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N-SUBSTITUTED 4-(5-INDOLYL)BENZOIC ACIDS. SYNTHESIS AND EVALUATION OF STEROID 5α-REDUCTASE TYPE I AND II INHIBITORY ACTIVITY

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Abstract: The synthesis of N-alkyl and N-arylalkyl substituted 4-(5-indolyl)benzoic acid derivatives as inhibitors of steroid 5α-reductases is described. For the human type II isozyme a benzyl substituent (IC_{50} 6.20 μM) and for the human type I isozyme a cyclohexanemethyl substituent (IC_{50} 2.10 μM) on the indole nitrogen proved to be most efficacious, thus providing interesting leads for the development of drugs for the treatment of benign prostatic hyperplasia (BPH).

Steroid 5α-reductase is a membrane bound enzyme, that in certain androgen dependent tissues catalyzes the irreversible conversion of testosterone (T) to the more potent androgen dihydrotestosterone (DHT) in the presence of NADPH. Using molecular biology methods two types of steroid 5α-reductase, named type I and II, have been isolated from human and rat prostatic cDNA libraries. These isozymes differ in tissue distribution (type I: peripheral skin and hair follicles; type II: prostate), pH optima and amino acid sequence[1].

An important step for the enzymatic reaction is the interaction of the enone moiety of the substrate (e.g. testosterone) with an electrophilic residue in the active site of the enzyme, yielding an enolate transition state, which permits the stereospecific transfer of an hydride from NADPH into the 5α position of the substrate (Figure 1). As DHT is associated with certain androgen dependent diseases like benign prostatic hyperplasia (BPH), the development of selective inhibitors of this enzyme has become the aim of several research groups[2]. As a soft therapeutic approach selective inhibition of this enzyme does not affect T-mediated physiological reactions and should therefore not be associated with undesired side effects. It is now hypothesized, that dual acting inhibitors are necessary for the treatment of BPH, as the limited clinical efficacy of Finasteride (Proscar®), a very potent type II selective steroidal inhibitor, might be due to its rather poor type I inhibitory potency[3].

A number of steroidal and nonsteroidal inhibitors[2], which can be considered as mimetics of the postulated enolate transition state have been developed and are showing competitive inhibitory patterns among them e. g.

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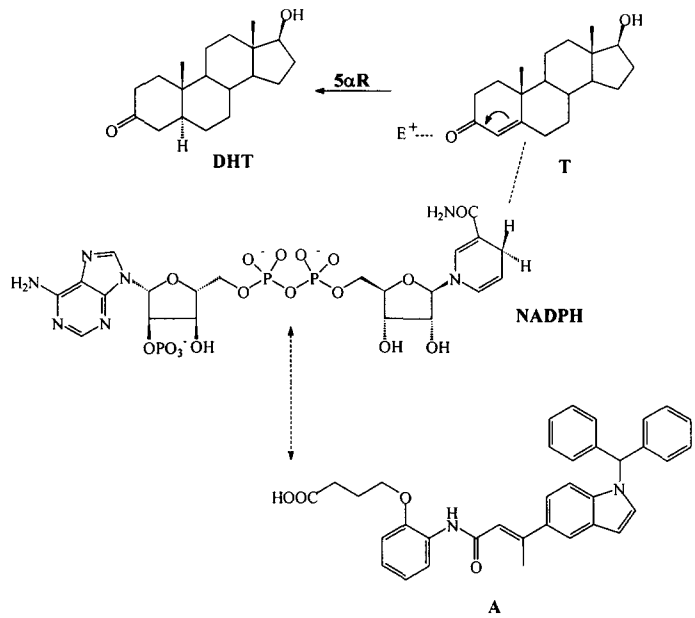
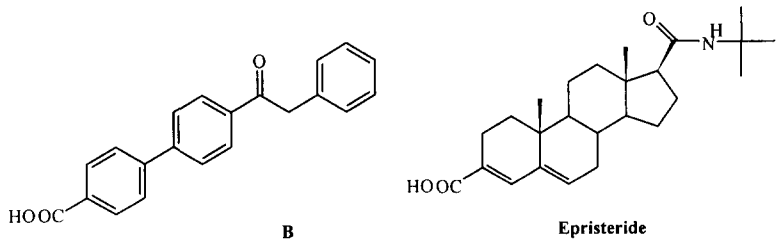
Figure 1



4- or 6-azasteroids[4]. Product-like inhibitors that interfere in an uncompetitive manner with the enzyme have also been designed (e.g. Epristeride and the biphenyl compound **B**[5], Figure 2).This type of inhibition could have advantages in vivo, as an uncompetitive inhibitor cannot directly be displaced by an increased substrate concentration, due to blockade of the metabolic pathway[6,10].

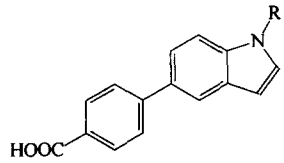
Figure 2



A third group of potent inhibitors like **ONO 3805**[7], compound **A**[8] (Figure 1), and **FK143**[9] having the butanoic acid moiety in common, can neither be considered as substrate nor as product-like inhibitors.

