# Quaternary ammonium salts
# XXXIII.* QSAR of antimicrobially active Niketamide derivatives

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*Dedicated to Professor P. Hrnčiar, DrSc., in honour of his 60th birthday*

Quantitative structure-activity relationships (QSAR) for 13 1-alkyl-3-
-($N,N$-diethylcarbamoyl)pyridinium bromides are reported. Effect of the
alkyl chain length ($m$) variation upon antimicrobial activity against *S.
aureus*, *E. coli*, and *C. albicans*, respectively, expressed as minimum inhibi-
tory concentration (MIC) and upon critical micellar concentration ($C_k$)
which was taken as a measure of lipophilicity of the compounds was
followed. Nonlinear relationships between MIC *vs. m* and $C_k$ were quan-
tified using the parabola and the bilinear model. The bilinear dependence
describes better the experimentally found data and the optimum values for
$C_k$ calculated from these regression equations show that only compounds
with $C_k$ in a certain narrow range around 1 mmol $dm^{-3}$ will exhibit maxi-
mum antimicrobial activity regardless of microorganism strain used in the
tests. This maximum is related to compounds containing 15 to 17 carbon
atoms in their long alkyl chain.

Приводятся количественные соотношения между строением и
активностью (QSAR) для 13 1-алкил-3-($N,N$-диэтилкарбамоил)-
пиридиний бромидов. Наблюдалось влияние изменения длины алкиль-
ной цепи ($m$) на антимикробную активность по отношению к *S. aureus*,
*E. coli* и *C. albicans*, выраженную как минимальная ингибирующая
концентрация (MIC), и на критическую мицеллярную концентрацию
($C_k$), принятую в качестве меры липофильности изучаемых соеди-
нений. Нелинейные зависимости между MIC и $m$, а также $C_k$ были
квантифицированы с использованием параболической и билинейной
модельных зависимостей. Билинейная модель лучше описывает эк-
спериментально полученные данные, и оптимальные значения $C_k$. вы-

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численные на основании регрессионных уравнений, показывают, что лишь соединения, $C_k$ которых находятся в определенном узком диапазоне около 1 ммоль дм$^{-3}$, проявляют максимальную антимикробную активность независимо от вида микроорганизма, используемого при тестировании. Максимальные значения относились к соединениям, содержащим от 15 до 17 углеродных атомов в своей длинной алкильной цепи.

Nicotinamide, which is the building block of nicotinamide coenzymes, seems to be one of the most important compounds for the living system. It can be found in each cell bound in $NAD^+$, $NADP^+$ or in the reduced form of the latter (NADPH). So it is not surprising that there exists an innumerable volume of papers dealing with the topic of nicotinamide. Moreover, after the invention of a new class of calcium channel-blocking agents — the 1,4-dihydropyridines — the interest in reduced or potentially reducible forms of different pyridine analogues is still growing.

It should be mentioned that the interest is stimulated not only by the aim to find new and more biologically active compounds, but also with the effort to gain access to structurally simple models which are close to the biological system.

Some of the interesting compounds from the class of reducible pyridinium salts are the derivatives of N,N-diethylnicotinamide (Niketamide). It is well known [1—7] that the 1-alkyl quaternary derivatives of Niketamide (1-alkyl-3--(N,N-diethylcarbamoyl)pyridinium salts) exhibit distinct biological activity which depends upon the structure of the alkyl chain.

Similarly, the antimicrobial activity of this type of compounds is alkyl chain length dependent. It was shown [8—12] that the most active compounds against e.g. Staphylococcus aureus, Salmonella typhosa, etc. possess a $C_{15}$—$C_{16}$ alkyl chain and that their antimicrobial activity can be related also to their physicochemical properties, e.g. surface activity [8, 10]. No one of the above-mentioned authors, however, has made any quantification in the matter.

