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GLYCOLIC ACID DERIVATIVES OF MEFENAMIC ACID AS POSSIBLE PRODRUGS: SYNTHESIS AND EVALUATION

JAMAL A. JILANI[1]*, SANA' A H. GHARIABEH[2], NAJI M. NAJIB[2]

Department of Medicinal Chemistry and Pharmacognosy[1]
Deparment of Pharmaceutical Technology[2]
Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

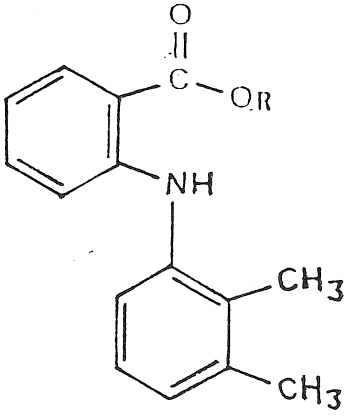
Three glycolic acid esters of mefenamic acid, namely carboxymethyl mefenamate, ethoxycarbonylmethyl mefenamate, and aminocarbonylmethyl mefenamate, (II-IV), were synthesized and evaluated as possible prodrugs. The hydrolysis of these compounds were performed in aqueous solutions of pHs 2 and 7.4, as well as in human plasma solutions at 37°C. Among the three synthesized compounds the aminocarbonylmethyl mefenamate (IV) shows the most promising in vitro results as possible prodrug. It shows $t_{1/2}$ of 36.1, 10.5 and 0.85 h in pHs 2, 7.4 and in human plasma respectively. It was evaluated in vivo showing serum concentrations of mefenamic acid comparable to control study after oral administration. On the other hand, carboxymethyl mefenamate (II) shows very slow rate of hydrolysis in both enzymatic and non-enzymatic media, while the ethoxycarbonylmethyl mefenamate (III), hydrolyzed in both enzymatic and non-enzymatic media with $t_{1/2}$ of 4.0, 4.3, and 1.25 h in pHs 2, 7.4 and in human plasma respectively.

Keywords : *Prodrug; Mefenamic acid; Anthranilic acid; Syhthesis; Hydrolysis*

Introduction

Mefenamic acid (I), is an acidic non-steroidal anti-inflammatory drug (NSAD) that is widely used as an analgesic anti-inflammatory agent (1). However, as with other NSAIDs, its major drawback is the preponderance of gastrointestinal (GI) side effects (2, 3, 4). Latentiation of the carboxylate moiety has been proposed as a promising mean to reduce or abolish the G.I.T. side effects (5, 6, 7) at the same time this latetiation could improve the delivery characteristics of the NSAIDs especially for topical applications (5).

Esterification of the NSAIDs represents a suitable approach to latentiate the carboxylate moiety and developing useful prodrugs. Many ester prodrugs of NSAIDs are commercially available or are under various phases of clinical trials (8, 9, 10). Among the various ester derivatives that have been evaluated as prodrugs for NSAIDs and showed promising results are the glycolamide ester derivatives (9,11). This work presents the synthesis and evaluation of three glycolic acid ester derivatives of mefenamic acid, namely, carboxymethyl mefenamate (II), ethoxycarbonylmethyl mefenamate (III), and aminocarbonylmethyl mefenamate (IV) (Fig. 1). The kinetics of hydrolysis under enzymatic and non-enzymatic conditions will be discussed. Preliminary in vivo studies on the amide derivative (IV) after oral administration will be presented.



(I) R = H
(II) R = CH_2CO_2H
(III) R = CH_2CO_2Et
(IV) R = CH_2CONH_2

Fig.1. Chemical structures of (I-IV)

