Novel 5α-Reductase Inhibitors: Synthesis, Structure−Activity Studies, and Pharmacokinetic Profile of Phenoxybenzoylphenyl Acetic Acids

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Novel substituted benzoyl benzoic acids and phenylacetic acids **1**−**14** have been synthesized and evaluated for inhibition of rat and human steroid 5α-reductase isozymes 1 and 2. The compounds turned out to be potent and selective human type 2 enzyme inhibitors, exhibiting IC_{50} values in the nanomolar range. The phenylacetic acid derivatives were more potent than the analogous benzoic acids. Bromination in the 4-position of the phenoxy moiety led to the strongest inhibitor in this class (**12**; $IC_{50} = 5$ nM), which was equipotent to finasteride. Since oral absorption is essential for a potential drug, **12** was further examined. In the parallel artificial membrane permeation assay (PAMPA) it turned out to be a good permeator, whereas it was a medium permeator in Caco2 cells. After oral administration (40 mg/kg) to rats a high bioavailability and a biological half-life of 5.5 h were observed, making it a promising candidate for clinical evaluation.

Introduction

5α-Reductase is a membrane-bound, NADPH-dependent enzyme (EC 1.3.99.5) that irreversibly catalyses the reduction of 4-ene-3-oxosteroids to the corresponding 5α-3-oxosteroids, particularly, testosterone (T) to the most potent androgen 5α-dihydrotestosterone (DHT).[1] Two isozymes, 5α-reductase 1 and 2, have been characterized[2] and confirmed by molecular cloning.[3] They show high contents of hydrophobic amino acids and a similar hydrophobicity pattern. However, the identity of their amino acid sequence is only about 50%, and different tissue distribution and biochemical properties were found for both isozymes. The type 1 isoform is the predominant enzyme in sebaceous glands and liver, whereas the type 2 enzyme is dominant in the prostate. An acidic pH of 5.5 is optimal for type 2 activity, whereas a pH between 6.0 and 8.5 is favorable for isozyme 1. The affinity for the substrate T is higher for isozyme 2 ($K_m \cong 0.4$ μM) than it is for isozyme 1 ($K_m \cong 10$ μM).[4] 5α-Reductase was shown to be involved in human disorders, e.g. benign prostatic hyperplasia (BPH),[5,6] alopecia,[7] acne,[8] and hirsutism,[9] and is proposed to play a role in prostate cancer.[10] BPH is the most common proliferative disease that affects males and causes obstructive uropathy. The use of surgery is effective in the treatment, but its high costs and postoperative complications leave the need for other treatments. Finasteride (Proscar, Propecia) and more recently dutasteride (Avodart, Avolve) are two steroidal 5α-reductase inhibitors already used for the treatment of BPH and male pattern baldness. Finasteride is a relatively selective 5α-reductase type 2 inhibitor capable of reducing the circulating DHT levels by approximately 70%,[11,12] whereas dutasteride, a potent dual inhibitor of both isozymes, reduces serum DHT by more than 90%; thus, it is a more efficient steroid 5α-reductase inhibitor than finasteride.[13] It is well-tolerated and side effects, e.g., sexual disorders and gynecomastia, are transient.[14,15] Fewer side effects might be exhibited by appropriate nonsteroidal inhibitors. Consequently, we and other groups have focused on the synthesis and evaluation of nonsteroidal inhibitors.[16−29] Recently, we have discovered a novel type of dual inhibition of 5α-reductase 1 and 2, a concept which is called hybrid inhibition.[30] Esters of substituted benzylidene-4-carboxylic acids are inhibitors of type 1 enzyme.[30] They act in the periphery and are cleaved in the prostate to the corresponding acids, which are potent type 2 inhibitors.[30] In a previous work,[28] we used compound **A** (Figure 1), described by Holt et al.,[29] as a potent 5α-reductase inhibitor as starting point for structural modifications aimed at the optimization of the compound. The most potent inhibitor obtained was 4-(4-(phenylaminocarbonyl)benzoyl)benzoic acid, showing an IC_{50} value of 820 nM for human type 2 enzyme. As this might not be sufficient for clinical efficacy, we describe in this paper the continuation of the former study, i.e., the synthesis, biological evaluation, and molecular modeling of novel phenoxybenzoyl benzoic and phenylacetic acids **1**−**14** (Figure 1). For the determination of biological activity in vitro, studies were performed using human and rat isozymes 1 and 2. Since besides potency the pharmacokinetic properties of a NCE are crucial, the most potent compound of this series was further investigated. To predict the oral availability of the most potent compound, the parallel artificial membrane permeation assay (PAMPA) and the Caco2 assay were used. Finally, the in vivo pharmacokinetic profile was determined in rats.[3]

Supporting the experimental findings, we applied a pharmacophore modeling approach that describes nicely the biological activities of the inhibitors investigated by us.

Chemistry

Terephthalic acid monomethyl ester was the precursor for the acids **1**−**4**, which were synthesized via two different routes: First, for the preparation of compounds **1**−**3**, the precursor was converted to the acid chloride, which then was reacted with anisole in a Friedel−Crafts acylation in the presence of $AlCl_3$ to yield the ketone **1c** as described.[32] Ether cleavage with $AlCl_3$ in benzene afforded the phenol **1b**, which was subjected to alkylation with different alkyl bromides in the presence of a base (NaH or K_2CO_3, respectively) to give the esters **1a**−**3a**. Alternatively, following the procedure of Goossen

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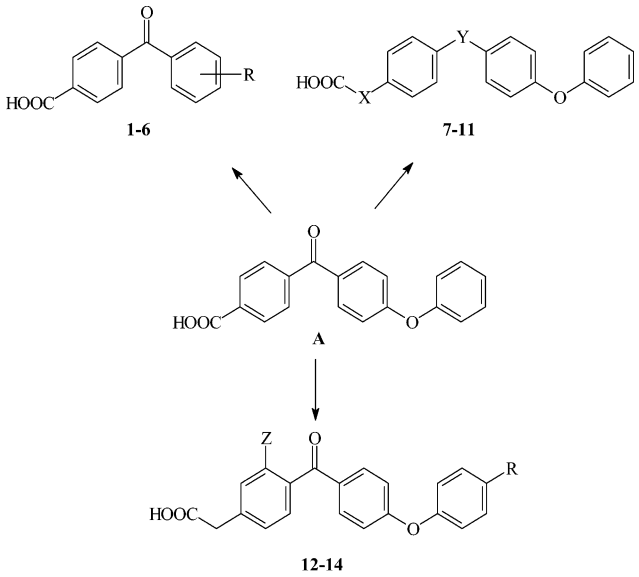
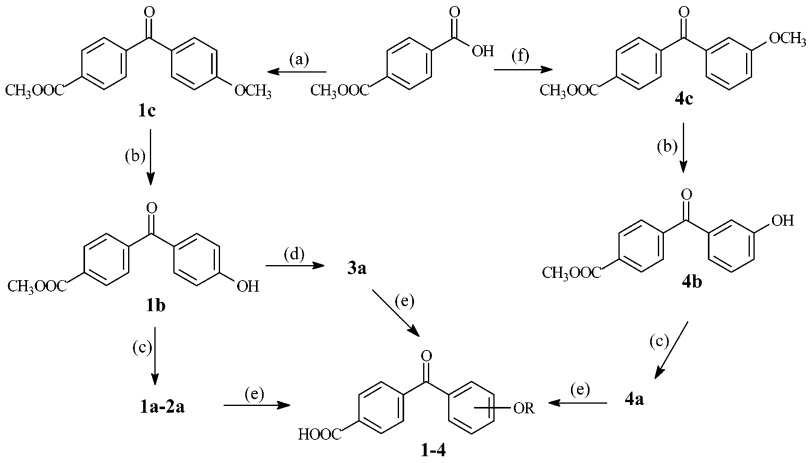


no	R	no	X, Y	no	Z, R
1	4-(Ph)CH_2O-	**7**	-CH_2-, C=O,	**12**	– , Br
2	4-(2,5-$(CF_3)_2$Ph)CH_2O-	**8**	-$(CH_2)_2$-, C=O,	**13**	F, –
3	4-$(Ph)_2$CHO-	**9**	-CH=CH-, C=O,	**14**	CH_3, –
4	3-(Ph)CH_2O -	**10**	-$(CH_2)_2$-, CH_2		
5	4-(Ph)CH_2NH-	**11**	-$(CH_2)_3$-, C=O		
6	4-(Ph)CONH-				

Figure 1. Lead compound (**A**) and structural optimizations (**1**−**14**).

