Synthesis and physical properties of mefenamic acid prodrugs

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Abstract

Purpose. Prodrugs of non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to suppress gastrointestinal (GI) toxicity. In this study, mefenamic acid prodrugs were synthesized and their solubilities and aqueous stabilities were investigated.

Methods. Compounds **1-6** were prepared by coupling the corresponding mefenamic acid prodrugs with paracetamol, solketal, 4-(2-hydroxyethyl)morpholine, 1-(2-hydroxyethyl)-2-pyrrolidinone, 1-(2-hydroxyethyl)-pyrrolidine, 1-(2-hydroxyethyl)-2-piperidine, and L-arginine in the presence of appropriate coupling agents. The solketal ester was hydrolysis using acetic acid to obtain compound **2**. The solubilities of all compounds were studied at pH 5, 7.4 and in pure water. Due to the highly lipophilicity of most compounds, partition coefficients (logP) of all compounds were calculated based on their structures. The hydrolysis of all compounds were investigated in aqueous buffer solutions pH 2, 5, 7.4 and 12 at 37°C.

Results. Compounds **1-4** are less soluble than mefenamic acid, due to the increase in size of the molecule and masking of the hydrophilic carboxyl of mefenamic acid. Compounds **5-6** are more soluble than mefenamic acid in pure water. Their solubilities in pure water are much higher than in aqueous buffer pH 7.4. Compound **7** is more soluble than mefenamic acid, due to the hydrophilic group of arginine. For aqueous stability studies, most prodrugs are stable at lower pHs of 2 and 5. Only compounds **1**, **3**, and **6** were degraded at pH 7.4. Compound **1** showed degradation at all pHs. All compounds, except compound **7**, were degraded at high pH of 12. Compound **7** which is the only prodrug with amide linkage is stable at all studied pHs.

Conclusion. The results from hydrolysis studies indicate that compouds **1-6** are prodrugs. Due to stability of amide bond, compound **7** is stable at all pHs, however, this compound might be prodrug if it can be hydrolyzed by enzyme. Further studies are

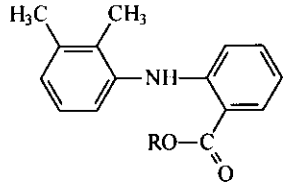
needed to investigate the enzymatic stability and the permeability across intestinal membranes of these compounds.

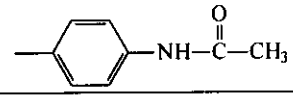
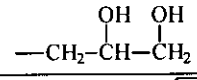
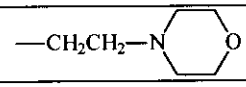
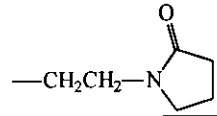
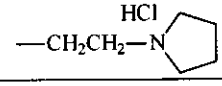
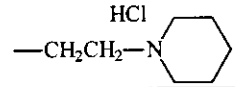
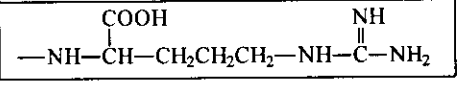
Keywords: Mefenamic acid, prodrugs, hydrolysis, solubility

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammatory diseases. The mechanism of action of NSAIDs involves the inhibition of cyclooxygenase (COX), the rate-limiting enzyme responsible for the conversion of arachidonic acid into prostaglandins. NSAIDs are frequently associated with high incidences of gastroenteropathy, ranging from mild gastric upset to life-threatening ulceration and hemorrhage (1). GI injury produced by NSAIDs is generally believed to be a result of two different mechanisms. The first mechanism involves a local irritant produced by acidic group of the NSAIDs. The second effect is attributed to blockage of prostagandin biosynthesis in the GI tract, which inhibits its cytoprotective effect.

Prodrugs which temporarily mask the acidic group of NSAIDs have been reported to suppress GI toxicity due to the local action mechanism (2). In this study, various mefenamic acid prodrugs (**1-7**) have been synthesized to decrease GI irritation. Mefenamic-paracetamol ester was also designed as a mutual prodrug since both compounds contain pharmacologic properties. Physicochemical properties and stability of these prodrugs were investigated.



Compound number	Compound	R
1	Paracetamol ester	
2	Glycerol ester	
3	Morpholino-ethyl ester	
4	Pyrrolidino-ethyl ester	
5	Pyrrolidinyl-ethyl ester	
6	Piperidinyl-ethyl ester	
7	Arginine amide	

2. Materials and Methods

2.1 Materials

N, N'-dimethylaminopyridine (DMAP), N, N'dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide, and L-arginine were obtained from Sigma. Solketal, 4-(2-hydroxyethyl)morpholine, 1-(2-hydroxyethyl)-2-pyrrolidinone, 1-(2-hydroxyethyl)-pyrrolidine, and 1-(2-hydroxyethyl)-2-piperidine were obtained from Fluka. Other reagents and solvents were purchased from common suppliers and were used as received.

