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Discovery and structure–activity relationships of pentanedioic acid diamides as potent inhibitors of 11β-hydroxysteroid dehydrogenase type I

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ABSTRACT

Benzylamides of pentanedioic acid were identified as inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) by high-throughput screening. Optimisation to 2-adamantyl amides yielded inhibitors with single digit nanomolar IC_{50}s on the 11β-HSD1 human isoform. The hydroxy adamantyl amide lead compound was selective against 11β-hydroxysteroid dehydrogenase type 2 (selectivity ratio >1000) and displayed good inhibition of 11β-HSD1 (IC_{50} < 0.1 μM) in a cellular model (3T3L1 adipocytes).
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Hydroxysteroid dehydrogenases are a family of enzymes that regulate ligand-binding to various steroid hormone receptors at the pre-receptor level, like a switch, converting steroid hormones to the inactive or precursor forms and vice-versa.[1] Among these, 11β-hydroxysteroid dehydrogenases 1 and 2 are enzymes modifying glucocorticoid chemistry, oxidizing the 11β-hydroxyl position or reducing the 11-oxo group, which determines whether or not steroids are able to bind to glucocorticoid (and mineralocorticoid) receptors.[2] Glucocorticoid receptor binding is restricted to active steroids with 11β-hydroxyl moiety, that is, mainly cortisol in humans and corticosterone in rodents. 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is expressed in tissues containing the mineralocorticoid receptor, predominantly kidney, but also gut and placenta.[3] 11β-HSD2 inactivates active glucocorticoids (such as cortisol and corticosterone) to cortisone or 11-dehydrocorticosterone.[2] On the other hand, the isoform 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activates inert precursors (cortisone, 11-dehydrocorticosterone) to active glucocorticoids by 11-oxoreductase activity in liver, adipose tissue, brain, skeletal muscle, vascular smooth muscle cells and other organs.[4] Several in vivo models and studies have documented the physiological importance of this glucocorticoid converting enzyme in insulin action. In particular, 11β-HSD1 knockout mice were shown to resist to hyperglycemia and to have increased hepatic insulin sensitivity.[5] In mirror of this, 11β-HSD1 overexpression in mouse adipose tissue resulted in development of visceral adiposity, hyperglycemia and insulin resistance.[6] In addition, 11β-HSD2 overexpression in mouse adipose tissue, leading to the inactivation of glucocorticoids, improved glucose tolerance and insulin sensitivity.[7]

This body of evidence strongly suggest that 11β-HSD1 inhibition is a potential therapeutic target for a broad range of disorders that could be improved by decreased intracellular glucocorticoid levels (i.e., cortisol), including the obesity-induced metabolic syndrome. Selectivity against the 11β-HSD2 isoform is expected to be crucial as deficiency or inhibition (i.e., licorice) of 11β-HSD2 are conditions allowing mineralocorticoid receptor to be occupied by cortisol, leading to the "essential" form of hypertension.[8] Therefore, many efforts towards the discovery and development of selective 11β-HSD1 inhibitors have been carried out.[9] In this report we describe such a new class of inhibitors.

Compound **1** derived from glutaric anhydride was identified as an initial hit by high-throughput screening. This compound displayed an interesting level of potency on the human isolated

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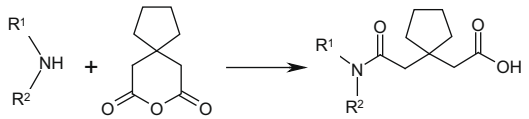
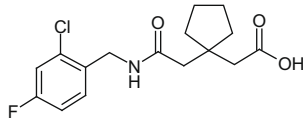


Scheme 1. Reagents and conditions: Et_3N, toluene, heat.



1
IC_{50} = 1.2 μM
MW = 327 Da

Figure 1. Initial hit discovered by HTS.