In 1968 Lien et al. [13] have quantitatively described the nonlinear relationship between antimicrobial activity (values taken from Ref. [8]) and the Hansch's $\pi$ hydrophobicity parameter by the 2nd order polynomial regression for a series of 1-alkyl-3-(N,N-diethylcarbamoyl)pyridinium chlorides (alkyl = = methyl to eicosyl). They found a significant correlation between antimicrobial activity and lipophilicity of the compounds. It is interesting to mention that Kourai et al. [14, 15] proved the dependence of the antimicrobial activity upon the chain length for a series of 1-alkyl-3-carbamoylpyridinium iodides, however, they report an approximately linear relationship between the critical micellar concentration ($C_k$, which can be used as one of hydrophobicity pa-

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rameters for different amphiphilic compounds [16]) and the minimum inhibitory concentration (MIC).

The purpose of the present investigation is to find out if there is a quantitative structure-activity correlation between the antimicrobial activity of a complete series of 1-alkyl-3-($N,N$-diethylcarbamoyl)pyridinium bromides (alkyl = hexyl to octadecyl) and structure (as a structural parameter the number of carbon atoms $m$ in the alkyl chain was used) and their lipophilicity (characterized by $C_k$). For quantification the parabolic as well as the bilinear approach was used to ascertain the model which more precisely describes the experimentally found data.

## Experimental

Infrared spectra were measured as a Nujol mull or in dichloromethane solutions on KBr windows using a Specord M 80 (Zeiss, Jena) apparatus. Ultraviolet spectra were measured in ethanol (compound $V$ also in methanol and acetonitrile) using a Specord M 40 (Zeiss, Jena) apparatus in silica cells. Results are shown in Table 1. The $R_f$ values are average from five measurements and were run on silica gel plates Silufol®. Developing system: acetone—HCl ($c$(HCl) = 1 mol dm$^{-3}$). Detection: Dragendorf's reagent in the Munier modification.

$C_k$ were determined by tensiometric and by conductometric method as described previously [17, 18]. For calculations the values determined conductometrically were chosen (Table 2).
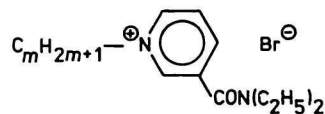
MIC expressed as the lowest concentration of a compound which still hindered the growth of microorganisms was determined by dilution test method according to [19] upon strains from the Czechoslovak State Collection of Type Cultures: *Staphylococcus aureus* Oxford Mau 29/58, *Escherichia coli* 377/79, and *Candida albicans* 45/53, respectively. Results are presented in Table 2.

The prepared compounds are characterized in Table 1. Analytical results of the elements C, H, N were indicated within ± 0.5 % for C and ± 0.2 % for H, N of the theoretical values, respectively. Melting points are uncorrected.

### *1-Alkyl-3-(N,N-diethylcarbamoyl)pyridinium bromides I—XIII*

In dry acetonitrile (30 cm³) $N,N$-diethylnicotinamide (0.1 mol) was dissolved and 1-bromoalkane (0.11 mol) was immediately added. The reaction mixture was warmed up for 12 to 24 h under reflux. After having distilled the solvent off *in vacuo* the drying of the product was completed by azeotropic distillation with toluene or benzene and the viscous residue was crystallized several times from dry ethyl acetate. White, crystalline, very (*I—III*) or slightly (*IV, V*) hygroscopic compounds soluble in water and in polar solvents, insoluble in nonpolar ones were obtained which should be stored in vacuum desiccator over $P_4O_{10}$.