Scheme 1. Synthesis of Compounds **1**−**4**[a]



1, 1a; R = 4-Benzyl-

2, 2a; R = 4-(2,5-Bis(trifluoromethyl)benzyl)-

3, 3a; R = 4-Diphenylmethyl-

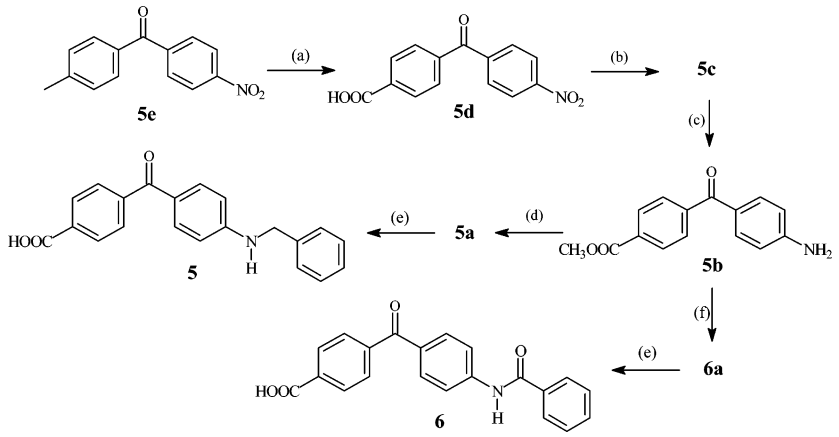
4, 4a; R = 3-Benzyl-

[a] Conditions: (a) 80%, (i) $(COCl)_2$, CH_2Cl_2/DMF, room temperature for 1 h; (ii) anisole, $AlCl_3$, CH_2Cl_2, room temperature for 24 h; (b) 81%, $AlCl_3$, benzene, reflux, 15−45 min; (c) 39−62%, R−Br, NaH, DMF, rt, 3−24 h; (d) 73%, diphenylmethyl bromide, K_2CO_3, DMF, 90 °C for 24 h; (e) 31−87%, K_2CO_3, MeOH/H_2O (9:1), reflux; (f) 37%, 3-methoxyphenylboronic acid, DSC, Pd(F_6-acac)$_2$, Pcy_3, Na_2CO_3, THF, 60 °C overnight.

et al.,[33] the palladium-catalyzed cross-coupling reaction of 3-methoxybenzeneboronic acid and terephthalic acid monomethyl ester using DSC (di-*N*-succinimidyl carbonate) as a coupling reagent proceeded smoothly and afforded the ester **4c**. Demethylation of **4c** and alkylation of the obtained phenol **4b** in analogy to the above-mentioned procedure provided the ester **4a**. Saponification of the esters **1a**−**4a** led to the target acids **1**−**4** (Scheme 1).

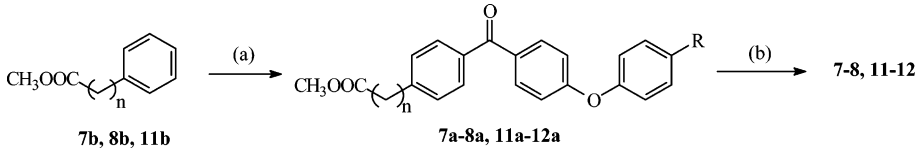
For the synthesis of compounds **5** and **6**, 4-(4-nitrobenzoyl)-benzoic acid **5d** was subjected to acid-catalyzed methylation to produce the ester **5c**. A mild reduction of the nitro group using stannous chloride following the procedure of Bellamy et

Scheme 2. Synthesis of Compounds **5** and **6**[a]



[a] Conditions: (a) 75%, glacial acetic acid, sulfuric acid, CrO_3, room temperature for 3 h; (b) 78%, MeOH, sulfuric acid, reflux for 18 h; (c) 93%, $SnCl_2 \cdot 2H_2O$, EtOH, 70 °C for 1 h; (d) 30%, benzyl bromide, DMF, room temperature, 20 h; (e) 48−54%, K_2CO_3, MeOH/H_2O (9:1), reflux for 1−2 h; (f) 49%, benzoyl chloride, triethylamine, CH_2Cl_2, room temperature for 2 h.

Scheme 3. Synthesis of Compounds **7**, **8** and **11**, **12**[a]



7b, 7a, 7; n = 1, R = H
8b, 8a, 8; n = 2, R = H
11b, 11a, 11; n = 3, R = H
12a, 12; n = 1, R = Br

[a] Conditions: (a) 15−64%; (i) $(COCl)_2$, $AlCl_3$, CH_2Cl_2, 5−10 °C for 15 min; (ii) 4-(un)substituted-diphenyl ether, $AlCl_3$, CH_2Cl_2, 24−40 °C for 1 h; (b) 31−83%, K_2CO_3, MeOH/H_2O (9:1), reflux for 1−2 h.

al.[34] afforded the amino derivative **5b** in excellent yield. Reaction of the amino group of **5b** with benzyl chloride or benzoyl chloride, followed by saponification of the formed esters **5a** and **6a**, gave access to the acids **5** and **6**, respectively (Scheme 2).

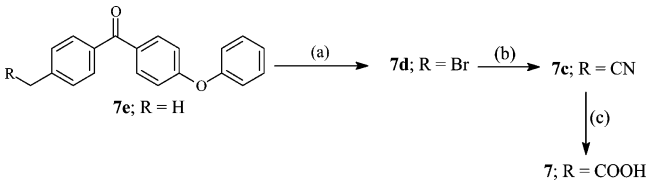
The phenylalkanoic acid methyl esters **7b**, **8b**, and **11b** were subjected to acylation[35] using oxalyl chloride/$AlCl_3$. The obtained acid chlorides were reacted with the corresponding diphenyl ethers in the presence of $AlCl_3$ to afford the alkanoic acid esters **7a**, **8a**, **11a**, and **12a**. The overall yield in this two-step reaction was in some cases below 20%, most probably due to the formation of positional isomers. Saponification of these esters resulted in the acids **7**, **8**, **11**, and **12** (Scheme 3).

Alternatively, bromination of the methyl group of (4-methylphenyl)(4-phenoxyphenyl)methanone **7e** using NBS in the presence of benzoyl peroxide, followed by sodium cyanide treatment of the obtained bromo derivative **7d**, provided the acetonitrile derivative **7c**, which after hydrolysis under basic conditions and subsequent acidification afforded the acid **7** in good yield (Scheme 4).

Heck coupling[36] of the iodide **9b** with methyl acrylate provided the cinnamic acid derivative **9a** in good yield. Hydrogenation of **9a** with H_2/Pd for 18 h resulted in both reduction of the keto group and saturation of the olefinic double bond, thus furnishing the propanoic acid methyl ester **10a**. Saponification of the esters **9a** and **10a** afforded the corresponding acids **9** and **10** (Scheme 5).

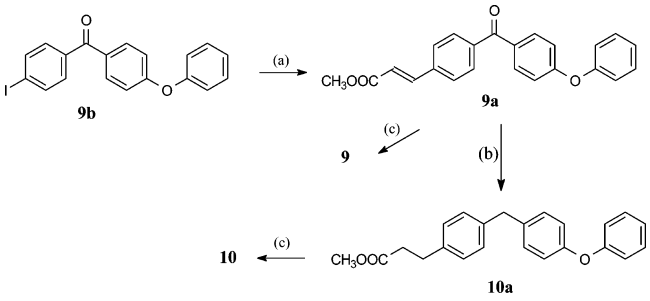
The synthesis of compounds **13** and **14** started from the benzoic acids **13c** and **14c**. Compound **13c** was afforded previously by oxidation of 4-bromo-2-fluorobenzaldehyde using a modified Lindgren reaction.[37,38] Friedel−Crafts acylation of

Scheme 4. Alternative Pathway for the Synthesis of Compound **7**[a]



[a] Conditions: (a) 44%, NBS, benzoyl peroxide, CCl_4, reflux; (b) 47%, NaCN, 1,4-dioxane/H_2O, reflux; (c) 63%, aq NaOH (40%), EtOH, reflux then 1 N HCl.

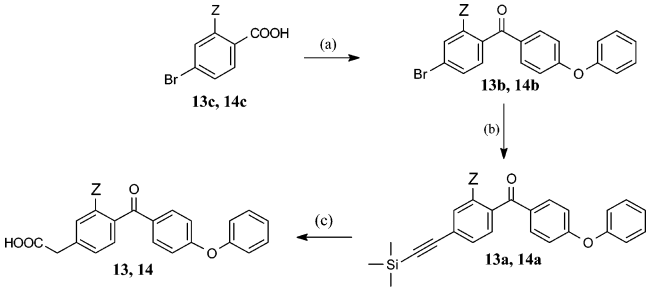
Scheme 5. Synthesis of Compounds **9** and **10**[a]



[a] Conditions: (a) 84%, methyl acrylate, $Pd(OAc)_2$, TBAC, DMF, 50 °C for 3 h; (b) 68%, Pd/C, H_2, MeOH, rt, 18 h; (c) 89%, K_2CO_3, MeOH/H_2O (9:1), reflux.

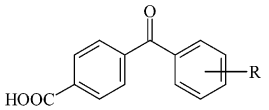
diphenyl ether with the acid chlorides of **13c** and **14c** respectively yielded the ketones **13b** and **14b**. The latter were subjected to a cross-coupling reaction with ethynyltrimethylsilane in the presence of $PdCl_2 \cdot 2PPh_3$ and cuprous iodide[39] to provide **13a** and **14a** in excellent yield. The hydroboration of the produced

Scheme 6. Synthesis of Compounds **13** and **14**[a]



[a] Conditions: (a) 60−70%; (i) $(COCl)_2$, CH_2Cl_2/DMF, room temperature for 1 h; (ii) diphenyl ether, $AlCl_3$, CH_2Cl_2, 24−40 °C for 1 h; (b) 84−88%, triethylamine/THF (5:1), $PdCl_2$•2PPh_3, CuI, trimethylsilylacetylene, 80 °C for 2−3 h; (c) 29−39%, BH_3, 1M in THF, 3 h at room temperature, MeOH, 3 N NaOH, 35% H_2O_2 at 40 °C.

