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Modulation of 11β-hydroxysteroid dehydrogenase type 1 activity by 1,5-substituted 1*H*-tetrazoles

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ABSTRACT

Inhibitors of 11β-hydroxysteroid dehydrogenase (11β-HSD1) show promise as drugs to treat metabolic disease and CNS disorders such as cognitive impairment. A series of 1,5-substituted 1*H*-tetrazole 11β-HSD1 inhibitors has been discovered and chemically modified. Compounds are selective for 11β-HSD1 over 11β-HSD2 and possess good cellular potency in human and murine 11β-HSD1 assays. A range of in vitro stabilities are observed in human liver microsome assays.

Increased circulating levels of the glucocorticoid hormone cortisol (Cushing's syndrome) causes central obesity, hyperglycaemia, dyslipidaemia and hypertension by acting in key metabolic tissues, including liver and adipose; and causes depression and cognitive impairments by acting in the CNS.[1] Elevated cortisol is thought to be important in metabolic syndrome and in age-related cognitive impairment.[2–5]

Glucocorticoid activity at the tissue level is modulated by the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, which interconvert inactive cortisone and cortisol (or dehydrocorticosterone and corticosterone in rodents), and thus control access of glucocorticoids to intra-cellular receptors.[6,7] Two isoforms of 11β-HSD are known. In the periphery, 11β-HSD1 is present predominantly in the liver and adipose tissue, while in the brain it is found mainly in the hippocampus and cortex, where it catalyses predominant reductase activity, regenerating glucocorticoids. 11β-HSD2, which catalyses the reverse reaction, is expressed mainly in the kidney where it protects mineralocorticoid receptors from glucocorticoid activation. Dysregulation of the 11β-HSDs is also thought important in metabolic syndrome.[2]

In mice, global knockout of 11β-HSD1 leads to enhanced hepatic insulin sensitivity and reduced gluconeogenesis and glycogenolysis, suggesting that inhibitors of 11β-HSD1 may be useful for the treatment of type 2 diabetes.[8] These mice also have low serum triglycerides and increased HDL cholesterol and apo-lipoprotein A1 levels, suggesting that 11β-HSD1 inhibition may prevent atherosclerosis.[9] 11β-HSD1 knockout mice are also protected against age-related cognitive impairment, suggesting that inhibitors may be useful in the treatment of diseases characterised by cognitive dysfunction, such as Alzheimer's disease.[10]

Substantial activity in the pharmaceutical industry has led to the discovery of numerous classes of non-steroidal 11β-HSD1 inhibitors.[11–13] These include triazoles such as compound 544 (**1**), sulphonamides like PF-915275 (**2**), adamantyl carboxamides (**3**) and thiazolones such as BVT116429 (**4**) (Fig. 1).[14–17] Compound **1** has been shown to produce beneficial effects in animal models of atherosclerosis and of type 2 diabetes, with lowering of plasma triglycerides, glucose, rate of body weight gain and food intake.[18] At high doses compound **4** lowers fasting plasma glucose in Ldlr 3KO mice, while compound **2** has been shown to alter plasma insulin levels in cynomolgus monkeys.[19,20]

We have discovered and modified a series of 1,5-substituted 1*H*-tetrazoles that display selective inhibition of 11β-HSD1 in vitro. Compounds were tested for inhibition in mammalian cells stably transfected with human 11β-HSD1 or human 11β-HSD2 using a scintillation proximity assay (SPA) at a 20 nM substrate concentration.[21] Selected compounds were also tested in cells expressing murine 11β-HSD1.

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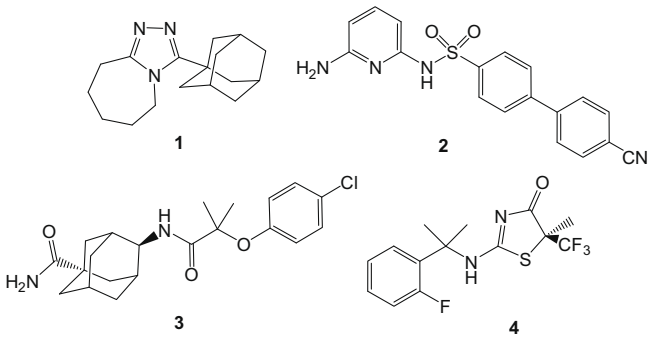


Figure 1. Selected non-steroidal 11β-HSD1 inhibitors.

