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Discovery of a Potent, Selective, and Orally Bioavailable Acidic 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) Inhibitor: Discovery of 2-[(3*S*)-1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]-3-piperidyl]acetic Acid (AZD4017)

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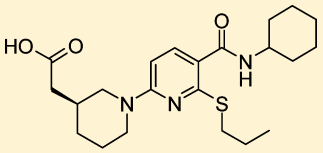
Supporting Information

ABSTRACT: Inhibition of 11β-HSD1 is an attractive mechanism for the treatment of obesity and other elements of the metabolic syndrome. We report here the discovery of a nicotinic amide derived carboxylic acid class of inhibitors that has good potency, selectivity, and pharmacokinetic characteristics. Compound **11i** (AZD4017) is an effective inhibitor of 11β-HSD1 in human adipocytes and exhibits good druglike properties and as a consequence was selected for clinical development.



11i

11β-HSD1 IC_{50} 0.007 μM

11β-HSD2 IC_{50} > 30 μM

Rat bioavailability F 100%

INTRODUCTION

The principal strategy in the management or prevention of type II diabetes and cardiovascular disease involves treatment of a collection of risk factors. When a patient exhibits central obesity in combination with two of the following risk factors, elevated fasting plasma glucose, dyslipidemia, hypertension, reduced high density lipoprotein (HDL) cholesterol, they are defined as having the metabolic syndrome.[1] The underlying causes of the metabolic syndrome are complex; however, the synthesis and metabolism of glucocorticoids have been proposed to play a key role.[2,3] Human patients with Cushing's syndrome, who exhibit elevated circulating glucocorticoid levels, display a phenotype similar to the metabolic syndrome.[4] Although patients with the metabolic syndrome do not exhibit elevated plasma glucocorticoid levels,[5] it has been hypothesized that elevated intracellular concentrations may play a crucial role. Therefore, the ability to reduce intracellular glucocorticoid concentrations has been proposed as an attractive therapeutic paradigm for the metabolic syndrome.[6−9]

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an NADPH dependent enzyme that is widely expressed, notably in liver, adipose tissue, and brain.[10] It catalyzes the interconversion of the inactive glucocorticoid hormone cortisone (**1**) to the active glucocorticoid hormone cortisol (**2**) (Figure 1) and therefore plays a key role in the regulation of intracellular cortisol concentrations.[11] 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2) is an NAD dependent oxidase expressed mainly in kidney and colon tissue that catalyzes the reverse reaction and prevents activation of mineralocorticoid receptors by **2**.[12] Inhibition of this enzyme has been associated with hypertension and other complications, and therefore, selectivity over this isoform is a key requirement for any potential therapeutic agent.[13]

Carbenoxolone (**3a**), originally developed as a treatment for esophageal ulceration and inflammation, is an example of an acidic compound that is a potent but nonselective inhibitor of both 11β-HSD1 and 11β-HSD2.[14] This has been shown to improve insulin sensitivity and reduce glucose production in patients with type II diabetes but is not used clinically for the treatment of the metabolic syndrome because of the associated hypertension risk.[15]

Reflecting the keen interest in the therapeutic potential of this target, over 250 patent applications from over 25 pharmaceutical companies and academic groups have now been published and comprehensively reviewed.[16−18] Several companies have now progressed compounds into development, and the structures of those compounds where disclosed are shown in Figure 2.

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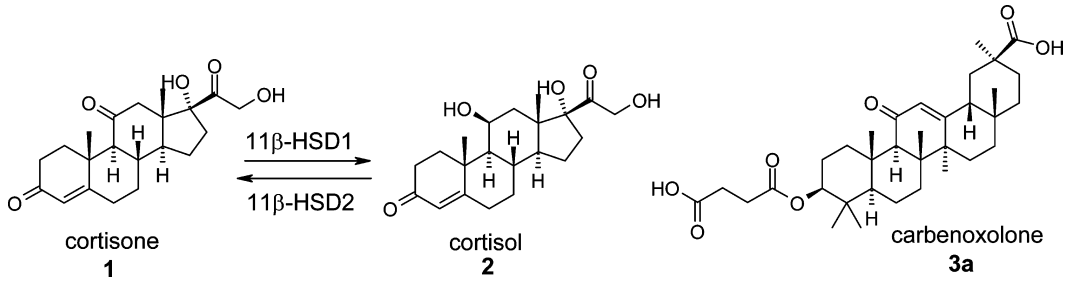


Figure 1. Interconverstion of cortisone (**1**) and cortisol (**2**) by 11β-HSD1 and 11β-HSD2 and the unselective inhibitor carbenoxolone (**3a**).

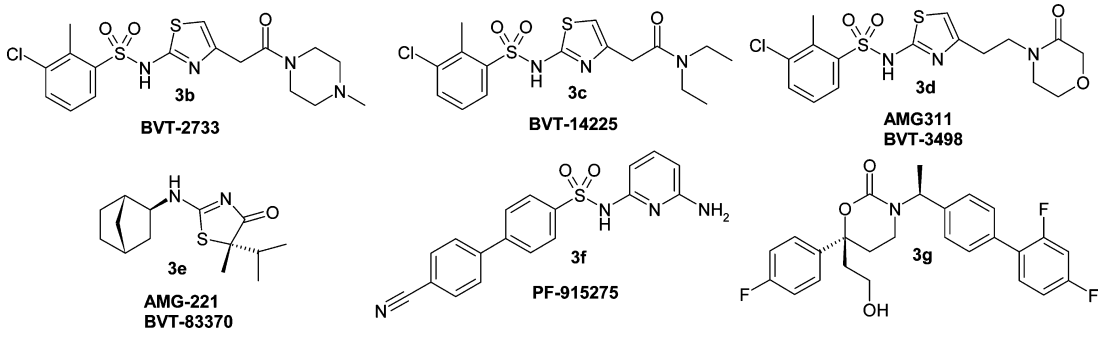
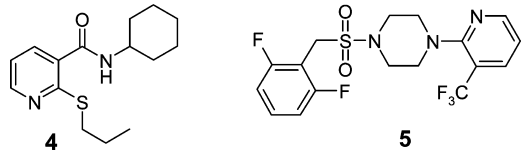


Figure 2. Selective 11β-HSD1 inhibitors disclosed in the literature.

Table 1. Properties of 2-Thioalkylnicotinamide 4 and Piperazinesulfonamide 5



compd	human 11β-HSD1 IC_{50} (μM)	log $D_{7.4}$	PPB rat (% free)	solubility (μM)	rat heps Cl_{int} ((μL/min)/10^6 cells)	permeability, CACO-2 P_{app} ($\times 10^{-6}$ cm s^{-1})
4	0.350	3.2	13.2	82	284	27
5	0.200	3.9	0.5	2	272	10

Biovitrum was the first company to disclose inhibitors such as BVT-2733 (**3b**) and BVT-14225 (**3c**) that were selective for 11β-HSD1 over 11β-HSD2.[19] They entered into a collaboration with Amgen, and this led to the development of the first compound to enter clinical trials, BVT-3498 (AMG-311)[20] (**3d**) reaching phase II in 2003. This was subsequently replaced by BVT-83370 (AMG-221)[21] which progressed into phase I trials, but development was halted in April 2011.[22] Merck has progressed two compounds into development, MK-0916, which entered phase II in 2004, and MK-0736, which was originally developed as a treatment for hypertension and entered phase II trials in 2005. Initial clinical data have now been reported showing no significant improvement in fasting plasma glucose at week 12 relative to placebo.[23,24] However, modest dose-dependent decreases in blood pressure and body weight were observed over the course of the study, together with a small but significant reduction of 0.3% in hemoglobin A1C (hBA1c) at week 12.[23,24] The structures of these candidates have not been disclosed. Pfizer took the inhibitor PF-915275 (**3f**)[25] into phase I trials in 2006. In 2007 it was announced that this compound had progressed to phase II; however, it was subsequently stopped because of tablet formulation issues.[26] Incyte has also taken a lead compound INCB-13739, the structure of which has not yet been disclosed, as far as phase II trials, and data in patients with type II diabetes have recently been reported[27] and subsequently reviewed.[28] The study showed that after 12 weeks as an addition to metformin monotherapy, doses of 200 mg resulted in significant reductions in HbA1c (−0.6%), fasting plasma glucose (−24 mg/dl), and homeostasis model assessment–insulin resistance (HOMA-IR) (−24%) compared with placebo. Vitae Pharmaceticals has been in collaboration with Boehringer Ingelheim, and a compound entered phase I trials in 2010. Inhibitors such as **3g** have been reported by Vitae,[29] although it is not known whether this represents the structure of their development compound. Other companies such as Bristol-Myers Squibb, Roche, and Lilly have also progressed compounds into the clinic, although to date the structures have not been disclosed.

RESULTS AND DISCUSSION

As part of a drug discovery program to identify a potent and selective inhibitor of 11β-HSD1, we undertook a high throughput screening campaign against the AstraZeneca compound collection. A number of chemotypes were found that inhibited 11β-HSD1, and these were profiled and developed to generate two distinct series that we felt represented promising leads. The series are exemplified by 2-thioalkylnicotinamide **4** and piperazinesulfonamide **5**, the properties of which are summarized in Table 1.