Partition coefficients were calculated using ClogP for Windows (Biobyte Corp.) All melting point were determined on a MEL-TEMP II capillary melting point apparatus and is uncorrected. Proton magnetic resonance spectra were recorded on a Varian Unity Inova (500 MHz) or a Bruker Spectrospin (300 MHz) spectrometers or a JEOL JNM-PMX 60SI. Chemical shifts are reported in parts per million (ppm, δ units) and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet. The IR spectra were recorded on a Perkin Elmer Model 1600 FT-IR spectrometer. Mass spectra (ESI) were measured on a Micromass Platform II mass spectrometer. Yields are of purified products and were not optimized. The purity of the esters was assessed by analytical HPLC as described below.

2.2 Synthesis of mefenamic acid prodrugs

Synthesis of paracetamol ester (**1**)

To a solution of mefenamic acid (3.0 g, 12.44 mmole) in dry CH_2Cl_2 (150 mL) was added a solution of paracetamol (1.87 g, 12.44 mmol) in dry THF, the mixture was cooled at 0°C, followed by the addition of DMAP (0.06 g, 0.5 mmole), and DCC (3.10 g, 15.08 mmole). The reaction mixture was stirred at room temperature overnight. The precipitated of N, N'-dicyclohexylurea (DCU) was removed by filtration. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography using CH_2Cl_2 as the eluent. The product was recrystallized from CH_2Cl_2/petroleum ether to afford 0.97 g (20.64%) of **1** as yellow crystals: > 99% purity; mp 131.5-132.3 ° C; IR (KBr) 1686, 1659 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.14 (s, 3H, ArCH_3), 2.17 (s, 3H, COCH_3), 2.29 (s, 3H, ArCH_3), 6.73 (m, 2H, aromatic), 7.58-7.55 (m, 6H, aromatic), 8.16 (m, 1H, aromatic), 9.14 (s, 1H, N*H*, broad); ESI MS: *m/z* 375 $[M+H]^+$

Synthesis of glycerol ester (**2**)

Compounds **2** were prepared by the same procedure as described for **1**. From mefenamic acid (1.0 g, 1.14 mmole) and solketal (1.09 g, 8.28 mmol) using ethylacetate:hexane (1:20) as the eluent and recrystallization from $MeOH/H_2O$ was obtained 0.88 g (59.52%) of solketal ester of mefenamic acid as a white solid: mp 58.3-61.4 °C; IR (KBr) 1689 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$,) δ 1.40 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.17 (s, 3H, ArCH_3), 2.29 (s, 3H, ArCH_3), 4.33-3.68 (m, 5H, CH_2CHCH_2), 6.56 (m, 2H, aromatic), 7.03-6.87 (m, 4H, aromatic), 7.9 (m, 1H, aromatic), 9.01 (s, 1H, N*H*, broad); ESI MS: *m/z* 378 $[M+Na]^+$, 356 $[M+H]^+$

A mixture of (0.99 g, 2.78 mmole) of solketal ester of mefenamic acid and 100 ml of 70% acetic acid was heated to 60° C for 1 hour. The reaction mixture was cooled to ambient temperature and extracted with CH_2Cl_2 (3 x 20 ml), the CH_2Cl_2 extracts were

wash with H_2O and brine. Precipitated solid was collected, washed with water, partially air dried, and dissolved in 50 ml CH_2Cl_2. The solution was dried with anhydrous sodium sulfate and the solvent was removed by evaporation under reduced pressure. The residual was recrystallized from methanol to obtain 0.30 g (34.06%) of **2** as white crystals: mp 60.6-62.4 °C; IR (KBr) 1680 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.08 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.42-3.50 (m, 2H, CH_2OH) 3.79-3.84 (m, 1H, CH_2CH), 4.19 (dd, J = 0.022, 0.013 Hz, 1H, OCH_2), 4.33 (dd, J = 0.022, 0.007 Hz, 1H, OCH_2), 4.70 (t, J = 0.011 Hz, 1H, OH), 5.02 (d, J = 0.01 Hz, 1H, OH), 6.65-6.73 (m, 2H, aromatic), 7.03-7.13 (m, 3H, aromatic), 7.30-7.33 (m, 1H, aromatic), 7.94-7.96 (m, 1H, aromatic), 9.12 (s, 1H, NH, broad); ESI MS: *m/z* 316 $[M+H]^+$

Synthesis of morpholino-ethyl ester (**3**)

