Synthesis and Evaluation of Ketorolac Ester Prodrugs for Transdermal Delivery

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ABSTRACT: Alkyl esters of ketorolac were synthesized as potential prodrugs for transdermal delivery and evaluated to determine the relationship between their skin permeation characteristics and their physicochemical properties. Solubility of the prodrugs in various vehicles was determined at room temperature while lipophilicity was obtained as 1-octanol/water partition coefficients (log*P*) and capacity factors (k') using HPLC. Metabolism of the prodrugs to ketorolac was studied both in rat skin homogenate and in plasma. Rat skin permeation characteristics of the prodrugs saturated in propylene glycol were investigated using the Keshary-Chien permeation system at 37°C. An increase in log*P* and capacity factor values of the prodrugs were observed in proportion to their alkyl chain length. Good linear relationship between the log*P* values and capacity factor was observed ($r^2 = 0.92$). Prodrugs were rapidly degraded to ketorolac both in the skin homogenate and in plasma following a first-order kinetics. To determine accurate amounts of prodrug permeated, both the prodrug and parent drug concentration in the receptor solution were determined in mole units. The skin permeation rate of the alkyl ester prodrugs was significantly higher with a shorter lag time than that of ketorolac. The permeation rate of ketorolac reached maximum in its 1-propyl ester form as 46.61 nmol/cm^2/h, and a parabolic relationship was observed between the permeation rate and the log*P* values of the prodrugs. Alkyl ester prodrugs of ketorolac having optimum lipophilicity could improve the transdermal delivery of ketorolac.

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Keywords: ketorolac; alkyl ester prodrug; transdermal delivery

INTRODUCTION

Ketorolac is a nonsteroidal anti-inflammatory drug (NSAID) for controlling moderate to severe pain by inhibiting the synthesis of prostaglandins.[1,2] Although oral bioavailability of ketorolac was reported to be 90% with a very low hepatic first-pass elimination, the biologic half-life of 4–6 h calls for frequent administration to maintain the therapeutic effect.[3] Long-term use of ketorolac may result in gastrointestinal ulceration and acute renal failure.[4]

Potential advantages associated with transdermal delivery are well documented, which include decrease in side effects, the avoidance of hepatic first-pass elimination, the relative ease of drug

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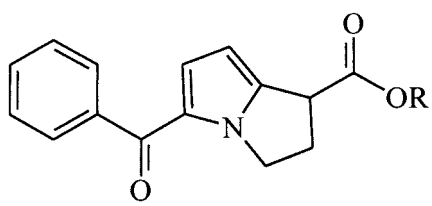
input termination in problematic cases, and the maintenance of constant therapeutic blood level.[5] Nevertheless, transdermal drug delivery has always been challenged by the formidable barrier property of the intercellular lipid bilayer in the stratum corneum. Drug candidates for transdermal delivery should have suitable molecular weight of around 200–500 dalton, appropriate lipophilicity, and high potency. Although ketorolac has high analgesic activity and a small molecular weight, its log*P* value is reported to be only 0.9, which is not lipophilic enough to pass through the lipid bilayer of the stratum corneum.[6] There have been several attempts to develop a transdermal delivery system of ketorolac using permeation enhancers,[7] various vehicle systems,[8,9] and electrotransport-facilitated transdermal systems.[10] However, these approaches were not very successful probably because of the inherently low lipophilicity of the drug itself. Roy et al. attempted to use the prodrugs approach and reported a relatively higher skin flux with the ethyl ester derivative of ketorolac.[11] Moreover, previous studies showed a characteristic parabolic relationship between the skin permeability and lipophilicity of various drugs with maximum permeability at a log*P* of approximately 3–4.[12,13] Herein we report on a systematic investigation of a series of alkyl ester prodrugs (methyl, ethyl, isopropyl, 1-propyl, isobutyl, 1-butyl, 1-pentyl ester) of ketorolac with emphasis on their correlation between the lipophilicity and the permeability (Figure 1).



R	NAME
-H	Ketorolac
$-CH_3$	k-001
$-CH_2CH_3$	k-002
$-CH(CH_3)_2$	k-003
$-CH_2CH_2CH_3$	k-004
$-CH_2CH(CH_3)_2$	k-005
$-CH_2(CH_2)_2CH_3$	k-006
$-CH_2(CH_2)_3CH_3$	k-007

Figure 1. Chemical structure of ketorolac and its alkyl ester prodrugs.

EXPERIMENTAL

Materials

Chemicals

Ketorolac and its alkyl ester prodrugs were synthesized in the Medicinal Chemistry Laboratory of Chonnam National University (Kwangju, South Korea). High-performance liquid chromatography grade methanol and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). Solvents for HPLC were filtered through 0.22-μm filters and thoroughly degassed in an ultrasonic bath before use. All other reagents were analytical grade and used without further purification.

