Synthesis and in Vitro Evaluation of Novel Morpholinyl- and Methylpiperazinylacyloxyalkyl Prodrugs of 2-(6-Methoxy-2-naphthyl)propionic Acid (Naproxen) for Topical Drug Delivery

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Various novel morpholinyl- (**3a,b**) and methylpiperazinylacyloxyalkyl (**3c**−**f**) esters of 2-(6-methoxy-2-naphthyl)propionic acid were synthesized and evaluated in vitro for topical drug delivery as potential prodrugs of naproxen (**1**). Compounds **3a**−**f** were prepared by coupling the corresponding naproxen hydroxyalkyl ester with the morpholinyl- or (4-methyl-1-piperazinyl)acyl acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP) and quantitatively hydrolyzed ($t_{1/2}$ = 1−26 min) to naproxen in human serum. Compounds **3c**−**f** showed higher aqueous solubility and similar lipophilicity, determined by their octanol−buffer partition coefficients (log P_{app}), at pH 5.0 when compared to naproxen. At pH 7.4 they were significantly more lipophilic than naproxen. The best prodrug **3c** led to a 4- and 1.5-fold enhancement of skin permeation when compared to naproxen at pH 7.4 and 5.0, respectively. The present study indicates using a methylpiperazinyl group yields prodrugs that are partially un-ionized under neutral and slightly acidic conditions, and thus, a desirable combination is achieved in terms of aqueous solubility and lipophilicity. Moreover, the resulting combination of biphasic solubility and fast enzymatic hydrolysis of the methylpiperazinylacyloxyalkyl derivatives gave improved topical delivery of naproxen.

Introduction

The topical application of drugs has recently received considerable attention due to its advantages over other drug delivery methods. Development of an efficient means of topical delivery can increase local soft-tissue and joint-drug concentrations while reducing the systemic distribution of a drug, thereby offsetting certain limitations of its oral use.[1−3] However, the barrier function of the skin limits the use of topical administration for most drugs. This limitation has led to the development of various strategies to enhance drug−skin permeation, such as modifying the lipophilicity of parent molecules to optimize partitioning into the skin and to maximize skin permeation.

2-(6-Methoxy-2-naphthyl)propionic acid (naproxen) is a nonsteroidal antiinflammatory drug (NSAID) that is widely used for the treatment of rheumatic diseases and related painful conditions.[4] Because the bioavailability of topically applied naproxen is only 1−2% in humans,[2,5−7] the temporary masking of the carboxylic acid via simple esterification has been investigated as a promising means of increasing the dermal permeation of naproxen.[8−10] These prodrugs permeate the skin in vitro markedly better than the parent drug, but they release naproxen very slowly in both human serum and skin−serum homogenate.[8] 1-Alkylazacycloalkan-2-one esters of naproxen possess the main requirements that are needed for topical prodrugs: e.g., enzymatic bioconversion to the parent drug and 3-fold greater in vitro skin permeation than naproxen itself from aqueous suspensions.[11]

Recently, we synthesized and evaluated a series of acyloxyalkyl and aminoacyloxyalkyl esters of naproxen, which readily hydrolyzed to naproxen in vitro and possessed highly variable aqueous solubilities and lipophilicities.[12,13] The highly lipophilic acyloxyalkyl esters did not enhance dermal permeation of naproxen, most probably due to the low aqueous solubilities.[12] However, the aminoacyloxyalkyl esters of naproxen had fast rates of enzymatic hydrolysis, in addition to a combination of adequate aqueous solubility and lipophilicity that resulted in a 3-fold increase in the in vitro skin permeation of naproxen.[13]

Amino acid esters bearing nitrogen heterocycles, e.g. the morpholine group attached to different drugs, have been prepared for oral drug delivery and demonstrated both high aqueous solubility and lipophilicity, with adequate chemical stability and a high susceptibility to undergo enzymatic hydrolysis.[14−17] On the basis of our earlier results[12,13] and the recent work of others,[14−18] we synthesized and evaluated a series of novel morpholinyl- and methylpiperazinylacyloxyalkyl esters as potential prodrugs of naproxen for topical drug delivery.

