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Synthesis of Piperazinylalkyl Ester Prodrugs of Ketorolac and their In Vitro Evaluation for Transdermal Delivery

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Ketorolac, an NSAID, has low intrinsic permeation capacity through the skin. In this work, seven piperazinylalkyl ester prodrugs of ketorolac were synthesized to enhance its skin permeation. The chemical hydrolysis and the stability in human serum at 37°C were investigated in buffer solutions (pH 5.0 and 7.4) and in 80% human serum (pH 7.4), respectively. The prodrugs were chemically more stable at pH 5.0 than at pH 7.4 with prodrug 8 being the most stable ($t_{1/2}$ = 119.75 h and 11.97 h at pH 5 and 7.4, respectively). The prodrugs' $t_{1/2}$ in human serum ranged from 0.79 to 3.92 min. The prodrugs' aqueous solubility was measured in buffer solution at pH 5.0 and 7.4 and Log P_{app} was measured by partitioning between buffer solution (pH 5.0 and 7.4) and *n*-octanol. The prodrugs were more lipophilic than ketorolac at pH 7.4. Skin permeation of ketorolac and prodrug 8, the most stable chemically, through rat skin was studied at pH 5.0 and 7.4. Prodrug 8 enhanced permeation by 1.56- and 11.39-fold at pH 5 and 7.4, respectively. This is attributed to higher lipophilicity at pH 7.4 and higher aqueous solubility at pH 5 compared to ketorolac.

Keywords Ketorolac; prodrug; piperazine; transdermal delivery; synthesis

INTRODUCTION

Transdermal administration of medicinal agents, although not new, is still an active area of pharmaceutical research. Transdermal delivery avoids the problems of gastric empting, the effects of pH, and the hepatic first-pass metabolism associated with the oral route. Also, this route spares the gastrointestinal tract form side effects such as local irritation and ulceration. In addition, the dose needed to attain therapeutic drug concentration can be lowered and patient compliance can be enhanced (Fourie et al., 2004).

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Ketorolac, (±)-5-benzoyl-1,2-dihydro-3*H*-pyrrolo-[*1,2-a*] pyrrole-1-carboxylic acid, is a nonselective *COX*-inhibitor and it is one of the most potent NSAIDs (Muchowski et al., 1985). Compared to other nonselective *COX*-inhibitors, it processes greater analgesic/anti-inflammatory ratio, but its oral administration is associated with a larger risk of gastric irritation (Buffum & Buffum, 2000; Macario & Lipman, 2001). The transdermal delivery of ketorolac can serve as an alternative route for its administration that can reduce gastrointestinal side effects and/or allow local application of the drug to certain areas of the body. Ketorolac itself exhibits low intrinsic permeation capacity through the skin (Cordero, Alarcon, Escribano, Obach, & Domenechx, 1997). Rautio et al. have improved naproxen's dermal delivery through synthesizing series of naproxen prodrugs, the most successful of which were some piperazinylalkyl ester as compared with alkyl esters or alkyl and aryl amides (Rautio et al., 1998, 1999, 2000a, 2000b; Tomi et al., 2001). This is because piperazinyl alkyl moieties impart lipophilicity due to their aliphatic backbone while they present ionizable nitrogen atoms, *p*Ka values around 9, which can facilitate dissolution in aqueous layers. Alkyl esters, aryl and alkyl amides, and polyoxyethylene glycol esters of ketorolac have been reported and their transdermal delivery was evaluated (Doh et al., 2003; Kim et al., 2005; Puglia et al., 2006; Roy & Manoukian, 1994). There are no ionizable prodrugs of ketorolac that have been reported earlier. In this work, a diverse selection of piperazinylalkyl ester prodrugs of ketorolac were synthesized, focusing on the nature of the piperazine ring rather than the length of the aliphatic chain between the carboxylate group and the piperazine ring (Rautio et al., 2000a). The piperazine rings chosen differ mainly in their lipophilicity and ionizability in order to manipulate aqueous solubility and lipophilicity; the main factors affecting dermal delivery (Beall & Sloan, 2001; Roberts & Sloan, 1999). Chemically, esterification of ketorolac is accomplished by simple acid catalysis rather than the more expensive utilization of coupling agents that might also complicate

reaction workup. Such acidic conditions have not been reported before in the synthesis of ketorolac esters.

MATERIALS AND METHODS

Materials

Reagents used such as the piperazines, bromoethanol, chlorobutanol in addition to the chemicals used in the preparation of the buffer were all of analytical grade and the solvents were high-performance liquid chromatography (HPLC)-grade, Acros Chemicals, Belgium. Ketorolac was obtained from Al-Hikma Pharmaceuticals, Amman, Jordan. Water used in the HPLC procedure was double distilled and deionized. Melting points were determined using Stuart Scientific-melting point apparatus (UK). ^{1}H and ^{13}C NMR spectra were obtained using a 400 MHz Bruker Avance Ultrashield Spectrometer (Switzerland). NMR data are reported in ppm using automatic calibration to the residual proton peak of the solvent, $CDCl_3$. The splitting pattern abbreviations are as follows: *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet; *dd*, doublet of doublet; *bs*, broad singlet. Atomic pressure chemical ionization (APCI) mass spectra of the prodrugs were obtained using Agilent 1100 series LC-MS (USA). IR spectra were recorded on Niclolet Avatar 360 FT-IR (USA) using KBr disks. TLC analysis was performed on Albet aluminum Thin Layer Chromatography (TLC) plate, Aluminum, Silica 60, and UV254 (Spain).

