

Research Article

Potential of Piperazinylalkylester Prodrugs of 6-Methoxy-2-Naphthylacetic Acid (6-MNA) for Percutaneous Drug Delivery

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Abstract. Piperazinylalkyl ester prodrugs (**4a–5d**) of 6-methoxy-2-naphthylacetic acid (6-MNA) (**1**) were synthesized and evaluated *in vitro* for the purpose of percutaneous drug delivery. These ionizable prodrugs exhibited varying aqueous solubilities and lipophilicities depending on the pH of the medium. The prodrugs (**4a–5c**) showed higher aqueous solubility and similar lipophilicity at pH 5.0 and lower aqueous solubility and higher lipophilicity at pH 7.4 in comparison to 6-MNA. The chemical and enzymatic hydrolyses of the prodrugs was investigated in aqueous buffer solutions (pH 5.0 and 7.4) and in 80% human serum (pH 7.4) at 37°C. The prodrugs showed moderate chemical stability ($t_{1/2}$ =6–60 h) but got readily hydrolyzed enzymatically to 6-MNA with half-life ranging from 10–60 min. In the *in vitro* permeation study using rat skin, the flux of 6-MNA and the prodrugs was determined in aqueous buffers of pH 5.0 and 7.4. The prodrug (**5b**) showed 7.9- and 11.2-fold enhancement in skin permeation compared to 6-MNA (**1**) at pH 5.0 and 7.4, respectively. It was concluded that the parent NSAIDs having favorable pharmacokinetic and pharmacodynamic properties coupled with increased skin permeability of their prodrugs could give better options for the treatment of rheumatic diseases.

KEY WORDS: 6-MNA; NSAID; piperazinylalkylester; prodrug; skin permeation.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drugs used for the treatment of rheumatic diseases and related painful conditions. Oral therapy using these agents is very effective but their clinical usage is often limited due to their adverse effects. Side effects of orally administered NSAIDs, such as ulceration potential and irritation of gastrointestinal mucosa (GI), have accelerated the development of alternative delivery systems for these drugs such as percutaneous drug delivery (1,2). Application of NSAIDs through percutaneous route can also lead to increase in drug concentration in the local soft tissue and joints while reducing their distribution and systemic toxicity (3,4). Unfortunately, the bioavailability of topically applied NSAIDs is only 1–2% in humans due to the barriers posed by the skin constituents (3,5–7).

Various strategies have been developed over the years to improve percutaneous delivery of the NSAIDs which include salt formation, chemical modifications and devising novel formulations for the drugs. In most of the formulations, penetration enhancers have been used to increase skin permeability

of the active ingredients. These penetration enhancers are chemicals that partition themselves into skin, interfere with the constituents of the skin layers and reduce the skin resistance for drug diffusion but may also lead to undesirable effects such as skin irritation, rashes and discomfort (8,9). Salt formation has been considered as an alternative strategy for the delivery of such drugs as it enhances skin permeation of these drugs without modifying the molecular structure of the active parent drug (10). Masking of the polar carboxylic acid functional group of the NSAIDs *via* esterification has been investigated as a promising strategy of increasing the dermal permeation. Various morpholinyl and methylpiperazinyl prodrugs of naproxen and ketorolac have been developed and found to be stable with improved permeation through the skin. These prodrugs got hydrolyzed rapidly *in vivo* to release the active drugs (1,5,11). Further, these prodrugs showed four- to nine-fold enhancement in skin permeation compared to the parent drugs.

Several current guidelines for management of osteoarthritis (OA) recommended topical NSAIDs, as safe and effective first line of treatment (12). In a recent study, nine commercially available topical NSAID formulations from the European Union have been studied for skin permeability, anti-inflammatory and analgesic responses. The study revealed that topical ketoprofen preparation showed superior results as compared to the other NSAID formulations (13). This study suggests that along with permeation enhancement, pharmacokinetic and pharmacodynamic properties of the NSAIDs are equally important parameters from the therapeutic point of view for the treatment of rheumatic diseases. A

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majority of the clinically used NSAIDs have short half lives, lack affinity towards the joints and possess large volume of distribution which adversely affect the treatment of chronic diseases such as arthritis. In order to obviate these drawbacks, we chose 6-methoxy-2-naphthylacetic acid (6-MNA), an active metabolite of nabumetone as the parent drug. It has certain advantages like having a long half-life (21–27 h), small volume of distribution (7.5 L), tendency to penetrate well into the synovial fluid, high binding to plasma proteins and absence of enterohepatic circulation. At the same time, it is a potent and selective inhibitor of cyclooxygenase-2 enzyme (14,15). Combining these ideal features of 6-MNA with the successful approach of converting the parent drugs into ester prodrugs for topical delivery, and keeping in view some recent work in the field, we planned to synthesize and evaluate a series of novel substituted piperazinylalkyl esters as prodrugs of 6-MNA for potential percutaneous drug delivery (11,16–18).

MATERIALS AND METHODS

All reagents and solvents required for synthesis were purified by general laboratory techniques before use. Purity of the compounds and completion of the reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60F₂₅₄; Merck), visualizing with ultraviolet light or iodine vapors. The yields reported here are unoptimized. Compounds were purified by column chromatography wherever required using neutral aluminum oxide (active) as stationary phase and a suitable solvent as eluent.

Melting points were determined using a Veego-make silicon oil bath-type melting point apparatus and are uncorrected. The IR spectra were recorded using KBr disc method in per centimeter on a Bruker FT-IR, Model 8300. The PMR and ¹³C NMR (ppm) spectra were recorded in CDCl₃/DMSO-*d*₆ on a Bruker 400 MHz spectrometer (chemical shifts in δ ppm, coupling constant *J* in Hz). λ_{max} was determined on Shimadzu 1800 UV spectrophotometer. Mass of the compounds was determined by LCMS using electron impact as source of ionization. Human serum was obtained from Baroda Blood Bank and Hospital, Vadodara. *In vitro* skin permeation studies were conducted using Franz-type diffusion cell using skin obtained from the whole dorsal area of a male Wistar rat (200±20 g). Animal experiments were performed with the approval of the Institutional Animal Ethical Committee (MSU/PHARM/IAEC/2011/14) in accordance with Laboratory Animal Welfare guidelines.

SYNTHESIS OF PRODRUGS

2-Bromoethyl 2-(6-Methoxy-2-Naphthyl)Acetate (2)

In a round-bottomed flask (250 ml), 6-MNA (**1**) (5.0 g, 23.14 mM) was dissolved in dry dichloromethane (DCM) (100 ml). The solution was cooled to 0–2°C followed by the addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (5.30 g, 27.77 mM) into it with stirring and dimethylaminopyridine (DMAP) (50 mg) was added into the reaction mixture as a catalyst followed by slow addition of 2-bromoethanol (4.90 ml, 69.44 mM). Stirring was continued till the reaction got completed. After completion of the

reaction, the reaction mixture was washed with chilled water (2×100 ml) followed by washing with acetic acid (10%, 2×50 ml) and later with saturated solution of sodium bicarbonate (2×100 ml) and chilled water (1×100 ml). The organic layer was separated and dried over anhydrous sodium sulphate, filtered and the solvent removed on rota evaporator to afford yellow product. The crude product so obtained was recrystallized from methanol after decolorization with activated charcoal to afford a fluffy white product. The solid product was dried under vacuum at 40°C/300 mmHg (3.5 g, 46.79%) m.p. 81–84°C; TLC (*R*_f): 0.74 (chloroform); UV (MeOH): 225 nm; IR (KBr, cm⁻¹): 1727 and 1214; PMR (CDCl₃): δ 7.71–7.67 (m, 3H, Ar-H), 7.39–7.37 (dd, 1H, Ar-H, *J*=1.76), 7.15–7.11 (m, 2H, Ar-H), 4.42–4.39 (t, 2H, -OCH₂, *J*=6.08), 3.91 (s, 3H, -OCH₃), 3.79 (s, 2H, -COCH₂) and 3.51–3.48 (t, 2H, -CH₂Br, *J*=6.08); Mass (*m/z*): 323.32 (M⁺).

