Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 3, pp. 227–234, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00018-6

Metabolism Resistant Isothiazolone Inhibitors of Cartilage Breakdown

Stephen W. Wright,† Joseph J. Petraitis,* Douglas G. Batt, Ronald L. Corbett, Susan V. Di Meo, Bruce Freimark, John V. Giannaras, Michael J. Orwat, Donald J. Pinto, Michael A. Pratta, Susan R. Sherk, Herman F. Stampfli, Jean M. Williams, Ronald L. Magolda and Elizabeth C. Arner

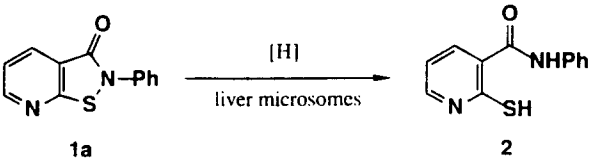
The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880, U.S.A.

Abstract—A series of 2-(arylmethyl)pyridoisothiazolones is reported that inhibit the IL-1 β induced breakdown of bovine nasal septum cartilage in an organ culture assay. The synthesis and preliminary SAR of these compounds are described. These compounds represent a novel, non-peptide lead series approach to the mediation of the chronic cartilage breakdown associated with arthritic disease. These compounds are relatively resistant to reductive metabolism by liver microsomal preparations and appear to inhibit cartilage breakdown by interfering with the proteolytic activation of matrix metalloproteinases.

Introduction

Osteoarthritis is characterized by the erosion of the cartilage pad during the progression of the disease.[1] This damage is thought to be triggered by a variety of stimuli, particularly cytokines.[2] IL-1β causes a net loss of proteoglycan from cartilage both *in vitro*[3] and *in vivo*.[4] This effect is due at least in part to its ability to stimulate stromelysin synthesis by articular chondrocytes[5] and other cells in connective tissues.[6] A number of approaches to the inhibition of cartilage breakdown have been described recently, including inhibition of cytokine production and inhibition of matrix metalloproteases.[7] There remains an unmet medical need for agents that will arrest or retard the cartilage loss associated with arthritis. To this end, we recently reported[8] a series of *N*-aryl pyridoisothiazolones (**1**) that inhibit the IL-1β induced breakdown of cartilage in a cartilage organ culture assay[9] in a dose-dependent manner while not affecting cartilage synthesis. We described several structural features that influence inhibitory activity and demonstrated that the pyridoisothiazolone moiety is critical to the potency observed. In this paper we report the results of our studies on the pharmacokinetics and metabolism of the *N*-aryl pyridoisothiazolones, and the synthesis, activity, and SAR of pyridoisothiazolones that are resistant to metabolism by liver microsomes.

Preliminary *in vivo* studies with some of the most potent *N*-aryl pyridoisothiazolones reported previously (**1a**, **1e** and **1g**) revealed that these compounds produced no inhibition of IL-1β induced cartilage breakdown in rabbit knees when dosed po, even at doses of up to 300 mg kg^{-1}. However, the compounds were effective inhibitors when dosed by intra-articular injection. To understand the discrepancy between *in vitro* and oral potencies, pyridoisothiazolone **1a** was selected for pharmacokinetic investigation. When plasma samples from animals dosed with **1a** were examined by HPLC, it was found that only a metabolic product of **1a** was observed. Subsequent incubation of **1a** with rabbit liver microsomes showed that **1a** was rapidly converted to a new product by the microsomal preparation ($t_{1/2}$ = *ca* 20 min, Scheme 1). This product was tentatively identified as **2** based on the pH dependence of its ultraviolet spectrum; this was confirmed by co-injection with an authentic sample of **2** on HPLC.[10] The plasma metabolite was likewise identified as **2**.



1a **2**

Scheme 1.

These results were not unique to the rabbit; similar results were obtained with rat liver microsomes. Incubation of other *N*-aryl pyridoisothiazolones (**1b–1g**) showed that all underwent reductive ring opening of the isothiazolone at a fairly rapid rate (Table 1). Those *N*-aryl pyridoisothiazolones which have electron-releasing substituents conjugated to the isothiazolone nitrogen (e.g. **1c** and **1g**), or in which the sulfur is sterically shielded (e.g., **1e** and **1f**), underwent reduction less rapidly but were still reduced at an unacceptable rate.

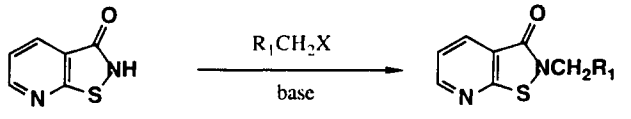
A variety of pyridoisothiazolones with diverse structures were surveyed in order to identify those that were less subject to rapid reduction by liver microsomes. The structural modification that was found to be most effective at preventing the microsomal reduction was deconjugation

†Present address: Pfizer Central Research, Eastern Point Road, Groton, CT 06340, U.S.A.

of the phenyl ring bonded to the isothiazolone nitrogen. The insertion of a methylene spacer into this bond afforded the *N*-benzyl analog **3a**, which was recovered unchanged (> 90%) following incubation (1 h, 37 °C) with microsomes.[11] This compound had potency similar to that of compounds in Table 1, and was selected for further analoging (Table 2).