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Nicotinamide **4** had modest potency (IC_{50} = 350 nM) and was suprisingly unbound (rat plasma protein binding (PPB), 13.2% free) given the measured log $D_{7.4}$ (3.2). Ligand efficiency was high as measured per heavy atom count (0.46) and moderate as measured by ligand lipophilicity efficiency[30] (LLE = pIC_{50} − logD = 3.3). Sulfonamide **5** was more potent (IC_{50} = 200 nM) although less impressive in terms of ligand efficiency per heavy atom count (0.33) or ligand lipophilicity efficiency (2.8). The permeability of both was high as measured in a CACO-2 assay with no evidence of efflux.

A key issue for both of these series was the lack of oral bioavailability in rat (F = 0%). The in vitro data suggested that metabolism may be a limiting factor, as both compounds were unstable in rat hepatocytes. In order to test this concept, we elected to carry out diagnostic DMPK experiments using a nonspecific inhibitor of the cytochrome P450 (CYP) enzymes, 1-aminobenzotriazole (ABT).[31] Our hypothesis was that if metabolism was the limiting factor, dosing of ABT should attenuate oxidative drug metabolism in vivo and improve the pharmokinetic parameters of the compounds. We elected to predose the ABT orally according to protocols described in the literature[32] to inhibit both gut wall and hepatic P450 metabolism. The results are shown in Table 2.

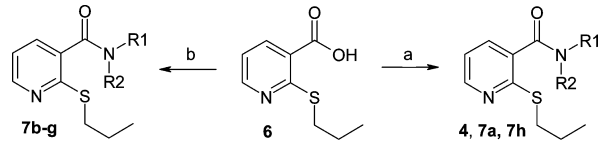
Table 2. DMPK Properties of 2-Thioalkylnicotinamide 4 and Piperazinesulfonamide 5 in the Absence and Presence of an ABT Predose

compd	expt	C_{max} (μg/mL)	AUC_{0-t} (μg·h/mL)	Cl (mL min^{-1} kg^{-1})
4	no ABT	0.039	0.08	42
4	po predose of ABT	0.514	2.51	7
5	no ABT	0	0	20
5	po predose of ABT	0	0	14

Upon oral predosing with ABT, 2-thioalkylnicotinamide **4** showed an improvement in AUC of approximately 30-fold relative to the compound with no ABT predose and a significant reduction in the clearance of the compound. This provided confidence that absorption was not an issue for this compound and that if the CYP mediated metabolic clearance could be reduced, it would lead to an improvement in oral levels. In contrast, piperazinesulfonamide **5** showed no observable compound levels following an ABT predose, leading us to conclude that the metabolism was not CYP mediated or that other factors, such as solubility, were restricting oral exposure. On the basis of these results, we elected to focus our optimization efforts on reducing metabolic clearance in the 2-thioalkylnicotinamide series. We were guided by metabolism identification (Met-ID) results that indicated that no amide bond cleavage was observed and the majority (>80%) of the metabolism was oxidative and occurring on the cyclohexyl ring with only moderate amounts on the pyridine and none being observed on the thioalkyl chain. Our medicinal chemistry strategy was to address the metabolism using amide modification. The compounds described in this optimization program were synthesized as described in Schemes 1−3.

An initial set of compounds (**7a−h**) was synthesized via amide couplings of the commercially available 2-thionicotinic acid **6** using either acid chloride chemistry to give compounds **4**, **7a**, and **7h** or HOBt/EDAC methodology to give compounds **7b−g** (Scheme 1).

Scheme 1[a]



[a]Reagents and conditions: (a) $(COCl)_2$, DMF, CH_2Cl_2, amine, 25 °C, 1 h, 16−54%; (b) HOBt, EDAC, $^iPr_2EtN$, CH_2Cl_2, amine, 25 °C, 18 h, 16−74%.

Synthesis of compounds substituted in the 6-position was achieved from 2,6-dichloronicotinic acid **8** via formation of the acid chloride and coupling with cyclohexylamine which proceeded in good yield (98%) to give amide **9**. In contrast to the results obtained with the corresponding ethyl ester,[33] we found that treatment of the amide **9** with sodium propylthiolate resulted in a completely regioselective displacement of the 2-chloro subsituent (no evidence of 6-chloro substitution evident in crude reaction mixtures) to afford key intermediate **10**. Subsequent treatment with amine nucleophiles with pendent ester groups and microwave heating using butyronitrile as solvent, followed by ester hydrolysis to the corresponding acids using lithium hydroxide, gave acidic compounds **11a−j** (Scheme 2).

Investigation of the SAR around the 2-substituent was carried out via treatment of intermediate **9** with propanol, propylamine, and *N*-methylpropylamine to give intermediates **12a**, **12b**, and **12c**, respectively, again with complete regioselectivity for displacement of the 2-chloro position. Treatment with methyl-(*S*)-3-piperidine acetate with microwave heating using butyronitrile as solvent followed by lithium hydroxide hydrolysis gave compounds **13a−c** (Scheme 2).

Compound **14** with methylene in place of sulfur was synthesized via a different approach involving construction of the pyridine ring. Starting from the known enamine ester **15**,[34] a Michael addition with methyl propiolate gave **16**. Then base mediated cyclization afforded the oxopyridine intermediate **17** in good yield. Treatment with phosphorus oxychloride to give **18** was followed by ester hydrolysis to give the chloro-substituted acid intermediate **19**. Subsequent amide coupling with cyclohexyalamine to form **20** and then treatment with methyl-(*S*)-3-piperidine acetate with microwave heating using butyronitrile as solvent gave compound **14** (Scheme 3).

Our efforts to address the metabolism by amide modification involved three approaches, namely, (i) introducing metabolic blocking groups (compound **7b**), (ii) lowering log $D_{7.4}$ by introduction of polar functionality (compounds **7c−g**), and (iii) reducing the levels of free drug by introducing acidic functionality with the aim of modulating the unbound clearance (compound **7h**). The results are summarized in Table 3.

Disappointingly, most of the putatively metabolism blocking or lower log $D_{7.4}$ compounds tested showed little or no improvement. In many cases, the clearance remained high in hepatocyctes and moderate to high in vivo, with no significant oral levels observed in rat. Significant increases in the free level of these compounds (in the cases of compounds **7c** and **7d** around 50% free) may have offset any reduction in lipophilicity driven metabolism. The introduction of a carboxylic acid functionality onto the cyclohexyl ring (compound **7h**), however, dramatically lowered the clearance (rat hepatocytes, <2 (μL/min)/10^6 cells) and when dosed in vivo showed an excellent pharmocokinetic profile in rat (Clp = 2.5 mL min^{-1}

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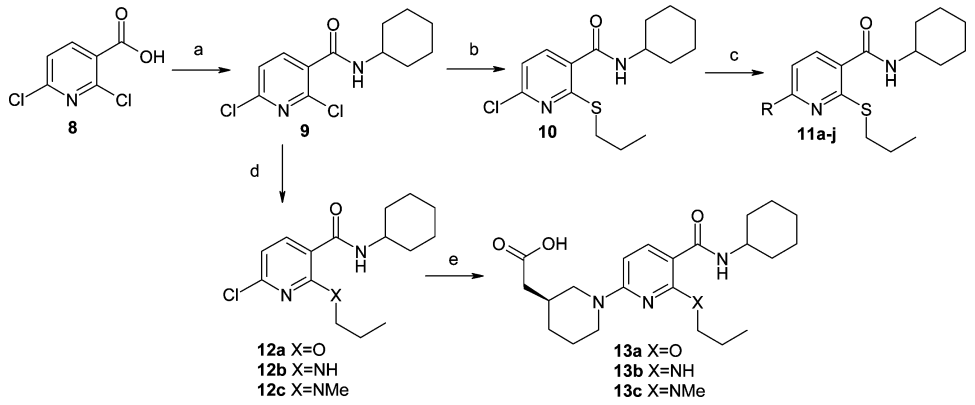
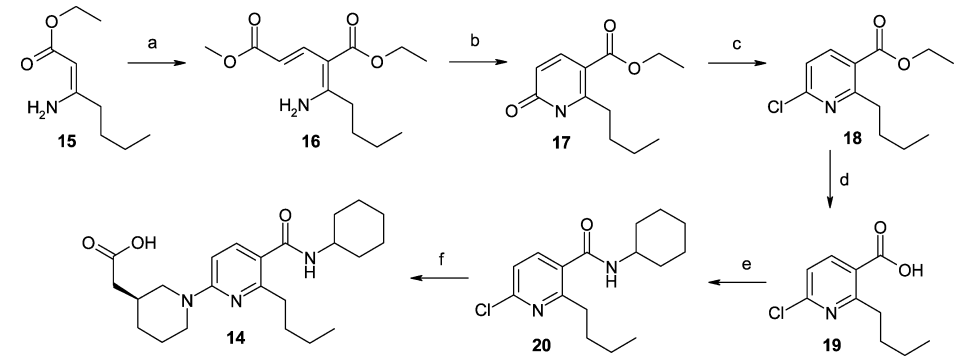
Scheme 2[a]



[a]Reagents and conditions: (a) (i) oxalyl chloride, DMF, CH_2Cl_2, 25 °C, 2 h; (ii) cyclohexylamine, CH_2Cl_2, 25 °C, 16 h, 98%; (b) ^{n}PrSH, NaHMDS, DMF, 25 °C, 2 h, 76%; (c) (i) RH, K_2CO_3, ^{n}BuCN, 170 °C, 2 h; (ii) LiOH, THF/H_2O, 25 °C, 3 h, 19−64%; (d) ^{n}PrXH, K_2CO_3, ^{n}BuCN, 150 °C, 1 h, 60−71%; (e) (i) methyl-(*S*)-3-piperidine acetate. HCl, K_2CO_3, ^{n}BuCN, 170 °C; (ii) LiOH, THF/H_2O, 25 °C, 16 h, 33−81%.

