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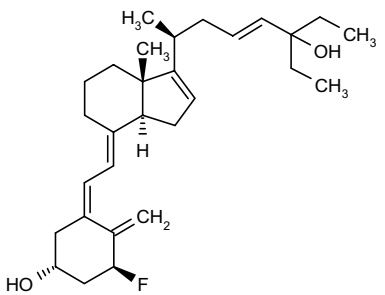
# Elocalcitol

Prop INN

*Vitamin D Receptor Agonist*
*Treatment of Benign Prostatic Hyperplasia*
*Treatment of Overactive Bladder*

BXL-628
Ro-26-9228

(23*E*)-1α-Fluoro-25-hydroxy-16,17,23,24-tetradehydro-26,27-bishomo-20-epivitamin $D_3$
(23*E*)-1α-Fluoro-25-hydroxy-16,17,23,24-tetradehydro-26,27-bishomo-20-epicholecalciferol




$C_{29}H_{43}FO_2$
Mol wt: 442.6489

CAS: 199798-84-0

EN: 259480

**Abstract**

Calcitriol is the active hormonal form of vitamin $D_3$, which regulates calcium and phosphorus levels in the blood and certain calcium-dependent processes, including bone formation, cell proliferation and differentiation, apoptosis and elements of the immune system. Calcitriol has shown potential in the treatment of various clinical conditions such as osteoporosis, secondary hyperparathyroidism, certain autoimmune diseases and cancer, but its therapeutic utility is limited by hypercalcemia at therapeutic concentrations. Elocalcitol is an analogue of calcitriol that possesses the desirable properties of calcitriol but does not cause hypercalcemia. Elocalcitol is in development for benign prostatic hyperplasia (BPH) and overactive bladder (OAB).

## Synthesis

Elocalcitol can be synthesized as follows:

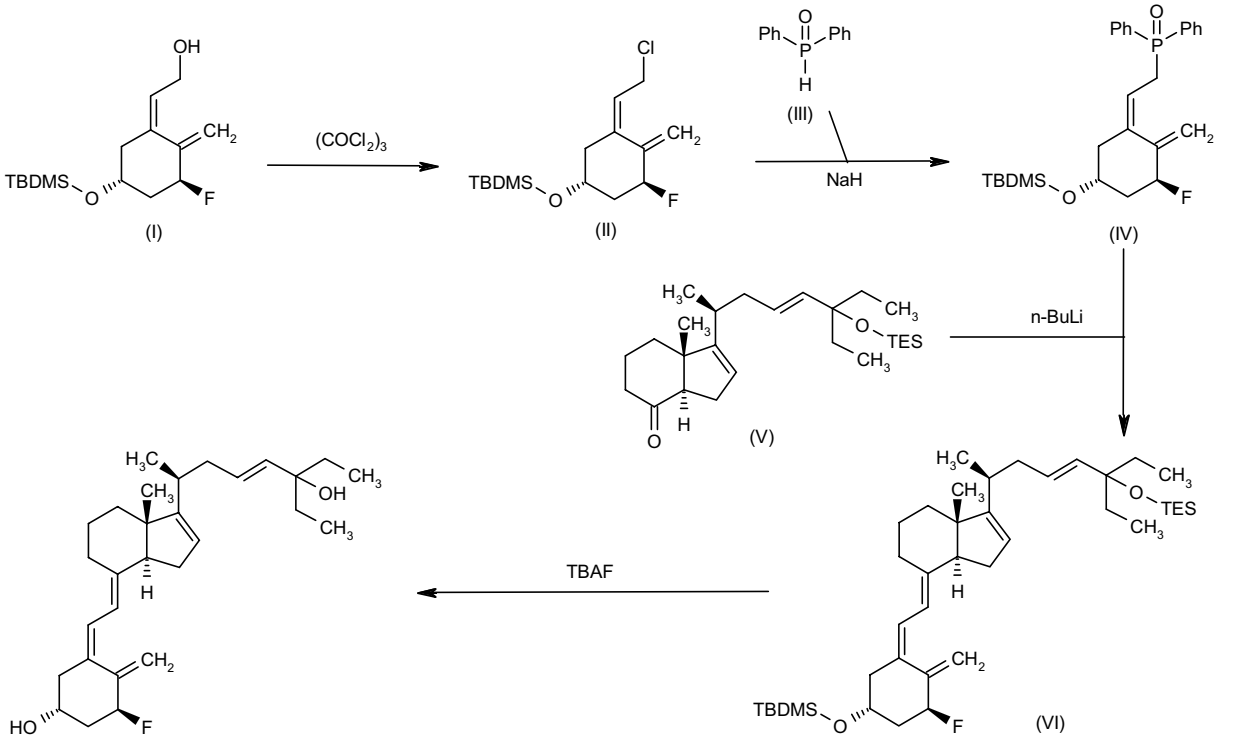
The reaction of the cyclohexylidene ethanol (I) with triphosgene gives the corresponding alkyl chloride (II), which is condensed with diphenylphosphine oxide (III) by means of NaH in DMF to yield the alkyldiphenylphosphine oxide (IV) (1). The condensation of (IV) with the hexahydroindenone intermediate (V) by means of BuLi in THF affords the disilylated precursor (VI), which is finally deprotected by means of TBAF in THF (1, 2). Scheme 1.

