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THE DEVELOPMENT OF NON-STEROIDAL DUAL INHIBITORS OF BOTH HUMAN 5α-REDUCTASE ISOZYMES

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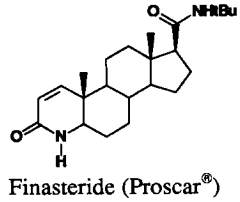
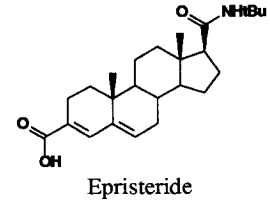
Abstract: The design, synthesis and biological properties of homochiral non-steroidal inhibitors of both isozymes of human 5α-reductase are described. The *o*-hydroxy aniline moiety of the initial lead (**1**) can be replaced by a 3-acyl indole isostere, whilst the minimum energy conformation of the benzyl ether in the potent inhibitor (**3**) is mimicked by the conformationally locked benzodioxolane system in the potent non-steroidal inhibitor (**7**). Pharmacokinetics and oral efficacy in a rat model of BPH are presented for (**3**) and (**7**).

5α-Reductase is a membrane bound, NADPH dependent, enzyme which converts testosterone to the more potent androgen dihydrotestosterone (DHT).[1] DHT has been implicated in the progression of benign prostatic hyperplasia (BPH), prostate cancer, acne and male pattern baldness.[2] Two isozymes of human 5α-reductase have been isolated,[3] 5α-reductase2 (5α-R2) is the predominant isozyme expressed in prostatic tissue, and is also present in liver[4] whilst 5α-reductase1 (5α-R1) is expressed primarily in the liver and skin.[5] Finasteride[6], a steroidal inhibitor of 5α-R2, has shown clinical application for the treatment of BPH whilst the steroidal acid epristeride[7] has demonstrated significant reduction of DHT in animal models of BPH. Selective inhibitors of 5α-R1 are of interest for the treatment of male pattern baldness.[8] In principal, a dual inhibitor of both isozymes of human 5α-reductase could lead to greater reduction of DHT levels in human prostate and plasma resulting in significantly greater reduction of prostate volume and improved clinical efficacy over the currently available agents for the treatment of BPH.[9] In addition, the use of a non-steroidal template reduces the potential for interaction with other enzymes in the steroidal pathway and reduces the complexity of target compound synthesis. This report describes our approach to the design and synthesis of non-steroidal, dual inhibitors of human 5α-R1 and 5α-R2 as well as evidence of their *in vivo* efficacy and good pharmacokinetic properties. Other non-steroidal inhibitors of 5α-reductase have recently been disclosed.[10]



Finasteride (Proscar®)



Epristeride

The non-steroidal *o*-hydroxyaniline (**1**)[11] proved a weak lead *in vitro* versus human 5α-R2 (Table 1). An initial SAR study revealed the importance of the O-linked butyric acid moiety and the amide linkage to the central

