Abstract: Pro-drugs of all non-steroidal anti-inflammatory drugs with free acid function derivatized with an ester group, which have the general structural formula given below (I) where A is aspirin, diflunisal, benorylate, ibufenac, diclofenac, indomethacin, sulindac, ketorolac, ibuprofen, naproxen, ketoprofen, fenoprofen, flurbiprofen, mefenamic acid, meclofenamic acid, flufenamic acid, and where in the ester group R can be a sugar (amongst which aldose, or ketose pentose, or eose selected from a group of D- and L-enantiomers of ribose, glucose, galactose, mannose, arabinose, xilose, allose, altrose, gulose, idose and talose and substituted derivatives thereof, such as glucosamine, galactosamine, iV-acetyl glucosamine, iV-acetyl galactosamine, N-acetyl ribosamine), a disaccharide, a trisaccharide or an oligosaccharide.
Published: without international search report and to be re-published upon receipt of that report (Rule 48.2(g))
GALACTOSYLATED PRO-DRUGS OF NON-STERoidal ANTI-INFLAMMATORYS WITH IMPROVED PHARMACOKINETIC CHARACTERISTICS AND REDUCED TOXICITY OF THE STARTING DRUG

Description

The present invention relates to galactosylated pro-drugs of non-steroidal anti-inflammatories with free acid function and the process of synthesis thereof, which enables minimization of the gastrointestinal toxicity of the starting anti-inflammatory by means of esterification of its carboxylic group.

Forming part of non-steroidal anti-inflammatory drugs with free acid function are: aspirin, aceclofenac, acemetacin, diflunisal, benorylate, ibufenac, diclofenac, indomethacin, sulindac, ketorolac, ibuprofen, naproxen, ketoprofen, fenoprofen, flurbiprofen, mefenamic acid, meclofenamic acid, flufenamic acid, niflumic acid.

Forming a further subject of the invention are pro-drugs of non-steroidal anti-inflammatories with free acid function derivatized with an ester group that have the general structural formula given below:

\[
\text{A} \quad \text{OR}
\]

where A is: aceclofenac, acemetacin, aspirin, dexketoprofen, diflunisal, etodolac, benorylate,
ibufenac, diclofenac, indomethacin, fenbufene, sulindac, ketorolac, ibuprofen, naproxen, ketoprofen, fenoprofen, flurbiprofen, mefenamic acid, meclofenamic acid, flufenamic acid, niflumic acid, tiaprofenic acid;

and

where in the ester group R can be a sugar (amongst which aldose, or ketose pentose, or esose, selected from a group of D- and L-enantiomers of ribose, glucose, galactose, mannose, arabinose, xilose, allose, altrose, gulose, idose and talose and substituted derivatives thereof, such as glucosamine, galactosamine, N-acetyl glucosamine, N-acetyl galactosamine, N-acetyl ribosamine), a disaccharide, a trisaccharide, or an oligosaccharide:

- where by disaccharide is meant a polymeric assemblage of two sugars to constitute both homopolymers (maltose and cellobiose) and heteropolymers (lactose and sucrose);

- where by trisaccharide is meant a polymeric assemblage of three sugars;

- where by oligosaccharides are meant polymers constituted by 4 to 10 residues; the polymer can be either homosaccharidic (the same sugar, that repeats) or heterosaccharidic (various sugars); and where the sugars are each bound to one another via a glycoside bond between Cl and C4 or alternatively between Cl and C3 or else between Cl and Cβ.

The ensuing description refers to some examples of embodiment for which both synthesis and clinical and pharmacological tests were carried out in order to
evaluate the anti-inflammatory, analgesic, and ulcerogenic activities of the pro-drug.

In particular, attention has been paid to the galactosylated derivative of ketorolac (hereinafter referred to as Ketogal):

![Chemical Structure](image.png)

(SII)

Said derivative is a pro-drug designed to overcome the obstacles, of a pharmacokinetic and pharmacological type, which render clinical use of ketorolac difficult, such as low oral availability, chemical instability, and toxicity at a gastrointestinal level.