Table 1. Inhibition of Human and Rat Steroid 5α-Reductase Types 1 and 2 in Vitro by Compounds **1**−**6**



1-6

		% inhibn (10 μM) or [IC_{50}, μM]			
		human		RVP	
compd	R	type 2[a,d]	type 1[b,e]	pH 5.5[c,d]	pH 6.6[c,d]
1	4-(Ph)CH_2O	[0.119]	ni	45	64
2	4-(2,5-$(CF_3)_2$Ph)CH_2O	[0.113]	12	29	69
3	4-$(Ph)_2CHO$	[0.79]	9	71	73
4	3-(Ph)CH_2O	[0.131]	ni	55	35
5	4-(Ph)CH_2NH	60	nd	nd	nd
6	4-(Ph)CONH	51	nd	nd	nd
A	4-(Ph)O	[0.053]	ni	33	53
finasteride		[0.005]	[0.051]	[0.011]	[0.01]

[a] Human prostate homogenates, 125 μg protein, pH 5.5. [b] DU 145 cell line. [c] Rat ventral prostate, 200−250 μg protein. [d] Substrate was [1β,2β-^{3}H]testosterone (210 nM). [e] Substrate was [^{3}H]androstenedione (5 nM). ni, no inhibition; nd, not determined.

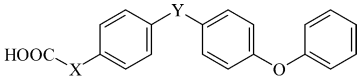
alkynes **13a** and **14a**, followed by oxidation with aqueous NaOH and hydrogen peroxide, led to the corresponding carboxylic acids **13** and **14** (Scheme 6).[40]

Biological Results

Inhibition of Human and Rat 5α-Reductase Isozymes 1 and 2 in Vitro. The inhibitory activities of compounds **1**−**14** were determined using rat prostate homogenates (pH 6.6 for type 1 and pH 5.5 for type 2), human prostate homogenate (BPH tissue for type 2) according to the method of Liang et al.,[41] and the DU145 cell line (for human type 1 enzyme) as described.[42−44] The percent inhibition values at a concentration of 10 μM or, for more potent compounds, the IC_{50} values are presented in Tables 1−3.

In a previous work,[28] we exchanged the phenoxy group of the potent and selective benzoyl benzoic acid **A**[29] (Table 1) by alkyl- and aryl carbamoyl groups to afford novel active and selective 5α-reductase inhibitors. However, the activities obtained did not reach the potency of the lead compound. In this study, we first performed further modifications of the phenoxy group, all of which are based on the insertion of a second atom between the two benzene nuclei, thus leading to an elongation of the molecule (compounds **1**−**6**, Table 1). Replacement of the phenyl group of compound **A** by a benzyl or diphenylmethyl group reduces inhibitory activity toward human type 2 enzyme by a factor of 2 and 15, respectively (compounds **1** and **3**). This finding confirms our previous observation[27] that the hydrophobic pocket, which also accommodates the substituents in the 17-position of the steroidal inhibitors, is limited in size. Introduction of two trifluoromethyl substituents into the benzyl group of **1**, mimicking the steroidal dual type 1 and 2 inhibitor dutasteride, does not have a significant impact on inhibitory activity (IC_{50} values: **1**, 119 nM; **2**, 113 nM). The displacement of the benzyloxy group from the para to the meta position decreases activity only marginally (IC_{50} values: **1**, 119 nM; **4**, 131 nM). On the other hand, exchange of the ether oxygen of compound **1** by an isosteric NH group reduces activity drastically. The resulting benzylamine compound **5** is similarly active as the benzoylamine **6** showing a reduction of activity by a factor of 100 compared to compound **1** (IC_{50} values: **1**, 119 nM; **6**, ca. 10 μM). From these results it has to be concluded that the phenoxy group in the para position of the benzoyl benzoic acid moiety should not be changed. In this class of compounds, this group is obviously appropriate for interaction with the lipophilic pocket of the human type 2 isozyme.

Table 2. Inhibition of Human and Rat Steroid 5α-Reductase Types 1 and 2 in Vitro by Compounds **7**−**11**

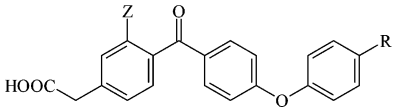


7-11

		% inhibn (10 μM) or [IC_{50}, μM]			
		human		RVP	
compd	X, Y	type 2[a,d]	type 1[b,e]	pH 5.5[c,d]	pH 6.6[c,d]
7	−CH_2−, C=O	[0.023]	ni	77	42
8	−$(CH_2)_2$−, C=O	[2.80]	ni	44	49
9	−CH=CH−, C=O	[0.47]	ni	63	61
10	−$(CH_2)_2$−, CH_2	12	ni	nd	nd
11	−$(CH_2)_3$−, C=O	10	nd	nd	nd

[a−e] See Table 1.

Table 3. Inhibition of Human and Rat Steroid 5α-Reductase Types 1 and 2 in Vitro by Compounds **12**−**14**



12-14

		% inhibn (10 μM) or [IC_{50}, μM]			
		human		RVP	
compd	Z, R	type 2[a,d]	type 1[b,e]	pH 5.5[c,d]	pH 6.6[c,d]
7	H; H	[0.023]	ni	77	42
12	H, Br	[0.005]	ni	55	59
13	F, H	[0.045]	ni	73	57
14	CH_3, H	[0.027]	ni	78	[1.45]

[a−e] See Table 1.

In the second step in the optimization process, variations in the carboxylic acid side chain by insertion of different spacers between the benzene ring and the carboxylic group were performed. These modifications were inspired by recent results of our group[27] and others[45] indicating that the protein shows conformational flexibility in the corresponding part of the active site. It becomes apparent from Table 2 that the insertion of a methylene spacer resulted in the phenylacetic acid compound **7** and an increase in inhibitory activity by a factor of 2 (IC_{50} values: compound **A**, 53 nM; compound **7**, 23 nM). Further elongation of the side chain with an ethylene (compound **8**) or propylene (compound **11**) spacer reduced inhibitory activity

dramatically (IC_{50} value: **8**, 2.8 μM) and led to an inactive compound (compound **11**, 10% inhibition at a concentration of 10 μM). Interestingly, enhancing the rigidity of the ethylene spacer in compound **8** by dehydrogenation, resulting in the cinnamic acid derivative **9**, increased activity by a factor of 6 (IC_{50} values: compound **8**, 2.8 μM; compound **9**, 0.47 μM). Reduction of the carbonyl group in the benzoyl moiety of compound **8** resulted in a loss of activity (compound **10**, 12% inhibition at a concentration of 10 μM). This indicates that a planar structure provided by the sp^2 carbon of the carbonyl linker between the two benzene nuclei in compound **8** is favorable as compared to the tetrahedral structure in compound **10**. Similar results have been obtained in the class of benzylidenepiperidine 5α-reductase inhibitors.[23]

Finally, the influence of aromatic substitution on rings A and C of compound **7** was investigated. Fluorine and methyl were selected, since both increase lipophilicity but differ in their electronic effects. They were introduced into the 3-position as substitution ortho to the carboxylic group is known to diminish inhibitory activity. As shown in Table 3, the fluoro substituent reduced inhibition by a factor of 2 (IC_{50} values: compound **13**, 45 nM; compound **7**, 23 nM). The methyl group did not increase the inhibitory activity either (compound **14**, 27 nM). Introduction of bromine into the 4-position of the C ring, however, markedly enhanced type 2 enzyme inhibitory activity. The resulting compound **12** exceeded compound **7** by a factor of 5 (IC_{50} values: compound **12**, 5 nM; compound **7**, 23 nM) and was equipotent to the steroidal inhibitor finasteride (IC_{50} value: 5 nM).

Regarding inhibition of the human type 1 enzyme, it is striking that the title compounds did not show inhibitory activity in this cellular assay (Tables 1−3). This cannot be explained by an insufficient permeation across the cell membrane of the DU145 cells due to their carboxylate group. As shown below, compound **12** shows a good permeation of artificial membranes (PAMPA assay), and other carboxylic acids have been shown to be able to inhibit the enzyme in the very same assay. Thus, it has to be concluded that the title compounds are selective type 2 inhibitors.

With respect to the inhibition of the rat isozymes, most of the compounds turned out to be dual inhibitors for both isoforms. However, the inhibitory activity was only weak. The most potent inhibitors toward rat type 2 enzyme were compounds **3**, **7**, **13**, **14** (71−78% inhibition at a concentration of 10 μM), whereas the most active inhibitor toward rat type 1 enzyme was compound **3** (73% inhibition). Due to this lack of activity for the rat enzymes, rats are not appropriate for the determination of the biological activity of the title compounds. However, they can be used for the determination of phamacokinetic properties.

