Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1093–1096

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ANTI-INFLAMMATORY/ANTIARTHRITIC KETONIC BISPHOSPHONIC ACID ESTERS

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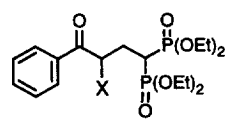
Received 29 December 1997; accepted 30 March 1998

Abstract: Bisphosphonate ester **2** is an inhibitor of inflammation, but is devoid of antiarthritic effects. SAR studies on a series of related bisphosphonate esters resulted in compounds **6e, 6i, 6j,** and **6m**, which exhibited excellent inhibition of an arthritis model, in addition to potent anti-inflammatory effects.

Bisphosphonic acids are potent antihypercalcemics with utility in therapeutic areas that involve abnormal calcium metabolism, such as Paget's disease, multiple myeloma of bone, and osteoporosis.[1] In addition, bisphosphonic acids are thought to be useful in controlling bone resorption and soft tissue inflammation associated with rheumatoid arthritis and osteoarthritis.[2] In an earlier publication, we described the anti-inflammatory and antiarthritic properties of ketonic bisphosphonate esters, such as **1**.[3] We are particularly interested in esters of these compounds because of their inability to adsorb to bone surfaces and their lack of significant activity in bone resorption assays. Thus, the anti-inflammatory activity of bisphosphonate esters appears to be unrelated to direct effects on calcium metabolism. Although **1** had both anti-inflammatory and antiarthritic activity, **2** failed to demonstrate any antiarthritic activity although it was more potent than **1** in a DTH model of chronic inflammation. Continued investigation has now identified derivatives of **2** that possess both anti-inflammatory and antiarthritic activity.

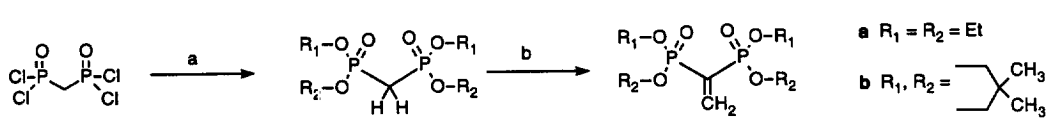


1 X = Ph
2 X = H

Chemistry

The tetraethyl vinylidene bisphosphonate **5a** was prepared from **4a** by literature procedure.[4] The importance of the ethyl esters was studied by preparing a cyclic ester analog with 2,2-dimethyl-1,3-propanediol. Methylenebisphosphonyl chloride **3**[5] was refluxed in chlorobenzene with 2,2-dimethyl-1,3-propanediol (2.2 equiv) for 20 h to yield **4b** in 25% yield after recrystallization from acetone (mp 193–194 °C).

Scheme 1



3 **4 a,b** **5 a,b**

a $R_1 = R_2 = Et$

b $R_1, R_2 =$

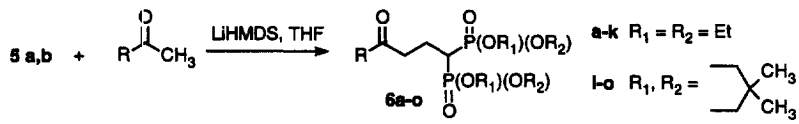
(a) 2,2 dimethyl-1,3-propanediol, PhCl, reflux, 20 h; (b) (i) $(CH_2O)_x$, Et_2NH, MeOH, reflux, 2.5 h; (ii) Amberlite IR120 (H^+), toluene, reflux, 1.5 h.

0960-894X/98/$19.00
PII: S0960-894X(98)00167-X

Compound **4b** was treated under Mannich conditions (5 equiv $(CH_2O)_x$ and 1.0 equiv of Et_2NH), then the crude material was azeotropically distilled from toluene in the presence of Amberlite IR-120(H^+) to yield **5b** in 40% yield after recrystallization from acetone (mp 193–194 °C).[6]

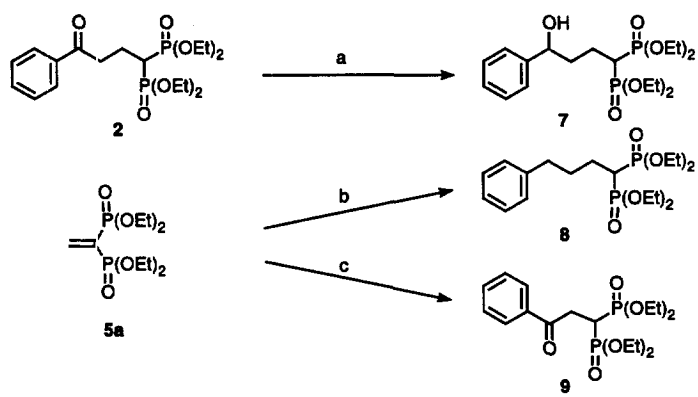
Ketonic bisphosphonates **6a–k** and **6l–o** were prepared through Michael addition of the appropriate methyl ketone with **5a** and **5b**, respectively (Scheme 2). Thus, the anion of acetophenone (1.2 equiv of LiHMDS at -78 °C) was treated with a solution of **5a** in THF to provide **2** after chromatography. Acetophenones, which contain a sulfonamide, carboxamide, or carbamate, and were insoluble in THF, were first dissolved in pyridine at 0 °C, then treated slowly with 2.2 equiv of LiHMDS. The suspension was stirred at 0 °C for 30 min, then treated with **5a** as before.

