Heteroaryl-Fused 2-Phenylisothiazolone Inhibitors of Cartilage Breakdown

Stephen W. Wright,*,† Joseph J. Petraitis, Matthew M. Abelman, Douglas G. Batt, Lori L. Bostrom, Ronald L. Corbett, Carl P. Decicco, Susan V. Di Meo, Bruce Freimark, John V. Giannaras, Alicia M. Green, James W. Jetter, David J. Nelson, Michael J. Orwat, Donald J. Pinto, Michael A. Pratta, Susan R. Sherk, Jean M. Williams, Ronald L. Magolda, and Elizabeth C. Arner

Inflammatory Diseases Research, The DuPont Merck Pharmaceutical Company, Wilmington, Delaware 19880-0353

Received April 12, 1994®

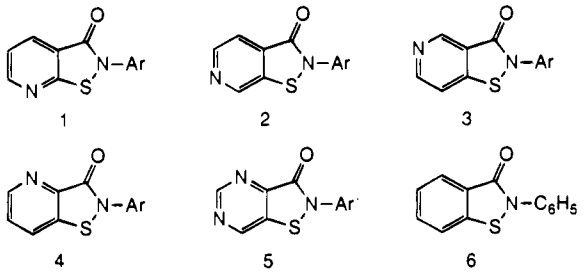
The synthesis, biological evaluation, and structure–activity relationships of a series of *N*-phenyl heteroaryl-fused isothiazolones are described. These isothiazolones have been shown to exhibit potent, dose-dependent inhibition of IL-1β-induced breakdown of proteoglycan in a cartilage organ culture assay. This effect is likely due to inhibition of MMP activation and a consequent reduction in MMP activity following IL-1β stimulation. Thus these compounds potentially represent simple, non-peptidic disease-modifying agents for the treatment of arthritic diseases. To examine the effects of structure on *in vitro* activity, three general features of the molecules were varied, substituents on the pendant *N*-phenyl group, the position of ring fusion to the isothiazolone, and substituents on the fused ring *peri* to the isothiazolone sulfur.

Introduction

There remains an unmet medical need for agents that are capable of arresting the progressive erosion of joint cartilage that is the hallmark of arthritic disease. This erosion is first characterized by the loss of proteoglycan from the cartilage.[1] It is thought to be triggered by a variety of stimuli, particularly cytokines such as the interleukins.[2] This loss of proteoglycan is caused in large measure by the digestion of the hyaluronic acid binding region of the proteoglycan core protein by the matrix metalloproteases (MMP), and particularly stromelysin (MMP-3).[3] Elevated levels of stromelysin have been found in the synovial fluid of patients with osteoarthritis and rheumatoid arthritis.[4] Stromelysin from human articular cartilage has been shown to break down proteoglycan[5] and is secreted by the cells lining the synovium.[6] Indeed, considerable efforts have been made to identify active site inhibitors of matrix metalloproteases. A number of agents, mostly peptidic in nature, have been identified as active site inhibitors of matrix metalloproteases.[7]

Interleukin-1 (IL-1) is a cytokine which is believed to play a role in the cartilage erosion observed in arthritis. IL-1β causes loss of proteoglycan from cartilage both *in vitro*[8] and *in vivo*.[9] This effect is due at least in part to its ability to stimulate stromelysin synthesis by articular chondrocytes[10] and other cells in connective tissues.[11] Thus, the IL-1β-stimulated proteoglycan breakdown in a cartilage organ culture system provides a more general model for evaluating compounds which may act to block cartilage breakdown at any number of possible steps.

In this paper, we describe studies on the ability of a series of *N*-aryl pyrido-fused isothiazolones to inhibit a cytokine-induced model of cartilage breakdown. This series includes the isomeric pyridoisothiazolones **1**–**4** and the pyrimidoisothiazolones **5**, all of which are formally analogs of the random screening lead *N*-phenylbenzisothiazolone (**6**). The synthesis, *in vitro* biological activity, structure–activity relationships, and preliminary mechanism of action results of these compounds are described herein.



Synthesis

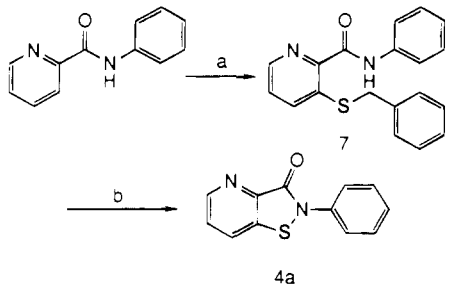
The synthesis of the pyridoisothiazolones (e.g., **1a**) was accomplished by the introduction of a protected sulfur atom into an appropriate synthetic precursor and oxidative cyclization of the resulting product to the desired pyridoisothiazolone.[12] Synthetic manipulations and the solubility of intermediates were considerably improved by protection of the sulfur atom. This also avoided the presence of a reactive thiol group or the use of relatively insoluble disulfides in synthetic sequences. Both benzyl and *tert*-butyl were found to be satisfactory protecting groups for sulfur, as both may be oxidatively deprotected with cyclization to afford the isothiazolone directly.[13] The benzyl and *tert*-butyl sulfides were conveniently prepared by either (1) the reaction of an organolithium species with the dialkyl disulfide or alkyl thiocyanate, (2) the alkylation of a commercially available mercaptopyridine, or (3) the displacement of an activated halide by the alkyl thiolate. Benzyl sulfides were simultaneously dealkylated and cyclized to the isothiazolone either by oxidation with sulfuryl chloride at 80 °C (method A, Scheme 1) or by oxidation to the sulfoxide with *m*-CPBA at 0 °C followed by treatment with trichloroacetic anhydride at 0 °C (method B, Scheme 2). *tert*-Butyl sulfides were oxidatively dealkylated and cyclized to the isothiazolone by oxidation to the sulfoxide with *m*-CPBA at 0 °C followed by thermolysis in refluxing toluene (method C, Scheme 3). The selection of cyclization conditions was generally deter-

† Present address: Pfizer Central Research, Eastern Point Rd., Groton, CT 06340.

® Abstract published in *Advance ACS Abstracts*, August 15, 1994.

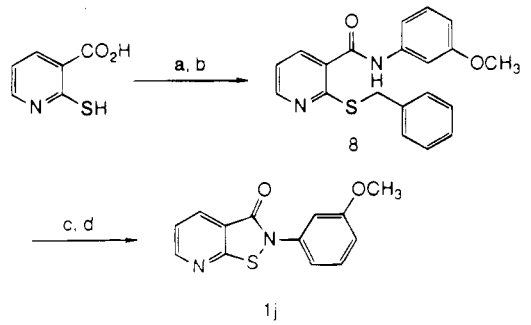
0022-2623/94/1837-3071$04.50/0

Scheme 1[a]



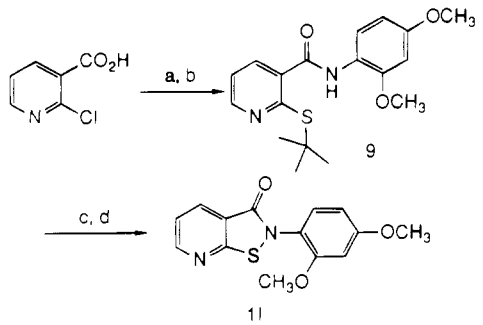
[a] (a) (i) *n*-BuLi, THF, −78 °C, (ii) BzSSBz; (b) SO_2Cl_2, CCl_4, 70 °C.

Scheme 2[a]



[a] (a) KOH, BzCl, 2-PrOH, 80 °C; (b) (i) Et_3N, *t*-BuCOCl, CH_2Cl_2, 45 °C, (ii) 3-$MeOC_6H_4NH_2$, 25 °C; (c) *m*-CPBA, CH_2Cl_2, 0 °C; (d) $(Cl_3CCO)_2O$, CH_2Cl_2, 25 °C.

Scheme 3[a]



[a] (a) (i) ClCOCOCl, CH_2Cl_2, 45 °C, (ii) 2,4-$(MeO)_2C_6H_3NH_2$, pyr, 0 °C; (b) *t*-BuSH, *t*-BuOK, *t*-BuOH, 85 °C; (c) *m*-CPBA, $CHCl_3$, 0 °C; (d) PhMe, pyr, 115 °C.

mined by the sensitivity of the anilide to chlorination or acid. Anilides with electron-releasing or benzylic substituents were often chlorinated by sulfuryl chloride. For these compounds, oxidative deprotection and cyclization via the benzyl sulfoxide method or the even milder *tert*-butyl sulfoxide method were preferred. A smaller number of pyrimidoisothiazolones (**5a−e**) were prepared by multistep functionalization of orotic acid (Scheme 4).

