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Ketorolac amide prodrugs for transdermal delivery: stability and in vitro rat skin permeation studies

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Abstract

Various amide prodrugs of ketorolac were synthesized and their rat skin permeation characteristics were determined. The solubility of the prodrugs in propylene glycol (PG) was determined at 37 °C while lipophilicity was obtained as 1-octanol/water partition coefficient (log *P*) and capacity factor (*k*′) using HPLC. Stability of the prodrugs in rat skin homogenate, plasma and liver homogenate was investigated to observe the enzymatic degradation. Rat skin permeation characteristics of the prodrugs saturated in PG were investigated using the Keshary-Chien permeation system at 37 °C. The log *P* value of the prodrugs increased up to 4.28 with the addition of various alkyl chain to ketorolac which has a log *P* of 1.04. Good linear relationship between log *P* and capacity factor was observed ($r^2 = 0.89$). Amide prodrugs were converted to ketorolac only in rat liver homogenate. However, the skin permeation rate of amide prodrugs did not significantly increase, probably due to their low aqueous solubility. Chemical modification of the ketorolac amide prodrug and/or the selection of proper vehicle to increase aqueous solubility would be necessary for an effective transdermal delivery of ketorolac.

Keywords: Ketorolac; Prodrug; Skin permeation; Amide; Transdermal delivery

1. Introduction

Ketorolac is a non-steroidal agent with potent analgesic and moderate anti-inflammatory activity.

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The mechanism of action involves the inhibition of prostaglandin synthesis (Dula et al., 2000; Guidera et al., 2001; Buckley and Brogden, 1990). In moderate to severe post-operative pain, clinical studies showed the single-dose efficacy of ketorolac to be greater than that of morphine, meperidine and pentazocine (Buckley and Brogden, 1990). However, despite having advantages like being free of potential addiction problems and respiratory depression and although the oral bioavailability is reported to be 90% with a very low hepatic first-pass metabolism, frequent dosing is required of ketorolac to maintain the therapeutic effect due to its short biological half-life (Buckley and Brogden, 1990). Long-term use of currently available dosage forms of ketorolac may result in gastrointestinal ulceration and acute renal failure (Reinhart, 2000).

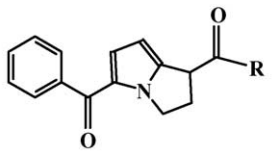
Transdermal delivery of ketorolac may, therefore, be the solution to reducing side effects associated with oral administration, and to maintaining constant therapeutic blood level for longer duration. Its high analgesic activity and low molecular weight makes ketorolac a good candidate for transdermal delivery. Several attempts to develop a transdermal delivery system of ketorolac using permeation enhancers, vehicles and iontophoresis have been reported. However, due to the inherently low lipophilicity of ketorolac, these approaches were not very successful (Yu et al., 1988; Roy et al., 1995; Roy and Manoukian, 1995). The log *P* value of 1.04 (Doh et al., 2003) is not suitable for the drug to pass through the lipid bilayer of the stratum corneum.

In our previous study, we synthesized and evaluated a series of alkyl ester prodrugs of ketorolac (methyl, ethyl, isopropyl, 1-propyl, isobutyl, 1-butyl and 1-pentyl ester), which brought about a significantly higher skin permeation rate with a shorter lag time than the parent drug (Doh et al., 2003). However, although the lipophilicity of ester prodrugs significantly increased, their physico-chemical instability due to hydrolysis was a hindrance to developing effective transdermal formulations. Generally, it is known that the amide bond exhibits greater physico-chemical stability than ester linkages. Herein, we report the synthesis and physicochemical properties of various ketorolac amide derivatives as potentially stable prodrugs for transdermal delivery.

2. Materials and methods

2.1. Materials

Ketorolac amide prodrugs were synthesized in the Medicinal Chemistry Laboratory of Chonnam National University. Ketorolac amide prodrugs were synthesized by adding *N*,*N*′-dicyclohexylcarbodiimide (DCC) to ketorolac together with the corresponding amines in the presence of 4-pyrrolidinopyridine (Bal-Tembe et al., 1989). The structures of these amides are shown in Fig. 1. Nuclear magnetic resonance spectra (^{1}H 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. HPLC grade methanol and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). Solvents for HPLC were filtered through 0.22 μm filters



R	NAME
-OH	Ketorolac
-NHC_6H_5	m-01
-$NHC_6H_5OCH_3$	m-02
-$NHCH_2C_6H_5$	m-03
-$NHCH_2C_6H_4OCH_3$	m-04
-$NHCH_2COOCH_3$	m-05
-$NHCH_2CH_2COOC_2H_5$	m-06
-$NHCH_2(CH_2)_2CH_3$	m-07
-NHC_6H_{10}	m-08
-$NHCH_2CH_2CH_3$	m-09
-$NHCH(CH_3)_2$	m-10
-$NH(CH_2)_3OCH_3$	m-11
-$NHCH_2CH{=}CH_2$	m-12

Fig. 1. Chemical structure of ketorolac and its amide prodrugs.

and degassed in an ultrasonic bath before use. All other reagents were analytical grade and used without further purification.