Results and Discussion

Chemistry. Morpholinyl- (**3a,b**) and methylpiperazinylacyloxyalkyl (**3c**−**f**) prodrugs of naproxen were prepared by coupling the corresponding hydroxyalkyl ester of naproxen **2a,b** with the morpholinyl- and (4-

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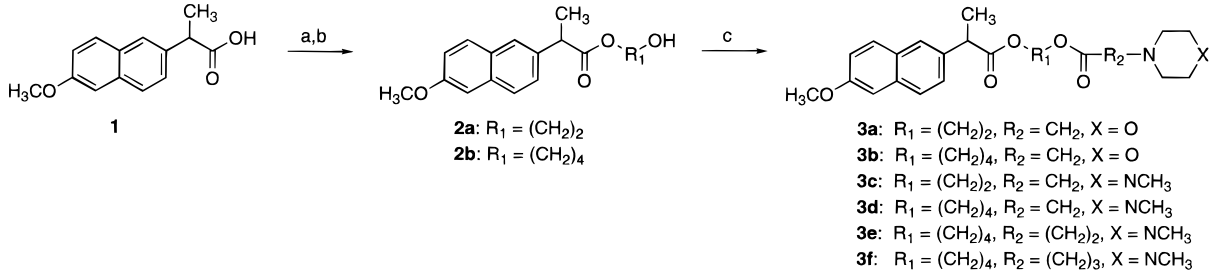
Scheme 1[a]



[a] Reagents: (a) NaOH; (b) Br-R_1-OH, DMF; (c) $HOOCR_2N(CH_2CH_2)_2X$, DCC, DMAP, CH_2Cl_2.

Table 1. Aqueous Solubility (mean ± SD; $n = 2-4$) and Apparent Partition Coefficient (log P_{app}, mean ± SD; $n = 2-3$) of Naproxen and Prodrugs

	aqueous solubility (mM)		log P_{app}[a]	
compd	pH 7.4	pH 5.0	pH 7.4	pH 5.0
naproxen	101.9 ± 1.3	0.40 ± 0.04	0.30 ± 0.03	2.38 ± 0.02
3a	0.07 ± 0.00	0.05 ± 0.00	2.14 ± 0.08	2.62 ± 0.00
3b	*b*	*b*	*b*	*b*
3c	4.1± 0.4	5.3 ± 0.9	1.16 ± 0.08	0.43 ± 0.05
3d	8.8 ± 0.9	16.2 ± 1.2	1.30 ± 0.05	0.94 ± 0.05
3e	432.4 ± 27.5	141.6 ± 27.0	3.04 ± 0.07	1.20 ± 0.06
3f	50.0 ± 1.9	61.0 ± 8.8	2.69 ± 0.06	1.41 ± 0.02

[a] P_{app} is the apparent partition coefficient between 1-octanol and phosphate buffer (pH 7.4 and 5.0) at room temperature. [b] Not determined.

methyl-1-piperazinyl)acyl acids in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP) in dry dichloromethane (Scheme 1).

Aqueous Solubility and Lipophilicity. Drug lipophilicity is a very important property for topical drug delivery because the stratum corneum, the major barrier to drug permeation, is lipophilic in nature and generally favors the permeation of lipophilic drugs. The aqueous solubility, or hydrophilicity, of a drug molecule has also been suggested to be as important a property as lipophilicity, especially for very lipophilic compounds.[19] Thus, a drug molecule should possess both hydrophilic and lipophilic properties to readily diffuse across the skin.[13,20-22] The two characteristics (expressed as the apparent partition coefficients (log P_{app}) between 1-octanol and phosphate buffer) are shown in Table 1 at pH 7.4 and 5.0 for naproxen and prodrugs.

Because of the acidic character of naproxen (pK_a 4.15), it is more water soluble at pH 7.4 than at pH 5.0. In contrast, the prodrugs **3c**−**d**,**f** are more soluble in acidic aqueous solutions due to the ionizable basic group in the promoiety. At pH 7.4, the prodrugs possess lower aqueous solubility (except **3e**) when compared to naproxen and showed a higher lipophilicity than naproxen, as indicated by the log P_{app} values (Table 1). The aqueous solubility of all methylpiperazine prodrugs (**3c**−**f**) was significantly greater at pH 5.0 compared to that of naproxen, while they maintained a log P_{app} value near 1. In the design of drugs for topical and transdermal use, it is often appropriate to have a log P value in the range 1−3.[23] The only tested morpholinylacyloxyalkyl prodrug (**3a**) was more lipophilic than naproxen and was poorly soluble at both pH values.

Chemical and Enzymatic Hydrolyses. Kinetics of the chemical and enzymatic hydrolyses of each naproxen prodrug followed pseudo-first-order kinetics over several half-lives. The rate of enzymatic hydrolysis of each

