Bioorganic & Medicinal Chemistry Letters 22 (2012) 6756–6761

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Optimisation of pharmacokinetic properties in a neutral series of 11β-HSD1 inhibitors

James S. Scott *, Adrian L. Gill, Linda Godfrey, Sam D. Groombridge, Amanda Rees, John Revill, Paul Schofield, Pernilla Sörme, Andrew Stocker, John G. Swales, Paul R. O. Whittamore

Cardiovascular & Gastrointestinal Innovative Medicines Unit, AstraZeneca Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK

ARTICLE INFO

Article history:
Received 31 July 2012
Revised 14 August 2012
Accepted 16 August 2012
Available online 5 September 2012

Keywords:
11β-HSD1 inhibitors
Pharmacokinetics
Reactive metabolites

ABSTRACT

11β-HSD1 is increasingly seen as an attractive target for the treatment of type II diabetes and other elements of the metabolic syndrome. In this program of work we describe how a series of neutral 2-thioalkyl-pyridine 11β-HSD1 inhibitors were optimized in terms of their pharmacokinetic properties to give compounds with excellent bioavailability in both rat and dog through a core change to pyrimidine. A potential reactive metabolite issue with 4-thioalkyl-pyrimidines was circumvented by a switch from sulfur to carbon substitution.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an NADPH dependent reductase expressed mainly in liver, adipose and brain tissue that converts the glucocorticoid inactive hormone cortisone to the glucocorticoid active hormone cortisol.[1] It has been postulated that elevated intracellular levels of the glucocorticoid hormone cortisol can lead to glucose intolerance and insulin resistance and could play a key role in the development of the metabolic syndrome.[2–4] Inhibition of 11β-HSD1 has been viewed as a potential therapeutic intervention that could reduce intracellular glucocorticoid concentrations and in turn have beneficial effects in patients with the metabolic syndrome.[5–7] A significant research effort by both academic and industrial groups has led to a diverse array of 11β-HSD1 inhibitors that has recently been reviewed.[8–10]

In previous publications, we have described our efforts to identify acidic inhibitors of 11β-HSD1 resulting in the development candidates AZD4017[11] and AZD6925 (Fig. 1).[12] In an attempt to increase the chemical diversity of our program, we sought to identify neutral inhibitors of the enzyme. Previously we had identified neutral ligands[11] but they had suffered from poor oral bioavailability in rat due to high metabolic clearance. Pyridine **1**, containing the *trans* 4-hydroxy adamantylamine amide, was one of the most potent compounds identified (IC_{50} 7 nM) and, in stark contrast to other neutral compounds profiled, showed at least some bioavailability in rat (F 11%). This was therefore chosen as the start point for our optimisation campaign. Our goals for this program were to obtain neutral compounds that had: (i) sufficient mouse potency to allow pharmacological evaluation in pre-clinical models; (ii) good bioavailability in two species (rat and dog) to facilitate robust dose to man predictions.

The synthetic routes towards the pyridines and pyrimidines reported in this study are described in Schemes 1–5.[13] 4-Thiopropyl pyrimidines with variation in the R1 position (Scheme 1) could be synthesized by a condensation between diethyl 2-(ethoxymethylene)malonate **31** and amidines to form dihydropyrimidines **32**. Chlorination with phosphorous oxychloride gave 4-chloropyrimidines **33** that could be displaced with propane thiolate to give 4-thiopyrimidines **34**. Ester hydrolysis under basic conditions to acids **35** was followed by HATU mediated amide coupling to give pyrimidines **2**, **4** and **10**.

6-Methyl pyridines (Scheme 2) were synthesized by conversion of 2-chloro-6-methylnicotinic acid **36** to amide **37** via formation of the acid chloride followed by addition of *trans*-4-aminoadamantan-1-ol. The 2-chloro substituent could be displaced with propane thiolate to give pyridine **3** or converted using Suzuki chemistry with cyclopropyl boronic acid to pyridine **19**.

An alternative strategy that allowed diversity at R1 to be introduced at the final step (Scheme 3) involved conversion of the dichloro pyridine **38** or pyrimidine **39** acid to the amides **40**, **41** via the acid chloride. Treatment with either propylthio or propoxy nucleophiles resulted in a completely regioselective displacement of the chloro group adjacent to the amide in both the pyridine and pyrimidine to give intermediates **42**–**45**. Displacement of the remaining chloro group gave pyridines **5**, **7**, **9** and **21** and pyrimidines **6**, **8** and **30**.