To a solution of mefenamic acid (5.0 g, 20.7 mmol) in CH_2Cl_2 (150 mL) was added 4-(2-hydroxyethyl)morpholine (2.86 g, 20.7 mmol) and DMAP (0.107 g, 0.83 mmol). The mixture was cooled to 0°C, followed by the addition of DCC (5.21 g, 25.11 mmol). The reaction was stirred for an additional 4 h at 0°C and stored overnight in the refrigerator. The precipitated DCU was removed by filtration. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography using ethyl acetate:hexane (20:80) as the eluent. The product was recrystallized from hexane to afford 4 g (57%) of **3** as pale yellow crystals: > 99% purity; mp 84.8 °C; IR (KBr) 1678 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.19 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.59 (m, 4H, N(CH_2)$_2$), 2.80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.72 (m, 4H, O(CH_2)$_2$), 4.47 (t, *J* = 5.8 Hz, 2H, OCH_2), 6.70-6.67 (m, 1H, aromatic), 6.76-6.75 (m, 1H, aromatic), 7.05-7.04 (m, 1H, aromatic) 7.18-7.10 (m, 2H, aromatic), 7.27-7.24 (m, 1H, aromatic), 7.98-7.96 (m, 1H, aromatic), 9.22 (s, 1H, NH, broad); ESI MS: *m/z* 355 $[M+H]^+$

Synthesis of pyrrolidino-ethyl ester (**4**)

This compound was prepared from mefenamic acid (5.0 g, 20.7 mmol) and 1-(2-hydroxyethyl)-2-pyrrolidinone (2.67 g, 20.7 mmol) as described for compound **3** and gave 4.6 gm (64%) of **4** as white crystals: > 99% purity; mp 66.7 °C; IR (KBr): 1682 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.05 (m, 2H, NC(O)CH_2CH_2 in pyrrolidone ring), 2.18 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.40 (t, *J* = 8.0 Hz, 2H, CH_2N in pyrrolidone ring), 3.56 (t, *J* = 7.5 Hz, 2H, NC(O)C$H_2$$CH_2$ in pyrrolidone ring), 3.71 (t, *J* = 5.5 Hz, 2H, CH_2N), 4.45 (t, *J* = 5.5 Hz, 2H, OCH_2-), 6.69-6.66 (m, 1H, aromatic), 6.76-6.73 (m, 1H, aromatic), 7.04-7.03 (m, 1H, aromatic), 7.16-7.09 (m, 2H, aromatic), 7.27-7.24 (m, 1H, aromatic), 7.94-7.92 (m, 1H, aromatic), 9.22 (s, 1H, NH, broad); ESI MS: *m/z* 353 $[M+H]^+$

Synthesis of pyrrolidinyl-ethyl ester HCl (**5**)

To a solution of mefenamic acid (3 g, 8.6 mmol) and N-hydroxysuccinimide (1.43 g, 8.6 mmol) in 1,2-dimethoxyethane (50 ml) was added DCC (2.56 g, 12.45 mmol). The mixture was stirred at 0°C for 1 h and stored overnight in the refrigerator. The precipitated DCU was removed by filtration. The filtrate was added to a solution of 1-(2-hydroxyethyl)-pyrrolidine (2.93 ml, 24.86 mmol) in 1,2-dimethoxyethane (15 ml). The mixture was stirred overnight at room temperature. The main product was purified by column chromatography using ethyl acetate:hexane (5:95) as the eluent which was concentrated in vacuo. The crude product was diluted with absolute ethanol (50 mL) and treated with gaseous hydrogen chloride to give (10 %) as a white solid: > 99%

purity; mp190-191°C; IR (KBr): 1681 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 1.91 (m, 2H, CH_2CH_2N in pyrrolidine ring), 2.01 (m, 2H, CH_2CH_2N in pyrrolidone ring), 2.09 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.11 (m, 2H, CH_2N), 3.61 (m, 4H, CH_2NCH_2 in pyrrolidine ring) 4.62 (t, J = 5.03 Hz, 2H, OCH_2CH_2N), 6.65-6.74 (m, 2H, aromatic), 7.05-7.15 (m, 3H, aromatic), 7.33-7.37 (m, 1H, aromatic), 8.06 (dd, J = 0.016, 0.003 Hz, 1H, aromatic), 9.14 (s, 1H, N*H*, broad); ESI MS: *m/z* 339 $[M+H]^+$

Synthesis of piperidinyl-ethyl ester HCl (**6**)