The indenone intermediate (V) is obtained as follows. The silylation of 3-ethyl-1-pentyn-3-ol (VII) with chlorotriethylsilane and DMAP in DMF gives the silyl ether (VIII), which, after metalation with *n*-BuLi in THF, is condensed with paraformaldehyde to yield the propargyl alcohol (IX). Partial reduction of (IX) with Red-Al in THF affords the allyl alcohol (X), which is treated with tosyl chloride and DMAP in dichloromethane to provide the allyl chloride (XI). The condensation of (XI) with the unsaturated ester (XII) (obtained by Wittig reaction of perhydroindanone [XIII] with triethyl phosphonoacetate [XIV] in the presence of NaOEt) by means of BuLi and dicyclohexylamine in HMPA gives the octenoic ester (XV), which is reduced with $LiAlH_4$, yielding the corresponding primary alcohol (XVI). The reaction of (XVI) with *p*-toluenesulfonyl chloride and pyridine affords the tosylate (XVII), which is reduced with $LiBHEt_3$ in THF to provide the hexahydroindenol (XVIII). Finally, this compound is oxidized with pyridinium dichromate (PDC) to yield the target hexahydroindenone intermediate (V) (3). Scheme 2.

The preparation of intermediate (I) has been reported utilizing (*S*)-carvone (XIX) as the starting material. Aldol condensation of ketone (XIX) with *tert*-butyl acetate in the presence of LDA and catalytic $CeF_3$ leads to the hydroxy ester (XX), which undergoes regioselective epoxidation to (XXI) by means of *tert*-butyl hydroperoxide and vanadyl acetylacetonate. Ozonolysis of the isopropenyl group of (XXI) in cold MeOH, followed by reductive workup with dimethyl sulfide, produces the acetyl derivative (XXII), which is subjected to Baeyer-Villiger oxidation with *m*-CPBA in EtOAc/hexane, yielding the acetate ester

P. Revill, N. Serradell, J. Bolós. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

**Scheme 1: Synthesis of Elocalcitol**




(XXIII). After methanolysis of acetate (XXIII), the resulting secondary alcohol (XXIV) is selectively protected as the corresponding silyl ether (XXV) employing TBDMS-Cl and imidazole. Subsequent dehydration of the tertiary alcohol (XXV) with $SOCl_2$ in cold pyridine gives the unsaturated ester (XXVI) with the undesired (*E*)-configuration. When the palladium-catalayzed isomerization of epoxide (XXVI) is conducted in the presence of 1,1,1,3,3,3-hexafluoro-2-phenyl-2-propanol, the allylic alcohol (XXVII) is obtained as the major compound. Then, fluorination of (XXVII) with DAST in trichloroethylene provides the $\alpha$-fluoride (XXVIII), which undergoes ester group reduction to the alcohol (XXIX) in the presence of DIBAL. Photochemical double bond isomerization of (XXIX) in *tert*-butyl methyl ether employing 9-fluorenone as photosensitizer furnishes the desired (*Z*)-allylic alcohol (I) (4). Scheme 3.

