New Quaternary Ammonium Oxicam Derivatives Targeted toward Cartilage: Synthesis, Pharmacokinetic Studies, and Antiinflammatory Potency

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Received July 19, 1999

Analogues of nonsteroidal antiinflammatory drugs (NSAIDs) oxicams, in which the active group was linked to a quaternary ammonium function [(4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-1,1-dioxide-3-carboxamido)2-methylpyridinium iodide or piroxicam-N^+ and [3-(4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-1,1-dioxide-3-carboxamido)propyl]trimethylammonium iodide or propoxicam-N^+] were synthesized. Compounds were labeled with tritium for piroxicam-N^+ and carbon-14 for propoxicam-N^+. Pharmacokinetic studies conducted on rats showed that these molecules were able to highly concentrate in joint cartilages but their bioavailability by the oral way was low. Only propoxicam-N^+ exhibited a sufficient water solubility to be administered intravenously. This molecule was able to restore proteoglycans biosynthesis in cultured articular chondrocytes treated with Interleukin-1β with an efficiency identical to that of indomethacin. These results suggest that the functionalization of oxicam derivatives by a quaternary ammonium group greatly increases their affinity toward articular cartilage without eliminating their pharmacological activity. New drugs synthesized according to this scheme could be useful to obtain a significant decrease of the efficient administered dose and consequently an attenuation of adverse effects such as digestive toxicity.

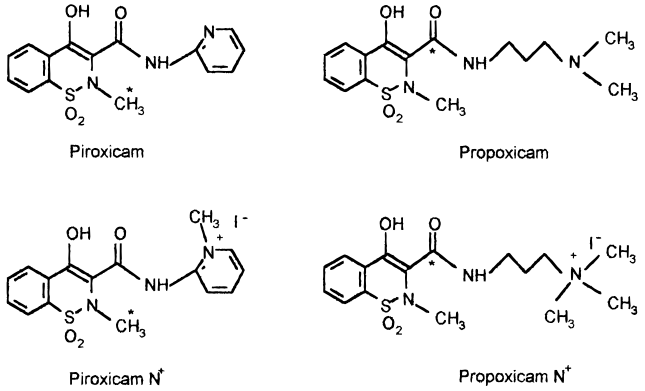
Introduction

A previous pharmacokinetic study performed in our laboratory on acetylcholinesterase-reactivating quaternary ammonium oximes[1−2] showed that these compounds were intensively and precociously concentrated in cartilage after intravenous injection. We have studied the mechanism of binding between quaternary ammonium and cartilage using cultured chondrocytes.[3] These cells are able to synthesize in vitro the cartilage constituents such as collagen and proteoglycans (PrGs) and, thus, to reproduce the in vivo biochemical mechanism. Then we have shown that quaternary ammonium cations are bound to PrGs by ionic interactions with sulfate and carboxyl anions, which corroborates similar observations about other cationic species.[4−6]

This property can be used to target therapeutic agents as nonsteroidal antiinflammatory drugs (NSAIDs) toward cartilage. We have chosen to synthesize new potential antiinflammatory drugs by binding pharmacologically active structures to quaternary ammonium salts. These new molecules could be able to highly concentrate in the cartilaginous tissues and thus allow a significant decrease of the efficient dose and therefore an attenuation of adverse effects such as gastrointestinal toxicity.

In this paper, we describe the synthesis and labeling by tritium or 14-carbon of quaternary ammonium−NSAIDs in which NSAIDs derived from the oxicam structure (piroxicam and propoxicam) were selected to obtain, respectively, piroxicam-N^+ and propoxicam-N^+ (Chart 1).

Chart 1



Using these labeled molecules, pharmacokinetic studies were conducted on rats to check their targeting toward cartilage. Moreover, a preliminary pharmacological test was performed in vitro with the intent of examining if the new quaternary ammonium−NSAIDs compared with clinically used NSAIDs, such as piroxicam and indomethacin, maintained antiinflammatory potency.

Chemistry

Among various known NSAIDs, piroxicam **(5a)** was selected as offering the possibility of [^{3}H]-labeling of the 2-methyl group and quaternarization of the pyridin moiety, leading to compound piroxicam-N^+ **(6a)**. However, it appeared that **6a** could not be injected by intravenous route, due to its very low water solubility. For this reason, the aliphatic compounds propoxicam **(5b)** and propoxicam-N^+ **(6b)** were finally prepared