3-Bromopropyl 2-(6-Methoxy-2-Naphthyl)Acetate (3)

The intermediate (**3**) was synthesized by the same procedure as mentioned for compound **2** using 6-MNA (**1**) (1.0 g, 4.62 mM), EDC (1.06 g, 5.55 mM), DMAP (50 mg) and 3-bromo-1-propanol (0.62 ml, 6.94 mM) to afford a yellow product. The crude product so obtained was recrystallized from methanol after decolorization with activated charcoal to afford a fluffy white product. The solid product (**3**) was dried under vacuum at 40°C/300 mmHg (0.8 g, 51.25%) m.p. 44–46°C; TLC (*R*_f): 0.81 (chloroform); UV (MeOH): 225 nm; IR (KBr, cm⁻¹): 1735 and 1267; PMR (CDCl₃): 7.71–7.68 (dd, 2H, Ar-H, *J*=4.28), 7.64 (s, 1H, Ar-H) 7.38–7.35 (m, 1H, Ar-H), 7.15–7.11 (m, 2H, Ar-H), 4.25–4.22 (t, 2H, -OCH₂, *J*=6.56), 3.91 (s, 3H, -OCH₃), 3.75 (s, 2H, -COCH₂), 3.40–3.36 (t, 2H, -CH₂Br, *J*=6.04) and 2.18–2.11 (m, 2H, -CH₂, *J*=6.56); Mass (*m/z*): 337.23 (M⁺).

2-(4-Methylpiperazin-1-yl)Ethyl 2-(6-Methoxy-2-Naphthyl)Acetate (4a)

The intermediate 2-bromoethyl 2-(6-methoxy-2-naphthyl)acetate (**2**) (0.5 g, 1.54 mM) was dissolved in dry acetone (20 ml) containing two drops of dimethyl formamide (DMF) in a pressure tube. *N*-Methylpiperazine (0.46 g, 4.64 mM) and potassium carbonate (0.5 g, 3.62 mM) were added to the above reaction mixture. The tube was sealed and heated on an oil bath at 70–80°C with stirring. The reaction was monitored for completion, filtered to remove K₂CO₃ and then solvent recovered under vacuum to get a brown oily product which was purified by column chromatography using hexane:ethyl acetate (1:1) as the mobile phase and active neutral alumina as the stationary phase to yield a yellowish brown oil (**4a**) which failed to crystallize. The oily product so obtained was dried in a vacuum oven at 40°C/300 mmHg (0.44 g, 83%); TLC (*R*_f): 0.43 (chloroform:methanol; 9:1); UV (H₂O): 226 nm; IR (KBr, cm⁻¹): 1730, 1264 and 1148; PMR (CDCl₃): 7.63–7.03 (m, 6H, Ar-H), 4.16–4.13 (t, 2H, -OCH₂, *J*=5.8), 3.83 (s, 3H, -OCH₃), 3.68 (s, 2H, -OCH₂), 2.54–2.51 (t, 2H, N-CH₂, *J*=5.8), 2.38–2.27 (m, 8H, piperazine-H) and 2.16 (s, 3H, -CH₃); ¹³C NMR (CDCl₃): 171.67 (1C, CO), 157.62–105.56 (10C, Ar-C), 62.31 (1C, OCH₂), 56.2 (O-CH₃), 55.3–54.94 (2C, piperazine), 52.93 (1C, -CH₂-N), 52.64–52.12 (2C, piperazine), 45.70 (Ar-CH₂-) and 41.20 (1C, -N-CH₃); Mass

(m/z): 342.5 (M^+); anal. calcd for $C_{20}H_{26}O_3N_2$: C 70.15, H 7.65, N 8.17; found: C 70.32, H 7.43, N 8.32; chromatographic purity (HPLC): >98.9%.

2-(4-Ethylpiperazin-1-yl)Ethyl 2-(6-Methoxy-2-Naphthyl) Acetate (4b)

The derivative (4b) was synthesized by the same procedure as mentioned for (4a) taking 2-bromoethyl 2-(6-methoxy-2-naphthyl)acetate (2) (0.5 g, 1.54 mM) and *N*-ethylpiperazine (0.21 g, 1.85 mM) to get an oily product which was purified by column chromatography using hexane:ethyl acetate (1:1) as the mobile phase and active neutral alumina as the stationary phase. The yellowish green oily product (4b) so obtained was dried in a vacuum oven at 40°C/300 mmHg (0.25 g, 45.45%); TLC (R_f): 0.53 (chloroform:methanol; 9:1); UV (H_2O): 226 nm; IR (KBr, cm^{-1}): 1731, 1264 and 1151; PMR ($CDCl_3$): 7.63–7.03 (m, 6H, Ar-H), 4.17–4.14 (t, 2H, $-OCH_2$, $J=5.76$), 3.83 (s, 3H, $-OCH_3$), 3.68 (s, 2H, $-COCH_2$), 2.54–2.52 (t, 2H, $-CH_2N$, $J=5.76$), 2.38–2.37 (m, 8H, piperazine-H), 2.31–2.26 (q, 2H, $-CH_2$, $J=7.2$) and 0.99–0.95 (t, 3H, $-CH_3$, $J=7.16$); Mass (m/z): 356.38 (M^+); anal. calcd for $C_{21}H_{28}O_3N_2$: C 70.76, H 7.91, N 7.85; found: C 70.96, H 7.74, N 7.68; chromatographic purity (HPLC): >97.8%.

2-(4-Acetylpiperazin-1-yl)Ethyl 2-(6-Methoxy-2-Naphthyl) Acetate (4c)

The derivative (4c) was synthesized by the procedure as mentioned for (4a) using 2-bromoethyl 2-(6-methoxy-2-naphthyl)acetate (2) (0.5 g, 1.54 mM) and *N*-acetylpiperazine (0.23 g, 1.84 mM) to afford an oily product which was purified by column chromatography using hexane: ethyl acetate (1:1) as the mobile phase and active neutral alumina as the stationary phase. The red brown oily product (4c) so obtained was dried in vacuum oven at 40°C /300 mmHg (0.17 g, 34.88%); TLC (R_f): 0.56 (chloroform:methanol; 9:1); UV (H_2O): 225 nm; IR (KBr, cm^{-1}): 1730, 1626, 1263 and 1150; PMR ($CDCl_3$): 7.63–7.03 (m, 6H, Ar-H), 4.16–4.13 (t, 2H, $-OCH_2$, $J=5.44$), 3.82 (s, 3H, $-OCH_3$), 3.67 (s, 2H, $-COCH_2$), 3.13–3.11 (t, 2H, N- CH_2 , $J=5.0$), 2.52–2.50 (m, 4H, piperazine-H), 2.29–2.19 (m, 4H, piperazine-H) and 1.90 (s, 3H, $-COCH_3$); Mass (m/z): 370.45 (M^+); anal. calcd for $C_{21}H_{26}O_4N_2$: C 68.09, H 7.07, N 7.55; found: C 68.28, H 7.24, N 7.75; chromatographic purity (HPLC): >97.4%.

