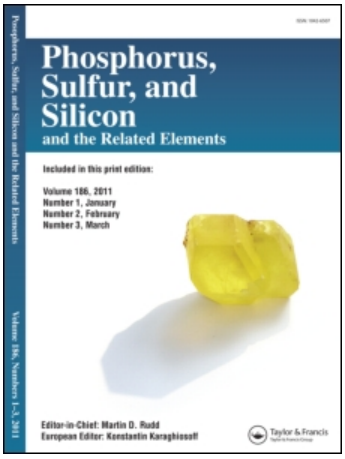

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NEW ANTI-INFLAMMATORY/ANTI-ARTHRITIC HETEROCYCLIC BISPHOSPHONATES

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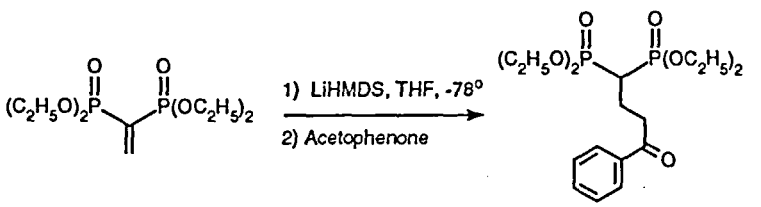
Abstract. In the course of research toward a safe and effective treatment for rheumatoid arthritis, we identified new pyrazolo[1,5-a]pyrimidine and 4-pyrimidinone bisphosphonate esters, which are potent inhibitors of a murine model of chronic, cutaneous inflammation (delayed type hypersensitivity granuloma) and a murine antigen induced arthritis model. **9a** has EC_{30} values of 0.01 and 0.005 mg/kg respectively and represents a new class of antiinflammatory/antiarthritic bisphosphonate ester.

Key Words: Bisphosphonate esters, antiarthritic, antiinflammatory, delayed type hypersensitivity, antigen induced arthritis

INTRODUCTION

Bisphosphonic acids are potent antihypercalcemics with utility in therapeutic areas which 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 the control rheumatoid and osteoarthritis. We have described the anti-inflammatory and anti-arthritic properties of ketonic bisphosphonate esters, such as **1**,[2] which are synthesized from **2**[3] and acetophenones. Since bisphosphonate esters do not bind bone and have weak or no effect in bone resorption assays, their anti-inflammatory activity appears to be unrelated to direct effects on calcium metabolism. We continued to search for other reactive methylenes which, with **2**, yield novel anti-inflammatory and anti-arthritic compounds.



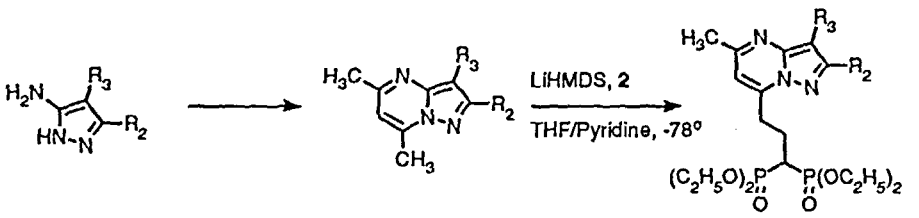
2 **1**

CHEMISTRY

Pyrazolo[1,5-a]pyrimidine Bisphosphonate Esters

The ring system was constructed by reacting a suitably substituted nitrile with triethylorthoacetate (or benzoate), then with hydrazine to give an amino pyrazole **3**. This was condensed with 2,4-pentanedione to yield the pyrazolo[1,5-a]pyrimidine **4**.

