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2,5-Diarylisothiazolone: Novel Inhibitors of Cytokine-Induced Cartilage Destruction

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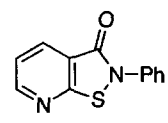
Abstract—A series of 2,5-diarylisothiazolones is reported that inhibit the IL-1β-induced breakdown of bovine nasal septum cartilage in an organ culture assay. The synthesis and preliminary SAR of these compounds are described. These compounds represent a novel, nonpeptide lead series approach to the 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. Copyright © 1996 Elsevier Science Ltd

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 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 synthesis, in vitro activity, SAR and preliminary mechanism of action results of a novel series of 2,5-diarylisothiazolones (**2**) that are the first isothiazolone cartilage breakdown inhibitors we have identified that are not hetero-aryl fused isothiazolones.



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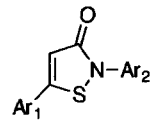

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Chemistry

The 2,5-diarylisothiazolones are almost unknown in the literature.[10] The 2,5-diarylisothiazolones (**2**) described in this work were prepared by the methods outlined in Schemes 1 and 2. The 2,5-diarylisothiazolones in which R_3 is hydrogen were conveniently prepared through the series of reactions (Method A) shown in Scheme 1. Thus, treatment of appropriately substituted phenylpropiolic acids (**3**)[11] with either thionyl chloride[12a] or trimethylacetyl chloride and triethylamine,[12b] followed by addition of appropriately substituted arylamines, afforded the N,3-diarylpropiolamides (**4**). Treatment of **4** with thiourea and *p*-toluenesulfonic acid monohydrate[13] in ethanol gave the isothiouronium *p*-toluenesulfonates **5**. Conventional hydrolysis of the isothiouronium salts with sodium hydroxide in aqueous ethanol, followed by acidification, resulted in the precipitation of the vinyl mercaptan **6**. The oxidative cyclization of **6** was best effected with iodine and triethylamine to afford the target 2,5-diarylisothiazolones **2**.

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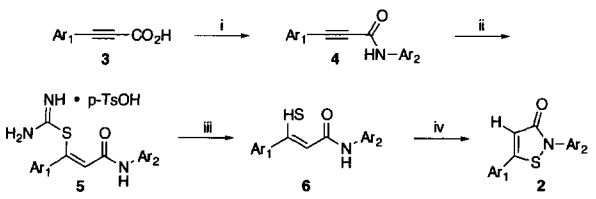


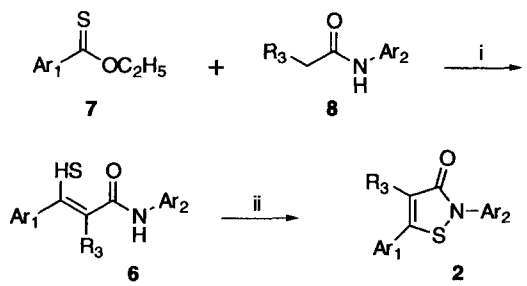
Scheme 1. (i) $SOCl_2$, PhH, reflux, then $ArNH_2$, PhH, 0 °C; (ii) thiourea, *p*-TsOH, EtOH, reflux; (iii) NaOH, H_2O, EtOH; (iv) I_2, Et_3N, CH_2Cl_2, 20 °C.

For those 2,5-diarylisothiazolones with a group R_3 other than hydrogen, a second route was employed that produced the vinyl mercaptan **6** via a Claisen-like condensation between an O-alkyl thionoester (**7**)[14] and an anilide (**8**) (Method B) as shown in Scheme 2. Treatment of **8** with lithium diisopropylamide, followed by addition of the thionoester **7** afforded the vinyl mercaptan **6** directly which upon oxidative cyclization with iodine and triethylamine gave the 4-substituted-2,5-diarylisothiazolones (**2**).

