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Inhibition of Cartilage Degradation by Isothiazoloquinolinones

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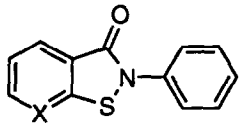
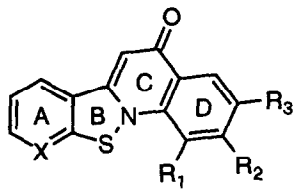
Abstract: Several aryl-fused isothiazoloquinolinones were prepared and were found to inhibit IL-1β induced degradation of bovine nasal cartilage in an organ culture assay. These compounds, derived conceptually from the benzisothiazolone ring system, represent a new, non-peptide class of inhibitors of cartilage degradation.

Osteoarthritis (OA) is a chronic joint disease which is characterized by the progressive degeneration of the articular cartilage in the afflicted joint[1]. Although the pathogenesis of OA remains largely unclear, increasing evidence indicates that cytokines, such as interleukin-1 (IL-1), and matrix metalloproteinases, such as collagenase and stromelysin, may play important roles. Recently, aryl-fused isothiazolones such as **1** have been found[2] to inhibit IL-1β induced degradation of bovine nasal cartilage in a dose-dependent manner in an organ culture assay. Accordingly, these compounds are expected to have utility in the treatment of diseases characterized by the erosion of cartilage tissue, such as OA[3]. As part of an effort to identify novel isothiazolones with similar or enhanced cartilage degradation inhibitory properties, we have prepared the tetracycles **2**. In this communication, we report the design, synthesis and results of preliminary biological evaluation of these novel heterocycles.



1



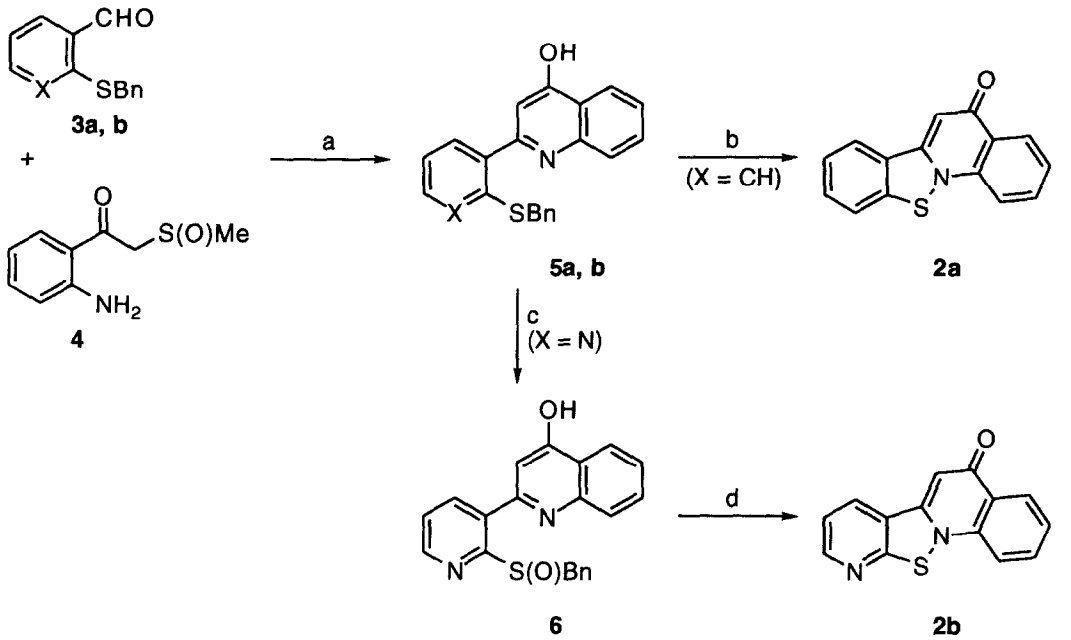
2

In seeking variants of the isothiazolone ring system, we envisioned restricting the rotational mobility of the N-aryl moiety. In doing so, however, we required maximum incorporation of the key pharmacophore of **1**[2] into the rigidifying construction. Thus, the rigidified compound had to contain the nitrogen-chalcogen linkage, maintain the electronic nature at nitrogen in an amide or amide-like function, and match as closely as possible the steric requirements of **1**. The aryl-fused isothiazolo[2,3-a]quinoline-5-one system **2** satisfied these requirements. Incorporated into this tetracyclic system is the key nitrogen-sulfur linkage as well as a

vinylogous amide, a functional group which is electronically similar to an amide. Furthermore, tetracycle **2** is comparable to **1** in its steric requirements.