*: To whom correspondence should be directed

Materials and Methods

Apparatus

The mefenamate esters (II-IV) were characterized by a variety of analytical techniques. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution with TMS as internal standard at 300 MHz by means of a *Bruker spectrometer.* Mass spectra were obtained using 707-E VG *spectrometer.* IR spectral data were recorded using a *Shimadzu,* IR-435 instrument and KBr disk. Melting points were determined using *Gallenkamp Electrothermal* melting point apparatus and were uncorrected. The high-performance liquid chromatography (HPLC) was performed using a *Spectra-Physics, Isochrom LC,* model pump, equipped with a variable wavelength detector; *Jasco 875-UV* intelligent uv/vis detector, *Jasco Corporation,* Japan and a 50 µl loop injection valve, *Altex Model 212 a, Beckman instruments inc., Berkele, USA.* In these methods a reversed-phase HPLC columns, *250x4 mm, Packed with Lichrosorb, RB-18 (5 µm particles), Merck, Germany* were used, the column effluent was monitored using *Spectra Physics, Model SP 4270, Spectra-Physics Inc., USA,* chromjet integrator.

Chemicals

Mefenamic acid was provided by *AL-Hikma pharmaceutical company, Jordan.* Ethylbromoacetate was purchased from *Fluka Chemie AG, Switzerland.* Chemicals and solvents used in kinetic study were of reagent grade.

Synthesis of prodrugs

Preparation of ethoxycarbonylmethyl mefenamate (III)

Sodium mefenamate (3 g, 0.0087 mol) and KI (0.15 g, 0.0009 mol) was suspended in DMF (20 ml), to the suspension ethylbromoacetate (1.7 g, 0.010 mol) was added, the suspension was then stirred for 4 h at 70°C in an oil bath. The reaction mixture was treated with EtOAC (50 ml) and the ppt formed was filtered off. The filtrate was washed with 5% solution of sodium thiosulfate (3x20 ml). Evaporation of the organic layer gave an oily liquid which was poured into ice water to yield a solid ppt. The solid ppt was taken by filtration and purified by recrystallization from aqueous MeOH to yield 2.2 g (77%) of (III). mp 65-66°C; IR (KBr) 1680 cm^{-1} (ArCOO-), 1750 cm^{-1} (EtOCO-); ^{1}H-NMR ($CDCl_3$), δ 1.2 (t,3H,CH_3), 2.1 (s,3H, -CH_3), 2.3 (s, 3H, CH_3), 4.2 (q, 2H, OCH_2), 4.8 (s, 2H, OCH_2), 6.6-8 (m, 7H, ArH), 9.1 (s, 1H, NH).

Preparation of carboxymethyl mefenamate (II)

To a solution of (III) (2 g, 0.0069 mol) in acetone (30 ml) was added a solution of K_2CO_3 (0.5 g) in H_2O (5 ml). The solution was stirred with reflux 24 h. Evaporation of the solvent under vacuo gave a pasty material which was poured onto acidified ice-water mixture yielding a solid ppt. the solid was collected by vacuum filtration and purified by recrystallization from aqueous ethanol yielding 1.8 g of (II) (87%). mp 155-157°C; IR (KBr) 1680 cm^{-1} (ArCOO-), 1730 cm^{-1} (-COOH) ^{1}H-NMR, ($CDCl_3$), δ 2.1 (s,3H,CH_3), 2.3 (s, 3H, CH_3), 4.9 (s, 2H, OCH_2), 6.6-8.1 (m,7H, ArH), 9(br, 1H, ArNH).

Preparation of aminocarbonylmethyl mefenamate (IV)

To a solution of (III) (1 g, 0.0029 mol) in methanol (15 ml) was added 2 ml of cocentrated ammonia solution. The reaction mixture was kept stirred at room temperature for 24 h. The solvent was evaporated in vacuo and the resulting residue was purified by column chromatography (hexane (6): EtOAc (2): HOAc: (0.25)) yielding 0.6 g (70%) of (IV) mp 125-126°C; IR (KBr) 1680 cm^{-1} (ArCOO-), 1690 cm^{-1} ($CONH_2$), ^{1}HNMR, ($CDCl_3$), 2.1 (s,3H,$ARCH_3$), 2.3 (s, 3H, $ArCH_3$), 4.8 (s,2H,OCH_2CO), 6.1 (br, 1H, NH_2), 6.2 (br, 1H, $CONH_2$), 6.6-8.0 (m, 7H, ArH) 9.2 (s, 1H, ArNH).