* Corresponding author. Tel.: +44 1625 232567; fax: +44 1625 516667.
E-mail address: jamie.scott@astrazeneca.com (J.S. Scott).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.070

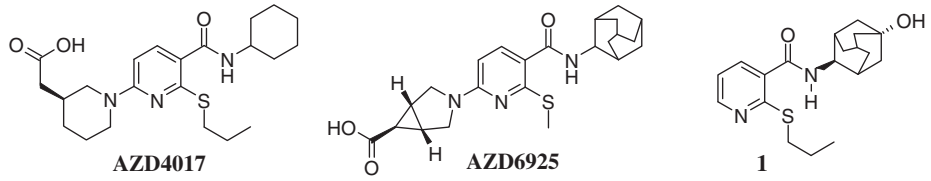
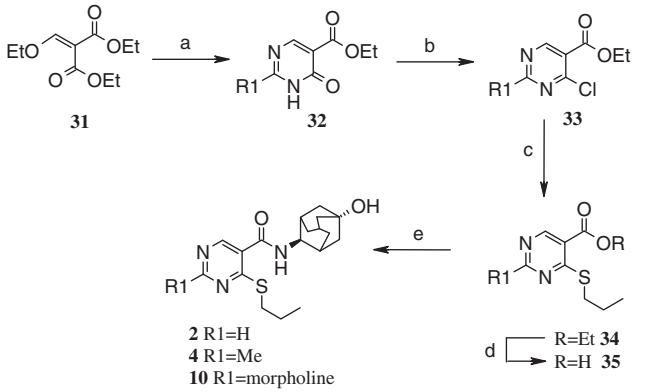
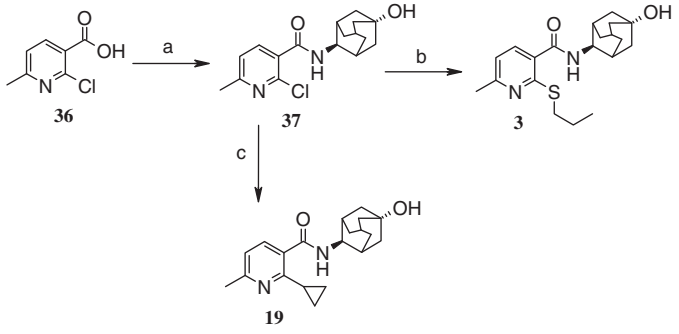


Figure 1. Structures of AZD4017, AZD6925 and pyridine **1**.



Scheme 1. Synthesis of pyrimidines **2**,**4** and **10**. Reagents and conditions: (a) R1C(=NH)NH_2, NaOMe, MeOH, Δ, 4 h, 42–64%; (b) $POCl_3$, Δ, 3 h, 45–100%; (c) ^{n}PrSH, Na_2CO_3, DMF, 60 °C, 30 min, 62–92%; (d) NaOH, MeOH/H_2O, 25 °C, 2 h, 65–100%; (e) *trans* 4-hydroxy adamantylamine, HATU, N(^{i}Pr)$_2$Et, DMF, 25 °C, 3 h, 16–74%.