Studies of Holt et al.[10] suggested for **ONO 3805** an interference with the active site of the enzyme according to figure 1. They hypothesized the butanoic acid moiety to be localised in the region of the phosphate groups of

Figure 3



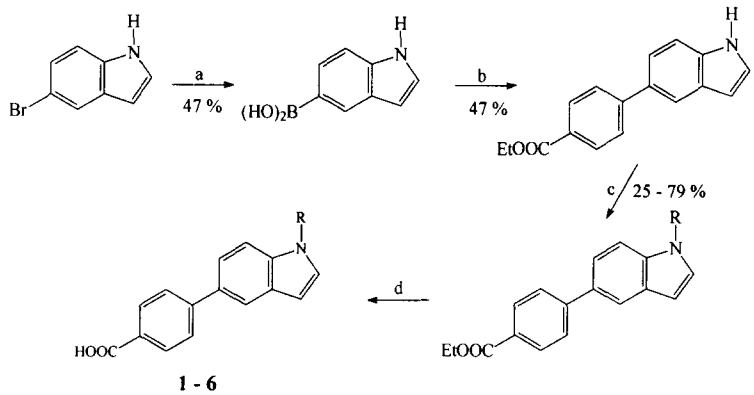
R = Benzyl, Benzhydryl
1-Butyl, 4-Heptyl
Cyclohexanemethyl
Cyclohexyl

1-6

NADPH. The lipophilic part could then be orientated in the region of the steroidal rings C and D and thus occupy the hydrophobic pocket of the enzyme, known from studies with steroidal inhibitors to be localised in the 17ß position[4b]. The fact, that these compounds act as noncompetitive inhibitors (vs T) and not as uncompetitive ones, supports this hypothesis[11]. On the basis of these findings it seemed very interesting to verify the activity of a series of N-alkyl and N-arylalkyl-substituted indole derivatives (Figure 3). In this class the aromatic carboxylic acid could interact with the supposed electropositive centre of the enzyme in a similar way as seen with the uncompetitive inhibitor Epristeride. As substituents on the indole-nitrogen, groups were chosen, which had proven to be very active in the series of type **A**[8] compounds.

The synthetic approach to the target compounds **1-6** is outlined in Scheme 1. First indole-5-boronic acid was synthesized by bromine-lithium exchange and reaction with $B(OMe)_3$ as an electrophilic agent according to a modified literature procedure[12]. Suzuki cross coupling reaction under anhydrous conditions in DMF yielded the

Scheme 1



1 - 6

Reagents and conditions: a) KH, 2 eq t-BuLi, THF, - 75°C, 5 min, then $B(OMe)_3$, 1h, -75 °C. b) 4-BrC_6H_4COOEt, K_2CO_3, DMF, 80°C, 14h. c) KOtBu, DMF, R-X (X = Br or I), varying reaction temperatures and times. d) NaOH (1N) / EtOH / 1,4-Dioxane (1/1/1), 24h, 25 °C, then HCl (1N); recrystallisation from petroleum ether (40 - 60 °C) / ethyl acetate, yields see ref. 13.

Table 1: Inhibition of **1-6** against human 5α reductases I and II and rat 5α reductase I.

Compound	R	Human 5αR Type 2[a,b] % inh (10 μM) [IC_{50} (nM)]	Human 5αR Type 1[c] % inh (10 μM) [IC_{50} (nM)]	Rat 5αR Type 1[b,d] % inh (10 μM) [IC_{50} (nM)]
1	Benzyl	60 [6200]	67	12
2	Benzhydryl	40	81 [2780]	26
3	1-Butyl	24	72	17
4	4-Heptyl	10	75 [3200]	43
5	Cyclohexanemethyl	50	81 [2100]	n.I.[f]
6	Cyclohexyl	36	n.d.[e]	n.I.[f]
Epristeride		[1-3]	[1100]	[40-45]

[a] Human prostate homogenates, [b] Substrate 1ß2ß ^{3}H testosterone 210 nM, [c] DU 145 cell culture, substrate ^{3}H androstenedione 5 nM, [d] Rat ventral prostate, pH 6.6, [e] not determined, [f] no inhibition.

biphenyl coupling product, which was alkylated using various alkylhalides, giving good to fair yields depending on the sterical nature of the alkyl groups. Finally saponification with sodium hydroxide gave the desired target molecules **1-6**[13].

The inhibitory potency of compounds **1-6** (Table 1) was evaluated using human prostate homogenates (human type II), DU 145 cell culture (human type I) and rat prostate homogenates (pH 6.6, type I). All compounds proved to be inactive or rather poor inhibitors of the rat type I isozyme although considerable inhibitory potency could be found, when tested for the human type I isozyme (spec. cpd **5**), an increase in activity being observed, when a bulkier substituent was introduced (cpds. **1/2** and **3/4**). These findings also reveal the difficulties using rat enzyme sources for an initial inhibitor evaluation and the impossibility using in these cases rats for in vivo testing. The most active inhibitor of the human type II enzyme was the benzyl substituted compound **1**, showing a mediocre inhibition. Interestingly the structurally related compound **B** (Figure 2) has shown potent inhibitory activity towards the human type II isozyme without affecting type I isozyme[5]. One important finding of the current study is the shift from selective type II inhibition to type I inhibition. For this reason the biphenyl compounds **1-6** and **B** could be valuable tools to elucidate the structural requirements for selective type I and II inhibition. Futhermore the compounds could be leads for the development of a drug for the treatment of BPH having the advantage of being nonsteroidal and thus avoiding some of the well-known side effects of steroidal drugs.

