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Acyloxymethyl acidic drug derivatives: in vitro hydrolytic reactivity

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Abstract

A series of acyloxymethyl drug derivatives of the NH-acidic drugs, phenytoin and theophylline and of the carboxylic acid drugs, thioctic acid and indomethacin, were prepared in order to determine the effect of varying the nature of the drug on the in vitro rate of hydrolysis catalyzed by porcine liver esterase and human plasma. The acyl portion was comprised of either valeric acid (val) or γ-linolenic acid (GLA). With the exception of some GLA prodrugs, the derivatives displayed first-order kinetics in both enzyme systems. The NH-acidic drug derivatives were hydrolyzed faster than the carboxylic drug derivatives by porcine liver esterase and human plasma. It was found that the short chain valeric acid derivatives were hydrolyzed faster than the GLA derivatives. The rates of hydrolysis for the relatively smaller prodrugs of theophylline and thioctic acid were greater than the rates of hydrolysis for the bulkier phenytoin and indomethacin prodrugs indicating steric hindrance was important. The lipophilicity index, log *K*, of the valeric acid drug derivatives was plotted against the logarithm of the hydrolysis rate constant, *k*, and it was observed that log *k* decreased with an increase in log *K*. A comparison of these results with those of previous studies where the alkyl and acyl moieties were varied of acyloxyalkyl theophylline derivatives has provided a rationale, based on lipophilicity, for the structure of a prodrug to be designed based on an in vitro desired rate of hydrolysis.

Keywords: Prodrugs; Fatty acids; Acyloxymethyl derivatives; Enzymatic hydrolysis; Porcine liver esterase; Human plasma

1. Introduction

The rationale of preparing lipophilic prodrugs of conventional drugs with the aim of providing better penetration into cells or across the skin, improved absorption from the gut or better trans-

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port across the blood–brain barrier is well established (Stella and Pocchopin, 1992).

Our interest in this area arose based on the idea of using polyunsaturated fatty acids (PUFA) as the lipophilic promoiety since general health improvements have been associated with certain PUFAs (Horrobin, 1992, 1996). Moreover, for saturated fatty acids a number of studies have been conducted looking at the improvement in oral bioavailability of drugs by making long chain saturated fatty acid ester derivatives. For example, palmitate and stearate esters of testosterone increased the duration and bioavailability of testosterone for several weeks (Stella and Pocchopin, 1992; Charman and Porter, 1996).

We have previously reported the in vitro hydrolysis using porcine liver esterase and human plasma of a variety of *N*-acyloxyalkyl derivatives of theophylline (Burke et al., 1997; Redden et al., 1998). We chose the *N*-acyloxyalkyl approach as a means of evaluating the fatty acid prodrug derivatives because of the increased flexibility in that both the alkyl (R′) and acyl (R″) portion could be varied systematically and whereby a single esterase hydrolysis step results in the eventual release of the parent drug as shown in Scheme 1. In this way the rate of hydrolysis can be specifically designed to suit an oral, iv or percutaneous application.

By utilizing these acyloxyalkyl derivatives we first demonstrated that as the steric hindrance of the alkyl group linking the drug theophylline and the unsaturated fatty acid, γ-linolenic acid (GLA) increased, the rate of ester hydrolysis decreased (Burke et al., 1997). Secondly, upon varying the acyl promoiety of *N*-acyloxymethyl derivatives of theophylline the classical parabolic relationship resulted when the logarithm of the hydrolysis rate constant was plotted versus the lipophilicity index of the derivative (Redden et al., 1998).

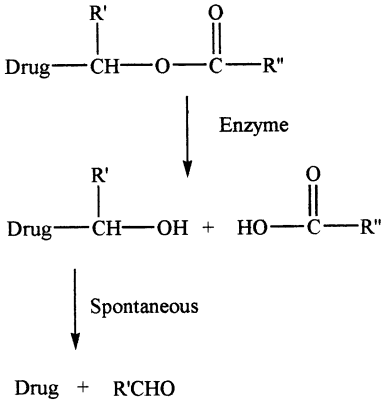
Since both of these studies utilized the same drug, theophylline, the next logical step was to examine the rates of hydrolysis by varying the nature of the drug. The drugs investigated include the NH-acidic drugs phenytoin and theophylline as well as the carboxylic acid drugs thioctic acid and indomethacin. Within each functional class, the drugs were arbitrarily chosen to represent varying degrees of steric hindrance. All the prodrugs were prepared with methylene as the alkyl portion since this linking group yielded acyloxyalkyl prodrugs which were readily hydrolyzed (Burke et al., 1997). Two carboxylic acids were examined for each drug, namely, valeric acid (val) and GLA. The rationale for choosing these acyl groups was that previously the valeric acid acyloxymethyl derivatives of theophylline were hydrolyzed the fastest overall, and the GLA derivatives were shown to be the fastest hydrolyzed polyunsaturated fatty acid derivatives (Redden et al., 1998). However the aim of this study is to determine the effect of the acidic drug portion of these acyloxymethyl derivatives rather than the acid moiety, on in vitro rates of hydrolysis.

