Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4837–4840
Bioorganic & Medicinal Chemistry Letters

Oxazolones as potent inhibitors of 11β-hydroxysteroid dehydrogenase type 1

Lori Sutin,* Sören Andersson, Lars Bergquist, Victor M. Castro, Eva Danielsson, Stephen James, Martin Henriksson, Lars Johansson, Christina Kaiser, Katarina Flyrén and Meredith Williams†

Biovitrum AB, SE-112 76 Stockholm, Sweden

Received 30 May 2007; revised 13 June 2007; accepted 13 June 2007
Available online 20 June 2007

Abstract—2,5,5-Trisubstituted oxazolones were identified as potent inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). The synthesis, structure–activity relationship and metabolic stability of these compounds are presented.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a biological target that has attracted much interest over the past few years.[1–3] This membrane-bound enzyme acts as an NADPH-dependent reductase and directly controls the interconversion of inactive cortisone to the receptor-active glucocorticoid cortisol (Fig. 1).[4]

It has been suggested, based on both animal and human studies, that glucocorticoid excess in tissues such as liver, adipose and skeletal muscle might be a contributing factor towards the onset of the metabolic syndrome.[5,6] Studies have shown that 11β-HSD1 knockout mice, compared to wild-type, demonstrate resistance to the development of diet-induced metabolic syndrome as well as displaying a lower fasting blood glucose level.[7] Furthermore, this phenotype was found to be closely tied to metabolic effects in the adipose tissue, where glucocorticoid-inducible transcripts encoding for leptin and TNF-α were reduced.[8] The enzyme, 11β-HSD1, therefore is thought to play a crucial role in diseases associated with the metabolic syndrome, such as obesity and diabetes. The type II isoform, 11β-HSD2, catalyzes the inactivation of cortisol using NAD as a cofactor. Whereas 11β-HSD1 is primarily expressed in the liver and adipose tissue, 11β-HSD2 is located mainly in the kidney.[9] Any potential drug aimed at inhibiting 11β-HSD1 should be selective towards this isoform, since inappropriate inhibition of 11β-HSD2 may lead to adverse side effects.[10]

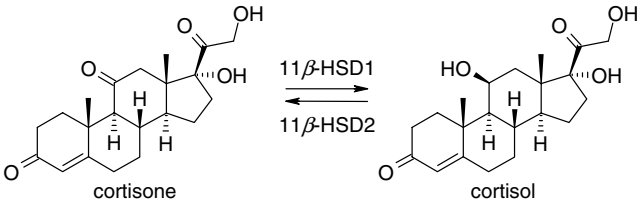


Figure 1. Interconversion of cortisone and cortisol by 11β-HSD1 and 11β-HSD2 enzymes.

Work from our laboratories has demonstrated that 2,5,5-trisubstituted 1,3-thiazol-4(5*H*)-ones (Fig. 2) are inhibitors of 11β-HSD1.[11] In particular, highly potent compounds were obtained with aliphatic mono-, bi- or tricyclic groups for R^1, in combination with short alkyl chains for R^2 and R^3.

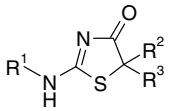


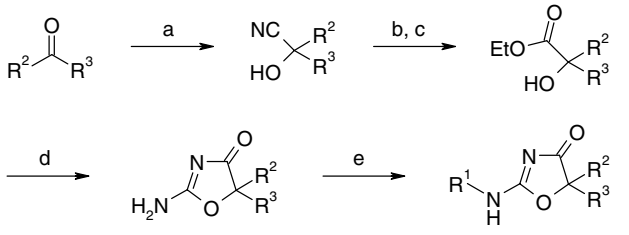
Figure 2. General structure of 11β-HSD1 active thiazolones.

