



GLOBAL JOURNAL OF MEDICAL RESEARCH
PHARMA, DRUG DISCOVERY, TOXICOLOGY AND MEDICINE
Volume 13 Issue 4 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Antimicrobial Profile Investigation of Potential Ultrashort Acting *Beta*-Adrenoceptor Blocking Compounds Containing *N*-Phenylpiperazine Moiety

By Ivan Malík, Marián Bukovský, Petr Mokřý & Jozef Csöllei

Faculty of Pharmacy/Comenius University, Slovakia

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GJMR-B Classification : NLMC Code: QS 679, QV 701



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Ivan Malík^α, Marián Bukovský^σ, Petr Mokry^ρ & Jozef Csöllei^ω

Abstract - The set of original, highly lipophilic ultrashort acting *beta*-adrenoceptor antagonists containing *N*-phenylpiperazine fragment, labelled as 1–4, was *in vitro* screened for the activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, respectively. Following the minimum inhibitory concentration (MIC) assay by the microdilution method, all the tested molecules were practically inactive against both selected Gram-positive and Gram-negative bacterial strains showing the MICs > 1.00 mg·mL⁻¹. From structural point of view, the presence of ester group and the position of carbamoyloxy moiety within the compounds 1–4 have appeared to be the most notable factors which have decisively influenced the effectiveness against *S. aureus* and *E. coli* compared to the importance of electronic or hydrophobic interactions, which have probably been involved by the presence of *N*-phenylpiperazine, with different membrane components of the bacteria. The current research has also pointed out that the increase in the lipophilicity has been regarded as favourable aspect for the potency of these compounds against *C. albicans*. From entire evaluated set, the molecule 4 has been considered the most active against mentioned yeast with MIC = 0.78 mg·mL⁻¹.

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I. INTRODUCTION

The term „non-antibiotics“ has been taken to include a variety of the compounds that have been neither antibiotics nor antimicrobial chemotherapeutic agents which have been employed in the management of pathological conditions of a non-infectious aetiology, but which have modified cell permeability and have shown broad-spectrum *in vitro* antimicrobial activity [1]. In addition, some of non-antibiotics have been found to enhance the *in vitro* -potency of certain antibiotics against specific bacteria to make them susceptible to previously ineffective

substances [2, 3]. An antimicrobial potential of drugs classified as general or local anaesthetics, diuretics, anti-inflammatory compounds, mucolytic agents, proton pump inhibitors, calcium antagonists, antihistamines or psychotherapeutic agents has been already observed and reported in a review [1]. An antimicrobial profile of the antagonists of *beta*-adrenergic receptors, have only been investigated sporadically, and their practical contribution to the management of microbial infections has not been intensively evaluated yet. Despite mentioned, the experimental investigations [4–6] have indicated that some of them have been able to inhibit the microbial growth. Similarly, the surveillance study of Drug Institute in Warsaw [7], which was performed on standard ATCC microbial strains, has revealed the efficiency of matipranolol, therapeutically used as an antiarrhythmic drug and an antiglaucomicum, against *Staphylococcus aureus* as well as certain antihypertensives (i.e. losartan or telmisartan) against *S. aureus* and *Escherichia coli*.

The current article is the continuation of methodical searching and characterising the *in vitro* antimicrobial activity of selected non-antibiotic drugs against mentioned Gram-positive and Gram-negative microbial strains as well as against *Candida albicans*. From structural point of view, the compounds under the study, labelled as 1–4, belong to the class of ultrashort acting *beta*-adrenoceptor blockers due to the presence of the ester bond and connecting 2-hydroxypropane-1,3-diyl fragment as well. As indicated in Table 1, another considerable feature within the structure of inspected molecules is the incorporation of unsubstituted *N*-phenylpiperazine moiety (or, to be more precise, substituted by hydrogen atoms only) which could play an essential role in terms of an antimicrobial efficiency due to possible electronic or hydrophobic interactions with different membrane components of the bacteria [8].

II. MATERIALS AND METHODS

a) Chemicals and Reagents

The evaluated compounds labelled as 1–4 (Table 1), chemically *N*-(2-hydroxy-4-oxa-5-oxy-5-(4-

Authors α ω : Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovak Republic. E-mail : malikivan001@gmail.com

Author σ : Department of Cell and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Kalinčiakova 8, 832 32 Bratislava, Slovak Republic.

Authors ρ ω : Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic.