The hydrolysis of these prodrugs will be examined using human plasma and porcine liver esterase. Porcine liver esterase (pig liver esterase) is a good model of the esterases found in the skin and, as such, is important in predicting the use of certain prodrugs as topical treatments (Bonina et al., 1991).

2. Materials and methods

2.1. Apparatus

The HPLC system consisted of a Beckman System Gold™ (Beckman Instruments, Palo Alto,



Scheme 1.

CA) system equipped with a programmable solvent module 126, autosampler 507 and either a diode array detector module 168 or programmable detector module 166. The analyses were performed using either a Beckman ODS reverse phase column (5 μ, 25 cm × 4.6 mm) or a Zorbax ODS reversed phase column (5 μ, 25 cm × 4.6 mm). The HPLC mobile phase had a flow rate of 1 ml/min and ranged from 70 to 98% methanol–water (vol/vol) for all derivatives with the exception of Indo-m-GLA (95% acetonitrile/5% 2-propanol) and Theo-m-GLA (90% 0.5% acetic acid/10% acetonitrile). UV detection was at 333 nm for thioctic acid and its derivatives and at 254 nm for all other drugs and derivatives.

^{1}H NMR analyses was performed using a Bruker AC 250 MHz spectrometer using $CDCl_3$ as the solvent. Chemical shifts are given in δ (ppm) values measured downfield from the tetramethylsilane internal standard.

Accurate mass measurements were determined by high resolution liquid secondary ion mass spectrometry (LSIMS) in positive ion mode using a Micromass AutoSpec Oatof Mass Spectrometer with resolution 8000, voltage scanning and 6 s/scan. The reference compound was polyethylene glycol (PEG) 600 and the matrix was 1:1 glycerol:*meta*-nitrobenzyl alcohol plus 0.1% trifluoroacetic acid.

2.2. Chemicals

Phenytoin, dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (4-DMAP), valeric acid, indomethacin, pyridine, thioctic acid, trizma base, porcine esterase, pancreatin and bile salts were purchased from Sigma–Aldrich (St. Louis, MO). All solvents used were of HPLC grade or better and supplied by either Fisher Scientific (Ottawa, ON, Canada) or BDH (Toronto, ON, Canada).

2.3. Synthesis

3-Hydroxy-5, 5-diphenylhydantoin and 3-valeroyloxymethyl-3-hydroxy-5, 5-diphenylhydantoin, Phy-m-val, **1**, were prepared as described previously (Yamaoka et al., 1983).

2.3.1. Preparation of 3-γ-linolenoyloxymethyl-3-hydroxy-5, 5-diphenylhydantoin, Phy-m-GLA, **2**

In a 50 ml round bottom flask equipped with a stirring bar were placed 3-hydroxy-5, 5-diphenylhydantoin (502 mg, 1.8 mmol), GLA (543 mg, 1.9 mmol) and 4-DMAP (214 mg, 1.8 mmol) in 2 ml chloroform ($CHCl_3$). In a separate vial, DCC (401 mg, 1.9 mmol) was added to 1 ml $CHCl_3$which was then added dropwise to the reaction mixture. The reaction was left overnight at room temperature with stirring and was monitored for completion by TLC in 50% diethyl ether (Et_2O)/hexane. The solvent was evaporated and the product purified by flash column chromatography using 250 ml of solvent starting with 5% Et_2O/hexane, increasing by 5% for each additional 250 ml of solvent, to yield Phy-m-GLA, **2**, as a clear oil, $R_f = 0.40$, 25% ethyl acetate/hexane. 0.73 g (76%). Analysis: ^{1}H NMR ($CDCl_3$) δ: 0.88-0.91 (3H, t, $CH_2C\underline{H}_3$), 1.25–1.42 (8H, m, $C\underline{H}_2$), 1.59–1.67 (2H, m, $CO_2CH_2C\underline{H}_2$), 2.04-2.09 (4H, q, $CH_2C\underline{H}_2CH$=), 2.32-2.36 (2H, t, $CO_2C\underline{H}_2$), 2.79-2.83 (4H, m, =$CHC\underline{H}_2CH$=), 5.32–5.44 (6H, m, $CH_2C\underline{H}$=$C\underline{H}$), 5.61 (2H, s, $NC\underline{H}_2CO_2$), 6.42 (1H, s, N$\underline{H}$), 7.36–7.41 (10H, m, aromatic H). ^{13}C NMR: 14.3, 22.8, 24.5, 25.8 (2), 27.0, 27.4, 29.2, 29.5, 31.7, 34.0, 62.0, 70.0, 127.0 (4), 127.8, 128.3, 128.5, 128.7, 129.1 (2), 129.2 (4), 129.7, 130.7, 138.8 (2), 154.6, 172 (2). HR-LSIMS MH^+ 543.7277 ± 0.0004, $C_{34}H_{43}O_4N_2$ requires 543.7281.