2.1.1. Synthetic experimental

Melting points were determined on an Electrothermal IA9200 melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian 300 spectrometer, using TMS as the internal standard; chemical shifts are reported in parts per million and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). IR spectra were recorded on a Perkin-Elmer 783 spectrometer and a Nicolet instrument using KBr pellets. Mass spectra were analyzed on Varian MS 1200.

2.1.1.1. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid phenyl amide (m-01). To a 250 ml round bottom flask that contains ketorolac (6.27 g, 25 mmol) and aniline (2.42 g, 25 mmol) in methylene chloride (80 ml) was added 25 ml of 1.0 M DCC in methylene chloride (25 mmol) and the reaction was refluxed for 30 min. After reaction was over, *N*,*N*-dicyclohexyl urea was removed by filtration. The filtrate was washed with water two times and then washed with 10% acetic acid after which the organic layer was washed with water again two times and then with brine and dried over sodium sulfate. After CH_2Cl_2 was evaporated off, the residue was separated by column chromatography on silica gel and recrystallized in ethanol to give compound m-01 as white solid (7.6 g, 92%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84–7.82 (2H, m, Ar–H), 7.81 (1H, b, NH), 7.55–7.43 (5H, m, Ar–H), 7.44–7.25 (2H, m, Ar–H), 7.14–7.12 (1H, m, Ar–H), 6.88 (1H, d, J = 4.0 Hz, H-9), 6.16 (1H, d, J = 4.0 Hz, H-10), 4.57–4.45 (2H, m, CH_2), 4.06 (1H, m, CH), 2.97 (2H, m, CH_2). IR (cm^{-1}) 3320 (NH), 1640 (CO). EIMS: *m*/*z* 330 (M^+, 100).

The following derivatives were prepared in a similar manner as described in the synthesis of m-01.

2.1.1.2. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid (4-methoxy-phenyl)-amide (m-02). Yield (82%), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84–7.81 (2H, m, Ar–H), 7.81 (1H, b, NH), 7.57–7.37 (5H, m, Ar–H), 6.88–6.82 (3H, m, Ar–H(2H), H-9), 6.16 (1H, d, J = 4.0 Hz, H-10), 4.59–4.40 (2H, m, CH_2), 4.07–4.02 (1H, m, CH), 3.77 (3H, s, OCH_3), 2.97 (2H, m, CH_2). IR (cm^{-1}) 3300 (NH), 1660 (CO). EIMS: *m*/*z* 360 (M^+, 56).

2.1.1.3. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid benzyl amide (m-03). Yield (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81–7.78 (2H, m, Ar–H), 7.53–7.18 (8H, m, Ar–H), 6.81 (1H, d, J = 4.0 Hz, H-9), 6.26 (1H, b, NH), 6.04 (1H, d, J = 4.0 Hz, H-10), 4.57–4.37 (4H, m, NCH_2), 3.96 (1H, m, CH), 2.86 (2H, m, CH_2). IR (cm^{-1}) 3280 (NH), 1640 (CO). EIMS: *m*/*z* 344 (M^+, 100).

2.1.1.4. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid (4-methoxy-benzyl)-amide (m-04). Yield (64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81–7.78 (2H, m, Ar–H), 7.55–7.50 (5H, m, Ar–H), 6.88–6.80 (3H, m, Ar–H(2H), H-9), 6.20 (1H, b, NH), 6.02 (1H, d, J = 4.0 Hz, H-10), 4.59–4.37 (4H, m, NCH_2), 3.93 (1H, m, CH), 3.78 (3H, s, OCH_3), 2.97 (2H, m, CH_2). IR (cm^{-1}) 3300 (NH), 1660, 1640 (CO). EIMS: *m*/*z* 374 (M^+, 87).

2.1.1.5. [(5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-amino]-acetic acid methyl ester (m-05). Yield (66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82–7.80 (2H, m, Ar–H), 7.57–7.43 (3H, m, Ar–H), 6.86 (1H, d, J = 4.0 Hz, H-9), 6.51 (1H, b, NH), 6.15 (1H, d, J = 4.0 Hz, H-10), 4.56–4.39 (2H, m, NCH_2), 4.06 (2H, m, CH_2CO), 4.02 (1H, m, CH), 3.76 (3H, s, CH_3), 2.91–2.89 (2H, m, CH_2). IR (cm^{-1}) 3277 (NH), 1753 ($COCH_3$), 1653 (CONH). EIMS: *m*/*z* 326 (M^+, 46).