In general, the pyridoisothiazolones are stable, nonhygroscopic crystalline materials. The pyridine ring nitrogen atom does not exhibit basic properties, as shown by the inability of these compounds to be extracted into mineral acids or form salts with hydrogen halides. They likewise resist alkylation at nitrogen or sulfur with methyl iodide and benzyl bromide, even under forcing conditions. They are stable to aqueous acids and to aqueous solutions of alkali metal carbonates and alkylamines but can be decomposed upon prolonged exposure to hydroxide solutions.

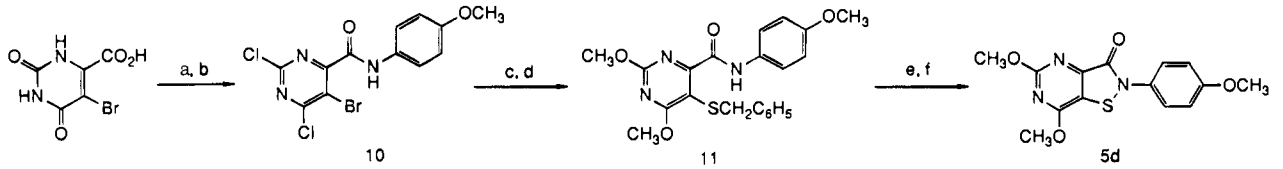
Biological Evaluation

The two series of isothiazolones were examined for their ability to inhibit the IL-1β-induced breakdown of cartilage in a cartilage organ culture assay.[14] IL-1β causes a time- and concentration-dependent stimulation of proteoglycan breakdown (as measured by reaction of the liberated glycosaminoglycans with 1,9-dimethylmethylene blue) and also inhibits proteoglycan resynthesis (as measured by uptake of $^{35}SO_4^{2-}$ by the cartilage). To evaluate inhibitors, bovine nasal septum cartilage slices were stimulated with a soluble, fully active recombinant human IL-1β[15] (500 ng mL^{-1}) for 40 h, which resulted in a submaximal effect on proteoglycan metabolism. Incubations were carried out at 37 °C in the presence or absence of test compounds. Initial screening was carried out with 30 μM inhibitor, with dose responses being determined for those compounds that exhibited >50% inhibition at this concentration. The isothiazolones inhibited the breakdown of proteoglycan in IL-1β stimulated cartilage in a dose-dependent manner (Tables 1 and 2). The viability of the cartilage slices at the end of the assay was conveniently monitored by the uptake of $^{35}SO_4^{2-}$ during the proteoglycan resynthesis analysis. As a class, the inhibitors **1−5** did not further inhibit cartilage resynthesis beyond that caused by IL-1β, indicating that they are not simply toxic to the cartilage. Control incubations of cartilage with **1−5** also showed no increase or decrease in $^{35}SO_4^{2-}$ incorporation from control cartilage incubated in the absence of **1−5**. The compounds **1−5** did not reverse the IL-1β-induced inhibition of cartilage synthesis, however, suggesting that they exert their action at a stage following the IL-1β signal transduction events.

These compounds were also tested for their ability to inhibit various enzymes thought to play a role in inflammatory diseases. They were found as a class to be inactive as inhibitors of cyclooxgenase[16] (bovine seminal vesicles, IC_{50} generally >750 μM), PLA_2[17] (*Croatalus adamanteus*, IC_{50} generally >1 mM), and 5-lipoxygenase[18] (rat basophilic leukemia cell line, IC_{50} generally >25 μM). They also did not inhibit the release of IL-1β by human monocytes *in vitro*.[19]

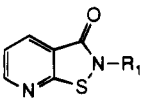
Preliminary data suggest that these compounds inhibit cartilage degradation by interfering with the normal activation of matrix metalloproteinases. For example, **11** did not inhibit plasmin-activated stromelysin,[20a] nor did it inhibit stromelysin activated by chymotrypsin. However, it was found that stromelysin activity was inhibited by 45% if **11** was present *during* the activation of prostromelysin by either plasmin or chymotrypsin. Control experiments indicated that **11** was not an inhibitor of either of the activating proteases,[20b] suggesting that **11** interacts with the prostromelysin in such a way as to inhibit subsequent proteolytic activation. Further experimentation revealed that the inhibition of stromelysin activity was enhanced by preincubation of **11** with prostromelysin prior to the addition of the activating protease. The inhibition of activation reached its maximal effect at approximately 6 h of preincubation and 80% of the maximal effect in 3 h. A summary of the effects of **11** and a more potent analog (**1m**) on stromelysin activation is presented in Table 3. Taken together, these data suggest that these compounds inhibit IL-1β-stimulated

Scheme 4[a]



[a] (a) $POCl_3$, 170 °C; (b) 4-$CH_3OC_6H_4NH_2$, PhMe, 0 °C; (c) $NaOCH_3$, DMF, 90 °C; (d) (i) NaH, THF, 25 °C, (ii) TMEDA, *n*-BuLi, −78 °C, (iii) $C_6H_5CH_2SCN$, 0 °C; (e) *m*-CPBA, CH_2Cl_2, 0 °C; (f) Cl_3CCOCl, CH_2Cl_2, 25 °C.

Table 1. Physical Data and *in Vitro* Activity for Isothiazolones 1



entry	R_1	mp[a] (°C)	yield[b] (%)	sulfur[c]	cycln[d]	anal.[e]	*in vitro* IC_{50}[f]
1a	C_6H_5	133	80	2	A	$C_{12}H_8N_2OS$	4.4
1b	2-ClC_6H_4	163	45	2	B	$C_{12}H_7ClN_2OS$	(0)
1c	3-ClC_6H_4	185	56	2	B	$C_{12}H_7ClN_2OS$	30
1d	4-ClC_6H_4	195	97	2	A	$C_{12}H_7ClN_2OS$	8.5
1e	4-FC_6H_4	200	89	2	A	$C_{12}H_7FN_2OS$	12.0
1f	4-BrC_6H_4	185	72	2	B	$C_{12}H_7BrN_2OS$	8.2
1g	4-IC_6H_4	198	77	2	B	$C_{12}H_7IN_2OS$	7.8
1h	4-$CF_3C_6H_4$	200	34	2	A	$C_{13}H_7F_3N_2OS$	(17)
1i	2-$MeOC_6H_4$	145	65	2	A	$C_{13}H_{10}N_2O_2S$	9.6
1j	3-$MeOC_6H_4$	114	96	2	B	$C_{13}H_{10}N_2O_2S$	3.5
1k	4-$MeOC_6H_4$	164	51	2	A	$C_{13}H_{10}N_2O_2S$	13.5
1l	2,4-$(MeO)_2C_6H_3$	178	94	2	C	$C_{14}H_{12}N_2O_3S$	9.5
1m	2,5-$(MeO)_2C_6H_3$	121	53	2	A	$C_{14}H_{12}N_2O_3S$	5.4
1n	2,6-$(MeO)_2C_6H_3$	164	41	2	B	$C_{14}H_{12}N_2O_3S$	(40)
1o	3,4-$(MeO)_2C_6H_3$	176	52	2	B	$C_{14}H_{12}N_2O_3S$	8.2
1p	3,5-$(MeO)_2C_6H_3$	124	67	2	B	$C_{14}H_{12}N_2O_3S$	4.9
1q	3,4,5-$(MeO)_3C_6H_2$	186	50	2	B	$C_{15}H_{14}N_2O_4S$	7.0
1r	2,4,6-$(MeO)_3C_6H_2$	179	84	2	B	$C_{15}H_{14}N_2O_4S$	13.0
1s	2,5-$(MeO)_2$-4-Cl-C_6H_2	217	41	2	A	$C_{14}H_{11}ClN_2O_3S$	11.0
1t	3-HOC_6H_4	215	35	2	B	$C_{12}H_8N_2O_2S$	21.0
1u	2,6-$Me_2C_6H_3$	112	88	2	A	$C_{14}H_{12}N_2OS$	8.0
1v	2,6-$Et_2C_6H_3$	110	28	2	B	$C_{16}H_{16}N_2OS$	9.0
1w	2,6-(*i*-Pr)$_2C_6H_3$	147	60	2	B	$C_{18}H_{20}N_2OS$	12.0
1x	3-$O_2NC_6H_4$	250	93	2	A	$C_{12}H_7N_3O_3S$	(37)
1y	4-$O_2NC_6H_4$	285	91	2	A	$C_{12}H_7N_3O_3S$	28.0
1z	3-$NH_2C_6H_4$	157	47	2	D	$C_{12}H_9N_3OS$	(34)
1aa	3-$AcNHC_6H_4$	176	49	2	D[g]	$C_{14}H_{11}N_3OS$	30.0
1bb	4-$AcNHC_6H_4$	228	50	3	C	$C_{14}H_{11}N_3O_2S$	28.0
1cc	4-$EtO_2CNHC_6H_4$	204	16	3	C	$C_{15}H_{13}N_3O_3S$	14.0
1dd	4-$MeNHCONHC_6H_4$	242	27	3	C	$C_{14}H_{12}N_4O_2S$	9.0
1ee	4-$MeSO_2NHC_6H_4$	226	59	3	C	$C_{13}H_{11}N_3O_3S_2$	(28)
1ff	2-$MeO_2CC_6H_4$	120	53	2	A	$C_{14}H_{10}N_2O_3S$	25.0
1gg	3-$MeO_2CC_6H_4$	209	26	2	A	$C_{14}H_{10}N_2O_3S$	(35)
1hh	4-$MeO_2CC_6H_4$	210	53	2	B	$C_{14}H_{10}N_2O_3S$	30.0
1ii	4-$EtO_2CC_6H_4$	156	44	3	A	$C_{15}H_{12}N_2O_3S$	20.0
1jj	4-$Me_2NCOC_6H_4$	176	51	2	B	$C_{15}H_{13}N_3O_2S$	17.0
1kk	4-NCC_6H_4	250	60	2	B	$C_{13}H_7N_3OS$	(40)
1ll	2-C_5H_4N[h]	210	5	2	A	$C_{11}H_7N_3OS$	(23)
1mm	1-$C_{10}H_7$	142	40	2	A	$C_{16}H_{10}N_2OS$	7.0
1nn	2-MeO-4-$MeO_2CC_6H_3$	147	98	3	C	$C_{15}H_{12}N_2O_4S$	3.0
1oo	2-MeO-5-$MeO_2CC_6H_3$	181	84	3	C	$C_{15}H_{12}N_2O_4S$	8.0
1pp	2-MeO-6-$MeO_2CC_6H_3$	145	70	3	C	$C_{15}H_{12}N_2O_4S$	8.0

