Bioorganic & Medicinal Chemistry Letters 20 (2010) 2897–2902

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The development and SAR of pyrrolidine carboxamide 11β-HSD1 inhibitors

Hengmiao Cheng [a,*], Jacqui Hoffman [a], Phuong Le [a], Sajiv K. Nair [a], Stephan Cripps [a], Jean Matthews [a], Christopher Smith [a], Michele Yang [a], Stan Kupchinsky [a], Klaus Dress [a], Martin Edwards [a], Bridget Cole [b], Evan Walters [b], Christine Loh [b], Jacques Ermolieff [a], Andrea Fanjul [a], Ganesh B. Bhat [a], Jocelyn Herrera [a], Tom Pauly [a], Natilie Hosea [a], Genevieve Paderes [a], Paul Rejto [a]

[a] Pfizer Global Research & Development, La Jolla Labs, 10770 Science Center Drive, San Diego, CA, 92121, United States
[b] Pfizer Global Research & Development, Research Technology Center, 620 memorial Drive, Cambridge, MA 02139, United States

ARTICLE INFO

Article history:
Received 2 February 2010
Revised 4 March 2010
Accepted 5 March 2010
Available online 10 March 2010

Keywords:
11β-HSD1
Inhibitor
Diabetes
Pyrrolidine carboxamide
PF-877423

ABSTRACT

The design and development of a series of highly selective pyrrolidine carboxamide 11β-HSD1 inhibitors are described. These compounds including PF-877423 demonstrated potent in vitro activity against both human and mouse 11β-HSD1 enzymes. In an in vivo assay, PF-877423 inhibited the conversion of cortisone to cortisol. Structure guided optimization effort yielded potent and stable 11β-HSD1 selective inhibitor **42**.
© 2010 Elsevier Ltd. All rights reserved.

11β-HSD1 catalyzes the intracrine conversion of inactive cortisone to the active glucocorticoid agonist cortisol.[1] The 11β-HSD1 knockout mouse is protected from hyperglycemia associated with stress or obesity through reduced hepatic expression of phosphoenol-pyruvate carboxy kinase (PEPCK), which controls the rate limiting step of gluconeogenesis, as well as other glucose mobilizing enzymes such as glucose-6-phosphatase (G6Pase).[2] Positive effects may also occur outside of the liver via improved β-cell function[3] and lipid modulation through adipose tissue.[4] In addition, transgenic mice that over-express 11β-HSD2, the enzyme that catalyzes the conversion of the active glucocorticoid cortisol back to inactive cortisone, have improved insulin sensitivity and glucose tolerance and are protected from weight gain on a high-fat diet.[5] Because 11β-HSD1 is highly expressed in the liver relative to other tissues,[6] inhibition of 11β-HSD1 activity provides an opportunity to reduce glucocorticoid levels specifically in the liver and splanchnic circulation.[7] Hence, inhibition of 11β-HSD1 activity in the liver is an attractive approach to treat diabetes.

Several classes of selective, non steroid 11β-HSD1 inhibitors have been published (Fig. 1). These include sulfonamides such as BVT.2733,[8] triazoles such as the Merck compound 544,[9] and adamantine amides from Abbott with generic structure **3**.[10] Here we report the development of a series of amides such as PF-877423[11], *N*-adamantan-2-yl-1-ethyl-D-prolinamide, that demonstrated high selectivity, potent in vitro activity against both human and mouse enzymes, and good in vivo activity inhibiting the conversion of cortisone to cortisol.

Cell based high throughput screening (HTS) was carried out to search for non-steroid 11β-HSD1 inhibitors, the adamantine carboxamide **5** was identified as a lead with K_i of 175 nM against human enzyme[12] and cell IC_{50} of 114 nM[13] (Fig. 2). Compound **5** also demonstrated very good selectivity against 11β-HSD2, with

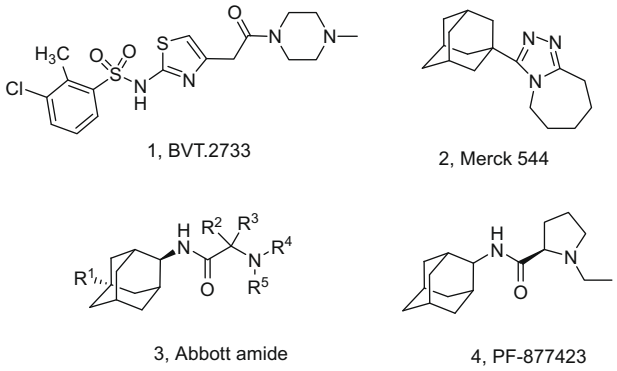


Figure 1. Non-steroid inhibitors of 11β-HSD1.

