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4,5-Disubstituted *cis*-pyrrolidinones as inhibitors of type II 17β-hydroxysteroid dehydrogenase. Part 3. Identification of lead candidate

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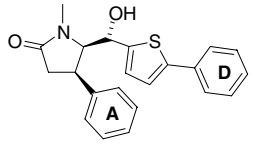
Abstract—A series of 4,5-disubstituted *cis*-pyrrolidinones was investigated as inhibitors of 17β-HSD II for the treatment of osteoporosis. Biochemical data for several compounds are given. Compound **42** was selected as the lead candidate.

The goal of this project was to identify an orally active inhibitor of 17β-hydroxysteroid dehydrogenase, type II (17β-HSD II),[1] an enzyme known to oxidize many steroid substrates. We aimed to test the hypothesis that such an inhibitor could increase local bone concentrations of estradiol resulting in maintenance of bone quality and strength in an osteoporosis model.[2] We previously reported on the discovery of 4,5-disubstituted *cis*-pyrrolidinone **1** as a 17β-HSD II inhibitor.[3] Here, we discuss further refinement of the series to identify a suitable compound for in vivo studies.

The screening cascade began with a biochemical assay that measured conversion of NAD to NADPH by recombinant human 17β-HSD II. Next, inhibition of estradiol to estrone conversion by intact osteoblast MG63 cells was measured. Advanced compounds were evaluated against 11β-HSD II, the closest structurally related hydroxysteroid dehydrogenase, and for absence of binding in several hormone receptor assays. Analogs of interest were resolved into enantiomers by chiral chromatography, and evaluated in plasma exposure studies.

The relative stereochemistry illustrated in Figure 1 was critical for activity against 17β-HSD II.[3] Further investigations were needed to understand the SAR involving the two phenyl groups. Related regioisomers of the 2-phenylthiophene show only slightly lower activity against 17β-HSD II, suggesting that the thiophene is in a large pocket or exposed to solvent (Table 1). Although benzothiophene **4** showed moderate activity, it was rapidly degraded by liver microsomes and was not pursued.

Due to the relatively large tolerance to modifications of the D-ring, we sought to improve potency along with



1

Figure 1. Pyrrolidinone **1** shown with relative stereochemistry.

Keywords: Pyrrolidinone; Hydroxysteroid dehydrogenase; Inhibitor; Osteoporosis.
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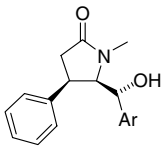
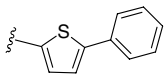
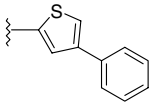
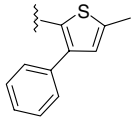
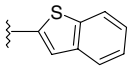
Table 1. Thiophene substitution pattern[4]



Compound	Ar	17β-HSD II IC_{50} (μM)
1		0.10
2		0.58
3		0.32
4		0.59

some of the biopharmaceutical properties, specifically solubility, by varying this position (Table 2). These analogs were prepared either by direct addition of an aryl thiophene to **5** as previously reported,[5] or, via Suzuki coupling of **6** with various boronic acids (Scheme 1).

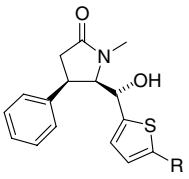
A wide variety of substituents such as mono-, di-, and tri-substituted phenyls, naphthyls, and biphenyls were investigated. Loss of activity was seen with some large or more lipophilic groups, however reasonable activity was maintained over a very diverse set of analogs.

Substituents at the *meta*-position of the phenyl ring did not greatly affect activity. Both electron-withdrawing (**10**) and electron-donating (**11** and **12**) groups caused a slight drop-off in activity. At the *para*-position, hydrophilic substituents generally showed much better activity than lipophilic groups. The *para*-position also seemed to be readily oxidized by liver microsomes. In comparing the active enantiomers of **1** (*para*-H) and **16** (*para*-F) after 20 min in rat microsomes, we found only 11% of (−)-**1** remaining while there was 94% of (−)-**16** remaining. The putative metabolite, phenol **14**, maintains moderate activity.

Most of the pyrrolidinones were poorly soluble in water. To improve water solubility, nitrogen heterocycles and acid derivatives were synthesized. Acids **13** and **21** were more soluble but unfortunately were much less active in the cell assay. The 2- and 3-pyridyl compounds, **19** and **20**, respectively, maintained activity in the cell however, they were still poorly soluble as a free base. Nevertheless, the pyridyl analogs had the option for salt formation at a later stage to aid solubility.