2-(4-Phenylpiperazin-1-yl)Ethyl 2-(6-Methoxy-2-Naphthyl) Acetate (4d)

The derivative (4d) was synthesized by the same procedure as mentioned for (4a) using 2-bromoethyl 2-(6-methoxy-2-naphthyl)acetate (2) (0.5 g, 1.54 mM) and *N*-phenylpiperazine (0.30 g, 1.85 mM) to afford a crude solid product which was successively recrystallized from methanol to yield white solid (4d). The product was dried in a vacuum oven at 60°C/300 mmHg (0.3 g, 47.91%) m.p. 91–93°C; TLC (R_f): 0.69 (chloroform:methanol; 9:1); UV (H_2O): 226 nm; IR (KBr, cm^{-1}): 1720, 1234 and 1147; PMR ($CDCl_3$): 7.70–7.22 (m, 6H, Ar-H), 7.15–6.80 (m, 5H, phenyl-H), 4.29–4.27 (t, 2H, $-OCH_2$, $J=5.56$), 3.90 (s, 3H, $-OCH_3$), 3.77 (s, 2H, $-COCH_2$), 3.02–2.95 (t, 4H, piperazine-H, $J=5.10$), 2.67–2.64 (t, 2H, N- CH_2 , $J=5.56$) and 2.52–2.49 (t, 4H, piperazine-H, $J=5.10$); Mass (m/z): 404.56 (M^+); anal. calcd for

$C_{25}H_{28}O_3N_2$: C 74.23, H 6.97, N 6.92; found: C 74.41, H 7.12, N 7.09; Chromatographic Purity (HPLC): >98.7%.

3-(4-Methylpiperazin-1-yl)Propyl 2-(6-Methoxy-2-Naphthyl) Acetate (5a)

Derivative (5a) was synthesized by the same procedure as mentioned for (4a) using the intermediate 3-bromopropyl 2-(6-methoxy-2-naphthyl)acetate (3) (0.3 g, 0.890 mM) and *N*-methylpiperazine (0.106 g, 1.068 mM) to afford a brown oily product. The oily product was purified by column chromatography using hexane:ethyl acetate (1:1) as the mobile phase and neutral alumina as the stationary phase to yield a brown oily product (5a) which failed to crystallize. The product so obtained was dried in a vacuum oven at 40°C/300 mmHg (0.15 g, 48.31%); TLC (R_f): 0.60 (chloroform:methanol; 10:0.5); UV (H_2O): 226 nm; IR (KBr, cm^{-1}): 1732, 1398 and 1163; PMR ($CDCl_3$): 7.63–7.03 (m, 6H, Ar-H), 4.07–4.04 (t, 2H, $-OCH_2$, $J=6.48$), 3.81 (s, 3H, $-OCH_3$), 3.64 (s, 2H, $-COCH_2$), 2.34–2.28 (m, 8H, piperazine-H), 2.27–2.23 (t, 2H, N- CH_2 , $J=7.24$), 2.16 (s, 3H, N- CH_3) and 1.75–1.69 (m, 2H, $-C-CH_2$, $J=7.04$); ^{13}C NMR ($CDCl_3$): 172.76, 168.92–105.56, 62.94, 55.32–21.31; mass (m/z): 356.42 (M^+); anal. calcd for $C_{21}H_{28}O_3N_2$: C 70.76, H 7.91, N 7.85; found: C 70.84, H 8.13, N 7.98; chromatographic purity (HPLC): >98.5%.

3-(4-Ethylpiperazin-1-yl)Propyl 2-(6-Methoxy-2-Naphthyl) Acetate (5b)

Derivative (5b) was synthesized by the same procedure as described for (4a) using the intermediate 3-bromopropyl 2-(6-methoxy-2-naphthyl)acetate (3) and *N*-ethylpiperazine (0.12 g, 1.0 mM) to afford brown oily product. The product was purified by column chromatography using hexane: ethyl acetate (1:1) as the mobile phase and neutral alumina as the stationary phase to yield brown oily product which failed to crystallize. The product (5b) so obtained was dried in a vacuum oven at 40°C/300 mmHg (0.20 g, 62.50%); TLC (R_f): 0.40 (chloroform:methanol; 10:0.5); UV (H_2O): 226 nm; IR (KBr, cm^{-1}): 1732, 1265 and 1156; PMR ($CDCl_3$): 7.55–6.95 (m, 6H, Ar-H), 3.99–3.96 (t, 2H, $-OCH_2$, $J=6.4$), 3.75 (s, 3H, $-OCH_3$), 3.54 (s, 2H, $-CH_2$), 2.50–2.47 (t, 2H, N- CH_2), 2.42–2.26 (m, 8H, piperazine-H), 2.21–2.19 (m, 2H, $-N-CH_2$), 1.58–1.55 (m, 2H, $-CH_2$, $J=4.88$) and 0.97–0.94 (m, 3H, $-CH_3$); Mass (m/z): 370.39 (M^+); anal. calcd for $C_{22}H_{30}O_3N_2$: C 71.32, H 8.16, N 7.55; found: C 71.56, H 8.45, N 7.72; chromatographic purity (HPLC): >98.3%.

3-(4-Acetylpiperazin-1-yl)Propyl 2-(6-Methoxy-2-Naphthyl) Acetate (5c)

Derivative (5c) was synthesized by the same procedure as described for (4a) using the intermediate 3-bromopropyl 2-(6-methoxy-2-naphthyl)acetate (3) and *N*-acetylpiperazine (0.22 g, 1.78 mM) to afford a brown oily product which was purified by column chromatography using hexane: ethyl acetate (1:1) as the mobile phase and neutral alumina as stationary phase to yield a brown oily product (5c) which failed to crystallize. The product thus obtained was dried in a vacuum oven at 40°C/300 mmHg (0.51 g, 89.47%); TLC (R_f): 0.51 (chloroform:methanol; 19:1); UV (H_2O): 226 nm;

IR (KBr, cm^{-1}): 1728, 1632, 1263 and 1150; PMR (CDCl_3): 7.70–7.11 (m, 6H, Ar-H), 4.17–4.13 (t, 2H, $-\text{OCH}_2$, $J=6.4$), 3.91 (s, 3H, $-\text{OCH}_3$), 3.73 (s, 2H, $-\text{COCH}_2$), 3.57–3.54 (t, 2H, N- CH_2 , $J=5.12$), 3.38–3.35 (t, 2H, piperazine -H), 2.33–2.27 (m, 6H, piperazine-H) 2.05 (s, 3H, $-\text{COCH}_3$), 1.80–1.77 (m, 2H, $-\text{CH}_2$, $J=5.12$); ^{13}C NMR (CDCl_3): δ 171.76 (1C, $-\text{CO}$), 168.92 (1C, $-\text{CO}$), 157.65–105.56 (10C, Ar-C), 62.94 (1C, $-\text{OCH}_2$), 55.32 (1C, $-\text{OCH}_3$), 54.57 (1C, N- CH_2), 53.05–52.60 (2C, piperazine-C), 46.04 (1C, $-\text{CH}_2\text{CO}$), 41.50–41.18 (2C, piperazine-C), 25.80 (1C, $-\text{CH}_2$) and 21.31 (1C, $-\text{CH}_3$); mass (m/z): 384.38 (M^+); anal. calcd for $\text{C}_{22}\text{H}_{28}\text{O}_4\text{N}_2$: C 68.73, H 7.34, N 7.28; found: C 68.92, H 7.16, N 7.09; chromatographic purity (HPLC): >97.8%.

