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Identification of oxazolidinediones and thiazolidinediones as potent 17β-hydroxysteroid dehydrogenase type 3 inhibitors

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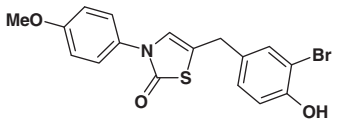
ABSTRACT

Novel and potent inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) were identified based on oxazolidinedione and thiazolidinedione derivatives, starting from a high-throughput screening hit, 5-(3-bromo-4-hydroxybenzyl)-3-(4-methoxyphenyl)-1,3-thiazol-2-one **1**. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-thiazolidin-4-one **21** exhibited a promising activity profile and demonstrated significant selectivity over the related 17β-HSD isoenzymes and nuclear receptors.

Prostate cancer is the most frequently diagnosed cancer in men. According to the American Cancer Society, an estimated 192,280 men were diagnosed with prostate cancer in the US during 2009. With an estimated 27,360 deaths in 2009, prostate cancer is the second-leading cause of cancer death in men.[1] For advanced prostate cancer, 10–60% of the patients experience biochemical recurrence associated with treatment failure, and no consensus exists on the optimal therapy.

The human prostate is a hormone-sensitive organ that depends on androgens for growth and development. The regulation of androgen biosynthesis or its action on the androgen receptor is central to the management of prostate cancer.[2] Production of androgens is controlled at two levels within the central nervous system; in addition, it is controlled locally in peripheral organs that are targeted by the hormones. The active androgens testosterone (T) and dihydrotestosterone (DHT) can be synthesized through the conversion of the inactive precursor 4-androstene-3,17-dione (Δ^4-dione) by 17β-hydroxysteroid dehydrogenases (17β-HSDs), which mediate the activation and deactivation of sex steroids involving redox reactions centered about the C17 area of the steroid backbone.[3]

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) catalyzes the final step in the steroidogenesis of the potent androgen T by selectively reducing the C17 ketone of Δ^4-dione with NADPH as a cofactor, in testis and prostate tissue.[4] 17β-HSD3 is expressed at high levels in these peripheral target tissues, suggesting its potential involvement in both gonadal and non-gonadal T biosynthesis. In addition, the expression of 17β-HSD3 mRNA in cancerous prostate biopsies was found to be 30-fold higher than in normal tissue.[5] Therefore, the role of 17β-HSD3 in T biosynthesis makes this enzyme an attractive molecular target of a small-molecule inhibitor for the treatment of prostate cancer.[6]



1

Figure 1. Structure of HTS hit compound **1**.

From a high-throughput screening (HTS) of a 200K-member of small-molecule compound library in search of inhibitors of the 17β-HSD3, we identified 5-(3-bromo-4-hydroxybenzyl)-3-(4-methoxyphenyl)-1,3-thiazol-2-one **1** with a submicromolar IC_{50} value (Fig. 1).

Compound **1** represented an attractive starting point for medicinal chemistry in consideration of its biochemical potency.

We focused on the central ring system of biaryl-substituted heterocycle series. The biaryl analogs were prepared following the synthetic procedure outlined in Scheme 1. Commercially available *p*-anisidine **2** was treated with Boc_2O, followed by addition of