As is known, pro-drugs are inert derivatives constituted by an active principle, bound chemically and in a reversible way to which is a vehicle group. In the embodiment according to the present invention that will be provided hereinafter, the drug is represented by ketorolac, the general structural formula of which is:

![Chemical Structure](image.png)

and the vehicle is represented by a galactose molecule.
Ketogal-is synthetized by esterifying the carboxyl group of ketorolac with the hydroxyl in position 6' of galactose.

According to the invention, the compound is synthetized by means of a reaction of esterification between ketorolac and 1,2,3,4-di-O-isopropylidene-\(\alpha\)-galactopyranose using JV-ethyl-3\(\cdot\)1\(\cdot\)7\(\cdot\)r-(3-dimethyl aminopropyl) carbodiimide (EDC) hydrochloride as condensing agent, and 4-(dimethyl amino) pyridine (DMAP) as catalyst, in anhydrous dichlororomethane:

The protected conjugate, Ia, is thus obtained with a yield of 75%. The ketals are completely removed by means of trifluoroacetic acid (TFA) in anhydrous dichloromethane so as to obtain Ketogal, with a yield of 65%.

The chemical stability of the pro-drug was measured both at pH 1 and pH 7.4 so as to verify its degree of manageability and the possibility of oral administration. The enzymatic stability was also measured, in plasma, with the purpose of ascertaining the reactivity of the pro-drug in an environment as
close as possible to the biological one (FIGURE 1).

From studies on gastrolesivity and pharmacokinetics, Ketogal has shown that it does not cause ulcers and has a better oral availability than ketorolac. Finally, studies of pharmacological activity have shown that Ketogal (0.163 mg/kg) has a better analgesic and anti-inflammatory activity than ketorolac (0.1 mg/kg), both after oral administration and after subcutaneous administration.

Further characteristics and advantages of the invention will emerge clearly from the ensuing detailed description and from the corresponding experimentation of some embodiments, with reference to the annexed plates of drawings, wherein:

Figure 1 shows the plasmatic concentration of ketorolac after administration of Ketogal and ketorolac as such;

Figures 2A and 2B show the concentration of ketorolac in the stomach, liver, and kidneys after administration of ketorolac as such (A) and of Ketogal (B);

Figure 3A shows the analgesic effect on writhing induced by acetic acid;

Figure 3B shows the analgesic effect on writhing induced by magnesium sulphate;

Figures 4A and 4B show the anti-inflammatory effect of the oral administration of ketorolac (A) and Ketogal (B).

CHEMICAL PART

Synthesis of Diacetone 6'-O-ketorolac-D-galactopyranoside (Ia)
1 g of ketorolac 1 (3.9 mmol), 1.015 g of 1,2,3,4-di-O-isopropylidene-D-α-galactopyranose (3.9 mmol), 748 mg of 4-(dimethyl amino) pyridine (DMAP) (0.19 mmol) were dissolved in anhydrous dichloromethane (10 mL). The reaction mixture was kept under electromagnetic stirring at room temperature for 12 hours. The organic phase was extracted several times with water and dehydrated with anhydrous sodium sulphate, filtered, and dried in vacuo. The reaction crude was purified on a chromatography column with silica gel using as eluent CHCl₃, to obtain 1.45 g of Ia as a white solid (yield 75%).

1H-NMR (CDCl₃): δ 1.30, 1.31, 1.40, 1.41 (4s, 12H, ketals); 2.80, 2.90 (m, 2H, 2-H); 3.30 (m, 1H, 1-H); 4.05 (m, IH, 4'-H)/ 4.15 (m, IH, 5'-H); -4.20 (m, 2H, 6'-H); 4.40 (m, IH, 2'-H); 4.50 (m, IH, 3'-H); 4.60 (m, 2H, 3-H); 5.35 (m, IH, 1'-H); 5.90 (d, IH, 7-H); 6.80 (d, IH, 6-H); 7.45 (m, 2H, 2,6-Ph); 7.55 (m, 1H, 4-Ph); 7.85 (m, 2H, 3,5-Ph). 13C-NMR (CDCl₃): δ 20 and 22 (4CH₂-ketals); 30 (C-2); 44 (C-3); 48 (C-I); 65 (C-6'); 66.7 (C-4'); 71 (C-5'); 71.5 (C-2'); 72 (C-3'); 97 (C-1'); 105 (C-7); 109 and 111 (C-ketals); 125 (C-6); 127 (C-5); 129 (3,5-Ph); 130 (2,6-Ph); 133 (4-Ph); 140 (C-8); 142 (1-Ph); 175 (ketonic CO); 185 (esteric CO), m/z: 498 M + H⁺.

