

# Vitamin D analogs as anabolic agents

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Several vitamin D metabolites and analogs, including  $1\alpha,25$ -dihydroxyvitamin  $D_3$  [ $1\alpha,25(OH)_2D_3$ ], have been investigated for efficacy in stimulating bone formation and increasing bone mass in animal models and osteoporotic patients.  $1,25(OH)_2D_3$  (calcitriol) and its prodrug,  $1\alpha$ -hydroxyvitamin  $D_3$  (alfacalcidol), have been reported to reduce vertebral and hip fractures in postmenopausal and senile osteoporosis patients. Sairanen et al.<sup>1</sup>, reported that  $1,25(OH)_2D_3$  (calcitriol) treatment for 4 years increases bone mineral density in lumbar spine and femoral neck in postmenopausal women resulting from reduced bone turnover (decreased serum bone markers of both formation and resorption). However, there is increasing evidence that vitamin D analogs may directly stimulate osteoblastic bone formation. Not only do osteoblasts possess abundant vitamin D receptors, but also calcitriol and alfacalcidol have been demonstrated to stimulate bone formation and increase bone mass and strength in animal models of osteopenia<sup>2-7</sup>. Due to hypercalcemia and hypercalciuria, calcitriol and alfacalcidol have a very narrow therapeutic window for the treatment of osteoporosis. Extensive research has focused on the identification of structurally distinct, novel vitamin D analogs that have an increased ability to stimulate bone formation and an improved therapeutic index with regard to increased urinary or serum calcium levels<sup>8-18</sup>.

Over 1,000 secosteroidal vitamin D analogs have been synthesized and characterized in the literature. It was reported that Ro-26-9228, a vitamin D analog, increased osteoblast surface and bone mass and inhibited bone resorption in an OVX rat model at doses that did not

increase serum and urine calcium<sup>9</sup>. ED-71 [ $2\beta$ -(3-hydroxypropoxy)- $1\alpha,25$ -dihydroxyvitamin  $D_3$ ], another vitamin D analog, was reported to restore bone mass back to sham control levels by maintaining bone formation and inhibiting bone resorption at 2 and 4 weeks post-OVX rats given daily treatment for 3 months<sup>10</sup>. However, markers of both resorption and formation were decreased at all time points in a recently published 1-year clinical study with ED-71<sup>11</sup>. Recently, a great number of non-secosteroidal VDR agonists have been reported in the scientific and patent literature<sup>8,14-18</sup>. Screening strategies have focused on evaluating the potential therapeutic index in cell models, typically Caco2 as a representative intestinal cell line, and an osteoblast cell line such as ros or MG63. Tissue selectivity has exceeded 1000-fold for bone versus intestine in these cell models, as for example with Lilly WO2004048309<sup>16</sup>. Chugai reported bone efficacy in the absence of changes in serum calcium in an OVX rat model with two novel non-secosteroid analogs, although from the data reported it is difficult to assess the magnitude of the TI observed *in vivo*<sup>18</sup>.

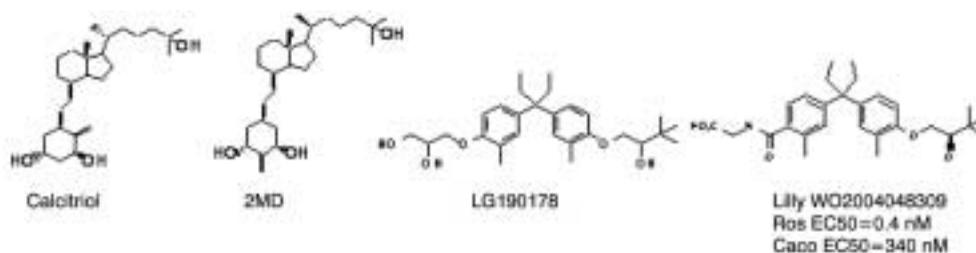
We have extensively characterized a new analog of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  discovered by Hector DeLuca and Deltanoid Pharmaceuticals, 2-methylene-19-nor-(20S)- $1\alpha,25(OH)_2D_3$  in an OVX rat model (abbreviated as 2MD, Figure 1). 2MD binds to the vitamin D receptor (VDR) with a high affinity, comparable to that of the endogenous ligand,  $1\alpha,25(OH)_2D_3$ <sup>12</sup>. However, 2MD is approximately 10-50 times more potent than calcitriol in transcriptional reporter cell assays using vitamin D responsive promoters in an osteoblastic cell line, MC3T3. 2MD is also vastly more potent and efficacious in stimulating mineralization of primary human osteoblasts in culture<sup>12</sup>.

In a long-term, dose-response study with 2MD using 0.5 ng/kg/d, 1 ng/kg/d, 2.5 ng/kg/d, 5 ng/kg/d and 10 ng/kg/d<sup>13</sup> 2MD significantly and dose-dependently increased total body bone mineral density, total body bone mineral content, bone density and total bone content of the distal femoral metaphysis (DFM), and trabecular bone volume of LV3. Bone strength testing revealed that 2MD significantly and dose-dependently increased maximal load and stiff-

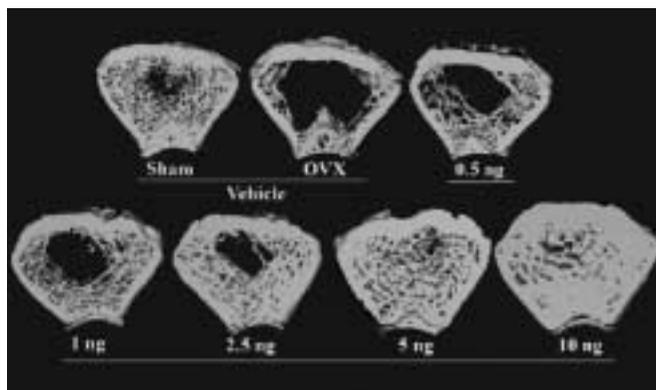
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**Figure 1.** Chemical structures of calcitriol and vitamin D analogs.



**Figure 2.** Characterization of a new analog of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  discovered by Hector DeLuca and Deltanoid Pharmaceuticals, 2-methylene-19-nor-(20S)- $1\alpha,25(OH)_2D_3$  in an OVX rat model, abbreviated as 2MD.  $\mu$ CT images of distal femur metaphases.

ness of femoral shaft (FS), maximal load and stiffness of femoral neck, and toughness, ultimate strength and stiffness of the fifth lumbar vertebral body (LV5). At doses of 0.5 ng/kg/d and 1 ng/kg/day many parameters had fully restored to sham control levels, whereas at doses greater than 2.5 ng/kg/d, most of the bone mass and bone strength related parameters were significantly higher in 2MD-treated OVX rats compared with sham controls. Serum calcium did not change significantly with 2MD at 0.5 or 1 ng/kg/d, while it was significantly increased at 2.5, 5 or 10 ng/kg/d. Bone histomorphometric analysis showed dose-dependent decreases in osteoclast number and osteoclast surface on trabecular bone surface, and a dose-dependent increase in periosteal bone formation associated with 2MD treatment.

In summary, in rodent pre-clinical models certain vitamin D analogs can, as illustrated in our studies with 2MD, restore trabecular and cortical bone mass and strength by stimulating periosteal bone formation and decreasing trabecular bone resorption in ovariectomized (OVX) rats with established osteopenia. These results indicate that vitamin D analogs such as 2MD may have therapeutic potential in human skeletal disorders such as osteoporosis.

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