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Synthesis and bioactivity of new Finasteride conjugate

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ABSTRACT

Finasteride is a synthetic 4-azasteroid compound that acts by inhibiting type II 5α-reductase, the enzyme that converts the androgen testosterone to 5α-dihydrotestosterone. It was approved by the US FDA for the treatment of benign prostatic hyperplasia and male pattern baldness. Here the acylation product of Finasteride C-18 amide *N*-polimod was synthesized by employing acylation reaction with polimod amide as a pivotal intermediate. The structure of the key intermediate and target molecule was confirmed by infrared spectrum, ^{1}H NMR and ^{13}C NMR spectra and mass spectrum, and the inhibition of the steroid 5α-reductase and the rats' benign prostatic hyperplasia by the new Finasteride conjugate and Finasteride was also determined. The inhibition of the Finasteride conjugate on 5α-reductase was stronger than that of Finasteride. Prostate hyperplasia of rats was reduced by Finasteride conjugate treatment similar to the Finasteride treatment. However, the Finasteride conjugate treated animals showed better viable condition than the Finasteride treated ones, suggesting the new compound may have improved toxicity profile than Finasteride.

Finasteride[1] a synthesized steroid chemical compound, is an idiosyncratic inhibitor of intracellular enzymes type II 5α-reductase produced in the process of androgen testosterone metabolizing into dihydrotestosterone (DHT)[2] which is applied to the treatment of benign prostatic hyperplasia (BPH).[3–8] Since structural modifications of steroid drugs by glycosidation and peptidation have been shown to have a dramatic effect on their physical, chemical, and biological properties biological activities and consequently benefit in their therapeutic applications[9,10], the synthesis of glycosidation and peptidation of steroidal conjugates are of significance for research and development of new steroid-based drugs.[9–15] Polimod (Pidotimod), a synthetic dipeptide, is an orally bioactive immune enhancer,[16] which promotes not only non-idiosyncratic immune reactions,[17] but also idiosyncratic immune reactions.[18] Under the inspiration of the glycosidation and peptidation of steroid, we examined the possibility of Finasteride and polimod could be joined into a new conjugate molecule, and studied the active suppression of the steroid 5α-reductase by the new conjugate. We also observed whether the new conjugates could possess the pharmacological effect of Finasteride, and reduce its side effects.

The acylation reaction of Finasteride and acetyl chloride was examined first in order to realize the peptide reaction between Finasteride and polimod. However, the result demonstrated that Finasteride and polimod could not produce compound **8** as expected under such a condition because of the inability of polimod to produce acyl-chlorine effectively. Therefore, a new synthetic route was designed, that polimod acyl-amine was produced first, which reacted with Finasteride. In this way, compound **8** was obtained. (Fig. 1)

When Testosterone (T) was used as substrate, the K_m value of 5α-reductase was 4.087 ± 0.102 μM, V_{max} was 0.651 ± 0.010 μM min^{-1}. The results showed that Finasteride inhibited 5α-reductase effectively. It's IC_{50} was 147.49 ± 2.84 nM (Fig. 2). The inhibition of the Finasteride and polimod conjugate (compound **8**) on 5α-reductase was stronger than that of Finasteride. It's IC_{50} was 87.98 ± 1.76 nM (Fig. 3).

We next examined the therapeutic effect of compound **8** on rat model of BPH and compared its effect with Finasteride treatment. A significant difference of the weight of prostates (Fig. 4) and prostate index was observed (data not shown, no significant differences in body weight were observed among difference groups of the animals) between the testosterone propionate (TP) control group and the normal control group (P <0.05). The increase of the size of prostate and an obvious hyperplasia exist as well through observation (data not shown). These results proved that the hypodermic injec-