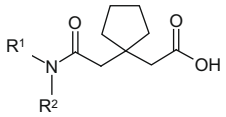
11β-HSD1 (IC_{50} = 1.2 μM) with regard to its small size (MW = 327 Da) (Fig. 1).

Due to the simplicity of the scaffold, a large collection of amines was used to prepare libraries of amides and explore rapidly the SAR around the R^1,R^2 variations (Scheme 1).

However the levels of inhibition obtained after screening these libraries were disappointing (selected data in Table 1). Furthermore, the activity of the benzylamide derivatives could not be confirmed (compounds **3** and **4**, Table 1). As anticipated based on the known hydrophobic nature of the 11β-HSD1 ligand binding pocket, the most active compounds were derived from hydrophobic bicyclic amines such as decahydroquinoline and 2-adamantyl amines (compounds **13** and **14**). The decreased activity of the corresponding 1-adamantyl regioisomer **12** underlined the requirement of both a perfect shape match and the presence of a hydrophobic group in this pocket to achieve high potency.

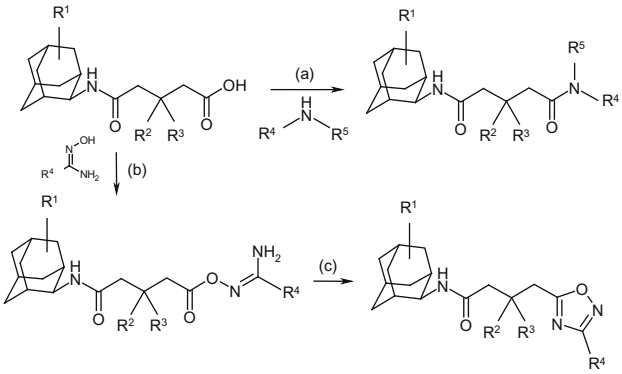
Therefore, efforts were focused on new analogues based on a pentanedioic 2-amino-*N*-(adamantan-2-yl) amide scaffold. To further explore the SAR, the free carboxylic acid moiety was replaced with an amide or an oxadiazole. The oxadiazole derivatives were prepared in 2 steps from the corresponding carboxylic acids according to Scheme 2. Amides appeared far superior to carboxylic acid derivatives in terms of potency (Table 2). Aromatic amides

Table 1
Variations of the amide substituents



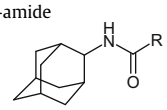
Compounds	R^1NR^2	h-HSD1% inhibition at 1 μM[a]
2	2-Cl-Ph-NH	0
3	2-Cl-Bn-NH	22
4	2,4-diCl-Bn-NH	27
5	cHex-CH_2–NH	7
6	cHex-NH	13
7	4-Cl,3-pyr-NH	0
8	4-OCH_3, 3-pyr-NH	7
9	*N*-Me-piperazine	0
10	7-(2-Methyl)-indole-NH	0
11	3-Pyr-CH_2–NH	11
12	1-Adamantyl-NH	8
13	Decahydroquinoline	34
14	2-Adamantyl-NH	50[b]

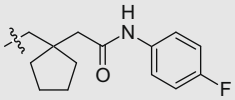
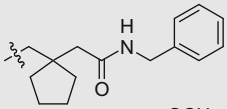
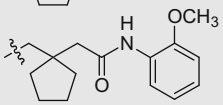
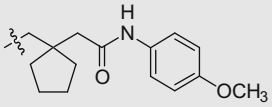
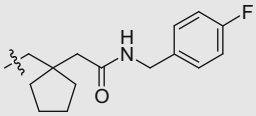
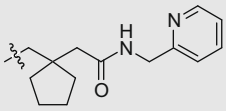
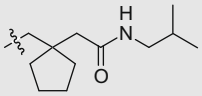
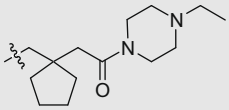
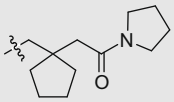
[a] IC_{50} values were only determined for compounds displaying >50% inhibition at 1 μM.
[b] This compound exhibited an IC_{50} of 1800 nM.



