ELSEVIER

Il Farmaco 57 (2002) 1009–1013

IL FARMACO

www.elsevier.com/locate/farmac

In vitro evaluation of thiazolyl and benzothiazolyl Schiff bases on pig cartilage

V. Cardile [a], A.M. Panico [b,*], A. Geronikaki [c], B. Gentile [b], G. Ronsisvalle [b]

[a] *Department of Physiological Sciences, University of Catania, Viale Andrea Doria 6, 95125 Catania, Italy*
[b] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Catania, Viale Andrea Doria 6, 95125 Catania, Italy*
[c] *Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, 54006 Thessaloniki, Greece*

Received 11 March 2002; accepted 8 June 2002

Abstract

A series of anti-inflammatory agents known as Schiff bases, combining thiazolyl and benzothiazolyl ring and vanillin moieties in the same molecule, was synthesized and evaluated for screening anti-degenerative activity on nasal pig cartilage cultures treated with interleukin 1β (IL-1β). The amount of glycosaminoglycans (GAGs), the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2), released into the culture medium, were detected. The tested Schiff bases decreased, dose-dependently, the NO and PGE_2 production and the GAGs release with respect to samples treated with IL-1β alone, showing a different behavior correlated to their structure. These results suggest that thiazolyl and benzothiazolyl Schiff bases in general, and particularly the Schiff base with bromine and methoxyl group in position three would protect cartilage matrix from degenerative factors induced by IL-1β.

Keywords: Cartilage; Glycosaminoglycans; Nitric oxide; Prostaglandines; Schiff bases

1. Introduction

Recently, we synthesized, using a computer aided prediction [1] a number of Schiff bases combining thiazolyl or benzothiazolyl ring with vanillin type moieties in the same molecule and we evaluated their anti-inflammatory activity in a range of in vitro and in vivo model systems [2]. The encouraging results on the anti-phlogistic–analgesic activity of these compounds led us to evaluate in vitro their effect on anti-degenerative activity of nasal pig cartilage treated with interleukin 1β (IL-1β), a cytokine released during inflammatory process [3,4]. Concerning anti-inflammatory activity, it has been observed that non-steroidal anti-inflammatory drugs (NSAIDs), in spite of their clinical effectiveness, may in their long term use cause development of erosive mucosal ulceration [5] and damage to joint articular cartilage [6–9]. Thus, modern pharmacology is interested in the development of new NSAIDs, that exhibit low gastrointestinal toxicity and have limited effects on articular cartilage function.

Nitric oxide (NO) and glycosaminoglycans (GAGs) can be considered as factors of cartilage destruction. Particularly NO, produced by NO synthase (NOS), is a multi-functional mediator which participates in inflammatory tissue destruction [10–13]. In addition, GAGs release is a consequence of matrix proteases increased activity leading to the cleavage of collagen and proteoglycans, fundamental constituents of cartilage. The catabolic effect of NO determines the inhibition of proteoglycans synthesis and stimulates the chondrocyte production of proenzymes which, converted into active enzymes (metalloproteinases), cause cartilage breakdown [14,15].

Moreover, during the inflammatory processes, PGE_2 production is increased and contributes to synovial inflammation by increasing local blood flow and by potentiating the effects of mediators such as bradykinin, which induce vasopermeability. PGE_2 has been shown to inhibit chondrocyte growth, trigger osteoclastic bone resorption, and upregulate interleukin 1β, suggesting that this molecule may also contribute to the pathophy-

* Corresponding author
E-mail address: panico@unict.it (A.M. Panico).

0014-827X/02/$ - see front matter
PII: S0014-827X(02)01290-9

siology of joint erosion in inflammatory disease (arthritis) [16,17]. Starting from the above considerations, we investigated, in the present work, the effect on NO, PGE_2 production and GAGs release of a series of derivatives Schiff bases with anti-inflammatory activity [1,2]. Their effects were evaluated in vitro on the metabolism of nasal pig cartilage treated with IL-1β. Parallel experiments in the presence of indomethacin were also carried out. The general formula of tested compounds **a**, **b**, **b_1**, **b_2**, is given in Table 1.