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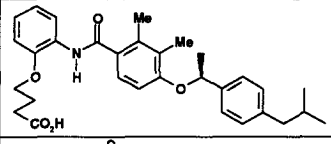
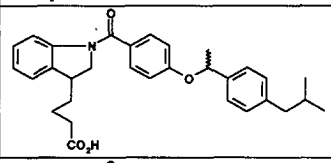
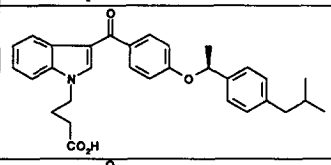
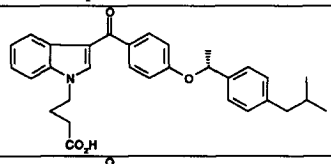
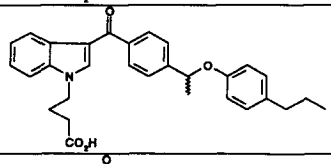
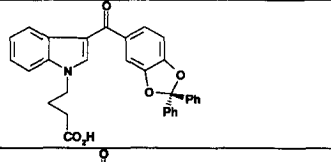
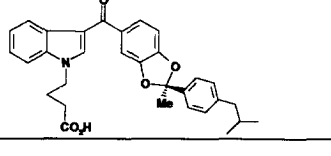
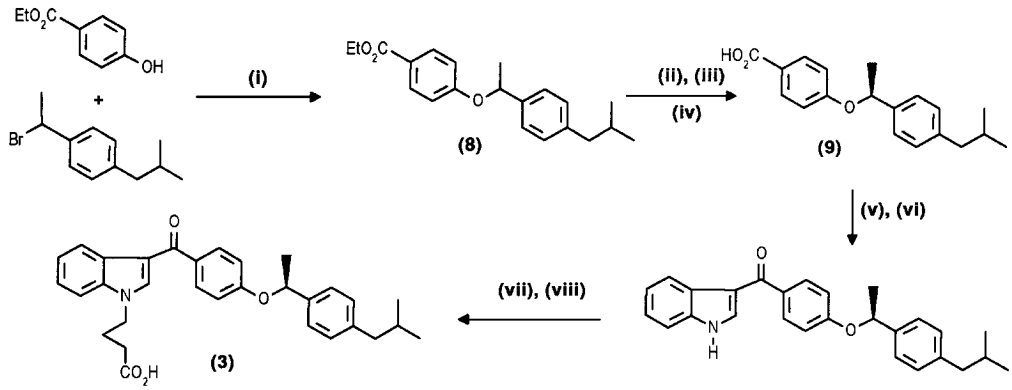
dimethylphenyl spacer. By contrast, the two methyl groups on the central phenyl spacer could be removed without significant loss in potency. We were keen, however, to replace the *ortho*-hydroxy aniline moiety and recognised the potential importance of an intramolecular H-bond between the aniline NH and the proximal ether oxygen. This thinking lead to synthesis of the racemic, conformationally restricted, dihydroindole (**2**).

Table 1[12]

No	Structure	Rat 5α-R nM[13]	Human 5α-R1 nM[13]	Human 5α-R2 nM[13]
(1)		1.7	-	256
(2)		24	113	481
(3)		1	40	4
(4)		30	574	69
(5)		5	8	10
(6)		3	23	34
(7)		9	25	23

A number of dihydroindole replacements were synthesised with the aim of improving potency and removing the C3-chiral centre of the dihydroindole. In particular, introduction of a double bond to form an indole proved advantageous and the C3-acyl-indole (**3**) demonstrated improved, balanced potency versus both 5α-reductase isozymes (Table 1). The α-methyl-benzyl chiral centre was essential for good *in vitro* potency with the *S*-enantiomer significantly more potent than its antipode (**4**). Preparation of homochiral (**3**) was achieved by a resolution after the key ether linkage had been formed (Scheme 1). Treatment of the crude reaction mixture from the hydrolysis of the ethyl ester (**8**) with (+)-ephedrine gave a single crystalline diastereomer from which the enantiomerically pure acid (**9**) could be obtained by acid treatment. The absolute configuration of (**9**) was determined by correlation (HPLC, Chiralpak AD column) of the final product (**3**) with material of known absolute configuration prepared by an alternative route (See Scheme 2).

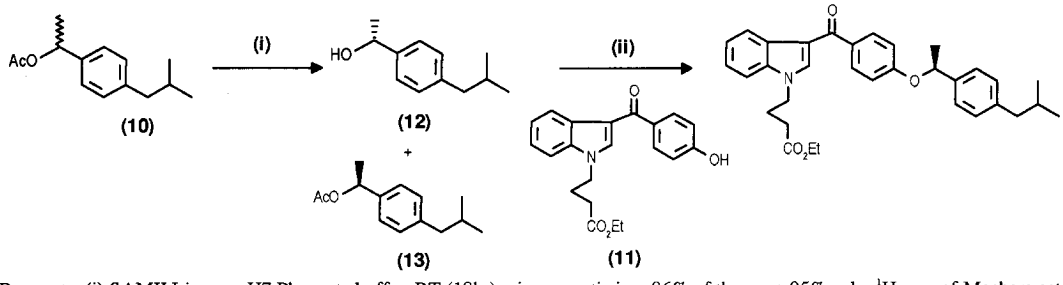
Scheme 1



Reagents: (i) K_2CO_3, 2-butanone, TBAB, 83%; (ii) NaOH, IMS, 94%; (iii) (+)-Ephedrine.HCl (0.5eq) 67% of theory. (iv) 1N HCl 99%; (v) $SOCl_2$, pyridine (vi) Indole (1.1eq), MeMgI (1.1eq), toluene, 42%; (vii) $Br(CH_2)_3CO_2Et$, K_2CO_3, 2-butanone, 71%; (viii) NaOH, IMS, 95%.