This compound was prepared from mefenamic acid (5.0 g, 20.7 mmol) and 1-(2-hydroxyethyl)-2-piperidine (8.25 ml, 62.17 mmol) as described for compound **5** and gave 2.12 gm (36 %) as a pale yellow solid: > 99% purity; mp 209-211°C; IR (KBr): 1681 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 1.38-1.39 (m, 1H, piperidine ring), 1.68-1.70 (m, 1H, piperidine ring), 1.79-1.83 (m, 4H, piperidine ring), 2.04 (m, 3H, CH_3), 2.25 (m, 3H, CH_3), 2.97-3.04 (m, 2H, CH_2N), 3.49-3.50 (m, 4H, piperidine ring), 4.67 (t, J = 0.01, 2H, OCH_2CH_2N), 6.65-6.74 (m, 2H, aromatic), 7.05-7.15 (m 3H, aromatic), 7.33-7.37 (m, 1H, aromatic), 7.99 (dd, J = 0.016, 0.003 Hz, 1H, aromatic), 9.12 (s, 1H, N*H*, broad); ESI MS: *m/z* 353 $[M+H]^+$

Synthesis of arginine amide (7)

To a solution of mefenamic acid (1.207 g, 5 mmol) and N-hydroxysuccinimide (0.58 g, 5 mmol) in 1,2-dimethoxyethane (20 ml) was added DCC (0.103 g, 5 mmol). The mixture was stirred at 0°C for 1 h and stored overnight in the refrigerator. The precipitated DCU was removed by filtration. The filtrate was added to a solution of L-arginine (0.871 g, 2.6 mmol) and $NaHCO_3$ (0.42 g, 5 mmol) in water (10 ml). After stirring at room temperature for 1 hour, 2-dimethoxyethane was evaporated in vacuo. The yellow solid was obtained after the aqueous layer was kept in the refrigerator overnight which was recrystallization from ethanol to afford 0.71 g (69 %) of **7** as pale yellow crystals: > 99% purity; mp 126-127°C; IR (KBr): 1685, 1623, 1580, 1510, 1468 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.53 (m, 2H, CH_2), 1.87-1.73 (m, 2H, CH_2,), 2.08 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 4.18 (m, 1H, C*H*), 6.59-6.62 (m, 1H, aromatic), 6.74-6.62 (m, 1H, aromatic), 6.87-6.89 (m, 1H, aromatic), 7.01-7.02 (m, 2H, aromatic), 7.10-7.13 (m, 1H, aromatic), 7.38 (m, 2H, NH_2, broad), 7.68-7.70 (m, 1H, aromatic), 8.32 (d, J = 0.015 Hz, 1H, N*H*CH), 9.25 (s, 1H, N*H*, broad), 9.70 (s, 1H, N*H*, broad); ESI MS: *m/z* 398 $[M+H]^+$

2.3 HPLC analysis

The HPLC system consisted of a Waters 600 Pump, a Waters 717 plus Autosampler, a Waters 486 Tunable Absorbance Detector, and a Waters 746 Data Module. A Rexchrom S5 octyl column (150 x 4.6 mm i.d., 5 μm) connected to a Nova-Pak C8 (3.9 x 20 mm, 4 μm, Waters, U.S.A.) guard column was used for all sample separation. In order to separate mefenamic acid from its prodrugs in each analysis, mobile phase mixtures of either acetonitrile or methanol and 0.05M buffer solution of pH 3-5.5, depending on compounds, were employed at a flow rate of 1.0 mL/min, the eluent was monitored at 280 nm. The mobile phases used for HPLC analysis and retention times obtained for each compound were listed in Table 1

2.4 Aqueous Solubility

The aqueous solubility of mefenamic acid prodrugs was determined in water and 0.05 M buffer solutions (phosphate pH 7.4, ionic strength 0.1 M adjusted with NaCl). Excess amounts of each compound were added to 1-2 ml of buffer in screw-capped glass vials. Samples were then tumbled at 20 rpm at 25°C until a constant solubility value was obtained. The saturated solutions were filtered through 0.45-μm cellulose acetate membrane (Sartorius, Germany) filters, quantitatively diluted with mobile phase, and analyzed by HPLC as previously described.

2.5 Chemical stability

The stability of prodrugs **1-7** were examined in 0.01M HCl (pH 2.0), 0.05M acetate buffer (pH 5.0), 0.05 M phosphate buffer (pH 7.4), 0.01 M NaOH (pH12). The ionic strength was maintained at 0.1 M by the addition of NaCl. The reaction samples were prepared by adding 20-40 μL of 2 mg/ml solution of prodrugs in acetonitrile or methanol to 5.0 mL prewarmed buffer solution, resulting in 0.5-20 μg/mL solutions with 1-2% of acetonitrile or methanol. The solutions were then placed into a thermostatically controlled water bath at 37°C. At appropriate times, 200-650 μl aliquots of samples were taken, stored at 4°C until assay by HPLC. Upon analysis, samples were thawed, added with 10 μL diclofenac solution (internal standard), diluted with mobile phase and analyzed by HPLC. Pseudo-first order rate constants (k) were determined from the slopes of linear plots of the logarithm of residual prodrug concentrations versus time. Triplicate samples were analyzed, and the mean value of the rate constant was reported. The corresponding half-life obtained from the equation: $t_{1/2} = 0.693/k$.

