Benign prostatic hyperplasia (BPH), a ubiquitous condition in aging males with susceptibility increasing from ~50% at age 60 to ~90% at age 85, may cause severe lower urinary tract symptoms (LUTS) leading to significant erosion of the quality of life (QoL). The available treatments like 5α-reductase inhibitors reduce these risks to some extent but provide slow symptomatic relief and require long treatment periods, often accompanied with undesirable side-effects. Phytotherapy is increasingly being preferred by BPH patients because of its minimal side-effects, safety in long-term use and effective improvement of urologic symptoms and flow measures. Clinical and experimental researches published till date clearly demonstrate that both androgens and estrogens play a crucial role in the normal development and functioning of prostate, as well as in the pathogenesis of prostatic diseases (BPH and CaP). However, management strategies mostly focus on disruption of signaling through the androgen receptor (AR). Since a critical balance between AR and ER signaling is now known to exist in prostatic cells for their normal proliferation and differentiation, CaP treatments targeting one set of receptors (e.g. AR) often result in the disturbance of this crucial equilibrium. Hence dual targeting of AR and ER is required for the therapeutic approaches towards prostatic hyperplasia. The present investigation is focused on discovering novel agents that could be used as therapeutic candidates for benign hyperplasia and/or cancer of the prostate. For a better correlation of data, the work has been divided into three major experimental studies summarized as below-

1. The first study involves scientific validation of anti-Benign Prostatic Hyperplasia (BPH) activity in natural compound(s) from the fruit extract of Cupressus sempervirens (CS), which has been traditionally used to treat BPH-like urinary symptoms in patients. The ethanolic fruit-extract of CS (CDRI code-4697, CS-EtOH) inhibited proliferation of human BPH-stromal cells and the activity was localized to
Investigation of novel synthetic molecules and natural products for prostatic hyperplasia

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Summary

its chloroform-soluble, diterpene-rich fraction (CS-EtOH-Chlor). Eight major diterpenes isolated from this fraction exhibited moderate to potent activity and the most active diterpene (labda-8(17),12,14-trien-19-oic acid); Compound B exhibited an IC₅₀ of 37.5 µM. It significantly inhibited activation (phosphorylation) of Signal transducer and activator of transcription 3 (Stat-3) in BPH-stromal cells and prevented transactivation of androgen sensitive Kallikrein-3 popularly known as Prostate-specific antigen (KLK3/PSA) and Transmembrane protease, serine 2(TMPRSS2) genes in LNCaP cells. Labda-8(17),12,14-trien-19-oic acid-rich CS-fraction prevented prostatic hyperplasia in rat model and caused TUNEL-labeling of stromal cells with lower expressions of IGF-I, TGF-β and PCNA, and bcl-2/bax ratio. Human BPH tissues exhibited precise lowering of stromal component after incubation in labda-8(17),12,14-trien-19-oic acid, ex vivo. On the basis of these results we conclude that labda-8(17),12,14-trien-19-oic acid contained in CS exhibits anti-BPH activity through inhibition of stromal-proliferation and suppression of androgen-action in the prostate, presenting a unique lead structure for further optimization of anti-BPH activity.

2. The second objective was to investigate and evaluate novel series of molecules that were designed with dual androgen receptor (AR) and estrogen receptor (ER) modulating capability by coalescing the crucial chemical moieties of some known anti-CaP molecules that act via modulation of AR and/or ER, viz. 3,3′diindolyl methane (DIM), mifepristone, toremifene, tamoxifen and raloxifene. N,N-diethyl-4-((2-(4-methoxyphenyl)-1H-indol-3-yl) methyl) aniline (DIMA) was identified as a novel molecule with most promising activity. DIMA increased annexin-V labelling, cell-cycle arrest and caspase-3 activity in LNCaP cells, which was accompanied by decreased expressions of the androgen receptor and the prostate specific antigen. On the other hand, DIMA increased ER-β, p21 and p27 protein levels in LNCaP cells in vitro and exhibited ~5 times more selective binding for ER-β than ER-α, in comparison to raloxifene. DIMA exhibited dose dependent ER-β agonism and ER-α
antagonism in classical gene reporter assay and decreased hTERT (catalytic subunit of telomerase) transcript levels in LNCaP at 3.0 μM (P<0.05). DIMA also dose dependently decreased telomerase activity in prostate cancer cells. Conclusively we can state that DIMA acts as a multi-steroid receptor modulator and effectively inhibits proliferation of prostate cancer cells through ER-β mediated cellular senescence, by countering actions of AR and ER-α. Its unique molecular design can serve as a lead structure for a new generation of potent agents against endocrine malignancies like the CaP.

3. The next study investigated the effectiveness of DIMA in BPH condition (in vitro and in vivo). DIMA exhibited a better activity profile against human BPH stromal cells in comparison to the well-known marketed compounds like Tamoxifen and Toremifene. In vivo administration of DIMA; markedly reversed the condition of BPH in rat model by extreme thinning of the prostatic epithelium, increase in acinar volume, disappearance of epithelial invagination, expansion of tubular area and reduction in stromal area. DIMA prevented prostatic hyperplasia in rat model and caused TUNEL-labeling of stromal cells with lower expressions of ER-α, IGF-I, TGF-β and PCNA, and the bcl-2/bax ratio, with increased expression of ER-β. Circulating hormone levels in the blood of treated as well as control BPH rats exhibits differential levels of steroid hormones presenting increased E2/T+DHT ratio in BPH rats (citral) and proficient decreased ratio in DIMA-treated in rats.

In summary, the study has discovered, characterized and mechanistically demonstrated a natural compound B (labda-8(17),12,14-trien-19-oic acid) and a synthetic compound created by ingenious drug-design DIMA in the rat model of prostatic hyperplasia, human ex vivo model of benign prostatic hyperplasia(stromal cells and tissues-explants) and human prostate cancer cell lines mimicking CaP in vitro. The studies have provided firm evidence that K031, a natural diterpene, interferes with BPH-stromal cell proliferation and supresses androgen action in prostate with precise inhibition of STAT3 phosphorylation. On the other hand DIMA, a rationally synthesized molecule, effectively inhibits the proliferation of Prostate cancer as well
as BPH cells by ER mediated cellular senescence via ability to refute the effects of AR and ERα. DIMA also changes the level of circulating steroid sex hormones to correct the circulating hormonal imbalance causing prostatic hyperplasia. In conclusion, K031 and DIMA, can serve as a promising lead molecules for further optimization and evaluation as promising therapeutic agents for the management of prostatic hyperplasia and prostate cancer.

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