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Inhibition of Cartilage Breakdown by Isothiazolones

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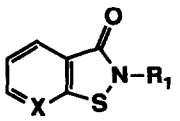
Abstract: Isothiazolones and isoselenazolones have been found to inhibit IL-1β induced breakdown of bovine nasal cartilage in an organ culture assay. The synthesis and preliminary SAR of these compounds are described. These compounds represent a novel, non-peptide lead series approach to the mediation of the chronic cartilage breakdown associated with arthritic disease.

Osteoarthritis is characterized by the progressive erosion of the cartilage pad during the progression of the disease.[1] This erosion is thought to be triggered by a variety of stimuli, particularly cytokines.[2] A number of approaches to the inhibition of cartilage breakdown have been described recently, including inhibition of cytokine production and inhibition of matrix metalloproteases.[3] There remains an unmet medical need for agents that will arrest or retard the cartilage loss associated with arthritis. We have found that aryl-fused isothiazolones (**1**) inhibit the IL-1β induced breakdown of cartilage in an organ culture assay[4] in a dose - dependent manner while not affecting cartilage synthesis.



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Preliminary studies were carried out on N-phenyl benzisothiazolone (**1a**) and N-phenyl pyrido[5,4-b]isothiazolone (**1b**). These compounds were prepared from the mercaptoacids **2a** and **2b** as shown in Scheme 1. The thiols were protected as the S-benzyl thioethers, after which the carboxylic acids were converted to the anilides **3a** and **3b**. Oxidative deprotection of the S-benzyl thioethers with sulfuryl chloride afforded the sulfenyl chlorides, which were treated *in situ* with DABCO to furnish the isothiazolones.[5]

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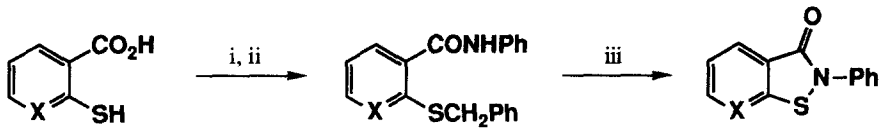
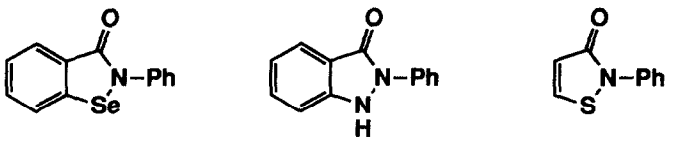
Scheme 1



2a (X = CH) **3a** (X = CH) **1a** (X = CH)
2b (X = N) **3b** (X = N) **1b** (X = N)

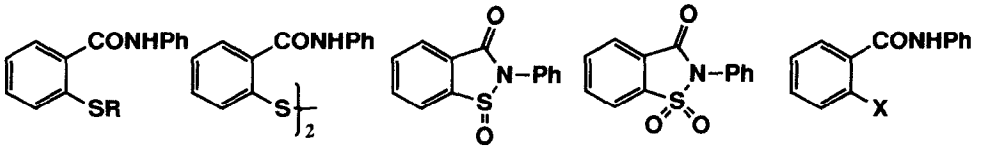
i: $PhCH_2Cl$, KOH, 2-PrOH, 80 °C; ii: $(CH_3)_3CCOCl$, Et_3N, $PhNH_2$, CH_2Cl_2, 25 °C; iii: SO_2Cl_2, C_6H_6, 80 °C, 2 h then DABCO, 25 °C.

A number of structurally similar compounds (**4**, **5**, and **6**) were examined to determine whether the inhibition of cartilage breakdown observed with the isothiazolones was associated exclusively with the presence of the benzisothiazolone and pyridoisothiazolone rings. Replacement of sulfur by selenium (**4**)[6] resulted in a decrease in potency (Table 1), while replacement of sulfur by nitrogen (**5**)[7] resulted in a complete loss of activity. Isothiazolones which lack the aryl-fused ring (e.g., **6**)[8] were likewise inactive.



4 **5** **6**

Because of the many oxidation states available to sulfur, we also examined reduced (**7**, **8**) and oxidized (**9**, **10**) derivatives of **1a**. The thiol **7**[9] and disulfide **8**[9] were less potent than **1a** *in vitro*.[10] The sulfoxide **9**[11a] and sulfone **10**[11b] were both completely inactive. The S - methyl derivative (**11**), oxygen analog (**12**), and nitrogen analog (**13**) were entirely without *in vitro* activity. These results suggest that the benzo- or pyrido-fused isothiazolone ring is the key pharmacophore.