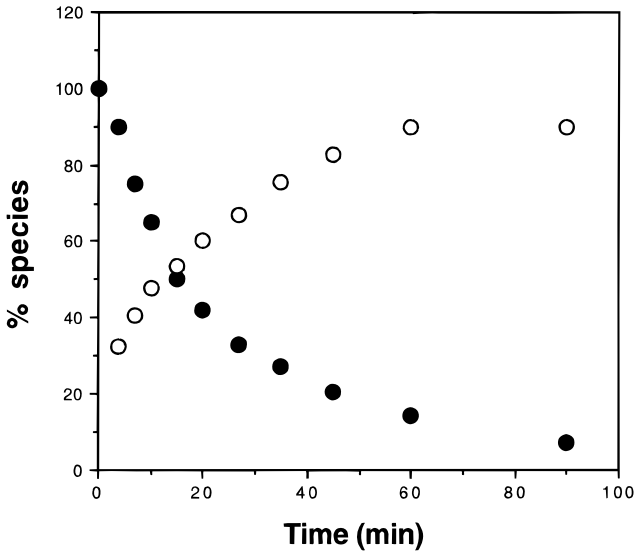


Figure 1. Time courses for naproxen 2-[(4-methyl-1-piperazinyl)acetyloxy]ethyl ester (**3c**) (●) and naproxen (○) during hydrolysis of the prodrug in 80% human serum (pH 7.4) at 37 °C.

Table 2. Hydrolysis Rates of Naproxen Prodrugs in Phosphate Buffer Solutions (pH 7.4 and 5.0) and 80% Human Serum (pH 7.4) at 37 °C

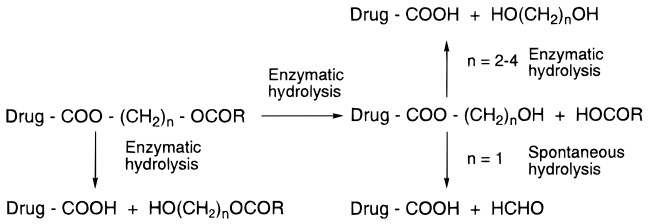
	$t_{1/2}$ (days)		$t_{1/2}$ (min)	$f_{50\%}$ (min)[a]
compd	buffer pH 7.4	buffer pH 5.0	80% human serum (mean; $n = 2$)[c]	80% human serum (mean; $n = 2$)[c]
3a	1.4	6.7	20 (24, 15)	25 (33, 17)
3b	*b*	*b*	13 (13, 12)	11 (11, 11)
3c	2.4	66.5	26 (30, 22)	22 (26, 19)
3d	7.6	79.9	3 (3, 3)	7 (8, 6)
3e	0.7	18.0	4 (3, 4)	5 (6, 4)
3f	2.0	37.2	1 (1, 1)	3 (4, 1)

[a] $f_{50\%}$ is the time by which 50% of total naproxen is formed (mean of two determinations). [b] Not determined. [c] Individual values are shown in parentheses.

prodrug was determined in 80% human serum, because human serum or plasma is a commonly used medium to determine the ester hydrolysis of prodrugs for topical drug delivery.[8,24]

The prodrugs were highly susceptible to enzymatic hydrolysis in serum and hydrolyzed quantitatively to naproxen (Figure 1) with half-lives ($t_{1/2}$) ranging from 1 to 26 min. The formation of naproxen is represented by the $f_{50\%}$ values (Table 2), indicating pseudo-first-order times at which 50% of total naproxen was formed.[25] These mean values also ranged from 3 to 25 min. The chemical stability of prodrugs in aqueous solutions was substantially higher at pH 5.0 than at pH 7.4, with half-lives ranging from 6.7 to 79.9 days and from 0.7 to 7.6

Scheme 2



days, respectively. Because the esters proved to be chemically stable at pH 5.0, they fulfill an important criterion for becoming an ideal prodrug: i.e., that of maintaining chemical stability while providing fast enzymatic degradation. However, the stability of these prodrugs at pH 5.0 is not good enough to prepare a commercial product at pH 5.0. Further studies are under way to stabilize the prodrugs.

The hydrolysis of acyloxymethyl esters is a two-step process (Scheme 2).[26,27] The first step, which is rate-determining, is the enzymatic hydrolysis of the terminal ester group with the formation of the hydroxymethyl ester as an unstable intermediate, which spontaneously dissociates to the parent drug. However, the hydroxyethyl and -butyl esters (**2a,b**), which are intermediates in the hydrolysis of **3a**−**f**, are stable in aqueous solution but hydrolyze to naproxen in human serum.[12] Moreover, the hydrolysis rates of **2a,b** ($t_{1/2}$ = 224 and 147 min, respectively) are much slower than those of **3a**−**f** ($t_{1/2}$ = 1−26 min) in human serum. A similar order of magnitude in $t_{1/2}$ and $f_{50\%}$ values of **3a**−**f** (Table 2) indicates that the formation of naproxen takes place at a similar rate as the loss of prodrug in human serum. Therefore, prodrugs **3a**−**f** may hydrolyze to naproxen without formation of the hydroxyalkyl ester intermediate, and thus hydrolysis of prodrugs **3a**−**f** may result from enzymatic attack on the carbonyl of the parent drug, rather than the carbonyl of the promoiety (Scheme 2).