Linking groups were investigated between the thiophene and the phenyl group (**22**–**26**). Again we found that a wide variety of groups were tolerated, however sulfone

Table 2. D-ring analogs[4]



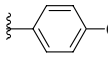
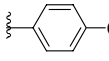
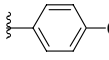
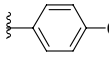
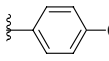
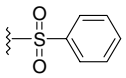
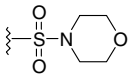
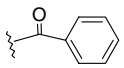
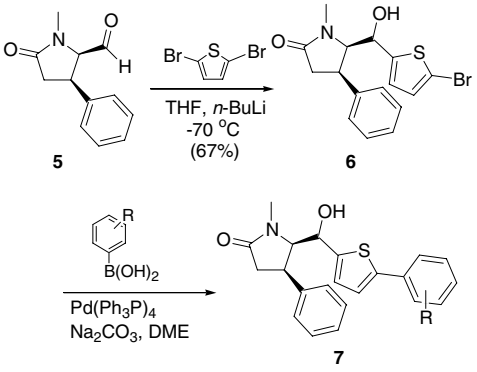
Compound	R	17β-HSD II IC_{50} (μM)
1		0.10
8		0.12
9		0.28
10		0.58
11		0.34
12		0.15
13		1.32
14		0.48
15		4.11
16		0.14
17		0.03
18		1.28
19		0.15
20		0.30
21		0.38
22		0.33
23		1.19

Table 2 (*continued*)

Compound	R	17β-HSD II IC_{50} (μM)
24		0.16
25		0.07
26		0.95

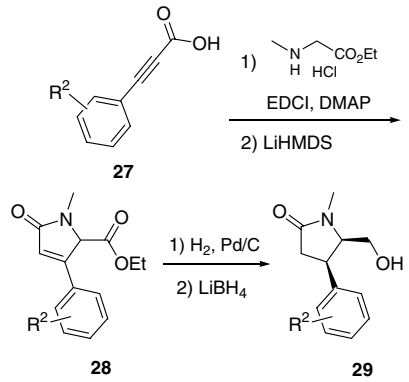


Scheme 1. Late stage modification of D-ring.

linkers were the most potent in the biochemical assay. Phenyl sulfones (**24**) gave compounds that were potent in the biochemical and cell assays. Sulfonamide **25** was very active against the enzyme, but lost activity 10-fold in the cellular assay.

Turning to the preparation of A-ring analogs, few changes could be made using the initial route.[5] However, an alternative synthesis based on an intramolecular Michael addition was developed that allows for the production of A-ring analogs (Scheme 2).[6]

In vitro evaluation of this series indicated that substitution at the *ortho*-position by small groups was neutral to beneficial while substitutents at the *meta*- and *para*-positions, for the most part diminished the 17β-HSD II



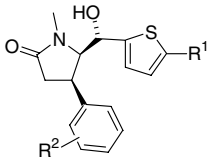
Scheme 2. Intramolecular Michael addition route.

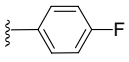
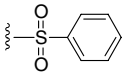
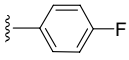
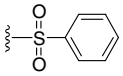
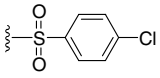
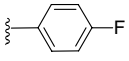
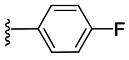
activity. An *ortho*-fluorine generally improved the activity 10-fold (**30**–**35**) (Table 3).

In summary, biochemical potency against 17β-HSD II was achieved with analogs from a novel, non-steroidal structural class (Tables 1–3). The majority of enzyme active compounds maintained activity in the cell estradiol conversion functional assay. The pyrrolidinones did not show appreciable binding to the estrogen, androgen or other steroid receptors and no tangible activity was seen in the counter-screens (11 β-HSD II, 17β-HSD I, and 17β-HSD III).