Methods

Synthesis

2-Bromoethyl-5-Benzoyl-1,2-Dihydro-3H-Pyrrolo-[1,2-a] Pyrrole-1-Carboxylate (1). Ketorolac (100 g, 0.39 mol) was dissolved in dichloromethane (750 mL), and to it benzenesulfonic acid (25 g, 0.1 mol) and 2-bromoethanol (145 g, 1.17 mol) were added, and the reaction mixture was allowed to stir at room temperature for 3 days. The reaction progress was followed with TLC (50% ethyl acetate in hexane). Upon completion of the reaction it was washed with distilled water (300 mL × 2), then with cold 0.5 M sodium hydroxide solution (300 mL × 3). The organic layer was then dried over Na_2SO_4 and the solvent was evaporated. The crude residue was crystallized from ethyl acetate and hexane to yield 98.62 g (70.0%) of light brown crystals. mp: 80–82°C. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (2H, *d*, *J* = 7.3 Hz, Ar-H_2), 7.55 (1H, *t*, *J* = 7.3 Hz, Ar-*H*), 7.47 (2H, *t*, *J* = 7.3 Hz, Ar-H_2), 6.85 (1H, *d*, *J* = 3.8 Hz, *H*-C = C-H), 6.19 (1H, *d*, *J* = 3.8 Hz, H-C = C-*H*), 4.62-4.43 (4H, *m*, NCH_2+ OCH_2), 4.13 (1H, *dd*, *J* = 8.6 and 5.8, C*H*CO), 3.55 (2H, *t*, *J* = 5.8, CH_2Br), 3.00–2.92 (1H, *m*, C*H*H), 2.87-2.81 (1H, *m*, CH*H*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 185.04, 170.81, 141.84, 139.18, 131.46, 128.91, 128.18, 127.28, 124.99, 103.37, 64.64, 47.56, 42.46, 30.88, 28.58. IR (KBr): 2890.5, 1746.0, 1618.9, 1266.5 cm^{-1}. LC-MS (APCI) *m/z*: MH^+ (362, 100%), MH^++1 (363, 21.43%), and MH^++2 (364, 76.19%).

General Procedure for the Synthesis of Prodrugs 2–7. To a solution of 2-bromoethyl ketorolac ester (1) (10 g, 27.7 mmol) in acetone (200 mL), the appropriate piperazine (4 equivalents of unsubstituted piperazine and 2 equivalents for the rest) and triethylamine (11.5 mL, 83.1 mmol) were added and the reaction mixture was allowed to stir at room temperature for 3 days. The progress of the reaction was followed by using TLC (10% methanol in dichloromethane). Upon completion of the reaction it was filtered and the solvent was evaporated. The oily residue was dissolved in ethyl acetate (100 mL) and was washed with water (100 mL), then with saturated sodium bicarbonate solution (100 mL × 2) and again with water (100 mL). The organic layer was dried over $MgSO_4$, and it was evaporated to obtain an oily residue that failed to crystallize. This residue was dissolved in absolute ethanol and excess amount of oxalic acid in absolute ethanol was added to it, upon which a precipitate was formed instantly. The precipitate was then filtered and crystallized from methanol. The prodrug in its free base form was always freshly prepared on-demand, which was done by dissolving the salt in cold distilled water followed by careful neutralization by sodium bicarbonate, which was monitored by a pH-meter. Extraction by ethyl acetate followed by drying ($MgSO_4$ or Na_2SO_4) and evaporation of the solvent yielded the prodrug in its free base form, and it was analyzed using HPLC to prove sufficient purity and ^{1}H-NMR and ^{13}C-NMR to confirm identity. Yields, melting points, IR, NMR and mass spectral characterization of compounds 2–7 are summarized in Table 1.

2-(4-Methyl-1-Piperazinyl)Butyl-5-Benzoyl-1,2-Dihydro-3H-Pyrrolo-[1,2-a]Pyrrole-1-Carboxylate (8) To a solution of ketorolac (20 g, 78.4 mmol), and benzenesulfonic acid (3.1 g, 19.6 mmol) in dichloromethane (150 mL), 4-chlorobutanol (16.94 g, 156.8 mmol) was added and the reaction mixture was allowed to stir at room temperature for 5 days. The reaction progress was followed up by TLC (25% ethyl acetate in hexane). Upon completion of the reaction it was washed with water (200 mL × 3) then with cold 0.5 M sodium hydroxide (200 mL × 2). The organic layer was dried over $MgSO_4$ and evaporated. The residue was used in the next reaction without further purification. An analytical sample was obtained by preparative TLC and its ^{1}H-NMR is shown below. To the crude residue dissolved in acetone, 1-methylpiperazine (15.7 g, 156.8 mmol), triethylamine (32.7 mL, 235.2 mmol), and sodium iodide (11.74 g, 78.4 mmol) were added and the reaction mixture was allowed to stir at room temperature for 3 days. The reaction progress was followed up by TLC (10% methanol in dichloromethane). Upon completion of the reaction, it was filtered and the solvent was evaporated and the oily residue was used immediately. The oily residue was dissolved in ethyl acetate (500 mL) and was washed with water (300 mL), saturated sodium bicarbonate solution (300 mL × 2) and again with water (300 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated. The crude product was dissolved in absolute ethanol (100 mL) and was added to excess amount

TABLE 1
Yields, Melting points, IR, 1H NMR, and Mass Spectral Characterization of Synthesized Prodrugs of Ketorolac

