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Hydrolysis kinetics and QSAR investigation of soft antimicrobial agents

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Abstract

Quaternary ammonium surfactants, such as benzalkonium chloride and cetylpyridinium chloride, are commonly used as antibacterial agents for disinfectants and for general environmental sanitation, as well as in surfactants, penetration enhancers and preservatives in pharmaceutical and cosmetic formulations. However, these agents are known to cause various side-effects and toxic reactions that are believed to be associated with their chemical stability. Soft analogues of the long-chain quaternary ammonium compounds were synthesized according to the soft drug approach and their physicochemical properties investigated, such as their hydrolytic rate constant, surface activity and lipophilicity. Structure–activity studies showed that the antimicrobial activity of the compounds was strongly influenced by their lipophilicity and chemical stability, the activity increasing with increasing lipophilicity and stability. However, in soft drug design structure–activity relationships are combined with structure–inactivation relationships during the lead optimization. The safety index (SI) of compounds was defined as the hydrolytic rate constant divided by the minimum inhibitory concentration. The SI of the soft antibacterial agents was found to increase with increasing lipophilicity but optimum SI was obtained when their hydrolytic $t_{1/2}$, at pH 6 and 60°C, was about 11 h. Optimization of the soft antibacterial agents through SI optimization resulted in potent but chemically unstable quaternary ammonium antibacterial agents.

Introduction

Cationic quaternary ammonium surfactants, such as benzalkonium chloride, cetylpyridinium chloride and cetrimonium bromide, possess a broad spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria as well as against some viruses and pathogenic species of fungi and protozoa (Petrocci 1983; Thorsteinsson et al 2003a). Quaternary ammonium agents have also been shown to have potent antimalarial activity (Ancelin et al 2003). Consequently, these surfactants are widely used as disinfectants and for general environmental sanitation. In addition, quaternary ammonium compounds are commonly used in various cosmetics (e.g. hair conditioners) and household products (e.g. fabric softeners), and as penetration enhancers in pharmaceutical products. They are considered non-toxic when applied topically to skin but can be highly toxic when ingested (Xue et al 2004). Most quaternary ammonium compounds used as antibacterial agents are chemically stable and relatively resistant towards biodegradation. Low sustained levels of these antibacterial agents in the environment will generate selective pressure on bacteria, allowing them to develop resistance (Sidhu et al 2002; Kümmerer et al 2004). Furthermore, studies have indicated that quaternary ammonium compounds may be one of the groups of compounds responsible for the increased prevalence of allergic airway diseases observed in recent years (Larsen et al 2004). It is possible that both the toxic side-effects and the environmental impact of quaternary ammonium antibacterial agents can be reduced through formation of biologically active but chemically unstable analogues of these agents.

The pharmacological and toxicological profile of a given drug is the combination of the intrinsic biological activity and toxicity of the drug itself and of its metabolites and reactive intermediates (Bodor & Buchwald 2004). In medicinal chemistry (Korolkovas 1988) non-metabolizable drugs or drugs that are metabolized to biologically active metabolites are sometimes referred to as 'hard drugs' (Ariëns 1980), and drugs that are inactivated in one

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metabolic step are called 'soft drugs' (Bodor 1977).[1] In the classical design of hard drugs, one starts with a lead compound, which has a well-defined structure of known biological activity. Structure–activity relationships are then applied to maximize the pharmacological effect. Cationic quaternary ammonium surfactants, such as benzalkonium chloride, are examples of hard antibacterial agents that, analogous to hard drugs, are slowly inactivated in the environment. In soft drug design structure–activity relationships are combined with structure–metabolism (or structure–inactivation) relationships during drug optimization (Thorsteinsson et al 2003a; Bodor & Buchwald 2004). Slow and easily saturable oxidative pathways are avoided in favour of hydrolytic deactivation pathways. The main advantages of this approach in drug design are as follows: first, improvement of the therapeutic index by eliminating the formation of biologically active metabolites and toxic intermediates; second, elimination of many metabolic drug interactions by avoiding saturable enzymatic processes; and third, simplification of the drug pharmacokinetics (Bodor & Buchwald 2003). This leads to the formation of drugs with an enhanced margin of safety (i.e. the soft drug will exhibit a wider therapeutic window compared to its hard analogue). This same approach has been applied in the design of environmentally safe, non-toxic chemicals ('green' chemistry) where structure–activity relationships are combined with structure–environmental deactivation relationships (Bodor 1996).