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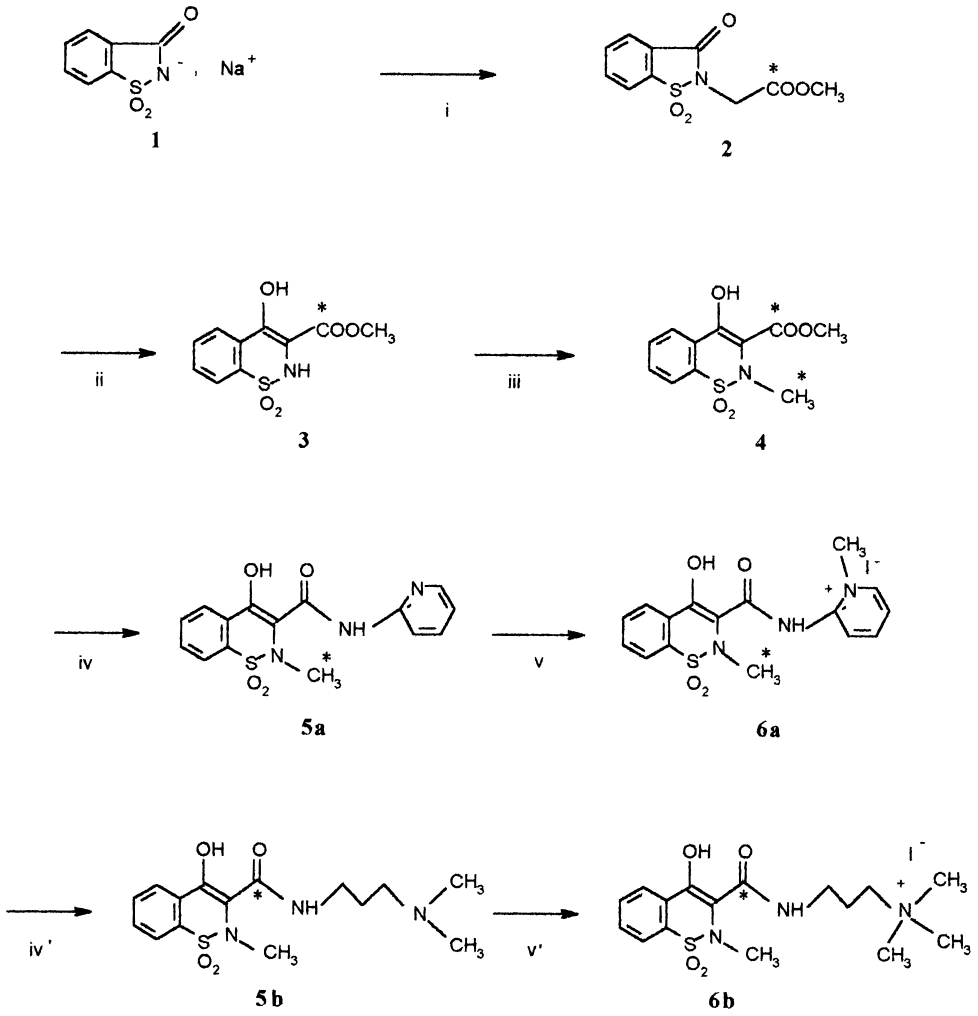
10.1021/jm991120o CCC: $18.00
Published on Web 11/25/1999

Scheme 1[a]



[a] Reagents: (i) $BrCH_2COOCH_3$ or $BrCH_2C^*OOCH_3$, (ii) CH_3ONa, (iii) CH^*_3I or CH_3I, (iv) 2-aminopyridine, (v) CH_3I, (iv′) 3-(dimethylamino)propylamine, (v′) CH_3I, where the asterisk (*) indicates the ^3H- and ^{14}C-labeling positions.

and labeled by 14-carbon on the 3-carbonyl group (Scheme 1).

The synthesis of compounds **5a** and **5b** was performed following the method of Lombardino et al.[7,8] The labeling of **5a** takes place at step iii, using [^{3}H]-CH_3I, whereas 1-[^{14}C]methyl bromoacetate is used at step i in view of obtaining labeled **6a**. Sodium salt of saccharin (**1**) was treated by 1-methyl bromoacetate or 1-[^{14}C]-methyl bromoacetate in mild hydroalcoholic solution to give 3-oxo-1,2-benzoisothiazoline-2-acetic acid methyl ester 1,1-dioxide (**2**). The rearrangement in basic media, sodium methoxide in methyl sulfoxide, of compound **2** was used to produce 4-hydroxy-*2H*-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide (**3**). *N*-methylation of **3** in **4**, using [^{3}H]-methyl iodide or methyl iodide, followed by reaction with either 2-aminopyridine or 3-(dimethylamino)propylamine at 130 °C under argon in xylene provides, respectively, *N*-(2-pyridyl)-2-[^{3}H]methyl-4-hydroxy-*2H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide or piroxicam (**5a**) or *N*-[3-(dimethylamino) propyl]-2-methyl-4-hydroxy-*2H*-1,2-benzothiazine-3-[^{14}C]-carboxamide 1,1-dioxide or propoxicam (**5b**).These compounds were converted into quaternary salts **6a** and **6b** by means of an excess of methyl iodide in acetone at 80 °C under argon with, respectively, 29% and 23% overall yields (obtained from 1-methyl bromoacetate). Their specific radioactivities were, respectively, 49 MBq/mmol for **6a** and 125 MBq/mmol for **6b**.