**Synthesis of ketorolac-D-galactos-6'-yl ester (Ketogal)**

Added to a solution of Ia (1.45 g; 2.9 mmol) in anhydrous dichloromethane (10 mL) were 2 mL of
trifluoroacetic acid (TFA), and the reaction mixture was kept under electromagnetic stirring at room temperature for 48 hours. By evaporating the solvent, a residue was obtained, which was purified on a chromatography column with silica gel using as eluent CHCl₃ in a gradient of CH₃OH to obtain 800 mg of Ketogal as a white solid (yield 65%). mp: 195 °C.

¹H-NMR (CD₃OD): δ 2.85 (m, 2H, 2-H); 3.50 (m, IH, 1-H); 3.75 (m, IH, 4'-H); 3.85 (m, IH, 5'-H); 4.20 (m, 2H, 6'-H); 4.30 (m, IH, 2'-H); 4.35 (m, IH, 3'-H); 4.50 (m, 2H, 3-H); 5.15 (m, IH, 1'-H); 6.15 (d, IH, 7-H); 6.80 (d, IH, 6-H); 7.45 (m, 2H, 2,6-Ph); 7.55 (m, IH, 4-Ph); 7.75 (m, 2H, 3,5-Ph). ¹³C-NMR (CD₃OD): δ 30 (C-2); 44 (C-3); 48 (C-I); 65.5 (C-6'); 68 (C-4'); 70 (C-5'); 73.1 (C-2'); 74.9 (C-3'); 94 and 99 (C-I'); 105 (C-7); 125 (C-6); 127 (C-5); 129 (3,5-Ph); 130 (2,6-Ph); 133 (4-Ph); 140 (C-8); 142 (1-Ph); 175 (ketonic CO); 185 (esteric CO), m/z: 418 (M + H)⁺.

Stability studies on Ketogal

For the stability study, the solutions of pro-drug were prepared by dissolving an aliquot of Ketogal in a phosphate buffer at pH 7.4, or else in a solution of HCl 0.1 N (pH 1), as regards chemical stability, or else in a plasma specimen, for enzymatic stability. The solutions were kept in the dark, at a temperature of 37°C, for 24 hours. After each hour an aliquot of specimen was taken. In the case of plasma, said aliquot was previously extracted with acetonitrile (1:2), "vortexed" and centrifuged at 3000 r.p.m. for 10 min. The supernatant was taken and used for analysis at HPLC.
with diode detector. The half-lives for chemical stability and enzymatic stability were calculated by quantizing, in time, the percentage of pro-drug that remained in solution. For the chromatographic separations, a 1090L HPLC (Hewlett-Packard, Palo Alto, USA) was used, coupled to a diode detector HP 1040A. A Phenomenex Luna C18 (250 x 4.6 mm, 5 µm) column was used. The wavelength used was 313 nm. The mobile phase was constituted by acetonitrile and an aqueous solution of phosphoric acid 1 mm (pH 3) in the ratio of 68:32. The flow was 1 mL/min, with an injection volume of 20 µl. All the reagents and the solvents used were of analytical degree. The distilled and deionized water was purified by means of a Milli Q system (Millipore).

The retention times of the compounds were: ketorolac: 6.7 min; Ketogal: 3.8 min. As internal standard tolmetin was used (9.3 min). The straight line of calibration for quantification of the compounds was constructed using standard solutions with concentrations of between 0.1 and 100 µg/ml. The linearity was obtained with a coefficient of regression ($R^2$) of approximately 0.998.

