Polish Journal of Microbiology
2014, Vol. 63, No 2, 231–236

ORIGINAL PAPER

Dibasic Esters of *ortho*-/*meta*-Alkoxyphenylcarbamic Acid Containing 1-Dipropylamino-3-piperidinopropan-1-yl and Their Antimicrobial Activity

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Submitted 24 November 2013, revised 5 March 2014, accepted 12 May 2014

A s t r a c t

In Europe, the presence of microorganisms that have become resistant to antimicrobials as the most significant disease threat has remained. The aim of the current research was to screen the *in vitro* susceptibility of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* to the series of dibasic esters of *ortho*-/*meta*-alkoxyphenylcarbamic acid previously known for their local anaesthetic effectiveness and to contribute for the structure – antimicrobial potency relationships study within that class of the compounds. The antimicrobial activity investigation involved determination of the minimum inhibitory concentration (MIC) by applying the microdilution method; quantitative screening was performed on a blood agar (*S. aureus*), Endo agar (*E. coli*) or on Sabouraud's agar (*C. albicans*). The activity against all the microorganisms tested was primarily influenced by the position of alkoxy side chain attached to lipophilic aromatic ring and by its length as well. Inspected *meta*-alkoxy substituted derivatives have shown higher efficiency against all chosen microorganisms than their *ortho*-alkoxy positional isomers. The most promising results were observed when investigating the activity of *meta*-alkoxy substituted molecules against *E. coli* with the estimated MICs in the range of 12–49 μg/ml. Furthermore, such potency was found to be quasi parabolically dependent on alkoxy chain length achieving a maximum for *meta*-hexyloxy derivative which has shown MIC = 12 μg/ml. Considered compound was also regarded as the most effective against *S. aureus* with MIC = 98 μg/ml. Evaluating the potency against *C. albicans*, it was revealed that no molecule within the tested set displayed MIC < 100 μg/ml.

K e y w o r d s: *Escherichia coli*, *Staphylococcus aureus*, alkoxyphenylcarbamic acid

Introduction

The incidence of invasive microbial infections caused by opportunistic pathogens, often characterized by high mortality rates, has been increased over the past two decades (Sharma *et al.*, 2009). The majority of antibiotics currently applied in therapy are connected with drug clasess discovered before 1970 (Livermore, 2011). Not surprisingly, the recent expansion of antibacterial and antifungal drug research has occurred because there is an incessant need for developing new compounds to fight life-threating infections (Ziemska *et al.*, 2013). The rational design of new drugs based on the relatively older molecules, the discovery of the agents with novel modes of action, seeking alternate drug targets or the innovative application of the principles of pharmacokinetics and pharmacodynamics aiming to improve the use of existing drugs could offer promising possibilities in the treatment of bacterial and fungal diseases (Enquist *et al.*, 2012; Livermore, 2011; Perez *et al.*, 2008; Ziemska *et al.*, 2013). Furthermore, many of the currently available drugs are toxic, they also enable recurrence because of being bacteriostatic/fungistatic and not bactericidal/fungicidal (Sharma *et al.*, 2009). In addition, recent years have brought the anthropomorphic problem of antimicrobial resistance which appears to be accelerating, accumulating and worldwide (Okele, 2009; Wise, 2008).

As a consequence of the above, the objective of the current study was to investigate *in vitro* the susceptibility of some selected clinically significant microbial strains, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, to dibasic esters of *ortho*-/*meta*-alkoxyphenylcarbamic acid. The evaluated substances, whose general chemical structure is drawn in Table I, belong to a broad class of compounds which have been previously known particularly due to their significant local anaesthetic activity (Pokorná, 1998). In terms of an

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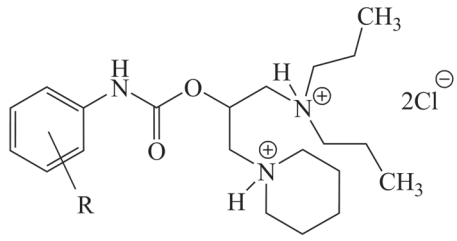
Table I
The *in vitro* activity of evaluated compounds CK-3624-CK-3637 against selected microbial strains



