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Coumarins as novel 17β-hydroxysteroid dehydrogenase type 3 inhibitors for potential treatment of prostate cancer

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ABSTRACT

The synthesis and SAR studies of 3- and 4-substituted 7-hydroxycoumarins as novel 17β-HSD3 inhibitors are discussed. The most potent compounds from this series exhibited low nanomolar inhibitory activity with acceptable selectivity versus other 17β-HSD isoenzymes and nuclear receptors.

Prostate cancer is among the most common causes of death from cancer in men, and accounts for 10% of all new cases of cancer in males worldwide. Between 10% and 60% of the patients experience biochemical recurrence associated with treatment failure. No consensus exists on the optimal therapy for advanced prostate cancer. The androgens testosterone (T) and dihydrotestosterone (DHT) are hormones that play an important role in the development of prostate cancer. The regulation of androgen biosynthesis or its action on the androgen receptor is central to the management of prostate cancer.[1] The 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 T and DHT can be synthesized directly through the conversion of the inactive precursor androstenedione (Δ^4-dione) by 17β-hydroxysteroid dehydrogenases (17β-HSDs) that mediate the final steps in the conversion of sex steroids in peripheral target tissues.[2] 17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) catalyzes the final step in the biosynthesis of the potent androgen T by selectively reducing the C17 ketone of Δ^4-dione with NADPH as a cofactor; in addition, it is expressed at high levels in testes and prostate tissue of some prostate tumors, suggesting its potential involvement in both gonadal and nongonadal T biosynthesis. The expression of 17β-HSD3 mRNA in cancerous prostate biopsies was found to be 30-fold higher than in normal tissue.[3] 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.[4]

A high-throughput screening (HTS) of a compound library (~220,000 compounds) led to the identification of 4-methylumbelliferone (**1**, Fig. 1)[5] as an inhibitor of 17β-HSD3. 4-MU was already known to exhibit weak inhibitory activity against 17β-HSD3. We studied new 17β-HSD3 inhibitors with high activity and selectivity around 4-MU as a seed compound. This report mainly discusses the SAR of the 3- and 4-positions of 7-hydroxycoumarin derivatives.

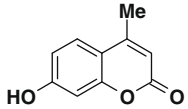

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Figure 1. Structure of 4-methylumbelliferone (4-MU).

4-MU analogs were prepared by the synthetic procedure outlined in Scheme 1. The condensation of resorcinol **2** and β-ketoesters in concentrated sulfuric acid at room temperature afforded the corresponding 7-hydroxycoumarins **3a–h**.[6] In the case of the synthesis of coumarin derivatives that afforded a complex reaction mixture under this condition, trifluoroacetic acid as weaker acid was used to improve the yields. The 4-thioether (**4c**, **4f–p**), 4-ether (**4b**,**r**), and 4-amino derivatives (**4d**,**e**) were synthesized by the nucleophilic substitution of various thiols, alcohols, or amines with 4-chloromethyl and 4-chloroethyl coumarins (**3g** and **3h**) that were obtained from 4-chloroacetoacetic acid ester and 5-chloro-3-oxo-pentanoic acid ester.[7] Moreover, the coupling of acetoacetic acid ester **5** with alkyl halides in the presence of 2 equiv base and ammonium salt (Aliquat 336) afforded γ-alkylated-β-ketoesters **6** in moderate yield, and subsequent treatment with resorcinol **2** provided the desired coumarins **4a** and **4q**. 3-Substituted coumarins **9b–e** were obtained from α-alkylated-β-ketoesters **8** that were prepared by using β-ketoesters **7** with alkyl halides in the presence of one equivalent sodium hydride. Other 3-substituted coumarins **9f–i** was synthesized from commercially available 2-halo or