## Background

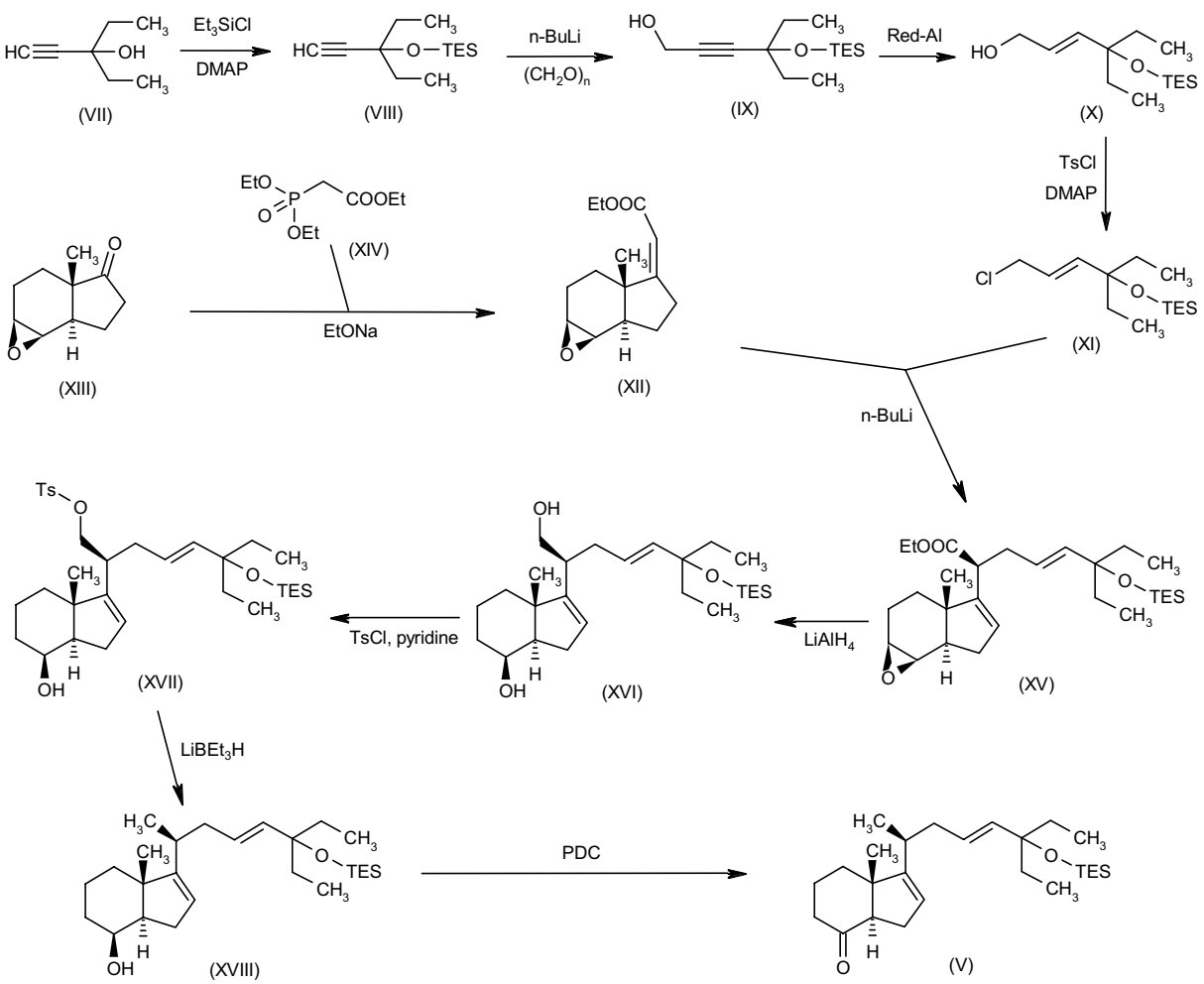
The active metabolite of vitamin $D_3$, 1,25-dihydroxyvitamin $D_3$ (calcitriol), has classically been known as a regulator of calcium and phosphorus homeostasis and bone metabolism. The hormone targets the vitamin D receptor (VDR) from the steroid/thyroid hormone/retinoid nuclear receptor superfamily which has been shown to be present in a number of other tissues and to regulate many other processes, including cell proliferation and differentiation, apoptosis and the dendritic cell and T-cell components of the immune system. Calcitriol and its analogues have demonstrated therapeutic potential in a broad range of conditions, including osteoporosis, secondary hyperparathyroidism, cancer, urological disorders such as benign prostatic hyperplasia (BPH), prostatitis and interstitial cystitis, and in conditions associated with chronic inflammation, including psoriasis. However, calcitriol is also associated with side effects such as hypercalcemia, limiting its therapeutic use, and significant effort has been concentrated on the identification of synthetic analogues with a wider therapeutic index (5-8).