Keywords: 11β-HSD1; Metabolic syndrome; Oxazolones.
* Corresponding author. Tel.: +46 8 6973540; fax: +46 8 6972320; e-mail: lori.sutin@biovitrum.com
† Present address: S*Bio Pte Ltd, 1 Science Park Road, #05-09 The Capricorn, Singapore Science Park II, Singapore 117528, Singapore.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.06.054

As an extension of this work, the analogous 1,3-oxazol-4(5*H*)-ones (henceforth abbreviated as oxazolones) were investigated. Synthesis of the 2-alkylamino-5,5-dialkyloxazolones was achieved in 5 steps from the corresponding ketones (Scheme 1). Addition of potassium cyanide to the ketones in 20% sulfuric acid gave the cyanohydrins.[12] The α-hydroxy esters were formed by hydrolysis of the cyanohydrins followed by esterification, and were then reacted with guanidine in the presence of base to give the corresponding 2-amino-5,5-dialkyloxazolones.[13] These key intermediates were then treated with primary amines[14] under microwave irradiation (typically at 180 °C for 1 h) to give the final products in modest yields (10–40%).[15]

Based on our experience of the SAR from the thiazolones (vide supra), a series of oxazolones (**1**–**17**) were synthesized incorporating alkyl, fluoroalkyl, alkylspiro or heteroalkylspiro moieties in the 5-position and mono-, bi- or tricycloalkylamines in the 2-position. The results, summarized in Table 1, include binding and inhibitory activity in human 11β-HSD1 SPA[16] and adipocyte[17] assays, respectively, as well as human and rat microsomal intrinsic clearance data.[18]

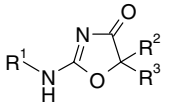


Scheme 1. Reagents and conditions: (a) KCN, 20% H_2SO_4, rt; (b) concd HCl, reflux; (c) EtOH, HCl, reflux; (d) guanidine hydrochloride, K_2CO_3, EtOH, reflux; (e) R^1NH_2, EtOH, MW 180 °C.

Results from the SPA assay reveal that several of the oxazolones are highly potent. For R^1, it appears that the potency is slightly enhanced with increased lipophilicity, manifested by the high activities of the adamantyl derivatives **8**, **10** and **12**–**14**. In accordance with this trend, the introduction of a 3-hydroxy substituent on the 1-adamantyl group (**16** and **17)** resulted in decreased activities when compared to the corresponding 1-adamantyl compounds **12** and **13**. For R^2 and R^3, 5,5-diethyl, 5-spiropentyl, 5-methyl-5-trifluoromethyl and 5-isopropyl-5-methyl substitution give essentially equipotent species within each R^1-series. The compounds employing heterospiro substitution at the oxazolone C5 position (**2**, **3** and **15**) exhibit lower binding activities, most markedly the *N*-benzyl-4-piperidine analogue **2**. To confirm selectivity, compound **5** was tested against 11 β-HSD2 and showed no appreciable activity ($K_i > 10$ μM).

The activities in adipocytes follow a similar trend as the SPA assay potencies, however, more dramatic differences are observed. This might indicate that some of the compounds are susceptible to poor cell permeability and/or efflux. For a majority of the compounds in Table 1, intrinsic clearance in rat microsomes is significantly lower than in human microsomes. However, an exception to this trend is observed with the

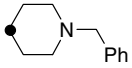
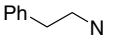
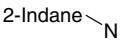
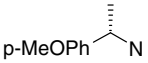
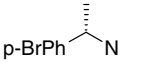
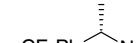
Table 1. SAR of 2-cycloalkylaminooxazolones



