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PREFORMULATION PRODRUG RESEARCH - CHEMICAL AND ENZYMATIC HYDROLYSIS KINETICS OF THE GLYCEROL, GLYCOLIC ACID AND MORPHOLINO ETHYL ESTER DERIVATIVES OF A DEVELOPMENTAL ANALGESIC AGENT (RS-82917)

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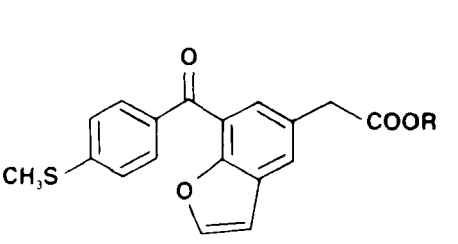
ABSTRACT

The chemical and enzymatic hydrolysis of the glycerol-(2), glycolic acid-(3), and morpholino ethyl-(4) ester derivatives of 7-(4-methylthiobenzoyl) benzofuran-5-yl-acetic acid (RS-82917, 1) were evaluated for potential application as prodrugs. In aqueous solution with pH from 1-10, all three ester derivatives hydrolyzed quantitatively to 1. Rate expressions were derived from the log(rate)-pH profiles for each ester, and pH-dependent intramolecular catalysis was proposed to account for the hydrolysis kinetics of all three esters. None of the ester aqueous solutions can provide two-year shelf-life at 25°C. The rate of hydrolysis was accelerated significantly by human and mouse plasma enzymes for 2 and 4 and by mouse liver homogenate for 2, 3 and 4.

INTRODUCTION

Esterification of acidic non-steroidal anti-inflammatory (NSAI) agents has been proved to be an effective way of reducing the GI irritation commonly encountered for these agents.[1-4] With proper design, the ester prodrugs can often have comparable bio-activity to that of the parent drug, thus providing a better therapeutic ratio. Many ester prodrugs of NSAI agents are

commercially available or are under various phases of clinical trials.[1] We are interested in the general application of the promoieties used in these successful examples to the new drugs under development. This paper evaluates the chemical and enzymatic hydrolysis kinetics of the glycerol (2), glycolic acid (3), and morpholino ethyl esters (4) for an investigational analgesic agent, 7-(4-methylthiobenzoyl)-benzofuran-5-yl-acetic acid (RS-82917, 1).[5,6]



R =

1. -H
2. $-CH_2CH(OH)CH_2OH$
3. $-CH_2COOH$
4. $-CH_2-N$ O $\cdot$ ½ H_2SO_4

EXPERIMENTAL

Materials

RS-82917 (1) and the acid chloride of 1 were prepared according to the method previously described.[7] Nanopure® water and high-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were used for the preparation of mobile phase. All other chemicals were regent grade and were used as received.

Preparation of 1-Glycerol Ester of RS-82917 (2)

The acid chloride of 1 (4.0 g, 0-01 M) was dissolved in 50 mL dichloromethane and the solution was added to a stirring mixture of 4.0 g (0.03M) solketal, 1.2 g (0.01 M) triethylamine and 50 mL CH_2Cl_2. After 16 hours the solution was extracted with water (2 x 100 mL) and the organic phase was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residual oil was dissolved in toluene and percolated over 60 g silica gel. Purified 1,3-dioxolan-4-yl methyl ester of 1 was isolated by evaporation of solvent under reduced pressure and recrystallization from cyclohexane. M.P. 88-90°C.

A mixture of 10.6 (0.024 M) of the 1,3-dioxolan-4-yl methyl ester of 1 and 100 mL 70% acetic acid/water was heated to 60°C for 1 hour. The reaction mixture was cooled to ambient temperature and diluted with 200 mL water. Precipitated solid was collected, washed with water, partially air dried, and dissolved in 50 mL dichloromethane. The solution was dried with anhydrous sodium sulfate and the solvent was removed by evaporation under reduced pressure. The residual oil slowly crystallized and the crude solid was recrystallized from ethyl acetate. M.P. 92-94°C; ^{1}HNMR consistent with structure. Anal. Calcd. for $C_{21}H_{20}O_6S$: C, 62.98; H, 5.03 Found: C, 62.60; H, 4.91.