Compound	Yield[a] (%)	Melting Range[a] (°C)	MS[b] (*m/z*, MH^+)	1H NMR[b] ($CDCl_3$, δ, ppm)	13C NMR[b] ($CDCl_3$, δ, ppm)	IR[b] (KBr, cm^{-1})
2	37.3	218–220	382 100%	7.83 (2H, *d*, *J* = 7.3 Hz, Ar-H_2), 7.54 (1H, *t*, *J* = 7.3, Ar-*H*), 7.46 (2H, *t*, *J* = 7.3, Ar-H_2), 6.83 (1H, *d*, *J* = 4.0 Hz, *H*-C=C-H), 6.13 (1H, *d*, *J* = 4.0 Hz, H-C=C-*H*), 4.62-4.55 (1H, *m*, NC*H*H), 4.50-4.44 (1H, *m*, NCH*H*), 4.32-4.28 (2H, *m*, OCH_2CH_2N), 4.09 (1H, *dd*, *J* = 8.8 and 5.5 Hz, C*H*), 2.99-2.91 (1H, *m*, C*H*H), 2.86-2.77 (1H, *m*, CH*H*), 2.67 (2H, *t*, *J* = 5.8 Hz, OCH_2CH_2N), 2.55 (4H, *bs*, $CH_2N^4CH_2$, 4H), 2.36 (4H, *bs*, $CH_2N^1CH_2$, 4H), 2.29 (3H, *s*, N-CH_3)	184.97, 171.15, 142.37, 139.30, 131.39, 128.88, 128.15, 127.20, 124.90, 103.20, 62.68, 56.48, 55.01 53.18, 47.59, 45.92, 42.62, 31.01	3,057.7, 2,937.2, 2,796.7, 1,735.1, 1,620.7, 1,267.0, 1,169.2
3	32.1	187–190	396 100%	7.79 (2H, *d*, *J* = 7.4 Hz, Ar-H_2), 7.50 (1H, *t*, *J* = 7.5, Ar-*H*), 7.43 (2H, *t*, *J* = 7.4, Ar-H_2), 6.80 (1H, *d*, *J* = 4.0 Hz, *H*-C=C-H), 6.11 (1H, *d*, *J* = 4.0 Hz, H-C=C-*H*), 4.58-4.52 (1H, *m*, NC*H*H), 4.46-4.40 (1H, *m*, NCH*H*), 4.29-4.25 (2H, *m*, OCH_2CH_2N), 4.06 (1H, *dd*, *J* = 8.8 and 5.6 Hz, C*H*), 2.94-2.87 (1H, *m*, C*H*H), 2.82-2.73 (1H, *m*, CH*H*), 2.67 (2H, *t*, *J* = 5.8 Hz, OCH_2CH_2N), 2.55-2.36 (8H, *bs*, $CH_2N^4CH_2$ and $CH_2N^1CH_2$), 2.38 (2H, *q*, *J* = 7.2 Hz, N-CH_2CH_3), 1.05 (3H, *t*, *J* = 7.2 Hz, N-CH_2CH_3)	184.91, 171.13, 142.39, 139.21, 131.38, 128.86, 128.14, 127.12, 124.90, 103.21, 62.70, 56.52, 53.30, 52.74, 52.27, 47.57, 42.59, 30.98, 11.98	3,057.6, 2,943.8, 2,811.9, 1,739.1, 1,621.8, 1,270.7, 1,172.2
4	63.3	195–197	368 100%	7.82 (2H, *d*, *J* = 7.8 Hz, Ar-H_2), 7.53 (1H, *t*, *J* = 7.8, Ar-*H*), 7.46 (2H, *t*, *J* = 7.8, Ar-H_2), 6.83 (1H, *m*, *H*-C=C-H), 6.13 (1H, *m*, H-C=C-*H*), 4.62-4.55 (1H, *m*, NC*H*H), 4.49-4.42 (1H, *m*, NCH*H*), 4.31-4.25 (2H, *m*, OCH_2CH_2N), 4.11-4.06 (1H, *m*, C*H*), 3.04-2.91 (1H, *m*, C*H*H), 2.88 (4H, *t*, *J* = 4.9, $CH_2N^4CH_2$), 2.84-2.75 (1H, *m*, CH*H*), 2.64 (2H, *t*, *J* = 5.8 Hz, OCH_2CH_2N), 2.50-2.47 (4H, *bs*, $CH_2N^1CH_2$), 2.00 (1H, *s*, N*H*)	184.96, 171.15, 142.39, 139.23, 131.41, 128.87, 128.16, 127.18, 124.90, 103.20, 62.56, 57.15, 56.50, 54.56, 53.28, 47.60, 46.01, 42.62, 31.00	3,013.4, 2,974.1, 2,838.9, 1,738.8, 1,619.9,1,270.0, 1,219.6

5	40.1	199–201	412 100%	7.82 (2H, *d*, *J* = 8.0 Hz, Ar-H_2), 7.55 (1H, *m*, Ar-*H*), 7.47 (2H, *m*, Ar-H_2), 6.83 (1H, *m*, *H*-C=C-H), 6.13 (1H, *m*, H-C=C-*H*), 4.59-4.56 (1H, *m*, NC*H*H), 4.50-4.44 (1H, *m*, NCH*H*), 4.33-4.30 (2H, *m*, OCH_2 CH_2N), 4.10 (1H, *m*, C*H*), 3.65-3.61 (2H, *m*, NCH_2CH_2OH), 2.99-2.88 (1H, *m*, C*H*H), 2.86-2.77 (1H, *m*, CH*H*), 2.68-2.64 (2H, *m*, N-CH_2CH_2-OH), 2.59-2.54 (8H, *m*, $CH_2N^4CH_2$ and $CH_2N^1CH_2$)	184.99, 171.17, 142.38, 139.20, 131.45, 128.89, 128.18, 127.18, 124.92, 103.20, 62.65, 59.19, 57.65, 56.49, 53.26, 52.79, 47.61, 42.62, 31.02	3,422.6, 2,942.8, 2,817.6, 1,731.8, 1,621.4, 1,265.2, 1,165.1
6	50.7	121–123	410 100%	7.77 (2H, *d*, *J* = 7.2 Hz, Ar-H_2), 7.49 (1H, *t*, *J* = 7.2, Ar-*H*), 7.42 (2H, *t*, *J* = 7.2, Ar-H_2), 6.79 (1H, *d*, *J* = 4.0 Hz, *H*-C=C-H), 6.08 (1H, *d*, *J* = 4.0 Hz, H-C=C-*H*), 4.57-4.50 (1H, *m*, NC*H*H), 4.44-4.38 (1H, *m*, NCH*H*), 4.29-4.25 (2H, *m*, OCH_2CH_2N), 4.05 (1H, *dd*, *J* = 8.7 and 5.4 Hz, C*H*), 3.54 (2H, *t*, *J* = 5.2 Hz, CH_2NCO), 3.38 (2H, *t*, *J* = 5.2, CH_2NCO), 2.92-2.85 (1H, *m*, C*H*H), 2.81-2.73 (1H, *m*, CH*H*), 2.62 (2H, *t*, *J* = 5.6 Hz, OCH_2CH_2N), 2.42 (4H, *m*, $CH_2N^1CH_2$), 2.02 (3H, *s*, $COCH_3$)	184.92, 171.71, 168.95, 142.33, 139.11, 131.46, 128.84, 128.17, 127.14, 124.97, 103.15, 62.31, 56.41, 53.20, 52.87, 47.57, 46.20, 42.57, 41.33, 30.97, 21.27	3,056.8, 2,949.6, 2,818.9, 1,733.3, 1,616.6, 1,267.7, 1,180.5
7	23.3	214–216	397 100%	7.83 (2H, *d*, *J* = 7.3 Hz, Ar-H_2), 7.54 (1H, *t*, *J* = 7.3, Ar-*H*), 7.46 (2H, *t*, *J* = 7.3, Ar-H_2), 6.83 (1H, *d*, *J* = 4.0 Hz, *H*-C=C-H), 6.13 (1H, *d*, *J* = 4.0 Hz, H-C=C-*H*), 4.62-4.55 (1H, *m*, NC*H*H), 4.50-4.44 (1H, *m*, NCH*H*), 4.31-4.28 (2H, *m*, OCH_2CH_2N), 4.09 (1H, *dd*, *J* = 8.8 and 5.4 Hz, C*H*), 2.97-2.91 (1H, *m*, C*H*H), 2.85-2.76 (1H, *m*, CH*H*), 2.67 (2H, *t*, *J* = 5.8 Hz, OCH_2CH_2N), 2.55 (4H, *bs*, CH_2NCH_2, 4H), 2.36 (4H, *bs*, CH_2NCH_2), 2.28 (3H, *s*, N-CH_3), 2.12 (1H, *bs*, N*H*)	184.98, 171.16, 142.39, 139.24, 131.40, 128.88, 128.16, 127.18, 124.92, 103.21, 62.70, 56.50, 55.06, 53.29, 47.59, 46.03, 42.62, 31.00	2,938.7, 2,798.2, 1,736.4, 1,620.8, 1,268.2, 1,167.6