* Corresponding author. Tel.: +1 858 622 3208; fax: +1 858 526 4151.
E-mail address: henry.cheng@pfizer.com (H. Cheng).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.032

K_i greater than 10 μM. However, compound **5** did not show measurable activity against murine enzyme at 100 nM. Initially a combinatorial chemistry approach was taken to further expand the SAR knowledge around **5** by investigating a series of amide libraries. Compound **6** was identified via this approach. Compound **6** demonstrated comparable in potency as **5** in the cell based assay but was 3 units lower in *c*log *P*. Effort continued by combining structural aspects of **5** and **6** to produce chimeric compounds of

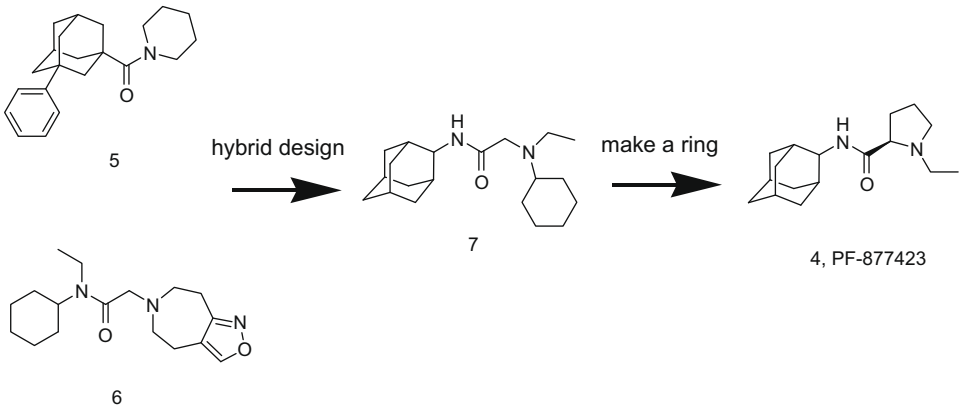


Figure 2. Discovery of PF-877423.

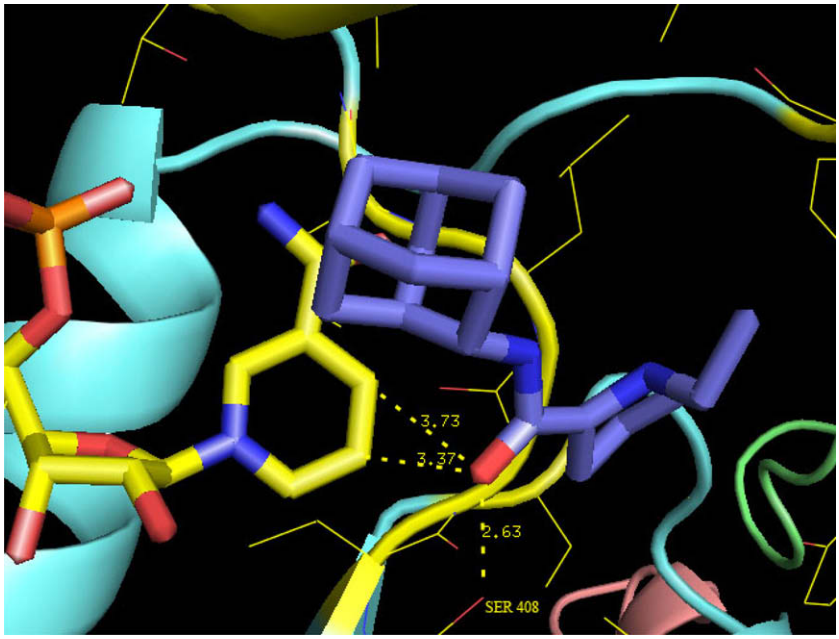
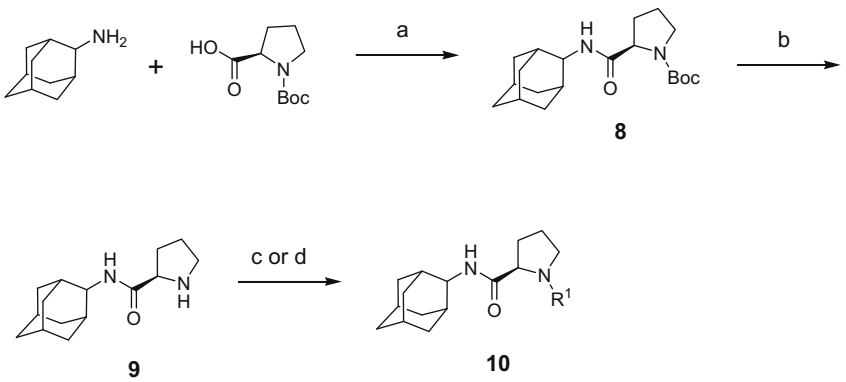
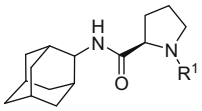