Certain 2,5-diarylisothiazolones (e.g., **2s** and **2ll**) contained functional groups that were incompatible with the reaction conditions used to form the 2,5-diarylisothiazolone nucleus. These groups were introduced by functional group manipulation following the cyclization of the isothiazolone ring (see Experimental). The reaction conditions used for the functional group transformations in the presence of the isothiazolone had to be carefully selected because of the lability of the isothiazolone ring in the presence of strong bases (e.g., NaOH), sulfur nucleophiles, and reducing agents (e.g., $NaBH_4$).

In vitro evaluation

The 2,5-diarylisothiazolones **2** 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



Scheme 2. (i) LDA, THF, 0 °C to reflux; (ii) I_2, Et_3N, CH_2Cl_2, 20 °C.

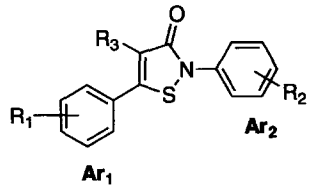
37 °C in the presence or absence of test compounds at 30 μM. The 2,5-diarylisothiazolones **2** inhibited the breakdown of proteoglycan in IL-1β stimulated cartilage (Table 1). As a class, the inhibitors **2** did not further inhibit cartilage resynthesis beyond that caused by IL-1β alone, indicating that they are not simply toxic to the cartilage. Control incubations of cartilage with **2** also showed no increase or decrease in $^{35}SO_4=$ incorporation from control cartilage incubated in the absence of **2**. Compound **2** 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 plasmin-activated stromelysin,[16] cyclooxgenase[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 **1** previously suggested that this compound inhibited cartilage degradation by interfering with the normal activation of matrix metalloproteinases.[8b] Studies carried out on **2a** suggest that compounds in this series act similarly to **1**. For example, **2a** 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 45% if **2a** was present *during* the activation of prostromelysin by either plasmin or chymotrypsin. Control experiments indicated that **2a** was not an inhibitor of either of the activating proteases,[21b] suggesting that **2a** 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 **2a** with prostromelysin prior to the addition of the activating protease. The inhibition of activation reached its maximal effect (65% inhibition of control stromelysin activity) 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 **2a** indicate that this compound produces inhibition of IL-1β-induced cartilage breakdown in rabbit knees when dosed by intraarticular injection.

Structure–activity relationships

Initially, 2,5-diphenylisothiazol-3-one (**2a**), 2,4-diphenylisothiazol-3-one (**2b**) and 2,4,5-triphenylisothiazol-3-one (**2c**) were prepared and screened. Due to the better inhibition observed with **2a**, coupled with the greater synthetic accessibility of analogues of **2a**, it was decided that the focus of our investigations should be on the 2,5-diarylisothiazolone series.

Table 1.