3. Results and Discussion

Aqueous solubility and lipophilicity are considerable properties for drug delivery. Drug molecules should possess optimal hydrophilic and lipophilic properties to readily solubilize and permeate membrane. The aqueous solubility and the calculated partition coefficients (logP) for mefenamic acid and its prodrugs are shown in Table2. Due to the high values of partition coefficients (logP) of most prodrugs, the octanol-water partition (logP) of prodrugs could not be determined to obtain a reliable result by experimentation (3). The logP value was therefore estimated using the ClogP program (Biobyte, CA), which calculates the value directly from its molecular structure.

The aqueous solubility of prodrugs may be influenced by two components. Firstly, by masking the carboxyl moiety which is the hydrophilic part of mefenamic acid may reduce the aqueous solubility to some extent. Secondly, aqueous solubility typically decreases with increasing size of the molecules. However, the aqueous solubility might be increased if the group linked to parent compound is hydrophilic. In this study, mefenamic acid prodrugs with various chemical structures show different aqueous solubilities. Most of the prodrugs have poorer water solubility than parent mefenamic acid, except compounds **5-7**, as shown in table 2. Forming HCl salt has several folds increased water solubility of compound **5** and **6**. The solubility of compounds **1-4** and **7** is comparable at pH 7.4, pH 5.0 and in water (pH of 6.8). Compounds **1, 2,** and **4** are neutral compounds, so its solubility is independent of pH. Compound **3** contains basic group which is completely protonated at both pH 5.0 and 7.4, so the solubility at these two pHs is not different. Compound **7** posseses both acidic and basic functions. The pKa value for carboxyl group of arginine is 1.82 (4)

which is substantially more acidic than acetic acid (pKa of 4.76) due to the protonated amino group of this amino acid. However, typically, the pKa for the terminal carboxyl group in proteins is about 3.1 (5), the approximate pKa value for carboxyl group of this prodrug should be in this range. Arginine contains the strongly basic guanidino group, corresponding to pKa 13.2 (3). Thus, at pH 5.0 and pH 7.4 the arginine moiety of prodrug exists in zwitterionic form, the carboxyl function is in ionized form and guanidine moiety is protonated. The solubility of this prodrug, **7**, therefore, show the comparable solubility at both pH 5.0 and 7.4. Interestingly, compounds **5** and **6** show 48 and 124 fold greater solubility in pure water than at pH 7.4, respectively. At pH 7.4 or in pure water, both are in completely protonated forms. The differences in solubilities might be affected by salts from buffer solution. Further studies are needed to explore this phenomenon.

At constant pH and temperature, the degradation rates of the prodrugs follow apparent first order kinetics for at least two half-lives. The apparent half-lives are shown in Table 3. Most prodrugs, except compound **1**, showed chemical stability at pH 2.0 and 5.0. At pH 7.4, compound **1**, **3** and **5** were degraded with half-lives ranging from 4 to 27 hours. Most prodrugs were degraded at pH 12, except compound **7**. The hydrolysis half-lives for compounds **2-6** were 0.41, 0.35, 0.66, 4.67, 2.78 hours, respectively. Compound **1** was degraded at all pHs, its degradation rate is faster with the increasing of pH. At pH 12, compound **1** was rapidly degraded, and the compound could not be detected from the solution after 5 min. Compound **7** which is the only prodrug with amide linkage is stable at all studied pHs. The degradation of each prodrug yielded mefenamic acid and the time courses for compound **1-6** and mefenamic acid during hydrolysis of the prodrug were shown in Fig 1-6.

Further studies are needed to investigate the enzymatic stability of mefenamic acid prodrugs in plasma and also the permeability or transport properties of these prodrugs across intestinal membranes.

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Figure Legends

Fig 1. Stability of compound **1** at pH 7.4 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

Fig 2. Stability of compound **2** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

Fig 3. Stability of compound **3** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

Fig 4. Stability of compound 4 at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

Fig 5. Stability of compound **5** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

Fig 6. Stability of compound **6** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

Table 1 Mobile phases used for HPLC analysis and retention times obtained of each compound.