Compound	R^1	R^2	R^3	R^2, R^3	SPA K_i[a] (nM)	Adipocytes IC_{50}[a] (nM)	CL_{int}[a,b] (μL/min/mg)	
							Human	Rat
1	Cycloheptyl	Me	^{i}Pr		22	1866	5	52
2	Cycloheptyl			–$(CH_2)_2$–N(Bn)–$(CH_2)_2$–	>10,000	nd	nd	nd
3	Cycloheptyl			–$(CH_2)_2$–O–$(CH_2)_2$–	1433	nd	nd	nd
4	Cyclooctyl	Me	CF_3		17	117	6	62
5	Cyclooctyl	Et	Et		10	nd	nd	nd
6	Cyclooctyl	Me	^{i}Pr		17	61	10	125
7	Cyclooctyl			–$(CH_2)_4$–	14	454	9	50
8	2-Adamantyl	Me	CF_3		7	59	5	29
9	2-Adamantyl	Et	Et		12	76	nd	nd
10	2-Adamantyl	Me	^{i}Pr		6	14	28	81
11	2-Adamantyl			–$(CH_2)_4$–	18	214	13	51
12	1-Adamantyl	Et	Et		4	nd	26	108
13	1-Adamantyl	Me	^{i}Pr		6	24	20	94
14	1-Adamantyl			–$(CH_2)_4$–	4	nd	nd	nd
15	1-Adamantyl			–$(CH_2)_2$–O–$(CH_2)_2$–	43	273	6	28
16	1-(3-Hydroxyadamantyl)	Et	Et		39	358	5	5
17	1-(3-Hydroxyadamantyl)	Me	^{i}Pr		25	66	3	9

[a] For chiral compounds, data reported for mixture of stereoisomers.
[b] Microsomal clearance.

Table 2. 11β-HSD1 SPA binding affinities of oxazolones with aryl-containing amines in the 2-position

R^1	Compound K_i (nM)		
			
	**18** 6451	nd	nd
	**19** 1761	nd	nd
	**20** 879	nd	nd
	**21** 369	**22**[a] 77	**23** 676
	nd	**24**[a] 871	**25** 1418
	**26** >10,000	nd	nd
	**27** >10,000	nd	nd
	**28** >10,000	nd	nd
	**29** >10,000	nd	nd
	**30** 2875	nd	nd
	**31** 19	**32**[a] 46	**33** 20

[a] Racemate.

1-(3-hydroxyadamantyl) derivatives **16** and **17** for which both human and rat clearance are low. Moreover, the clearance values of **16** and **17** are significantly lower than those of the corresponding 1-adamantyl analogues **12** and **13**. This result is not unexpected since it is known that increased polarity of the substrate often decreases the affinity to metabolizing CYP enzymes.[19] Unfortunately, the 11β-HSD1 activities for **16** and **17** in adipocytes are modest.

To further investigate the SAR at the 2-position of the oxazolone, a series of compounds featuring aryl-containing substituents on the exocyclic nitrogen were synthesized. As shown in Table 2, the 2-benzylamine derivative (**19**) is more active than its one-carbon homologue (**18**), but both are considerably less potent than their cycloalkyl analogues presented in Table 1. Increased activity is observed with the derivatives incorporating indane-2-amine (**20**), and more significantly, (*S*)-methylbenzylamine (**21**).

A direct comparison of the (*S*)-methylbenzylamine derivatives **22** and **23** to their (*R*)-stereoisomers **24** and **25** clearly indicates that there is a stereochemical preference at the binding site for the (*S*)-configuration. This observation was also made for corresponding compounds in the thiazolone series.[20] Encouraged by this result, a series of oxazolones based on commercially available (*S*)-alkylbenzylamines were investigated. Notably, replacing the methyl group in **21** with either ethyl (**26**) or hydroxymethyl (**27**) results in loss of activity. Poor potency is also shown for the naphthyl derivative **28**. The series of substituted (*S*)-methylbenzylamines **29**–**31** reveal that the binding affinity is highly dependent on the substituent. The most potent compounds in the series are the *ortho*-CF_3 derivatives **31**–**33**. Notably, the *N*-benzyl-4-piperidine compound **33** exhibits surprisingly high activity when compared to the analogous species **23** and **25**. Even more striking is the difference in activity between **33** and its cycloheptyl analogue **2** (Table 1). This suggests that the SAR for the compounds with aryl-containing amines in the oxazolone 2-position cannot be directly extrapolated from that of the 2-cycloalkylamine series presented in Table 1.