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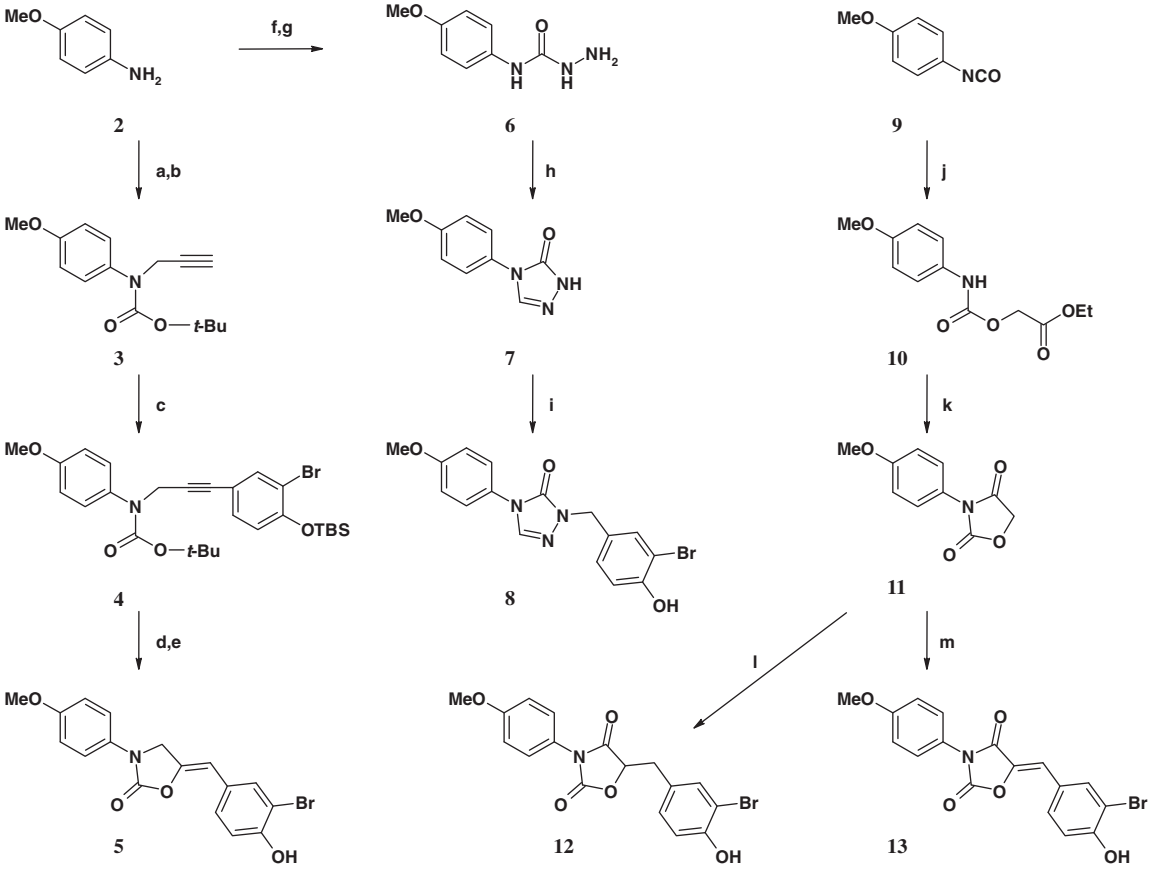


Scheme 1. Reagents and conditions: (a) Boc_2O, THF, rt, 5 h, 82%; (b) propargyl bromide, NaH, DMF, rt, 1 h, 71%; (c) 2-bromo-4-iodo-1-(*t*-butyldimethylsilyloxy)benzene, $Pd(OAc)_2$, CuI, PPh_3, TEA, toluene, rt, 5 h, 68%; (d) $AuPPh_3Cl$, $AgSbF_6$, CH_2Cl_2, rt, 1.5 h, 43%; (e) TBAF, THF, rt, 0.5 h, 72%; (f) PhOCOCl, pyridine, AcOEt, rt, 1 h, 100%; (g) hydrazine, DMF, rt, 3 h, 79%; (h) formamidine acetate, DMF, 80 °C, 5 h, 59%; (i) 3-bromo-4-acetoxybenzyl chloride, K_2CO_3, DMF, 80 °C, 5 h, then water, 50 °C, 1 h, 47%; (j) $HOCH_2CO_2Me$, DMF, rt, 3 h, 91%; (k) NaOMe, toluene, 100 °C, 1 h, 85%; (l) 3-bromo-4-(*t*-butyldimethylsilyloxy)benzyl chloride, $MeOMgOCO_2Me$, DMF, 85 °C, 3 h, 42%; and (m) 3-bromo-4-hydroxybenzaldehyde, β-alanine, AcOH, 120 °C, 4 h, 54%.

