L-Carnitine Esters as "Soft", Broad-Spectrum Antimicrobial Amphiphiles

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A new class of antimicrobial, "soft", quaternary ammonium L-carnitine esters, of the type $(CH_3)_3N^+$-CH_2-$CHOCO(R_1)$-CH_2-$COO(R_2)$ Cl^-, has been designed, with R_1 and R_2 being in general long-chain alkyl substituents. The series shows good activity against a wide range of bacteria, yeasts, and fungi. Lipophilicity has been measured by RP-HPLC method to give the logarithm of the experimental capacity factor (log k'), and a quantitative relationship has been determined between log k' and the theoretical partition coefficient (CLOGP); also, bond−dipole descriptors have been introduced into calculations by accounting for polar moieties present within the apolar cores of the molecules, giving a more refined calculated capacity factor (log k'_{calcd}). Finally the latter has been related to the antimicrobial activity (MIC values). The proposed models are predictive for the best broad-spectrum antimicrobial compound within the series.

Introduction

Amphiphilic compounds generally are regarded as effective antimicrobial agents;[1] in particular, surface-active quaternary ammonium salts (QUATs) exhibit high activity against Gram-positive bacteria, but their efficacy is lower against Gram-negative bacteria, yeasts, and fungi. Most analogues with increased broad-spectrum antimicrobial activity also have an increased cytotoxicity, which prevents their use in practice;[2] moreover, their resistance to environmental biodegradation advises against any employment in household and agriculture formulations. To overcome these limits, new antimicrobial, "soft" QUATs have been designed, featuring amide or ester structural functionalities:[3] this accounts for their low systemic toxicity, as compared to that of "hard" analogues. It is also important to note that many studies have been recently published which focus their attention on the physicochemical characteristics of QUATs, to better understand their interactions with biological membranes.[4]

In the search of a new class of antimicrobial compounds especially devoted to cure dermatologic infections, hence designed to fight against bacteria and fungi while maintaining low mammalian cell toxicity, L-carnitine[5] (L-carnitine hydrochloride; Chart 1, structure **1**) appeared to us the right candidate to represent the common polar structural motif of quaternary ammonium amphiphiles.[6]

This paper describes design, lipophilicity, and QSAR (quantitative structure−activity relationship) of amphiphilic L-carnitine esters, with long alkyl chains present at either of the oxygenated functions of carnitine itself. The predictable metabolism of these compounds is supposed to produce harmless moieties, thus allowing to classify them as "soft" drugs.[2] The compounds studied are reported in Chart 1. They have been grouped into two sets: members are labeled with **a** or **b** according to the absence or presence, respectively, of dipolar moieties at the alkyl carnitine substituents. The antimicrobial activity of the L-carnitine QUATs was tested with favorable results against Gram-positive and Gram-negative bacteria, yeasts, and fungi. Some polarity variations in the amphipathy of the chains (set **b**) and their effect on the physicochemical properties and antimicrobial activity have also been examined. Moreover, the compounds examined show, as expected, low in vitro cytotoxicity and good in vivo dermal tolerance (data given as Supporting Information).

Chemistry

The preparation of all acylcarnitine alkyl esters (ACAE), with the exception of **4b** and **5b**, has been routinely performed by reaction of the selected *O*-acylcarnitoyl chloride with the appropriate alcohol.[7] The synthetic route which affords **4b** and **5b** is presented in Scheme 1: thus, treatment of nonafluoro-1-iodobutane (**A**) with 5-hexen-1-ol (**B**) (a radical mechanism is supposed) provided a mixture of the required condensation product **C**, as expected on the basis of an earlier report dealing with different substrates,[8] along with the iodo derivative **D** and the olefin **E**. The whole mixture was then subjected to dehydrohalogenation conditions followed by catalytic hydrogenation to afford pure **C**. Finally, condensation of **C** with the appropriate carnitine derivatives gave esters **4b** and **5b**.

Lipophilicity

Although the literature of QUATs is vast,[1−4] not many compounds have been evaluated for lipophilicity parameters. The shake-flask methodology, which is routinely used to calculate the logarithm of the partition

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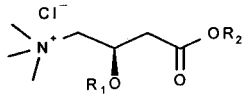
Chart 1. Chemical Structures of L-Carnitine QUATs