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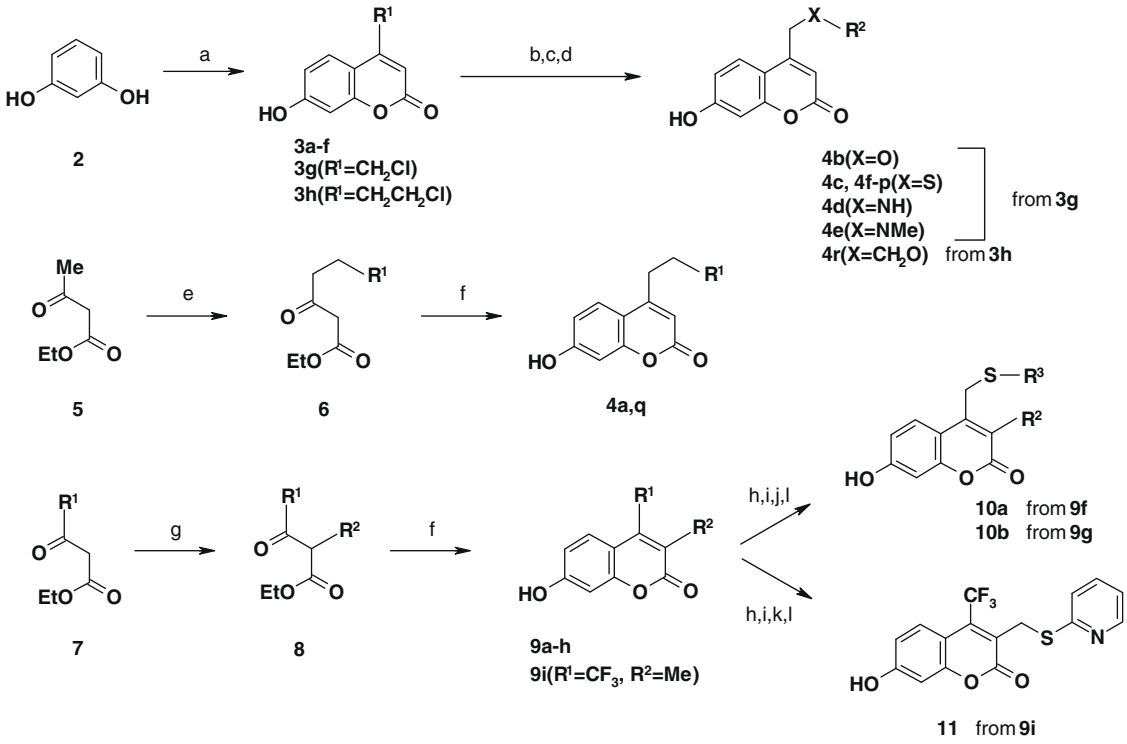


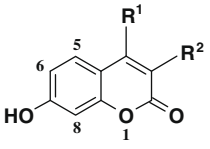
Scheme 1. Reagents and conditions: (a) $R^1COCH_2CO_2Et$, concd H_2SO_4 or TFA, rt–reflux, 3–15 h, 30–50%; (b) Ac_2O, reflux or Ac_2O, pyridine, rt, 3–8 h, 80–100%; (c) R^2SH or R^2OH or R^2NHR(R = H or Me), TEA or NaH or pyridine, DMF, rt, 1–8 h, 50–95%; (d) 1 N NaOH, MeOH, rt, 1–3 h, 70–90%; (e) R^1CH_2Cl, NaH, n-BuLi, THF, −10 °C–rt, 1 h, 40–50%; (f) **2**, $MeSO_3H$ or TFA, rt––reflux, 3–15 h, 20–40%; (g) R^2Br, NaH, Aliquat 336, toluene, 0 °C–rt, 1–3 h, 50–70%; (h) Ac_2O, reflux or Ac_2O, pyridine, rt, 3–8 h, 80–100%; (i) NBS, BPO, CCl_4, reflux, 5 h, 70–80%; (j) R^3SH, NaH, DMF, 0 °C–rt, 1 h, 75–80%; (k) 2-mercaptopyridine, NaH, DMF, 0 °C–rt, 3 h, 45%; and (l) 1 N NaOH, MeOH, rt, 1–3 h, 75–90%.

2-cyano β-ketoesters. Another thioether derivatives **10a**,**b** and **11** were prepared from **9f**,**g** and **9i** followed by allylic bromination by treatment with *N*-bromosuccinimide (NBS) and benzoyl peroxide (BPO).[8] Moderate yields were obtained by the protection of the 7-hydroxyl group with acetyl group to afford 2-pyridyl compounds **4f**, **4p–4r,** and **10a**,**b** that required a strong base such as sodium hydride for the substitution reaction.

The initial result for the inhibition of 17β-HSD3 using our 7-hydroxycoumarin analogs suggested that the nature of the substituent at the 4-position affects the activity in a cell-based assay[9] that SCH-391[10] was used as reference inhibitor (Table 1, compounds **3a–f** and **4a**). The order of potency cannot be easily explained based on the physical parameters, but the 4-phenethyl derivative **4a** indicated that the steric allowance at the 4-position preferred more than two carbon chain. The introduction of the alkyl group at the 3-position improved the activity (**9b–d**). When electron-withdrawing groups were incorporated at the 3-position (**9f** and **9h**), the activities were similar to 4-MU **1**, and 3-chloro derivative **9g** was 10-fold more potent than 4-MU **1**. Moreover, 4-trifluoromethyl analog **9e** was found to be favorable and resulted in a sevenfold increase in potency as compare to **9d**.