In Vitro Skin Permeation Study. Excised postmortem human skin was used to examine the permeation of naproxen and prodrugs **3a**,**c**−**f**. Prodrug suspensions in phosphate buffer were applied to maintain constant diffusion and maximum flux, and each compound was applied in isotonic phosphate buffer (0.05 M) at both pH 7.4 and 5.0.

Cumulative amounts of permeated naproxen (in nmol/cm^2), intermediate, or intact prodrug are shown in Figure 2. The steady-state flux (J_{ss}) was calculated from the slope of the linear portions of these plots, and the values are presented in Table 3. The permeability coefficients (K_p) for the steady-state delivery were obtained by dividing the steady-state flux by the solubilities of the compounds in the corresponding vehicle (Table 3).

Passive diffusion is driven by high drug concentration in the aqueous vehicle, and therefore, naproxen itself showed a 28-fold greater flux (6.5 ± 0.6 nmol/cm^2·h) across human skin from saturated suspension than from a 5.0 mM solution at pH 7.4 (0.23 ± 0.03 nmol/cm^2·h).[12] Because different pH values in donor or receptor sides do not cause different degrees of damage to the skin,[28] the higher flux of naproxen from a saturated solution of pH 7.4, compared to that from a saturated solution of pH 5.0, is due to increased drug concentration in the applied vehicle. Naproxen afforded a 4-fold greater flux at pH 7.4 (6.5 ± 0.6 nmol/cm^2·h) than at pH 5.0 (1.6 ± 0.2 nmol/cm^2·h) despite decreased ionization and an increased partition coefficient of naproxen at pH 5.0. This increased flux is attributed to a 250-fold greater aqueous solubility of naproxen at pH 7.4 than at pH 5.0.

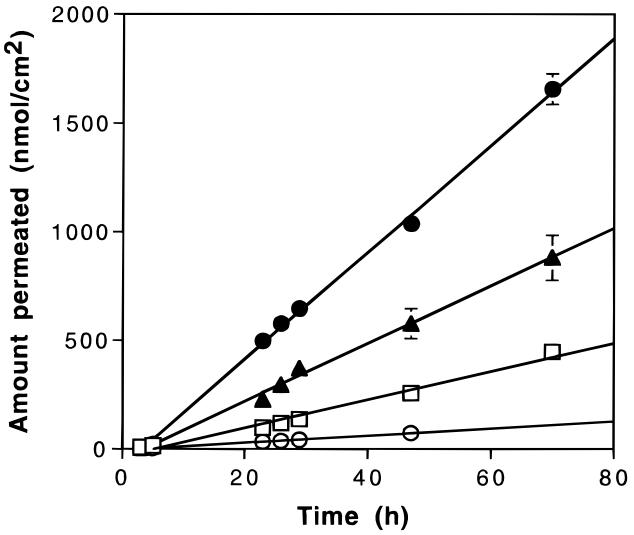


Figure 2. Permeation profiles (mean ± SE; n = 3−12) for naproxen (□) and prodrugs **3c** (●) and **3f** (▲) through excised human skin in 0.05 M phosphate buffer (pH 7.4) vehicle and for naproxen (○) in 0.05 M phosphate buffer (pH 5.0) vehicle. The data represent the sum of naproxen, the intermediate, and its prodrug.

Table 3. Steady-State Fluxes (J_{ss}) and Permeability Coefficients (K_p) (mean ± SE; n = 3−12) for Delivery of Total Naproxen Species through Excised Human Skin in Vitro in Isotonic Phosphate Buffer (0.05 M, pH 7.4 and 5.0) at 37 °C

	J_{ss} (nmol/cm^2·h)		$K_p \times 10^6$ (cm/h)	
compd	pH 7.4	pH 5.0	pH 7.4	pH 5.0
naproxen	6.5 ± 0.6	1.6 ± 0.2	0.06 ± 0.01	4.07 ± 0.47
3a	0.7 ± 0.0[a]	0.6 ± 0.0[a]	9.59 ± 0.27[a]	12.56 ± 0.77[a]
3c	24.6 ± 1.0[a]	2.2 ± 0.1	6.00 ± 0.25[a]	0.41 ± 0.02[a]
3d	7.2 ± 0.1	0.2 ± 0.0[a]	0.82 ± 0.01[a]	0.02 ± 0.00[a]
3e	7.7 ± 1.3	0.6 ± 0.0[a]	0.02 ± 0.00[a]	0.004 ± 0.000[a]
3f	13.2 ± 1.6[a]	1.2 ± 0.2	0.27 ± 0.03[a]	0.02 ± 0.00[a]

[a] p < 0.05 compared to naproxen (ANOVA, Fisher's PLSD test).