Animal Model

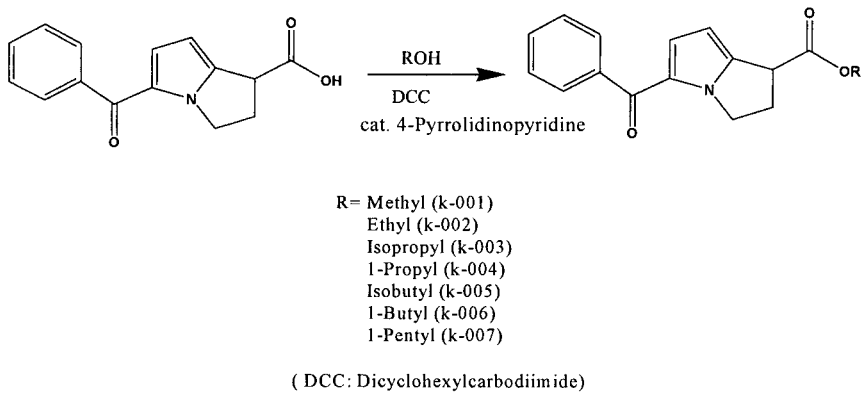
Male Sprague-Dawley rats were obtained from Dae-Han Laboratory Animal Research Center Co. (Dae-Jeon, South Korea). These rats, weighing approximately 220–250 g, were used both in the *in vitro* skin permeation studies and in the stability studies in the skin homogenate and rat plasma.

Synthesis of Ketorolac Ester Prodrugs

Ketorolac alkyl esters were synthesized by adding *N,N′*-dicyclohexylcarbodiimide (DCC) to ketorolac together with the corresponding alcohols in the presence of 4-pyrrolidinopyridine, as shown in Scheme 1.[14] Nuclear magnetic resonance spectra (^{1}H-NMR, ^{13}C-NMR) were recorded on a Varian 300 spectrometer, using TMS as the internal standard; chemical shifts were reported in parts per million (δ) and ^{1}H-NMR signals were quoted as s (singlet), d (doublet), t (triplet), and m (multiplet). IR spectra were recorded on a Perkin-Elmer 783 spectrometer and a Nicolet instrument. Solvents were routinely distilled prior to use. Column chromatography was performed on Merck silica gel 60 (70-230 mesh). TLC was carried out using plates coated with silica gel 60F 254 purchased from Merck Co. Reagents were obtained from commercial suppliers and were used without purification. Ketorolac sodium was dissolved in water and acidified with *c*-HCl to give the suspension, which was collected and dried *in vacuo* to give ketorolac as a white solid.

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid methyl ester (k-001)

To a stirred solution of ketorolac (7.0 g, 27.4 mmol) in CH_2Cl_2 (50 mL), *N,N′*-dicyclohexylcarbodii-



Scheme 1. Synthesis of ketorolac ester prodrugs.

mide (1.0 M in CH_2Cl_2, 34 mmol) and 4-pyrrolidinopyridine (50 mg) and MeOH (2.6 g, 80 mmol) were added consecutively at room temperature. The reaction mixture was stirred for 30 min and the resulting suspension (*N,N′*-dicyclohexyl urea) was filtered off. The filtrate was washed with water and 10% acetic acid. The organic layer was washed again with brine and dried over sodium sulfate. The concentrated residue was purified by column chromatography on SiO_2 with CH_2Cl_2 to give 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid methyl ester (6.6 g, 89%) as yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.82–7.42$ (5H, *m*, Ar–H), 6.82 (1H, *d*, $J = 4.0$ Hz, CH=CH), 6.10 (1H, *d*, $J = 4.0$ Hz, CH=CH), 4.61–4.53 (2H, *m*, NCH_2–), 4.48–4.39 (1H, m, –CH–), 3.76 (3H, s, –OMe), 2.96–2.78 (2H, m, CH_2). ^{13}C-NMR (75.5 MHz, $CDCl_3$) $\delta = 184.9$, 171.7, 142.3, 139.2, 131.4, 128.9, 128.8, 128.2, 128.1, 124.9, 103.1, 52.6, 47.5, 42.4, 33.9, 31.0. IR (neat) $cm^{-1} = 1740$ (C=O).

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid ethyl ester (k-002)

The ethyl ester (k-002) was prepared as described for k-001. Ketorolac (1.301 g, 5.1 mmol) in CH_2Cl_2 (50 mL) with the addition of *N,N′*-dicyclohexylcarbodiimide (6 mL of 1.0 M in CH_2Cl_2, 6 mmol), 4-pyrrolidinopyridine (16 mg) and EtOH (580 mg, 12.6 mmol) gave 5-benzoyl-2, 3-dihydro-1*H*-pyrrolizine-1-carboxylic acid ethyl ester (1.126 g, 78%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.81–7.40$ (5H, *m*, Ar–H), 6.81 (1H, *d*, $J = 4.0$ Hz, CH=CH), 6.10 (1H, *d*, $J = 4.0$ Hz, CH=CH), 4.57–4.40 (2H, *m*, NCH_2), 4.24–4.16 (2H, *m*, OCH_2), 4.06–4.01 (1H, *m*, –CH–), 2.94–2.74 (2H, *m*, CH_2) 1.28 (3H, *t*, $J = 7.1$ Hz, H-17). ^{13}C-NMR (75.5 MHz, $CDCl_3$) $\delta = 187.7$, 174.0, 145.4, 142.1, 134.2, 131.5, 131.0, 130.0, 127.8, 105.9, 64.3, 50.4, 45.4, 34.4, 17.0. IR (neat) $cm^{-1} = 1735$ (C=O).

