The hydrolysis kinetics of monobasic and dibasic aminoalkyl esters of ketorolac

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Abstract
Six aminoethyl and aminobutyl esters of ketorolac containing 1-methylpiperazine (MPE and MPB), N-acetyl-piperazine (APE and APB) or morpholine (ME and MB), were synthesized and their hydrolysis kinetics were studied. The hydrolysis was studied at pH 1 to 9 (for MPE, APE and ME) and pH 1 to 8 (for MPB, APB and MB) in aqueous phosphate buffer (0.16 M) with ionic strength (0.5 M) at 37°C. Calculation of kₜ, construction of the pH-rate profiles and determination of the rate equations were performed using KaleidaGraph 4.1. The hydrolysis displays pseudo-first order kinetics and the pH-rate profiles shows that the aminobutyly esters, MPE, APB and MB, are the most stable. The hydrolysis of the ethyl esters MPE, APE and ME, depending on the pH, is either fast and catalyzed by the hydroxide anion or slow and uncatalyzed for the diprotonated, monoprotonated and nonprotonated forms. The hydrolysis of the butyl esters showed a similar profile, albeit it was also catalyzed by hydroxide anion. In addition, the hydroxide anion is 10⁵ more effective in catalyzing the hydrolysis than the hydronium cation. The hydrolysis pattern of the aminoisopropyl esters is affected by the number and pKₐ of its basic nitrogen atoms. The monobasic APE and ME show a similar hydrolysis pattern that is different than the dibasic MPE. The length of the side chain and the pKₐ of the basic nitrogen atoms in the aminoalkyl moiety affect the mechanism of hydrolysis as the extent of protonation at a given pH is directly related to the pKₐ.

Keywords: ester prodrugs, hydrolysis kinetics, ketorolac, pH-rate profile, stability

Introduction
Ketorolac, (±)-5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, is a NSAID that was introduced by Syntex Research, Institute of Organic Chemistry, California in 1981. It is a nonselective Cyclooxygenase inhibitor as it inhibits both Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2). Inhibition of COX-2 results in an anti-inflammatory effect, while inhibition of COX-1 causes the gastrointestinal (GI) toxicity.

Ketorolac is one of the most potent NSAIDs but its use is limited due to its gastric side effects. So, many ketorolac prodrugs were investigated to overcome this side effect such as simple alkyl esters, amides, polyethylene glycol esters, fatty esters, amino acid amides and N-piperazinylalkyl esters. We have previously reported some ketorolac piperazineylethyl and piperazinylbutyl esters and studied their hydrolysis at pH 5 and 7.4. The piperazinyl...
moieties in the ethyl esters were 1-methylpiperazine, 1-ethylpiperazine, 1-(2-hydroxyethyl)piperazine, piperazine, 4-methyl-1-aminopiperazine and \(N\)-acetyl-piperazine and in the butyl ester was 1-methylpiperazine. The results showed that the ethyl esters (i.e. with an ethyl spacer between ketorolac and the piperazine ring) had a much faster hydrolysis rates than the one with a butyl spacer. On the other hand, all the prodrugs were more stable at pH 5 than at pH 7.4\textsuperscript{12}. 1-Methylpiperazinylbutyl ketorolac ester (MPB) have shown promising results as a transdermal agent\textsuperscript{13}, while both 1-methylpiperazinylbutyl and 1-methylpiperazinylethyl ketorolac esters (MPB and MPE, respectively) have been shown to be potential oral agents with reduced gastric toxicity\textsuperscript{14}.

It is worth mentioning that aminoalkyl and piperazinylalkyl ester prodrugs have been reported for other NSAIDs. For example, Tammara et al. (1993) synthesized morpholynylalkyl esters of naproxen and indomethacin and evaluated their oral delivery in vitro and in vivo\textsuperscript{15}. In 2000, a detailed study by Rautio et al. reported some 1-methylpiperazinylalkyl and morpholynylalkyl esters of naproxen with study of their chemical stability at pH 5 and 7.4 and evaluation of their transdermal permeation\textsuperscript{16}. Also, some \(N\),\(N\)-disubstituted amino alcohol esters of some NSAIDs like ketorolac, naproxen, ibuprofen, aspirin, diclofenac were reported to possess anticholinergic activity and liberate the NSAID component via metabolism. In addition to decreasing the gastric irritation via masking the carboxylate group as an ester, the anticholinergic action was proposed to inhibit gastric secretions and gastric motility\textsuperscript{17–19}.

The ester linkage present in these prodrugs is susceptible to acid and base-catalyzed hydrolysis\textsuperscript{20}. There are many factors affecting the rate of ester hydrolysis such as temperature (usually the rate of hydrolysis is proportional to temperature\textsuperscript{12,23}), pH of the media, buffer concentration (usually increasing the buffer’s concentration increases the hydrolysis rate), the type of buffer (e.g. highly nucleophilic buffer species can attack the ester linkage and increase the rate of ester hydrolysis) and ionic strength.