3-(4-Phenylpiperazin-1-yl)Propyl 2-(6-Methoxy-2-Naphthyl) Acetate (5d)

Derivative (5d) was synthesized by the same procedure as described for (4a) using the intermediate 3-bromopropyl 2-(6-methoxy-2-naphthyl)acetate (3) and *N*-phenylpiperazine (0.28 g, 1.78 mM) to afford solid crude product which was repeatedly recrystallized from methanol to yield a light brown solid (5d). The product was dried in vacuum oven at 60°C/300 mmHg (0.3 g, 48.32%) m.p. 78–80°C; TLC (R_f): 0.73 (chloroform:methanol; 1:0.05); UV (H_2O): 227 nm; IR (KBr, cm^{-1}): 1736, 1267 and 1155; PMR (CDCl_3): δ 7.63–7.15 (m, 6H, Ar-H), 7.07–6.75 (m, 5H, phenyl-H), 4.11–4.07 (t, 2H, $-\text{OCH}_2$, $J=6.44$), 3.81 (s, 3H, $-\text{OCH}_3$), 3.66 (s, 2H, $-\text{CH}_2\text{CO}$), 3.06–3.04 (t, 4H, piperazine-H, $J=4.88$), 2.43–2.40 (t, 4H, piperazine-H, $J=5.04$), 2.32–2.28 (t, 2H, N- CH_2 , $J=7.2$) and 1.77–1.73 (m, 2H, $-\text{CH}_2$, $J=7.64$); mass (m/z): 418.14 (M^+); anal. calcd for $\text{C}_{26}\text{H}_{30}\text{O}_3\text{N}_2$: C 74.61, H 7.22, N 6.69; found: C 74.39, H 7.41, N 6.88; chromatographic purity (HPLC): >99.6%.

HPLC Analysis

High-performance liquid chromatography (HPLC) was used for analysis. The analytical system consisted of an LC system (LC-20AD, Shimadzu USA) and UV-visible detector (SPD 20A, Shimadzu). The chromatographic separation was carried out under isocratic reverse phase conditions on purospher RP-18C column (250×4.6 mm, 5 μ , Merck Germany) with column temperature 25–28°C. The injection volume was 20 μL and the detection wavelength was 227 nm. The mobile phase consisted of a mixture of 15 mM phosphate buffer and acetonitrile of pH 5.0–5.5 and the flow rate was 0.9 ml/min.

Aqueous Solubility

The aqueous solubility of 6-MNA and its prodrugs were determined in phosphate buffer (0.16 M) at pH 5.0 and 7.4 at room temperature. Excess amounts of the prodrugs were added to phosphate buffer (0.5–1 ml), the mixture stirred and centrifuged at 8000 rpm and filtered (Millipore 0.45 μm). The concentrations of each prodrug in their saturated solutions were analyzed by HPLC. The pH of the solutions was held constant throughout the experiment (1,7).

Apparent Partition Coefficient ($\log P_{\text{app}}$)

The apparent partition coefficients ($\log P_{\text{app}}$) of 6-MNA and its prodrugs were determined at room temperature between 1-octanol and phosphate buffer (0.16 M) at pH 5.0 and 7.4 using shake flask method. 1-Octanol was saturated with phosphate buffer (0.16 M) for 24 h by stirring vigorously before use. A known concentration of the compound in phosphate buffer was shaken with a suitable fixed volume of 1-octanol. After shaking, both the phases were separated by centrifugation at 8000 rpm for 5–6 min. The concentrations of the compounds in the buffer phase and in the 1-octanol phase were determined by HPLC (1,7).

Hydrolysis in Aqueous Solution

Stability study or the rates of chemical hydrolysis of the prodrugs were carried out in aqueous phosphate buffer solutions (0.16 M, ionic strength was adjusted to 0.5 with NaCl) of pH 5.0 and 7.4 at $37\pm 1^\circ\text{C}$. An appropriate amount of the prodrug was dissolved in phosphate buffer (5 ml) and the solutions were placed in a thermostatically controlled water bath at temperature $37\pm 1^\circ\text{C}$. Depending upon stability of each prodrug, samples were withdrawn at appropriate time intervals and analyzed for the intact amount of the prodrug and the liberated 6-MNA using HPLC. The experiments were run in triplicates for each compound. Half-lives ($t_{1/2}$) for the hydrolysis of the different prodrugs were calculated from the slope of the linear portion of the plotted logarithm of the remaining concentrations of the prodrugs versus time (1,7). The first-order equation ($t_{1/2}=0.693/k$) was used to calculate the half-lives as the prodrugs were found to degrade by pseudo-first-order degradation kinetics.

Hydrolysis in Human Serum

The rates of enzymatic hydrolysis for prodrugs were studied in human serum at $37\pm 1^\circ\text{C}$. The human serum was diluted to 80% with 0.16 M phosphate buffer, pH 7.4. The study was performed by dissolving an appropriate amount of prodrug in phosphate buffer and human serum mixture. The solutions were maintained at $37\pm 1^\circ\text{C}$ in water bath and 0.5 ml of serum/buffer mixture were withdrawn at appropriate time intervals and added to 1.0 ml of ethanol to precipitate the proteins from the serum. Then, the mixture was centrifuged for 10 min at 10,000 rpm, the supernatant was analyzed for the released 6-MNA and the remaining prodrug by HPLC. The pseudo-first-order times, at which 50% total parent compound had been formed were determined from the linear slope of the logarithm of formed parent compound over various time intervals (1,5).

In Vitro Skin Permeation Studies

In vitro skin permeation studies of 6-MNA and prodrugs were performed by using glass Franz diffusion cells and skin from the whole dorsal area of a male Wistar rat. The skin specimens were dried and frozen prior to use and rehydrated before being mounted in the diffusion cell. The receptor medium (0.05 M phosphate buffer saline solution of pH 7.4) was stirred and kept at $37\pm 1^\circ\text{C}$ throughout the study. The

prodrugs were applied as solutions in phosphate buffer (0.05 M) at both pH 5.0 and 7.4. Infinite dose technique was used to determine the permeation profile of 6-MNA and the prodrugs in which excess amount of the test compound was used and the increase in the amount of the prodrug and/or parent along the experiments time frame was measured (19).

The receiver medium (0.05 M phosphate buffer saline solution of pH 7.4) was stirred and kept at $37 \pm 1^\circ\text{C}$ throughout the study. The prodrugs were applied as solutions in phosphate buffer of pH 5.0 and 7.4. At specified time intervals, aliquots (0.25 ml) were withdrawn from the receptor compartment and replaced with fresh buffer. The drug concentrations were analyzed by HPLC. The steady-state flux for 6-MNA (**1**) and its prodrugs were determined by plotting the cumulative amount of the parent drug and the intact prodrug as measured in the receiver phase against time, and dividing the slope of the steady-state position by the surface area of the diffusion cell (4.906 cm^2). The permeability coefficients (K_p) for the steady-state delivery were obtained by dividing the steady-state flux (J_{ss}) by the solubilities of the prodrugs in the corresponding vehicle (1,7).