2.1.1.6. 3-[(5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-amino]-propionic acid ethyl ester (m-06). Yield (57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.81 (2H, m, Ar–H), 7.54–7.43 (3H, m, Ar–H), 6.85 (1H, d, J = 4.0 Hz, H-9), 6.53 (1H, b, NH), 6.07 (1H, d, J = 4.0 Hz, H-10), 4.57–4.42 (2H, m, NCH_2), 4.16 (2H, q, J = 7.1 Hz, CH_2O), 3.91 (1H, t, J = 7.1 Hz, CH), 3.56–3.52 (2H, m, $NHCH_2$), 2.89–2.86 (2H, m, CH_2), 2.58–2.52 (2H, m, CH_2CO), 1.25 (3H, t, J = 7.1 Hz, CH_3). IR (cm^{-1}) 3292 (NH), 1726 ($COCH_2$), 1654 (CONH). EIMS: *m*/*z* 354 (M^+, 100).

2.1.1.7. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid butyl amide (m-07). Yield (76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84–7.81 (2H, m, Ar–H), 7.55–7.44 (3H, m, Ar–H), 6.85 (1H, d, J = 4.0 Hz, H-9), 6.07 (1H, d, J = 4.0 Hz, H-10), 5.84 (1H, b, NH), 4.57–4.42 (2H, m, NCH_2), 3.92 (1H, m, CH), 3.32 (2H, m, $NHCH_2$), 2.88–2.86 (2H, m, CH_2), 1.50–1.45 (2H, m, CH_2), 1.36–1.31 (2H, m, CH_2), 0.92 (3H, t, J = 7.2 Hz, CH_3). IR (cm^{-1}) 3284 (NH), 1632 (CONH). EIMS: *m*/*z* 310 (M^+, 94).

2.1.1.8. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid cyclohexyl amide (m-08). Yield (61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84–7.81 (2H, m, Ar–H), 7.55–7.44 (3H, m, Ar–H), 6.85 (1H, d, J = 4.0 Hz, H-9), 6.05 (1H, d, J = 4.0 Hz, H-10), 5.72 (1H, b, NH), 4.56–4.40 (2H, m, NCH_2), 3.89 (1H, m, CH), 3.78 (1H, m, NHCH), 2.00–1.10 (10H, m, CH_2). IR (cm^{-1}) 3302 (NH), 1648 (CONH). EIMS: *m*/*z* 336 (M^+, 100).

2.1.1.9. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid propyl amide (m-09). Yield (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.80 (2H, m, Ar–H), 7.54–7.43 (3H, m, Ar–H), 6.85 (1H, d, J = 4.0 Hz, H-9), 6.07 (1H, d, J = 4.0 Hz, H-10), 5.89 (1H, b, NH), 4.54–4.42 (2H, m, NCH_2), 3.92 (1H, m, CH), 3.28–3.20 (2H, m, $NHCH_2$), 2.94–2.86 (2H, m, CH_2), 1.50 (2H, q, J = 7.2 Hz, CH_2), 0.91 (3H, t, J = 7.2 Hz, CH_3). IR (cm^{-1}) 3324 (NH), 1630 (CONH). EIMS: *m*/*z* 296 (M^+, 45).

2.1.1.10. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid isopropyl amide (m-10). Yield (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.80 (2H, m, Ar–H), 7.54–7.43 (3H, m, Ar–H), 6.85 (1H, d, J = 4.0 Hz, H-9), 6.05 (1H, d, J = 4.0 Hz, H-10), 5.71 (1H, s, NH), 4.54–4.38 (2H, m, NCH_2), 4.13–4.08 (1H, m, NHCH), 3.88 (1H, m, CH), 2.92–2.84 (2H, m, CH_2), 1.16 (3H, d, J = 12 Hz, CH_3), 1.14 (3H, d, J = 12 Hz, CH_3). IR (cm^{-1}) 3324 (NH), 1630 (CONH). EIMS: *m*/*z* 296 (M^+, 77).