Pharmacokinetic Evaluation in Vitro and in Vivo. Compound **12** showed a high permeability in PAMPA (flux rate = 91 ± 3% and recovery 81%). Thus, it is most likely that compound **12** is able to cross biological membranes, indicating a good oral absorption. Evaluation of compound **12** on Caco2 cells revealed a P_{app} value of 6.6 ± 0.7 cm/s, which is indicative of a medium permeator.[46] In this assay, ketoprofen was used as a "high permeator" (P_{app} = 28.9 ± 3.0 cm/s) and ranitidine as a "low permeator" reference (P_{app} = 0.07 ± 0.02 cm/s) according to the FDA guidance.[47]

To get an idea about the in vivo properties of compound **12**, the pharmacokinetics was determined after oral administration in the rat. These results should be well transferable to the human situation, since it has been demonstrated that there is a great similarity in the extent of gastrointestinal absorption between human and rat.[48]

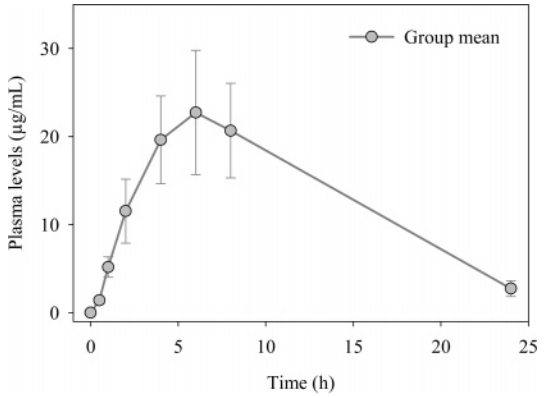


Figure 2. The mean profile (±SEM; n = 5) of absolute plasma levels (μg/mL) in rats versus time after a single oral dose (40 mg/kg) of compound **12**.

Table 4. Pharmacokinetic Parameters of Compound 12 in Male Rats after Oral Application (40 mg/kg)

parameters[a]	value	parameters	value
dose po (mg/kg)	40	$t_{1/2z}$ (h)	5.5
$C_{max\ obs}$ (μg/mL)	22.7	CL (L/kg/h)	0.12
C_z (μg/mL)	2.7	AUC_{0-t_z} (μg h/mL)	314.5
$t_{max\ obs}$ (h)	6.0	$AUC_{0-\infty}$ (μg h/mL)	336.1
t_z (h)	24		

[a] $C_{max\ obs}$, maximal measured concentration; C_z, last analytical quantifiable concentration; $t_{max\ obs}$, time to reach the maximum measured concentration; t_z, time of the last sample which has an analytical quantifiable concentration; $t_{1/2z}$, half-life of the terminal slope of a concentration−time curve; AUC_{0-t_z}, area under the concentration−time curve up to the time t_z of the last sample; $AUC_{0-\infty}$, area under the concentration−time curve extrapolated to infinity; Cl, body clearance.

After a single po administration of compound **12** to male rats (40 mg/kg), plasma samples were collected over 24 h and plasma concentrations were determined by HPLC−MS/MS. Individual profiles of five rats showed a continuous increase of plasma levels over time. In these rats, maximal plasma concentrations (C_{max}) were in the range between 21 and 48 μg/mL. The time of maximal plasma concentration (t_{max}) was measured 6−8 h postdosing in four out of five rats. In one rat, the C_{max} value was measured 4 h after application. Subsequently, plasma concentrations decreased again. Plasma levels were low but still measurable 24 h after application in all rats. The mean profile of absolute plasma levels of **12** is illustrated in Figure 2.

The pharmacokinetic parameters of **12** evaluated on absolute group mean values are presented in Table 4. After oral administration, **12** was slowly absorbed, as shown by the t_{max} of 6 h. In addition, the elimination was medium, as indicated by a half-life ($t_{1/2}$) of 5.5 h and by a clearance rate of 0.12 L/kg/h, which was considered as low, relative to an estimated hepatic plasma flow of 2.16 L/kg/h in the rat.[49]

Discussion and Conclusion

Aiming at the discovery of a potent, selective, and in vivo active 5α-reductase type 2 inhibitor, compound **A** was structurally modified. An important structural feature for the enhancement of inhibitory activity was the extension of the carboxylic acid side chain with a methylene spacer (compounds **7** and **12**−**13**). However, further elongation of the side chain reduced the activity markedly (compounds **8**−**11**).

For structural elucidation, we employed the 5α-reductase type 2 pharmacophore model, which was recently described by Chen

et al.[50] The model consists of five pharmacophoric features: two hydrogen-bond acceptors (HBA1−2) and three hydrophobic groups (HP1−3). For any two of the five features a distance contraint is given (see Supporting Information, Table S1). In this context, a compound fits well to the pharmacophore model if it has all features present and if the distance constraints are within the range claimed by the model. In this work, we applied our inhibitors **7**−**9** and **12** to the pharmacophore model of Chen et al. Therefore, we used the lowest energy conformations of compounds **7**−**9** and **12** to calculate the distances between any two of the five features of the pharmacophore model. These conformations were calculated by a simulated annealing molecular dynamics approach followed by an ab initio electronic structure geometry optimization. This approach allows us to get highly reliable minimum structures of our inhibitors. For more details, see the molecular modeling procedure in the Experimental Section below. The results are summarized in Table S1 located in the Supporting Information. This table shows that the calculated values of the highly active inhibitors **7** and **12** (IC_{50} values: 23 and 5 nM) fit very well in the model, whereas in case of the less active inhibitors **8** and **9** (IC_{50} values: 2800 and 470 nM) marked deviations were observed. This means that the structure−activity relationship of our compounds are very well described by the pharmacophore model of Chen et al.

In the context of the finding that the compounds of this study did not show a significant inhibition of the type 1 enzyme, it is worth mentioning that in a recent QSAR study we have obtained results suggesting a different hydrogen-bonding pattern in the binding pockets of both isoforms as well as a lower content of aromatic amino acids in the binding site of the type 2 isozyme.[51] Another important finding was the observation that a bromo substituent in the 4-position of the phenoxy group of compound **7** leads to a further increase in activity (compound **12**). This was a little surprising since in case of compound **1** disubstitution in the 2,5-positions with CF_3, which like Br is also lipophilic and electronegative, did not enhance activity (compound **2**). Being as active as finasteride, compound **12** is one of the most potent human type 2 inhibitors known so far.

To evaluate its potential for gastrointestinal absorption, compound **12** was passed through three assays.[31] The assays were developed to identify those compounds out of a series that are able to permeate artificial and biological membranes and to enter blood circulation in animals. Compound **12** was predicted to be a high permeator in the PAMPA and a medium permeator in Caco2 cells. These results were verified by the pharmacokinetic evaluation in the rat. After peroral administration of compound **12** at the dose of 40 mg/kg, plasma levels of the compound were measurable for at least 24 h. This showed that compound **12**, given orally, was able to cross the gastrointestinal tract and reached the general circulation, as predicted in the preceding in vitro tests. The pharmacokinetics of compound **12** was characterized by both a slow absorption and a slow elimination. The slow elimination rate of **12**, resulting in a long plasma half-life, might be explained by a strong binding to plasma proteins and/or by a high metabolic stability, which are presently being further investigated. It is worth mentioning that no apparent signs of discomfort could be observed in the rats after injection of **12** during the duration of the experiment.

As the compound is less potent in vitro on the rat enzymes, it is not feasible to investigate its in vivo potency in our in vivo rat model.[27] However, since a great similarity between the gastrointestinal absorption between human and rat has been demonstrated, it can be expected that **12** shows favorable pharmacokinetic properties in humans. The results of compound **12** in vitro and in vivo demonstrate that it is a very promising new 5α-reductase inhibitor. Further preclinical and clinical studies will elucidate whether this compound is an alternative to existing steroidal and nonsteroidal inhibitors. Presently, structure modifications are being performed to examine whether our hybrid inhibition concept[30] can also be introduced into this class of compounds.

Experimental Section

Chemicals and solvents obtained from commercial suppliers (Aldrich, Acros, Fluka, Lancaster) were used without further purification. Solvents for reactions under anhydrous conditions were dried according to standard procedures. All reactions, except those involving water as a reagent, were performed under nitrogen atmosphere. All reactions were monitored by thin-layer chromatography (TLC) using Alugram silica G/UV254 (purchased from Macherey-Nagel). Column chromatography (CC) was performed on Merck Kieselgel 60 [40−63 μm (flash column chromatography, FCC) or 50−200 μm, respectively]. Melting points were determined on a Reichert Thermometer hot stage microscope and are uncorrected. ^{1}H NMR spectra were measured on a Bruker AM 400 at 400 MHz or DRX 500 (500 MHz) instrument using the indicated solvent. Chemical shifts are reported in δ (ppm) downfield from TMS (δ: 0 ppm). IR spectra were performed with KBr disks or films, as indicated, on a Perkin-Elmer 398 infrared spectrometer. Wavenumbers are given in cm^{-1}. The purity of the compounds was verified using HPLC (Agilent 1100, Agilent, Waldbronn, Germany) under the following conditions: UV-DAD; Nucleodur 100−3 C18ec colum, 3 μm, 125 × 3 mm; solvent A, H_2O with 0.1% TFA; solvent B, acetonitrile; gradient, 0.0−3.0 min, 90% A and 10% B; 3.0−10.0 min from 90% A to 0% A; 10−13 min from 0% A to 100% A; flow rate, 0.7 mL/min, temperature, 40 °C; pressure, 18.0 MPa ± 0.1. Elemental analyses (C, H, N) were within ±0.4% of the theoretical values and were performed by the Institute of Inorganic Chemistry, Saarland University, Saarbrücken, Germany. ^{1}H NMR and IR spectra as well as elemental analysis (Table S2) are available in the Supporting Information.

