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4,5-Disubstituted *cis*-pyrrolidinones as inhibitors of type II 17β-hydroxysteroid dehydrogenase. Part 2. SAR

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Abstract—4,5-Disubstituted *cis*-pyrrolidinones were investigated as inhibitors of type II 17β-hydroxysteroid dehydrogenase (17β-HSD). Early structure–activity relationship patterns for this class of compounds are discussed.

It is well recognized that steroids have an important influence on bone physiology. Many participate in the sexual dimorphism of the skeleton and play a key role in mineral homeostasis during reproduction. Such compounds are essential in maintaining bone balance in adults, where insufficient regulation of certain steroids can lead to bone loss and osteoporotic fractures.[1] The regulation of sex hormone levels is an important therapeutic approach for a variety of diseases. Estrogen has been historically implicated as an important steroid in preventing osteoporosis in women.[1] Hormone replacement therapy, using estrogen or estrogen–progesterone combinations, has been used as a treatment for this disease.[2,3] Currently, raloxifene,[4] a selective estrogen receptor modulator (SERM), is approved for the treatment of osteoporosis in postmenopausal women.[5] The related sex hormones, androgen and estrogen, have received recent interest as targets for cancer[6] and osteoporosis,[7] respectively.

The mechanism by which estrogen preserves bone mass is complex. Mature bone formation results from the mineralization of a collagen-rich environment that is synthesized by heterogeneous osteoblastic cells.[8] Since osteoblasts express estrogen receptors and respond to estrogen treatment, it is believed that estrogen has a direct effect on maintenance of bone mineral density.[9] The recombinant type II 17β-hydroxysteroid dehydrogenase (HSD) converts 17β-hydroxysteroids such as estradiol, testosterone, and dehydrotestosterone to their ketone counterparts.[10] The interconversion of estradiol to estrone by bone cells has been hypothesized as a possible mechanism for the local regulation of intracellular ligand supply.[9] Biologically active 17β-estradiol has between 3- and 50-fold greater affinity than estrone for the estrogen receptors ERα and ERβ.[9] Inhibition of oxidative type II 17β-HSD activity could help maintain local levels of estradiol in bone tissue when circulating estradiol levels drop. Thus, the identification of a non-steroidal small molecule that modulates steroid levels through type II 17β-HSD inhibition could provide a novel approach to treating osteoporosis.[10]

Pyrrolidinones **1** and **2** served as leads from high-throughput screening for type II 17β-HSD inhibition to initiate our medicinal chemistry effort (Fig. 1). In addition to reasonable potency in the primary conversion assay (IC_{50} = 700 and 400 nM, respectively), compounds **1** and **2** were inactive at the estrogen and

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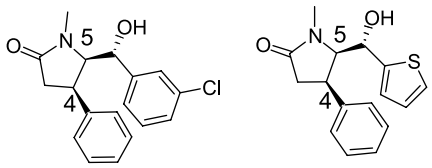


1 2

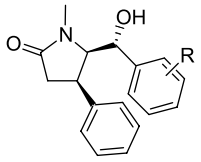
Figure 1. *cis*-4,5-Disubstituted pyrrolidinone leads.

selected nuclear receptors (androgen, progesterone, and glucocorticoid), and at type I and III 17β-HSDs.[11] It is noteworthy that the stereochemistry of the C(4) and C(5) substituents on the pyrrolidinone ring must be cis to maintain good activity against type II 17β-HSD (trans stereochemistry $IC_{50} > 10\ \mu M$). All compounds described have the C(4)–C(5) cis configuration. The detailed synthesis of **1**, **2**, and related target molecules has been previously reported, including Part 1 of this effort.[12,13] To summarize, they were readily prepared by the addition of a metalated aromatic ring into an advanced aldehyde precursor (Scheme 1). This report will focus on our early SAR effort derived from intermediate **A**.[12,13]

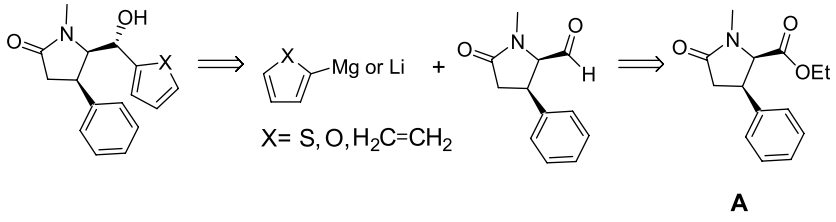
A number of substituted 5-[hydroxy(phenyl)methyl] pyrrolidinones of type **1** were synthesized (Table 1).[14] In general, substitution at the *meta* position was favored over the *para* position although the electronic effects were not significant. The 3-phenyl analog **7** ($IC_{50} = 420$ nM) and the 3,4-dichloro analog **11** ($IC_{50} = 440$ nM) were moderately better than the lead.