[a]Values determined for the oxalate salt.
[b]Values determined for the free base.

of oxalic acid in ethanol. The precipitated salt was filtered and then was crystallized from methanol to yield 8.37 g (46.76%) as off-white crystals. The prodrug in its free base form was always freshly prepared on-demand, which was done by dissolving the salt in cold distilled water followed by careful neutralization by sodium bicarbonate, which was monitored by a pH-meter. Extraction by ethyl acetate followed by drying ($MgSO_4$ or Na_2SO_4) and evaporation of the solvent yielded the prodrug in its free base form and it was analyzed using HPLC to prove sufficient purity and ^{1}H-NMR and ^{13}C-NMR to confirm identity. *Crude ketorolac 4-chlorobutyl ester*: ^{1}H-NMR ($CDCl_3$, 400MHz): δ 7.81 (2H, *d*, *J* = 7.3 Hz, Ar-H_2), 7.52 (1H, *t*, *J* = 7.3, Ar-*H*), 7.44 (2H, *t*, *J* = 7.3, Ar-H_2), 6.82 (1H, *d*, *J* = 3.8 Hz, *H*-C = C-H), 6.09 (1H, *d*, *J* = 3.8 Hz, H-C = C-*H*), 4.58-4.53 (1H, *m*, NC*H*H), 4.47-4.41 (1H, *m*, NCH*H*), 4.19 (2H, *bs*, OCH_2CH$_2$CH$_2$CH$_2$Cl), 4.06 (1H, *dd*, *J* = 8.8 and 5.6 Hz, C*H*), 3.55 (2H, *m*, OCH$_2$(CH$_2$)$_2$CH_2Cl), 2.95-2.90 (1H, *m*, C*H*H), 2.84-2.77 (1H, *m*, CH*H*), 1.7 (4H, *m*, OCH$_2$(CH_2)$_2$CH$_2$Cl). Oxalate salt: mp: 210–212°C; Free base, **8**: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.80 (2H, *d*, *J* = 7.6 Hz, Ar-H_2), 7.51 (1H, *t*, *J* = 7.6, Ar-*H*), 7.44 (2H, *t*, *J* = 7.6, Ar-H_2), 6.80 (1H, *d*, *J* = 4.0 Hz, *H*-C = C-H), 6.08 (1H, *d*, *J* = 4.0 Hz, H-C = C-*H*), 4.56–4.53 (1H, *m*, NC*H*H), 4.46-4.40 (1H, *m*, NCH*H*), 4.16 (2H, *t*, *J* = 6.4 Hz, OCH_2(CH$_2$)$_2$CH$_2$N), 4.05 (1H, *dd*, *J* = 8.8 and 5.6 Hz, C*H*), 2.94-2.87 (1H, *m*, CH*H*), 2.82-2.68 (1H, *m*, C*H*H), 2.68-2.36 (8H, *m*, CH_2N^{4}CH_2 and CH_2N^{1}CH_2), 2.35 (2H, *t*, *J* = 7.2 Hz, OCH$_2$(CH$_2$)$_2$CH_2N), 2.26 (3H, *s*, N-CH_3), 1.72-1.56 (2H, *m*, OCH$_2$CH_2CH$_2$CH$_2$N), 1.58-1.51 (2H, *m*, OCH$_2$CH$_2$CH_2CH$_2$N). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 184.80, 171.14, 142.41, 139.14, 131.33, 128.80, 128.08, 127.03, 124.89, 103.05, 65.30, 57.90, 54.97, 53.03, 47.52, 45.95, 42.54, 30.80, 26.56, 23.16. IR (KBr): 2940.19, 2796.37, 1734.6, 1623.1, 1269.1, 1167.9 cm^{-1}. LC-MS (APCI) *m/z*: MH$^+$ (410, 99.60%).

HPLC Analysis

HPLC was used for analysis. The analytical (HPLC) system consisted of an LC system (LC-10AD vp, Shimadzu, USA), a UV-visible detector (SPD-10AV vp, Shimadzu, USA), auto injector (SIL-10AD vp, Shimadzu, USA) and degasser (DGU-12A, Shimadzu, USA), a system controller (SCL-10A vp, Shimadzu, USA), and was connected to a computer furnished with the appropriate software (ClassVP, V 6.2, Shimadzu, USA). The chromatographic separation was carried out under isocratic reversed-phase conditions on purospher RP-18C column (125 × 4 mm, 5 μm) (Merck, Germany). The injection volume was 25 μL and the detection wavelength was 314 nm (Wang & Avram, 2001). The mobile phase was a mixture of 0.02 M phosphate buffer and acetonitrile (65:35 vol/vol, pH 5.4) and the flow rate was 1.5 mL/min. The mobile phase was filtered through 0.45 μm membrane filters. In the same chromatographic run, the prodrug and the parent drug (ketorolac) were detected with different retention times. The retention time for ketorolac was 1.97 min and was for prodrug **2**: 3.56 min, prodrug **3**: 4.1 min, prodrug **4**: 3.43 min, prodrug **5**: 3.28 min, prodrug **6**: 8.18 min, prodrug **7**: 4.04 min and for prodrug **8**: 4.54 min. This method has been recently reported and validated (Qandil, Tashtoush, Al-Taani, Al-Nabulsi, & Al-Zogoul, 2008).