[a] All melting points represent the lower value of a 2 °C melting range. [b] All yield represent recrystallized products. [c] Method by which protected sulfur was introduced into substance (see Synthesis). [d] Method by which benzyl or *tert*-butyl sulfide was oxidatively cyclized to isothiazolone (see Synthesis). [e] All compounds gave satisfactory ^1H NMR, mass spectra, and elemental analyses for C, H, and N. [f] IC_{50} (μM) in IL-1β-stimulated cartilage organ culture assay; data in parentheses represent percent inhibition at 30 μM inhibitor for compounds whose IC_{50} was not determined. Standard errors ± 15%. [g] Prepared by acetylation of **1z** with Ac_2O. [h] See *J. Heterocycl. Chem.* **1985**, *22*, 1353−1356, for preparation.

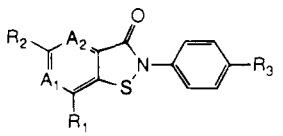
cartilage breakdown by interfering with prostromelysin activation. Studies are currently in progress to further elucidate the biochemical mechanism of this inhibition.[20c]

Structure−Activity Relationships

Previous work[21] had shown that an aryl-fused *N*-arylisothiazolone was required for inhibition of IL-1β-induced cartilage breakdown *in vitro*. Comparison of the data in Tables 1 and 2 reveals that, for the parent pyridoisothiazolones **1a**, **2a**, **3a**,[22] and **4a**, the *in vitro* potency was largely independent of the position of the pyridine−isothiazolone ring fusion. The pyrimido-fused isothiazolone **5a** was a relatively weak inhibitor.

Within the [5,4-*b*]pyridoisothiazolone series (1), the *in vitro* effects of a substantial number of substituents

Table 2. Physical Data and *in Vitro* Activity for Isothiazolones **2**, **4**, and **5**



entry	R_1	R_2	R_3	A_1	A_2	mp[a] (°C)	yield[b] (%)	sulfur[c]	cycln[d]	anal.[e]	*in vitro* IC_{50}[f]
2a	H	H	H	N	CH	168	73	1	B	$C_{12}H_8N_2OS$	4.4
2b	MeO	H	H	N	CH	146	89	1	B	$C_{13}H_{10}N_2O_2S$	2.9
2c	MeO	H	CF_3	N	CH	159	38	1	B	$C_{14}H_9F_3N_2O_2S$	9.0
2d	MeO	H	MeO	N	CH	184	50	1	B	$C_{14}H_{12}N_2O_3S$	(20)
2e	Cl	H	H	N	CH	130	61	1	B	$C_{12}H_7ClN_2OS$	(38)
2f	Cl	H	CF_3	N	CH	135	13	1	B	$C_{13}H_6ClF_3N_2OS$	14
2g	Cl	H	MeO	N	CH	170	55	1	B	$C_{13}H_9ClN_2O_2S$	30
2h	MeO	MeO	H	N	CH	160	67	1	B	$C_{14}H_{12}N_2O_3S$	(39)
4a	H	H	H	CH	N	198	59	1	B	$C_{12}H_8N_2OS$	6.0
4b	MeO	H	H	CH	N	88	10	1	B	$C_{13}H_{10}N_2O_2S$	4.0
5a	H	H	H	N	N	187	48	3	D	$C_{11}H_7N_3OS$	30
5b	MeO	MeO	H	N	N	210	48	3	D	$C_{13}H_{11}N_3O_3S$	(40)
5c	MeO	MeO	MeO	N	N	217	50	3	D	$C_{14}H_{13}N_3O_4S$	(50)
5d	MeO	MeO	CF_3	N	N	201	67	3	D	$C_{14}H_{10}F_3N_3O_3S$	(39)
5e	HO	HO	MeO	N	N	312	86	3	D	$C_{12}H_9N_3O_4S$	(49)

[a] All melting points represent the lower value of a 2 °C melting range. [b] All yields represent recrystallized products. [c] Method by which protected sulfur was introduced into substance (see Synthesis). [d] Method by which benzyl or *tert*-butyl sulfide was oxidatively cyclized to isothiazolone (see Synthesis). [e] All compounds gave satisfactory ^1H NMR, mass spectra, and elemental analyses for C, H, and N. [f] IC_{50} (μM) in IL-1β-stimulated cartilage organ culture assay; data in parentheses represent percent inhibition at 30 mM inhibitor for compounds whose IC_{50} was not determined. Standard errors ± 15%.

Table 3. Inhibition of Stromelysin Activation by Sample Pyridoisothiazolones **1**

entry	R_1	cartilage IC_{50}[a]	inhibition of activation[b] 0 h	inhibition of activation[b] 3 h	activation IC_{50}[c]
1l	2,4-$(MeO)_2C_6H_3$	9.5	45	65	62
1m	2,5-$(MeO)_2C_6H_3$	5.4	51	68	53

[a] IC_{50} (μM) in IL-1β-stimulated cartilage organ culture assay, standard errors ± 15%. [b] Expressed as percent inhibition of control stromelysin activity following preincubation of compounds with prostromelysin for 0 or 3 h prior to activation by plasmin and assay (see Experimental Section). Standard errors ± 10%. [c] IC_{50} (μM) for inhibition of prostromelysin activation with no preincubation period, standard errors ± 10%.

on the pendant phenyl ring were examined. The introduction of a chlorine into this ring caused the activity to decrease considerably in the order 4 > 3 ≫ 2 (**1b–d**). The substitution of other halogens for chlorine at the 4-position resulted in activity increasing in the order F < Cl, Br, I (**1d–g**), suggesting that activity decreases as the 4-substituent becomes more electronegative. Likewise, a loss in activity is noted with the 4-trifluoromethyl derivative **1h** and the nitro derivatives[23] **1x,y** which are significantly less potent than the halo-substituted compounds and **1a**.