From the results obtained, illustrated in Table 1, it is possible to envisage for Ketogal a good stability, which also enables administration thereof by oral route. In this way, ketorolac will not be released fast at a gastric level, causing the classic side effects, and may perform its pharmacological action once the circulatory system has been reached.
Table 1. Chemical stability and enzymatic stability of Ketogal

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>pH 7.4 T&lt;sub&gt;50&lt;/sub&gt; (HOURS)</th>
<th>pH 1 T&lt;sub&gt;50&lt;/sub&gt; (HOURS)</th>
<th>Plasma T&lt;sub&gt;50&lt;/sub&gt; (HOURS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketogal</td>
<td>&gt;4</td>
<td>2.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 1. Chemical stability and enzymatic stability of Ketogal

PHARMACOLOGICAL PART

All the tests were conducted in due respect to the Italian guidelines (D.L. [Order of the Executive] No.116 of January 27, 1992) and of the European Community (Directive of November 24, 1986, 86/609/ECC). All the surgical procedures were approved by the Ministry for Scientific Research and were in compliance with the "International Association for the Study of Pain".

TEST 1 - Ulcerogenicity studies

The gastric lesions were evaluated in male Swiss mice. To the fasted animals (16-18 h) were administered, by oral route, ketorolac (10 mg/kg), Ketogal (16.3 mg/kg), or the vehicle. After 4 hours, the mice were sacrificed and the stomach removed, cut along the major curvature, washed with physiological solution, and the mucous was examined for evaluation of the presence of petechias and/or gastric lesions. A "score" of 1 was assigned to the petechias, and a "score" based on the diameter (a "score" of 5 for lesions of a diameter of less than 1 mm; a "score" of 10 for lesions with a diameter of more than 1 mm) was, instead, assigned to the lesions.
The study of the ulcerogenic activity showed for Ketogal a clear reduction in the gastrolesiye properties, as compared to ketorolac, following upon equimolar administration of the two compounds; in fact Ketogal showed only a slight irritation of the gastric mucosa, with an almost total absence of ulcers (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oral dose (mg/kg)</th>
<th>Number of ulcers</th>
<th>Degree of ulcerogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CMC 0.5%)</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>ketorolac</td>
<td>10.0</td>
<td>4±0.4</td>
<td>10</td>
</tr>
<tr>
<td>Ketogal</td>
<td>16.3</td>
<td>1±0.4</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Ulcerogenicity

TEST 2 - Pharmacokinetic studies

The pharmacokinetic profile of Ketogal and of ketorolac was evaluated in Wistar rats by measuring the concentration of the two compounds in the plasma, in the stomach, in the kidney, and in the liver. The rats received ketorolac (0.100 mg/kg) or Ketogal (0.163 mg/kg) by oral route and were sacrificed after 1 h, 2 h, 4 h, and 6 h from administration. The various specimens were treated with acetonitrile, and the suspension was "vortexed" and centrifuged at 3000 r.p.m. for 10 min. The organic phase was injected in the same HPLC system used for the stability assays. The results are given in Figures 2A and 2B.

From the results obtained it emerged that the introduction of galactose increased the oral availability of ketorolac. In fact, the plasma levels
of the drug, following upon administration of Ketogal, were higher than the ones observed following upon administration of the drug as such (Figure 1). In the plasma, moreover, the pro-drug was not present, which is indicative of the fact that hydrolysis occurs at a gastric level. In the stomach, in fact, (Figures 2A and 2B) it is evident how Ketogal (Figure 2B) hydrolyses progressively, releasing ketorolac, the concentration of which remains roughly constant over time and even higher, after 2 hours, than the one obtained following upon administration of the drug as such. The kidney showed a profile similar to that of the stomach, with the difference that the greater concentration of ketorolac, after administration of Ketogal, was found after three hours, with respect to the administration of the drug as such (Figure 2A). There were, moreover, no traces of the pro-drug.

Instead, in the liver (Figures 2A and 2B), Ketogal reappeared, which is sign of a likely entero-hepatic recycling of the pro-drug, and the concentration of ketorolac was always higher following upon administration of Ketogal (Figure 2B) as compared to that of the drug alone (Figure 2A).