Compounds **31**–**33** were investigated further with regard to 11 β-HSD1 potency in human adipocytes and for microsomal intrinsic clearance (Table 3). The results show that 11β-HSD1 activity in cells is high for **32** and **33**, and somewhat lower for **31**. The table also shows that the 5,5-dialkyl-substituted oxazolones **31** and **32** are metabolically more stable in both rat and human microsomes when compared to the spiro-*N*-benzylpiperidine analogue **33**. Furthermore, in comparing **31** and **32** to the corresponding 2-cycloalkylaminooxazolones (**1**, **6**, **10**, **12** and **13** in Table 1), rat microsomal metabolic stability is clearly improved. This implies that benzylic amines on the oxazolone 2-position might provide a versatile alternative to the cycloalkylamines in identifying compounds with a beneficial preclinical profile.

In conclusion, a novel class of potent 11β-HSD1 inhibitors has been identified. Oxazolone compounds incorporating cycloalkylamines or aryl-containing amines in

Table 3. 11β-HSD1 activity and intrinsic clearance data of oxazolones incorporating the *o*-(trifluoromethyl)phenyl group

Compound	SPA K_i (nM)	Adipocytes IC_{50} (nM)	CL_{int} (μL/min/mg)	
			Human	Rat
31	19	95	6	6
32[a]	46	12	23	15
33	20	29	193	293

[a] Racemate.

the 2-position and alkyl, fluoroalkyl, spiroalkyl or heterospiroalkyl substituents in the 5-position have been investigated for human 11β-HSD1 activity in SPA and adipocyte assays. Potent compounds with low intrinsic clearance in human and rat microsomes have been demonstrated.

