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A Novel Class of Inhibitors for Steroid 5α-Reductase: Synthesis and Evaluation of Umbelliferone Derivatives

Gao-jun Fan,[a,b] Woongchon Mar,[a] Man Ki Park,[b] Eun Wook Choi,[a] Kinam Kim[a] and Sanghee Kim[a,*]

[a]*Natural Products Research Institute, Seoul National University, 28 Yungun, Jongro, Seoul 110-460, South Korea*
[b]*College of Pharmacy, Seoul National University, San 56-1, Shillim, Kwanak, Seoul 151-742, South Korea*

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Abstract—A series of umbelliferone derivatives was prepared and their 5α-reductase type 1 inhibitory activities were evaluated in cell culture systems. Our studies have identified a new series of potent 5α-reductase type 1 inhibitors and provided the basis for further development for the treatment of human endocrine disorders associated with overproduction of DHT by 5α-reductase type 1. The preliminary structure–activity relationship was described to elucidate the essential structural requirements.

Introduction

Steroid 5α-reductase (5αR) is an NADPH-dependent enzyme that catalyzes the reduction of testosterone (T) into the more potent androgen, dihydrotestosterone (DHT) (Fig. 1).[1] Two different 5α-reductase isozymes, type 1 and type 2 (5αR-1 and 5αR-2) have been cloned, expressed, and characterized.[1,2] These two isozymes are differently distributed in the human tissues. While 5αR-1 is present mainly in hair follicles, sebaceous glands of the skin (including the scalp) and liver, 5αR-2 is found predominantly in the prostate, genital skin, seminal vesicles, epididymis, and liver.[2,3] It was reported that many human endocrine diseases such as acne, male pattern baldness, alopecia in men and hirsutism in women are due to elevated levels of DHT.[4]

A number of different classes of steroidal compounds that inhibit 5αR have been designed and synthesized as therapeutic agents for the treatment of DHT-related pharmacological disorders.[5,6] In designing these inhibitors, mimics of the proposed enolate intermediate of the natural substrate in the reduction were usually employed.[6,7] This approach led to the introduction of finasteride[8] (Proscar®), a 4-azasteroid inhibiting mainly 5αR-2 (Fig. 1). Recently, a number of simpler, non-steroidal inhibitors have also been designed based on the structures of the related steroidal inhibitors. Selected examples of these nonsteroidal inhibitors are benzophenone carboxylic acids,[9] indole derivatives,[6] benzoquinolizinones[10] and hydrophenanthren-2-ones.[11] More recently, the evaluation of 6-substituted 1*H*-quinolin-2-ones (Fig. 1) as a new class of nonsteroidal inhibitors of 5αR was reported by Hartmann.[12]

In the course of our study to find a potent 5αR-1 inhibitor, we focused our attention on nonsteroidal inhibitors to avoid some of the well-known side effects of steroidal drugs. On the basis of the transition state

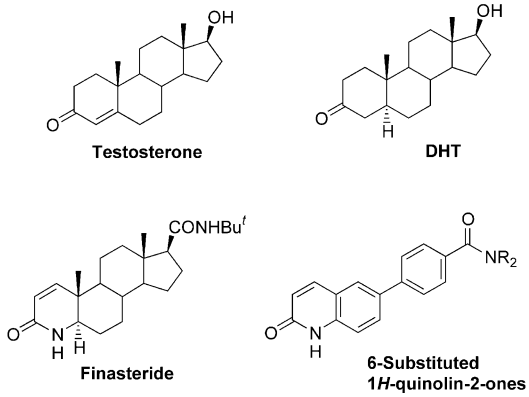

Figure 1. Chemical structures of testosterone, dihydrotestosterone (DHT), finasteride and 6-substituted 1*H*-quinolin-2-ones.

*Corresponding author. Tel.: +82-2-740-8913; fax: +82-2-762-8322; e-mail: pennkim@snu.ac.kr

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paradigm along with Hartmann's results,[12] we opted for the synthesis and biochemical evaluation of the B-ring substituted coumarin derivatives. To the best of our knowledge, no coumarin derivatives have been reported as inhibitors of 5αR. The coumarin skeleton can be considered as a mimetic of the proposed transition state of the natural substrate as well as bioisostere of quinolin-2-one. We designed and prepared a series of derivatives of umbelliferone (7-hydroxycoumarin), and screened their 5αR inhibitory activities. Herein, we report our preliminary results of these studies.

Synthesis

The synthesis of derivatives of umbelliferone **1** was outlined in Scheme 1. Propargyl ether **2** was obtained by heating the solution of umbelliferone **1** and propargyl bromide together with potassium carbonate and potassium iodide in DMF. Compound **3** was prepared according to a method of Pettus et al.[13] Reduction of **3** under H_2 gas in the presence of Lindlar catalyst and quinoline in EtOAc provided compound **4**.

