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Full Paper

Bioreversible Derivatives of Phenol. 2. Reactivity of Carbonate Esters with Fatty Acid-like Structures Towards Hydrolysis in Aqueous Solutions

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Abstract: A series of model phenol carbonate ester prodrugs encompassing derivatives with fatty acid-like structures were synthesized and their stability as a function of pH (range 0.4 – 12.5) at 37°C in aqueous buffer solutions investigated. The hydrolysis rates in aqueous solutions differed widely, depending on the selected pro-moieties (alkyl and aryl substituents). The observed reactivity differences could be rationalized by the inductive and steric properties of the substituent groups when taking into account that the mechanism of hydrolysis may change when the type of pro-moiety is altered, e.g. *n*-alkyl vs. *t*-butyl. Hydrolysis of the phenolic carbonate ester 2-(phenoxycarbonyloxy)-acetic acid was increased due to intramolecular catalysis, as compared to the derivatives synthesized from ω-hydroxy carboxylic acids with longer alkyl chains. The carbonate esters appear to be less reactive towards specific acid and base catalyzed hydrolysis than phenyl acetate. The results underline that it is unrealistic to expect that phenolic carbonate ester prodrugs can be utilized in ready to use aqueous formulations. The stability of the carbonate ester derivatives with fatty acid-like structures, expected to interact with the plasma protein human serum albumin, proved sufficient for further *in vitro* and *in vivo* evaluation of the potential of utilizing HSA binding in combination with the prodrug approach for optimization of drug pharmacokinetics.

Introduction

A major cause of failures in early drug development is, together with toxicological issues, poor drug pharmacokinetics. The high affinity ligands generated in drug discovery are rarely endowed with physical chemical properties favorable for drug transport to the target site within the body. In recent years efforts have been made to improve the drug-like properties of the leads generated in the drug screening facilities [1-5]. However, concurrent optimization of pharmacodynamic and pharmacokinetic properties may not always be achievable. In such cases, the prodrug approach [6-11] may be feasible, enabling transient modification of pharmacokinetic properties and thus improving the drug performance of chemical entities characterized by suitable *in vitro* activity but poor transport properties. Human serum albumin (HSA) is the major plasma protein, interacting with a variety of endogenous and exogenous substances, and in particular with lipophilic organic anions such as fatty acids and non-steroidal anti-inflammatory drugs [12-17]. It is generally recognized that HSA binding plays a major role in determining the *in vivo* fate of many drug substances [17-20]. Despite this fact, reversible drug-HSA binding has been used deliberately to improve drug pharmacokinetics a few cases only, e.g. [21-25].

With the overall objective of assessing the potential of utilizing plasma protein binding interactions in combination with the prodrug approach for improving pharmacokinetics we have studied a series of bioreversible derivatives of phenol [26]. Phenolic drugs may be subject to extensive first-pass metabolism [27]. Thus, phenol was selected as a model drug substance because the metabolism has been studied extensively [28-31]. Further, it has been used previously as a model compound in prodrug related studies aiming at the circumvention of first-pass metabolism [32-35]. A series of carbonate ester bioreversible derivatives of phenol (Table 1) was synthesized, including derivatives possessing a fatty acid-like structure as a means for building in affinity for HSA in the structures. Since carbonate esters have been subject of much less attention than carboxylic acid ester derivatives in the prodrug context, it was found of interest to investigate in more detail the hydrolysis kinetics and reactivity of phenolic carbonate ester derivatives of potential interest in prodrug design. In the current work we present the synthesis procedures and the obtained pH rate profiles for the hydrolysis of a series of carbonate esters of phenol encompassing derivatives with fatty acid-like structures. Aspects pertaining to the binding of the carbonate esters derivatives to HSA and the effects of HSA on the carbonate ester stability are reported in the accompanying communication [26].

Results and Discussion

Synthesis

The carbonate esters with fatty acid-like structure were synthesized from phenyl chloroformate and the appropriate ω-hydroxycarboxylic acids or ethyl ester of the ω-hydroxycarboxylic acids, essentially following the procedures described by King *et al.* [36] (see Experimental section for details). In addition to the carbonate esters with fatty acid-like structure listed in Table 1, attempts were made to synthesize 4-(phenoxycarbonyloxy)-butyric acid. These efforts were however unsuccessful, presumably due to rapid formation of γ-butyrolactone.