Although the aminoalkyl esters of NSAIDs and specifically the piperazinylalkyl esters have been investigated in many reports, their pH-rate profile have never been reported. Hence, further study of their hydrolysis kinetic is needed in order to understand the mechanism of their hydrolysis as a well determined pH-rate profile is a key step in studying reaction mechanisms\textsuperscript{20,23}. This work details the synthesis and characterization of representative aminoethyl and aminobutyl esters of ketorolac containing both monobasic and dibasic six member heterocyclic rings namely, 1-methylpiperazine, morpholine and \(N\)-acetyl-piperazine, Figure 1. In addition, the chemical hydrolysis of these compounds was studied in 0.16 M phosphate buffer at constant ionic strength (0.5M) over a pH range 1–9 or 1–8 in a thermostatically controlled water bath at 37°C. The constructed pH-rate profile allowed the determination of the rate law dependence on [pH] and a general mechanism was proposed for the hydrolysis of each of the synthesized esters. (Figure 1)

**Experimental**

Ketorolac was obtained from Al-Hikma Pharmaceuticals, Amman, Jordan. Reagent grade and fine chemicals were obtained from Aldrich Chemical Company, St. Louis, MO, USA (www.sigmaaldrich.com), ACROS Chemicals, Belgium (www.acros.com) and Scharlau Chemicals, Spain (www.scharlau.com). Bulk solvents were purchased through local vendors.

Melting points were determined using Stuart Scientific melting point apparatus (Stuart Scientific, Stone, Staffordshire, UK) and were uncorrected. IR spectra were recorded on IRAfinity-1 FT-IR (Shimadzu, Kyoto, Japan) using KBr disks and absorptions are reported in cm\(^{-1}\). NMR spectra were obtained on a Bruker Advance Ultrashield 400-MHz instrument, (Bruker, Fallanden, Switzerland) and chemical shifts (\(\delta\)) are reported in ppm relative to automatic calibration to the residual proton peak of the solvent used namely CDCl\(_3\). TLC analysis was performed on Merck aluminum TLC plates, Silica 60, F\(_254\) (Merck, Darmstadt, Germany). Mass spectra were obtained by Agilent 1100 series LC-MSD-Trap instrument (Agilent, Santa Clara, CA, USA) at Princess Haya Biotechnology Center, Jordan University of Science and Technology.

**Synthesis**

The target compounds were synthesized according to published procedure\textsuperscript{12}.

2-Bromoethyl-5-benzoyl-1,2-dihydro-3H-pyrrole-[1,2-\(a\)]pyrrole-1-carboxylate (1)

Benzenesulfonic acid (2.75 g, 15.67 mmol) was added to a stirring solution of ketorolac (20.00 g, 78.35 mmol) in 300-mL dichloromethane in a 500-mL round bottom flask. Then to the mixture, 2-bromoethanol (30.90 g, 0.25 mol) was added and the reaction mixture allowed to stir at room temperature for 5 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), then with cold 0.5N NaOH solution (300 mL x 3), then again with distilled water (300 mL).
The organic layer was then dried over MgSO₄ and the solvent was evaporated. The crude residue was crystallized from ethyl acetate:hexane giving 17.18 g (60.54%) of brown crystals. ¹H-NMR (CDCl₃, 400 MHz): δ = 7.83 (2H, d, J = 7.3 Hz, Ar-H2), 7.55 (1H, t, J = 7.3 Hz, Ar-H), 7.47 (2H, t, J = 7.3 Hz, Ar-H), 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, NCH₂ + OCH₂), 4.13 (1H, dd, J = 8.6 and 5.8, CHCO), 3.55 (2H, t, J = 5.8, CHBr), 3.00-2.92 (1H, m, CHH), 2.87-2.81 (1H, m, CHH) and they were identical to a previous report.¹²

General procedure for the synthesis of ketorolac aminoalkyl esters as the oxalic acid salts MPE-Ox, APE-Ox and ME-Ox

To a solution of 2-bromoethyl ketorolac ester 1 (5.00 g, 12.42 mmol) in 100 mL acetonitrile in 250 mL round bottom flask, the appropriate piperazine or morpholine (in excess amount) and triethylamine (5.30 mL, 38.03 mmol) were added and the reaction mixture was stirred and heated at 40°C for 3 days. The progress of the reaction was followed by TLC (10% methanol in dichloromethane). Upon completion of the reaction it was filtered and solvent was evaporated. The oily residue was dissolved in ethyl acetate (100 mL) and then washed with water (100 mL x 3). The organic layer was dried over MgSO₄ and it was evaporated to obtain an oily residue. The residue was dissolved in absolute ethanol and oxalic acid in absolute ethanol was added to it and the formed precipitate was filtered and crystallized from methanol in case of MPE-Ox and from ethylacetate:ether in case of APE-Ox and ME-Ox. For analysis, the free base was always prepared on-demand by dissolving the oxalate salt in cold distilled water followed by careful neutralization by sodium bicarbonate. Extraction by ethyl acetate from the formed precipitate gave the free base. All ¹H-NMR, ¹³C-NMR and IR spectra in addition to the Mass Spectrometric analysis were obtained using the free bases, MPE, APE and ME.