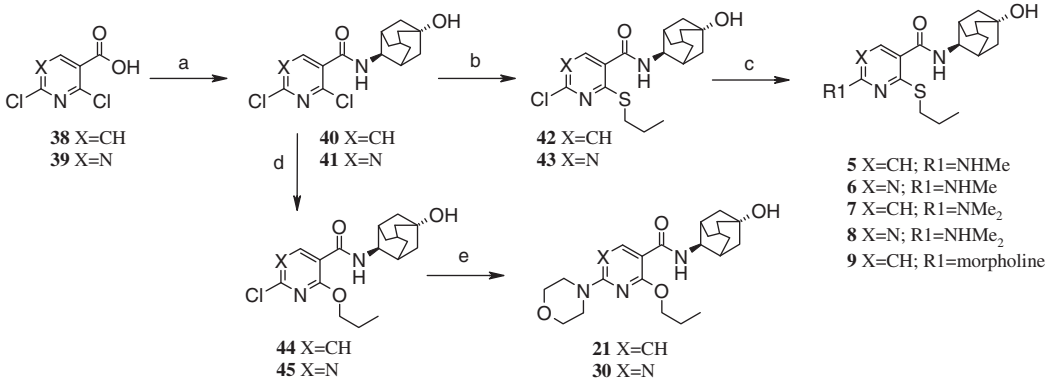


Scheme 2. Synthesis of pyridines **3** and **19**. Reagents and conditions: (a) (i) Oxalyl chloride, DMF, CH_2Cl_2, 20 °C, 16 h; (ii) *trans*-4-aminoadamantan-1-ol, (^{i}Pr)$_2$NEt, THF, 20 °C, 16 h, 31%; (b) ^{n}PrSH, Na_2CO_3, DMA, 60 °C, 3 h, 54%; (c) ^{c}PrB(OH)$_2$, $Pd(OAc)_2$, PCy_3, K_3PO_4, toluene/H_2O, 100 °C, 16 h, 27%.

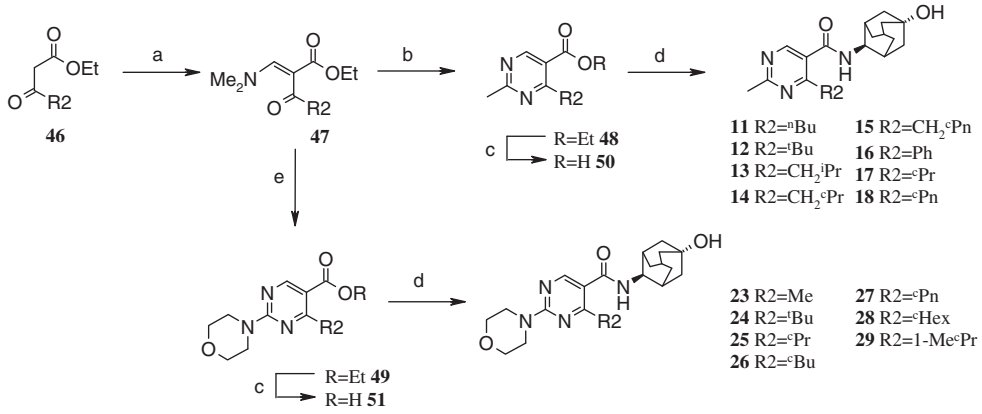
For pyrimidines containing a carbon substituted group at R2 (Scheme 4), the ring was constructed by taking β-keto esters of type **46** and condensing them with *N*,*N*-dimethylformamide dimethyl acetal to form enamines of type **47**. Subsequent condensation with either methyl or morpholino substituted amidines formed pyrimidines of type **48** and **49**, respectively. Base mediated hydrolysis of the ester groups gave acids of type **50** and **51** which could be coupled with *trans*-4-aminoadamantan-1-ol under HATU mediated coupling conditions to give pyrimidines **11**–**18** and **23**–**29**.

In the case of pyridine **20**, the ring was constructed by conversion of β-keto ester **52** to enamine **53** followed by a Michael reaction with methyl propiolate to give dienyl ester **54**. Condensation under basic conditions afforded the pyridone **55** which could be converted to the chloropyridine **56** using phenyl dichlorophosphate. Ester hydrolysis to acid **57** was followed by HATU mediated coupling with *trans*-4-aminoadamantan-1-ol to form amide **58**. Displacement of the chloro group with morpholine under microwave irradiation gave pyridine **20** in good yield.

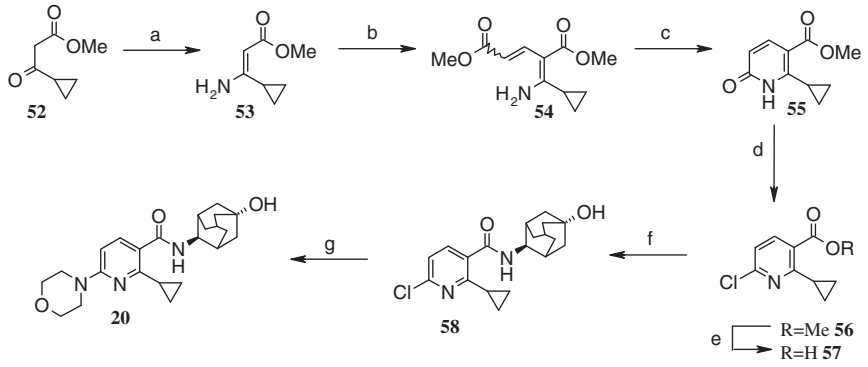
Based on crystallographic structural information and prior structure–activity relationships (SAR),[11] we knew that substitution at the 6-pyridyl position was tolerated and elected to explore this in both pyridine and pyrimidine sub-series as shown in Table 1. In terms of human potency, the SAR for R1 substituents **1**–**10** was notably flat across both sub-series, with arguably the NMe and NMe_2 groups showing small increases in potency. In the case of potency against the mouse enzyme however, dramatic improvements (>30-fold) were seen upon 6-substitution in the pyridine series with NHMe **5**, NMe_2 **7** and morpholine **9** and to a lesser extent (fourfold) with methyl **3**. The pyrimidine sub-series showed the same trend in terms of rank order of substituents (NHMe, NMe_2, morpholine ≫ Me > H) although these were always less potent than the corresponding pyridine matched pairs. Pleasingly, and in contrast to the unsubstituted compounds **1** and **2**, methyl (**3**, **4**) and morpholine (**9**, **10**) substitution resulted in good pharmacokinetic properties in both the pyridine and pyrimidine sub-series whereas the NHMe **5** and NMe_2 **7** pyridines showed more modest improvements.