The methylpiperazinylacyloxyalkyl prodrugs of naproxen (**3c**−**f**) permeated better from saturated solutions at pH 7.4 than at pH 5.0. Because the aqueous solubilities of prodrugs **3c**−**f** generally showed small differences at different pH values except for **3e**, the higher partition coefficients at pH 7.4 favor permeation at this pH rather than at pH 5.0 (Table 1). At pH 7.4 the best prodrug (**3c**) afforded almost a 4-fold higher flux when compared to naproxen itself. At pH 5.0 the methylpiperazinylacyloxyalkyl prodrugs **3c**−**f** permeated the skin with an order of magnitude similar to that of naproxen itself at pH 5.0. The only tested morpholinylacyloxyalkyl prodrug (**3a**) exhibited similar fluxes at pH 5.0 and 7.4 which were, however, lower than the flux of naproxen. This is most probably due to the poor aqueous solubility of **3a**.

It is interesting to note that the flux of naproxen at pH 7.4 is lower than that of **3c,** although a 25 times higher concentration of naproxen was used in the donor compartment, due to a higher intrinsic aqueous solubil-

ity of naproxen. The permeability coefficients of the prodrugs which, unlike flux, are independent of donor concentration were 0.3−160- and 0.001−3-fold higher than that of naproxen at pH 7.4 and 5.0, respectively. The permeability of these compounds in aqueous solutions increased as the solubility decreased. Thus, **3a**, having the lowest aqueous solubility among all prodrugs, showed the highest permeability coefficient while its value for flux was much lower than that for any other compound. Therefore, the flux, which measures the mass of material transported through the skin, is a more relevant parameter, therapeutically, than the permeability coefficient. Finally, comparing the flux and solubilities of these compounds confirms earlier observations which indicated that an effective prodrug for topical drug delivery must possess good biphasic solubility characteristics: i.e., adequate aqueous solubility as well as lipophilicity over the parent drug.[21,22,29]

In conclusion, the present study shows that the permeation of naproxen through human skin can be markedly improved by using bioreversible methylpiperazinylacyloxyalkyl prodrugs of naproxen. The ionizable basic prodrugs combine the desirable aqueous solubility and lipophilicity for skin permeation, which can vary widely by changing the acyl group. Furthermore, these prodrugs were rapidly bioconverted to the parent drug in human serum. These properties make these novel methylpiperazinylacyloxyalkyl esters promising prodrugs for improved topical delivery of naproxen.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 WB operating at 400.1 MHz, and chemical shifts are reported in parts per million (δ) using TMS as the internal standard. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, qui = quintet, m = multiplet, dt = doublet of triplets, bs = broad singlet, bm = broad multiplet. Electron impact (EI) mass spectra of the prodrugs were determined by a VG 70-250SE magnetic sector mass spectrometer (VG Analytical, Manchester, U.K.). Flash chromatography was accomplished using silica gel (30−60 μm, J. T. Baker 7024-02). Thin-layer chromatography (TLC) analyses of reactions were run on aluminum foil plates coated with silica gel 60 F_{254} (Merck). Elemental analysis was carried out by a Perkin-Elmer Series II CHNS/O Analyzer 2400.

All reagents were obtained from commercial suppliers and were used without further purification.The hydroxyalkyl esters of naproxen (**2a**,**b**) were synthesized and identified as described earlier.[12]

2-[(4-Morpholinyl)acetyloxy]ethyl 2-(6-Methoxy-2-naphthyl)propanoate (3a). To a solution of 2-hydroxyethyl 2-(6-methoxy-2-naphthyl)propanoate (**2a**) (0.32 g, 1.2 mmol), 4-morpholinylacetic acid (0.18 g, 1.2 mmol), and DMAP (40 mg, 0.2 mmol) in dry CH_2Cl_2 (15 mL) was added DCC (0.41 g, 2.0 mmol) and the mixture was stirred at 30 °C for 3 days. The precipitated dicyclohexylurea (DCU) was filtered, and the filtrate was evaporated. The resulting residue was purified by flash silica gel column chromatography, eluting with 5% MeOH in CH_2Cl_2 and affording **3a** as a white solid (0.19 g, 40%): mp 84.9−85.6 °C; TLC R_f 0.56 (5% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72−7.11 (6H, m, aromatic), 4.27 (4H, m, OCH_2CH_2O), 3.91 (3H, s, CH_3O) 3.86 (1H, q, J = 7.1 Hz, C*H*Me), 3.69 (4H, m, CH_2OCH_2), 3.00 (2H, s, −CH_2COO), 2.43 (4H, m, CH_2NCH_2), 1.58 (3H, d, J = 7.1 Hz, CH_3C); HRMS *m*/*z* 401.1918, calcd for $C_{22}H_{27}NO_6$ 401.1838. Anal. C: calcd, 65.82; found, 66.74; H, N.

