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ANTIMICROBIAL ACTIVITY OF NOVEL 1-[(2,4-(DI-TERT-BUTYLPHENOXY))-3-DIALKYLAMINO-2-PROPANOL] DERIVATIVES

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Keywords: *amino-propanol derivatives, antimicrobial activity, bacteria, biofilm.*

Introduction. *The microbial biofilm-forming ability is one of the major aspects of the emerging issue of antibiotic resistance, which makes them tolerant to antibiotics and host defense systems and other external stresses, thus contributing to persistent chronic infections. A series of relevant studies confirmed the high efficiency of aminopropanol derivatives as potential antibacterial and antifungal agents. This present study was aimed to evaluate the antimicrobial activity of new 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives on the planktonic bacterial/fungal cells and biofilms.*

Material and methods. *The minimum inhibitory concentrations (MIC) of the new compounds were determined by a standard method, along with their effects on biofilms estimated via the gentian violet adsorption-desorption assay.*

Results*. The KVM-219 compound showed the most pronounced effect on planktonic bacterial and fungal cells. The MIC values ranged between 0.78 µg/mL to 12.5 µg/mL, depending on the microbial strain. The KVM-316 compound exhibited the strongest inhibitory effect on biofilms, thus preventing their formation by S. aureus (96.1%), E. coli (57.2%), and P. aeruginosa (96.1%).*

Conclusions. *The 15 newly synthesized 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives revealed marked antibacterial and antifungal effects on planktonic microorganisms. Most of these compounds showed a strain-specific inhibition of biofilm formation by at least 50% for S. aureus 222, E. coli 311, P. aeruginosa 449 and C. glabrata 404 strains.*

Cuvinte cheie: *derivați de amino-propanol, activitate antimicrobiană, bacterii, biofilm.*

ACTIVITATEA ANTIMICROBIANĂ A NOILOR DERIVAȚI DE 1 - [(2,4-(DI-TERT-BUTYLPHENOXY)) - 3-DIALKILAMINO-2-PROPANOL]

Introducere. *Scopul studiului l-a constituit evaluarea efectelor antimicrobiene ale derivaților 1 - [(2,4 (di-terț- butylphenoxy)) - 3- dialkylamino-2-propanol] asupra celulelor planctonice bacteriene/fungice și asupra biofilmelor.*

Material și metode. *Concentrațiile minime inhibitorii (CMI) ale compușilor noi au fost determinate printr-o metodă standard, activitatea antibiofilm a fost testată prin absorbția violetului de gențiană pe structuri formate pe plăci de polistiren, urmată de resolubilizare cu solvent organic și resazurină ca indicator redox.*

Rezultate. *Efectul cel mai pronunțat asupra celulelor planctonice bacteriene și fungice l-a demonstrat compusul KVM -219, CMI 0,67 µg/ml - 12,5 µg/ml, în funcție de microorganism, iar asupra biofilmelor - compusul KVM-316. KVM-316 a prevenit formarea biofilmelor de către S. aureus (96,1%), E. coli (57,2%) și P. aeruginosa (96,1%).*

Concluzii. *Cei 15 derivați nou sintetizați ai 1 - [(2,4- (di- tert-butylphenoxy)) - 3- dialkylamino -2-propanol] au prezentat efecte antibacteriene și antifungice pronunțate asupra microorganismelor planctonice. Majoritatea acestor compuși au inhibat în mod specific formarea biofilmelor de către tulpinile clinice S. aureus 222, E. coli 311, P. aeruginosa 449 și C. glabrata 404 cu cel puțin 50%.*

INTRODUCTION

Over the decades, antimicrobial agents have been considered the primary suppressing means for bacterial infections. However, their irrational use led to the emergence and spread of antimicrobial-resistant strains (1, 2). The antimicrobial drug resistance has led to a decrease of infection prevention and control measures and lowered the therapeutic effectiveness thus resulting in a prolonged patient's hospital stay and increased treatment costs. The antibiotic resistance has been regarded nowadays as a major threat to public internal safety across many countries. Therefore, a global action plan to overcome antimicrobial resistance has been developed by World Health Assembly (3). In 2017, WHO presented a list of 12 bacterial species that pose a threat to human health, which are classified into three categories of pathogens, namely critical, high and medium priority, according to the urgency of need for new antibiotics (4). The goal of the global action plan is to ensure, for as long as possible, continuity of successful treatment and prevention of infectious diseases by effective and safe medicines that are quality-assured, used in a responsible way and accessible to all who need them. To achieve this goal, five strategic objectives have been set out: to improve awareness and understanding of antimicrobial resistance; to strengthen knowledge through surveillance and research; to reduce the incidence of infection; to optimize the use of antimicrobial agents; to develop the economic case for sustainable investment that takes account of the needs of all countries; and to increase investments in new medicines, diagnostic tools, vaccines and other interventions (3). Considering all of the above mentioned, the most promising approach is to search for potentially novel antimicrobial agents for combatting antimicrobial resistance.