Chemistry

The benzylic isothiazolones **3** were prepared by any one of five methods. Most were prepared by the alkylation of 2-*H*-isothiazolo[5,4-*b*]pyridin-3-one (**4**)[12] by the corresponding benzylic bromide (Scheme 2, X = Br) or methanesulfonate (X = OSO_2CH_3) under basic conditions. This was accomplished by the use of either NaH in THF (method A) or Hunig's base in EtOH (method B). The use of NaH in THF tended to afford significant quantities of *O*-alkylated product in addition to the desired *N*-alkylated product. This side reaction was best minimized by the use of Hunig's base in EtOH; however, in these reactions some of the alkylating agent was lost to solvolysis by EtOH.

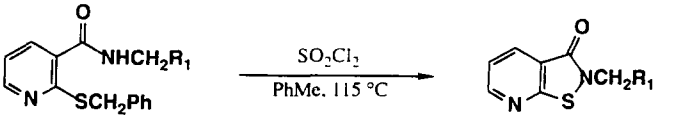


4 3

Scheme 2.

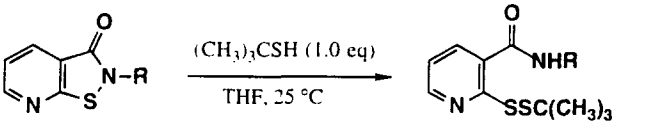
An alternate means of preparing these compounds, which was particularly useful when the appropriate benzylic amine ($R_1CH_2NH_2$) was readily available and/or the corresponding benzylic halide would be unstable, was to synthesize the 2-benzylthionicotinamide **5** by standard methods and subsequently convert **5** to **3** (Scheme 3). The oxidative cyclization of **5** to **3** was carried out in one step by heating with sulfuryl chloride (method C).[13]

In order to avoid the need to test the stability of each new compound toward liver microsomes, we devized a means of chemically modeling this reduction. We found that the reaction of these isothiazolones with *t*-butyl mercaptan provided a useful model for the microsomal reduction. *t*-Butyl mercaptan reacts with **1** or **3** to form the corresponding ring-opened mixed disulfides **6** (Scheme 4).



5 3

Scheme 3.



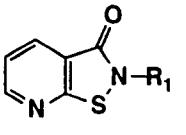
1, R = Aryl
3, R = CH_2Aryl

6

Scheme 4.

We found that the rate of reaction of isothiazolones with Me_3CSH to form the mixed disulfides **6** was proportional to their rate of reduction by the microsomal preparation to form the thiols **2**. For instance, **1a** underwent essentially complete reaction with Me_3CSH (1.0 eq. Me_3CSH, THF, 25 °C, 0.5 M reactants) within 20 min. The more resistant **1c** and **1f** required 1 h for complete reaction with Me_3CSH. However, **3a** failed to undergo significant reaction (< 10%) with Me_3CSH after 24 h as judged by HPLC and ^1H NMR. A similar correlation between reactivity towards liver microsome reduction and Me_3CSH disulfide formation was found for all of the isothiazolones **1** and **3** that were tested in both systems.[14] The rate of reduction of isothiazolones to form **2** by liver microsomes was slower than the rate of reaction of isothiazolones with Me_3CSH to form **6** in all cases examined; thus it was proposed that compounds resistant to Me_3CSH for 24 h

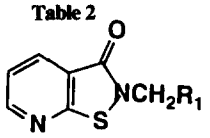
Table 1



Entry	R_1	mp,[c] °C	*In Vitro*[a] Activity	*In Vivo* Activity[b] ia	po	Microsomal[c] Stability
1a	C_6H_5	135	77 (4.4)	75	0	10
1b	4-ClC_6H_4	195	68 (8.5)	nt[d]	nt	10
1c	4-$CH_3OC_6H_4$	165	68 (13.5)	nt	nt	50
1d	4-$O_2NC_6H_4$	285	7	nt	nt	0
1e	2,6-$(CH_3)_2C_6H_3$	112	72 (8.0)	47	0	60
1f	2,6-$((CH_3)_2CH)_2C_6H_3$	147	83 (12.0)	nt	nt	50
1g	2,4-$(CH_3O)_2C_6H_3$	178	86 (9.5)	67	0	60

[a]Expressed as percent inhibition of control cartilage breakdown at 30 μM dose. Standard errors ± 15%; IL-1 β stimulated bovine nasal cartilage assay as outlined in Ref. 9. IC_{50} (μM) values in parentheses. [b]Expressed as percent inhibition of control cartilage breakdown: ia = intra-articular injection of 5 μg drug in 0.2 mL PBS simultaneously with IL-1 β injection; po = oral dose of 100 mg kg^{-1} (see Experimental section). [c]Expressed as percent recovery of drug following incubation with liver microsomes (see Experimental section). [d]Not tested. [e]Mp were within a 2 °C range with the lower value reported.

