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SYNTHESIS AND 5α-REDUCTASE INHIBITORY ACTIVITIES OF BENZOFURAN DERIVATIVES WITH A CARBAMOYL GROUP

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Abstract: A series of 2-phenylbenzofuran derivatives with a diphenylmethylcarbamoyl group at the 5 or 6 position of the benzofuran ring were synthesized and evaluated for rat and human testosterone 5α-reductase inhibitory activities *in vitro*. They had inhibitory activities against both enzymes and the 6-carbamoyl derivatives tended to be more potent than the 5-carbamoyl compounds.

Benign prostatic hyperplasia (BPH) is an ailment afflicting a large proportion of aging men. Although the etiology of BPH is unclear, dihydrotestosterone (DHT) is recognized as a principal mediator of this disease and its primary role in the hyperplastic growth of the prostate is well established.[1)] DHT is derived from a major circulating androgenic hormone testosterone (T) and the conversion of T to DHT is catalyzed by testosterone 5α-reductase. Recently, several classes of 5α-reductase inhibitors have been reported, including steroidal inhibitors[2)] and nonsteroidal ones,[3)] and one of the steroidal inhibitors, finasteride (Proscar®), was launched as a medicine for BPH.

Although steroidal inhibitors show potent activities, various hormonal actions that they might show have to be considered. From that point of view, nonsteroidal inhibitors have recently been investigated and some potent inhibitors, including FK 143[3g)] and ONO 3805[3f)], were found. In the course of our study to find a potent testosterone 5α-reductase inhibitor, we focused our attention on a nonsteroidal 5α-reductase inhibitor. Simple fatty acids such as γ-linolenic acid, have been reported to have weak inhibitory activity against rat 5α-reductase.[3a)] On the other hand, we reported that the C-17 *N*-diphenylmethylcarbamoyl substituent of the androstane-3-carboxylic acid compounds plays an important role for high inhibitory potency against 5α-reductase.[4)] Based on the structural features of these two classes of compounds, we designed several simple ω-carbamoylfatty acids, which have both a carboxy group and a ω-diphenylmethylcarbamoyl group, and evaluated them for 5α-reductase inhibitory activity. Among the compounds, 4-carboxy-4'-(*N*-diphenylmethyl)carbamoyl-*trans*-stilbene showed weak inhibitory activity. Then, the stillbene structure was converted to a 2-phenylbenzofuran structure. After some modification of the functional groups, 5- or 6-diphenylmethylcarbamoyl-2-phenylbenzofuran derivatives with butyric acid group at the 2 position in the 2-phenyl moiety were found to have potent 5α-reductase inhibitory activities.

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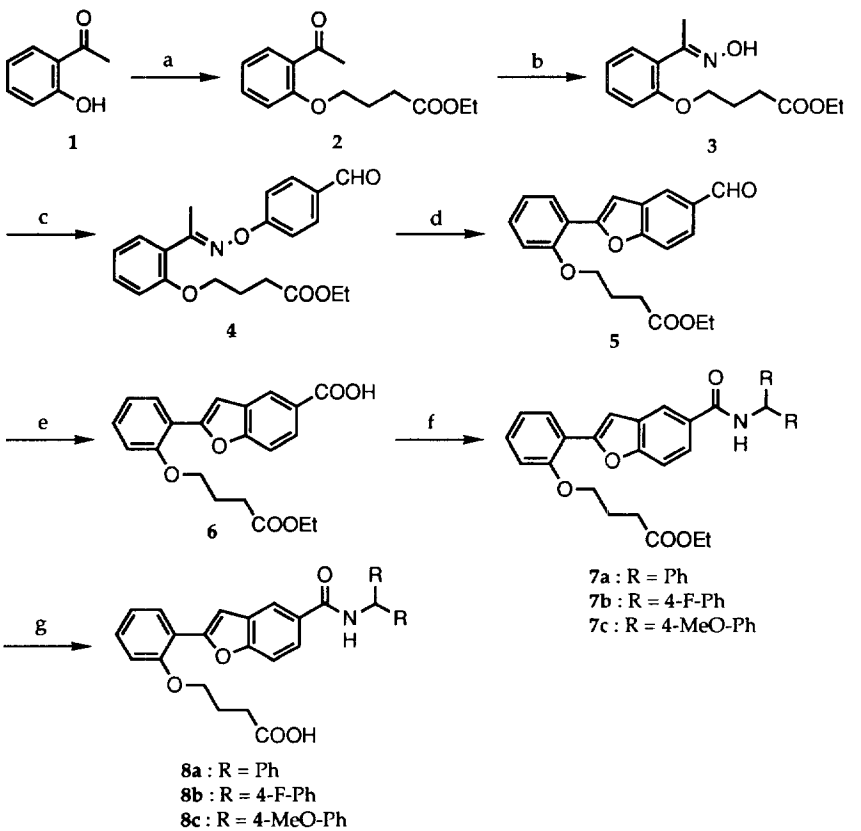
Scheme 1