Statistical Analysis

For testing the statistical significance of the data, Graph Pad Prism software was used. Data is presented as mean \pm standard deviation ($n=3-6$). Groups were compared using analysis of variance (ANOVA) one-way test. Differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Synthesis of Piperazinylalkyl Ester Prodrugs of 6-MNA

6-MNA (**1**) required for the synthesis of the prodrugs has been prepared by the reported procedure (20,21). Scheme 1 was employed to synthesize piperazinylalkyl ester prodrugs (**4a-5d**) of 6-MNA (**1**). The ester intermediates (**2**, **3**) were prepared by coupling the corresponding bromoalkanol with 6-MNA (**1**) using EDC as the coupling agent. The structures of the intermediates were confirmed by IR and NMR spectroscopy. Intermediate (**2**) showed strong absorption peak at 1727 cm^{-1} in the IR spectrum due to carbonyl stretching of the ester group. Its PMR spectrum showed multiplet at δ 7.71–7.11 of six naphthalene protons (Ar-H), a triplet at δ 4.42–4.39

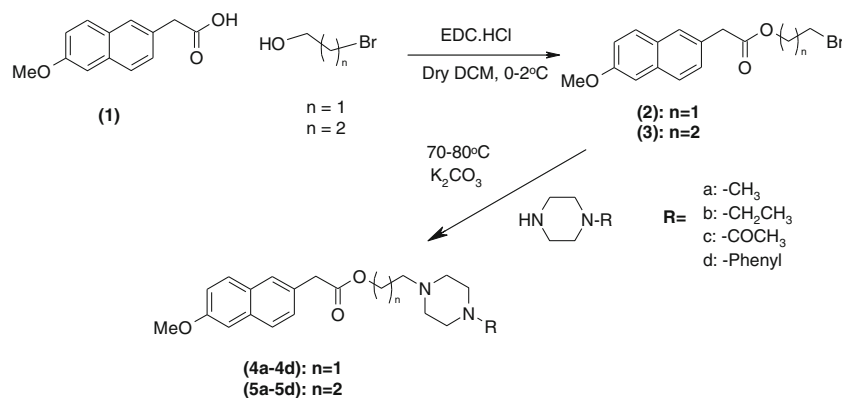
due to methylene protons ($-\text{CH}_2$) with coupling constant equal to 6.08 Hz. Methoxy protons ($-\text{OCH}_3$) appeared at δ 3.91 as singlet and methylene protons attached to aromatic ring ($-\text{CH}_2$) appeared at δ 3.79. A triplet due to methylene protons ($-\text{CH}_2$) was observed at δ 3.51–3.48 with coupling constant equal to 6.08 Hz.

Similarly, another intermediate (**3**) showed strong absorption peak at $1,735\text{ cm}^{-1}$ due to carbonyl stretching of the ester group. The PMR spectrum of intermediate (**3**) showed multiplet at δ 7.71–7.11 for the six naphthalene protons (Ar-H) and methylene protons ($-\text{CH}_2$) appeared at δ 4.25–4.22 as a triplet with coupling constant equal to 6.4 Hz. Methoxy protons ($-\text{OCH}_3$) showed a singlet at δ 3.91 and a singlet of methylene protons attached to aromatic ring (Ar- CH_2) appeared at δ 3.75. Other methylene protons ($-\text{CH}_2$) appeared at δ 3.40–3.36 as a triplet with coupling constant equal to 6.04 Hz. Multiplet at δ 2.18–2.11 appeared due to methylene protons ($-\text{CH}_2$) with coupling constant equal to $J=6.56$ Hz. These bromoalkyl ester intermediates (**2**, **3**) were further treated with appropriate piperazine derivatives (**a-d**) to get piperazinylalkyl ester prodrugs (**4a-5d**) of 6-MNA (**1**).

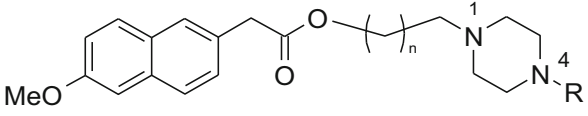
The IR spectrum of the ester (**4a**) showed absorption peak at 1730 cm^{-1} due to the carbonyl stretching of the ester group. The peaks at 1264 and 1148 cm^{-1} are due to C-O and C-N stretching vibrations, respectively. Its PMR spectrum showed multiplet at δ 7.63–7.03 for six naphthalene protons (Ar-H) and a triplet at δ 4.16–4.13 due to methylene ($-\text{CH}_2$) protons with coupling constant equal to 5.8 Hz. A singlet due to methoxy protons ($-\text{OCH}_3$) appeared at δ 3.83 and of methylene (Ar- CH_2) at δ 3.68. The other methylene protons (N- CH_2) showed triplet at δ 2.54–2.51 with coupling constant equal to 5.8 Hz. The piperazine protons appeared as multiplet at δ 2.38–2.27 and N-methyl protons ($-\text{CH}_3$) at δ 2.16 as a singlet.

Its ^{13}C NMR spectrum shows peak at 171.67 for carbonyl carbon. Aromatic carbons of naphthalene ring appear in the range of δ 157.62–105.56. Methoxy carbon appears at δ 56.2 and $-\text{OCH}_2$ at δ 62.31. Four piperazine carbons were observed at δ 52.12–55.3. Other aliphatic carbons appeared at δ 52.93 (N- CH_2), δ 45.70 (Ar- CH_2) and δ 41.20 (1C, N- CH_3). Mass spectrum shows molecular ion peak at 342.5 (M^+) and also M+2 peak at 344.0 which is the base peak. The compound showed high chromatographic purity ($>98.2\%$) by HPLC.

Representative compound (**5a**) from the second series was confirmed in a similar way and its IR spectrum showed a strong absorption peak at $1,732\text{ cm}^{-1}$ due to carbonyl



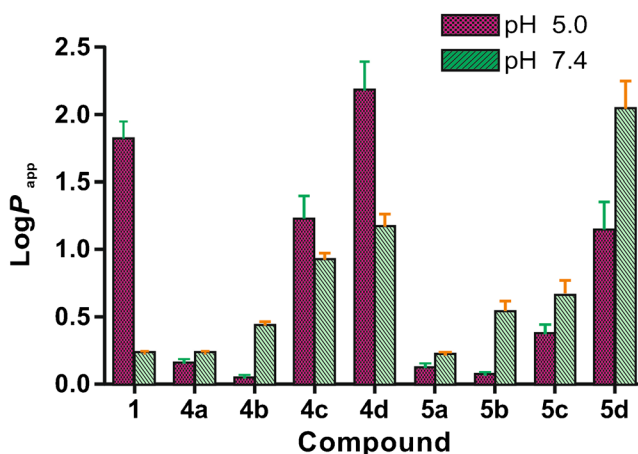
Scheme 1. Synthesis of piperazinylalkyl ester prodrugs