2-(4-Methyl-1-piperazinyl)ethyl-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2-α]pyrrole-1-carboxylic acid salt (ME-Ox)

Morpholine (4.40 g, 50.50 mmol). ME-Ox (oxalate salt): 3.64 g of pale-yellow crystals (63.93%). mp: 135–137°C. ME (free base): 1H-NMR (CDCl₃, 400 MHz): δ = 7.76 (2H, d, J = 8.0 Hz, Ar-H), 7.47 (1H, t, J = 8.0, Ar-H), 7.39 (1H, t, J = 8.0, Ar-H), 6.75 (1H, d, J = 4.0, H-C-C-H), 6.07 (1H, d, J = 4.0, H-C-C-H), 4.45 (2H, m, COCH₂), 4.23 (2H, m, -COOH), 4.03 (1H, m, -COCH), 3.61–3.63 (4H, bs, CH₂NCH₂), 2.80 (2H, m, CH₂N(CH₃)₂), 2.59 (2H, t, J = 4.0, COOH₂), 2.43 (4H, bs, CH₂OCH₂). ¹³C-NMR (CDCl₃, 100 MHz): δ = 33.63, 54.24, 50.26, 56.35, 59.61, 64.91, 69.51, 105.86, 127.52, 129.80, 130.84, 131.51, 134.09, 141.85, 145.05, 173.77 and 187.52. IR (KBr): 2953.0 (conjugated C=C), 1735.9 (O=C-O), 1647.2 (N-C=O), 1622.1 (Ar-C=O) and 1197.7 (aliphatic tertiary amine) cm⁻¹. LC-MS (APCI) m/z: 410 ([M+1], 100%).

2-(4-Acetyl-1-piperazinyl)ethyl-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2-α]pyrrole-1-carboxylic acid salt (APE-Ox)

Morpholine (3.25 g, 25.35 mmol). APE-Ox (oxalate salt): 2.56 g of off-white crystals (41.26%). mp: 128–130°C. APE (free base): 1H-NMR (CDCl₃, 400 MHz): δ = 7.77 (2H, d, J = 7.2 Hz, Ar-H2), 7.49 (1H, t, J = 7.2, Ar-H), 7.42 (2H, t, J = 7.2, Ar-H2), 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, NCH₂), 4.44–4.38 (1H, m, NCH₂), 4.29–4.25 (2H, m, OCH₂CH₂N), 4.05 (1H, dd, J = 8.7 and 5.4 Hz, COCH₂), 3.54 (2H, t, J = 5.2 Hz, CH₂NCO), 3.38 (2H, t, J = 5.2, CH₂NCO), 2.92–2.85 (1H, m, COCH₂CH₂), 2.81–2.73 (1H, m, COCH₂CH), 2.62 (2H, t, J = 5.6 Hz, OCH₂CH₂N), 2.42 (4H, m, CH₂NCH₂), 2.02 (3H, s, COCH₂). ¹³C-NMR (CDCl₃, 100 MHz): δ = 184.92, 171.71, 171.10, 168.95, 142.33, 139.11, 131.46, 128.84, 121.87, 124.14, 124.97, 103.15, 62.31, 56.41, 53.20, 52.87, 47.57, 46.20, 42.57, 41.33, 30.97, 21.27. IR (KBr): 2953.0 (conjugated C=C), 1735.9 (O=C-O), 1647.2 (N-C=O), 1622.1 (Ar-C=O) and 1197.7 (aliphatic tertiary amine), and 1116.7 (aliphatic C-O-C) cm⁻¹. LC-MS (APCI) m/z: 369 ([M+1], 100%).
General procedure for the synthesis of ketorolac aminobutyl esters as the oxalic acid salts MPB-Ox, APB-Ox and MB-Ox.

To a solution of 4-chlorobutyl ketorolac ester (5.00g, 24.96mmol), sodium iodide (13.14mmol) and triethylamine (5.50 mL, 39.46 mmol) in 100 mL acetone, 1-Methylpiperazine (2.50 g, 24.96 mmol). MPB-Ox (oxalate salt): 2.79 g of light-gray crystals (36.57%). mp: 119–120°C.

APB (free base): 1H-NMR (CDCl3, 400 MHz): δ = 7.81 (2H, d, J = 8.0 Hz, Ar-H2), 7.53 (1H, t, J = 8.0, Ar-H), 7.47 (2H, t, J = 8.0 Hz, Ar-H), 6.82 (1H, d, J = 4.0, H-C=C-H), 6.09 (1H, d, J = 4.0, H-C=C-H), 4.56 (2H, m, NCH2), 4.05–4.20 (3H, bs, CHCOO and OC=O), 4.05–4.20 (3H, bs, CHCOO and OC=O), 3.54 (4H, bs, CH2N), 2.92 (2H, m, COHCH2), 2.17 (2H, m, OCH2CH2CH2N), 2.04 (3H, s, COCH3), 1.65–1.72 (4H, bs, OCH2CH2CH2N), 1.25 (2H, m, OCH2CH2CH2N). 13C-NMR (100 MHz, CDCl3): δ = 187.67, 173.94, 171.6, 145.05, 141.82, 134.18, 131.56, 130.89, 129.86, 127.60, 105.75, 67.73, 60.34, 55.72, 52.55, 50.28, 48.36, 45.30, 43.51, 33.62, 29.11, 25.33, 23.96. IR (KBr): 2981.9 (conjugated C=C), 1732.0 (O=C=O), 1649.1 (N=C=O), 1624.0 (Ar=C=O), and 1199.7 (aliphatic tertiary amine) cm⁻¹. LC-MS (APCI) m/z: 438 ([M + 1], 100%).