2.1.1.11. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid (3-methoxypropyl)-amide (m-11). Yield (76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.80 (2H, m, Ar–H), 7.54–7.43 (3H, m, Ar–H), 6.84 (1H, d, J = 4.0 Hz, H-9), 6.71 (1H, s, NH), 6.08 (1H, d, J = 4.0 Hz, H-10), 4.54–4.41 (2H, m, NCH_2), 3.93 (1H, t, J = 7.2 Hz, CH), 3.48–3.37 (4H, m, $NHCH_2CH_2O$), 3.39 (3H, s, OCH_3), 2.88 (2H, q, J = 7.2 Hz, CH_2), 1.80–1.72 (2H, m, CH_2). IR (cm^{-1}) 3324 (NH), 1630 (CONH). EIMS: *m*/*z* 326 (M^+, 100).

2.1.1.12. 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid allyl amide (m-12). Yield (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.80 (2H, m, Ar–H), 7.55–7.43 (3H, m, Ar–H), 6.85 (1H, d, J = 4.0 Hz, H-9), 6.09 (1H, d, J = 4.0 Hz, H-10), 5.96 (1H, s, NH), 5.82 (1H, m, CH), 5.19–5.12 (2H, m, CH=CH_2), 4.51–4.43 (2H, m, NCH_2), 3.98–3.87 (3H, m, CHCO and $NHCH_2$), 2.93–2.86 (2H, m, CH_2). IR (cm^{-1}) 3324 (NH), 1630 (CONH). EIMS: *m*/*z* 294 (M^+, 65).

2.2. Animal model

Male Sprague–Dawley rats (220–250 g) were obtained from Dae-Han Laboratory Animal Research Center Co. (Dae-Jeon, Korea). The rats had free access to food and water until they were used for experiments. These rats were used both in the in vitro skin permeation studies and in the stability studies in the skin homogenate, plasma and liver homogenate.

2.3. Determination of lipophilicity

2.3.1. Partition coefficient (log P)

Methanolic solution of ketorolac or its prodrugs (1 mg/ml) was placed in a glass tube. After completely evaporating the methanol, 1 ml of each saturated solvent was added to the tubes. After shaking the stoppered tube for 24 h at 150 rpm at 37 °C by shaking incubator (SI-600R, JEIOtech, Korea), the phases were separated by centrifugation at 4000 × g for 20 min. The concentration of the compound in each phase was determined by HPLC after appropriate dilution with methanol when necessary. Calculated log P values were obtained by using the ChemDraw program.

2.3.2. Capacity factor (k')

The k' values of ketorolac and its amide prodrugs were determined isocratically (50% methanol in acetate buffer, pH 3.6, 1.0 ml/min, UV 314 nm) using HPLC. Retention times of each compound were measured, and k' value were calculated from the following

equation:

$$k' = \frac{t_i - t_0}{t_0}$$

where t_0 is the retention time of methanol and t_i is the retention time of each compound (Nowotnik et al., 1993).

2.4. Determination of melting point

The melting points of the amide prodrugs were determined by thermal analysis (Perkin-Elmer Pyris series, Perkin-Elmer, UK), which was calibrated with indium (calibration standard, purity >99.999%). About 10 mg samples were layered evenly over the bottom of a standard aluminum pan. A heating rate of 10 °C/min was employed in the range of 20–220 °C using an empty pan as a reference. Analysis was performed under a nitrogen purge (50 ml/min). There were no significant differences in the thermograms for each compounds from run to run.

2.5. Determination of solubility

The solubility of ketorolac and its amide prodrugs in propylene glycol (PG) were determined at 37 °C. Excess amount of each compound was added to 1 ml of PG. The solution was placed in a water bath maintained at 37 °C and was stirred for 24 h to reach equilibrium. After filtering though a Minisart RC 4 filters (0.45 μm, Satorius, Germany), the filtrate was appropriately diluted with methanol and was analyzed by HPLC (Rautio et al.,1999).

2.6. Stability studies

Stability studies were conducted to confirm the conversion of various amide prodrugs to the parent drug, ketorolac, during the skin permeation process and/or in blood circulation. Skin homogenate was prepared by homogenizing freshly excised rat skin with 10-fold isotonic phosphate buffer (IPB, pH = 7.4) for 15 min in an ice bath. The supernatants were obtained after centrifugation for 20 min at 9000 × *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 × *g*. Liver homogenate was prepared by homogenizing freshly collected rat liver with IPB (pH = 7.4) in ice bath to obtain 20% (w/v) tissue suspension. The supernatants were obtained after centrifugation for 20 min at 9000 × *g* (Yang et al., 1995). Gentamicin (0.01%, w/v) was added to all solutions used for stability studies to inhibit the bacterial degradation of the ketorolac prodrugs. Due to the poor aqueous solubility of the amide prodrugs, 20% PG was added to solubilize the prodrugs in the media for stability studies. Amide prodrugs were spiked to make 1.5 μg/ml concentration in each solution. These solutions were placed in a shaker (shaking incubator SI-600R, 150 rpm) at 37 °C. At predetermined time intervals, 150 μl solution was taken and mixed with the same amount of DMSO/acetonitrile (5:95, v/v) solution in order to stop the enzymatic reaction and to precipitate protein. After immediate mixing and centrifugation for 2 min, the resulting clear supernatants were analyzed by HPLC. Considering the initial molar concentration of alkyl amide prodrugs as 100%, the remaining molar percentage of prodrugs in each solution was determined as a function of time.