Scheme 2. Reagents and conditions: (a) DCC, HOBt, THF, HNR_4R_5, rt; (b) DCC, HOBt, DMF, rt; (c) DMF, heat.

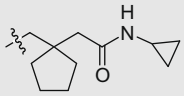
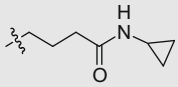
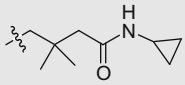
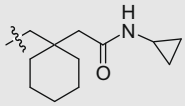
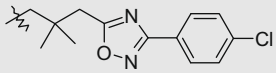
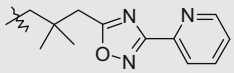
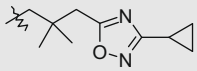
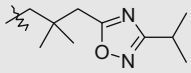
Table 2
Variations on the 2-adamantyl-amide



Compounds	R	h 11β-HSD1 IC_{50}[a] (nM)	BEI[b]
15		17	18.8
16		19	18.3
17		9	19.0
18		48	17.2
19		18	18.2
20		16	19.0
21		9	21.5
22		160	16.4
23		48	19.6

(*continued on next page*)

Table 2 (*continued*)

Compounds	R	h 11β-HSD1 IC_{50}[a] (nM)	BEI[b]
24		60	20.1
25		>1000	–
26		150	20.5
27		340	17.4
28		200	15.6
29		57	18.3
30		12	22.1
31		8	22.5

[a] 11β-HSD1 enzyme activity was assessed in 30 mM Hepes buffer, pH 7.4, containing 1 mM EDTA, 1 mM G-6P, and a substrate mixture cortisone/NADPH (200 nM/200 μM). Reaction was initiated by addition of 3 μg of human recombinant 11β-HSD1 expressed in *E. coli* and terminated with addition of 18β-glycyrrhetinic acid stop solution after 150 min incubation at 37 °C. Determinations of cortisol levels were monitored by HTRF assay (Cisbio International).

[b] Binding efficiency index = pK_i or pIC_{50}/molecular weight (kDa), pIC_{50} was used in this study.

such as 4-fluoro-phenyl **15**, 4-fluoro-benzyl **19** or pyridine analogue **20** all showed a 2 log improvement in potency, with the best member of the series achieving 9 nM (2-methoxy-phenyl derivative **17**). Interestingly, amides derived from *sec*- and *tert*-alkyl amines remained in the same range of activity (compounds **21–24**), with compound **21** reaching the same potency and a better Binding Efficiency Index[10] (BEI) due to a smaller molecular weight when compared to the most potent aromatic amide (compound **17**). In this optimisation program, BEI was consistently considered to identify and focus on chemical functionalities carrying the strongest binding contributions.

Despite the high potency of aromatic amides, aliphatic derivatives were preferred due to the potential toxicity of the aniline group, these *N*-alkyl derivatives possessing the additional advantage of improving the physico-chemical properties.

Furthermore, the unsubstituted, *gem*-dimethyl and cyclohexyl derivatives **25–27** were prepared in order to explore the influence of the R^2/R^3 linker substitution (hydrophobic interaction and/or conformational effect through Thorpe-Ingold restriction).[11] The presence of a chain-constraining group was clearly necessary to achieve potency, as the unsubstituted analogue **25** failed to show satisfactory inhibition. This evidence was further supported by docking studies which showed an orientation of the pentanoic chain towards the hydrophobic pocket exploited by other 11β-HSD1 inhibitors (Fig. 2).

Amide replacement with the oxadiazole bioisoster led to potent 11β-HSD1 inhibitors, compounds **30** and **31** being the most potent with respective IC_{50} of 12 and 8 nM and an improved BEI. However, optimisation of these compounds was not pursued due to their poor metabolic stability (data not shown).