Table 1. Rate constants for the hydrogen ion catalyzed (k_H), the spontaneous (k_o), and hydroxide ion catalyzed (k_{OH}) hydrolysis of various carbonate esters and phenyl acetate and estimated ionization constants at 37°C and $\mu = 0.50$ M.
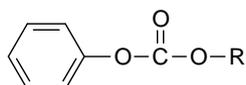
Compound	R	k_H $(M^{-1}s^{-1})$	k_o (s^{-1})	k_{OH} $(M^{-1}s^{-1})$	pK_a
1	-C$_2$H$_5$	1.4×10^{-6}	6.8×10^{-8}	1.3	
2	-C(CH$_3$)$_3$	1.8×10^{-3}	4.1×10^{-4}	3.3×10^{-2}	
3	-C$_6$H$_5$		1.2×10^{-5}	12.6	
4	-CH$_2$COOH		4.7×10^{-6}	1.0	2.6; 2.42[b]
			$3.7 \times 10^{-4 [a]}$		
5	-(CH$_2$)$_5$COOH	1.7×10^{-6}	6.5×10^{-8}	1.0	4.70[b]
6	-(CH$_2$)$_7$COOH			1.0	4.79[b]
7	-(CH$_2$)$_{11}$COOH			1.0	
8	-(CH$_2$)$_{15}$COOH				
9	Phenyl acetate	3.2×10^{-4}	3.9×10^{-8}	4.1	

a) k'_o (cf. Equation 2)

b) pK_a was determined by capillary electrophoresis at 25°C and μ = 0.050 M

Kinetics of chemical hydrolysis

As a part of the pharmaceutical chemical characterization the kinetics of hydrolysis of the bioreversible derivatives of phenol was investigated in aqueous buffer solutions of ionic strength (μ) 0.50 M at 37°C. The stability of carbonate esters has been subject to less attention than carboxylic acid esters. Thus, the influence of pH on hydrolysis in aqueous solutions was studied in some detail. The structures of the compounds included in the study are depicted in Table 1 and comprise eight carbonate esters and phenyl acetate (**9**). Hydrolysis experiments were started by adding an acetonitrile stock solution to the preheated buffer solutions providing initial carbonate ester concentrations of about $1 \times$

10^{-4} M. The stability of bioreversible derivatives **1-5** was investigated in the pH range 0.4 – 12.5. Due to solubility limitations, derivatives **6** and **7** were only studied in alkaline solution (pH ranges 8.4 – 12.4 and 10 – 12.4, respectively) whereas hydrolysis studies of **8** were not performed.

Under the experimental conditions employed the hydrolysis reactions were found to obey first-order kinetics. In most cases general acid/base catalysis of the buffer substances were negligible. In cases where buffer catalysis was observed the pseudo-first-order rate constants at zero buffer concentration (k) were determined from the y-intercept of linear plots of the observed pseudo-first-order rate constants versus total buffer concentration. The pH of the hydrochloric acid and sodium hydroxide solutions was calculated according to [37]. Rate constants obtained by the UV spectrophotometric method and HPLC agreed within 5%. Especially the fatty acid-like structure carbonate esters with long alkyl chains may be expected to be endowed with amphiphilic properties. However, the adherence to first-order kinetics indicates that aggregation or micellization was not occurring in the concentration range studied or did not affect aqueous stability. The effect of pH on hydrolysis rates at 37°C is depicted in Figure 1. The stability of the bioreversible derivatives in solution at 37°C varied widely as reflected in the half-lives (in hours) of the esters **1-5** and **9** at their rate minimum in the pH range investigated, which were 2800 (120 days), 0.47, 17, 30, 3000 (125 days), and 3700 (150 days), respectively.

Figure 1. pH-rate profiles for the hydrolysis of compounds **1** ($\triangle$), **2** ($\triangledown$), **3** ($\blacksquare$), **4** ($\bullet$), **5** ($\boldsymbol{+}$), and **9** ($\bigcirc$) in aqueous solution at 37°C ($\mu = 0.50$ M).
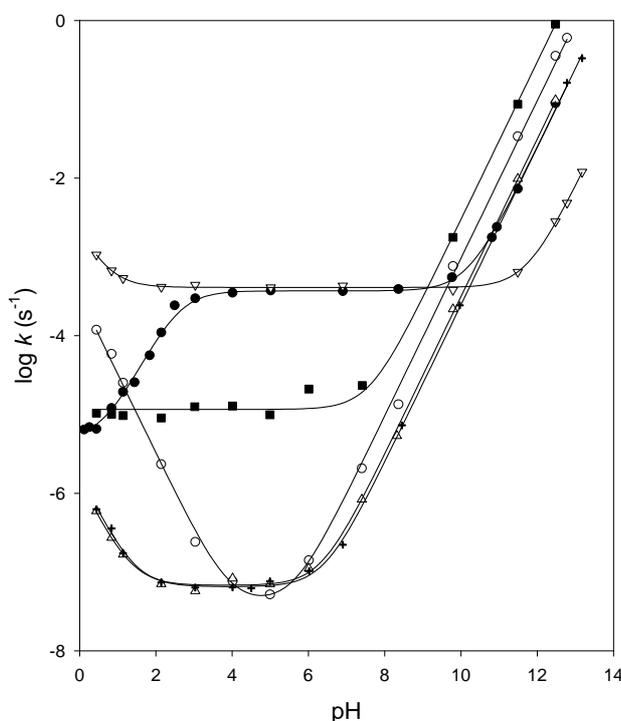
In the pH range investigated, the shape of the pH-rate profiles of compounds **1, 2** and **9** indicates that the pseudo-first-order rate constant at zero buffer concentration can be adequately described by specific acid, specific base, and spontaneous or water catalyzed hydrolysis reactions:

$$k = k_H a_H + k_o + k_{OH} \frac{K_w}{a_H} \tag{1}$$

where a_H is the hydrogen ion activity, K_w is the autoprotolysis constant of water, and k_o, is the rate constant for the spontaneous reaction. k_H and k_{OH} refer to the second-order rate constants for the hydrogen ion and hydroxide ion catalyzed reactions, respectively. The hydroxide ion activity, a_{OH}, is given by the ratio K_w/a_H. For diphenyl carbonate (**3**) specific acid catalysis was not observed in the investigated pH range, thus, only the last two terms of Equation 1 was used to describe the pH-rate profile.