MB-Ox: (free base): 1H-NMR (CDCl3, 400 MHz): 1H-NMR (CDCl3, 400 MHz): δ = 7.78 (2H, d, J = 4.0 Hz, Ar-H2), 7.50 (1H, t, J = 8.0, Ar-H), 7.42 (2H, t, J = 8.0 Hz, Ar-H2), 6.79 (1H, d, J = 4.0, H-C=C-H), 6.06 (1H, d, J = 4.0, C=C-H), 4.45 (2H, m, NCH2), 4.21, (1H, t, J = 4.04, CHCOO), 4.04 (2H, t, J = 4.0, H-C=C-H), 3.66 (4H, bs, CH2OCH2), 2.89 (2H, m, COCH2), 2.45 (4H, bs, CH2N), 2.34 (2H, m, OCH2CH2CH2N), 1.68 (2H, m, OCH2CH2CH2N), 1.54 (2H, m, OCH2CH2CH2N). 13C-NMR (100 MHz, CDCl3): 187.62, 173.91, 145.11, 141.87, 134.10, 131.56, 130.84, 129.82, 127.60, 105.75, 96.56, 68.00, 61.02, 56.30, 50.26, 45.30, 33.57, 29.20, 25.51. IR (KBr): 2981.9 (conjugated C=C), 1732.0 (O=C=O), 1649.1 (N=C=O), 1624.0 (Ar=C=O), and 1199.7 (aliphatic tertiary amine) cm⁻¹. LC-MS (APCI) m/z: 397 ([M + 1], 100%).

Analysis of ketorolac aminoalkyl esters in the hydrolysis study.

High-performance liquid chromatography (HPLC).

A validated chromatographic separation and quantitative analysis method developed by Qandil et al. (2008) was used. The high-performance liquid chromatography (HPLC) system used consisted of UV-visible detector, auto injector and degasser and was connected to computer furnished with the appropriate software (Shimadzu, Canby, OR, USA). An isocratic reversed-phase RP-18C column (125 × 4 mm, 5 µm) (ACE, UK) was...
used. The injection volume was 25 µL and the detection wavelength was 314 nm. The mobile phase was a mixture of 0.02 M phosphate buffer and acetonitrile (65:35 v/v) 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 ester (prodrug) and the parent drug (ketorolac) were detected with different retention time.

Preparation of standard solutions for calibration curves
Stock solutions of the oxalate salts of the prodrugs were prepared by dissolving an accurately weighed 25 mg of the salt in methanol (HPLC grade) in a 50-mL volumetric flask to obtain 500-µg/mL stock solutions. Then the standard solutions for each salt were prepared by serial dilution of the stock solution to obtain final concentrations in range of 5–100 µg/mL.

Then calibration curves for ketorolac and the oxalate salts (MPE-Ox, APE-Ox, ME-Ox, MPB-Ox, APB-Ox and MB-Ox) were constructed by injecting samples from the standard solutions then plotting the area under the curve that result for each standard solution versus concentration.

Hydrolysis of the ketorolac esters MPE, APE, ME, MPB, APB and MB in aqueous solution
The rate of chemical hydrolysis of the ketorolac prodrugs MPE, APE, ME, MPB, APB and MB were investigated in aqueous 0.16 M phosphate buffer solution at 37 °C ± 0.1 over pH 1 to 9 (for MPE, APE and ME) and pH 1 to 8 (for MPB, APB and MB) with constant ionic strength that was adjusted to 0.5 M with NaCl.

The reactions were initiated by preparing 100 µg/mL solution of the oxalate salts (MPE-Ox, APE-Ox, ME-Ox, MPB-Ox, APB-Ox and MB-Ox) in 0.16 M phosphate buffer with constant ionic strength of 0.5 M over the pH range in a thermostatically controlled water bath at 37 °C ± 0.1. Then according to the prodrug’s stability, samples were taken at appropriate time intervals and analyzed immediately using HPLC for the remaining prodrug and the released ketorolac. The experiments were run in triplicates for each prodrug at each pH.

The rate of hydrolysis was determined using KaleidaGraph software version 4.0 by plotting the remaining concentration of the prodrug versus time.

Statistical analysis
Statistical analysis (ANOVA and Fisher’s least-significant-difference tests) of the $k_{obs}$ values for all esters together and for each ester alone at the studied pH range was performed using SYSTAT version 5.0.

Molecular modeling and pKa estimation
Hyperchem 7.5 (Hypercube, Gainesville, FL, USA) was used to calculate the proton affinities and to find the minimum energy conformation with intramolecular hydrogen bonding. ME-H, MPE and MPB were imported into Hyperchem prepared by dissolving an accurately weighed 25 mg of the salt in methanol (HPLC grade) in a 50-mL volumetric flask to obtain 500-µg/mL stock solutions. Then the standard solutions for each salt were prepared by serial dilution of the stock solution to obtain final concentrations in range of 5–100 µg/mL.

The reactions were initiated by preparing 100 µg/mL solution of the oxalate salts (MPE-Ox, APE-Ox, ME-Ox, MPB-Ox, APB-Ox and MB-Ox) in 0.16 M phosphate buffer with constant ionic strength of 0.5 M over the pH range in a thermostatically controlled water bath at 37 °C ± 0.1. Then according to the prodrug’s stability, samples were taken at appropriate time intervals and analyzed immediately using HPLC for the remaining prodrug and the released ketorolac. The experiments were run in triplicates for each prodrug at each pH.