no.	R_1	R_2	formula[a]
1	H-	-H	
1a	*n*-$C_6H_{13}CO$-	*n*-$C_{10}H_{21}$	$C_{24}H_{48}ClNO_4$
2a	*n*-$C_8H_{17}CO$-	*n*-C_8H_{17}	$C_{24}H_{48}ClNO_4$
3a	*n*-$C_6H_{13}CO$-	*n*-$C_{11}H_{23}$	$C_{25}H_{50}ClNO_4$
4a	*n*-$C_6H_{13}CO$-	*n*-$C_{12}H_{25}$	$C_{26}H_{52}ClNO_4$
5a	*n*-$C_7H_{15}CO$-	*n*-$C_{11}H_{23}$	$C_{26}H_{52}ClNO_4$
6a	*n*-$C_6H_{13}CO$-	*n*-$C_{13}H_{27}$	$C_{27}H_{54}ClNO_4$
7a	*n*-$C_7H_{15}CO$-	*n*-$C_{13}H_{27}$	$C_{28}H_{56}ClNO_4$
8a	*n*-$C_8H_{17}CO$-	*n*-$C_{13}H_{27}$	$C_{29}H_{58}ClNO_4$
9a	*n*-$C_{10}H_{21}CO$-	*n*-$C_{11}H_{23}$	$C_{29}H_{58}ClNO_4$
10a	*n*-$C_{10}H_{21}CO$-	*n*-$C_{13}H_{27}$	$C_{31}H_{62}ClNO_4$
11a	$(CH_3)_2CHCH_2CO$-	*n*-C_8H_{17}	$C_{20}H_{40}ClNO_4$
12a	$(CH_3)_2CHCH_2CO$-	*n*-$C_{10}H_{21}$	$C_{22}H_{44}ClNO_4$
13a	$(CH_3)_2CHCH_2CO$-	*n*-$C_{11}H_{23}$	$C_{23}H_{46}ClNO_4$
14a	$(CH_3)_2CHCO$-	*n*-$C_{12}H_{25}$	$C_{23}H_{46}ClNO_4$
15a	$(CH_3)_2CHCH_2CO$-	*n*-$C_{12}H_{25}$	$C_{24}H_{48}ClNO_4$
16a	$(CH_3)_2CH(CH_2)_2CO$-	*n*-$C_{11}H_{23}$	$C_{24}H_{48}ClNO_4$
17a	$(CH_3)_2CHCH_2CO$-	*n*-$C_{13}H_{27}$	$C_{25}H_{50}ClNO_4$
18a	$H_3(CH_2)_3CH(CH_3)CO$-	*n*-$C_{13}H_{27}$	$C_{27}H_{54}ClNO_4$
19a	$(CH_3)_2CHCH_2CO$-	*n*-$C_{16}H_{33}$	$C_{28}H_{56}ClNO_4$
20a	$(CH_3)_2CHCH_2CO$-	-CH(*n*-$C_5H_{11})_2$	$C_{23}H_{46}ClNO_4$
1b	$Br(CH_2)_3CO$-	*n*-$C_{11}H_{23}$	$C_{22}H_{43}BrClNO_4$
2b	$(CH_3)_2CHCH_2CO$-	-$(CH_2)_{11}Br$	$C_{23}H_{45}BrClNO_4$
3b	$Br(CH_2)_9CO$-	*n*-C_7H_{15}	$C_{24}H_{47}Br_2NO_4$
4b	$(CH_3)_2CHCH_2CO$-	-$(CH_2)_6C_4F_9$	$C_{22}H_{35}F_9ClNO_4$
5b	*n*-$C_{10}H_{21}CO$-	-$(CH_2)_6C_4F_9$	$C_{28}H_{47}F_9ClNO_4$
6b	*n*-$C_{10}H_{21}CO$-	-$(CH_2)_2C_8F_{17}$	$C_{28}H_{39}F_{17}ClNO_4$
7b	*n*-$C_{11}H_{23}CO$-	-$(CH_2)_2C_6F_{13}$	$C_{27}H_{41}F_{13}ClNO_4$
8b	$(CH_3)_2CHCH_2CO$-	-$(CH_2)_9CH{=}CH_2$	$C_{23}H_{44}ClNO_4$
9b	H-	*n*-$C_{11}H_{23}$	$C_{18}H_{38}ClNO_3$
10b	H-	*n*-$C_{16}H_{33}$	$C_{23}H_{48}ClNO_3$
11b	*n*-$C_{17}H_{35}CO$-	-H	$C_{23}H_{46}ClNO_4$
12b	$C_6H_5O(CH_2)_2CO$-	*n*-C_7H_{15}	$C_{22}H_{36}ClNO_5$
13b	*n*-$C_{10}H_{21}CO$-	-$(CH_2)_2OC_6H_5$	$C_{26}H_{44}ClNO_5$
14b	*n*-$C_{11}H_{23}CO$-	-$(CH_2)_2OC_6H_5$	$C_{27}H_{46}ClNO_5$

[a] All compounds were analyzed within +/- 0.4% of the theoretical value.

coefficient between 1-octanol and water (log *P* values) of most bioactive compounds,[9] fails with surface-active compounds because of the formation of intractable foams. As this was the case with our carnitine derivatives, we resorted to RP-HPLC to calculate the logarithm of the capacity factors (log *k*′) which, for many classes of compounds, is linearly related to the log *P* values.[10] The capacity factors *k*′ were thus determined on an ODS (octadecylsilane) column, by using 75% acetonitrile−phosphate buffer, under isocratic conditions. To take the silanol effect into account, also the k'_{oct} constants were determined by doping the eluent with 0.25% 1-octanol.[11] The values of log k'_{oct} are listed in Table 1; they have been used throughout the course of the present research.