Table I. Predicted Protonated Forms Obtained for Derivatives (4a–5d) at pH 5.0, 7.4 and Predicted pK_a of 6-MNA (1) and its Derivatives at Physiological pH 7.4 Using H₂O as Solvent


| Entry ID | <i>n</i> | <i>R</i> | Protonation of nitrogen | | | | Predicted pK_a | |
|----------|----------|----------------------------------|-------------------------|----------------|----------------|----------------|------------------|----------|
| | | | pH 5.0 | | pH 7.4 | | pK_a 1 | pK_a 2 |
| | | | N ¹ | N ⁴ | N ¹ | N ⁴ | | |
| 1 | – | – | – | – | – | – | 4.316 | – |
| 4a | 1 | –CH ₃ | + | + | -- | -- | 3.572 | 7.719 |
| 4a-1 | 1 | –CH ₃ | – | – | + | -- | – | – |
| 4a-2 | 1 | –CH ₃ | – | – | -- | + | – | – |
| 4a-3 | 1 | –CH ₃ | – | – | + | + | – | – |
| 4b | 1 | –CH ₂ CH ₃ | + | + | -- | -- | 3.657 | 7.863 |
| 4b-1 | 1 | –CH ₂ CH ₃ | – | – | + | -- | – | – |
| 4b-2 | 1 | –CH ₂ CH ₃ | – | – | -- | + | – | – |
| 4b-3 | 1 | –CH ₂ CH ₃ | – | – | + | + | – | – |
| 4c | 1 | –COCH ₃ | + | -- | + | -- | 6.335 | – |
| 4d | 1 | –Ph | + | -- | + | -- | 3.49 | 7.324 |
| 4d-1 | 1 | –Ph | + | + | – | – | – | – |
| 5a | 2 | –CH ₃ | + | + | -- | -- | 4.214 | 7.749 |
| 5a-1 | 2 | –CH ₃ | – | – | + | -- | – | – |
| 5a-2 | 2 | –CH ₃ | – | – | -- | + | – | – |
| 5a-3 | 2 | –CH ₃ | – | – | + | + | – | – |
| 5b | 2 | –CH ₂ CH ₃ | + | + | -- | -- | 4.299 | 7.893 |
| 5b-1 | 2 | –CH ₂ CH ₃ | – | – | + | -- | – | – |
| 5b-2 | 2 | –CH ₂ CH ₃ | – | – | -- | + | – | – |
| 5b-3 | 2 | –CH ₂ CH ₃ | – | – | + | + | – | – |
| 5c | 2 | –COCH ₃ | + | -- | + | -- | 6.592 | – |
| 5d | 2 | –Ph | + | + | + | -- | 3.681 | 7.581 |
| 5d-1 | 2 | –Ph | + | -- | – | – | – | – |

+ Protonated, -- not protonated, – not applicable

stretching of ester group. The peaks at 1,398 and 1,163 cm⁻¹ are due to C–O and C–N stretching vibrations, respectively. The PMR signals of compound (5a) appeared at δ 7.62–7.02 for six naphthalene protons (Ar-*H*), a triplet at δ 4.07–4.04 was due to methylene (–CH₂) protons. A singlet due to methoxy protons (–CH₃) appeared at δ 3.81 and of methylene protons

**Fig. 1.** Comparison of log P_{app} values at pH 5.0 and pH 7.4, $p < 0.05$ compared to 6-MNA (1) (ANOVA, Bonferroni's test)

(Ar-CH₂) at δ 3.64. The other methylene protons (N-CH₂) showed triplet at δ 2.27–2.23, the piperazine protons appeared as multiplet at δ 2.34–2.28 and N-methyl protons (–CH₃) appeared at δ 2.16 as a singlet.

Its ¹³C NMR spectrum shows peaks at 172.76 for carbonyl carbon. Aromatic carbons of naphthalene ring appear in the range of 168.92–105.56, and other aliphatic carbons appeared at δ 62.94, 55.32–21.31. The mass spectrum of compound (5a) shows peak at 356.03 (M⁺) which is also the base peak. The compound also showed high chromatographic purity (>98%) by HPLC.

Aqueous Solubility

Due to the biphasic nature of skin, an ideal percutaneous prodrug should exhibit adequate lipid as well as aqueous solubility. Aqueous solubility of 6-MNA and its prodrugs were determined at the physiological pH 7.4 and at 5.0, the pH of the outer surface of the skin (pH 4.2–6.5) (3). Generally, an ionized molecule is more water soluble than its unionized form. Because of the acidic character of 6-MNA (pK_a 4.16) it is more soluble in aqueous medium at pH 7.4 than at pH 5.0. On the other hand, piperazinylalkyl ester prodrugs (4a–5b) which possess basic groups (N¹ and/or N⁴) are expected to be more ionized at pH 5.0 than at pH 7.4. The predicted pK_a of prodrugs (4a–5d) has been shown in Table I. pK_a values for these structures were calculated using EPIC software at physiological pH using water as solvent.

Table II. Aqueous Solubility of 6-MNA (1) and Prodrugs (4a–5d) at pH 5.0 and pH 7.4

| Compound | Aqueous solubility (mM) | | Log P_{app} | |
|----------|-------------------------|-------------|---------------|-----------|
| | pH 5.0 | pH 7.4 | pH 5.0 | pH 7.4 |
| 1 | 0.78±0.18 | 63.34±0.24 | 1.82±0.12 | 0.23±0.02 |
| 4a | 23.41±0.16 | 21.58±0.23 | 0.10±0.08 | 0.23±0.01 |
| 4b | 26.01±0.23 | 12.10±0.17 | 0.41±0.02 | 0.43±0.03 |
| 4c | 1.22±0.01 | 1.49±0.01 | 1.21±0.18 | 0.91±0.01 |
| 4d | 0.29±0.02 | 0.31±0.02 | 2.17±0.22 | 1.16±0.10 |
| 5a | 28.41±0.19 | 20.09±0.16 | 0.12±0.03 | 0.22±0.02 |
| 5b | 16.30±0.14 | 6.20±0.03 | 0.69±0.02 | 0.53±0.08 |
| 5c | 6.07±0.02 | 3.11±0.01 | 0.37±0.07 | 0.67±0.01 |
| 5d | 0.26±0.01 | 0.019±0.002 | 1.15±0.21 | 2.04±0.21 |

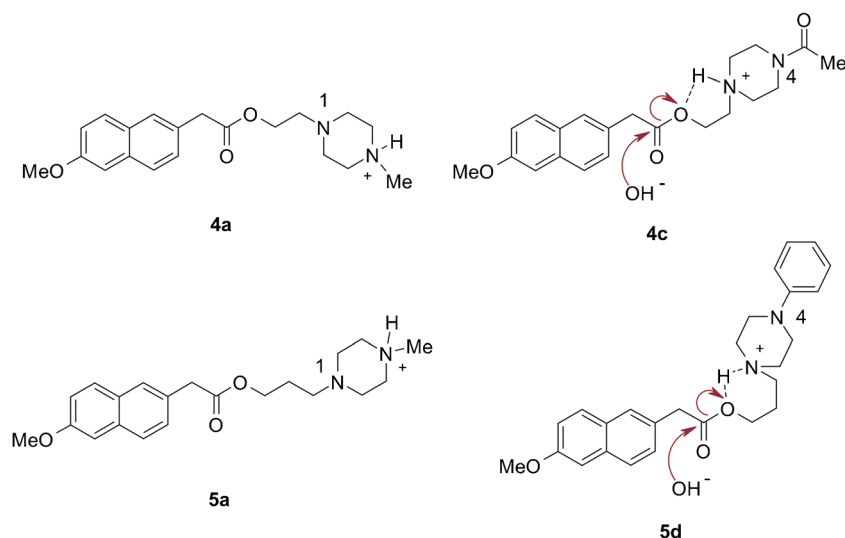


Fig. 2. The chemical structure of prodrugs (**4a**, **5a**) protonated at N-4 and the intramolecular hydrogen bonding in prodrug (**4c**, **4d**) because of exclusive protonation at N-1

Due to the presence of dibasic groups in prodrugs along with various electron withdrawing or donating substituents at N-4, it is difficult to specify the ionization of these prodrugs at pH 5.0 and 7.4. For understanding, predicted protonated forms of these prodrugs have been determined using Quick prop 32 software and the result obtained has been shown in Table I.