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Results and discussion
Chemistry
The target compounds were synthesized following a previously published procedures. Scheme 1. First, ketorolac was treated with 2-bromoethanol or 4-chlorobutanol in presence of benzenesulfonic acid as a catalyst to obtain the desired ester 1 and 2 respectively. Then the target compounds MPE-Ox, APE-Ox and ME-Ox were then obtained by heating 2-bromoethyl ester with excess N-methylpiperazine, N-acetylpirpiperazine or morpholine in the presence of triethylamine in acetone for 3 days to obtain aminooethyl ester MPE, APE and ME as oily residues which were purified by converting them to the corresponding oxalate acid salts MPE-Ox, APE-Ox and ME-Ox by dissolving each ester in absolute ethanol followed by addition of a solution of oxalic acid in absolute ethanol. The resultant precipitates were crystallized from methanol in case of MPE-Ox or ethylacetate-ether in case of APE-Ox and ME-Ox. In case of ester MPB-Ox, APB-Ox and MB-Ox, similar procedure was used but to effect the reaction with the cyclic amine to obtain aminobutyl ester MPB, APB and MB, the presence of sodium iodide in addition to maintenance at reflux for 3 days was required. For esters 1 and 2 which have been previously reported only 1H-NMR was obtained to verify the chemical identity. On the other hand, all final compounds were characterized using 1H-NMR, 13C-NMR, Mass Spectrometry (MS) and Fourier Transformer-Infrared Spectrometry (FT-IR) of the free bases MPE, APE, ME, MPB, APB and MB, even though three of them have been reported earlier.

Hydrolysis study
Standard solutions of ketorolac, MPE-Ox, APE-Ox, ME-Ox, MPB-Ox, APB-Ox and MB-Ox were prepared by serial dilution of their respective stock solutions to obtain final concentrations in range 5–100 µg/mL. The calibration curves of ketorolac, MPE-Ox, APE-Ox,
ME-Ox, MPB-Ox, APB-Ox and MB-Ox were constructed by plotting the concentration versus the resultant area under curve (AUC) for each standard solution. As an example, the calibration curve for MB-Ox is shown in Figure 2. The R^2 for the calibration curves are in the range 0.9978–0.9999.

The rates of hydrolysis of the ketorolac esters were studied in 0.16 M phosphate buffer solution at 37°C ± 0.1 over pH 1 to 9 (for MPE, APE and ME) and pH 1 to 8 (for MPB, APB and MB). All hydrolysis studies were carried out in solutions with constant ionic strength that was adjusted to 0.5 M with NaCl in triplicate trials and almost in all cases for five half-lives. The k_{obs} and half-lives (t_1/2) for each compound are presented in Table 1. The hydrolysis of all esters displays pseudo-first-order kinetics. The observed rate constant k_{obs} for the overall hydrolysis of each trial was calculated by a non-linear fitting of the data using KaleidaGraph version 4.0 based on equation (1).

\[ C_t = C_0 \times \exp\left(-k_{obs} \times t\right) \]  

(1)

Where \( C_t \) is the remaining concentration of the ester at time \( t \), \( C_0 \) is the initial concentration of the ester, \( k_{obs} \) is the observed first-order hydrolysis rate constant. The average \( k_{obs} \) from the three trials were calculated and from it, the half-life (t_{1/2}) for each ester was calculated.

The hydrolysis behavior of MPE, APE, ME, MPB, APB and MB at the studied pH range was investigated by plotting the obtained \( k_{obs} \) versus pH for each compound as seen in Figure 3.

As seen in Figure 3, the average \( k_{obs} \) value for MPE at pH 1 is almost two fold that at pH 2, and seems constant in the pH range 2–4 and starts to increase significantly from pH 5 to 9. The average \( k_{obs} \) value for MPB decreases as the pH increases in the pH range 1–3, almost the same at pH 3 and pH 4, then starts to increase as the pH increases in the pH range 5–8. And finally the average \( k_{obs} \) value for both APB and MB decreases as the pH increases from pH 1 to 4 reaching its lowest value at pH 4, then increases as the pH increases in the pH range 5–9.

It is clear that both esters APE and ME have similar hydrolysis pattern that is different than that of MPE. Compounds APE and ME have the same electronic character in their neutral form since both have one basic nitrogen atom, while compound MPE contains two basic nitrogen atoms as shown in Figure 4. Similar distinction in the hydrolysis behaviors of esters APB and MB vs. MPB can also be seen.

In solution, the protonation status of these esters is determined by pH and the pKa of the ionizable groups, namely the basic nitrogen atoms. Esters MPE and MPB can be either diprotonated at N¹ and N⁴ (MPE-H² and MPB-H²), monoprotonated at either of these nitrogen atoms (MPE-H and MPB-H) or nonprotonated (MPE-H⁻ and MPB-H⁻).
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and MPB), while APE, ME, APB and MB can either be monoprotonated (APE-H, ME-H, APB-H and MB-H) or nonprotonated (APE, ME, APB and MB). In general, at the pH range where esters MPE and MPB are mainly diprotonated, esters APE, ME, APB and MB will mainly be monoprotonated, while in the pH range where esters MPE and MPB are mainly monoprotonated, esters APE, ME, APB and MB will mainly be nonprotonated. It is also

Table 1. Average observed rate constants and half-lives for all esters at the studied pH range.