Biological Studies

Using labeled compounds, tissue distribution was studied in rats after oral administration of a 30 μmol/kg dose of **5a**, **6a**, **5b**, and **6b**, and intravenous administration of a 30 mmol/kg dose of **6b**. Tissue radioactivity was measured by counting of aliquots with a liquid scintillation spectrometer after combustion and direct counting of whole-body slices using a computer-controlled multiwire proportional counter (Ambis 4000).

A pharmacological test was performed using the properties of NSAIDs to restore the PrGs biosynthesis inhibited by interleukin-1β(IL-1β), a proinflammatory agent known to promote neutral proteases and prostaglandin E_2 release and to inhibit collagen and PrGs synthesis in cultured chondrocytes.[9−11] The amount of synthesized PrGs in a culture medium and cell layer was measured by metabolic labeling using [^{35}S]-sulfate.

Results and Discussion

The results of the pharmacokinetic experiments performed with **5a**, **6a**, **5b**, and **6b** are shown in Figures 1−4. After oral administration of a 30 μmol/kg dose of

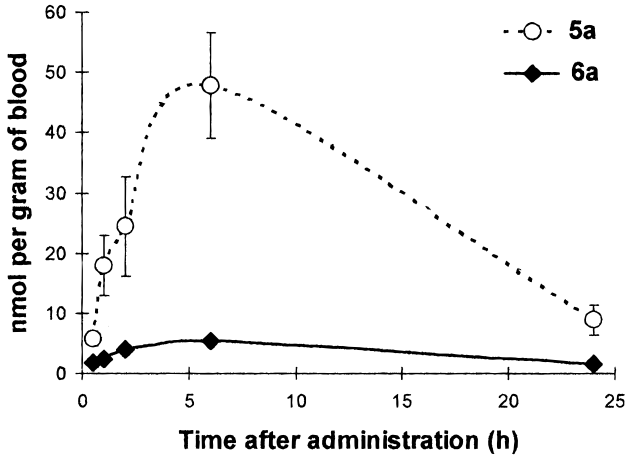


Figure 1. Radioactive concentration in blood after oral administration to rats of a 30 μmol/kg dose of ^{3}H-**5a** (7.8 μCi) and ^{3}H-**6a** (8.0 μCi). Values are the mean of five animals ± SD.

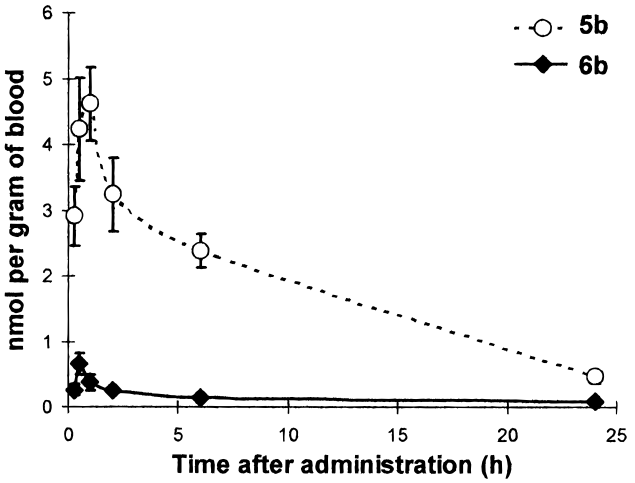


Figure 2. Radioactive concentration in blood after oral administration to rats of a 30 μmol/kg close of ^{14}C-**5b** (6.8 ?Ci) and ^{14}C-**6b** (7.0 ?Ci). Values are the mean of five animals ± SD.

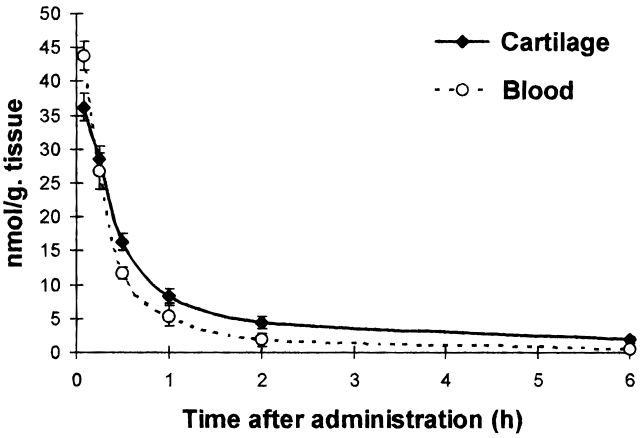


Figure 3. Radioactive concentrations in blood and cartilage after intravenous administration to rats of 30 μmol/kg dose of ^{14}C-**6b** (7.0 μCi). Values are the mean of five animals ± SD.

