Benzophenone- and Indolecarboxylic Acids: Potent Type-2 Specific Inhibitors of Human Steroid 5α-Reductase

Dennis A. Holt,*[†,‖] Dennis S. Yamashita,[†] Arda L. Konialian-Beck,[†] Juan I. Luengo,[†] Andrew D. Abell,[§] Derk J. Bergsma,[‡] Martin Brandt,[†] and Mark A. Levy[†]

Departments of Medicinal Chemistry and Molecular Genetics, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, Pennsylvania 19406

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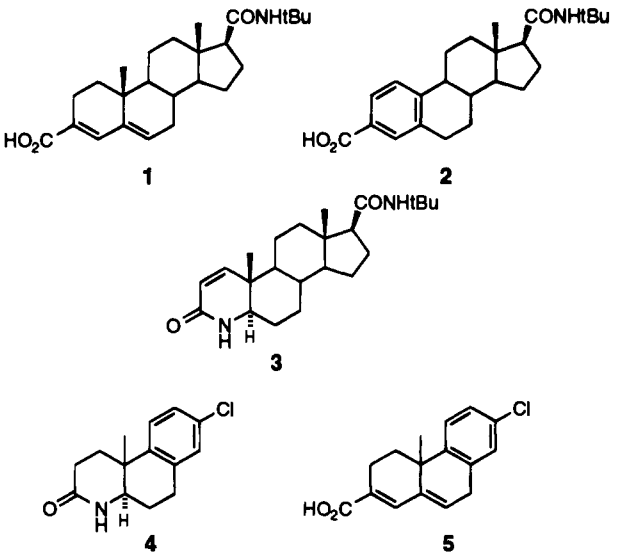
Dihydrotestosterone (DHT), a metabolite of the predominant circulating androgen testosterone (T), is recognized as a principal mediator of benign prostatic hyperplasia (BPH), an ailment afflicting a remarkably large percentage of aging males.[1] DHT also has been implicated in the etiology of prostatic cancer[2] and several disorders of the skin including acne,[3] androgenic alopecia,[4] and hirsutism.[5] As a consequence, a search has ensued for potent inhibitors of the NADPH-dependent enzyme responsible for the production of DHT, steroid 5α-reductase (SR).[6] Several classes of steroidal inhibitors designed as transition state mimics of SR have been described, most notably the C-3 carboxylic acids[7] exemplified by **1** (epristeride) and **2** and the 4-aza class[8] exemplified by finasteride (**3**) which is currently marketed worldwide as a treatment for BPH. Following the discovery of these inhibitors, the existence of two different genes encoding unique isozymes of SR was reported.[9] The roles of these two isozymes in DHT-dependent pharmacology has yet to be fully elucidated. Interestingly, **1**, **2**, and **3** all selectively inhibit the isozyme predominantly expressed in the prostate, type-2, although analogs of **3** bearing alternative amide side chains inhibit both type-1 and type-2 isozymes.[10–12] A more recent report describes C-ring aromatic, nor-D-ring tricyclic analogs of the 4-aza-steroids (e.g., **4**) as potent and selective inhibitors of type-1 SR.[13] Subsequently, we reported that a similar tricyclic analog of epristeride (e.g., **5**) is a modest inhibitor of SR and is selective for the type-2 isozyme.[14]

Prompted by the promising activities of the D-ring-deleted analogs **4** and **5**, we began a selected screening program of nonsteroidal aryl carboxylic acids which retain the key A-ring features central to the enolate transition state mimetic design strategy and thus might functionally mimic **2** as SR inhibitors. This initial screening effort uncovered two classes of very potent *type-2 selective* SR inhibitors. The *p*-toluoylbenzophenonecarboxylic acid (**6**, Table 1) exhibited an apparent inhibition constant of 10 nM against recombinant human type-2 SR, and the 5-(benzyloxy)indole-2-carboxylic acid (**28**, Table 2) inhibited the type-2 SR with a $K_{i,app}$ of 40 nM.[15] Neither compound significantly inhibited recombinant human type-1 SR at concentrations approaching their solubility limits. In this report we describe the results of a preliminary structure–activity study around these two classes of inhibitors.[16]



