

# Identification of biphenyl-carboxylic acid derivatives as a novel class of bone resorption inhibitors

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Many common diseases of the skeleton including osteoporosis, Paget's disease, and cancer-associated bone disease are characterised by excessive bone loss due to increased osteoclastic bone resorption. Whilst several inhibitors of osteoclastic bone resorption are available for clinical use, they are incompletely effective and fall into few mechanistic classes, indicating the need to identify novel antiresorptive agents. We have developed a series of butanediol ester derivatives that have potent inhibitory effects on osteoclastic bone resorption in vitro and on ovariectomy induced bone loss in vivo.

Compounds were initially tested on mouse osteoblasts and osteoclasts in vitro. Osteoblast viability was measured using Alamar Blue, and osteoclasts were identified by TRAcP staining. Bone resorption was measured in co-cultures performed on dentine slices, using a reflected light microscope linked to an image analysis system. The most potent compounds were finally tested in the murine ovariectomy (OVX)-induced bone loss system. Bones were analysed using a Skyscan 1072  $\mu$ CT scanner.

The most potent compound tested was the butanediol ester of biphenyl carboxylic acid (ABD056), which inhibited osteoclast formation and activity with a half maximal effect at 22  $\mu$ M. ABD056 did not affect osteoblast viability or alkaline phosphatase expression. Structure activity-analysis showed that the terminal hydroxyl moiety, 4-membered diol and the biaryl moieties of ABD056 were crucial for its activity. Treatment by IP injection with ABD056 (10 mg/kg/day) completely prevented OVX- induced bone loss in mice. However, oral administration of ABD056 (30 mg/kg/day) only partially prevented OVX-induced bone loss. This is probably due to the fact that ABD056 is an ester, and therefore susceptible to degradation by stomach acid and esterases. We therefore developed ABD068, where the ester bond in ABD056 is replaced by a non-hydrolysable ketone link. ABD068 was as potent as ABD056 in inhibiting osteoclast formation and activity in vitro, and had no inhibitory effects on osteoblast viability or differentiation. Unlike ABD056, oral administration of ABD068 (30 mg/kg/day) completely prevented bone loss in OVX mice.

In conclusion, ABD056 and ABD068 are potent osteoclast inhibitors that are effective both in vitro and in vivo. This identifies biphenyl carboxylic acid derivatives as a novel class of antiresorptive agents which may be of clinical value in the prevention and treatment of diseases characterized by increased osteoclastic bone resorption such as osteoporosis, cancer associated bone disease and Paget's disease of bone.