5a and **6a**, a maximal blood concentration of 47.8 ± 8.7 nmol/g for **5a** and 5.5 ± 1.5 nmol/g for **6a** was obtained 6 h after dosing (Figure 1). On the other hand, the ratio of the cartilage radioactive concentration/blood radioactive concentration was significantly higher for the quaternarized molecule **6a** (Figure 4A). These results clearly indicate that on the one hand only a small part of the administered drug is absorbed through the gastrointestinal tract and on the other hand the quaternarization of the pyridinium group greatly increases the affinity of the drug for cartilage. Similar results were obtained with propoxicam **5b** and its quaternarized analogue **6b** (Figures 2 and 4B). When **6b** was administered through the intravenous route, it was highly concentrated in cartilage 5 min after injection but was rapidly eliminated from this tissue, the radioactive concentration decreasing from 44.2 ± 3.5 nmol/(g of tissue) to 7.5 ± 1.2 nmol/(g of tissue) between 5 min and 1 h after administration (Figure 3). However, the ratio of the cartilage concentration/blood concentration was >1 for all times following administration, which indicates a high affinity of this molecule for cartilage. Figure 5 shows the image of a whole-body slice of a rat given a 30 μmol/kg of **6b**, obtained 15 min after intravenous administration, using the proportional counter Ambis 4000. This result confirms the findings obtained by radioactivity measurements of tissue aliquots proves that this compound is highly concentrated in the articular cartilages and in the kidney and that its concentration in other tissues, including the liver, was low. Quantitative analysis of the radioactivity, plotted in Figure 6, shows that maximal cartilage concentration is obtained between 5 and 15 min, followed by a fast decrease, 75% of the radioactivity being eliminated between 15 min and 1 h.

The results of the in vitro pharmacological test performed with **5a**, **5b**, **6a**, and **6b** are plotted in Figure 7. Treatment of cultured articular chondrocytes with IL-1β mimics inflammatory conditions in joints. The main effect of this cytokine is an inhibition of PrGs biosynthesis, and NSAIDs are known to restore this biosynthesis.[9,10] After treatment with IL-1β, PrGs biosynthesis was strongly inhibited in the cell layer (Figure 7A, b) and secretion in the culture medium was also diminished (Figure 7B, b). After treatment with different molecules, only **6b** and Indomethacin were able to partially restore PrGs biosynthesis, especially for those secreted in the culture medium (Figure 7B, f, g). These results show that the quaternarized propoxicam **6b** is able to partially restore PrGs biosynthesis inhibited by IL1-β as efficiently as indomethacin. On the other hand, no significant difference was observed between piroxicam **5a** and its quaternarized analogue **6a**.

In conclusion, this study proves that the introduction of a quaternary ammonium function on oxicam structures greatly increases their affinity for the cartilaginous tissues and confirms previous findings obtained on other quaternary ammoniums[1−3] but decreases their bioavailability by the oral route. Only the quaternarized propoxicam **6b** is water-soluble enough to be administered by the intravenous route, contrary to the quaternarized piroxicam **6a**. This striking difference between the two functionalized NSAIDs, **6a** and **6b**, could be explained by the formation of an internal salt between the basic quaternary ammonium function and the acidic function of the oxicam cycle. This formation is possible on the piroxicam-N^+ molecule (**6a**) (cycle with 6 C) but not on the propoxicam-N^+ molecule (**6b**) (cycle with 9