Figure 3. Crystal structure of guinea pig 11β-HSD1 with PF-877423. Resolution is 1.84 Å.



Scheme 1. Reagents and conditions: (a) HATU, Et_3N, CH_2Cl_2; or EDC, HOBt, Et_3N, CH_2Cl_2; (b) TFA; (c) R^1-X, Et_3N, CH_2Cl_2; (d) R′CHO, $NaBH(OAc)_3$, THF.

Table 1
Summarized data for compounds **4**–**7**



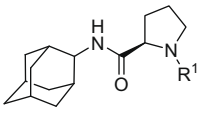
Compound	K_{iapp} (nM)		IC_{50} (nM)	*c*log *P*
	Human	Mouse		
5	175	6% @100 nM	261	4.58
6	15	1.75% @100 nM	290	1.63
7	3.1		2.2	2.99
4	1.4	0.63	4.2	4.22

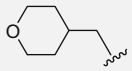
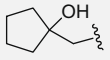
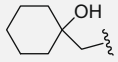
5 and **6**. A 10-fold increase in potency was achieved following this strategy as exemplified by **7** (Table 1).

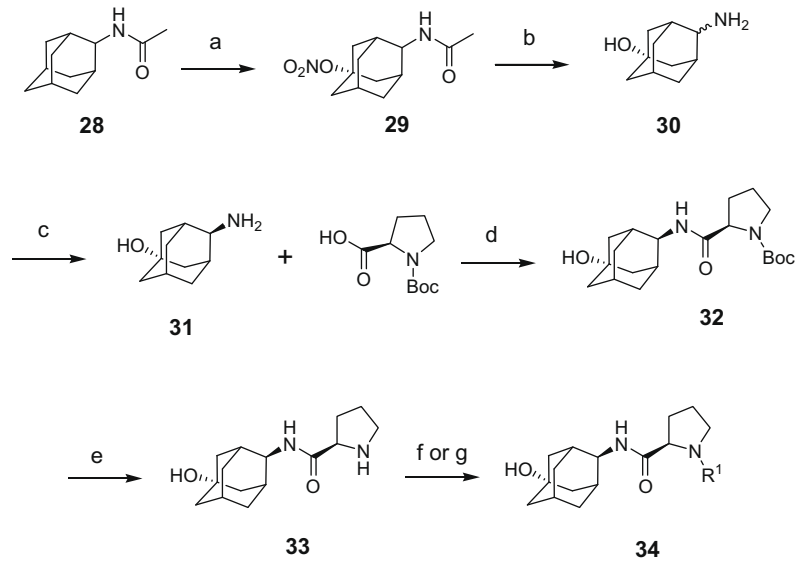
Significant chemical space around **7** had already been explored via our combinatorial chemistry approach thus a new design strategy was investigated—'make a ring, break a ring'. These efforts identified **4**, PF-877423, which gave moderate metabolism in HLM (Clint 44 mL/min/Kg), good potency in the cell and potent inhibition of the mouse 11β-HSD1 enzyme. PF-877423 is selective over 11β-HSD2, with K_i greater than 10 μM.

PF-877423 is highly ligand efficient with ligand efficiency (LE) of 0.57 based on the cell IC_{50}. The cocrystal structure of PF-877423 with guinea pig 11β-HSD1 enzyme was determined to understand how it binds in the enzyme active site (Fig. 3).

