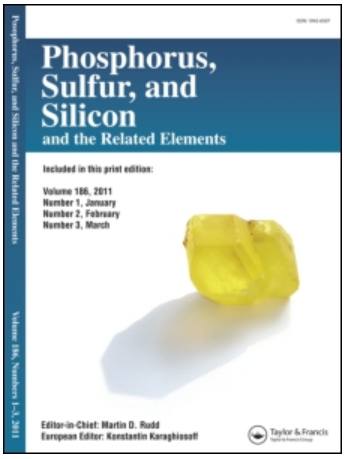

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Amino-Substituted *GEM*-Bisphosphonates

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In 1986, a chemistry program was initiated to discover a more potent bisphosphonate as a follow-up to pamidronate for the treatment of osteoporosis and metastatic bone disease. Three subclasses of bisphosphonates were investigated: 1) analogues of pamidronate with different alkyl and phenyl-X-alkyl-substituents (X = O, S) at the nitrogen (tables 1 and 2); 2) hydroxy bisphosphonates with a five-membered heteroaromatic substitutent linked via a methylene bridge to C1 (table III); 3) aminomethylene bisphosphonates with a five-membered heteroaromatic substitutent (table IV). **Zoledronate** was selected for development based on its high antiresorptive activity, good dissociation from inhibition of bone mineralization and excellent renal tolerability. Preliminary results from Phase I clinical trials have confirmed the compound's predicted profile.

Keywords: bisphosphonate; zoledronate

INTRODUCTION

In the early 1960s Fleisch and Neuman studied the role of pyrophosphate in bone mineralization [1]. Inorganic pyrophosphate was isolated from serum, urine, synovial fluid and saliva. It inhibits calcification of soft tissue as well as bone mineralization and resorption *in vitro*. However, pyrophosphate is inactive when given orally, due to its rapid hydrolysis by phosphatases. Replacement of the P-O-P backbone of pyrophosphate led to the *geminal* bisphosphonates with a P-C-P structure, which renders them resistant to hydrolysis. The first bisphosphonates to be used therapeutically were etidronate and clodronate. A marked increase in potency was achieved with pamidronate, which has a basic amino group in its side chain.

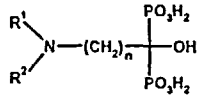
The mode of action of bisphosphonates on bone resorption is still not completely elucidated. It is generally agreed that bisphosphonates act either directly, or indirectly via osteoclast recruitment, on the osteoclast [2]. Bisphosphonates accumulate preferentially under the osteoclast, where high concentrations can be reached [3]. The biochemical mechanism by which they inhibit osteoclastic activity is still not clear. Recently bisphosphonates have been found to induce osteoblasts to release an inhibitor of osteoclast recruitment [4].

STRUCTURE-ACTIVITY RELATIONSHIP OF GEMINAL BISPHOSPHONATES

In 1986, we initiated a chemistry program to find a follow-up to pamidronate for the treatment of osteoporosis and metastatic bone disease. The activity of bisphosphonates on bone resorption is highly dependent on small modifications of the structure, which is not the case for the inhibition of mineralization. Therefore initial screening relied on assessing potency in an in vivo bone resorption model. Compounds were tested in thyro-parathyroidectomized (TPTX) rats with hypercalcemia induced by 1,25-dihydroxy vitamin D_3 [5].

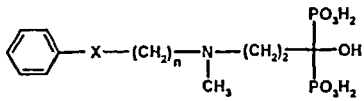
It was generally found that high antiresorptive potency required a hydroxyl group at the carbon atom between the two phosphonate groups (C1). Pamidronate showed clearly that derivatives with an amino group at the end of the side chain have improved potency compared with the early bisphosphonates. The length of the linker between C1 and the amino group in the side chain is optimal for n = 3 (alendronate; cf. table 1). N-Acety-

TABLE 1



R^1	R^2	n	TPTX-VitD3 ED_{50} [µg/kg]	
H	H	1	150	
H	H	2	85	Pamidronate
H	H	3	8	Alendronate
H	H	4	20	
H	H	5	60	Neridronate
Ac	H	2	> 1000	
Me	Me	2	12	Olpadronate
Pentyl	Me	2	1.1	Ibandronate