8-Allyl-7-hydroxycoumarin **7** was a key intermediate which can easily be obtained from umbelliferone **1** in two steps. Treatment of umbelliferone **1** with allyl bromide in H_2O/CH_2Cl_2 two-phase solution in the presence of sodium hydroxide and tetrabutylammonium iodide afforded **5** in 82% yield, which was then heated with *p*-xylene in sealed tube at 260 °C to afford mainly the C-8 Claisen rearrangement product **7** together with its C-6 isomer **6** in a ratio of 9:1.[14]

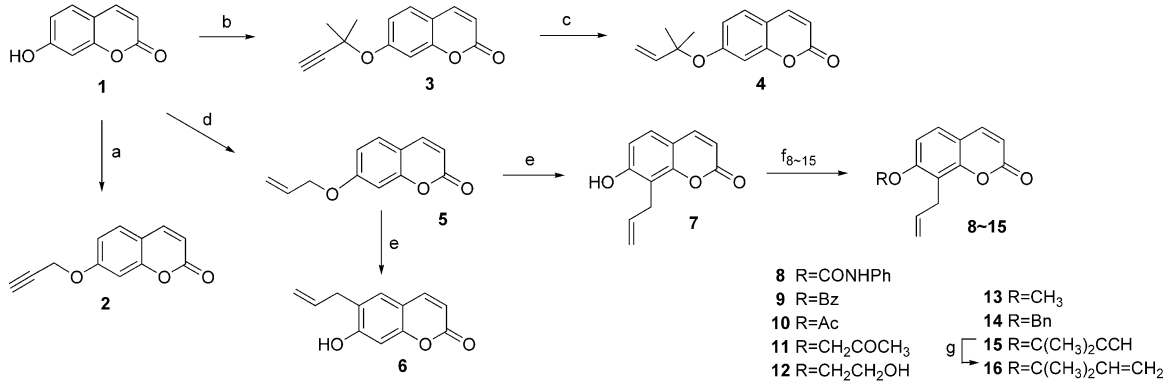
Compound **7** was condensed with phenylisocyanate in the presence of pyridine in CH_2Cl_2 at room temperature to furnish **8**. Stirring a solution of **7**, benzoyl chloride and pyridine in CH_2Cl_2 at 0 °C gave the corresponding ester **9**. Acetylation of **7** using acetyl chloride and pyridine in THF at room temperature afforded ester **10**. Compounds **11**, **12**, **14**, and **15** were synthesized according to a general procedure[15] using **7** and its corresponding alkylhalide or benzylhalide together with potassium carbonate in DMF or acetone. Methylation of coumarin **7** with dimethyl sulfate in aqueous ethanol solution resulted in **13**. Hydrogenation of **15** was conducted in the same manner for compound **4** to afford **16**.

Enzyme Inhibitory Activity and Discussion

LNCaP cells are the androgen-sensitive human prostatic cancer cell line widely available for use in preclinical investigations[16] and it has been reported that 5αR-1 is expressed in LNCaP cells but not 5αR-2.[17] The existence of androgen sensitivity as well as 5α-reductase activity in LNCaP cells offered us the opportunity to study the effects of inhibitors in the enzymatic and in vitro functional LNCaP responses to androgens in a single human cell line.

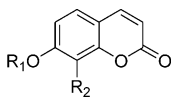
The compounds were evaluated for their steroid 5αR-1 inhibitory activities in cell culture system using LNCaP cells according to a documented procedure with some modification.[18] Inhibitory effects were represented with the concentration (μg/mL) giving 50% inhibition (IC_{50}) relative to the control (0.5% DMSO). In the case of highly active compounds, IC_{50} values are also presented as μM. The obtained results were summarized in Table 1.

Umbelliferone **1** and 7-alkoxycoumarins **2**, **3**, and **5** do not display any inhibitory activity. However, 1′,1′-dimethylallyloxycoumarin **4** shows potent inhibitory activity ($IC_{50} = 1.3\,\mu M$) for the 5αR-1. The considerably different activity between **1**–**3**, **5**, and **4** is possibly a result of conformational effects of geminal dimethyl group (the Thorpe–Ingold effect).[19] This observation led us to investigate the compounds **6** and **7** which are the Claisen rearrangement products of **5**. To our delight, 8-allyl-7-hydroxycoumarin **7** exhibits a potent inhibitory activity ($IC_{50} = 0.99\,\mu M$) against 5αR-1. The compound