**TEST 3 - Analgesic activity**

The analgesic activity was studied using the "writhing test" using acetic acid and using magnesium sulphate. To the mice there was administered by intraperitoneal route 1 mL of solution at 1% of acetic acid, and the number of writhing events was evaluated for 20 min, after 5 min from administration of the
solution of acetic acid. Writhing is defined as a contraction of the abdominal muscles accompanied by elongation of the body and of the rear legs. Ketorolac and Ketogal were administered by oral route 60 min prior to administration of the solution of acetic acid.

The analgesic effect is expressed in number of writhing events as compared to the control.

Ketorolac (0.01-10 mg/kg, by oral route) caused a dose-dependent analgesic effect. In particular, the effect was significant at the dosages of 1 and 10 mg/kg (p<0.01). Ketogal, (0.0163-16.3 mg/kg, by oral route) showed a dose-dependent analgesic effect of higher than 0.163 mg/kg (Figure 3A).

In order to determine whether ketorolac and Ketogal are able to inhibit visceral pain, the effects of these compounds on "writhing" induced by magnesium sulphate were tested. Magnesium sulphate induces a reversible response when injected by intraperitoneal route in the mouse. The administration of 120 mg/kg of magnesium sulphate induces an average of 6 ± 0.9 episodes of "writhing" in the mouse (n=6). Both ketorolac (0.01 to 10 mg/kg) and Ketogal (in equimolar dose as compared ketorolac) administered 1 hour before the magnesium sulphate are able to inhibit this response in a dose-dependent way (Figure 3B).

TEST 4 - Anti-inflammatory activity

Anti-inflammatory activity was tested by means of the carrageenan oedema test. The mice were divided into groups of six. The initial volumes of the leg of all the animals were measured using a plethysmometer. The
Oedema is induced in the leg by means of sub-plantar injection of 50 µl of saline solution containing 1% of carrageenan. The volume of the leg was measured at different time intervals. Said experimental model consists of two phases: a first, acute, phase lasting 6 hours and a second, chronic, phase, up to 72 hours. Treatment with Ketogal (0.163-16.3 mg/kg oral), 1 h prior to injection of carrageenan, considerably reduces the oedema of the leg in a time-dependent and dose-dependent way.

During the first phase, all the doses tested inhibited formation of oedema, whereas the lowest dose (0.1 mg/kg) of ketorolac had no effect. Finally, the effect of Ketogal continued also in the second phase. (Figure 4A and 4B).

**Further studies and investigations**

In order to broaden the study of the stability and of the possible applications of blocking of the carboxylic function of a starting drug, which seems to be responsible for its gastrolesivity, further examples of synthesis and characterization of other galactosylated derivatives are illustrated.

**Synthesis of Flugal**

The galactosylated derivative of flurbiprofen (Flugal)
is a pro-drug in which the drug is represented by flurbiprofen

and the vehicle by a galactose molecule.

Flugal is synthetized by esterifying the carboxyl group of flurbiprofen with the hydroxyl in position 6\' of galactose, as described above for Ketogal.

**Chemical Part**

**Synthesis of Diacetone 6'-O-Flurbiprofen-D-galactopyranoside (2a)**

1 g of flurbiprofen  
2 (4.1 iranol), 1.067 g of  
1,2,3,4-di-O-isopropylidene-D-\(\alpha\)-galactopyranose  
25 (4.1 mmol), 786 mg of  
\(N\)-ethyl-\(N'\)-(3-dimethyl aminopropyl) carbodiimide (EDC) HCl (4.1 mmol), and  
25.2 mg of 4-(dimethyl amino) pyridine (DMAP)
(0.205 iratol). were dissolved in anhydrous dichloromethane (10 inL). The reaction mixture was kept under electromagnetic stirring at room temperature for 12 hours. The organic phase was extracted several times with water and dehydrated with anhydrous sodium sulphate, filtered and dried in vacuum. The reaction crude was purified on a chromatography column with silica gel using CH2Cl2 as eluent, to obtain 1.21 g of 2a as a white solid (yield 61%) m/z: 487 (M + H)+.

