Synthesis and Evaluation of Morpholinoalkyl Ester Prodrugs of Indomethacin and Naproxen

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Morpholinoalkyl esters (HCl salts) of naproxen 1 and indomethacin 3 were synthesized and evaluated *in vitro* and *in vivo* for their potential use as prodrugs for oral delivery. Prodrugs were freely soluble in simulated gastric fluid (SGF) and pH 7.4 phosphate buffer and showed a minimum of a 2000-fold increase in solubility over the parent drugs. All prodrugs were more lipophilic than parent drugs as indicated by *n*-octanol/pH 7.4 buffer partition coefficients but less lipophilic in terms of *n*-octanol/SGF partition coefficients. Potentiometrically determined pK_a's for prodrugs were in the range of 6.89 to 8.62 at 25°C. All prodrugs were quantitatively hydrolyzed to their respective parent drugs by enzymatic and/or by chemical means. An increase in carbon chain length rendered the prodrugs more stable at pH 7.4 but less stable in SGF. The esters were generally found to be hydrolyzed rapidly in rat plasma at 37°C, the half-lives being in the range of 1.2–31.0 min. Based on *in vitro* results, prodrugs 2c and 4c were chosen to evaluate solid-state stability, *in vivo* bioavailability, and ulcerogenicity. At elevated temperatures, the solid-state decomposition of 2c and 4c followed biphasic kinetics, with rapid decomposition occurring initially. The prodrugs were 30–36% more bioavailable orally than the parent drugs following a single equimolar solution dose in rats. Prodrugs 2c and 4c were significantly less irritating to gastric mucosa than parent drugs following single-dose and chronic oral administration in rats.

KEY WORDS: indomethacin; naproxen; prodrugs; hydrolysis kinetics; solubility; partition coefficient; ulcerogenicity; bioavailability.

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs)[3] are a diverse group of drugs, used mainly in the treatment of acute and chronic painful disorders of the locomotor system (1). Gastrointestinal (GI) side effects constitute the most frequent of all adverse reactions of NSAIDs (2–4). These reactions range in both severity and frequency from relatively mild to the more serious and potentially life-threatening states, such as GI ulceration and hemorrhage (5,6).

GI mucosal injury produced by NSAIDs is generally believed to be caused by two different mechanisms (7–12): The first mechanism involves a local action comprising of a direct contact mechanism and an indirect effect on the GI mucosa. The direct contact effect can be attributed to a combination of local irritation produced by the acidic group of the NSAIDs and local inhibition of prostaglandin synthesis in the GI tract. The indirect effect can be attributed to a combination of an ion-trapping mechanism of NSAIDs in mucosal cells and back diffusion of hydrogen ions from the lumen into the mucosa. The second mechanism is based on a generalized systemic action occurring after absorption, which can be demonstrated following intravenous dosing.

Recently, considerable attention has been focused on the development of bioreversible derivatives, such as prodrugs, to mask temporarily the acidic group of NSAIDs as a promising means of reducing or abolishing the GI toxicity due to localized effect. Shanbhag *et al.* (13) have reported the antiinflammatory activity and GI toxicity of aminophenyl esters, aminoalkyl esters, and amides of ibuprofen and naproxen 1. They observed that the esters exhibited significantly reduced gastric toxicity compared to the parent drugs and their amide prodrugs. The antiinflammatory activity of all the prodrugs was found to be greater than the parent drugs. Kahns *et al.*(14) investigated the stability of a glycolamide ester of 3 in aqueous buffers and observed that the ester exhibited maximum stability at pH 4.7. However, at this pH, the ester had a half-life of only 43 days.

Gu *et al.* (15) evaluated the kinetics of chemical and enzymatic hydrolysis of glycerol, glycolic acid, and morpholinoethyl esters of a developmental analgesic agent. The aqueous shelf lives of all the esters were less than 2 years at all pH values studied. No GI toxicity was reported for these prodrugs. Venuti *et al.* (16) reported the synthesis and biological evaluation of (*N,N,N*-trialkylammonium) alkyl esters and thioesters of 1,3, and other NSAIDs. In general, these prodrugs exhibited moderate to greatly reduced GI toxicity, but significantly reduced analgesic potencies. The aqueous shelf-lives of all the esters were less than two years.

N,N-Disubstituted glycolamide esters of naproxen 1 and indomethacin 3 were evaluated (17) and found to hydrolyze very rapidly in human plasma. These esters were found to be stable in aqueous solutions for at least a year, but showed poor aqueous solubility. Moreover, no data have been published on the antiinflammatory activity or gastrointestinal toxicity of these prodrugs.

Perisco *et al.* (18) have reported that the glycine amide of tolmetin produced lower peak plasma tolmetin levels than an equivalent dose of tolmetin sodium, but plasma concentrations were sustained for a longer period of time. Cioli *et al.* (19) reported the toxicological and pharmacological profile of the guaiacol ester of ibuprofen and observed that the ester exhibited less toxicity than ibuprofen on both the gastric and the intestinal mucosa. However, the peak concentration of ibuprofen was delayed upon oral administration of the ester, due to slow enzymatic conversion rate of the ester to parent compound.

The present work was initiated to develop prodrugs of

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[3] *Abbreviations used:* nonsteroidal antiinflammatory drug, NSAID; gastrointestinal, GI; naproxen, 1; indomethacin, 3; naproxen ethylmorpholino ester, 2a; naproxen propylmorpholino ester, 2b; naproxen butylmorpholino ester, 2c; indomethacin ethylmorpholino ester, 4a; indomethacin propylmorpholino ester, 4b; indomethacin butylmorpholino ester, 4c; simulated gastric fluid, SGF; propyl paraben, PP; butyl paraben, BP; diclofenac, DF; mefenamic acid; MA; partition coefficient, PC; solubility, Sol.

