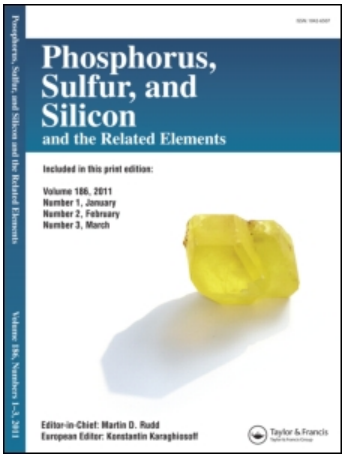

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Elucidation of a Pharmacophore for the Bisphosphonate Mechanism of Bone Antiresorptive Activity

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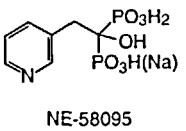
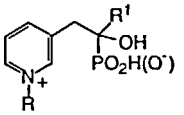
Abstract Our recent discoveries in the field of phosphonate bone antiresorptive agents include analogs of the pyridine containing bisphosphonate NE-58095 (Risedronate) such as the pyridinium bisphosphonate NE-10575, the pyridine phosphonocarboxylate NE-10790, and the pyridine phosphonophosphinate NE-10864. We believe these analogs differ at key recognition sites in the putative cellular pharmacophore and thus offer important structure-activity learnings.

INTRODUCTION

The structure-activity relationships of the bisphosphonate series of bone antiresorptive agents continue to be intriguing to the medicinal chemist. The work of several laboratories has now demonstrated the importance of basic nitrogen moieties in the design of potent drugs.[1] For example, we have studied the pyridyl class of bisphosphonates, such as Risedronate (NE-58095), in preclinical models,[2] and as a useful agent in the clinic for the treatment of osteoporosis.[3] We have also previously reported new modifications at the P-C-P moiety, which is known to be the primary chemical function responsible for bone affinity. Coupled with the growing biochemical evidence indicating cellular involvement, these structure-activity relationships suggest the bisphosphonate (BP) antiresorptive mechanism *in vivo* involves both bone targeting and a subsequent specific cellular recognition event.[4]



NE-58095



R = H, $R^1 = PO_3H_2$, NE-58019
R = CH_3, $R^1 = PO_3H_2$, NE-10575
R = H, $R^1 = CO_2H$, NE-10790
R = H, $R^1 = PO_2H(CH_3)$, NE-10864

FIGURE 1. Risedronate and Several Key Phosphonate Derivatives.

RESULTS AND DISCUSSION

Understanding the Nature of the BP-Nitrogen Pharmacophore

We have been interested in better characterizing the key elements of the putative BP cellular pharmacophore. For example, what type of interaction is occurring at the nitrogen binding site? Previous evidence suggested that a basic nitrogen was required for high potency in these molecules. Recently, we hypothesized that the protonated form of nitrogen was optimal in the pharmacophore (i.e.,

NE-58019). Thus, it could be interacting in an electropositive form through a coulombic attraction to an electronegative binding site. Therefore, a quaternized nitrogen functionality such as a pyridinium species should be an effective or improved moiety at the nitrogen site on the bisphosphonate molecule. To test this hypothesis, NE-10575 (Figure 1), the methyl pyridinium analog of NE-58095 was synthesized.

NE-10575 was synthesized through standard aqueous methylation conditions from Risedronate and methyl iodide.[5] and evaluated in the growing rat or Schenk Model.[2] Since the potency observed for NE-10575 (lowest effective dose (LED) = 0.0001 mg P/kg) was similar to that of Risedronate (0.0003) this rationale for the design of a potent new bisphosphonate was a success. In fact, since NE-10575 also demonstrated reduced bone affinity *in vitro* vs. Risedronate (NE-58095), we may have designed an analog with significantly improved cellular activity.

From this initial finding in the pyridyl series, we initiated the synthesis of several pyridinium analogs derived from a range of known pyridyl bisphosphonates with varying potency. We reasoned that if a structure-activity correlation existed, it would be likely that the pyridinium series was deriving its antiresorptive potency from the same cellular mechanism by utilizing the same nitrogen binding interaction as the pyridyl series. Figure 2 lists the series studied to initiate this comparison, with the corresponding antiresorptive potency reported in parentheses (LED in mg P/kg).

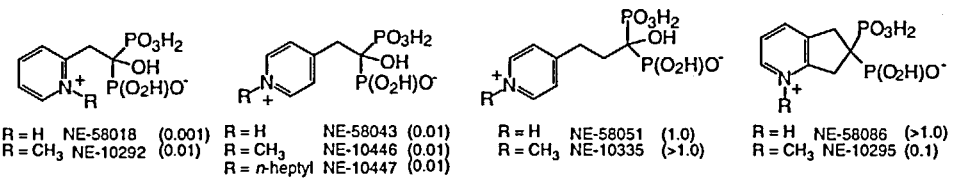


FIGURE 2. Lowest Effective Doses for a Variety of Substituted Pyridinium Bisphosphonic acids.

As demonstrated in Figure 2, potent pyridine containing BP's maintained high levels of potency when converted to the corresponding pyridinium analogs. This also included tolerance of more bulky, longer chain pyridinium species, such as NE-10447. Also, the two pyridinium examples derived from low potency BP's, NE-10335 and NE-10295, led to correspondingly low potency pyridinium analogs. Thus, this preliminary evidence suggests the pyridinium series is in fact involving the same antiresorptive mechanism as the parent pyridyl series.