*Table 1*

Characterization of 1-alkyl-3-(*N,N*-diethylcarbamoyl)pyridinium bromides

$$C_mH_{2m+1}-\overset{\oplus}{N}\quad Br^{\ominus}$$
$$CON(C_2H_5)_2$$

| Compound m | Formula $M_r$ | Yield % | M.p. /°C $R_f$ | IR$^a$, $\tilde{v}$/cm$^{-1}$ ν(C—H) | ν(C=O) | ν(N—C)$_{aliph}$ | γ(C—H)$_{ring}$ | UV$^b$ $\lambda_{max}$ nm | log{ε} |
|---|---|---|---|---|---|---|---|---|---|
| *I* | C$_{16}$H$_{27}$N$_2$OBr | 88 | —$^c$ | — | 1625 | 1208 | 820 | 271 | 3.51 |
| 6 | 343.31 | | 0.66 | 3040$^c$ | 1647$^c$ | 1208$^c$ | — | | |
| *II* | C$_{17}$H$_{29}$N$_2$OBr | 90 | 43—46$^d$ | — | 1628 | 1208 | 812 | 271 | 3.58 |
| 7 | 357.34 | | 0.66 | 3040$^c$ | 1647$^c$ | 1208$^c$ | — | | |
| *III* | C$_{18}$H$_{31}$N$_2$OBr | 92 | 75—77$^d$ | 3076 | 1630 | 1191 | 824 | 271 | 3.60 |
| 8 | 371.37 | | 0.66 | | | | | | |
| *IV* | C$_{19}$H$_{33}$N$_2$OBr | 98 | 89—92$^d$ | 3070 | 1628 | 1209 | 814 | 271 | 3.59 |
| 9 | 385.40 | | 0.65 | | | | | | |
| *V* | C$_{20}$H$_{35}$N$_2$OBr | 88 | 85—87 | 3074 | 1628 | 1189 | 822 | 271 | 3.60 |
| 10 | 399.42 | | 0.65 | 3038$^c$ | 1646$^c$ | 1208$^c$ | — | 271$^f$ | 3.60$^f$ |
| | | | | | | | | 272$^g$ | 3.56$^g$ |
| *VI* | C$_{21}$H$_{37}$N$_2$OBr | 90 | 86.5 | 3069 | 1628 | 1189 | 822 | 271 | 3.61 |
| 11 | 413.45 | | 0.63 | | | | | | |
| *VII* | C$_{22}$H$_{39}$N$_2$OBr | 91 | 84—85 | 3072 | 1628 | 1189 | 822 | 271 | 3.62 |
| 12 | 427.48 | | 0.63 | | | | | | |
| *VIII* | C$_{23}$H$_{41}$N$_2$OBr | 88 | 77—79 | 3076 | 1628 | 1191 | 822 | 271 | 3.62 |
| 13 | 441.50 | | 0.56 | | | | | | |
| *IX* | C$_{24}$H$_{43}$N$_2$OBr | 84 | 74 | 3070 | 1628 | 1189 | 824 | 271 | 3.62 |
| 14 | 455.53 | | 0.56 | | | | | | |
| *X* | C$_{25}$H$_{45}$N$_2$OBr | 89 | 73—75 | 3072 | 1628 | 1189 | 824 | 271 | 3.63 |
| 15 | 469.56 | | 0.49 | | | | | | |

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*Table 1* (Continued)

| Compound $m$ | Formula $M_r$ | Yield % | M.p. /°C $R_f$ | IR$^a$, $\tilde{v}$/cm$^{-1}$ | | | | UV$^b$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $v$(C—H) | $v$(C=O) | $v$(N—C)$_{aliph}$ | $\gamma$(C—H)$_{ring}$ | $\lambda_{max}$ nm | log$\{\varepsilon\}$ |
| *XI* 16 | $C_{26}H_{47}N_2OBr$ 483.59 | 94 | 72—73 0.43 | 3074 | 1630 | 1195 | 818 | 271 | 3.63 |
| *XII* 17 | $C_{27}H_{49}N_2OBr$ 497.61 | 98 | 78—79 0.43 | 3074 | 1630 | 1189 | 824 | 271 | 3.62 |
| *XIII* 18 | $C_{28}H_{51}N_2OBr$ 511.64 | 95 | 75—78 0.38 | 3070 | 1628 | 1189 | 820 | 271 | 3.67 |
| Niketamide | $C_{10}H_{14}N_2O$ 178.23 | — — | — | 3032 | 1630 | 1185 | 816 | 257 262 270$^h$ | 3.59 3.59 — |

*a*) Nujol mull; *b*) ethanol; *c*) not determined due to very high hygroscopicity; *d*) capillary tube; *e*) dichloromethane; *f*) methanol; *g*) acetonitrile; *h*) shoulder.