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naproxen 1 and indomethacin 3, possessing a high enzymatic bioconversion rate and favorable physicochemical properties. Morpholinoethyl esters of different drugs have been prepared and shown to combine high water solubility and lipophilicity, with adequate stability and a high susceptibility to undergo enzymatic hydrolysis in plasma (15,20). A series of morpholinoalkyl esters (HCl salts) of 1 and 3 was synthesized and evaluated *in vitro* and *in vivo* for their potential use as prodrugs for oral delivery. The physicochemical properties, stability, plasma-catalyzed hydrolysis, bioavailability, and GI toxicity of these derivatives are reported.

MATERIALS AND METHODS

Naproxen [1; $[\alpha]^{25}$ + 66°(c1, $CHCl_3$)] and indomethacin 3 were obtained from Aldrich and Sigma Chemical Company, respectively, and were used as received. All other chemicals and reagents were either analytical or reagent grade and were used as received. Distilled deionized water was used in the preparation of buffer solutions and mobile phases. Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5M × FT spectrometer. NMR spectra were recorded as 6% (w/v) solutions on a JEOL FX-90Q spectrometer. Analytical data were obtained from Oneida Research Services, Inc., Whitesboro, New York. UV spectroscopic analysis was performed on a Gilford spectrophotometer. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. pH measurements were performed using a digital-type Orion Research microprocessor pH/millivolt meter 811. Adult male Sprague–Dawley rats (180–250 g) were used in the ulcerogenicity and bioavailability studies. Tissue examinations were performed with a Bausch and Lomb (2 × 2) binocular magnifier.

Synthesis of Hydroxyalkylmorpholines

The synthesis of 4-(1-hydroxypropyl) morpholine is representative of the general method. A suspension of lithium aluminum hydride ($LiAlH_4$; 11.4 g, 300.2 mmol) in tetrahydrofuran (THF; 150 mL) was treated in a dropwise fashion with a solution of ethyl 3-(4-morpholino) propanoate (21) [bp 84–87°C, 0.1 mm Hg; lit. (21) bp 138–140°C, 25 mm Hg] and refluxed under a N_2 atmosphere for 12 hr. The solution was cooled and the excess $LiAlH_4$ was decomposed by the addition of water. The suspension was filtered, and the THF was evaporated under reduced pressure. The clear solution was extracted with CH_2Cl_2 (3 × 50 mL), and the combined CH_2Cl_2 extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), filtered, and evaporated to yield an oil. Vacuum distillation gave 12.77 g (75%) of 4-(1-hydroxypropyl) morpholine[bp 94–97°C, 0.15 mm Hg; lit. (22) bp 134–136°C, 24 mm Hg]. This method was used to prepare 4-(1-hydroxybutyl) morpholine[bp 101–103°C, 0.15 mm Hg; lit. (23) pb 116.5–117°C, 5 mm Hg].

Synthesis of Morpholinoalkyl Esters (HCl Salts) of 1 and 3

Synthesis of 2-(morpholinoethyl)2-(6-methoxy-2-naphthalyl) propionate hydrochloride (**2a**) is representative of the general procedure (24). A solution of 1 (5.0 g, 21.7 mmol) in dry toluene (150 mL) was heated to reflux and treated in a dropwise manner with thionyl chloride (16.5 mL). After refluxing for 2 hr, the excess thionyl chloride was azeotropically removed with dry toluene under reduced pressure. The crude acid chloride was dissolved in tetrahydrofuran (150 mL) and treated with 4-(1-hydroxyethyl) morpholine (5.7 g, 43.42 mmol) in a dropwise fashion. A white precipitate formed almost immediately, and the reaction mixture was stirred continuously overnight at room temperature. The precipitated 4-(1-hydroxyethyl) morpholine hydrochloride was removed by filtration, and the filtrate was evaporated under reduced pressure to give a yellow oil. The oily product was dissolved in methylene chloride (150 mL) and washed with 5% sodium bicarbonate (3 × 50 mL) and water (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain an oil. The oil was dissolved in a minimum quantity of ethanolic hydrochloride and, upon cooling overnight, gave a white solid, which upon recrystallization from ethanol/ether gave analytically pure **2a**. This method was used to prepare **2a**, **2b**, **2c**, **4a**, **4b**, and **4c**. Physical and analytical data for the prodrugs are given in Table I. The ^1H NMR spectra of the compounds were consistent with their structures.

HPLC Analysis

A reversed-phase HPLC procedure was used for the quantitative determination of the esters and parent compounds. HPLC studies were performed on a Varian 5000 pump equipped with a Waters 484 variable-wavelength detector, a Valco C10W injector, and a Shimadzu CR501 Chromatopac data module. A reversed-phase μBondapak C_{18} column was used for the analysis of all compounds. A mobile phase consisting of acetronitrile and phosphate buffer (60:40, pH 7.22), with 0.004 *M* triethylamine was used for the analysis of 1 and its esters. The flow rate was 1.0 mL/min and the column effluent was monitored at 232 nm, using propyl paraben (PP) as the internal standard. The retention times for **1**, **2a**, **2b**, **2c**, and PP were 3.6, 7.8, 9.7, 11.8, and 5.4 min, respectively. For analysis in rat plasma a mobile phase consisting of acetonitrile and phosphate buffer (40:60, pH 3.26), using diclofenac (DF) as the internal standard, was used. The retention times for **1**, **2a**, **2b**, **2c**, and DF were 9.8, 5.0, 5.5, 6.3, and 12.0 min, respectively. For the analysis of 3 and its esters, the ratio of solvents used was 70:30 (pH 7.32), and the column effluent was monitored at 257 nm, with butyl paraben (BP) as the internal standard. The retention times for **3**, **4a**, **4b**, **4c**, and BP were 3.2, 7.8, 9.8, 11.9, and 5.2 min, respectively. For analysis in rat plasma, the ratio of solvents used was 50:50 (pH 3.40), using mefenamic acid (MA) as the internal standard. The retention times for **3**, **4a**, **4b**, **4c**, and MA were 9.7, 6.9, 7.6, 8.6, and 14.0 min, respectively.