From the data, it could be concluded that at pH 5.0, prodrugs (**4a**, **4b**, **5a**, **5b**) get protonated on both the nitrogens (N-1,4), **4c**, **5c** get protonated only at one nitrogen (N-1) and prodrugs (**4d**, **5d**) get protonated at N-1 or N-4 or both nitrogens. At pH 7.4, prodrugs (**4a**, **4b**, **5a**, **5b**) get protonated either at N-1 or N-4 or both nitrogens and prodrugs (**4c**, **5c**) get protonated only at one nitrogen (N-1) due to the presence of a strong electron withdrawing group at N-4. From this data it is concluded that piperazinyl prodrugs (**4c**, **4d**, **5c**, **5d**) with electron withdrawing groups at N⁴ have only one basic ionizable group that makes them less soluble at pH 5.0 with no significant differences observed at pH 7.4. The aqueous solubility of 6-MNA is higher at pH 7.4 and for piperazinyl prodrugs (**4a**, **4b**, **5a**, **5b**) it is higher at pH 5.0. Overall, substituted piperazinylalkyl prodrugs exhibited higher aqueous solubilities compared to 6-MNA (**1**) at pH 5.0 except for **4d** and **5d**. Aqueous solubility of all piperazinylalkyl prodrugs of 6-MNA at pH 7.4 was found to be comparatively lesser than 6-MNA as shown in Table II.

The data showed that aqueous solubility of piperazinyl prodrugs was pH dependent and lowering the pH generally increased aqueous solubility of the prodrugs. Substituted piperazinyl promoieties seemed to be suitable for maintaining or increasing the aqueous solubility of 6-MNA in the pH range of 5.0 to 7.4.

Determination of Apparent Partition Coefficient ($\log P_{app}$)

Lipid solubility, *i.e.* lipophilicity plays a crucial role in determining skin permeability of a compound because stratum corneum (SC) the major barrier to drug permeation is essentially lipoidal in nature and generally favors permeation of

lipophilic drugs. The apparent partition coefficients of 6-MNA (**1**) and prodrugs (**4a–5d**) were determined by partitioning them between phosphate buffer (0.16 M) and saturated 1-octanol at pH 5.0 and pH 7.4 using the shake flask method. The apparent partition coefficients of 6-MNA (**1**) and prodrugs are listed in Table II.

All of the prodrugs of 6-MNA (**1**) have retained more or less comparable lipophilicity at pH 5.0 except for derivative (**4d**, $\log P_{app}=2.17\pm 0.05$) which is even more lipophilic than 6-MNA (**1**) ($\log P_{app}=1.82\pm 0.02$) and derivative (**5b**) at this pH. At pH 7.4, all the prodrugs showed somewhat increased $\log P_{app}$ values compared to 6-MNA with a maximum increase in lipophilicity of about ten-fold for prodrug (**5d**) as shown in Fig. 1.

Although the lipophilicity of most of the prodrugs were lower at pH 5.0 than at pH 7.4, some of them maintained good lipophilicity ($\log P_{app}=0.10–2.17$) at pH 5.0. The lipophilicity of all the prodrugs of 6-MNA (**1**) varied widely due to changes in promoieties. For an effective dermal penetration, the

Table III. Half-Lives of the Prodrugs in Phosphate Buffer at pH 5.0, 7.4 and in Human Serum

| Compound | $t_{1/2}$ | | |
|-----------|---------------------------------------|--------------------|--|
| | Phosphate buffer ($t_{1/2}$ in h) | | Human serum (80%) ($t_{1/2}$ in min) |
| | pH 5.0 | pH 7.4 | |
| 4a | 79.18 | 17.39 | 46.29±3.1 |
| 4b | 250.76 | 21.96 | 37.61±2.6 |
| 4c | 27.60 | 27.86 | 11±1.5 |
| 4d | 6.9 | 7.5 | 7.8±1.2 |
| 5a | 376.14 | 23.50 | 60.18±2.1 |
| 5b | 601.82 | 45.59 | 29.79±1.8 |
| 5c | 158.3 | 30.39 | 10±1.9 |
| 5d | <10.0 ^a | <10.0 ^a | 8.2±2.3 |

^a Complete disappearance of derivative within this time interval

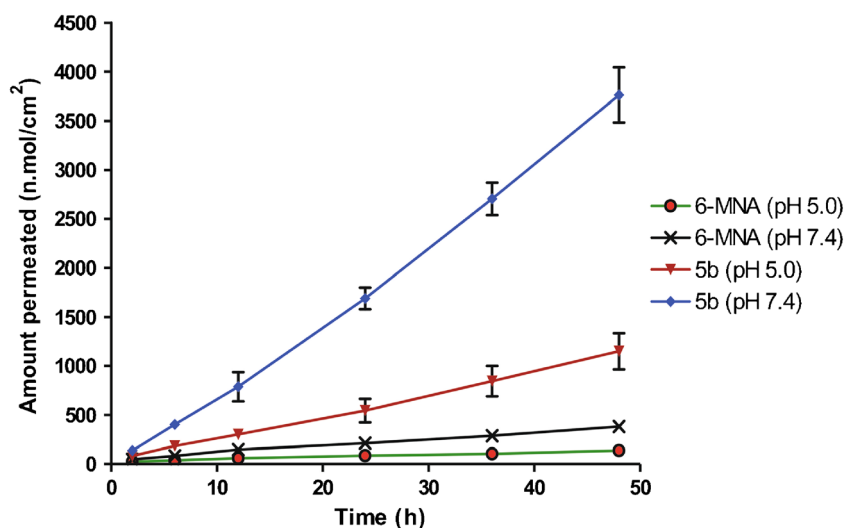


Fig. 3. Permeation profiles (mean \pm SEM, $n=3-6$) for (1) and prodrug (5b) at pH 5.0 and 7.4

prodrugs should possess not only high lipophilicity but also adequate aqueous solubilities which got improved in this case by piperazinyl promoieties.

Hydrolyses Kinetics Studies

Chemical hydrolysis of prodrugs was studied both at physiological pH 7.4 and in acidic pH 5.0 at constant temperature $37 \pm 1^\circ\text{C}$ in aqueous buffer solution. The enzymatic hydrolysis kinetics study of the prodrugs was carried out in 80% human serum. The chemical and enzymatic hydrolyses of all of the 6-MNA prodrugs, followed pseudo-first-order kinetics over several half lives. The aqueous stability of piperazinyl prodrugs was pH dependent and stabilities were substantially greater at pH 5.0 than at pH 7.4. The reason for high instability at pH 7.4 is quite obvious as the esters are more prone to hydrolysis in alkaline medium due to higher concentration of hydroxyl nucleophile. Prodrugs **4c**, **4d**, **5c**, **5d** exhibited lowest stability as compared to prodrugs **4a**, **4b**, **5a**, **5b**. This can be attributed to the fact that prodrugs **4c**, **4d**, **5c** and **5d** contain electron withdrawing groups at N-4.