HPLC analytical methods

The columns were eluted at ambient temperature with mixtures of acetonitrile and 0.006 M sodium-acetate buffer (pH 4.0 or PH 4.5). The composition of the eluant for each compound was adjusted to provide an appropriate retention times and resolution of ester, related intermediate and mefenamic acid. The flow rate was 2 ml/min., and the column effluent was monitored spectrophotometrically at 280 nm. Quantitation of the compounds was done by measurement of the peak heights in relation to those of standards chromatographed under the same conditions.

Hydrolysis kinetics in aqueous buffer solutions

The synthesized compounds (II-IV) were incubated at 37 0.2°C in 0.1N HCl (pH 1.0) and 0.02 M phosphate buffer (pH 7.4). The reactions were initiated, by addition of 25.0 µl of a 1.0 mg/ml stock acetonitrile solution of each derivative to 5 ml of preheated and freshly prepared buffer solution in hermetically sealed flasks. The final concentrations of the compounds being 5.0 µg/ml. The solutions were then kept in a shaking water-bath at 37 0.2°C and at appropriate intervals samples of 100 µl were withdrawn and added to 100 µl of stock solution of the predetermined internal standard, and mixed. A 50 µl aliquot portion of the mixtures were immediately chromatographed using the prescribed HPLC system for each derivative. The kinetic rate constants for the degradation of the compounds were determined from the linear plots of the logarithm of the residual amount function against time.

Hydrolysis kinetics in human plasma

Human plasma was obtained by centrifugation of blood samples containing 0.3% citric acid at 3000 xg for 15-20 minutes. Incubations were performed at 37 0.2°C using a shaking water-bath. The reactions were initiated by adding 25 µl of a 1.0 mg/ml stock acetonitrile solution to 5 ml of preheated plasma in hermetically sealed flasks. The final concentrations

being 5.0 µg/ml. At the appropriate time points, aliquots (100 µl) were withdrawn and deproteinized by addition of an equal volume of acetonitrile, aliquot (100 ml) of stock of the appropriate internal standard was also added. The solutions were mixed on a vortex mixture for 30 seconds, and centrifuged for 1 minute at 1100 xg, 50 µl of the clear supernatant were chromatographed as described above. The amounts of the remaining intact prodrug as well as the parent acid, and related intermediate, were plotted as a function of incubation time as described above under buffer hydrolysis.

In vivo study

The potential of the mefenamate ester derivatives as prodrugs was assessed in vivo using rabbits as an animal model. One adult female rabbit weighing 4 kg was used in a two phase cross-over bioavailability experiments with interval of one week for washing out. Food was not given to the rabbit for 12 h prior to and during the experiment, but water was allowed *ad libitum*. Mefenamic acid (10 mg/kg) and equimolar doses of the glycolamide ester (IV) (12.5 mg/kg) were administered orally as a capsule dosage form to the same rabbit by gastric intubation, the dosage form was followed with 10 ml of water.

Blood samples (1.5 ml) were collected just prior to drug administration and at 15-30 minutes time intervals post administration for 6 h. The blood was obtained from the marginal ear veins into an eppendorf tubes by dripping procedure. The blood samples were immediately centrifuged and the obtained serum samples were freezed pending assay. At the time of assay samples were prepared for HPLC analysis by adding 200 µl of acetonitrile spiked with internal standard to an equivalent volume of serum sample to precipitate the proteins. The samples were vortexed for 0.5 minute and centrifuged at 11000 rpm for 1.0 minute. An appropriate volume of the resulting clear supernatant was injected on the HPLC column.

Results and Discussion

Chemistry

In a pervious work on hydroxyethyl mefenamate we observed a relatively high stability of hydroxyethyl mefenamate versus other hydroxyethyl esters at different pHs at 37°C (12). Thes finding suggests that in general anthranilic acid diesters can be selectively de-blocked under mild acidic or basic conditions. Based on these findings we prepare the glycolote ethyl ester of mefenamic acid (III) and utilized it in the synthesis of (II) and (IV). The differences in the electrophilicity of the two carbonyls in (III) allow selective hydrolysis and aminolysis to prepare (II) and (IV) respectively, (Fig. 2).