propargyl bromide, giving carbamate **3**. The phenol moiety was incorporated by Sonogashira cross-coupling with 2-bromo-4-iodo-1-(*t*-butyldimethylsilyloxy)benzene to afford an intermediate **4** in a moderate yield. Intramolecular cyclization of **4** to benzylidene oxazolidinone **5** was achieved by treatment with Au catalyst and tetrafluoroantimonide.[7] Conversion of aniline **2** to phenylcarbamate, followed by addition of hydrazine, provided (4-methoxyphenyl)semicarbazide **6**. Then, addition of formamidine acetate in DMF afforded (4-methoxyphenyl)triazolone **7**. Alkylation of **7** with benzylchloride, followed by deprotection of the acetyl group, gave the corresponding target compound **8**. 4-Methoxyphenylisocyanate **9** was treated with glycolate ester to give carbamate **10**. Then, addition of catalytic sodium methoxide in toluene, followed by azeotropic removal of the generated alcohol under reflux, provided the cyclized product **11**. A direct alkylation of **11** with 3-bromo-4-(*t*-butyldimethyl silyloxy)benzyl chloride using magnesium methyl carbonate (MMC) and subsequent deprotection of TBDMS in situ gave benzyloxazolidinedione **12**.[8] Knoevenagel condensation of **11** with benzaldehyde in the presence of β-alanine in acetic acid afforded the corresponding benzylidene derivative **13**.[9]

Biological activities of the synthesized compounds were evaluated in a human cellular assay, which measured the ability of the compounds to inhibit 17β-HSD3. The IC_{50} values were calculated for the inhibition at each concentration (1, 10, 100 nM, and 1 μM) of each compound, by measured concentration of T converted from Δ^4-dione with human 17β-HSD3 expressing HeLa cells.

As revealed by a comparison of biological activities between **1** and **5**, replacement of the benzylthiazolone with benzylideneoxazolidinone resulted in a similar potency (Table 1). 5-Benzyloxazolidinedion **12** maintained a potent inhibitory activity. Based on this result, introduction of a polar functional group at the 4-position of 5-benzylideneoxazolidinone **5** was expected to provide a substantial boost in potency. On combining the oxazolidinone **12** with structural rigidity by an exo-olefin, the resultant 5-benzylideneoxazolid inedione **13** exhibited a potent inhibitory activity with IC_{50} value of 23 nM. On the other hand, the activity of compound **8** was much weaker than that of the others.

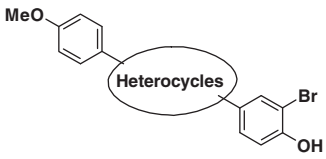
Molecular modeling overlay indicated the differences in shape between **1** and **13**, based on the structures: in compound **1**, the benzyl group was bent at the sp^3 carbon of the methylene; on the other hand, the benzylidene oxazolidinedione part of compound **13** was nearly planar. In both, it was found that the methoxyphenyl group was practically located on the same plane as the five-membered heterocyclic ring (Fig. 2).

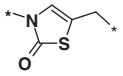
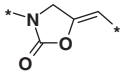
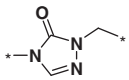
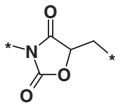
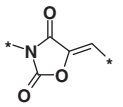
With the identification of the benzylidene oxazolidine-dione as a new active scaffold, we explored the replacement of oxygen on the heterocyclic ring system to study further modification. The methodology used to synthesize compounds **14**–**18** has been previously descried.[10] Compounds **19**–**23** were prepared in an analogous fashion as that is described for its close analog **13**. Condensation of oxazolidinediones and thiazolidinediones with 4-hydroxybenzaldehyde was achieved by Knoevenagel reaction (Scheme 2).

As a result, benzylidene oxazolidinedione and thiazolidinedione derivatives showed good inhibitory activity; in particular, 2-thioxo-1,3-oxazolidin-4-one **19** and rhodanine **21** improved in vitro activity, with IC_{50} values of 13 and 14 nM, respectively.

Compound **20** substituted with sulfur at the 1-position of the oxazolidinedione reduced the activity to one-fourth of the original

Table 1
SAR on the thiazolone ring of compound **1**



Compound	Heterocyclic ring	HSD IC_{50}[a] (μM)
1		0.57
5		0.67
8		5.7
12		0.30
13		0.023

[a] Inhibition of 17β-HSD3 in human cellular assay. Data shown were obtained by performing the tests in duplicate. The deviations were within ±5%.