Stability of prodrugs **4c**, **4d**, **5c** and **5d** was surprisingly the lowest in acidic pH because these dibasic prodrugs are not likely to be completely diprotonated at pH 5.0 and remain monoprotonated at this pH. The prodrugs **4a**, **4b**, **5a**,

5b would be protonated first at N-4 because it is relatively more accessible and makes them more stable, whereas for prodrugs **4c**, **4d**, **5c** and **5d** containing the electron withdrawing groups at N-4 have only N-1 to be protonated. Taking this into consideration, it is possible that these compounds may undergo intramolecular hydrogen bonding between the hydrogen on the protonated nitrogen and the oxygen of the ester group as shown in Fig. 2. Such hydrogen bonding will make the alkoxy moiety a better leaving group facilitating the process of hydrolysis (11,22).

All prodrugs were found to be highly susceptible to enzymatic hydrolysis in human serum *in vitro* as compared to chemical hydrolysis. They exhibited first-order degradation kinetics and hydrolyzed quantitatively to the corresponding parent drugs. Half-life ($t_{1/2}$) values of the prodrugs are summarized in Table III.

In Vitro Skin Permeation Study

In vitro skin permeation studies were performed by using skin obtained from the whole dorsal area of a male Wistar rat in a Franz-type diffusion cell. Skin specimens were rehydrated

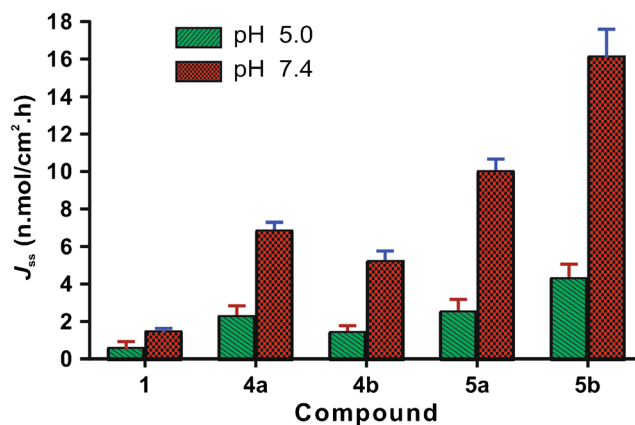


Fig. 4. *In vitro* steady-state flux (J_{ss}) of 6-MNA (1) and the prodrugs (4a-5b) at 37°C

Table IV. *In vitro* Steady-State Flux (J_{ss}) and Permeability Coefficient (K_p) of 6-MNA (1) and the Prodrugs (4a-5b) at 37°C

| Compound | $K_p \times 10^6$ (cm/h) | | J_{ss} (nmol/cm ² h) | |
|-----------|--------------------------|-----------------|-----------------------------------|-----------------|
| | pH 5.0 | pH 7.4 | pH 5.0 | pH 7.4 |
| 1 | 0.67 ± 0.03 | 0.03 ± 0.01 | 0.54 ± 0.1 | 1.44 ± 0.2 |
| 4a | 0.10 ± 0.01 | 0.32 ± 0.02 | 2.23 ± 0.14 | 6.80 ± 0.3 |
| 4b | 0.05 ± 0.01 | 0.43 ± 0.01 | 1.37 ± 0.15 | 5.17 ± 0.4 |
| 5a | 0.09 ± 0.01 | 0.50 ± 0.02 | 2.48 ± 0.2 | 9.96 ± 0.7 |
| 5b | 0.26 ± 0.02 | 2.59 ± 0.10 | 4.26 ± 0.4 | 16.10 ± 1.5 |

$p < 0.05$ compared to 6-MNA (ANOVA)

before being mounted in the diffusion cell. The receiver medium (0.05 M phosphate buffer saline solution of pH 7.4) was stirred and kept at $37 \pm 1^\circ\text{C}$ throughout the study. The selected prodrugs (**4a–5b**) were applied as solutions in phosphate buffer (0.05 M) at both pH 5.0 and 7.4.

The diffusion experiments showed that both 6-MNA (**1**) and its prodrugs were able to permeate rat skin *in vitro*. For different prodrugs, the cumulative amounts of permeated 6-MNA, or intact prodrug through skin were plotted against time as shown in Fig. 3. A steady-state flux (J_{ss}) was calculated by dividing the slope of the linear portion of the graph by surface area of the diffusion cell (1,7). The obtained permeability coefficient (K_p) for the steady-state delivery and steady-state flux (J_{ss}) values of 6-MNA (**1**) and prodrugs are given in Table IV. All the prodrugs have shown higher flux values than the parent 6-MNA (**1**).

Amongst all of the prodrugs, prodrug (**5b**) has shown the highest steady-state flux and **4b** the lowest. Prodrug (**5b**) with the highest flux had 7.9- and 11.2-fold higher flux than 6-MNA at pH 5.0 and 7.4, respectively. Prodrugs (**4a** and **5a**) have shown intermediate flux values with 4.1- and 4.6-fold higher flux at pH 5.0 and 4.7- and 6.9-fold higher flux at pH 7.4, respectively, than 6-MNA as shown in Fig. 4. The results showed that lipophilic piperazinyl prodrugs with adequate aqueous solubility increased the flux of 6-MNA (**1**). Permeability coefficient (K_p) is independent of donor concentration and a useful parameter to evaluate the permeation potential of a compound. The K_p value of all the prodrugs was enhanced several folds compared to 6-MNA at pH 7.4, however at pH 5.0 all of the prodrugs exhibited low values than 6-MNA because permeability of the compounds from aqueous solution increased as the solubility of the compounds in these solutions decreased. The high K_p value of **5b** can be explained by its very low aqueous solubility compared to the other compounds.

The results clearly demonstrated that those prodrugs of 6-MNA (**1**) which possessed balanced lipid and aqueous solubility, permeated better through the skin than those which had low lipophilicity and poor aqueous solubility. While making a comparison with the previously published work on percutaneous drug delivery, it is concluded that prodrug formation with piperazinylalkyl moieties improves skin permeability significantly (1,23,24).

CONCLUSION

The present study shows that the permeation of 6-MNA (**1**) through rat skin can be markedly improved by derivatizing it into piperazinylalkyl prodrugs. The ionizable basic prodrugs combine the desired properties for skin permeation like high aqueous solubility and lipophilicity. Both of these properties can be modulated by changing the substituents on the piperazinyl ring and by varying the alkyl chain length. Increased skin permeability of these prodrugs can be attributed to the combined effect of aqueous solubility and lipophilicity. From the flux and permeability coefficient (K_p) values it can be concluded that the prodrug (**5b**) is the most promising prodrug to improve percutaneous drug delivery of 6-MNA. From this study, it is concluded that an NSAID like 6-MNA having favourable pharmacokinetic and pharmacodynamic properties coupled with its increased

permeability through skin by converting it into suitable prodrug like **5b** offer better options for the treatment of rheumatic diseases.

Declaration of Interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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