Stability in Aqueous Phosphate Buffer

The rates of the chemical hydrolysis of the prodrugs were determined in aqueous phosphate buffer solution at pH 5 and 7.4 (0.16 M, ionic strength was adjusted to 0.5 with NaCl) at 37°C (Bonina et al., 2001; Mantyla et al., 2004). The reactions were initiated by preparing 100 μg/mL solutions of the prodrugs in 0.16 M phosphate buffer at pH 5 and 7.4, and the solutions were placed in a thermostatically controlled water bath at 37°C. At appropriate time intervals, depending on the stability of each prodrug, samples were taken and analyzed immediately for the remaining prodrug and the liberated ketorolac using HPLC. The experiments were run in triplicates for each compound. The rate of hydrolysis of each ester was determined from the slope of the linear plot of the residual prodrug concentration against time.

Hydrolysis in Human Serum

The rates of hydrolysis for ketorolac prodrugs in 80% human serum diluted with 0.16 M phosphate buffer were determined at 37°C, pH 7.4. The reactions were initiated by dissolving an appropriate amount of the prodrug in phosphate buffer and adding it to human serum, which was kept at 37°C. The solutions were kept in a water bath at 37°C, and 0.5 mL of serum/buffer mixture were withdrawn at certain time intervals and added to 1.0 mL absolute ethanol to precipitate the protein from the serum. Immediately after mixing and centrifugation for 5 min at 1073 g, the supernatant was analyzed for the remaining prodrug and the liberated ketorolac by HPLC.

Aqueous Solubility

The aqueous solubility of ketorolac and its prodrugs was determined at room temperature in aqueous phosphate buffer solution (0.16 M) at both pH 5 and 7.4. Excess amounts of each compound were added to 1 mL aqueous phosphate buffer at pH 5 and 7.4 and were continuously stirred for a maximum of 25 min using a magnetic stirrer. This time was chosen to prevent the hydrolysis of the ester prodrugs (Rautio et al., 2000a). Shorter times, such as 10 min, were also reported previously to determine the solubility of ketorolac esters to prevent their hydrolysis (Doh et al., 2003). The solutions were centrifuged at 1073 g for 5 min, and then filtered using millipore filters 0.45 μm and the concentration of each compound was determined by using HPLC.

Apparent Partition Coefficient

The apparent partition coefficients expressed as log P_{app} of ketorolac and its prodrugs were determined at room temperature between *n*-octanol and aqueous phosphate buffer solution (0.16 M)

at both pH 5 and 7.4. *n*-Octanol was firstly saturated with aqueous phosphate buffer solution by vigorously stirring them together using a magnetic stirrer for 24 h. Log P_{app} was measured by dissolving 5 mg of each compound in 2 mL aqueous phosphate buffer solution in glass screw-capped test tubes. One milliliter of the solution was taken for analysis of the initial concentration of each compound using HPLC, and the remaining 1 mL was added to 1 mL pre-saturated *n*-octanol and shaken for 30 min (pH 5), and 15 min (pH 7.4). The same rationale for choosing short times for the determination of solubility applies here. After centrifugation at 1073 g at room temperature for 5 min, the final concentration of the compound in the aqueous layer was analyzed using HPLC. The experiments were done in triplicates. The concentration of each compound in the *n*-octanol layer was calculated by subtracting the final concentration of the compound in the aqueous layer from its initial aqueous layer concentration. The partition coefficient was calculated by dividing the concentration of the compound in the *n*-octanol layer by its final concentration in aqueous phase.

In Vitro Skin Permeation Study

Diffusion studies of ketorolac and prodrug 8 were carried out using glass Franz diffusion cells (O-ring system, 2 mm ID, Pyrex, Thomas scientific, USA). Skin was obtained from the whole dorsal area of a male Sprague–Dawley rat (200 ± 10 g) after hair removal by hand to avoid skin damage that can be caused by electric shavers. The receiver solution used in all diffusion experiments was isotonic phosphate buffer solution (pH 7.4, 0.05 M). Solutions of ketorolac and prodrug 8 containing 1000 μg/mL were prepared by dissolving 50 mg of each compound in 50 mL isotonic buffer solution (0.05 M) at pH 5 and 7.4. The experiments were initiated by adding 10 mL of aqueous phosphate buffer solutions of ketorolac or prodrug 8 to the donor compartment. In general, prodrugs of ketorolac or naproxen were added to the donor compartment in the form of a suspension, saturated solution, or 50 mM solution all of which are, in general, applied in larger concentrations than the one used in this study (Doh et al., 2003; Rautio et al., 1999, 2000a). At appropriate intervals, 200 μL aliquots were accurately taken and analyzed using HPLC and 200 μL of fresh isotonic buffer solution (pH 7.4, 0.05 M) was added to compensate. The experiment was continued for 50 h. The steady-state flux (J_{ss}) of ketorolac and its prodrug 8 was determined by plotting the cumulative amount of the compound (nmol) as measured in the receiver phase against time, and dividing the slope of the steady-state by the surface area of the diffusion cell (3.14 cm^2). The permeability coefficients (K_p) of ketorolac and prodrug 8 were calculated by dividing the steady-state flux by the solubility values of the compounds in the applied vehicle (Mantyla et al., 2004; Sloan, 1992). Lag time, which is the time needed for the permeated compound to reach the steady-state, was measured from the *X*-axis intercept of the linear plot of the amount permeated against time.

