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SHORT COMMUNICATION

Steroid 5α-Reductase Inhibition by Polymethoxyflavones

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Abstract Steroid 5α-reductase inhibition was studied using eleven polymethoxyflavones (PMFs). Homologous eleven PMFs showed wide range of steroid 5α-reductase inhibitory effects. Among the tested PMFs, 5-hydroxy-7,4'-dimethoxyflavone (5) was found to be the most potent inhibitor of steroid 5α-reductase with the IC_{50} of 20 μM. These results showed PMFs could be used as a non-steroidal steroid 5α-reductase for the treatment of benign prostatic hyperplasia.

Keywords benign prostatic hyperplasia · *Kaempferia parviflora* · polymethoxyflavones · steroid 5α-reductase inhibitor

Benign prostatic hyperplasia (BPH) is a progressive disease of older men, and more than 50% of men aged in the 50s are affected by BPH (Berry et al., 1984). The patients are diagnosed by non-malignant enlargement of the prostate gland and developed lower urinary tract symptoms (LUTS) leading to increased urinary frequency, slow and weak stream, terminal dribble, and sense of incomplete emptying. Other than surgical treatments recommended for the severe cases, α_{1A}-adrenoceptor blockage and 5α-reductase inhibition are major targets for pharmaceutical interventions. However, due to the severe side effects of androgen blockade, including gynaecomastia and diarrhea, 5α-reductase inhibitor such as finasteride has been clinically used (Dutkiewics, 2001) recently. Steroid 5α-reductase converts testosterone into 5α-dihydrotestosterone in the presence of NADPH (nicotinamide adenine dinucleotide phosphate) (Fig. 1), and the steroid 5α-reductase inhibitor lowers available cellular concentration of androgen, 5α-dihydrotestosterone. However, currently prescribed drugs have also shown serious side effects such as reduced libido, impotence, and increased risk of prostate cancer (Polat et al., 1997). Therefore, discovery of new steroid 5α-reductase inhibitors is sought. In some countries, phytotherapy-utilizing plant extracts been a choice for BPH treatment due to lack of side effects (Buck, 1996).

We have been studying natural product chemistry of *Kaempferia parviflora* (Wongsrikaew et al., 2011; 2012). This perennial plant belongs to a Zingiberaceae family, and the rhizome part has been used as a traditional folk medicine in southeastern Asian countries for the treatments of gout, aphthous ulcer, abscesses, and allergy (Yenjai et al., 2004). Interestingly, *K. parviflora* has also been used as an aphrodisiac herb by local people, and Chaturapanich et al. (2008) claimed it enhanced male function by rectifying impotence. Therefore, we tested steroid 5α-reductase inhibition by *K. parviflora* extracts prepared with EtOH and supercritical CO_2 fluid as solvents. Homogenate of the ventral prostate of male Sprague-Dawley rats was used for the steroid 5α-reductase inhibition study (Kim et al., 2012; Supplementary Material). Whereas the ethanol extracts (0.32 mg/mL) inhibited steroid 5α-reductase by 83.3±5.5%, the supercritical extract showed 100% inhibition at the same concentration. Because it is known that polymethoxyflavones (PMFs) are the only components of the supercritical extract from our previous study (Wongsrikaew et al., 2011), we decided to compare the steroid 5α-reductase inhibitions of each PMF from *K. parviflora*. PMF compounds were isolated by conventional column chromatography (Supplementary Material), and tested again for the inhibition study. Structurally related eleven PMFs showed wide range of steroid 5α-reductase inhibitory effects (Table 1). Most PMFs, except 5-hydroxy-3,7,4'-trimethoxyflavone (7), inhibited steroid 5α-reductase significantly. Especially, 5-hydroxy-7,4'-dimethoxyflavone (5) showed 20 μM of IC_{50}, which was comparable to the *in vitro* positive control,

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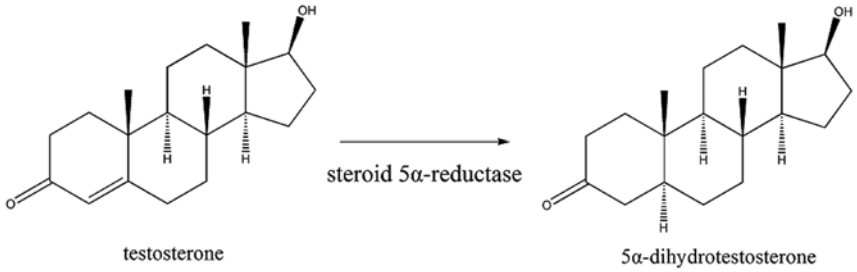

Fig. 1 Testosterone reduction by steroid 5α-reductase.