Determination of Ionization Constants

Ionization constants were determined potentiometrically (25) at 25 ± 0.2°C. The pK_a's were determined by titrating aqueous solutions of prodrugs (0.01 *M*) with 0.1 *N* KOH. Titrations were carried out in a nitrogen atmosphere. A digital pH meter equipped with a combination electrode was used to measure the pH. Triplicate samples were analyzed, and the mean value of pK_a was calculated.

Table I. Physical and Analytical Characteristics of 1, 3, and Their Prodrugs

Compound	% yield	Melting point (°C)		Analysis (%) Calculated	Found	Formula	$[\alpha]_D^{25}$ (c 1, $CHCl_3$)
1	—	157–158		—		$C_{14}H_{14}O_3$	+65.8
3	—	155–162		—		$C_{19}H_{16}ClNO_4$	—
2a	48[a]	165–167	C	63.23	63.01	$C_{20}H_{26}ClNO_4$	+43.2
			H	6.90	6.96		
			N	3.69	3.65		
2b	42[a]	139–141	C	64.03	63.78	$C_{21}H_{28}ClNO_4$	+33.1
			H	7.16	7.19		
			N	3.56	3.58		
2c	38[b]	140–142	C	64.77	64.41	$C_{22}H_{30}ClNO_4$	+27.7
			H	7.41	7.40		
			N	3.43	3.32		
4a	49[a]	197–199	C	59.17	58.83	$C_{25}H_{28}Cl_2N_2O_5$	—
			H	5.56	5.68		
			N	5.52	5.37		
4b	52[a]	175–177	C	59.89	59.90	$C_{26}H_{30}Cl_2N_2O_5$	—
			H	5.80	5.79		
			N	5.37	5.34		
4c	46[a]	205–207	C	60.56	60.39	$C_{27}H_{32}Cl_2N_2O_5$	—
			H	6.02	6.02		
			N	5.23	5.20		

[a] Ethanol/ether.
[b] Isopropyl alcohol.

Determination of Solubility in SGF and pH 7.4 Phosphate Buffer

The solubilities of esters and the parent compounds were determined in SGF and pH 7.4 phosphate buffer at 25 ± 0.2°C. The esters were freely soluble in both aqueous media. The solubility of 1 and 3 was determined at 25 ± 0.2°C by adding excess amounts of compounds to the aqueous media in screw-capped test tubes. The mixtures were shaken on a mechanical shaker for 48 hr to ensure equilibrium. Upon filtration through 0.45-μm nylon filters, an aliquot of the filtrate was diluted with mobile phase, mixed with appropriate internal standard, and analyzed by HPLC. Six determinations were made to calculate the mean solubility.

Determination of Partition Coefficients

The apparent partition coefficients (PC) of 1 and 3 and their prodrugs were determined in *n*-octanol/SGF and *n*-octanol/pH 7.4 buffer at 25 ± 0.2°C. Mutually presaturated phases were used. The initial aqueous concentrations of all the prodrugs were 5×10^{-4} *M*. The PCs were calculated by the equation

$$PC = (C_i - C_w)/C_w$$

where C_i and C_w represent the solute concentrations in the aqueous phase before and after equilibrium, respectively. Insignificant (≤5%) hydrolysis of prodrugs was observed during these experiments. Triplicate samples were analyzed, and the mean value of the PC was calculated.

Kinetics of Hydrolysis in Aqueous Solutions

Reactions were initiated by adding 100 μL of a 0.01 *M* stock solution of prodrugs to 10 mL of SGF or pH 7.4 buffer in screw-capped vials preequilibrated at 37 ± 0.5°C. The reaction was monitored by HPLC for residual prodrug and parent drug concentrations. Psuedo-first-order rate constants for the hydrolysis of prodrugs were determined from the slopes of linear plots of the logarithm of residual prodrug concentration versus time. Triplicate samples were analyzed, and the mean value of the rate constant was calculated.

Kinetics of Hydrolysis in Rat Plasma

The hydrolysis of prodrugs was examined in rat plasma at 37 ± 0.5°C. The reactions were initiated by adding 5 μL of stock solution (0.01 *M*) of the prodrugs in acetonitrile to 5.0 mL of preheated plasma. The solutions were kept in a water bath at 37 ± 0.5°C, and at appropriate time intervals samples of 25 μL were withdrawn and added to 1 mL of acetonitrile spiked with internal standard. After immediate mixing and centrifugation for 5 min at 6000 rpm, the clear supernatant was diluted with mobile phase and 30 μL of clear supernatant was analyzed by HPLC for residual prodrug and parent drug. The pseudo-first-order rate constants for the hydrolysis of the prodrugs were determined by linear regression of the logarithm of peak height ratios versus time plots. Triplicate samples were analyzed, and the mean value of rate constant was calculated.

Solid-State Stability of 2c and 4c

Individual samples of about 5 mg each of 2c and 4c were

stored in open vials in constant-temperature ovens (26), maintained at 40 ± 0.5, 60 ± 0.5, and 80 ± 0.5°C. At each assay point, samples were removed and analyzed immediately by HPLC.

