Drugs of the Future 2002, 27(10): 935-941

CCC: 0377-8282/2002

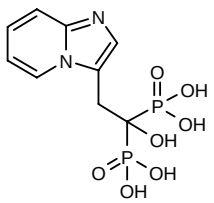
Minodronic Acid

Prop INN

Treatment of Osteoporosis
Treatment of Bone Metastasis
Treatment of Multiple Myeloma

Ono-5920
YH-529
YM-529

1-Hydroxy-2-(imidazo[1,2-*a*]pyridin-3-yl)ethane-1,1-bis(phosphonic acid)



$C_9H_{12}N_2O_7P_2$
Mol wt: 322.1488
CAS: 180064-38-4
CAS: 155648-60-5 (as hydrate)
EN: 160070

Abstract

Osteoporosis is the most common type of metabolic bone disease affecting an estimated 200 million women worldwide. The disorder is characterized by low bone mass and structural deterioration of bone tissue which leads to bone fragility and an increased risk for fractures. The majority of the agents currently available for the treatment of osteoporosis decrease bone resorption (*e.g.*, estrogens, selective estrogen modulators, calcitonin and bisphosphonates), although some agents increase bone formation (*e.g.*, fluoride and parathyroid hormone). Bisphosphonates are synthetic analogues of the endogenous mineral deposition inhibitor pyrophosphate and are the most widely used for the treatment of osteoporosis, Paget's disease and tumor-related bone disease. Some of these compounds have also shown efficacy against myeloma. Recently, minodronic acid was shown to have bone resorptive activity in a number of preclinical models. The agent also showed activity against tumor-induced osteolysis in rats and mice and antimyeloma effects *in vitro*. Due to its excellent preclinical activity profile, minodronic acid was selected for further development. and is currently undergoing phase III trials for the treatment of osteoporosis, multiple myeloma and bone metastasis of breast/lung cancer.

Synthesis

Cyclization of 2-aminopyridine (I) with 4-bromo-3-oxobutyric acid ethyl ester (II) by means of $NaHCO_3$ in hot dioxane gives 2-(imidazo[1,2-*a*]pyridin-3-yl)acetic acid ethyl ester (III), which is hydrolyzed at the ester function with KOH in ethanol to yield the free acid (IV) (1). Finally, the free acid (IV) is treated with phosphorous acid and phosphorous trichloride in either chlorobenzene at 110-5 °C (2, 3) or toluene at 80 °C (4), followed by hydrolysis with HCl (2-4). Scheme 1.

Introduction

Osteoporosis is a skeletal disorder characterized by low bone mass and structural deterioration of bone tissue resulting in bone fragility and increased susceptibility to fractures, particularly the hip, spine and wrist. It is the most common type of metabolic bone disease and a major health concern affecting 200 million women worldwide. An estimated 10 million Americans have osteoporosis with another 18 million suffering from low bone mass, thus having an increased risk for the disease. In addition, an estimated 1-2 million men in the U.S. have osteoporosis and 8-13 million suffer from low bone mass (5, 6).

Osteoporosis develops when the rate of bone resorption is too rapid or new bone formation is too slow. The process of modeling and remodeling of the skeleton is a highly controlled and specialized process where new bone is formed and old bone is resorbed so that the entire skeleton is renewed about every 10 years. However, after age 30, the rate of bone resorption exceeds that of new bone formation. By age 50, 1-3% of bone mass is lost by both men and women. In women, bone loss is apparent throughout the entire postmenopausal period, although it