The rate expression necessary to describe the overall hydrolysis of **5** is given by:

$$k = k_H a_H \frac{a_H}{a_H + K_a} + k_o \frac{a_H}{a_H + K_a} + k'_o \frac{K_a}{a_H + K_a} + k_{OH} \frac{K_w}{a_H} \frac{K_a}{a_H + K_a} \tag{2}$$

where K_a is the apparent ionization constant of the carbonate ester, k_H is the second-order rate constant for the hydrogen ion catalyzed reaction of the neutral form of the ester, and k_{OH} represents the second-order rate constant for the hydroxide ion catalyzed reaction of the ionized form of the ester. k_o and k'_o are macro reaction constants [38] with k_o related to the two kinetically equivalent reactions, viz. spontaneous reaction of the neutral form of the ester and specific acid catalyzed hydrolysis of the anionic species; k'_o is associated to the following kinetically equivalent reactions, viz. the spontaneous reaction of the deprotonated form of the ester and the specific base catalyzed hydrolysis of the neutral form. From the present data, it is not possible to distinguish between the various processes associated with k_o and k'_o. However, the obtained rate data may suggest that the neutral and anionic (**5**) exhibit quite similar susceptibility to undergo hydrolysis in the pH range 3-6, most likely because the carboxylic acid group of the hexanoic acid derivative (**5**) is placed far away from the reactive center of the molecule. Thus, the constants presented in Table 1 for **5** were obtained by fitting to Equation 1. For the acetic acid derivative (**4**), specific acid catalysis of hydrolysis was not observed in the pH range studied, thus only the three last terms of Equation 2 were required to describe the hydrolysis kinetics of **4**. For the derivative **4,** stability was assessed in hydrochloric solutions with $\mu > 0.5$ M. Rate and ionization constants were determined from regression analysis and are compiled in Table 1.

Reactivity of carbonate esters

The reactivity differences observed among the carbonate esters must be ascribed to either polar or steric effects of the R-groups. The proposed mechanisms for hydrolysis of the carbonate esters catalyzed by specific acid, water, and specific base are outlined in Scheme 1. Apparently, the hydroxide ion catalyzed hydrolysis has been studied the most. The hydrolysis of alkyl carbonates in the presence of hydroxide ions has been shown to be a two-step process; a second-order reaction is followed by a slow first-order decomposition of a monoalkyl carbonate [39-41].

A similar reaction scheme has been proposed for diaryl carbonates, though, the cleavage of the monophenyl carbonate anions was suggested to be extremely fast [42]. Cooper *et al*. [43] determined the second-order rate constants for the alkaline hydrolysis of a series of symmetric substituted diaryl carbonates. The rate of reaction was increased by the presence of electron-withdrawing and decreased

by electron-donating substitutents, respectively. Rate data for diaryl esters with *meta* and *para* substituents were fitted to the Hammett equation [44]. The significance of steric effects were apparent by comparison of hydrolysis rates of *ortho* carbonate esters with *meta* and *para* substituted carbonates [43]. As for carboxylate esters [45], carbonate ester hydrolysis is expected to proceed through a tetrahedral intermediate by a stepwise mechanism [46]. The rate limiting step being the formation of the tetrahedral intermediate by addition of the hydroxide ion [46,47].