Entry[a]	R_1	R_2	R_3	Ar_1	Ar_2	mp (°C)	Yield	Activity[b]
2a	H	H	H	Ph	Ph	89–91	67	70
2b	—	H	Ph	H	Ph	196 (dec)	1	56
2c	H	H	Ph	Ph	Ph	175–176	73	42
2d	H	H	H	Ph	c-C_6H_{10}	265 (dec)	8	29
2e	H	H	H	c-C_6H_{10}	Ph	79–81	84	3
2f	H	H	H	Ph	2-bzth[c]	201–202	36	76
2g	H	H	H	Ph	2-thz[d]	136–137	74	54
2h	H	H	H	Ph	2-pyr[e]	122–124	80	30
2i	H	H	H	Ph	3-pyr[f]	109–111	55	61
2j	H	H	H	(4-Ph)Ph	Ph	144–145	68	37
2k	H	H	H	2-nap[g]	Ph	135–136	44	50
2l	H	5-CH_3	H	2-thph[h]	Ph	104–105	2	28
2m	H	H	H	Ph	CH_2Ph	157–158	31	33
2n	H	H	H	Ph	CH_2CH_2Ph	96–98	6	24
2o	H	2-CH_3	H	Ph	Ph	114–116	69	34
2p	H	3-CH_3	H	Ph	Ph	106–108	87	74
2q	H	4-CH_3	H	Ph	Ph	110–112	52	62
2r	H	2-NH_2	H	Ph	Ph	145–146	49	19
2s	H	4-NH_2	H	Ph	Ph	163–166	34	32
2t	H	4-$NHCOCH_3$	H	Ph	Ph	232–234	38	15
2u	H	2-Cl	H	Ph	Ph	122–124	52	33
2v	H	3-Cl	H	Ph	Ph	109–111	577	63
2w	H	4-Cl	H	Ph	Ph	134–135	53	80
2x	H	2-OCH_3	H	Ph	Ph	147–150	57	63
2y	H	3-OCH_3	H	Ph	Ph	76–77	40	60
2z	H	4-OCH_3	H	Ph	Ph	121–123	55	44
2aa	H	2-CF_3	H	Ph	Ph	149–150	58	25
2bb	H	3-CF_3	H	Ph	Ph	112–113	59	85
2cc	H	4-CF_3	H	Ph	Ph	162–164	58	76
2dd	H	2-NO_2	H	Ph	Ph	190–192	26	43
2ee	H	3-NO_2	H	Ph	Ph	187–189	86	62
2ff	H	4-NO_2	H	Ph	Ph	207–210	21	31
2gg	H	2-CO_2Et	H	Ph	Ph	143–145	89	44
2hh	H	2-CO_2H	H	Ph	Ph	228–230	84	26
2ii	H	3-CO_2Et	H	Ph	Ph	96–98	55	83
2jj	H	3-CO_2H	H	Ph	Ph	263–265	72	22
2ll	H	4-CO_2Et	H	Ph	Ph	134–135	68	81
2mm	H	4-CO_2H	H	Ph	Ph	238–240	34	43
2nn	H	4-CO_2-n-hexyl	H	Ph	Ph	110–112	42	11
2oo	H	4-CO_2Bn	H	Ph	Ph	131–132	35	19
2pp	H	4-$CONH_2$	H	Ph	Ph	219–220	67	42
2rr	H	4-$CONMe_2$	H	Ph	Ph	188–189	79	66
2ss	H	4-CN	H	Ph	Ph	190–193	49	79
2tt	2-Cl	H	H	Ph	Ph	114–115	44	79
2uu	3-Cl	H	H	Ph	Ph	107–108	74	54
2vv	4-Cl	H	H	Ph	Ph	138–139	70	66
2ww	2-OCH_3	H	H	Ph	Ph	124–126	35	55
2xx	3-OCH_3	H	H	Ph	Ph	109–110	26	27
2yy	4-OCH_3	H	H	Ph	Ph	170–172	49	15
2zz	2-CF_3	H	H	Ph	Ph	112–113	66	78
2aaa	3-CF_3	H	H	Ph	Ph	110–112	38	72
2bbb	4-Cf_3	H	H	Ph	Ph	179–180	72	25
2ccc	H	H	OCH_3	Ph	Ph	96–98	5	24
2ddd	H	H	Br	Ph	Ph	114–117	19	37
2eee	H	H	CO_2Et	Ph	Ph	102–103	38	51
2fff	H	H	CN	Ph	Ph	135–136	24	72
2ggg	2-Cl	4-CN	H	Ph	Ph	176–178	73	89
2hhh	2-Cl	4-CO_2Et	H	Ph	Ph	143–145	34	87
2iii	2-Cl	4-CF_3	H	Ph	Ph	185–187	73	83

[a]All compounds gave satisfactory ^1H NMR, CIMS, and elemental analyses.
[b]Expressed as per cent inhibition of control cartilage breakdown at 30 μM dose. Standard errors ± 15%; IL-1β stimulated bovine nasal cartilage assay as outlined in ref 9.
[c]2-Benzothiazolyl; [d]2-Thiazolyl; [e]2-Pyridyl; [f]3-Pyridyl; [g]2-Naphthyl; [h]2-Thiophenyl.