Scheme 3. Synthesis of pyridines **5**, **7**, **9**, **21** and pyrimidines **6**, **8** and **30**. Reagents and conditions: (a) (i) Oxalyl chloride, DMF, CH_2Cl_2, 20 °C, 16 h; (ii) *trans*-4-aminoadamantan-1-ol, (^{i}Pr)$_2$NEt, THF, 0 °C, 4 h, 66%; (b) ^{n}PrSH, Na_2CO_3, DMA, 25 °C, 4 h, 49–90%; (c) R1H, THF, 150 °C, μW, 6 h, 54–96%; (d) ^{n}PrOH, NaHMDS, 150 °C, μW, 2 h, 82–85%; (e) morpholine, 150 °C, μW, 10 h, 34–83%.



Scheme 4. Synthesis of pyrimidines **11**–**18** and **23**–**29**. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, 1,4-dioxane, 100 °C, 7 h, 93–99%; (b) MeC(=NH)NH_2·HCl, NaOMe, MeOH, 80 °C, 4 h, 61–67%; (c) NaOH, MeOH/H_2O, 60 °C, 2 h, 81–96%; (d) *trans*-4-aminoadamantan-1-ol, HATU, N(^{i}Pr)$_2$Et, DMF, 20 °C, 3 h, 14–72%; (e) (*N*-morpholino)C(=NH)NH_2·HBr, NaOMe, MeOH, 80 °C, 6 h, 61–67%.



Scheme 5. Synthesis of pyridine **20**. Reagents and conditions: (a) NH_4OAc, MeOH, 25 °C, 48 h, 90%; (b) CH≡CCOOMe, toluene, 100 °C, 20 h, 31%; (c) NaO^{t}Bu, *N*-methyl-2-pyrrolidone, 145 °C, 30 h, 71%; (d) $PhOP(=O)Cl_2$, 180 °C, 10 min, 53%; (e) NaOH, MeOH/H_2O, 25 °C, 3 h, 100%; (f) *trans*-4-aminoadamantan-1-ol, HATU, N(^{i}Pr)$_2$Et, DMF, 25 °C, 2 h, 85%; (g) morpholine, THF, 150 °C, μW, 10 h, 83%.

The methyl and morpholine compounds were evaluated in a dog pharmacokinetic study as shown in Table 4. The methyl pyrimidine **4** showed an excellent pharmacokinetic profile whereas the corresponding pyridine **3** showed much lower exposure despite similar clearance values. The morpholine compounds **9** and **10** showed higher clearances with the pyrimidine **10** having low bioavailability in contrast to the pyridine **9** which had no detectable oral levels. The methyl pyrimidine **4** was therefore chosen as an initial lead on the basis of having good pharmacokinetics in both rat and dog with the key challenge being to improve the mouse potency.

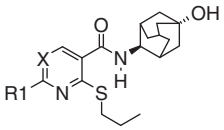
A metabolism-ID study on pyrimidine **6** indicated that the majority of the metabolism (86% by peak area) was oxidation of the ^{n}Pr group with no glucuronidation of the hydroxyl adamantyl group observed. Alarmingly however, small amounts of compounds corresponding to glutathione trapping and thio de-alkylation were observed indicating bioactivation of the thiopropyl group (Scheme 6). This was in direct contrast to the pyridine series where no such metabolism had been observed and was presumably a consequence of the increased electron deficiency of the pyrimidine ring. We felt that this presented a potential toxicological risk and therefore decided to explore replacing the thio-group with a carbon based substituent in order to circumvent this issue (Table 2).