The SAR of the linker part was studied further with an emphasis on the 4-phenethyl derivative **4a** that had potent activity to explore more potent compounds (Table 2). The replacement of the methylene with oxygen and nitrogen led to poor 17β-HSD3 inhibitory activity (**4b** and **4d**,**e**); on the other hand, the thioether (**4c**) continued to exhibit good potency. Then, heterorings were optimized as follows with thioether as the linker part. As a result, various heterorings showed good inhibitory activity, in particular, 2-pyridyl (**4f**) exhibited potent activity. In addition, the substituents on a pyridine ring were studied; 6-methyl-2-pyridyl derivative **4p** substituted with electron-donating group exhibited IC_{50} = 1.5 nM and was found to be the most potent 17β-HSD3 inhibitor in this series. The basicity of nitrogen at a suitable position was considered to be important for high inhibitory activity (as revealed by a comparison between **4f** and **4p**). On combining the aryl part with the linker part, ethylene derivative **4q** similarly exhibited low nanomolar activity. However, the introduction of a halogen atom at the third position (**10a**,**b**) reduced the activity to one-tenth of the original. Substitution of the 4-trifluoromethyl derivative with

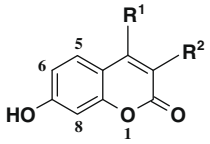
Table 1
Inhibition of 17β-HSD3 by 4-MU analogs in human cellular assay



Compounds	R^1	R^2	HSD IC_{50} (μM)
1 (4-MU)	Me	H	1.0
3a	Et	H	0.10
3b	*n*-Pr	H	0.059
3c	CF_3	H	0.19
3d	CH_2OMe	H	5.0
3e	Ph	H	1.0
3f	CH_2Ph	H	10
4a	$(CH_2)_2Ph$	H	0.020
9a	H	Me	1.0
9b	Me	Me	0.21
9c	Me	*n*-Pr	0.24
9d	Me	CH_2Ph	0.20
9e	CF_3	CH_2Ph	0.030
9f	Me	F	1.0
9g	Me	Cl	0.10
9h	Me	CN	1.0
SCH-391[a]			0.10

[a] See Ref. 10 for reference inhibitor.

Table 2
Inhibition of 17β-HSD3 by 4-phenethyl-7-hydroxycoumarin derivatives in human cellular assay



Compounds	R^1	R^2	HSD IC_{50} (μM)
4a	$(CH_2)_2$-Phenyl	H	0.020
4b	CH_2O-Phenyl	H	10
4c	CH_2S-Phenyl	H	0.093
4d	CH_2NH-Phenyl	H	4.0
4e	CH_2NMe-Phenyl	H	6.0
4f	CH_2S-2-Pyridiyl	H	0.0030
4g	CH_2S-4-Pyridyl	H	5.0
4h	CH_2S-2-Pyrimidinyl	H	1.0
4i	CH_2S-2-Thienyl	H	0.42
4j	CH_2S-1,3,4-Thiadiazol-2-yl	H	0.200
4k	CH_2S-2-Thiazolyl	H	0.010
4l	CH_2S-2-Thiazolidinyl	H	0.096
4m	CH_2S-1-Methyl-2-imidazolyl	H	0.091
4n	CH_2S-5-Nitro-2-pyridyl	H	0.088
4o	CH_2S-5-Trifluoromethyl-2-pyridyl	H	0.23
4p	CH_2S-6-Methyl-2-pyridyl	H	0.0015
4q	$(CH_2)_2$-6-Methyl-2-pyridyl	H	0.0080
4r	$(CH_2)_2O$-6-Methyl-2-pyridyl	H	1.0
10a	CH_2S-6-Methyl-2-pyridyl	F	0.027
10b	CH_2S-6-Methyl-2-pyridyl	Cl	0.047
11	CF_3	CH_2S-2-pyridyl	0.32

2-pyridylthiomethyl at the 3-position resulted in reduced activity (**11**).