2. Results

2.1. Chemistry

Compounds were synthesized according to method described in our previous paper [1].

2.2. Pharmacological results and discussion

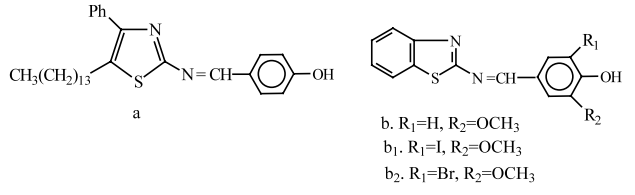
The anti-degenerative effect of thiazolyl and benzothiazolyl Schiff bases on cartilage metabolism was evaluated at different concentrations (1, 10, 100 μg/ml) by determining GAGs release, NO and PGE_2 production in vitro. In our experimental model, thiazolyl and benzothiazolyl Schiff bases at various concentrations did not affect normal cartilage metabolism when added alone into the culture medium (data not shown). On the other hand every Schiff base combined with IL-1β reduced in vitro the degradation of pig cartilage in a different manner. This cytokine was used to simulate the inflammatory process. It is, in fact, well known that, during the inflammatory processes, monocytes and macrophages release IL-1β, which reduces proteoglycans synthesis by the articular chondrocytes, increasing PGE_2 production, that destroys the cartilage. This effect was also confirmed by our experimental results, as it can be observed in Figs. 1–3 by comparing with control data the remarkably increased release of NO, GAGs and PGE_2, after treatment with IL-1β at concentration of 10 ng/ml. Furthermore, indomethacin (10^{-5} M) was used as a reference drug and samples treated with indomethacin combined with IL-1β showed a significant decrease on NO production, GAGs release and PGE_2 production (20, 12, 76.6%, respectively) with respect to the IL-1β treated samples.

In Fig. 1, the levels of NO are reported. The thiazolyl and benzothiazolyl Schiff bases at different concentrations (1, 10 and 100 μg/ml) when combined with IL-1β exhibited a significant reduction of NO release compared to the samples treated with IL-1β. The effect of compounds '**b_2**', '**b_1**' and '**b**' at 100 μg/ml was higher than the one observed with indomethacin and IL-1β, while compound '**a**' had a similar behavior to indomethacin. Regarding the activity on GAGs release (Fig. 2), compounds '**a**', '**b**', '**b_1**' and '**b_2**', combined with IL-1β, showed inhibitory effect, but only compound '**b_2**' exhibited a significantly better effect (37.5%) than the one observed in samples treated with indomethacin and IL-1β (12%). Under these experimental conditions, '**b_2**' compound induced a similar decrease in PGE_2 production (76.4%) as showed in Fig. 3. These experiments suggest that benzothiazolyl Schiff bases inhibit NO production, PGE_2 and GAGs release. This favorable effect may be used in inflammatory disease to decrease the severity of the damage induced into joint articular cartilage [18].

In particular, this study showed that all compounds inhibited the effect of IL-1β on the NO and PGE_2 production, whereas only compound '**b_2**' inhibited effect of IL-1β on NO and PGE_2 production, and GAGs levels.

From a structural point of view its activity seems to be correlated with the presence of benzothiazolyl ring, and of the different substituent in R_1 and R_2 (Table 1) in the aromatic ring. The substitution in position three of the aromatic ring with bromine and methoxyl group seems necessary for this type of pharmacologic activity. In fact, this compound presented a better action in preventing IL-1β harmful effects on cartilage, reducing NO production and GAGs release level, and inhibiting the PGE_2 production. According to our results obtained in the model of pig cartilage culture, this compound may provide a new tool for protecting cartilage from degradative factors and may be identified as a potential candidate for the treatment of inflammatory diseases.

Table 1
Structure of tested compounds



3. Experimental

3.1. Synthesis of tested compounds

Thiazolyl and benzothiazolyl Schiff bases (**a**, **b**, **b_1**, **b_2**) (Table 1) were synthesized as described by Geronikaki et al. [1].

The compounds were dissolved in dimethylsulphoxide 10%, diluted in culture medium and used at a concentration of 1, 10 and 100 μg/ml.