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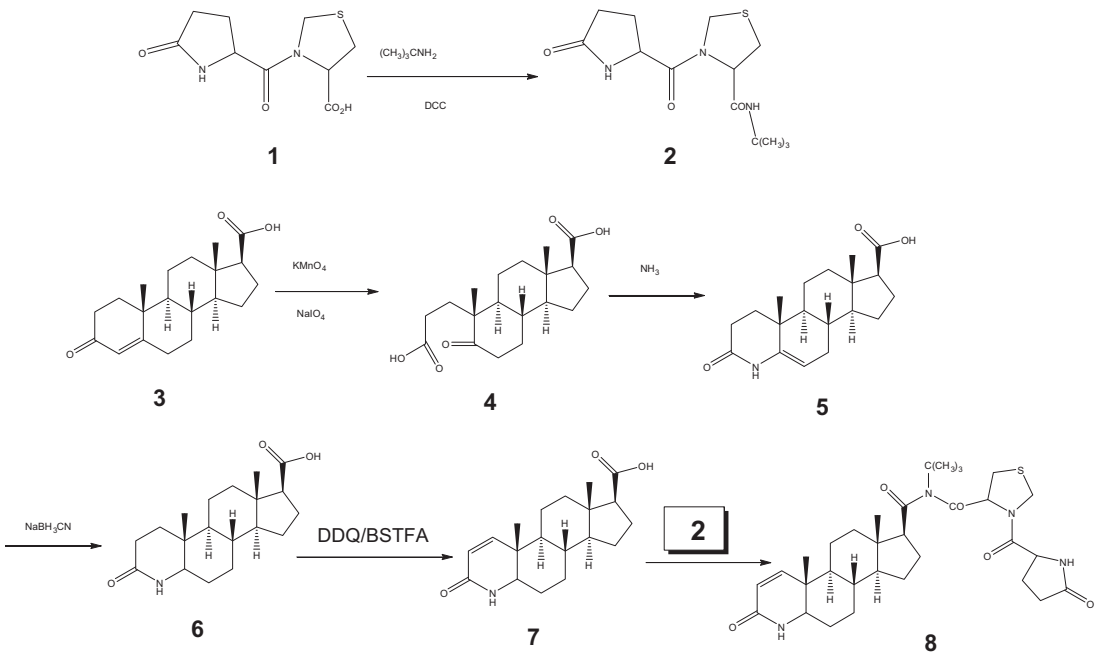

Figure 1. The synthetic route of Finasteride conjugates (compound **8**).

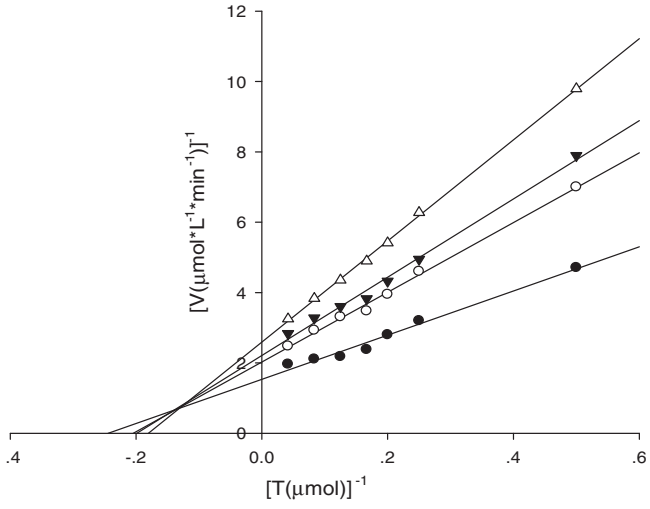

Figure 2. Lineweaver–Burk plot of Finasteride inhibition of 5α-reductase.

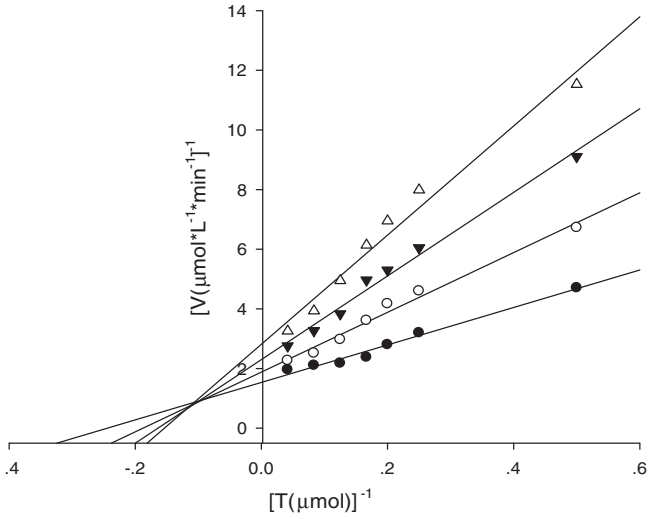

Figure 3. Lineweaver–Burk plot of Finasteride-polimod inhibition of 5α-reductase.

tions of testosterone propionate promoted the prostate hyperplasia of rats successfully.

Compared with the castrated negative control group, the growth of prostate and seminal vesicle had been greatly stimulated in TP control group (Fig. 4). After administration of treatment with Finasteride conjugate with increased dose (2, 10, 50 mg kg^{-1} d^{-1}, 14 d), the prostate and seminal vesicle's weight in the treatment group declined significantly, showing the inhibitory effect on benign prostatic hyperplasia in Finasteride conjugate group was similar to Finasteride group (Fig. 4A), while no statistical differences were found between Finasteride conjugate groups and Finasteride group although the absolute weight value appear higher in the Finasteride conjugate group of the same dosage (Fig. 4A).

We next performed histochemistry of the prostates of the animals for a closer look of the effects of the Finasteride and Finasteride conjugate treatment. The prostate gland of the rats in the normal control group arranged orderly. The columnar epithelium appeared single-layered, and a few basal cells and basement membrane were observed. There was little luminal gland secretion, and nor expansion in the gland cavity. No hyperplasia was found in interstitial tissues (Fig. 5A).