The structures of compounds (II-IV) were confirmed using different spectroscopic techniques including ^1H NMR. The addition of D_2O to $CDCl_3$ solution of the compounds allows the assignment of amide and other exchangeable protons.

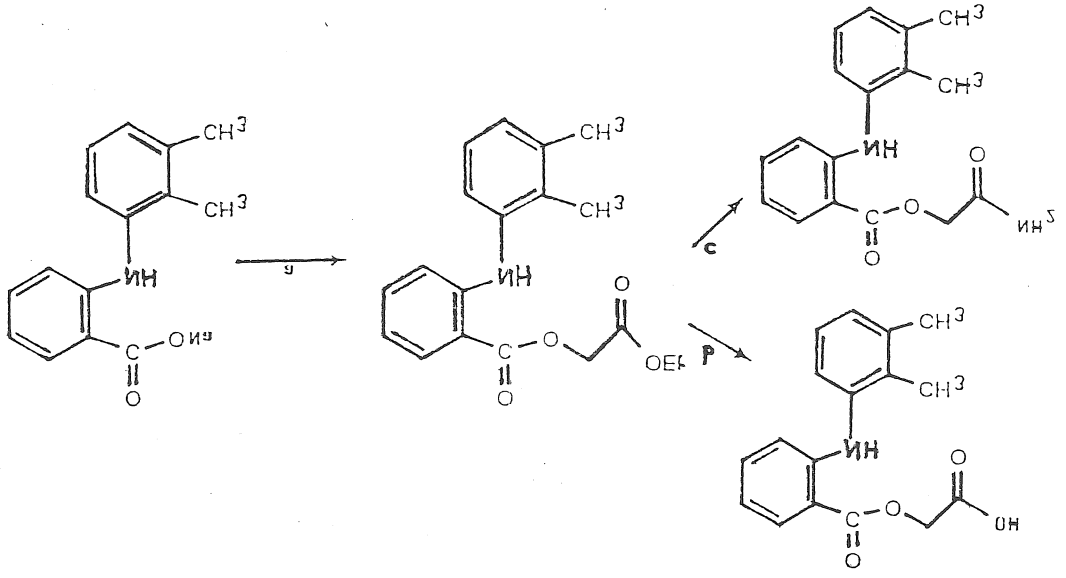


Fig.2. Synthesis of the compounds (II-IV). Keys: a, Ethyl bromoacetate/DMF; b, K_2CO_3/H_2O/Acetone; c, NH_3/MeOH