Several alternative methods for the introduction of the α-methyl benzyl chiral centre were examined including lipase mediated kinetic resolution of the acetate (**10**)[14] (Scheme 2) followed by Mitsunobu coupling with the phenol (**11**) accompanied by inversion of stereochemistry. Enantiomeric purity and assignment of the absolute configuration of alcohol (**12**) and its antipode (**13**) were achieved by formation of the Mosher's ester[15] and ^{1}H n.m.r. correlation according to the method of Yamaguchi.[16] This route provided homochiral (*S*)-(**3**) of known absolute configuration on a small scale.

Scheme 2



Reagents: (i) SAMII Lipase, pH7 Phospate buffer, RT (18hr), vigorous stirring, 86% of theory, >95%ee by ^{1}H nmr of Moshers ester derivative; (ii) 1eq (**11**), DEAD, PPh_3, THF rt.

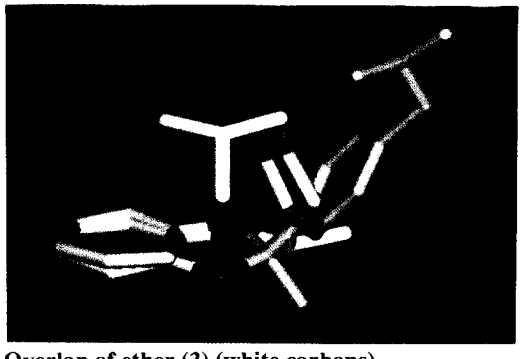
Compound (**3**) exhibited a long half life and good oral bioavailability in rat and dog (Table 2) and was progressed to a rat *in vivo* model of prostate shrinkage,[17] where it demonstrated a 35% reduction in prostate weight after 10 days (1mg/kg p.o.). In summary, the weak lead (**1**) has been developed into a non-steroidal dual inhibitor of both human 5α-R isozymes which demonstrates good efficacy after once daily oral dosing in an animal model of BPH. However compound (**3**) is 10-fold weaker versus 5α-R1 compared to 5α-R2 and we were keen to explore tactics for increasing potency versus 5α-R1 in this series.

Table 2 UK-117,025 Pharmacokinetics

	Rat	Dog
$T_{1/2}$	3.7hr	1.9hr(β), 10hr (terminal)
Vd	3.6L/kg	0.9L/kg
Clearance	11.0ml/min/kg	2.4ml/min/kg
Bioavailability	56%	86%

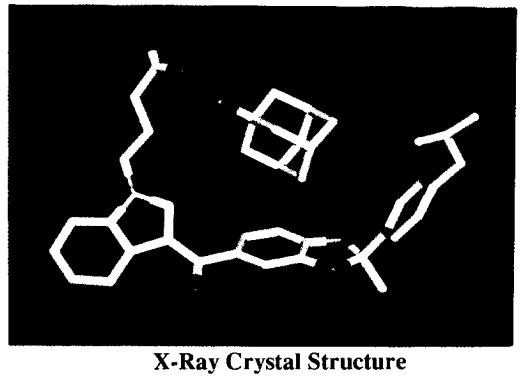
Computer modelling of the benzyl ether of compound (**3**) indicated a minimum energy conformation as depicted in Scheme 3 with the methyl group occupying the least hindered transoid position relative to the central phenyl spacer. We postulated that this minimum conformation may represent that required for inhibition of both isozymes of human 5α-reductase. Indeed, reversal of the ether linkage (compound **5**, Table 1) gave a compound of equivalent overall conformation and potency indicating that the ether link provided a conformational preference rather than a specific binding motif. We therefore attempted to lock the compound into this favoured orientation by constructing a suitable ring system. Molecular modelling studies predicted that a pendant aromatic ring of the benzodioxolane system (**7**) would readily adopt a pseudoaxial conformation due to the lack of eclipsing diaxial inteactions in the five membered ring. Indeed, overlap of the rigid benzodioxolane structure with the predicted minimum conformation of (**3**) suggested that the pendant methyl and *para*-isobutyryl phenyl groups of (**7**) should adopt a similar spatial arrangement to the α-methyl-*p*-isobutyrylphenylmethyl ether of (**3**) (Scheme 3). This prediction was borne out by the good *in vitro* potency of the prototype benzodioxolane (**6**) and its close analogue (**7**) versus both isozymes of human 5α-reductase (Table 1). In addition, an X-ray crystal structure analysis of (**7**) as its adamantylamine salt showed an overall cup conformation of the molecule with the pendant *p-iso*butyrylphenyl ring of the benzodioxolane system in a pseudoaxial position butressing the adamantylamine counterion (Scheme 4).[18]