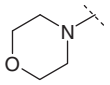
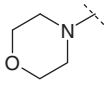
The direct replacement of the sulphur atom present in **4** with carbon (compound **11**) resulted in a dramatic loss of potency as expected based on previous SAR.[11] Efforts to increase steric bulk by moving to a *tert*-butyl substituent **12** resulted in a loss of activity and further exploration of branched alkyl chains (**13**–**15**) resulted in minimal improvements. Phenyl **16** was poorly tolerated however, cyclopropyl **17** resulted in an increase in potency in both human (IC_{50} 395 nM) and mouse (IC_{50} 8.2 μM). Expanding the ring size to cyclopentyl **18** brought further improvements in human potency (IC_{50} 68 nM) but a loss of mouse activity.

The pyridine variant **19** of the cyclopropyl compound was found to be potent in human (IC_{50} 107 nM) and was significantly more active against the mouse enzyme (IC_{50} 139 nM) than any of the pyrimidines. All of the six compounds tested showed good pharmacokinetics in rat. The two most potent compounds **18** and **19** were also profiled in dog (Table 4). The pyrimidine again showed superior pharmacokinetics relative the pyridine with significantly lower clearance being observed.

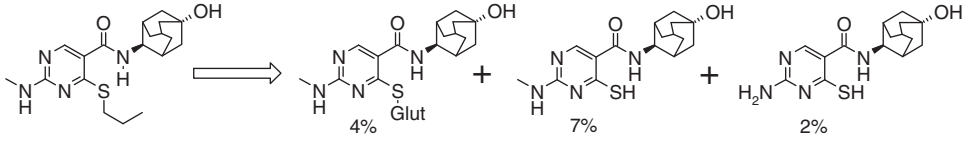
The SAR of non thioalkyl substituents was also examined in the *N*-morpholino substituted pyridine and pyrimidine series (Table 3) in an effort to increase potency against the mouse enzyme. In the morpholino-pyridine series, cyclopropyl **20** was more potent than its matched pair **19** in the methyl-pyridine series and was the first compound in this series to be more active in mouse than in human. The direct replacement of the sulfur atom present in **9** with oxygen (compound **21**), showed slightly reduced potency in human but retained mouse potency. Notably, the measured lipophilicty of the ether **21** ($\log D_{7.4}$ 3.8) was surprisingly higher than the

Table 1



Entry	X	R1	Human 11β-HSD1 IC_{50}[a] (μM)	Mouse 11β-HSD1 IC50[a] (μM)	log$D_{7.4}$	Rat heps clint (μL/min/10^6 cells)	Rat Cl (mL/min/kg)	Rat bioavailability (%)
1	C		0.007	0.922	2.3	49	110	11
2	N		0.009	2.049	2.0	87	100	0
3	C		0.007	0.233	2.8	44	13	63
4	N		0.010	1.509	2.5	11	14	74
5	C		0.003	0.019	3.3	34	36	25
6	N		0.004	0.029	2.9	69	–	–
7	C		0.002	0.026	3.8	73	30	24
8	N		0.004	0.335	3.4	29	–	–
9	C		0.004	0.025	2.9	6	17	64
10	N		0.011	0.191	2.8	3	11	83

[a] Homogeneous time resolved fluorescence assay (HTRF) assay based on mean n >3 results.



Scheme 6. Metabolism-ID on pyrimidine **6** in rat hepatocytes indicating bioactivation of the thiopropyl group.

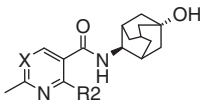
corresponding thioether **9** (log$D_{7.4}$ 2.9). This could be attributable to the potential formation of a 6-membered ring intramolecular hydrogen bond between the ether oxygen and the amide NH reducing the polarity of both the ether and the amide NH substituents. In the morpholine-pyrimidine series, the methyl and *tert*-butyl substituents **23** and **24** had poor potency against the human and mouse isoforms. Carbocyclic rings (3–6 membered) **25**–**28** were profiled and showed interesting species differences. In terms of human potency, increasing rings size (3→5) adds potency with the cyclopentyl **27** being optimal (IC_{50} 22 nM). Further expansion to the cyclohexyl **28** resulted in a decrease in human potency. In terms of mouse potency, the SAR was orthogonal in that the smallest substituent, cyclopropyl **25** was optimal and increasing ring size (3→6) reduced mouse potency. Attempts to further substitute the cyclopropyl ring **29** had little effect on human potency but resulted in an order of magnitude reduction in mouse potency. The oxygen analogue **30** in the morpholine-pyrimidine series was found to have reasonable activity against both human and mouse, but had only mediocre rat pharmacokinetics and was not progressed further.