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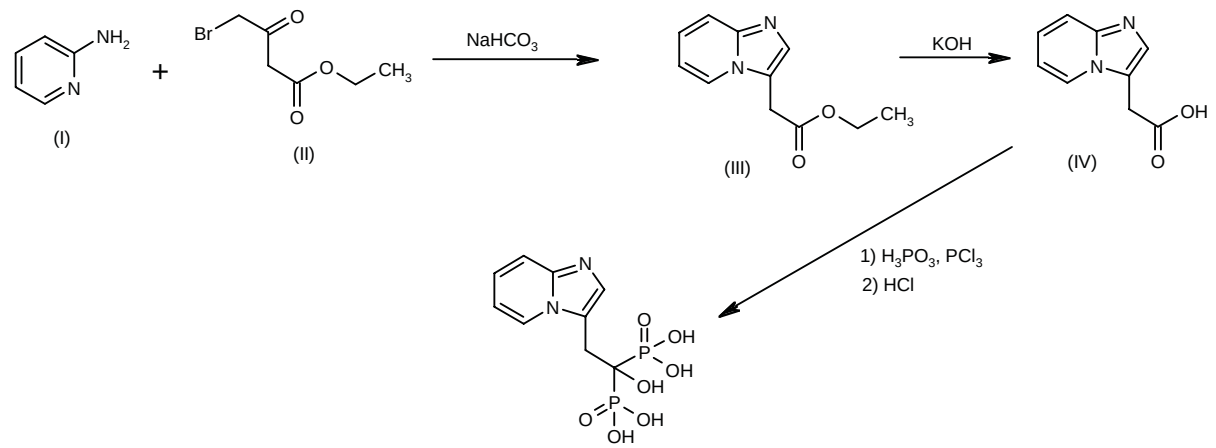
Scheme 1: Synthesis of Minodronic Acid



is highly accelerated during the first 5-10 years after menopause. The accelerated and severe bone loss that is evident in some postmenopausal women is classified as type I (postmenopausal) osteoporosis while that associated with normal aging in both women and men is referred to as type II (senile) osteoporosis. Type I and II osteoporosis are considered primary disorders related to decreased gonadal function or aging. However, osteoporosis may also be a secondary disorder occurring in patients administered glucocorticoids or other agents and in individuals suffering from other conditions such as malignancies, inflammatory bowel disease, hypothyroidism and cystic fibrosis (5, 7).

Osteoporosis can be prevented and is routinely averted through calcium and vitamin D supplementation combined with good nutrition, weight-bearing exercise and reductions in tobacco, alcohol and caffeine intake. However, restoration of bone loss in those patients with established osteoporosis is much more difficult. The majority of the agents currently available for the treatment of osteoporosis decrease bone resorption and they include estrogens, selective estrogen modulators, calcitonin and bisphosphonates. Other agents increase bone formation such as fluoride and parathyroid hormone (5).

Antiresorptive therapies have been shown to be particularly effective in the treatment of osteoporosis, although they generally do not induce formation of new bone. To date, bisphosphonates are the most widely used and effective antiresorptive agents. These compounds are synthetic analogues of the endogenous mineral deposition inhibitor pyrophosphate and have shown efficacy in the treatment of osteoporosis, Paget's disease and tumor-associated bone disease. Bisphosphonates prevent bone resorption and increase bone mineral density, although their exact mechanism of action has not been fully elucidated. Studies have shown that bisphosphonates are specifically internalized by osteoclasts where they inhibit osteoclast activity and induce apoptosis. Bisphosphonates such as clodronate and etidronate that are structurally similar to pyrophosphate are thought to be metabolized to cytotoxic analogues of ATP which are responsible for inhibition of osteoclast activity. Nitrogen-containing bisphosphonates such as pamidronate, ibandronate, alendronate and zoledronate induce osteoclast apoptosis by inhibiting enzymes of the mevalonate pathway (*e.g.*, farnesyl diphosphate [FPP]) and prevent isopenylation of small GTP-binding proteins such as Ras and Rho. Several bisphosphonates are currently available or under active development as a treatment for osteoporosis and other indications and are shown in Table I (5, 8-10).

In addition to their efficacy in bone disorders, bisphosphonates have also displayed potent antimyeloma activity. Although their direct mechanism of action on myeloma cells is not known, it appears that the agents reduce interleukin 6 (IL-6) production from myeloma and stromal cells, induce apoptosis of myeloma cells via reductions in mevalonic acid, inhibit angiogenesis and stimulate gammadelta T-cells (11-13).

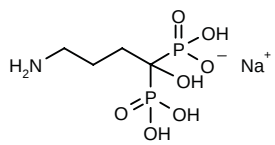
Recently, minodronic acid (YM-529, Ono-5920, YH-529), a third-generation aza-heteroarylbisphosphonate derivative, was synthesized and shown to have 100- to 1000-fold higher bone resorption activity than pamidronate in addition to activity against myeloma and tumor-associated osteolysis. Due to its excellent preclinical activity profile, minodronic acid was selected for further development as a treatment for osteoporosis as well as for the treatment of bone metastasis from breast/lung cancer and for the treatment of multiple myeloma (2).