Scheme 3



Overlap of ether (3) (white carbons) and benzodioxolane (7) (green carbons)

Scheme 4

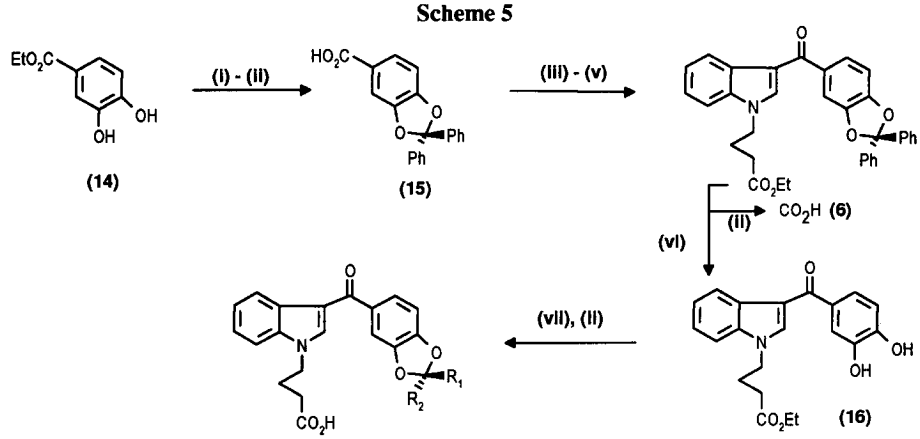


X-Ray Crystal Structure (7).Adamantylamine salt

Preparation of the diphenylbenzodioxolane ring (**15**) was initially achieved by fusing a mixture of the catechol (**14**) and diphenyldichloromethane at 170°C.[19] Subsequent ester hydrolysis, indole acylation and N-functionalisation gave the prototype benzodioxolane inhibitor (**6**). Analogues bearing a range of substituents at the 2 position of the benzodioxolane ring were prepared by acidic removal of the diphenyl benzodioxolane ring

to reveal the catechol (**16**). The required benzodioxolanes could be reformed by heating the catechol at reflux in toluene with preformed dimethylketals or dimethylacetals (Scheme 5). Compounds bearing a chiral centre at the benzodioxolane 2-position could be resolved by preparative chiral HPLC (Chiralpak AD column).