Scheme 3[a]



[a]Reagents and conditions: (a) methyl propiolate, toluene, 100 °C, 96 h, 81%; (b) NaO^{t}Bu, NMP, 180 °C, 4 h, 78%; (c) $POCl_3$, 120 °C, 2 h, 81%; (d) NaOH, MeOH, 25 °C, 16 h, 86%; (e) HOBt, EDAC, NEt_3, CH_2Cl_2, cyclohexylamine, 25 °C, 16 h, 75%; (f) methyl-(*S*)-3-piperidine acetate. HCl, K_2CO_3, ^{n}BuCN, 150 °C, 96 h, 25%.

kg^{-1}; F = 81%). Notably this compound had high free drug levels for an acid (rat PPB, 41% free) with considerably reduced lipophilicity (log $D_{7.4}$ < 0.5) relative to the original lead (log $D_{7.4}$ = 3.2). Unfortunately, when tested in the 11β-HSD1 assay, the activity of the compound was below the reporting threshold (IC_{50} > 30 μM).

Encouraged by the fact that **3a**, a known inhibitor of 11β-HSD1, contains acidic functionality, we sought to introduce an acidic group in our series in a position that would be tolerated by the enzyme. The structure of **3a** in complex with 11β-HSD1 was published in 2004 (PDB code 2BEL).[35] The active site was analyzed using SiteMap probes, as implemented in the Schrödinger software package. This identified two primary positions for hydrogen bond acceptors: at the active site residues S170 and Y183 and near the backbone NH of L217. The site analysis suggested a slight concavity near L217, which would promote a hydrogen bond interaction, and indeed, **3a** utilizes this hydrogen bond position. Similarly, there is a significant region of the pocket that is predicted to favor hydrophobic groups and that is occupied by the steroid scaffold of **3a**. We speculated that a potential binding mode for our compound could involve the amidecarbonyl interacting with Y183. We therefore chose to explore substitution of the pyridine core as a potential way to access this hydrophobic pocket with the hope of finding a suitable way to position an acidic group. The results of a series of 6-substituted pyridyl compounds are presented in Table 4.

Consistent with our hypothesis and in contrast with compound **7h**, compounds **11a−k** showed inhibition of the enzyme while retaining the favorable DMPK properties associated with the introduction of acidic functionality. Compounds **11a** and **11b** demonstrated that an NH linker was tolerated, while efforts to incorporate an *O*-aryl linker (**11c**, **11d**) were less successful. Moving the position of the acid around a piperidine ring (**11e−g**) revealed that the 3- and 4-positions were particularly favored (**11f**, **11g**). Chain homologation at these positions (**11h−j**) resulted in the identification of compound **11i** which was the most potent acidic compound identified within this series (IC_{50} = 7 nM).

The observation that compound **11i** was >10-fold more potent than its enantiomer **11h** suggested that a specific interaction was being made by the acidic group with the protein. In order to understand the nature of this protein−ligand interaction, we obtained a crystal structure of compound **11i** in complex with human 11β-HSD1. Figure 3 shows a view of **11i** bound in the active site, with associated ligand density.

We observed an interaction between the amidecarbonyl group and the hydroxyl side chain of Y183. There is a hydrogen bond between the carboxylate group and the backbone NH of L217. The carboxylate is also in proximity to the side chain of

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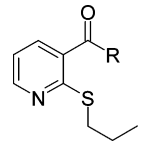
Table 3. Enzyme Potencies and Physicochemical and DMPK Properties for Pyridineamides



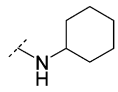
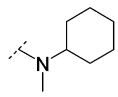
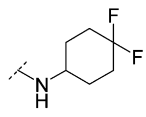
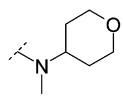
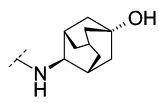
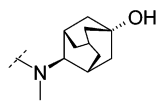
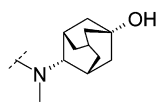
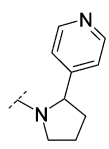
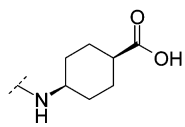
Compound	R	human 11β-HSD1 IC_{50} (μM)	$logD_{7.4}$	Rat PPB (%free)	Rat Heps Clint (uL/min/10^6cells)	Rat Cl (mL/min/kg)	Rat Bioavailability (%)
4		0.350	3.2	13	100	41	0
7a		0.016	3.4	10	>350	48	0
7b		0.860	3.0	29	130	30	0
7c		0.430	1.6	49	90	46	0
7d		0.007	2.3	>50	49	110	11
7e		0.012	2.5	20	140	24	0
7f		0.180	2.5	41	140	-	-
7g		0.170	2.0	13	120	39	0
7h		>30	<0.5	41	<2	2.5	81

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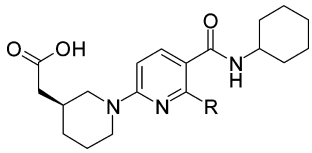
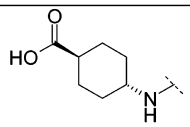
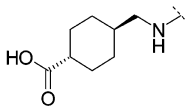
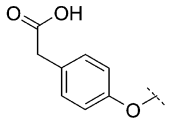
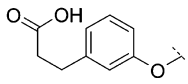
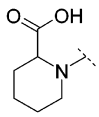
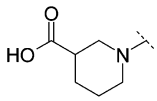
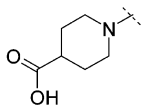
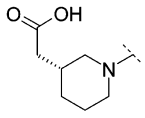
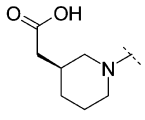
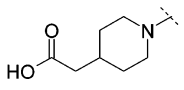
Table 4. Enzyme Potencies and Physical and DMPK Properties for Acidic Compounds



Compound	R	human 11β-HSD1 IC_{50} (μM)	$logD_{7.4}$	Rat PPB (%free)	Rat Heps Clint (uL/min/10^6cells)	Rat Cl (mL/min/kg)	Rat Bioavailability (%)
11a		0.120	2.0	2.9	<2	6	>100[a]
11b		0.099	2.6	1.5	8	8	81
11c		0.700	1.8	0.3	<2	-	-
11d		0.350	1.7	0.6	15	-	-
11e		2.100	1.4	1.9	10	12	>100[a]
11f		0.140	1.7	2.3	6	4	45
11g		0.220	1.6	3.0	<2	8	>100[a]
11h		0.096	2.2	0.6	8	1	79
11i		0.007	2.2	1.7	<2	9	>100[a]
11j		0.200	2.0	3.2	6	4	90

[a]Bioavailabilities of >100% are believed to reflect enterohepatic recirculation of acylglucuronide metabolite.