Preparation of Glycolic Acid Ester of RS-82917 (3)

To a solution of glycolic acid (2.3 g, 0.03 M) and triethylamine (8.5 mL, 0.061M) in 50 mL THF at ice bath temperature was added 5.58 g of the acid chloride of 1 in 75 mL THF over a period of 30 min. The mixture was warmed to room temperature (RT) after the addition, stirred for additional 16 hours, added to H_2O (2 x 100 mL) and acidified with 10% HCl. The mixture was then extracted with EtOAC, dried with $MgSO_4$ and evaporated to yield 6.2 g of crude product. The crude product was chromatographed on a silica gel column (EtOAc: hexane: HOAc (40/60/2)) followed by recrystallization from acetone/hexane to yield 3.7 g of compound 2. M.P. 142-144 °C; ^{1}HNMR consistent with structure. Anal. Calcd. for $C_{19}H_{16}O_6S$: C, 62.49; H, 4.20. Found: C, 62.53; H, 4.25.

Preparation of Morpholinoethyl Ester Sulfate (4)

Acid chloride of 1 (11.2 g, 0.031 M) was dissolved in 120 mL of CH_2Cl_2 and the solution was added dropwise over a period of 30 min to an ice bath cooled solution of 4-morpholinoethanol (8.75 g, 0.067 moles) in 150 mL of CH_2Cl_2. The mixture was warmed to RT after the addition and stirred for an additional 16 hours. The reaction solution was then added to 250 mL of 10% HCl and extracted with CH_2Cl_2. The organic layer was washed with H_2O, 10% Na_2CO_3 aqueous solution and dried over Na_2SO_4 and evaporated to yield

13.5 g of the crude product. The crude product was chromatographed on a silica gel column in acetone: hexane (50/50) to yield 11.5 g of morpholino ethyl ester of 1.

To the solution of 2-morpholinoethyl ester of 1 (4.0 g, 0.091 M) in 80 mL CH_2Cl_2/2-propanol (50/50) was added slowly H_2SO_4 (1.0 g, 0.010 M) in 16 mL 2-propanol solution. The CH_2Cl_2 in the mixture was then removed to yield 5.0 g of the crude product. Recrystallization from methanol yield 4.0 g of compound 4. m.p. 104-106°C; ^{1}HHMR consistent with structure. Anal. Calcd. for $C_{48}H_{52}N_2O_{14}S_2$; C, 59.0; H, 5.36; N, 2.87; Found: C, 55.6; H, 5.59; N, 2.96.

HPLC Methods

HPLC was performed using a Spectra-Physics Model 8700 pump, Kratos 757 variable wavelength UV detector, Micromiretics 728 autosampler and a Spectra-Physics 4100 computing integrator. The detection wavelength was 320nm and at a sensitivity of 0.05 AUFS. A C18 (Whatman Partisil 5 μ) column was used and the flow rate was controlled at 1.0 mL/min. Mobile phase A which contained MeOH/H_2O/HOAc (70/30/1) was used for the analysis of compounds 2 and 3 in the presence of 1. Mobile phase B which contained MeOH/H_2O (0.01 M phosphate buffer; 0.01 M heptane sulfonic acid)/THF (47.5/40/12.5) was used for the analysis of compound 4 in the presence of 1.

Kinetics in Aqueous Solution

Buffers were prepared at 0.020 M total concentration using acetate, phosphate, or carbonate. NaCl was added to adjust the ionic strength to 0.50. The pH of each buffer solution was measured at the reaction temperature. For the very acidic and basic solutions, aqueous HCl and KOH solutions were used to obtain the desired pH. Typically, 1.0 mL of a 1.0 mg/mL stock methanol solution of the derivative was added to 100 mL of a freshly prepared buffer solution. Aliquots (5 mL) of this solution were removed and placed in 5 mL clear glass ampules. The ampules were sealed and stored in a constant temperature water bath at the desired temperature. At predetermined time periods, the samples were removed and assayed by HPLC.

Kinetics in Biological Media

A solution containing 5 μg/mL of the derivatives was prepared in 0.01 M pH 7.4 phosphate buffer (μ = 0.5) containing 80% (v/v) mouse plasma, 10% mouse liver homogenate or 80% human plasma. At the appropriate time points, aliquots were withdrawn and added to an equal volume of CH_3CN. The solutions were mixed on a Votex mixture for 30 sec and centrifuged for 3 min. The supernatant was diluted with mobile phase (1:1) and analyzed by HPLC.

RESULTS AND DISCUSSION

Hydrolysis in Aqueous Solution

The hydrolysis of compounds 2 - 4 in aqueous solution was studied at 25°C and 37°C from pH 1 to 10. The extent of the reaction was followed by reverse phase HPLC. All reactions gave strictly first order kinetics and parent drug 1 was the quantitative product in each case. The effect of buffer concentrations on the rate of hydrolysis for compounds 2 - 4 was examined at pH 7.4 using phosphate buffer (0.02-0.10 M) and was found to be negligible.

