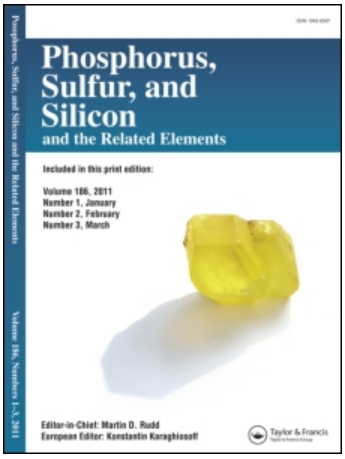

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The Design and Synthesis of Sulfur Containing Bisphosphonic Acids for the Treatment of Arthritis

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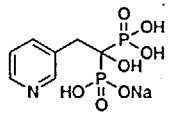
The sulfur containing bisphosphonic acids have been examined as a chondroprotective therapy for the treatment of arthritis. Several compounds have demonstrated both *in vitro* and *in vivo* activity. The newly synthesized compounds all contain a latent or a free thiol group which may bind to the zinc atom in the active site of the matrix metalloproteases. The *in vivo* activity of some of these bisphosphonic acids in the rat adjuvant model of arthritis is also discussed.

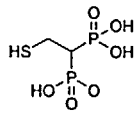
Keywords: bisphosphonate; arthritis; antiinflammatory

INTRODUCTION

The bisphosphonates, especially, the nitrogen containing analogs, have been demonstrated to be potent inhibitors of bone resorption, and highly selective bone targeting agents.[1] Our group[2] and others[3] have shown the utility of bisphosphonates in models of arthritis, particularly rheumatoid. In our research, we have attempted to utilize the high bone/joint specificity of this class of compounds together with other chemical moieties with potential anticatabolic pharmacology in the design of specialized antiarthritic agents.[4] Recently, we have focused our attention on the sulfur containing bisphosphonic acids as a chondroprotective therapy for the treatment of arthritis. Our initial leads in this area, Risedronate, and HSEDP both have demonstrated remarkable activity in the rat adjuvant arthritis model.



Risedronate

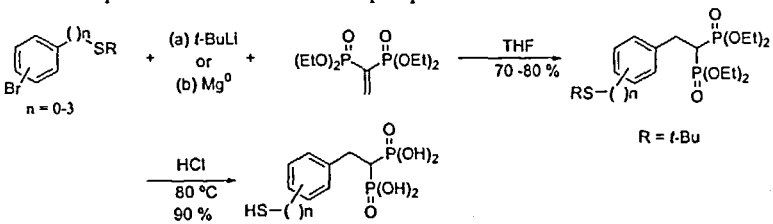


HSEDP

CHEMISTRY

The thio-bisphosphonates were designed to contain a latent (or free) thiol group which may bind to the Zinc atom in the active site of the matrix metalloproteases (MMPs).[5,6] The most interesting series prepared utilizes a rigid aromatic group with a bisphosphonic acid as a potential "bone hook" and a pendant thiol chain present for interaction with the enzyme systems. The synthesis of the these compounds was accomplished from the corresponding aromatic bromides via lithium halogen exchange and subsequent addition of the aryl lithium species to vinyl diphosphonate (VDP).[7] Alternatively, the grignard reagent could be prepared by addition of magnesium metal to the corresponding aromatic bromide and then addition of this species to VDP. The Michael addition of the organometallic species to VDP proceeded in high yield to give the desired bisphosphonate. Hydrolysis of the resulting bisphosphonate with concentrated hydrochloric acid affords the desired bisphosphonic acid in high yield.

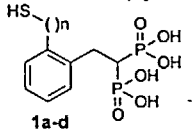
General Preparation of Aromatic Thio Bisphosphonates



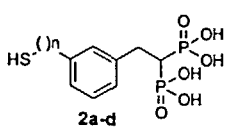
RESULTS AND DISCUSSION

The bisphosphonic acids were evaluated *in vitro* for inhibition of the matrix metalloproteases (collagenase-1 and stromelysin-1) and for *in vivo* activity in the rat adjuvant model of arthritis (see Table below). The thio-bisphosphonic acids proved to be weak inhibitors of both collagenase (MMP-1) and stromelysin (MMP-3). The most potent inhibitors contained a 1 carbon linkage between the thiol group and the aromatic ring (see **1b, 2b, 3b**). Extending the length of the alkyl chain resulted in diminished inhibition against MMP-1. Several of the compounds were also tested for *in vivo* activity in the adjuvant arthritis model. All of the thio-bisphophonic acids tested demonstrated statistically significant activity in the adjuvant model of arthritis at doses of 1 mg P/kg.[8] No significant correlation was observed between the observed enzyme activity and the *in vivo* activity in the rat adjuvant model. Compounds which had almost no activity against MMP-1 (compounds **1c** and **1d**) demonstrated significant activity in the adjuvant model. Removal of the free thiol group (replacement with a tosyl group), or capping the free thiol with a methyl group to form a sulfide completely eliminated the enzyme activity and also resulted in diminished activity in the adjuvant model.