**Synthesis of flurbiprofen-D-galactos-6'-yl ester (Flugal)**

Added to a solution of 2a (1.21 g; 2.5 mmol) in anhydrous dichloromethane (10 inL) were 2 mL of trifluoroacetic acid (TFA), and the reaction mixture was kept under electromagnetic stirring at room temperature for 48 hours. By evaporating the solvent, a residue was obtained, which was purified on a chromatography column with silica gel using CHCl3 as eluent in a gradient of CH3OH to obtain 538 mg of Flugal as a white solid (yield 53%).

**1H-NMR (CD3OD):**

- 1.5 (d, 3H, -CH3); 3.45 (m, IH, 4'-H);
- 3.70 (m, IH, -CH); 3.8 (m, IH, 5'-H); 4.20 (m, 2H, 6'-H);
- 4.30 (m, IH, 2'-H); 4.40 (m, IH, 3'-H); 5.15 (m, IH, 1'-H);
- 7.17 (m, 2H, 6,5-Biph); 7.36 (m, IH, 3-Biph);
- 7.43 (m, 3-H, 9,10,12-Biph); 7.52 (m, 2-H, 8,12-Biph). **13C-NMR (CD3OD):**

- 18 (CH3); 45 (CH); 64 (C-6'); 68 (C-4'); 70 (C-5'); 73 (C-2'); 74 (C-3'); 93 and 98 (C-I'); 115 (3-Biph); 124 (5-Biph); 127 (10-Biph); 128 (8,12-Biph); 129 (9,11-Biph); 131 (6-Biph); 135 (4-Biph); 142 (7-Biph); 159 (1-Biph); 160 (2-Biph); 174
(esteric CO) : m/z : 407 \((M + H)^+\).

**Stability studies on Flugal**

For the study of the stability, the solutions of pro-drug were prepared by dissolving an aliquot of Flugal in a phosphate buffer at pH 7.4, as regards chemical stability, or else in a plasma specimen, for enzymatic stability. The solutions were kept in the dark, at a temperature of 37°C, for 24 hours. After each hour an aliquot of specimen was taken. In the case of the plasma said amount was previously extracted with acetonitrile (1:2), "vortexed", and centrifuged at 3000 r.p.m. for 10 min. The supernatant was taken and used for analysis at HPLC with diode detector. The half-lives for the chemical stability and enzymatic stability were calculated by quantizing, in time, the percentage of pro-drug that remained in solution. A 1090L HPLC (Hewlett-Packard, Palo Alto, USA) coupled to a diode detector HP 1040A was used for the chromatographic separations. The column used was a Supelcosil LC-18, 250 x 4.6 mm, particle size 5 mm. The volume injected was 20 mL and the flow of 1 mL/min. The mobile phase was constituted by a mixture of aqueous CH3CN/H3PO4 1 mM (pH 3) [32:68]. For the quantitative determination reading was made at 250 nm. The retention times of the various compounds involved in the analysis were: for Flugal 6 min and for flurbiprofen 11 min. All the reagents and the solvents used were of analytical degree. The distilled and deionized water was purified by means of a Milli Q system (Millipore). The straight line of calibration for the quantification of the
compounds was constructed using standard solutions with concentrations of between 0.1 and 100 µg/ml. Linearity was obtained with a coefficient of regression ($R^2$) of approximately 0.998. From the results of said analysis (illustrated in Table 3) it emerges that the pro-drug Flugal has a good chemical stability and a good susceptibility to enzymatic hydrolysis.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>pH 7.4</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flugal</td>
<td>$T_{48}$ (HOURS)</td>
<td>$T_{95}$ (HOURS)</td>
</tr>
<tr>
<td></td>
<td>&gt; 8</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Chemical stability and enzymatic stability of Flugal

Synthesis of the galactosylated derivative of acetylsalicylic acid (Asagal)

The galactosylated derivatives of acetylsalicylic acid (Asagal) is a pro-drug in which the drug is represented by acetylsalicylic acid and the vehicle by a galactose molecule.

Asagal is synthetized by esterifying the carboxyl group of acetylsalicylic acid with the hydroxyl in position 6' of galactose.
as given above and so far described for Ketogal and/or Flugal.