Next we turned our attention to possible heterocyclic replacements for the 5-[hydroxy(2-thienyl)methyl] moiety in compound **2**. Substitution changes on the thiophene moiety revealed that we could achieve good potency across a small subset of analogs (Table 2).

Table 1. SAR of 5-[(3-chlorophenyl)(hydroxy)methyl] analogs



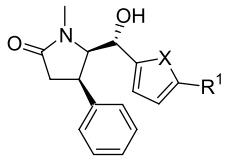
Compound	R	17β-HSD II IC_{50}, μM[15]
1	3-Cl	0.7
3	H	5.0
4	3-Me	1.52
5	3-CF_3	0.88
6	3-OCH_3	0.81
7	3-Ph	0.42
8	3-(4-F-C_6H_4)	2.78
9	3,4-OCH_2O–	0.96
10	4-Cl	1.96
11	3,4-Di-Cl	0.44
12	4-Me	1.82
13	4-Ph	1.02
14	4-(4-F-C_6H_4)	1.26
15	4-OCH_3	1.23
16	4-$N(CH_3)_2$	1.65
17	4-(4-OH-C_6H_4)	1.13



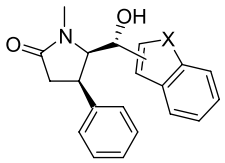
A

Scheme 1. Retrosynthesis of target molecules.

Table 2. SAR of heterocyclic replacements



2-23



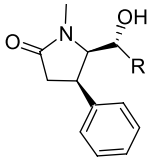
24-27

Compound	X	R^1	17β-HSD II IC_{50}, μM[15]	Compound	X	Position	17β-HSD II IC_{50}, μM[15]
2	S	H	0.40	**24**	S	2	0.59
18	S	Me	0.56	**25**	S	3	2.66
19	S	Ph	0.10	**26**	O	3	1.16
20	S	Br	1.2	**27**	NH	2	>10
21	O	H	3.18				
22	O	Br	1.27				
23	O	Ph	3.24				

Phenyl thiophene **19** (IC_{50} = 100 nM), like compound **7**, showed a considerable increase in potency. Replacements with furan and substituted furans showed a notable decrease in activity as exemplified in compound **23** IC_{50} = 3.24 μM. Benzothiophene analog **24** was comparable in activity to the substituted thiophenes. The corresponding 3-benzothiophene showed weak activity and was not further pursued. Additionally, the benzofuran and indole replacements were also less active.

Alkyl and alkyne analogs were studied as suitable replacements for the aromatic phenyl and thiophene groups (Table 3). There was a significant drop in activity for alkyl modified analogs as indicated by *tert*-butyl **28** (IC_{50} > 10 μM) and the methylene homolog of the lead **29** (IC_{50} = 2.39 μM). The extended analog **30** was weak (IC_{50} = 1.83 μM); however, many substituted alkynes maintained potency comparable to the initial leads, for example, **33** (IC_{50} = 300 nM). Reduction of the alkyne to the *trans* olefin or alkyl group, as demonstrated in the compound series **39**, **41**, **31**, resulted in a moderate loss of activity. Although further potency optimization may be possible in the alkyne series, exploration was abandoned due to the poor PK properties of compound **34**.