Elocalcitol (BXL-628, Ro-26-9228) is a new analogue of calcitriol that possesses the desirable properties of the natural hormone but is devoid of a hypercalcemic effect. Elocalcitol is in phase II clinical trials for BPH/lower urinary tract symptoms (LUTS) and overactive bladder (OAB) (7, 9-12).

## Preclinical Pharmacology

Using a rat model of ovariectomy-induced osteoporosis, elocalcitol was identified as having a bone-protecting effect, significantly increasing femoral bone mineral density (BMD) and inhibiting bone resorption at oral doses of 3 $\mu$g/kg/day and above, compared to 0.2 $\mu$g/kg/day for calcitriol. Moreover, elocalcitol did not induce hypercalcemia at up to the maximum tested dose of 14 $\mu$g/kg/day, whereas calcitriol induced hypercalcemia at the dose required for bone protection. The beneficial effects of elo-

**Scheme 2: Synthesis of Intermediate (V)**

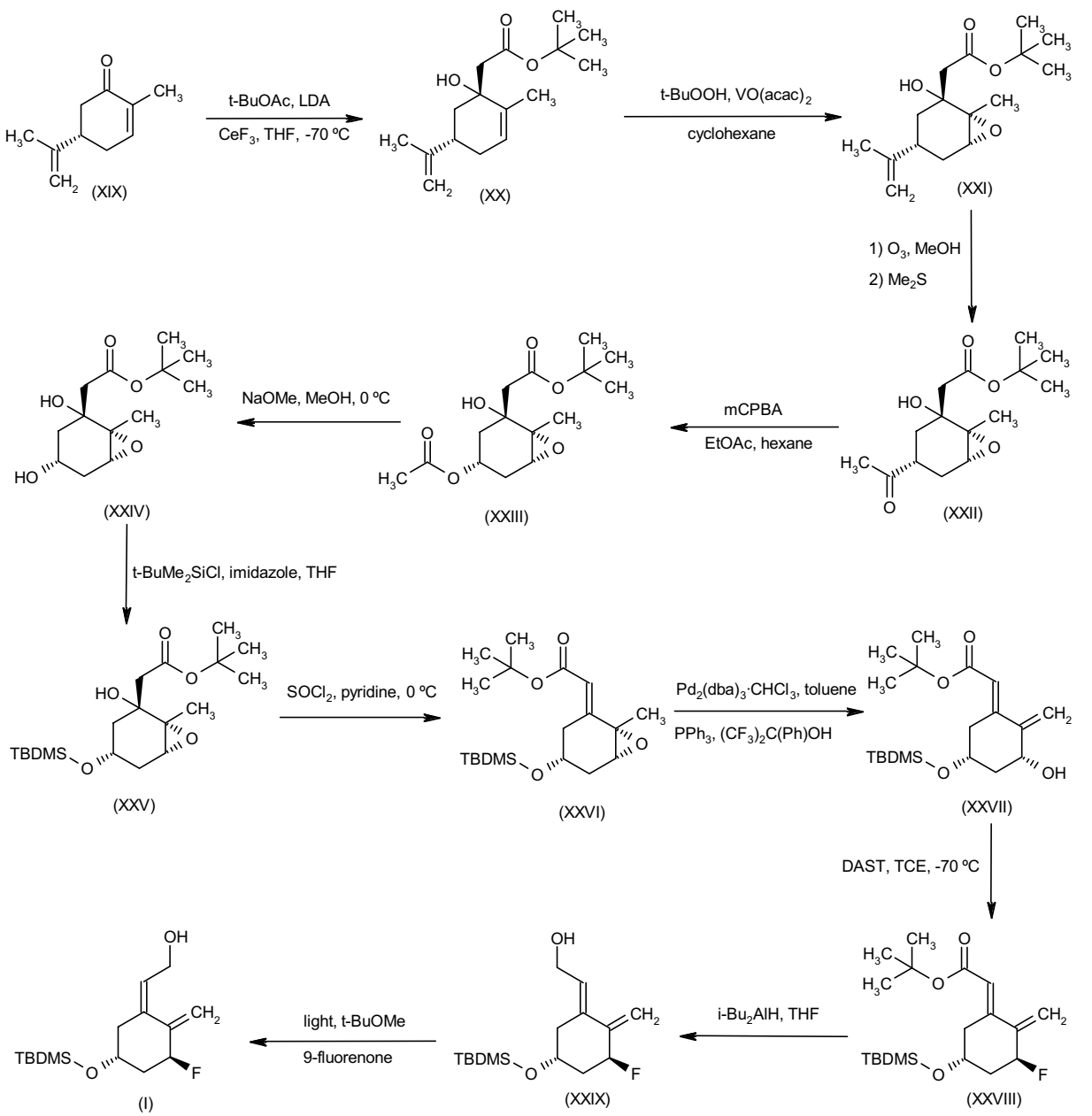



calcitol appeared to involve upregulation of osteoblast-specific genes and bone growth factors coupled with a lack of effect on genes in the duodenum, unlike calcitriol (12). Experiments in human osteoblast-like ROS 17/2.8 and hFOB cells and osteosarcoma MG-63 cells demonstrated that both calcitriol and elocalcitol promoted the expression of osteopontin and osteocalcin genes, with $ED_{50}$ values of 0.8-5.5 nM. On the other hand, in human intestinal Caco-2 cells, the $ED_{50}$ values for inducing osteocalcin gene expression were 2 and 120 nM, respectively, for calcitriol and elocalcitol, and respective values for inducing the expression of calbindin D 9K mRNA were 5-8 and 100-265 nM (12-14). The molecular mechanism of this selective action of elocalcitol has been suggested to involve differential activation of cellular VDR by the drug, affecting the interaction of elocalcitol-VDR complexes with co-activator proteins (14-17).

In rat models of BPH, elocalcitol (10-300 μg/kg p.o.) decreased prostate growth in intact rats and in castrated rats supplemented with testosterone, with similar potency to finasteride (10 and 40 mg/kg). Further experiments demonstrated that elocalcitol abrogated the proliferative effects of testosterone without affecting serum luteinizing hormone (LH) or testosterone levels. In prostate cells isolated from patients undergoing surgery, elocalcitol inhibited cell proliferation more potently than calcitriol ($-\log IC_{50}$ = 15.8 *vs.