-alkoxycarbamoylphenyl)-*N*-phenyl-*N*-piperazinium chlorides, were purchased from Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. The estimation of their physicochemical properties, i.e. solubility profile, dissociation constant pK_a , surface activity γ and lipophilicity descriptors (the $\log k'$ s from RP-HPLC, the R_M s from RP-TLC), with appropriate readouts has been previously published in the paper [9].

b) The In Vitro Antimicrobial Activity Assay

Microorganisms. An antimicrobial profile of the compounds **1–4** was investigated against Gram-positive bacteria *S. aureus* ATCC 6538 (*Micrococcaceae*), Gram-negative bacteria *E. coli* CNCTC 377/79 (*Enterobacteriaceae*) and yeast *C. albicans* CCM 8186 as well. The 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. For a cultivation of the microorganisms, listed in the previous section of this paper, a blood agar, Endo agar and Sabouraud's agar (Imuna, Šarišské Michaľany, Slovak Republic) were used. Blood agar was prepared by adding 10% of defibrine sheep's blood to melted and cooled (50°C) competent components.

Determination of minimum inhibitory concentration (MIC). The MIC values of presently investigated compounds **1–4** were carried out by following the procedure previously published in literature [8, 10]. The respective tested molecules have been dissolved in dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) due to their very limited solubility in distilled water. Standard suspension of bacteria was prepared from their 24 h cultures which were cultivated on a blood agar (Gram-positive bacteria) and Endo agar (Gram-negative bacteria). Standard suspension of *Candida* was prepared from its 48 h cultures cultivated on Sabouraud's agar.

Prepared suspension contained the concentration of 5×10^7 colony forming unit (CFU) per mL of bacteria and 5×10^5 CFU·mL⁻¹ of *Candida*, respectively. The UV/VIS spectrophotometry was used for the determination of the microorganisms concentration, all evaluated suspensions were adjusted to the absorbance output of 0.35 at the wavelength of 540 nm.

The suspension of microorganisms was added in the amount of 5 microL into the solutions of inspected compounds (100 microL) 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 50.00 mg of respective compound per mL of distilled water. These stock solutions (5%) were then serially diluted by a half and final concentrations were 25.00, 12.50, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 mg·mL⁻¹, respectively. Antibacterial effect of present DMSO in thus diluted final testing medium was completely lost.

The quantitative 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 microL of evaluated suspension has been taken from each well by using transferring tool and cultured on a blood agar (*S. aureus* ATCC 6538), Endo agar (*E. coli* CNCTC 377/79) or on Sabouraud's agar (*C. albicans* CCM 8186), respectively. Petri dishes were then incubated for 24 h at 37 °C.

Positive control using only an inoculation of the microorganisms and negative control using only DMSO were realized parallelly. Both DMSO and nutrient concentrations remained stable in each well, only the concentration of inhibitory compound has changed. All experiments were performed in duplicate. The MIC was regarded as the lowest concentration of antimicrobial agent required to inhibit the visible growth of microorganism after incubation [11]. The MIC was dependent on the presence/absence of the culture on used solid media after the transfer of 5 microL of suspension from each well. The values of MIC which have been estimated for tested compounds as well as for DMSO (due to comparison) are reported in Table 1 in mg·mL⁻¹ units.

III. RESULTS AND DISCUSSION

Possible structural and physicochemical aspects of *beta*-adrenergic receptors antagonists under the study (Table 1) which could substantially affect their antimicrobial properties were: (i) the position of carbamoyloxy (NHCOO) group which has not been inserted between 2-hydroxypropane-1,3-diyl connecting chain and the aromate; (ii) the presence of carboxy (COO) group directly attached to lipophilic aromatic ring; (iii) possible electronic and hydrophobic effects which have been induced by the substituent forming basic part of the molecule; (iv) the lipohydrophilic properties.

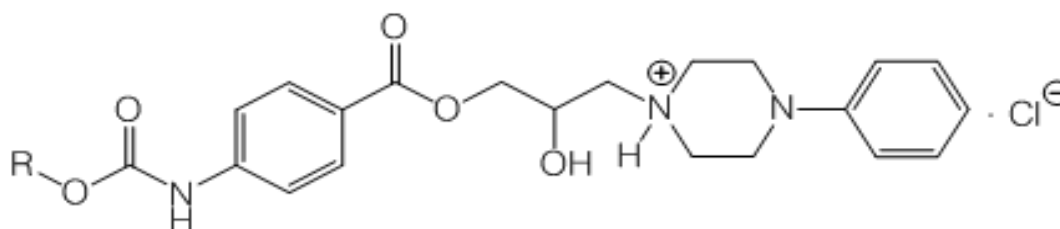
Following the quantification of an antibacterial efficiency which has already been published in a paper [12], the entire set of currently inspected compounds **1–4** has been regarded as completely inactive against both tested bacterial strains showing the MICs in the range of 6.25–25.00 mg·mL⁻¹ for *S. aureus* and 6.25–12.50 mg·mL⁻¹ for *E. coli*, respectively (Table 1). Previously performed experiments [8] have pointed out that the incorporation