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid isopropyl ester (k-003)

The isopropyl ester (k-003) was prepared as described for k-001. Ketorolac (166 mg, 0.65 mmol) in CH_2Cl_2 (10 mL) and *N,N′*-dicyclohexylcarbodiimide (0.8 mL of 1.0 M in CH_2Cl_2, 0.8 mmol), 4-pyrrolidinopyridine (16 mg), and isopropanol (120 mg, 2 mmol) gave 5-benzoyl-2, 3-dihydro-1*H*-pyrrolizine-1-carboxylic acid isopropyl ester (144 mg, 74%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.80–7.77$ (5H, *m*, Ar–H), 7.42 (1H, *d*, $J = 4.0$ Hz, CH=CH), 6.77(1H, *d*, $J = 4.0$ Hz, CH=CH), 5.02 (1H, *m*, –CH–), 4.53–4.34 (2H, m, $-NCH_2-$), 3.96 (1H, *dd*, $J = 8.9$, $J = 6.0$ Hz, –CH–), 2.87–2.65 (2H, m, $-CH_2-$), 1.25–1.16 (6H, *m*, $-CHMe_2$). ^{13}C-NMR (75.5 MHz, $CDCl_3$) $\delta = 183.9$, 169.9, 142.2, 138.8, 130.7, 128.3, 127.5, 126.5, 124.2, 102.4, 68.2, 47.0, 42.2, 30.0, 21.2. IR (neat) $cm^{-1} = 1733$ (C=O).

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid propyl ester (k-004)

The 1-propyl ester (k-004) was prepared as described for k-001. Ketorolac (510 mg, 2 mmol) in CH_2Cl_2 (20 mL) with *N,N′*-dicyclohexylcarbodiimide (2.4 mL of 1.0 M in CH_2Cl_2, 2.4 mmol), 4-pyrrolidinopyridine (5 mg), and *n*-propanol (380 mg, 6.34 mmol) gave 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid propyl ester (490 mg, 82%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.80–7.77$ (2H, *m*, Ar–H), 7.46–7.36 (3H, *m*, Ar–H), 6.78 (1H, *d*, $J = 4.0$ Hz, CH=CH), 6.08 (1H, *d*, $J = 4.0$ Hz, CH=CH), 4.52–4.38 (2H, *m*, $-NCH_2-$), 4.08–4.06 (2H,

m, OCH_2), 4.04–4.00 (1H, *m*, –CH–), 2.88–2.68 (2H, *m*, –CH_2–), 1.66–1.63 (2H, *m*, –CH_2–), 0.94–0.89 (3H, *t*, $J = 7.4$ Hz, CH_3). ^{13}C-NMR (75.5 MHz, $CDCl_3$) $\delta = 184.1, 170.6, 142.1, 138.7, 130.8, 128.3, 127.6, 126.5, 124.3, 102.5, 66.4, 47.0, 42.0, 30.3, 21.4, 9.8$. IR (neat) $cm^{-1} = 1732$ (C=O).

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid isobutyl ester (k-005)

The isobutyl ester (k-005) was prepared as described for k-001. Ketorolac (510 mg, 2 mmol) in CH_2Cl_2 (20 mL) with *N,N′*-dicyclohexylcarbodiimide (2.4 mL of 1.0 M in CH_2Cl_2, 2.4 mmol), 4-pyrrolidinopyridine (5 mg), and isobutanol (370 mg, 5.0 mmol) gave 5-benzoyl-2, 3-dihydro-1*H*-pyrrolizine-1-carboxylic acid isobutyl ester (460 mg, 74%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.82–7.79$ (2H, *m*, Ar–H), 7.50–7.40 (3H, *m*, Ar–H), 6.81(1H, *d*, $J = 4.0$ Hz, CH=CH), 6.10 (1H, *d*, $J = 4.0$ Hz, CH=CH), 4.56–4.54 (1H, *m*, –CH–), 4.07–4.05 (1H, *m*, –CH–), 3.96–3.94 (2H, *m*, OCH_2), 2.96–2.76 (2H, *m*, –CH_2–), 2.02–1.92 (1H, *m*, –CH–), 0.94 (6H, *d*, $J = 6.7$ Hz, CH_3). IR (neat) $cm^{-1} = 1737$ (C=O).

