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Discovery of a novel hybrid from finasteride and epristeride as 5α-reductase inhibitor

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ABSTRACT

Finasteride and epristeride both inhibit 5α-reductase with high potency via competitive and non-competitive mechanism, respectively. A new hybrid of finasteride and epristeride was designed as a new 5α-reductase inhibitor based on combination principles in medicinal chemistry. Human 5β-reductase was chosen as a plausible surrogate of 5α-reductase type II and the results indicate that although the hybrid compound possesses the main bulk of epristeride, its inhibitory mechanism is same as of finasteride. The hybrid turned out to be a potent 5α-reductase inhibitor in low IC_{50} ranges.

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Benign Prostatic Hyperplasia (BPH) is an andron-related disorder common in old male. Typical symptoms include increased frequency or urgency to urinate, which results from increased pressure on the urethra. It has been well established that growth of the prostate is stimulated by androgens, and it now appears that 5α-dihydrotestosterone (DHT) plays primary role in the trophic support of this organ.[1,2] Steroid 5α-reductase (5-AR) is an enzyme responsible for the conversion of testosterone (T) into DHT.[3] Thus selective inhibition of steroid 5-AR could offer an alternative therapy for BPH. There are two isoforms of human 5-AR identified as type I and type II.[4–6] In normal human skin the type I reductase is the predominant type expressed, whereas 5-AR type II is the major form found in the prostate.[7]

Finasteride **1** is a selective inhibitor of the type I and type II isoforms of steroid 5-AR. It is currently marketed worldwide as a drug for BPH, and is also used in the treatment of hair loss[8,9] and in the prevention of prostate cancer.[10] A few years later, epristeride, behaving as an uncompetitive inhibitor, was also launched in 2000 as a therapy for BPH.[11] On the basis of their different modes of action, a combination of finasteride and epristeride may prove similarly beneficial. It is worthwhile to design and synthesize a novel hybrid which not only combine important pharmacophore structures of finasteride and epristeride, but also retain both inhibition activities of 5-AR. We hypothesized that keeping the A and B ring of epristeride, and moving the A ring of finasteride to replace the D ring of epristeride would increase the inhibition activity of 5-AR (Fig. 1). This article reports the synthesis of this new series of compounds and the results we have obtained using this approach.

The hybrid **3** was synthesized by using the procedure described in Figure 2. Oppenauer oxidation of *trans*-dehydroandrosterone **4** led to yield compound **5**, which was sequentially sulfanilated to

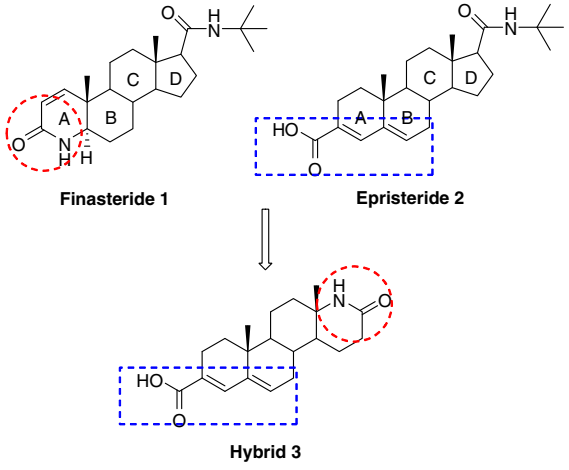

Figure 1. Structures of finasteride **1**, epristeride **2** and their hybrid **3**.