1-(Theophyllin-7-yl)methyl valerate, Theo-m-val, **3**, and 1-(Theophyllin-7-yl)methyl γ-linolenate, Theo-m-GLA, **4,** were prepared as described previously (Redden et al., 1998).

2.3.2. Preparation of valeroyloxymethyl-6, 8-thioctate, Thio-m-val, **5**

In a 4 ml conical vial equipped with stirring bar were added thioctic acid (502 mg, 2.4 mmol), α-chloromethyl valerate (Bodor et al., 1980; 372 mg, 2.5 mmol), triethylamine (TEA; 336 μl, 2.42 mmol) and pyridine (969 μl). The reaction mixture was heated at 80°C overnight and monitored for completion by TLC using 25% ethyl acetate (EtOAc)/hexane. The mixture was evaporated and the product purified by flash column chromatography using 750 ml of 5% EtOAc/hexane, to yield

Thio-m-val, **5**, as a yellow oil, $R_f = 0.57$, 25% ethyl acetate/hexane. 0.29g (37%). Analysis: ^{1}H NMR ($CDCl_3$) δ: 0.98–0.93 (3H, t, $C\underline{H}_3$), 1.30–1.39 (2H, m, $C\underline{H}_2CH_3$), 1.41–1.54 (2H, m, $C\underline{H}_2$), 1.58–1.72 (6H, m, $C\underline{H}_2$), 1.86–1.94 (1H, m, ring H), 2.34–2.37 (2H, t, $CO_2C\underline{H}_2$), 2.35–2.39 (2H, t, $CO_2C\underline{H}_2$), 2.42–2.50 (1H, m, ring H), 3.07–3.21 (2H, m, $SC\underline{H}_2$), 3.52–3.59 (1H, m, $SC\underline{H}$), 5.74 (2H, s, $CO_2C\underline{H}_2CO_2$). ^{13}C NMR: 13.9, 22.3, 24.5, 26.8, 28.8, 33.88, 33.93, 34.8, 38.7, 40.4, 56.5, 79.3, 172.3, 172.7. HR-LSIMS MH^+ 321.4831 ± 0.0007, $C_{14}H_{25}O_4S_2$ requires 321.4837.

2.3.3. Preparation of γ-linolenoyloxymethyl-6, 8-thioctate, Thio-m-GLA, **6**

In a 4-ml conical vial equipped with stirring bar were added thioctic acid (515 mg, 2.5 mmol), α-chloromethyl γ-linolenate (Burke et al., 1997; 791 mg, 2.42 mmol), TEA (336 μl, 2.42 mmol) and pyridine (969 μl). The reaction mixture was heated at 80°C overnight and monitored by TLC using 25% EtOAc/hexane. The mixture was evaporated and the product purified by flash column chromatography using 500 ml of 5% EtOAc/hexane, to yield Thio-m-GLA, **6**, as a yellow oil, $R_f = 0.70$, 25% ethyl acetate/hexane. 0.39g (32%). Analysis: ^{1}H NMR ($CDCl_3$) δ: 0.88–0.91 (3H, t, $CH_2C\underline{H}_3$), 1.24–1.52 (12H, m, $C\underline{H}_2$), 1.62–1.74 (4H, m, $C\underline{H}_2CH_2CO_2$), 1.87–1.95 (1H, m, ring H), 2.03-2.12 (4H, m, $CH_2C\underline{H}_2CH$=), 2.36-2.40 (4H, d of t, $CO_2C\underline{H}_2$), 2.43–2.50 (1H, m, ring H), 2.79-2.82 (4H, m, =$CHC\underline{H}_2CH$=), 3.09–3.21 (2H, m, $SC\underline{H}_2$), 3.53–3.60 (1H, m, $SC\underline{H}$), 5.31–5.44 (6H, m, $CH_2C\underline{H}$=$C\underline{H}$), 5.75 (2H, s, $CO_2C\underline{H}_2CO_2$). ^{13}C NMR: 14.3, 22.8, 24.5, 25.8, 27.0, 27.4, 28.8, 29.2, 29.5, 31.7, 33.9, 34.0, 34.8, 38.7, 40.4, 56.5, 79.3, 127.8, 128.2, 128.6, 128.7, 129.6, 130.7, 172.3, 172.5. HR-LSIMS MH^+ 497.7865 ± 0.0005, $C_{27}H_{45}O_4S_2$ requires 497.7860.