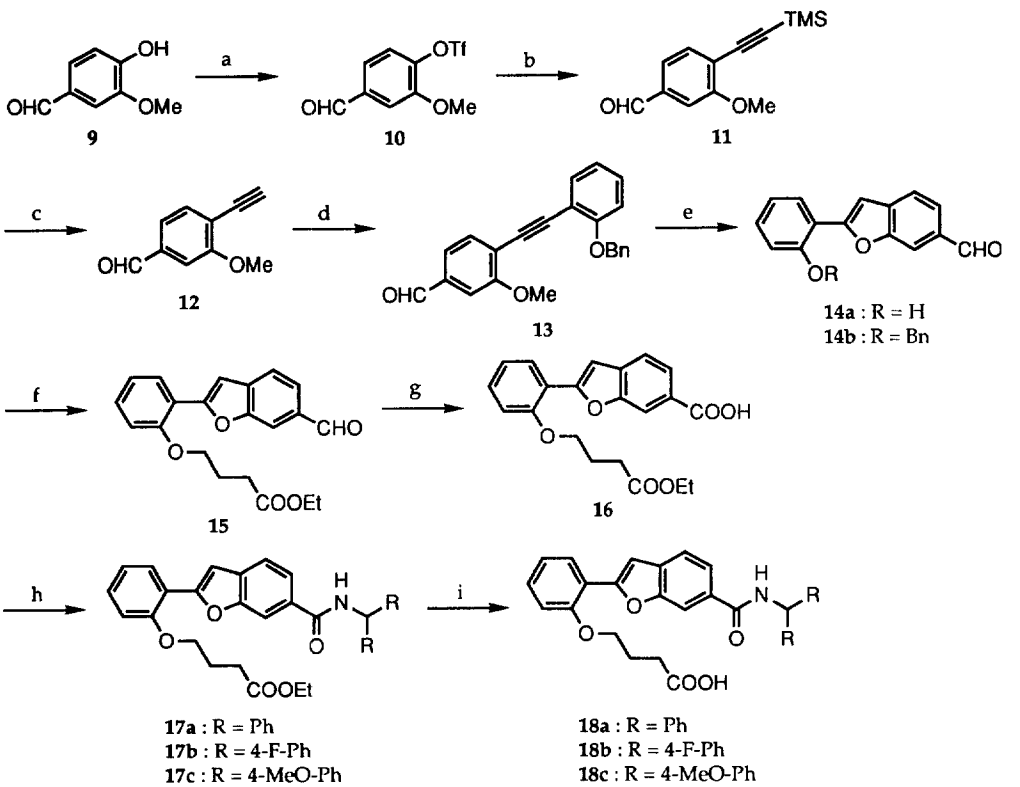


Reagents : (a) NaH, $Br(CH_2)_3COOEt$, DMF, 50°C ; (b) NH_2OH·HCl, NaOAc, EtOH-H_2O, reflux ; (c) NaH, 4-fluorobenzaldehyde, THF-DMSO, r.t. ; (d) HCl-AcOH, 100°C ; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, DMA-H_2O, r.t. ; (f) amines, 2,4,6-triisopropylbenzenesulfonyl chloride, Et_3N, DMAP, $CHCl_3$, r.t. ; (g) KOH, dioxane-H_2O, reflux

We herein describe the synthesis and 5α-reductase inhibitory activities of novel 5- or 6-carbamoylbenzofuran derivatives.