2.7. Skin permeation studies

In vitro rat skin permeation studies were carried out with Keshary-Chien diffusion cells at 37 °C. Freshly excised skin was mounted between the donor and receptor cells with the effective diffusion area of 2.14 cm^2. 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 facing the stratum corneum were each filled with a saturated solution of amide 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 to maintain constant volume. The samples were analyzed by HPLC.

2.8. Drug analysis

The concentrations of ketorolac and its amide 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™ 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.

3. Results

3.1. Physico-chemical characteristics

The physico-chemical properties of ketorolac, and its amide prodrugs are shown in Table 1. The molecular weights of ketorolac and its prodrugs ranged from 255.27 to 374.44 g/mol. Lipophilicity of ketorolac and its prodrugs were determined as the *n*-octanol/water partition coefficient (log *P*) and the capacity factor (*k*′). The experimentally determined log *P* values of amide prodrugs ranged from 1.96 to 4.28. The plot of log *P* versus capacity factor showed a good linear relationship with a correlation coefficient (r^2) of 0.89 (Fig. 2). Determining the capacity factors instead of the log *P* values may be a more convenient alternative to estimating the lipophilicity of future analogs. As shown in Table 1, the solubility of amide prodrugs in PG ranged from 1.28 to 15.12 mg/ml, which is much lower than that of ester prodrugs (Doh et al., 2003). The melting point of amide prodrugs, determined by thermal analysis (Perkin-Elmer Pyris series, Perkin-Elmer, UK), ranged from 124.01 to 198.87 °C.

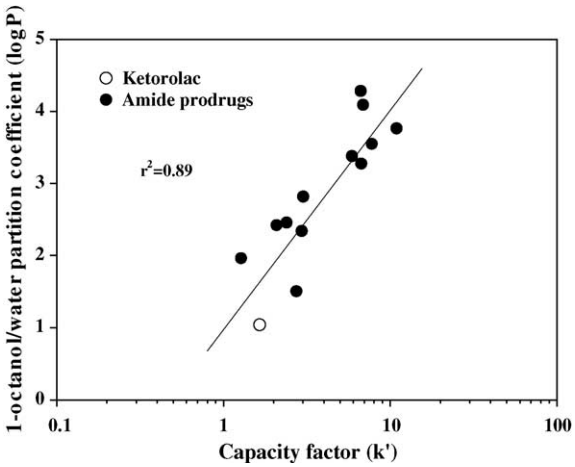


Fig. 2. Relationship between the apparent 1-octanol/water partition coefficient (log *P*) and the capacity factor (*k*′) of ketorolac amide prodrugs.

3.2. Stability of ketorolac prodrugs

The enzymatic hydrolysis of ketorolac prodrugs by the skin homogenate, plasma and liver homogenate

Table 1
Physico-chemical properties of ketorolac and its amide prodrugs

	Side chain	Molecular weight (g/mol)	Solubility in PG (mM)	Calculated log *P*	Experimental log *P*	Melting point (°C)
Ketorolac	–	255.27	52.85 (±5.95)	1.64	1.04 (±0.01)	158.68
m-01	Phenyl	330.38	6.66 (±0.48)	2.89	3.55 (±0.02)	170.36
m-02	4-Methoxy-phenyl	360.41	3.55 (±0.39)	2.76	4.09 (±0.04)	195.17
m-03	Benzyl	344.41	12.43 (±1.07)	2.96	4.28 (±0.07)	179.70
m-04	4-Methoxy-benzyl	374.44	5.10 (±1.04)	2.83	3.28 (±0.02)	137.71
m-05	Acetic acid methyl ester	326.35	16.09 (±1.78)	0.75	1.96 (±0.04)	124.01
m-06	Propionic acid ethyl ester	350.40	6.39 (±0.34)	1.38	2.34 (±0.03)	124.51
m-07	*n*-Butyl	310.39	27.06 (±2.35)	2.46	3.38 (±0.06)	145.71
m-08	Cyclohexyl	336.43	7.76 (±1.75)	2.77	3.77 (±0.06)	198.87
m-09	*n*-Propyl	296.36	37.15 (±3.44)	2.05	2.82 (±0.05)	153.80
m-10	Isopropyl	296.36	22.00 (±1.86)	1.88	2.53 (±0.04)	180.33
m-11	3-Methoxy-propyl	326.39	46.32 (±6.34)	1.17	2.42 (±0.03)	111.51
m-12	Allyl	294.35	46.71 (±1.49)	1.92	2.46 (±0.04)	162.64