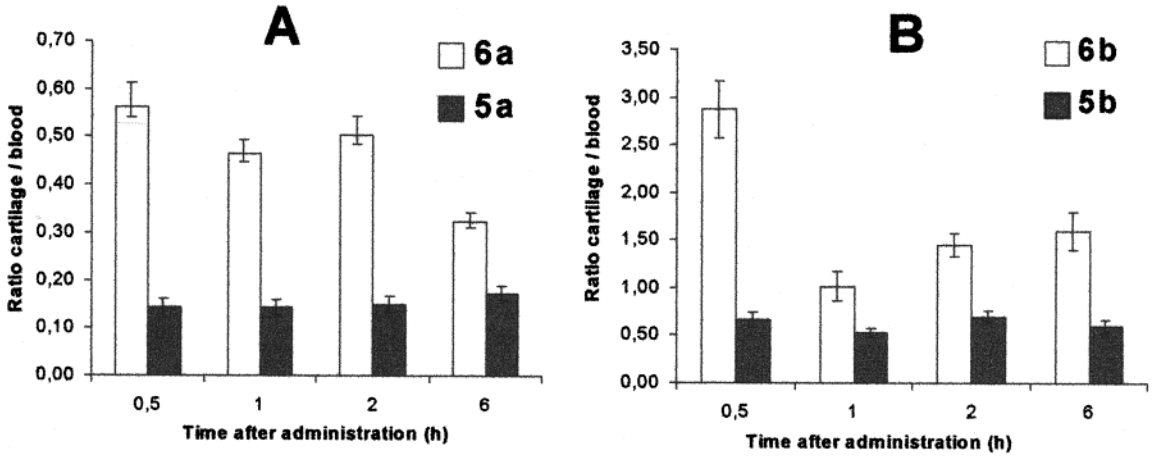


Figure 4. Ratiocartilage radioactivity/blood radioactivity after oral administration to rats of a 30 μmol/kg dose of ^{3}H-**5a** (7.8 μci) and ^{3}H-**6a** (8.0 μCi) (A) and a 30 μmol/kg dose of ^{14}C-**5b** (6.8 μCi) and ^{14}C-**6b** (7.0 μCi) (B). Values are the mean of five animals ± SD.

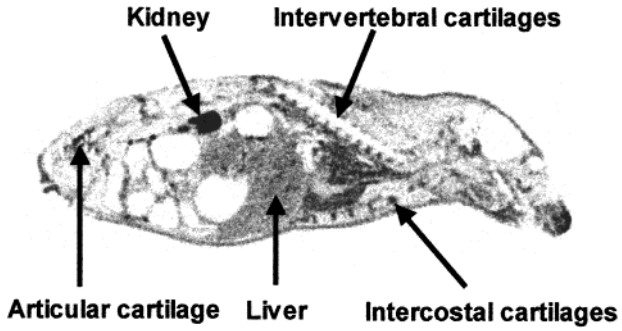


Figure 5. Two-dimensional unhenhanced radioisotopic image obtained with AMBIS 4000 multiwire proportional counter of a 40 μm whole-body slice of a rat 15 min after intravenous administration of a 30 μmol/kg dose of ^{14}C-**6b** (20 μCi).

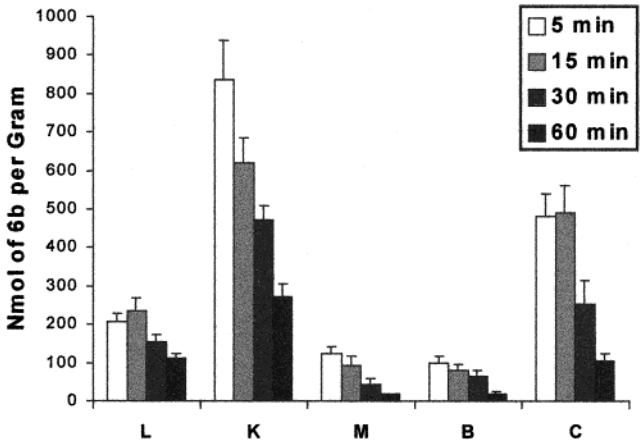


Figure 6. Biodistribution profile after intravenous injection of a 30 μmol/kg dose of ^{14}C-**6b** (20 ?Ci) to rats. L = liver, K = kidney, M = muscle, B = bone, C = cartilage. Tissue radioactivity was measured by AMBIS 4000 counting. Values are the mean of five 40 μm whole-body slices ± SD.

C). Results of the pharmacological test show that the quaternarization of propoxicam (**6b)** increases its efficiency compared to its nonquaternarized analogue (**5b**). On the other hand, this effect was not observed for piroxicam (**5a)** and its quaternarized derivative (**6a**), probably because of the neutralization of the quaternary ammonium by formation of an internal salt. The results obtained with propoxicam (**5b**) and its derivative (**6b**) demonstrate that the presence of a quaternary ammonium function does not abolish the pharmacological properties of the oxicam group. Indeed, the acidic pK_a value necessary for the pharmacological activity is not significantly modified by the functionalization. As it was demonstrated for other water-soluble quaternary ammonium salts, it is possible for these molecules to enter cartilage, via synovial fluid or plasma, to bind to proteoglycans and to counteract the effects of interleukin in inflammation.

This study suggests that a quaternary ammonium group linked to an NSAID can help to increase the drug concentration in joints and could permit a decrease in the administered dose to diminish the secondary effects of these drugs, such as digestive toxicity. Pharmacological studies on arthropathy models in animals are actually in progress with a purpose of determining the increase of the therapeutic efficiency as compared with known NSAIDs.