The first soft antibacterial agents were synthesized by Bodor (Bodor 1977; Thorsteinsson et al 2003a). Since then several soft analogues of long-chain quaternary ammonium compounds have been synthesized, such as soft analogues of cetylpyridinium chloride and benzalkonium chloride (Bodor et al 1980; Thorsteinsson et al 2003b), and chemically labile L-carnitine (Calvani et al 1998) and betaine (Ahlström et al 1999a, b) esters. It has been shown that adequate antibacterial activity can only be obtained if the soft analogues have sufficient chemical stability to allow the soft analogues to express their activity for a sufficient duration of time (Thorsteinsson et al 2003b; Loftsson et al 2004). However, antibacterial agents can only be classified as soft and environmentally friendly if they are deactivated (through chemical or enzymatic degradation) within a relatively short time. Thus, the requirements for adequate antibacterial activity and rapid deactivation appear to contradict each other.

Previously we have described the synthesis and antibacterial and antiviral activity of soft quaternary compounds (Thorsteinsson et al 2003b; Loftsson et al 2004). The purpose of this present study is to investigate further the physicochemical properties of the soft long-chain quaternary ammonium compounds, their hydrolytic mechanism, surface activity and lipophilicity as well as their structure–activity and structure–deactivation relationships.

[1]These are by definition totally unrelated to the classification of illegal dugs into highly addictive hard drugs, e.g. cocaine and heroin, and presumably less harmful soft drugs, e.g. cannabis and LSD.

Materials and Methods

Materials

Synthesis and purification of the soft antibacterial agents (compounds **2**–**3**, **6**–**13**, **15**, **16**, **18**–**22** and **24**–**25**) have previously been described (Thorsteinsson et al 2003b). Lauroylcholine chloride **1**, cetylpyridinium chloride **4** and benzalkonium chloride **26** were purchased from Sigma-Aldrich (Germany). Compounds **14**, **17** and **23** are commercially available intermediates used during the synthesis of the soft agents. All other chemicals used in this study were commercially available and of analytical or regent grade.

Chromatographic conditions

The quantitative determination of the soft antibacterial agents was performed on HPLC equipment from Merck-Hitachi, equipped with a Model AS-4000 autosampler and a temperature-controlled sample rack, and variable wavelength detector operated at 230 or 254 nm. The flow rate was 1.50 mL min^{-1}. The columns and mobile phases were as follows: compound **5**, 150 (length) × 4.6 mm (inner diameter), 5 μm (bead size), C18 RP column, acetonitrile (40–100% v/v), 0.015% w/v octasulfonic acid mobile phase; compound **6**, LiChrosorb-NH2, 5 μm column, water, methanol (80:20), 0.015% w/v octasulfonic acid mobile phase; compound **8**, 150 × 4.6 mm, 5 μm, C18 RP column, acetonitrile, acetic acid, water (90:0.5:9.5); compounds **9**–**15**, 75 mm, 4.6 mm, 3 μm, C18 RP, methanol, 1% v/v acetic acid in water (55:45, 70:30 or 75:25) mobile phases; compounds **16**–**19**, cyanocolumn 150 mm, 4.6 mm, 5 μm, methanol, 0.05 M aqueous phosphoric acid (92:8) mobile phase; compounds **20** and **21**, 75 mm, 4.6 mm, 3 μm, C18 RP column, methanol, 1% v/v acetic acid in water (68:32 and 81:19, respectively) mobile phases. The retention times ranged from 2 to 4 min. For other chromatographic conditions see Thorsteinsson et al (2003b).