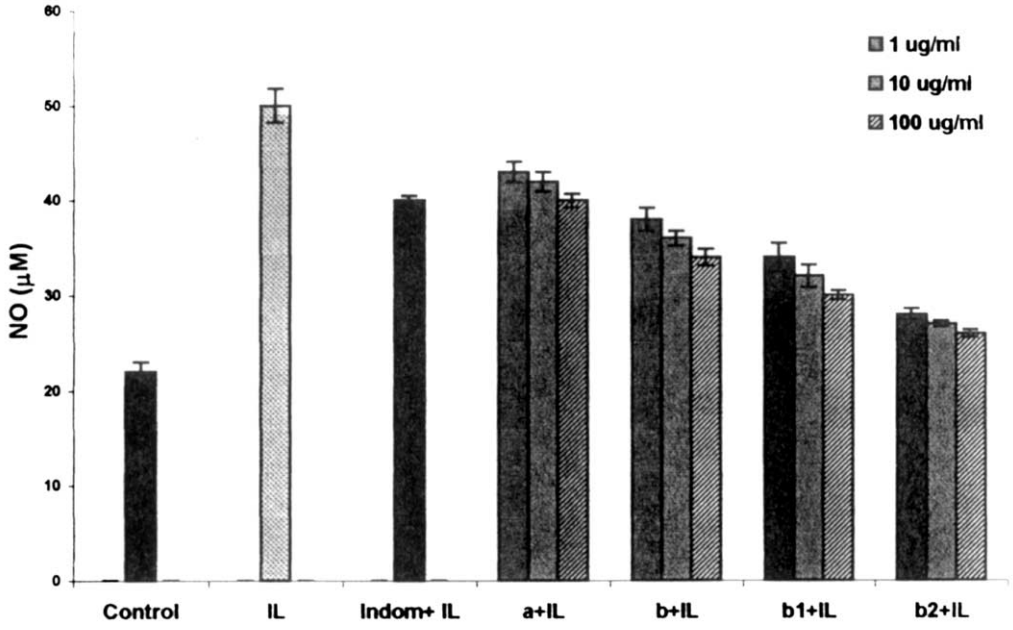


Fig. 1. NO production (means±SEM) from porcine nasal cartilage into the culture medium 120 h after the addition of Schiff bases derivatives (100 μg/ml) or indomethacin with IL-1β. Values are expressed as μM.

3.2. Preparation of cartilage

Nasal pig cartilage was obtained from a local abattoir and washed in Hank's balance salt solution containing antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin). Cartilage was sliced in disks (3–4 mm in diameter) and weighted. The samples were placed into 24 well plates containing 1 ml phenol red free Dulbecco's modified Eagle's medium (Sigma, Italy), glutamine (10 mM), penicillin/streptomycin (50 U/ml and 50 μg/ml, respectively) and 10% heat inactivated fetal calf serum. Cartilage pieces were incubated at 37° C in a humidified 5% CO_2/95% air incubator. After 24 h the medium was removed and cartilage samples were treated for 120 h as follows: (a) control medium; (b) IL-1β 10 ng/ml; (c) 10^{-5} M indomethacin; (d) 10^{-5} M indomethacin and 10 ng/ml IL-1β; (e) compounds **a**, **b**, $\mathbf{b_1}$, $\mathbf{b_2}$ (1,10 and 100 μg/ml); (f) compounds **a**, **b**, $\mathbf{b_1}$, $\mathbf{b_2}$ and IL-1β.

3.3. Determination of GAGs

GAGs were tested in the culture medium by utilizing a spectrophotometric assay using 1,9-dimethylmethylene blue at a wavelength of 535 nm [19,20]. Shark chondroitin sulphate was used to obtain a standard curve. The values were expressed as μg/ml culture medium.