Recent Analysis of the P-C-P moiety

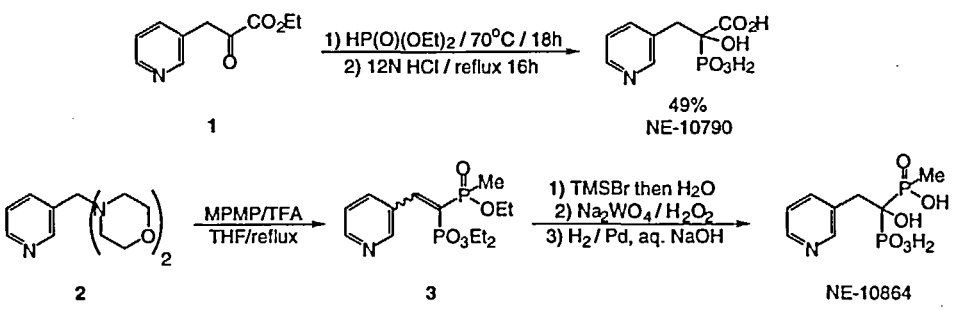
We have also studied, in more depth, the SAR of the P-C-P moiety of the bisphosphonates.[6] In recent years, with the evolution of more potent analogs, this moiety has been associated with the physicochemical mechanisms of accumulation of drug on the bone (hydroxyapatite) surface.[7] Based on new *in vitro* evidence, we now believe the phosphonate moieties of bisphosphonates have more than just a targeting function.[8,9]

Two new classes of phosphonate analogs with lower affinities for hydroxyapatite than bisphosphonates have been designed and studied. These are the pyridylethane hydroxy-phosphonocarboxylate (PC) and pyridylethane hydroxyphosphonomethylphosphinate, (PAP) analogs of Risedronate, NE-10790 and NE-10864, respectively (Figure 1).

The synthesis of NE-10790 was initiated with the condensation of pyridine 3-carboxaldehyde and N,N-dimethylglycine ethyl ester to give the α-ketoester reported (1) by Horner and Reuth[10] in suitable yield. Reaction of 1 with 4.5 equivalents of diethylphosphite at 70°C for 18 hours afforded the phosphonocarboxylate triethyl ester as an impure, viscous syrup in 78% yield. Acid hydrolysis in boiling concentrated HCl overnight led to a 49% yield (from 1) of 2-hydroxy-2-phosphono-3-(3-pyridinyl)propanoic acid (NE-10790).

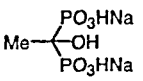
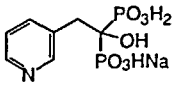
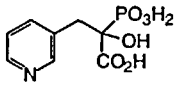
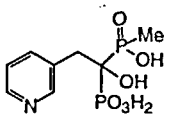
The synthesis of NE-10864 was began with the interesting acid mediated condensation of pyridylaminal 2 with methylenephosphonomethylphosphinic acid triethyl ester (MPMP) in 41% yield.[11] Vinyl bisphosphonate 3 was converted to the desired NE-10864 in the 3 step hydrolysis (99%), epoxidation (52%), and hydrogenation (61%) sequence shown in Scheme 1.

SCHEME 1



In our previous studies in the fetal rat long bone *in vitro* antiresorptive assay,[8] the potency of earlier low affinity, PAPs were devoid of any activity in these organ culture models at concentrations up to 5 mM. The phosphonocarboxylate (NE-10790) analog of Risedronate also displayed low bone affinity, but its antiresorptive activity was similar to that of an early generation bisphosphonate, etidronate, with an IC_{50} of around 50 μM. The PAP analog of Risedronate, NE-10864, which represents the first member of this class reported with a central geminal P-C-P carbon OH substituent was found to have bone affinity similar to or slightly higher than that of NE-10790. Although it is now the first PAP to demonstrate some antiresorptive activity *in vitro*, its activity (200 μM) is reduced compared to the corresponding PC, NE-10790 (Table 1). Thus, molecules differing in the structure of the phosphonate moiety, but with similar affinities for hydroxyapatite, and with the same nitrogen containing side chains, demonstrated markedly different antiresorptive effects. These findings provide additional evidence that the phosphonate moiety, beyond determining the affinity of the molecule for mineral surfaces, plays a key role in the pharmacophore of the cellular mechanism(s) of the bisphosphonate class of compounds.

TABLE 1. Antiresorptive Activity (*in vivo*-entry 1, *in vitro*-entry 2) of Bisphosphonates and Low Affinity Phosphonates.

	 Etidronate BP	 Risedronate BP (NE-58095)	 NE-10790 PC	 NE-10864 PAP
LED (mg P/kg)[1]	1.0	0.0003	1.5	-
IC_{50} (10^{-6}M)[7]	30-50	3.5	50	200

CONCLUSION

The alkylated/quaternized pyridinium analogs of Risedronate and other pyridine containing bisphosphonates have been discovered to be potent antiresorptive agents *in vivo*. Preliminary studies indicate a correlation between the structure-activity relationships of the pyridine and pyridinium bisphosphonates suggesting that these new analogs inhibit bone resorption through similar cellular mechanisms. The P-C-P moiety, in addition to its role as a bone targeting function, also appears to be important in the molecular mechanism by which bisphosphonates affect cell function.

ACKNOWLEDGMENTS

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