Scheme 1. Reagents and conditions: (a) propargyl bromide, K_2CO_3, KI, DMF, 80 °C, 2 h, 98%; (b) $(CF_3CO)_2O$, $(CH_3)_2C(OH)C{\equiv}CH$, DBU, then **1**, $CuCl_2.H_2O$, DBU, rt, 2 h, 61%; (c) H_2, quinoline, Lindlar cat., EtOAc, rt, 1 h, 73%; (d) allyl bromide, NaOH, KI, cat. $(n\text{-}Bu)_4NI$, H_2O, CH_2Cl_2, rt, 2 h, 82%; (e) *p*-xylene, 260 °C, 17 h (90% for **7**, 10% for **6**); (f_8) $C_6H_5N{=}C{=}O$, pyr, CH_2Cl_2, rt, 2 days, 85%; (f_9) C_6H_5COCl, pyr, CH_2Cl_2, 0 °C, 5 h, 87%; (f_{10}) CH_3COCl, pyr, THF, rt, overnight, 89%; (f_{11}) CH_3COCH_2Cl, K_2CO_3, acetone, reflux, 4 h, 84%; (f_{12}) $BrCH_2CH_2OH$, K_2CO_3, acetone, reflux, 1 day, 91%; (f_{13}) $(CH_3)_2SO_4$, NaOH, C_2H_5OH, H_2O, rt, 5 h, 63%; (f_{14}) BnBr, K_2CO_3, DMF, 60 °C, 3 h, 98%; (f_{15}) $CHCC(CH_3)_2Cl$, K_2CO_3, DMF, 90 °C, 26 h, 56%; (g) H_2, quinoline, Lindlar cat., EtOAc, rt, 5 h, 90%.

Table 1. Steroid 5α-reductase type 1 inhibitory activities in cell culture systems using LNCaP cells



Compound	R_1	R_2	IC_{50}[a] (μg/mL) [μM]
Finasteride			19.8 [53]
1	H	H	>20
2	CH≡CCH$_2$	H	>20
3	CH≡CC(CH$_3$)$_2$	H	>20
4	CH$_2$=CHC(CH$_3$)$_2$	H	0.3 [1.3]
5	CH$_2$=CHCH$_2$	H	>20
6	(see Scheme 1)	H	1.7 [8.4]
7	H	CH$_2$=CHCH$_2$	0.2 [0.99]
8	C$_6$H$_5$NHCO	CH$_2$=CHCH$_2$	0.2 [0.62]
9	C$_6$H$_5$CO	CH$_2$=CHCH$_2$	0.15 [0.49]
10	CH$_3$CO	CH$_2$=CHCH$_2$	0.2 [0.82]
11	CH$_3$COCH$_2$	CH$_2$=CHCH$_2$	>20
12	HOCH$_2$CH$_2$	CH$_2$=CHCH$_2$	>20
13	CH$_3$	CH$_2$=CHCH$_2$	>4
14	C$_6$H$_5$CH$_2$	CH$_2$=CHCH$_2$	>4
15	HC≡CC(CH$_3$)$_2$	CH$_2$=CHCH$_2$	>4
16	H$_2$C=CHC(CH$_3$)$_2$	CH$_2$=CHCH$_2$	13.6 [50.3]

[a]Duplicate samples were treated at nontoxic doses to obtain IC_{50} values.

6, 6-allyl-7-hydroxycoumarin (IC_{50} = 8.4 μM) is also more active than finasteride (IC_{50} = 53 μM) against 5αR-1, but less active than the corresponding C-8 isomer **7** by about 10-fold.

In the investigation of the substituent effects on the 7-hydroxyl group of **7**, the introduction of carbonyl groups (such as in compounds **8**, **9**, and **10**) resulted in a slight enhancement of 5αR-1 inhibitory. However, these compounds are about 100 times more potent than finasteride. The introduction of alkyl groups into the 7-hydroxyl position was not suitable for increasing inhibitory potency (**13**–**16**). Replacement of the hydrophobic alkyl group with an acetonyl group (**11**) or ethanol group (**12**) also caused a drop in potency. From these results, the existence of a carbonyl group-containing side chain at the C-7 hydroxyl position of 8-allylcoumarin appears to be desirable for potent inhibitory activity against 5αR-1.

In conclusion, we have designed and evaluated the B-ring substituted umbelliferone derivatives as 5αR-1 inhibitors. Several compounds showed potent inhibitory activity towards 5αR-1 isozyme. This new series of potent 5αR-1 inhibitors could be leads for the development of a drug for the treatment of human endocrine disorders associated with overproduction of DHT by 5αR-1. Further studies for more potent inhibitors based on the above findings are in process in our laboratory.

Acknowledgements

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