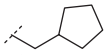
Compounds **20** and **25** were identified as the most promising candidates in terms of potency across both species and rat pharmacokinetics, and were profiled in dog (Table 4). Despite low in vivo clearance, pyridine **20** showed only moderate pharmacokinetics, however, the profile of pyrimidine **25** was excellent with low clearance (Clp 8.6 mL/min/kg) and complete bioavailability (F 100%).

Further profiling of compound **25** demonstrated an attractive profile. The compound was inactive (<25% inhibition at 10 μM) against five isoforms of the cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) and showed no detectable activity in the hERG IonWorks assay (Table 5). Plasma protein binding showed very high free levels that compensated for the moderate enzyme potency. The solubility as measured on crystalline material was 240 μM and when coupled with good cellular permeability as measured in an MDCK assay (P_{app}(A–B) 19×10^{-6} cm s^{-1}; efflux ratio 1.7), it was predicted that this would lead to good absorption in vivo. Importantly, compound **25** displayed excellent selectivity against the related anti-target 11-βHSD2 (IC_{50} >30 μM)[14] indicating that this compound was unlikely to lead to hypertensive effects associated with inhibition of this enzyme.[15]

In conclusion, we have optimised a neutral pyrimidine series of 11β-HSD1 inhibitors to obtain compounds with good potency against human and mouse isoforms and with good pharmacokinetics in rat and dog as exemplified by compound **25**. A potential reactive metabolite issue with 4-thioalkyl-pyrimidines was identified

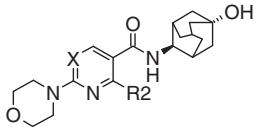
Table 2



Entry	X	R	Human 11β-HSD1 IC_{50}[a] (μM)	Mouse 11β-HSD1 IC_{50}[a] (μM)	log $D_{7.4}$	Rat heps clint (uL/min/10^6cells)	Rat Cl (mL/min/kg)	Rat bioavailability (%)
11	N		2.10	>30	1.6	3	9.5	98
12	N		>8.0	17.8	1.9	<2	4.4	84
13	N		0.644	>30	1.4	—	13	100
14	N		1.48	>30	1.2	5	—	—
15	N		0.847	>30	2.1	7	17	50
16	N		14.8	>30	1.1	11	—	—
17	N		0.395	8.2	1.2	<2	—	—
18	N		0.068	>30	2.0	7	6.6	77
19	C		0.107	0.139	1.6	<2	8.6	75

[a] Homogeneous time resolved fluorescence assay (HTRF) assay based on mean *n* >3results.

Table 3



Entry	X	R2	Human 11β-HSD1 IC_{50}[a] (μM)	Mouse 11β-HSD1 IC_{50}[a] (μM)	log $D_{7.4}$	Rat heps clint (uL/min/10^6cells)	Rat Cl (mL/min/kg)	Rat bioavailability (%)
20	C		0.021	0.006	2.0	<2	21	100
21	C		0.014	0.019	3.8	15	33	31
23	N		5.67	3.45	1.2	<2	—	—
24	N		3.18	17.0	2.6	<2	4.8	100
25	N		0.102	0.095	2.1	<2	8.1	100
26	N		0.060	0.264	2.4	<2	11	45
27	N		0.022	0.784	3.0	3	7.6	67
28	N		0.080	0.831	3.2	6	5.8	31
29	N		0.137	1.11	1.8	<2	—	—
30	N		0.028	0.078	3.0	9	14	36

[a] Homogeneous time resolved fluorescence assay (HTRF) assay based on mean *n* >3results.