Placement of electron-releasing methoxy substituents on this ring causes the activity to decrease 3 > 2, 4 (**1i–k**). Noting the activity of the 3-methoxy compound **1j**, we evaluated the effects of increasing the number of methoxy substituents. None of the dimethoxy and trimethoxy derivatives examined were more potent than **1j**, but the data suggest that the 3-methoxy substitution is most favorable. For example, the 2,5-dimethoxy and 3,4-dimethoxy (**1m,o**, both with one *meta* methoxy group) and the 3,5-dimethoxy (**1p**, with two *meta* methoxy groups) analogs are generally more potent than those dimethoxy analogs that lack a *meta* methoxy group (**1l,n**). Placement of a strongly electron releasing substituent such as OH (**1t**) or NH_2 (**1z**) at the 3-position resulted in a substantial decrease in activity, which could be somewhat attenuated by N-acetylation (**1aa**). Placement of the *N*-acetyl group at the 4-position (**1bb**) resulted in similarly low potency. Replacement of the 4-*N*-acetyl group by an ethyl carbamate (**1cc**) or methylurea (**1dd**) led to some recovery of potency, while the sulfonamide **1ee** was inactive.

Replacement of the pendant phenyl ring by a naphthyl ring (**1mm**) was tolerated, but replacement with a pyridine ring (**1ll**) led to a substantial loss in activity. Increasing the steric crowding about the isothiazolone, and simultaneously increasing the out-of-plane rotation of the pendant phenyl ring, was examined by placing increasingly large alkyl substituents at the 2- and 6-positions (**1u–w**). This resulted in some loss of potency (**1w**) compared to **1a,i**. Substitution of carbomethoxy groups on this ring also caused potency to decrease in the order 2, 4 > 3 (**1ff–hh**).

The effect of placing substituents on the fused pyridine ring, *peri* to the isothiazolone sulfur, was also of interest. This was synthetically feasible with the pyridoisothiazolones **2** and **4**. Here it was found that placement of a methoxy group next to the isothiazolone sulfur atom led to somewhat improved potency (**2b**, **4b**) compared to the parent compounds. Placing an additional methoxy group in the pyridine ring resulted in a substantial loss of activity (**2h**). Likewise, the addition of two methoxy groups to the pyrimido-fused series (**5b–d**) also resulted in decreased potency. Placing a chlorine *peri* to the isothiazolone sulfur led to a considerable loss of activity (**2e**).

Taken together with previous findings,[21] these data suggest that the key pharmacophore is a pyrido-fused *N*-arylisothiazolone. The observed *in vitro* activity is relatively insensitive to the position of the pyridine–isothiazolone ring fusion, but it is sensitive to the nature of the substituents placed on either the pyridyl ring or the *N*-aryl ring. In particular, optimum *in vitro* activity is achieved with substituents on the *N*-aryl ring which are capable of only modestly affecting the electron density in the ring.

Table 4. Data for Standard Drugs and Selected Pyridoisothiazolones

compound	IC_{50} (μM)[a]
indomethacin	>30
naproxen	>30
phenidone	>30
tetracycline	>30
BBT-16	3.0
1a	4.4
1j	3.5
2b	2.9
4b	4.0

[a] IC_{50} in IL-1β-stimulated cartilage organ culture assay, standard errors ± 15%.

Summary

A comparison of selected pyridoisothiazolones with some standard drugs is given in Table 4. It will be noted that conventional anti-inflammatory drugs, such as indomethacin and naproxen, as well as tetracycline (a collagenase inhibitor),[24] are ineffective at blocking the IL-1-stimulated breakdown of cartilage in vitro, as is the dual 5-LO/CO inhibitor phenidone. By way of further comparison, a typical peptidic inhibitor of stromelysin[25] (BBT-16, IC_{50} = 20 nM in enzyme assay) was shown to be approximately equipotent to **1a,j** and **2b** in the organ culture assay.

In conclusion, pyridoisothiazolones represent simple, non-peptidic small molecule structures that inhibit the IL-1β-stimulated breakdown of cartilage tissue in an organ culture system. These compounds are equally or more potent at inhibiting cartilage destruction in a tissue-based assay than other anti-inflammatory agents. In general, it would appear that potency in the cartilage organ culture assay is mediated to some extent by the electron-releasing or electron-withdrawing nature of substituents placed on the pendant phenyl ring, with the best potency observed with those compounds that have limited excess electron density in this ring. The observed structure–activity relationships must be interpreted with some care, as the *in vitro* data represent the product of independent structural contributions to solubility, tissue penetration, and cell penetration, as well as contributions to intrinsic potency. Studies are in progress to further elucidate the mechanism of action of these compounds and their *in vivo* biological properties, particularly their effects upon models of arthritic diseases.

Experimental Section

^{1}H NMR spectra were recorded on Varian Gemini 200 (200 MHz) or IBM 200 SY (200 MHz) spectrometers using tetramethylsilane as an internal standard. Infrared spectra were recorded as neat films or KBr pellets as noted on a Perkin-Elmer 1710 FT spectrometer. Mass spectral data were recorded on Finnigan-MAT 8230 or DuPont DP-1 instruments, using the indicated ionization techniques. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ, and were within 0.4% of the calculated values. Thin layer chromatography was carried out with E. Merck 15327 silica gel plates.

All reactions were carried out with continuous magnetic stirring under an atmosphere of dry nitrogen. All solutions were dried over anhydrous magnesium sulfate unless otherwise noted; all evaporations were carried out on a rotary evaporator at ca. 30 Torr. Commercial reagents and solvents were generally used as received without additional purification. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. 3-Chloroperbenzoic acid samples were assayed by iodometric titration before use.[26]

Preparation of Pyridoisothiazolones by Oxidative Cyclization with Sulfuryl Chloride (Method A). 2-Phenylisothiazolo[4,5-*b*]pyridin-3-one (4a). Step 1: Pyridine-2-carboxylic acid phenylamide[27] (1.00 g, 5 mmol) was dissolved in 20 mL of THF and cooled to −78 °C while *n*-BuLi (2.5 M, 4.4 mL, 11 mmol) was added. After 30 min at −78 °C, the reaction mixture was warmed to −23 °C and kept at −23 °C for 45 min. The mixture was then cooled to −78 °C and treated with 1.48 g (6 mmol) of dibenzyl disulfide in THF. The reaction mixture was then allowed to warm to 25 °C over 1 h and then quenched with saturated NH_4Cl solution. The mixture was extracted with $CHCl_3$, and the extracts were dried and concentrated to give a residue that was chromatographed on silica gel eluting with $CHCl_3$ to give 1.23 g (77%) of 3-(benzylsulfanyl)pyridine-2-carboxylic acid phenylamide (**7**) as white crystals, mp 152–154 °C. ^{1}H NMR ($CDCl_3$): δ 10.10 (br s, 1 H), 8.31 (d, 1 H), 7.76 (m, 3 H), 7.46 (d, 2 H), 7.38–7.24 (m, 6 H), 7.14 (m, 1 H), 4.16 (s, 2 H). MS (NH_3, CI): *m/z* 321 (M + H^+, 100).

Step 2: The benzyl sulfide **7** (0.15 g, 0.47 mmol) was dissolved in 10 mL of CCl_4 with heating to 70 °C. A solution of SO_2Cl_2 in CCl_4 (1 M, 1.87 mL, 1.87 mmol) was added, and the mixture was heated at 70 °C for 45 min. The mixture was then cooled to 25 °C, the reaction was quenched with saturated $NaHCO_3$ solution, and the mixture was extracted with $CHCl_3$. The organic extracts were dried, concentrated, and chromatographed on silica gel eluting with $CHCl_3$:CH_3OH to give 0.063 g (59%) of **4a**, mp 198–200 °C. ^{1}H NMR ($CDCl_3$): δ 8.83 (d, 1 H), 8.04 (d, 1 H), 7.72 (d, 2 H), 7.58 (m, 1 H), 7.50 (m, 2 H), 7.36 (m, 1 H). MS (NH_3, CI): *m/z* 229 (M + H^+, 100). Anal. Calcd for $C_{12}H_8N_2OS$: C, H, N.