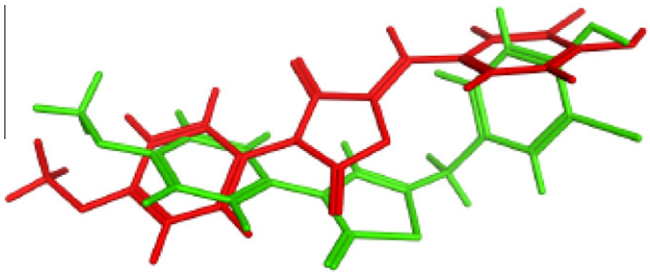
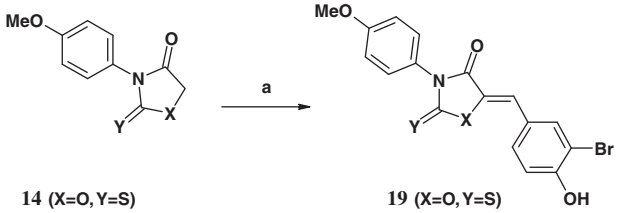


Figure 2. Overlay of a low energy conformer of compound **13** (red) onto HTS hit compound **1** (green).

13. On the other hand, hydantoins (**22** and **23**) led to lack of cellular activity (Table 2). When the rhodanine derivative **21** was evaluated against testes homogenate 17β-HSD3, it was found to possess IC_{50} values of 6.0 (human), 40 (mouse), and 0.6 nM (marmoset).

In an effort to determine specificity, compound **21** was evaluated against the oxidative isoenzyme 17β-HSD2 or 17β-HSD1 which convert estrone (E1) into estradiol (E2). This compound did not show any undesired inhibition of estrogen-biosynthesis. In selectivity over nuclear receptors such as androgen receptor (AR), estrogen receptor alpha (ERα), and glucocorticoid receptor (GR), this inhibitor did not possess the transcriptional activity against any of these nuclear receptors. Compound **21** showed excellent selectivity over 17β-HSD isoenzymes and nuclear receptors (Table 3).

Superimposition of the rhodanine derivative **21** with Δ^4-dione, which is an endogenous substrate, was performed by a molecular structure alignment method using Hopfield neural network.[11] Figure 3 shows that **21** overlaps well with Δ^4-dione in the hydrophobic regions and hydrogen-bonding functional groups: the C-3 and C-17 carbonyl groups of Δ^4-dione correspond to the hydroxyl

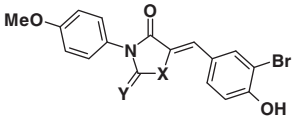


14 (X=O, Y=S)
15 (X=S, Y=O)
16 (X=S, Y=S)
17 (X=NH, Y=O)
18 (X=NMe, Y=O)

19 (X=O, Y=S)
20 (X=S, Y=O)
21 (X=S, Y=S)
22 (X=NH, Y=O)
23 (X=NMe, Y=O)

Scheme 2. Synthesis of oxazolidinedione and thiazolidinedione derivatives. Reagents and conditions: (a) 3-bromo-4-hydroxybenzaldehyde, ammonium acetate or β-alanine, AcOH, 120 °C, 2–6 h, 50–90%.

Table 2
Inhibition of 17β-HSD3 by oxazolidinedione and thiazolidinedione derivatives in human cellular assay



Compound	X	Y	HSD IC_{50}[a] (nM)
13	O	O	23
19	O	S	13
20	S	O	90
21	S	S	14
22	NH	O	na
23	NMe	O	na

[a] Human cell-based assay. Data shown were obtained by performing the tests in duplicate. The deviations were within ±5%. na = not active.

Table 3
The specificity of 5-(3-bromo-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-thiazolidin-4-one **21**

Human cell-based assay IC_{50}[a]	17β-HSD isoenzyme		
	Type 1	Type 2	Type 3
Reporter gene assay agonist/antagonist[b]	na	na	14 nM
	Nuclear receptor		
	AR	ERα	GR
	na/na	na/na	na/na

[a] Data shown were obtained by performing the tests in duplicate. The deviations were within ±5%. na = not active.
[b] na = not active.

group and the polar group on the heterocyclic ring of inhibitor **21**, respectively. The phenyl group of the phenol moiety overlaps onto C and D-ring of the steroid backbone; in addition, the bridge part is located on B-ring. In a brief structure–activity relationship (SAR) surrounding compound **21**, the methoxyphenyl group which is nearly perpendicular to the rhodanine ring is important to exhibit a potent inhibitory activity, and deletion of the methoxyphenyl group resulted in a dramatic loss in potency (inhibition of 23% at 10 μM). The corresponding hydrophobic binding pocket of the enzyme was observed to affect a key interaction with this chemotype.