CHEMICAL PART

Synthesis of diacetone 6-O-acetylsalicylic-D-galactopyranoside (3a)

1 g of acetylsalicylic acid 3 (5.5 mmol), 1.43 g of 1,2,3,4-di-0-isopropylidene-D-α-galactopyranose (5.5 mmol), 1.08 g of 1-l'carbonyl di-imidazole (CDI) (6.6 mmol) were dissolved in anhydrous dichloromethane (10 mL). The reaction mixture was kept under electromagnetic stirring at room temperature for 12 hours. The organic phase was extracted several times with water and dehydrated with anhydrous sodium
sulphate, filtered and dried in vacuo. The reaction crude was purified on a chromatography column with silica gel using CHCl₃ as eluent, to obtain 1.89 g of 3a as a white solid (yield 70%).

Synthesis of acetylsalicyli σ-D-galactos-δ'-yl ester (Asagal)

Added to a solution of 3a (1.89 g; 3.8 mmol) in anhydrous dichloromethane (10 mL) were 2 mL of trifluoroacetic acid (TFA), and the reaction mixture was kept under electromagnetic stirring at room temperature for 48 hours. By evaporating the solvent, a residue was obtained, which was purified on a chromatography column with silica gel using CHCl₃ as eluent in a gradient of CH₃OH to obtain 830 mg of Asagal as a white solid (yield 63%).

Synthesized for the first time in the present invention is a galactosylated derivative (Ketogal) of an non-steroidal anti-inflammatory (ketorolac). The process of blocking of an active drug in a pro form, by means of the esterification of the carboxyl group of the drug in question with the hydroxyl in position 6 of galactose, has enabled maintenance of the potent anti-inflammatory effect of the starting drug, on the other hand minimizing its negative effects.

The process of synthesis of the galactosylated derivatives has been repeated successfully for the creation of a second pro-drug (Flugal) and a third pro-drug (Asagal) derived from further non-steroidal anti-inflammatories (flurbiprofen and acetylsalicylic acid) in which the vehicle is always represented by a
Various studies were conducted on chemical stability under physiological conditions (pH 7.4) and enzymatic stability (plasma) of the pro-drugs for verifying and testing their capacity for releasing the derivation drug.

In order to evaluate the anti-inflammatory, analgesic, and ulcerogenic activities of the pro-drug, numerous pharmacological analyses were conducted in which the results clearly highlight how the administration of Ketogal minimizes gastrointestinal toxicity, maintaining its anti-inflammatory and analgesic activity unaltered.

Furthermore, pharmacokinetic studies have highlighted how the galactosylated derivative of the non-steroidal anti-inflammatory produces a delayed and slower release, which is a fundamental process for the prevention of the formation of ulcers, a side effect common to all non-steroidal anti-inflammatories in so far as it has been shown how the administration of the pro-drug allows release of the drug into the blood plasma in therapeutic but non-toxic concentrations, as compared to the non-galactosylated form.

On the basis of these results, it may be stated that the synthesis of a galactosylated derivative of a non-steroidal anti-inflammatory forming the subject of the present invention enables minimization of gastrointestinal toxicity by means of esterification of the carboxyl group of the drug in question, preserving in any case the anti-inflammatory power and the analgesic activity of non-steroidal anti-
inflammatories.

REFERENCES


CLAIMS

1. A process of synthesis of galactosylated derivatives of all non-steroidal anti-inflammatory drugs with free acid function, characterized in that it envisages esterification of the carboxyl group with the hydroxyl in position 6 of galactose, using as condensing agents both 1,1'-carbonyl di-imidazole (CDI) and \(N\)-ethyl-\(N'\)-((3-dimethyl amino- propyl) carbodiimide (EDC) HCl, with subsequent deprotection of the sugar using trifluoroacetic acid (TFA) in anhydrous dichloromethane.

2. The process of synthesis of galactosylated derivatives of all non-steroidal drugs with free acid function as per the preceding claim, characterized in that said drugs are chosen in the following group: aspirin, diflunisal, benorylate, ibufenac, diclofenac, indomethacin, sulindac, ketorolac, ibuprofen, naproxen, ketoprofen, fenoprofen, flurbiprofen, mefenamic acid, meclofenamic acid, flufenamic acid, niflumic acid.