Table 2



Entry[a]	R_1	mp,[p] °C	Yield	Method	Activity[b]
3a	C_6H_5	89	82	A	73
3b	4-$NO_2C_6H_4$	172	25	B	< 20
3c	4-$CH_3O_2CC_6H_4$	169	15	A	59
3d	3-$CH_3O_2CC_6H_4$	95	36	A	47
3e	2-$CH_3O_2CC_6H_4$	124	18	A	41
3f	3-CH_3O_2-4-ClC_6H_3	109	8	A	40
3g	4-NCC_6H_4	187	27	A	47
3h	3-NCC_6H_4	143	26	A	49
3i	2-NCC_6H_4	162	30	A	42
3j	4-ClC_6H_4	164	21	B	61
3k	3-ClC_6H_4	115	54	B	58
3l	2-ClC_6H_4	155	57	B	31
3m	1-$C_{10}H_7$	116	17	B	68
3n	4-$CF_3C_6H_4$	119	62	B	50
3o	4-$CH_3OC_6H_4$	107	41	B	52
3p	3-$CH_3OC_6H_4$	97	14	B	49
3q	2-$CH_3OC_6H_4$	92	24	B	71
3r	2,5-$(CH_3O)_2C_6H_3$	109	20	B	70
3s	4-$C_6H_5C_6H_4$	183	13	B	67
3t	3-$C_6H_5C_6H_4$	106	23	B	45
3u	4-(2-$CH_3O_2CC_6H_4$)C_6H_4	141	45	B	58
3v	4-(2-$HN_4CC_6H_4$)C_6H_4[c]	214	27	B	63
3w	4-((2-$CH_3O_2CC_6H_4$)CO)C_6H_4	160	25	B	71
3x	4-($C_6H_5N(CH_3)CO$)C_6H_4	145	28	B	70
3y	3-($C_6H_5N(CH_3)CO$)C_6H_4	144	7	B	53
3z	2-C_5H_4N[d]	117	77	C	64
3aa	3-C_5H_4N	136	91	C	56
3bb	4-C_5H_4N	129	61	C	40
3cc	2-$C_4H_3N_2$[e]	134	27	B	43
3dd	2-(6-CH_3O_2C)C_5H_3N[f]	134	41	B	54
3ee	2-(5-CH_3O_2C)C_5H_3N	149	14	B	43
3ff	2-(3-CH_3O_2C)C_5H_3N	168	35	B	32
3gg	4-(3-CH_3O_2C)C_5H_3N	159	25	B	78
3hh	3-C_4H_3O[g]	100	31	B	55
3ii	3-(2-CH_3O_2C)C_4H_2O[h]	74	27	B	49
3jj	2-(3-CH_3O_2C)C_4H_2O	163	25	B	54
3kk	2-(4-CH_3O_2C)C_4H_2O	96	20	B	52
3ll	2-(5-CH_3O_2C)C_4H_2O	142	43	B	72
3mm	3-(2-NC)C_4H_2O	167	13	B	46
3nn	2-(3-NC)C_4H_2O	164	12	B	51
3oo	2-(5-NC)C_4H_2O	176	20	B	44
3pp	2-(5-HO_2C)C_4H_2O	258	80	B	60
3qq	2-(5-HN_4C)C_4H_2O	255	80	B	94
3rr	2-C_8H_5O[i]	146	28	B	73
3ss	2-C_4H_3S[j]	126	30	A	50
3tt	3-C_4H_3S	125	21	A	40
3uu	3-(2-$CH_3CH_2O_2C$)C_4H_2S[k]	124	34	A	61
3vv	2-(5-$CH_3CH_2O_2C$)C_4H_2S	110	13	A	69
3ww	3-(2-NC)C_4H_2S	172	64	B	40
3xx	2-(5-NC)C_4H_2S	159	43	B	67
3yy	2-(5-HO_2C)C_4H_2S	211	13	B	80
3zz	2-(5-HN_4C)C_4H_2S	275	23	A	87
3aaa	2-(5-$(CH_3)_2NCO$)C_4H_2S	141	64	B	45
3bbb	2-C_8H_5S[l]	168	17	B	58
3ccc	3-C_8H_5S	137	23	B	44
3ddd	4-C_3H_2NS[m]	137	17	A	57
3eee	2-C_7H_4NS[n]	139	51	B	70

[a]All compounds gave satisfactory ^1H NMR, CIMS, and elemental analyses. [b]Expressed as percent inhibition of control cartilage breakdown at 30 μM dose. Standard errors ± 15%; IL-1 β stimulated bovine nasal cartilage assay as outlined in Ref. 9. [c]Tetrazolyl = HN_4C. [d]Pyridyl = C_5H_4N. [e]Pyrimidyl = $C_4H_3N_2$. [f]Substituted pyridyl. [g]Furyl = C_4H_3O. [h]Substituted furyl. [i]Benzofuranyl. [j]Thiophenyl = C_4H_3S. [k]Substituted thiophenyl. [l]Benzothiophenyl. [m]Thiazolyl = C_3H_2NS. [n]Benzothiazolyl. [p]Mp were within a 2 °C range with the lower value reported.

should be resistant to liver microsomes for 1 h (considering only the reductive metabolism of the isothiazolone). Indeed, all of the benzylic isothiazolones **3** reported in Table 2 were found to be relatively unreactive (< 10% reaction in 24 h) towards *t*-butyl mercaptan.