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Figure 3. The pH-rate profile of the ketorolac aminoalkyl esters (A) MPE, (B) APE, (C) ME, (D) MPB, (E) APB and (F) MB.
unlikely that esters MPE and MPB are going to exist in a totally nonprotonated form in the pH range from 1–8. It can be argued that for MPB, the protonation of either N1 or N4 will have minimum effect on the mechanism of hydrolysis since it is far from the hydrolysis center, that is, the ester group. On the other hand, the situation is expected to be different in the case of the MPE. Hence, gas-phase proton affinity calculation for each of the nitrogen atoms was determined applying the equation

\[ PA = (H_f(MPE) + H_p) - H_f(MPE-H), \]

where PA is the proton affinity and \( H_f(MPE) \), \( H_p \) and \( H_f(MPE-H) \) are the heat of formation of the nonprotonated form (MPE), proton’s heat of formation in gas phase and the heat of formation of the monoprotonated form (MPE-H), respectively. \( H_f(MPE) \) and \( H_f(MPE-H) \) were calculated using PM3 semiempirical computations as implemented in Hyperchem 7.5. The gas-phase proton affinity \( H_p \) used for these calculations is the experimental value of 367.2 Kcal/mol. The calculations revealed that the proton affinities for N4 and N1 are 184.9092 and 185.4151 Kcal/mol respectively. Although this might not reflect the case in aqueous media, it is still an indication that there is no strong preference for protonating one nitrogen atom over the other and hence MPE-H can be found either as MPE-HN1 or MPE-HN4.

According to Figure 3a, it can be proposed that at pH range 6–9 the hydrolysis of MPE is base-catalyzed since the hydrolysis rate increases linearly as the [H+] decreases (pH increase). At pH <6, the hydrolysis reaction seems to be uncatalyzed (neutral solution) since the rate of hydrolysis became almost constant and at pH 2–4 where this ester appears to be most stable. Although subtle, but there is a visible inflection point at pH 8 in the rate pH profile of MPE, Figure 3a, indicating the pKa of MPE will be close to 8, so the diprotonated (MPE-H2) will be predominate at ≥pH 3 and while the monoprotonated (MPE-H) form will predominate at pH 6 and above. This means that the hydrolysis of MPE-H is faster and mainly catalyzed by the hydroxide anion at pH 6–9. While at pH <6, both MPE-H2 and MPE-H are slowly hydrolyzed without catalysis. A visible decrease MPE’s stability at pH 1 may indicate that acid-catalyzed hydrolysis occurs, but since the hydrolysis was not studied at pH values lower than 1, this observation is non-conclusive. The hydrolysis pathways of ester MPE is shown in Figure 5.

Based on the hydrolysis pathway, the value of \( k_{obs} \) consists of three rate constants \( k_1 \), \( k_1' \) and \( k_2 \) according to equation 2.

\[ k_{obs} = \left( k_1 K_a + k_1' [H^+] \right) / \left( K_a \left[ H^+ \right] \right) + k_2 K_w / [H^+] \] (2)

Where

\( k_{obs} \) is the observed pseudo-first order hydrolysis rate constant.

\( k_1 \) is the rate constant for uncatalyzed hydrolysis for the monoprotonated form (MPE-H).

\( k_1' \) is the rate constant for uncatalyzed hydrolysis for the diprotonated form (MPE-H2).

\( k_2 \) is the rate constant for base-catalyzed hydrolysis.

\( K_a \) is the acid dissociation constant for the ester.

\( K_w \) is the dissociation constant for water at 37°C, which equals 2.512 × 10^-14.

The rate constants \( k_1 \), \( k_1' \), \( k_2 \), the experimental Ka (Ka_{exp}) and experimental pKa (pKa_{exp}) that were obtained from the obtained equations and the estimate pKa (pKa_{exp}) that was obtained in silico for each of the synthesized esters are presented in Table 2.

In case of APE, Figure 3b, at pH range 5–9 the hydrolysis shows a more complex profile than that of MPE. Two important differences can be seen here; first, there