Compound	Mobile phase	Retention time (min)		
		Prodrug	Mefenamic acid	Diclofenac[a]
1	Phosphate buffer pH3 45% Acetonitrile 55%	12.6	7.6	-
2	Ammonium acetate pH 5 40% Methanol 60%	18.1	12.1	-
3	Acetate buffer pH 4.5 57% Acetonitrile 43%	22.0	16.1	5.6
4	Acetate buffer pH 4.1 55% Acetonitrile 45%	16.2	11.3	6.7
5	Ammonium acetate pH 4.1 50% Acetonitrile 50%	17.6	8.7	6.1
6	Ammonium acetate pH 4.1 50% Acetonitrile 50%	15.5	9.6	6.6
7	Heptane sulfonic acid (0.1%) in acetate buffer pH 4.1 53% Acetonitrile 47%	14.6	17	8.84

[a]internal standard

Table 2 Aqueous solubility and calculated partition coefficient (clog P) of mefenamic acid and its prodrugs

Compound	Aqueous solubility (μg/mL)			Clog P
	Phosphate buffer pH 7.4	Acetate buffer pH 5.0	H_2O	
Mefenamic acid	a	a	67.00	4.777[b]
1	0.334	a	0.652[c]	5.733
2	5.02	a	6.02	3.497
3	3.61	3.92	3.15	5.276
4	7.43	7.64	6.44	4.80
5	36.6	269.7	1787	5.986[d]
6	15.7	a	1961	6.545[d]
7	95.3	101.6	94.1	-0.085

[a]Not determined. [b]Experimental logP is 5.12. [c]With 2% v/v ethanol as cosolvent. [d]Value as of free base

Table 3 Half-lives of mefenamic acid prodrugs in aqueous buffers

Compound	Half-lives (hrs) (mean ± S.D.)			
	0.01 M HCl pH 2	Acetate buffer pH 5.0	Phosphate buffer pH 7.4	0.01 M NaOH pH12
1	18.7 ± 2.59	5.79 ± 1.05	4.19 ± 0.78	a
2	b	b	b	0.411 ± 0.01
3	b	b	4.30 ± 2.64	0.346 ± 0.02
4	b	b	b	0.663 ± 0.085
5	b	b	b	4.67 ± 1.2
6	b	b	26.5 ± 2.97	2.78 ± 0.64
7	b	b	b	b

[a]Rapidly degraded. [b]No degradation was observed during 24 hour-incubation.

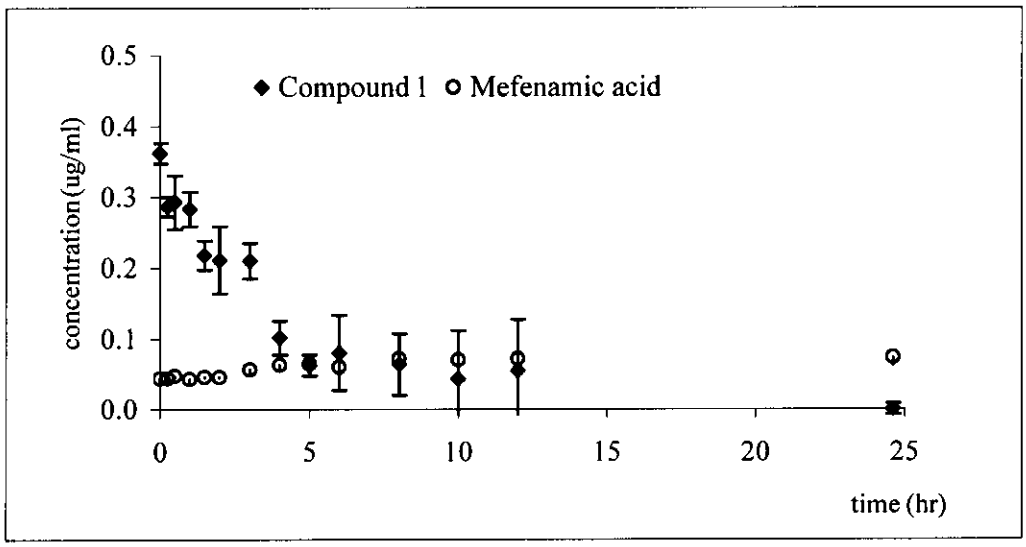

Fig 1. Stability of compound **1** at pH 7.4 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