The prostate gland of the rats in the TP control group arranged relatively tight, and the size of the gland cavity significantly decreased. The columnar gland epithelium was single-layered, however, most of which was pseudostratified, with its thickness increased, and arranged disorderly. Some of the epithelium stretched out papillarily or serratedly towards lumen, and the fibrosis of the interstitial tissues was observed (Fig. 5B).

In the Finasteride group, the prostate gland of the rats arranged relatively in order, and the gland cavity recovered to the normal size. The pseudostratified epithelia reduced, and the stretched gland epithelial reduced and the hyperplasia of the interstitial tissues decreased (Fig. 5C).

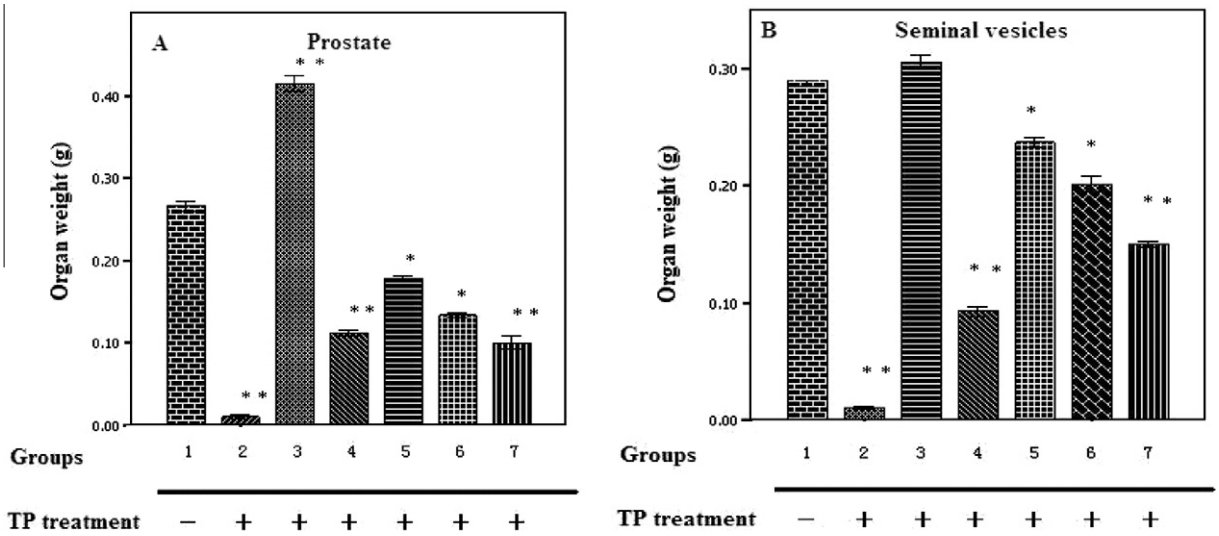

Figure 4. The inhibition of BPH in castrated rats by Finasteride and Finasteride-polimod conjugate. (A) The weight of prostate in each group. (B) The weight of seminal vesicles in each group. Group 1: normal control, Group 2: negative control, Group 3: TP control, Group 4: Finasteride (10 mg kg^{-1} d^{-1}) treated, Group 5–7: Finasteride conjugate (2, 10, 50 mg kg^{-1} d^{-1}) treated. TP (5 mg kg^{-1} d^{-1}) was injected everyday except the normal group. The value of each group was shown as mean ± SE (n = 8), $^{*}P$ <0.05, $^{**}P$ <0.01 versus the control group.