![Diagram](image-url)
is clear inflection at pH 6–7 and hence, the pKa of these esters is expected to be 6–7. Second, the hydrolysis rate stayed relatively higher than that of MPE even after reaching a relatively lower pH value, that is, pH 5. With a pKa close to 6, APE will be present mostly as APE-H until pH 6, then the APE fraction starts to increase. Accordingly, at pH 6–8, APE is hydrolyzed by hydroxide anion (base-catalyzed hydrolysis), while at pH 5–6 the hydrolysis of both forms (APE-H and APE) will be uncatalyzed and at pH <5 where APE-H predominates the hydrolysis will also be uncatalyzed. In general, at pH <5 the hydrolysis rate becomes much slower and almost constant. It can also be noticed that there is a visible decrease in stability at pH 1 which might, again, indicate the start of an acid-catalyzed hydrolysis. The deacetylated analog of APE (DeAc-APE) was synthesized and its HPLC profile was determined in a study, which might, again, indicate the start of an acid-catalyzed hydrolysis. The hydrolysis profile of ester ME, is similar to that of APE and there is also a clear inflection around pH 6–7. Consequently it will be present mostly in the ME-H form until pH 5 or even 6 where the nonprotonated (ME) fraction starts to increase. The hydrolysis rate is low and becomes almost constant at pH <5. The hydrolysis pathways and the equation that determines the $k_{\text{obs}}$ for APE and ME are the same as those of MPE with the consideration that $k_1$ is the rate constant for uncatalyzed hydrolysis for the nonprotonated forms, APE or ME, and $k'_1$ is the rate constant for uncatalyzed hydrolysis for the monoprotonated forms APE-H or ME-H. The terms for the rate equations are summarized in Table 2. A comparison between the hydrolysis rate of three esters discussed earlier at pH 5 and 6 were APE-H or ME-H are expected to be protonated at their only basic nitrogen (N1) and MPE can be protonated at either of its basic nitrogen atoms (N1 and N4) is worthwhile. At this range the hydrolysis of the monobasic esters is 3.4 to 12.3 folds faster. This might be due to the formation of an intramolecular hydrogen bond which we have hinted to its possibility earlier. Molecular modeling revealed the possibility of forming two different intramolecular hydrogen bonding as seen for ME-H, Figure 6. Figure 6a and 6b, shows hydrogen bond formation leading to five-membered ring. Such hydrogen bond will facilitate the leaving of alcohol during ester hydrolysis. While Figure 6c and 6d shows an intramolecular hydrogen bonding leading to seven-membered ring. This hydrogen bond will render the carbonyl more nucleophilic and hence facilitate its attack by nucleophiles. Both of these possible hydrogen bonds can, theoretically, facilitate the hydrolysis of the ester linkage.

The pH-rate profile of MPB, Figure 4d, shows that at pH 5–8, the hydrolysis is relatively fast and the rate decreases as the pH decreases indicating a base-catalyzed hydrolysis for the monoprotonated form. In the pH range from 3 to 5, the hydrolysis is mainly uncatalyzed for both forms, MPB-H$_1$ and MPB-H and the hydrolysis rate starts to become relatively constant and its rate reaches its lowest value. At pH <3, MPB will be predominantly in the diprotonated form, MPB-H$_2$. In this region as $[\text{H}^+]$ increases (pH decreases) the hydrolysis rate increases significantly, which indicates a predominant acid-catalyzed hydrolysis for MPB-H$_2$. The hydrolysis pathways for MPB are shown in Figure 6 and accordingly, the value of $k_{\text{obs}}$ consists of four rate constants $k_1$, $k'_1$, $k_2$ and $k_3$ as shown in equation 3;

$$k_{\text{obs}} = (k_1K_a + k'_1[H^+])/(K_a + [H^+]) + k_2K_a/[H^+] + Kk_a[H^+]$$

where $k_{\text{obs}}$ is the observed pseudo-first order hydrolysis rate constant.

$k_1$ is the rate constant for uncatalyzed hydrolysis for the monoprotonated form (MPB-H$_1$).

$k'_1$ is the rate constant for uncatalyzed hydrolysis for the diprotonated form (MPB-H$_2$).

$k_2$ is the rate constant for base-catalyzed hydrolysis.

$k_3$ is the rate constant for acid-catalyzed hydrolysis.

$K_a$ is the acid dissociation constant for the prodrug.

$K_k$ is the dissociation constant for water at 37°C, which equals $2.512 \times 10^{-14}$.

The rate constants ($k_1$, $k'_1$, $k_2$, $k_3$), the experimental Ka ($K_a\text{[exp]}$) and experimental pKa (p$K_a\text{[exp]}$) that were obtained from the obtained equations and the estimate pKa (p$K_a\text{[est]}$) that was obtained in silico for each of the synthesized esters are presented in Table 2.

The hydrolysis profiles of the ester APB and MB, Figure 4e and 4f respectively, show one very important difference when compared to their ethyl analogs APE and ME. It was expected that the pKa values of esters APB and MB are going to be similar to those of their ethyl analogs APE and ME respectively, but the situation was puzzling to its possibility earlier. Molecular modeling revealed the possibility of forming two different intramolecular hydrogen bonding as seen for ME-H, Figure 6. Figure 6a and 6b, shows hydrogen bond formation leading to five-membered ring. Such hydrogen bond will facilitate the leaving of alcohol during ester hydrolysis. While Figure 6c and 6d shows an intramolecular hydrogen bonding leading to seven-membered ring. This hydrogen bond

### Table 2. The rate constants $k_i$, $k'_i$, $k_2$, $k_3$, $K_a\text{[exp]}$, p$K_a\text{[exp]}$ and p$K_a\text{[est]}$ for all esters.