Theoretical partition coefficients (see Table 1), useful to correlate with the capacity factors, were calculated

Table 1. Hydrophobicity and Electronic Parameters of L-Carnitine QUATs

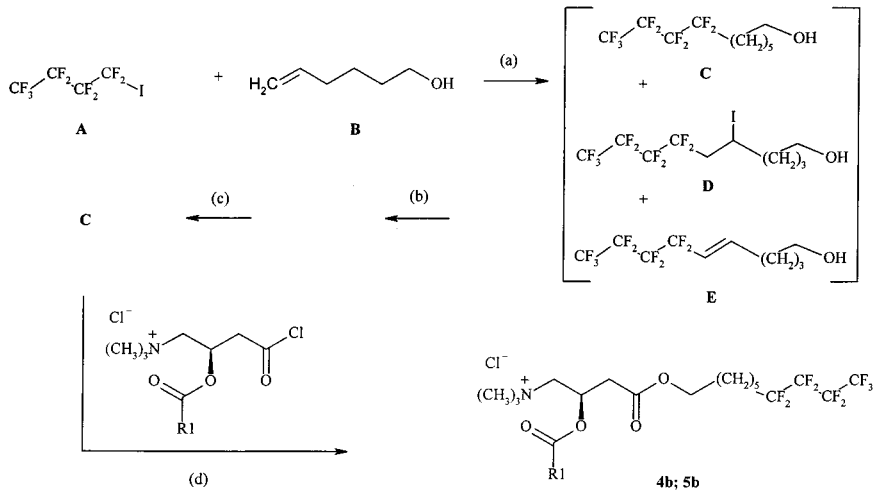
compd	log k'_{oct}	CLOGP[a]	BD_{R1}	BD_{R2}	log k'_{calcd}[a]
1a	0.31	10.00	1.466	0.626	0.39
2a	0.30	10.00	1.466	0.626	0.39
3a	0.35	10.53	1.466	0.626	0.50
4a	0.56	11.06	1.466	0.626	0.62
5a	0.55	11.06	1.466	0.626	0.62
6a	0.68	11.59	1.466	0.626	0.74
7a	0.83	12.12	1.466	0.626	0.86
8a	1.01	12.65	1.466	0.626	0.97
9a	0.96	12.65	1.466	0.626	0.97
10a	1.25	13.70	1.466	0.626	1.20
11a	−0.27	7.66	1.490	0.626	−0.13
12a	0.06	8.81	1.490	0.626	0.12
13a	0.30	9.34	1.490	0.626	0.24
14a	0.19	9.25	1.477	0.626	0.22
15a	0.39	9.87	1.490	0.626	0.35
16a	0.39	9.87	1.457	0.626	0.36
17a	0.51	10.40	1.490	0.626	0.47
18a	0.79	11.37	1.482	0.626	0.69
19a	0.98	11.99	1.490	0.626	0.82
20a	0.15	9.12	1.490	0.690	0.18
1b	0.23	8.80	0.663	0.626	0.25
2b	0.31	9.20	1.490	−1.321	0.38
3b	0.41	9.85	0.663	0.626	0.48
4b	0.01	7.90	1.490	−0.283	0.00
5b	0.61	11.20	1.466	−0.283	0.73
6b	0.67	10.03	1.466	−0.319	0.48
7b	0.57	10.09	1.466	−0.321	0.49
8b	0.16	8.80	1.490	0.479	0.13
9b	0.08	7.02	−0.278	0.626	0.00
10b	0.63	9.67	−0.279	0.626	0.59
11b	0.63	10.58	1.466	0.538	0.52
12b	−0.24	7.27	2.389	0.626	−0.36
13b	0.18	9.27	1.466	0.647	0.22
14b	0.24	9.80	1.466	0.647	0.34

[a] Calculated not taking into consideration the charged nitrogen atom.

by applying the Hansch−Leo fragmental procedure,[12] and according to the procedure used, they are indicated as CLOGP. It should be taken into account that we were forced not to consider into calculations the contribution of the charged nitrogen atom, since the correct parameters relevant to the quaternary ammonium moiety are not available. As a consequence, the numerical values of CLOGPs do not have any absolute validity: nevertheless the whole family of the CLOGP descriptor, being to an approximation the same for all members, can be used for correlations with experimental physicochemical parameters. We have also checked the correctness of this procedure by trying to correlate the log k'_{oct} constants (obtained under our chromatographic conditions) of simple reference QUATs such as pure benzalkonium chlorides with their CLOGPs (neglecting the charged nitrogen atom contribution);[13] results were encouraging since an almost perfect correlation was obtained, with a coefficient of 0.997. With L-carnitine-based QUATs indeed, the CLOGP values correlate fairly well with log k'_{oct} (correlation coefficient 0.88; see Table 2), as shown by the plot in Figure 1, elaborated over all 34 compounds. It is important to note that a much better relationship could be found by leaving out from calculations all the compounds of the **b** group ($r^2 = 0.96$; see Table 2).