Determination of the hydrolytic rate constants

A stock solution (0.1 mg mL^{-1}) of the compound to be tested was prepared in ethanol and 30 μL of the drug stock solution was added to 1.5 mL buffer solution, previously equilibrated to the desired temperature in a temperature-controlled sample rack and mixed thoroughly. The initial concentration of the test compounds in the aqueous buffer solution was 2 μg mL^{-1} (about 10^{-6} M). All reactions were run under pseudo-first-order conditions. Aliquots (10 μL) were injected onto the HPLC column at various time intervals, and the observed pseudo-first-order degradation rate constants (k_{obs}) were determined from the disappearance of the compound by linear regression of the natural logarithm of the peak area vs time plots. The aqueous buffer solutions used in this study were hydrochloric acid (pH 1–2), 10 to 100 mM acetate buffer (pH 3–5), 50 to 200 mM phosphate buffer (pH 6–7.5), borate buffer (pH 8–9) and sodium hydroxide (pH 10) solutions. The ionic strength varied from 0.2 to 0.6.

The rate constants for specific acid (k_H), specific base (k_{OH}) and water hydrolysis (k_O) were determined from the V-shaped pH–rate profiles (Connors et al 1986).

The entropy ($\Delta H^{\ddagger}$) and the enthalpy ($\Delta S^{\ddagger}$) of activation were determined from linear plots of ln(k/T), where k is k_{obs}, vs 1/T based on the Eyring equation:

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{R} \cdot \frac{1}{T} \quad (1)$$

where T is the absolute temperature, k_B denotes Bolzmann's constant, h is Planck's constant and R is the gas constant.

The deuterium solvent isotope effects were measured in D_2O and H_2O at pD/pH 7.0 and pD/pH 8.0, 50 mM phosphate buffer, ionic strength (μ) 0.2 (NaCl) at 70.0 ± 0.2°C. The dielectric constant solvent effects were measured in phosphate buffer pH 8.0 (200 mM, $\mu = 0.6$) and pH 2.0 (200 mM, $\mu = 0.6$), at 70.0 ± 0.2°C and from 0 to 30% (v/v) dioxane. The dielectric constant of the dioxane–water mixture was calculated according to the *CRC Handbook of Chemistry and Physics* (1989).

The pKa values were determined by titration of 0.1 M solutions of the compounds in water with 0.1 M NaOH (i.e. from titration curves) at room temperature.

Determination of the critical micelle concentration

The critical micelle concentration (CMC) was calculated by determining the surface tension of aqueous solutions of the compounds in a digital-tensiometer K9 (Krüss GmbH, Germany) using the Lecomte Du Noüy ring method. The compound to be tested was dissolved in purified water. A series of dilutions was made and the surface tension was determined at room temperature (22°C).

Calculation of the octanol/water partition coefficient and data fitting for the structure–property relationship

The octanol/water partition coefficient (Clog P) was calculated on-line (www.syrres.com) according to structure. Fitting of statistical models and regression analysis of the Clog P values, degradation rate values and antimicrobial activity data to obtain the structure–property relationships was done using the R programming environment for data analysis and graphics developed by W. N. Venables and D. M. Smith and the R development core team. This software is available from www.r-project.org.

Statistical analysis

Statistical analysis of the increasing concentrations of dioxane on the degradation rate of compounds **5** and **8** was performed using the Kruskal–Wallis rank sum test. This test was also used to investigate the difference between specific base-catalysed hydrolyses of the pyridinium acetic acid esters of fatty alcohols. A significance level of $P < 0.05$ denoted significance in all cases. Statistical analysis was done in the R programming environment.

Results

The soft antibacterial agents were designed according to the soft analogue approach by introducing a hydrolytic sensitive linkage into the structure of known hard antibacterial agents (Bodor & Buchwald 2004). The soft compounds consisted of a hydrophilic quaternary ammonium head group, a spacer group and a lipophilic alkyl chain (Table 1).