Entry	*R*	MIC (μg/ml)		
		S. aureus	*E. coli*	*S. aureus*
CK-3624	*ortho*-OC_4H_9	6250	781	6250
CK-3625	*ortho*-OC_5H_{11}	6250	1563	6250
CK-3626	*ortho*-OC_6H_{13}	3125	3125	781
CK-3627	*ortho*-OC_7H_{15}	6250	6250	391
CK-3634	*meta*-OC_4H_9	195	49	3125
CK-3635	*meta*-OC_5H_{11}	391	24	1563
CK-3636	*meta*-OC_6H_{13}	98	12	391
CK-3637	*meta*-OC_7H_{15}	781	49	391

antimicrobial inspection of such molecules, the first preliminary reference has propably come from the research paper of Mlynarčík and Čižmárik (1976). Their results have shown that some piperidinoethyl esters seemed to be effective inhibitors of microbial growth, in particular Gram-positive bacteria, yeasts and fungi. Those authors also reported that the most active substance against *S. aureus* exhibited MIC = 0.5 μg/ml.

Given those results, the need for more extensive profile of antimicrobial effectiveness of another series of variously substituted alkoxyphenylcarbamic acid-based compounds (Fig. 1) continuously has arisen (Čižmárik *et al.*, 1983; 1986; Malík *et al.*, 2012; Mlynarčík and Čižmárik, 1979). Furthermore, the eventual antimicrobial efficiency of such compounds which have shown local anaesthetic activity would be highly welcome in terms of the health status of patients and would supplement primary pharmacological effect as well. Additionally, another advantage might be their inhibitory effect towards microorganisms in a concrete drug form or at site of application (Mlynarčík *et al.*, 1991).

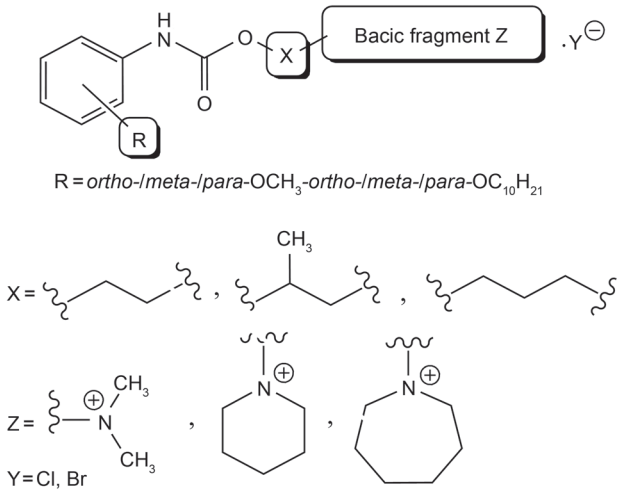


Fig. 1. General chemical structure of previously antimicrobially *in vitro* investigated monobasic esters of *ortho*-/*meta*-/*para*-alkoxyphenylcarbamic acid.

Experimental

Materials and Methods

Chemistry. The preparation of currently *in vitro* evaluated compounds labelled as **CK-3624**-**CK-3627** and **CK-3634**-**CK-3637** (Table I), chemically 1-(dipropylamino-3-piperidinopropan-1-yl)-2-/3-alkyloxyphenylcarbamates with alkyloxy side chain represented by butoxy-heptyloxy substituent in *ortho*- or in *meta*-position, local anaesthetic activities as well as acute toxicity indices has already been published (Csöllei *et al.*, 1993).

The determination of some fundamental physicochemical parameters of these molecules, *i.e.* solubility profile, dissociation constant pK_a and lipophilicity descriptors (log P_{exp} estimated by shake-flask method in the octan-1-ol/buffer medium with pH = 7.3, log k' from RP-HPLC, R_M from RP-TLC), with appropriate readouts can be found in an earlier paper (Malík *et al.*, 2007).