Experimental Section

Chemistry. *General Comments.* Melting points (mp), uncorrected, were determined in open capillaries with an electrothermal digital apparatus. Analytical thin-layer chromatography (TLC) was conducted on precoated silica gel plates (Merck 60F-254, 0.2 mm thick) with detection by both UV at 254 nm and visualization by iodine using the following eluents: A, dichloromethane/ethyl alcohol (98/2); B, dichloromethane/methyl alcohol/NH_4OH 2 N (50/48/2). The radioactivity of the compounds was assessed using a Packard 4550 Scintillation counter. Radiochemical purities were measured using the AMBIS 4000 detector (Braun Sciencetec France). Infrared absorption spectra were recorded using a Brüker Vector 22 spectrometer. Proton−carbon correlations were obtained from 2D-NMR Spectra which were performed on a Brüker AM 200 (4.5 T) spectrometer; chemical shifts (δ) are reported in parts per million relative to the tetramethylsilane internal standard. Electrospray ionization mass spectra (ESI-MS) were performed by CNRS, Service Central d'Analyses, Vernaison (France). Elemental analyses for carbon, hydrogen, and nitrogen were within ±0.4% of the theoretical values unless otherwise indicated; pK_a values were measured in H_2O with 0.5% DMSO. [^{3}H]-CH_3I was purchased from Amersham (370 MBq, code TRK 706). The radioactive precursor 1-[^{14}C]-methyl bromoacetate was prepared in the laboratory from Na-[^{14}C]N (CMM61- CEA, 3700 MBq) with a specific radioactivity of 125 MBq/mmol according to the published methods of Cox et al.[15,16] and Saunders et al.[17,18]

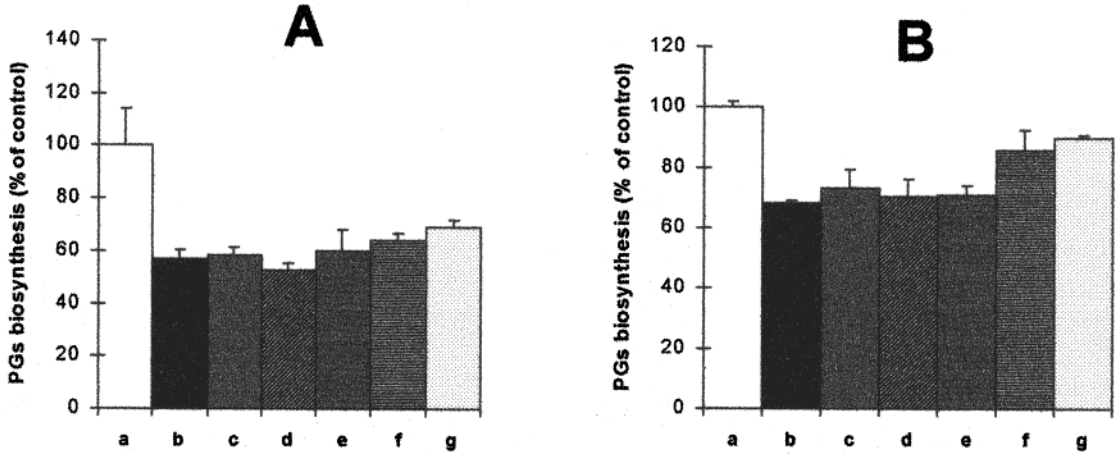


Figure 7. Proteoglycans synthesis by cultured chondrocytes measured in the cell layer (A) and culture medium (B) after simultaneous treatment with IL-1β and NSAIDs.

2-Methyl-4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylic Acid Methyl Ester 1,1-Dioxide (4). Steps i, ii, and iii were performed according to Lombardino et al.[7,8] with, respectively, 86%, 68%, and 85% yields. To prepare the tritiated compound **4**, 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide, **3** (2 g; 7.84 mmol), is reacted with [^{3}H]-CH_3I (370 MBq; 3.15 TBq/mmol) introduced through a vacuum ramp and then diluted with CH_3I (0.35 mL; 5.6 mmol). After 24 h of agitation at room temperature, excess CH_3I (1.5 mL; 24 mmol) is added and the mixture is agitated again, leading to 1.80 g (85%) of the tritiated title compound **4**; specific radioactivity 49 MBq/mmol; mp 160−162 °C; R_f 0.7 (A); ^{1}H NMR ($CDCl_3$) δ 2.95 (3H, s, NCH_3), 3.96 (3H, s, OCH_3), 7.71−8.05 (4H, m, C_6H_4), 12.05 (1H, s, OH).

***N*-(2-Pyridyl)-2-[^{3}H]-methyl-4-hydroxy-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide or [^{3}H]-Piroxicam (5a).** According to the general procedure,[7,8] the [^{3}H]-labeled compound **4** (1.76 g; 6.54 mmol) was reacted with 2-aminopyridine (0.75 g; 8 mmol) to yield 1.65 g (74%) of [^{3}H]-piroxicam **(5a)**; specific radioactivity 49 MBq/mmol.; mp 200−202 °C (dec); R_f 0.90 (B); ^{1}H NMR ($CDCl_3$) δ 2.96 (3H, s, N−CH_3), 7.73−7.95 (4H, m, Ph), 7.15, 8.06, 8.28, 8.37 (4 × 1H, mmdd, Pyr), 9.06 (1H, s, NH), 13.30 (1H, s, OH); ^{13}C NMR ($CDCl_3$) δ 39.9 (N−CH_3), 111.5 (=*C*−CO), 114.3, 120.6, 126.6, 148.1, 150.1 (Pyr), 124.8, 132.5, 133.0, 134.6, 138.4 (Ph), 159.9 (=COH), 166.9 (C=O); pK_a 5.9. Anal. ($C_{15}H_{13}N_3O_4S$) C, H, N.