2.3.4. Preparation of valeroyloxymethyl-indomethacin, Indo-m-val, **7**

In a 4-ml conical vial equipped with stirring bar were added indomethacin (415 mg, 1.5 mmol), α-chloromethyl valerate (215 mg, 1.40 mmol), TEA (194 μl, 1.40 mmol), and pyridine (559 μl). The reaction mixture was heated at 80°C overnight and monitored by TLC using 25% EtOAc/hexane. The mixture was evaporated and the product purified by flash column chromatography using 500 ml each of 5 and 10% EtOAc/hexane to yield Indo-m-val, **7**, as white crystals, $R_f = 0.45$, 25% ethyl acetate/hexane. 0.34g (51%). Analysis: ^{1}H NMR ($CDCl_3$) δ: 0.87–0.91 (3H, t, $CH_2C\underline{H}_3$), 1.26–1.36 (2H, m, $C\underline{H}_2CH_3$), 1.52–1.60 (2H, m, $C\underline{H}_2CH_2CH_3$), 2.28–2.32 (2H, t, $COC\underline{H}_2CH_2$), 2.38 (3H, s, $CC\underline{H}_3$), 3.72 (2H, s, $CC\underline{H}_2CO_2$), 3.85 (3H, s, $OC\underline{H}_3$), 5.77 (2H, s, $CO_2C\underline{H}_2CO_2$), 6.67–6.70 (1H), 6.88–6.95 (2H), 7.47–7.49 (2H), 7.66–7.68 (2H). ^{13}C NMR: 13.6, 13.8, 22.4, 26.8, 30.3, 33.8, 55.9, 79.6, 101.4, 111.9, 112.0, 115.2, 129.4 (2), 130.6, 131.0, 131.4 (2), 134.0, 136.4, 139.6, 156.31, 168.5, 169.8, 172.6. HR-LSIMS MH^+ 472.9464 ± 0.0004, $C_{25}H_{27}O_6NCl$ requires 472.9467.

2.3.5. Preparation of γ-linolenoyloxymethyl-indomethacin, Indo-m-GLA, **8**

In a 4-ml conical vial equipped with stirring bar were added indomethacin (503 mg, 1.4 mmol), α-chloromethyl γ-linolenate (456 mg, 1.4 mmol), TEA (194 μl, 1.40 mmol) and pyridine (559 μl). The reaction mixture was heated at 80°C overnight and monitored by TLC using 25% EtOAc/hexane. The mixture was evaporated and the product purified by flash column chromatography using 100 ml each of 5, 7.5, 10, 12.5 and 15% EtOAc/hexane plus 1 ml TEA, to yield Indo-m-GLA, **8**, as a yellow oil, $R_f = 0.57$, 25% ethyl acetate/hexane. 0.74g (82%). Analysis: ^{1}H NMR ($CDCl_3$) δ: 0.87–0.91 (3H, t, $CH_2C\underline{H}_3$), 1.24–1.38 (8H, m, $C\underline{H}_2$), 1.58–1.62 (2H, m, $CO_2CH_2C\underline{H}_2$), 2.03–2.09 (4H, m, $CH_2C\underline{H}_2CH$=), 2.28–2.32 (2H, t, $COC\underline{H}_2CH_2$), 2.38 (3H, s, $CC\underline{H}_3$), 2.80–2.82 (4H, m, =$CHC\underline{H}_2CH$=), 3.71 (2H, s, $CC\underline{H}_2CO_2$), 3.84 (3H, s, $OC\underline{H}_3$), 5.34–5.39 (6H, m, $CH_2C\underline{H}$=$C\underline{H}$), 5.77 (2H, s, $CO_2C\underline{H}_2CO_2$), 6.66–6.69 (1H), 6.87–6.94 (2H), 7.47–7.49 (2H), 7.66–7.68 (2H). ^{13}C NMR: 13.5, 14.4, 21.2, 22.8, 24.3, 25.8, 27.0, 27.4, 29.1, 29.5, 30.3, 31.7, 34.0, 55.9, 79.7, 101.4, 111.8, 112.0, 115.2, 127.8, 128.2, 128.6, 128.7, 129.4 (2), 129.6, 130.6, 130.7, 131.0, 131.4 (2), 134.0, 136.4, 139.6, 156.3, 168.5, 169.7, 172.4. HR-LSIMS MH^+ 649.2495 ± 0.0005, $C_{38}H_{47}O_6NCl$ requires 649.2490.