In Vivo Bioavailability Studies

Male Sprague–Dawley rats (220–250 g) were used. The rats were fasted for a 12-hr period prior to the administration of drug solutions. Food was withdrawn during the first 4 hr of the experiment. Water was available ad libitum throughout the experiment. The following drug solutions in pH 7.4 phosphate buffer were subjected to testing: (i) the sodium salt of 1, (ii) 2c, (iii) 3, and (iv) 4c. The solutions were administered at a dose equivalent to 7.14 mg/kg of 1 and 1.1 mg/kg of 3, using a gavage needle and washed down with 0.2 mL of distilled water. Blood samples (75 μL) were withdrawn from the tail vein into a microsyringe prerinsed with heparin solution and stored in heparinized tubes. Blood sampling was performed at 0, 5, 20, 35, 50, 60, 80, 100, 120, 150, 180, and 240 min postadministration and the samples were analyzed immediately. Six determinations were made at each time interval for each preparation. The samples were prepared for HPLC analysis by adding 200 μL of acetonitrile spiked with internal standard to blood samples to precipitate the proteins. The samples were vortexed for 2 min and centrifuged at 6000 rpm for 7 min; the supernatant was separated and filtered through a 0.45-μm nylon filter. One hundred microliters of the filtrate was added to 100 μL of mobile phase, and 30 μL of this solution was injected onto the HPLC.

The blood concentration–time curves were plotted and the area under the curve $(AUC)_{0-4}$ was determined by the trapezoidal method.

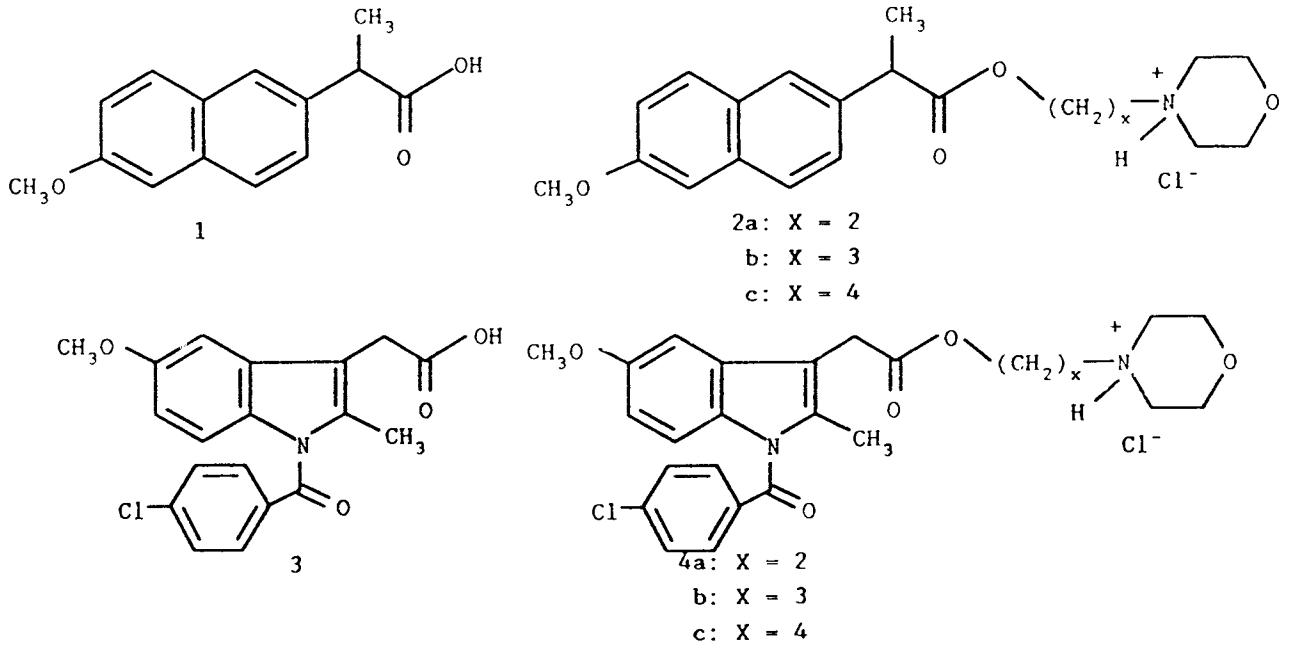
Statistical analysis (Student *t* test) was performed to test the significance of difference in C_{max} and AUC between the prodrugs and the parent drugs.

In Vivo Ulcerogenicity Studies

Male Sprague–Dawley rats (180–210 g) were used. The rats were fasted for 12 hr prior to administration of drug solutions and for 4 hr postdosing. Food was available at all other times, and free access to water was provided throughout the experiment. One group of rats (control) received no drug treatment, while other groups received either the parent drug or the prodrug. The following solutions in saline were administered orally: (1) the sodium salt of 1, (2) 2c, (3) the sodium salt of 3, and (4) 4c. Doses equivalent to 40 mg/kg of 1 and 6.75 mg/kg of 3 (19,27,28) were used. The rats were subjected to a single-dose and chronic-dose regimen, which utilized the same daily dose for 4 days. At 24 hr following the last dose of chronic treatment and 4 hr after the single dose, the rats were sacrificed in a carbon dioxide chamber. The stomach was dissected out of the body along with the first 5 cm of the intestine, then rinsed with saline, and the contents of the stomach were emptied. The stomach and the intestine were then excised open along the greater curvature and gently wiped clean with a swab dipped in saline. The mucosal damage was examined grossly under a binocular magnifier. The severity of mucosal damage was assessed by modification of a previously reported rating scale (27).

Observation	Score
No lesions	0.0
Punctiform lesions (lesions less than 1 mm)	0.5
Five or more punctiform lesions	1.0
One to five small ulcers (1–2 mm)	2.0
More than five small ulcers or one large ulcer (2–4 mm)	3.0
More than one large ulcer (greater than 4 mm)	4.0

Based on the severity of the mucosal damage, the specimen was assigned an ordinal score as per the scoring scheme. For example, a specimen with five punctiform lesions, two small ulcers, and one large ulcer was assigned a score of 3.0. However, the control specimens did not exhibit



Scheme I. Chemical structures of naproxen 1, indomethacin 3, and their hydroxyalkylmorpholine (HCl) ester prodrugs.