If we assume the log k'_{oct} parameter likely to be of value in designing a set of QUATs for general antimicrobial activity, all the structural variations, particularly those introducing dipolar differentiations within the "apolar core" common to all molecules, are to be

Scheme 1. Preparation of L-Carnitine Esters **4b** and **5b**[a]



[a] R_1 is defined in Chart 1. Reagents: (a) Zn powder, CH_2Cl_2, 25 °C, 1 h; (b) CH_3CO_2H, HCl concentrated, 25 °C, 1 h; (c) H_2, Pd/C (10%), EtOH, rt, 4 h; (d) CH_2Cl_2, rt, 3 h.

Table 2. Correlation Parameters (Refer to eq 1) for the Relationship between log k'_{oct} and CLOGP for L-Carnitine QUATs

parameter[a]	compounds **1a**−**20a** (dipolar contributions not considered)	all compounds (dipolar contributions not considered)	all compounds (dipolar contributions considered[b])
a	−2.090	−1.673	−1.535
b	0.244	0.210	0.221
c			−0.158
d			−0.090
r^2	0.963	0.883	0.937
s	0.074	0.120	0.091
r^2_{CV}	0.956	0.865	0.906

[a] r^2, square of the correlation coefficient; s, standard deviation; r^2_{CV}, correlation coefficient calculation by the cross-validation method (leave three out). [b] Dipolar contributions treated according to TSAR 2.4 program.

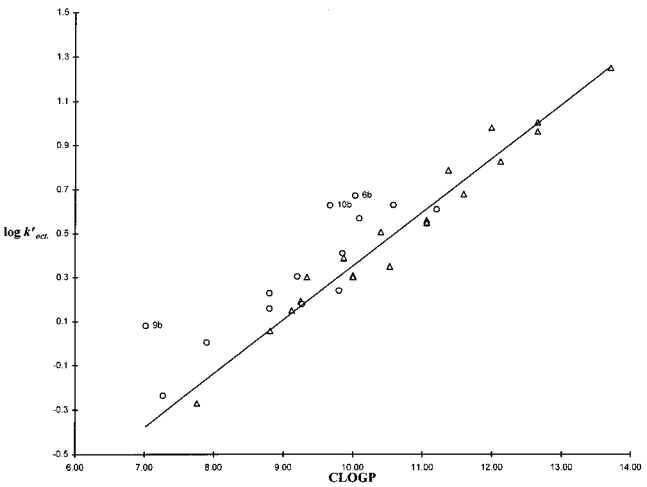


Figure 1. Correlation of the logarithms of the experimental capacity factors, taken on an ODS column doping the eluent with 0.25% 1-octanol, and CLOGP for the 34 L-carnitine esters (set **a**, Δ; set **b**, ○; refer to Table 1 for compound identification). The line refers only to compounds of set **a**.

taken into account. Within the plot of Figure 1, most of the points scattering upward with respect to the line belong to the group **b** compounds: in particular, the highly fluorinated compound **6b** and those carrying the free hydroxy group at C-3 of the carnitine skeleton (**9b** and **10b**) do not show a correctly predicted partition coefficient. It seems plausible that the structural features of these compounds may affect lipophilicity, and hence biological potency, and need, therefore, to be more appropriately described.

To take this phenomenon into account, we have introduced polarity effects into the correlation function between log k'_{oct} and CLOGP by treating the bond−dipole (BD) contribution of each substituent according to the TSAR 2.4 program. Thus BD_{R1} and BD_{R2} descriptors, according to each R_1 and R_2 substituent, have been calculated (see Table 1). It is worth noting that the numerical values of the BD contributions of the pure alkyl substituents are practically constant, only slightly varying according to the branched nature of some of them. Equation 1 is finally derived, which correlates reasonably well log k'_{oct} with all the theoretical parameters ($r^2 = 0.963$; see Table 2):

$$\log k'_{oct} = a + b\mathrm{CLOGP} + c\mathrm{BD}_{R1} + d\mathrm{BD}_{R2} \quad (1)$$

The importance of our original approach to the theoretical evaluation of the polar contributions to lipophilicity parameters of quaternary ammonium salts is pointed out by comparison of the experimental log k'_{oct} values with the predicted capacity factors, which have been calculated according to eq 1. These new parameters, which we refer to as log k'_{calcd}, are reported in Table 1. A new plot is thus obtained and given in Figure 2. Accordingly, we are now able to treat most of the subtle or more evident structural variations present at the substituents of L-carnitine-based QUATs, which exert their effect onto amphipathy and hence chromatographic retention times.

Biological Results and QSAR Studies

Antimicrobial activity of carnitine-based QUATs was measured against several representative strains of Gram-positive and Gram-negative bacteria, yeasts, and fungi. As can be seen from Table 3, most of our L-carnitine amphiphiles are indeed broad-spectrum inhibitors of microorganism growth; their efficacy, however, is inferior against Gram-negative bacteria,