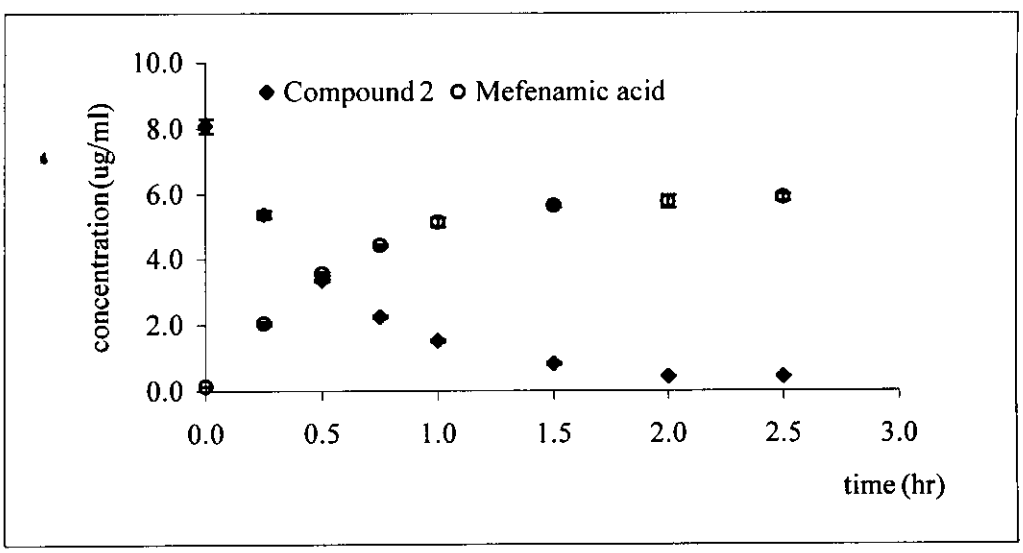

Fig 2. Stability of compound **2** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

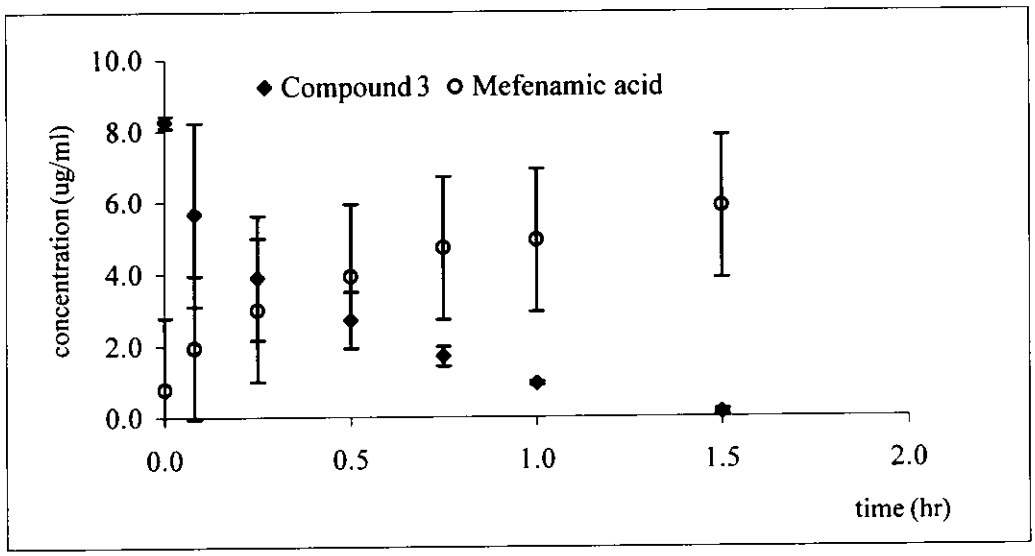

Fig 3. Stability of compound **3** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