* 10.2) and induced apoptosis, even in the presence of testosterone (7, 18). Similarly, elocalcitol was antiproliferative and proapoptotic in stromal cells isolated from the bladder neck of patients undergoing surgery for BPH, with $-\log IC_{50}$ values against basal, testosterone- and growth factor-stimulated growth of 13.9-14-8. This effect was associated with a decreased expression of markers of smooth muscle remodeling. These findings supported the potential of elocalcitol for alleviating BPH-related lower urinary tract symptoms (LUTS), as well as OAB (7, 8).

In a female rat model of bladder outlet obstruction, bladder wall hypertrophy was induced to an equal extent

**Scheme 3: Synthesis of Intermediate (I)**




in rats treated with either elocalcitol or vehicle. However, in the elocalcitol-treated rats contractile function was maintained, whereas function decreased in the vehicle-treated animals. *Ex vivo* analysis of bladder muscle strips showed that the bladder muscle of elocalcitol-treated rats remained responsive to nerve stimulation, whereas that from vehicle-treated rats had reduced contractile responses. These results showed that although elocalcitol does not prevent the bladder hypertrophy associated with outlet obstruction, it does have the potential to preserve bladder function (19).

Results from *in vitro* and *in vivo* studies in human and rat bladder showed that elocalcitol inhibits RhoA/Rho kinase (ROK) signaling, further supporting its potential in the treatment of OAB and LUTS (20, 21).

Elocalcitol was also evaluated in a model of experimental autoimmune prostatitis in nonobese diabetic (NOD) mice. Administration of nonhypercalcemic doses (100 μg/kg p.o. 5 days/week for 2 weeks) reduced leukocyte infiltration into the prostate and the levels of the proinflammatory cytokines interferon gamma and IL-17 in T-cells. Cell proliferation was decreased and apoptosis

increased (22-24). Nerve-induced contractile responses of prostate preparations were restored to levels comparable to in control mice following treatment with elocalcitol (25).