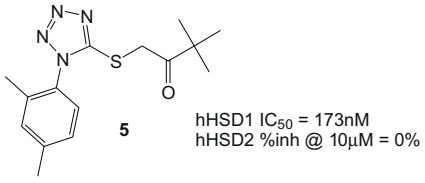


Figure 2. Initial hit from the primary in vitro screen.

An 11β-HSD1 inhibitor containing a 5-sulphanyl 1*H*-tetrazole moiety was identified following a primary in vitro compound screen on a selection of putative 11β-HSD1 inhibitors identified from a pharmacophore field-based in silico screen.[22,23] Compound **5** displays sub-micromolar potency in cellular assays and is selective for 11β-HSD1 (Fig. 2).

We initially sought to explore SAR at the 1-position of the tetrazole and at the position adjacent to the carbonyl group of the 5-thioethanone moiety. A number of compounds were synthesised according to Scheme 1. A selection of 1-substituted 1*H*-tetrazole-5-thiols were reacted with a range of acylbromides in the presence of sodium methoxide to yield compounds **6a**–**w**.

Compound **5** was treated with oxone in aqueous methanol to yield the sulphoxide **7** and the sulphone **8** (Scheme 1).

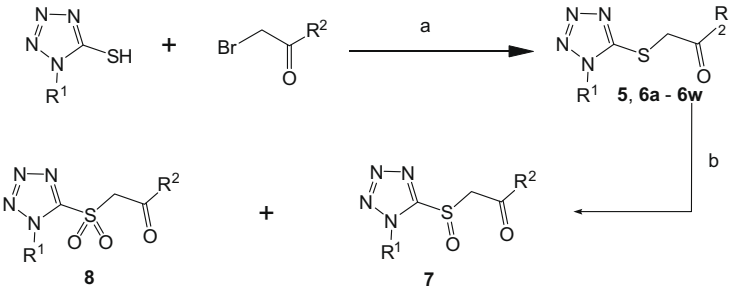
A number of analogues were generated by forming the tetrazole moiety from a series of 4-oxo-butyramide derivatives as shown in Scheme 2A. A series of isomeric tetrazoles was also generated by forming the 'reverse' amide from benzoyl chloride and 3-amino-1-phenylpropan-1-one, followed by reaction with azidotrimethyl silane (Scheme 2B).

A 1-phenyl-1*H*-tetrazol-5-oxy linked compound was prepared by reacting 5-chloro-1-phenyl-1*H*-tetrazole with 2-hydroxyacetophenone (Scheme 2C).

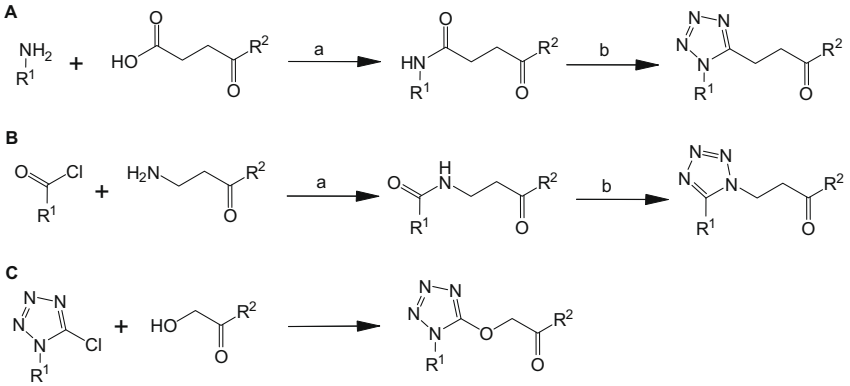
Our next goal was to explore SAR in the sulphanyl tetrazole series. The results indicated that a hydrophobic group directly linked to the ketone, such as *tert*-butyl, benzyl and phenyl, conferred good potency suggesting that this part of the scaffold occupies a lipophilic binding site within the enzyme. Substitution on the phenyl group with fluorine or methoxy groups (compounds **6a**–**g**) was generally detrimental to good cellular potency (Table 1). However, in contrast to mono substitution, 3,4-dimethoxy substitution conferred approximately 10-fold greater potency (compound **6h**). In general compounds were highly selective for 11β-HSD1 over 11β-HSD2, although the heterocyclic analogues, **6j** and **6k**, inhibited 11β-HSD2 substantially and were not progressed further.