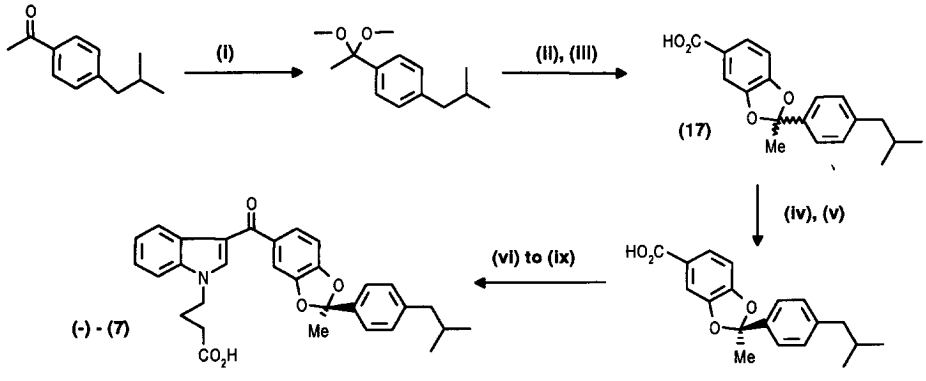
Scheme 5



Reagents: (i) $(Ph)_2CCl_2$, 170°C, 10min, 95%; (ii) NaOH, MeOH/THF, 95%; (iii) $(COCl)_2$,CH_2Cl_2/DMF, 95%; (iv) Indole(2eq), MeMgI(2eq),toluene, 64% (v) $Br(CH_2)_3CO_2Et$, K_2CO_3, 2-butanone, 89% (vi) gAcOH, 80°C, 5hr; (vii) $R_1R_2C(OMe)_2$, pTSA, toluene.

Compound (**7**) has an improved pharmacokinetic profile in the dog compared to (**3**) and was chosen for further progression into animal models of benign prostatic hyperplasia. The compound caused a 48% reduction in rat prostate weight after 10 day oral dosing (10mg/kg b.i.d).[17] In addition, (**7**) had a clean toxicological profile in rat up to 150mg/kg dosing and dog up to 300mg/kg dosing. Resolution *via* chiral HPLC proved impractical for the large scale preparation of this material; therefore a resolution precedure was developed for the intermediate acid (**17**) using L-(-)-α-methylbenzylamine (Scheme 6). The absolute configuration of the desired diastereomer of the salt was determined to be (*R*) by X-ray crystal structure analysis.

Scheme 6



Reagents: (i) $(MeO)_3CH$, MeOH, pTSA 90%, (ii) Ethyl-3,4-dihydroxybenzoate, (iii) NaOH, aqIMS [71% for (ii) and (iii)]; (iv) L-(-)-α-methylbenzylamine, EtOAc/acetone (v) aq HCl slurry, 54% of theory; (vi) $SOCl_2$, Pyridine, Toluene (vii) Indole (2.2eq), EtMgBr(2.2 eq) -40°C, 69% (viii) $Br(CH_2)_3CO_2Et$, K_2CO_3, 2-butanone, reflux, 90%; (ix) NaOH, aq IMS, 99%.

In summary, compound (**7**) is an equipotent, dual inhibitor of 5α–R1 and 5α–R2. Like compound (**3**), it has a long half life, high oral bioavailability and demonstrates good oral efficacy in an animal model of BPH. Further exploration of this inhibitor series will be discussed in subsequent publications.

Acknowledgements:
We thank Prof C. Crout (Univ of Warwick) for assistance in developing the lipase mediated resolution of (**10**) and Dr J. Bordner (Central Research, Pfizer Inc, Groton, CT 06340) for X-ray crystal structure analysis of (**7**). J.A. Appleton, S. Baber, M. Berry, S. J. Cambers, G. Corless, L. L. Foster, B. Learmouth, J. MacKay, M. Metcalfe, M. E. Price, M. E. Glanfield, D. Falconer, D. S. Senior, V. Sethi, F. Stewart, L. A. Turner and the Physical Sciences Department are thanked for their valued contribution to the work described above.

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12. All compounds were analytically pure and had satisfactory ^{1}H n.m.r and mass spectral data. For full experimental details see PCT Int. Appl.: WO 9302050 A1 and WO 9317014 A1.
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15. Mosher, H. S. *J. Org. Chem.*, **1969**, *34*, 2543.
16. Yamaguchi, S. *Tetrahedron*, **1976**, *32*, 1363.
17. Test compounds were suspended in 0.25% methyl cellulose aqueous solution and administered to rats (c.a. 250g bodyweight, 8 rats per treatment group) by oral gavage once or twice daily (as specified in text) for 10 days. At the end of this period each animal was killed by cervical dislocation and the ventral lobe of the prostate removed, cleaned of capsular material and immediately weighed. Prostate weights were expressed relative to bodyweight (mg/ 100 g) and were compared with the values obtained from the control group which received dose-vehicle alone. The 10-fold increase in dose for compound (**7**) relative to compound (**3**) is consistent with the relative *in vitro* potencies of the two compounds versus rat 5α-R (Table 1).
18. Full X-ray crystallographic data has been submitted to the Cambridge Crystallographic Data Centre.
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