dx.doi.org/10.1021/jm300592r | J. Med. Chem. 2012, 55, 5951−5964

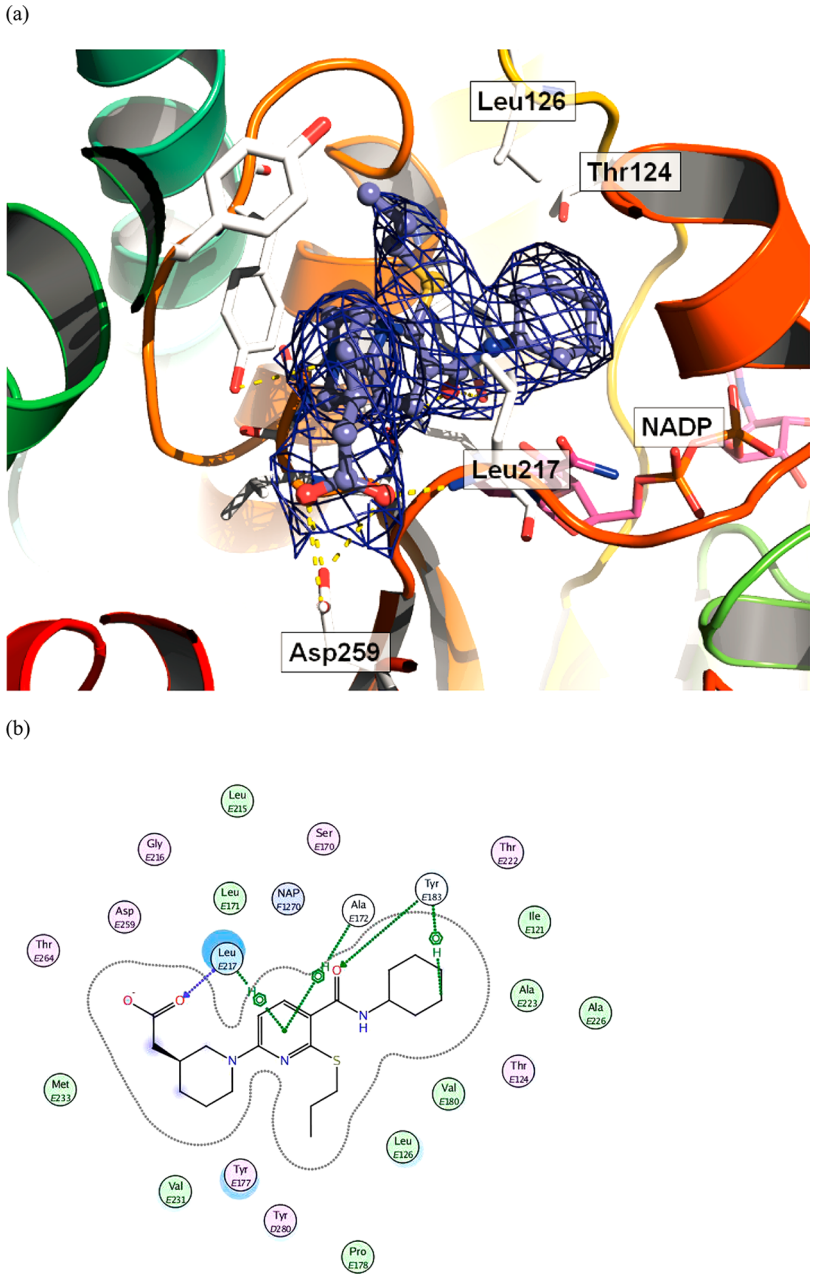


Figure 3. (a) Binding mode of compound **11i** in human 11β-HSD1, with associated $2F_o - F_c$ density contoured at 1σ. The ligand was cocrystallized along with the NADP cofactor, but there are no contacts between the two molecules. (b) Interaction map of compound **11i** in the 11β-HSD1 active site. Blue arrows indicate a backbone hydrogen bond. Green arrows indicate a side chain hydrogen bond. L217 and A172 show hydrophobic packing against the pyridyl group. Labels D, E, and F refer to the protein chain indices. The dotted line marks the protein surface. Purple patches indicate solvent exposure. The interaction map was generated using the MOE software package.

D259, although the O···O separation is slightly longer than expected for a hydrogen bond interaction. The pyridyl ring is sandwiched between the hydrophobic side chains of L217 and A172. The *S*-propyl and cyclohexyl groups are packed within the hydrophobic region of the pocket, with some limited exposure to solvent for the cyclohexyl. There is no contact with the $NADP^+$ cofactor, which remains in situ on binding the inhibitor. Overall, the high affinity of compound **11i** may be explained both from a complementary shape match and burial of hydrophobic groups and from two hydrogen bond interactions: first, an essential interaction with the active site residue Y183 and second, an optional, but energetically favorable, interaction with the backbone at L217.

The nature of the 2-substituent was examined to determine if the thioalkyl subsituent was optimal by varying the atom directly attached to the pyridyl ring while keeping the rest of the molecule constant (Table 5). The oxo analogue **13a** was less potent and more lipophilic, perhaps because of the potential for formation of an internal H-bond with the amide NH. Amino analogues **13b** and **13c** were isolipophilic but had reduced potency. Switching to the carbon analogue **14** that has no potential to form an internal H-bond resulted in a reduction in lipophilicity, a drop in potency, and an overall less ligand efficient compound. All compounds showed good oral bioavailability in rat. On the basis of these results, the thioalkyl substituent was determined to be optimal and compound **11i**

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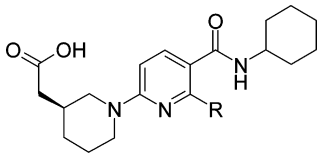
Table 5. Enzyme Potencies and Physical and DMPK Properties for Acidic Compounds



Compound	R	human 11β-HSD1 IC_{50} (μM)	$logD_{7.4}$	Rat PPB (%free)	Rat Heps Clint (uL/min/10^6cells)	Rat Cl (mL/min/kg)	Rat Bioavailability (%)
11i		0.007	2.2	1.7	<2	9	>100[a]
13a		0.037	2.8	0.6	9	9	>100[a]
13b		2.400	2.3	2.9	7	4	72
13c		0.120	2.3	1.8	12	15	39
14		0.520	1.6	6.0	<2	4	57

[a]Bioavailabilities of >100% are believed to reflect enterohepatic recirculation of acylglucuronide metabolite.

Table 6. Physical Properties of Compound 11i

aqueous solubility,[a] pH 7.4 (μM)	mouse PPB[b] (% free)	rat PPB[b] (% free)	dog PPB[b] (% free)	human PPB[b] (% free)	MDCK[c] permeability P_{app} ($\times 10^{-6}$ cm·s^{-1})
144 (n = 2)	2.9 (n = 2)	1.8 (n = 4)	2.3 (n = 2)	0.8 (n = 3)	29 (A–B), 22 (B–A) (n = 2)

[a]Solubility of compounds in aqueous phosphate buffer at pH 7.4 after 24 h at 25 °C (μM). [b]PPB was assessed by equilibrium dialysis in the appropriate species plasma at 37 °C. Free and bound concentrations were quantified by LC–UV–MS. [c]Compounds were incubated at 10 μM in cultured MDCK cells. Permeability was measured in both the A to B and B to A direction.

was selected for further profiling. The LLE for **11i** is 6.0, significantly higher than those of all of the other acidic analogues and a considerable improvement over the initial lead **4**.

Further profiling of compound **11i** indicated that the properties of this compound made it suitable for further development (Table 6). The compound was inactive (<25% inhibition at 10 μM) against five isoforms of the cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). Plasma protein binding showed good free levels for an acid (0.8–2.9% free across species), perhaps structurally related to the high free levels of the initial lead. The aqueous solubility as measured on crystalline material was 144 μM, and when coupled with good cellular permeability as measured in an MDCK assay (P_{app}(A–B) = 29 × 10^{-6} cm·s^{-1}; efflux ratio of 0.8), it was predicted that this would lead to good absorption in vivo.

The pharmacokinetic profile of compound **11i** was examined in other species (mouse, rat, dog), and the results are shown in Table 7. The compound showed consistently good properties with moderate clearances and high bioavailabilities (F > 60%) across all three species. The compound had notably high volumes of distribution across species (mouse, 4.2 L/kg; rat, 2.6 L/kg; dog, 0.9 L/kg), which was viewed as surprising for an acidic compound. Additionally in mouse and dog (but not rat), secondary peaks were observed in the pharmacokinetic profiles, leading to the hypothesis that the high volumes could be resulting from enterohepatic recirculation (EHC) whereby an

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Table 7. Pharmacokinetic Parameters of Compound 11i[a]

species	Clp (mL min^{-1} kg^{-1})	Vd_{ss} (L/kg)	iv half-life (h)	oral half-life (h)	bioavailability (%)
mouse	16	4.2	4.2	3.2	>100[b]
rat	9	2.6	4.4	4.7	>100[b]
dog	7	0.9	6.2	4.9	60

[a]Compounds were dosed at 1−3 mg/kg in either solution (DMSO/hydroxy-β-cyclodextrin) or suspension (HPMC/Tween). [b]Bioavailabilities of >100% are believed to reflect enterohepatic recirculation of acylglucuronide metabolite.

acylglucuronide of the acid was being formed and then subsequently cleaved leading to release of the parent compound. In mouse and dog, species that possess a gall bladder, it was postulated that the secondary peaks observed were a result of parent compound being released into the GI tract, whereas these were not evident in the rat, a species that lacks a gall bladder.[36] It was hypothesized that this was the effect responsible for the high volumes of distribution observed.

Met-ID results in hepatocytes (rat, dog, human) revealed that conversion of the acid to the acylglucuronide was indeed the major route of metabolism in all three species (Table 8).

Table 8. Metabolism-ID Summaries for Selected Compounds 11i

species	parent (%)	[+Glu] (%)	[+O] (%)	[+Glu]/[O]
rat	66	23	11	2.1
dog	34	53	13	4.1
human	93	6	1	6

While this is a normal metabolic fate for acids, acylglucuronides have been associated in some cases with idiosyncratic toxicity.[37] It is postulated that the initially formed 1β-isomer of the acylglucuronide metabolite may lead directly to drug−protein conjugates while Amadori rearrangement can result in the formation the 2-O- and 4-O-acylated products which can in turn lead to protein adducts through Schiff base formation with the amino groups of proteins.[38] To assess the potential risk associated with this, ^{14}C radiolabeled compound **11i** was prepared and dosed orally to a rat to assess the degree of covalent binding. Pleasingly this compound did not show any significant covalent binding in plasma or liver tissue when dosed to male rats at 20 mg/kg (40 μCi/kg). Less than 25 pmol/mg protein was detected after 2 h, and no detectable binding was observed after 24 h. This suggested that this compound would be unlikely to give rise to idiosyncratic toxicity as a result of acylglucuronide formation.