4-(4-Methoxybenzoyl)benzoic Acid Methyl Ester (1c). Compound **1c** was synthesized as previously described.[32] as colorless crystals, mp 161−162 °C (lit.[32] mp 158 °C).

4-(3-Methoxybenzoyl)benzoic Acid Methyl Ester (4c). A mixture of terephthalic acid monomethyl ester (0.9 g, 5.0 mmol), palladium(II)-1,1,1,5,5,5-hexafluoroacetylacetonate (78 mg, 0.15 mmol), tricyclohexylphosphine (0.126 g, 0.45 mmol), Na_2CO_3 (1.04 g, 10 mmol), and di-*N*-succinimidyl carbonate (1.67 g, 6.5 mmol) in anhydrous THF (15 mL) was purged with N_2, degassed, and stirred at 60 °C for a few minutes until the formation of CO_2 ceased. The solution was cooled to room temperature and a solution of 3-methoxybenzeneboronic acid (0.91 g, 6.0 mmol) in anhydrous THF (15 mL) was added. The reaction mixture was stirred at 60 °C overnight, poured into water (300 mL), and extracted with ethyl acetate (100 mL × 3). The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by FCC using hexane/ethyl acetate (17:3) as eluent to give light brown crystals in 37% yield, mp 94−95 °C.

4-[4-(Hydroxybenzoyl)]benzoic Acid Methyl Ester (1b). A mixture of **1c** (1.0 g, 3.70 mmol) and $AlCl_3$ (1.97 g, 14.8 mmol) in benzene (25 mL) was stirred at room temperature for 15 min and then heated under reflux for further 15 min. The mixture was cooled to room temperature, poured on ice, and extracted with ethyl acetate. The organic layer was washed with aqueous $NaHCO_3$ (5%, 20 mL), water, and brine. After drying over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was purified by FCC eluting with hexane/ethyl acetate (4:6) to give colorless crystals in 81% yield, mp 184−185 °C.

Compound **4b** was prepared following the same procedure.

4-[3-(Hydroxybenzoyl)]benzoic Acid Methyl Ester (4b). Compound **4b** was synthesized starting from the ester **4c**. The mixture was heated under reflux for 45 min. The residue was purified using

FCC eluting with hexane/ethyl acetate (7:3) to give light yellow crystals in 71% yield, mp 157−158 °C.

4-[4-(Benzyloxy)benzoyl]benzoic Acid Methyl Ester (1a). A solution of **1b** (0.512 g, 2 mmol) and NaH (60% in mineral oil, 88 mg, 2.2 mmol) in anhydrous DMF (15 mL) was stirred at room temperature for 15 min. Benzyl bromide (0.376 g, 2.2 mmol) was added and stirring was continued for 24 h at room temperature. The mixture was poured on ice and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water and brine. After drying over $MgSO_4$, the solvent was evaporated under reduced pressure. The residue was purified using FCC, eluting with hexane/ethyl acetate (17:3) to give colorless crystals in 62% yield, mp 169−170 °C.

Compounds **2a** and **4a** were prepared following the same procedure as for **1a**.

4-(4-{[2,5-Bis(trifluoromethyl)benzyl]oxy}benzoyl)benzoic Acid Methyl Ester (2a). Compound **2a** was synthesized starting from **1b** and 2-(bromomethyl)-1,4-bis(trifluoromethyl)benzene. The mixture was stirred at room temperature for 3 h. Purification was achieved by FCC using hexane/ethyl acetate (19:1) as eluent to give colorless crystals in 79% yield, mp 120−121 °C.

4-[3-(Benzyloxy)benzoyl]benzoic Acid Methyl Ester (4a). Compound **4a** was synthesized starting from **4b** and benzyl bromide. The mixture was stirred at room temperature for 3 h. The residue was purified by FCC using hexane/ethyl acetate (9:1) as eluent. Further purification was achieved by recrystallization from hexane to give colorless crystals in 39% yield, mp 109−110 °C.

4-[4-(Benzhydryloxy)benzoyl]benzoic Acid Methyl Ester (3a). A solution of diphenylmethyl bromide (0.31 g, 1.25 mmol) in anhydrous DMF (5 mL) was added to a mixture of **1b** (0.32 g, 1.18 mmol) and K_2CO_3 (0.189 g, 1.31 mmol) in DMF (5 mL). The mixture was heated at 90 °C for 24 h, cooled to room temperature, poured on ice, and extracted with ethyl acetate (50 mL × 4). The combined organic layers were washed with water and brine and were dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the residue was purified by FCC using hexane/ethyl acetate (8:2) as eluent to give a white powder in 73% yield, mp 150−151 °C.

4-(4-Nitrobenzoyl)benzoic Acid (5d). To an ice-cooled mixture of (4-methylphenyl)(4-nitrophenyl)methanone[49] (**5e**, 2.2 g, 9.12 mmol) in glacial acetic acid (17 mL) was added sulfuric acid (1.4 mL) slowly to keep the temperature below 5 °C. Chromium(VI) oxide (2.74 g, 27.4 mmol) was added in small portions at such a rate that the temperature did not exceed 10 °C. The reaction slurry was stirred at room temperature for 3 h, poured on ice, and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water to neutral pH and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, a crude product was obtained which was purified by recrystallization from ethyl acetate to give a yellowish white powder in 75% yield, mp 254 °C (lit.[50] mp 257−258 °C).

4-(4-Nitrobenzoyl)benzoic Acid Methyl Ester (5c). A solution of **5d** (6.0 g, 22.1 mmol) in anhydrous methanol (50 mL) and sulfuric acid (5 mL) was heated under reflux for 4 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. It was poured into a cold aqueous solution of sodium bicarbonate (5%) and extracted with ethyl acetate (200 mL × 3). The combined organic layers were washed with small amounts of water and brine and were dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, **5c** was obtained as light yellow crystals in 78% yield, mp 174−175 °C. It was pure enough to be used in the next synthetic step without further purification.

4-(4-Aminobenzoyl)benzoic Acid Methyl Ester (5b). A mixture of **5c** (5.0 g, 17.5 mmol) and $SnCl_2 \cdot 2H_2O$ (19.7 g, 87.5 mmol) in ethanol (60 mL) was heated at 70 °C for 1 h. After cooling to room temperature, the mixture was poured on ice and the pH was adjusted to slightly alkaline (pH 7−8) using $NaHCO_3$ solution (5%). The aqueous phase was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with brine and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded **5b** as a yellow powder in 93% yield, mp 135 °C. It could be used in the next synthetic step without further purification.

4-[4-(Benzylamino)benzoyl]benzoic Acid Methyl Ester (5a). A solution of **5b** (0.1 g, 0.39 mmol) and benzyl bromide (0.14 mL, 1.17 mmol) in DMF (10 mL) was stirred at room temperature for 20 h. The reaction was quenched by the addition of water and the product was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by FCC using hexane/ethyl acetate (3:1) as eluent to give a yellow powder in 30% yield, mp 113−114 °C.

4-[4-(Benzoylamino)benzoyl]benzoic Acid Methyl Ester (6a). To an ice-cooled solution of **5b** (0.50 g, 1.96 mmol) and triethylamine (0.26 mL, 1.96 mmol) in anhydrous CH_2Cl_2 was added benzoyl chloride (0.23 mL, 1.96 mmol). The mixture was stirred for 2 h at room temperature, poured on ice, and extracted with ethyl acetate (75 mL × 3). The combined organic layers were washed with brine and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the compound was purified by recrystallization from hexane/ethyl acetate to give colorless crystals in 49% yield, mp 199 °C.

(4-Methylphenyl)(4-phenoxyphenyl)methanone (7e). Compound **7e** was prepared as previously described[52] as colorless crystals, mp 80−81 °C (lit.[52] mp 79.8−80 °C).

[4-(Bromomethyl)phenyl](4-phenoxyphenyl)methanone (7d). A solution of **7e** (2.0 g, 7.0 mmol), NBS (1.25 g, 7.0 mmol), and a catalytic amount of benzoyl peroxide in anhydrous CCl_4 (30 mL) was stirred at room temperature for 10 min and then heated under reflux for 4 h. Succinimide was filtered off and washed with CCl_4. The combined organic phases were washed with a small amount of water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the compound was purified by FCC eluting with hexane/ethyl acetate (19:1) to give colorless crystals in 44% yield, mp 85−86 °C.