The dependences of the hydrolysis kinetics on the solution pH for the glycerol ester 2 and glycolic acid ester 3 are shown in Figures 1 and 2 respectively. The shapes of the log(rate)-pH profiles of these two esters are similar and indicate the occurrence of specific acid-catalyzed (k_H), neutral or water catalyzed (k_o), and specific base-catalyzed (k_{OH}) processes according to the following rate expression:

$$k_{obs} = k_H a_H + k_o + k_{OH} a_{OH} \quad (1)$$

where a_H and a_{OH} are the hydrogen ion and hydroxide ion activity at the reaction temperature. The best fit rate constants derived for compounds 2 and 3 using a non-linear curve fitting method and equation (1) are summarized in Table 1. The solid curves in Figures 1 and 2 were constructed from these derived rate constants.

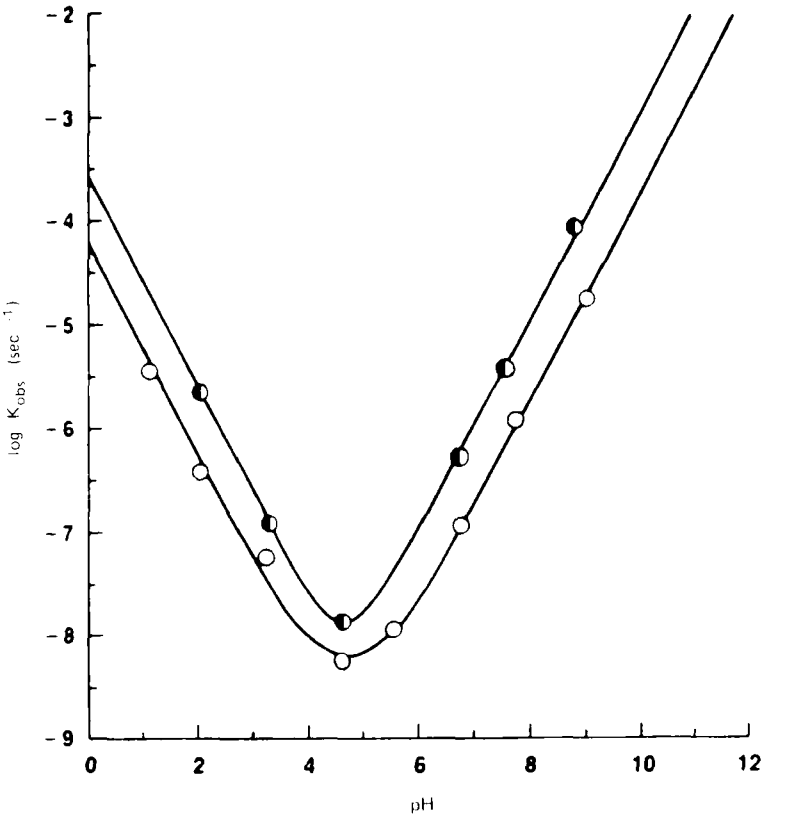


Figure 1. Log(rate)-pH profile for the hydrolysis of compound 2 in H_2O at 25°C (○) and 37°C (◐). The lines for k_{obs} are constructed from the best fit values of k_H, k_o and k_{OH} obtained by non-linear regression analysis using equation 1.

The acid-catalyzed (k_H) and base-catalyzed (k_{OH}) rate constants for 2 are each about 4 fold greater than that of 3. The higher k_{OH} of 2 can be attributed to the electronic effect, i.e. the neutral glycerol (pK_a = 14.4) (68) is expected to be less basic than the glycolate anion and thus is a better leaving group. To explain the higher k_H of 2, a neighboring electronic stabilization of the protonated 2 (2a) provided by the hydroxyl group is suggested (Scheme 1). Similar stabilization, however, is unavailable for compound 3 which exists mainly in the free acid form at the pH range where the acid-catalyzed process dominates.