Table 4
Dog pharmacokinetic parameters

Entry	Dog heps clint (uL/min/10^6cells)	Clp[a] (mL/min/kg)	Vdss (L/kg)	C_{max} (μM)	PO half-life (h)	IV half-life (h)	Bioavailability (%)
3	18	11	0.4	0.15	0.7	0.5	5
4	5.8	9.9	0.3	0.49	0.6	0.4	110
9	40	18	0.5	—	—	0.4	0
10	12	17	0.7	0.04	0.9	0.7	10
18	4.6	3.5	0.9	0.17	6.7	3.5	58
19	<2	17	0.3	0.26	1.7	0.3	37
20	24	7.9	0.6	0.04	5.7	1.1	25
25	5.3	8.6	0.9	0.36	2.7	1.7	100

[a] Compounds were dosed IV/PO at 1 mg/kg in 5% DMSO: 95% hydroxypropyl β cyclodextrin. Data is mean of two animals (male and female) in a single experiment.

Table 5
Physical properties of compound **25**

Aqueous solubility[a] pH 7.4 (μM)	hERG IC_{50} (μM)	Rat PPB[b] (% free)	Dog PPB[b] (% free)	Human PPB[b] (% free)	MDCK[c] permeability Papp ($\times 10^{-6}$ cm s^{-1})
240	>100	>57	43	38	19 (A–B); 30 (B–A)

[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–UVMS.
[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.

and circumvented by a switch from sulphur to carbon substitution followed by optimsation of this substituent. A key challenge in this program was the co-optimisation of human and mouse potency with the observed SAR being divergent.

Acknowledgements

Nidhal Selmi is thanked for synthetic contributions and Gemma Convey is thanked for the generation of biological data.

References and notes

1. Edwards, C. R. W.; Benediktsson, R.; Lindsay, R. S.; Seckl, J. R. *Steroids* **1996**, *61*, 263.
2. Björntorp, P.; Rosmond, R. *Nutrition* **2000**, *16*, 924.
3. Björntorp, P.; Holm, G.; Rosmond, R. *Diabet. Med.* **1999**, *16*, 373.
4. Walker, B. R. *Eur. J. Endocrinol.* **2007**, *157*, 545.
5. Tomlinson, J. W.; Walker, E. A.; Bujalska, I. J.; Draper, N.; Lavery, G. G.; Cooper, M. S.; Hewison, M.; Stewart, P. M. *Endocr. Rev.* **2004**, *25*, 831.
6. Thieringer, R.; Hermanowski-Vosatka, A. *Expert Rev. Cardiovasc. Ther.* **2005**, *3*, 911.
7. Wamil, M.; Seckl, J. R. *Drug Discov. Today* **2007**, *12*, 504.
8. Webster, S. P.; Pallin, T. D. *Expert Opin. Ther. Patents* **2007**, *17*, 1407.
9. Boyle, C. D.; Kowalski, T. J. *Expert Opin. Ther. Patents* **2009**, *19*, 801.
10. Morgan, S. A.; Tomlinson, J. W. *Expert Opin. Investig. Drugs* **2010**, *19*, 1067.
11. Scott, J. S.; Bowker, S. S.; deSchoolmeester, J.; Gerhardt, S.; Hargreaves, D.; Kilgour, E.; Lloyd, A.; Mayers, R. M.; McCoull, W.; Newcombe, N. J.; Ogg, D.; Packer, M. J.; Rees, A.; Revill, J.; Schofield, P.; Selmi, N.; Swales, J. G.; Whittamore, P. R. O. *J. Med. Chem.* **2012**, *55*, 5951.
12. Scott, J. S.; Barton, P.; Bennett, S. N. L.; deSchoolmeester, J.; Godfrey, L.; Kilgour, E.; Mayers, R. M.; Packer, M. J.; Rees, A.; Schofield, P.; Selmi, N.; Swales J. G.; Whittamore, P. R. O. *Med. Chem. Commun*. in press, http://dx.doi.org/ 10.1039/C2MD20154B.
13. For experimental details of compounds described in this study see; Gill, A.; Leach, A.; Packer, M. J.; Scott, J. S.; Sorme, P.; Swales, J. G.; Whittamore, P. R. O. WO2009/130496.
14. Wilson, R. C.; Dave-Sharma, S.; Wei, J.; Obeyesekere, V. R.; Li, K.; Ferrari, P.; Krozowski, Z. S.; Shackleton, C. H. L.; Bradlow, L.; Wiens, T.; New, M. I. *Proc. Nat. Acad. Sci.* **1998**, *95*, 10200.
15. Kotelevtsev, Y.; Brown, R. W.; Fleming, S.; Kenyon, C.; Edwards, C. R. W.; Seckl, J. R.; Mullins, J. J. *J. Clin. Invest.* **1999**, *103*, 683.