In vitro antimicrobial activity assay

Microorganisms. The antimicrobial activity of **CK-3624**-**CK-3627** and **CK-3634**-**CK-3637** was investigated against Gram-positive bacterium *S. aureus* ATCC 6538 (*Micrococcaceae*), Gram-negative bacterium *E. coli* CNCTC 377/79 (*Enterobacteriaceae*) and the yeast *C. albicans* CCM 8186 as well. These tested bacterial strains were purchased from American Type Culture Collection (Manassas, United States of America) and Czech National Collection of Type Cultures (Prague, Czech Republic), yeast was obtained from Czech Collection of Microorganisms (Brno, Czech Republic).

Culture media. Blood agar, Endo agar and Sabouraud's agar (Imuna, Šarišské Michaľany, Slovak Republic) were used for cultivation of the microorganisms listed in the previous section of this paper. Blood agar was prepared by adding 10% of defibrinated sheep blood to the melted and cooled (50°C) competent components.

Determination of minimum inhibitory concentration (MIC). The MIC values of investigated compounds were carried out by following the modified procedure described previously (Malík *et al.*, 2012). The respective test compounds were dissolved in distilled water. Standard suspension of bacteria was prepared from their 24 h

cultures which were cultivated on blood agar (Gram-positive bacteria) and Endo agar (Gram-negative bacteria). Standard suspension of *Candida* was prepared from 48 h cultures cultivated on Sabouraud's agar.

Prepared suspension contained of 5×10^7 colony forming unit (CFU) *per* ml of bacteria and 5×10^5 CFU/ml of *Candida*, respectively. UV/VIS spectrophotometry was used for the determination of the microorganisms concentration. For the measurements, the UV/VIS range Jenway spectrophotometer, model 6305 (United Kingdom) was used. All evaluated suspensions were adjusted to the absorbance value of 0.35 at the wavelength of 540 nm.

A suspension of the microorganisms was added in amount of 5 μl into the solutions of evaluated substances (100 μl) and to double concentrated peptone broth medium (8%) for bacteria or to Sabouraud's medium (12%) for *Candida*. The peptone broth and Sabouraud's media were purchased from Imuna (Šarišské Michaľany, Slovak Republic).

Starting concentration of prepared stock solutions was 50 000 μg of respective compound *per* 1 ml of distilled water. These stock solutions (5 %) were then serially diluted twofold and the final concentrations were 25 000, 12 500, 6 250, 3 125, 1 563, 781, 391, 196, 98, 49, 25, 12 and 6 μg/ml, respectively.

Quantitative screening. The screening was performed using sterile 96-well plastic microtiter plates (with round-bottomed wells) with matching covers. Microorganisms were incubated in each well at 37°C for 24 h. Upon completion of this process, the volume of 5 μl of evaluated suspension was taken from each well by using a transferring tool and cultured on blood agar (*S. aureus*), Endo agar (*E. coli*) or on Sabouraud's agar (*C. albicans*). Petri dishes were then incubated for 24 h at 37°C.

The positive control using only an inoculation of microorganisms and the negative control of solvent were realized parallelly. The nutrient concentration remained stable in each well, only the concentration of inhibitory compound changed. All experiments were performed in duplicate. The MIC was considered to be the lowest concentration of the tested compound which inhibited visible microbial growth (Andrews, 2001). The MIC was dependent on the presence/absence of the culture on used solid media after the transfer of 5 μl of suspension from each well. The values of MIC were reported in Table I in μg/ml units.

Results and Discussion

From the chemical viewpoint, the common feature of the alkoxyphenylcarbamic acid-based molecules, which have been the objectives of previously published papers (Čižmárik *et al.*, 1983; 1986; Mlynarčík and Čižmárik, 1979), has been the introduction of only one nitrogen atom within their basic part (Fig. 1).