The synthesis of the 5-carbamoylbenzofuran derivatives is shown in Scheme 1. 2-Hydroxyacetophenone (**1**) was alkylated with ethyl 4-bromobutyrate in the presence of NaH in DMF to give **2** (86% yield). Treatment of **2** with hydroxylamine afforded the oxime **3** (92%), the sodium salt of which was reacted with 4-fluorobenzaldehyde in THF-DMSO to give the *O*-phenyl oxime **4** (50%). According to the method of Mooradian *et al.*,[5] heating of the oxime **4** in HCl-AcOH solution caused cyclization of the furan ring to give the 2-phenyl-5-formylbenzofuran compound **5** (61%). **5** was oxidized with $NaClO_2$ to afford the carboxylic acid **6** (90%), and then **6** was reacted with amines in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride and triethylamine to give the corresponding amides **7a~c** (67~97%).[6]

Scheme 2



Reagents : (a) Tf_2O, pyridine, CH_2Cl_2, 0°C, ; (b) trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 90°C ; (c) K_2CO_3, MeOH r.t. ; (d) 2-benzyloxyiodobenzene, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 110°C ; (e) pyridinium chloride, 200°C ; (f) NaH, $Br(CH_2)_3COOEt$, DMF, r.t. ; (g) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, DMA-H_2O, r.t. ; (h) amines, 2,4,6-triisopropylbenzenesulfonyl chloride, Et_3N, DMAP, $CHCl_3$, r.t. ; (i) KOH, dioxane-H_2O, reflux

Hydrolysis of the ester groups of **7a~c** with KOH in aqueous dioxane afforded the 2-phenyl-5-carbamoylbenzofuran derivatives **8a~c** (92~96%).

The 6-carbamoylbenzofuran derivatives were synthesized by the route described in Scheme 2. First, the diphenylacetylene compound **13** was synthesized as a key intermediate. The triflate **10**, prepared from vanillin (**9**), was reacted with trimethylsilylacetylene in the presence of a catalytic amount of $PdCl_2(Ph_3P)_2$ in DMF containing triethylamine at 90°C to afford the cross-coupling product **11** (74%). Desilylation of **11** gave the alkyne **12** (88%), which was reacted with 2-benzyloxyiodobenzene in the presence of $PdCl_2(Ph_3P)_2$ in DMF at 110°C to give the diphenylacetylene compound **13** (34%).

Then, cyclization of the furan ring was attempted. According to the method of Hiroya *et al.*,[7] by heating of **13** with LiCl at 160°C in DMPU (1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone), demethylation of the methoxy group, followed by furan ring cyclization, occurred to give the 2-phenyl-6-formylbenzofuran derivative **14b** (21%), but the yield was low. Demethylation using some other

dealkylating reagents was attempted and pyridinium chloride was the most effective. By heating a mixture of **13** and pyridinium chloride at 200°C, demethylation, followed by cyclization, occurred to give the cyclization product **14a** in 45% yield. In this reaction, debenzylation of the benzyloxy group occurred simultaneously.

Finally, introduction of a butyric acid group was accomplished. Alkylation of the sodium salt of **14a** with ethyl 4-bromobutyrate provided **15** (75%), which was oxidized to the carboxylic acid **16** (75%). Condensation reaction of **16** with several amines using 2,4,6-triisopropylbenzenesulfonyl chloride gave the amide products **17a~c** (81~94%). Hydrolysis of the ester groups of **17a~c** afforded the 2-phenyl-6-carbamoylbenzofuran derivatives **18a~c** (85~95%).

The molecular structure of the compound **18c** was confirmed by X-ray crystallographic analysis[8] and shown in Figure 1. The benzofuran ring and the 2-phenyl group was approximately on the same plane. The oxygen atom of the furan ring and the ether-oxygen of the carboxypropyloxy group are on the opposite sides against the linkage combining the 2-phenyl group with the benzofuran ring. This is thought to be due to the electrical repulsion of two oxygen atoms.

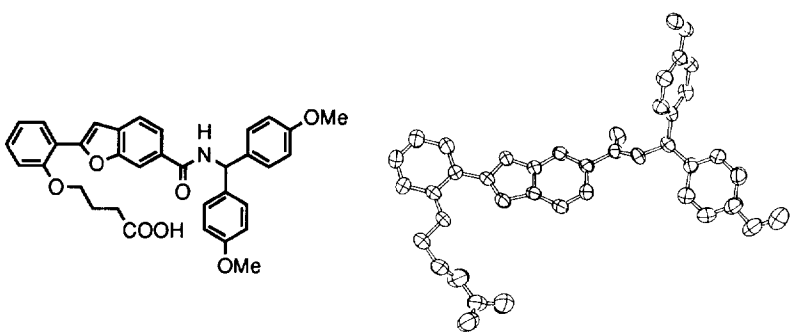


Figure 1. The molecular structure of **18c**.