2.4. Hydrolysis by porcine esterase

The derivatives were prepared as 0.1 M or 0.05 M solutions in acetonitrile and added to 0.025 M phosphate buffer pH = 7.4 with an ionic strength of 0.5 (KCl) equilibrated in a shaker water bath at 37°C. Porcine esterase (170 units/mg protein) was diluted with 0.025 M phosphate buffer and added to the buffer solution to give a final volume of 5 ml, 8×10^{-4} M prodrug concentration and 1.3 units of enzyme/ml. At appropriate time intervals, 100 μl aliquots were removed and added to 250 μl acetonitrile in order to quench the enzyme activity. The amount of derivative remaining was determined by reversed-phase HPLC as described above. Pseudo-first order rate constants and half-lives were determined from the slopes of the linear plots of the logarithm of residual derivative versus time.

2.5. Hydrolysis by human plasma

For the hydrolysis experiments in plasma, a total of 120 ml of human plasma from 30 volunteers was pooled, aliquoted into 10-ml vials and kept at −20°C until used. To 1.98 ml of 80% human plasma in phosphate buffer equilibrated in a 37°C shaker bath was added 16 μl of 0.1 M substrate solution, or 32 μl of 0.05 M substrate solution. At appropriate time intervals, 100 μl aliquots were removed and added to 250 μl acetonitrile in order to quench the enzyme activity. The samples were vortexed and centrifuged for 10 min at 10 000 rpm. The clear supernatant was removed and analyzed by HPLC as described above.

2.6. Determination of lipophilicity index

The lipophilicity indices of the prodrugs were determined by reversed phase HPLC as described above. The mobile phase was 85% methanol/water. The lipophilicity index, log K, for each prodrug was determined by Eq. (1) where t_r is the retention time of the prodrug and t_o is the solvent front retention time.

$$\log K = \log [(t_r - t_o)/(t_o)] \quad (1)$$

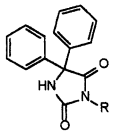
The lipophilicity index for each of the eight prodrugs were determined as follows: Phy-m-val, **1**, −0.484; Phy-m-GLA, **2,** 1.11; Theo-m-val, **3**, −0.831; Theo-m-GLA, **4**, 0.878; Thio-m-val, **5**, 0.0635; Thio-m-GLA, **6**, 1.55; Indo-m-val, **7**, 0.323; Indo-m-GLA, **8**, 1.75.

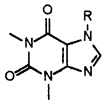
3. Results

3.1. Synthesis

The general structures of the acyloxymethyl drug derivatives examined in this study are shown in Fig. 1. The objective of this report was to determine the effect of varying the drug portion on the rate of hydrolysis catalyzed by porcine liver esterase and human plasma. The *N*-acyloxymethyl derivatives of phenytoin, **1**, **2** and theophylline, **3**, **4** were readily prepared by first making the respective hydroxymethyl derivatives and esterifying with the desired acid, either valeric acid or GLA, in the presence of DCC and 4-DMAP. For the preparation of the thioctic acid, **5**, **6**, and indomethacin, **7**, **8**, prodrugs, the α-chloromethyl ester of valeric acid or GLA was prepared by reacting the appropriate acid chloride with paraformaldehyde. The α-chloromethyl ester was then added to the drug in pyridine containing TEA to give the desired derivative. The derivatives which were observed to undergo hydrolysis displayed pseudo first-order kinetics indicating that the initial concentration was below the saturation level of the enzymes. The rate constants and corresponding half lives are given in Table 1.

The stability of all prodrugs studied were examined in the absence of the enzyme to determine whether the prodrugs were subject to non-enzymatic degradation reactions of any kind, including hydrolysis. For example, GLA and its derivatives are potentially susceptible to auto-oxidation due to the unsaturation in the GLA portion. However, all prodrugs including the GLA derivatives were found to be stable for the length of the assay.



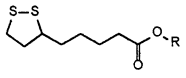


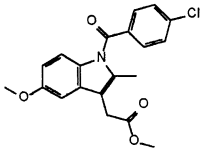
1 R=$CH_2O_2C(CH_2)_3CH_3$ Phy-m-val

2 R=CH_2OGLA Phy-m-GLA

3 R=$CH_2O_2C(CH_2)_3CH_3$ Theo-m-val

4 R=CH_2OGLA Theo-m-GLA





5 R=$CH_2O_2C(CH_2)_3CH_3$ Thio-m-val

6 R=CH_2OGLA Thio-m-GLA

7 R=$CH_2O_2C(CH_2)_3CH_3$ Indo-m-val

8 R=CH_2OGLA Indo-m-GLA

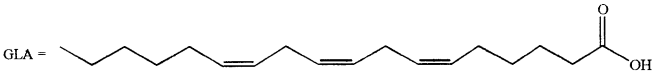


Fig. 1. Acyloxymethyl derivatives of phenytoin, theophylline, thioctic acid and indomethacin.