On the contrary, the currently inspected compounds from both series **CK-3624**-**CK-3627** and **CK-3634**-**CK-3637** were considered unique in terms of the presence of a dibasic 1-dipropylamino-3-piperidinopropan-1-yl fragment. That aspect could significantly influence their activity against selected the microorganisms *S. aureus*, *E. coli* and *C. albicans* as well. Besides being clinically significant microbial strains, these microorganisms were chosen for current *in vitro* evaluation due to the possibility to compare the acquired data related to the substances **CK-3624**-**CK-3627** and **CK-3634**-**CK-3637**, respectively, to the results from previous effectiveness determination of monobasic esters of alkoxyphenylcarbamic acid. In the experiments carried out previously, the susceptiblity of the same bacterial strains as well as the same yeast to mentioned esters was *in vitro* screened.

The results of current antimicrobial evaluation are indicated in Table I. In general, all the investigated compounds have shown relatively improved activity against Gram-negative bacteria when compared to their potency against the Gram-positive one or anticandidacidal efficiency.

Assuming the presence of two basic centres within the structure of inspected compounds **CK-3624**-**CK-3627** and **CK-3634**-**CK-3637**, respectively, their activity against *S. aureus* could be influenced by at least these factors: (i) by the position of alkoxy side string (positional isomerism) directly attached to lipophilic aromatic moiety and (ii) by their acidobasic (pK_a values) and lipohydrophilic ($\log P_{exp}$ data) properties.

Current experimental findings revealed that the steric and the electronic effects, which were connected with the alkoxy side chain positional isomerism, were found to be the crucial factors conducive to the antimicrobial activity of the studies molecules. As indicated, *meta*-alkoxy substituted compounds, **CK-3634**-**CK-3637**, with the substituent attached to the position 3 of the lipophilic phenyl ring, exhibited the MICs against *S. aureus* in the range of 98–781 μg/ml. In addition, such derivatives were considerably more active than their *ortho*-positional isomers **CK-3624**-**CK-3627**, containing alkoxy substituent attached to the position 2 of the aromate (Table I).

From the chemical viewpoint, the explanation of different level of an antibacterial efficacy could be in the fact that the proximity of *ortho*-alkoxy side chain to carbamoyloxy group (NHCOO) meant the twist of an aromatic ring plain towards given moiety. Described process then resulted in the planarity violation of considered compounds which led to subsequent conjugation of aromatic ring π-bonds over the amino fragment up to carbonyl.

The outcome of introduced process was different electronic density (charge) on carbonyl moiety which could be one of the possible binding sites to reactive *S. aureus* membrane locations. The transfer of alkoxy chain to *meta*-position meant the avoidance of mentioned secondary steric phenomenon.

The cell wall of *S. aureus* consists of some fundamental units (Dmitriev *et al.*, 2004; Endl *et al.*, 1983; Schneewind *et al.*, 1995): murein, teichoic acids and wall-associated surface proteins. It was documented (Mlynarčík *et al.*, 1991) that structurally similar alkoxyphenylcarbamic acid esters were bonded to phospholipids and proteins forming the cell wall of *S. aureus* by hydrophobic bonds and after the incorporation into the membrane bilayer by electrostatic bonds. It could be assumed that the compounds under the study interacted with the membrane phospholipids and the planarity maintenance of the aromate within the structure of the substances **CK-3634**-**CK-3637**, *i.e.* the presence of the areas with negative electrostatic potentials, provided possible electron donor sites. In addition, at physiological p*H*, phosphate and carboxylate groups of membrane´s phospholipid and lipoprotein domains are negatively charged (Remko and Van Duijnen, 1983).

In agreement with the knowledge about the physicochemical properties of currently investigated derivatives, firstly the protonization of aliphatic amine (dipropylamino group) proceeded followed by the protonization of cyclic amine (piperidin-1-yl fragment). At considered acidobasic conditions, the basic moieties of evaluated compounds were partially ionized, as reported in a previous paper by Malík *et al.* (2007).

Consequently, the interaction of the negatively charged phosphates of the phospholipids with polar proton donor groups could represent one of the possible types of drug-receptor interactions (Remko and Van Duijnen, 1983). The higher number of protonated basic (amino) centers within the chemical structure of the inspected compounds, the more likely will be their interaction with negatively charged carboxylate or phosphate fragments.