Pharmacological Actions

A study comparing the inhibitory activity of nitrogen-containing bisphosphonates on the mevalonate pathway

Table I: Bisphosphonates launched or under active development (from Prous Science Integrity®).

Compound	Source	Indication	Phase
1. Alendronate Sodium	Gentili/Merck & Co.	Osteoporosis	Launched
	Merck & Co.	Paget's disease	Launched
2. Cimadronate Sodium	Yamanouchi	Hypercalcemia of malignancy	Launched
	Yamanouchi	Periodontitis	II
3. Clodronate Disodium	Leiras	Malignant osteolytic bone diseases	Launched
	Abiogen	Osteoarthritis	II
	Aventis Pharma/Roche/Abiogen	Tumor-induced hypercalcemia	Launched
	Abiogen	Osteoporosis	Launched
	Kissei	Bone metastasis of breast cancer	II
4. Etidronate Disodium	Procter & Gamble	Paget's disease	Launched
	Procter & Gamble/Sumitomo	Osteoporosis	Launched
	Sumitomo	Ossification of the posterior longitudinal ligament	II
5. Ibandronate Sodium Hydrate	Roche	Hypercalcemia of malignancy	Launched
	Roche/GlaxoSmithKline	Osteoporosis	Prereg-2002
	Roche	Bone metastasis of breast/prostate cancer	Prereg-2002
	Roche	Opioid-resistant metastatic bone pain	Clinical
6. Minodronic Acid	Ono	Osteoporosis	III
	Yamanouchi	Bone metastasis of breast/lung cancer	III
	Yamanouchi	Multiple myeloma	III
7. Neridronic Acid	Abiogen	Paget's disease	III
	Abiogen	Osteoporosis	Clinical
	Abiogen	Osteogenesis imperfecta	Launched
8. Olpadronate Sodium	Gador	Paget's disease	III
9. Pamidronate Sodium	Gador	Paget's disease	Launched
	Novartis	Tumor-induced hypercalcemia	Launched
10. Risedronate Sodium	Aventis Pharma	Paget's disease	Launched
	Procter & Gamble/Aventis Pharma/ Takeda/Ajinomoto	Osteoporosis	Launched
11. Tiludronate Disodium	Sanofi-Synthélabo	Paget's disease	Launched
12. Zoledronic Acid Monohydrate	Novartis	Neuropathic pain	Preclinical
	Novartis	Bone metastasis of breast/renal/lung/prostate cancer or multiple myeloma	Rec. Appr.-2002
	Novartis	Tumor induced hypercalcemia	Launched
	Novartis	Paget's disease	II
	Novartis	Osteoporosis	III



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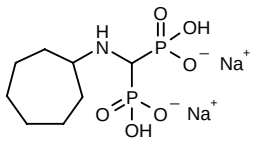

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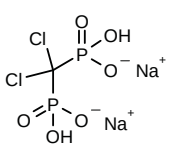

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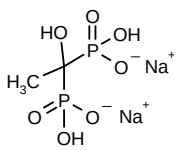

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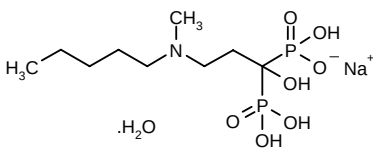

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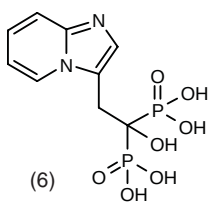

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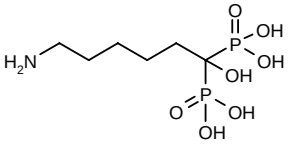

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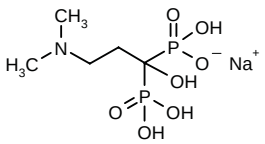

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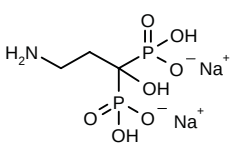