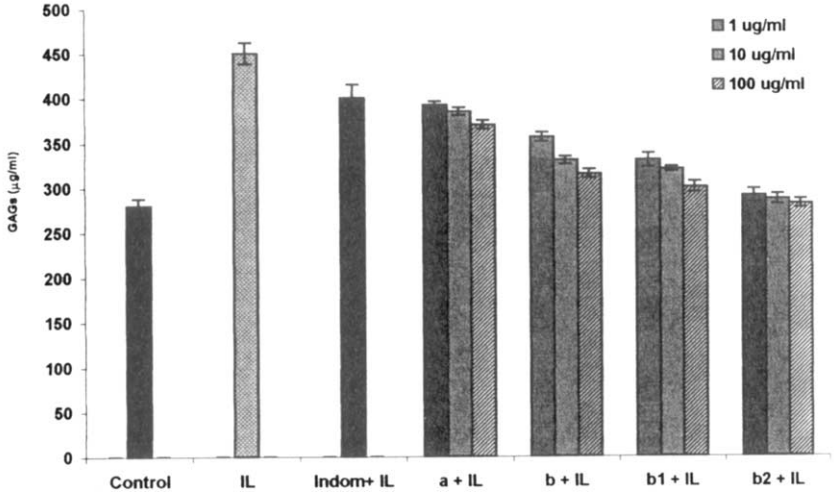


Fig. 2. GAGs release (means±SEM) from porcine nasal cartilage into the culture medium 120 h after the addition of Schiff bases derivatives (100 μg/ml) or indomethacin with IL-1β. Values are expressed as μg/ml.

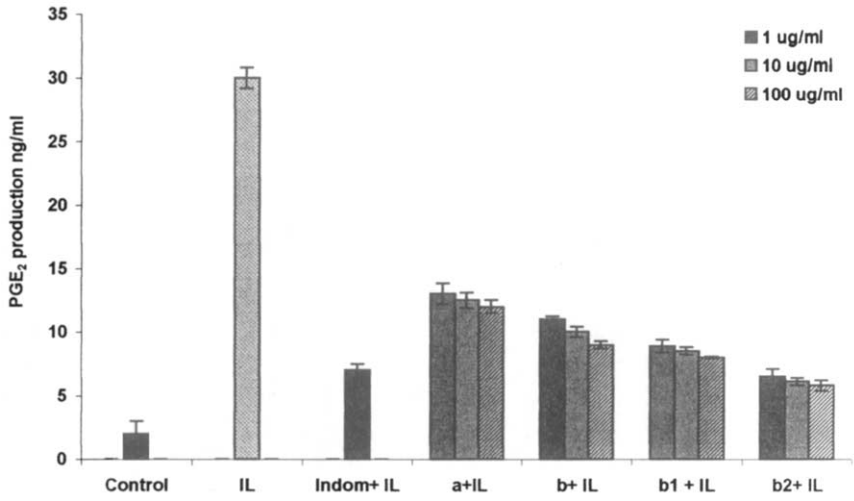


Fig. 3. PGE_2 production (means ± SEM) from porcine nasal cartilage into the culture medium 120 h after the addition of Schiff bases derivatives (100 μg/ml) or indomethacin with IL-1β. Values are expressed as ng/ml.

3.4. Determination of PGE_2

PGE_2 was determined in the culture supernatant using RIA (Sigma, Italy), according to the manufacture's instructions. The detection limit is 1 pg/ml. The values were expressed as ng/ml culture medium.

3.5. Determination of NO

NO production was determined in the tissue culture media by the nitrite release direct measurement using Griess reaction [21]. The assay is based on the addition of equal volumes of sulphanilamide (1% w/v) ethylendiamine (1% w/v) and culture media into 96-well plates. The absorbance was measured at λ 570 nm and finally the nitrite concentration determined from a sodium nitrite standard curve (0–120 μM). The values were expressed as μM nitrite released into culture medium.

3.6. Statistical analysis

All the presented results are means ± SEM of three experiments performed on quadruplicate samples. All the statistical analyses were performed using the statistical software package SYSTAT. A difference was considered significant at $P < 0.05$.