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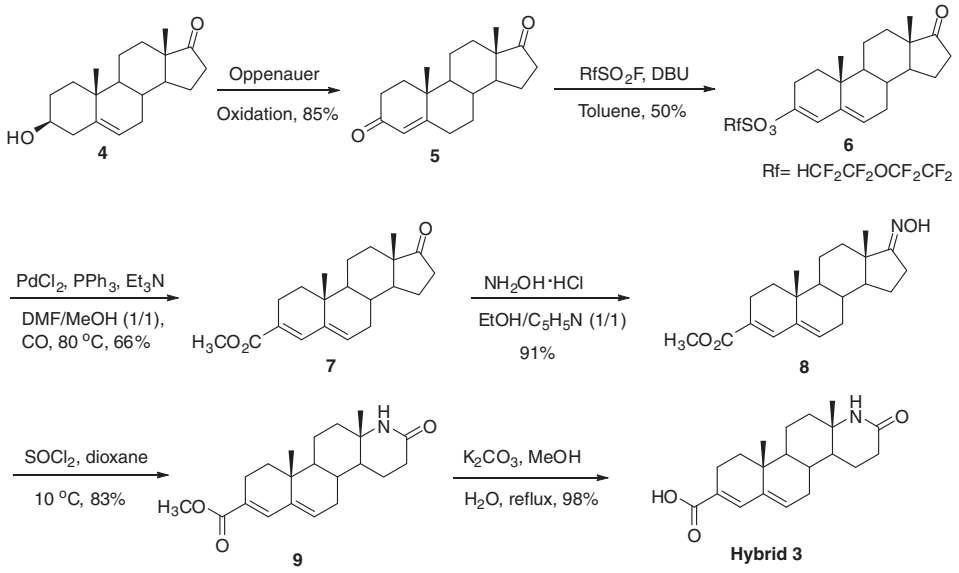

Figure 2. The synthesis of hybrid **3**.

afford **6** in 50% yield. The $RfSO_3$ group of **6** was transformed to carboxylic ester via Pd catalyzed CO addition reaction. After reaction with NH_2OH, Beakmann rearrangement[12] was performed with $SOCl_2$ to afford compound **9**, which was rapidly saponified with K_2CO_3 to give hybrid **3** in 98% yield.

To evaluate the function of A ring of compound **3**, we replace the A ring with the phenyl. The synthesis of compound **13** was described in Figure 3. Starting with the estrone, compound **11** was synthesized in 99% yield. Then Beckmann rearrangement was performed with $SOCl_2$ to afford compound **12**, which was rapidly sulfation with sulfamic acid to give **13** in 50% yield.

The inhibitory effects of compounds **3** and **13** on human prostatic 5α-reductase were investigated with an in vitro incubation technique.[13] Finasteride was used as a reference compound. IC_{50} values and relative inhibitory efficiencies as compared with the IC_{50} of finasteride are presented in Table 1. The investigated steroid **3** exhibit promising 5α-reductase inhibitory effect. The IC_{50} value of compound **3** is 71 nM. The relative inhibitory effect of the compound **3** is 0.49. As concerns the effects of replacement at A ring in **3**, the introduction of phenyl group (**12** and **13**) caused a drop-off in inhibition ability of 5α-reductase.

Currently, for both of 5α-reductase type I and type II, there are no crystal structures available in the RCSB Protein Data Bank (PDB). Conversely, for 5β-reductase, several crystal structures, binding with different steroids and same cofactors (NADP+ or NADPH), have been deposited in PDB. The structure of the AKR1D1·NADP+·finasteride was determined with a high resolution of 1.70 Å (PDB ID 3G1R)[14] and was used as a surrogate of 5α-reductase type II in this modeling study based on the following reasons: (1) The reduction of the Δ^4-ene in humans, the initial step in steroid hormone metabolism, is mediated by 5α-reductases or 5β-reductase to yield the subsequent 5α- or 5β-dihydrosteroids, respectively,[15] which means 5α-reductases or 5β-reductase have same or at least similar enzymatic functions when metabolizing steroid hormones. (2) The sequence identities between 5α- and 5β-reductase are very low, mainly because these two kinds of reductases are encoded by completely unrelated genes. This hampers the developing of a reliable homology model. So using 5β-reductase as a surrogate of 5α-reductases is more reasonable than developing a homology model to investigate how 5α-reductase inhibitors to interact with the enzyme.