Table 2



Compound	R^1	K_{iapp} (nM) or % inhibition @100 nM		HEK IC_{50} (nM)	*c*log *P*	HLM Clint (ml/min/kg)
		Human	Mouse			
4	Et	1.3	0.63	4.15	4.22	43.9
11	Me	2.4	5.4	22	3.69	22.2
12	Pr	1.4	1	1.2	4.75	93.3
13	*i*-Pr	1.6	9.4	3.5	4.53	ND
14	Cyclopentylmethyl	ND	1	0.03	5.67	75.3
15	Isobutyl	ND	ND	0.03	5.15	79.3
16	Cyclobutylmethyl	ND	ND	0.03	5.11	85.8
17	Cyclohexylmethyl	2.4	3.5	0.32	6.23	62.8
18	CF_3CH_2	1	35.2	0.32	4.48	ND
19	$CF_3CH_2CH_2$	1	3.6	0.32	4.46	ND
20	*p*-Cl-Benzyl	2	6.3	0.32	6.12	ND
21	*p*-CN-Benzyl	ND	ND	0.03	4.84	73.2
22	*m*-CN-Benzyl	1	170	0.32	4.84	ND
23	Hydroxyethyl	3.6	ND	20	3.12	ND
24	2-Pyridyl	1	40	5	3.91	79.2
25		1	16	10	3.83	20.3
26		ND	17	0.34	4.58	86.2
27		ND	48	0.03	5.14	64.6



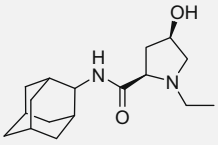
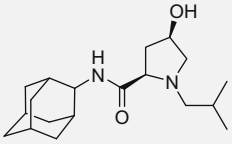
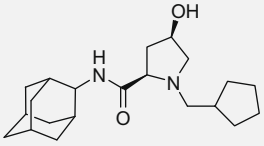
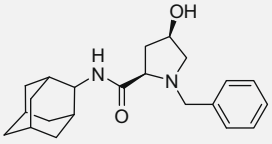
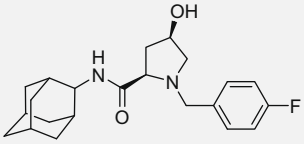
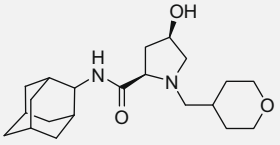
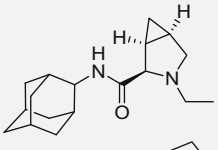
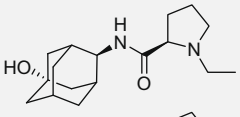
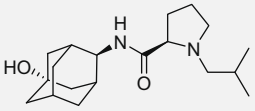
Scheme 2. Reagents and conditions: (a) HNO_3, room temperature, 63%; (b): 6 N HCl, water, reflux, 60%; (c) chromatography separation of the two isomers; (d) HATU, Et_3N, CH_2Cl_2; or EDC, HOBt, Et_3N, CH_2Cl_2; (e) TFA; (f) R^1-X, Et_3N, CH_2Cl_2; (g) R′CHO, $NaBH(OAc)_3$, THF.

PF-877423 interacts with NADP and several active site residues, most notably Ser408, Tyr469, Leu364, Ile418, Leu 455, Tyr469 and Tyr415. The adamantyl of PF-877423 packs against the nicotinamide ring of the NADP and the pyrrolidine ring is stacked with the ring of Tyr415. Most of the interactions are hydrophobic in nature with the exception of a hydrogen bond between the amide oxygen of PF-877423 and the side chain of Ser408.

Structure analysis indicated that there is space to accommodate a variety of side chains off the pyrrolidine nitrogen. In order to explore the SAR, as illustrated in Scheme 1, different substituents were placed on the pyrrolidine nitrogen by either SN2 displacement or reductive amination. Listed in Table 2 are in vitro biochemical data such as human and mouse enzyme K_{iapp} and cellular IC_{50}, *c*log *P* and human liver microsomal clearance. The in vitro cell potency is correlated to *c*log *P* of the compounds. For example, when the R^1 group is smaller alkyl groups as in compounds **4**, **11**, **12**, and **13**, these compounds exhibited cell IC_{50} greater than 1 nM. When the R^1 group is larger groups as in compounds **14** through **22**, these compounds exhibited higher *c*log *P* and demonstrated cell IC_{50} less than 1 nM. The data from the table also shows that human liver microsome intrinsic clearance is inversely correlated to *c*log *P*. Interestingly, compound **25** with lower *c*log *P* demonstrated good in vitro activity and metabolic stability.