Table 3. In Vitro Antimicrobial Activity of Tested Compounds

	MIC,[a] μg/mL								
	Gram+ organisms		Gram− organisms		yeasts		fungi		
compd	*S. aureus*	*S. faecalis*	*E. coli*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*	*A. fumigatus*	*A. niger*	*M. mucedo*
1a	3.12	3.12	25	50	3.12	3.12	25	25	50
2a	3.12	3.12	25	50	3.12	3.12	12.5	6.25	25
3a	3.12	1.56	25	100	3.12	3.12	3.12	3.12	6.25
4a	3.12	1.56	>100	>100	3.12	3.12	3.12	6.25	12.5
5a	3.12	3.12	25	>100	3.12	>100	3.12	3.12	6.25
6a	6.25	3.12	>100	>100	6.25	3.12	6.25	6.25	12.5
7a	6.25	6.25	>100	>100	3.12	3.12	6.25	3.12	12.5
8a	12.5	6.25	>100	>100	12.5	12.5	100	25	50
9a	>100	3.12	>100	>100	>100	>100	>100	>100	>100
10a	50	25	>100	>100	25	25	>100	100	50
11a	50	50	>100	>100	>100	>100	>100	>100	>100
12a	12.5	6.25	50	>100	3.12	3.12	50	25	100
13a	6.25	3.12	25	50	3.12	3.12	12.5	12.5	50
14a	6.25	3.12	25	100	3.12	6.25	12.5	12.5	25
15a	3.12	3.12	100	>100	1.56	1.56	6.25	6.25	12.5
16a	3.12	1.56	25	>100	1.56	3.12	6.25	6.25	12.5
17a	3.12	1.56	100	100	1.56	3.12	3.12	6.25	6.25
18a	6.25	6.25	>100	>100	3.12	3.12	3.12	6.25	6.25
19a	6.25	3.12	>100	>100	3.12	3.12	6.25	12.5	>100
20a	12.5	3.12	100	>100	25	12.5	>100	50	100
1b	6.25	3.12	50	100	3.12	3.12	12.5	12.5	25
2b	12.5	6.25	100	100	6.25	>100	25	50	50
3b	3.12	3.12	50	100	3.12	>100	6.25	6.25	25
4b	12.5	6.25	>100	100	12.5	25	100	50	50
5b	12.5	12.5	>100	>100	12.5	12.5	25	12.5	50
6b	>100	>100	>100	>100	>100	>100	>100	>100	>100
7b	>100	>100	>100	>100	>100	>100	>100	>100	>100
8b	12.5	3.12	25	50	6.25	6.25	12.5	6.25	12.5
9b	12.5	25	>100	>100	>100	>100	>100	>100	>100
10b	6.25	3.12	>100	>100	6.25	>100	6.25	3.12	6.25
11b	>100	25	>100	>100	>100	>100	>100	>100	>100
12b	>100	>100	>100	>100	>100	>100	>100	>100	>100
13b	6.25	3.12	25	50	3.12	12.5	25	25	50
14b	6.25	3.12	50	100	3.12	3.12	12.5	12.5	25

[a] See the Experimental Section.

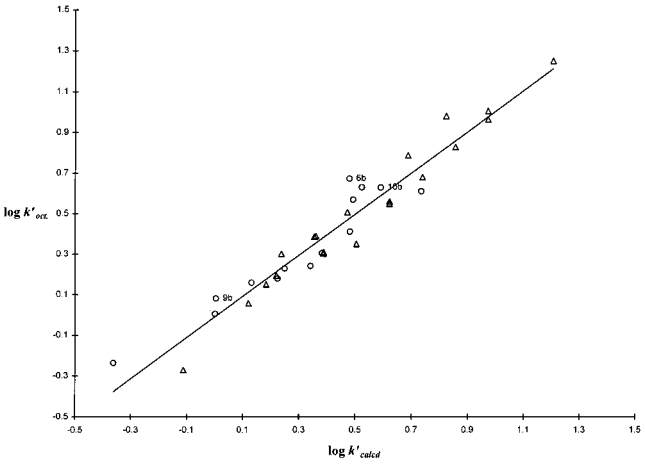


Figure 2. Plot of the logarithms of the experimental capacity factors (log k'_{oct}) vs calculated capacity factors (log k'_{calcd}) according to eq 1 for the 34 L-carnitine esters (set **a**, Δ; set **b**, ○; refer to Table 1 for compound identification).

especially against *Proteus vulgaris*, as compared to the other microorganisms. In general, the activity of the most effective analogues is comparable to, if not largely higher than, that of other "hard" and "soft" amphiphilic compounds.[2] From Table 3, it is also readily apparent that activity in the **a** series is strictly related to the number of carbon atoms in the alkyl and acyl chains. Compounds **3a** and **17a**, characterized by substituents with a total of 18 carbon atoms, demonstrate much higher levels of antimicrobial potency in comparison to the analogues with shorter or longer substituents (**3a** is also active against Gram-negative bacteria).

In general, dealing with compounds with an equal, total number of carbon atoms, it is not possible to correlate the actual potency with the lipophilic contribution relative to the length of each chain (compare, for instance, **1a** with **2a** or **8a** with **9a**), but a possible hint is also that a branched, short acyl group in R_1 seems to increase the in vitro activity (compare **1a** or **2a** with **15a**: activity against yeasts and fungi). In any case, judicious esterification of the carboxy function is required; in **11b** in fact, where the carboxyl is not esterified, activity is null. Conversely, a free hydroxy group at position 3 of the carnitine skeleton is tolerated (compare **10b** with **1a**−**2a** and with **13a**−**14a**). The direct consequence of this behavior is that carnitine QUATs do not lose their antimicrobial efficacy when hydrolyzed at the level of the acyl substituents (compare, for instance, structures and activities of **19a** and **10b**). The products of a possible biodeterioration process which cleaves acylcarnitines are thus still active.