Previously the pseudo-first-order hydrolytic rate constant of all the soft compounds was determined in aqueous 10 mM phosphate buffer at pH 6.0 and 60.0 ± 0.2°C (Thorsteinsson et al 2003b). In this present study the hydrolytic cleavage of selected soft antibacterial agents was studied further. First, the catalytic effect of buffer ions (i.e. general acid/base catalysis) was evaluated in aqueous 10 to 100 mM acetate buffers (pH 3.0) and 50 to 200 mM aqueous phosphate buffers (pH 7.0). The buffer salts did not accelerate the hydrolysis of compounds **9** and **10** and the effects on compounds **5**, **8** and **20** were insignificant. Thus, the values of the rate constants were not corrected for possible buffer catalysis. The experimentally determined pH–rate profiles were V-shaped with a specific acid-catalysed region at low pH and a specific base-catalysed region at pH above 4 (Figure 1). The experimentally determined pH–rate profiles could be fitted (at pH 1 to 8) to the general equations:

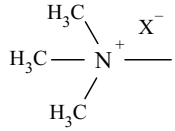
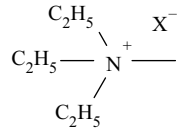
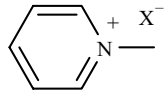
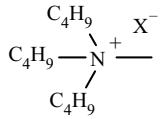
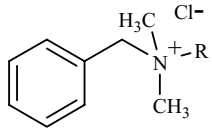
$$k_{obs} = k_H[H^+] + k_O + k_{OH}[OH^-] \quad (2)$$

where k_O is the rate constant for spontaneous (i.e. uncatalysed) water hydrolysis, and k_H and k_{OH} are the specific acid- and specific base-catalysed rate constants for the hydrolysis. The values of k_O, k_H and k_{OH} shown in Table 2 were calculated from the experimentally determined pseudo-first-order rate constants. The observed slope change at pH about 7 (Figure 1) is due to decreased reactivity of compound **8**, which could be due to ionization of the ethylene bridge or reversible hydroxylation of the pyridinium ring (Bunting 1980; Mikhailovskaya et al 2001). Compound **6** was the only observed degradation product of compounds **7–11**. Compound **6** was then slowly degraded to form pyridine. Other compounds degrade at their most vulnerable site, at the ester or amide linkage, forming their original building blocks. Compound **12** was chemically stable.

The entropy of activation ($\Delta H^{\ddagger}$) and the enthalpy of activation ($\Delta S^{\ddagger}$) were calculated from the k_{obs} values determined at 40, 50, 60 and 70°C and pH 7.0 and 8.0. The values of $\Delta S^{\ddagger}$ were almost identical for all the compounds containing an ester linkage at about 186 J mol^{-1} K^{-1} at both pH 7.0 and pH 8.0. The values of $\Delta H^{\ddagger}$ were also very similar for all compounds containing an ester linkage, about 75 kJ mol^{-1} at pH 7.0 and about 50 kJ mol^{-1} at pH 8.0. The relatively high negative $\Delta S^{\ddagger}$ value may indicate a bimolecular reaction.

The deuterium solvent isotope effects (k_{H_2O}/k_{D_2O}) were determined for compounds **5** and **8**. At pH 2.0 and 7.0 the k_{H_2O}/k_{D_2O} ratio was determined to be about 2 for both compounds, indicating that an O–H bond is cleaved in the rate-determining step of the reaction. However, for

Table 1 Compounds evaluated in this study (Thorsteinsson et al 2003a)

