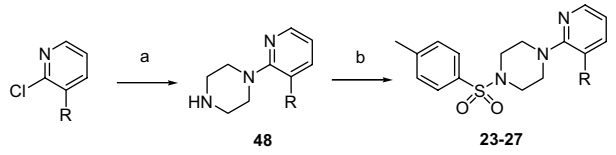
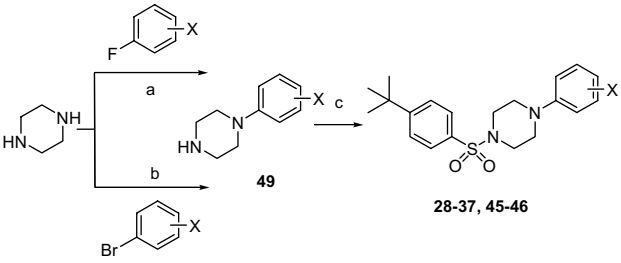


Scheme 1. Reagents and conditions: (a) piperazine, 120 °C, 74%; (b) RSO_2Cl, Et_3N, CH_2Cl_2, 51–90%; (c) benzoic acid, HBTU, HOBT, NMM, DMF, 63%; (d) phenyl isocyanate, $CHCl_3$, 82%; (e) benzaldehyde, $NaBH(OAc)_3$, acetic acid, dichloroethane, 90%.



Scheme 2. Reagents and conditions: (a) piperazine, 120 °C, 78–91%; (b) 4-methylbenzenesulfonyl chloride, Et_3N, CH_2Cl_2, 75–90%.



Scheme 3. Reagents and conditions: (a) piperazine, 120 °C, (X = 2-CF_3, 4-NO_2, 2-NO_2), 60–78%; (b) $Pd(OAc)_2$, tritolyphosphine, NaO*t*Bu, toluene, 110 °C, 42–63%, (X = 2-Me, 2-Cl, 2-F, 4-Me, 4-Cl, 4-F, 4-OMe); (c) 4-*t*-butylbenzenesulfonyl chloride, Et_3N, CH_2Cl_2, 80–95%.

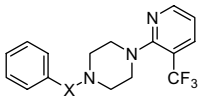
In a similar fashion, compounds **28–37, 45**, and **46** were obtained simply by reaction of *tert*-butylbenzesulfonyl chloride and arylpiperazines **49**, which were synthesized by either direct nucleophilic displacement or palladium mediated coupling reaction between the appropriate aryl halide and piperazine (Scheme 3).

Compounds were evaluated for inhibition of human and mouse 11β-HSD1 enzymes, as well as in cell-based assays. 11β-HSD1 enzyme activity was determined by measuring the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol. Product [^{3}H]-cortisol, captured by an anti-cortisol monoclonal antibody conjugated to scintillation proximity assay (SPA) beads, was quantified with a microscintillation plate reader. Biochemical enzyme assays were performed with Baculovirus-produced recombinant full-length human or mouse 11β-HSD1 as the enzyme source and NADPH as cofactor. Cell-based enzyme assays (h-293) utilized HEK293 cells stably expressing recombinant human full-length 11β-HSD1 as the enzyme source without supplementation of NADPH. IC_{50} values for enzyme inhibition were calculated with a dose response curve fitting algorithm with at least duplicate sets of samples.

Initial optimization of **1** began with the replacement of the sulfonamide functionality (Table 1). Replacement of the sulfonamide moiety with amide, urea, and methylene groups (**3**, **4**, and **5**) resulted in a significant loss of potency in both human and mouse enzymatic assays, as well as in the human cell-based assay as compared to **2**. However, insertion of a methylene between the sulfonamide and the phenyl ring (**6**) led to a 2-fold increase in human potency, suggesting that the sulfonamide moiety is an important feature for binding of this class of inhibitors to 11β-HSD1.

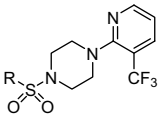
We then turned our attention to modification of the sulfonamide aryl ring (Table 2). Gradually increasing the alkyl substituent size at the *para*-position of the sulfonamide aryl ring led to the *tert*-butyl analog **10**, which had fourfold increase in human biochemical potency and a twofold increase of cellular potency over **1**, respectively. These data suggest that lipophilicity in the 4-substituent is beneficial to 11β-HSD1 inhibition. However, incorporation of a chloro, trifluoromethyl, or methoxy group (**12**–**14**) did not improve potency, while fluoro, nitro, or cyano groups (**11**, **15**, and **16**) led to approximately a threefold reduction of potency. Similarly, introduction of a phenyl group at the *para*-position (**17**) brought a slight decrease in potency, indicating a steric limit to substituents in this position. Interestingly, the incorporation of an additional fused benzene ring (**18**, **19**) resulted in potency similar to compound **10**. Introduction of an additional chlorine atom at the 2-po-