To determine whether the aryl rings at the 2- and 5-positions were necessary, both **2d** (2-cyclohexyl) and **2e** (5-cyclohexyl) were synthesized. The diminution of inhibitory activity seen with both compounds versus **2a** suggested a preference for aryl substituents simultaneously at positions 2 and 5 of the isothiazolone nucleus.

A variety of other aromatic residues were used to replace the phenyl rings at the 2 or 5 position. At the 2-aryl position, a 2-thiazole (**2g**), 2-pyridine (**2h**) and 3-pyridine (**2i**) all proved to be less effective inhibitors compared with **2a**, while only 2-benzothiazole (**2f**) showed any improved inhibitory effect.

Turning to the 5-aryl position, other aromatic residues used to replace the phenyl ring found in **2a** were 4-biphenyl (**2j**), 2-naphthyl (**2k**) and 2-(5-methyl)thiophene (**2l**). Again, the inhibitory effects resulting from these substitutions were lower than those seen for the parent compound **2a**, suggesting phenyl to be a relatively optimal substitution at both the 2- and 5-aryl positions.

The effects of methylene spacer groups between the 2-position nitrogen and the phenyl ring found in **2a** were also investigated. Both methylene (**2m**) and ethylene (**2n**) spacer groups were employed and were found to result in compounds that were less effective inhibitors of cartilage degradation in comparison to **2a**.

We examined the effects of placing a variety of substituents at the *ortho*-, *meta*- and *para* positions of both the 2- and 5-aryl rings. Of those substituents placed on the 2-aryl ring, those showing the greatest inhibitory effects tended to be small, noncharged, electron withdrawing groups residing at the *meta* or *para* position. Examples of this are **2w** (4-Cl), **2bb** (3-CF_3), **2cc** (4-CF_3), **2ii** (3-CO_2Et), **2ll** (4-CO_2Et), and **2ss** (4-CN). It is interesting to note that the ester **2ll** becomes far less potent if the size of the ester alkyl residue is increased as in **2nn** (n-hexyl) and **2oo** (benzyl). Also interesting is the low inhibitory activity of the *para*-nitro substituted compound **2ff**.

A similar study was carried out to examine the effect on inhibitory activity by placing various substituents on the phenyl residing at the 5-aryl position of **2a**. In this case small, noncharged electron-withdrawing groups residing at the *ortho* and *meta* positions appeared to give rise to the greatest inhibitory effects. Exemplifying this trend were **2tt** (2-Cl), **2zz** (2-CF_3) and **2aaa** (3-CF_3).

The effects of placing various substituents at the 4-position of **2a** were also examined. Again, electron-withdrawing groups appeared to confer the greatest potency as seen with **2fff** (4-CN). However, even **2fff** appeared little more effective than **2a** in vitro.

Finally, we examined the combination of optimal 2-aryl residue substituents and optimal 5-aryl residue substituents to afford **2ggg–2iii**. All three compounds showed increased in vitro activity when compared with **2a** or their mono-substituted counterparts. Of the three, the greatest improvement in potency was observed with **2ggg**.

In conclusion, substituted 2,5-diphenylisothiazol-3-ones were designed as cartilage protectants based on the structure of 2-phenyl [5,4-*b*]pyridoisothiazolone. Substitution on the 2-phenyl ring with small, noncharged electron-withdrawing groups at the *meta* and *para* positions tended to give rise to the best activity. Similar substituents placed at the *ortho* and *meta* positions of the 5-phenyl ring of **2a** also gave rise to improved potency. Finally, a combination of the above substituents produced our most potent cartilage protectant within this series of isothiazolones, **2ggg**.

Summary

A comparison of in vitro data for selected 2,5-diarylisothiazolones with some standard drugs is given in Table 2. 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 **2a**, **2rr** and **2aaa** in the organ culture assay.