References and notes

1. Walker, B. R.; Seckl, J. R. *Exp. Opin. Ther. Targets* **2003**, *7*, 771.
2. Fotsch, C.; Askew, B. C.; Chen, J. J. *Exp. Opin. Ther. Patents* **2005**, *15*, 289.
3. Barf, T.; Williams, M. *Drugs Future* **2006**, *31*, 231.
4. Krozowski, Z. *Mol. Cell. Endocrinol.* **1992**, *84*, C25.
5. Rosmond, R. *Psychoneuroendocrinology* **2005**, *30*, 1.
6. Asensio, C.; Muzzin, P.; Rohner-Jeanrenaud, F. *Int. J. Obes. Relat. Metab. Disord.* **2004**, *28*(Suppl. 4), S45.
7. 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.
8. Morton, N. M.; Paterson, J. M.; Masuzaki, H.; Holmes, M. C.; Staels, B.; Fievet, C.; Walker, B. R.; Flier, J. S.; Mullins, J. J.; Seckl, J. R. *Diabetes* **2004**, *53*, 931.
9. Kataoka, S.; Kudo, A.; Hirano, H.; Kawakami, H.; Kawano, T.; Higashihara, E.; Tanaka, H.; Delarue, F.; Sraer, J.-D.; Mune, T.; Krozowski, Z. S.; Yan, K. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 877.
10. Steward, P. M.; Wallace, A. M.; Valentino, R.; Burt, D.; Shackleton, C. H. L.; Edwards, C. R. W. *Lancet* **1987**, *2*, 821.
11. Henriksson, M.; Homan, E.; Johansson, L.; Vallgårda, J.; Williams, M.; Bercot, E.; Fotsch, C. H.; Li, A.; Cai, G.; Hungate, R. W.; Yuan, C. C.; Tegley, C.; St. Jean, D.; Han, N.; Huang, Q.; Liu, Q.; Bartberger, M. D.; Moniz, G. A.; Frizzle, M. J. WO 05116002, 2005.
12. Decourcy, M. S. Eur. Patent 1,371,632, 2003.
13. Traube, W.; Ascher, R. *Chem. Ber.* **1913**, *46*, 2077.
14. Those primary amines not commercially available were synthesized from the corresponding carboxylic acids via a modified Curtius rearrangement according to Ninomiya et al., followed by acid-mediated deprotection. Ninomiya, K.; Thioiri, S.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.
15. Lee, C.-M.; Horrom, B. W.; Michaels, R. J.; Rasmussen, R. M.; Plotnikoff, N. P. *J. Med. Chem.* **1972**, *15*, 1252.
16. Human 11β-HSD1 scintillation proximity assay (SPA) was carried out in replicon plates each replica containing 10 μL of diluted compound, 40 μL assay buffer, 10 μL of 11 mM G-6-P, and 25 μL substrate mixture [^{3}H]cortisone/NADPH (175 nM/200 μM). Reactions were initiated by the addition of 25 μL 11β-HSD1 as raw microsome fraction from recombinant *Pichia pastoris* (the final concentration of protein used was varied between 0.057 and 0.11 mg/mL, depending on the batch). Following mixing, the plates were incubated on a shaker for 30–60 min at rt. The reactions were terminated with 10 μL stop solution (1 mM GA in ethanol). Monoclonal mouse antibody was then added (10 μL of 1.92 μM working solution) followed by 50 μL of YSi SPA beads. As reference substance, carbenoxolone was run in each plate. The plates were sealed with plastic film (Perkin-Elmer, Top Seal-A) and incubated on a shaker for 30 min at rt before counting. The amount of [^{3}H]cortisol captured on the beads was determined in a microplate liquid scintillation counter. Kinetic constants were calculated employing the Microsoft Excel integrated application XLfit (Version 5.3.0.19, ID Business Solutions Ltd) using the sigmoidal dose–response model 205 which is based on the non-linear curve fitting based on Levenberg–Marquardts algorithm.
17. Compounds were dissolved in 100% DMSO to a final concentration of 10 mM and diluted in DMSO followed by a dilution in adipocyte medium. Cells were subjected to compounds serially diluted in eight steps (nine concentrations) ranging from 10 μM to 0.15 nM. The resulting compound solutions were added to cells in presence of 100 nM cortisone. All samples were made and analyzed in triplicate. Human primary subcutaneous adipocytes from ZenBio (#SP-F-1) were propagated and differentiated according to the protocol. Induction with compounds was made for 5 h and the cortisol level for each compound in the harvested media was determined with a Cortisol Immuno Assay Kit from Assay Designs (Correlate #ADI-901-071). Percent inhibition was calculated from absorbance (A_{405}) raw data of the samples relative to the positive control. Dose–response curves were generated by plotting percent inhibition against compound concentrations and IC_{50} values were calculated as the inflection point at 50% inhibition, using 4-Parameter Logistic Model in ExcelFit.
18. Incubations were performed at 37 °C using 1 μM compound concentration, 1 mg/mL microsomal protein and 1 mM NADPH in a total volume of 150 μL of 100 mM KPO_4 buffer, pH 7.4. Parent compound remaining (at six different time points from 0 to 40 min) is measured with LC-MS/MS after incubation with human and rat liver microsomes (microsomal protein concentration in incubation 1 mg/mL). In vitro half-life of the compound was calculated and from this CL_{int} (μL/min/mg) was derived.
19. Testa, B. *Biochemistry of Redox Reactions*; Academic Press, 1995, pp 31–32.
20. St. Jean, D. J., Jr.; Yuan, C.; Bercot, E. A.; Cupples, R.; Chen, M.; Fretland, J.; Hale, C.; Hungate, R. W.; Komorowski, R.; Veniant, M.; Wang, M.; Zhang, X.; Fotsch, C. *J. Med. Chem.* **2007**, *50*, 429.