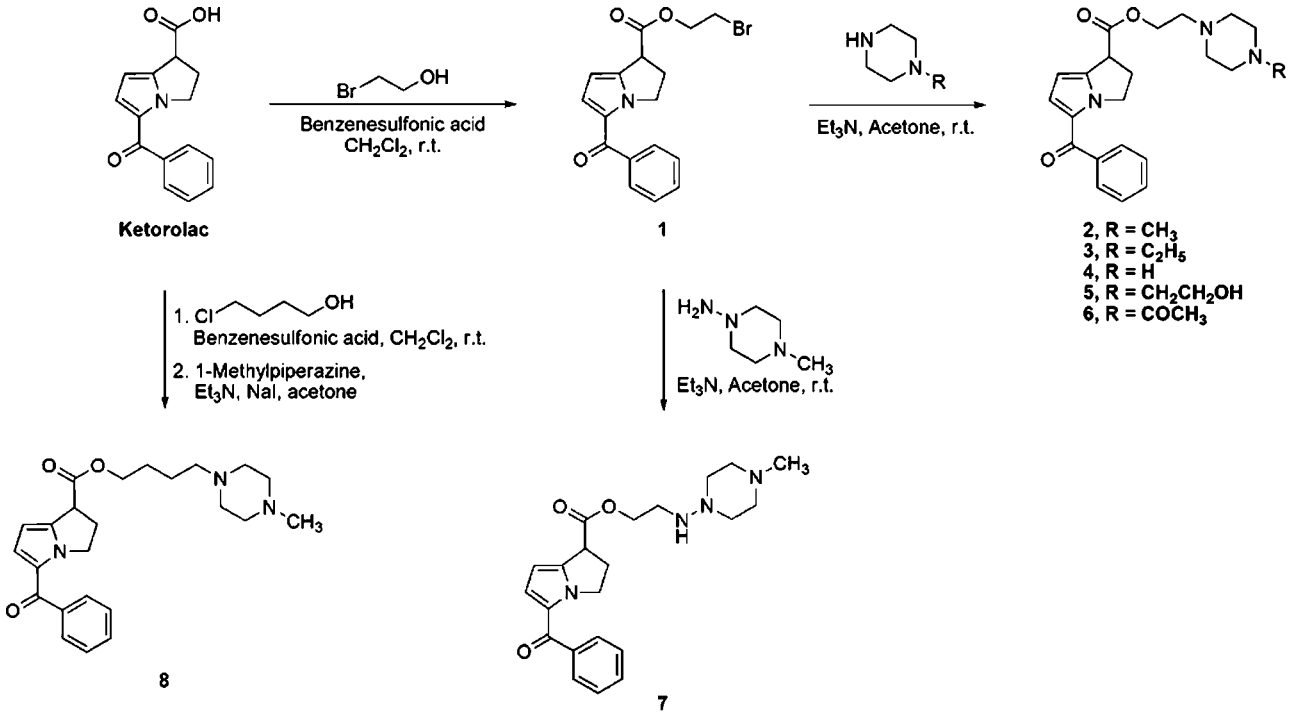
This procedure was approved by the Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology.

RESULTS AND DISCUSSION

Chemistry

Previous syntheses of ketorolac esters and amides were effected using *N*,*N*′-dicyclohexylcarbodiimide, DCC, as a coupling agents (Doh et al., 2003; Kim et al., 2005; Puglia et al., 2006). Preparation of the of the 2-bromoethyl ester of ketorolac allows for the subsequent connection of various piperazine derivatives or any nucleophilic species. The synthesis of 2-bromoethyl ester of ketorolac is illustrated in Scheme 1, and it was effected using an acid catalyst and without the need of a coupling agent such as DCC. 2-Bromoethanol and ketorolac were both dissolved in dichloromethane and benzenesulfonic acid, as a catalyst, was added to the solution at room temperature. Reaction follow up revealed a slow but steady formation of a lone product. Attempts to accelerate the reaction by heating or by using a stronger acid were detrimental, and hence, the reaction was subsequently done at room temperature and the reaction time was extended to 3 days. The resultant 2-bromoethyl ester was used subsequently as a starting material in the synthesis of the 2-(1-piperazinyl)ethyl (**4**), 2-(4-methyl-1-piperazinyl)ethyl (**2**), 2-(4-ethyl-1-piperazinyl)ethyl (**3**), 2-(4-(2-hydroxethyl)-1-piperazinyl)ethyl (**5**), 2-(4-acetyl-1-piperazinyl)ethyl (**6**), and 2-(4-methyl-1-piperazinylamino) ethyl (**7**) esters of ketorolac as shown in Scheme 1. Attempts to crystallize the ester prodrugs proved to be futile. The oily residue obtained after reaction workup failed to crystallize regardless of the solvent system used. Alternative methods of purification such as flash column chromatography were not practical because of the basicity of the ester and their chemical instability on silica gel. Formation of the oxalate salt for each prodrug followed by crystallization from methanol was the most practical and cost effective way of purification. The free bases were obtained on-demand and were analyzed using HPLC to assure sufficient purity and ^{1}H-NMR and ^{13}C-NMR to confirm identity.

Synthesis of 4-(4-methyl-1-piperazinyl)butyl ester of ketorolac was employed in a similar manner to the other esters. First, ketorolac was esterified with 4-chlorobutanol in the presence of benzenesulfonic acid. Then, after proper workup, the crude 4-chlorobutyl ester was stirred with a solution of 1-methylpiperazine in acetone in the presence of triethylamine and sodium iodide as a catalyst (Scheme 1). The crude residue was converted to the oxalate salt and the resultant salt was crystallized from methanol. Like the other free bases, prodrug 8 was obtained on-demand and was analyzed using HPLC to prove sufficient purity and ^{1}H-NMR and ^{13}C-NMR to confirm identity.



SCHEME 1. Synthesis of ketorolac piperazinylalkyl ester prodrugs 2–8.

Stability in Aqueous Phosphate Buffer

Chemical hydrolysis of ketorolac prodrugs in aqueous buffer solutions was investigated and the results are reported in Table 2. The chemical hydrolysis of each prodrug followed *pseudo* first-order kinetics in which a linear relationship between time and the logarithm of remaining prodrug concentration was found. Chemically, each prodrug was hydrolyzed quantitatively to ketorolac as seen for prodrug 8 at pH 5, as an example, Figure 1.

The prodrugs were more stable at pH 5 than at pH 7.4, with prodrug 8 exhibiting the highest stability at both pH values. This can be attributed to the fact that prodrug 8 contains the longest side chain, which makes nucleophilic attack more difficult; in addition, it is the least water soluble. The $t_{1/2}$ values for prodrug 8 at pH 5 and 7.4 were 119.75 and 11.97 h, respectively.