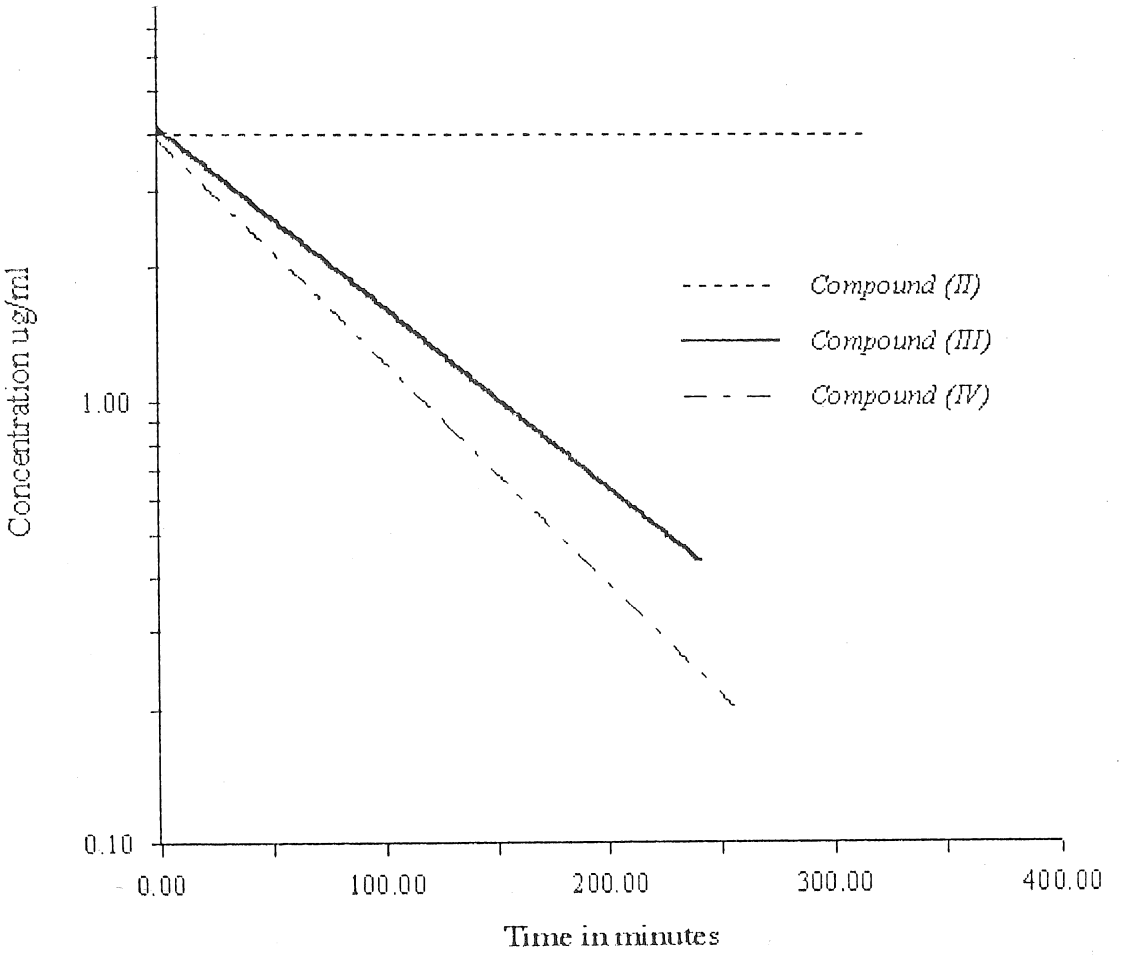


Fig.3. First order plots for the hydrolysis of (II), (III) and (IV) in human plasma solutions at 37°C.

In vitro study

The hydrolytic stability of the derivatives (II-IV) was examined in aqueous solutions at 2.0 and 7.4 pHs, as well as in human plasma at 37°C. The appearance of the degradation products was monitored using a specially developed precise, accurate and selective HPLC method as described in the experimental section.

The progress of hydrolysis in human plasma of (II), (III) and (IV) is illustrated in Fig.3.

Table 1. Kinetic data for the in vitro hydrolysis of compounds (III-IV)

Compound No.	$K_{obs}x10^{-3}$ (min^{-1})			$t_{1/2}$, h		
	In human plasma	In 7.4 buffer	In 0.1N HCl	In human plasma	In 7.4 buffer	In 0.1 N HCl
II	0.096	0.052	0.050	120	222	231
III	9.34	2.70	2.90	1.25	4.30	4.00
IV	13.00	0.32	1.10	0.85	10.50	36.10

The first-order rate constants (K_{obs}) calculated from the slopes of linear plots of the logarithm of remaining ester against time, and the corresponding half-lives obtained from the identity $t_{1/2}=0.693/K_{obs}$.

Among the prepared compounds, (II) represents a monoester containing free carboxylate moiety. It hydrolyses very slowly in both enzymatic and non-enzymatic media as reflected by its $t_{1/2}$(Table 1). Its high stability in non-enzymatic media can be attributed, at least partially, to the low electrophilicity of the ester carbonyl due to possible resonance effect (12). While the stability of (II) against enzymatic hydrolysis can be attributed to the presence of polar, negatively charged carboxylate group (11, 13, 14). On the other hand, each of the compounds (III) and (IV) contains two electrophilic sites susceptible to hydrolysis, thus introducing some complexity concerning hydrolysis pathways as shown in Fig.4.