Table II. Physicochemical Properties of 1, 3, and Their Prodrugs

Compound	Sol (M/L)[a]	Sol (M/L)[b]	pK_a	PC[c]	PC[d]
1	$5.93 \times 10^{-5} \pm 1.5 \times 10^{-6}$	$1.73 \times 10^{-2} \pm 6.34 \times 10^{-4}$	4.2 reported	29.6 ± 2.2	2.26 ± 0.17
3	$1.33 \times 10^{-5} \pm 1.05 \times 10^{-6}$	$6.69 \times 10^{-3} \pm 3.21 \times 10^{-4}$	4.5 reported	100.64 ± 6.77	10.13 ± 0.09
2a	Freely soluble	Freely soluble	7.75 ± 0.029	1.41 ± 0.04	1370.0 ± 34.97
2b	Freely soluble	Freely soluble	8.03 ± 0.03	1.95 ± 0.07	1819.05 ± 5.2
2c	Freely soluble	Freely soluble	8.62 ± 0.02	2.83 ± 0.08	3409.9 ± 90.3
4a	Freely soluble	Freely soluble	6.89 ± 0.02	21.25 ± 0.74	403.8 ± 0.09
4b	Freely soluble	Freely soluble	7.26 ± 0.01	27.87 ± 0.77	705.45 ± 26.18
4c	Freely soluble	Freely soluble	7.71 ± 0.03	33.74 ± 1.32	1322.56 ± 32.10

[a] In simulated gastric fluid (pH 1.3).
[b] In pH 7.4 phosphate buffer.
[c] In octanol/simulated gastric fluid (pH 1.3).
[d] In octanol/pH 7.4 phosphate buffer.

the formation of lesions or ulcers and accordingly had a score of 0. The scores were averaged and the mean score was tabulated as the severity index for the drug solution administered. Six determinations were made at each dose, for each solution.

Statistical analysis (Student *t* test) was performed to test the significance of difference in severity index, among the prodrugs and parent drugs.

RESULTS AND DISCUSSION

Chemical structures of 1, 3, and their prodrugs are shown in Scheme I. The percentage yield, melting points, analytical data, and optical rotation of prodrugs and parent drugs are listed in Table I. All prodrugs of 1 were prepared from the (+)-enantiomer and were optically active, showing the same sign of optical rotation as that of 1. No attempt was made to determine the enantiomeric purity of these prodrugs.

The physicochemical properties of 1, 3, and their prodrugs are shown in Table II. The pK_a's determined potentiometrically were in the range of 6.89 to 8.62 (Table II). An increase in carbon chain length resulted in an increase in the pK_a value, at least by 0.5 unit, which could be attributed to reduced acidity. The aqueous solubility of prodrugs at pH 1.3 (SGF) and pH 7.4 phosphate buffer increased a minimum of 2000-fold over the parent drugs. As expected from pK_a's between 6.89 and 8.62, the PC data listed in Table II indicate that all prodrugs are appreciably lipophilic at pH 7.4, but less lipophilic at pH 1.3, compared to the parent drugs. An increase in carbon chain length rendered the prodrugs more lipophilic. There is a two- to threefold increase in PC from the ethyl to the butyl derivative for both drugs in both pH media. The PCs determined for 1 and 3 at pH 7.4 agreed well with the reported values (29).

The kinetics of chemical and enzymatic hydrolysis of all prodrugs displayed pseudo-first-order kinetics over several half-lives. The half-lives and the rate constants for prodrug hydrolyses are listed in Table III. As can be seen from the rate data, an increase in carbon chain length rendered the prodrugs more stable at pH 7.4 but less stable at pH 1.3. At pH 1.3 all prodrugs exist primarily as protonated species. Hence, an increase in the carbon chain length probably facilitates hydrolysis due to a change in the transition state for hydrolysis and an added inductive effect. The inductive effect increases with an increase in carbon chain length between the ester moiety and the morpholine function. At pH 1.3, increasing the carbon chain length from ethyl to propyl (for both 1 and 3) rendered the prodrug more labile to hydrolysis, which was indicated by a two- to threefold increase in the rate constant. A further increase in carbon chain length from propyl to butyl did not indicate a significant change in rate constant, presumably due to an increase in steric hindrance thus offsetting any additional rate increasing effects of the inductive effect. Also, increases in chain length beyond propyl have very remote (from the ester carbonyl) inductive effects and thus fail to affect hydrolysis significantly. Conversely, at pH 7.4, an increase in carbon chain length from ethyl to propyl rendered the prodrug more stable; there is a five- to sixfold decrease in rate constant (only twofold for 3). On the other hand, an increase in carbon

Table III. Kinetic Parameters of Prodrugs of 1 and 3 at 37°C

Compound	K_{obs} (hr^{-1})[a]	$t_{1/2}$ (hr)[a]	K_{obs} (hr^{-1})[b]	$t_{1/2}$ (hr)[b]	K_{obs} (min^{-1})[c]	$t_{1/2}$ (min)[c]
2a	0.0103	67.30	0.057	12.16	0.423	1.64
2b	0.0258	26.86	9.9×10^{-3}	70.00	0.169	4.10
2c	0.0261	26.35	4.7×10^{-3}	147.45	0.601	1.15
4a	0.0103	66.79	0.056	12.38	0.029	23.9
4b	0.0281	24.66	0.034	20.38	0.022	31.5
4c	0.0303	22.85	0.0227	30.53	0.034	20.4

[a] In simulated gastric fluid (pH 1.3).
[b] In pH 7.4 phosphate buffer.
[c] In rat plasma.