Table 1
Inhibition of 11β-HSD1 by selected analogs: Sulfonamide replacements



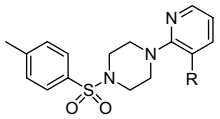
Compound	X	h-HSD1 IC_{50} (nM)	h-293 IC_{50} (nM)	m-HSD1 IC_{50} (nM)
2	$-SO_2-$	45	724	122
3	$-C(O)-$	262	>1000	>1000
4	$-NHC(O)-$	>1000	—	—
5	$-CH_2-$	550	1930	>1000
6	$-CH_2SO_2-$	20	574	47

Table 2
Sulfonamide aryl modification



Compound	R	h-HSD1 IC_{50} (nM)	h-293 IC_{50} (nM)	m-HSD1 IC_{50} (nM)
1	4-Me-Ph	16	461	32
7	4-Et-Ph	18	510	53
8	4-Pr-Ph	16	613	106
9	4-*i*Pr-Ph	15	556	67
10	4-*t*Bu-Ph	4	201	21
11	4-F-Ph	46	667	211
12	4-Cl-Ph	14	518	40
13	4-CF_3-Ph	14	727	75
14	4-OMe-Ph	19	456	32
15	4-NO_2-Ph	37	922	311
16	4-CN-Ph	50	978	546
17	4-Ph-Ph	74	603	>1000
18	2-naphthyl	13	547	11
19	3-naphthyl	13	329	80
20	3-Cl-Ph	55	>1000	58
21	3-CF_3-Ph	64	>1000	53
22	2,4-diCl-Ph	7	241	25

Table 3
Pyridyl ring substitution



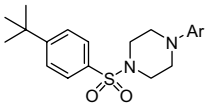
Compound	R	h-HSD1 IC_{50} (nM)	h-293 IC_{50} (nM)	m-HSD1 IC_{50} (nM)
1	CF_3	16	461	32
23	H	>1000	>1000	>1000
24	CH_3	152	>1000	240
25	Cl	117	>1000	146
26	NO_2	291	>1000	—
27	CN	628	>1000	—

sition (**22**) also led to a significant increase in potency, while substitution at the 3-position resulted in less active compounds (**20** and **21**) in comparison with **12** and **13**. In general this set of analogs showed a significant shift in potency in human biochemical and cellular 11β-HSD1 assays.

We next turned to substituent modification and replacement of the *N*-aryl ring (Tables 3 and 4). Replacing the trifluoromethyl group in **1** by a variety of subtituents resulted in a substantial reduction in activity (**23**–**27**). Removal of the 3-trifluoromethyl group led to a significant reduction in potency, while substitution at the 3-position with other groups (**24–27**) was more potent than the unsubstituted analog **23**, but they were less potent than parent compound **1**. Replacement of the trifluoromethylpyridyl moiety with either *meta* or *para*-substituted phenyl groups decreased HSD1 inhibition (**28**–**37**). In the best case, incorporation of a 4-nitrophenyl group (**33**) showed a two-fold reduction in human biochemical potency and a slight loss in cellular potency compared to **10**. However, compound **33** displayed significant improvement in *in vitro* metabolic stability as compared to **10** (90% vs. 61% remaining in human liver microsomes @ 10 min). The rat *in vivo* PK profile of **33** featured a moderate clearance, although oral bioavailability was poor (CL = 1.0 L/h/Kg; %F = 7).

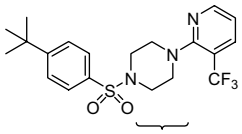
Our investigation into the 11β-HSD1 inhibition pharmacophore then moved to limited modification of the piperazine ring, including replacement by homopiperazine and a set of substituted piperazines (Table 5). Replacement of the piperazine ring with homopiperazine (**38**) resulted in a slight increase in human biochemical potency, while both 2-methyl and 2-ethyl piperazine

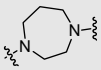
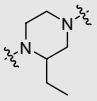
Table 4
N-Aryl replacements



Compound	Ar	h-HSD1 IC_{50} (nM)	h-293 IC_{50} (nM)	m-HSD1 IC_{50} (nM)
10	3-CF_3-2-pyridyl	4	201	21
28	2-CF_3-Ph	15	971	73
29	2-Me-Ph	32	702	204
30	2-Cl-Ph	95	>1000	184
31	(2-F)Ph	106	>1000	98
32	2-NO_2-Ph	37	839	133
33	4-NO_2-Ph	10	247	161
34	4-Cl-Ph	87	>1000	>1000
35	4-Me-Ph	390	>1000	>1000
36	4-F-Ph	42	>1000	186
37	4-OMe-Ph	234	>1000	—

Table 5
Piperazine modifications



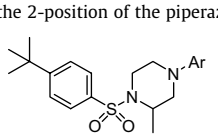
Compound	Ring	h-HSD1 IC_{50} (nM)	h-293 IC_{50} (nM)	m-HSD1 IC_{50} (nM)
10		4	201	21
38		3	315	84
39		9	632	4
40		17	>1000	6
41		58	>1000	—
42		62	638	9

(**39** and **40**) showed a small loss in human potency but improved mouse potency relative to **10**. However, further substitution to give either the 2,6- or 2,2-dimethyl analog (**41** and **42**) was detrimental for activity.