Compound*	Head group§	Spacer	Alkyl chain
1		$-CH_2CH_2-$	$-OOC(CH_2)_{10}CH_3$
2		$-CH_2CH_2-$	$-OOC(CH_2)_{10}CH_3$
3	–	$-CH_2CH_2-$	$-OOC(CH_2)_{14}CH_3$
4		$-CH_2CH_2-$	$-CH_2(CH_2)_{14}CH_3$
5	–	$-CH_2-$	$-OOC(CH_2)_{10}CH_3$
6	–	$-CH_2CH_2-$	–OH
7	–	$-CH_2CH_2-$	$-OOC(CH_2)_6CH_3$
8	–	$-CH_2CH_2-$	$-OOC(CH_2)_{10}CH_3$
9	–	$-CH_2CH_2-$	$-OOC(CH_2)_{14}CH_3$
10	–	$-CH_2CH_2-$	$-OOC(CH_2)_{16}CH_3$
11	–	$-CH_2CH_2-$	$-OOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$
12	–	$-CH_2CH_2-$	$-HNOC(CH_2)_{14}CH_3$
13	–	$-CH_2CH_2CH_2-$	–OH
14	–	$-CH_2CH_2CH_2-$	$-OOC(CH_2)_{10}CH_3$
15	–	$-CH_2CH_2CH_2-$	$-OOC(CH_2)_{14}CH_3$
16	–	$-CH_2CO-$	–OH
17	–	$-CH_2CO-$	$-OCH_2(CH_2)_4CH_3$
18	–	$-CH_2CO-$	$-OCH_2(CH_2)_{14}CH_3$
19	–	$-CH_2CO-$	$-OCH_2(CH_2)_{16}CH_3$
20	–	$-CH_2CO-$	$-HN(CH_2)_{11}CH_3$
21	–	$-CH_2CO-$	$-HN(CH_2)_{17}CH_3$
22	–	$-CH_2CH_2CO-$	–OH
23	–	$-CH_2CH_2CO-$	$-OC(CH_2)_{14}CH_3$
24		$-CH_2CH_2-$	$-OOC(CH_2)_{10}CH_3$
25	–	$-CH_2CH_2-$	$-OOC(CH_2)_{14}CH_3$
26		$R = C_8H_{17}$ to $C_{18}H_{37}$	

*Reference hard antibacterial agents: cetylpyridinium chloride (**4**) and benzalkonium chloride (**26**). §X^- = chloride or bromide anion.

compound **5** an almost negligible effect ($k_{H_2O}/k_{D_2O} = 1.05$) was observed at pH 8.0.

The effect of the dielectric constant of the reaction medium on the hydrolytic rate of compounds **5** and **8** is shown in Table 3. The observed rate constants decrease with decreasing dielectric constant of the medium, indicating formation of a charged transition state (i.e. decreased stabilization of the transition state with decreasing dielectric constant of the medium). The effect was significant except for compound **5** at pH 8.0. For

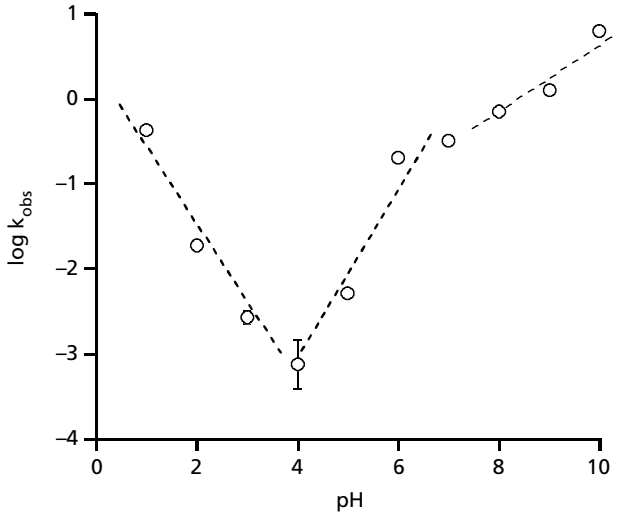


Figure 1 Experimentally determined pH–rate profile for hydrolysis of compound **8** (n = 3) in aqueous buffer solution at 60.0 ± 0.2°C.

Table 2 The rate constants for the specific acid-catalysed hydrolysis (k_H), the specific base-catalysed hydrolysis (k_{OH}) and the uncatalysed hydrolysis (k_O) of the soft antibacterial agents in aqueous buffer solutions at 60.0 ± 0.2°C

Compound	$k_H \times 10^3$ ($M^{-1}\,min^{-1}$)	$k_O \times 10^4$ (min^{-1})	$k_{OH} \times 10^{-3}$ ($M^{-1}\,min^{-1}$)
5	58.4	4.8	54.1
6	0.4	0.3	0.6
8	71.4	0.2	5.6
9	28.0	4.2	1.6
10	19.0	1.5	1.8
11	10.0	0.6	3.3
17	4.5	4.5	63.1
18	19.6	19.6	74.7
19	20.0	20.0	88.8
21	6.0	6.0	3.0

$pK_w = 13.02$ at 60.0°C.

compound **8** a greater effect was observed at pH 2.0 than at pH 8.0.

