Synthesis and Pharmacological Evaluation of Oligoethylene Ester Derivatives as Indomethacin Oral Prodrugs

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Abstract □ Five indomethacin oligoethylene ester derivatives (**3–7**) were synthesized and evaluated for their anti-inflammatory, analgesic, and ulcerogenic activity after oral administration. The molecular weight of the oligoethylene glycols used for synthesizing esters **3–7** ranged from 106 to 282. The chemical and enzymatic stabilities of esters **3–7** were evaluated in pH 7.4 and 2.0 buffers and in human plasma, respectively. All the prodrugs showed a good stability both in pH 7.4 phosphate buffer and in pH 2.0 buffer, and they were readily hydrolyzed by human plasma. Esters **3–7** showed an anti-inflammatory activity, determined as the percent inhibition of carrageenan-induced edema, similar to that of indomethacin, although at higher doses. From writhing test results, we observed that all the prodrugs exhibited better or similar analgesic activity compared to indomethacin. Esters **3–7** were significantly less irritating to the gastric mucosa than indomethacin, after oral administration, and esters **3–5** did not show any ulcerogenic activity, although they were administered at higher doses than indomethacin.

The many acidic nonsteroidal anti-inflammatory drugs (NSAIDs) constitute the principal class of agents for controlling the pain and inflammation of rheumatic disease.[1]

The considerable gastrointestinal distress associated with chronic use of these compounds and their low half-life constitute the main disadvantages in clinical use of NSAIDs.[2,3]

The gastrochemical side effects are the result of a direct contact effect and a systemic effect which may be also manifested after iv dosing.[4] Of these effects, the direct contact mechanism appears to play a determinant role in the production of gastrointestinal lesions,[5] and it is probably a combination of local irritation produced by the free carboxylic acid group of the NSAIDs and local inhibition of the cytoprotective action of prostaglandines on gastric mucosa.[6] In order to decrease the gastrointestinal toxicity of NSAIDs, prodrugs, where the carboxylic moiety has been temporarily masked, have been developed.[7,8]

One compound can be regarded as a potential NSAID prodrug when it maintains the desired activity of the parent drug while unwanted side effects are eliminated or notably reduced. To achieve such a pharmacological profile a NSAID prodrug should exhibit some important requirements: (1) the prodrug should show a good stability in aqueous solutions and in the gastrointestinal fluid so as to temporarily mask the acidic group of the NSAIDs prior to absorption in the gastrointestinal tract, (2) the prodrug should have suitable water solubility and lipophilicity to ensure absorption by the oral route, and (3) the prodrug should be readily hydrolyzed following gastric absorption to release the parent drug.[7]

Since simple alkyl and aryl ester prodrugs do not show the requirements mentioned above (they are not sufficiently labile *in vivo* to ensure a high rate of prodrug conversion[9]), different promoieties have been used to design NSAID prodrugs.

So, recently Shanbhag et al.[6] synthesized various ester and amide prodrugs of ibuprofen and naproxen, and some of them showed good chemical stability in simulated gastric fluid and better or similar anti-inflammatory activity together with decreased gastrointestinal side effects with respect to the parent drug. The better therapeutic ratio of these prodrugs was attributed by the authors to their intact absorption in the gastrointestinal tract.

Furthermore, very interesting N,N-disubstituted glycol amides[10,11] have been proposed as a potentially useful biolabile prodrug type for carboxylic acid agents and particularly for NSAIDs. These esters combined a high susceptibility to undergo enzymatic hydrolysis in plasma with a high stability in aqueous solution.

In order to design potential NSAID prodrugs, we think that oligoethylene glycols are attractive promoieties since (1) they are known to have a good biocompatibility,[12] (2) they could give prodrugs with enhanced aqueous as well as lipid solubility compared to the parent drug so as to increase gastrointestinal absorption,[9] and (3) they should lengthen NSAID lifetime since they prevent enzymes from attacking the drug.[13]

Previously, Cecchi et al.[12] have synthesized and pharmacologically evaluated three polyoxyethylene esters of ibuprofen using polyoxyethylenic chains of different length (Mw 194, 1000, and 2000) as promoieties. Among these three prodrugs the derivative with the lowest molecular weight showed a remarkably increased initial bioavailability, probably due to better gastrointestinal absorption and better anti-inflammatory activity with a comparable or somewhat lower ulcerogenic potency. However, the authors did not report any data about the chemical stability of these esters in aqueous solution or in gastric fluid.