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid butyl ester (k-006)

The 1-butyl ester (k-006) was prepared as described for k-001. Ketorolac (510 mg, 2 mmol) in CH_2Cl_2 (20 mL) with *N,N′*-dicyclohexylcarbodiimide (2.4 mL of 1.0 M in CH_2Cl_2, 2.4 mmol), 4-pyrrolidinopyridine (5 mg), and *n*-butanol (370 mg, 5.0 mmol) gave 5-benzoyl-2, 3-dihydro-1*H*-pyrrolizine-1-carboxylic acid butyl ester (500 mg, 80%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.80–7.77$ (2H, *m*, Ar–H), 7.46–7.36 (3H, *m*, Ar–H), 6.79 (1H, *d*, $J = 4.0$ Hz, CH=CH), 6.08 (1H, *d*, $J = 4.0$ Hz, CH=CH), 4.52–4.50 (1H, *m*, –CH–), 4.37–4.39 (1H, *m*, –CH–), 4.12–4.14 (2H, *m*, OCH_2), 4.03–4.00 (1H, *m*, –CH–), 2.87–2.69 (2H, *m*, –CH_2–), 1.59–1.57 (2H, *m*, –CH_2–), 1.40–1.33 (2H, *m*, –CH_2–), 0.93–0.88 (3H, *t*, $J = 7.2$ Hz, CH_3). ^{13}C-NMR (75.5 MHz, $CDCl_3$) $\delta = 184.0, 170.5, 141.9, 138.6, 130.6, 128.2, 127.4, 126.4, 124.2, 102.4, 64.5, 46.9, 41.9, 30.1, 29.9, 18.4, 13.0$. IR (neat) $cm^{-1} = 1736$ (C=O).

5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid pentyl ester (k-007)

The 1-pentyl ester (k-007) was prepared as described for k-001. Ketorolac (510 mg, 2 mmol) in CH_2Cl_2 (20 mL) with *N,N′*-dicyclohexylcarbodiimide (2.4 mL of 1.0 M in CH_2Cl_2, 2.4 mmol), 4-pyrrolidinopyridine (5 mg), and *n*-pentanol (440 mg, 5.0 mmol) gave 5-benzoyl-2, 3-dihydro-1*H*-pyrrolizine-1-carboxylic acid pentyl ester (450 mg, 69%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 7.82–7.79$ (2H, *m*, Ar–H), 7.51–7.41 (3H, *m*, Ar–H), 6.81 (1H, *d*, $J = 4.0$ Hz, CH=CH), 6.10 (1H, *d*, $J = 4.0$ Hz, CH=CH), 4.57–4.54 (1H, *m*, –CH–), 4.47–4.43 (1H, *m*, –CH–), 4.17–4.14 (2H, *m*, OCH_2), 4.06–4.04 (1H, *m*, –CH–), 2.91–2.79 (2H, *m*, –CH_2–), 1.68–1.64 (2H, *m*, –CH_2–), 1.35–1.28 (4H, *m*, –CH_2–), 0.92–0.88 (3H, *t*, CH_3). IR (neat) $cm^{-1} = 1736$ (C=O).

Determination of Lipophilicity

*Partition Coefficient (log**P**)*

Apparent 1-octanol/water partition coefficient (log*P*) of ketorolac and its alkyl ester prodrugs were determined at 32°C, as reported in the literature.[15] A 1-octanol/water mutual saturation was prepared for 12 h with gentle mechanical stirring, and then each phase was separated. Methanolic solution of ketorolac or its prodrugs (1 mg/mL) was placed in a glass tube appropriately. After completely evaporating the methanol, 1 mL of each saturated solvent was added to the tubes. After shaking the stoppered tube for 20 min at 150 rpm at 32°C (Shaking Incubator SI-600R), the phases were separated by centrifugation at 5000 g for 5 min. The concentration of the compound in each phase was determined by HPLC after appropriate dilution with methanol.

Capacity Factor (k′)

The k' values of ketorolac and its ester prodrugs were determined isocratically (50% methanol in acetate buffer, 1.0 mL/min, UV 314 nm) using HPLC. Retention times of each compound were measured, and k' values were calculated from the following equation:

$$k' = (t_i - t_0)/t_0$$

where t_0 is the retention time of methanol and t_i is the retention time of each compound.[16]

Determination of Solubility

The solubility of ketorolac and its ester prodrugs in water, isotonic phosphate buffer (IPB), and propylene glycol (PG) were measured at room temperature. Excess amounts of each compound were

added to 1 mL of each vehicle and the mixtures were vortexed for 10 min. After filtering through Minisart RC 4 filters (0.45 μm, Satorius, Germany), solutions were analyzed by HPLC after appropriate dilution with methanol.[17]