In vitro metabolic identification studies with PF-877423 indicated that the adamantly ring and the pyrrolidine ring were oxidized by HLM. In order to reduce the clearance by either block the metabolic soft spots or lower *c*log *P* by introducing polar groups such as hydroxyl group, the cocrystal structure of PF-877423 with the protein was further investigated. Around the pyrrolidine ring, there is space to introduce small functional groups such as hydroxyl and methyl. Introduction of a hydroxyl group to the adamantly ring is also viable since this hydroxyl group can occupy the space next to

Table 3

Compound	Structure	K_{iapp} (nM) or % inhibition @100 nM		HEK IC_{50} (nM)	*c*log *P*	HLM Clint (ml/min/kg)
		Human	Mouse			
35		1.4	23	34	3.02	ND
36		1	3.3	0.8	3.94	ND
37		1	12	0.173	4.5	72
38		1	129	1	4.15	ND
39		1	ND	0.03	4.3	ND
40		2.6	ND	30	2.66	84.2
41		1	3.8	19.4	3.98	65
42		ND	8.4	23	1.09	11.8
43		ND	ND	31.3	2.02	60.2

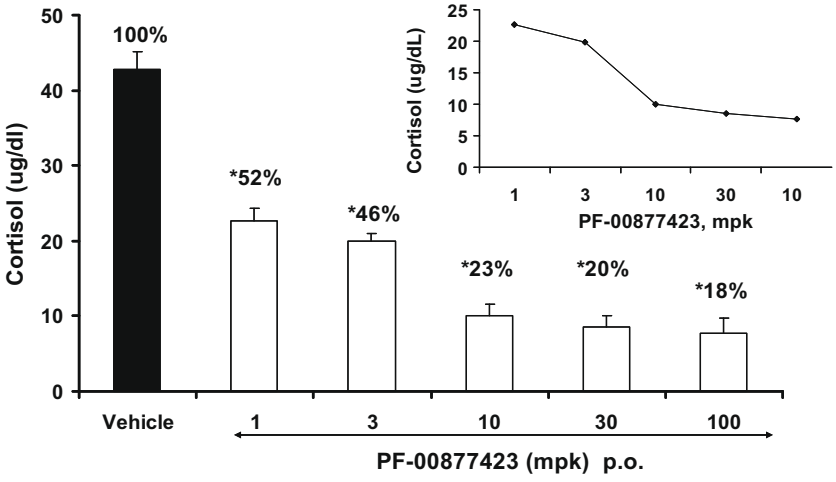


Figure 4. In vivo inhibition of 11β-HSD1 activity in normal C57Bl/6 mice by PF-877423.

the phosphate group of NADP. Subsequently, the hydroxyproline derivatives were prepared following similar synthetic methods as outlined in Scheme 1 using the commercially available *N*-Boc-hydroxyproline. The cyclopropane fused proline derivative **39** was also prepared following the same synthetic route. The synthesis of the hydroxyl-adamantyl ring is described in Scheme 2.[15]

The data for the hydroxylated amide derivatives is summarized in Table 3. Introduction of hydroxyl group to the proline core lowered the *c*log *P*. Compounds with lipophilic substituents such as compounds **35**–**41** demonstrated good in vitro potency. However, their metabolic stability was not improved. The in vitro clearance for the cyclopropane fused proline derivative **41** is also high. Interestingly, the hydroxyl adanmantyl compound **42**, which has cell IC_{50} of 23 nM, has significantly lower *c*log *P* and is metabolically stable in the in vitro human liver microsomal assay. Replacement of the ethyl by isobutyl increased *c*log *P* by one unit as in compound **43**. This compound showed increased in vitro potency, but high in vitro clearance.