Compound **11i** displayed excellent selectivity versus the related enzymes 11-βHSD2, 17β-HSD1, 17β-HSD3 (all IC_{50} > 30 μM) and showed no measurable activity against the glucocorticoid and mineralocorticoid receptors. Further profiling revealed that **11i** was nonmutagenic in a two-strain Ames test and negative in a mouse lymphoma assay. It had IC_{50} > 100 μM in the hERG IonWorks assay and was assessed in a guinea pig MAP assay with no changes observed in any of the parameters. No significant induction of CYP450 enzymes was observed following administration to rats for 1 month. A good selectivity profile was observed versus a panel of 119 unrelated enzymes and receptors with the only two weak hits being angiotensin (AT_2) receptor and choleocystokinin (CCK_2) receptor. One stable anhydrous crystalline form was identified following a polymorph screen, providing confidence that solid-state issues should not hinder development.

Despite having high potency for the human form of 11β-HSD1, compound **11i** showed much reduced activity across species as shown in Table 9 with the exception of cynomolgous

Table 9. In Vitro Potencies for Compound 11i

IC_{50} (μM)					
human enzyme	mouse enzyme	rat enzyme	dog enzyme	cyno enzyme	human adipocyte
0.007	0.750	4.06	3.77	0.029	0.002

monkey (IC_{50} = 0.029 μM). Additionally, as we believed adipose is a key target organ, inhibition of 11β-HSD1 activity was measured in isolated human adipocytes from nondiabetic volunteers. The compound was shown to be a potent inhibitor in this key target tissue (IC_{50} = 0.002 μM) in good agreement with the enzyme potency, thus providing some confidence that the compound was not restricted from adipose tissue by the fact that it was acidic.

Since **11i** had lower potency against the mouse enzyme, only a limited number of preclinical pharmacodynamic measurements were performed. An ex vivo assay measuring the inhibition of conversion of ^3H-cortisone to ^3H-cortisol was conducted in epididymal adipose tissue. Figure 4 shows the

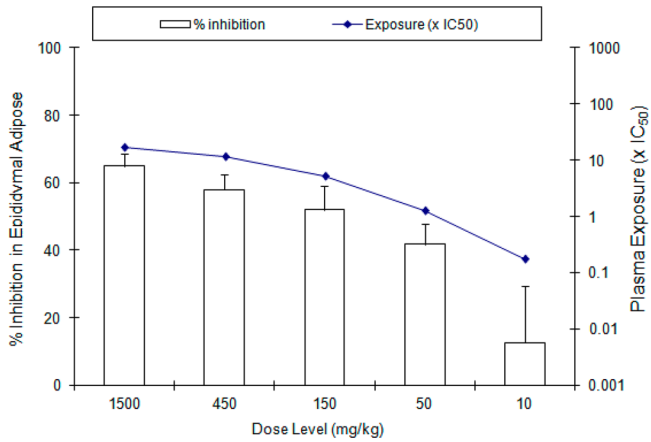

Figure 4. Inhibition of 11-βHSD1 in mouse epididymal adipose tissue ex vivo for compound **11i**.

effects of compound **11i** dosed orally to lean mice (10−1500 mg/kg). At 1 h postdose, at a dose of 50 mg/kg compound **11i**, 50% inhibition was observed. This corresponded to a plasma exposure equivalent to the IC_{50} in the mouse. Increasing the dose further led to a maximal effect of approximately 70% inhibition at 1500 mg/kg, equivalent to 10 × IC_{50} in the mouse, demonstrating the dose dependent inhibition of 11β-HSD1 by compound **11i** in this model.

In summary, we have discovered novel acidic inhibitors of 11β-HSD1 that show excellent pharmacokinetic profiles. On the basis of these encouraging results and the overall profile, compound **11i** was selected for development as the clinical candidate AZD4017. Further details of these investigations will be reported in due course.

EXPERIMENTAL SECTION

All solvents and chemicals used were reagent grade. Anhydrous solvents tetrahydrofuran (THF), benzene, dimethoxyethane (DME)

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were purchased from Aldrich. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep, Biotage, or Crawford and elution was with an Isco Companion system. ^{1}H NMR data were recorded on a Varian Gemini 2000 (300 MHz) or a Bruker Avance DPX400 (400 MHz) instrument and were determined in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference, and coupling constant (J) values are reported in hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, no. 5715) were used for TLC analysis. The purity of compounds submitted for screening was >95% as determined by UV analysis of liquid chromatography−mass spectrometry (LC−MS) chromatograms at 254 nm and substantiated using the TAC (total absorption chromatogram). Further support for the purity statement was provided using the MS TIC (total ion current) trace in ESI +ve and −ve ion modes and HRMS and NMR analysis. Solutions were dried over anhydrous magnesium sulfate, and the solvent was removed by rotary evaporation under reduced pressure.

***N*-Cyclohexyl-2-propylsulfanylpyridine-3-carboxamide (4).** Oxalyl chloride (0.332 mL, 3.81 mmol) was added dropwise to 2-(propylthio)nicotinic acid (0.751 g, 3.81 mmol) and *N*,*N*-dimethylformamide (0.014 g, 0.19 mmol) in CH_2Cl_2 (50 mL) over a period of 2 min. The resulting solution was stirred for 2 h at ambient temperature and then added dropwise to cyclohexylamine (0.755 g, 7.61 mmol) in CH_2Cl_2 (20 mL) and stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and washed sequentially with saturated $NaHCO_3$ (25 mL), water (25 mL), and saturated brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to afford crude product that was purified by flash silica chromatography, with elution gradient from 0% to 50% EtOAc in isohexane. Pure fractions were evaporated to dryness to give a yellow solid. This was triturated with Et_2O (10 mL) and then the suspension filtered to afford *N*-cyclohexyl-2-(propylthio)nicotinamide (0.435 g, 41%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (3H, t, J = 7.2), 1.02−1.38 (5H, m), 1.50−1.90 (6H, m), 3.06 (2H, t, J = 7.2), 3.60−3.76 (1H, m), 7.13 (1H, dd, J = 7.5, 4.9), 7.63 (1H, dd, J = 7.5, 1.8), 8.24 (1H, d, J = 7.8), 8.46 (1H, dd, J = 4.9, 1.8). HRMS (EI) for $C_{15}H_{23}ON_2S$ (MH^+): calcd, 279.1526; found, 279.1525.

***N*-Cyclohexyl-*N*-methyl-2-propylsulfanylpyridine-3-carboxamide (7a).** **7a** was prepared according to the procedure of **4** from *N*-methylcyclohexylamine in 16% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85−1.15 (4H, m), 1.30−1.85 (11H, m), 2.65 (1.5H, s, rotamers), 2.93 (1.5H, s), 3.01−3.21 (2.5H, m), 4.44−4.58 (0.5H, m), 6.90−6.98 (1H, m), 7.24−7.35 (1H, m), 8.33−8.40 (1H, m). HRMS (EI) for $C_{16}H_{25}ON_2S$ (MH^+): calcd, 293.1682; found, 293.1681.

***N*-(4,4-Difluorocyclohexyl)-2-propylsulfanylpyridine-3-carboxamide (7b).** 2-(Propylthio)nicotinic acid (0.130 g, 0.66 mmol), 1-hydroxybenzotriazole (0.105 g, 0.78 mmol), and *N*,*N*-diisopropylethylamine (0.314 mL, 1.80 mmol) was added to 4,4-difluorocyclohexanamine hydrochloride (0.103 g, 0.60 mmol) in CH_2Cl_2 (5 mL) followed by *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.150 g, 0.78 mmol). The resulting suspension was stirred at ambient temperature for 18 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with water (5 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to afford crude product that was purified by flash silica chromatography, with elution gradient from 0% to 10% EtOAc in CH_2Cl_2. Pure fractions were evaporated to dryness to afford *N*-(4,4-difluorocyclohexyl)-2-(propylthio)nicotinamide (0.139 g, 74%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (3H, t, J = 7.5), 1.52−1.68 (4H, m), 1.81−2.16 (6H, m),, 3.07 (2H, t, J = 7.8), 3.85−4.00 (1H, m), 7.15 (1H, dd, J = 7.5, 4.8), 7.66 (1H, dd, J = 7.5, 1.7), 8.38 (1H, d, J = 7.5), 8.48 (1H, dd, J = 4.8, 1.7). HRMS (EI) for $C_{15}H_{21}ON_2F_2S$ (MH^+): calcd, 315.1337; found, 315.1337.

***N*-Methyl-2-propylsulfanyl-*N*-tetrahydropyran-4-yl-pyridine-3-carboxamide (7c).** **7c** was prepared according to the procedure of **7b** from *N*-methyltetrahydropyran-4-amine in 66% yield. ^{1}H NMR (500 MHz, DMSO-d_6, 373 K) δ 0.97 (3H, t, J = 7.5), 1.59−1.70 (4H, m), 1.78−1.89 (2H, m), 2.78 (3H, s), 3.18 (2H, t, J = 7.5), 3.21−3.33 (3H, m), 3.86−3.96 (2H, m), 7.15 (1H, dd, J = 7.5, 4.8), 7.49 (1H, dd, J = 7.5, 1.7), 8.46 (1H, dd, J = 4.8, 1.7). HRMS (EI) for $C_{15}H_{23}O_2N_2S$(MH^+): calcd, 295.1475; found, 295.1474.