In conclusion, these 2,5-diarylisothiazolones (**2**) represent simple, nonpeptidic small molecule structures that inhibit the IL-1β stimulated breakdown of cartilage tissue in an organ culture system. These compounds are equal to 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 electronic nature of the 2- and 5-aryl rings, with small, noncharged electron-withdrawing groups yielding the best activity.

Clearly the structure–activity relationships are not entirely additive and must be interpreted with some

Table 2. Data for standard drugs and selected 2,5-diarylisothiazolones (**2**)

Compound	In vitro activity[a]
Indomethacin	<20
Naproxen	<20
Phenidone	<20
BBT-16	73
2a	70
2ll	81
2iii	83
2hhh	87
2ggg	89

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

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 or IBM 200 SY spectrometers using tetramethylsilane as an internal standard. IR 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, New Jersey, and were within 0.4% of the calculated values. TLC 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: 5-phenyl-2-*p*-tolyl-isothiazol-3-one (2q)

Step 1: 3-phenylprop-2-ynoic acid *p*-tolyl-amide. A mixture of 14.61 g (0.1 mol) of 3-phenylpropiolic acid, 14.28 g (0.12 mol) of thionyl chloride and 50 mL benzene was stirred and heated at 70 °C for 3 h. The reaction was allowed to cool to room temperature and the solvent was evaporated. The reaction flask was charged with 50 mL of fresh benzene and the mixture cooled in an ice bath. A solution of 21.43 g (0.2 mol) of 4-toluidine in 50 mL benzene was then added dropwise. After the addition was complete, the ice bath was removed and the mixture stirred at room temperature for 1 h. The mixture was poured into 200 mL of cold water and the layers were separated. The aqueous layer was extracted with three 100 mL portions of ethyl acetate. The combined organic layers were then washed sequentially with 100 mL portions of 5% HCl, water, 5% Na_2CO_3 and water. The organic layer was dried, filtered and evaporated. The resulting solid was recrystallized twice from ethanol/water to afford 11.58 g (0.0492 mol, 49.2%) of 3-phenylprop-2-ynoic acid p-tolylamide (**4q**) as a white powder, mp 139–141 °C. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H), 7.1–7.6 (m, 10H); IR (Nujol): 2214, 1636 cm^{-1}. High resolution mass spectrum: Calcd: 236.107539. Measured: 236.107101. Anal. calcd for $C_{16}H_{13}NO$: C, 81.70; H, 5.53; N, 5.96. Found: C, 81.55; H, 5.40; N, 6.05.

Step 2: 3-mercapto-3-phenyl-*N*-*p*-tolyl-acrylamide. A mixture of 4.70 g (20 mmol) of **4q**, 1.52 g (20 mmol) of thiourea, 3.80 g (20 mmol) of *p*-toluenesulfonic acid monohydrate and 40 mL absolute ethanol was stirred under reflux for 3 h. The reaction was allowed to cool to room temperature and 100 mL of Et_2O was added. The resulting solid was filtered to provide 8.26 g (17 mmol, 85%) of *S*-(1-phenyl-2-((4-methylphenyl)carbamoyl)vinyl)isothiuronium-*p*-toluenesulfonate (**5q**) as a white powder, mp 204–206 °C. ^{1}H NMR (DMSO-d_6): 2.25 (s, 3H), 2.3 (s, 3H), 7.05–7.6 (m, 13H), 9.2 (b s, 4H), 10.1 (s, 1H).

A mixture of 8.02 g (16.6 mmol) of **5q**, 2.66 g (33.2 mmol) of 50% aqueous NaOH and 200 mL of 50% aqueous EtOH was stirred at reflux for 2 h. The mixture was allowed to cool to room temperature and was added to 200 mL of water. The mixture was washed with three 100 mL portions of Et_2O and the aqueous layer was cooled in an ice bath. The solution was acidified to pH 3 with concentrated HCl. The resulting solid was filtered, thoroughly washed with water and air-dried overnight to give 2.68 g (9.9 mmol, 60%) of 3-mercapto-3-phenyl-N-*p*-tolyl-acrylamide (**6q**) as a pale yellow powder, mp 113–114 °C. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H), 6.15 (s, 1H), 7.1 (b s, 1H), 7.15–7.6 (m, 9H), 8.45 (b s, 1H). IR (Nujol): 1626 cm^{-1}. High-resolution mass spectrum: Calcd: 270.095261. Measured: 270.094538. Anal. calcd for $C_{16}H_{15}NOS$: C, 71.38; H, 5.58; N, 5.20; S, 11.90. Found: C, 71.29; H, 5.53; N, 5.29; S, 11.80.