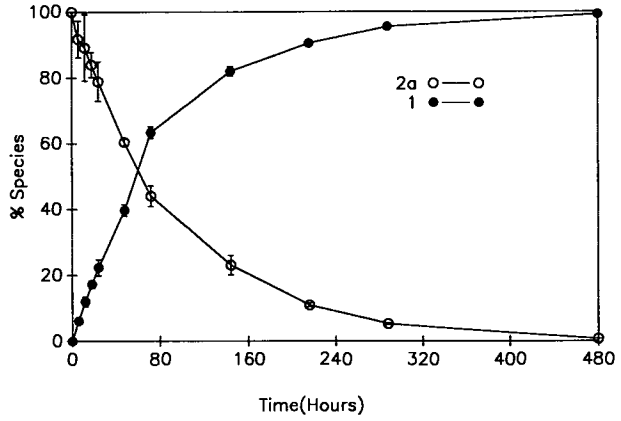


Fig. 1. Time courses for the disappearance of naproxen hydroxyethylmorpholine (HCl) ester 2a (○) and the appearance of naproxen 1 (●) during hydrolysis of the ester in SGF at 37°C.

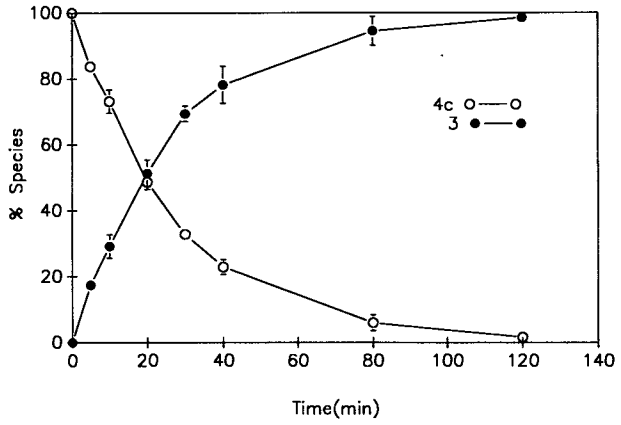


Fig. 2. Time courses for the disappearance of indomethacin hydroxybutylmorpholine (HCl) ester 4c (○) and the appearance of indomethacin 3 (●) during hydrolysis of the ester in rat plasma at 37°C.

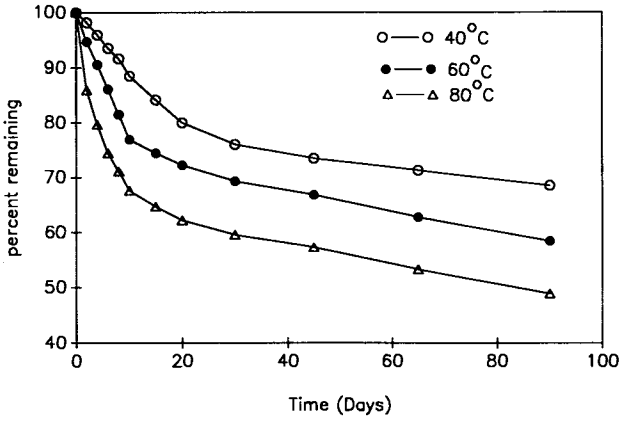


Fig. 3. Solid-state degradation of naproxen hydroxybutylmorpholine (HCl) ester 2c at elevated temperatures.

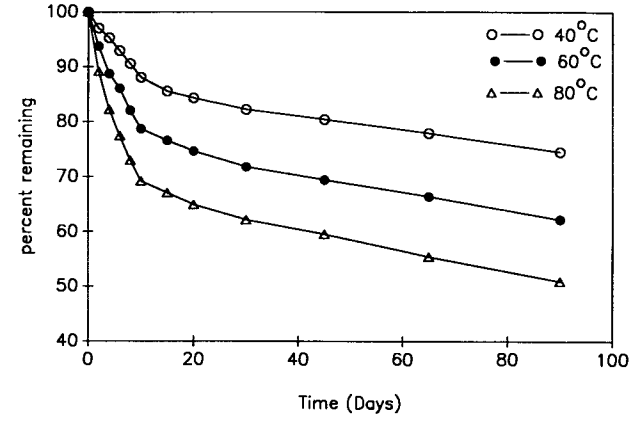


Fig. 4. Solid-state degradation of indomethacin hydroxybutylmorpholine (HCl) ester 4c at elevated temperatures.

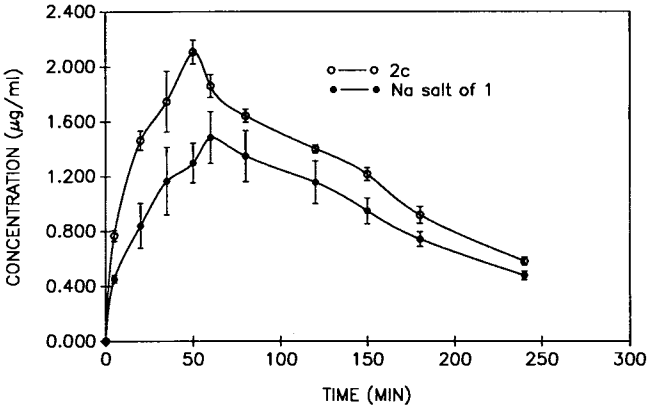


Fig. 5. Plasma concentrations of naproxen 1 in rats following peroral administration of naproxen sodium (●) and the naproxen hydroxybutylmorpholine (HCl) ester 2c (○) at a dose equivalent to 7.14 mg/kg of 1.