4-[(4-Morpholinyl)acetyloxy]butyl 2-(6-Methoxy-2-naphthyl)propanoate (3b). 3b was prepared as described for **3a** from **2b** (0.96 g, 3.2 mmol) and 4-morpholinylacetic acid (0.46 g, 3.2 mmol). Flash chromatography (5% MeOH in CH_2Cl_2) gave **3b** as a viscous oil (0.27 g, 20%): TLC R_f 0.68 (5% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72−7.11 (6H, m, aromatic), 4.10 (2H, t, J = 6.1 Hz, OCH_2−), 4.06 (2H, t, J = 6.1 Hz, OCH_2−), 3.92 (3H, s, CH_3O), 3.85 (1H, q, J = 7.1 Hz, C*H*Me), 3.74 (4H, t, J = 4.7 Hz, CH_2OCH_2), 3.15 (2H, s, −CH_2-COO), 2.55 (4H, t, J = 4.6 Hz, CH_2NCH_2), 1.62 (4H, m, CCH_2CH_2C), 1.58 (3H, d, J = 7.2 Hz, CH_3C); HRMS *m*/*z* 429.2281, calcd for $C_{24}H_{31}NO_6$ 429.2151. Anal. ($C_{24}H_{31}NO_6$·0.5H_2O) C, H, N.

2-[(4-Methyl-1-piperazinyl)acetyloxy]ethyl (6-Methoxy-2-naphthyl)propanoate (3c). 3c was prepared as described for **3a** from **2a** (0.39 g, 1.4 mmol) and (4-methyl-1-piperazinyl)-acetic acid (0.24 g, 1.5 mmol), DMAP (70 mg, 0.6 mmol), and DCC (0.44 g, 2.1 mmol). The flash chromatography (20% MeOH in CH_2Cl_2) provided **3c** as a viscous oil (0.41 g, 71%): TLC R_f 0.45 (50% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71−7.11 (6H, m, aromatic), 4.29 (4H, m, OCH_2CH_2O,), 3.91 (3H, s, CH_3O), 3.84 (1H, q, J = 7.1 Hz, C*H*Me), 3.02 (2H, s, −CH_2COO), 2.54 (8H, bm, N(CH_2CH_2)$_2$N), 2.33 (3H, s, NCH_3), 1.56 (3H, d, J = 7.1 Hz, CH_3C); HRMS *m*/*z* 414.2208, calcd for $C_{24}H_{31}NO_6$ 414.2155. Anal. ($C_{23}H_{30}N_2O_5$·0.8H_2O) C, H; N: calcd, 6.53; found, 5.97.

4-[(4-Methyl-1-piperazinyl)acetyloxy]butyl 2-(6-Methoxy-2-naphthyl)propanoate (3d). 3d was prepared as described for **3a** from **2b** (0.85 g, 2.8 mmol) and (4-methyl-1-piperazinyl)acetic acid (0.44 g, 2.8 mmol). Flash chromatography (10% MeOH in CH_2Cl_2) gave **3d** (0.50 g, 40%) as a viscous oil: TLC R_f 0.42 (50% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71−7.10 (6H, m, aromatic), 4.07 (4H, m, OCH_2−), 3.91 (3H, s, CH_3O), 3.84 (1H, q, J = 7.1 Hz, C*H*Me), 3.16 (2H, s, −CH_2COO), 2.6−2.5 (8H, bm, N(CH_2CH_2)$_2$N), 2.33 (3H, s, NCH_3), 1.62 (4H, m, CCH_2CH_2C), 1.56 (3H, d, J = 7.1 Hz, CH_3C); HRMS *m*/*z* 442.2569, calcd for $C_{25}H_{34}N_2O_5$ 442.2468. Anal. ($C_{25}H_{34}N_2O_5$·0.5H_2O) C, H, N.

4-[3-(4-Methyl-1-piperazinyl)propionyloxy]butyl 2-(6-Methoxy-2-naphthyl)propanoate (3e). 3e was prepared as described for **3a** from **2b** (2.0 g, 6.6 mmol) and 3-(4-methyl-1-piperazinyl)propionic acid (1.2 g, 6.9 mmol). Flash chromatography (50% MeOH in CH_2Cl_2) gave **3e** (2.45 g, 54%) as a viscous oil: TLC R_f 0.39 (50% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72−7.11 (6H, m, aromatic), 4.10 (1H, t, J = 6.4 Hz, CO_2CH_2−), 4.09 (1H, t, J = 6.1 Hz, CO_2CH_2−), 4.02 (2H, t, J = 6.1 Hz, CH_2O_2C−), 3.91 (3H, s, CH_3O) 3.85 (1H, q, J = 7.2 Hz C*H*Me), 2.66 (2H, t, J = 7.3 Hz, −CH_2-COO), 2.45 (2H, t, J = 7.5 Hz, CH_2N), 2.6−2.3 (8H, bm, N(CH_2-CH_2)$_2$N), 2.27 (3H, s, NCH_3), 1.7−1.5 (4H, m, CCH_2CH_2C), 1.57 (3H, d, J = 7.0 Hz, CH_3C); HRMS *m*/*z* 456.2728, calcd for $C_{26}H_{36}N_2O_5$ 456.2624. Anal. ($C_{26}H_{36}N_2O_5$·0.7H_2O) C, H, N.