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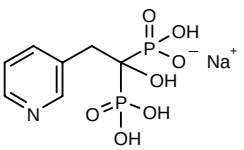

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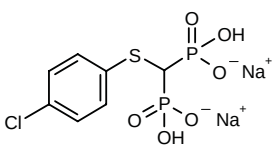

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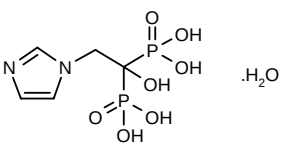

(12)

concluded that FPP synthase is the major pharmacological target of minodronic acid and other bisphosphonates. Minodronic acid potently inhibited recombinant human FPP synthase activity in vitro at concentrations of 1 nM or greater in a manner similar to zoledronic acid (IC_{50} = 0.003 μM for both agents). The order of potency for this assay was zoledronic acid > minodronic acid > risedronate> ibandronate > incadronate > alendronate > pamidronate. The inhibitory activity of the bisphosphonates in this assay closely correlated with the ability of these agents to inhibit prenylation in cell-free extracts (rabbit reticulocyte lysates) and in purified osteoclasts and to inhibit bone resorption *in vivo* in rats (lowest effective dose for minodronic acid = 0.0001 mg phosphorous/kg) (14).

Further examination of the mechanism of action of bisphosphonates revealed that these agents not only have inhibitory activity on osteoclasts, but also have effects on osteoblastic function. An *in vitro* study using an osteoblastic cell line (MC3T3-E1) examined the effects of bisphosphonates on protein tyrosine phosphatases (E1-PTP1, -2 and -3) purified from the cytosolic fraction of these cells. Results showed that the agents inhibited both E1-PTP-1 and -2 but had no effects on E1-PTP-3. The order of potency for inhibition of E1-PTP-1 was minodronic acid > etidronate> incadronate > alendronate; the order of potency for inhibition of E1-PTP-2 could not be clearly ascertained. Thus, minodronic acid and other bisphosphonates may influence the activity of osteoblastic cell via inhibition of PTP activity (15).

Minodronic acid was shown to have potent activity in a number of *in vivo* models. The agent displayed potent inhibitory activity in reducing serum Ca^{2+} concentrations in rats (the PIH model). In this model, minodronic acid or other bisphosphonates were administered for 3 days (s.c. or p.o.) after which rats were treated with synthetic human parathyroid hormone 1-34 (PTH; 30 μg/kg i.v.). The minimum effective doses (MEDs) for minodronic acid in this assay were 0.003 mg/kg s.c. and 1 mg/kg or less p.o. as compared to 0.3 mg/kg s.c. and 300 mg/kg p.o. for pamidronate and 0.03 mg/kg s.c. and 30 mg/kg p.o. for alendronate. These results suggest that minodronic acid has more potent s.c. activity and better oral bioavailability as compared to the other agents (2). Minodronic acid was the most effective agent examined in the immobilization bone atrophy model in rats. After immobilizing the left forelimb in this assay, rats were administered the bisphosphonates p.o. every day for 2 weeks. The dry weight of the left humerus was then obtained after it was removed, dehydrated and defatted. Minodronic acid (MED = 1 mg/kg p.o.) was 30- and 100-fold more potent than alendronate (30 mg/kg p.o.) and pamidronate (100 mg/kg p.o.) (2).

Minodronic acid (0.04 mg/kg/day s.c. for 10 days starting 3 days before tail suspension) was found to be effective in preventing disuse bone atrophy induced by tail suspension (7 days) in rats. Treatment with the agent significantly prevented the decrease in calcium and phosphorous content in the femur on days 5 and 7 and significantly increased these levels in the humerus. Moreover, minodronic acid-treated animals had significantly lower urinary deoxypyridoline excretion, a marker for bone resorption. It was concluded that minodronic acid inhibited development of bone atrophy and increased systemic bone mineralization. After examining urinary excretion of corticosterone and epinephrine from suspended and non-suspended rats, it was also suggested that treatment with minodronic acid may enhance the adrenomedullary response to stress load (16-19).