Multiple fluorine atoms at the lipophilic moiety of surface-active compounds are supposed to increase their emulsifying power.[14] Even (perfluoroalkyl)acylcarnitines themselves have been synthesized in view of their possible biomedical use.[15] Consequently, carnitine-based QUATs with a high degree of fluorination at the alkyloxy chain have been synthesized by us and tested; also, extensive modifications at both chains have been carried out, keeping the total number of carbon

atoms in the same range of the most active, unmodified derivatives (see Chart 1, derivatives belonging to the **b** series).

As to the fluoro derivatives, in compounds where the total number of carbon atoms is the same, a decrease in activity is associated with the extent of fluorination (compare **5b** and **6b**). The bromo derivatives **1b**−**3b** are fairly active, and the presence of one bromine atom in the alkyl chain does not seem to alter markedly the antimicrobial activity (compare **1b** with **12a** and **2b** with **13a**). The introduction of a terminal double bond in the alkyloxy chain has the effect of a slight increase of the efficacy against fungi, while this is lower against yeasts (see **8b** and **13a**). The most interesting results have been obtained when introducing a phenoxy group at the terminus of the alkyl chains. This kind of modification at the acyl substituent resulted in almost total loss of activity (derivative **12b**), reasonably due to its very low degree of lipophilicity (see Figure 1), while the presence of the phenoxy group at the other substituent has a favorable effect especially against Gram-negative bacteria (derivatives **13b** and **14b**).

In accordance with the proposed model of the mode of action of antimicrobial amphiphilic compounds, as membrane-active compounds, the measure of lipophilicity is the most reliable tool for a correct prediction of their activity. The partition coefficient is a widely used lipophilicity parameter in QSAR studies;[16] the nature of surface-active compounds for our L-carnitine esters and the inclusion of polar groups within the alkyl chains inhibit the use of both experimental and calculated log *P* values. Nevertheless, the studies exposed in the preceding paragraph allow the application to the QSAR approach of both the experimental and calculated log k' (indicated respectively as log k'_{oct} and log k'_{calcd}) as lipophilicity measures.

As already pointed out, our MIC results clearly show a nonlinear dependence of biological activity on chemical structure. We found that the best mathematical description of our data is obtained by a parabolic model (eq 2),[17,18] where antimicrobial efficacy (MIC), expressed as *C* (mol/L), is related to the calculated measure of lipophilicity:

$$\log 1/C = a(\log k'_{calcd})^2 + b \log k'_{calcd} + c \qquad (2)$$

Figure 3 shows the relevant plot for a Gram-positive bacterium, a yeast, and a fungus; the statistical parameters for all microorganisms (data for Gram-negative bacteria are poor for a thorough description) are reported in Table 4. In all cases, primarily for compounds of set **a**, results appear significant, as the overall trend is that biological activity increases with increasing lipophilicity, and such a behavior is reversed if a certain level of lipophilicity is exceeded (typically, the range of log k'_{calcd} for the most active compounds is 0.3−0.7). Only a few points are reported in the right sides of the curves since all the most lipophilic compounds of the series (log $k'_{calcd} > 1.1$) are devoid of antimicrobial activity (MIC > 100 μg/mL).[19] Also, the two sides of the parabola are more curved in the cases of fungi, while the curvature is less pronounced with yeasts. This different behavior is clearly to be ascribed to the peculiar architectures of exterior cell membranes of different