On the contrary, the stability of prodrug 6 was surprisingly the lowest at acidic pH, with $t_{1/2}$ 1.31 h. Although this ester was not expected to be more stable than the others, it was not expected to be five times less stable either. The main difference between prodrug 6 and the rest of the prodrugs that have same spacer size, i.e., 2, 3, 4, and 5, is that it is monobasic while the others are dibasic. The dibasic prodrugs are not likely to be completely diprotonated at pH 5.0 and likely to be completely mono-protonated at the pH 7.4 (Akre & Gaikar, 2006; Armarego & Perrin, 1996; Lin, Liao, Chen, & Lin, 2003). For

TABLE 2
The First-Order Degradation Rate Constants (k) and Half-Lives of Prodrugs 2–8 (pH 5 and 7.4) and in 80% Human Serum (pH 7.4) at 37°C

	pH 5		pH 7.4		Human Serum	
Prodrug	k (h^{-1})	$t_{1/2}$ (h)	K	$t_{1/2}$	k (min^{-1}))	$t_{1/2}$ (min)
2	0.071 ± 0.001	9.784 ± 0.083	0.051 ± 0.008 min^{-1}	13.480 ± 2.312 min	0.19 ± 0.01	3.75 ± 0.19
3	0.058 ± 0.000	12.031 ± 0.000	0.026 ± 0.000 min^{-1}	26.605 ± 0.361 min	0.18 ± 0.03	3.84 ± 0.64
4	0.082 ± 0.0005	8.414 ± 0.048	0.021 ± 0.001 min^{-1}	33.894 ± 1.309 min	0.87 ± 0.04	0.79 ± 0.04
5	0.084 ± 0.002	8.262 ± 0.153	0.040 ± 0.002 min^{-1}	17.281 ± 0.849 min	0.44 ± 0.00	1.59 ± 0.00
6	0.529 ± 0.003	1.309 ± 0.007	0.034 ± 0.001 min^{-1}	20.433 ± 0.562 min	0.29 ± 0.05	2.44 ± 0.39
7	0.0501 ± 0.001	13.820 ± 0.208	0.031 ± 0.002 min^{-1}	22.509 ± 1.407 min	0.59 ± 0.48	1.18 ± 0.10
8	0.006 ± 0.000	119.75 ± 0.000	0.058 ± 0.001 h^{-1}	11.969 ± 0.128 h	0.18 ± 0.01	3.92 ± 0.19

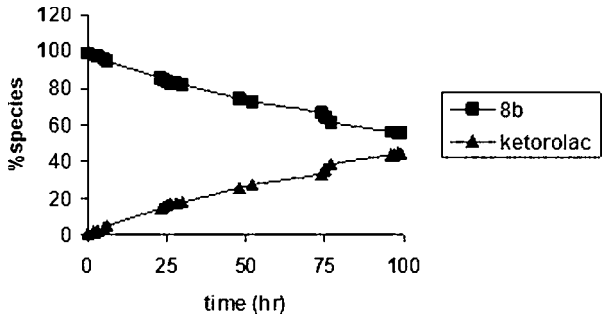


FIGURE 1. Plot showing the release of ketorolac (■) from prodrug 8 (▲) at pH 5.

example, for prodrug 2 it is expected that N_4, Figure 2, will be protonated first because it is relatively more accessible, while for prodrug 6 which contains the *N*-acetylpiperazinyl moiety only N_1 will be protonated. Taking this into consideration, it is possible that this compound will have a higher chance of having an intramolecular hydrogen bond between the hydrogen on the protonated nitrogen and the sp^3 oxygen of the ester group as depicted in Figure 2. Such hydrogen bonding will make the alkoxy moiety a better leaving group, and hence facilitates hydrolysis. Although this is probable, further studies are still needed for any conclusive explanation.

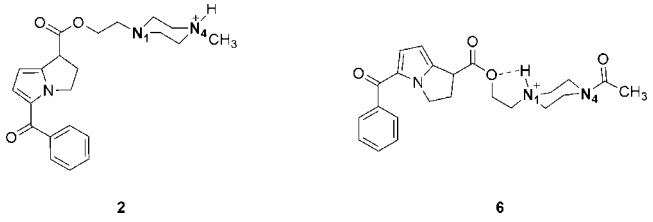


FIGURE 2. The chemical structure of prodrug 2 protonated at N_4 and the intramolecular hydrogen bonding in prodrug 6 because of exclusive protonation at N_1.

Hydrolysis in Human Serum

The hydrolysis of the prodrugs in human plasma was conducted to confirm that the prodrug can effectively release the parent drug when they reach the blood. The rates of hydrolysis of ketorolac prodrugs were studied in 80% human serum at 37°C at pH 7.4 (Table 2).

It can be seen that the hydrolysis in human serum is much faster than chemical hydrolysis, which is presumably because of enzymatic hydrolysis. Here, it appears that there is a correlation between lipophilicity and stability in human serum. The most lipophilic prodrugs, 2, 3, and 8 are 2–3 times more stable than the most hydrophilic prodrugs 4 and 5. The hydrolysis rates of the prodrugs were in the range of 0.79–3.92 min, which indicated rapid release of the parent drug in systemic circulation. Only the amount of remaining prodrugs were used to measure stability of esters in human serum. The amount of ketorolac detected did not represent the actual extent of degradation because it is expected to be more than 99% protein bound and ethanol addition was insufficient to liberate all the protein-bound ketorolac (Chaudhary, Gangwal, Jindal, & Khanna, 1993).

Aqueous Solubility and Lipophilicity

The aqueous solubility and lipophilicity (evaluated by drug partitioning between *n*-octanol and phosphate buffer) of ketorolac and its ester prodrugs at pH 5 and 7.4 are summarized in Table 3. The time scale of solubility measurements and log P_{app} measurement was no longer than 25 min, taking into consideration the chemical hydrolysis rates of the prodrugs; hence. the solubility and the partition coefficient in basic and acidic media will be named solubility$_{(25\ min)}$ and Log $P_{app(15\ min)}$ or Log $P_{app(30\ min)}$, respectively.

The prodrugs were more aqueous soluble at pH 5 than at pH 7.4. This is attributed to the protonation of the basic amino groups at pH 5 leading to 10 fold improvement of solubility compared to pH 7.4 in which the extent of protonation is

TABLE 3
Apparent Partition Coefficient (Log P_{app}) and Aqueous Solubility
($M \pm SD$; n = 2–3) of Ketorolac and its Prodrugs (2–8)