Table 1
5α-Reductase inhibition by Hybrid **3**, **12** and **13**

Compound	IC_{50} value (nM)	Relative inhibition (finasteride vs compound)
Finasteride	35	1
3	71	0.49
12	>1000	<0.055
13	>1000	<0.055

The crystal structure of 3G1R was downloaded from the PDB website, and the monomer B was kept and treated using Schrö-

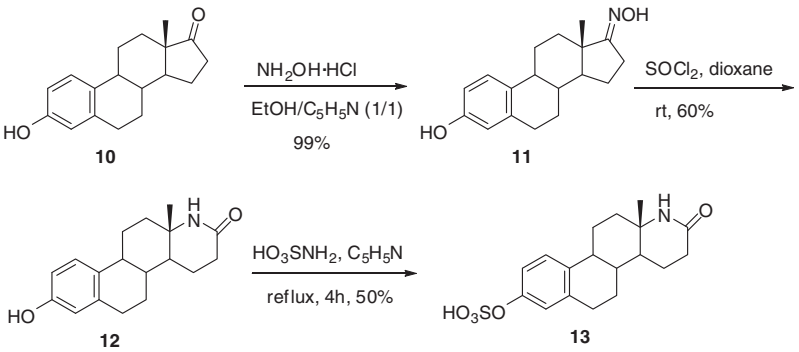

Figure 3. The synthesis of compound 13.

Table 2
The calculation results of docking and Prime MM-GBSA

Compound	Glide XP GScore (kcal/mol)	Prime MM-GBSA ΔG binding (kcal/mol)
Finasteride	−15.57	−34.75
Epristeride	−12.63	−14.43
Hybrid	−13.11	−30.32

dinger's Protein Preparation Wizard[16] to adjust the protein, the cofactor, and the ligand. Receptor grids were generated for further docking studies. The three compounds, finasteride, epristeride, and hybrid, were processed using LigPrep 2.4[17], a robust collection of tools designed to prepare 3D structures for drug-like molecules, and then docked to the active site of 5β-reductase, represented by the receptor grids using Glide 5.6[18] extra precision (XP) mode. The ligand binding energies of the three docked compounds were calculated using Prime MM-GBSA.[19] When calculating, the protein structure was relaxed by setting the 'Size of flexible region in Å' to 'Small (8)'.

Finasteride mainly interacts with 5β-reductase by hydrophobic interactions and hydrogen bonds. The active site, composed of Tyr 26, Tyr 58, Trp 89, Glu 120, Tyr 132, Trp 140, Trp 230, Met 313, and Trp 314, is very hydrophobic and so can easily accommodate hydrophobic compounds such as finasteride. The C3 carbonyl oxygen of finasteride forms two hydrogen bonds with the phenolic hydroxyl group of Tyr 58 and the neutral anti-oriented conformer of the carboxylic acid side chain of Glu 120. Additionally, three hydrogen bonds forming with water molecules are observed. Our docking studies and Prime MM-GBSA calculations reproduced this scenario: the best scored docking conformation of finasteride has a heavy atom RMSD of 0.461 Å relative to its crystallographic orientation (see Table 2 and Fig. 4A).

Epristeride could be docked into the active site with similar interactions with the protein: the main hydrophobic bulk forms hydrophobic interactions in the hydrophobic active pocket; the negative charged carboxylic acid group forms two hydrogen bonds with Glu 120 and Tyr 58. Two hydrogen bonds are observed with the surrounding water molecules. Although similar interactions as finasteride, the docking score and calculated Prime MM-GBSA ΔG is worse than those calculated ones of finasteride, indicating epristeride maybe have a worse binding affinity than finasteride to 5β-reductase.