Although the adamantyl group has been used quite often in the synthesis of bioactive molecules,[12] its high hydrophobicity renders it prone to oxidation in living organism, which could lead to a rapid elimination of the drug. However, substituted-adamantanes have been used recently to prepare metabolically stable 11β-HSD1

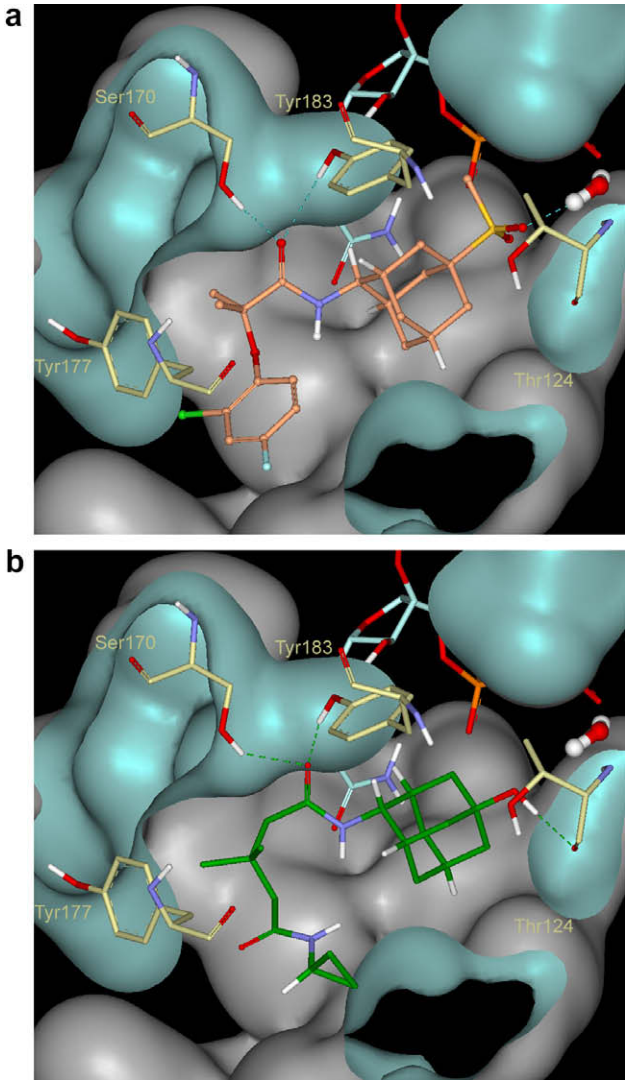
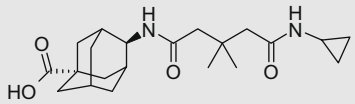
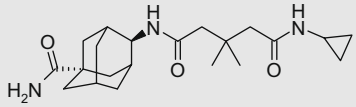
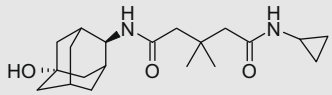
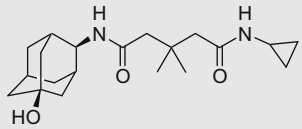


Figure 2. View of the already published steroid substrate binding site of human 11β-HSD1 (PDB code 2ILT)[15] with (a) the adamantane sulfone compound from the experimental structure and (b) docked compound **34**; for the sake of clarity, only Thr124, Tyr177 and catalytic residues Ser170 and Tyr183, and only polar hydrogen atoms have been displayed; colour coding by atoms is as follows: oxygen in red, nitrogen in blue, hydrogen in white, fluorine in light blue, chlorine in light green, phosphorus in orange; carbon atoms are coloured in light orange for the experimental ligand (a), in green for compound **34** (b), in light yellow for the protein and in light blue for the NADP(H) cofactor (protonation state not determined in the X-ray structure).