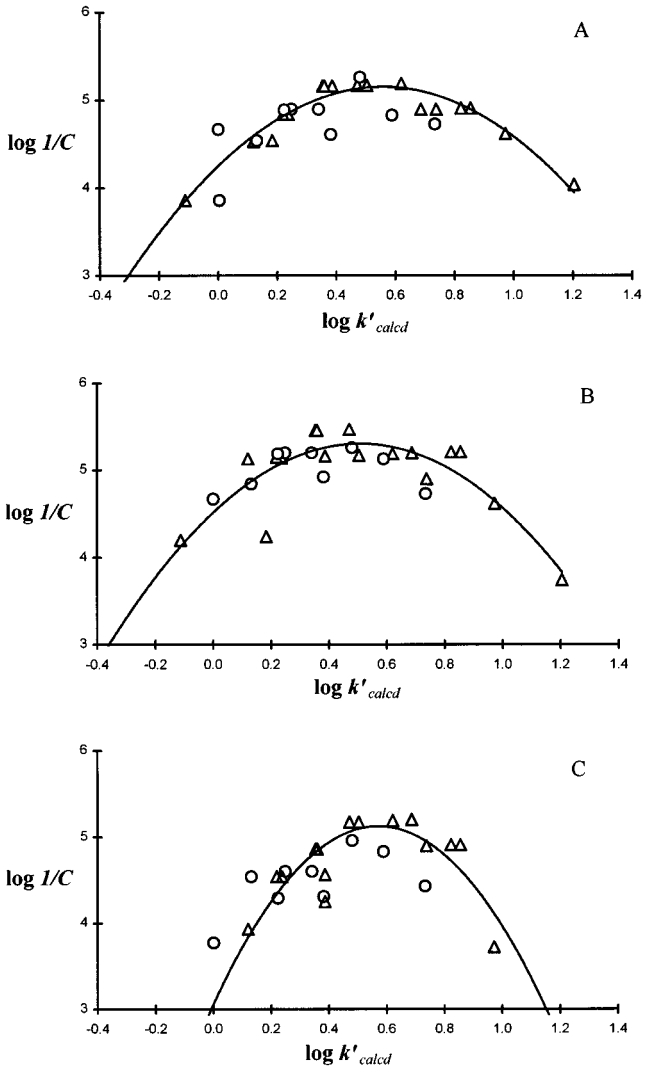


Figure 3. Correlation graphs between calculated capacity factors (log k'_{calcd}) of L-carnitine QUATs and antimicrobial activity against *S. aureus* (A), *C. albicans* (B), and *A. fumigatus* (C) (set **a**, Δ; set **b**, ○; refer to Tables 1 and 3 for compound identification). Data are treated according to eq 2.

microorganisms: in the cases of fungi, appropriate selection of the lipophilic contribution of carnitine QUATs is a more critical point.

Conclusions

In summary, by pursuing the project of new antimicrobial agents for dermatological infections, we have designed a new class of "soft",[3] surface-active, quaternary ammonium salts. The common framework of these amphiphilic compounds is represented by the carnitine moiety responsible for the polar portion of the molecules and two lipophilic chains that esterify both the oxygenated functions of carnitine itself. This class of compounds revealed to possess an effective, broad-spectrum antimicrobial potency, particularly those members characterized by alkyl chains with a total of 16−18 carbon atoms. Some "polar" inclusions within the alkyl chains are also tolerated and, sometimes, even concur to increase their effectiveness. We have also demonstrated that the lipophilic character of our carnitine-based QUATs can be conveniently measured by the logarithms of their capacity factors (log k'_{oct} values)[20] or predicted

Table 4. Correlation Parameters (Refer to eq 2) for the Relationship between Antimicrobial Activity and log k'_{calcd} of L-Carnitine QUATs

		parameters[a]						
strain	compd	*a*	*b*	*c*	*n*	r^2	*s*	log k'_{calcd}(opt.)
S. aureus	set **a** + **b**	−2.63	2.91	4.29	29	0.77	0.19	0.90
	set **a**	−2.80	3.11	4.29	19	0.93	0.11	0.90
S. faecalis	set **a** + **b**	−2.10	2.31	4.61	31	0.45	0.30	0.91
	set **a**	−2.59	2.94	4.52	20	0.79	0.19	0.88
C. albicans	set **a** + **b**	−2.70	2.55	4.68	27	0.81	0.17	1.06
	set **a**	−2.88	2.76	4.68	18	0.88	0.16	1.04
S. cerevisiae	set **a** + **b**	−2.09	2.29	4.58	23	0.63	0.18	0.91
	set **a**	−1.81	1.87	4.75	16	0.77	0.13	0.97
A. fumigatus	set **a** + **b**	−4.22	4.70	3.64	25	0.58	0.29	0.90
	set **a**	−6.14	6.99	3.14	16	0.70	0.27	0.88
A. niger	set **a** + **b**	−2.66	3.12	3.99	26	0.51	0.28	0.85
	set **a**	−3.33	4.00	3.78	17	0.70	0.23	0.83
M. mucedo	set **a** + **b**	−1.85	2.40	3.79	25	0.35	0.30	0.77
	set **a**	−3.14	4.25	3.29	16	0.64	0.26	0.74

[a] *n*, numbers of compounds taken into account; r^2, square of regression coefficient; *s*, standard error of the regression model; log k'_{calcd}(opt.), apex of the parabola. Only compounds with MIC values ≤100 have been considered.

by the theoretical partition coefficients (CLOGP).[21] In this latter case, where "polar" effects have been present in the lipophilic core of the molecules, bond−dipole descriptors have been used to improve the algorithm efficacy and thus the accordance with the experimental lipophilicity parameters. This more refined calculated measure of carnitine-based QUAT lipophilicity (log k'_{calcd}) has been quantitatively related to their antimicrobial activity, revealing a parabolic dependence with a maximum of activity. Our approach makes it possible to design a single carnitine-based QUAT molecule, having a broad spectrum of antimicrobial activity by just measuring its capacity factor or even calculating its theoretical lipophilicity.

Experimental Section

Chemistry. ^{1}H NMR spectra were determined in the indicated solvent on a Varian VXR-300 spectrometer and are reported as δ units (parts per million) downfield from tetramethylsilane as the internal reference. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Syntheses of common intermediate **C** and of compounds **4b** and **5b** have been described, according to Scheme 1.