***N*-[(1*R*,3*S*)-5-Hydroxy-2-adamantyl]-2-propylsulfanylpyridine-3-carboxamide (7d).** **7d** was prepared according to the procedure of **7b** from (1*R*,3*S*)-5-hydroxy-2-adamantylamine in 45% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (3H, t, J = 7.8), 1.27−1.37 (2H, m), 1.53−1.76 (8H, m), 1.90−2.11 (5H, m), 3.08 (2H, t, J = 7.8), 3.87−3.95 (1H, m), 4.39 (1H, s), 7.10−7.17 (1H, m), 7.59−7.66 (1H, m), 8.20 (1H, d, J = 6.9), 8.43−8.50 (1H, m). HRMS (EI) for $C_{19}H_{27}O_2N_2S$(MH^+): calcd, 347.1788; found, 347.1785.

***N*-[(1*R*,3*S*)-5-Hydroxy-2-adamantyl]-*N*-methyl-2-propylsulfanylpyridine-3-carboxamide (7e).** **7e** was prepared according to the procedure of **7b** from (1*R*,3*S*)-*N*-methyl-5-hydroxy-2-adamantylamine in 20% yield. ^{1}H NMR (300 MHz, DMSO-d_6, 373 K) δ 0.98 (3H, t, J = 7.2), 1.46−1.56 (2H, m), 1.59−1.73 (8H, m), 1.93−2.04 (2H, m), 2.09−2.18 (1H, m), 2.32−2.40 (2H, m), 3.00 (3H, s), 3.18 (2H, t, J = 7.8), 3.98 (1H, s), 4.00−4.04 (1H, m), 7.14 (1H, dd, J = 7.5, 4.9), 7.49 (1H, dd, J = 7.5, 1.8), 8.45 (1H, dd, J = 4.9, 1.8). HRMS (EI) for $C_{20}H_{29}O_2N_2S$(MH^+): calcd, 361.1944; found, 361.1943.

***N*-[(1*R*,3*R*)-5-Hydroxy-2-adamantyl]-*N*-methyl-2-propylsulfanylpyridine-3-carboxamide (7f).** **7f** was prepared according to the procedure of **7b** from (1*R*,3*R*)-*N*-methyl-5-hydroxy-2-adamantylamine in 16% yield. ^{1}H NMR (300 MHz, DMSO-d_6, 373 K) δ 0.98 (3H, t, J = 7.2), 1.51−1.73 (10H, m), 1.92−2.09 (3H, m), 2.39−2.48 (2H, m), 2.94 (3H, s), 3.18 (2H, t, J = 7.8), 3.95 (1H, s), 4.02−4.06 (1H, m), 7.14 (1H, dd, J = 7.5, 4.9), 7.49 (1H, dd, J = 7.5, 1.8), 8.45 (1H, dd, J = 4.9, 1.8). HRMS (EI) for $C_{20}H_{29}O_2N_2S$(MH^+): calcd, 361.1944; found, 361.1943.

(2-Propylsulfanyl-3-pyridyl)-[2-(4-pyridyl)pyrrolidin-1-yl]-methanone (7g). **7g** was prepared according to the procedure of **7b** from 4-pyrrolidin-2-ylpyridine in 62% yield. ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers in a 2:1 ratio) δ 0.97 (3H, dt), 1.51−1.99 (5H, m), 2.32−2.57 (1H, m), 2.98−3.11 (0.67H, m), 3.19 (1.34H, t), 3.27−3.41 (0.67H, m), 3.48−3.56 (0.67H, m), 3.77 (0.67H, t), 4.72 (0.34H, dd), 5.14 (0.67H, dd), 6.86 (0.34H, dd), 6.98 (0.67H, d), 7.17−7.26 (1H, m), 7.41 (1.34H, d), 7.74 (0.67H, dd), 8.31 (0.34H, dd), 8.33−8.38 (0.67H, m), 8.50−8.56 (2H, m). HRMS (EI) for $C_{18}H_{22}ON_3S$(MH^+): calcd, 328.1478; found, 328.1477.

4-[(2-Propylsulfanylpyridine-3-carbonyl)amino]-cyclohexanecarboxylic Acid (7h). Oxalyl chloride (0.496 g, 3.91 mmol) was added dropwise to 2-(propylthio)nicotinic acid (0.154 g, 0.78 mmol) and *N*,*N*-dimethylformamide (6.05 μL, 0.08 mmol) in CH_2Cl_2 (5 mL). The resulting solution was stirred for 2 h at ambient temperature and then concentrated under reduced pressure and azeotroped with toluene (10 mL). The residue was suspended in THF (1 mL) and added to a solution of (1*S*,4*S*)-4-aminocyclohexanecarboxylic acid (0.112 g, 0.78 mmol) in 1 M NaOH in water (1.56 mL, 1.56 mmol) simultaneously with 1 M NaOH in water (0.78 mL, 0.78 mmol). The reaction mixture was stirred at ambient temperature overnight. The pH was adjusted to 5 with 1 M hydrochloric acid, and all volatiles were removed under reduced pressure. The resultant solid was triturated with water (10 mL), filtered and the solid washed with water (5 mL), Et_2O (5 mL), then isohexane (5 mL) to afford (1*S*,4*S*)-[(2-propylsulfanylpyridine-3-carbonyl)amino]cyclohexanecarboxylic acid (0.137 g, 54%) as an off white solid. ^{1}H NMR (300 MHz, DMSO-d_6, 373 K) δ 0.98 (3H, t, J = 7.3), 1.55−1.75 (8H, m), 1.84−2.04 (2H, m), 2.35−2.45 (1H, m), 3.13 (2H, t, J = 7.2), 3.80−3.96 (1H, m), 7.10 (1H, dd, J = 7.5, 4.8), 7.62 (1H, dd, J = 7.5, 1.7), 7.82−7.98 (1H, m), 8.44 (1H, dd, J = 4.8, 1.7), 11.56 (1H, s). HRMS (EI) for $C_{16}H_{23}O_3N_2S$(MH^+): calcd, 323.1424; found, 323.1423.

2-[(3*R*)-1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]-3-piperidyl]acetic Acid (11h) [General Procedure for SNAr and Ester Hydrolysis]. 6-Chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** (1.88 g, 6.03 mmol), methyl-(*R*)-3-piperidine acetate hydrochloride (1.17 g, 6.03 mmol), K_2CO_3 (2.50 g, 18.08 mmol), and butyronitrile were mixed in a microwave tube and stirred at 170 °C for 2 h. The solvent was evaporated under reduced pressure. Then water (20 mL) was added and the product was extracted with

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EtOAc (2 × 40 mL), washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give an orange oil. Purification by flash column chromatography (SiO_2, eluent gradient from 0% to 50%, hexane/EtOAc) afforded a slightly yellow oil which crystallized to give a white solid (1.44 g, 55%). The solid was dissolved in THF (20 mL), and water was added (10 mL) followed by LiOH (281 mg). The mixture was stirred at room temperature for 3 h. The solution was acidified with 2 N HCl between pH 4 and pH 5 and the product extracted in EtOAc (2 × 40 mL). The solution was washed with brine (10 mL), dried over $MgSO_4$ and the organic phase evaporated under reduced pressure to give a white solid (1.38 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.6), 1.18−1.48 (6H, m), 1.51−1.65 (2H, m), 1.68−1.78 (5H, m), 1.89−2.13 (4H, m), 2.25−2.37 (2H, m), 2.81 (1H, dd, J = 10.0, 13.1), 2.95−3.21 (3H, m), 3.92−4.05 (1H, m), 4.16−4.32 (2H, m), 6.36 (1H, d, J = 8.8), 6.57 (1H, d, J = 7.7), 7.80 (1H, d, J = 8.8). HRMS (EI) for $C_{22}H_{34}O_3N_3S$ (MH^+): calcd, 420.2314; found, 420.2317.

4-[[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]amino]cyclohexane-1-carboxylic Acid (11a). **11a** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and *E*-4-aminocyclohexylcarboxylic acid methyl ester hydrochloride in 48% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (3H, t, J = 7.2), 1.15 (2H, m), 1.24 −1.30 (6H, m), 1.37−1.44 (2H, m), 1.57−1.66 (3H, m), 1.70−1.78 (4H, m), 1.95−2.03 (4H, m), 2.15−2.25 (1H, m), 2.97 (2H, t, J = 7.2), 3.64 (1H, s), 3.71−3.77 (1H, m), 6.14 (1H, d, J = 8.8), 7.48 (1H, d, J = 8.8), 7.63 (1H, d, J = 7.2). HRMS (EI) for $C_{22}H_{34}O_3N_3S$ (MH^+): calcd, 420.2315; found, 420.2314.