Scheme 1. Proposed reaction mechanisms for (A) specific acid catalyzed, (B) water catalyzed, and (C) specific base catalyzed hydrolysis of carbonate esters.
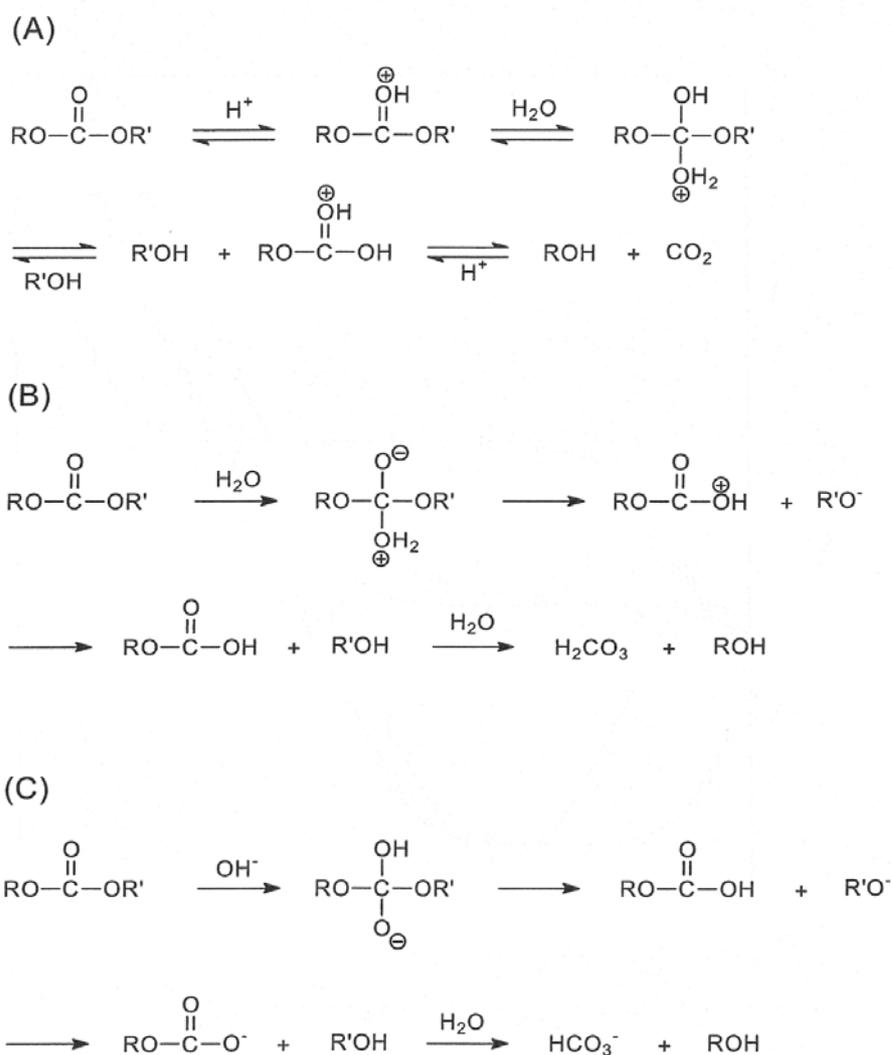
Tentatively, inductive effects of the R-groups may be suggested to account for the reactivity of the carbonate esters towards hydroxide ion catalyzed hydrolysis shown in Table 1. To aid interpretation of the rate data, the Taft polar substituent parameter σ^* (values taken from [48]) of the R-moieties may be useful. Ethyl phenyl carbonate (**1**, $\sigma^* = -0.10$) is slightly more susceptible to alkaline hydrolysis than **5**, **6**, and **7**, which have larger electron-donating capabilities (the σ^*-value of **5-7** were taken to be similar to that of the n-alkyl substituents with similar number of carbons, i.e. around -0.16). The *t-*

butyl derivative is the least reactive of the derivatives, this may be expected due to the bigger electron-donating power of branched alcohols ($\sigma^* = -0.30$). However, the main contribution to the stability of **2** may be steric hindrance. The high reactivity of **3** as compared to **1** is expected from the electron-withdrawing properties of the phenyl ring ($\sigma^* = -0.60$) and in excellent agreement with the work of Cooper and Williams [42]. The k_{OH} obtained for the acetic acid derivative (**4**) is similar to that of **5**, **6**, and **7**; from the Taft polar substituent constant σ^* ($\sigma^* = -0.06$ for CH_2COO^-) reactivity was expected to be higher than that of **1**, however, steric hindrance and electrostatic repulsion due to the nearby carboxylate anion may most likely lower the reactivity. The carboxylate anions of **5**, **6**, and **7** are thought to be positioned too far away from the carbonyl carbon to provide a significant shielding effect.

Analogous accounts for the structure-reactivity relations found for the hydroxide ion catalyzed hydrolysis of various alkyl aryl and diaryl carbonate esters have been set forward. The reactivity of ethyl *p*-nitrophenyl carbonate, ethyl phenyl carbonate and diethyl carbonate was found to be governed by the polar effects of the alcohol and phenol moieties, as reflected by their pK_a values [49]. The relative hydrolysis rates of four alkyl carbonate esters of salicylic acid at pH 12 were rationalized by the electron-donating capabilities of the alkyl substituents [50]. The ethyl derivative was slightly more reactive (~20-30% more reactive) than esters possessing longer alkyl chains as found in the present study. Similarly differences in the specific base catalyzed hydrolysis of four alkyl carbonates of acetaminophen (paracetamol) were accounted for by the inductive properties of the alcohol moieties [51], as was the hydrolysis of 11 carbonate esters of acetaminophen studied at pH 7.4 [52].