Preparation of Pyridoisothiazolones by Oxidative Cyclization with *m*-CPBA and Trichloroacetic Anhydride (Method B). 2-(3-Methoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-one (1j). Step 1: 2-Mercaptonicotinic acid (23.2 g, 150 mmol) was added to a solution of KOH pellets (85%, 19.8 g, 300 mmol) in 200 mL of 2-propanol and 40 mL of H_2O. The mixture was heated to reflux, and benzyl chloride (17.3 mL, 150 mmol) was added dropwise. The mixture was heated for 30 min and then cooled, and half of the solvent was evaporated. The residue was diluted with 100 mL of water and acidified with AcOH (8.6 mL, 150 mmol). The precipitated acid was filtered, dried, and recrystallized from EtOH to give 28.7 g (78%) of 2-(benzylsulfanyl)nicotinic acid, mp 192–194 °C. ^{1}H NMR ($CDCl_3$): δ 8.67 (t, 1 H), 8.21 (d, 1 H), 7.35 (m, 6 H), 4.39 (s, 2 H). MS (NH_3, CI): *m/z* 246 (M + H^+, 100).

Step 2: The above acid (2.50 g, 10.2 mmol) was suspended in 100 mL of CH_2Cl_2 and treated with Et_3N (1.6 mL, 11.5 mmol). The acid dissolved, and the mixture was stirred for 5 min before being treated with 1.4 mL (11.4 mmol) of trimethylacetyl chloride. The mixture was stirred at 45 °C for 1 h and then cooled to 25 °C and treated with 1.1 mL (11.6 mmol) of 3-methoxyaniline. This mixture was then stirred at 25 °C for 21 h and the reaction quenched with 50 mL of 1 M NaOH, and the mixture was partitioned between brine and $CHCl_3$. The layers were separated, and the aqueous phase was extracted with $CHCl_3$. The combined organic extracts were dried, concentrated, and recrystallized from EtOAc:hexane to give 2.6 g (72%) of white crystals of 2-(benzylsulfanyl)-*N*-(3-methoxyphenyl)nicotinamide (**8**), mp 130–132 °C. ^{1}H NMR ($CDCl_3$): δ 8.58 (d, 1 H), 7.95 (br s, 1 H), 7.91 (d, 1 H), 7.42–7.37 (m, 3 H), 7.30–7.20 (m, 4 H), 7.12 (m, 1 H), 7.03 (d, 1 H), 6.71 (d, 1 H), 4.51 (s, 2 H), 3.82 (s, 3 H). MS (NH_3, CI): *m/z* 351 (M + H^+, 100).

Step 3: One gram (2.85 mmol) of **8** was dissolved in 25 mL of CH_2Cl_2 and cooled to 0 °C. 3-Chloroperbenzoic acid (60%, 0.82 g, 2.85 mmol) was added, and the mixture was stirred for 15 min at 0 °C. The reaction was quenched with 2 M NaOH and the mixture stirred. The reaction mixture was filtered, and the organic phase was dried and concentrated. The residue was combined with the collected precipitate to give 0.85 g (82%) of 2-(benzylsulfinyl)-*N*-(3-methoxyphenyl)nicotinamide as a white powder, mp 195–197 °C. ^{1}H NMR ($CDCl_3$): δ 10.70 (br s, 1 H), 8.89 (d, 1 H), 8.31 (d, 1 H), 7.77 (m, 1 H),

7.39–7.24 (m, 8 H), 6.76 (m, 1 H), 4.48 (d, 1 H), 4.16 (d, 1 H), 3.78 (s, 3 H). MS (NH_3, CI): m/z 367 ($M + H^+$, 100).

Step 4: A suspension of the sulfoxide (0.82 g, 2.3 mmol) in 50 mL of CH_2Cl_2 was cooled to 0 °C and treated with trichloroacetic anhydride (0.47 mL, 2.6 mmol). The sulfoxide dissolved in 15 min, and the mixture was stirred for 3.5 h while being allowed to warm to 25 °C. The reaction mixture was quenched with 2 M NaOH and the mixture separated. The aqueous phase was extracted with $CHCl_3$, and the combined organic phases were dried and concentrated. The residue was chromatographed on silica gel eluting with EtOAc:hexane to give 0.57 g (97%) of white crystals of **1j**, mp 114–116 °C. ^1H NMR ($CDCl_3$): δ 8.82 (d, 1 H), 8.16 (d, 1 H), 7.39 (m, 2 H), 7.32 (m, 1 H), 7.25 (m, 1 H), 6.87 (d, 1 H), 3.83 (s, 3 H). MS (NH_3, CI): m/z 259 ($M + H^+$, 100%). Anal. Calcd for $C_{13}H_{10}N_2O_2S$: C, H, N.

Preparation of Pyridoisothiazolones by Oxidative Cyclization with *m*-CPBA and Thermolysis (Method C). 2-(2,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-one (1l). Step 1: A suspension of 2-chloronicotinic acid (3.15 g, 20 mmol) in 40 mL of CH_2Cl_2 was treated with 0.8 mL of DMF followed by a solution of oxalyl chloride (1.83 mL, 21 mmol) in CH_2Cl_2 (2 mL) with cooling in ice over 5 min. The mixture was then heated under reflux for 40 min until all material dissolved. The solution was cooled to 0 °C and treated with a solution of freshly distilled 2,4-dimethoxyaniline (3.22 g, 21 mmol) in pyridine (10 mL). The mixture was stirred for 90 min at 0 °C and then washed with 3 M HCl, water, 1 M $NaHCO_3$, and brine, dried, and concentrated. The residue was recrystallized from *n*-BuCl to give 4.97 g (85%) of 2-chloro-*N*-(2,4-dimethoxyphenyl)nicotinamide as white crystals, mp 98–100 °C. ^1H NMR ($CDCl_3$): δ 8.69 (br s, 1 H), 8.50 (d, 1 H), 8.39 (d, 1 H), 8.21 (d, 1 H), 7.39 (m, 1 H), 6.55 (m, 1 H), 6.53 (s, 1 H), 3.89 (s, 3 H), 3.83 (s, 3 H). MS (NH_3, CI): m/z 293 ($^{35}ClM + H^+$, 100), 295 ($^{37}ClM + H^+$, 33).

Step 2: A solution of 1.82 g (16.2 mmol) of KO*t*-Bu and 1.83 mL (16.2 mmol) of *tert*-butyl mercaptan in 32 mL of *t*-BuOH was heated under reflux while 3.17 g (10.8 mmol) of the chloroamide was added in portions through the condenser. The mixture was heated for 2 h and then cooled to 25 °C and stirred overnight with a solution of 2.85 g (8.6 mmol) of $K_3Fe(CN)_6$.[28] The reaction mixture was diluted with water and extracted three times with EtOAc. The EtOAc extracts were washed twice with water and brine, dried, and concentrated. Recrystallization from *n*-BuCl gave 4.12 g (90%) of white crystals of 2-(*tert*)-butylsulfanyl-*N*-(2,4-dimethoxyphenyl)nicotinamide (**9**), mp 104–106 °C. ^1H NMR ($CDCl_3$): δ 9.27 (br s, 1 H), 8.57 (d of d, 1 H), 8.42 (d, 1 H), 8.12 (d, 1 H), 7.19 (m, 1 H), 6.52 (m, 2 H), 3.88 (s, 3 H), 3.82 (s, 3 H), 1.57 (s, 9 H). MS (NH_3 CI): m/z 347 ($M + H^+$, 100).

Step 3: A solution of **9** (3.46 g, 10 mmol) in 15 mL of $CHCl_3$ was cooled to −10 °C and treated with a cold (0 °C) solution of *m*-CPBA (57%, 3.03 g, 10 mmol) in 30 mL of $CHCl_3$. The progress of the reaction was monitored by TLC (1:1 EtOAc:hexane). Upon completion of the reaction, 3 mL of Me_2S was added to destroy any remaining peracid. The solution was extracted with 1 M $NaHCO_3$, washed with water and brine, dried, and concentrated to an oil that began to deposit crystals (4.49 g). This was used directly in the next step without attempting to remove the residual $CHCl_3$ in the crude product. ^1H NMR ($CDCl_3$): δ 8.88 (d, 1 H), 8.71 (br s, 1 H), 8.23 (d, 1 H), 8.18 (d, 1 H), 7.54 (m, 1 H), 6.52 (m, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H), 1.29 (s, 9 H). MS (NH_3, CI): m/z 289 ($M + H^+ - C_4H_8$, $- H_2O$, 100); 363 ($M + H^+$, 7).