In Vitro 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. 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 $^{35}SO_4$ = by the cartilage). To evaluate inhibitors, bovine nasal septum cartilage slices were stimulated with a soluble, fully-active recombinant human IL-1β (500 ng mL^{-1}) for 40 h, which resulted in a submaximal effect on proteoglycan metabolism.[15] Incubations were carried out at 37 °C in the presence or absence of test compounds (at 30 μM inhibitor). The novel isothiazolones **3** inhibited the breakdown of proteoglycan in IL-1β stimulated cartilage (Table 2). As a class, the inhibitors **3** did not further inhibit cartilage resynthesis beyond that caused by IL-1β alone, indicating that they are not simply toxic to the cartilage. Control incubations of cartilage with **3** also showed no increase or decrease in $^{35}SO_4$ = incorporation from control cartilage incubated in the absence of **3**. The compounds **3** did not reverse the IL-1β induced inhibition of cartilage synthesis, 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 plasmin-activated stromelysin,[16] cyclooxygenase[17] (bovine seminal vesicles, IC_{50} generally > 750 μM), PLA_2[18] (*Croatalus adamanteus*, IC_{50} generally > 1 mM) and 5-lipoxygenase[19] (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*.[20] Data generated for **1g** previously suggested that this compound inhibited cartilage degradation by interfering with the normal activation of matrix metalloproteinases.[8] Studies carried out on **3a** suggest that compounds in this series act similarly to **1g**. For example, **3a** did not inhibit plasmin-activated stromelysin,[21a] nor did it inhibit stromelysin activated by chymotrypsin. However, it was found that stromelysin activity was inhibited by 20% if **3a** was present *during* the activation of prostromelysin by either plasmin or chymotrypsin. Control experiments indicated that **3a** was not an inhibitor of either of the activating proteases,[21b] suggesting that **3a** 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 **3a** with prostromelysin prior to the addition of the activating protease. The inhibition of activation reached its maximal effect at approximately 3 h of preincubation. Taken together, these data suggest that these compounds inhibit IL-1β stimulated cartilage breakdown by interfering with prostromelysin activation. Studies are currently in progress to further elucidate the biochemical mechanism of this inhibition.[21c] Preliminary *in vivo* studies with **3a** indicate that this compound produces inhibition of IL-1β induced cartilage breakdown in rabbit knees when dosed by intra-articular injection or po. A summary of the effects of **3a** and one of the previously reported pyridoisothiazolones (**1g**) is presented in Table 3.

Structure–Activity Relationships

Previous work[8a] had shown that the pyrido-fused *N*-benzyl isothiazolone is the key pharmacophore for inhibition of IL-1β induced cartilage breakdown *in vitro*. Thus, attention was turned to the benzyl group of **3a** for optimization of *in vitro* activity. The effects of adding various functional groups to the benzyl moiety and of changing the phenyl ring to various heterocyclic rings, as well as combinations of these two strategies, were studied.

The addition of various functional groups, such as nitro, ester, amide, halogen, nitrile, and methoxy, to the benzyl moiety of **3a** generally led to decreased activity *in vitro*. For example, the methyl esters **3c–e** were all less potent than **3a**. A similar trend was observed for chloro (**3j–3l**) and nitrile (**3g–3i**) substituents. For these series of substituents, the activity also decreased in the order 4 ≥ 3 > 2. The methoxy-substituted compounds **3o** and **3p** were likewise less potent than **3a**, but the 2-isomer **3q** and the 2,5-dimethoxy compound **3r** were equipotent to **3a**, resulting in an activity trend of 2 > 3 ≥ 4, the opposite of the trend noted with Cl, CN and CO_2CH_3. The addition of a highly lipophilic 4-phenyl substituent **3s** resulted in activity similar to **3a**, but the 3-phenyl substituted compound **3t** suffered a significant loss of activity, suggesting that steric effects are also important. The

Table 3. Inhibition of stromelysin activation by pyridoisothiazolones

Entry	R_1	Cartilage[a]	Inhibition of Activation[b]		Activation[c]	*In Vivo* Activity[d]	
		IC_{50}	0h	3h	IC_{50}	ia	po
2g	2,4-$(MeO)_2C_6H_3$	9.5	45	65	62	67	0
3a	$CH_2C_6H_5$	5.4	20	50	99	77	30

[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 h 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%. [d]Expressed as percent inhibition of control cartilage breakdown: ia = intra-articular injection of 5 μg; po = oral dose of 100 mg kg^{-1} (see Experimental section).

highly lipophilic *para* *N*-methyl anilide **3x** was likewise equipotent to **3a** and **3s**, in contrast to the simple methyl ester **3c**. Again, movement of the lipophilic functional group to the 3-position (**3y**) resulted in a loss of activity. The addition of functional groups (ester and tetrazole) to the 4-phenyl substituent of **3s** (**3u** and **3v**) led to a decrease in activity as was observed for **3a**.

Replacement of the phenyl ring by other aromatic residues was also examined. The pyridyl series (**3z–3bb**) was less potent than **3a**, with activity decreasing only marginally as the pyridyl nitrogen was moved away from the methylene group (2 ≥ 3 ≥ 4). The pyrimidyl analog **3cc** was relatively poor, similar to **3bb**. Similarly, the furyl (**3hh**), thiophenyl (**3ss** and **3tt**), and thiazolyl (**3ddd**) analogs were also less potent than **3a**. Fused bicyclic aromatic residues were also examined. Not surprisingly, the lipophilic 1-naphthyl group **3m** was similarly potent to **3a**, as were the 2-benzofuran **3rr** and 2-benzothiazole **3eee**. However, the benzothiophene isomers **3bbb** and particularly **3ccc**, which would be expected to be comparable in terms of lipophilicity and steric demands, were considerably less potent than **3a**, **3m**, **3rr**, or **3eee**.