As for carboxylic acid esters, the catalytic coefficient for the specific acid catalyzed process is much smaller than that of the base catalyzed reaction. The hydrogen ion catalyzed hydrolysis is thought to occur through a multistage process ($A_{Ac}2$ [53]) (Scheme 1A). A rapid preequilibrium protonation step is followed by a slow bimolecular reaction, the attack of a water molecule, and carbonyl-oxygen fission [54-56] and subsequently by a fast unimolecular acyl-oxygen fission of the alkyl hydrogen carbonate [55,57]. Ethyl phenyl carbonate [42] has previously been found to be subject to specific acid catalysis. However, in contrast to the earlier studies [42,54] conducted in mixed organic solvent-aqueous solutions and elevated temperature, specific acid catalysis was not observed for diphenyl carbonate in the present study. Specific acid catalyzed hydrolysis of carboxylic acid esters is governed primarily by steric effects [58]. Thus, in contrast to the obtained results, the hydrogen ion catalyzed hydrolysis of **1** was expected to be slightly higher than that for **5**. The rate constant determined for the *t*-butyl derivative **2** is several orders of magnitude higher than expected from steric considerations. However, acid catalyzed hydrolysis of *t*-butyl carboxylic acid esters has been found to proceed through a different mechanism than esters of primary and secondary alcohols involving cleavage of the alkyl-oxygen bond ($A_{AL}1$) [58-60]. An alternative mechanism may also be the cause of the high susceptibility of carbonate ester **2** towards hydrogen ion catalyzed hydrolysis.

The spontaneous or water catalyzed hydrolysis of carbonate esters has been proposed to proceed through the same mechanism as the hydroxide ion catalyzed reaction ($B_{Ac}2$) (Scheme 1B), a water molecule being the nucleophile instead of the hydroxide ion [42,43]. Water catalyzed hydrolysis of *meta* substituted [43] and *para* substituted [46] diaryl carbonates has been found to fit the Hammett equation, i.e. reactivity is governed by polar effects in a fashion similar to the hydroxide ion catalyzed

hydrolysis [43]. However, the spontaneous reactivity of the investigated carbonate esters in Table 1 does not parallel the reactivity towards specific base catalysis for various reasons. Compared to the other derivatives, the *t*-butyl derivative (**2**) is relatively more susceptible to water catalyzed hydrolysis than specific base catalysis, i.e. the k_{OH}:k_o ratio is much smaller. The hydrolysis of *t*-butyl acetate has been found proceed through a different mechanism ($B_{AL}1$) involving the formation of *t*-butyl carbonium ion [61]. Thus, increased reactivity of **2** might be ascribed to the hydrolysis reaction proceeding through a different mechanism. The increased spontaneous degradation of 2-(phenoxycarbonyloxy)-acetic acid (**4**) is presumably due to intramolecular catalysis by the carboxylate anion. Intramolecular catalysis of carbonate ester hydrolysis by the carboxylate anion may also be the cause of the high lability of the salicylic acid carbonate esters studied by Dittert *et al.* at pH 7.4 as compared to pH 12 [50]. Other examples of intramolecular catalysis involved in carbonate ester hydrolysis can be found [47,62]. Further investigations are required to distinguish whether the increased rate of hydrolysis is due to intramolecular nucleophilic catalysis or general base catalysis involving the carboxylic acid group. This topic has been discussed elsewhere, e.g. [63-66]. Attempts to synthesize 4-(phenoxycarbonyloxy)-butyric acid were unsuccessful most probably due to the favored cyclization of the ω-hydroxy butyric acid leading to γ-butyrolactone. For the hexanoic acid derivative (**5**), the spontaneous hydrolysis proceeded with similar rates at the plateau around the pK_a value of the compound (4.70), indicating that the reactivity of the neutral and ionized form of the carbonate ester is similar.

The effects of structural variations in carbonate esters on reactivity in aqueous solution have been found largely to parallel the effects of similar variations in the alcohol moiety of carboxylic acid esters [51,52]. Phenyl acetate (**9**) was included in the study allowing comparison between the two ester types. It appears that the carboxylic acid ester is more prone to specific acid and base catalysis than the corresponding carbonate esters, e.g. **1** and **5**, but less sensitive towards water catalyzed hydrolysis. In a study designing prodrugs of the opioid analgesic ketobemidone for buccal delivery 4 carboxylic acid and 5 carbonate ester prodrugs were investigated. The same trend in reactivity, as observed here, was found for the ethyl carbonate and acetate ester of the phenolic drug [67]. In line with these findings are the rate constants for the hydroxide ion catalyzed hydrolysis of carbonate and carboxylic acid ester prodrugs of paracetamol [51]. The ethyl and *i*-butyl carbonates being more stable than the acetate and butyrate esters, the k_{OH} being 68 and 70 versus 310 and 155 M^{-1} min^{-1}, respectively.