Step 4: The crude sulfoxide was dissolved in 5 mL of PhMe and concentrated (<40 °C) to remove excess $CHCl_3$. The sulfoxide was then dissolved in 11 mL of PhMe and 4 mL of pyridine. This solution was then added dropwise to a refluxing mixture of 23 mL of PhMe and 8 mL of pyridine with azeotropic removal of water. The mixture was heated for an additional 30 min and then cooled and concentrated to dryness. The residue was digested with *n*-BuCl, cooled, and filtered to give 2.72 g (94% from **9**) of **1l** as white crystals, mp 178–180 °C. ^1H NMR ($CDCl_3$): δ 8.79 (d, 1 H), 8.33 (d, 1 H), 7.38 (m, 1 H), 7.35 (d, 1 H), 6.58 (s, 1 H), 6.55 (m, 1 H), 3.86 (s, 3 H), 3.82 (s, 3 H). MS (NH_3, CI): m/z 289 ($M + H^+$, 100). Anal. Calcd for $C_{14}H_{12}N_2O_3S$: C, H, N.

Preparation of Pyrimidoisothiazolones: 5,7-Dimethoxy-2-[4-(trifluoromethyl)phenyl]isothiazolo[4,5-*d*]pyrimidin-3-one (5d). Step 1: A mixture of 25.0 g (106 mmol) of 5-bromoorotic acid[29] and 500 mL of $POCl_3$ was placed in a sealed, glass-lined steel bomb and heated to 170 °C for 6 h. After cooling to 25 °C, the solution was poured into 500 mL of PhMe and filtered through a sintered glass frit. The $POCl_3$ was removed by azeotropic distillation with PhMe (re-evaporating with an additional 2 × 500 mL of PhMe) under reduced pressure. The resulting brown oil was filtered through a short plug of Celite. The crude material was dissolved in 500 mL of PhMe, transferred to a 3 L flask equipped with a mechanical stirrer, and cooled to 0 °C. *p*-Anisidine (66.0 g, 0.54 mol) in 400 mL of PhMe was added cautiously over 40 min. The resulting slurry was poured into 500 mL of H_2O, and an additional 1 L of PhMe was added. The PhMe was separated, washed with 1 M NaOH (1 × 200 mL), H_2O (1 × 500 mL), and brine, and dried. The solvent was evaporated to give a semisolid that was triturated with hexane to afford after drying 22.0 g (55%) of **10** as a yellow solid, mp 285 °C dec. ^1H NMR (DMSO-d_6): δ 10.55 (s, 1 H), 7.55–7.59 (d, 2H), 6.95–7.00 (d, 2 H), 3.75 (s, 3 H). MS (NH_3, CI): m/z 378 ($M + H^+$, 100).

Step 2: To 15.0 g (40 mmol) of **9** in 100 mL of DMF was added 150 g (2.78 mol) of NaOMe. The solution was heated to 90 °C for 1 h and cooled and then poured into H_2O and extracted with EtOAc. The EtOAc was separated, washed with H_2O and brine, dried, and evaporated. The residue was purified by SiO_2 chromatography (4:1 hexane:EtOAc) to give 11.5 g (78%) of the dimethoxypyrimidine, mp 140–141 °C. ^1H NMR (DMSO-d_6): δ 10.55 (d, 2 H), 7.58 (d, 2 H), 6.94 (d, 2 H), 4.03 (s, 3 H), 3.95 (s, 3 H), 3.75 (s, 3 H). MS (NH_3, CI): m/z 368, 370 ($M + H^+$, Br isotopes, 100).

Step 3: To a stirred solution of 10.0 g (27 mmol) of the product from step 2 in 500 mL of THF under N_2 was added 3.30 g (136 mmol) of NaH. The resulting solution was stirred for 2 h at 25 °C. TMEDA (4.1 mL, 27 mmol) was added, and the reaction mixture was cooled to −78 °C followed by the addition of 20.5 mL of *n*-BuLi (2.5 M, 51.2 mmol). The mixture was stirred for an additional 10 min at −78 °C. The reaction vessel was then placed in an ice bath and allowed to reach 0 °C. To the thick mixture was added 7.86 g (52.7 mmol) of benzyl thiocyanate in one portion. After 10 min, the reaction was quenched by adding MeOH and the resulting solution was poured into 200 mL of H_2O. The organic material was extracted with PhMe (3 × 500 mL). The combined PhMe extracts were washed with H_2O (2 × 200 mL), 1 M HCl (1 × 200 mL), and brine and dried. Concentration gave an oil that was purified by SiO_2 chromatography (eluted first with 500 mL of hexane and then ramped to 4:1 hexane:EtOAc and finally EtOAc) to give a solid that was recrystallized from *n*-BuCl to afford 6.50 g (59%) of **11**, mp 95–99 °C. ^1H NMR (DMSO-d_6): δ 10.37 (s, 1 H), 9.59 (d, 2 H), 7.16–7.25 (m, 5 H), 6.95 (d, 2 H), 3.98 (s, 2 H), 3.93 (s, 3 H), 3.88 (s, 3 H), 3.75 (s, 3H). MS (NH_3, CI) m/z 412 ($M + H^+$, 42).

Step 4: To 4.11 g (10 mmol) of **11** in 100 mL of CH_2Cl_2 was added *m*-CPBA (72.5%, 2.38 g, 10 mmol) in 50 mL of CH_2Cl_2. Excess *m*-CPBA was quenched by addition of $NaHSO_3$, and the resulting solution was poured into H_2O. The CH_2Cl_2 layer was separated, washed with 10% NaOH, H_2O, and brine, dried, and evaporated to a solid that was recrystallized from EtOAc:hexane to give 2.81 g (66%) of sulfoxide. ^1H NMR ($CDCl_3$): δ 9.24 (s, 1 H), 7.59 (d, 2 H), 7.30–7.41 (m, 5 H), 6.92 (d, 2 H), 4.52 (d of d, 2 H), 4.10 (s, 3 H), 3.99 (s, 3 H), 3.80 (s, 3 H).

Step 5: A solution of the sulfoxide (2.00 g, 4.7 mmol) in 100 mL of CH_2Cl_2 was added to a solution of 2.10 mL (18.8 mmol) of trichloroacetyl chloride in 80 mL of CH_2Cl_2 over 1 h at 25 °C. The reaction was allowed to continue for an additional 10 min, and then the mixture was poured into H_2O and neutralized with $NaHCO_3$. The CH_2Cl_2 layer was separated, washed with H_2O and brine, dried, and evaporated to give a solid which was recrystallized from *n*-BuCl:CH_2Cl_2 to afford 1.00 g (67%) of **5d**, mp 217–218 °C. ^1H NMR ($CDCl_3$): δ 7.53 (d, 2 H), 7.00 (d, 2 H), 4.18 (s, 3 H), 4.16 (s, 3 H), 3.85 (s, 3 H). MS

(NH_3, CI): m/z 320 ($M + H^+$, 100). Anal. Calcd for $C_{14}H_{13}N3O_4S$: C, H, N.

2-Phenylisothiazolo[4,5-c]pyridin-3-one (3a). A solution of 4-[(2-methylprop-2-yl)sulfinyl]pyridine[30] (0.37 g, 2.0 mmol) in 3 mL of THF was added dropwise to 2.2 mmol of LDA in 4 mL of THF at −78 °C. The mixture was stirred at −78 °C for 30 min, during which time a white suspension formed. Redistilled phenyl isocyanate (0.24 mL, 2.2 mmol) was added, and the mixture was stirred overnight at 20 °C. The reaction was quenched with saturated NH_4Cl, and the mixture was extracted three times with $CHCl_3$. The $CHCl_3$ extracts were washed with water and brine, dried, and concentrated to a brown oil. Chromatography on silica gel (95:5 CH_2Cl_2:2-PrOH) gave 0.07 g (15%) of **3a**, mp 141−143 °C, after recrystallization from *n*-BuCl. ^1H NMR ($CDCl_3$): δ 9.29 (d, 1 H), 8.74 (d, 1 H), 7.67 (d, 2 H), 7.56 (d, 1 H), 7.50 (t, 2 H), 7.38 (d, 1 H). MS (NH_3, CI): m/z 229 ($M + H^+$, 100). Anal. Calcd for $C_{12}H_8N_2OS$: C, H, N.