Compound	Log $P_{app\,(30\,min)}$ (pH 5)	Log $P_{app\,(15\,min)}$ (pH 7.4)	Solubility$_{(25\ min)}$ (mmol/L) (pH 5)	Solubility$_{(25\ min)}$ (mmol/L) (pH 7.4)
Ketorolac	0.404 ± 0.192	–0.833 ± 0.076	1.147 ± 0.017	70.869 ± 7.89
2	–0.573 ± 0.021	1.73 ± 0.049	507.568 ± 17.362	63.587 ± 8.110
3	–0.403 ± 0.084	2.090 ± 0.070	460.845 ± 23.885	38.594 ± 6.220
4	–0.545 ± 0.035	0.400 ± 0.0141	109.144 ± 15.744	49.825 ± 8.261
5	–0.633 ± 0.163	1.385 ± 0.035	586.949 ± 67.217	141.700 ± 32.607
6	1.140 ± 0.135	1.850 ± 0.096	19.434 ± 0.320	11.453 ± 1.626
7	–0.445 ± 0.049	2.750 ± 0.095	670.792 ± 59.277	62.515 ± 6.495
8	0.06 ± 0.028	2.153 ± 0.086	438.610 ± 22.721	35.639 ± 3.162

expected to be lower. These differences in aqueous solubility are much more pronounced than similar naproxen prodrugs (Rautio et al., 2000a). Prodrugs 4 and 6 did not show clear difference in solubility at different pH values. Prodrug 4 has a secondary nitrogen (N_4) and prodrug **6**, is monobasic. Although ionization is the most important factor affecting the solubility of these prodrugs, the trend in the solubility of the rest of the prodrugs is consistent with their inherent lipophilicities. At pH 7.4 the inherent lipophilicity plays a more prominent role in determining water solubility except for prodrug 6 which was the least water soluble mainly because it is monobasic. So in accordance, the higher lipophilicity of prodrugs 3 compared to prodrug 5 and 2 and that of prodrug 8 compared to prodrug 2 translates proportionally to difference in aqueous solubility.

At pH 5, where the free bases are significantly ionized they exhibited lower log P values than ketorolac. Partitioning depends on the unionized fraction of the prodrug. Since prodrug 6 is monobasic, its chances to be unionized is more than the other prodrugs and hence it will have a higher partition coefficient. For the rest of the prodrugs the trend clearly correlates with the chemical structure. In this context, the least lipophilic prodrugs were 2, 4, 5 and 7. Prodrug 3 was more lipophilic and prodrug 8 was the most lipophilic.

At pH 7.4, ionization is less prominent than at pH 5 and hence all compounds tested showed higher Log P values compared to ketorolac. The most lipophilic prodrugs were 7, 3 and 8. The least lipophilic was clearly prodrug 4. Prodrugs 2, 5 and 6 showed Log P values that were between those of the two groups above.

TABLE 4
Study-State Flux (J_{SS}), Lag Time and Permeability Coefficient (K_p) Values for the Delivery Ketorolac and Prodrug 8 Through Hairless Rat Skin In Vitro in Phosphate Buffer (0.05 M, pH 5 and 7.4) at 37°C

	Compound	
pH values	Ketorolac	8
pH 5		
Flux, J_{ss} (nmol/cm^2.h)	28.65 ± 2.69	44.60 ± 12.14
Lag time (h)	2.07 ± 0.64	3.06 ± 0.42
$K_p \times 10^3$ (cm/h)	24.98 ± 2.35	0.10 ± 0.03
pH 7.4		
Flux, J_{ss} (nmol/cm^2.h)	2.12 ± 1.08	24.15 ± 3.50
Lag time (h)	1.25 ± 0.71	2.20 ± 0.09
$K_p \times 10^3$ (cm/h)	0.03 ± 0.02	0.68 ± 0.10

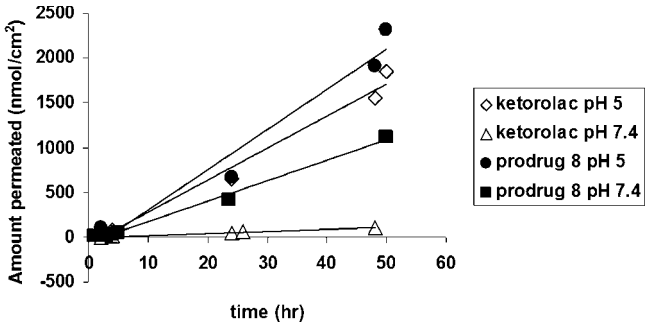


FIGURE 3. Plot showing the permeation profiles for ketorolac (◇) and prodrug 8 (•) through rat skin, after hair removal, in 0.05 M phosphate buffer at pH 5 and for ketorolac (△) and prodrug 8 (■) through in 0.05 M phosphate buffer at pH 7.4.

In Vitro Skin Permeation Study

Infinite-dose technique was used to determine the permeation profile of ketorolac and prodrug 8 in which excess amount of the test compound was used and the decrease in the concentration of the test compound in the donor compartment or increase in the amount of the test compound and/or parent, in the case of a prodrug, along the experiment's time frame was caused by diffusion only (Walters, 2002). The permeation of prodrug 8 through the skin was studied because it exhibited acceptable chemical stability ($t_{1/2}$ values are 119.75 h and 11.97 h at pH 5 and 7.4, respectively), good aqueous solubility and sufficient lipophilicity.

The steady-state flux values lag times, and permeability coefficients for ketorolac and prodrug 8 at pH 5 and 7.4 are shown in Table 4. The permeation profiles for ketorolac and prodrug 8 at pH 5 and 7.4 are shown in Figure 3.

As seen in Table 4 the steady-state flux for ketorolac was higher at pH 5 (28.65 nmol/cm^2.h) than at pH 7.4 (2.12 nmol/cm^2.hr) because of lower ionization of ketorolac at pH 5. The experiments were continued for 50 h. With regard to prodrug 8 which have a $t_{1/2}$ that is less than 12 h at pH 7.4, the experiment was continued to 50 h because it was observed the there was permeation over this time scale. This might be due to the fact that the permeation study was done using 10 times the concentration that was used for the determination of aqueous stability. Consequently, this high concentration allows for the presence of enough amounts of the prodrug that will be available for permeation even after 12 h. Prodrug 8 showed 1.56-fold enhancement in permeation compared to ketorolac at pH 5 which can be attributed to its higher aqueous solubility (aqueous solubility values are 1.15 and 438.61 mmol/L for ketorolac and prodrug 8 respectively).

The flux enhancement was more evident for prodrug 8 at pH 7.4. At this pH value, prodrug 8 showed 11.39-fold enhancement in permeation compared with ketorolac. This enhancement can be attributed to the higher lipophilicity of the prodrug (log P values are –0.83 and 2.15 for ketorolac and prodrug 8, respectively).

In conclusion, prodrug 8 has demonstrated enhanced transdermal permeation compared to the parent drug at pH 5 and 7.4. Also, prodrug 8 showed fast degradation in human serum. The transdermal permeation of the rest of the prodrugs can be

evaluated if they are administered in vehicles that enhance their chemical stability. The results shown earlier are considered promising and warrant further permeation studies.

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