Conclusions

The obtained results confirm that carbonate ester derivatives may constitute a feasible platform for designing prodrugs of phenolic drug substances and that widely varying hydrolysis rates in aqueous solutions may be achieved depending on the selected pro-moiety. The observed reactivity may be rationalized from the inductive and steric effects exerted by the substituent groups when considering that the mechanism of hydrolysis may vary depending on the specific pro-moieties. Hydrolysis of the phenolic carbonate ester 2-(phenoxycarbonyloxy)-acetic acid was facilitated by intramolecular catalysis in contrast to derivatives prepared from ω-hydroxy carboxylic acids with longer alkyl chains. The carbonate esters appear to be less reactive towards specific acid and base catalysis than

corresponding carboxylic acid ester derivatives (phenyl acetate). Results underline that it is very unlikely that phenolic carbonate ester prodrugs can be utilized in ready to use aqueous drug formulations. However, the stability of the carbonate ester derivatives with fatty acid-like structure is sufficient for further evaluation *in vitro* and *in vivo* of the potential of utilizing HSA binding in combination with the prodrug approach for optimization of drug pharmacokinetics.

Experimental

Chemicals

Ethyl phenyl carbonate was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethyl glycolate and phenyl acetate were obtained from Acros Organics (Geel, Belgium). Diphenyl carbonate, ethyl 6-hydroxyhexanoate, 12-hydroxydodecanoic acid, 16-hydroxyhexadecanoic acid, phenol, phenyl chloroformate, 20 % technical grade solution poly(diallyldimethylammonium chloride) (PDMAC) M_w 400,000-500,000, 25 % technical grade solution poly(vinylsulfonate) sodium salt (PVS) M_w 4,000-6,000, and *t*-butyl phenyl carbonate were obtained from Aldrich-Chemie (Steinheim, Germany). 8-Hydroxyoctanoic acid and pyridine were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Silica gel 60 was obtained from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical grade or better. Purified water from a Milli-Q deionization unit (Millipore, Bedford, MA, USA) was used throughout.

Apparatus

An Aquarius CE7200 UV-spectrophotometer (Cecil Instruments, Cambridge, England) equipped with a thermostatted cell compartment, using 10 mm quartz cuvettes, was used for kinetic measurements. Data was collected and transferred to Microsoft Excel using HyperAccess Ver. 8.4 software (Hilgraeve, Monroe, MI, USA). HPLC was generally performed using a Merck-Hitachi L-6200 pump, a L-4000 UV-detector, and a D-2000 Chromato-Integrator (Merck-Hitachi, Tokyo, Japan) equipped with a Rheodyne 7125 injection valve with a 20 μL loop. ChromSpher C18 (150 × 4.6 mm; 5 μm particles) columns (Chrompack Varian, The Netherlands) were used. The mobile phases consisted of acetonitrile and 0.1 % v/v H_3PO_4 in suitable proportions (10 to 70 % v/v of acetonitrile). The flow rate set at 1 ml/min and the effluent was monitored at 200 nm. Melting points were obtained on a capillary melting-point apparatus and are uncorrected. Measurements of pH were conducted with a Metrohm 744 pH Meter (Metrohm Ltd., Herisau, Switzerland). CE experiments were performed on a Hewlett-Packard ^{3D}CE (Avondale, PA, USA) equipped with a diode-array detector. Mass spectrometry (MS) was performed on an ion trap (MSD Trap) from Agilent Tecnologies equipped with an electrospray ion source operated in positive ion mode with the following settings: Capillary voltage: -3000 V; drying gas temperature: 325°C; nebulizer gas 10 psi; drying gas: 4 L/min; skimmer 1: 15 V; trap drive: 40. ^{1}H-NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) instrument in $CDCl_3$ solutions. Spectra were analyzed using gNMR 5.0.

Preparation of carbonate esters

The compounds were synthesized by procedures modified from those reported by King *et al.* [36]. HPLC was used to monitor progress of reactions.

2-(Phenoxycarbonyloxy)-acetic acid (**4**). Phenyl chloroformate (5.0 mL, 39.8 mmol) and ethyl glycolate (4.15 mL, 39.8 mmol) were added to diethyl ether (200 mL) under continuous stirring in an ice bath. Pyridine (3.2 mL, 39.8 mmol) in diethyl ether (200 mL) was then added dropwise over 3 h. The mixture was stirred overnight at room temperature. The reaction mixture was filtered to remove pyridine hydrochloride followed by rotary evaporation. The obtained residue was dissolved in 0.5 M HCl-acetone (1:1, v/v, 200 mL) at 60°C under stirring. Progress of hydrolysis to obtain the desired product was monitored by HPLC. Upon completion acetone was removed by rotary evaporation. The remaining aqueous solution was extracted twice with diethyl ether. After drying over anhydrous sodium sulphate the organic phase was evaporated in vacuo. The obtained residue was recrystallized from diethyl ether – petroleum ether to give the title compound. m.p. 95.5-96.5°C (lit. [68]100-102°C); Anal. calc. for $C_9H_8O_5$: C, 55.11; H, 4.11; found: C, 55.12; H, 4.00; N, 0; MS: $[M + Na]^+$ m/z = 219.0; calculated monoisotopic mass m/z = 219.0; ^{1}H-NMR: δ 7.21 (2H, m, *J* = 8.7, 0.5, 1.1, 2.0 Hz), 7.40 (2H, m, *J* = 8.7, 7.8, 0.5, 2.0 Hz), 7.27 (1H, m, *J* = 7.8, 1.1 Hz), 4.81 (2H, s).