Table 3
Substituted adamantane derivatives

Compounds	Structures	h 11β-HSD1 IC_{50}[a] (nM)	BEI[b]
32		>1000	—
33		8	21.6
34		8	23.3
35		550	18.0

[a] h 11β-HSD1 experimental protocol as described in Table 2.
[b] BEI as described in Table 2.

inhibitors[13,12c] and DPP-4 inhibitors[14] suitable for oral administration. We therefore explored the influence of substituting the adamantane ring on the potency, physicochemical properties and metabolic stability of our best compounds.

Gratifyingly, not only the activity of the naked adamantyl compound **26** was preserved, but it was improved with the *trans*-carboxamide and *trans*-hydroxy isomers **33** and **34** (Table 3). In comparison, the *cis*-hydroxy isomer **35** was moderately active while the carboxylic acid adamantyl was inactive.

In order to investigate the influence of the hydroxy substituent of the adamantane on potency, compound **34** was docked in the already published human 11β-HSD1–adamantane sulfone co-crystal structure (PDB code 2ILT)[15] and results are depicted in Figure 2.[16] The carbonyl of the adamantyl-amide established hydrogen bonds with hydroxyl groups of residues Ser170 and Tyr183, while the adamantane occupied the same position in compound **34** docking pose and in the ligand from the original crystal structure. In addition to its potential influence on PK properties, the hydroxyl group carried by the adamantyl group contributed to the binding of compound **34** to the protein through a hydrogen bond with residue Thr124. As a consequence, compound **34** was identified as the compound having the best binding efficiency in this series (BEI of 23.3).

Potential of compounds **33** and **34** to complete lead candidate criteria was further evaluated (Table 4). Both **33** and **34** were potent inhibitors of human, mouse and rat 11β-HSD1, with a weaker potency of compound **34** in rat. Both compounds were highly selective against human 11β-HSD2 with IC_{50} selectivity ratio >1250. Compound **34** was more potent in the 3T3L1 cell-based assay and had better pharmacokinetic properties, with a bioavailability of 30% in a low-dose PK cocktail study. However both compounds suffered from a short half-life and a medium (compound **34**) to high clearance (compound **33**). In addition, higher p.o. recovery of unchanged compound in the feces for compound **33** (RF results) suggested that poor absorption contributed to its low bioavailability (F, % of the dose), while compound **34** was well absorbed (similar RF after i.v. and p.o. administration).

In conclusion, optimisation of a 1.2 μM hit led to the discovery of lead compound **34**. This compound displayed excellent activity on human and murine 11β-HSD1, was selective over 11β-HSD2, had good inhibition on mouse 11β-HSD1 (IC_{50} < 0.1 μM) in a cellular model (3T3L1 adipocytes), exhibited a decent PK profile and therefore looks promising for in vivo evaluation. Further optimisation of this compound is currently ongoing.

Table 4
Profile of the two lead compounds

Compounds	**33**	**34**
h 11β-HSD1 IC_{50}[a]	8 nM	8 nM
m 11β-HSD1 IC_{50}[a]	10 nM	88 nM
r 11β-HSD1 IC_{50}[a]	31 nM	980 nM
m 11β-HSD1 3T3L1 cellular IC_{50}[b]	<1 μM	<0.1 μM
h 11β-HSD2 IC_{50}[c]	>10 μM	>10 μM
h 11β-HSD2/HSD1 IC_{50} selectivity ratio	>1250	>1250
Water solubility	491 μg/mL	597 μg/mL
Plasma $t_{1/2}$[d]	0.6 h	1.2 h
F[d]	10%	30%
CL[d]	3.3 L/h/kg	1.8 L/h/kg
Vss[d,e]	3.1 L/kg	2.7 L/kg
RF[f]	24% (iv) 50% (po)	20% (iv) 22% (po)
h SPB[g]	<20%	40%