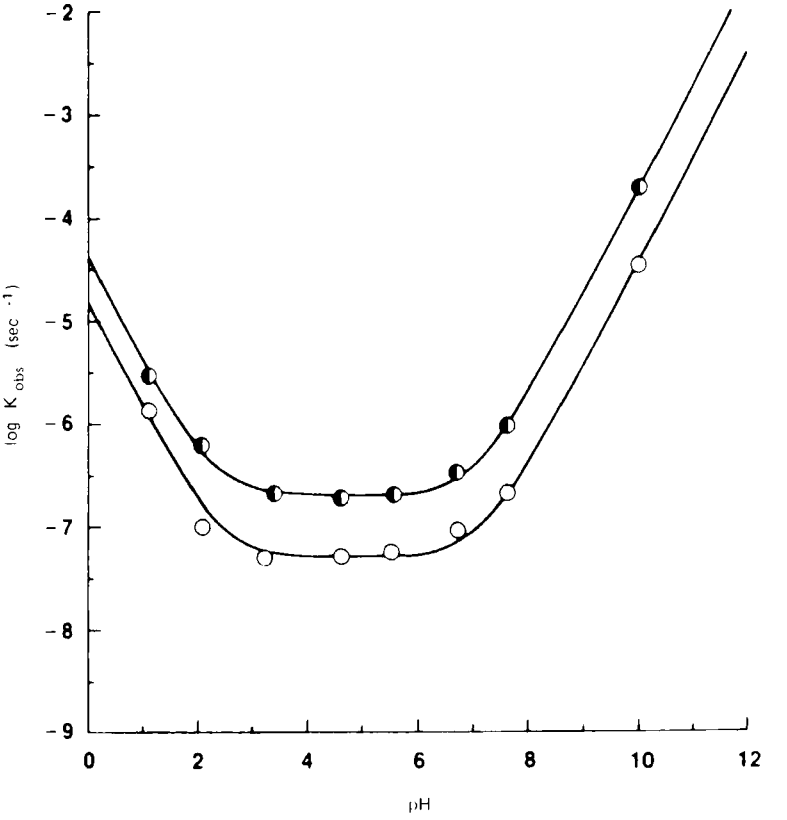
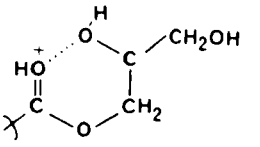


Figure 2. Log(rate)-pH profile for the hydrolysis of compound 3 in H_2O at 25°C (○) and 37°C (◐). The lines for k_{obs} are constructed from the best fit values of k_H, k_o and k_{OH} obtained by non-linear regression analysis using equation 1.

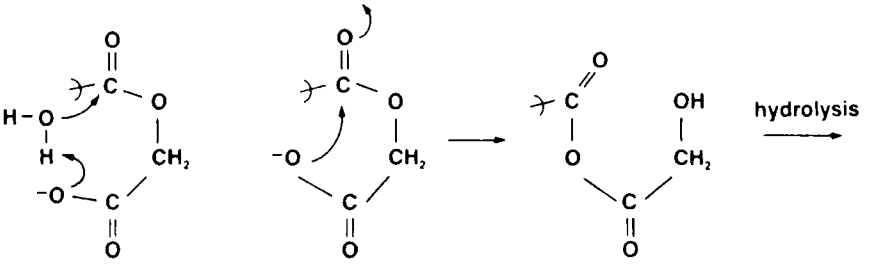
Table 1 Kinetic Analysis of Hydrolysis of Various Ester Derivatives of 1 in Aqueous Solution

Compound	Temp °C	k_H $x10^5, M^{-1}s^{-1}$	k_o $x10^7, s^{-1}$	k'_o $x10^5, s^{-1}$	k_{OH} M^{-1}, s^{-1}	pK_a
2	25	5.5	0.043	--	1.8	--
	37	25	[a]	--	4.5	--
3	25	1.4	0.50	--	0.38	--
	37	3.9	2.0	--	0.78	--
4	25	1.2	1.33.0	1.0		6.34
	37	4.8	4.0	7.6	2.6	6.24

[a]Negligible



Scheme 1



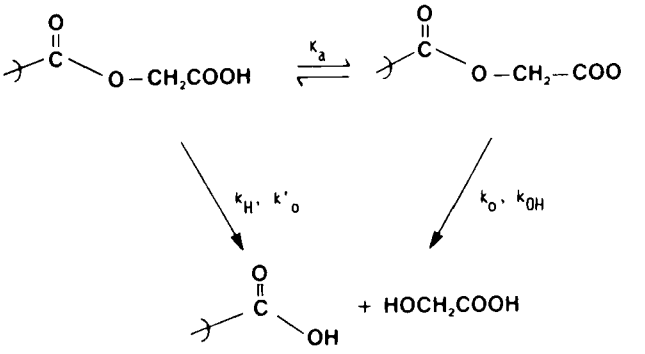
a b

Scheme 2 Suggested Intramolecular Catalysis Pathways for the Hydrolysis of 3

The k_o value of 3, on the other hand, is ~ 12 fold greater than that of 2 at 25°C. Two possible mechanisms (Scheme 2) may be suggested: (a) an intramolecular general base catalysis by the carboxylate anion on water attack of the ester bond, and (b) a kinetically equivalent neucleophilic catalysis on the ester bond followed by rapid hydrolysis of the anhydride intermediate.