In two rodent models of chronic cystitis (ovalbumin- and cyclophosphamide-induced cystitis), oral treatment with elocalcitol at nonhypercalcemic doses reduced bladder inflammation, improved bladder capacity and reduced the number and amplitude of nonvoiding bladder contractions. Fewer leukocytes infiltrated the bladder tissue of the elocalcitol-treated animals, and the expression and release of inflammatory markers, including mast cell proteases, were reduced (26, 27).

As a demonstration of the immunomodulating properties of elocalcitol, the agent inhibited acute and chronic allograft rejection in two mouse models, and was more potent than calcitriol. In the vascularized heart transplantation model of acute rejection, elocalcitol extended the mean survival time from 9 days on vehicle to 22 days, and in the aortic transplant model of chronic rejection, elocalcitol caused an 80% reduction in vascular intimal thickening compared with vehicle controls. This latter effect was associated with a reduction in leukocyte recruitment to the site of the allograft (most notably $CD11b^+$ and $CD11c^+$ cells) and reduced expression of genes involved in muscle development in the allograft tissue (28).

The potential of elocalcitol for the treatment of androgen-independent prostate cancer was suggested by results from experiments using cultures of the androgen-independent human prostate cancer cell line DU 145. Cells were incubated with keratinocyte growth factor (KGF) in the presence or absence of elocalcitol. The growth factor stimulated both the proliferative and invasive capacity of these cells, and brief exposure to elocalcitol suppressed these effects. Further mechanistic studies showed that the agent inhibited autophosphorylation of the KGF receptor, and this in turn prevented activation of the downstream phosphatidylinositol 3-kinase (PI3K)/Akt cell survival pathway (29, 30).

## Clinical Studies

In a double blind, randomized study of the effects of elocalcitol in postmenopausal osteoporotic women, 101 subjects received 1.2 g/day calcium and 400 IU vitamin D/day together with elocalcitol (150 μg/day p.o.) or placebo for 90 days. Elocalcitol treatment was associated with a significant increase in whole-body BMD, a decrease in markers of bone formation and resorption, and no change in serum calcium content compared to placebo. At day 30, the 24-h urine calcium content had increased by 33% compared to 6% in the control group (31).

In a multicenter, double-blind, parallel-group phase IIa study of the efficacy and safety of elocalcitol in the treatment of patients with BPH, 119 men aged 50 years or over and with a prostatic volume of 40 ml or more were randomized (1:1) to receive elocalcitol (150 μg once daily) or placebo for 12 weeks. Active treatment was associated with a 2.9% reduction in prostate volume, whereas the prostate continued to increase in size in the placebo-treated group (+4.3%). The prevalence of adverse events was 5.3%, with no significant difference between the placebo and the active treatment groups. There were no serious events in the active treatment group, and no patient dropped out due to adverse events. Neither serum nor urinary calcium levels changed significantly in either group from baseline to the end of therapy. There was no effect of active treatment on hormone levels or sexual function (7, 32, 33). BioXell is currently conducting a phase IIb trial of elocalcitol for the treatment of BPH (9-11).

In a placebo-controlled, double-blind phase IIa trial, 114 patients with OAB were treated with elocalcitol for 3 months, which led to a 21.5% increase in the mean volume voided per micturation (the primary endpoint) compared to a 10.9% increase in patients on placebo. The agent was well tolerated (9). A phase IIb clinical trial is planned (10, 11).

Preliminary results from a randomized, double-blind, placebo-controlled, parallel-group phase IIa study in 129 patients with nonbacterial chronic prostatitis/chronic pelvic pain syndrome indicated that elocalcitol was no different from placebo with respect to the primary endpoint of pelvic pain. However, there was a 55% reduction in the number of patients on active treatment needing to urinate > 10 times/day, compared with a 14% reduction in the placebo group. Levels of the proinflammatory cytokine IL-8 were also significantly reduced in the seminal fluid of patients in the active treatment group. Although elocalcitol will not be developed further for urological conditions where pain is a central component, the results from the secondary endpoint analysis were considered encouraging for the ongoing trials of elocalcitol in BPH and OAB (11, 23).

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