[a] h 11β-HSD1 experimental protocol as described in Table 2; for murine (m) or rat (r) 11β-HSD1, 2.5 μg of microsome fractions from liver (Tebu) were used in the assay.
[b] 3T3L1 cells were differentiated (5 days) in adipocytes and cultured (4 days) in a 24 well plate format before incubation with test compounds (10^{-5}–10^{-7} M) for 150 min. Cortisol in the culture supernatant was then measured for 11β-HSD1 activity in HTRF assay (see Table 2).
[c] 11β-HSD2 enzyme activity was assessed in 50 mM Tris buffer, pH 7.8, containing 1 mM $MgCl_2$, 0.5 mM NAD, and 1 μg of human recombinant 11β-HSD2 expressed in *E. coli*. Reaction was initiated by addition of cortisol 20 nM and terminated with addition of carbenoxolone 10 μM stop solution after 60 min incubation at room temperature. Determinations of cortisol levels were monitored by HTRF assay (Cisbio International).
[d] Dosed i.v. (0.2 mg/kg) and p.o. (0.5 mg/kg) in male rats in a low dose cocktail study; mean values over 3 rats.
[e] Volume of distribution at steady state.
[f] Recovery of unchanged drug in feces (0–24 h collection).
[g] Human serum protein binding.

References and notes

1. Nobel, S.; Abrahmsen, L.; Oppermann, U. *Eur. J. Biochem.* **2001**, *268*, 4113.
2. Tomlinson, J. W.; Walker, E. A.; Bujalska, I. J.; Draper, N.; Lavery, G. G.; Cooper, M. S.; Hewison, M.; Stewart, P. M. *Endocrinol. Rev.* **2004**, *25*, 831.
3. (a) Seckl, J. R.; Walker, B. R. *Endocrinology* **2001**, *142*, 1371; (b) Kotelevtsev, Y. V.; Brown, R. W.; Fleming, S.; Kenyon, C.; Edwards, C. R. W.; Seckl, J. R.; Mullins, J. J. *J. Clin. Invest.* **1999**, *103*, 683.
4. (a) Brereton, P. S.; van Driel, R. R.; Suhaimi, F.; Koyama, K.; Dilley, R.; Krozowski, Z. *Endocrinology* **2001**, *142*, 1644; (b) Bujalska, I. J.; Kumar, S.; Stewart, P. M. *Lancet* **1997**, *349*, 1210; (c) Jellinck, P. H.; Pavlides, C.; Sakai, R. R.; McEwen, B. S. *J. Steroid. Biochem. Mol. Biol.* **1999**, *71*, 139; (d) Moisan, M. M.; Seckl, J. R.; Edwards, C. R. W. *Endocrinology* **1990**, *127*, 1450; (e) Ricketts, M. L.; Verhaeg, J. M.; Bujalska, I. J.; Howie, A. J.; Rainey, W. E.; Stewart, P. M. *J. Clin. Endocrinol. Metab.* **1998**, 1325; (f) Whorwood, C. B.; Mason, C. B.; Ricketts, M. L.; Howie, A. J.; Stewart, P. M. *Mol. Cell. Endocrinol.* **1995**, *110*, R7.
5. Morton, N. M.; Holmes, M. C.; Fiévet, C.; Staels, B.; Tailleux, A.; Mullins, J. J.; Seckl, J. R. *J. Biol. Chem.* **2001**, *276*, 41293.
6. Masuzaki, H.; Paterson, J.; Shinyama, H.; Morton, N. M.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. *Science* **2001**, *294*, 2166.
7. Kershaw, E. E.; Morton, N. M.; Dhillon, H.; Ramage, L.; Seckl, J. R.; Flier, J. S. *Diabetes* **2005**, *54*, 1023.
8. White, P. C.; Mune, T.; Agarwal, A. K. *Endocrinol. Rev.* **1997**, *18*, 135.
9. See for example: (a) Wang, H.; Ruan, Z.; Li, J. J.; Simpkins, L. M.; Smirk, R. A.; Wu, S. C.; Hutchins, R. D.; Nirschl, D. S.; Van Kirk, K.; Cooper, C. B.; Sutton, J. C.; Ma, Z.; Golla, R.; Seethala, R.; Salyan, M. E.; Nayeem, A.; Krystek, J.; Sheriff, S.; Camac, D. M.; Morin, P. E.; Carpenter, B.; Robl, J. A.; Zahler, R.; Gordon, D. A.; Hamann, L. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3168; (b) Zhu, Y.; Olson, S. H.; Graham, D.; Patel, G.; Hermanowski-Vosatka, A.; Mundt, S.; Shah, K.; Springer, M.; Thieringer, R.; Wright, S.; Xiao, J.; Zokian, H.; Dragovic, J.; Balkovec, J. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3412; (c) Sun, D.; Wang, Z.; Di, Y.; Jaen, J. C.; Labelle, M.; Ma, J.; Miao, S.; Sudom, A.; Tang, L.; Tomooka, C. S.; Tu, H.; Ursu, S.; Walker, N.; Yan, X.; Ye, Q.; Powers, J. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3513; (d) Aster, S. D.; Graham, D. W.; Kharbanda, D.; Patel, G.; Ponpipom, M.; Santorelli, G. M.; Szymonifka, M. J.; Mundt, S. S.; Shah, K.; Springer, M. S.; Thieringer, R.; Hermanowski-Vosatka, A.; Wright, S. D.; Xiao, J.; Zokian, H.; Balkovec, J. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2799; (e) Zhu, Y.; Olson, S. H.; Hermanowski-Vosatka, A.; Mundt, S.; Shah, K.; Springer, M.; Thieringer, R.; Wright, S.; Xiao, J.; Zokian, H.; Balkovec, J. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*,