*Table 2*

Aggregation properties and antimicrobial activity of 1-alkyl-3-($N,N$-diethylcarbamoyl)pyridinium bromides used for correlations

| Compound | $-\log(C_k/(\text{mol dm}^{-3}))^a$ | $\log(\text{MIC}^{-1}/(\text{dm}^3 \text{ mol}^{-1}))$ | | |
|---|---|---|---|---|
| | | *S. aureus* | *E. coli* | *C. albicans* |
| *I* | — | $< 2.5357^b$ | $< 2.5357^b$ | $< 2.5357^b$ |
| *II* | 0.3410 | 2.5531 | $< 2.5531^b$ | $< 2.5531^b$ |
| *III* | 0.9787 | 2.7247 | $< 2.5698^b$ | $< 2.5698^b$ |
| *IV* | 1.1906 | 2.9838 | 2.5859 | 2.5859 |
| *V* | 1.3179 | 3.3004 | 2.7563 | 2.9993 |
| *VI* | 1.7020 | 3.9176 | 3.1393 | 3.8383 |
| *VII* | 2.0068 | 4.7282 | 3.3298 | 3.9318 |
| *VIII* | 2.3010 | 4.8665 | 3.7418 | 4.3439 |
| *IX* | 2.5294 | 5.6576 | 3.8133 | 4.0565 |
| *X* | 2.7544 | 5.7212 | 4.0696 | 5.0706 |
| *XI* | 3.1919 | 6.0000 | 3.8392 | 5.7212 |
| *XII* | 3.4005 | 5.6990 | 3.3959 | 5.3979 |
| *XIII* | 3.8632 | 5.1079 | 2.7090 | 4.7077 |
| Ajatin | 2.1079 | 4.5850 | 3.5850 | 4.5850 |
| Septonex | 3.2218 | 5.3279 | 4.3279 | 5.7212 |

*a*) Determined conductometrically; *b*) not included in calculations.

## Results and discussion

The preparation of ammonium salts was performed in acetonitrile. An advantage of this solvent in comparison to benzene, toluene, xylene recommended by literature [8, 10, 11, 20] is that the reaction is completed in 12 to 24 h compared with 34—35 h of reported methods and the yields of final products are very good.

The purity of the products was proved besides of elemental analysis and TLC also by IR spectroscopy (Table 1). The quaternary ammonium salts were compared with the starting $N,N$-diethylnicotinamide. The quaternization of the pyridine nitrogen influences the intensity as well as the position of characteristic bands $\nu(\text{C—H})$, $\nu(\text{C=C})$, which is a consequence of decreasing electron density of the pyridinium ring after quaternization [21—23]. The characteristic band of $\nu(\text{C=O})$ of the amide group is not influenced.

The polarity change of the solvent has shifted the amide carbonyl band to higher wavenumbers (by *ca.* 20 cm$^{-1}$, Table 1). In the spectra of quaternary salts we observed a new band in the region of $\tilde{\nu} = 1189$—1209 cm$^{-1}$ which, according to [22, 23], belongs to stretching mode of N—C$_{\text{aliph}}$.

The UV spectra of 1-alkyl-3-($N,N$-diethylcarbamoyl)pyridinium bromides were also compared with those of starting $N,N$-diethylnicotinamide (Table 1). Niketamide has two intense bands with the maximum at $\lambda = 257$ and $262$ nm, respectively, and a shoulder at *ca.* 270 nm. The $N^1$-alkylation simplifies the spectra and with quaternary salts we detected only one intense band at $\lambda = 271$ nm. *Kosower* has reported [24] in the spectra of 1-alkylpyridinium iodides a second long-wave band which he has ascribed to the electron donor--acceptor interaction of the pyridinium ring with the iodide anion. With polarity change of the solvent this band was significantly shifted. With our compounds we could not find any long-wave band and no solvent effect was observed with the *e.g.* decyl derivative *V* (Table 1) using solvents of different polarity (methanol, ethanol, acetonitrile). It should be mentioned that, according to *Ciusa* and *Lipparini* [25], there is no significant influence of diethylamide group upon the position and intensity of the characteristic band of the quaternary salts investigated. However, an exact interpretation of pyridinium salts spectra is quite difficult and requires a more detailed study [26] which has not been the goal of this work.