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<th>$k'_1$ (hr$^{-1}$)</th>
<th>$k_2$ (hr$^{-1}$M$^{-1}$)</th>
<th>$k_3$ (hr$^{-1}$M$^{-1}$)</th>
<th>$K_a\text{[exp]}$</th>
<th>p$K_a\text{[exp]}$</th>
<th>p$K_a\text{[est]}$</th>
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significantly as the [H+] increases (pH decreases) in this range with a relatively small fraction of this form is subjected to uncatalyzed hydrolysis at pH 6. At pH <6, the protonated form, APB-H, is expected to be predominant. Accordingly, at pH range 3–6, the hydrolysis reaction will be mainly uncatalyzed and then at pH <3, the hydrolysis will become mainly acid-catalyzed as the hydrolysis rate increases significantly as [H+] increases (pH decreases). APB was found to be most stable at pH 3–5. The hydrolysis pathways of APB is similar to those expected for MPB, Figure 5. Figure 4f shows that the profile for MB is similar to that of APB and hence the hydrolysis pathways are similar, Figure 5. Accordingly, the observed rate constant (k_{obs}) of the overall hydrolysis for APB and MB can be expressed with the equation 7 with the consideration that k_1 is the rate constant for uncatalyzed hydrolysis for the nonprotonated forms (APB or MB) and k_1' is the rate constant for uncatalyzed hydrolysis for the monoprotonated forms (APB-H or MB-H). After substituting for the constants in equation.

It is clear from Table 2 that k_1 (the rate constant for the uncatalyzed hydrolysis of the forms MPE-H, APE, ME, MPB-H, APB and MB) is higher than that of k_1' (the rate constant for the uncatalyzed hydrolysis of the forms MPE-H, APE-H, ME-H, MPB-H, APB-H and MB-H). This means that the hydrolysis of the former forms is much faster and contribute significantly to the overall observed rate constant. In addition, it can be seen that as the fraction of these forms increases the hydrolysis rate will also increase. On the other hand, k_2 (rate constant of base-catalyzed hydrolysis of the forms MPE-H, APE, ME, MPB-H, APB and MB) is the highest rate constant in the hydrolysis reactions of all the esters, which means that the base-catalyzed hydrolysis is the fastest. The values of k_3 (the rate constant of the acid-catalyzed hydrolysis of the forms MPB-H2, APB-H and MB-H), which is only found in the rate equations of the butyl esters is higher than that of the k_2, k_1' but much lower than that of k_1. This means that acid-catalyzed hydrolysis plays a more important role in the hydrolysis of these esters than the uncatalyzed hydrolysis, but less important than the base-catalyzed hydrolysis. For these esters, the rate of the base-catalyzed hydrolysis (pH 6–8) is of the same order as the acid-catalyzed reaction (pH 1–3) even though k_2 is at least 10^5 higher than k_1. This can be explained by the fact that both the base-catalyzed reaction and the acid-catalyzed reaction have second order kinetics, which means that the rate is dependent on hydroxide anion concentration or proton concentration. For example for MPB at pH 8, k_{obs} was 0.0938 h^{-1} and k_2 (2.914 \times 10^4) which if multiplied by K_w/[H^+] (2.512 \times 10^{-6}) affords 0.0732 h^{-1}M^{-1} as the rate of the overall base-catalyzed reaction. While at pH 1, k_{obs} was 0.0515 h^{-1} and k_3 (0.4957) which if multiplied by [H^+] (1.000 \times 10^{-1}) affords 0.0496 h^{-1}M^{-1} as the rate of the overall base-catalyzed reaction equals. This ultimately mean that hydroxide anion concentrations in the order of 10^{-6} is as effective as proton concentrations in the order of 10^{-1}, that is, hydroxide anion is 10^5 more effective in catalyzing the hydrolysis than hydronium cation.

Acid-catalyzed hydrolysis is obvious in the case of the butyl esters MPB, APB and MB but not the ethyl esters ME, APE and ME. This is might be due to the fact that acid-catalyzed hydrolysis requires protonation of the carbonyl group of the ester linkage which is closer to the protonated nitrogen atom in the ethyl ester than the butyl ester, hence the larger charge separation in the butyl esters allows for more carbonyl protonation and hence more prominent acid-catalyzed hydrolysis.

With regard to the base-catalyzed hydrolysis it can be seen that k_2 is 10-times higher for the ethyl esters than the butyl esters. This can be attributed to the fact that base-catalyzed hydrolysis is govern by a nucleophilic attack by
the hydroxide anion on the carbonyl which seems to be less accessible in the case of butyl esters which may be due to the bulkiness of the side chain or to lower degree of solvation of the longer side chain.

Conclusion

The overall pseudo-first order rate constant of the ethyl esters (MP, APE and ME) was much higher than that of the butyl esters (MPB, APB and MB) at any given pH. The hydrolysis of the ethyl esters is either uncatalyzed or base-catalyzed depending on the pH. While, in case of butyl esters, the hydrolysis is either uncatalyzed, base-catalyzed or acid-catalyzed depending on the pH. It is also clear that the base-catalyzed hydrolysis of all the esters plays a more important role than the uncatalyzed hydrolysis or the acid-catalyzed hydrolysis. Finally, esters containing methylpiperazine (MPE and MPB), which are dibasic, shows a distinct pH-rate profile compared to their monobasic acetylpirperazine (APE and APB) and morpholine (ME and MB) containing analogs. Further investigation at higher pH values than 8(9) and lower pH values than 1 at suitable conditions is planned for the near future. In addition, the effect of buffer type and concentration will also be investigated.

Acknowledgments

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Declaration of interest

The authors report no conflict of interest.

References