The SAR in the pyrrolidinone series differed greatly between human and rat enzyme; this was not unexpected since rat 17β-HSD II has only a 60% homology to the human protein.[7] Since SAR from biochemical assays using monkey enzyme correlated with the human results, it was felt a proof of principle experiment should be conducted in Cynomolgus macaques. In preparation for this study, select analogs were separated into enanti-

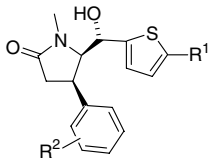
Table 3. Substituted A-ring analogs[4]



Example	R^1	R^2	17β HSD II IC_{50} (μM)
30[a]		2-F	0.01
31		2-F	0.06
32		2-F	0.06
33		2-OCH_3	0.12
34		2-OCH_3	0.04
35		2-OCH_3	0.19
36		3-F	0.48
37		3-OCH_3	2.55
38		4-F	0.76
39		4-OCH_3	0.88

[a] Single enantiomer.

Table 4. Resolved enantiomers[9]



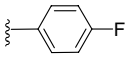
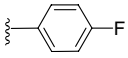
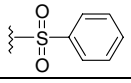
Example	R^1	R^2	17β-HSD II IC_{50} (μM)	MG63 cell IC_{50} (μM)
40		H	0.12	0.10
41		2-F	0.22	0.06
42		H	0.05	0.09
43		2-F	0.03	0.03
44		H	0.06	0.48

Table 5. Exposure studies

	Sprague–Dawley rat 20 mg/kg, po	Cynomolgus macaque 30 mg/kg, po
AUC_{norm} (g h/L)	76.1	179
$C_{max,\ norm}$ (g/L)	28.7	36.4

Compound **42** was dosed as a suspension in CMC/Tween 80/Raspberry crystal light (0.5/0.5/99).

omers using chiral HPLC.[8] Compounds as single enantiomers were prioritized in rat serum exposure studies (po) before moving to Cynomolgus macaque exposure studies. Representative compounds are shown in Table 4.

Compound **43**, when dosed as the hydrochloride salt, had very low exposure level in the rats and so was not considered further. Compounds **40**, **41**, **42**, and **44** advanced to plasma exposure studies in macaques. In all cases except for **42**, the exposure level was 2–10-fold lower in macaques than in rats, based on AUC. A parallel trend was observed in microsomal incubations, that is, compounds **40**, **41**, and **44** were more stable in rat microsomal assays than in the macaque, while **42** was more stable in the macaque assay. Table 5 outlines the plasma levels obtained for **42**. The hydrochloride salt of **42** was also profiled in plasma studies and did not show significantly different plasma levels than the parent. Based on its overall in vitro profile and the Cynomolgus macaque exposure profile, compound **42** was selected as the lead to establish non-human primate proof-of-concept.

Acknowledgments

We thank L. Musza for his structural NMR work. We also thank Dr. Rolf Grosser and Dr. J. Brice for chiral HPLC method development and support. We thank Dr. D. Mueller and G. Medvedeff for providing ample human 17β-HSD II enzyme for the biochemical assay. We thank T. Alebic, S. Jaworski, and M. Prevost for plasma data and D. Young and L. Zadjura for microsomal stability data. We thank Dr. Y.-C. Tseng and J. Luong for formulation support. We thank Dr. Robert Brommage and his team at Wake Forest University for collecting the primate plasma samples.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.041.

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4. In Tables 1–3 biochemical data are given, compounds are racemic unless otherwise noted.
5. (a) Hartwig, W.; Born, L. *J. Org. Chem.* **1987**, *52*, 4352; (b) Wood, J. E.; Baryza, J. L.; Brennan, C. R.; Choi, S.; Cook, J. H.; Dixon, B. R.; Ehrlich, P. P.; Gunn, D. E.; McAlexander, I.; Liu, P.; Lowe, D. B.; Redman, A. M.; Scott, W. J.; Wang, Y. US Patent 6,784,167 B2, 2004.
6. Cook, J. H.; Baryza, J.; Brennan, C.; Lowe, D.; Wang, Y.; Redman, A.; Scott, W. J.; Wood, J. E. *Tetrahedron Lett.* **2005**, *46*, 1525.
7. The human and the rat 17β-HDS II (Accession # NM_002153 and BC088134, respectively) are 60% homologous at the protein level using Omiga software.
8. Alcohol **29** could also be resolved by HPLC and used as a late stage intermediate.
9. Absolute stereochemistry as shown was determined by NMR studies on the methoxy-phenylacetic esters of both enantiomers of one analog in the *cis*-pyrrolidinone series.