3405; (f) 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-Nii, Y.; Ohman, B.; Alberts, P.; Abrahmsen, L. *J. Med. Chem.* **2002**, *45*, 3813; (g) Coppola, G. M.; Kukkola, P. J.; Stanton, J. L.; Neubert, A. D.; Marcopulos, N.; Bilci, N. A.; Wang, H.; Tomaselli, H. C.; Tan, J.; Aicher, T. D.; Knorr, D. C.; Jeng, A. Y.; Dardik, B.; Chatelain, R. E. *J. Med. Chem.* **2005**, *48*, 6696; (h) Johansson, L.; Fotsch, C.; Bartberger, M. D.; Castro, V. M.; Chen, M.; Emery, M.; Gustafsson, S.; Hale, C.; Hickman, D.; Homan, E.; Jordan, S. R.; Komorowski, R.; Li, A.; McRae, K.; Moniz, G.; Matsumoto, G.; Orihuela, C.; Palm, G.; Veniant, M.; Wang, M.; Williams, M.; Zhang, J. *J. Med. Chem.* **2008**, *51*, 2933; (i) Xiang, J.; Wan, Z. K.; Li, H. Q.; Ipek, M.; Binnun, E.; Nunez, J.; Chen, L.; McKew, J. C.; Mansour, T. S.; Xu, X.; Suri, V.; Tam, M.; Xing, Y.; Li, X.; Hahm, S.; Tobin, J.; Saiah, E. *J. Med. Chem.* **2008**, *51*, 4068.