Stability Studies

Stability studies were conducted to confirm the conversion of various alkyl ester prodrugs to the parent drug, ketorolac, during the skin permeation process and/or in blood circulation. Skin homogenate was prepared from the freshly excised rat skin by adding 10-fold IPB (pH 7.4), and homogenized for 15 min in an ice bath. The supernatants were obtained after centrifugation for 20 min at $9000 \times g$. Rat plasma was withdrawn by heart puncture using a heparinized syringe and transferred into the heparinized tube. The supernatants were obtained after centrifugation for 20 min at $9000 \times g$. The degradation of prodrugs during the course of *in vitro* skin permeation study was also investigated using the skin extracts. Extractions of rat skin were conducted using Valia-Chien permeation cells as previously reported.[18] Briefly, freshly excised skin specimen was mounted between the two half-cells, with the stratum corneum facing the donor half-cell and the dermis facing the receptor half-cell. The donor half-cell was filled with PG, and the receptor half-cell was filled with IPB/ polyethylene glycol 400 mixture (60:40, v/v). The extraction of enzymes from the skin was carried out for 24 h, after which the donor and receptor solutions from each pair of half-cell were separately combined and stored in the freezer until used. Gentamicin (0.01%, w/v) was added to all solutions used for stability studies to inhibit the bacterial degradation of the ketorolac prodrugs. Alkyl ester prodrugs were spiked to make 10 μg/mL concentration in IPB (as control), skin homogenate, and plasma. These solutions were placed in a shaker (Shaking Incubator SI-600R, 150 rpm) at 37°C. At predetermined time intervals, the concentration of ester prodrugs in each solution was measured by HPLC. Skin homogenate and plasma samples were added with acetonitrile to precipitate protein. After immediate mixing and centrifugation for 1 min, the resulting clear supernatants were analyzed by HPLC. Considering the initial molar concentration of alkyl ester prodrugs as 100%, the remaining molar percentage of prodrugs in each solution was determined as a function of time.

Table 1. Physicochemical Properties of Ketorolac and Its Ester Prodrugs

Drugs	Side Chain	Molecular Weight (g/mol)	Solubility (μg/mL)			Log*P*	
			Water	IPB	PG	Calculated	Experimental
Ketorolac	—	255.27	183.01 (±10.79)	10509.41 (±153.40)	13.49 (±1.52) × 10^3	1.64	1.04 (±0.01)
k-001	Methyl	269.30	177.84 (±4.26)	135.54 (±2.39)	102.39 (±4.37) × 10^3	1.90	2.81 (±0.03)
k-002	Ethyl	283.32	35.77 (±1.36)	34.99 (±5.50)	112.70 (±6.13) × 10^3	2.24	2.97 (±0.02)
k-003	Isopropyl	297.35	15.13 (±0.49)	12.08 (±0.61)	129.37 (±1.59) × 10^3	2.56	3.55 (±0.04)
k-004	1-Propyl	297.35	33.49 (±3.46)	27.27 (±1.31)	136.37 (±1.63) × 10^3	2.73	3.73 (±0.01)
k-005	Isobutyl	311.37	1.23 (±0.23)	6.18 (±0.27)	196.20 (±12.44) × 10^3	3.12	4.10 (±0.01)
k-006	1-Butyl	311.37	4.15 (±0.75)	8.22 (±0.20)	133.15 (±3.17) × 10^3	3.14	4.22 (±0.02)
k-007	1-Pentyl	325.40	2.08 (±0.07)	3.05 (±0.02)	292.58 (±17.90) × 10^3	3.56	4.52 (±0.17)

Each data represents the mean ± SD of three determinations.

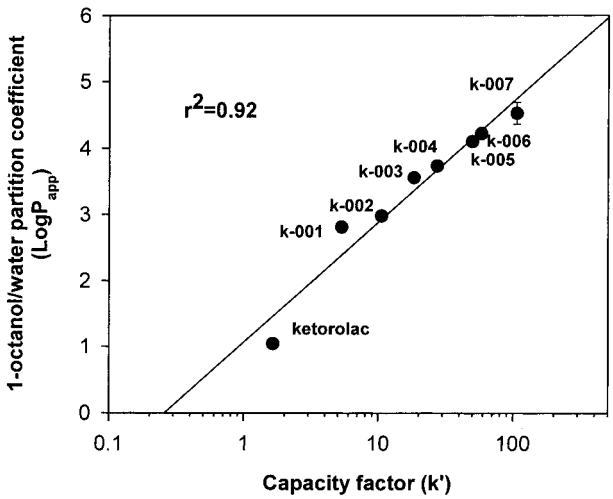


Figure 2. Relationship between the apparent 1-octanol/water partition coefficient (LogP_{app}) and the capacity factor (k') of ketorolac alkyl ester prodrugs.

Skin Permeation Studies

In vitro rat skin permeation studies were carried out with Keshary-Chien diffusion cells at 37°C. The effective area of diffusion was 2.14 cm^2. Freshly excised skin was mounted between the donor and receptor cells. The dermal side of the skin was exposed to the receptor solution (IPB: PEG 400 = 60:40), which was stirred magnetically and kept at a constant temperature of 37°C. After equilibration with the receptor solution for at least 30 min, the donor cells, faced with the stratum corneum surface, were filled with each saturated solution of ketorolac or its ester prodrugs in PG (3 mL). The donor cells were occluded with parafilm to prevent the invasion of other materials and vehicle evaporation. At predetermined time intervals, 1 mL of receptor solution was withdrawn and refilled with the same volume of fresh receptor solution. The samples were analyzed by HPLC.