Indomethacin is a potent anti-inflammatory agent whole clinical use is strongly limited by its gastrointestinal side effects and its short half-life.

Notwithstanding that the prodrug approach has been extensively used to obtain indomethacin prodrugs, such as indomethacin farnesil[14] and acemethacin,[15] none of the synthesized derivatives has resulted in an ideal prodrug.

In this paper we report the synthesis, the chemical and enzymatic hydrolysis rates, the preliminary anti-inflammatory and analgesic activities, and the gastrointestinal toxicity of five oligoethylene ester prodrugs of indomethacin (see Scheme 1) in which the active principle is bound to the end of oligoethylene chain promoieties of relatively low molecular weight.

Experimental Section

Materials and Methods—Melting points were taken on Büchi 510 capillary melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer infrared spectrophotometer model 281 using sodium chloride plates for neat liquid compounds and potassium bromide plates for the solid compound. ^{1}H-NMR and

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Table 1—Physical Properties of Indomethacin Prodrugs 3-7

compd	Yield, %	IR Wavenumbers, cm^{-1} -CO-OR	IR Wavenumbers, cm^{-1} -CO-N<	^{1}H-NMR (δ, $CDCl_3$) Chemical Shifts, ppm[a,b]	^{13}C-NMR (δ, $CDCl_3$) Chemical Shifts, ppm[a,b]
3	80.3	1740	1678	3.48 (t, 2H, f), 3.66–3.55 (m, 6H, a, c, g), 4.28 (t, 2H, b)	72.21 (f), 68.89 (c), 63.93 (b), 61.60 (g), 30.18 (a)
4	90.0	1738	1680	3.72–3.50 (m, 12H, a, c, d, e, f, g), 4.27 (t, 2H, b)	72.50 (f), 70.55 and 70,28 (d, e), 69.08 (c), 64.06 (b), 61.71 (g), 30.19 (a)
5	84.1	1740	1681	3.70–3.48 (m, 16H, a, c, d, e, f, g), 4.28 (t, 2H, b)	72.44 (f), 70.46, 70.36 (2C), and 70,13 (d, e), 68.95 (c), 63.99 (b), 61.56 (g), 30.05 (a)
6	71.2	1737	1679	3.69–3.45 (m, 20H, a, c, d, e, f, g), 4.22 (t, 2H, b)	72.50 (f), 70.35, 70.27 (4C), and 70,00 (d, e), 68.85 (c), 63.93 (b), 61.35 (g), 29.95 (a)
7	53.3	1735	1682	3.75–3.52 (m, 24H, a, c, d, e, f, g), 4.25 (t, 2H, b)	72.52 (f), 70.45, 70.39 (6C), and 70,11 (d, e), 68.94 (c), 64.05 (b), 61.57 (g), 30.08 (a)

[a] The letters a–g refer to Scheme 1. [b] The assignation of all hydrogen and carbon resonances was achieved by a HETCOR experiment on compound **3**.

^{13}C-NMR spectra were recorded on a Bruker WM 250 and on a Bruker AMX 500, respectively, using $CDCl_3$ as solvent. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (1%) as internal standard. Elemental analysis was performed on a Carlo Erba model 1108 elemental analyzer. The HPLC consisted of a Waters model 600 pump with a model 486 VU–vis detector, an automatic sample injection module (Wisp model 712), a Waters C_{18} μBondapack 4.6 mm ×30 cm reverse phase column, and a NEC Power Mate SX Plus computer.