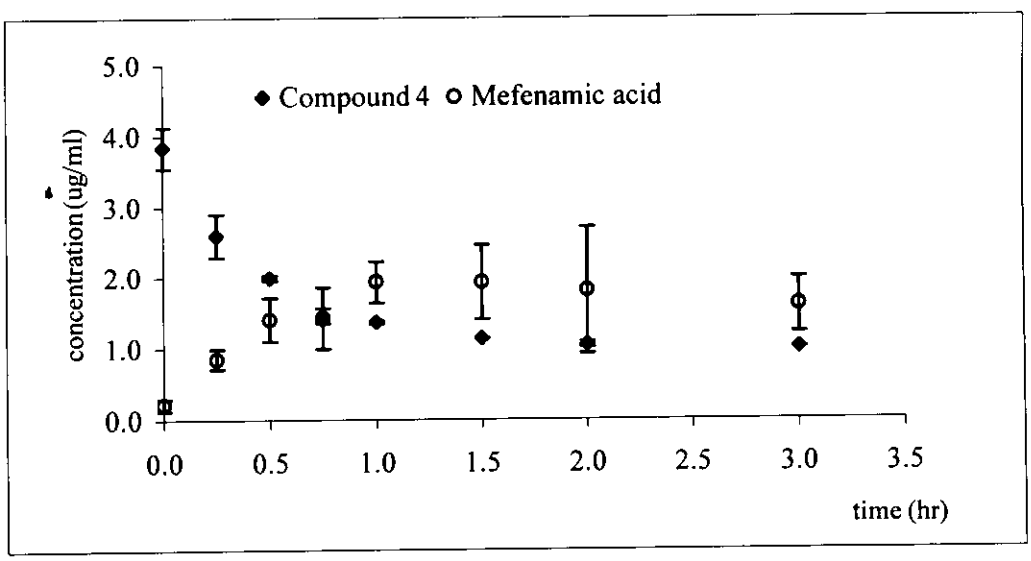

Fig 4. Stability of compound 4 at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

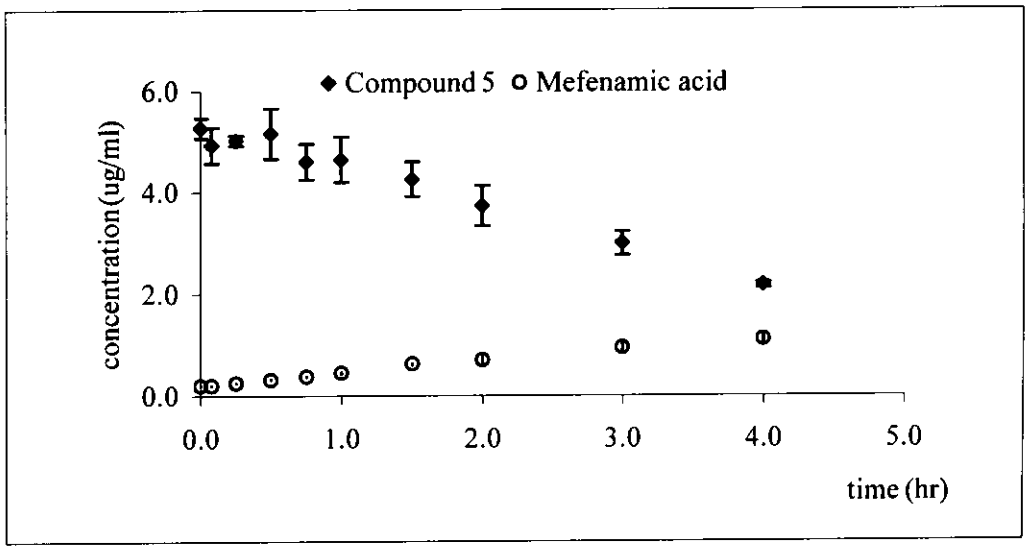

Fig 5. Stability of compound **5** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).

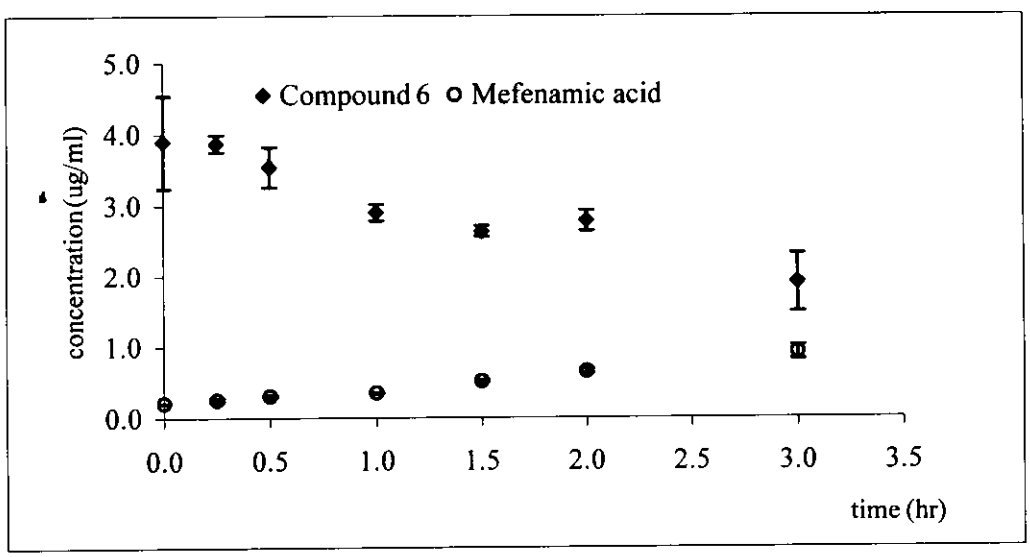

Fig 6. Stability of compound **6** at pH 12 (37°C), showing the time course of the disappearance of prodrug and the appearance of mefenamic acid. Experiments were performed in triplicate (average ± SD).