The pKa of pyridinium acetic acid (compound **16**) was determined to be 2.3, that of pyridinium-2-propionic acid (compound **22**) to be 3.5 and that of pyridinium-2-ethanol (compound **6**) to be 9.4 at room temperature (approx. 22°C).

The calculated octanol/water partition coefficients (Clog P), the CMC values and the logarithm of the degradation constants (log k_{obs}) in hours are displayed in Table 4. However, due to their instability, we were unable to determine the CMC values for compounds **17**, **18** and **19**.

Discussion

As expected, the relative degradation rates of the compounds were greatly affected by the nature of the hydrolytic-sensitive linkage introduced into the structure of the hard parent compound (Table 4). In general, the specific base-catalysed hydrolyses of the pyridinium acetic acid esters of fatty alcohols (compounds **17**, **18** and **19**) are faster than those of the pyridinium methyl esters of fatty acids (compounds **5** and **7**–**11**), the amides are more stable than the esters and the further away the ester or amide linkage is from the quaternary ammonium group the more stable is the compound (Table 5). These observations together with the isotope effect, $\Delta S^{\ddagger}$ values and the effect of the dielectric constant indicate that the hydrolysis proceeds through the conventional $A_{AC}2$ (specific acid-catalysed acyl-oxy cleavage) and $B_{AC}2$ (specific base-catalysed acyl-oxy cleavage) mechanisms. For fatty acid esters the electron-withdrawing effect of the cationic quaternary ammonium group accelerates the $B_{AC}2$ hydrolytic reaction but has an insignificant effect on the $A_{AC}2$ reaction (Table 2). The electron-withdrawing effect of the quaternary ammonium group lowers the pKa values of the quaternary ammonium derivatives of carboxylic acids (pKa 2.3 for compound **16** and 3.5 for compound **22**), making them better leaving groups than the fatty acids (pKa ~ 4.8). Thus, the ester linkage of the fatty acid derivatives is stronger and, consequently, is hydrolysed at a slower rate than the ester linkage of compounds **16** and **22**. The solvent isotope effect for the hydrolysis of compound **5** at pH 7 is about 2 but at pH 8.0 it is close to unity, indicating that the reaction mechanism changes when the pH of the aqueous buffer solution is increased from 7.0 to 8.0. The ammonium-

Table 3 The effect of the dielectric constant (ε) on hydrolysis (n = 3, except n = 2 for compound **5** at pH 8) in aqueous 200 mM phosphate buffer at 70.0°C (± 0.2°C) and ionic strength 0.6 M

Dioxane (% v/v)	ε	k_{obs} (h^{-1}) for compound **5**		k_{obs} (h^{-1}) for compound **8**	
		pH 2.0	pH 8.0	pH 2.0	pH 8.0
0	64.61	0.157 ± 0.002	32.30 ± 1.79	0.080 ± 0.002	1.204 ± 0.026
10	56.44	0.131 ± 0.005	30.91 ± 0.07	0.055 ± 0.002	1.036 ± 0.014
20	48.31	0.103 ± 0.010	30.36 ± 2.07	0.034 ± 0.003	1.002 ± 0.053
30	40.28	0.087 ± 0.004	29.62 ± 0.59	0.012 ± 0.003	0.946 ± 0.045

Table 4 The logarithm of the calculated octanol/water partition coefficient (Clog P), the measured CMC, the logarithm of their half-life in hours (log $t_{½}$, n = 3) in aqueous buffer solution at pH 6.0 and 60°C, their MIC* against *Staphylococcus aureus* and their SI calculated according to equation 4