Since the 3,4-dimethoxy phenyl group conferred good potency when combined with phenyl at the 1-position of the central tetrazole, a series of 3,4-dimethoxy phenyl analogues was prepared according to Scheme 1 in an effort to improve potency.

Only marginal increases in potency relative to compound **6h** were achieved within the group of compounds tested. Replacement of the 1-phenyl group with alkyl groups such as *tert*-butyl

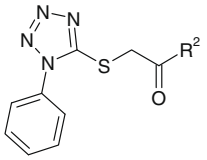


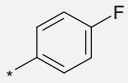
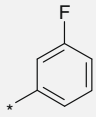
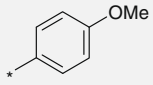
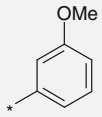
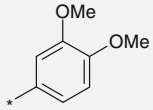
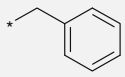
Scheme 1. Reagents and conditions: (a) MeOH, NaOH, 60 °C, 4 h; (b) MeOH, $KHSO_5$, rt, 18 h.



Scheme 2. Reagents and conditions: (A) a—DCM, HATU, DIPEA, rt, 20 min; b—DCM, PCl_5, N_2, rt, 15 min then TMS-N_3, rt, 18 h; (B) a—DCM, DIPEA, rt, 1 h; b—DCM, PCl_5, N_2, rt, 15 min then TMS-N_3, rt, 48 h; (C) DMF, NaH, rt, N_2, rt, 1 h.

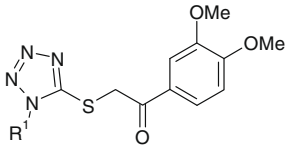
Table 1
Human 11β-HSD1 and 11β-HSD2 inhibition for compounds **6a**–**k**

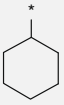
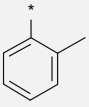
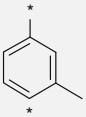
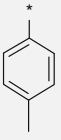
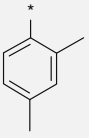
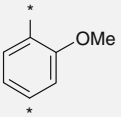
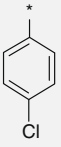
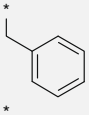
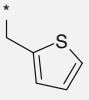


Compound	R^2	hHSD1 IC_{50}, nM	hHSD2 %inh at 10 μM
6a		14,500	nd
6b		252	9
6c		257	7
6d		1300	nd
6e		1340	0
6f		1050	38
6g		1600	nd
6h		116	nd
6i		289	18
6j		485	69
6k		1700	100

nd, not determined.

Table 2
Human 11β-HSD1 and 11β-HSD2 inhibition for compounds **6l**–**w**



Compound	R^1	hHSD1 IC_{50}, nM	hHSD2 %inh at 10 μM
6l		2180	0
6m		954	nd
6n		1660	0
6o		183	0
6p		1450	28
6q		93	nd
6r		2450	0
6s		1000	3
6t		1600	nd
6u		1040	nd
6v		860	0
6w		1700	0

nd, not determined.

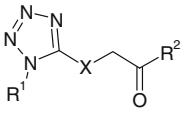
and isopropyl led to a loss in potency, while substitution at the *ortho*- and *para*-positions of the 1-phenyl group was generally detrimental to potency (Table 2). Only methyl substitution at the *meta*-position led to a minor increase in potency suggesting that the 1-phenyl group occupies a confined binding pocket within the enzyme.

The microsomal stability of several 5-sulphanyl-tetrazole analogues was assessed in vitro using human liver microsomes (data not shown). The results suggested that microsomal stability was poor in this series. To investigate whether the sulphanyl group represented the main metabolically labile group, we oxidised the sulphanyl group and isolated both the sulphoxide and sulphone derivatives of compound **5**. We also targeted oxygen, nitrogen and carbon replacements of the sulphur atom linked to the 5-position of the tetrazole (Scheme 2).

The results from our initial studies showed that the ether, sulphone and sulphoxide analogues possessed lower potency than the corresponding sulphanyl analogues (Table 3). In addition, microsomal stability was lower for these compounds. In contrast, replacement of the 5-sulphanyl group with methylene led to only a small drop in potency and a large increase in microsomal stability. These results suggested that carbon-linked tetrazoles offered an improved profile for in vivo dosing and further optimization.