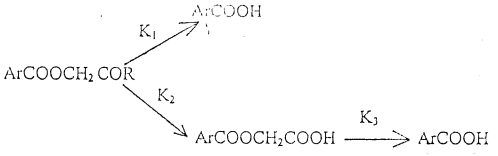


Fig.4. Hydrolysis pathway for diesters

In Fig.4., K_1-K_3 refer to the 1 st-order rate constants for the processes indicated. Since K_3 is much smaller than K_1&K_2 as reflected by the long $t_{1/2}$ of (II), the overall rate constant for degradation (K_{obs}) will be the summation of K_1&K_2 and can be calculated according to the following equations

$$K_{obs} = K_1 + K_2$$

$$K_1 = (\%\ \text{mefenamic acid formed})K_{obs}\ /100$$

$$K_2 = K_{obs} - K_1$$

The values of K_1, K_2 and K_{obs} are listed in Table 2. From the data obtained, one can conclude that compound (III) is a poor candidate as a prodrug for mefenamic acid, since it dosen't show acceptable selective hydrolysis to mefenamic acid in addition to its relatively poor stability in non-enzymatic media.

Table 3 shows the percentage of mefenamic acid (I) and the hydrolysis intermediate (II) formed after incubation of (III) and (IV) with human plasma. From this table one can see that the amide (IV), unlike (III), transformed selectively to mefenamic acid. The time courses of degradation of (III) and (IV) in human plasma are shown in Figs. 5 and 6 respectively.

The apparent difference in hydrolysis selectivity between (III) and (IV) although reflects enzymatic selectivity, the non-enzymatic effect of the plasma media should not be excluded since compound (III) is less stable in pH 7.4 solution than (IV) (Table 1). In pH 7.4 the selectivity (site of hydrolysis) factor depends on the difference in the susceptibility of the two carbonyls to nucleophilic attack. The diester (III) is expected to hydrolyze

Table 2. Kinetic analysis of hydrolysis of (III) and (IV) in human plasma at 37°C

Compound	K_{obs} x10^{-3}	K_1x10^{-3}	K_2x10^{-3}
III	9.34	6.82	2.52
IV	13.00	12.87	0.13

Table 3. Percentage of mefenamic acid (I) and (II) formed upon degradation of (III) and (IV) in human plasma 37°C