The simulated conformation of the hybrid compound 3 in the active site is similar to the crystallographic orientation of finasteride (see Fig. 4C): the C3 carbonyl oxygen forms two hydrogen bonds with Glu 120 and Tyr 58; the N4 group engages a hydrogen bond with an environmental water molecule. The difference is the carboxylic acid group, which is on the rim of the active site and forms two hydrogen bonds with Arg 134 and a water molecule. The docking score and calculated binding affinity are a little bit worse than the ones of finasteride, but are much better than the ones of epristeride. The overlapping of the simulated conformations of these three studied compounds shows that the carboxylic acid group of epristeride sticks out (see Fig. 4D), which may increase the torsion energies of Glu 120 and Tyr 58 of the protein and then lead to worse binding affinity.

In summary, a new hybrid **3** from finasteride and epristeride was designed as a 5α-reductase inhibitor, and synthesized through a convenient route. 5β-reductase was chosen as a plausible surrogate of human 5α-reductase type II because of their similar enzymatic functions. Furthermore, finasteride can inhibit both of these two enzymes, and NADP+ is an identical cofactor of both of them. The results indicate, although the hybrid compound possesses the main bulk of compound **2**, its inhibitory mechanism is same as of compound **1**.

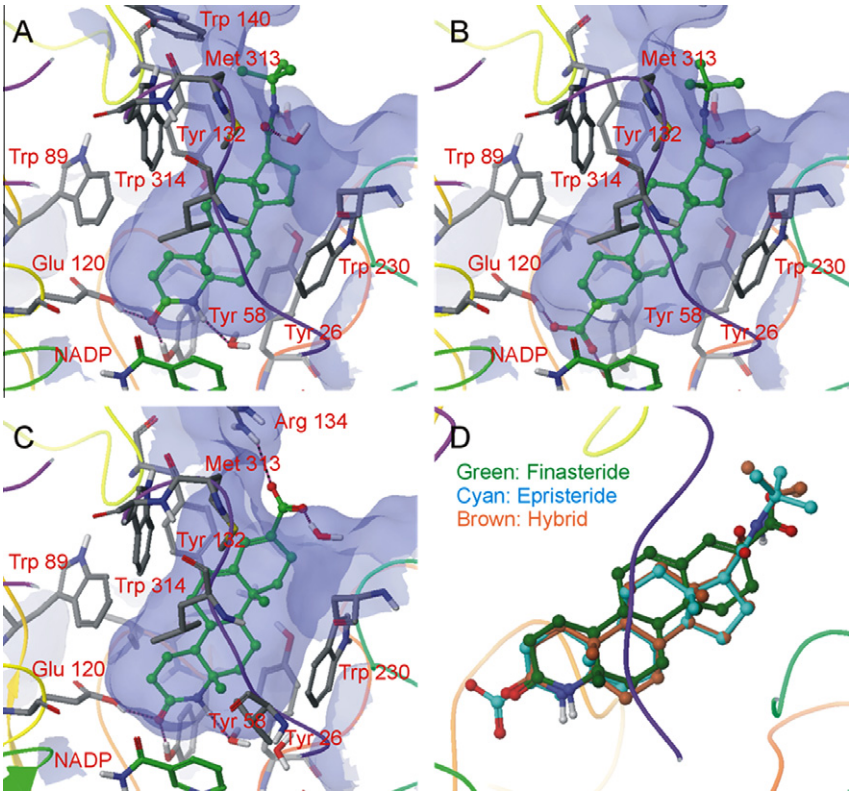

Figure 4. The binding modes predicted by Glide XP docking and Prime MM-GBSA. (A) Finasteride; (B) epristeride; (C) hybrid; (D) the overlapping of the simulated conformations of these three compounds. The protein was presented as a thin tube, and the active site was presented as a light blue molecular surface.

The hybrid **3**, closely resembling structure features of finasteride and epristeride, exhibited promising 5α-reductase inhibitory effect in vitro. The aromatization of the A ring and the replacement of the carboxylic group of the hybrid compound **3** led to diminished activities. This fully elaborated hybrid provides us a novel template for the development of pharmacokinetically improved analogues that can selectively bind to 5α-reductase, and may act as potential therapeutic agents for the treatment of BPH.

Acknowledgments

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