3.2. Effect of functional group

As can be seen from Table 1, trends exist when comparing the rate of hydrolysis of drugs in one functional class to another. For example, for the hydrolysis in porcine esterase, the NH-acidic prodrugs of phenytoin, **1**, **2** and theophylline, **3**, **4** hydrolyzed faster than the carboxylic acid prodrugs of thioctic acid, **5**, **6** and indomethacin, **7**, **8**. The rate constants for the NH-acidic prodrugs ranged from 0.0779 to 1.82 min^{-1}, whereas those of the carboxylic acid prodrugs did not undergo hydrolysis under our conditions with the exception of Thio-m-val which gave a rate constant of 0.0642 min^{-1}.

Similarly, the NH-acidic prodrugs were hydrolyzed faster then the carboxylic acid prodrugs in human plasma. The rate constants for the NH-acidic valeric acid prodrugs ranged from 0.0254 to 3.65 min^{-1}, whereas the valeric acid derivatives of the carboxylic acid drugs were only 0.0825 and 0.00559 min^{-1}, respectively.

3.3. Effect of steric size

Within each class of drugs studied, the drugs were arbitrarily chosen in terms of varying steric size. Keeping the alkyl and acyl promoieties constant the bulkier phenytoin prodrugs were hydrolyzed slower than the theophylline prodrugs. For example the hydrolysis rate constants, k, for the valeric acid prodrugs, in porcine esterase, were 1.82 min^{-1} for Theo-m-val, **3**, compared to 0.462 min^{-1} for Phy-m-val, **1**, and in human plasma, 3.65 min^{-1} compared to 0.365 min^{-1}, respectively.

Similarly, for the carboxylic acid drug class the valeric acid prodrug of thioctic acid, **5**, was hydrolyzed faster than the relatively larger indomethacin prodrug, **7**. In fact, in porcine esterase no hydrolysis of Indo-m-val, **7**, was observed under our experimental conditions. Also the hydrolysis rate constants for Thio-m-val, **5**, and Indo-m-val, **7**, in human plasma were 0.0825 and 0.00559 min^{-1}, respectively.

Table 1
Hydrolysis rate constants, k, and half-lives, $t_{1/2}$, for the valeric and γ-linolenic acid acyloxymethyl derivatives of phenytoin, theophylline, thioctic acid and indomethacin obtained using porcine liver esterase and 80% human plasma

Prodrug		Porcine esterase		80% human plasma	
		k (min^{-1})	$t_{1/2}$ (min)	k (min^{-1})	$t_{1/2}$ (min)
Phy-m-val	**1**	0.462	1.5	0.365	1.9
Phy-m-GLA	**2**	N/H[c]	–	–	<13%[a]
Theo-m-val	**3**	1.82[b]	0.38[b]	3.65[b]	0.19[b]
Theo-m-GLA	**4**	0.0779[b]	8.9[b]	0.0254[b]	27.3[b]
Thio-m-val	**5**	0.0642	10.8	0.0825	8.4
Thio-m-GLA	**6**	N/H[c]	–	N/H[c]	–
Indo-m-val	**7**	N/H[c]	–	0.00559	124
Indo-m-GLA	**8**	N/H[c]	–	N/H[c]	–

[a] Unable to determine $t_{1/2}$, value shown is% degradation after 7 h.
[b] From Burke et al. (1997).
[c] No hydrolysis observed after 7 h.

3.4. Effect of carboxylic acid derivatizing moiety

Although no hydrolysis was observed for certain GLA prodrugs, a trend still emerges (Table 1) showing that the valeric acid prodrugs hydrolyzed faster then the longer GLA prodrugs. For example for the theophylline prodrugs the rate constants for Theo-m-val, **3**, in porcine esterase and in human plasma were 1.82 and 3.65 min^{-1}, respectively, whereas the rate constants for Theo-m-GLA, **4**, were 0.0779 and 0.0254 min^{-1}, respectively.