Compound **11** possessed both moderate potency and high microsomal stability. Replacement of the 1-phenyl group with cycloalkyl groups such as cyclohexyl or adamantyl resulted in a loss of potency (Table 4). Substitution on the 1-phenyl ring at the *ortho*- and *para*-positions generally lowered potency. However,

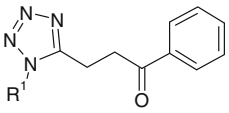
Table 3
Human 11β-HSD1 and liver microsomal stability for compounds **6c** and **7**–**11**

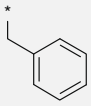
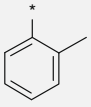
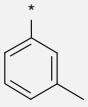
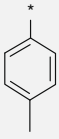
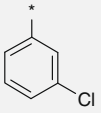
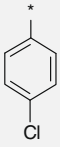
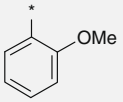
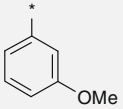
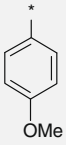
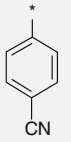
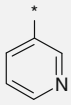
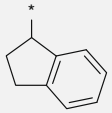
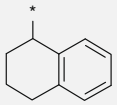


Compound	X	R^1	R^2	hHSD1 IC_{50}, nM	HLM[a] % parent
6c	S	Ph	Ph	257	34
7	S(=O)	Ph	*t*-Bu	1230	25
8	$S(=O)_2$	Ph	*t*-Bu	943	4
9	O	Ph	Ph	971	4
10	NMe	Ph	Ph	Inactive	nd
11	CH_2	Ph	Ph	359	88

[a] Percentage parent remaining after 30 min incubation with human liver microsomes (HLM).

Table 4
Human 11β-HSD1 and liver microsomal stability for compounds **12a**–**r**



Compound	R^1	hHSD1 IC_{50}, nM	HLM[a] % parent
12a		2100	33
12b		683	22
12c		948	2
12d		588	2
12e		114	52
12f		575	34
12g		2200	42
12h		304	6
12i		426	12
12j		686	37
12k		3900	81
12l		1500	1
12m		1100	7
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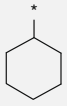
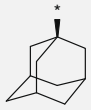
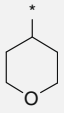
12o		987	nd
12p		915	14
12q		1500	nd
12r		2400	nd

nd, not determined.
[a] Percentage parent remaining after 30 min incubation with human liver microsomes (HLM).

the introduction of a *meta*-chloro group increased cellular potency approximately 3-fold relative to compound **11**. This compound (**12e**) also possessed moderate microsomal stability.

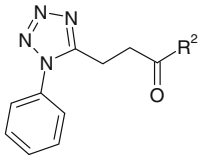
The SAR adjacent to the carbonyl group of compound **11** was also investigated. The majority of substitutions on the phenyl ring failed to improve either the potency or the microsomal stability relative to the unsubstituted compound, with only the *meta*-methoxy analogue (compound **13g**) showing a slight increase in potency (Table 5). Replacement of the phenyl moiety with alkyl and cycloalkyl groups also tended to reduce potency.

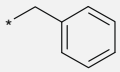
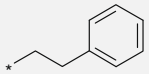
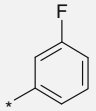
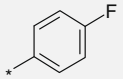
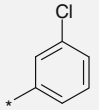
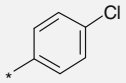
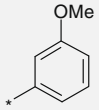
Since compound **12e** displayed acceptable levels of potency versus human and murine 11β-HSD1 (IC_{50} = 114 nM and IC_{50} = 302 nM, respectively) we investigated its inhibition profile in vivo in C57/Bl6 mice.[24] The compound displayed good levels of inhibition in liver, subcutaneous adipose tissue and brain following an oral dose suggesting that **12e** was orally bioavailable (Table 6).

Since ketones are potentially reactive in vivo a suitable replacement for the carbonyl group was desirable. A wide array of analogues containing amido, hydroxyl, hydroxyamino and sulphanyl groups were prepared. In addition, a variety of non-conventional heterocyclic ketone isosteres were also synthesised. However, these analogues were either inactive or displayed low inhibition and no suitable replacement for the ketone was identified.