In order to establish the correlation between in vitro activity and in vivo inhibition, PF-877423 was progressed to the in vivo biomarker assay methodology using exogenous substrate cortisone conversion to cortisol in normal mice.[14]

As shown in Figure 4, oral treatment of normal mice with 1–100 mg/kg PF-877423 dose dependently reduced the generation of cortisol thirty minutes following compound administration with a maximum inhibition of 82% in animals treated with 100 mpk. As shown in the figure inset, the calculated dose to inhibit 50% 11β-HSD1 in vivo activity was 5.0 mpk of PF-877423.

Additional studies with PF-877423 indicated that blocking adipogenesis with selective 11β-HSD1 inhibitor may represent a novel approach to treat obesity in patients with MS.[16]

In summary, we have discovered a series of pyrrolidine carboxamides with good selectivity over 11β-HSD2 and potent in vitro activity against both human and mouse 11β-HSD1 enzymes. PF-877423 was progressed to in vivo biomarker conversion assay, and demonstrated in vivo activity. Introduction of the hydroxyl group to the adamantly ring afforded compound **42** that is stable in the in vitro human liver microsomal assay.

Accession code: The coordinates for PF-877423 with Guinea Pig 11β hydroxysteroid dehydrogenase have been deposited, and the PDB ID code is 3LZ6.

References and notes

1. (a) Amelung, D.; Hubener, H. J.; Roka, L.; Meyerheim, G. *J. Clin. Endocrinol. Metab.* **1953**, *13*, 1125; (b) Lakshmi, V.; Monder, C. *Endocrinology* **1988**, *123*, 2390; (c) Edwards, C. R. W.; Stewart, P. M.; Burt, D.; Brett, L.; McIntyre, M. A.; Sutanto, W. S.; De Kloet, E. R.; Monder, C. *Lancet* **1988**, *2*, 986; (d) Funder, J. W.; Pearce, P. T.; Smith, R.; Smith, A. I. *Science* **1988**, *242*, 583.
2. Kotelevtsev, Y.; Holmes, M. C.; Burchell, A.; Houston, P. M.; Schmoll, D.; Jamieson, P.; Best, R.; Brown, R.; Edwards, C. R. W.; Seckl, J. R.; Mullins, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14924.
3. Davani, B.; Khan, A.; Hult, M.; Martensson, E.; Okret, S.; Efendic, S.; Jornvall, H.; Oppermann, U. *J. Biol. Chem.* **2000**, *275*, 34841.
4. Masuzaki, H.; Paterson, J.; Shinyama, H.; Morton, N.; Mullins, J.; Seckl, J.; Flier, J. *Science* **2001**, *294*, 2166.
5. Kershaw, E.; Morton, N.; Dhillon, H.; Ramage, L.; Seckl, J.; Flier, J. *Diabetes* **2005**, *54*, 1023.
6. Fotsch, C.; Liu, Y.; Nakagawa, Y.; Wang, Y.; Sakurai, R.; Tripathi, P.; Lutfy, K.; Friedman, T. *Diabetes* **2005**, *54*, 32.
7. Basu, R.; Singh, R.; Basu, A.; Chittilapilly, E.; Johnson, C.; Toffolo, G.; Cobelli, C.; Rizza, R. *Diabetes* **2004**, *53*, 2051.
8. Barf, T.; Vallgarda, J.; Emond, R.; Haggstrom, C.; Kurz, G.; Nygren, A.; Larwood, V.; Mosialou, E.; Axelsson, K.; Olsson, R.; Engblom, L.; Edling, N.; Ronquist-Nil, Y.; Ohman, B.; Alberts, P.; Abrahmsen, L. *J. Med. Chem.* **2002**, *45*, 3813.
9. Olson, S.; Aster, S. D.; Brown, K.; Carbin, L.; Graham, D. W.; Hermanowski-Vosatka, A.; LiGrand, C. B.; Mundt, S. S.; Robbins, M. A.; Schaeffer, J. M.; Slossberg, L. H.; Szymonifka, M. J.; Thieringer, R.; Wright, S. D.; Balkovec, J. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4359.
10. (a) Rohde, J. J.; Pliuschev, M. A.; Wodka, D.; Sorensen, B. K.; Yong, H.; Madar, D. J.; Shuai, Q.; Patel, J. R.; Jae, H.; Sarris, K. A.; Wang, J.; Fung, S.; Monzon, K.; Chiou, W.; Pan, L.; Deng, X.; Chovan, L. E.; Longenecker, K. L.; Judge, R. A.; Qin, W.; Wagaw, S. H.; Engstrom, K. M.; Kerdesky, F. A. J.; Stolarik, D. F.; Imade, H. M.; Marsh, K. C.; Beno, D. W. A.; Fey, T. A.; Droz, B. A.; Brune, M.; Camp, H.; Sham, H. L.; Frevert, E. U.; Jocobson, P.; Link, J. T. *J. Med Chem.* **2007**, *50*, 149; (b) Patel, J. R.; Shuai, Q.; Dinges, J.; Winn, M.; Pliushchev, M.; Fung, S.; Monzon, K.; Cjiou, W.; Wang, J.; Pan, L.; Wagaw, S.; Engstrom, K.; Kerdesky, F. A.; Longenecker, K.; Judge, R.; Qin, W.; Imade, H. M.; Stolarik, D.; Beno, D. W. A.; Brune, M.; Chovan, L. E.; Sham, H. L.; Jacobson, P.; Link, J. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 750.
11. Cheng, H.; Smith, C. R.; Wang, Y.; Parrott, T. J.; Dress, K. R.; Nair, S. K.; Hoffman, J. E.; Le, P. T. Q.; Kupchinsky, S. W.; Yang, Y.; Cripps, S. J.; Huang, B. PCT Int. Appl. WO 2005108359, **2005**. The preparation, ^{1}H NMR and MS data for the compounds that are discussed in this article are described in this patent.
12. The enzyme assay is described in Ref. 11.
13. *HEK293-11β-HSD1/GRE-Luciferase cell-based assay*: EC_{50} determination/inhibition of 11β-HSD1 enzyme activity was measured using human kidney HEK293 stable transfected cells, over-expressing human 11β-HSD1, and a reporter plasmid containing DNA sequences for specific recognition of glucocorticoid-activated glucocorticoid receptors (GRE). These sequences were fused to a luciferase reporter gene (Luc) allowing for quantification of 11β-HSD1 enzyme modulation. Cortisol (but not cortisone) binds and activates glucocorticoid receptors (GR), which will result in activation of luciferase and production of light (assay readout). A compound with the capability of inhibiting 11β-HSD1 will reduce the luciferase signal, compare to cortisone control (enzyme substrate). Cells were plated in 384 well flat bottom white polystyrene TC-treated microplates, at 20,000 cell/well at a volume of 40 μL/well, in serum-free DME Medium. Plates were incubated at 37 °C, 5% CO_2 overnight before addition of inhibitor compounds. Different concentrations of inhibitor compounds were added in 10% (v/v) dimethylsulfoxide (5 μL/well), followed by addition of 3 μM cortisone (5 μL/well), and cells were incubated at 37 °C (5% CO_2) for six hours. At the end of the incubation, 25 μL/well SteadyLite HTS were added and plates were incubated 10 min at room temp on shaker. Plates were then read on Top Count using 384HSD1 program. The concentration of inhibitor compound causing 50% inhibition of light signal was determined via