Drug Analysis

The concentrations of ketorolac and its ester prodrugs were determined using an HPLC system equipped with a binary pump system (Gilson Model 305 and 306) and an automatic injector (Gilson Model 234). A Chromolith^{TM} column (RP-18e 100–4.6 mm, Merck, Germany) was used as an analytical column at ambient temperature. The mobile phase was a combination mixture of two organic solvents (methanol:acetonitrile = 10:25) and acetate buffer (0.05 M, pH 3.6). The ratio of mobile phase composition was controlled to accommodate the different retention times of prodrugs. The flow rate of mobile phase was 1.0 mL/min and all solutions to be analyzed were injected at a volume of 20 μL. The variable wavelength UV detector (Gilson Model 118) was set at 314 nm. Retention times of ketorolac and its alkyl ester prodrugs (methyl, ethyl, isopropyl, 1-propyl, isobutyl, 1-butyl, 1-pentyl) were 3.1, 5.7, 7.0, 11.5, 14.1, 20.1, 21.8, and 25.5 min, respectively, in a 50% buffer mixture.

RESULTS AND DISCUSSION

Physicochemical Characteristics

The physicochemical properties of ketorolac and its various ester prodrugs are summarized in Table 1. The molecular weights of ketorolac and its prodrugs ranged from 255.27 to 325.40 g/mol, which fell in the appropriate range for transdermal delivery.[19] Their lipophilicity were determined as

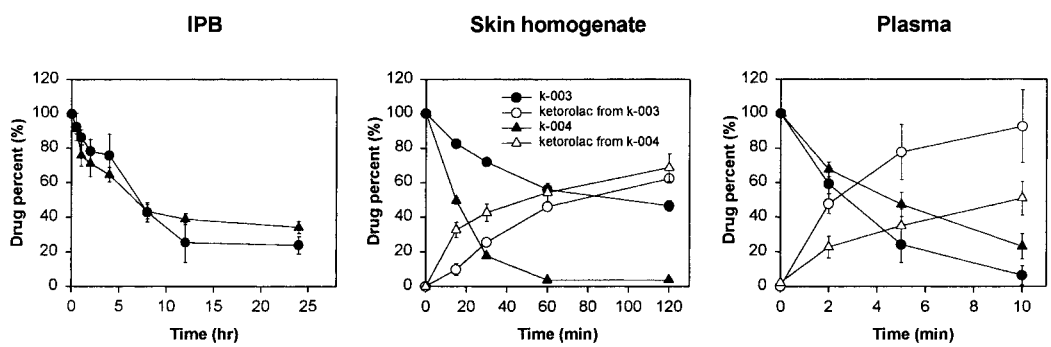


Figure 3. Stability of isopropyl prodrug (k-003) and 1-propyl prodrug (k-004) of ketorolac in rat skin homogenate and plasma compared to that in isotonic phosphate buffer (IPB, pH 7.4) at 37°C.

both the 1-octanol/water partition coefficients (log*P*) and the capacity factors (k'). The log*P* value of ketorolac was 1.04 (±0.01) which was close to the value of 0.9 (±0.1) found in literature.[6] As expected, as the alkyl chain length of the prodrug increased, the log*P* value of the alkyl ester prodrugs proportionally increased (methyl < ethyl < isopropyl < 1-propyl < sobutyl < 1-butyl < 1-pentyl). These fit well with the objective of this study, which was to synthesize more lipophilic prodrugs of ketorolac by attaching alkyl chains to improve the penetration in the stratum corneum, the structural barrier to the transdermal drug delivery. Similar to results obtained previously with testosterone ester derivatives,[20] the plot of log*P* versus capacity factor showed a good linear relationship with a correlation coefficient (r^2) of 0.92 (Figure 2). Determining the capacity factors instead of the log*P* values may be a more convenient alternative to estimating the lipophilicity of future analogs.

The solubility of ketorolac and its ester prodrugs in various vehicles was determined by HPLC after only 10 min vortexing to prevent the hydrolysis of the ester prodrugs. As expected, the alkyl ester prodrugs were less soluble in water as the alkyl chain length increased. The solubility in IPB showed a similar pattern to that in water. In PG, the solubility of ketorolac and its prodrugs increased in proportion to the alkyl chain length opposite to aqueous vehicles, and the solubility were 10^3-fold higher than in IPB and water. Compared to μg unit solubilities in water or IPB, this mg unit solubility of alkyl prodrugs in PG was enough to conduct the skin permeation study. Thus, PG was chosen as a vehicle for the following skin permeation study.

Stability of Ketorolac Ester Prodrugs

The conversion of ester prodrugs to the parent drug, ketorolac, was confirmed by the stability studies in the skin homogenate and plasma at 37°C using IPB as a control. Because all alkyl ester prodrugs were poorly soluble in aqueous vehicles, 10% PG was added to solubilize the prodrugs in the media for stability studies. The degradation of ester prodrugs of ketorolac followed first-order kinetics, and quantitatively converted to ketorolac. Figure 3 shows the representative stability of k-003 and k-004 in IPB, in skin homogenate, and in plasma. Table 2 shows the half-lives of the prodrugs in IPB, in skin homogenate, and in plasma. Half-lives of ketorolac prodrugs ranged from 0.21 to 2.45 h in the skin homogenate. In plasma, k-001, k-003, k-004 rapidly converted to ketorolac with half-lives of less than 5 min. It was not possible to determine the half-life of the other prodrugs in plasma since they converted to ketorloac in less than 1 min. Moreover, no degradation was observed for the parent drug ketorolac for 24 h under the same conditions in a preliminary study (data not shown). Although the conversion of alkyl ester prodrugs to ketorolac in the skin and plasma is proven from these results, the relationships between the stability of prodrugs and alkyl chain length were not apparent and needed further evaluation.