Analogs **9**–**22** in the benzoylbenzophenone class were prepared as shown in Scheme 1. Biphenyl compound **15** was obtained as a reaction byproduct in the synthesis of **14**. Compounds **6** and **7** were prepared as a separable mixture arising from $KMnO_4$ oxidation of 4-methyl-4′-(*p*-toluoyl)benzophenone. Compounds **8** and **23** were obtained through addition of [4-[[(*tert*-butyldimethylsilyl)oxy]methyl]phenyl]lithium to 4-cyanobenzaldehyde followed by DIBAL reduction, addition of phenyl- or benzylmagnesium bromide, and finally Jones oxidation. Compound **24** was derived from addition of (4-phenoxyphenyl)magnesium bromide to 4-cyanobenzaldehyde followed by DIBAL reduction of the nitrile and Jones oxidation of the resulting aldehyde alcohol to the desired keto acid. Compounds **25** and **26** were obtained from **24** upon sequential reduction with $NaBH_4$ and catalytic hydrogenation using palladium on carbon. Biphenyl derivative **27** was prepared from 4,4′-biphenyldicarboxylic acid via a five-step sequence involving LAH reduction, monosilylation, TPAP oxidation to the aldehyde, benzyl Grignard addition, and finally Jones oxidation.

Analogs **28**, **30**, and **32**–**34** in the indolecarboxylic acid series (Table 2) were purchased as acids (or as esters and hydrolyzed to acids) from Sigma Chemical Co. or Aldrich Chemical Co. Compounds **29** and **31** were prepared via methylation of the parent indoles **28** and **30**, respectively.

In the benzoylbenzophenone series, C-ring substitution is well tolerated (Table 1), although no significant correlations between activity and σ or π parameters are readily apparent. The C-ring can also be saturated without loss of activity (compound **19**). The carbonyl linker between the B- and C-rings can be replaced by ether, methylene, or oxoethylene linkers without loss of potency for type-2 SR (**20**–**27**). In the case of the B–C ether, a 5–8-fold increase in potency was observed (**8** vs **24** and **12** vs **20**). A direct bond between the B- and C-rings is also tolerated (compound **15**). Substitution of the carbonyl linker between the A- and B-rings with methylene or hydroxymethylene results in a loss of activity by 7–12-fold (compounds **25** and **26**), while

† Department of Medicinal Chemistry.
‡ Department of Molecular Genetics.
‖ Current address: ARIAD Pharmaceuticals, 26 Landsdowne St., Cambridge, MA 02139-4234.
§ Visiting Scholar, Department of Chemistry, University of Canterbury, Christchurch, New Zealand.

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Scheme 1

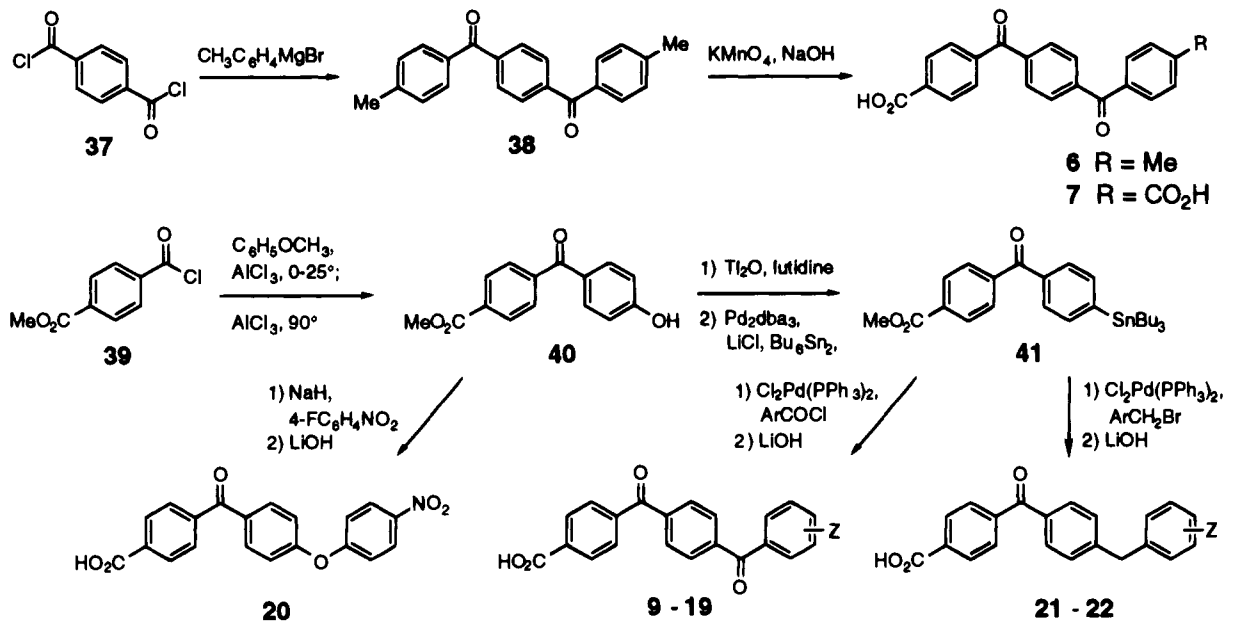
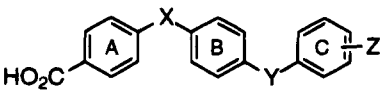