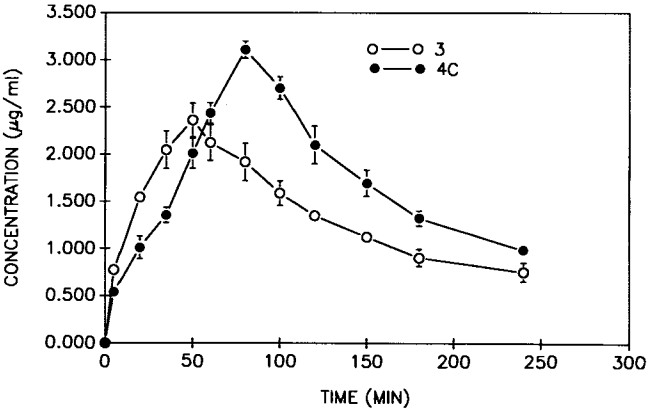


Fig. 6. Plasma concentrations of indomethacin 3 in rats following peroral administration of indomethacin (○) and indomethacin hydroxybutylmorpholine (HCl) ester 4c (●) at a dose equivalent to 1.1 mg/kg of 3.

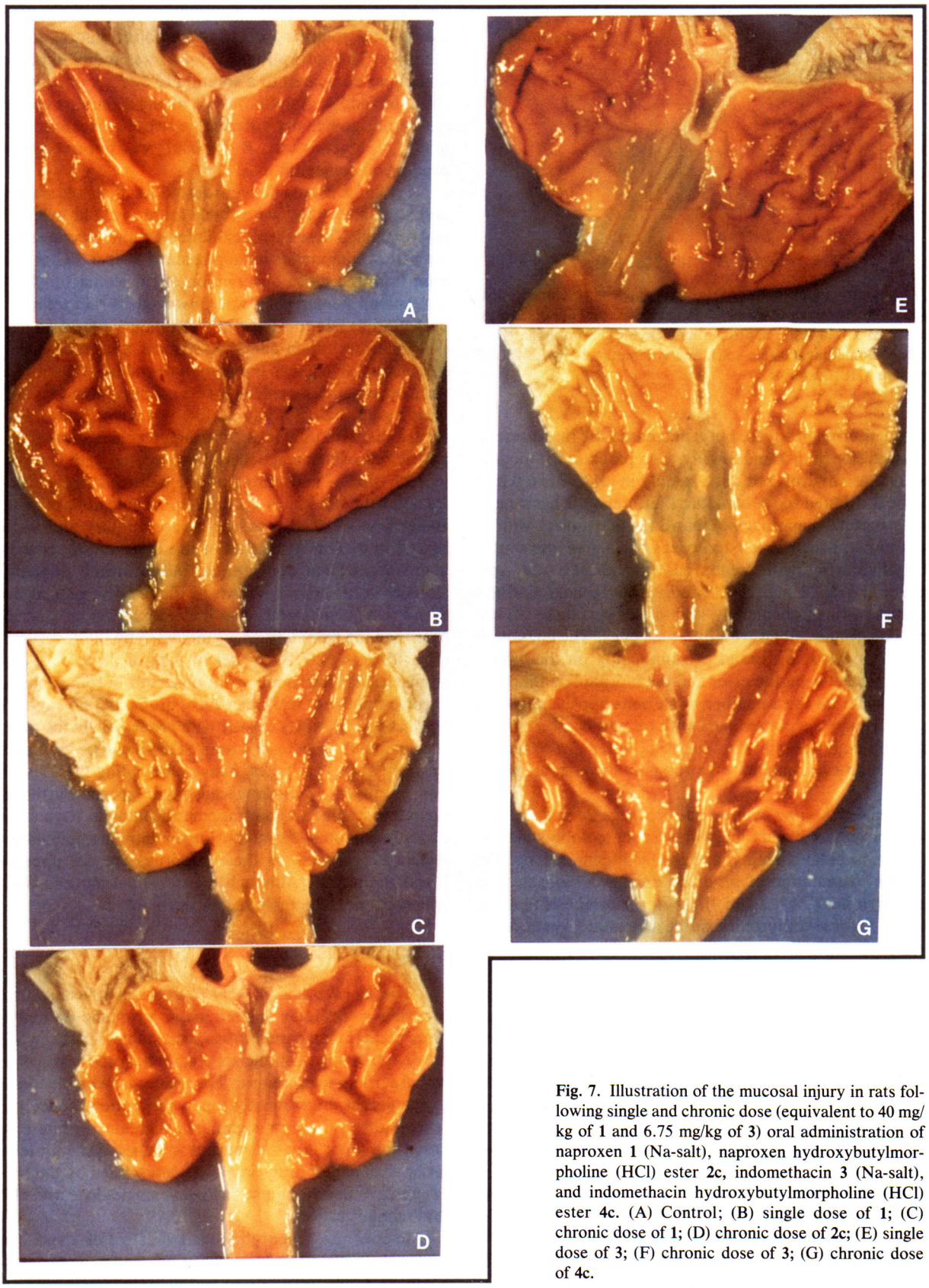


Fig. 7. Illustration of the mucosal injury in rats following single and chronic dose (equivalent to 40 mg/kg of **1** and 6.75 mg/kg of **3**) oral administration of naproxen **1** (Na-salt), naproxen hydroxybutylmorpholine (HCl) ester **2c**, indomethacin **3** (Na-salt), and indomethacin hydroxybutylmorpholine (HCl) ester **4c**. (A) Control; (B) single dose of **1**; (C) chronic dose of **1**; (D) chronic dose of **2c**; (E) single dose of **3**; (F) chronic dose of **3**; (G) chronic dose of **4c**.