In order to compare the rates of hydrolysis of the various drug derivatives it is useful to utilize the lipophilicity index (or log K) as determined by reversed-phase HPLC. Log K is linearly related to log P, the n-octanol/water partition coefficient, however, log K is a convenient alternative to the task of measuring n-octanol/water solubility normally required for determining log P (Bonina et al., 1995). As we have previously shown a plot of log k versus log K will reveal any structure activity relationships (Burke et al., 1997; Redden et al., 1998). Hence log k obtained using porcine liver esterase and human plasma, for the four valeric acid drug derivatives, **1**, **3**, **5** and **7** versus their respective lipophilicity index, log K, is shown in Fig. 2. Although only seven points are represented a linear trend still emerges whereby log k decreases as the lipophilicity index, log K increases.

4. Discussion

With the exception of some of the GLA drug derivatives, all derivatives displayed first-order hydrolysis kinetics using porcine liver esterase and human plasma. A possible explanation why there was no observed hydrolysis of some of these GLA derivatives may be that they are not substrates for the enzyme. Alternatively, it may be that these very lipophilic derivatives are not sufficiently soluble in the medium used for hydrolysis. This may

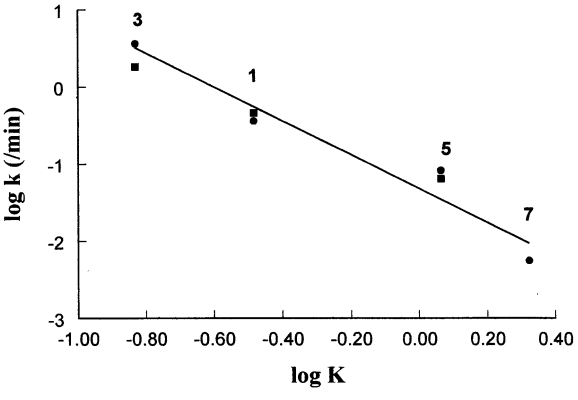


Fig. 2. Logarithm of the hydrolysis rate constant (log k) versus the lipophilicity index (log K) of the valeroyloxymethyl derivatives of phenytoin, **1**, theophylline, **3**, thioctic acid, **5**, and indomethacin, **7** using porcine liver esterase (+) and human plasma (*). The solid line represents the best exponential fit through the data.

also be the case for Indo-m-val, **7**, where we did not observe any hydrolysis using porcine liver esterase.

When comparing the functional classes of the prodrugs, namely the NH-acidic and carboxylic acid classes, definite trends appear in their hydrolysis rates. For instance, in porcine esterase, the NH-acidic prodrugs were observed to be hydrolyzed faster than the carboxylic acid prodrugs. In human plasma, the NH-acidic prodrugs also were hydrolyzed faster than the carboxylic acid prodrugs. The slower rates of hydrolysis, using porcine esterase, observed for the larger prodrugs of phenytoin and indomethacin compared to the smaller prodrugs of theophylline and thioctic acid can be explained by examining their molecular volume and linear parameters. For instance, in a study examining the hydrolysis of cytarabine-N^4-carboxylate and succinamate ester prodrugs by porcine esterase, it was shown that the steric parameters such as the Van der Waals volume of the ester side chain as well as the molecular bulkiness and linear dimensions influence the rate of hydrolysis. This study reported a correlation of the rate of hydrolysis of the ester prodrugs with an increase in the molecular bulkiness but an increase in the linear parameter led to a decrease in the hydrolysis rate. It was suggested that for porcine liver esterase there is a need for a bulky ester group to increase the molecular bulkiness of the prodrug and hence the rate of hydrolysis, bearing in mind that a concomitant increase in the linear parameter outside the boundary of the pocket of the active site retards hydrolysis significantly (Kawaguchi et al., 1996). Now, in this present study, for the four valeric acid prodrugs, the rates of hydrolysis in both porcine esterase and human plasma are from fastest to slowest as follows: Theo-m-val, Phy-m-val, Thio-m-val, and Indo-m-val. For these results using porcine esterase, this order is as would be expected based on an examination of the empirical length of the molecules. Theophylline is shorter than phenytoin which are both markedly shorter than thioctic acid and indomethacin. Therefore, this increase in length for these latter drugs may explain the observed slower rates of hydrolysis. Additionally, in all cases, the rates of hydrolysis were faster for the valeric acid prodrugs compared to the GLA prodrugs presumably because GLA, although bent and twisted like part of a coil in a spring, increases the length of the prodrugs significantly compared to valeric acid, giving rise to a reduced rate of hydrolysis. A similar order of hydrolysis rates was observed in human plasma and although there are numerous esterases in plasma the linear parameter of the larger drugs is responsible for the decreased rates.