Furthermore, the lipophilicity of *meta*-alkoxy substituted molecules **CK-3634**-**CK-3637** was not found as the factor proportionally influencing their potency against *S. aureus*. The hexyloxy derivative **CK-3636** was regarded as the most active (MIC = 98 μg/ml) despite the fact that it was not the most lipophilic one from the whole analyzed set. For illustration, previously estimated log P_{exp} readout (Malík *et al.*, 2007) in the partition system octan-1-ol/phosphate buffer medium (p*H* = 7.3) for **CK-3636** was set to 4.00, however, heptyloxy substituted molecule **CK-3637** was slightly more lipophilic (log P_{exp} = 4.13).

Anyway, the current statement based on the experiments was not quite completely in line with previous observations of the Čižmárik research team (Čižmarik *et al.*, 1987). Čižmárik and coworkers suggested that more lipophilic piperidinoethyl esters of alkoxyphenylcarbamic acid, with a suitable position of alkoxy side chain, displayed relatively higher efficiency.

In terms of possible further practical applications, the compound **CK-3636** would be probably less effective than vancomycin or telavacin (Gould *et al.*, 2011; Hsu *et al.*, 2008; Putnam *et al.*, 2010), well-known drugs currently used in therapeutic practice. Vancomycin has long been regarded as conventional and the most-used option for initial treatment for severe methicillin-resistant *S. aureus* infections.

Current MIC breakpoints define the vancomycin susceptibility lower than 2 μg/ml, as referred in paper of Gould *et al.* (2011). On the other hand, limited outputs also supported the use of a clinical vancomycin breakpoint of 0.5 μg/ml for broth microdilution (Hsu *et al.*, 2008).

Following the conclusions from an international antimicrobial resistance program (Putnam *et al.*, 2010), telavacin has been considered highly active against *S. aureus* across several geographic regions (Asia-Pacific region, Europe, Latin America, North America) with reported MIC_{50} = 0.12 μg/ml and MIC_{90} = 0.25 μg/ml, respectively.

Similarly, the substance **CK-3636** would be probably less effective than ceftaroline (Poon *et al.*, 2012), the only US Food and Drug Administration-approved cephalosporin with activity against multidrug-resistant strains of *S. aureus* showing MIC_{90} in the range of 0.5–2.0 μg/ml.

As might be deduced from actual experimental data (Table I), the factors, which would play an essential role in terms of the activity of such dibasic alkoxyphenylcarbamic acid esters against *E. coli*, could correspond to the ones which have been relevant for effectiveness against *S. aureus*: (i) the steric and the electronic aspects primarily induced by the position of alkoxy side chain, (ii) the acidobasic and the lipohydrophilic properties of inspected derivatives and (iii) the electronic interactions evoked mainly by the fragments containing protonated basic centres.

From the structural viewpoint, *ortho*-/*meta*-alkoxy side chain isomerism appears to be the decisive factor for the activity maintenance of tested compounds against *E. coli*. In the series of *ortho*-alkoxy substituted substances, only **CK-3624** exhibited relatively acceptable level of the potency (MIC = 781 μg/ml), other structures were weakly active or inactive (Table I).

A completely opposite situation occurred when evaluating the set **CK-3634**-**CK-3637**, where these compounds have shown MIC readouts ranging from 12 μg/ml to 49 μg/ml. The efficiency of individual members from a given homological series against the Gram-negative bacteria progressively increased with the number of carbon atom forming alkoxy side chain up to a critical point, which was represented by the derivative

CK-3636 with MIC = 12 μg/ml, beyond which the next homolog was less potent (Table I).

Such observed phenomena were previously defined as the cut-off effect. Balgavý and Devínsky (1996) have extensively reviewed several hypotheses of that aspect in biological activities as well as experimental evidences which supported them.

Previous pharmacological testing has shown that the compound **CK-3636** exhibited 60-fold higher index in surface anaesthesia than applied standard cocaine. Furthermore, another standard used, procaine, was 600-fold less potent than **CK-3636** in such type of anaesthesia (Csöllei *et al.*, 1993). Similarly, cocaine was regarded as 33-fold less potent and procaine even 60-fold less effective than the considered molecule **CK-3636** in infiltrative anaesthesia (Csöllei *et al.*, 1993).