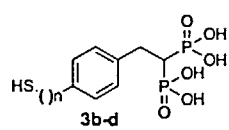
TABLE: Inhibitory profile of aromatic thio-bisphosphonates.



1a-d



2a-d



3b-d

Compound	n	*In Vitro Studies* IC_{50} (μM) MMP-1[a]	MMP-3[a]	Adjuvant Studies % Reduction[b]
Risedronate	-	-	-	55[c]
HSEDP	-	63	33	65[d]
1a	0	787	314	-
1b	1	76.1	48.6	65
1c	2	>1000	168	67
1d	3	383	92.4	75
2a	0	102	561	-
2b	1	11.1	110	81
2c	2	45.1	112	68
2d	3	>333	324	-
3b	1	92.9	124	70
3c	2	56	119	75
3d	3	59.4	130	-

[a]All in vitro binding data were obtained as single determinations. [b]All compounds dosed at 1 mg P/kg unless otherwise indicated. %Reduction in paw volume versus control. [c]Dosed at 0.03 mg P/kg. [d]Dosed at 0.3 mg P/kg.

CONCLUSION

The thio-bisphosphonic acids were determined to be moderate inhibitors of the matrix metalloproteases and potent agents in the rat adjuvant model of arthritis. The observed activity in the adjuvant model appears to be independent of the *in vitro* activity. The bisphosphonic acids which contain a one-carbon linkage between the free thiol group and the aromatic ring were found to be most potent in the *in vitro* assay.

EXPERIMENTAL SECTION

Preparation of 2b

3-(*t*-Butylthio)benzyl bromide. The 3-bromobenzyl bromide (24.9 g, 99.6 mmol) was added to a solution of *t*-butyl mercaptan (12.4 mL, 9.88 g, 109.6 mmol, 1.1 equiv), ethanol (150 mL), and potassium *t*-butoxide (12.30 g, 109.6 mmol, 1.1 equiv) at room temperature. The reaction mixture was stirred at room temperature for 3 hours and then poured into water. The solution was extracted with EtOAc (3 × 150 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated to an oil. No further

purification of the oil was needed. Spectroscopic data: ^{1}H-NMR ($CDCl_3$) δ 7.53 (m, 1 H), 7.46 (m, 1 H), 7.25 (m, 1 H), 7.09 (m, 1 H), 3.89 (s, 2 H), 1.39 (s, 9 H).

Tetraethyl (3-[*t*-butylthiomethyl]phenyl)ethylidenebisphosphonate. The aromatic bromide (3.05 g, 11.77 mmol) was stirred at -78 °C in THF and then *t*-BuLi (13.84 mL, 23.53 mmol, 2.0 equiv) was added. The reaction mixture was kept below -78 °C during the addition. The resulting mixture was stirred for 10 minutes and then the bisphosphonate (3.53 g, 11.77 mmol) was added in one portion. The reaction mixture was stirred for 10 minutes at -78 °C and then quenched by the addition of saturated ammonium chloride solution. The mixture was warmed to room temperature and then extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to an oil under reduced pressure. Purification of the oil was accomplished by chromatography on silica gel using 80/20/1 hexanes/acetone/EtOH as the eluent to afford 3.80 g (77%) of the desired product as a colorless oil. Spectroscopic data: ^{1}H-NMR ($CDCl_3$) δ 7.05 (m, 4 H), 4.02 (m, 4 H), 3.61 (s, 2 H), 3.18 (dt, J = 6.1, 14.0), 2.61 (tt, J = 6.1, 18.0), 1.37 (s, 9 H), 1.23 (m, 6 H). ^{13}C-NMR ($CDCl_3$) δ 139.69, 138.22, 129.38, 128.20, 127.29, 127.03, 62.40, 42.67, 38.86, 37.10, 33.15, 30.80, 16.20. ^{31}P-NMR ($CDCl_3$) δ 23.58.

(2-(3-Thiomethylphenyl)ethylidene)bisphosphonic acid 2b. The bisphosphonate (2.00 g, 4.16 mmol) was heated in concentrated HCl (50 mL) at 80 °C for 18 h. The water was then removed under reduced pressure to leave the product as a yellow oil. The last traces of solvent were removed under high vacuum to leave 1.20 g (92%) of the desired product as a crusty yellow solid. Analytical data for : ^{1}H-NMR (D_2O) δ 7.16 (m, 4 H), 3.63 (s, 2 H), 3.11 (dt, J = 6.1, 14.0), 2.58 (tt, J = 6.1, 18.0, 1 H). ^{13}C-NMR (D_2O) δ 141.58, 140.13, 128.77, 128.20, 127.25, 125.99, 39.57, 30.46, 27.53. ^{31}P-NMR (D_2O) δ 22.12. MS (ion spray): 311 (M - H^+). Anal. Calcd. for $C_9H_{14}O_6P_2S$: C, 34.62; H, 4.52. Found C, 34.81, H, 4.81.

Acknowledgments

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