Table 2
Rates of metabolism of ketorolac amide prodrugs in skin homogenate, plasma and liver homogenate at 37 °C

Drugs	Skin homogenate $t_{1/2}$ (h)	Plasma $t_{1/2}$ (min)	Liver homogenate $t_{1/2}$ (h)
m-01	ND[a]	ND[a]	0.67 (±0.14)
m-02	ND[a]	ND[a]	0.47 (±0.03)
m-03	ND[a]	ND[a]	4.41 (±0.79)
m-04	ND[a]	ND[a]	8.16 (±0.43)
m-05	19.91 (±0.46)	20.83 (±1.89)	0.15 (±0.03)
m-06	8.54 (±1.39)	ND[b]	ND[b]
m-07	ND[a]	ND[a]	7.24 (±0.50)
m-08	ND[a]	ND[a]	9.47 (±5.34)
m-09	ND[a]	ND[a]	5.92 (±0.33)
m-10	ND[a]	ND[a]	11.17 (±0.33)
m-11	ND[a]	ND[a]	9.25 (±2.06)
m-12	ND[a]	ND[a]	12.17 (±0.38)

[a] Not determined due to high chemical stability.
[b] Not determined due to fast degradation.

was evaluated at 37 °C. Table 2 shows the half-lives of the amide prodrugs in skin homogenate, plasma and liver homogenate. Fig. 3 shows the representative stability of the *n*-propyl ester prodrug (k-004) (Doh et al., 2003), and *n*-propyl amide prodrug (m-09) in skin homogenate, plasma and in liver homogenate. The kinetics of the enzymatic hydrolysis of each prodrug followed first-order kinetics and converted to ketorolac. Previous study indicates that the half-lives of ester prodrugs ranged from 0.21 to 2.45 h in the skin homogenate, while in plasma they converted to ketorolac with half-lives of less than 5 min. Moreover, in liver homogenate, half-lives of ester prodrugs were not determined due to rapid degradation (Doh et al., 2003). Amide prodrugs, on the contrary, showed higher enzymatic stability in skin homogenate and plasma except m-05 and m-06, which have ester bonds in the side chain. However, the amide prodrugs hydrolyzed quantitatively to ketorolac in liver homogenate with half-lives ranging from 0.47 to 12.17 h.

3.3. In vitro skin permeation study

Fig. 4 shows the rat skin permeation profiles of ketorolac and its amide prodrugs saturated in PG, using Keshary-Chien permeation cells at 37 °C. Table 3 summarizes the skin permeation parameters of ketorolac amide prodrugs. Prodrugs were saturated in PG in order to keep a constant driving force with maximum thermodynamic activity. Considering the degradation of prodrugs during the skin permeation and/or in the receptor solution, the cumulative amount of both the prodrug

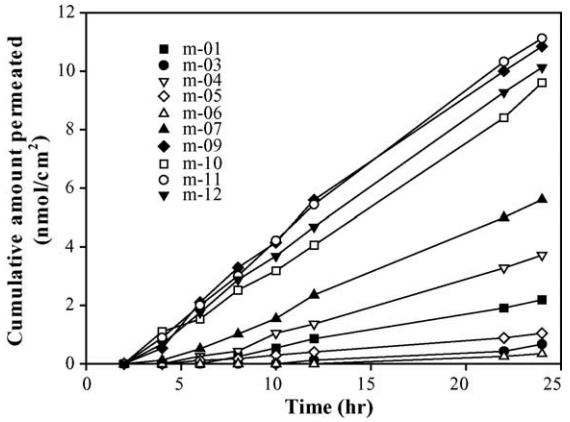


Fig. 4. Rat skin permeation profiles of ketorolac amide prodrugs saturated in propylene glycol at 37 °C.