A number of isomeric 1,5-substituted tetrazoles were produced to explore SAR around the central tetrazole group (Table 7). In

Table 5
Human 11β-HSD1 and microsomal stability for compounds **13a–i**



Compound	R^2	hHSD1 IC_{50}, nM	HLM[a] % parent
13a		820	nd
13b		987	nd
13c		597	21
13d		4600	nd
13e		310	8
13f		539	9
13g		215	14
13h		>10,000	nd
13i		506	11

nd, not determined.
[a] Percentage parent remaining after 30 min incubation with human liver microsomes (HLM).

Table 6
Ex vivo pharmacodynamic inhibition of 11β-HSD1 for compound **12e**

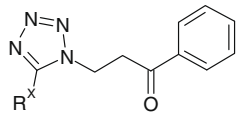
Route	% inhibition in liver 2 h	% inhibition in adipose 2 h	% inhibition in brain 2 h
ip	61 ± 19	52 ± 4	25 ± 1
po	38 ± 11	35 ± 8	22 ± 13

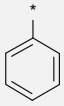
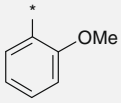
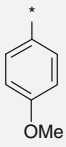
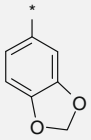
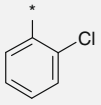
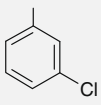
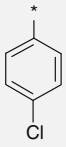
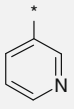
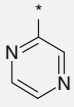
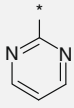
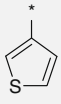
Compound was administered at 10 mg/kg.

general, the regioisomeric 5-phenyl tetrazole analogues were less potent than the 1-phenyl tetrazoles and SAR was not transferable between each series.

An in silico model, generated using Glide and based on the crystal structure of human 11β-HSD1 (PDB: 2BEL) was prepared (Fig. 3). The model places the tetrazole group in approximately the same orientation as the A ring of the steroid substrate.[25] The phenyl-tetrazole ring stacks close to Y177 and is orientated towards the mouth of the pocket. The key hydrogen-bonding interaction is made by the ketone moiety and the catalytic residues S170 and Y183. The phenyl-ketone ring is orientated towards the back of the binding pocket

Table 7
Human 11β-HSD1 and microsomal stability for compounds **14a–k**



Compound	R^X	hHSD1 IC_{50}, nM	HLM[a] % parent
14a		843	29
14b		751	9
14c		493	18
14d		722	11
14e		1800	5
14f		1100	20
14g		>10,000	nd
14h		3900	81
14i		1900	31
14j		4500	53
14k		1100	6

nd, not determined.
[a] Percentage parent remaining after 30 min incubation with human liver microsomes (HLM).

where there is only limited space for further substitution. The modelling is in keeping with our in vitro results, which indicate limited scope for significant modification of the phenyl groups.

The majority of the compounds prepared in the carbon-linked tetrazole series possessed only low to moderate microsomal stability. To investigate this further metabolite identification studies on compound **12e** were undertaken. The compound was incubated

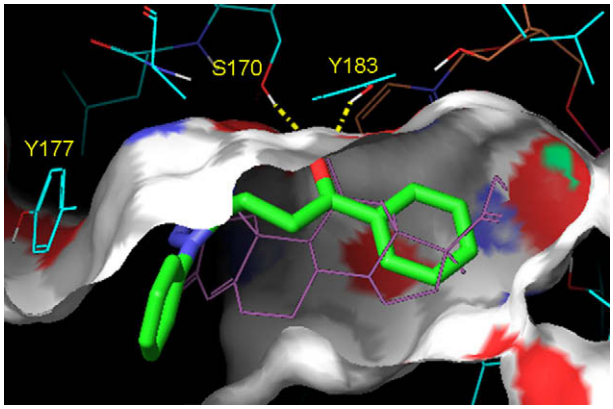


Figure 3. In silico model of compound **11** bound to human 11β-HSD1.

with human liver microsomes in the presence of NADPH and the metabolites analysed using LC–MS/MS. The results showed that two metabolites were formed with masses equal to (parent+2) and (parent+16). Our analysis of the spectra for the (parent+16) peak suggested that this corresponded to a hydroxylated species, with hydroxylation taking place on the phenyl ring adjacent to the propanone linker. This ring is unsubstituted and may be liable to CYP450-mediated oxidation. Analysis of the (parent+2) peak suggested that this corresponded to a species where the carbonyl moiety has been reduced, since the breakdown pattern in the MS/MS spectrum was identical to a hydroxypropyl reference compound (data not shown). Each of the metabolic processes is dependent on NADPH since no metabolism occurred in the absence of this cofactor.