Compound	%Mefenamic acid formed	%Intermediate formed (II)
III	73	27
IV	99	1

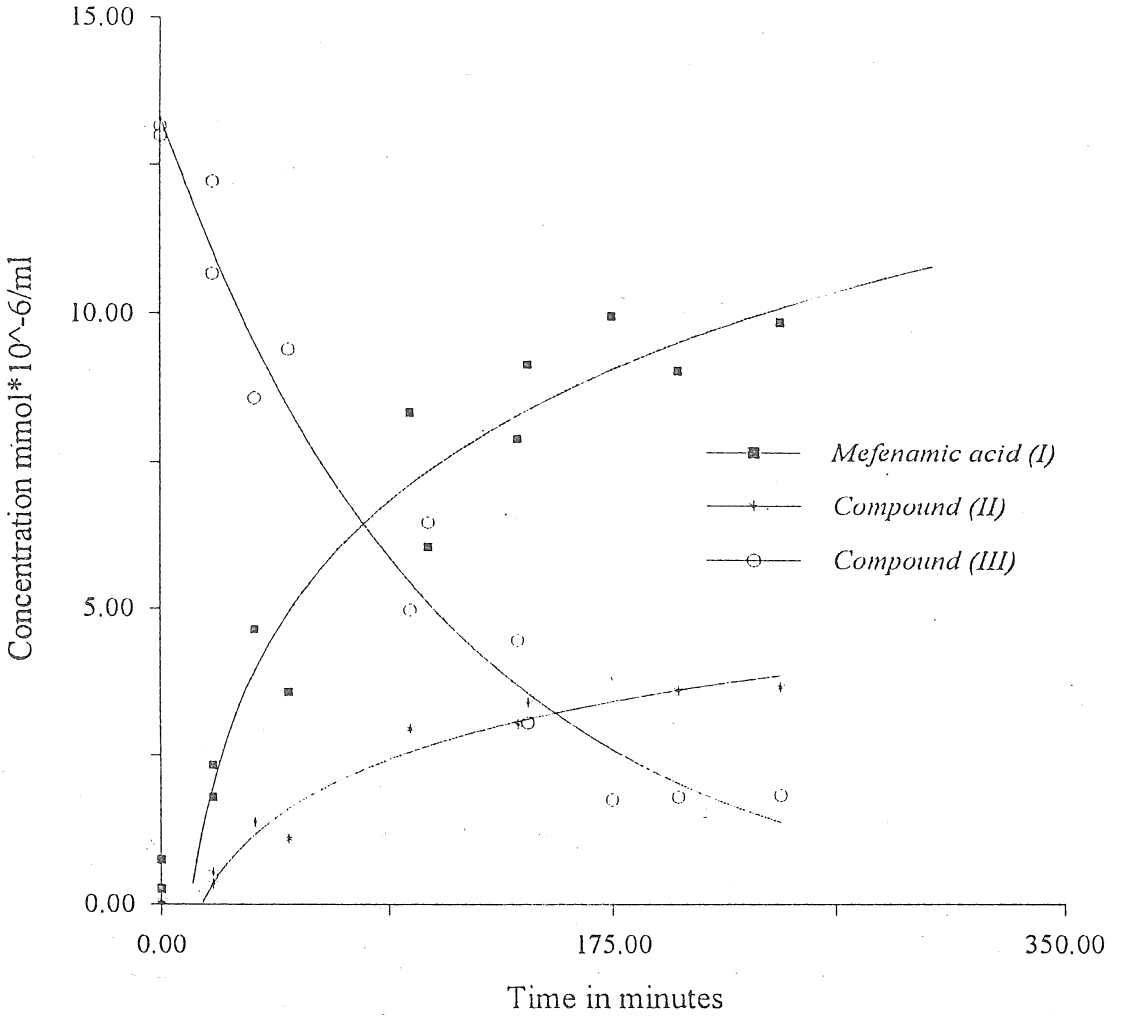


Fig.5. Time courses for compounds (III), (I) and (II) during the degradation of compound (III) in human plasma at 37°C.

at the terminal aliphatic carbonyl which is more susceptible than the anthranilic carbonyl; it is also more susceptible than the amide carbonyl. This assumption can be validated by inspecting the hydrolysis pattern of the compounds (III) and (IV) in non-enzymatic media i.e. pH 7.4. Unfortunately, compounds (III) and (IV), although degraded under such conditions at different rates (Table 1), we could not detect the formation of either mefenamic acid (1) nor the intermediate (II) to quantify the rates of their productions. This observation suggests different, unexpected way of degradation of (III) and (IV) in non-enzymatic media.

In vivo study

Based on the in vitro results, compound (IV), was chosen for the in vivo evaluation. In this study, mefenamic acid (I) and (IV) were given to the same rabbit in an equimolar doses to study the possible in vivo conversion of the prodrug (IV) to the parent compound. The plasma concentration-time profiles are illustrated in Fig.7. A very rapid appearance of mefenamic acid (I) in the blood after prodrug administration indicated rapid absorption and hydrolysis of (IV) in vivo to mefenamic acid (Fig.7). The overall time-courses of the mefenamic acid resulted from the hydrolysis of (IV) and control mefenamic acid were similar in nature, providing comparable