Some combinations of phenyl ring replacements with functional group substituents were also examined. These groups were mostly carboxylic acids and esters, nitriles, and tetrazoles that were introduced to provide a substituent that could be expected to increase the water solubility of these compounds, which might otherwise have very low solubility in aqueous media. In the 2-pyridyl series, the addition of a methyl ester (**3dd–3ff**) resulted in a decrease in activity as compared to the parent pyridine **3z**, similar to that previously observed with the benzyl series **3c–3e**. Unlike **3c–3e**, however, the activity decreased as the ester substituent was moved away from the pyridine ring nitrogen (2,6 > 2,5 > 2,3). However, the SAR must not be assumed to be entirely additive, as the methyl ester **3gg** was *more* potent than **3a**. In the furan and thiophene series, the nitriles **3mm–3oo**, **3ww** and **3xx** were all less potent than **3a**, but not necessarily less potent than the parent furan and thiophene compounds. In marked contrast to previous observations with the carbomethoxy substituted benzyl series **3c–3e** and the pyridines **3dd - 3ff**, the addition of ester groups to the furan (**3ii–3ll**) and thiophene (**3uu** and **3vv**) series resulted in an *increase* in activity when compared to the parent furan and thiophene compounds. Like the pyridines **3dd–3ff**, the activity of the furans **3ii–3ll** decreased as the carbomethoxy group was moved away from the ring heteroatom (2,5 > 2,4 ≥ 2,3). Having noted previously that a tetrazole can be more potent than the corresponding methyl ester (**3v**), as well as being potentially more water soluble, the 2,5-furan tetrazole **3qq** and the 2,5-thiophene tetrazole **3zz** were prepared and found to be very active inhibitors of cartilage breakdown (94% and 87% inhibition of IL-1β induced breakdown, respectively).

Summary

A comparison of *in vitro* data for 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),[22] are ineffective at blocking the IL-1β 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[23] (BBT-16, IC_{50} = 20 nM in activated stromelysin enzyme assay) was shown to be approximately equipotent to **3a**, **3r** and **3gg** in the organ culture assay. The tetrazoles **3qq** and **3zz** were the most active compounds evaluated.

Table 4. Data for standard drugs and selected pyridoisothiazolones **3**

Compound	*In Vitro* Activity[a]
Indomethacin	< 20
Naproxen	< 20
Phenidone	< 20
BBT-16	73
3a	73
3r	70
3gg	78
3qq	94
3zz	87

[a]Expressed as percent inhibition of control cartilage breakdown at 30 μM dose. Standard errors ± 15%; IL-1β stimulated bovine nasal cartilage assay as outlined in Ref. 9.

In conclusion, these pyridoisothiazolones **3** 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 effective at inhibiting cartilage destruction in a tissue-based assay than other anti-inflammatory agents. In general, it would appear that activity 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 benzyl ring. Steric effects are important as evidenced by the changes in activity noted as a substituent is moved about the pendant ring. Lipophilicity also appears to be important, but is not sufficient by itself to impart high *in vitro* activity. The higher activity noted with the 2-methoxy and 2-pyridyl compounds as compared to their positional isomers might suggest that a hydrogen bond acceptor *ortho* to the methylene tether is helpful, but this interpretation is somewhat clouded by the varied results obtained for ester derivatives. Clearly the structure–activity relationships are not entirely additive, and must be interpreted with some care, as the *in vitro* data represent the product of structural contributions to solubility, tissue penetration, and cell penetration, as well as contributions to intrinsic potency. Studies are in progress to determine the mechanism of action of these compounds and to further profile their *in vivo* biological properties, particularly their effects upon models of arthritic diseases.

Experimental

^{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 was recorded on Finnigan-MAT 8230 or Du Pont 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.

*Method A: 4-(3-oxo-3*H*-isothiazolo[5,4-*b*]pyridin-2-ylmethyl)-benzonitrile, **3g***

Sodium hydride (80%, 0.90 g, 32.8 mmol) was washed free of mineral oil with hexane, suspended in 100 mL of dry THF, and treated with 5.00 g (32.8 mmol) of **4**. The mixture was stirred for 30 min at 20 °C until gas evolution ceased. 4-Cyanobenzyl bromide (7.06 g, 36 mmol) was added in one portion and the mixture was heated under reflux for 24 h. The mixture was then cooled, poured into 10% K_2CO_3 solution, and separated. The aqueous phase was extracted twice with EtOAc, and the combined organic extracts were washed with water and brine, dried, and concentrated. The residue was recrystallized from 9:1 *n*-BuCl:MeCN to give 2.32 g (27%) of **3g**, mp 185–187 °C. ^{1}H NMR ($CDCl_3$): 8.77 (*d*, 1H), 8.32 (*d*, 1H), 7.66 (*d*, 2H), 7.46 (*d*, 2H), 7.40 (*d* of *d*, 1H); 5.13 (*s*, 2H). MS (NH_3Cl): m/z = 268 (M + H$^+$, 100%). Analysis: calcd for $C_{14}H_9N_3OS$: C 62.91%, H 3.39%, N 15.72%. Found: C 63.04%, H 3.29%, N 15.70%.