In general, the compounds from the series of **CK-3634**-**CK-3637** were notably more potent than previously *in vitro* tested corresponding *meta*-alkoxyphenylcarbamic acid esters with incorporated aliphatic dimethylammonium (Mlynarčík and Čižmárik, 1976), cyclic piperidinium (Mlynarčík and Čižmárik, 1979) or perhydroazepinium (Mlynarčík and Čižmárik, 1976) moiety, respectively (Fig. 1).

Similarly to the structural and physicochemical properties requirements which were essential for the potency of currently screened molecules against *S. aureus*, except from *meta*-alkoxy substitution and relatively high lipophilicity, it could be concluded that the presence of more than one protonated atom of nitrogen positively influenced activity against *E. coli*.

Although a possible mechanism of alkoxyphenylcarbamic acid esters action against *E. coli* was briefly proposed previously (Malík *et al.*, 2012), the purpose of this research nevertheless was also to provide a more detailed perspective. Based on the above, presumably the electronic effects induced by the protonated nitrogen centres of **CK-3634**-**CK-3637** were primarily responsible for better interaction of these compounds with the Gram-negative outer membrane. It could be supposed that the cationic fragments under consideration would be the most eligible proton donors in specific (drug-receptor) interactions between exposed phosphoryl and carboxyl groups of highly negatively charged outer face. Due to the negative charge of phosphate or carboxylate, the hydrogen bond would be fairly strong. That bond type could potentially lead to conformational changes within membrane. Analogical conclusions also resulted from the paper of Remko and Van Duijnen (1983) wherein the *ab initio* investigations of local anaesthetic-phospholid model membrane interactions were studied.

In more detail, cyclic piperidin-1-yl within the basic compartment of the tested compounds could pose a steric constraint for favorable hydrogen bond formed by such group. Following current experimental results and the conclusions from the paper which dealt with structurally similar molecules (Remko *et al.*, 1997), it could be proposed that the protonated form of the compounds **CK-3634**-**CK-3637** would be recognized and bound to the negatively charged carboxylate part of the receptor. In a subsequent step, the interaction between carbonyl group (polar oxygen region, specifically) of the carbamoyloxy moiety and positively charged fragment of the membrane might follow.

Furthermore, according to current results (Table I) it could be also pointed out that the presence of more than one basic centrum of protonation within the structure of concerned compounds would mean a more notable impact on their effectiveness against *E. coli* than against *S. aureus*.

In addition, considering the neutral base of the evaluated substances, it could be suggested that they could reach a receptor *via* hydrophobic pathways and a cationic receptor site would prefer the interaction by means of their polar oxygen areas of carbonyl group (Remko *et al.*, 1997).

The indirect comparison of the efficiency against *E. coli* between the most perspective compound **CK-3636** and a broad spectrum of the comparators which activities were determined within the Tigecycline Evaluation and Surveillance Trial between the years 2004–2009 and which were published in a paper by Andrasevic and Dowzicky (2012), revealed that **CK-3636** could be regarded as more promising than amoxicillin/clavulanic acid combination, ampicillin, ceftriaxone or levofloxacin therapy, respectively. On the contrary, tigecycline with its $MIC_{90} = 0.5$ μg/ml or meropenem, which has shown $MIC_{90} \leq 0.06$ μg/ml, could be considered more active.

The activity of the inspected compounds against *C. albicans* was not dependent only on the position of the alkoxy side chain but also on its length, as the results in Table I indicated. Generally, the introduction of *meta*-alkoxy group slightly favored the compounds **CK-3634**-**CK-3637** compared to those containing the *ortho*-alkoxy one.

In the set **CK-3624**-**CK-3627**, only the potency of the *ortho*-hexyloxy derivative (MIC = 781 μg/ml) and the *ortho*-heptyloxy one (MIC = 391 μg/ml) could be taken into consideration. Other substances within the mentioned series were practically inactive.