a custom made Excel Macro. All results were compared to 100% activation control, that is, cells treated only with cortisone (no inhibitors added).
14. Normal mice were used to develop an acute mechanistic biomarker model. For this mechanistic biomarker study, normal 8–10 weeks old C57Bl mice (Charles River Laboratories, Wilmington, MA) were dosed orally with vehicle (0.5% methyl cellulose) or with 1–100 mg/kg of PF 00877423. Thirty minutes after the vehicle or drug treatment, mice were dosed orally with cortisone (Cat# C2755, Sigma-Aldrich, St Louis, MO, 10 mg/kg in 0.5% methyl cellulose). Plasma samples were collected 60 min later. Plasma cortisol levels were measured using ELISA assay kit (Cat# 1850, Alpha Diagnostic International, San Antonio, TX). Background value from no cortisone treated mice sample was subtracted from all cortisone treated samples.
15. The authors would like to thank Wuxi Apptec Co. for the preparation of intermediate compound **30**.[17]
16. Bujalska, I. J.; Gathercole, L. L.; Tomlinson, J. W.; Darimont, C.; Ermolieff, J.; Fanjul, A. N.; Rejto, P. A.; Stewart, P. M. *J. Endocrinol.* **2008**, *197*, 297.
17. Geluk, H. W. *Synthesis* **1972**, 374.