The presence of the keto-reduced species in the microsomal extracts was a concern, since 11β-HSD1 is also present in human liver microsomes. The ketone moiety had previously been shown to be necessary for inhibitory activity. Therefore, it was possible that these compounds were acting as substrates for the enzyme rather than inhibitors. Indeed, previous work has demonstrated that carbonyl-containing compounds are able to serve as substrates for 11β-HSD1.[26] Several tetrazoles were incubated in the presence of recombinant enzyme and incubates analysed for the presence of the keto-reduced species. Analysis suggested that 11β-HSD1 was responsible for the reduction of compound **12e** in human liver microsomes since the compound was also reduced in the presence of recombinant 11β-HSD1 enzyme (Table 8). However, the analyses of a wide array of compounds from the carbon-linked tetrazole series suggested that the main route of metabolism in the series was not 11β-HSD1-mediated since many compounds with low microsomal stability did not serve as substrates for the enzyme (Table 8).

Table 8
Metabolic stability data for selected tetrazoles

Compound	hHSD1 IC_{50}, nM	HLM[a] % parent	% ketone reduction by 11β-HSD1[b]
12e	114	52	31
12f	575	34	0
12j	686	37	0
13e	310	8	0
13f	539	19	5
13g	215	14	0
13h	934	4	1
14c	493	18	0

[a] Percentage parent remaining after 30 min incubation with human liver microsomes (HLM).
[b] Percentage of ketone reduced in the presence of recombinant 11β-HSD1 enzyme after 30 min at 37 °C.

In summary, we have discovered moderately potent and selective inhibitors of 11β-HSD1 that display inhibition in vivo. However, certain compounds have been shown to act as substrates and inhibit the enzyme by competing with the substrate.

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21. In vitro cellular enzyme inhibition was determined using a scintillation proximity assay (SPA). Human 11β-HSD1 enzyme inhibition was assessed in HEK293 cells stably transfected with the full length human hsd11b1 gene. HEK293 cells were plated in 96-well poly-D-Lys coated flat-bottomed microplates in DMEM containing 1% glutamine, 1% penicillin and streptomycin. Compounds were added to plates such that the final concentration of DMSO was 1%. Tritiated cortisone was added at a final concentration of 20 nM and the cells incubated at 37 °C in 5% CO_2, 95% O_2 for 2 h. The assay solutions were transferred to a scintillation microplate and mixed with a solution of anti-mouse YSi SPA beads and anti-cortisol antibody in assay buffer (50 mM Tris–HCl, pH 7.0; 300 mM NaCl, 1 mM EDTA, 5% glycerol). The plate was incubated for 2 h at room temperature and read on a scintillation counter. The percentage inhibition was determined relative to a non-inhibited control and the median inhibitory concentration (IC_{50}) determined by plotting fractional inhibition against log compound concentration. Data were fitted to the four parameter logistic equation. Murine 11β-HSD1 enzyme inhibition was assessed in CHO cells stably transfected with the full length murine hsd11b1 gene. Enzyme inhibition was determined as described for human 11β-HSD1 following a 4-h incubation of cells, compound and substrate.
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24. Male C57BL/6 mice (25–30 g in weight) were group housed and allowed free access to food and water. Compounds was dissolved in 5% DMSO, 3% ethanol, 4 mM cyclodextrin. Animals ($n = 3$ per group) were dosed

intraperitoneally or orally with vehicle or compound at 12-hourly intervals. At 2 h following the third dose mice were killed by cervical dislocation. Liver, adipose and brain samples were removed and frozen until analysis was performed. Inhibition of 11β-HSD1 in each tissue was determined by incubating homogenates with 20 nM tritiated cortisone, 2 mM NADPH and 0.2% glucose in Krebs buffer, pH 7.4. Cortisone and cortisol levels were measured by HPLC and the percentage inhibition determined relative to vehicle treated tissue.

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