10. Abad-Zapatero, C.; Metz, J. T. *Drug Discovery Today* **2005**, *10*, 464.
11. (a) Eliel, E. L. In *Stereochemistry of Carbon Compounds*; McGraw-Hill: New York, 1962; pp 197–202; (b) Jung, M. E.; Gervay, J. *J. Am. Chem. Soc.* **1991**, *113*, 224.
12. (a) Iyazaki, A.; Tsuda, Y.; Fukushima, S.; Yokoi, T.; Vantus, T.; Bokonyi, G.; Szabo, E.; Horvath, A.; Keri, G.; Okada, Y. *J. Med. Chem.* **2008**, *51*, 5121; (b) Papanastasiou, I.; Tsotinis, A.; Kolocouris, N.; Prathalingam, S. R.; Kelly, J. M. *J. Med. Chem.* **2008**, *51*, 1496; (c) Rohde, J. J.; Pliushchev, M. A.; Sorensen, B. K.; Wodka, D.; Shuai, Q.; Wang, J.; Fung, S.; Monzon, K. M.; Chiou, W. J.; Pan, L.; Deng, X.; Chovan, L. E.; Ramaiya, A.; Mullally, M.; Henry, R. F.; Stolarik, D. F.; Imade, H. M.; Marsh, K. C.; Beno, D. W. A.; Fey, T. A.; Droz, B. A.; Brune, M. E.; Camp, H. S.; Sham, H. L.; Frevert, E. U.; Jacobson, P. B.; Link, J. T. *J. Med. Chem.* **2007**, *50*, 149; (d) Van der Mey, M.; Boss, H.; Hatzelmann, A.; Van der Laan, I. J.; Sterk, G. J.; Timmerman, H. *J. Med. Chem.* **2002**, *45*, 2520; (e) Stilz, H. U.; Guba, W.; Jablonka, B.; Just, M.; Klingler, O.; Koenig, W.; Wehner, V.; Zoller, G. *J. Med. Chem.* **2001**, *44*, 1158; (f) Kolocouris, N.; Kolocouris, A.; Foscolos, G. B.; Fytas, G.; Neyts, J.; Padalko, E.; Balzarini, J.; Snoeck, R.; Andrei, G.; De Clercq, E. *J. Med. Chem.* **1996**, *39*, 3307; (g) Kolocouris, N.; Foscolos, G. B.; Kolocouris, A.; Marakos, P.; Pouli, N.; Fytas, G.; Ikeda, S.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 2896; (h) Gerzon, K.; Krumins, E. V.; Brindle, R. L.; Marshall, F. J.; Root, M. A. *J. Med. Chem.* **1963**, *6*, 760.
13. (a) Olson, S.; Aster, S. D.; Brown, K.; Carbin, L.; Graham, D. W.; Hermanowski-Vosatka, A.; LeGrand, 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; (b) Yeh, V. S. C.; Kurukulasuriya, R.; Madar, D.; Patel, J. R.; Fung, S.; Monzon, K.; Chiou, W.; Wang, J.; Jacobson, P.; Sham, H. L.; Link, J. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5408; (c) Patel, J. R.; Shuai, Q.; Dinges, J.; Winn, M.; Pliushchev, M.; Fung, S.; Monzon, K.; Chiou, 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.
14. (a) Vielhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K. A.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774; (b) Kirk, J. K.; Oldham, E. C. *Metab. Imm. Drug Discovery* **2008**, *2*, 162.
15. Sorensen, B.; Winn, M.; Rohde, J.; Shuai, Q.; Wang, J.; Fung, S.; Monzon, K.; Chiou, W.; Stolarik, D.; Imade, H.; Pan, L.; Deng, X.; Chovan, L.; Longenecker, K.; Judge, R.; Qin, W.; Brune, M.; Camp, H.; Frevert, E. U.; Jacobson, P.; Link, J. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 527.
16. Docking was performed using the program GOLD 4.0.[17] The population size and number of operations per docking were set to auto with a maximum search efficiency (200%). Water molecules 4288 and 4285 were set to toggle and spin, and a maximum of 20 poses per ligand was set, using the Chemscore scoring function.[18]
17. (a) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727; (b) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. *Proteins* **2003**, *52*, 609.
18. (a) Murray, C. W.; Auton, T. R.; Eldridge, M. D. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 503; (b) Verdonk, M. L.; Berdini, V.; Hartshorn, M. J.; Mooij, W. T. M.; Murray, C. W.; Taylor, R. D.; Watson, P. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 793.