The results presented here when coupled with our previous work (Burke et al., 1997; Redden et al., 1998) describing the effects of the linking and derivatizing promoieties in acyloxyalkyl esters provide some useful information in the rational development of these types of prodrugs. For example our previous study examined the rates of hydrolysis by both porcine esterase and human plasma by maintaining the drug (theophylline) and the acyl (GLA) portions constant while varying the alkyl linking moiety (Burke et al., 1997). For non-branched alkyl linking groups, there was a good correlation between the logarithm of the hydrolysis rate constant, log k, and the lipophilicity index log K. This is depicted diagrammatically in Fig. 3(a), based on experimental data from Burke et al., 1997, as a line with a negative slope for alkyl moieties derived from formaldehyde through to valeraldehyde.

Our second study maintained the alkyl (methylene) and drug (theophylline) portions constant but varied the acyl derivatizing agent (Redden et al., 1998). In this case, a parabolic relationship was observed between log k and log K and is depicted in Fig. 3(b), again based on experimental data from Redden et al., 1998. The rate of hydrolysis was slowest for acetic acid derivatives reaching a maximum between the valeric and non-anoic acid derivatives and then diminishing again for the fatty acid derivatives consisting of 18 and 20 carbon atom chains.

In this present study the alkyl (methylene) and acyl (val or GLA) moieties were maintained constant but the drug was varied. A decreasing relationship was observed as shown in Fig. 2 and is depicted diagrammatically in Fig. 3(c), when plotting log k for the hydrolysis of the different prodrugs versus log K. In this case the faster rates

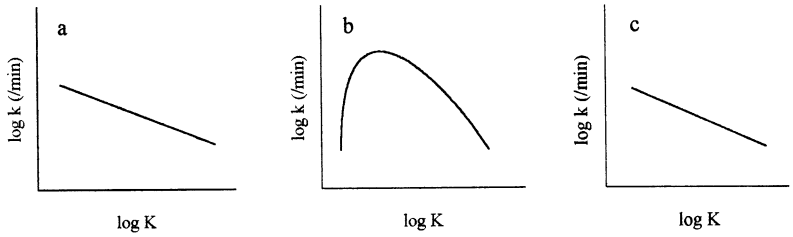


Fig. 3. Diagrammatic representation of the logarithm of the hydrolysis rate constant (log k) in porcine liver esterase and human plasma versus the lipophilicity index (log K) for acyloxyalkyl drug derivatives (a) maintaining the drug (theophylline) and acyl (γ-linolenic acid) portions constant while varying the alkyl moiety; based on experimental data from Burke et al. (1997), (b) maintaining the drug (theophylline) and alkyl (methylene) portions constant while varying the acyl moiety, based on experimental data from Redden et al. (1998) and (c) maintaining the acyl (valeric acid) and alkyl (methylene) portions constant while varying the drug, based on the present experimental data.

of hydrolysis were obtained for prodrugs with the lower lipophilicity indices.

Therefore, the general in vitro hydrolytic reactivity of acyloxyalkyl acidic drug derivatives using porcine liver esterase or human plasma is dependent on the drug, alkyl linking group and acyl carboxylic acid derivatizing moieties as depicted diagrammatically in Fig. 3 and summarized as follows:

- For acyloxyalkyl acidic drug derivatives as the size of the alkyl linking group increases, so does the lipophilicity of the derivative, but the rate of hydrolysis decreases. This reduced rate of hydrolysis presumably results from the increased steric hindrance towards the esterase enzymes.
- For acyloxyalkyl acidic drug derivatives the hydrolysis rate depends parabolically on the size, or lipophilicity, of the acyl carboxylic acid derivatizing moiety. The rate of hydrolysis increases as the size, or in this case, the length, of the carboxylic acid increases to a maximum, however as the lipophilicity increases further, the rate of hydrolysis decreases again. This reduced rate is attributed to an increased length of the derivative resulting in a decreased interaction with the pocket of the active site of the enzyme.
- For acyloxyalkyl acidic drug derivatives the rate of hydrolysis tends to decrease as the lipophilicity of the parent drug increases. This decreased rate of hydrolysis may be attributed to the increased steric nature of the drug. A similar observation and explanation was noted with amidomethyl esters of carboxylic acid drugs where the rate of hydrolysis decreased with an increasing lipophilicity (Iley et al., 1997).

These observations are, perhaps, not surprising when looking empirically at the results of systematically changing the parts of the acyloxyalkyl prodrug. However, the combined results provide the investigator with a rationale, based on the lipophilicity of the prodrug, for determining the structure of a prodrug to be designed in order to obtain a desired rate of hydrolysis, in vitro. Clearly, the position of the line in all three of the log k versus log K plots of Fig. 3 will depend on the drug of interest. For additional drugs there would be a series of multiple lines for each of the panels in Fig. 3, moved horizontally and/or vertically depending on the lipophilicity and rate of hydrolysis, respectively.

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