4-[4-(4-Methyl-1-piperazinyl)butyryloxy]butyl 2-(6-Methoxy-2-naphthyl)propanoate (3f). 3f was prepared as described for **3a** from **2b** (1.0 g, 3.3 mmol) and 4-(4-methyl-1-piperazinyl)butyric acid (0.61 g, 3.3 mmol). Flash chromatography (MeOH) gave **3f** (1.17 g, 75%) as a viscous oil: TLC R_f 0.35 (50% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72−7.11 (6H, m, aromatic), 4.10 (1H, t, J = 6.4 Hz, CO_2CH_2−), 4.09 (1H, t, J = 6.1 Hz, CO_2CH_2−), 4.00 (2H, t, J = 6.2 Hz, CO_2CH_2−), 3.91 (3H, s, CH_3O) 3.85 (1H, q, J = 7.2 Hz, C*H*Me), 2.6−2.3 (8H, bm, N(CH_2CH_2)$_2$N), 2.33 (2H, t, J = 7.3 Hz, −CH_2-COO), 2.29 (2H, m, CH_2N), 2.27 (3H, s, NCH_3), 1.78 (2H, m, J = 7.4 Hz, CCH_2C), 1.7−1.5 (4H, m, CCH_2CH_2C), 1.57 (3H, d, J = 7.3 Hz, CH_3C); HRMS *m*/*z* 470.2593, calcd for $C_{27}H_{38}N_2O_5$ 470.2781. Anal. ($C_{27}H_{38}N_2O_5$·0.5H_2O) C, H, N.

4-Morpholinylacetic Acid. Morpholine (5.5 g, 63 mmol) in 10 mL of benzene was added dropwise to a solution of ethyl bromoacetate (5.5 g, 33 mmol) in 10 mL of benzene and the solution was refluxed for 30 min.[30] After cooling, morpholine hydrobromide was filtered and the filtrate was evaporated to provide ethyl 4-morpholinylacetate (5.2 g, 91%) as a yellowish liquid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (3H, t), 2.58 (4H, t), 3.21 (2H, s), 3.75 (4H, t), 4.19 (2H, q). A portion of the above ester (3.3 g, in 75 mL of water) was refluxed for 30 h. Water was evaporated and the residue was recrystallized form

dichloromethane−methanol providing (2.42 g, 87%) white crystals: mp 161.5−161.9 °C (lit. mp 160−162 °C);[31] ^{1}H NMR (CD_3OD) δ 3.31 (4H, bs), 3.61 (2H, s), 3.92 (4H, bm).

(4-Methyl-1-piperazinyl)acetic acid was prepared from 4-methylpiperazine and ethyl bromoacetate using the procedure described above. The product was recrystallized from methanol−ether: mp 160−161 °C (lit. mp 159.5−161 °C);[32] ^{1}H NMR (CD_3OD) δ 2.56 (3H, s), 2.95 (4H, bm), 3.09 (4H, bm), 3.37 (2H, s).

3-(4-Methyl-1-piperazinyl)propanoic acid was prepared from methyl acrylate and 4-methylpiperazine by utilizing the same procedure as for 4-morpholinylpropanoic acid:[33] ^{1}H NMR (CD_3OD) δ 2.36 (3H, s), 2.52 (2H, t), 2.61 (4H, bm), 2.79 (4H, bm), 2.83 (2H, t).

3-(4-Methyl-1-piperazinyl)butyric acid was prepared by utilizing the same procedure as for 4-morpholinylacetic acid from 4-methylpiperazine and ethyl 4-bromobutyrate: mp 98 °C; ^{1}H NMR ($CDCl_3$) δ 1.82 (2H, qui) 2.35 (3H, s), 2.41 (2H, t), 2.56 (2H, t), 2.65 (8H, bm).

HPLC Analysis. The analytical high-performance liquid chromatography (HPLC) system consisted of a Merck Hitachi L-6200A intelligent pump, a Hewlett-Packard HP1046A programmable fluorescence detector (exitation 226 nm; emission 368 nm), a Merck Hitachi D-6000A interface module, a Merck Hitachi AS-2000 autosampler, and a Merck LaChrom column oven L-7350. For all sample separations a Purospher RP-C18 column (125 × 4 mm, 5 μm) was used. A mobile phase mixture of acetonitrile and a 0.02 M phosphate buffer solution of pH 5.0−5.5 at a flow rate of 1.2 mL/min were used. The ratio of solvents varied according to the compound.