Scheme 2



The alcohol **7** was prepared by reduction of **2** with $NaBH_4$ in ethanol. Attempts to prepare the alkyl bisphosphonate **8** from the methylene bisphosphonate **4a** resulted in mixtures of mono- and bis-alkylated products that were difficult to separate. To avoid this difficulty, **5a** was treated with 1.2 equiv of a freshly prepared, etheral phenylethyl magnesium bromide which gave **8** in good yield. A shortened analog of **2** was prepared by addition of 2-(trimethylsilyloxy)-2-phenylacetonitrile[7] to **5a** to give **9**.[8]

Scheme 3



a) $NaBH_4$, THF/H_2O (7:1), 1 hr; b) $PhCH_2CH_2MgBr$, THF, -20 °C, 2 h; c) PhCH(OTMS)(CN), LiHMDS, -78 °C, 30 min then 22 °C for 4 h.

Results and Discussion

An initial evaluation of anti-inflammatory activity was performed in the delayed type hypersensitivity granuloma (DTH granuloma), a model of chronic, cutaneous inflammation.[9] Previously, we have shown this model is unaffected by traditional nonsteroidal anti-inflammatory drugs, such as indomethacin or ibuprofen,[10,11] but can be suppressed by bisphosphonic acids and esters.[12,13] Several compounds were also tested in a murine

model of antigen-induced arthritis (AIA).[3] The articular pathology of AIA involves an initial intense inflammatory synovitis followed by chronic inflammation and severe erosion of articular cartilage and subchondral bone, resembling the pathology seen in human rheumatoid arthritis.[14] This arthritis model can be suppressed by potent immunosuppressive drugs and bisphosphonic acids and esters.[15,16]

Although bisphosphonate esters such as **1** are effective, though not potent anti-inflammatory and antiarthritic agents, the simpler **2** did not possess any antiarthritic activity. As is demonstrated in Table 1, the lack of antiarthritic activity among this series of compounds is not unique to **2**. The benzamides **6a** and **6b** have activity in the DTH granuloma, but like **2**, are devoid of antiarthritic activity in the AIA assay. The carbamate **6c** showed only modest anti-inflammatory activity. Finally, we found that the methanesulfonamide **6d**, while having moderate anti-inflammatory activity, suppressed AIA to a significant degree.

Table 1. Anti-inflammatory and antiarthritic activity

Compd	R	% Yield	Mp (°C)	DTH Granuloma % Inhibition [a,b]	Antigen Induced Arthritis % Inhibition [a,b]
Clodronate				48[c] (30)	42[c]***(50)
1				65[c] (50)	54[c]***(50)
2	Ph	63	Oil	51 (0.1)	10
6a	4-BzNH Ph	45	110–112	63 (0.13)	4
6b	4-AcNH Ph	21	96–97	75	10
6c	4-EtO_2CNH Ph	61	96–97	43	18
6d	3-MsNH Ph	61	Oil	37	33***
6e	3-F Ph	88	Oil	44 (0.1)	40*** (0.1)
6f	3-Cl Ph	79	Oil	10	26*
6g	3-Br Ph	68	Oil	59	22*
6h	3-I Ph	84	Oil	5	ND[d]
6i	2-pyridyl	83	Oil	43 (0.05)	47***
6j	3-pyridyl	56	Oil	51 (0.05)	61***
6k	2-furanyl	56	Oil	23	11
6l	Ph [e]	56	199–200	36	ND
6m	3-F Ph [e]	73	201–202	57 (<0.01)	48*** (0.01)
6n	3-furanyl [e]	65	247–248	45	16
6o	3-pyridyl [e]	45	183–184	38 (0.05)	28* (0.4)
7		82	Oil	39	ND
8		39	Oil	44	11
9		35	Oil	7	11

[a]Drugs were administered po at 10 mg/kg and results are from single experiments with 10 animals. Values in parenthesis refer to ED_{30} values (mg/kg) obtained in separate experiment. [b](***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$. [c]Dosed at 100 mg/kg. [d]ND = not determined. [e]Cyclic ester.

Compound **6e** significantly inhibited DTH granuloma formation, with an EC_{30} identical to **2**. More importantly however, it suppressed AIA, with an EC_{30} that matched that observed in the DTH granuloma assay. Notably, **6e** was the only compound in a series of 3-halophenyl derivatives, which had activity in both assays. **6g** suppressed DTH granuloma formation, but lacked good antiarthritic activity. The heterocyclic compounds **6i** and **6j** showed significant activity in both assays, however, **6k** demonstrated that dual activity is not universal to all heterocycles.

To improve the physical properties of these compounds, we investigated the crystalline, cyclic ester analogs of the more notable compounds described above. Compound **6m** was as active as **6e** at 10 mg/kg, it was more potent, as shown in the ED_{30} values, and antiarthritic activity was maintained. In contrast to **6k**, **6n** significantly inhibited DTH induced granuloma formation, however there was no significant activity in the AIA assay. While **6o** had potent suppressive activity in the DTH assay, its antiarthritic activity was inconsistent.

The carbonyl group in **2** does not appear to play a crucial role in the anti-inflammatory activity, since reduction to **7** or removal of the carbonyl entirely as in **8** did not alter its ability to inhibit the DTH granuloma assay. However shortening the chain length from the bisphosphonate to the aromatic group by just a single carbon, as in **9**, dramatically reduced anti-inflammatory activity.

Conclusion

The activities of **2** and **6a–c** in the two in vivo assays indicate that the molecular mechanism whereby bisphosphonate esters suppress chronic inflammation is distinct from that which results in antiarthritic activity. We were able to prepare compounds such as **6m** and **6o**, which preserved both the anti-inflammatory and antiarthritic properties of the tetraethyl esters. Compounds **6m** and **6o** displayed EC_{30}s in both assays of <0.01 mg/kg. Finally, the activity demonstrated by these and other bisphosphonate esters suggests that the anti-inflammatory and/or antiarthritic activity associated with bisphosphonic acids may be due to a pharmacological mechanism that is independent from their direct effects on bone resorption.

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