*Method B: 3-(3-oxo-3*H*-isothiazolo[5,4-*b*]pyridin-2-ylmethyl)-benzoic acid methyl ester, **3d***

A solution of **4** (2.00 g, 13.0 mmol) in 50 mL of dry MeOH was treated successively with 3-carbomethoxybenzyl bromide (3.89 g, 17.0 mmol) and *N,N*-diisopropylethylamine (2.5 mL, 14.4 mmol). The reaction mixture was stirred at 25 °C for 16 h, then was poured into 200 mL of 1 M HCl and extracted with EtOAc. The EtOAc extract was washed with water and brine and dried, and concentrated and the residue chromatographed on silica (1:1 hexane:EtOAc) to give 1.40 g (36%) of **3d**, mp 95–97 °C after recrystallization from *n*-BuCl:hexane. ^{1}H NMR ($CDCl_3$): 8.75 (*d*, 1H), 8.29 (*d*, 1H), 8.03 (*s*, 1H), 7.58–7.46 (*m*, 3H), 7.38 (*m*, 1H), 5.13 (*s*, 2H), 3.92 (*s*, 3H). MS (NH_3Cl): m/z = 301 (M + H$^+$, 100%). Analysis: calcd for $C_{15}H_{12}N_2O_3S$: C 59.99%, H 4.03%, N 9.33%. Found: C 59.97%, H 3.96%, N 9.21%.

*Method C: 2-pyridin-3-ylmethyl-isothiazolo[5,4-b]-pyridin-3-one, **3aa***

A suspension of 2-benzylsulfanyl-*N*-pyridin-3-ylmethyl-nicotinamide[24] (3.00 g, 8.94 mmol) in PhMe (100 mL) was treated with SO_2Cl_2 (2.16 mL, 26.8 mmol) and heated at reflux for 4 h. The mixture was then cooled, filtered, and the solid was digested with 150 mL of EtOH at reflux for 1 h. The mixture was cooled, filtered, and dried to give 1.40 g (56%) of **3aa** as the HCl salt, mp > 250 °C.[25] This solid (0.28 g, 1 mmol) was dissolved in 5 mL of H_2O and treated with saturated $NaHCO_3$. The mixture was extracted with EtOAc and the EtOAc was dried and concentrated to afford 0.22 g (90%) of **3aa**, mp 134–136 °C. ^{1}H NMR ($CDCl_3$): 8.76 (*d*, 1H), 8.66 (*s*, 1H), 8.59 (*d*, 1H), 8.31 (*m*, 1H), 7.72 (*m*, 1H), 7.38 (*m*, 1H), 7.30 (*m*, 1H); 5.08 (*s*, 2H). MS (NH_3Cl): m/z = 244 (M + H$^+$, 100%). Analysis: calcd for $C_{12}H_9N_3OS$: C 59.24%, H 3.73%, N 17.27%. Found: C 58.98%, H 3.64%, N 17.04%.

Liver microsome stability studies

A sample of 2.5 μg of test compound was incubated with 200 μL of rat liver microsomes (1 mg protein mL^{-1})[26] in 0.1 M pH 7.4 phosphate buffer containing NADPH (1 mM) in a final volume of 2 mL for 1 h at 37 °C. At the end of incubation, metabolism was stopped by extraction with either CH_3CN or CH_2Cl_2. Samples were analyzed by HPLC using a μ-Bondpack column (15 cm × 4.6 mm) with a mobile phase of CH_3CN:0.01 M H_3PO_4 (35:65, pH 3.0) at a flow rate of 1 mL min^{-1}. Peaks were identified by retention time with reference to standard compounds and quantitated using peak area.

Chemical modeling of stability

A sample of 0.1 mmol of test compound in 100 μL of THF and 100 μL of 1 M Me_3CSH in THF were combined in a micro vial under nitrogen and closed. The reaction was kept at 25 °C and monitored by HPLC, and at the end of 24 h the mixture was evaporated to dryness and the ^{1}H NMR spectrum was recorded. The extent of reaction was determined by peak area integration (HPLC) and by comparison of the Me_3C integrals in the ^{1}H NMR spectrum against the rest of the spectrum. Authentic samples of reduced products were prepared by $NaBH_4$–EtOH reduction of the test compound.

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[27] and cut into eighths. Cartilage pieces were then weighed and each placed into a well of a 96-well culture dish containing 180 mL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum, penicillin (100 IU mL^{-1}), streptomycin (100 μg mL^{-1}), amphotericin B (0.25 μg mL^{-1}), and neomycin (50 μg mL^{-1}). 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%.[28] Cartilage was incubated in the absence or presence of IL-1β (500 ng mL^{-1}), with or without drug. Under these conditions, 500 ng of IL-1β resulted in submaximal stimulation of proteoglycan breakdown,[9] 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^{-1} of ^{35}S-sulfate. The samples were incubated an additional 2 h, and the media was removed. The cartilage was digested with papain, the proteoglycan precipitated with cetylpyridinium chloride, and the precipitates counted for ^{35}S. GAGs in the culture media were measured from the amount of polyanionic material reacting with 1,9-dimethylmethylene blue, using shark chondroitin sulfate as a standard.[29] Results were reported as μg GAG per mg wet weight of cartilage. ^{35}S-Sulfate incorporation was determined as dpm per mg wet weight of cartilage.