(2-[^{3}H]-Methyl-4-hydroxy-2*H*-1,2-benzothiazine-1,1-dioxide-3-carboxamido)-2-methylpyridinium Iodide or [^{3}H]-Piroxicam-N$^+$ (6a). In a 50 mL tight-stopped flask, **5a** (1 g; 3.02 mmol), excess methyl iodide (3 mL) and acetone (30 mL) were heated at 80 °C for 24 h. After the mixture was cooled, the pale yellow precipitate **6a** was filtered, washed with acetone, and dried under vacuum. Yield 1.15 g (80%); mp 202−203 °C; R_f 0.69 (B); specific radioactivity 49 MBq/mmol; IR (cm^{-1}) 3425, 3370, 3006, 1660, 1350, 1172; ^{1}H NMR (DMSO-d_6) δ 2.79 (3H, s, N−CH_3), 4.07 (3H, s, $^+$N−CH_3), 5.50 (3H, s, OH + NH + 0.5 H_2O), 7.69−8.06 (4H, m, Ph), 7.28, 8.19, 8.53, 8.91 (4 × 1H, ttdd, Pyr);^{13}C NMR (DMSO- d_6) δ 38.6 (N−CH_3), 43.7 ($^+$N−CH_3), 111.1 (=*C*−CO), 118.9, 120.1, 143.6, 144.3, 149.7 (Pyr), 123.2, 126.7, 131.8, 132.6, 135.0, 140.3 (Ph), 159.0 (=COH), 163.1 (C=O); ESI-MS *m*/*z* 346.2 (M$^+$); pK_a 5.5. Anal. ($C_{16}H_{16}N_3O_4SI$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-2-methyl-4-hydroxy-2*H*-1,2-benzothiazine-3-[^{14}C]carboxamide 1,1-Dioxide or Propoxicam (5b).** The [^{14}C]-labeled compound **4** (1.72 g, 6.4 mmol) was refluxed for 24 h under argon in the presence of 0.65 g (6.4 mmol) of 3-(dimethylamino)propylamine in 125 mL of anhydrous xylene. After removal of the solvent, the solid residue was washed with ethanol to give **5b** (1.23 g; 57%) as a white powder; mp 199−200 °C (dec); R_f 0.25 (B); specific radioactivity 125 MBq/mmol;^{1}H NMR (DMSO-d_6) δ 1.6−1.75 (2H, m, CH_2−CH_2−CH_2), 2.35 (6H, s, N−$(CH_3)_2$), 2.50 (2H, t, CH_2N), 2.75 (3H, s, N−CH_3), 3.35 (2H, m, NH−CH_2), 7.6−8.1 (4H, m, Ph), 8.50 (1H, t, NH), 13.2 (1H, s, OH);^{13}C NMR (DMSO-d_6) δ 26.0 (CH_2−*C*H_2−CH_2), 35.9 (NH−CH_2), 43.6 (N−CH_3), 52.2 (CH_2−N), 55.6 (N−$(CH_3)_2$), 109.5 (=*C*−CO), 120.3, 126.3, 129.0, 130.7, 132.4, 134.6 (Ph), 160.5 (−C−OH), 168.3 (C=O); pK_a 6.4. Anal. ($C_{15}H_{21}N_3O_4S$) C, H, N.

[3-(2-Methyl-4-hydroxy-2*H*-1,2-benzothiazine-1,1-dioxide-3-[^{14}C]carboxamido)propyl]trimethylammonium Iodide or Propoxicam-N$^+$ (6b). The conversion of 1.15 g (3.39 mmol) of **5b** to **6b** was achieved as described for **6a**, giving 1.74 g (80%) of a yellow powder; mp 220−222 °C (dec); R_f 0.10 (B); specific radioactivity 125 MBq/mmol; IR (cm^{-1}) 3274, 1629, 1179, 1335; ^{1}H NMR (DMSO-d_6) δ 1.98−2.09 (2H, m, CH_2−CH_2−CH_2), 2.82 (3H, s, N−CH_3), 3.12 (9H, s, $^+$N$(CH_3)_3$), 3.35−3.43 (4H, m, NH−CH_2 + $^+$N−CH_2), 7.88−8.02 (4H, m, Ph), 8.82 (1H, t, NH), 14.30 (1H, s, OH); ^{13}C NMR (DMSO-d_6) δ 22.6 (CH_2−*C*H_2−CH_2), 35.9 (NH−CH_2), 38.7 (N−CH_3), 52.2 ($^+$N−$(CH_3)_3$), 63.3 (CH_2−N$^+$), 111.15 (=*C*−CO), 124.0, 125.9, 127.9, 132.6, 133.3, 133.9 (Ph), 156.0 (=C−OH), 168.2 (C=O); solubility 10 g/L (H_2O) at 20 °C, 20 g/L (H_2O−DMSO 5%) at 20 °C; ESI-MS *m*/*z* 354.3 (M$^+$); pK_a 5.85; Anal. ($C_{16}H_{24}N_3O_4$-SI·0.5H_2O) C, H, N.