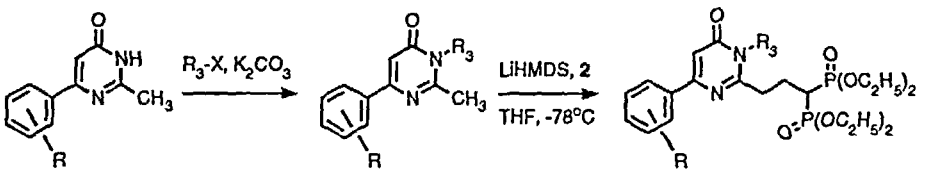
Attempts to deprotonate 4 with LiHMDS in THF at -78°C gave only an incomplete reaction. The problem was found to be the insolubility of 4 in THF at -78°C, but with the addition of pyridine as co-solvent, 4 completely deprotonated and added to give 5.



3 4 5

4-Pyrimidinone Bisphosphonate Esters

4-Pyrimidinones were synthesized by treating the appropriate β-keto ester with acetamidine to give 6. Alkylation of the ring nitrogen with an alkyl halide and K_2CO_3 in methanol yielded 7, though large alkyl groups gave more O-alkylation. 7 was successfully deprotonated with LiHMDS in THF at -78°C, then treated with 2 to give 8.



6 7 8

RESULTS AND DISCUSSION

Delayed-Type Hypersensitivity Granuloma

The pyrazolopyrimidines were initially screened in a model of chronic inflammation, the delayed type hypersensitivity granuloma (DTH-GRA).[4,5] 5a-h gave significant inhibition at the standard dose of 10 mg/kg (Table I). The data indicate that methyl is preferred at C-2 over phenyl while bromine and iodine (5g-h) are preferred at the C-3 position. In further studies, 5a was found to have an EC_{30} of 0.1 mg/kg.

TABLE I

Cmpd	R_2	R_3	% Inhibition (10 mg/kg)[6]	mp (°C)	Cmpd	R_2	R_3	% Inhibition (10 mg/kg)	mp (°C)
5a	Me	CN	46	49-50	5e	Ph	H	47	51-52
5b	Me	Br	44	oil	5f	Ph	Cl	23	66-68
5c	Me	NO_2	41	oil	5g	Ph	Br	31	46-48
5d	Ph	CN	26	107	5h	Ph	I	53	81-82

4-Pyrimidinones were also investigated in the DTH-GRA (Table II), where we studied two changes to the pyrimidinone ring, modification of the aromatic ring at C-6 and of the alkyl group at N-3. Introducing alkyl groups at either the 3 or 4 position of the phenyl group (**8b-c**) had little effect, but an electron withdrawing group, **8d**, dramatically reduced the activity. Electron donating groups at the 4 position gave mixed results (**8e-g**), but the data show that activity increases with increasing size. Varying the alkyl group at N-3 gave mixed results (**8a**, **8h-j**), but methyl appeared to be preferred. The EC_{30} for **8a** was determined to be 0.01 mg/kg.

TABLE II

Cmpd	R	R_3	% Inhibition (10 mg/kg)[6]	mp (°C)	Cmpd	R	R_3	% Inhibition (10 mg/kg)	mp (°C)
8a	H	Me	60	83-84	**8f**	4-OEt	Me	46	94-96
8b	3-Me	Me	40	90-91	**8g**	4-NMe_2	Me	63	92-94
8c	4-Me	Me	38	90-92	**8h**	H	Et	12	79-81
8d	3-CF_3	Me	6	77-79	**8i**	H	n-Pr	41	oil
8e	4-OCH_3	Me	22	81-83	**8j**	H	Bn	17	72-74

Antigen Induced Arthritis (AIA)

The pathology of AIA involves an initial intense inflammatory synovitis followed by chronic inflammation and severe erosion of articular cartilage and subchondral bone, resembling human rheumatoid arthritis.[7] **5a** and **8a** were tested for their ability to control soft tissue inflammation, pannus formation, and cartilage and bone erosion. **5a** has an EC_{30} of 10 mg/kg, while the EC_{30} of **8a** was 0.005 mg/kg.

CONCLUSION

The DTH-GRA and AIA models have been successfully controlled only by steroids, potent immunosuppressive agents, and bisphosphonates. The bisphosphonate ester **8a**, which has EC_{30} values of 0.01 and 0.005 mg/kg respectively, is the most potent bisphosphonate found so far in these assays and offers the potential to successfully treat inflammatory joint disease in man.