Cartilage Inhibitor Studies. Nasal septa were removed from bovine noses obtained at the time of slaughter. Uniform cartilage discs (1 mm thick × 8 mm diameter) were prepared[31] and cut into eighths. Cartilage pieces were then weighed and each placed into a well of a 96-well culture dish containing 180 μL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum, penicillin (100 IU/mL), streptomycin (100 μg/mL), amphotericin B (0.25 μg/mL), and neomycin (50 μg/mL). Paired explants from the same disc were used to compare the effects of various experimental conditions. Eight replicates per treatment group were run for each experiment, and a well containing medium but no cartilage was included as a negative control for each group. Cultures were incubated for 40 h at 37 °C in an atmosphere of 95% air and 5% CO_2. Drugs were dissolved in DMSO to provide 10 mM stock solutions and then further diluted with DMEM to the required final concentrations. DMSO concentrations in the assay media never exceeded 1%.[32] Cartilage was incubated in the absence or presence of IL-1β (500 ng/mL), with or without drug. Under these conditions, 500 ng of IL-1β resulted in submaximal stimulation of proteoglycan breakdown,[33] thus allowing the observation of either inhibition or augmentation of the effects of IL-1β by the added drug. When included, drugs were present throughout the culture period. At the end of the incubation, the media were removed for glycosaminoglycan (GAG) analysis and replaced with Ham's F-12 media, containing 20 μCi/mL [^{35}S]sulfate. The samples were incubated an additional 2 h, and the media were removed. The cartilage was digested with papain, the proteoglycan was precipitated with cetylpyridinium chloride, and the precipitates were counted for ^{35}S. GAGs in the culture media were measured from the amount of polyanionic material reacting with 1,9-dimethylmethylene blue,[34] using shark chondroitin sulfate as a standard. Results were reported as μg of GAG/mg wet weight of cartilage. [^{35}S]sulfate incorporation was determined as dpm/mg wet weight of cartilage.

Stromelysin Activation Studies. Incubation mixtures were prepared from 3.3 μL of prostromelysin (31.25 μg mL^{-1}),[35] 3.3 μL of plasmin (6.25 μg mL^{-1}), and 10 μL of drug stock solution (prepared by dilution of a DMSO stock solution with an appropriate volume of water). DMSO concentrations never exceeded 1%. Drugs were assayed at 100, 30, 10, and 1 μM. Control assays employed 10 μL of H_2O instead of drug stock solution. Activation was allowed to proceed for 2 h at 37 °C, after which the stromelysin activity was assayed as described below.

Preincubation experiments were conducted by combining the prostromelysin and drug solutions (or water for controls) as described above and allowing the mixture to stand for the desired preincubation period at 25 °C. Plasmin was then added, and activation was carried out at 37 °C, after which the stromelysin activity was assayed as described below.

Stromelysin Assay. Stromelysin activity was assessed using a [^{3}H]transferrin substrate.[36] Following proteolytic activation with plasmin for 2 h at 37 °C as described above, the incubation mixtures were treated with 3.3 μL of 500 μM PACK-II, 10 μL of water, 10 μL of 3X, pH 7.8, buffer, and 10 μL of [^{3}H]transferrin to give a total volume of 50 μL. The mixtures were incubated for 4 h at 37 °C, then the reactions were quenched with 200 μL of 3.3% Cl_3CCO_2H, and the mixtures were centrifuged. An aliquot of the supernatant (100 μL) was added to 5 mL of scintillation cocktail for LSC.

Acknowledgment. We are grateful to P. E. Crawford, R. A. Quas de Penno, and K. F. Blom for their assistance in obtaining spectral data.

References

(1) Mankin, H. J.; Lipiello, L. Biochemical and Metabolic Abnormalities in Articular Cartilage from Osteoarthritic Hips. *J. Bone Jt. Surg.* **1970**, *52A*, 424−434.

(2) Shinmei, M.; Masuda, K.; Kikuchi, T.; Shimomura, Y. Interleukin-1, Tumor Necrosis Factor, and Interleukin-6 as Mediators of Cartilage Destruction. *Semin. Arthritis Rheum.* **1989**, *18* (3, Suppl. 1), 27−32.

(3) (a) Palmoski, M. J.; Brandt, K. D. Hyaluronate Binding by Proteoglycans: Comparison of Mildly and Severely Osteoarthritic Regions of Human Femoral Cartilage. *Clin. Chim. Acta* **1976**, *79*, 87−95. (b) Sandy, F. D. Analysis of the Catabolism of Aggrecan in Cartilage Explants by Quantitation of Peptides from the Three Globular Domains. *J. Biol. Chem.* **1991**, *266*, 8198−8205. (c) Fosang, A. J. The Interglobular Domain of Cartilage Aggrecan is Cleaved by PUMP, Gelatinases, and Cathepsin B. *J. Biol. Chem.* **1992**, *267*, 19470−19474.

(4) (a) Walakovits, L. A.; Moore, V. L.; Bhardwaj, N.; Gallich, G. S.; Lark, M. W. Detection of Stromelysin and Collagenase in Synovial Fluid from Patients with Rheumatoid Arthritis and Posttraumatic Knee Injury. *Arthritis Rheum.* **1992**, *35*, 35−42. (b) Pelletier, J.-P.; Martel-Pelletier, J.; Cloutier, J. M.; Woessner, J. F., Jr. Proteoglycan Degrading Acid Metalloprotease Activity in Human Osteoarthritic Cartilage, and the Effect of Intra-articular Steroid Injections. *Arthritis Rheum.* **1987**, *30*, 541−548.

(5) (a) Woessner, J. F., Jr.; Selzer, M. G. Two Latent Metalloproteases of Human Articular Cartilage that Digest Proteoglycan. *J. Biol. Chem.* **1984**, *259*, 3633−3638. (b) Gunja-Smith, Z.; Nagase, H.; Woessner, J. F., Jr. Purification of the Neutral Proteoglycan Degrading Metalloproteinase from Human Articular Cartilage Tissue and its Identification as Stromelysin. *Biochem. J.* **1989**, *258*, 115−119.

(6) Okada, Y.; Takeuchi, N.; Tomita, K.; Nakanishi, I.; Nagase, H. Immunolocallsation of Matrix Metalloproteinase 3 (Stromelysin) in Rheumatoid Synovioblasts (B Cells): Correlation with Rheumatoid Arthritis. *Ann. Rheum. Dis.* **1989**, *48*, 645−653.

(7) For a recent review, see: Van Wart, H. E.; Schwartz, M. A. Synthetic Inhibitors of Bacterial and Mammalian Interstitial Collagenases. *Prog. Med. Chem.* **1992**, *29*, 271−334.

(8) (a) Saklatvala, J.; Sarsfield, S. J.; Pilsworth, L. M. C. Characterization of Proteins from Human Synovium and Mononuclear Leukocytes that Induce Resorption of Cartilage Proteoglycan In Vitro. *Biochem. J.* **1983**, *209*, 337−344. (b) Tyler, J. A. Chondrocyte − Mediated Depletion of Articular Cartilage Proteoglycans In Vitro. *Biochem. J.* **1985**, *225*, 493−507. (c) Tyler, J. A. Articular Cartilage Cultured with Catabolin Synthesizes a Decreased Number of Normal Proteoglycan Molecules. *Biochem. J.* **1985**, *227*, 869−878. (d) Arner, E. C.; Pratta, M. A. Independent Effects of Interleukin-1 on Proteoglycan Breakdown, Proteoglycan Synthesis, and Prostaglandin E_2 Release from Cartilage in Organ Culture. *Arthritis Rheum.* **1989**, *32*, 288−297.