The synthesized 5- or 6-carbamoylbenzofuran derivatives were evaluated for inhibitory activities against rat testosterone 5α-reductase *in vitro* using the standard method.[4b] The preparation of recombinant human type 1 and type 2 5α-reductase and the 5α-reductase assay using human enzyme were carried out according to the protocol described below.[9] IC_{50}s of the derivatives are shown in Table 1. In the 5-carbamoyl derivatives, against rat enzyme, the diphenylmethylcarbamoyl compound **8a** and the fluoro-substituted compound **8b** showed moderate inhibitory activities and introduction of a methoxy group into the phenyl moiety of **8a** increased the inhibitory potency (the compound **8c**). In the 6-carbamoyl derivatives, against rat enzyme, the diphenylmethylcarbamoyl compound **18a**, the fluoro-substituted compound **18b**, and the methoxy-substituted compound **18c** had potent inhibitory activities, and they tended to be more active than the 5-carbamoyl derivatives. **8a** had moderate inhibitory activity against human type 1 enzyme but no activity against human type 2 enzyme. **18a**, **18b**, and **18c** showed potent inhibitory activity against human type 1 enzyme and moderate activity against human type 2 enzyme. **18a~18c** were 4~7 times more potent against type 1 enzyme than against type 2 enzyme. Also against

Table 1. Inhibitory Activities of 5- or 6-carbamoylbenzofuran derivatives against rat and human 5α-reductase

Compound	IC_{50} [nM]		
	rat	human type1	human type2
8a	155	310	$>10^5$
8b	279	N.T.	N.T.
8c	26.3	N.T.	N.T.
18a	28.5	62	270
18b	37.9	50	340
18c	35.5	130	930
FK 143	118	3.0	11

N.T. : not tested

human enzyme, the 6-carbamoyl derivatives tended to be more active than the 5-carbamoyl derivatives. The presence of a diphenylmethylcarbamoyl group in the 6-position of the benzofuran ring is thought to be desirable for potent inhibitory activity against rat and human 5α-reductase.

In conclusion, a series of novel benzofuran derivatives with both carboxy and 5- or 6-diphenylmethylcarbamoyl groups were synthesized, and their inhibitory activities against rat and human testosterone 5α-reductase were tested *in vitro*. The derivatives showed inhibitory activities against both enzymes and were more active against human type 1 enzyme than against type 2 enzyme. The 6-carbamoyl derivatives tended to be more potent than the 5-carbamoyl ones.

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8) Crystal data of **18c**; $C_{34}H_{31}NO_7$, MW=565.6, T=298K, Cu Kα radiation, λ=1.5418Å, monoclinic, C2/c, a=34.838(5)Å, b=5.061(5)Å, c=34.808(5)Å, β=109.69(5)°, V=5788(5)Å^3, Z=8, R=0.067, Rw=0.066. Full X-ray crystallographic data will be deposited with the Cambridge Crystallographic Data Centre.

9) *cDNA cloning and expression of human 5α-reductase type 1 and type 2 in COS-1 cells* ; The cDNA of human 5α-reductase type 1 and type 2 were obtained by polymerase chain reaction method from human cDNA library of liver and prostate (CLONTECH Laboratories, Inc.), respectively. They were ligated into pME18s expression vector. Transient transfections of COS-1 cells were carried out by electroporation method (Gene PulserTM, Bio-Rad Laboratories, 960 μFD, 200 ohm, 300 V) with type 1 or type 2 expression vector. The cells were harvested 48 h after transfection and were broken by freezing and thawing in 20 mM potassium phosphate buffer, pH 7.4, containing 10% glycerol, 0.33 M sucrose, 50 μM NADPH and 0.001% PMSF. Then broken cells were homogenized by POLYTRON (KINEMATICA GmbH) and centrifuged at 10000xg. The pellet was resuspended in buffer and stored at -80°C until assay. These crude membrane fraction were used as human 5α-reductase type 1 and type 2.

5α-reductase assay ; The reaction solution of type 1 was 40 mM potassium phosphate buffer, pH 7.5, containing 1 μM [^{14}C] testosterone, 1mM dithiothreitol and 0.5 mM NADPH, and that of type 2 was 100 mM Tris-citrate buffer, pH 5.5, containing 1 μM [^{14}C] testosterone, 1 mM dithiothreitol and 1mM NADPH. Test sample was added in 5 mM of dimethyl sulfoxide (DMSO) and the control tube received the same volume of DMSO. The reaction was carried out for 15 min at 37°C and then stopped with 2 ml of ethyl acetate containing testosterone, 5α-dihydrotestosterone and androstenedione (10 μg each). The following procedure was the same as that described in the reference 4b.