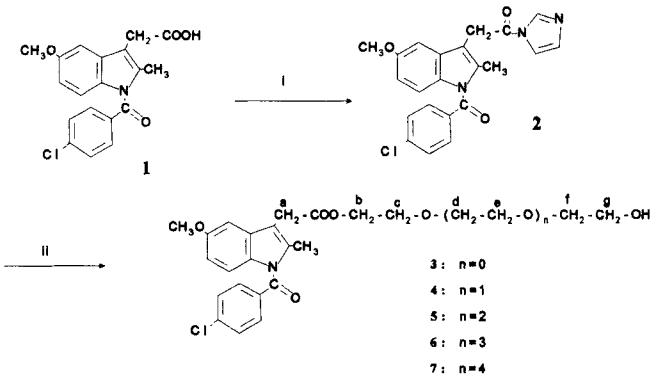
The progress of all the reactions was monitored by thin layer chromatography (TLC) that was performed on Whatman K6F glass plates. The chromatograms were developed using $CHCl_3$–CH_3OH (99:1) as eluant and were viewed under UV light (254 nm). For flash column chromatography, Merck silica gel (230–400 mesh) was used. Indomethacin was purchased from Sigma (St. Louis, MO). Diethylene glycol, Triethylene glycol, Tetraethylene glycol, Pentaethylene glycol, and Hexaethylene glycol were obtained from Fluka. 1,1′-Carbonyldiimidazole was obtained from Aldrich Chimica. LC grade acetonitrile and water were used in HPLC procedures and were obtained from Carlo Erba. All other chemicals were of reagent grade.

Synthesis of Oligoethylene Esters of Indomethacin (3–7)—The derivatives **3–7** were prepared using a slight modification of the method reported by Cecchi et al.[12] As reported in Scheme 1, the imidazolide **2** was prepared by dissolving indomethacin (**1**) (4.3 g, 0.012 mol) in dry ($CaCl_2$) alcohol-free chloroform (250 mL), adding portionwise 1,1′-carbonyldiimidazole (2.42 g, 0.015 mol) and stirring for 45 min at room temperature, until effervescence ceased. The intermediate imidazolide (**2**) was not isolated, but the appropriate oligoethylene glycol (0.04 mol) was added to its solution. The reaction mixture was stirred at room temperature for 24 h, then it was washed with water (2 × 100 mL), aqueous 0.1 N HCl (2 × 100 mL), water (2 × 100 mL), aqueous 0.1 N NaOH (2 × 100 mL), and water (2 × 100 mL), dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness *in vacuo*. The product was then purified by flash chromatography using chloroform as eluent for compound **3** and ethyl acetate for compounds **4–7**. The products obtained were all oils and they failed to crystallize, except the compound **3**, which was crystallized from *n*-hexane (mp 92–93 °C).

Elemental analyses (C, H, N) were within ±0.3% of the theoretical values. Yields, IR data, and ^{1}H-NMR and ^{13}C-NMR chemical shifts of esters **3–7** are listed in Table 1.

Chemical and Enzymatic Hydrolysis—The chemical stability of esters **3–7** as solutions in isotonic phosphate buffer, pH 7.4, and in a pH 2.0 buffer was determined at 32 °C, by following the disappearance of the esters with the HPLC method described below. Human plasma fractions (4 mL) were diluted with 1 mL of isotonic phosphate buffer, pH 7.4 (80% plasma). Plasma samples were thermostated at 37 ± 0.2 °C during the experiments. The reactions were started by adding 100 μL of a stock solution of esters **3–7** (1.0 mg/mL in methanol) to 5 mL of prethermostated plasma. Aliquots (300 μL) were withdrawn at intervals and deproteinized by mixing with 600 μL of 0.01 N HCl in methanol. After centrifugation at 5000*g* for 5 min, 25 μL of the clear supernatant was chromatographed as described below. The hydrolysis rate of esters **3–7** was monitored following the disappearance of the ester in the plasma samples. Pseudo-first-order rate constants for the chemical and enzymatic hydrolysis were determined from the slopes of linear plots of the logarithm of residual indomethacin esters against time.

HPLC Analysis—Indomethacin and esters **3–7** were determined by HPLC using a convex gradient starting with acetonitrile–0.1 M acetic acid 40:60, changing to acetonitrile–0.1 M sodium acetate 40:60 over 5 min and then to acetonitrile–0.1 M sodium acetate 60:40 over 5 min, and then returning to the initial conditions over 10 min. The flow rate was 1.8 mL/min and effluent was continuously monitored at 250 nm.



Scheme 1—Synthesis of Prodrugs **3–7**. Key: (i) carbonyldiimidazole/$CHCl_3$; (ii) $HO(CH_2CH_2O)_nH/CHCl_3$.