The efficacy of long-term treatment with minodronic acid (6, 30 or 150 μg/kg p.o. for 12 months) in preventing bone loss and increasing mechanical strength was demonstrated in a study using ovariectomized rats. Treatment with the agent starting the day of ovariectomy dose-dependently and significantly ameliorated the decrease in lumbar vertebrae bone mineral density (BMD) so that at 12 months, lumbar vertebrae BMD increased 15.1, 23.3 and 30.8% for the respective doses. In addition, minodronic acid-treated animals showed dose-dependent and significant increases in femur and tibia BMD; the order of potency for increasing BMD was lumbar > femur > tibia. Treatment also significantly increased the ultimate compressive load of the fifth lumbar vertebral body and the ultimate bending load of the femoral shaft. Urinary deoxypyridinoline and serum osteocalcin levels were significantly decreased in minodronic acid-treated animals as compared to controls (20).

The efficacy of minodronic acid (0.02, 0.1 and 0.5 mg/kg/day p.o. for 12 months) in influencing trabecular and cortical bone turnover and bone mass and strength was examined in ovariohysterectomized beagles given a restricted calcium diet (0.14 *vs.* 1.4% in a standard diet). In this model, although the effects of ovariohysterectomy on bone mass and bone turnover were found to be small, it was concluded that long-term treatment with minodronic acid maintained mass and strength of lumbar bone via a reduction in bone resorption. Minodronic acid was less effective in cortical bone as compared to trabecular bone (21).

Minodronic acid (p.o.) was effective in preventing inflammation-induced bone loss and deterioration of bone microstructure in rats with collagen-induced arthritis. Prophylactic treatment starting immediately after sensitization and continuing for 8 weeks was found to be more effective than therapeutic treatment which was initiated 2 week after sensitization. Marked inhibition of arthritis-induced increases in osteoclast number and bone resorption with accelerated bone turnover were observed in animals treated with minodronic acid. In addition, cancellous bone loss was inhibited and microstructure was maintained with treatment (22).

Several studies have reported that minodronic acid was effective against tumor-induced osteolysis in nude mice with bone metastases. Two studies demonstrated the efficacy of the agent in BALB/c nu/nu mice with osteolytic metastases induced by intracardiac injection of a human breast cancer cell line (MDA231). In one study, both minodronic acid and zoledronic acid (0.1 mg/kg i.v.)

dose-dependently and significantly decreased the number of osteoclasts and the ratio of osteoclast surface to bone surface at each tumor site; comparable significant activity was also observed with pamidronate although a dose of 10 mg/kg i.v. was required. In the second study, treatment with minodronic acid (0.2, 2 and 20 μg/mouse/day s.c. for 4 weeks) markedly inhibited the number of osteoclasts and the size of tumors at metastatic sites. In addition, the agent potently reduced bone metastases and the progression of established metastatic foci (23, 24).

Similarly, minodronic acid was effective against tumor-induced osteolysis in nude mice and rats with bone metastases induced by intracardiac injection of human melanoma cells (A375). Treatment with minodronic acid (3-10 mg/kg p.o. single dose in rats; 0.03-3 mg/kg/day for 7 days in mice starting at 4 weeks after inoculation) dose-dependently decreased osteoclast number and the ratio of osteoclast surface. Significant effects were observed with doses of 10 and 30 m/kg in rats and 0.3 and 3 mg/kg for 7 days in mice. When osteoclasts from treated groups were observed, morphological abnormalities were detected suggesting possible apoptosis. It was also noted that treatment of mice with the 3 mg/kg dose for 7 days significantly induced the reduction in ash weight of femurs (25).

Minodronic acid has been reported to have another important effect which is inhibition of growth of myeloma cells. This antimyeloma activity has been demonstrated in 2 studies. An *in vitro* study compared the direct effects of minodronic acid with pamidronate and incadronate (50, 100 and 500 μM for 72 h) on proliferation of 3 myeloma cell lines (RPMI8226, U266 and IL-6-dependent KPMM2). The 500 μM dose for all agents significantly decreased the number of viable cells in all cell lines. However, minodronic acid was the most potent agent followed by incadronate and pamidronate. Minodronic acid was confirmed to significantly induce apoptosis in all cell lines and inhibit proliferation (26).