EXPERIMENTAL SECTION

(3-(3-Cyano-5-methyl-2-phenyl-pyrazolo[1,5-a]pyrimidin-7-yl)-propylidene)-bisphosphonic acid tetraethyl ester **5d**. The pyrazolopyrimidine **4d**[8] (621 mg, 2.50 mmol) in pyridine (5.0 ml) at 0°C was treated with LiHMDS (1M in THF, 2.6 ml, 2.6 mmol) and stirred for 30 min. The deep red solution was treated with **2** (750 mg, 2.50 mmol) in THF (0.5 ml). After stirring for 1 hour at 22°C, the reaction was poured onto 10% HCl and extracted 3x CH_2Cl_2. The organics were washed with 1N HCl, $NaHCO_3$, and NaCl, dried with $MgSO_4$, and concentrated *in vacuo*. The crude material was purified by chromatography (CH_2Cl_2, CH_2Cl_2/acetone 9:1, then 1:9) to give **5d**:

600 mg (1.09 mmol, 49%), mp 107°C (methyl t-butyl ether); ^{1}H NMR ($CDCl_3$) δ 8.2 (m, 2 H), 7.5 (m, 2 H), 6.86 (s, 1 H), 4.2 (m, 8 H), 3.54 (t, J = 7.3 Hz, 2 H), 2.68 (s, 3 H), 2.5 (m, 3 H), 1.32 (m, 12 H); IR (mull) 2212, 1624, 1556, 1413, 1393, 1250, 1241, 1072, 1046, 1027, 980, 965, 849, 763, 699 cm^{-1}; MS (EI) *m/z* (rel. intensity) 548 (M+, 12), 301 (12), 289 (11), 288 (99), 261 (43), 260 (9), 233 (11), 218 (7), 177 (7), 152 (29); Anal. Calcd for $C_{25}H_{34}N_4O_6P_2$ x H_2O: C, 53.00; H, 6.40; N, 9.89. Found: C, 53.05; H, 6.49; N, 9.92.

(3-(2-(3-Methyl-4-oxo-6-phenyl-4(3H)-pyrimidinyl))-propylidene)bisphosphonic acid tetraethyl ester, **8a**. To a solution of LiHMDS (1.0 M in THF, 83 ml, 83 mmol) at -78°C was added dropwise a solution of **7a**[9] (15.0 g, 75.0 mmol) in THF (50 ml). After stirring for 30 min at -78°C, **2** (24.75 g, 82.5 mmol) was added and the reaction warmed to 22°C for 1 hour. The reaction was quenched with NH_4Cl, then extracted 3x EtOAc. The organics were washed 2x NaCl, dried with $MgSO_4$, and concentrated *in vacuo*. The crude was recrystallized to give **8a**: 29.1 g (58.1 mmol, 78%), mp 85-86°C (methyl tert-butyl ether); ^{1}H NMR ($CDCl_3$) δ 8.02 (m, 2 H), 7.45 (m, 3 H), 6.81 (s, 1 H), 4.20 (m, 8 H), 3.59 (s, 3 H), 3.22 (t, J = 7.2 Hz, 2 H), 2.83 (tt, J = 23.7, 6.5 Hz, 1 H), 2.58 (m, 2 H), 1.34 (m, 12 H); IR (mull) 1668, 1571, 1551, 1441, 1257, 1240, 1218, 1077, 1034, 1012, 982, 969, 840, 804, 704 cm^{-1}; MS (EI) *m/z* (rel. intensity) 500 (M+, 19), 363 (19), 301 (15), 288 (18), 240 (6), 214 (19), 213 (99), 200 (8), 68 (18), 44 (8); Anal. Calcd for $C_{22}H_{34}N_2O_7P_2$: C, 52.80; H, 6.85; N, 5.60. Found: C, 52.55; H, 6.73; N, 5.53.

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