Anti-Inflammatory Activity—The anti-inflammatory activity of derivatives **3–7** was assessed by the carrageenan-induced rat paw edema assay. Young male rats (180–220 g) were used in groups of five. The rats were fasted with free access to water for 12 h prior to the test. Derivatives **3–7** were orally administered by gastric probe as suspensions in 10% gum arabic solution, at three different concentrations (40, 20, and 10 mg/kg). Also, indomethacin was administered orally by gastric probe, in the same vehicle used for derivatives **3–7**, at the dose of 5 mg/kg. One hour after the dose, 0.2 mL of a 1% carrageenan solution in normal saline was injected subcutaneously under the plantar surface of the right hind paw. The volume of the paw was measured immediately and after 3 h, using a plethysmometer.

The differences in the anti-inflammatory activity (percent inhibition) observed after the administration of derivatives **3–7** and indomethacin were analyzed by Student's *t* test for unpaired samples and are expressed as means ± standard deviations of the mean.

Analgesic Activity—Male mice (18–25 g) were used in groups of five. Parent drug or prodrugs **3–7** were administered orally by gastric probe , in the same vehicle and at the same concentrations described above, to mice 1 h before the intraperitoneal (ip) injection of 0.25 mL of a 0.5% acetic acid in 0.9% saline solution.

The number of writhes for each mouse was counted for 25 min after the acetic acid injection. The percentage writhing induced by acetic acid in the presence of indomethacin and esters **3–7** was calculated according the following expression:

$$\text{percent writhing} = \frac{\text{(average number of writhes with drug)}}{\text{(average number of writhes with control)}} \times 100$$

Gastric Ulceration Assay—Gastrointestinal toxicity was measured in male rats, weighting 180–220 g, in groups of 10. Parent drug or prodrug **3–7** were administered orally, by gastric probe, daily

Table 2—Chemical and Enzymatic Hydrolysis of Prodrugs 3–7

Compd	Half-Life (h)		
	pH 7.4 Buffer	pH 2.0 Buffer	Human Plasma
3	533	539	3.4
4	695	691	3.8
5	540	535	3.1
6	602	610	3.5
7	562	569	2.8

for 4 days, using the same vehicle and the same concentrations reported above. The control animals were given only the vehicle (10% gum arabic solution). Animals were fasted after the last dose. Six hours after the final dose, they were sacrificed by decapitation, and the stomachs were removed, opened, and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a 2 × 2 binocular magnifier, with all the ulcers >0.5 mm being recorded.

Results and Discussion

Chemical and Enzymatic Hydrolysis—As shown in Table 2, esters **3**–**7** had a notable chemical stability in phosphate buffer at pH 7.4. No significant differences in the chemical hydrolysis rate of esters **3**–**7** was observed as the length of the oligoethylene chain increased. Since, as previously mentioned, NSAID prodrugs should show good stability in aqueous acidic media to decrease the gastric toxicity due to the direct contact effect, we studied the chemical hydrolysis of esters **3**–**7** using buffer at pH 2.0 to simulate gastric fluid. As may be seen in Table 2, esters **3**–**7** showed good stability at pH 2.0, and the hydrolysis rate was very close to that observed in phosphate buffer at pH 7.4.

Since an essential requisite for prodrug effectiveness is their ability to readily release the parent drug after oral administration, we assessed the enzymatic hydrolysis of esters **3**–**7** in human plasma. From the data reported in Table 2, it can be noted that all the esters were readily hydrolyzed by human plasma, and no significant difference in hydrolysis rate was observed as the length of the oligoethylene chain increased. Preliminary experiments, carried out using porcine esterase, showed that the enzymatic hydrolysis of ester **3**–**7** was catalyzed by esterases, and the cleavage of the promoiety regenerated the parent drug directly (data not shown).

Anti-Inflammatory Activity—The percent inhibition values of carragenaan-induced edema formation by indomethacin esters **3**–**7** are shown in Table 3. Indomethacin inhibited significantly ($p < 0.01$) edema formation by 82% of the control, while esters **3**–**7** gave a dose-dependent inhibition of edema formation. At 40 mg/kg doses all the esters showed the same anti-inflammatory activity of the parent drug at 5 mg/kg, while at 20 mg/kg doses only ester **4** maintained a notable activity ($p < 0.01$), and at the lowest dose (10 mg/kg) all the esters were significantly less active than indomethacin at 5 mg/kg ($p < 0.05$). These results indicate that, after oral administration, esters **3**–**7** are able to elicit the same anti-inflammatory activity of the parent drug, although at higher doses.

Analgesic Activity—The analgesic activity of indomethacin and esters **3**–**7**, expressed as percent inhibition of acetic acid-induced writhing in mice, is reported in Table 3.

