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

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Abstract

Finasteride and epristeride both inhibit 5 α -reductase with high potency via competitive and non-competitive mechanism, respectively. A new hybrid of finasteride and epristeride was designed as a new 5 α -reductase inhibitor based on combination principles in medicinal chemistry. Human 5 β -reductase was chosen as a plausible surrogate of 5 α -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 5 α -reductase inhibitor in low IC₅₀ ranges.

Keywords

Hybrid; Finasteride; Epristeride; 5 α -reductase inhibitor; Combination principles

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 5 α -dihydrotestosterone (DHT) plays primary role in the trophic support of this organ.^{1,2} Steroid 5 α -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

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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 afford **6** in 50% yield. The RfSO₃ group of **6** was transformed to carboxylic ester via Pd catalyzed CO addition reaction. After reaction with NH₂OH, Beckmann rearrangement¹² was performed with SOCl₂ to afford compound **9**, which was rapidly saponified with K₂CO₃ 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 SOCl₂ 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 5 α -reductase were investigated with an in vitro incubation technique.¹³ Finasteride was used as a reference compound. IC₅₀ values and relative inhibitory efficiencies as compared with the IC₅₀ of finasteride are presented in Table 1. The investigated steroid **3** exhibit promising 5 α -reductase inhibitory effect. The IC₅₀ 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 5 α -reductase.

Currently, for both of 5 α -reductase type I and type II, there are no crystal structures available in the RCSB Protein Data Bank (PDB). Conversely, for 5 β -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 5 α -reductase type II in this modeling study based on the following reasons: (1) The reduction of the ⁴-ene in humans, the initial step in steroid hormone metabolism, is mediated by 5 α -reductases or 5 β -reductase to yield the subsequent 5 α - or 5 β -dihydrosteroids, respectively,¹⁵ which means 5 α -reductases or 5 β -reductase have same or at least similar enzymatic functions when metabolizing steroid hormones. (2) The sequence identities between 5 α - and 5 β -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 5 β -reductase as a surrogate of 5 α -reductases is more

reasonable than developing a homology model to investigate how 5 α -reductase inhibitors to interact with the enzyme.

The crystal structure of 3G1R was downloaded from the PDB website, and the monomer B was kept and treated using Schrödinger's Protein Preparation Wizard¹⁶ 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 2.4¹⁷, a robust collection of tools designed to prepare 3D structures for drug-like molecules, and then docked to the active site of 5 β -reductase, represented by the receptor grids using Glide 5.6¹⁸ 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 5 β -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 G is worse than those calculated ones of finasteride, indicating epristeride maybe have a worse binding affinity than finasteride to 5 β -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 5 α -reductase inhibitor, and synthesized through a convenient route. 5 β -reductase was chosen as a plausible surrogate of human 5 α -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**.

The hybrid **3**, closely resembling structure features of finasteride and epristeride, exhibited promising 5 α -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 5 α -reductase, and may act as potential therapeutic agents for the treatment of BPH.

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

Figure 2.
The synthesis of hybrid 3.

Figure 3.
The synthesis of compound 13.

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.

Table 15 α -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

Table 2

The calculation results of docking and Prime MM-GBSA

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