TABLE 2

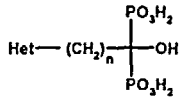


X	n	TPTX-VitD3 ED_{50} [µg/kg]
-	2	1.4
-	3	1
O	2	1.5
O	3	0.5
O	4	4
S	2	0.7
S	3	0.33

lation of pamidronate destroys the activity almost completely. Alkylation of the amino group increases antiresorptive potency as seen in olpadronate and ibandronate (table 1). Phenylalkyl- and phenyl-X-alkyl-substituents (X = O, S) at the nitrogen lead to some very potent bisphosphonates. A distance of 2-5 atoms between the nitrogen and the phenyl group proved to be optimal (cf. table 2).

The most active bisphosphonates were found in the series in which heteroaromatic substituents with at least one nitrogen in the ring are linked via a single methylene to C1. Extension of the linker causes a marked loss of activity. Of particular interest are compounds containing an imidazole residue. The highest activity is obtained when the imidazole is attached via one of the nitrogen atoms (**zoledronate**; cf. table 3).

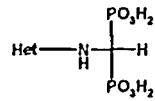
TABLE 3

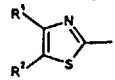


Het	n	TPTX-VitD3 ED_{50} [µg/kg]	
	1	0.3	
	2	20	
	1	0.6	
	1	0.072	**zoledronate**
	2	45	

High activity is also observed for aminomethylene bisphosphonates with nitrogen-containing heteroaromatic substituents. The best ring systems are thiazoles and imidazoles (cf. table 4). A methyl group ortho to the nitrogen of the thiazole ring decreased

TABLE 4



Het	R^1	R^2	TPTX-VitD3 ED_{50} [µg/kg]
	H	H	5
	Me	H	100
	H	Me	1.5
	-	-	5

the activity substantially, whereas the same substituent ortho to the sulfur atom brought a slight increase. However, aminomethylene bisphosphonates had a less favorable profile in a renal tolerability model [6].

PRECLINICAL AND CLINICAL DATA OF ZOLEDRONATE

Due to its high potency in the TPTX-VitD_3 model, zoledronate was selected for further preclinical profiling [5]. In vitro calvarial cultures were used to confirm a direct, potent inhibition of bone resorption (IC_{50} = 0.002 μM), widely dissociated from any effect on bone mineralization (IC_{50} = 30 μM). Short-term treatment of young rats with zoledronate dose-dependently increased the radiographic density of the proximal tibia (ED_{50} = 1.7 μg/kg s.c.), as well as increasing the calcium and hydroxyproline content of the femoral trabeculae (ED_{50} values = 0.17 and 1.1 μg/kg s.c., respectively). Inhibition of bone resorption and preservation of trabecular architecture were confirmed by histomorphometric analysis of the proximal tibial metaphysis of intact rats [6]. In a renal tolerability model [7] zoledronate showed the highest therapeutic index of any bisphosphonate tested. In long-term (16 months) studies in ovariectomized monkeys [8], weekly injections of zoledronate (0.5 - 12.5 μg/kg, s.c.) prevented the bone loss due to estrogen deficiency and maintained bone strength, thus demonstrating the compound's potential to treat postmenopausal osteoporosis.

The phase I clinical program comprised studies in patients with tumor-induced hypercalcemia, Paget's disease or osteolytic bone metastases. Intravenous administration of zoledronate (24 - 2000 μg) was generally well tolerated, a minority of patients experienced adverse events of a type similar to those reported previously for other potent amino-bisphosphonates, e.g. mild hypocalcemia and hypophosphatemia, transient fever, arthralgia, and bone pain. Single infusions of zoledronate (200 - 2000 μg) dose-dependently reduced biochemical markers of bone turnover, thereby confirming the clinical potency predicted from the preclinical profile. Zoledronate is now in phase II clinical development for the treatment of tumor-induced hypercalcemia, osteolytic bone metastases, Paget's disease and osteoporosis, and has the potential to be a highly effective therapy for all these indications.

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