The general synthetic approach to heterocycles **2** is exemplified by the preparation of the parent compound **2a** and the corresponding aza analog **2b** (Scheme 1). Condensation of aldehydes **3a**[4,5] or **3b**[5] with β-ketosulfoxide **4**[8,9] provided compounds **5a** and **5b** in 55 % and 26 % yields, respectively, in analogy with the literature method for the preparation of 2-aryl-4-hydroxyquinolines[8]. On treatment with sulfuryl chloride, compound **5a** was converted to a putative sulfenyl chloride intermediate which, on exposure to DABCO *in situ*, cyclized to afford 5H-[1,2]benzisothiazolo[2, 3-a]quinoline-5-one (**2a**) in 37 % yield. Alternatively, the key oxidative cyclization could be realized through application of methodology described recently for the preparation of derivatives of **1**[10]. Thus, when crude sulfoxide **6**, obtained on *m*-CPBA oxidation of **5b**, was exposed to the action of trichloroacetic anhydride in methylene chloride, 5H-pyrido[3', 2': 4, 5]isothiazolo[2, 3-a]quinoline-5-one (**2b**) was obtained in 80 % yield. Presumably, the reaction proceeds through an intermediate activated sulfoxide, which cyclizes spontaneously under the reaction conditions with loss of trichloroacetic acid and benzyl trichloroacetate. All isothiazoloquinolinones used in this study were prepared using these methods[11].

Scheme 1



Key: **a** series, X = CH; **b** series, X = N; (a) piperidine, toluene, reflux (**5a**, 55 %; **5b**, 26 %); (b) SO_2Cl_2, CH_2Cl_2, -15°C then DABCO, 25°C (37 %); (c) *m*-CPBA, CH_2Cl_2, 0°C; (d) $(Cl_3CCO)_2O$, CH_2Cl_2, 0°C (80 % for two steps).

The results of evaluation of several derivatives of **2** as inhibitors of IL-1β induced cartilage degradation are summarized in Table 1[12,13,14]. The parent tetracycle **2a** proved to be essentially equipotent with the benzisothiazolone **1a**, demonstrating that locking the aryl ring at the 2-position of **1a** into coplanarity with the amide moiety is not detrimental to *in vitro* activity. Substitution in the D-ring appeared to be well tolerated, as compounds **2c**, **2d**, and **2e** were all effective inhibitors of cartilage degradation. However, substitution of the A-ring at the position ortho to sulfur (compound **2f**) clearly had an adverse effect on activity. Further, the pyridyl derivative **2b** was somewhat less potent than both **2a** and the corresponding isothiazolone derivative **1b**. This finding is interesting in light of recent results[2] which showed that the *in vitro* activity of derivatives of **1b** was generally equal to or greater than that in the corresponding derivatives of **1a**. Although no conclusions can be drawn at this time, the diminished activity of both **2b** and **2f** implies that alteration of the environment about sulfur in aryl-fused isothiazoloquinolinones can have pronounced effects on biological activity.