Table 1. Inhibition of Human Type-2 Steroid 5α-Reductase by Benzophenonecarboxylic Acids

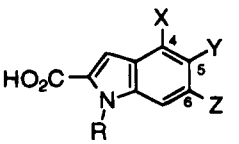


no.	X	Y	Z	$K_{i,app}$ type-2 (nM)
6	C=O	C=O	4-CH_3	10
7	C=O	C=O	4-CO_2H	1000
8	C=O	C=O	H	40
9	C=O	C=O	4-OH	22
10	C=O	C=O	4-Cl	35
11	C=O	C=O	4-CF_3	30
12	C=O	C=O	4-NO_2	100
13	C=O	C=O	4-tBu	110
14	C=O	C=O	2,4-Cl_2	35
15	C=O		2,4-Cl_2	85
16	C=O	C=O	3,4-Cl_2	35
17	C=O	C=O	3-CF_3	38
18	C=O	C=O	3,4-(OCH_2O)	120
19	C=O	C=O	hexahydro	30
20	C=O	O	4-NO_2	20
21	C=O	CH_2	4-CH_3	15
22	C=O	CH_2	4-CF_3	30
23	C=O	(C=O)CH_2	H	40
24	C=O	O	H	5
25	CHOH	O	H	60
26	CH_2	O	H	35
27		(C=O)CH_2	H	60

an A–B biphenyl analog retains potency (**23** vs **27**). Clearly, both the A-ring carboxylic acid and the C-ring are critical, as benzophenonecarboxylic acid is only a relatively weak inhibitor ($K_{i,app}$ = 840 nM) and methyl ester analogs of Table 1 compounds are inactive against the type-2 isozyme at concentrations approaching their aqueous solubility limit (5–10 μM; data not shown). None of the benzophenone analogs of Table 1 exhibited any inhibition of type-1 SR at concentrations of 10 μM.

Fewer compounds have been examined in the indole series; nevertheless, an interesting structure–activity profile has emerged. *N*-Methylation of the 5-benzyloxy derivative results in an 8-fold diminution of type-2 inhibitory activity (Table 2, **28** vs **29**). The 5-methoxy 6-benzyloxy analog (**30**) exhibits comparable potency to

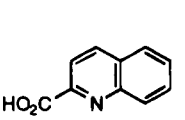
Table 2. Inhibition of Human Type-1 and Type-2 Steroid 5α-Reductases by Indolecarboxylic Acids



					$K_{i,app}$ (nM)	
no.	R	X	Y	Z	type-1	type-2
28	H	H	OCH_2Ph	H	>2500	40
29	CH_3	H	OCH_2Ph	H	>2500	310
30	H	H	OCH_3	OCH_2Ph	460	20–30
31	CH_3	H	OCH_3	OCH_2Ph	500	10
32	H	OCH_2Ph	H	H	>2500	550
33	H	H	H	H	NI[a]	230
34	CH_3	H	H	H	NI	NI[a]

[a] NI: no inhibition at 10 μM.