of polar carbamoyloxy group between lipophilic aromatic ring and 2-hydroxypropane-1,3-diyl connecting chain has been considered very essential for the activity maintenance. On the contrary, the absence of direct covalent bond between carbamoyloxy moiety and given connecting string has led to the loss of the potency, as current experimental results have indicated. Identical conclusions have been also reported in previously published article of Malik et al. [10].

Furthermore, current experimental data could lead to the assumption that ester bond within the structure of tested compounds **1–4** would be splitted due to the enzymatic equipment of both tested bacterial strains. Possible electronic or hydrophobic interactions, induced by integrated *N*-phenylpiperazine moiety, with certain membrane elements of the bacteria have been

previously considered important [8] but the presence of direct bond between polar carbamoyloxy moiety and connecting chain has seemed to be more significant factor in terms of the activity against *S. aureus* and *E. coli* as well. It could be suggested that possible isosteric replacement of carboxy moiety for etheric bridge (the bond which would probably be more resistant to enzymatic splitting) could improve an antibacterial profile of such designed compounds.

All evaluated structures **1–4** have been regarded as highly lipophilic because of bearing two aromatic rings and hydrocarbon chain as well. Their lipophilicity enhancement due to alkyl substituent elongation has meant the decrease in the MIC values for *S. aureus*. However, as indicated in Table 1, no MIC entry has been lower than 1.00 mg·mL⁻¹.



Entry	R	MIC (mg·mL ⁻¹)		
		<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
1	CH ₃	25.00	6.25	3.13
2	C ₂ H ₅	25.00	12.50	3.13
3	C ₃ H ₇	12.50	6.25	1.56
4	C ₄ H ₉	6.25	6.25	0.78
DMSO	–	25.00	25.00	6.25

Table 1 : The *in vitro* antimicrobial activity of investigated structures **1–4** against selected microbial strains

It has been already reported that the parameters characterising the lipophilicity have been linearly related to the inhibitory activity against *C. albicans* for structurally similar set of the compounds bearing *meta*-alkoxyphenylcarbamoyloxy fragment directly bonded to 2-hydroxypropane-1,3-diyl connecting chain [8]. Additionally, the presence of highly lipophilic, sterically bulky substituent, which has shown primarily electron-withdrawing effect, attached to *N*-phenylpiperazine (trifluoromethyl group into *meta*-position) has been considered favourable, leading to more effective molecules with their MIC outputs in the interval of 0.10–0.20 mg·mL⁻¹. Following current experimental readouts, the increase in the lipophilicity of tested series **1–4** has meant a slight increase in the activity against mentioned yeast. The maximum of the effectiveness has been noted for the compound **4**, as indicated in Table 1 (MIC=0.78 mg·mL⁻¹). Furthermore, it could be assumed that eventual incorporation of i.e. trifluoromethyl substituent into *meta*-position of *N*-

-phenylpiperazine fragment within the structure of investigated set **1–4** would even lead to more active compounds against *C. albicans*.

IV. CONCLUSION

The results of current study have pointed out that the presence of polar ester group directly attached to 2-hydroxypropane-1,3-diyl moiety, which has been integrated within the structure of evaluated prospective *beta*-adrenergic receptor blockers, has probably been responsible for the complete loss of their activity against both tested bacterial strains, *S. aureus* and *E. coli*. Furthermore, assuming the position maintenance of ester (carboxy) moiety within currently inspected compounds, the nature of basic fragment, (substituted) *N*-phenylpiperazin-1-yl, and consequent electronic and hydrophobic interactions with specific components of bacterial membrane as well as the increase in the lipophilicity could be regarded as very substantial but probably not decisive factors which have positively

influenced the activity of such molecules against aforementioned tested microorganisms. On the contrary, relatively highly lipophilic antagonists of *beta*-adrenergic receptors would be promising in terms of their efficiency against *C. albicans*.

V. ACKNOWLEDGEMENT

The authors are very grateful to Slovak Grant Agency for Science for supporting by the VEGA Grant Projects No. 1/0039/12 and No. 1/0055/11. The authors deeply thank the anonymous reviewers for their valuable comments and helpful revision suggestions.

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