References

[1] A. Geronikaki, V. Poroikov, D. Hadjipavlou-Litina, D. Filimonov, A. Lagunin, R. Mgonzo, Computer aided predicting the biological activity spectra and experimental testing of new thiazole derivatives, Quant. Struct.-Act. Relat. 18 (1999) 16–25.
[2] D.J. Hadjipiavlou-Litina, A.A. Geronikaki, Thiazolyl and benzothiazolyl Schiff bases as novel possible lypooxygenase inhibitors and anti inflammatory agents. Synthesis and biological evaluation, Drug Des. Discov. 15 (1998) 199–206.
[3] M.A. Pratta, N.R. Ackerman, E.C. Arner, Effect of Ebselen on IL-1 induced alteration in cartilage metabolism, Inflamm. Res. 47 (1998) 115–121.
[4] M.D. Smith, A. Parker, P.J. Robert Thomson, M.J. Ahern, M. Coleman, Production of IL-1 and IL-1RA in different stages of arthritis, Rev. Prat. 48 (1998) S16–S23.
[5] M.R. Griffin, J.M. Piper, J.R. Daugherty, M. Snowden, W.A. Ray, Non-steroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons, Ann. Intern. Med. 114 (1991) 257–263.
[6] F.M. Desa, C.L. Chander, D.W. Howat, A.R. Moore, D.A. Willoughby, Indomethacin and cartilage breakdown, J. Pharm. Pharmacol. 40 (1988) 667.
[7] M.J. Shield, Anti-inflammatory drugs and their effects on cartilage synthesis and renal function, Eur. J. Rheumatol. Inflamm. 13 (1993) 7–16.
[8] E.V. Hess, J.H. Herman, Cartilage metabolism and anti-inflammatory drugs in osteoarthritis, Am. J. Med. 81 (1986) 36–43.
[9] M.J. Palmoski, K.D. Brandt, Relationship between matrix proteoglycans content and the effects of salicylate and Indomethacin on articular cartilage, Arthritis Rheum. 26 (1983) 528–531.
[10] C. Cipolletta, J.Y. Jouzeau, P.G. Pottie, N. Presle, K. Bordji, P. Netter, B. Terlain, Modulation of IL-1-induced cartilage injury by NO synthase inhibitors: a comparative study with rat chondrocytes and cartilage entities, Br. J. Pharmacol. 124 (1998) 1719–1727.
[11] M. Lotz, The role of nitric oxide in articular cartilage damage, Rheum. Dis. Clin. North Am. 25 (1999) 269–280.
[12] D. Taskiran, M. Stefanovic Racic, H. Georgescu, C.H. Evans, Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1, Biochem. Biophys. Res. Commun. 200 (1994) 142–148.
[13] D. Jang, G.A.C. Murrell, Nitric oxide in arthritis, Free Radic. Biol. Med. 9 (1998) 1511–1519.
[14] T. Cawston, Matrix metalloproteinases and TIMPs: properties and implications for the rheumatic diseases, Mol. Med. Today (1998) 130–137 (March).
[15] K.M. Bottonley, W.H. Jhonson, D.S. Walter, Matrix metalloproteinase inhibitors in arthritis, J. Enzyme Inhib. 13 (1998) 79–101.

[16] P. Needleman, P.T. Manning, Interactions between the inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) pathways: implications for therapeutic intervention in osteoarthritis, Osteoarthritis Cartilage 7 (1999) 367–370.
[17] E.C. Arber, M.A. Pratta, Independent effects of interleukin-1 on proteoglycan breakdown, proteoglycan synthesis, and prostaglandin E2 release from cartilage in organ culture, Arthritis Rheum. 32 (1989) 288–297.
[18] J.R. Hubbard, J.J. Steinberg, M.S. Bednar, C.B. Sledge, Effect of purified human interleukin-1 on cartilage degradation, J. Orthop. Res. 6 (1988) 180–187.
[19] R.W. Farndale, D.J. Buttle, A.J. Barrett, Improved quantification and discrimination of sulphated glycosamminoglycans by use of dimethylmethylene blue, Biochem. Biophys. Acta 883 (1986) 173–177.
[20] J.E. Stone, N. Akttar, S. Botchway, C.A. Pennock, Interaction of 1,9-dimethylmethylene blue with glycosaminoglycans, Ann. Clin. Biochem. 31 (1994) 147–152.
[21] L.C. Green, D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnock, S.R. Tannenbaum, Analysis of nitrate, nitrite, and [^{15}N] nitrate in biological fluids, Anal. Biochem. 126 (1982) 131–138.