Stromelysin activation studies

Incubation mixtures were prepared from 3.3 μL of prostromelysin (31.25 μg mL^{-1}),[30] 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.[31] 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 quenched with 200 μL of 3.3% Cl_3CCO_2H and centrifuged. An aliquot of the supernatant (100 μL) was added to 5 mL of scintillation cocktail for LSC.

Intra-articular injection of IL-1β

Male New Zealand white rabbits (2.3–3.0 kg) were injected intra-articularly through the suprapatellar ligament into the joint space with 5 ng of human recombinant IL-1β[15] in a volume of 0.2 mL of phosphate buffered saline (PBS). In initial studies, injection of 0.2 mL of PBS into the knees of rabbits (vehicle control) caused no difference in the amount of GAG released into the synovial fluid or cell number as compared with untreated, naive control knees. Therefore, the contralateral uninjected knees were used as paired controls in these studies. Animals were euthanized 18 h following a single injection of IL-1β. Under these conditions, 5 ng of IL-1β gave approximately half-maximal stimulation of GAG release into the synovial fluid.

Inhibitor studies

Drugs were administered 1 h prior to IL-1β challenge as a suspension or solution in methyl cellulose vehicle via gavage in a volume of < 5 mL. Following euthanasia, 1 mL of sterile PBS was injected into the joint space and the joints were articulated. The diluted synovial washes were taken for cell count and the cell-free joint fluids were then stored at –70 °C for assay of sulfated glycosaminoglycans (GAG). Aliquots of the synovial fluid wash were digested with 250 μg mL^{-1} of papain in 0.1 M pH 6.5 phosphate buffer containing 0.005 M cysteine HCl and 0.005 M EDTA at 65 °C for 2 h. Sulfated GAG concentrations of the digests were measured from the amount of polyanionic material reacting with 1,9-dimethylmethylene blue, using shark chondroitin sulfate as a standard.[29]

References and Notes

1. Dieppe, P.; Hutton, C.; Campion, G. *Monographs in Medicine — Rheumatology*, p. 1, PharmaLibri; Morristown, NJ 1986.

2. (a) Shinmei, M.; Masuda, K.; Kikuchi, T.; Shimomura, Y. *Semin. Arthritis Rheum.* **1989**, *18*, 27; (b) Richardson, H. J.; Elford, P. R.; Sharrard, R. M.; Russell, R. G. G. *Cell. Immunol.* **1985**, *90*, 41.

3. (a) Saklatvala, J.; Sarsfield, S. J.; Pilsworth, L. M. C. *Biochem J.* **1983**, *209*, 337; (b) Tyler, J. A. *Biochem J.* **1985**, *225*, 493; (c) Tyler, J. A. *Biochem J.* **1985**, *227*, 869.

4. (a) Pettipher, E. R.; Higgs, G. A.; Henderson, B. *Proc. Natl Acad. Sci. U.S.A.* **1986**, *83*, 8749; (b) Pettipher, E. R.; Henderson, B.; Hardingham, T.; Ratcliffe, A. *Arthritis Rheum.* **1989**, *32*, 601; (c) Arner, E. C.; DiMeo, T. M.; Pratta, M. A. *Agents Actions* **1989**, *27*, 254; (d) O'Byrne, E. M.; Blancuzzi, V.; Wilson, D. E.; Wong, M.; Jeng, A. Y. *Arthritis Rheum.* **1990**, *33*, 1023; (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. *Arthritis Rheum.* **1992**, *35*, 799.

5. Gowen, M.; Wood, D. D.; Ihrie, E. J.; Meats, J. E.; Russell, R. G. G. *Biochem. Biophys. Acta* **1984**, *797*, 186.

6. Richardson, H. J.; Elford, P. R.; Sharrard, R. M.; Meats, J. E.; Russell, R. G. G. *Cell Immunol.* **1985**, *90*, 41.

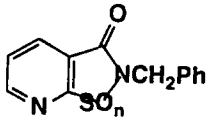
7. (a) Bender, P. E.; Lee, J. C. *Ann. Rep. Med. Chem.* **1990**, *25*, 185; (b) Henderson, B.; Docherty, A. J. P.; Beeley, N. R. A. *Drugs of the Future* **1990**, *15*, 495.

8. (a) 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. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2875; (b) Wright, S. W.; Petraitis, J. J.; Abelman, M. M.; Batt, D. G.; Bostrom, L. L.; Corbett, R. L.; Decicco, C. P.; Di Meo, S. V.; Freimark, B.; Giannaras, J. V.; Green, A. M.; Jetter, J. W.; Nelson, D. J.; Orwat, M. J.; Pinto, D. J.; Pratta, M. A.; Sherk, S. R.; Williams, J. M.; Magolda, R. L.; Arner, E. C. *J. Med. Chem.* **1994**, *37*, 3071.

9. Arner, E. C.; Pratta, M. A. *Arthritis Rheum.* **1989**, *32*, 288.

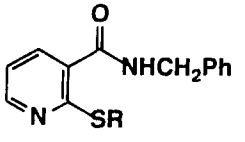
10. Compound **2** was prepared by reduction of **1a** ($NaBH_4$, EtOH, 20 °C), Monge, A.; Martinez-Merino, V. *J. Heterocycl. Chem.* **1985**, *22*, 1353.