6-(Phenoxycarbonyloxy)-hexanoic acid (**5**). Phenyl chloroformate (5.0 mL, 39.8 mmol) and ethyl 6-hydroxyhexanoate (6.5 mL, 39.8 mmol) were added to diethyl ether (200 mL) under continuous stirring on an ice bath. Pyridine (3.2 mL, 39.8 mmol) in diethyl ether (200 mL) was then added dropwise over 4 h. The mixture was stirred overnight at room temperature. The reaction mixture was filtered to remove pyridine hydrochloride, followed by rotary evaporation. The residue was dissolved in acetone (120 mL), a portion of 1M HCl (40 mL) was added and the solution was heated to 70°C for 4 h under stirring. The reaction mixture was poured into toluene (100 mL), the organic phase washed twice with water (50 mL) and once with saturated sodium chloride solution (20 mL) followed by drying over anhydrous sodium sulphate. Precipitation occurred upon standing. Dry column flash chromatography [69] (glass filter Ø 45 mm pore size 3, silica gel 60) was performed on the product the fractions were eluted with ethyl acetate – hexane mixtures. The fractions containing the products were collected and evaporated *in vacuo*, followed by recrystallization from hot diethyl ether; m.p. 68.5-71°C; Anal. calc. for $C_{13}H_{16}O_5$: C, 61.90; H, 6.39; found: C, 62.05; H, 6.46; N, 0; MS: $[M + Na]^+$ m/z = 275.2; calculated monoisotopic mass m/z = 275.1; ^{1}H-NMR: δ 7.18 (2H, m, *J* = 8.7, 0.0, 1.3, 2.4 Hz), 7.39 (2H, m, *J* = 8.7, 7.9, 0.0, 2.8 Hz), 7.24 (1H, m, *J* = 7.9, 1.3 Hz), 4.26 (2H, t, *J* = 6.6 Hz), 2.40 (2H, t, *J* = 7.7 Hz), 1.74-1.79 (2H, m, *J* = 6.6, 6.6 Hz), 1.71 (2H, m; *J* = 7.7, 6.6 Hz), 1.28-1.35 (2H, m, *J* = 6.6, 6.6 Hz).

8-(Phenoxycarbonyloxy)-octanoic acid (**6**). Phenyl chloroformate (0.39 mL, 3.12 mmol) and 8-hydroxyoctanoic acid (0.50 g, 3.12 mmol) were added to diethyl ether (60 mL) under continuous stirring on an ice bath. Pyridine (0.25 mL, 3.12 mmol) in diethyl ether (200 mL) was added dropwise over 4 h. The mixture was stirred overnight at room temperature. The reaction mixture was filtered to

remove pyridine hydrochloride followed by rotary evaporation. The obtained solid was subjected to dry column flash chromatography [69] (glass filter Ø 45 mm pore size 3, silica gel 60) was performed on the product the fractions were eluted with ethyl acetate – hexane mixtures. The fractions containing the products were collected and evaporated in vacuo, followed by recrystallization from hot diethyl ether; m.p. 51-52°C; Anal. calc. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; found: C, 664.32; H, 7.23; N, 0; MS: $[M + H]^+$ m/z = 281.2; $[M + Na]^+$ m/z = 303.3; calculated monoisotopic mass m/z = 281.1 (303.1); ^{1}H-NMR: δ 7.18 (2H, m, J = 8.1, 0.6, 1.1, 2.6 Hz), 7.38 (2H, m, J = 8.1, 7.8, 0.6, 1.8 Hz), 7.24 (1H, m, J = 7.8, 1.1 Hz), 4.25 (2H, t, J = 6.9 Hz), 2.36 (2H, t, J = 7.5 Hz), 1.75 (2H, m, J = 6.6, 6.9 Hz), 1.65 (2H, m, J = 7.5, 6.6 Hz), 1.35-1.45 (6H, m).

12-(Phenoxycarbonyloxy)-dodecanoic acid (**7**). Carbonate ester **7** was prepared as **6**, using 12-hydroxydodecanoic acid (4.83 g, 22.3 mmol) initially dissolved in diethyl ether (200 mL); m.p. 67.5-70°C; Anal. calc. for $C_{19}H_{28}O_5$: C, 67.83; H, 8.39; found: C, 67.86; H, 8.50; N, 0; MS: $[M + H]^+$ m/z = 337.4; $[M + Na]^+$ m/z = 359.3; calculated monoisotopic mass m/z = 337.2 (359.2); ^{1}H-NMR: δ 7.18 (2H, m, J = 9.1, 1.1, -0.1, 2.7 Hz), 7.39 (2H, m, J = 9.1, 7.6, -0.1, 1.6 Hz), 7.24 (1H, m, J = 7.6, 1.1 Hz), 4.25 (2H, t, J = 6.9 Hz), 2.36 (2H, t, J = 7.8 Hz), 1.74 (2H, m, J = 6.9, 6.6 Hz), 1.63 (2H, m, J = 7.8, 6.6 Hz), 1.35-1.45 (14H, m).