[4-(4-Phenoxybenzoyl)phenyl]acetonitrile (7c). A solution of NaCN (0.049 g, 1.0 mmol) in H_2O (5 mL) was added to **7d** (0.180 g, 0.5 mmol), dissolved in 1,4-dioxane (5 mL). The resulting mixture was heated under reflux for 1 h. After cooling to room temperature the solution was acidified cautiously with diluted hydrochloric acid and extracted with ethyl acetate (50 mL × 4). The combined organic layers were washed with aqueous $NaHCO_3$ solution (5%) and brine. After drying over $MgSO_4$ and removing the solvent under reduced pressure, the compound was purified by FCC eluting with hexane/ethyl acetate (7:3) to give colorless crystals in 47% yield, mp 119−120 °C.

[4-(4-Phenoxybenzoyl)phenyl]acetic Acid (7). A mixture of **7c** (0.27 g, 0.862 mmol), aqueous sodium hydroxide solution (40%, 6 mL), and ethanol (5 mL) was heated under reflux for 1 h. After cooling to room temperature, the mixture was acidified with 1 N hydrochloric acid and extracted with ethyl acetate (75 mL × 3). The combined organic layers were washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent, the compound was purified by recrystallization from hexane/ethyl acetate to give a white powder in 63% yield, mp 137−138 °C, t_R = 11.3 min.

4-[4-(4-Phenoxybenzoyl)phenyl]butanoic Acid Methyl Ester (11a). To an ice-cooled solution of 4-phenylbutanoic acid methyl ester (**11b**, 0.18 g, 1.0 mmol) and oxalyl chloride (0.17 mL, 2.0 mmol) was added $AlCl_3$ (0.4 g, 3.0 mmol) in portions to keep the temperature below 5 °C. The mixture was stirred for 15 min at 5−10 °C and then poured on ice. The aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic phases were washed with brine and dried over $MgSO_4$. After concentrating the organic layer under reduced pressure, the residue was dissolved in dichloromethane (30 mL) and added dropwise to a mixture of diphenyl ether (0.17 g, 1.0 mmol) and $AlCl_3$ (0.6 g, 4.5 mmol) in dichloromethane (30 mL). The mixture was heated under reflux for 1 h. After cooling, the reaction slurry was poured on ice and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with water and brine, and dried over $MgSO_4$. After evaporation of the organic solvent, a crude product was

obtained which was purified by FCC using hexane/ethyl acetate (8:2) as eluent to give white waxy crystals in 64% yield, mp 32−33 °C.

Compounds **7a**, **8a**, and **12a** were synthesized following the procedure for compound **11a**.

[4-(4-Phenoxybenzoyl)phenyl]acetic Acid Methyl Ester (7a). Compound **7a** was synthesized starting from phenylacetic acid methyl ester **7b**. The reaction mixture was stirred at room temperature for 1 h. The product was purified by FCC using hexane/ethyl acetate (8:2) to give a colorless oil in 18% yield.

3-[4-(4-Phenoxybenzoyl)phenyl]propanoic Acid Methyl Ester (8a). Compound **8a** was synthesized starting from 3-phenylpropanoic acid methyl ester **8b**. The mixture was stirred at room temperature for 1 h. Purification of the product was achieved by FCC using hexane/ethyl acetate (17:3) to give colorless crystals in 18% yield, mp 96 °C.

{**4-[4-(4-Bromophenoxy)benzoyl]phenyl**}**acetic Acid Methyl Ester (12a).** Compound **12a** was synthesized starting from **7b** and 1-bromo-4-phenoxybenzene. The crude product was purified by FCC using hexane/ethyl acetate (17:3) as eluent to give colorless crystals in 15% yield, mp 67−68 °C.

(4-Iodophenyl)(4-phenoxyphenyl)methanone (9b). A solution of 4-iodobenzoyl chloride (2.66 g, 10 mmol) in anhydrous dichloromethane (50 mL) was added dropwise over 30 min to a stirred mixture of diphenyl ether (1.70 g, 10 mmol) and $AlCl_3$ (2.67 g, 20 mmol) in anhydrous dichloromethane (50 mL). The mixture was stirred under reflux for a further 2 h. After cooling, the reaction slurry was poured on ice and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with aqueous $NaHCO_3$ solution (5%), water, and brine and were dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by recrystallization from ethyl acetate to give colorless crystals in 68% yield, mp 161−162 °C.

(2*E*)-3-[4-(4-Phenoxybenzoyl)phenyl]acrylic Acid Methyl Ester (9a). To a degassed solution of **9b** (0.527 g, 1.0 mmol) in DMF (4 mL) were successively added palladium(II) acetate (11.0 mg, 0.05 mmol), methyl acrylate (0.17 g, 2.0 mmol), tetrabutylammonium chloride (0.28 g, 1.0 mmol), and K_2CO_3 (0.346 g, 2.5 mmol). The reaction mixture was stirred under nitrogen at 50 °C for 3 h. After cooling, the reaction mixture was concentrated under reduced pressure and diluted with diethyl ether (20 mL). The mixture was washed with water (10 mL), aqueous $NaHCO_3$ solution (5%), and brine. After drying the organic layer over $MgSO_4$, the solvent was removed under reduced pressure. Purification was achieved by FCC using hexane/ethyl acetate (8:2) as eluent to give colorless crystals in 84% yield, mp 184−185 °C.

3-[4-(4-Phenoxybenzyl)phenyl]propanoic Acid Methyl Ester (10a). Compound **9a** (0.519 g, 1.5 mmol), dissolved in methanol (30 mL), was subjected to hydrogenation under atmospheric pressure for 18 h using Pd/C 10% as a catalyst. After filtration, the solvent was evaporated under reduced pressure. The crude product was purified by FCC using hexane/chloroform (9:1) as eluent to give a colorless oil in 68% yield.

4-Bromo-2-fluorobenzoic Acid (13c). To an ice-cooled mixture of 4-bromo-2-fluorobenzaldehyde (2.0 g, 0.01 mol) in acetonitrile (30 mL) were slowly added, one by one, aqueous NaH_2PO_4 (0.24 g in 10 mL of water, 0.002 mol), H_2O_2 (1.36 mL, 0.014 mol), and a solution of $NaClO_2$ (1.3 g, 0.014 mol) in H_2O (20 mL). The mixture was stirred overnight at room temperature. At the end of the reaction, a voluminous precipitate was formed. A small amount of Na_2SO_3 was added to destroy unreacted HOCl and H_2O_2. After acidification of the mixture with aqueous HCl (10%), the product was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with water and dried over $MgSO_4$. After evaporation of the solvent, the product, a white powder (82% yield, mp 211 °C), was used without further purification.

(4-Bromo-2-fluorophenyl)(4-phenoxyphenyl)methanone (13b). To a suspension of **13c** (1.80 g, 8.19 mmol) in anhydrous dichloromethane (25 mL) containing a few drops of DMF was added oxalyl chloride (0.79 mL, 9.0 mmol) carefully via syringe. After the effervescence has ceased, the reaction mixture was stirred for 1 h at room temperature. The solvent and the unreacted oxalyl chloride were removed under reduced pressure. The residual acid chloride was dissolved in anhydrous dichloromethane (20 mL) and added dropwise to a mixture of diphenyl ether (1.39 g, 8.19 mmol) and $AlCl_3$ (2.73 g, 20.4 mmol) in anhydrous dichloromethane (50 mL) at room temperature. The mixture was refluxed for 1 h, cooled to room temperature, poured on ice, and extracted with diethyl ether (3 × 200 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (5%, 30 mL), water, and brine. After drying over $MgSO_4$, the solvent was evaporated under reduced pressure. Purification by FCC using hexane/dichloromethane (7:3) as eluent gave colorless crystals in 60% yield, mp 74−75 °C.

Compound **14b** was synthesized following the procedure of compound **13b**.

(4-Bromo-2-methylphenyl)(4-phenoxyphenyl)methanone (14b). The starting material was 4-bromo-2-methylbenzoic acid **14c**. The reaction mixture was stirred at room temperature for 1 h. The crude product was purified by FCC using hexane/chloroform (9:1) to give a colorless oil in 70% yield.

[2-Fluoro-4-(trimethylsilanylethynyl)phenyl](4-phenoxyphenyl)methanone (13a). Under nitrogen, ethynyltrimethylsilane (0.1 g, 1.0 mmol) was added to a mixture of **13b** (0.371 g, 1.0 mmol), bis-(triphenylphosphine)palladium(II) chloride (14.0 mg, 0.02 mmol) and copper(I) iodide (8.0 mg, 0.04 mmol) in anhydrous triethylamine/THF (4:1). The reaction mixture was heated to 80 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in chloroform (60 mL). The solution was washed with water (2 × 3 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure. Flash column chromatography using hexane/ethyl acetate (9:1) and subsequent crystallization from hexane yielded colorless crystals in 84% yield, mp 98−99 °C.

Compound **14a** was synthesized following the procedure for compound **13a**.