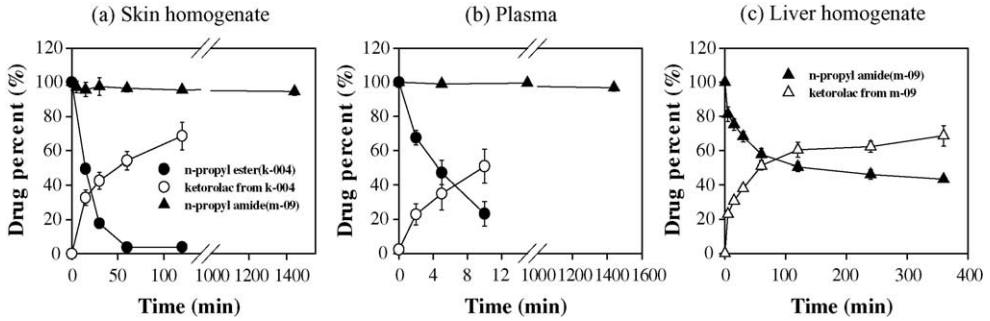


Fig. 3. Stability of *n*-propyl ester prodrug (k-004) and *n*-propyl amide prodrug (m-09) of ketorolac in (a) rat skin homogenate, (b) plasma, and (c) liver homgenate at 37 °C. Key: Prodrug k-004 was not determined in rat liver homogenate due to fast degradation. Data of k-004 were from Doh et al. (2003).

Table 3
Rat skin permeation parameters of ketorolac and amide prodrugs saturated in propylene glycol at 37 °C

Drugs	Permeation rate (nmol/(cm^2 h))	Lag time (h)	Permeability coefficient (cm/h) × 10^5
Ketorolac	3.86 (±1.35)	4.86 (±1.58)	7.31 (±2.56)
m-01	0.15 (±0.06)	7.57 (±4.06)	2.20 (±0.86)
m-02	ND	ND	ND
m-03	0.05 (±0.02)	12.34 (±1.83)	0.42 (±0.15)
m-04	0.24 (±0.15)	5.95 (±5.58)	4.68 (±3.04)
m-05	0.05 (±0.00)	2.56 (±2.05)	0.33 (±0.02)
m-06	0.03 (±0.00)	12.11 (±0.05)	0.44 (±0.04)
m-07	0.28 (±0.03)	4.57 (±0.06)	1.04 (±0.11)
m-08	ND	ND	ND
m-09	0.57 (±0.10)	3.55 (±1.34)	1.54 (±0.27)
m-10	0.50 (±0.05)	1.43 (±0.43)	2.26 (±0.21)
m-11	0.56 (±0.14)	3.48 (±0.60)	1.21 (±0.31)
m-12	0.52 (±0.08)	4.10 (±0.87)	1.11 (±0.17)

ND: not determined due to low permeation.

and the parent drug (ketorolac) was to be determined and combined in mole units. However, the prodrug was so stable that no parent drug was detected with the prodrug. The skin permeation rate of amide prodrugs was unexpectedly lower than that of ester prodrugs ranging from 0.03 to 0.57 nmol/(cm^2 h), and did not increase in proportion to the increase in lipophilicity.

4. Discussion

Our previous study showed that the ester prodrugs of ketorolac exhibited low physiochemical stability, although they significantly increased in vitro skin permeation rate of ketorolac (Doh et al., 2003). Thus, various amide prodrugs of ketorolac were synthesized and evaluated as potentially more stable prodrugs for transdermal delivery formulation. For stability studies, because all prodrugs were poorly soluble in aqueous vehicles, 20% PG was added to solubilize the prodrugs in the media. Unlike the ester prodrugs, which were rapidly degraded in the skin homogenate and in plasma, the amide prodrugs exhibited greater enzymatic stability in these media. As shown in Fig. 3, no significant degradation of amide prodrugs in the skin homogenate and plasma were noted for 24 h. However, in rat liver homogenate, the amide prodrugs were converted to ketorolac with half-lives of 0.47–12.17 h. Thus, it could be expected that the amide prodrugs would be converted to ketorolac in systemic circulation once they are absorbed through the skin. Amide bond is known to be relatively more stable than the ester bond (Holum, 1994), and these results fit well with the objective of this study, which was to synthesize more stable lipophilic prodrugs of ketorolac. Compounds m-05 and m-06 have both ester and amide bonds in side chain, thus was more rapidly hydrolyzed in liver homogenate than the other amide prodrugs. Various unidentified intermediates were observed in the stability study of these two compounds (data not shown), which suggests that prodrug-to-drug conversion is via intermediates, and that the rate of ester hydrolysis is faster than that of the amide hydrolysis.

Drugs for transdermal delivery should have suitable molecular weight of around 200–500 Da (Goldsmith, 1991). The molecular weight of amide prodrugs synthesized in this study fell in the appropriate range for transdermal delivery. Also, optimal log P value for maximal skin permeation for NSAIDs has been reported to be between two and three (Yano et al., 1986). A characteristic parabolic relationship between the skin permeability and lipophilicity of various drugs has been previously reported with the maximum permeability at log P of approximately three to four (Diez et al., 1991; Kim et al., 2000). However, although the lipophilicity of amide prodrugs fit well with the objective of this study, it is interesting to note that the skin permeation rates of the amide prodrugs did not increase in proportion to the increase in lipophilicity. For example, the skin permeation rate of the *n*-propyl amide prodrug (m-09, log P = 2.82) was only 0.57 nmol/(cm^2 h), while that of *n*-propyl ester prodrug (k-004, log P=3.73) was 46.61 nmol/(cm^2 h) (Doh et al., 2003). Although the amide prodrug m-08 had a similar log P value of 3.77, its skin permeation rate was too low to be determined.