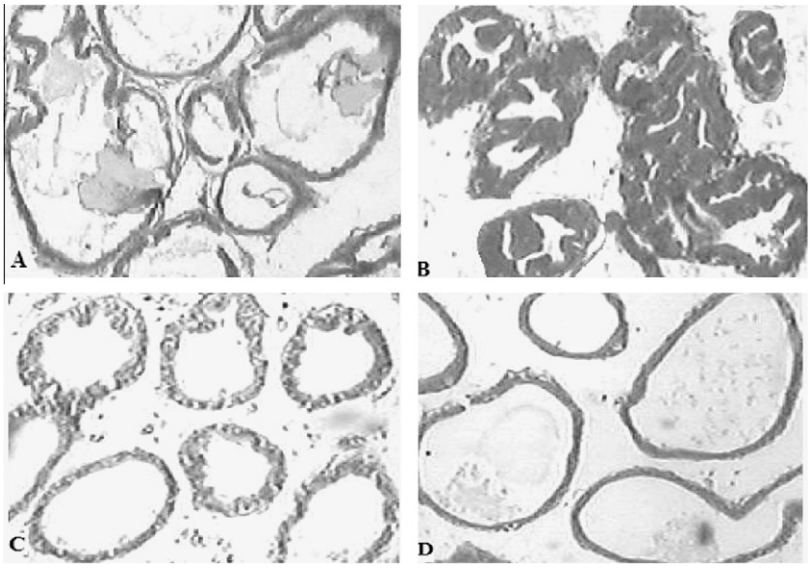

Figure 5. The histochemistry of the rats' prostate slices in each group. (A) normal control group (HE × 40). (B) TP control group (HE × 40). (C) Finasteride treated group (HE × 40). (D) Finasteride conjugate treated group (HE × 40). TP (5 mg kg^{-1} d^{-1}) was injected everyday except the normal group. The dose used in Finasteride and the Finasteride conjugate treatment group was 10 mg kg^{-1} d^{-1}.

When used at same dose as the Finasteride group, the Finasteride conjugates treatment group significantly inhibited prostatoplasia. The prostate gland arranged orderly similar to the animals in the normal control group. The prostate gland arranged orderly, and the pseudostratified epithelia and the epithelial protrusions disappeared. The hyperplasia of the interstitial tissues were also decreased (Fig. 5D). Taken together, these histochemistry results for the Finasteride conjugates showed slightly improvement compared to the Finasteride group.

We also observed that the rats in the Finasteride conjugate group were more active than those in the Finasteride group. The rats in the castrated control group and Finasteride group were less active, their skin elasticity reduced, and their fur color was dark and gloomy. However, the rats in the treatment group had almost no such appearance (Table 1).

Prostate disease is becoming the most prominent health issue in the elderly men. Benign prostatic hyperplasia (BPH) is the most common prostate disease.[19] It leads to symptoms of urinary hesitancy, frequent urination, dysuria, increased risk of urinary tract infections, and urinary retention. A safe, effective and relatively inexpensive treatment for BPH is in urgent need in clinical research.

In vivo, 5α-reductase is an NADPH-dependent enzyme that catalysis the reduction of testosterone to the more biologically active dihydrotestosterone (DHT), and the accumulation of DHT in prostate tissue is main cause for BPH. The inhibition of 5α-reductase activity can reduce the DHT levels in vivo, so as to achieve the effect of inhibition of BPH.[20–23] Thus 5α-reductase inhibitors can be used as an effective drug for the treatment of BPH.[24,25]

Finasteride is a selecting type II 5α-reductase inhibitor which has been approved for clinical use. Polimod (Pidotimod) is a synthetic dipeptide molecule with biological and immunological activity on both the adaptive and the innate immune responses. It may thus reduce the toxic effect in where the original drug (Finasteride) effects. The results show that comparing with Finasteride,

Table 1
Vital signs in each group of animals

Group	Activity (number of animals)			Hair lose, skin elasticity and fur color (number of animals)		
	Normal	Reduced activity	Significantly reduced activity	Normal	Spotted hair lose, skin elasticity reduction, normal fur color	Patched hair lose, severe skin elasticity reduction, dark fur color
Normal	8	0	0	8	0	0
Negative	1	4	3	2	5	1
Positive	3	4	1	4	3	1
Finasteride	0	2	6	0	3	5
Finasteride conjugate ($2\ mg^{-1}\ kg^{-1}\ day^{-1}$)	1	3	4	0	4	4
Finasteride conjugate ($10\ mg^{-1}\ kg^{-1}\ day^{-1}$)	3	4	1	3	3	2
Finasteride conjugate ($20\ mg^{-1}\ kg^{-1}\ day^{-1}$)	1	5	2	3	4	1

Note: The total number is animals in each group is 8.

Finasteride conjugate had better inhibition on activity of 5α-reductase in vitro and showed improved outcome in vivo, in the rat model with BPH (Fig. 4). The tissue-specific expression patterns of the 5α-reductase isozymes have been reported previously. The 5α-reductase type II isozyme was detected in the epididymis, seminal vesicle, prostate, and liver. The expression level of this isozyme in the seminal vesicle is lower than that in the prostate[26] which may account for the fact that Finasteride and Finasteride conjugate were more effective in reducing the organ weight of the prostate than that of the seminal vesicles in the TP treated rats in our test (Fig. 4). The rats in the Finasteride conjugate group also showed better vital signs such as activity, skin elasticity, and fur color than those in the Finasteride group (Table 1). These suggest that the Finasteride conjugate may have other function on the animals than that of Finasteride, possibly with improved immune response or reduced toxicity. It would be of interest to further investigate the immune modulation activity of the Finasteride conjugate and compare it with Polimod. In addition, it is also important to study the pharmaco kinetics and metabolism of this compound and see if the Polimod moiety is cleaved in vivo. Our results warrant further research of this new inhibitor of 5α-reductase in the treatment of benign prostate hyperplasia.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.102.

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