A second study using 12 human myeloma cell lines (KMM-1, KMS-11. KMS-12PE, KMS-12BM, KMS-18, KMS-20, KMS-21PE, KMS-21BM, KMS-26, KMS-27, KMS-28PE and KMS-28BM) further confirmed the antimyeloma effects of minodronic acid (1, 10, 30, 50, 100 and 300 μM for 72 h). Significant dose-dependent growth inhibition was observed with cells accumulating in the [2n≪4n] fraction of the cell cycle and formation of an apoptotic sub-G_1 fraction detected later. The study also demonstrated that the inhibitory effects of minodronic acid (10 μM) were enhanced when it was combined with all-*trans* retinoic acid (0.1 μM), thalidomide (100 μM) or interferon-α (1000 U/ml). However, minodronic acid had no significant effects on mRNA expression for angiogenic factors, cell cycle regulators or cytokine related to myeloma cells (27).

An HPLC method for determination of minodronic acid in plasma, urine and bone was described and validated using plasma from patients participating in low-dose clinical trials. Two methods were presented with the more sensitive procedure involving direct precipitation of the drug before deproteinization of plasma. This method had a limit of determination of 0.05 ng/ml, 0.05 ng/ml and 5 ng/g in plasma, urine and bone, respectively (28).

Potential minodronic acid aqueous formulations have been evaluated for stability with results showing that citrate and tartrate buffers maintained stability and prevented precipitation after storage for up to 4 weeks at 60 °C. Stability of the agent increased slightly with increasing buffer concentration. The best stabilizer was concluded to be citrate buffer. Particle formation was avoided with citrate buffer at pH 3-5 when samples were stored at 40 °C for 6 months or 60 °C for 3 months. Particle formation was observed at pH 6 and 7 and when storage temperature was reduced to 25 °C. Analysis of the precipitate showed that particles were a complex of minodronic acid and aluminum ions possibly leached from the glass vials. Complex formation was determined to be exothermic (29, 30).

Clinical Studies

The efficacy of minodronic acid (0.05, 0.5 and 1.5 mg/day for 24 weeks) was examined in a phase II, multicenter open study involving osteopenic subjects (postmenopausal, ovariectomized or other women with amenorrhea and men with a BMD of less than the peak bone mass of –2.5 SD); all subjects received calcium lactate supplementation. Administration of minodronic acid increased BMD. At 24 weeks, the increased rate of BMD for the respective dose groups were 1.53, 3.76 and 7.82%. In addition, significant and dose-dependent reductions in urinary pyridinoline, deoxypyridinoline and hydroxyproline and reductions in serum bone alkaline phosphatase and N-terminal osteocalcin were observed at 24 weeks. Serum calcium, phosphate, 25(OH)D, $1,25(OH)_2$ and $24,25(OH)_2D$ were unaltered throughout the treatment period (31).

The efficacy and safety of minodronic acid (0.5, 1 and 1.5 mg/day for 36 weeks) were shown in a multicenter, randomized, placebo-controlled trial involving 352 Japanese postmenopausal women who were diagnosed with osteoporosis; all patients were supplemented with calcium lactate (0.8 g). Significant increases in lumbar 2-4 BMD as compared to the placebo were observed after 36 weeks (105.65, 106.42 and 105.93% *vs.* 100.72% in placebo). Urinary deoxypyridinoline/creatinine and NTX/creatinine significantly decreased after 4 weeks and serum bone specific alkaline phosphatase significantly decreased after 12 weeks in minodronic acid-treated patients as compared to placebo. No serious adverse events were reported from the 351 patients evaluated for safety. The incidence of adverse events was 23.3, 22.9 and 27.3% for the respective dose groups as compared to 13.2% in placebo. In particular, the incidence of gastrointestinal events was 12.6, 6.3 and 11.1% for the respective dose groups as compared to 0% in placebo (32).

Minodronic acid is undergoing phase III testing for the treatment of osteoporosis with a preregistration scheduled for 2004 in Japan. In addition, the compound is currently in phase III development for the treatment of bone metastasis of breast/lung cancer and for the treatment of multiple myeloma (33, 34).

Source

Yamanouchi Pharmaceutical Co., Ltd. (JP); licensed to Ono Pharmaceutical Co., Ltd. (JP).

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