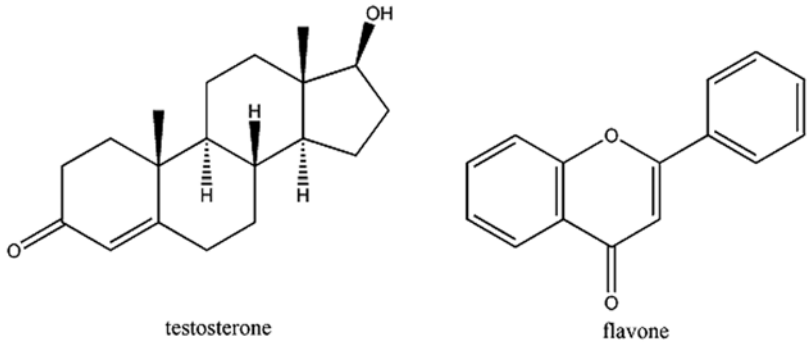

Fig. 2 Molecular structures of testosterone and flavone.

riboflavin (Nakayama et al., 1990).

Qualitatively, hydroxyl group at C-5 and methoxy group at C-4' position of flavone skeleton appeared to be essential for steroid 5α-reductase inhibition. 5-Hydroxy-7,4'-dimethoxyflavone (5) showed strong activity, whereas structurally similar 5,7,4'-trimethoxyflavone (2) and 5-hydroxy-7-methoxyflavone (4) showed no and marginal inhibitions, respectively. In addition, structural comparison of the flavone B-ring revealed that PMFs with two methoxy groups at the 3',4'-position enhanced the inhibitory effect, compared to the PMFs with one methoxy group at the 4'-position. For examples, 5,7,3',4'-tetramethoxyflavone (3), 5-hydroxy-3,7,3',4'-tetramethoxyflavone (8), and 3,5,7,3',4'-pentamethoxyflavone (11) were more effective than 5,7,4'-trimethoxyflavone (2), 5-hydroxy-3,7,4'-trimethoxyflavone (7), and 3,5,7,4'-tetramethoxyflavone (10), respectively.

When the molecular structures of testosterone and flavone were compared with each other, both structures could properly be superimposed (Fig. 2). Hence, PMFs molecules are suggested to bind at the substrate binding site of steroid 5α-reductase and inhibit its reaction.

While studying the phytoestrogenic activity and biotransformation of flavonoids (Kim et al., 2009; 2010a; 2010b; Park et al., 2011), we extended our interest to steroid 5α-reductase inhibition by PMFs. Although plant extracts containing polyphenols, synthetic derivatives, and isoflavonoids were tested (Hiipakka et al., 2002; Kumar et al., 2011; Bae et al., 2012). To the best of our knowledge steroid 5α-reductase inhibition by PMFs has never been reported. In this communication, we reported steroid 5α-reductase inhibition

Table 1 Steroid 5α-reductase inhibition by polymethoxyflavones (PMFs)

Compound	R_1	R_2	R_3	R_4	IC50 (μM)
5,7-Dimethoxyflavone (1)	H	OCH_3	H	H	880
5,7,4'-Trimethoxyflavone (2)	H	OCH_3	H	OCH_3	>1000
5,7,3',4'-Tetramethoxyflavone (3)	H	OCH_3	OCH_3	OCH_3	290
5-Hydroxy-7-methoxyflavone (4)	H	OH	H	H	470
5-Hydroxy-7,4'-dimethoxyflavone (5)	H	OH	H	OCH_3	20
5-Hydroxy-3,7-dimethoxyflavone (6)	OCH_3	OH	H	H	960
5-Hydroxy-3,7,4'-trimethoxyflavone (7)	OCH_3	OH	H	OCH_3	>1000
5-Hydroxy-3,7,3',4'-tetramethoxyflavone (8)	OCH_3	OH	OCH_3	OCH_3	210
3,5,7-Trimethoxyflavone (9)	OCH_3	OCH_3	H	H	200
3,5,7,4'-Tetramethoxyflavone (10)	OCH_3	OCH_3	H	OCH_3	440
3,5,7,3',4'-Pentamethoxyflavone (11)	OCH_3	OCH_3	OCH_3	OCH_3	250
Riboflavin (positive control)					5

by PMFs and discussed structural requirements for the activity. Lam et al. (2011) reported that steroid 5α-reductase inhibitors also have a potential application for the treatment of androgenetic alopecia (male pattern hair loss). Therefore, further study on PMF could result in the lead compound for the treatment of BPH and androgenetic alopecia.

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