7,7,8,8,9,9,10,10,10-Nonafluorodecyl Alcohol (C). To 4.53 g (0.045 mol) of 5-hexen-1-ol (**B**) in 30 mL of CH_2Cl_2 were added 3.6 g (0.049 mol) of Zn powder and 15.36 g (0.045 mol) of nonafluoro-1-iodobutane. The mixture was stirred for 2 h at room temperature. An additional 5 mL of CH_2Cl_2 was added, and 45 mL of a solution of water/acetic acid/concentrated HCl (6:3:1) was added dropwise over a period of 2 h. The aqueous phase was separated, the organic phase was diluted with CH_2Cl_2 and washed with 10% NaS_2O_3 (3×) and water, and then it was dried (Na_2SO_4) and evaporated to dryness in vacuo to yield 8 g (58%) of **C** as a pure product (single peak) by HPLC: SCX-SGE column, eluting with CH_3CN/phosphate buffer, 50 mM, pH 4.0, 35:65, v/v; ^{1}H NMR ($CDCl_3$) δ 1.40 (m, 4 H), 1.60 (m, 4 H), 2.50 (m, 2 H), 3.62 (t, 2 H). Anal. ($C_5H_{13}OF_9$) C,H.

Isovaleryl-L-carnitine Chloride 7,7,8,8,9,9,10,10,10-Nonafluorodecyl Ester (4b) and Undecanoyl-L-carnitine Chloride 7,7,8,8,9,9,10,10,10-Nonafluorodecyl Ester (5b). To 0.01 mol of L-acylcarnitine (acyl = isovaleryl (**4b**), undecanoyl (**5b**)), suspended in 15 mL of CH_2Cl_2, was added 0.014 mol of oxalyl chloride. The mixture was stirred at room temperature for 4 h and then evaporated to dryness in vacuo. The residue was dissolved in 15 mL of CH_2Cl_2 and added dropwise under stirring to 0.014 mol of pure **C**. The mixture was then stirred at room temperature for 4 h under N_2 flux and finally concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/CH_3OH to yield **4b** and **5b** as pure products. **4b**: 3.5 g (60%); purity was checked by HPLC on μ-Bondapack C18 column eluting with CH_3CN/phosphate buffer, 50 mM, pH 4.0, 60:40, v/v; $[\alpha]_D^{25} = -12.0$ ($c = 1$, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.9 (d, 6 H), 1.4 (m, 4 H), 1.6 (m, 4 H), 2.05 (m, 3 H), 2.2 (d, 2 H), 2.85 (ddd, 2 H), 3.50 (s, 9 H), 4.05 (m, 2 H), 4.30 (d, 2 H), 5.65 (dd, 1 H). Anal. ($C_{22}H_{35}NO_4F_9Cl$) C,H,N,F,Cl. **5b**: 4.2 g (63%); purity was checked by HPLC on μ-Bondapack C18 column eluting with CH_3CN/phosphate buffer, 50 mM, pH 4.0, 70:30, v/v; $[\alpha]_D^{25} = -10.7$ ($c = 1$, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H), 1.25 (br s, 16 H), 1.35 (m, 4 H), 1.55 (m, 4 H), 2.05 (m, 2 H), 2.30 (t, 2 H), 2.80 (m, H), 3.45 (s, 9 H), 4.00 (m, 2 H), 4.30 (d, 2 H), 5.65 (dd, 1 H). Anal. ($C_{28}H_{47}NO_4F_9Cl$) C,H,N,F,Cl.

Lipophilicity. All chemicals were analytical or chemical grade. Analytical samples were prepared at the concentration of 1 mg/mL in the mobile phase (vide infra), and 20 μL of this solution was then injected for analyses. HPLC runs were performed on a Varian 9010 chromatograph, equipped with a Reodyne 7125 injection port and a Varian 2050 UV spectrophotometer interfaced with a Merck-Hitachi d-2000 recorder. The stationary phase was a μ-Bondapack ODS (particle size 10 μm, 300 mm × 3.9 mm i.d.). The mobile phase was CH_3CN/phosphate buffer, 50 mM, pH 2.5, 75:25, v/v, and 1-octanol was added at a concentration of 0.25%. Flow rate was constantly 1 mL/min.

In Vitro Susceptibility Tests. MICs of the compounds were determined by a microtiter broth dilution assay as detailed previously.[6] The bacterial strains considered were *Staphylococcus aureus* WT (wild-type clinical isolates), *Streptococcus faecalis* WT, *Escherichia coli* WT, *Proteus vulgaris* WT, *Candida albicans* WT, *Saccharomyces cerevisiae* ATCC (American Type Culture Collection) 7752, *Aspergillus fumigatus* ATCC 28212, *Aspergillus niger* ATCC 16404, and *Mucor mucedo* ATCC 7941.

Supporting Information Available: Other bacterial strains assayed (*B. pumilus* WT, *C. tropicalis* WT, *C. krusei* WT, *Fusarium sp* WT, *Penicillium sp* WT, *S. typhimurium* WT, *K. oxytoca*) and in vitro cytotoxicity and in vivo dermal tolerance of representative compounds (3 pages). Ordering information is given on any current masthead page.

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