Step 3: 5-phenyl-2-*p*-tolyl-isothiazol-3-one (2q). A mixture of 1.35 g (5 mmol) of **6q** and 100 mL of CH_2Cl_2 was stirred at 20 °C, while 1.27 g (5 mmol) of I_2 was added in portions, followed by 1.0 mL (excess) of Et_3N and the mixture was stirred for 1 h. The solvent was evaporated and the residue was triturated with EtOAc. The mixture was filtered and the filtrate was evaporated. The resulting residue was purified by flash chromatography using hexanes:EtOAc (3:2) as eluant to give 0.70 g (2.6 mmol, 52%) of 5-phenyl-2-*p*-tolyl-isothiazol-3-one (**2q**) as a pale yellow powder, mp 110–112 °C. ^{1}H NMR ($CDCl_3$): 2.4 (s, 3H), 6.6 (s,1H), 7.25–7.6 (m, 9H). IR (Nujol): 1650 cm^{-1}. High-resolution mass spectrum: Calcd: 268.079611. Measured: 268.078308. Anal. calcd for $C_{16}H_{13}NOS$: C, 71.91; H, 4.49; N, 5.24; S, 11.98. Found: C, 71.85; H, 4.69; N, 5.21; S, 11.86.

Method B: 2,4,5-triphenylisothiazol-3-one (2c)

Step 1: 3-mercapto-2,3,*N*-triphenylacrylamide. A mixture of 1.27 g (6.02 mmol) of 2,N-diphenylacetamide in 20 mL of THF was stirred at 0 °C while lithium diisopropylamide (4.4 mL of a 1.5 M solution in cyclohexane, 6.62 mmol) was added dropwise. The solution was stirred for 15 min at 0 °C, followed by stirring at 20 °C for 15 min. The mixture was then cooled to 0 °C and 1.00 g (6.02 mmol) of *O*-ethylthionobenzoate in 10 mL of THF was added dropwise. After the addition was complete, the ice bath was removed and the

solution was stirred for 30 min at 20 °C. The reaction mixture was then heated under reflux for 48 h. The solution was allowed to cool to room temperature and was poured into 50 mL of water. The aqueous layer was acidified with concentrated HCl to yield a reddish-orange solid. The solid was recrystallized from ethanol to provide 0.159 g (0.479 mmol, 8%) of 2,3,N-triphenyl-3-mercaptopropenamide (**4c**) as a pale yellow solid, mp 144–146 °C (dec). ^{1}H NMR ($CDCl_3$): 7.41–7.07 (m, 16H), 5.20 (s, 1H). IR (KBr): 3400, 3302, 1658 cm^{-1}. MS (CH_4 CI): *m/e* 332 ($M+H^+$). Anal. calcd for $C_{21}H_{17}NOS$: C, 76.10; H, 5.17; N, 4.23; S, 9.67. Found: C, 75.94; H, 5.10; N, 4.16; S, 9.83.