11. No isothiazolone oxidation products (e.g. **7** or **8**) were detected upon incubation of isothiazolone **3a** with liver microsomes as determined by co-injection with authentic samples of **7** and **8** (prepared by oxidation of **1a** with 1.0 eq. and 2.3 eq. of *m*-CPBA in CH_2Cl_2 at 0 °C, respectively). Likewise, very little (< 5%) of the potential reduction products **9** (prepared by $NaBH_4$ reduction of **3a**) or **10** (prepared from **9** with KOH and MeI) were detected in these experiments. The disulfide of **9** (prepared from **9** and I_2) was also detected only in trace amounts.



7, n = 1

8, n = 2



9, R = H

10, R = CH_3

12. Wright, S. W.; Corbett, R. L. *OPPI* **1993**, *25*, 247.

13. Wright, S. W.; Abelman, M. M.; Bostrom, L. L.; Corbett, R. L. *Tetrahedron Lett.* **1992**, *33*, 153 and references cited therein.

14. A total of 10 pyridoisothiazolones **1** and 20 pyridoisothiazolones **3** were tested in both the liver microsomal and Me_3CSH assays to validate the Me_3CSH model results.

15. Huang, J. J.; Newton, R. C.; Pezzella, K.; Covington, M. B.; Tamblyn, T.; Rutledge, S. J.; Kelley, M.; Gary, J.; Lin, Y. *Mol. Biol. Med.* **1987**, *4*, 169.

16. Okada, Y.; Nagase, H.; Harris, Jr E. D. *J. Biol. Chem.* **1986**, *261*, 14245.

17. (a) White, H. L.; Glassman, A. T. *Prostaglandins* **1974**, *7*, 123; (b) Vigdahl, R. L.; Tukey, R. H. *Biochem. Pharmacol.* **1977**, *26*, 307.

18. (a) Patriarca, P.; Beckerdite, S.; Elsbach, P. *Biochim. Biophys. Acta* **1972**, *260*, 593; (b) Davidson, F. F.; Dennis, E. A.; Powell, M.; Glenney, Jr J. R. *J. Biol. Chem.* **1987**, *262*, 1698.

19. (a) Jakschik, B. A.; Lee, L. A.; Shuffer, G.; Parker, C. W. *Prostaglandins* **1978**, *16*, 733; (b) Jakschik, B. A.; Sun, F. F.; Lee, L. H.; Steinhoff, M. M.; Jakschik, B. A.; Sun, F. F.; Lee, L. H.; Steinhoff, M. M. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 103; (c) Jakschik, B. A.; DiSantis, D. M.; Sankarappa, S. K.; Sprecher, H. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 624.

20. Newton, R. C. *J. Leukocyte Biol.* **1986**, *39*, 299.

21. (a) Okada, Y.; Nagase, H.; Harris, Jr E. D. *J. Biol. Chem.* **1986**, *261*, 14245; (b) the isothiazolones **1** and **3** 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** and **3** may occur through a mechanism involving a covalent modification of prostromelysin via ring opening of the isothiazolone. The results of these experiments will be reported elsewhere.

22. Greenwald, R. A. *Drug News & Perspectives* **1990**, *3*, 161.

23. Campion, C.; Davidson, A. H.; Dickens, J. P.; Crimmins, M. J. *PCT/GB89/01399*, 23 November, 1989.

24. This compound (mp 111–112 °C) was prepared in 71% yield (recrystallized, EtOH) from 2-benzylsulfanylnicotinic acid by (i) ClCOCOCl (1.2 eq.), DMF (cat.), THF, 0 °C; (ii) 3-$H_2NCH_2C_5H_4N$, Et_3N (2.0 eq.), THF, 0 °C. ^1H NMR ($CDCl_3$): 8.57 (*s*, 1H), 8.52 (*t*, 2H), 7.84 (*d*, 1H), 7.70 (*d*, 1H), 7.35 (*m*, 2H), 7.25 (*m*, 4H), 7.07 (*d*, 1H), 6.79 (*br s*, 1 H), 4.60 (*d*, 2H), 4.46 (*s*, 2H). MS (NH_3Cl): m/z = 336 ($M + H^+$, 100%). Analysis: calcd for $C_{19}H_{17}N_3OS$: C 68.04%, H 5.11%, N 12.53%. Found: C 68.15%, H 5.08%, N 12.57%.

25. Analysis: calcd for $C_{12}H_{10}ClN_3OS$: C 51.52%, H 3.60%, N 15.02%, Cl 12.67%. Found: C 51.74%, H 3.63%, N 14.63%, Cl 12.67%.

26. See Lake, B. G. *Biochemical Toxicology: A Practical Approach*, pp. 185–187, Snell, K.; Mulloch, B. Eds.; IRL Press; Oxford, UK, 1987.

27. Steinberg, J.; Sledge, C. B.; Noble, J.; Stirrat, C. R. *Biochem. J.* **1979**, *180*, 403.

28. At this concentration DMSO had no effect on cartilage proteoglycan metabolism.

29. Farndale, R. W.; Sayers, C. A.; Barrett, A. J. *Connect. Tissue Res.* **1982**, *9*, 247.

30. Purified prostromelysin from human rheumatoid synovial fibroblasts was a kind gift from Dr Hideaki Nagase (University of Kansas Medical School).

31. Nagase, H.; Okada, Y.; Harris, Jr E. D. *J. Biol. Chem.* **1986**, *261*, 14245.

(*Received in U.S.A.* 14 *November* 1994; *accepted* 9 *December* 1994)