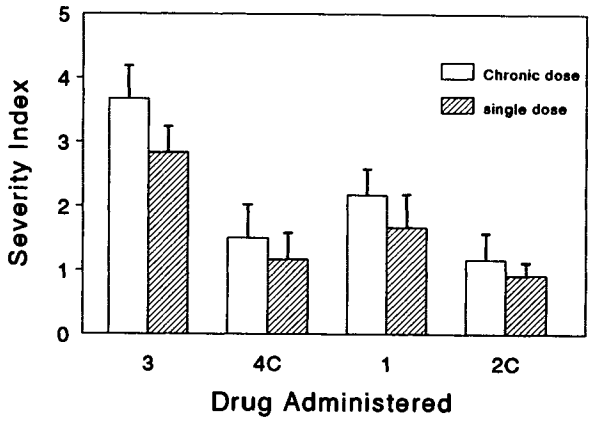


Fig. 8. Severity index in rats following single and chronic dose (equivalent to 40 mg/kg of 1 and 6.75 mg/kg of 3) oral administration of naproxen 1 (Na-salt), naproxen hydroxybutylmorpholine (HCl) ester 2c, indomethacin 3 (Na-salt), and indomethacin hydoxybutylmorpholine (HCl) ester 4c.

chain length from propyl to butyl decreased the rate constant by twofold (for both 1 and 3) only. Compared to esters of 3, esters of 1 are hydrolyzed quite rapidly in rat plasma, which could be attributed to the structural difference. 1 has a more planar structure (naphthalene) than 3 (Indole moiety with an *N*-acyl moiety), enabling 1 to access the active site on the hydrolytic enzyme with greater ease. As can be seen from the plots in Figs. 1 and 2, all prodrugs were quantitatively hydrolyzed either chemically and/or enzymatically to the parent compound, albeit with different rates. The enzymatic reactivity appears to depend predominantly on the carbon chain length between the ester and the morpholine function. Prodrugs with an even number of carbons appear to hydrolyze faster than the compounds with an odd number of carbons. This could possibly be due to a better fit of the prodrugs with an even number of carbons to the enzyme active site.

Based on *in vitro* evaluation, prodrugs 2c and 4c were chosen for further evaluation with respect to solid-state stability, *in vivo* bioavailability, and *in vivo* ulcerogenicity. The solid-state degradation of prodrugs exhibited biphasic degradation profiles (Figs. 3 and 4). A similar profile of biphasic degradation for levothyroxine in the solid state was recently reported by Won (26). Correlation coefficients of no less than 0.997 were obtained for linear portions of the first-order plots. The initial rate of degradation is much greater than that of the second phase and is more pronounced at higher temperatures. The faster initial degradation rate is consistent with partial solid-state hydrolysis, apparently due to limited amounts of water, an exhaustible solid constituent (30). Thermal degradation may account for the slower decay of the later phase. With an increase in temperature from 40 to 80°C, there is a two- to threefold increase in the initial rate of degradation.

Prodrug 2c was found to be 33.73 ± 8.4% (Student *t* test, $P < 0.001$) more bioavailable orally than parent drug (Na salt), following a single equimolar solution dose (Fig. 5) in rats. The rate of absorption of prodrug 2c appears to be faster than 1, although t_{max} was not significantly different. An approximately twofold increase in C_{max} (Student *t* test, $P < 0.001$) was seen after the administration of 2c compared to 1. Similarly, prodrug 4c appears to be 34.29 ± 3.35% (Student *t* test, $P < 0.0001$) more bioavailable orally than the parent drug, following a single equimolar solution dose (Fig. 6) in rats. The absorption rate for 4c was found to be slower initially as compared to a solution of 3. T_{max} observed for 3 (solution) and 4c (solution) were 50 and 80 min, respectively, and were significantly different. A two- to threefold increase in C_{max} (Student *t* test, $P < 0.0001$) was observed with the prodrug as compared to 3.

Prodrugs 2c and 4c were tested for their ulcerogenicity potential, since they were stable for at last 4 hr in simulated gastric fluid. These esters exhibited a rapid bioconversion to the parent compounds in rat plasma at 37 ± 0.5°C. As can be seen from the gross observation of rat stomachs (**7b, 7e**) a widespread hemorrhage was observed in rats treated with parent drugs due to gastric mucosal injury following a single dose. Further, upon administration of a chronic dose pale stomachs with a paper-thin structure and large ulcers (**7c, 7f**) were observed. The paleness was probably due to excessive bleeding during the study period of 4 days. It was also observed that rats treated with indomethacin 3 showed greater gastric mucosal damage than those treated with naproxen 1, in both single- and chronic-dose treatments. But the rats treated with either a single dose or chronic dose of prodrugs showed no significant gastric mucosal injury and were observed to be similar to that of the control (**7a**). Since they were identical in appearance, only chronic dose-treated stomachs (**7d, 7g**) are shown in Fig. 7. The prodrugs were found to be significantly less irritating to the rat gastric mucosa than the parent drugs, as indicated by the severity index (Fig. 8) ($P < 0.001$). A weight loss of 25 ± 5 g, bloated stomach, and soft stools were observed in rats subjected to chronic treatment with the parent drugs, but no such effects were observed in rats treated with prodrugs. As the prodrugs remain intact for at least 4 hr in SGF, it can be assumed that they are absorbed intact, hence eliminating the local irritation produced by the free carboxylic group. Furthermore, the indirect effect of ion trapping resulting in back diffusion of hydrogen ions from the lumen into the mucosal cells should be minimal, as these prodrugs are not appreciably lipophilic in SGF. Although the intestinal mucosa was also observed, no detectable ulcers were noted.

CONCLUSIONS

The *in vitro* and *in vivo* evaluation indicated that the prodrugs were freely soluble, more lipophilic (in neutral or basic media) than parent drugs, and stable enough in SGF to be absorbed intact. In the solid state the prodrugs were very stable at room temperature. The prodrugs were bioconverted to the parent drugs rapidly, upon absorption, hence the extent of absorption was significantly greater in rats as compared to parent drugs. Prodrugs were significantly less irritating than parent drugs in rats as determined by the severity of gastric mucosal injury, following single-dose and chronic oral administration.

In conclusion, morpholinoalkyl esters of 1 and 3 represent potentially useful derivatives to increase the aqueous solubility of the parent drugs and to decrease gastrointestinal side effects without altering the pharmacological profile of

the parent compounds. These properties make the novel esters promising prodrug forms for naproxen and indomethacin to improve oral delivery.

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