It is quite surprising that although the surface-active properties of this type of compounds were investigated [2, 8, 10, 25] in a certain range of concentrations no $C_k$ were reported. The $C_k$ (Table 2) *vs. m* follow a linear relationship and as shown by statistical analysis this regression equation is significant at high confidence limits

$$\log\{C_k\} = (1.562 \pm 0.111) - (0.295 \pm 0.009)m$$

$$n = 12,\ r = 0.996,\ s = 0.103,\ F = 1185.6$$

(Deeper insight into the aggregation properties of these compounds will be published elsewhere.)

From the results of antimicrobial activity (Table 2) clearly follows the nonlinear pattern in relation to structure as well as lipophilicity. Nonlinear relationships between lipophilicity or structure of congeneric drugs and their biological activities are well documented. For quantification purposes the parabolic Hansch's model or Kubinyi's bilinear model were adopted (see *e.g.* [27—31]). The parabolic model has an approximative character due to experimentally proved fact that the ascending part of the relationship is quite linear — and when working with an appropriate complete set of compounds, this holds also for the descending side of the curve. Because the parabola is curved in its ascending as well as descending part one will in the linear parts always find systematic deviations of experimentally observed values from those calculated. First *Franke* [32], then *McFarland* [33, 34] and finally *Kubinyi* [35—37] tried to overcome these difficulties. The Kubinyi's bilinear equation

*Table 3*

Regression coefficients for parabolic (P) and bilinear (BL) relationships between antimicrobial activity, lipophilicity, and structure for 1-alkyl-3-($N$,$N$-diethylcarbamoyl)pyridinium bromides

($\log(1/\text{MIC}) = f(m)$ (*), $\log(1/\text{MIC}) = f(\log C_k)$ (§))

$$\log(1/\text{MIC}) = A + Bx + Cx^2 \quad x = m, \log C_k$$
$$\log(1/\text{MIC}) = Am + B\log(\beta 10^m + 1) + C$$
$$\log(1/\text{MIC}) = A\log C_k + B\log(\beta C_k + 1) + C$$

| Eqn | Relationship | $A$ | $B$ | $C$ | $\log \beta$ | $n$ | $r$ | $s$ | $F$ | Optimum $m$ $C_k$/(mol dm$^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | | | | | | | | | | |
| 1 | (*) BL | 0.448 $\pm 0.023$ | −1.068 $\pm 0.138$ | −0.868 $\pm 0.269$ | −16.061 | 12 | 0.990 | 0.201 | 219.6 | 15.9 |
| 2 | (*) P | −4.185 $\pm 1.651$ | 1.113 $\pm 0.279$ | −0.031 $\pm 0.011$ | — | 12 | 0.959 | 0.403 | 51.1 | 17.7 |
| 3 | (§) BL | 6.692 $\pm 1.500$ | −8.301 $\pm 1.600$ | 33.471 $\pm 6.042$ | 3.861 | 12 | 0.976 | 0.305 | 92.2 | −3.2424 $5.7 \times 10^{-4}$ |
| 4 | (§) P | 1.076 $\pm 0.573$ | −2.372 $\pm 0.591$ | −0.303 $\pm 0.135$ | — | 12 | 0.939 | 0.488 | 33.4 | −3.9162 $1.2 \times 10^{-4}$ |
| *E. coli* | | | | | | | | | | |
| 5 | (*) BL | 0.268 $\pm 0.012$ | −0.892 $\pm 0.041$ | 0.152 $\pm 0.146$ | −15.491 | 10 | 0.994 | 0.067 | 277.7 | 15.1 |
| 6 | (*) P | −7.692 $\pm 1.720$ | 1.636 $\pm 0.263$ | −0.058 $\pm 0.010$ | — | 10 | 0.929 | 0.223 | 21.9 | 14.1 |
| 7 | (§) BL | 3.045 $\pm 0.222$ | −4.255 $\pm 0.287$ | 14.814 $\pm 0.814$ | 3.212 | 10 | 0.986 | 0.099 | 125.2 | −2.8113 $1.5 \times 10^{-3}$ |