4-[[[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]amino]methyl]cyclohexane-1-carboxylic Acid (11b). **11b** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and aminomethylcyclohexane carboxylic acid methyl ester hydrochloride in 33% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94−0.99 (5H, m), 1.10−1.22 (1H, m), 1.23−1.40 (6H, m), 1.53−1.69 (4H, m), 1.71−1.81 (6H, m), 1.93−2.01 (2H, m), 2.10−2.16 (1H, m), 2.94−2.96 (2H, t, J = 7.2), 3.16 (2H, d, J = 6.4), 3.55−3.65 (1H, m), 6.14 (1H, d, J = 8.4), 7.15 (1H, s), 7.47 (1H, d, J = 8.4), 7.69 (1H, d, J = 8.0), 12.20 (1H, s). HRMS (EI) for $C_{23}H_{36}O_3N_3S$ (MH^+): calcd, 434.2471; found, 434.2468.

2-[4-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]oxyphenyl]acetic Acid (11c). **11c** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and methyl 4-hydroxyphenylacetate in 38% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73 (t, 3H, J = 7.5), 1.14−1.50 (m, 7H), 1.55−1.81 (m, 3H), 1.95−2.07 (m, 2H), 2.75 (t, 2H, J = 7.5), 3.66 (s, 2H), 3.91−4.07 (m, 1H), 6.33 (d, 1H, J = 7.8), 6.62 (d, 1H, J = 8.4), 7.08 (d, 2H, J = 8.4), 7.31 (d, 2H, J = 8.4), 7.92 (d, 1H, J = 8.1). MS m/z (M^+ + H) 429. HRMS (EI) for $C_{23}H_{29}O_4N_2S$ (MH^+): calcd, 429.1842; found, 429.1841.

2-[3-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]oxyphenyl]acetic Acid (11d). **11d** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and methyl 3-hydroxyphenylacetate in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 (t, 3H, J = 7.2), 1.14−1.49 (m, 7H), 1.56−1.66 (m, 1H), 1.68−1.80 (m, 2H), 1.95−2.06 (m, 2H), 2.74 (t, 2H, J = 7.2), 3.65 (s, 2H), 3.92−4.06 (m, 1H), 6.35 (d, 1H, J = 7.2), 6.62 (d, 1H, J = 8.4), 7.01−7.09 (m, 2H), 7.14 (d, 1H, J = 7.6), 7.35 (t, 1H, J = 8.0), 7.91 (d, 1H, J = 8.0). HRMS (EI) for $C_{23}H_{29}O_4N_2S$(MH^+): calcd, 429.1842; found, 429.1841.

1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]piperidine-2-carboxylic Acid (11e). **11e** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and methyl 3-hydroxyphenylacetate in 45% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (3H, t, J = 7.2), 1.08−1.13 (1H, m), 1.21−1.33 (4H, m), 1.41−1.47 (1H, m), 1.52−1.67 (4H, m), 1.68−1.80 (4H, m), 2.19−2.28 (2H, m), 2.91 (2H, t, J = 7.2), 3.09−3.17 (2H, m), 3.60−3.66 (2H, m), 4.11 (1H, s), 5.02 (1H, s), 6.42 (1H, d, J = 8.8), 7.59 (1H, d, J = 8.8), 7.78 (1H, d, J = 7.6). HRMS (EI) for $C_{21}H_{32}O_3N_3S$(MH^+): calcd, 406.2159; found, 406.2157.

1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]piperidine-3-carboxylic Acid (11f). **11f** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and ethyl nipecotate in 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (3H, t, J = 7.2), 1.11−1.37 (6H, m), 1.46−1.57 (2H, m), 1.65−1.78 (5H, m), 1.92−1.97 (2H, m), 2.03−2.07 (1H, m), 2.50−2.57 (1H, m), 3.02−3.12 (3H, m), 3.42 (1H, q, J = 7.2), 3.88−3.95 (1H, m), 4.03 (1H, m), 4.36−4.41 (1H, m), 6.33 (1H, d, J = 8.8), 6.49 (1H, d, J = 7.6), 7.73 (1H, d, J = 8.8). HRMS (EI) for $C_{21}H_{32}O_3N_3S$(MH^+): calcd, 406.2159; found, 406.2158.

1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]piperidine-4-carboxylic Acid (11g). **11g** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and methyl isonipecotate in 19% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (3H, t, J = 7.6), 1.05−1.35 (5H, m), 1.43−1.93 (10H, m), 2.44−2.60 (2H, m), 2.94 (2H, t, J = 7.2), 2.99−3.10 (2H, m), 3.58−3.71 (1H, m), 4.21−4.31 (2H, m), 6.53 (1H, d, J = 8.8), 7.62 (1H, d, J = 8.8), 7.81 (1H, d, J = 7.6), 12.28 (1H, s). HRMS (EI) for $C_{21}H_{32}O_3N_3S$ (MH^+): calcd, 406.2159; found, 406.2157.

2-[(3*S*)-1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]-3-piperidyl]acetic Acid (11i). **11i** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and methyl-(*S*)-3-piperidine acetate hydrochloride in 50% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95 (3H, t, J = 7.6), 1.05−1.18 (1H, m), 1.15−1.35 (5H, m), 1.41−1.49 (1H, m), 1.55−1.90 (10H, m), 2.12−2.26 (2H, m), 2.71−2.77 (1H, m), 2.85−3.01 (3H, m), 3.63−3.66 (1H, m), 4.15−4.34 (2H, m), 6.47 (1H, d, J = 8.8), 7.61 (1H, d, J = 8.8), 7.79 (1H, d, J = 7.9), 12.15 (1H, s). HRMS (EI) for $C_{22}H_{34}O_3N_3S$ (MH^+): calcd, 420.2314; found, 420.2317.

2-[1-[5-(Cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]-4-piperidyl]acetic Acid (11j). **11j** was prepared according to the procedure of **11h** from 6-chloro-*N*-cyclohexyl-2-propylsulfanylpyridine-3-carboxamide **10** and 2-(piperidin-4-yl)acetic acid ethyl ester in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (3H, t, J = 7.6), 1.11−1.42 (7H, m), 1.49−1.59 (1H, m), 1.62−1.71 (4H, m), 1.77−1.81 (2H, m), 1.91−2.08 (3H, m), 2.19−2.29 (2H, m), 2.83−2.90 (2H, m), 3.06 (2H, t, J = 7.6), 3.92 (1H, m), 4.24−4.33 (2H, m), 6.29 (1H, d, J = 8.8), 6.47 (1H, d, J = 7.6), 7.72 (1H, d, J = 8.8). HRMS (EI) for $C_{22}H_{34}O_3N_3S$(MH^+): calcd, 420.2314; found, 420.2312.

2-[(3*S*)-1-[5-(Cyclohexylcarbamoyl)-6-propoxypyridin-2-yl]-3-piperidyl]acetic Acid (13a). 6-Chloro-*N*-cyclohexyl-2-propoxynicotinamide **12a** (211 mg, 0.71 mmol), methyl-(*S*)-3-piperidine acetate hydrochloride (276 mg,1.42 mmol), and potassium carbonate (393 mg, 2.84 mmol) were stirred in butyronitrile (5 mL). The mixture was sealed and heated in a microwave (Biotage initiator, 300 W) at 170 °C for 4 h. The mixture was diluted with dichloromethane (25 mL), washed with water (20 mL), brine (20 mL), and dried over $MgSO_4$, then filtered and the solvent removed in vacuo. Chromatography SiO_2, eluting with ethyl acetate/isohexane 20−60%, gave a clear gum (215 mg). The gum was dissolved in MeOH (15 mL). Then 2 M NaOH (540 μL, 1.07 mmol) was added and the mixture was stirred for 32 h. Solvent was removed in vacuo and the residue was taken up in water (10 mL) and pH adjusted to ∼3 to give 2-[(3*S*)-1-[5-(cyclohexylcarbamoyl)-6-propoxypyridin-2-yl]-3-piperidyl]acetic acid (167 mg, 81%) as a white powder after trituration with ether/isohexane. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02 (3H, t, J = 7.6), 1.15−1.90 (17H, m), 2.13−2.26 (2H, m), 2.77−2.83 (1H, m), 2.93−3.05 (1H, m), 3.71−3.83 (1H, m), 4.07−4.18 (1H, m), 4.23−4.35 (3H, m), 6.43 (1H, d, J = 8.8), 7.70 (1H, d, J = 7.6), 8.00 (1H, d, J = 8.8), 12.17 (1H, s). HRMS (EI) for $C_{22}H_{34}O_4N_3$ (MH^+): calcd, 404.2544; found, 404.2542.