7 (R = H) **8** **9** **10** **12** (X = OH)
11 ($R = CH_3$) **13** ($X = NH_2$)

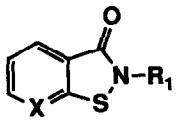
Table 1

Entry	IC_{50}, μM[a]	Entry	IC_{50}, μM[a]
4	13.0	**9**	> 30
5	> 30	**10**	> 30
6	> 30	**11**	> 30
7	18.2	**12**	> 30
8	10.0	**13**	> 30

[a] Standard errors ± 25%; IL-1β stimulated bovine nasal cartilage assay as outlined in Ref. 4.

In general, the pyridoisothiazolones are potent inhibitors of cartilage breakdown and are preferred over their benzisothiazolone counterparts. The isothiazolone nitrogen substituent (R_1) was modified to determine some preliminary structure - activity relationships for these compounds. Results are summarized in Table 2. The addition of either strongly electron - donating or strongly electron - withdrawing substituents to R_1 generally results in decreased *in vitro* activity (**1e**, **1f**, **1g**, **1h**). This effect is more pronounced in the benzo-fused series (**1e**, **1g** vs. **1f**, **1h**). Insertion of a methylene substituent between the isothiazolone nitrogen and the aromatic ring of R_1 results in a slight decrease in activity, which again is more pronounced in the benzo-fused series (**1i** vs. **1j**). Rotation of the R_1 aryl ring out of the plane of the isothiazolone ring also results in decreased *in vitro* activity (**1k**, **1l**).

Table 2



Entry[a]	X	R_1	mp, °C	IC_{50}, μM[b]	Entry[a]	X	R_1	mp, °C	IC_{50}, μM[b]
1a	CH	C_6H_5	141	3.0	**1b**	N	C_6H_5	135	3.0
1c	CH	4-ClC_6H_4	130	3.6	**1d**	N	4-ClC_6H_4	195	3.7
1e	CH	4-$CH_3OC_6H_4$	146	> 30	**1f**	N	4-$CH_3OC_6H_4$	165	13.5
1g	CH	4-$O_2NC_6H_4$	238	> 30	**1h**	N	4-$O_2NC_6H_4$	285	28.0
1i	CH	$CH_2C_6H_5$	98	> 30	**1j**	N	$CH_2C_6H_5$	89	5.0
1k	CH	2,6-$Me_2C_6H_3$	156	17.0	**1l**	N	2,6-$Me_2C_6H_3$	112	9.0

[a] All compounds gave satisfactory ^1H NMR, CIMS, and elemental analyses. [b] See footnote A in Table 1.

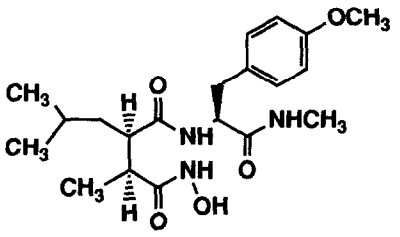
A comparison of selected isothiazolones with some standard drugs is given in Table 3. It will be noted that conventional non-steroidal anti-inflammatory drugs (NSAID), such as indomethacin and naproxen, as well as tetracycline (a collagenase inhibitor),[12] do not block the IL-1 stimulated breakdown of cartilage *in vitro*, while the peptidic stromelysin inhibitor **14**[13] is approximately equipotent to **1b** and **1d**.

In conclusion, benzisothiazolones and particularly pyridoisothiazolones, represent non - peptidic structures that inhibit the IL-1 stimulated breakdown of cartilage tissue in an organ culture system. These compounds are equally or more potent at inhibiting cartilage destruction than other anti-inflammatory agents.

The aryl-fused N-phenyl isothiazolone heterocycle is the key pharmacophore. The related aryl-fused isoselenazolones are likewise active *in vitro*. Studies are currently in progress to examine the biological properties of these compounds *in vivo* and to determine the mechanism by which these compounds exert their *in vitro* effects.

Table 3

Compound	IC_{50}, μM[a]
Indomethacin	> 30
Naproxen	> 30
Tetracycline	> 30
1b	3.0
1d	3.7
14	3.0



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[a] See footnote A in Table 1.

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10. The disulfide **8** is in equilibrium with **1a** and **7** in solution; see McClelland, E. W.; Bartlett, R. G. *J. Chem. Soc.*, **1934**, 818 - 821. Aromatic thiols and disulfides that cannot cyclize to benzisothiazolones, such as diphenyl disulfide or dibenzyl disulfide, are inactive *in vitro*. Sulfides (RSR') are also inactive. During the 40 h incubation period of the assay, the thiol **7** likely undergoes air oxidation to **8.**

11. (a) Prepared from **1a** by oxidation with 1 eq. mCPBA. (b) Beringer, F. M.; Brierly, A.; Drexler, M.; Gindler, E. M.; Lumpkin, C. C. *J. Am. Chem. Soc.*, **1953**, 75, 2708 - 2712.

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