Quantitative structure-permeability relationships (QSPRs) have been derived by many researchers to model the passive, diffusion-controlled, percutaneous penetration of exogenous chemicals (Moss et al., 2002). They indicated that molecular size (as molecular weight) and hydrophobicity (as the octanol/water partition coefficient) are the main determinants of transdermal delivery. Lipophilicity is very important for dermal permeation because the stratum corneum, the major barrier to drug permeation, is lipid in nature and generally favors permeation by lipophilic drugs. How-

ever, it has also been reported that an effective dermal prodrug should possess not only optimum lipophilicity, but also adequate aqueous solubility (Kerr et al., 1998; Taylor and Sloan, 1998; Rautio et al., 1999, 2000). The dependence of flux on aqueous solubility has been proposed in previous literatures (Roberts and Sloan, 1999; Waranis and Sloan, 1987). The enhancement of aqueous solubility seems to be equally important at the molecular level, because the lipid domain of the stratum corneum consists of multilamellar bilayers, and the transporting species must be able to repetitively cross lipid–aqueous phase interfaces. Lipophilic derivatization to obtain prodrugs increases partitioning into the stratum corneum, forming reservoir, but the subsequent transport into the aqueous milieu beneath may be limited by both prodrug aqueous solubility and the ability of epidermal enzymes to convert the prodrug into a more polar metabolite. Therefore, prodrugs for transdermal delivery should possess suitable water as well as lipid solubility, as proposed by Sloan (1989).

Bonina et al. (2001) found that the most permeable prodrugs of NSAIDs possessed both an adequate aqueous solubility and lipophilicity over the parent drug; optimal prodrugs showed similar lipophilicity as well as higher water solubility compared to the parent drug. Furthermore, these findings agreed well with their previous results (Bonina et al., 1994, 1995a,b; Palagiano et al., 1997) and other report (Sloan, 1989) in which a homologous series of prodrugs with similar lipophilicity compared to the parent drug revealed that the highest flux through the skin was achieved by the derivatives with the highest water solubility. Mendes et al. (2002) also found that the aminocarbonyloxymethyl derivative as prodrugs evaluated, exhibited a lower flux than the corresponding parent carboxylic acid. They suggested that poor permeation observed for prodrug is most probably due to their high partition coefficient and, more especially, their low aqueous solubility. Similar poor skin permeation has also been reported for neutral pivaloyloxymethyl derivative of naproxen (Rautio et al., 1998).

Generally, molecules having high melting point show strong intermolecular crystalline self-association. Such molecules have little tendency to dissolve in the organic phase and, consequently, their partitioning into the lipoidal barrier phases of the skin is minimal. Their permeation rates tend to be low even from their saturated solutions (Potts and Guy, 1995). Clearly, a low capacity to dissolve in the transport phases is a major obstacle to transdermal delivery.

Thus, the poor skin permeation rate observed for amide prodrugs of ketorolac in this study is most probably due to their low aqueous solubility and high melting point compared to the ester prodrugs, which ranged from −80.47 to −26.12 °C (unpublished data). Similar result has been reported for benzyloxycarbonyl and pentyloxycarbonyl derivatives as mitomycin C prodrugs (Sezaki et al., 1985), where derivatives with higher melting points and lower biphasic solubility were not able to adequately permeate through the skin.

5. Conclusions

Various amide prodrugs of ketorolac were successfully synthesized, whose log *P* value ranged between 2.0 and 4.3. Unlike the ester prodrugs which degraded rapidly in the rat skin homogenate and plasma, the amide prodrugs exhibited greater chemical stability than ester prodrugs. Since the amide prodrugs rapidly degraded to ketorolac in rat liver homogenate, it was confirmed that they would convert to ketorolac in the blood circulation once absorbed through the skin. However, skin permeation rate of amide prodrugs were significantly lower than that of the ester prodrugs with the same side chain or similar lipophilicity. The poor skin permeation rate of amide prodrugs is most probably due to their low aqueous solubility and/or high melting point. Thus, modifying the ketorolac amide prodrug into a more soluble analogue and/or the selection of a proper vehicle to increase the solubility would be necessary in order to further increase the transdermal delivery of ketorolac derivatives.

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