2-[(3*S*)-1-[5-(Cyclohexylcarbamoyl)-6-propylaminopyridin-2-yl]-3-piperidyl]acetic Acid (13b). 6-Chloro-*N*-cyclohexyl-2-(propylamino)nicotinamide **12b** (180 mg, 0.61 mmol), methyl-(*S*)-3-piperidine acetate hydrochloride (148 mg, 0.76 mmol), potassium carbonate (210 mg, 1.52 mmol) in butyronitrile (4 mL) were sealed in

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a microwave tube and heated (Biotage Initiator 300 W) at 150 °C for a total of 8 h. The mixture was diluted with water (20 mL) and extracted with dichloromethane (2 × 20 mL). The combined extracts were washed with brine (20 mL), dried over $MgSO_4$, then filtered, and the solvent was removed in vacuo. Chromatography (SiO_2), eluting with ethyl acetate/isohexane 0−40%, gave a clear gum (158 mg). The gum was stirred in methanol (3 mL), and 2 M sodium hydroxide (475 μL, 0.95 mmol) was added. The mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue taken up in water (10 mL) and acidified to pH ≈ 3 with 2 M HCl. The resulting precipitate was filtered, washed with water, and dried to give 2-[(3*S*)-1-[5-(cyclohexylcarbamoyl)-6-propylaminopyridin-2-yl]-3-piperidyl]-acetic acid as a white solid (126 mg, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (3H, t, *J* = 7.6), 1.03−1.95 (17H, m), 2.10−2.24 (2H, m), 2.65−2.75 (1H, m), 2.84−2.96 (1H, m), 3.27 (2H, t, *J* = 6.7), 3.60−3.76 (1H, m), 4.10−4.28 (2H, m), 5.92 (1H, d, *J* = 8.8), 7.58 (1H, d, *J* = 7.6), 7.75 (1H, d, *J* = 8.8), 8.76 (1H, bs), 12.12 (1H, bs); LRMS *m*/*z* (M^+ + H) 403.

2-[(3*S*)-1-[5-(Cyclohexylcarbamoyl)-6-(methylpropylamino)-pyridin-2-yl]-3-piperidyl]acetic Acid (13c). **13c** was prepared according to the procedure of **13b** from 6-chloro-*N*-cyclohexyl-2-[methyl(propyl)amino]nicotinamide **12c** and methyl-(*S*)-3-piperidine acetate hydrochloride in 33% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82 (3H, t, *J* = 7.6), 1.08−1.81 (17H, m), 2.10−2.22 (2H, m), 2.64−2.71 (1H, m), 2.76 (3H, s), 2.83−2.93 (1H, m), 3.14−3.25 (2H, m), 3.63−3.68 (1H, m), 4.05−4.25 (2H, m), 6.18 (1H, d, *J* = 8.4), 7.49 (1H, d, *J* = 8.4), 8.22 (1H, d, *J* = 8.4), 12.16 (1H, s). HRMS (EI) for $C_{23}H_{37}O_3N_4(MH^+)$: calcd, 417.2860; found, 417.2858.

2-[(3*S*)-1-[6-Butyl-5-(cyclohexylcarbamoyl)pyridin-2-yl]-3-piperidyl]acetic Acid (14). 2-Butyl-6-chloro-*N*-cyclohexylnicotinamide **20** (310 mg, 1.05 mmol), methyl-(*S*)-3-piperidine acetate hydrochloride (500 mg, 2.58 mmol), and potassium carbonate (436 mg, 3.15 mmol) were stirred in butyronitrile (20 mL) under nitrogen at 150 °C for 96 h. Solvent was removed. The residue was dissolved in CH_2Cl_2 (20 mL), and water (20 mL) was added. The mixture was acidified, and the organic layer was separated. The aqueous layer was extracted with further dichloromethane (2 × 20 mL). The combined extracts were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. Chromatography (SiO_2), eluting with 5% MeOH/EtOAc 0−100%, gave 2-[(3*S*)-1-[6-butyl-5-(cyclohexylcarbamoyl)pyridin-2-yl]-3-piperidyl]acetic acid (106 mg, 25%) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (3H, t, *J* = 7.6), 1.02−1.48 (10H, m), 1.55−1.88 (9H, m), 2.11−2.27 (2H, m), 2.65−2.79 (3H, m), 2.84−2.94 (1H, m), 3.61−3.73 (1H, m), 4.13−4.26 (2H, m), 6.57 (1H, d, *J* = 8.8), 7.42 (1H, d, *J* = 8.8), 7.84 (1H, d, *J* = 8.0), 12.07 (1H, s). HRMS (EI) for $C_{23}H_{36}O_3N_3(MH^+)$: calcd, 402.2751; found, 402.2749.

Measurement of 11β-HSD1 Activity. The conversion of cortisone to the active steroid cortisol by 11β-HSD1 oxo-reductase can be measured using a cortisol competitive homogeneous time-resolved fluorescence assay (HTRF) assay (CisBio International, R&D, Administration and Europe Office, In Vitro Technologies, HTRF/Bioassays BP 84175, 30204 Bagnols/Cèze Cedex, France). The evaluation of compounds was carried out using a baculovirus expressed N terminal 6-His tagged full length human, rat, dog, or cynomolgous monkey 11β-HSD1 enzyme. The enzyme was purified from a detergent solubilized cell lysate, using a copper chelate column. Inhibitors of 11β-HSD1 reduce the conversion of cortisone to cortisol, which is identified by an increase in signal, in the above assay. The assay incubation was carried out in black 384-well plates (Matrix, Hudson, NH, U.S.), consisting of cortisone (Sigma, Poole, Dorset, U.K., 160 nM), glucose 6-phosphate (Roche Diagnostics, 1 mM), NADPH (Roche Diagnostics, 100 μM), glucose 6-phosphate dehydrogenase (Roche Diagnostics, 12.5 μg/mL), EDTA (Sigma, Poole, Dorset, U.K., 1 mM), assay buffer (K_2HPO_4/KH_2PO_4, 100 mM), pH 7.5, recombinant 11β-HSD1 (1.5 μg/mL) plus test compound in a total volume of 20 μL. The assay plates were incubated for 25 min at 37 °C, and the reaction was stopped by the addition of 10 μL of 0.5 mM glycerrhetinic acid (Sigma) plus cortisol-XL665. Then 10 μL of anti-cortisol cryptate was added, and the plates were incubated for 2 h at room temperature. Following excitation at 320 nm, fluorescence at 665 and 620 nm was measured and the (665 nm)/(620 nm) ratio calculated using an Envision plate reader. This data were then used to calculate IC_{50} values for each compound (Origin 7.5, Microcal software, Northampton, MA, U.S.).

Measurement of 11-βHSD1 Activity in Isolated Human Adipocytes. Subcutanesous (sc) adipose tissue was obtained through a needle biopsy from the lower part of the abdomen after dermal local anesthesia with lidocaine (Xylocain; AstraZeneca, Södertälje, Sweden) from nondiabetic volunteers. Adipocytes were isolated from sc adipose tissue by collagenase digestion. In brief, adipocytes were isolated from the adipose tissue following shaking in medium 199 (Invitrogen, U.K.) supplemented with 5.6 mmol/L glucose, 4% BSA (Sigma-Aldrich, Poole, U.K.), and 0.6 mg/mL collagenase (Roche, Burgess Hill, U.K.) at 37 °C for ∼60 min. After filtration through a 250 μm nylon mesh the adipocytes were washed 4 times with medium 199 supplemented with 5.6 mmol/L glucose and 1% BSA. Isolated adipocytes were diluted to approximately 3−5% lipocrit containing DMEM (6 mmol/L glucose, 10% FCS (Invitrogen, UK), 1% penicillin/streptomycin (Invitrogen, U.K.)) and incubated in six-well plates in duplicate for 6 h with ^{3}H-cortisone (20 nmol/L, 1 μCi/mL, Amersham, Chalfont St. Giles, U.K.) containing DMEM (6 mmol/L glucose, 100 nmol/L cortisone (Sigma-Aldrich, Poole, U.K.), 10% FCS, 1% PEST) at 37 °C, 5% CO_2. Following incubations, media samples were collected and cortisone to cortisol conversion was analyzed. Radiolabeled steroids were extracted using ethyl acetate. Samples were evaporated to dryness under nitrogen and resuspended in mobile phase for HPLC analysis (methanol/H_2O, 50:50), adapted from Napolitano et al.[39] Radiolabeled steroids were separated using reversed phase HPLC, Agilent 1200 HPLC instrument using a Kromasil C18 5 μm column, 4.6 mm × 25 0 mm (Crawford Scientific, Lanarkshire, U.K.) with methanol/H_2O (50:50) at a flow rate of 1.5 mL/min. Radioactivity was measured using a flow scintillation analyzer (Radiomatic series 500TR, Perkin-Elmer Analytical Instruments) with FLO-ONE software.

Measurement of Inhibition of 11β-HSD1 Activity in the Mouse. Male C57Bl6J mice maintained on a chow diet were dosed by oral gavage with compound suspended in HPLC/Tween. At 1 h after dose, animals were euthanized using a rising concentration of CO_2. Blood samples were taken via cardiac puncture for compound determination, and epididymal fat pads were removed and snap-frozen in liquid N_2. Adipose tissue (50−100 mg) was cut into 2−3 mm^3 pieces using scissors and incubated in 24-well plate with medium (DMEM Ham F12 medium supplemented with 1% penicillin/streptomycin/ampicillin and 10% FCS) containing 20 nM ^{3}H-cortisone for 1 h at 37 °C. Following incubation, medium was removed and steroid extracted and HPLC performed as per adipocyte assay.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the syntheses of intermediates and crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS USED

11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; ABT, 1-aminobenzotriazole; AG, acylglucuronide; Clp, plasma clearance; CYP, cytochrome P450; DMPK, drug metabolism and pharmacokinetics; EDAC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; HDL, high density lipoprotein; HOBT, *N*-hydroxybenzotriazole; LLE, ligand lipophilicity efficiency; MDCK, Madin–Darby canine kidney cell line; NAD, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; PPB, plasma protein binding; SAR, structure–activity relationship

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