16-(Phenoxycarbonyloxy)-hexadecanoic acid (**8**). Carbonate ester **8** was prepared as **6**, using 16-hydroxyhexadecanoic acid (5.01 g, 18.3 mmol) initially suspended in diethyl ether (200 mL); m.p. 81-82°C; Anal. calc. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24; found: C, 70.15; H, 9.21; N, 0; MS: $[M + H]^+$ m/z = 393.4. $[M + Na]^+$ m/z = 415.4; calculated monoisotopic mass m/z = 393.3 (415.2); ^{1}H-NMR: δ 7.18 (2H, m, J = 9.1, 1.4, 0.0, 2.9 Hz), 7.39 (2H, m, J = 9.1, 7.4, 0.0, 2.2 Hz), 7.24 (1H, m, J = 7.4, 1.4 Hz), 4.25 (2H, t, J = 6.9 Hz), 2.35 (2H, t, J = 7.7 Hz), 1.74 (2H, m, J = 6.9, 6.6 Hz), 1.63 (2H, m, J = 7.7, 6.6 Hz), 1.32-1.40 (22H, m).

Kinetic measurements

Stability of the phenol derivatives was studied in aqueous buffer solutions at 37 ± 0.2°C. Buffers used were acetate, phosphate, borate and carbonate at total concentrations between 0.01 and 0.10 M. At strongly acidic and alkaline pH, hydrochloric acid and sodium hydroxide solutions were used, respectively. A constant ionic strength (μ) of 0.50 M was obtained by adding a calculated amount of potassium chloride. Degradation was followed by direct UV-spectrophotometry or HPLC. Reactions followed by UV-spectrophotometry in a thermostatted quartz cuvette containing aliquots (2.5 mL) of preheated buffer solution, were initiated by addition of a 25 μL stock solution. Progress of reactions was monitored by measuring the increase in absorbance at 269 or 287 nm, local absorption maxima for phenol and phenolate, respectively. Pseudo-first-order rate constants were determined from slopes of linear plots of ln (A_∞ – A_t) versus time t, where A_∞ and A_t are the absorbance measured at time infinity and t, respectively [70] or by applying Guggenheim´s method to the absorbance readings [70,71]. For experiments followed by HPLC, the reaction solutions were kept at constant temperature in a water bath, and at appropriate time intervals samples were withdrawn and chromatographed immediately.

The experiments were initiated by addition of stock solutions in acetonitrile to buffer solutions giving an organic solvent concentration of 1% or less, the initial carbonate ester concentration was $1 \cdot 10^{-4}$ M. Pseudo-first-order rate constants were determined by linear plots of the logarithm of residual derivative versus time or by using the initial rate method [70]. Rate constants were obtained from measurements done in (at least) triplicate. The relative standard deviation was below 5% in most cases.

Determination of pK$_a$ values by capillary electrophoresis

Fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA) and dynamically coated with PDMAC and PVS as described previously [72]. The PDMAC/PVS coated capillary of 32 cm × 50 μm ID, with a length of 24 cm to the detector was used. UV detection was performed at 200, 214, 230, and 250 nm. Injection was performed from the short end of the capillary by applying pressure (50 mbar) for 2 s. The capillary cassette temperature was set to 20°C. The applied voltage in the normal polarity mode was adjusted (6.5-20 kV) to give a mean capillary temperature of 25°C as calculated by the method of Kok [73]. Between measurements the capillary was flushed with 0.1 % PVS solution and electrophoresis buffer solution for 1 min each. Stock solutions of the carbonate esters were prepared in dimethylsulfoxide (DMSO). Sample solutions were obtained by dilution of the stock solutions with water to give a DMSO content of 1 % (v/v) and carbonate ester concentrations of ~20 μg/mL. Buffer solutions covering the range from pH 1.3 to 8.0 were prepared by mixing stock solutions to give the desired pH and an ionic strength of 0.05 M essentially as described by [74]. CE buffer solutions were filtered through 0.45 μm nylon filters (Chromacol LTD, Herts, UK) prior to use.

The effective electrophoretic mobility μ$_{eff}$ was calculated from

$$\mu_{eff} = \frac{l_c l_d}{V}\left(\frac{1}{t} - \frac{1}{t_0}\right) \tag{3}$$

where l_c is the total capillary length; l_d is the length of the capillary from the inlet end to the detector; V is the applied voltage; t and t_0 are the measured migration times of the analyte and the electroosmotic flow, respectively . Subsequently, the pK_a values were determined from Eq. 4 by non-linear regression analysis using the SigmaPlot 2000 software package (SPSS Inc. Chicago, IL, USA).

$$\mu_{eff} = \frac{10^{(pH - pK_a)}\mu_{B^-}}{1 + 10^{(pH - pK_a)}} \tag{4}$$

where pK_a is the apparent ionization exponent and μ$_{B^-}$ is the electrophoretic mobility of the fully deprotonated species.

Acknowledgements

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