[2-Methyl-4-(trimethylsilanylethynyl)phenyl](4-phenoxyphenyl)methanone (14a). 14b was used as starting material. The reaction mixture was heated at 80 °C for 3 h. The crude slurry was purified by FCC using hexane/dichloromethane (8:2) as eluent to give a colorless oil in 88% yield.

[3-Fluoro-4-(4-phenoxybenzoyl)phenyl]acetic Acid (13). To a solution of **13a** (0.32 g, 0.81 mmol) in anhydrous THF was added BH_3 (1 M in THF, 0.89 mL, 0.89 mmol) at 0 °C. After stirring for 3 h at room temperature, methanol (0.41 mL) was added carefully and the solution was oxidized at 40 °C with H_2O_2 (35%, 0.41 mL) in aqueous NaOH solution (3 N, 0.41 mL). The mixture was stirred at room temperature for 1 h, acidified with diluted hydrochloric acid, and extracted with ethyl acetate (75 mL × 3). The combined organic layers were washed with water and brine. After drying over $MgSO_4$, the solvent was evaporated under reduced pressure. Column chromatography of the residue using hexane/ethyl acetate (9:1) afforded the analytically pure product as pale yellow crystals in 39% yield; mp 109−110 °C, t_R = 11.3 min.

Compound **14** was synthesized following the procedure of compound **13**.

[3-Methyl-4-(4-phenoxybenzoyl)phenyl]acetic Acid (14). 14a was used as starting material. The product was purified by CC using hexane/ethyl acetate (8:2) as eluent to give cream colored crystals in 29% yield; mp 113−114 °C, t_R = 11.7 min.

4-[4-(Benzhydryloxy)benzoyl]benzoic Acid (3). A mixture of **3a** (0.297 g, 0.70 mmol) and K_2CO_3 (0.290 g, 2.10 mmol) in methanol/H_2O (9:1, 15 mL) was heated under reflux for 4 h. After cooling to room temperature, the mixture was acidified with diluted hydrochloric acid and extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with water and brine. After drying over $MgSO_4$, the solvent was evaporated under reduced pressure. The residue was recrystallized from ethyl acetate to afford the title compound as a white powder in 87% yield; mp 204−205 °C, t_R = 12.3 min.

Compounds **1**, **2**, **4**−**8**, **11**, and **12** were synthesized by following the procedure for compound **3**.

4-[4-Benzyloxy)benzoyl]benzoic Acid (1). The starting material was **1a**. The procedure gave colorless crystals in 57 % yield; mp 251−252 °C, t_R = 11.6 min.

4-(4-{[2,5-Bis(trifluoromethyl)benzyl]oxy}benzoyl)benzoic Acid (2). The starting material was **2a**. The mixture was heated under reflux for 3 h. Purification was achieved by recrystallization from ethyl acetate to give colorless crystals in 31% yield; mp 183−184 °C, t_R = 12.7 min.

4-[3-(Benzyloxy)benzoyl]benzoic Acid (4). The starting material was **4a**. The mixture was refluxed for 2 h. The residue was purified by recrystallization from methanol to give a white powder in 57% yield; mp 285−286 °C, t_R = 11.6 min.

4-[4-(Benzylamino)benzoyl]benzoic Acid (5). The starting material was **5a**. The mixture was heated under reflux for 2 h. The residue was purified by recrystallization from hexane/ethyl acetate to give yellow crystals in 54% yield; mp 173−174 °C, t_R = 10.7 min.

4-[4-(Benzoylamino)benzoyl]benzoic Acid (6). The starting material was **6a**. The mixture was heated under reflux for 1 h. The residue was purified by recrystallization from ethyl acetate to give a white powder in 48% yield, mp 278−279 °C.

4-(4-Phenoxybenzoyl)phenylacetic Acid (7). The starting material was **7a**. The mixture was heated under reflux for 2 h to give a white powder in 76% yield; mp 137−138 °C, t_R = 11.3 min.

3-[4-(4-Phenoxybenzoyl)phenyl]propanoic Acid (8). The starting material was **8a**. The mixture was heated under reflux for 2 h. The product was purified by recrystallization from hexane/ethyl acetate to give colorless crystals in 83% yield; mp 161 °C, t_R = 11.7 min.

4-(4-(4-Phenoxybenzoyl))phenylbutyric Acid (11). The starting material was **11a**. The mixture was heated under reflux for 2 h. Purification of the product was achieved by recrystallization from hexane/ethyl acetate to give colorless crystals in 76% yield; mp 114−115 °C, t_R = 12.0 min.

{**4-[4-(4-Bromophenoxy)benzoyl]phenyl**}**acetic Acid (12).** The starting material was **12a**. The mixture was heated under reflux for 1 h to give colorless crystals in 31% yield; mp 168 °C, t_R = 12.1 min.

3-[4-(4-Phenoxybenzyl)phenyl]propanoic Acid (10). A mixture of **10a** (0.21 g, 0.6 mmol) in methanol/aqueous KOH (2 M) (1:1, 10 mL) was heated under reflux for 1 h. The crude product was purified by recrystallization from hexane to give colorless crystals in 89% yield; mp 105−106 °C, t_R = 12.3 min.

Compound **9** was synthesized following the procedure of compound **10**.

(2*E*)-3-[4-(4-Phenoxybenzoyl)phenyl]acrylic Acid (9). The starting material was **9a**. The mixture was refluxed for 2 h. The crude product was purified by recrystallization from ethyl acetate to give colorless crystals in 89% yield; mp 235 °C, t_R = 11.8 min.

Enzyme Inhibition Test. Preparation of Tissue. Rat prostatic enzyme was prepared according to the method of Liang et al.[41] with slight modifications.[20] Male rats were sacrificed and prostates were taken within 5 min and put in ice-cold 0.9% aqueous NaCl solution. All the following steps were performed at 0−4 °C. The prostates were dissected free from fat and connective tissue, cut into pieces, and weighed. Per 1 g of tissue, 3 mL of 20 mM phosphate buffer, pH 6.5, containing 0.32 mM sucrose and 1 mM dithiothreitol (DTT) was added. The tissue was homogenized by 10-s strokes at 20 500 rpm of an ultraturax (IKA) in 60-s intervals, filtered through cheesecloth, and centrifuged for 60 min at 105 000*g*. The pellet obtained was resuspended in phosphate buffer. The centrifugation was repeated, and the final pellet was resuspended in a minimum volume of phosphate buffer and stored in 300-μL portions at −70 °C. The 105 000*g* pellet contains nuclei, mitochondria, and microsomes and is referred to as the enzyme preparation. The protein content was determined[55] and was in the range of 15−25 mg/mL. Human prostatic tissue from BPH patients was processed in the same way using citrate buffer, pH 5.5.

Incubation Procedure. The assay was performed as described[41] with modifications.[20] All values were run in duplicate. The incubation was carried out for 30 min at 37 °C in a total volume of 250 μL. In the case of rat enzyme preparation, phosphate buffer (40 mM, pH 6.6 for type 1) or citrate buffer (40 mM, pH 5.5 for type 2) was used. In the case of human enzyme preparation, citrate buffer (40 mM, pH 5.5) was employed. The incubation mixture contained approximately 250 μg of rat protein (125 μg human protein), 200 μM NADPH (human enzyme, 100 μM NADPH), 0.21 μM T including 100 nCi [1β,2β-^{3}H]T, and 2% DMSO with or without test compound (10 μM). In cases exceeding 60% inhibition, three concentrations were chosen for the determination of IC_{50} values. The reaction was started by adding the prostatic enzyme preparation and terminated by addition of 50 μL aqueous solution of NaOH (10 M). The steroids were extracted with diethyl ether (500 μL) by shaking for 10 min. Subsequent centrifugation was performed for 10 min at 4000 rpm. The water layer was frozen and the ether layer was decanted in fresh tubes and evaporated to dryness.

Human Type 1 Inhibition: DU 145-Assay.[43,44] Intact human prostatic carcinoma DU145 cells were used as the source of type 1 5α-reductase.[42] The inhibitory potencies of the compounds were determined by monitoring the conversion of the tritiated substrate androstenedione (5 nM) to androstanedione during an incubation period of 6 h. A day before the experiment, DU145 cells were seeded in a 24-well plate at a density of 180 000 cells/well and allowed to become adherent overnight in 1 mL of RPMI-1640 medium (with 10% FCS). Appropriate concentrations (10 μM final concentration at initial tests) of inhibitors dissolved in dimethyl sulfoxide (DMSO) were applied in duplicates. Growth medium was replaced by 500 μL of fresh medium containing 5 μL of the inhibitor and 5 nM [^{3}H]androstenedione as substrate. Inhibitors were first screened at concentrations of 10 μM in an initial test, and in cases exceeding 80% inhibition, three concentrations were chosen for measurement of IC_{50} values. As control of conversion (typically about 35% under these conditions) a triplicate of wells without inhibitors was used and finasteride (80, 60, 40, 20 nM) was a positive control for inhibition. After a 6-h incubation period in 5% CO_2 at 37 °C, the medium samples were extracted twice with 1 mL of diethyl ether and the steroids were separated by HPLC. Results are expressed as the amount of formed androstanedione as a percentage of control values.