Table 2. Degradation Half-Life ($t_{1/2}$) of Ketorolac Ester Prodrugs in Rat Skin Homogenate, Plasma and Isotonic Phosphate Buffer (IPB, pH7.4) Solution at 37°C

Drugs	IPB $t_{1/2}$ (h)	Skin Homogenate $t_{1/2}$ (h)	Plasma $t_{1/2}$ (min)
k-001	23.25 (±1.95)	0.57 (±0.01)	1.65 (±0.13)
k-002	12.93 (±1.46)	0.28 (±0.03)	ND
k-003	7.20 (±0.79)	1.88 (±0.16)	2.57 (±0.91)
k-004	9.07 (±0.69)	0.21 (±0.00)	4.43 (±1.21)
k-005	8.47 (±0.79)	0.65 (±0.06)	ND
k-006	10.99 (±0.97)	1.32 (±0.13)	ND
k-007	23.78 (±7.10)	2.45 (±0.63)	ND

Each data represents the mean ± SD of three determinations.

ND; not determined due to rapid degradation (less than one minute).

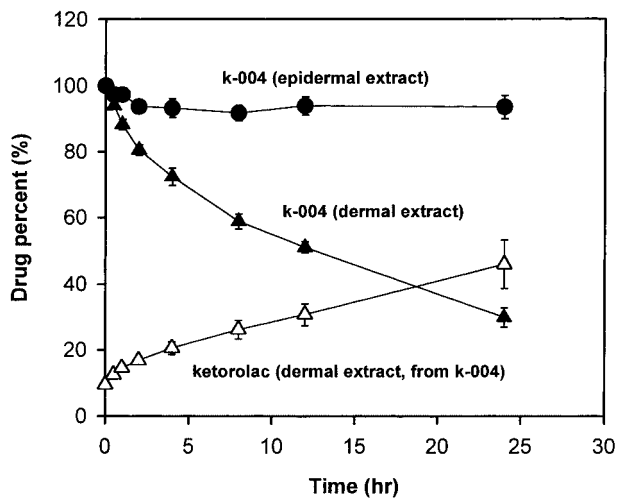


Figure 4. Stability of 1-propyl prodrug of ketorolac (k-004) in rat skin extracts for the skin permeation studies.

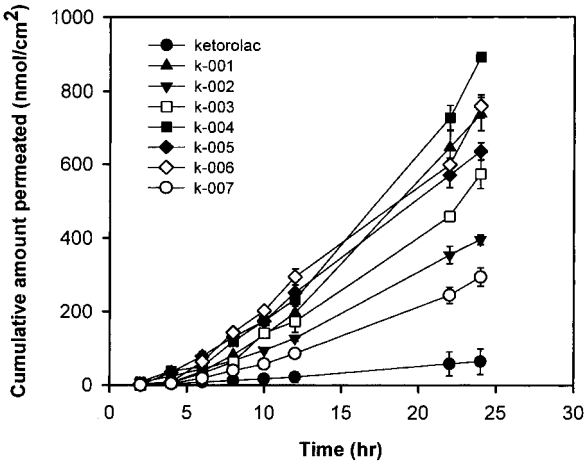


Figure 5. Rat skin permeation profiles of ketorolac and its alkyl ester prodrugs saturated in propylene glycol at 37°C.

The degradation of ester prodrugs during the course of *in vitro* skin permeation study was investigated using the skin extracts (Figure 4). Ketorolac ester prodrugs were stable in the epidermal extract for at least 24 h. Thus, no prodrug degradation in the donor cells was anticipated during the *in vitro* skin permeation study. However, ester prodrugs were unstable in the dermal extract, with half-lives ranging from 9.79 (k-005) ~43.26 (k-001) h. Measuring the cumulative amount of prodrugs in the receptor solution would not be accurate because part of the prodrugs, which permeated earlier, would already have been hydrolysed. Therefore, it is more reasonable to determine both the prodrug and the parent drug (ketorolac) in mole units from the receptor solution.