Similarly, from the series **CK-3634**-**CK-3637**, only those with *meta*-hexyloxy and *meta*-heptyloxy side chain appeared to be relatively effective (MIC = 391 μg/ml in both cases).

However, the current experimental observations indicated that the existence of more than one centrum of protonation within the basic compartment leads to a decrease in the activity against *C. albicans*.

Comparing the currently estimated MICs to the data published in the literature (Mlynarčík and Čižmárik,

1976; 1979), the tested compounds were less effective than the previously investigated *ortho*-/*meta*-alkoxy positional isomers containing dimethylammonium, piperidinium or perhydroazepinium group, respectively.

Similarly, the introduction of propane-1,3-diyl connecting chain instead of ethane-1,2-diyl or 1-methylethane-1,2-diyl with simultaneous presence of piperidinium moiety (Fig. 1) led to more potent substances (Králová *et al.*, 1995).

It could be suggested that the relatively higher density of positive charge in the basic part of inspected derivatives of both series disabled their smooth internalisation into a given eukaryotic pathogen potentially causing a perturbation of its membranes. That process was reflected in relatively higher MIC values which were estimated in the range of 391–6250 μg/ml (Table I). For the clarification, possible mechanism of action of alkoxyphenylcarbamates against given yeast were suggested in the paper of Malík *et al.* (2012).

Acknowledgments

The research has been supported by Slovak Grant Agency for Science, Grant Project VEGA No. 1/0039/12. That financial support has been gratefully acknowledged.

Literature

Andrasevic A.T. and M.J. Dowzicky. 2012. *In vitro* activity of tigecycline and comparators against Gram-negative pathogens isolated from blood in Europe (2004–2009). *Int. J. Antimicrob. Agents* 39: 115–123.

Andrews J.M. 2001. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.* 48: 5–16.

Balgavý P. and F. Devínsky. 1996. Cut-off effects in biological activities of surfactants. Adv. *Colloid Interface Sci.* 66: 23–63.

Csöllei J., Ľ. Búčiová, J. Čižmárik and L. Kopáčová. 1993. Studies of local anaesthetics CXII. Preparation and activity of dibasic alkylesters of 2-, and 3-alkoxy-substituted phenylcarbamic acids (In Slovak). *Českoslov. Farm.* 42: 127–129.

Čižmárik J., J. Trupl and M. Pešák. 1983. Korrelation zwischen der antimikrobiellen Aktivität quartärer Ammoniumsalze des Heptacains gegenüber *Staphylococcus aureus* der Extraktionskonstante der Ionenpaare (In German). *Pharmazie* 38: 789–790.

Čižmárik J., J. Trupl and M. Pešák. 1986. Beziehung zwischen antimikrobieller Wirkung auf *Staphylococcus aureus* und den Konstanten σ und π einiger p-substituierter Derivate der Phenylcarbamidsäure (In German). *Pharmazie* 41: 442–443.

Čižmárik J., J. Trupl and M. Pešák. 1987. A correlation between the antimicrobial activity to *Staphylococcus aureus* and selected physico-chemical parameters in the series of hydrochlorides piperidinoethylesters alkoxy-substituted phenyl-carbamic acids (In Slovak). *Českoslov. Farm.* 36: 345–348.

Dmitriev B.A., F.V. Toukach, O. Holst, E.T. Rietschel and S. Ehlers. 2004. Tertiary structure of *Staphylococcus aureus* cell wall murein. *J. Bacteriol.* 186: 7141–7148.

Endl J., H.P. Seidl, F. Fiedler and K.H. Schleifer. 1983. Chemical composition and structure of cell wall teichoic acids of staphylococci. *Arch. Microbiol.* 35: 215–223.

Enquist P.-A., A. Gylfe, U. Hägglund, P. Lindström, H. Norberg-Scherman, Ch. Sundin and M. Elofsson. 2012. Derivatives of 8-hydroxyquinoline – antibacterial agents that target intra- and extracellular Gram-negative pathogens. *Bioorg. Med. Chem. Lett.* 22: 3550–3553.