Table 1. Inhibition of IL-1β induced degradation of bovine nasal cartilage by isothiazoloquinolinones **2**

Compound	X	R_1	R_2	R_3	IC_{50}, μM (or % Inhibition at 30 μM)
1a[2]	CH	---	---	---	3.0*
1b[2]	N	---	---	---	3.0*
2a	CH	H	H	H	8.0*
2b	N	H	H	H	22*
2c	CH	OCH_3	H	H	(74 %)*
2d	CH	OCH_3	OCH_3	OCH_3	(80 %)*
2e	CH	H	H	Cl	(84 %)*
2f	$C(OCH_3)$	H	H	H	(30 %)

* Significantly different from cartilage stimulated with IL-1 alone at $P \leq 0.05$

A potential problem associated with these tetracycles is their relatively poor solubility in the assay medium[12]. As a result, no reliable IC_{50} values could be determined for compounds **2c** - **2f**. Despite this drawback, aryl-fused isothiazoloquinolinones are clearly effective inhibitors of IL-1β induced cartilage degradation *in vitro*, and represent a new, non-peptide class of cartilage degradation inhibitors. Identification of structural modifications for improving solubility, as well as further development of the SAR in this novel series, await further investigation.

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References and Notes

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5. (a) 2-Benzylthiobenzaldehyde (**3a**) and 2-benzylthiopyridine-3-carboxaldehyde (**3b**) were prepared from thiosalicylic acid and 2-mercaptonicotinic acid, respectively, by the sequence (i) BnBr (2.2 eq.), K_2CO_3 (2.5 eq.), 2-butanone, reflux; (ii) $LiAlH_4$, THF, 0°C to RT; (iii) DMSO, ClCOCOCl, CH_2Cl_2, -78°C, then Et_3N, warm to RT[6]. (b) 2-benzylthio-3-methoxybenzaldehyde (used in the preparation of **2f**) was prepared from *m*-anisaldehyde by the sequence (i) *p*-TsOH·H_2O, $(MeO)_3CH$, MeOH, 0°C; (ii) t-BuLi, Et_2O, 0°C[7] then BnSSBn, RT; (iii) *p*-TsOH·H_2O, 9:1 acetone/H_2O, RT.

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7. Metalation of *m*-anisaldehyde dimethyl acetal was performed by the literature method. See Plaumann, H. P.; Keay, B. A.; Rodrigo, R. *Tetrahedron Lett.* **1979**, 4921.

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9. All ketosulfoxides used in this study were prepared by application of the literature method for the preparation of *o*-amino-ω-(methylsulfinyl)acetophenones. See ref. 8.

10. Wright, S. W.; Abelman, M. M.; Bostrom, L. L.; Corbett, R. L. *Tetrahedron Lett.* **1992**, *33*, 153.

11. Compounds **2a** - **2f** all gave satisfactory ^1H NMR (300 MHz), IR, CIMS, and elemental analyses.

12. Biological assay conditions: Compounds were dissolved in dimethyl sulfoxide (DMSO) at 10 mM and then diluted with culture media to the final concentration. DMSO concentrations in the culture media never exceeded 1 % and at this concentration DMSO had no effect on cartilage degradation. Bovine nasal cartilage was incubated with or without IL-1 (500 ng/mL) +/- inhibitor in Dulbecco's modified Eagle's medium supplemented with 5 % heat-inactivated fetal calf serum and antibiotics at 37°C, 95 % air/5 % CO_2 for 40 hr. Cartilage degradation was determined by assessing proteoglycan breakdown products released into the media using a dimethylmethylene blue colorimetric assay for sulfated glycosaminoglycans. IL-1 alone stimulates proteoglycan breakdown, and compounds were evaluated for the ability to inhibit this stimulated breakdown. See (a) Arner, E. C.; Pratta, M. A. *Arthritis Rheum.* **1991**, *34*, 1006; (b) Arner, E. C.; Pratta, M. A. *Arthritis Rheum.* **1989**, *32*, 288.

13. Compounds **2a-f** do not appear to block cartilage degradation by inhibition of IL-1β. See Arner, E. C.; Pratta, M. A.; Freimark, B. L.; Lischwe, M.; Trzaskos, J.; Magolda, R. L.; Wright, S. W., manuscript in preparation.

14. For comparison, the non-steroidal antiinflammatory drugs (NSAIDs) indomethacin, naproxen, ibuprofen, and phenylbutazone, and the antiarthritis drugs levamisole and hydrocortisone do not inhibit IL-1β induced cartilage degradation *in vitro*. The antiarthritis drugs chloroquine and razoxane show significant inhibition, but also inhibit proteoglycan synthesis. See reference 12b.

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