Acknowledgment:

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13. Analysis of compounds **1 - 6**: NMR spectra were recorded with a Bruker AM-400 using d6-DMSO as solvent, all compounds gave satisfactory elemental analysis within ± 0.4 % of the calculated values.

4-(N-Benzyl-5-indolyl)benzoic acid (1) (59 %) mp 252 °C

δ_H : 5.47 (s; 2H, Ar-$\underline{CH_2}$); 6.58 (d; 1H, ^3J = 3.08 Hz, H-3); 7.21-7.57 (m; 8H, H-2, H-6, H-7, $\underline{Ar}$-CH_2); 7.79 and (a.) 7.98 (AA´BB´; 4H, ^3J = 8.18 Hz); 7.93 (s; 1H, H-4).

4-(N-Benzhydryl-5-indolyl)benzoic acid (2) (38 %) mp 251-252 °C

δ_H : 6.58 (d; 1H, ^3J = 3.08 Hz, H-3); 7.08 (d; 1H, ^3J = 3.52 Hz, H-2); 7.18-7.41 (m; 11H, Ar-CH-Ar); 7.47 (d; 1H, ^3J = 8.40 Hz, H-6); 7.55 (d; 1H, ^3J = 8.84 Hz, H-7); 7.79 a. 7.98 (AA´BB´; 4H, ^3J = 7.96 Hz); 7.94 (s; 1H, H-4); 12.71 (s; 1H, COOH).

4-[N-(n-Butyl)-5-indolyl]benzoic acid (3) (61 %) mp 214 °C

δ_H : 0.89 (t; 3H, ^3J = 7.3 Hz, N-$CH_2CH_2CH_2\underline{CH_3}$); 1.25 (sext; 2H, ^3J = 7.52 Hz, N-$CH_2CH_2\underline{CH_2}CH_3$); 1.75 (quint; 2H, ^3J = 7.28 Hz, N-$CH_2\underline{CH_2}CH_2CH_3$); 4.21 (t; 2H, ^3J = 6.87 Hz, N-$\underline{CH_2}CH_2CH_2CH_3$); 6.51 (d; 1H, ^3J = 3.08 Hz, H-3); 7.43 (d; 1H, ^3J = 3.12 Hz, H-2); 7.51 (dd; 1H, ^3J = 8.40 Hz, ^4J = 1.76 Hz, H-6); 7.58 (d; 1H, ^3J = 8.84 Hz, H-7); 7.80 a. 8.00 (AA´BB´; 4H, ^3J = 8.40 Hz); 7.92 (s; 1H, H-4); 12.86 (s; 1H, COOH).

4-[N-(4-Heptyl)-5-indolyl]benzoic acid (4) (55 %) mp 170 °C

δ_H : 0.78-1.89 (m; 14H, CH($\underline{CH_2CH_2CH_3}$)$_2$); 4.47 (m; 1H, N-CH); 6.56 (d; 1H, ^3J = 3.08 Hz, H-3); 7.49 (m; 2H, H-2 a. H-6); 7.63 (d; 1H, ^3J = 8.4 Hz, H-7); 7.80 a. 8.00 (AA´BB´; 4H, ^3J = 7.96 Hz); 7.89 (s; 1H, H-4); 12.83 (s; 1H, COOH).

4-[N-Cyclohexanemethyl-5-indolyl]benzoic acid (5) (58 %) mp 241 °C

δ_H : 0.98-1.81 (m; 11H, Cyclohexane H); 4.04 (d; 2H, ^3J = 7.04 Hz, N-CH_2); 6.50 (d; 1H, ^3J = 2.64 Hz, H-3), 7.39 (d; 1H, ^3J = 3.12 Hz, H-2); 7.49 (d; 1H, ^3J = 8.40 Hz, H-6); 7.58 (d; 1H, ^3J = 8.40 Hz, H-7); 7.80 a. 7.99 (AA´BB´; 4H, ^3J = 8.40 Hz); 7.90 (s; 1H, H-4); 12.84 (s; 1H, COOH).

4-[N-Cyclohexyl-5-indolyl]benzoic acid (6) (55 %) mp 232 °C

δ_H : 0.83-1.97 (m; 10H, Cyclohexan H); 4.30 (m; 1H, N-CH); 6.52 (m; 1H, H-3); 7.50 (d; 1H, ^3J = 8.84 Hz, H-6); 7.54 (d; 1H, ^3J = 3.12 Hz, H-2); 7.65 (d; 1H, ^3J = 8.40 Hz, H-7); 7.80 a. 7.99 (AA´BB´; 4H, ^3J = 7.74 Hz); 7.91 (s; 1H, H-5).