Gould I.M., R. Cauda, S. Esposito, F. Gudiol, T. Mazzei and J. Garau. 2011. Management of serious methicillin-resistant *Staphylococcus aureus* infections: what are the limits? *Int. J. Antimicrob. Agents* 37: 202–209.

Hsu D.I., L.K. Hidayat, R. Quist, J. Hindler, A. Karlsson, A. Yusof and A. Wong-Beringer. 2008. Comparison of method-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *Int. J. Antimicrob. Agents* 32: 378–385.

Králová K., H. Bujdáková and J. Čižmárik. 1995. Antifungal and antialgal activity of piperidinopropyl esters of alkoxy substituted phenylcarbamic acids. *Pharmazie* 50: 440–441.

Livermore D.M. 2011. Discovery research: the scientific challenge of finding new antibiotics. *J. Antimicrob. Chemother.* 66: 1941–1944.

Malík I., M. Bukovský, F. Andriamaity and J. Gališinová. 2012. Antimicrobial activity of *meta*-alkoxyphenylcarbamates containing substituted *N*-phenylpiperazine. *Braz. J. Microbiol.* 43: 959–965.

Malík I., E. Sedlárová, J. Csöllei, F. Andriamainty and J. Čižmárik. 2007. Relationship between physicochemical properties, lipophilicity parameters, and local anesthetic activity of dibasic esters of phenylcarbamic acid. *Chem. Papers* 61: 206–213.

Mlynarčík D., J. Bittererová, J. Čižmárik and L. Masárová. 1991. The effect of piperidinoethylesters of n-alkoxyphenyl-carbamic acids on bacterial cells (In Slovak). *Česk. Farm.* 40: 25–28.

Mlynarčík D. and J. Čižmárik. 1976. Antimicrobial efficiency of ω-piperidinoethyl esters of *n*-alkoxy-phenylcarbamic acids. *Folia Microbiol.* 21: 75–76.

Mlynarčík D. and J. Čižmárik. 1979. Antimikrobielle Eigenschaften einiger basischer Ethylester von Alkoxyphenylcarbaminsäuren (In German). *Pharmazie* 34: 575.

Okele I.N. 2009. The tragedy of antimicrobial resistance: achieving a recognition of necessity. *Curr. Sci.* 97: 1564–1572.

Perez F., R.A. Salata and R.A. Bonomo. 2008. Current and novel antibiotics against resistant Gram-positive bacteria. *Infect. Drug Resist.* 1: 27–44.

Pokorná M. 1998. Relationship of the structure and local anaesthetic effect in the group of esters of alkoxy-substituted phenylcarbamic acid (In Czech). *Čes. a Slov. Farm.* 47: 14–20.

Poon H., M.H. Chang and H.B. Fung. 2012. Ceftaroline fosamil: A cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. *Clin. Ther.* 34: 743–765.

Putnam S.D., H.S. Sader, G.J. Moet, R.E. Mendes and R.N. Jones. 2007. Worldwide summary of telavacin spectrum and potency against Gram-positive pathogens: 2007 to 2008 surveillance results. *Diagn. Microbiol. Infect. Dis.* 67: 359–368.

Remko M., K.R. Liedl and B.M. Rode. 1997. Theoretical study on the local anaesthetic-receptor interaction. *Chem. Papers* 51: 234–241.

Remko M. and P.T. Van Duijnen. 1983. *Ab initio* investigations of local anesthetic-phospholipid model membrane interactions. *Theochem, J. Mol. Struct.* 104: 451-457.

Schneewind O., A. Fowler and K.F. Faull. 1995. Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. *Science* 268: 103–106.

Sharma D., B. Narasimhan, P. Kumar, V. Judge, R. Narang, E. De Clerq and J. Balzarini. 2009. Synthesis, antimicrobial and antiviral evaluation of substituted imidazole derivatives. *Eur. J. Med. Chem.* 44: 2347–2353.

Wise R. 2008. The worldwide threat of antimicrobial resistance. *Curr. Sci.* 95, 181-187.

Ziemska J., A. Rajnisz and J. Solecka. 2013. New perspectives on antibacterial drug research. *Cent. Eur. J. Biol.* 8: 943–957.