Both types of mechanisms have been found in the hydrolysis of O-acylalicylic acids.[9] General base catalysis probably is more likely for 3 considering the relatively small catalysis (e.g. ≃ 12X) observed (8). The lack of a sigmoid curvature at pH near pK_a (= 3.15[9]) in the log(rate)-pH profile (Figure 2) may result from an accidental compensation between k_o and k'_o (Scheme 3).

Attempts trying to fit the k_{obs} data of compound 3 to Scheme 3 resulted in large uncertainties on both the k_o and k'_o values due to the lack of



Scheme 3 Suggested Kinetic Pathways for the Hydrolysis of 3

sufficient data points near pK_a. These values are therefore not reported here.

The dependence of the hydrolysis kinetics on the solution pH for the morpholino ethylester 4 is shown in Figure 3.

Although other kinetically competent systems are not ruled out, the profiles in Figure 3 can be described by Scheme 4 and fit to the following formula:

$$k_{obs} = (k_H a_H + k_o)\left(\frac{a_H}{a_H + K_a}\right) + (k'_o + k_{OH} a_{OH})\left(\frac{K_a}{K_a + a_H}\right) \qquad (2)$$

where $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of protonated and unionized 4 respectively.

The values of various rate constants and dissociation constants derived using non-linear curve fitting method and equation (2) are summarized in Table 1. The kinetic pK_a of 4 at 25°C was 6.34, in good agreement with that obtained from the solubility method(pK_a = 6.4)[10]. The solid curves drawn in Figure 3 were constructed from kinetically obtained rate constants and pKas. That the value of k'_o ~200 fold greater than k_o suggests that

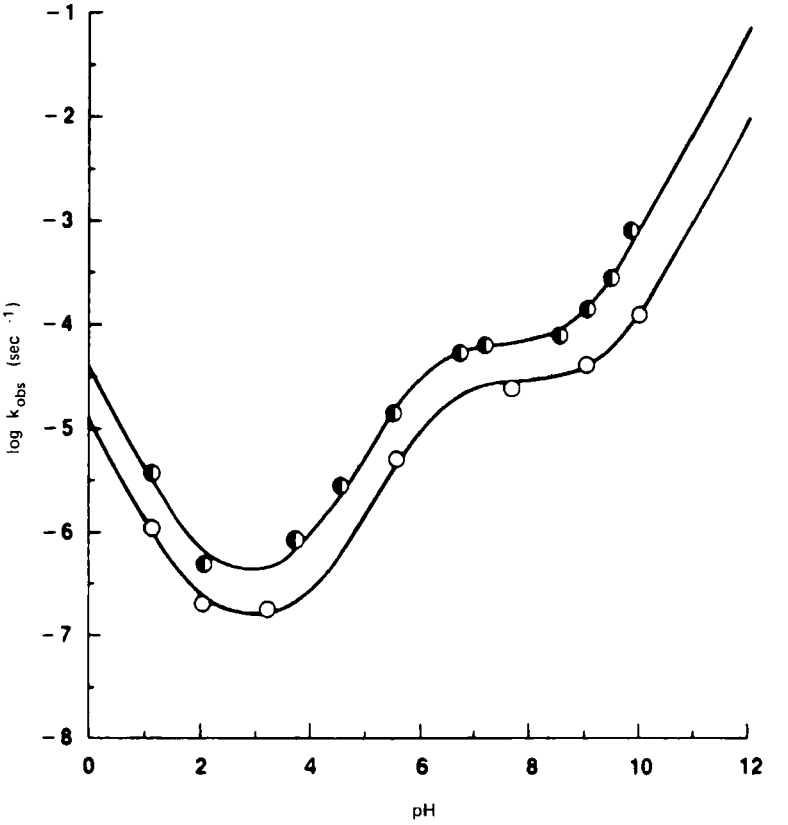
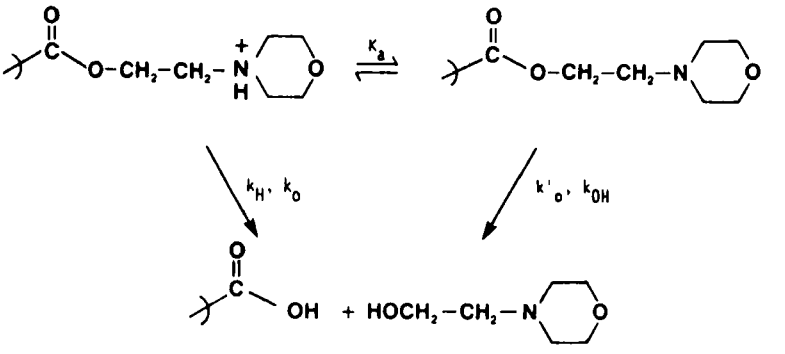
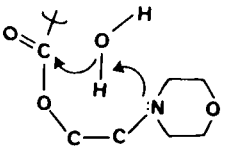