Starting from an HTS hit, benzylthiazolone **1**, the SAR studies on replacement of the central ring-system culminated in the discovery of benzylidene oxazolidinedione and thiazolidinedione derivatives, highly potent and selective inhibitors of 17β-HSD3. Although compound **21** exhibited a promising activity profile, the compound

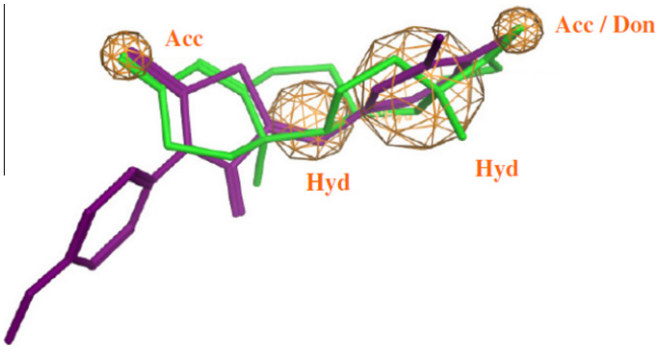


Figure 3. Alignment and shape similarity. In silico overlap of characteristic functional groups of **21** (purple) and androstenedione (green); sphere Acc is a hydrogen-bonding acceptor; Acc/Don is a hydrogen-bonding acceptor/donor; Hyd is a hydrophobic group.

displayed very low aqueous solubility and a poor pharmacokinetic (PK) profile characterized by a lack of oral bioavailability (data not shown). We are continuing to explore more potent compounds with improved oral exposure and which exhibit in vivo activity. The detailed SAR development of these compounds will be reported elsewhere.

Acknowledgment

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References and notes

1. Jeanne, C.; Carmen, R. *Cancer Facts & Figures 2009*.
2. van Bokhoven, A.; Varella-Garcia, M.; Korch, C.; Johannes, W. U.; Smith, E. E.; Miller, H. L.; Nordeen, S. K.; Miller, G. J.; Lucia, M. S. *Prostate* **2003**, *57*, 205.
3. Donald, P. *Curr. Med. Chem.* **2003**, *10*, 453.
4. Adamski, J.; Jakob, F. *J. Mol. Cell. Endocrinol.* **2001**, *171*, 1.
5. Koh, E.; Noda, T.; Namiki, M. *Prostate* **2002**, *53*, 154.
6. (a) Timothy, J. G.; Yi-Tsung, L.; Ronald, J. D.; Ani, S.; Viyyoor, M. G.; Jonathan, A. P. U.S. Patent US20040138226, 2004.; (b) Maltais, R.; Luu-The, V.; Poirier, D. *J. Med. Chem.* **2002**, *45*, 640; (c) Fink, B. E.; Gavai, A. V.; Tokarski, J. S.; Goyal, B.; Misra, R.; Xiao, H. Y.; Kimball, S. D.; Han, W. C.; Norris, D.; Spires, T. E.; You, D.; Gottardis, M. M.; Lorenzi, M. V.; Vite, G. D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1532; (d) Nigel, V.; Christopher, M. S.; Wesley, B. H.; Ana, M. R.; Gonzalez, H. V.; Bailey, A. S.; Jeremy, S. S.; Joanna, M. D.; Helena, J. T.; Michael, J. R.; Atul, P.; Barry, V. L. P. *Mol. Cell. Endocrinol.* **2009**, *301*, 259.
7. Michael, H.; Chaofeng, H. *Synlett* **2008**, 1399.
8. Finkbeiner, H. *J. Org. Chem.* **1965**, *30*, 3414.
9. Jones, G. *Org. React.* **1967**, *15*, 204.
10. (a) Liu, K.; Lu, H.; Hou, L.; Qi, Z.; Teixeira, C.; Barbault, F.; Fan, B.; Liu, S.; Jiang, S.; Xie, L. *J. Med. Chem.* **2008**, *51*, 7843; (b) Sahu, M.; Garnaik, B. K.; Behera, R. *Indian J. Chem., Sect B* **1987**, *26B*, 779; (c) Mitsubishi Paper Mills, Ltd, Japan. Jpn. Kokai Tokkyo Koho. JP56034675, 1981.; (d) Kim, S. C.; Kwon, B. M. *Synthesis* **1982**, *9*, 795; (e) Maggi, R.; Schlosser, M. *J. Org. Chem.* **1996**, *61*, 5430.
11. Arakawa, M.; Hasegawa, K.; Funatsu, K. *J. Comput. Aided Chem.* **2001**, *2*, 29.