Next, replacement of the 7-hydroxyl group and substitution at the 6- and 8-positions were studied; various functional groups such as hydrogen-bonding donor/acceptor (amino, carboxyl, amide, cyano, fluoro, etc.) at the 7-positon resulted in a considerable decrease in enzymatic activity. A polar group, alkyl, or halogen at the 6-position decreased the activity or made it disappear completely, in a manner similar to substitution at the 8-position (data not shown). The introduction of a substituent near the phenolic hydroxyl group appeared to prevent the access of the hydroxyl group to the hydrogen-binding acceptor of the ligand-binding site in the enzyme.

Another brief SAR of this chemotype was generated. 7-Hydroxy-4-methylquinoline and the N-methylated was not tolerated (data not shown). It was suggested that the lactone part had low steric allowance or that it was unsuitable for a hydrogen-donor moiety.

Selectivity over 17β-HSD isoenzymes and nuclear receptors is shown in Table 3. The inhibitory activity to types 1 and 2 was not observed for compounds **4p**, **4q,** and **9e**. In selectivity over the nuclear receptors, compound **9e** exhibited AR antagonist activity and ERα agonist activity. Affinity of the nuclear receptors was not observed in the case of compounds **4p** and **4q**; 4-substituted coumarins had better selectivity than the 3-substituted ones.

Superimposition of the 6-methyl-2-pyridylethyl derivative **4q** with 4-androstene-3,17-dione (Δ^4-dione) which is an endogenous ligand was performed using Hopfield Neural Network (HNN) method.[11] These compounds were sterically rigid, and therefore, the active conformer is nearly equal to the low-energy conformation. Figure 2 shows that **4q** overlaps well with Δ^4-dione in the hydrophobic regions and hydrogen-bond functions; the two carbonyl groups of Δ^4-dione correspond to the 7-hydroxyl group and the nitrogen atom of the pyridine ring at the 4-position of coumarin.

Table 3
IC_{50} value of the subtype of 17β-HSD and nuclear receptors for compounds **4p**, **4q,** and **9e**

Compounds	**4p**	**4q**	**9e**
AR antagonist[a]	50 μM	na	6.0 μM
agonist[a]	na	na	na
ER antagonist[a]	na	na	na
agonist[a]	50 μM	na	1.0 μM
GR antagonist[a]	na	na	na
agonist[a]	na	na	na
17-HSD type 1[b]	na	na	na
17-HSD type 2[b]	na	na	na

na = not active.
[a] Reporter gene assay.
[b] Human cell-based assay.

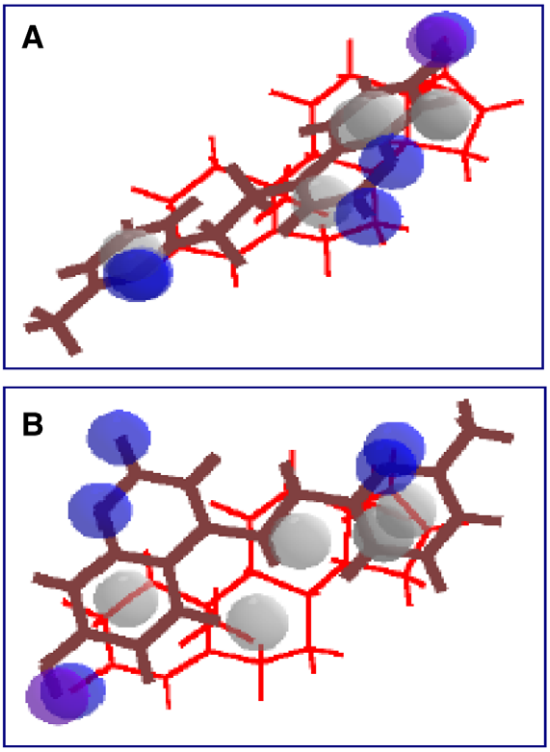


Figure 2. (A) In silico overlap of low-energy conformation of **4q** (brown) with androstenedione (red). (B) Reverse orientation of upper panel (A): blue: hydrogen-bonding acceptor; purple: hydrogen-bonding donor/acceptor; gray: hydrophobic group.

The basicity of the nitrogen of the pyridine ring appeared to affect the interaction with the enzyme.

7-Hydroxy-4-substituted coumarins represent a potent class of 17β-HSD3 inhibitors. Preliminary SAR of this series led to the discovery of novel and selective 7-hydroxy-4-(2-pyridylethyl) coumarin **4q** and 7-hydroxy-4-(2-pyridylthiomethyl) coumarin **4p**. These compounds will be considered for further in vitro and in vivo evaluation.

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