Figure 3. Log(rate)-pH profile for the hydrolysis of compound 4 in H_2O at 25°C (O) and 37°C (◐). The lines for k_{obs} are constructed from the best fit values of k_H, k_O and k_{OH} obtained by non-linear regression analysis using equation 2.



Scheme 4 Suggested Kinetic Pathways for the Hydrolysis of 4



Scheme 5

Table 2 Half-lives of Hydrolysis of Various Ester Derivatives of 1 in 0.10 N HCl and in 0.05M pH 7.4 Phosphate Buffer, Mouse Plasma (80%), Mouse Liver Homogenate (13%), or Human Plasma (80%) at 37°C.

	Hydrolysis Half-Life				
Substrate	pH 1[a]	pH 7.4[a]	80% Mouse Plasma[b]	13% Mouse Live Homogenate[b]	80% Human Plasma[b]
2	7.1 h	81h	2.6 min.	< 0.5 min.	9.0 min.
3	43 h	408h	10.2 h	0.88 min.	65 h
4	39 h	3.6 h	1.4 min.	6.1 min.	4.8 min.

[a]Extropolated from the log (rate)-pH profile for each compound; see Figures 1-3. [b]In 0.05 M pH 7.4 phosphate buffer.

intramolecular general base catalysis from the amino group onto the water molecule is occurring[12], as shown in Scheme 5.

Enzymatic Hydrolysis

The hydrolysis of compounds 2-4 was also investigated at pH 7.4 and 37°C in phosphate buffer/mouse plasma (20/80), phosphate buffer/mouse liver homogenate (90/10) or phosphate buffer/human plasma (20/80). In all cases 1 was found to be the quantitative product by HPLC and Table 2 summarizes the half-lives obtained. For comparison, the non-catalyzed chemical degradation rates of 2-4 in phosphate buffer at 37°C are also included in Table 2.

Human plasma enzymes accelerate the hydrolysis of esters 2, 3 and 4, by factors of 540, 6.3 and 45 respectively and mouse plasma was 3-6 fold more effective than the human plasma. The unfavorable electric repulsion between the negatively charged 3 and esterase[13,14] at physiological pH of 7.4 may

have caused the smaller catalytic effect observed for the hydrolysis of 3 (Table 2).

Finally, liver enzymes appeared to be less selective as all three esters hydrolyzed to the parent drug 1 rapidly (Table 2).

CONCLUSIONS

The promoieties in compounds 2-4 have been used in several prodrug applications for the purpose of reducing GI toxicities of the parent drugs. Therefore, compounds 2-4 and their cleaved promoieties are not expected to introduce any unexpected GI toxicities. Chemically, all three esters were reasonably stable at pH 1-7.4 and 37°C (Table 2) suggesting that they could potentially be absorbed as the prodrug form. The glycerol ester 2 and morpholino ethyl ester 4 were also found to hydrolyze rapidly (t 1/2 < 10 min) and quantitatively to the parent drug (1) in plasma and in liver homogenate. Although the glycolic acid ester 3 has a t 1/2 of < 1 min in liver homogenate, its slow conversion in plasma may prevent its potential use for a prodrug especially if a rapid and/or complete plasma level of the parent drug is desired for therapeutic reasons. The aqueous shelf-lives (t_{90}) of all three ester derivatives at 25°C are less than two years ($k_{obs} > 9 \times 10^{-10}$ sec^{-1}) at all pHs studied (Figures 2-4). Lyophilization may prove to be necessary if an injectable formulation is to be developed for these ester prodrugs.

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