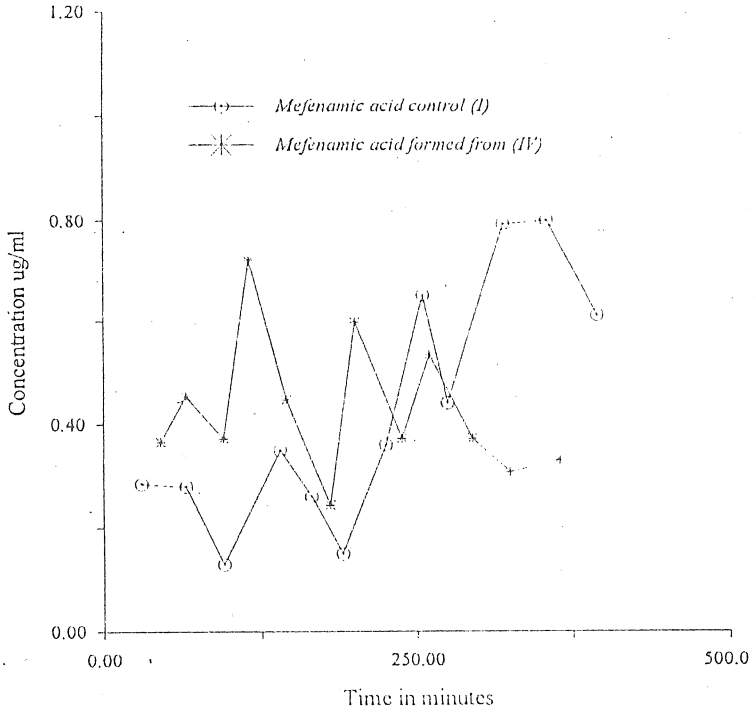


Fig.6. Time courses for compounds (IV) and (I) during the degradation of compound (IV) in human plasma at 37°C

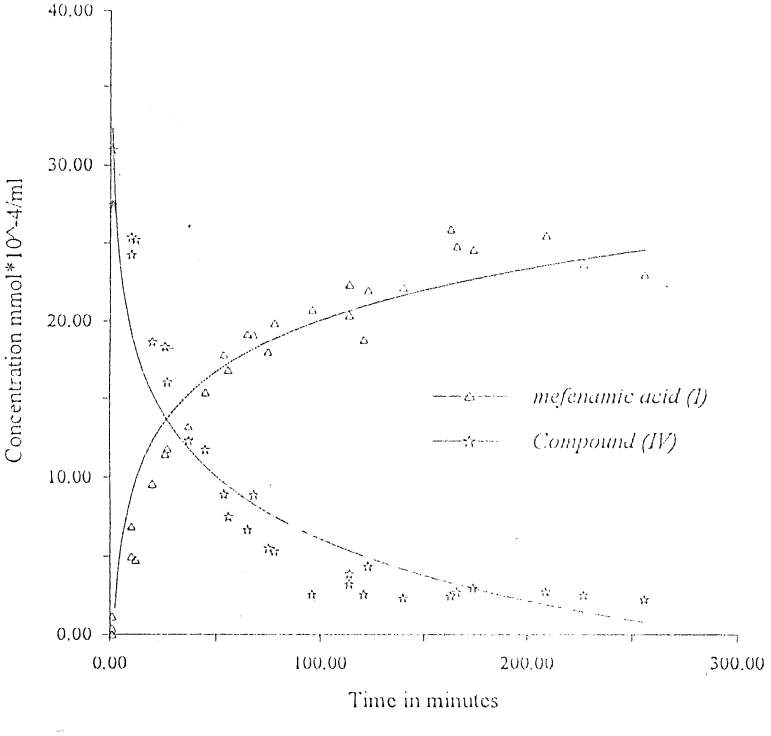


Fig.7. Plasma concentrations of mefenamic acid (I) in rabbit following peroral administration of (I) and (IV)

mefenamic acid plasma levels. However, the number of rabbits used in this study is not sufficient to conclude a very dependable conclusions or statistically significant values. The study should be expanded to a larger number of animals and to include the GIT toxicity in addition to evaluating the compound (IV) after topical application.

Conclusion

The major objective of the present work was to study the kinetics of hydrolysis of some derivatives of mefenamic acid with different physico-chemical properties to evaluate them as prodrugs. Glycolamide (IV) which is an ester-amide showed promising results after oral administration. One can conclude that glycolamide may represent a useful promoiteies for developing prodrugs for drugs containing anthranilic acid moiety. The desired in vitro stability of such compounds is due to the low nucleophilic susceptibility of the two carbonyls. Also the glycolamide moiety allows modifications in physico-chemical properties by modifying the N-substituents.

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