Aqueous Solubility. The aqueous solubility of naproxen and its prodrugs was determined at room temperature in phosphate buffer (0.16 M) at the physiological pH 7.4 and at pH 5.0. The pH of 5.0 was selected for the aqueous solubility and partition coefficient determinations due to acidic conditions (pH $\sim$ 5)[34] on the outer surface of the skin. Excess amounts of each compound were added to 1 mL of buffer; the mixtures were stirred for either 60 min (pH 5.0) or 30 min (pH 7.4), filtered (Millipore 0.45 μm), and analyzed by the HPLC. The pH of the mixtures was held constant throughout the study.

Apparent Partition Coefficients. The apparent partition coefficients (log P_{app}) of naproxen and its prodrugs were determined at room temperature by a 1-octanol−phosphate buffer system at both pH 5.0 and 7.4. Before use, the 1-octanol was saturated with phosphate buffer for 24 h by stirring vigorously. A known concentration of compound in phosphate buffer was shaken for either 30 min (pH 5.0) or 15 min (pH 7.4), with a suitable volume of 1-octanol. After shaking, the phases were separated by centrifugation at 14000 rpm for 4 min. The concentrations of the compounds in the buffer phase before and after partitioning were determined by HPLC.

Hydrolysis in Aqueous Solution. The rates of chemical hydrolysis of prodrugs were studied in aqueous phosphate buffer solutions of pH 7.4 and 5.0 (0.16 M, ionic strength 0.5) at 37 °C. An appropriate amount of prodrug was dissolved in 10 mL of preheated buffer and the solutions were placed in a thermostatically controlled water bath at 37 °C. At appropriate intervals, samples were taken and analyzed for remaining prodrug by the HPLC. Pseudo-first-order half-time ($t_{1/2}$) for the hydrolysis of prodrug was calculated from the slope of the linear portion of the plotted logarithm of remaining prodrugs versus time.

Hydrolysis in Human Serum. The rates of enzymatic hydrolysis for naproxen prodrugs were studied in human serum at 37 °C (Institute of Public Health, University of Kuopio) which was diluted to 80% with 0.16 M phosphate buffer of pH 7.4. The reactions were initiated by dissolving an appropriate amount of prodrug in phosphate buffer, and preheated human serum was added. The solutions were kept in a water bath at 37 °C, and 0.5-mL aliquots of serum/buffer mixture were withdrawn and added to 1.0 mL of ethanol to precipitate protein from the serum. After immediate mixing and centrifugation for 10 min at 14000 rpm, the supernatant was analyzed for remaining prodrug and released naproxen by the HPLC. Pseudo-first-order half-time ($t_{1/2}$) for the hydrolysis of prodrug was calculated from the slope of the linear portion of the plotted logarithm of remaining prodrugs against time. The pseudo-first-order times, at which 50% of total parent compound had been formed ($f_{50\%}$), were determined from the linear slope of the logarithm of unformed parent compound ($\log(\text{parent compound}_{max} - \text{parent compound}_t)$) over time.[23]

In Vitro Skin Permeation. Samples of human skin were obtained from the abdominal region of adult human cadavers from the Kuopio University Hospital (Kuopio, Finland). The epidermis was isolated from the underlying dermis by heat separation at 60 °C in distilled water for 2 min, after which the skin specimens were dried and frozen prior to use. The permeation studies were carried out using the Franz-type diffusion cell (PermeGear, Inc., Riegel, PA) as previously described.[12] Skin specimens were rehydrated before being mounted in the diffusion cell. The receptor medium (0.05 M isotonic phosphate buffer solution of pH 7.4) was stirred and kept at 37 °C throughout the study. The compounds were applied as suspensions in 0.05 M phosphate buffer of pH 5.0 and 7.4 which had been prepared in the same way that the suspensions for determining the aqueous solubility had been prepared. At specified time intervals 250-μL aliquots were withdrawn from the receptor compartment and replaced with fresh buffer. The drug concentrations were assayed by HPLC. The steady-state flux for naproxen and its selected prodrug (**3a**,**c**−**f**) was determined by plotting the cumulative amount (in nmol) of the parent drug, intermediates and intact prodrug as measured in the receptor phase against time and dividing the slope of the steady-state position by the surface area of the diffusion cell (0.71 cm^2). The permeability coefficients of naproxen and selected prodrugs were calculated by dividing the steady-state flux by the saturation solubility of the compound in the corresponding vehicle.

Statistical Analysis. A one-factor analysis of variance (ANOVA factorial) was used to test the statistical significance of differences between the fluxes of naproxen and prodrugs. Significance in the differences in the means was tested using Fisher's protected least significance difference (PLSD) at 95% confidence.

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