3. Pro-drugs of all non-steroidal anti-inflammatory drugs with free acid function derivatized with an ester group, which have the general structural formula given below:

\[
\begin{align*}
A & \quad \text{OR} \\
\text{O} & \\
\end{align*}
\]

(I)
where A is: aspirin, diflunisal, benorylate, ibufenac, diclofenac, indomethacin, sulindac, ketorolac, ibuprofen, naproxen, ketoprofen, fenoprofen, flurbiprofen, mefenamic acid, meclofenamic acid, fiufenamic acid, niflumic acid, and where in the ester group R can be a sugar (amongst which aldose, or ketose pentose, or ifose selected from a group of D- and L-enantiomers of ribose, glucose, galactose, mannose, arabinose, xilose, allose, altrose, gulose, idose and talose and substituted derivatives thereof, such as glucosamine, galactosamine, N-acetyl glucosamine, N-acetyl galactosamine, N-acetyl ribosamine), a disaccharide, a trisaccharide, or an oligosaccharide.

4. Pro-drugs of all non-steroidal anti-inflammatory drugs with free acid function as per Claim 3, where by disaccharide is meant a polymeric assemblage of two sugars, constituting both homopolymers (maltose and cellobiose) and heteropolymers (lactose and sucrose).

5. Pro-drugs of all non-steroidal anti-inflammatory drugs with free acid function as per Claim 3, where by trisaccharide is meant a polymeric assemblage of three sugars.

6. Pro-drugs of all non-steroidal anti-inflammatory drugs with free acid function as per Claim 3, where by oligosaccharides is meant polymers constituted by 4 to 10 residues; the polymer can be either homosaccharidic (the same sugar that repeats) of heterosaccharidic (different sugars), the sugars being each bound to another via a glycoside bond between Cl
and C4 or alternatively between Cl and C3 or else between Cl and Cβ.

7. Galactosylated derivatives of ketorolac having the following general structural formula:

![Formula II](image)

8. Galactosylated derivatives of flurbiprofen having the following general structural formula:

![Formula III](image)

9. Galactosylated derivatives of acetylsalicylic acid having the following general structural formula:

![Formula IV](image)
10. Pharmaceutical compositions with analgesic and anti-inflammatory activity comprising as active ingredient a derivative according to Claims 1-9 in a mixture with an appropriate vehicle.

11. Pharmaceutical compositions with analgesic and anti-inflammatory activity comprising as active ingredient a conjugate according to Claims 1-9 in a mixture with an appropriate vehicle.

12. Use of the derivatives of Claims 1-9 for the preparation of medicaments for pain therapy or inflammatory diseases.

13. Use of the conjugate of Claims 1-9 for the preparation of medicaments for pain therapy or inflammatory diseases.

14. Pharmaceutical compositions containing a derivative according to Claims 1-9 in a mixture with one or more excipients and/or vehicles.

15. Pharmaceutical compositions containing a conjugate according to Claims 1-9 in a mixture with one or more excipients and/or vehicles.

16. Pharmaceutical compositions comprising the derivatives and/or the conjugates according to Claims 1-9 in a technical form suitable for cutaneous, oral, sub-lingual, nasal, parenteral, rectal, intrathecal, bronchial, and intra-ocular administration.

17. The derivatives and/or conjugates according to Claims 1-9 formulated as syrup, elixir, tablets, capsules, parenteral and injectable solutions, nasal solutions, collyria, powders, granulates, controlled-release capsules, emollient creams, ointments,
impregnated bandages, controlled-release liposoluble plasters, or suppositories.
FIG. 1

Plasma concentration of Ketorolac

Ketorolac
Ketogal

Time (hours)

FIG. 2A

A

\[ \mu g/g \]

stomach  liver  kidney

1h  2h  4h  6h

FIG. 2B

B

\[ \mu g/g \]

stomach  liver  kidney

1h  2h  4h  6h
FIG. 4A

FIG. 4B