Step 2: 2,4,5-triphenylisothiazol-3-one (2c). A solution of 0.150 g (0.45 mmol) of **4c** in 25 mL of methylene chloride was stirred at 20 °C, while I_2 (0.115 g, 0.45 mmol) and triethylamine (0.116 g, 0.45 mmol) were added. The mixture stirred for 1 h, after which the solvent was evaporated and the residue was partitioned between 50 mL of EtOAc and 50 mL of H_2O. The EtOAc was separated and washed with three 50 mL portions of H_2O, dried and concentrated. The resulting solid was recrystallized from ethanol:water to provide 0.108 g (73%) of **2c** as a tan solid, mp 175–176 °C. ^{1}H NMR ($CDCl_3$): 7.72–7.30 (m, 15H). IR (KBr): 1656 cm^{-1}. MS (CH_4 CI): *m/e* 330 ($M+H^+$). Anal. calcd for $C_{21}H_{15}NOS$: C, 76.57; H, 4.59; N, 4.25; S, 9.73. Found: C, 76.25; H, 4.40; N, 4.19, S, 9.81.

4-Bromo-2,5-diphenyl-isothiazol-3-one (2ddd). A mixture of 0.76 g (3 mmol) of **2a**, 0.30 g (3 mmol) of Et_3N and 10 mL of EtOAc was stirred at −5 °C. A solution of 0.48 g (3 mmol) of Br_2 in 5 mL of EtOAc was added dropwise. The mixture was stirred at −5 °C for 30 min, followed by stirring at 20 °C for 3 h. The solvent was evaporated and the residue was purified by flash chromatography (2:1, hexanes:EtOAc) to afford 0.19 g (0.57 mmol, 19%) of **2ddd** as a very pale yellow powder, mp 114–117 °C. ^{1}H NMR ($CDCl_3$): 7.3–7.8 (m, 10H). IR (Nujol): 1662 cm^{-1}. High-resolution mass spectrum: calcd (for 81Br isotope): 333. 972426. Measured: 333.972673. Anal. calcd for $C_{15}H_{10}BrNOS$: C, 54.22; H, 3.01; N, 4.22; S, 9.64; Br, 24.10%. Found: C, 54.11; H, 3.16; N, 4.30; S, 9.58; Br, 23.91.

4-(3-Oxo-5-phenyl-3*H*-isothiazol-2-yl)-benzoic acid ethyl ester (2ll)

Step 1: 4-(3-oxo-5-phenyl-3H-isothiazol-2-yl)-benzoic acid (2mm). A mixture of 1.449 g (5.00 mmol) of N-(4-carboxyphenyl)-3-mercapto-3-phenylpropenamide,[12a] 3 mL (excess) of triethylamine, 100 mL of water and 100 mL of ethanol was stirred at 20 °C while 1.269 g (5.00 mmol) of I_2 was added. The mixture was stirred at 20 °C for 3 h. The solution was filtered and the filtrate was acidified to pH 1 with concentrated HCl. The resulting solid was filtered and recrystallized twice from ethanol:water to provide 0.350 g (1.18 mmol, 23.5%) of **2mm** as a white powder, mp 238–240 °C. ^{1}H NMR ($CDCl_3$): 8.2 (d, 2H), 7.9 (d, 2H), 7.5–7.6 (m, 5H), 6.6 (S, 1H); IR (Nujol): 3481, 1698, 1619 cm^{-1}. High-resolution mass spectrum: calcd: 298.053790. Measured: 298.053472. Anal. calcd for $C_{16}H_{11}NO_3S$: C, 64.65; H, 3.70; N, 4.71; S, 10.77. Found: C, 64.52; H, 3.64; N, 4.59; S, 10.75.

Step 2: 4-(3-oxo-5-phenyl-3*H*-isothiazol-2-yl)-benzoic acid ethyl ester (2ll). A mixture of 0.120 g (4.04 mmol) of **2mm**, 1.00 g (excess) of sodium bicarbonate, 2 mL (excess) of iodoethane and 25 mL of N,N-dimethylformamide was stirred at 70 °C for 3 h. The mixture was poured into water and extracted with three 50 mL portions of ethyl acetate. The organic layers were combined, dried, concentrated, and the residue was purified by flash chromatography on silica gel (2:1 hexane:EtOAc) to give 90 mg (0.277 mmol, 68%) of **2ll** as a pale yellow powder, mp 134–135 °C. ^{1}H NMR ($CDCl_3$): 8.15 (d, 2H), 7.8 (d, 2H), 7.55 (m, 4H), 6.6 (s, 1H), 4.4 (q, 2H), 1.4 (t, 3H); IR (Nujol): 1703, 1664 cm^{-1}. High-resolution mass spectrum: calcd: 325.077265. Measured: 325.077132. Anal. calcd for $C_{18}H_{15}NO_3S$: C, 66.46; H, 4.62; N, 4.31; S, 9.85. Found: C, 66.35; H, 4.71; N, 4.22; S, 9.90.