At 40 and 20 mg/kg doses, all the esters exhibited significantly better activity than the parent drug at 5 mg/kg while at 10 mg/kg doses they showed an activity comparable to indomethacin's ($p < 0.01$). We think that these results are of particular interest, since other authors,[16] in order to synthesize indomethacin prodrugs without gastrolesive effects, obtained derivatives which retained anti-antiflammatory activity but significantly diminished analgesic properties compared to the parent drug.

Table 3—Anti-Inflammatory, Analgesic, and Ulcerogenic Activity of Indomethacin and Prodrugs 3–7

Compd[b]	Dose		% Inhibition[a]		% of Animals with Ulcers
	mg/kg	mmol/kg	Edema Formation (3rd hour)	Writhing	
1	5	0.0139	82 ± 7.4	51.8 ± 3.1	60
	40	0.0897	84 ± 8.5	80.2 ± 9.3	20
3	20	0.0448	53 ± 6.4	69.6 ± 5.4	0
(80.2)	10	0.0224	28 ± 8.1*	50.8 ± 6.6	0
	40	0.0816	81 ± 7.9	74.8 ± 5.9	20
4	20	0.0408	72 ± 8.2	61.3 ± 4.8	0
(73.0)	10	0.0204	45 ± 8.8	47.1 ± 6.4	0
	40	0.0749	83 ± 6.9	70.8 ± 8.3	20
5	20	0.0374	58 ± 4.3	60.8 ± 5.0	0
(67.0)	10	0.0187	38 ± 9.7*	46.4 ± 9.4	0
	40	0.0691	80 ± 7.1	70.5 ± 6.7	20
6	20	0.0345	54 ± 6.0	60.2 ± 5.4	20
(61.0)	10	0.0173	25 ± 6.8*	45.8 ± 7.3	10
	40	0.0642	72 ± 6.6	70.6 ± 7.5	20
7	20	0.0321	56 ± 3.7	60.6 ± 8.1	20
(57.5)	10	0.0161	21 ± 6.2*	47.0 ± 6.3	10

[a] All the values were significant at $p < 0.01$, except the asterisked values, which were significant at $p < 0.05$. [b] Molar % of indomethacin in prodrugs 3–7 is in parentheses.

Ulcerogenic Activity—The percentages of animals showing ulcers greater than 0.5 mm in the gastric mucosa after oral administration of indomethacin and esters **3**–**7** are reported in Table 3. All the esters were significantly less irritating to the gastric mucosa than indomethacin, at all doses tested. It is of worth to note that esters **3**–**5**, at 20 and 10 mg/kg doses, did not show any ulcerogenic activity, notwithstanding that they were administered at molar doses higher than indomethacin. These results could be explained on the basis of the inhibition of both direct contact and systemic gastrolesive effects. Since esters **3**–**7** are stable in simulated gastric fluid, it can be assumed that they are absorbed intact; therefore, the direct contact gastrointestinal irritation is inhibited. Inhibition of the systemic gastrolesive effect could be ascribed to changes in the absorption and metabolism patterns of the prodrugs compared to those of the parent drug. In preliminary studies to better understand the pharmacokinetic profile of the prodrugs synthesized in this paper, notably lower but therapeutically effective indomethacin blood concentrations were obtained after oral administration of esters **3**–**7** compared to indomethacin itself (unpublished data): this low indomethacin plasmatic level could explain the lack of systemic gastrolesivity. A similar profile of indomethacin plasmatic concentration has been obtained by Ogiso et al.[17] in the pharmacokinetic evaluation of indomethacin octyl ester prodrugs.

Conclusions

Esters **3**–**7** showed the requisites to be regarded as useful indomethacin prodrugs for oral administration since (1) they were stable in aqueous solution and in simulated gastric fluid, (2) they were readily hydrolyzed in human plasma, (3) depending on the dose administered, they maintained the same anti-inflammatory activity of the parent drug, (4) they showed better or equal analgesic activity, at all doses tested compared to indomethacin, and (5) they inhibited completely,

or at least notably, the gastrointestinal irritation produced by the parent drug. Further studies are on-going to investigate the pharmacokinetic profile of these prodrugs in order to elucidate their metabolic and absorption pattern so as to explain the interesting pharmacological results obtained in this work.

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