In Vitro Skin Permeation Study

Figure 5 shows the rat skin permeation profiles of ketorolac and its alkyl ester prodrugs saturated in PG, using Keshary-Chien permeation cells at 37°C. Saturated drugs in PG were used to keep a constant driving force with maximum thermodynamic activity. As shown in Figure 5, skin permeation of ester prodrugs was significantly enhanced compared to ketorolac. The skin permeation rates obtained from the slope of the linear portions of each plot are summarized in Table 3, together with the lag time and permeability coefficient. The maximum permeation rate of 46.61 nmol/cm^2/h was achieved for k-004 (1-propyl ester prodrug), which was 12-fold higher than that of ketorolac. Isopropyl ester prodrug (k-003) showed shorter lag time (0.17 h) than that of ketorolac (4.86 h) with significantly higher permeation rate. The ethyl ester prodrug (k-002) also showed a 10.46-fold higher permeation rate than ketorolac, which was significantly higher than that reported by Roy et al.[11]

It is interesting to note that the skin permeation rates of the prodrugs did not increase in proportion to the increase in lipophilicity. The skin is composed of a comparatively lipophilic stratum corneum and hydrophilic viable skin (epidermis and dermis). Although highly lipophilic drugs can easily get into the stratum corneum, the first structure barrier, they may not be able to escape out of the stratum corneum into the viable epidermis. Parabolic relationships between the skin permeability and log*P* values have been reported.[12,13] In this study, a similar parabolic relationship between the skin permeation rate and log*P* value was obtained (Figure 6). However, the isoforms of the ester prodrugs (k-003, k-005) showed a

Table 3. Rat Skin Permeation Parameters of Ketorolac and Its Prodrugs Saturated in Propylene Glycol at 37°C

Drugs	Permeation Rate (nmol/cm^2/h)	Lag Time (h)	Permeability Coefficient (cm/h) $\times 10^5$
Ketorolac	3.86 (±1.35)	4.86 (±1.58)	7.31 (±2.56)
k-001	19.51 (±1.05)	4.17 (±0.29)	5.13 (±0.28)
k-002	40.40 (±2.77)	6.09 (±0.11)	10.16 (±0.70)
k-003	30.19 (±2.05)	0.17 (±0.02)	6.94 (±0.47)
k-004	46.61 (±1.03)	5.80 (±0.09)	10.16 (±0.22)
k-005	36.37 (±0.81)	4.21 (±0.20)	5.77 (±0.13)
k-006	30.77 (±1.07)	3.68 (±0.13)	7.03 (±0.26)
k-007	14.77 (±1.19)	5.07 (±0.05)	1.64 (±0.13)

Each data represents the mean ± SD of three determinations.

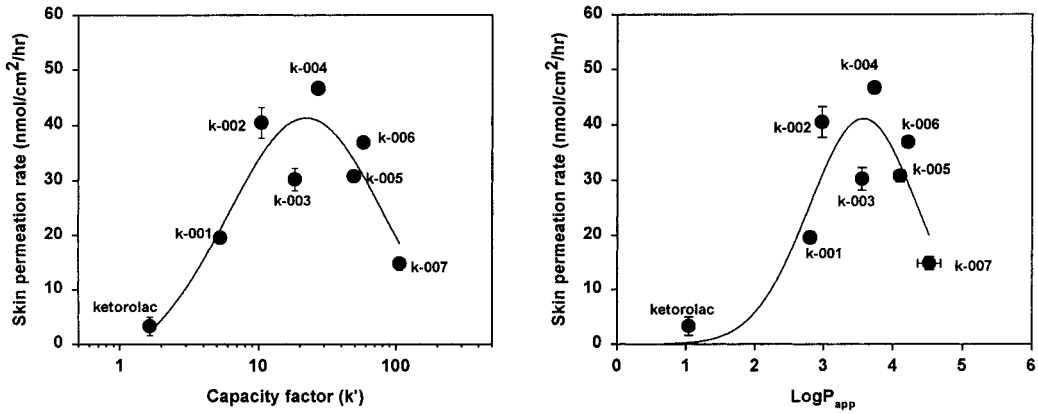


Figure 6. Relationship between the lipophilicity and the rat skin permeation rates of ketorolac ester prodrugs.

slight deviation from the parabolic relationship. The deviation may be due to the steric bulk of these isomers. For example, the lipophilicity constants (π) obtained by Hansch for the propyl and isopropyl moieties are reported to be 1.55 and 1.53, respectively, while the steric constants (Es) are -1.60 and -1.71, respectively.[21] Further study is being pursued to interpret the permeation profiles in terms of lipophilicity and steric properties.

CONCLUSIONS

Alkyl ester prodrugs of ketorolac were successfully synthesized. The lipophilicity of prodrugs increased as the alkyl chain length increased. The capacity factor (k') determined by HPLC showed good relationship with the apparent 1-octanol/water partition coefficient (log$P$). All alkyl ester prodrugs were rapidly converted to ketorolac in both the skin homogenate and plasma. In other words, alkyl ester prodrugs of ketorolac rapidly degraded to the parent form, ketorolac, during the skin permeation and/or in the blood circulation. However, prodrugs were stable in the skin extract of the epidermal side during the *in vitro* skin permeation study. Skin permeation of prodrugs significantly increased compared to the parent drugs. The maximum permeation rate was obtained with k-004 (1-propyl ester prodrug), which had a log$P$ value of 3.73. From these results, not only did we improve the transdermal delivery of ketorolac by synthesizing a series of alkyl ester prodrugs, but also observed that the alkyl ester prodrugs showed a parabolic relationship between the log$P$ values and the skin permeation rate.

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