The stereochemistry of substituents at the 2-position in the piprazine ring was also examined (Table 6). In the 3-trifluoro-2-pyridyl series, very little difference was observed between enantiomers (**43**, **44**), while in the 4-nitrophenyl series, the *R* enantiomer had an 11β-HSD1 IC_{50} = 3 nM, about 10 times more potent than the corresponding *S* isomer in both the biochemical and cellular assays (**45** vs. **46**).

The structure of a representative arylsulfonylpiperazine inhibitor bound to human 11β-HSD1 was determined by X-ray crystallography (Fig. 2) to a resolution of 2.2 Å.[14] The co-crystal structure of compound **45** with human 11β-HSD1 reveals that

Table 6
Effect of stereochemistry at the 2-position of the piperazine ring



Compound	Config	Ar	h-HSD1 IC_{50} (nM)	h-293 IC_{50} (nM)	m-HSD1 IC_{50} (nM)
43	*R*	3-CF_3-2-pyridyl	11	762	4
44	*S*	3-CF_3-2-pyridyl	9	569	17
45	*R*	4-NO_2-Ph	3	57	53
46	*S*	4-NO_2-Ph	29	527	37

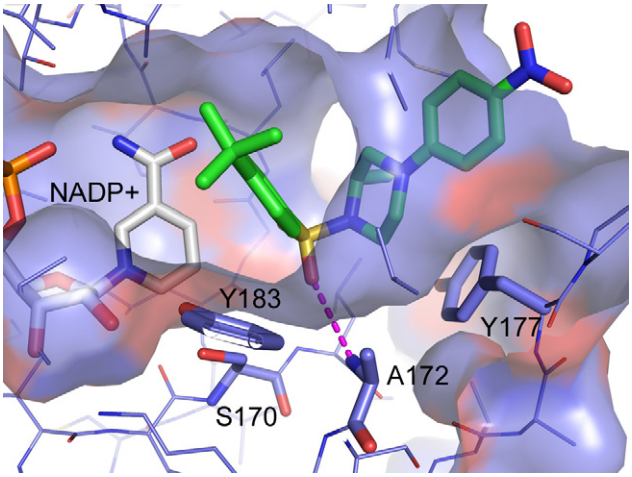


Figure 2. Co-crystal structure of compound **45** in human 11β-HSD1. The protein is shown in both stick and molecular surface representations which are color coded (red for oxygen atoms, blue for nitrogen, orange for sulfur, and slate for carbon). The inhibitor and the cofactor NADP+ are shown in sticks and color coded grey for carbon atoms in NADP+ and green for the inhibitor. The hydrogen bond is shown in magenta dashed line.

the inhibitor binds to the substrate site in a V-shape with its *tert*-butyl phenyl group pointing toward the cofactor NADP+ side. The central sulfonyl group makes a hydrogen bond from one of its oxygen atom to the backbone amide of Ala172, as well as VDW contacts with Ser170 in the catalytic site.

In conclusion, we have identified a series of novel and selective arylsulfonylpiperazine inhibitors of 11β-HSD1 through screening of a small-molecule library. SAR studies resulted in a significant improvement of human biochemical and cellular potencies, and established features of the pharmacophore for 11β-HSD1 inhibition in the series. The sulfonamide functionality was found to be important for 11β-HSD1 inhibition, while modification of the sulfonamide aryl ring via substituent replacement resulted in a fourfold increase of human biochemical potency and a twofold increase of cellular potency. Most of potent arylsulfonylpiperazine inhibitors of 11β-HSD1 showed little activity toward 11β-HSD2. Stereochemical studies at the 2-position of the methylpiperazine showed that *R* and *S* enantiomers can have a significant difference in properties, which may be important for future studies to optimize both ADME and potency parameters. Replacement of the *N*-aryl moiety by various substituted phenyl groups demonstrated that some changes were allowed in this region. This finding encouraged us to explore the replacement of the *N*-aryl moiety with non-aromatic groups to improve water solubility and metabolic stability, as well as cellular activity. These results will be reported in subsequent publications.

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13. All compounds gave satisfactory ^{1}H NMR, HPLC, and MS data in full agreement with their proposed structures, and purity (>95%) was determined by HPLC analysis.
14. The atomic coordinate has been deposited in the Protein Data Bank under an accession code 3CZR.