Table 3. Aryl replacements

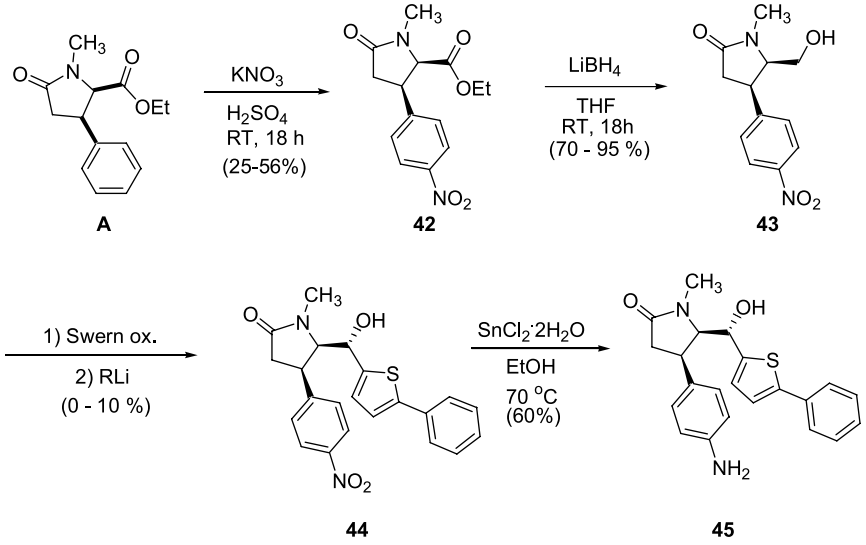


Compound	R	17β-HSD II IC_{50}, μM[15]
28	*tert*-Bu	>10
29	CH_2(3-Cl-C_6H_4)	2.39
30	CH_2CH_2Ph	1.83
31	CH_2CH_2(4-F-C_6H_4)	1.53
32	CC-Ph	0.43
33	CC-(2-Cl-C_6H_4)	0.30
34	CC-(2-F-C_6H_4)	0.32
35	CC-(3-OH-C_6H_4)	1.76
36	CC-(3-OCH_3-C_6H_4)	1.09
37	CC-(3-NH_2-C_6H_4)	0.37
38	CC-(4-Me-C_6H_4)	0.80
39	CC-(4-F-C_6H_4)	0.48
40	CC-2-Pyr	1.50
41	(4-F)Ph	1.40

Advanced intermediate **A** was also used to examine a small number of analogs at the 4-position of the pyrrolidinone. Several direct conversion methods such as chlorination, acetylation, and formylation on intermediate **A** proved problematic, however compound **A** could be nitrated to produce **42** (Scheme 2). Following a transformation of the ester to the desired heteroaromatic side chain, the nitro group could be reduced using tin chloride to give the *p*-amino phenyl analog **45**. The amine was converted to several analogs using standard chemistry. Only small, electron donating groups at the 4-position maintained or slightly increased activity as shown in Table 4. Later in the program, *ortho* and *meta* substituents were prepared through independent syntheses. These results will be the subject of a later paper.

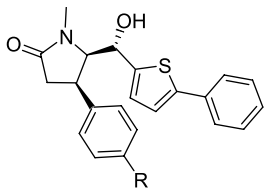
Modification of the secondary alcohol was also investigated. The alcohols **2** and **7** could be converted to ethers **52** (IC_{50} = 3.91 μM) and **53** (IC_{50} = 0.9 μM), acetate **54** (IC_{50} = 1.37 μM), fluorine **55** (IC_{50} = 2.98 μM), or removed **56** (IC_{50} = 2.18 μM) and maintain some activity (Table 5). However, when the alcohol is oxidized to a ketone as in **58** and **59**, converted into the epimeric alcohol **60** or extended as in **57**, the compounds lose activity.

Finally, modifications to the pyrrolidinone core were examined. Reduction of the carbonyl to give the pyrrolidine **61** eliminated all activity. A thio lactam **62** gave a concomitant loss of activity. The *N*-substitution of the



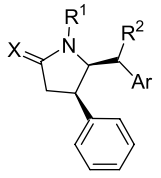
Scheme 2. Functionalization of the C-4 phenyl ring.

Table 4. (C-4) Phenyl modifications



Compound	R	17β-HSD II IC_{50}, μM[15]
44	NO_2	>10
45	NH_2	0.19
46	OH	0.22
47	OMe	0.89
48	NHMe	1.67
49	NHAc	>10
50	$NHSO_2CH_3$	>10
51	$N(Me)_2$	2.43

Table 5. Functional modifications



Compound	X	R^1	R^2	Ar	17β-HSD II IC_{50}, μM[15]
52	O	Me	OMe	2-Thienyl	3.91
53	O	Me	OMe	(3-Cl-C_6H_4)	0.90
54	O	Me	OAc	2-Thienyl	1.37
55	O	Me	–F	2-Thienyl	2.98
56	O	Me	H_2	(3-Cl-C_6H_4)	2.18
57	O	Me	OCH_2CH_2OH	2-Thienyl	>10
58	O	Me	=O	(3-Cl-C_6H_4)	>10
59	O	Me	=O	2-Thienyl	>10
60	O	Me	◂OH	(3-Cl-C_6H_4)	>10
61	H,H	Me	OH	2-Thienyl	>10
62	S	Me	OH	2-Thienyl	>10
63	O	H	OH	2-Thienyl	>10
64	O	Bn	OH	2-Thienyl	4.18

lactam was critical for activity, as de-alkylation or increasing the size both afforded inactive compounds.

In summary, we have discovered a novel non-steroid class of compounds that are potent inhibitors of type II 17β-hydroxysteroid dehydrogenase. Several features of lead molecules **1** and **2** were determined to play a role in activity. Aromatic modifications at the 5-[hydroxy(aryl)methyl] moiety were found to modulate activity. Additionally, substitutions on the aryl ring at the C4-position of the pyrrolidinone were tolerated with small electron-donating groups. Overall, we found the potency of the initial leads was improved by adding a second aromatic group to the 5-[hydroxy(aryl)methyl] side chain as shown by compound **19**, the most potent analog identified in the series. Further investigations surrounding this lead will be the topic of subsequent reports.