Table 3 (Continued)

| Eqn | Relationship | $A$ | $B$ | $C$ | $\log \beta$ | $n$ | $r$ | $s$ | $F$ | Optimum $m$ $C_k/(\text{mol dm}^{-3})$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | (§) P | −0.781 ±0.483 | −3.521 ±0.420 | −0.670 ±0.084 | — | 10 | 0.956 | 0.176 | 37.0 | −2.6287 $2.4 \times 10^{-3}$ |
| C. albicans | | | | | | | | | | |
| 9 | (∗) BL | 0.396 ±0.043 | −1.719 ±0.456 | −0.881 ±0.553 | −17.161 | 10 | 0.965 | 0.300 | 46.7 | 16.6 |
| 10 | (∗) P | −6.032 ±3.205 | 1.277 ±0.490 | −0.036 ±0.018 | — | 10 | 0.931 | 0.415 | 22.7 | 17.5 |
| 11 | (§) BL | 5.640 ±1.850 | −7.249 ±2.022 | 28.783 ±7.506 | 3.862 | 10 | 0.957 | 0.330 | 37.8 | −3.3173 $4.8 \times 10^{-4}$ |
| 12 | (§) P | −0.694 ±1.108 | −3.350 ±0.963 | −4.800 ±0.192 | — | 10 | 0.934 | 0.405 | 24.0 | −3.4906 $3.2 \times 10^{-4}$ |

$n$ — Number of data points used in deriving the regression equations; $s$ — standard deviation from regression; $r$ — correlation index; $F$ — values of the Fischer—Snedecor test. The values in parentheses are the 95 % confidence intervals.

$$\log(1/C) = a \log P - b \log(\beta P + 1) + c$$

where $C$, $P$, $\beta$ are the biological response, the apparent partition coefficient, the nonlinear parameter and $a$, $b$, $c$ are linear parameters, can be used for description of all nonlinear dependences where two linear sides with different slopes are separated by a nonlinear part. Indeed the bilinear model and its modifications are ideally suited to describe nonlinear structure-activity relationships and almost in every case they are superior to the parabolic ones.

From the results shown (Table 3) one can see:

*a*) all relationships are nonlinear in nature;

*b*) both approaches *viz.* parabolic and bilinear describe the relationships at high confidence limits;

*c*) the bilinear model is — based on statistical analysis — the superior one, however, in some cases the differences between parabola and bilinear relationships are not too great (*e.g.* eqns *3, 4* and *11, 12*, respectively).

Nevertheless, by more detailed inspection of the data and relationships one can see that the optimum values calculated using the bilinear model are much more close to experimental ones than those calculated from equations for parabola.

Because the antimicrobial activity of quaternary ammonium type surfactants can entirely be ascribed to their influence upon membranes and processes related to membranes [38] the differences in the microorganisms membrane architecture will influence also the activity of the congeners. This is demonstrated by a shift of maximum in antimicrobial activity observed with *C. albicans* and *E. coli* (compared to *S. aureus*) (Table 3). However, the shift is not too great.

With optimum $C_k$ values (used as a measure of lipophilicity of the compounds) this quantitative study has proved again our finding published in 1985 [39] namely that regardless of microorganism strain used only compounds with $C_k$ values within a certain, however, narrow range around 1 mmol dm$^{-3}$ will possess maximum antimicrobial activity. These values were observed with compounds having 15 to 17 carbon atoms in their long chain. The 1-alkyl-3- -(*N,N*-diethylcarbamoyl)pyridinium bromides are not — from this point of view — exceptions from the above-mentioned generalization. (For more detailed study on nonlinear relationships of antimicrobially active surfactants, the so- -called "cut-off" effect, see Ref. [40].)

The most antimicrobially active compounds (Table 2) are superior to benzyldodecyldimethylammonium bromide (Ajatin) as well as to [1-(ethoxycarbonyl)pentadecyl]trimethylammonium bromide (Septonex), both disinfectants broadly used in different medicinal preparations for external use.

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