(9) (a) Pettipher, E. R.; Higgs, G. A.; Henderson, B. Interleukin-1 Induces Leukocyte Infiltration and Cartilage Proteoglycan Degradation in the Synovial Joint. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8749−8753. (b) Pettipher, E. R.; Henderson, B.; Hardingham, T.; Ratcliffe, A. Cartilage Proteoglycan Depletion in Acute and Chronic Antigen − Induced Arthritis. *Arthritis Rheum.* **1989**, *32*, 601−607. (c) Arner, E. C.; DiMeo, T. M.; Pratta, M. A. In Vivo Studies on the Effects of Human Recombinant Interleukin-1β on Articular Cartilage. *Agents Actions* **1989**, *27*, 254−257. (d) O'Byrne, E. M.; Blancuzzi, V.; Wilson, D. E.; Wong, M.; Jeng, A. Y. Elevated Substance P and Accelerated Cartilage Degradation in Rabbit Knees Injected with Interleukin-1 and Tumor Necrosis Factor. *Arthritis Rheum.* **1990**, *33*, 1023−1028. (e) McDonnell, J.; Hoerrner, L. A.; Lark, M. W.; Harper, C.; Dey, T.; Lobner, J.; Eiermann, G.; Kazazis, D.; Singer, I. I.; Moore, V. L. Recombinant Human Interleukin-1β − Induced Increase in Levels of Proteoglycans, Stromelysin and Leukocytes in Rabbit Synovial Fluid. *Arthritis Rheum.* **1992**, *35*, 799−805.

(10) Gowen, M.; Wood, D. D.; Ihrie, E. J.; Meats, J. E.; Russell, R. G. G. Stimulation by Human Interleukin-1 of Cartilage Breakdown and Production of Collagenase and Proteoglycanase by Human Chondrocytes but Not Human Osteoblasts In Vitro. *Biochem. Biophys. Acta* **1984**, *797*, 186−193.

(11) Richardson, H. J.; Elford, P. R.; Sharrard, R. M.; Meats, J. E.; Russell, R. G. G. Modulation of Connective Tissue Metabolism by Partially Purified Human Interleukin-1. *Cell Immunol.* **1985**, *90*, 41–51.
(12) For a discussion of methods used to prepare pyridoisothiazolones, see: Monge, A.; Martinez-Merino, V.; Fernandez-Alvarez, E. Synthesis of 2-Substituted 3-Oxoisothiazolo[5,4-b]pyridines. *J. Heterocycl. Chem.* **1985**, *22*, 1353–1356 and references cited therein.
(13) For a discussion of the oxidative cyclization of benzyl and *tert*-butyl sulfoxides to isothiazolones, see: Wright, S. W.; Abelman, M. M.; Bostrom, L. L.; Corbett, R. L. Benzyl and t-Butyl Sulfoxides as Sulfenyl Halide Equivalents: A Convenient Preparation of Benzisothiazolones *Tetrahedron Lett.* **1992**, *33*, 153–156.
(14) Arner, E. C.; Pratta, M. A. Modulation of Interleukin-1 Induced Alteration in Cartilage Proteoglycan Metabolism by Activation of Protein Kinase C. *Arthritis Rheum.* **1991**, *34*, 1006–1013.
(15) Huang, J. J.; Newton, R. C.; Pezzella, K.; Covington, M. B.; Tamblyn, T.; Rutledge, S. J.; Kelley, M.; Gary, J.; Lin, Y. High Level Expression in Escherichia Coli of a Soluble and Fully Active Recombinant Interleukin-1β. *Mol. Biol. Med.* **1987**, *4*, 169–181.
(16) (a) White, H. L.; Glassman, A. T. Simple Radiochemical Assay for Prostaglandin Synthetase. *Prostaglandins* **1974**, *7*, 123–129. (b) Vigdahl, R. L.; Tukey, R. H. Mechanism of Action of Novel Anti-inflammatory Drugs Diflumidone and R-805. *Biochem. Pharmacol.* **1977**, *26*, 307–311.
(17) (a) Patriarca, P.; Beckerdite, S.; Elsbach, P. Phospholipases and Phospholipid Turnover in Escherichia coli Spheroplasts. *Biochim. Biophys. Acta* **1972**, *260*, 593-600. (b) Davidson, F. F.; Dennis, E. A.; Powell, M.; Glenney, J. R., Jr. Inhibition of Phospholipase A_2 by "Lipocortins" and Calpactins. An Effect of Binding to Substrate Phospholipids. *J. Biol. Chem.* **1987**, *262*, 1698–1705.
(18) (a) Jakschik, B. A.; Lee, L. A.; Shuffer, G.; Parker, C. W. Arachidonic Acid Metabolism in Rat Basophilic Leukemia (RBL-1) cells. *Prostaglandins* **1978**, *16*, 733–748. (b) Jakschik, B. A.; Sun, F. F.; Lee, L. H.; Steinhoff, M. M. Calcium Stimulation of a Novel Lipoxygenase. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 103–110. (c) Jakschik, B. A.; DiSantis, D. M.; Sankarappa, S. K.; Sprecher, H. Delta-Four Acetylenic Acids – Selective Inhibitors of the Formation of Slow Reacting Substance. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 624–629.
(19) Newton, R. C. Human Monocyte Production of Interleukin-1: Parameters of the Induction of Interleukin-1 Secretion by Lipopolysaccharides. *J. Leukocyte Biol.* **1986**, *39*, 299–311.
(20) (a) Okada, Y.; Nagase, H.; Harris, E. D., Jr. A Metalloproteinase from Human Rheumatoid Synovial Fibroblasts that Digests Connective Tissue Matrix Components. *J. Biol. Chem.*, **1986**, *261*, 14245–14255. (b) The isothiazolones **1–5** were as a class inactive as inhibitors of plasmin or chymotrypsin. (c) It is recognized that the inhibition of prostromelysin activation by the isothiazolones **1–5** may occur through a mechanism involving a covalent modification of prostromelysin by the isothiazolone via ring opening of the isothiazolone. The results of these experiments will be reported elsewhere.
(21) Wright, S. W.; Petraitis, J. J.; Abelman, M. M.; Bostrom, L. L.; Corbett, R. L.; Green, A. M.; Kindt, R. M.; Sherk, S. R.; Magolda, R. L. Inhibition of Cartilage Breakdown by Isothiazolones. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2875–2878.
(22) The synthesis of this previously unknown compound is described separately in the Experimental Section; it had $IC_{50} = 5.5\ \mu M$ in the cartilage organ culture assay.
(23) The 2-nitro isomer could not be isolated in satisfactory purity for testing.
(24) Greenwald, R. A. Tetracycline Derivatives as Potential Inhibitors of Connective Tissue Degradation. *DN&P* **1990**, *3*, 161–166.
(25) Campion, C.; Davidson, A. H.; Dickens, J. P.; Crimmins, M. J. PCT/GB89/01399, November 23, 1989.
(26) Vogel, A. *Practical Organic Chemistry*" 4th ed.; Longmans: London, 1987; p 308.
(27) Engler, C. On the Esters and Amides of the Pyridinecarboxylic Acids. *Chem. Ber.* **1894**, *27*, 1784–1789.
(28) This treatment with $K_3Fe(CN)_6$ serves to oxidize the liberated *tert*-butyl mercaptan back to the less malodorous disulfide. Overnight oxidation is necessary because of the relatively slow rate of oxidation.
(29) Berthold, R. V.; Crosby, D. G. 5-Bromoorotic Acid. *J. Org. Chem.* **1958**, *23*, 1377–1378.
(30) Snieckus, V.; Perrier, H.; Aubert, T.; Iihama, T.; Quesnelle, C. The tert-Butyl Sulfoxide Directed Ortho – Metalation Group. *Tetrahedron Lett.* **1992**, *33*, 2625–2628.
(31) Steinberg, J.; Sledge, C. B.; Noble, J.; Stirrat, C. R. A Tissue – Culture Model of Cartilage Breakdown in Rheumatoid Arthritis. *Biochem. J.* **1979**, *180*, 403–412.
(32) At this concentration, DMSO had no effect on cartilage proteoglycan metabolism.
(33) See ref 8d.
(34) Farndale, R. W.; Sayers, C. A.; Barrett, A. J. A Direct Spectrophotometric Microassay for Sulfated Glycosaminoglycans in Cartilage Cultures. *Connect. Tissue Res.* **1982**, *9*, 247–248.
(35) Purified prostromelysin from human rheumatoid synovial fibroblasts was a kind gift from Dr. Hideaki Nagase (University of Kansas Medical School).
(36) Nagase, H.; Okada, Y.; Harris, E. D., Jr. A Metalloproteinase from Human Rheumatoid Synovial Fibroblasts that Digests Connective Tissue Matrix Components. *J. Biol. Chem.* **1986**, *261*, 14245–14255.