HPLC Procedure. Steroid separation was performed[20] similarly to the method of Cook et al.[56] The steroids were dissolved in 50 μL of methanol, and 25 μL was injected into the computer-controlled HPLC system, which was checked before using labeled reference controls. Radioactivity was measured by employing a Berthold LB 506C monitor, using methanol/water (55/45, w/w) for T and DHT with a flow of 0.4 mL/min and an additive flow of 1.0 mL for scintillator. Baseline separation of T and DHT was achieved within 20 min. For the steroids androstenedione and androstanedione, methanol/water (50/50, w/w) was used and the retention times were 11.2 and 17.5 min, respectively.

Calculation Procedure. The amount of DHT formed was calculated (% DHT). The zero value was subtracted from the control (cv) and inhibition (iv) values (cv_{corr} and iv_{corr}). Inhibition (I) was calculated using the following equation: $\% I = (1 - iv_{corr}/cv_{corr})\text{-}100$.

Parallel Artificial Membrane Permeation Assay (PAMPA). The procedure according to Zhu et al. [57] was used. The membrane was built up by pipetting a solution of lipids in an organic solvent on a supporting filter material in 96-well plates. For all compounds, stock solutions at concentrations of 5 mM were prepared (test compounds in DMSO, reference compounds in ethanol). The stock solutions were subsequently diluted in Tris-buffer (0.05 M, pH 7.4) to a final concentration of 250 μM. Permeation rates of all test compounds were measured in triplicates. Diffusion time across the artificial membrane was 16 h. Reference values without lipid layer were individually determined for all compounds. The concentrations in the acceptor compartments were measured by UV difference spectroscopy using a microtiter plate reader (Spectramax Plus^{384}, Molecular Devices). For the compounds the λ_{max} values were determined in a previous run. The permeation rates are expressed

as flux rates, which were calculated according to

$$\text{flux (\%)} = \text{OD(test well)/OD(control well)} \times 100$$

For measuring recovery, the OD of the starting solution was determined and the recovery calculated according to

$$\text{recovery (\%)} = 2 \times \text{OD(control well)} \times \text{OD (starting solution)} \times 100$$

Three internal standards were used, one with low and two with high flux rates (chlorothiazide, metoprolol, and coumarin, respectively[57]).

Caco2 Assay. Cell Culture. Caco2 cells were obtained from DSMZ, Braunschweig, Germany. Cells were maintained in 25 cm^2 flasks in Dulbecco's modified Eagle medium (ccpro, Neustadt, Germany) containing high glucose (4.5 g/L), 10% FCS, 0.1 mM nonessential amino acids, 2 mM l-glutamin, 0.1 mg/mL streptomycin, and 100 U/mL penicillin at 37°C, 5% CO_2, and 95% relative humidity. Cells utilized for the transport studies were always between passage 4 and 15 after receipt from the culture collection. For transport studies, Caco2 cells were grown on Costar Transwell Plates (polycarbonate membrane, 0.4 μm pore size) for 21 days. Growth and differentiation were monitored by the measurement of the transepithelial electrical resistance (TEER) during culture. The permeation assay was performed after the TEER of a monolayer had reached a value around 400 Ω/cm^2.

Transport Assay. The transport assay was performed as described.[46] The appropriate drug solutions (50 μM) were added to the apical side at time zero, and samples from the apical side were taken at 0 and 60 min and from the basolateral side at 15, 30, and 60 min. The removed sample volume was replaced each time by fresh basolateral buffer. The concentrations of the compounds in the samples were determined using LC−MS/MS. The integrity of the monolayer was checked at the end of the experiment by assessing the transport of the paracellular marker lucifer yellow at a concentration of 100 μM. Each experiment was performed in triplicate.

The apparent permeability coefficients (P_{app}) for each well were calculated using the following equation

$$P_{app}\ (\text{cm/s}) = (\Delta c V_R/\Delta t A) c_o$$

where $\Delta c/\Delta t$ is the cumulative amount transported as a function of time (seconds), V_R is the volume of the receiver chamber (mL), A is the surface area of the cell monolayer (cm^2), and c_o is the concentration of compound at $t = 0$.

In Vivo Pharmacokinetics. Male Wistar rats weighing 300−330 g (Harlan Winkelmann) were housed in a temperature-controlled room (20−22 °C) and maintained in a 12h light/12h dark cycle. Food and water were available ad libitum. They were anaesthetized with a ketamine (135 mg/kg)/xylazine (10 mg/kg) mixture and cannulated with silicone tubing via the right jugular vein and attached to the skull with dental cement.[58] Prior to the first blood sampling, animals were connected to a counterbalanced system and tubing to perform blood sampling in the freely moving rat.

The oral administration of **12** at the dose of 40 mg/kg body weight was performed in awake rats ($n = 5$) by using a feeding needle. The compound was dissolved in 0.5% CMC and given at a volume of 5 mL/kg. Blood samples (0.3 mL) were taken at 0, 0.5, 1, 2, 4, 6, 8, and 24 h postdose and collected in heparinized tubes. They were centrifuged at 3000*g* for 10 min, and plasma was harvested and kept at −20 °C until analyzed.

The mean of absolute plasma concentrations (±SEM) was calculated for the five rats and the regression was performed on group mean values. The pharmacokinetic analysis was performed using a noncompartment model (PK Solutions 2.0, Summit Research Services).

HPLC−MS/MS analysis and quantification of the samples was carried out on a Surveyor-HPLC-system coupled with a TSQ Quantum (ThermoFinnigan) triple quadrupole mass spectrometer equipped with an electrospray interface (ESI).

The HPLC for the Caco2 samples was run isocratically on a EC50/3 Nucleodur 110−3 C18 ec-column (Macherey Nagel) with a flow rate of 300 μL/min. The HPLC for the serum samples was run on a CC 30/2 Nucleosil 100-3 C18 HD column (Macherey Nagel) in gradient mode with a flow rate of 300 μL/min. The mobile phase for the Caco2 samples consisted of a mixture of methanol/water (75/25) containing 0.1% formic acid and 10 mM ammonium acetate. For the pharmacokinetic study, the mobile phase consisted of 10 mM ammonium acetate in water and methanol containing 10 mM ammonium acetate. The proportion of methanol was programmed to linearly increase from 20 to 80% over 7 min and subsequently to decrease within 0.5 min to 20% again. For re-equilibration of the column, the proportion was subsequently held at this concentration for 1.5 min. MS detection was run in positive electrospray mode. The tandem mass spectrometer was used in multiple reaction mode (MRM) to detect parent and product ions of compound **12** and its internal standard.

Sample Preparation. Primary stock solutions in methanol containing up to 20% of DMSO were diluted in buffer or rat plasma to prepare calibration standards in the range from 0.15 to 75 μM for Caco2 samples or from 0.05 to 500 μM for serum samples. All samples were extracted for LC−MS/MS analysis to avoid ion suppression effects by matrix components during MS detection. Consequently, samples were acidified using hydrochloric acid and extracted with diethyl ether.

Validation. For validation of the liquid/liquid extraction method, recoveries in triplicates at three different concentrations (high, medium, low) in the range of the calibration curve were determined. The precision of the measurements was high, which was expressed by correlation coefficients higher than 0.96. The lowest calibration standards were chosen as the limit of quantification.

Molecular Modeling Procedure. Geometry Optimization. The geometry optimization (minimization) of compounds has been carried out using the Molecular Mechanics ff99 force field implemented in the AMBER 7 suite of programs.[59] The simulation systems were minimized by 5000 steps of steepest descent followed by 10 000 steps of conjugate gradient.

Simulated Annealing Molecular Dynamic Simulation. After the minimization described above, the following simulated annealing steps were repeated 10 times: First, the simulation system was heated from 200 to 1500 K at run time of 10 ps (step size 0.5 fs, 20 000 steps). Afterward, 30 ps of molecular dynamic simulation (step size 0.5 fs, 60 000 steps) at constant temperature (1500 K) was performed. Finally, the system was cooled to 200 K (step size 0.5 fs, 60 000 steps). Temperature was regulated by coupling to an external bath (Berendsen's method[60]) using a bath coupling constant of 1.2 ps. The generalized Born model was applied to account for solvation effects.[61] This procedure led to 10 low-energy structures which were superimposed and grouped into families by their rms values. An average structure of the family containing the most members was calculated and used for further investigations.

Ab Initio Electronic Structure Calculations. The average structure of the family obtained in the last step was further enhanced by performing an ab initio geometry optimization using the B3LYP density functional method.[62] The 6-31G** basis set of Pople et al.[63] with d-polarization functions for non-hydrogens and p-polarization functions for hydrogens was used. All ab initio electronic structure calculations were performed by the Gaussian 98 (Rev. A.7)[64] suite of programs.

The overall procedure leads to a most likely lowest energy conformation of the inhibitors studied in this publication.

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Supporting Information Available: Calculation of distances between five features of compounds **7**−**9** and **12**, using simulated annealing techniques and ab initio calculations (Table S1), results from elemental analysis (Table S2), and routine ^{1}H NMR and IR spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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