Compound	$SI \times 10^{-3}$ (m^{-1} h^{-1})	log SI			Experimental values and Clog P				
		Obs.	Calc.	Δ	log MIC	Clog P	log k_{obs}	CMC (mg mL^{-1})	log $t_{½}$
1	4.13	3.62	3.72	−0.11	−4.3	3.41	−0.69 ± 0.01	0.036	0.53 ± 0.01
2	5.27	3.72	4.26	−0.53	−4.4	4.9	−0.68 ± 0.01	0.036	0.52 ± 0.01
3	39.40	4.60	4.98	−0.38	−5.3	6.86	−0.74 ± 0.03	0.017	0.58 ± 0.03
4	~0	–	–	–	−5.9	–	–	0.005	Stable
5	2.48	3.39	3.11	0.28	−3.9	2.03	−0.48 ± 0.02	0.046	0.32 ± 0.02
7	0.41	2.61	2.17	0.44	−2.8	0.01	−0.21 ± 0.00	–	0.05 ± 0.00
8	12.57	4.10	3.21	0.89	−4.8	1.97	−0.69 ± 0.02	0.101	0.53 ± 0.02
9	24.16	4.38	4.05	0.34	−5.6	3.94	−1.26 ± 0.05	0.010	1.10 ± 0.05
10	40.65	4.61	4.38	0.23	−5.7	4.92	−1.06 ± 0.03	0.011	0.90 ± 0.03
11	2.77	3.44	4.31	−0.87	−4.8	4.7	−1.33 ± 0.03	0.012	1.18 ± 0.03
12	~0	–	–	–	−5.3	–	–	0.002	Stable
14	0.96	2.98	3.25	−0.27	−4.9	2.46	−1.97 ± 0.07	0.135	1.81 ± 0.07
15	4.33	3.64	3.51	0.13	−6.1	4.43	−2.46 ± 0.25	0.100	2.30 ± 0.25
17	0.01	1.00	1.84	−0.85	−1.3.	−1.13	−0.31 ± 0.02	–	0.15 ± 0.02
18	0.15	2.17	2.25	−0.09	−1.4.	3.78	0.72 ± 0.03	–	−0.88 ± 0.03
19	0.25	2.40	2.21	0.19	−1.5.	4.76	0.93 ± 0.09	–	−1.08 ± 0.09
20	7.95	3.90	3.79	0.11	−4.9	3.27	−1.03 ± 0.07	0.097	0.87 ± 0.07
21	170.09	5.23	4.35	0.89	−6.2	4.86	−0.97 ± 0.04	0.019	0.81 ± 0.04
23	1.05	3.02	3.70	−0.67	−3.2	4.27	−0.21 ± 0.01	–	0.05 ± 0.01
24	90.89	4.96	4.63	0.33	−5.4	6.37	−0.43 ± 0.02	0.018	0.27 ± 0.02
25	145.38	5.16	5.22	−0.05	−5.4	8.34	−0.28 ± 0.04	0.008	0.12 ± 0.04
26	~0	–	–	–	1	–	–	0.006	Stable

Data generated in this present study and from Thorsteinsson et al (2003b). *The MIC values were determined in a single dilution series. The dilution was 1:1 for each step.

methyloxy function in compound **5** is weakly acidic and this change in isotope effect could be due to formation of anion at higher pH and consequent spontaneous hydrolysis of compound **5** (Loftsson & Fridriksdottir 1990).

Previously we reported that there is no correlation with surface activity and that 1/MIC (minimum inhibitory concentration) is positively correlated with Clog P and $t_{½}$ (Thorsteinsson et al 2003b). The main objective in soft drug design is improvement of the therapeutic index, defined as the ratio between median toxic dose and media effective dose, rather than maximizing the pharmacological effect (Bodor & Buchwald 2004). Higher doses of less potent but much less toxic soft drugs are often preferred over lower doses of more potent but more toxic drugs. Likewise the main objective in the design of safer, non-toxic chemicals is improvement in the safety index (SI). The SI can be defined as the ratio between the median toxic level and the median effective level of a given compound in the environment. For a homologue series of quaternary antibacterial agents that degrade to biologically inactive products the environment impact is proportional to the degradation rate. Thus the SI is calculated as:

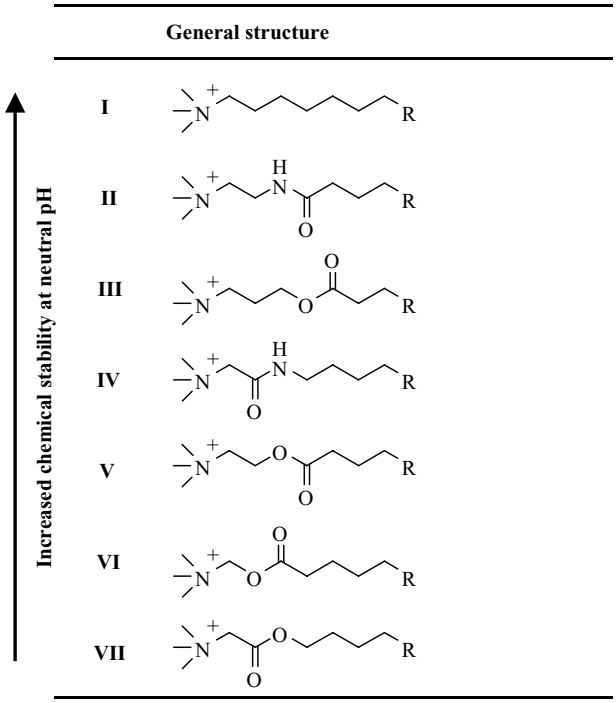
$$SI = \frac{\text{the observed hydrolytic rate constant at pH 6.0 and 60°C } (h^{-1})}{MIC} \quad (3)$$

The SI values of the soft quaternary compounds are listed in Table 4. The best fit for a structure–property relationship for log SI was with a first-order fit for Clog P and a second-order fit for log k_{obs} (data from Table 4; n = 19, s = 0.557, $R^2 = 0.788$, $F_{(3,15)} = 18.6$, $p = 2.60 \times 10^{-5}$):

$$\log SI = 1.95\ (\pm 0.29) + 0.358\ (\pm 0.06)\text{Clog P} - 1.12\ (\pm 0.26)\log k_{obs} - 0.460\ (\pm 0.135)(\log k_{obs})^2 \quad (4)$$

Thus the SI and the antimicrobial activity will increase with increasing Clog P and decreasing log k_{obs}. It has been shown that for the parent 'hard' antimicrobial compounds where the optimum antimicrobial activity is obtained when the alkyl chain is between C12 and C18 (Petrocci 1983), the 'hard' parent compound has an optimum Clog P. The antimicrobial activity of the soft compounds is proportional to Clog P and inversely related to log k_{obs}. According to equation 4 SI has an optimum when the hydrolytic $t_{½}$, at pH 6 and 60°C, is about 11 h (log $k_{obs} = 0.06$). Two of the soft quaternary antibacterial agents in this series have log SI greater than 5, the pyridinium acetamide **21** (log SI = 5.23) and tributyl ammonium ethyl ester **25** (log SI = 5.16). Other compounds tested have low SI due to low antibacterial activity (**5**, **7**, **17**, **18**, **19** and **23**) or relatively high chemical stability (**14** and **15**) or both (**1**, **2** and **8**).

Table 5 The general relationship between chemical stability and the structure of the hydrolytic sensitive linkage under specific base-catalysed conditions (neutral or weakly basic conditions) at 60°C



Based on results presented in this study and from Thorsteinsson et al (2003b).

Conclusions

The soft drug approach was successfully applied in the design of soft quaternary ammonium antibacterial agents. Structure–activity studies showed that the antimicrobial activity of the compounds was strongly influenced by their lipophilicity and chemical stability, the activity increasing with increasing lipophilicity and increasing stability. However, their SI was inversely proportional to their chemical stability and their MIC. Optimization of the soft antibacterial agents through a combination of structure–activity relationship and structure–inactivation relationship resulted in potent but chemically unstable quaternary ammonium antibacterial agents.

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