the 5-benzyloxy lead (**28**), but *N*-methylation of **30** results in a 2–3-fold enhancement of inhibitory activity (compound **31**). The disubstituted variants, **30** and **31**, also exhibit a weak but significant level of inhibitory activity against type-1 SR. The preference of B-ring substitution in the 5 or 6 position is demonstrated by the decreased potencies of the unsubstituted 2-carboxyindole (**33**) and the 4-benzyloxy analog (**32**). Curiously, while the parent carboxyindole **33** inhibits at submicromolar concentrations, the *N*-methylated derivative **34** shows no measurable inhibition at 10 μM. Quinolinecarboxylic acid (**35**) did not inhibit at 2.5 μM, and the related (benzyloxy)naphthylenecarboxylic acid (**36**) has only very weak inhibitory activity, underscoring the dramatic effects that subtle steric and/or electronic changes in the A-ring impart upon binding affinity to type-2 SR.



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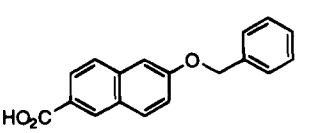

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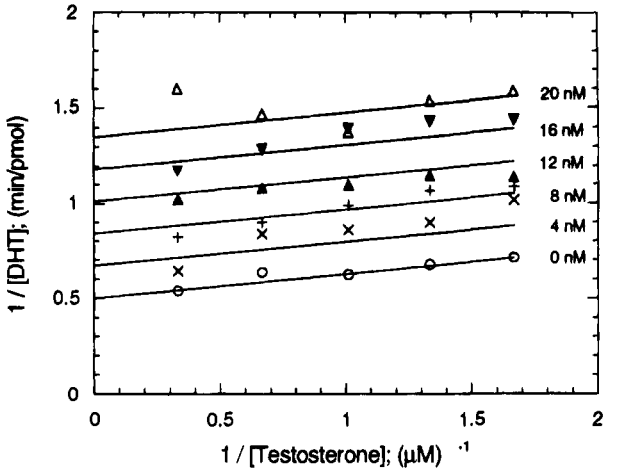

Figure 1. Inhibition of recombinant human steroid 5α-reductase type-2 by compound **6**. The activity of human type-2 steroid 5α-reductase expressed in CHO cells[11] was evaluated at variable concentrations of [^{14}C]testosterone and compound **6** in 50 mM sodium citrate buffer, pH 5.0, containing 0.2 mM NADPH and a cofactor-regenerating system (supplementary material). Reactions were initiated by addition of enzyme solution and incubated at 30 °C for 30 min. Concentrations of inhibitor **6** represented in the figure are 0 (○), 4 (×), 8 (+), 12 (▲), 16 (▼), and 20 (△) nM . Data was fit to the equation describing a linear, uncompetitive model[16] to give kinetic constants of $K_m = 0.33 \pm 0.05$ μM and $K_{ii} = 11.6 \pm 0.7$ nM.

We have proposed for steroidal compounds that charge complementarity between the A-ring functionality and the nicotinamide moiety of the cofactor is a critical determinant in the mechanism of inhibition.[6] Inhibitors such as carboxylates **1** and **2**, which are anionic at physiologic pH, preferentially bind to the enzyme–NADP$^+$ complex and demonstrate uncompetitive dead-end inhibition kinetics versus T, while neutral compounds such as **3** exhibit competitive inhibition versus T and bind preferentially to the enzyme–NADPH complex.[17] Compound **6** exhibits uncompetitive steady state kinetics versus T (Figure 1), suggesting that this class of anionic nonsteroidal inhibitors behaves analogously to the steroidal carboxylic acids, perhaps having a similar binding mode within the enzyme active site which allows for a charge–charge interaction with the oxidized cofactor, NADP$^+$.

In summary, substituted benzophenone- and indolecarboxylic acids have been identified as potent and selective inhibitors of type-2 human steroid 5α-reductase. Work to further characterize the structure–activity relationships within these nonsteroidal classes and the kinetic mechanisms of the inhibition is ongoing.

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Supplementary Material Available: Experimental procedures, NMR spectra, and analytical data for the final products (16 pages). Ordering information is given on any current masthead page.

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