Acknowledgments

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10. Poirer, D. *Curr. Med. Chem.* **2003**, *10*, 453. Of the eight isoforms of 17β-hydroxysteroid dehydrogenase known, five isoforms have been isolated and cloned. Types 1, 3, and 5 generally are reductases while types 2 and 4 are oxidases. These enzymes have only a 15–30% sequence identity to each other. They are present throughout the body with each isoform having unique tissue specificities. The hydroxysteroid dehydrogenases are a family of enzymes that catalyze the stereospecific oxido-reduction reactions of alcohols or carbonyls using NAD(P)H or NAD(P) as a cofactor. Among the different HSDs, 3α-, 3β-, 11β-, 17β-, and 20α-HSDs are found in humans. 17β-HSDs catalyzing the last reaction of the biosynthesis of most estrogens and androgens, such as estradiol, 5-androsterone-3β,17β-diol (Δ^5-diol), testosterone, and dihydrotesterone.
11. Selectivity data generated during the medicinal chemistry effort correlates with the lead structures.
12. (a) Hartwig, W.; Born, L. *J. Org. Chem.* **1987**, *52*, 4352; (b) Hartwig, W. U.S. Patent 4,731,456, 1988; (c) Hartwig, W. U.S. Patent 4,751,315, 1988; (d) Hartwig, W. U.S. Patent 4,803,285, 1989.
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14. All compounds listed are racemic (cis) unless otherwise noted. Compound structures were consistent by ^{1}H NMR and LC–MS analysis (>95% purity).

15. Type II 17β-HSD enzyme assay conditions (based on the conversion of the cofactor, NAD to NADPH): After titration, the 17β-HSD II preparation, generated with a standard protocol,[16] was diluted to the appropriate concentration (generally 1/15 dilution) and 55 μL of assay buffer (50 mM Hepes, pH 8.0) was added to all wells of a 96-well plate. DMSO (5 μL, 5%) were added to wells A1–C1, A2–C2, F1–H1, and F2–H2. A 10 μL aliquot of a 10 mM DMSO stock of each compound well was diluted in 190 μL of assay buffer. The plate with these compound dilutions was then mixed for 15 min on a shaker and a 5 μL aliquot from each well was added to the test plate. This was followed by 10 μL of HSD 2 enzyme (1/15 dilution)/estradiol mix in assay buffer containing 1% Triton X-100. The estradiol mix was prepared from a 20 mM estradiol DMSO stock solution, which was diluted 1/20 (50 μg/mL) in 50 mM Hepes buffer containing 1% Triton X-100. All wells, except F1–H1 and F2–H2, also received 10 μL of 25 mg/mL NAD (Sigma) in 100 mM Hepes, pH 9. The blank wells F1–H1 and F2–H2 received 10 μL of NAD buffer and 20 μL of PMS–MTS solution (100 μL PMS into 1800 μL MTS, Promega) was added to all wells. The plate was covered with aluminum foil and incubated at room temperature for 1.5 h. The plate was analyzed at 490 nm on a Hewlett–Packard 8453 spectrophotometer and results were calculated using an in-house analysis program and the Hill slope method.

16. Type II 17β-HSD protein purification: The method for purification of baculovirus-expressed HSD II was standardized as follows. The cell pellet containing recombinant HSD II was lysed in 2.5 vol of lysis buffer (40 mM Tris, pH 7.5, 1 mM EDTA, 2 mM DTT, 10 μM NAD) containing 500 mM AEBSF, 10 μg/mL aprotinin, 1 μM pepstatin A, and 100 μM leupeptin. The pellet was nutated for 30 min in this buffer. Subsequently, the pellet was homogenized with 15 strokes using a glass Teflon potter. The volume was doubled with a 2× sucrose/NaCl solution (500 mM sucrose, 300 mM NaCl in lysis buffer). After centrifugation at 10,000*g* for 30 min, the supernatant was decanted and stored at −20 °C. The pellet was resuspended in 2.5 vol of lysis buffer containing 250 mM sucrose and 150 mM NaCl and 1% Triton X-100. The suspension was nutated for 20 min and then homogenized with five strokes. Subsequently, the suspension was centrifuged at 30,000*g* for 1 h and the supernatant was decanted and poured into a chromatographic column. Sephadex G-25 (fine) was added in small amounts, allowing approximately 15 min after each addition for complete swelling. Sephadex addition was continued until all liquid was absorbed. The protein was eluted with one void volume of lysis buffer and the eluate was monitored at 280 nm optical density. The chromatography was repeated and an equal volume of glycerol was added to the final eluate.