2-(2-Amino-phenyl)-5-phenyl-isothiazol-3-one (2r). A mixture of 0.560 g (1.88 mmol) of **2dd**, 0.450 g (excess) of iron powder, 1 mL of glacial acetic acid and 15 mL of abs ethanol was stirred under reflux for 1 h. The mixture was cooled, poured into 150 mL of water and extracted with three 100 mL portions of ethyl acetate. The organic layers were combined, dried and concentrated. The resulting solid was recrystallized from ethanol:water to provide 0.250 g (49%) of **2r**, mp 145–146 °C. ^{1}H NMR ($CDCl_3$): 6.8–7.6 (m, 9H), 6.6 (s, 1H), 4.15 (bs, 2H). IR (Nujol): 3358, 3216, 1658 cm^{-1}. High-resolution mass spectrum: calcd: 269.074860. Measured: 269.074265. Anal. calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44; S, 11.95. Found C, 66.76; H, 4.43; N, 10.19; S, 12.09.

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[24] 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%.[25] 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.[26] 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 mg mL^{-1}),[27] 3.3 μL of plasmin (6.25 μg mL^{-1}), and 10 mL 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 mL 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.[28] 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 3× 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.

References and Notes

1. Dieppe, P.; Hutton, C.; Campion, G. *Monographs in Medicine-Rheumatology* **1986**, 1–41; PharmaLibri: Morristown, New Jersey, 1986; pp 1–41.

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; (c) Wright, S. W.; Petraitis, J. J.; Batt, D. G.; Corbett, R. L.; Di Meo, S. V.; Freimark, B.; Giannaras, J. V.; Orwat, M. J.Pinto, D. J.; Pratta, M. A.; Sherk, S. R.; Stampfli, H. F.; Williams, J. M.; Magolda, R. L.; Arner, E. C. *Bioorg. Med. Chem.* **1995**, 227.

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

10. (a) Faust, J. *Z. Chem.* **1968**, *8*, 170; (b) Scott, M. D. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1432; (c) Le Coustumer, G.; Mollier, Y. *Bull. Soc. Chim. Fr.* **1970**, *8–9*, 3076; (d) Faust, J. *Z. Chem.* **1975**, *15*, 478; (e) Goerdeler, J.; Roegler, M. *Chem. Ber.* **1970**, *103*, 112.

11. For general synthetic procedures for the synthesis of substituted phenylpropiolic acids see: (a) Chimichi, S.; De Sio, F.; Donati, D.; Pepino, R.; Rabatti, L.; Sarti-Fantoni, P. *J. Heterocyc. Chem.* **1983**, *20*, 105; (b) Reimer, M. *J. Am. Chem. Soc.* **1942**, *64*, 2510.

12. (a) Yokoyama, A.; Ashida, K.; Tanaka, H. *Chem. Pharm. Bull.* **1964**, *12*, 690; (b) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer–Verlag: New York, 1984; p 105.

13. See ref 12.

14. Lawesson, S. O.; Clausen, K.; Scheibye, S.; Pedersen, B. S. *Bull Soc. Chim. Belg.* **1978**, *87*, 293.

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, E. D. Jr *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, J. R. Jr *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, E. D. Jr *J. Biol. Chem.* **1986**, *261*, 14245; (b) The isothiazolones **1** and **2** 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 **2** 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.

22. Greenwald, R. A. *DN&P* **1990**, *3*, 161.

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

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

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

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

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

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

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