Pharmacology. *Materials*. Collagenase was obtained from Worthington. Dulbecco's modified Eagle's medium (DMEM) and glutamine were purchased from Gibco (Uxbridge, U.K.). Foetal calf serum (FCS) was from Seromed. Protease inhibitors, Interleukin-1β (IL-1β) and Indomethacin, were purchased from Sigma Chemical Co. (Poole, U.K.) and guanidinium hydrochloride (GuHCl) from Merck (Darmstadt, Germany). [^{35}S]-Sodium sulfate (carrier-free) in aqueous solution (39 GBq/mmol) was obtained from Amersham (U.K.). Sprague−Dawley rats weighing 100−120 g were purchased from Iffa-Credo (L'Arbresle, France).

In Vitro Experiments. (*1) Cell Cultures*. Articular chondrocytes were obtained from the knees of a 1 month-old rabbit (Fauve de Bourgogne, Elevage Scientifique des Dombes-France) by enzymatic digestion overnight at 37 °C, with 0.2% collagenase.[12] Cells were cultured in DMEM supplemented with 10% FCS, 2 mM glutamine, 8 μg/mL gentamycin at 37 °C in a 5% CO_2 atmosphere. Experiments were performed on primary confluent culture to avoid differentiation of the chondrocytes.

(2) Pharmacological Test. Chondrocytes were cultured in 9.6 cm^2 Petri dishes, and used at confluence.The pharmacological experiment was performed in incubating cells with IL-1β(200 pg/dish) in the presence or absence of the drugs dissolved in dimethyl sulfoxide (DMSO) (final concentration in the medium 0.125% v/v) at 10^{-4} M in DMEM with 10% FCS.[13] The products and medium were changed every second day. At the fifth day, ^{35}S-sulfate (5 μCi/dish) was added for 24 h to label the newly synthesized PGs. The amounts of labeled PGs were measured in the cell layer and cell culture supernatant as

described in a previous paper.[3] Briefly, the cell layer was extracted overnight at 4 °C by 4 M GuHCl in buffer B (0.05 M sodium acetate, pH 5.8 containing a cocktail of protease inhibitors). The samples were dissolved in soluene and their radioactivity measured by liquid scintillation counting. The supernatant was dialyzed for 48 h at 4 °C against buffer A (0.5 M sodium acetate, pH 6.8 containing a cocktail of protease inhibitors), and the amount of labeled PGs was measured as above. Controls were performed by incubating cells with 0.125% DMSO for 6 or 8 days in the same conditions. We have verified that this low DMSO concentration did not alter cell morphology and metabolism.

In Vivo Experiments. *(1) Pharmacokinetic Studies.* Groups of five rats were orally given a 30 μmol/kg dose of each labeled compound in a solution containing 10% DMSO and 1% carboxymethyl cellulose. Only **6b** exhibited sufficient water solubility to be administered intravenously in a solution of 5% H_2O/DMSO. The radioactivity per rat was 7.8 μCi for **5a**, 8.0 μCi for **6a**, 6.8 μCi for **5b**, and 7.0 μCi for **6b**. Five animals per time were sacrificed by ether inhalation at 5, 15, and 30 min and 1, 6, and 24 h post administration of the drug. Blood was collected by cardiac puncture, and aliquots of liver, kidney, muscle, bone, and cartilage were taken. The radioactivity was measured with a Wallac Win-Spectral 1414 liquid scintillator after combustion in a Packard 306 sample oxidizer. In other experiments, rats were administered intravenously a 30 μmol/kg dose of **6b** (20 μCi/rat). At several times following administration, animals were sacrificed by ether inhalation, immediatly frozen in liquid nitrogen, and sliced (40 μm) using a cryomicrotome. The tissue radioactive concentration was determined with a computer-controlled multiwire proportional counter (Ambis 4000). The number of particles per time and surface unit obtained with the gaseous detector is a linear function of the radioisotope concentration. Counts are recorded in 1 064 448 discrete detection points from which the composite image is displayed on a high-resolution color monitor so that regions of interest may be directly quantitated.[14]

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