The microbial biofilm-forming ability is one of the major aspects of the emerging issue of antibiotic resistance, which makes them tolerant to antibiotics and host defence systems and other external stresses, thus contributing to persistent chronic infections (5). Several studies confirmed the high efficiency of aminopropanol derivatives as potential antibacterial and antifungal agents, which actually drew our interest to compounds of this group (6, 7, 8).

The purpose of the present study was to evaluate the antimicrobial activity of new 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives.

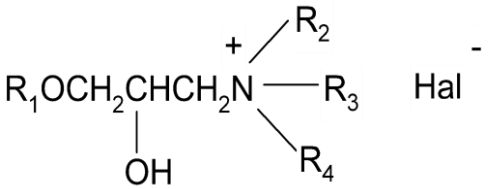
MATERIAL AND METHODS

Bacterial strain and growth conditions

The study was conducted on the gram-positive (*Staphylococcus aureus subsp. aureus* (ATCC® 25923™), *S. aureus* 222 (*MRSA*)) and gram-negative (*Escherichia coli* (ATCC® 25922™), *E. coli* 311, *Pseudomonas aeruginosa* (ATCC® 27853 ™), *P. aeruginosa* 449) bacterial strains, and yeasts (*Candida albicans* NTCC 885/653, *C. glabrata* 404). The bacterial strains were subcultured in Mueller-Hinton broth (HiMedia™ Laboratories Pvt Ltd) and Tryptic Soy Broth (TSB) (Merck Millipore) (pH 7.3), yeasts –in Saburo dextrose broth (HiMedia™ Laboratories Pvt Ltd) (pH 5.6).

Chemicals

The new derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] were first synthesised at the Institute of Organic Chemistry NAS of Ukraine. These compounds were synthesized by using the same procedure (9). Its general structural formula is shown in Figure 1.



R_1 – (2,4-di-tert-butylphenyl); R_2,R_3 – alkyl, dialkyl, cycloalkyl; R_4 – methyl, benzyl, 4-nitrobenzyl, 4-metoxybenzyl, 4-chlorobenzyl, 4- fluorobenzyl, 4-methylbenzyl; Hal – Cl-, (I-).

Figure 1. Structural formula of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] new derivatives.

Minimum Inhibitory Concentration (MIC) determination

Synthesized compounds antimicrobial activity (**I – XV**) was tested by the twofold serial dilution method (10, 11) against gram-positive (*S. aureus* ATCC 25923) and gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) bacteria, and yeasts (*C. albicans* NTCC 885/653). Inoculum density was 1-2×10^5 CFU/mL culture medium (bacteria) and 1-2×10^4 CFU/mL (yeasts). The 96-well microtiter plates with bacterial cultures were incubated for 18-24 h, while yeasts – for 24-48 h at 35-37°C. Mueller-Hinton broth and Saburo dextrose broth were used for minimal inhibitory concentration (MIC) determination. The lowest compound concentration inhibiting the microbial growth was considered as the MIC. All assays were performed in triplicate for control of culture growth (as a positive) and cultural media (as a negative).

Quantitative biofilm assay

The anti-biofilm activity of the tested compounds was determined by using the microtiter plate for biofilm formation assay described by O'Toole (12). The overnight cultures were diluted 100-fold with fresh TSB medium (bacteria) or Saburo dextrose broth (yeasts). Cell suspensions (100 µL) were transferred into individual wells of sterile polystyrene 96-well plate. The anti-biofilm effect was estimated by growing strains in media with or without test compounds (2.0×MIC) at 37°C for 24 hours. After incubation, the media were discarded, and plates were rinsed thrice with distillate water to remove nonadherent cells. Adherent cells were stained for 10-15 min with 0.1% crystal violet. The dye was extracted with ethanol for 15 min to quantify biofilm formation. The optical density was measured at 630 nm via the Absorbance Microplate Reader (model ELx800, BioTek, USA). The measurements were performed in six replications and repeated for at least three times; the values were then averaged.

Statistical Analysis

Newman-Keils (ANOVA) and Kruskal-Wallis criteria were used to assess the results via the STATISTICA, version 6.0 (StatSoft. Inc., USA) (13). Data are presented as M±m, where M is the mean value and m is the standard error of the mean.

RESULTS

The studies of antibacterial and antifungal activities confirmed that 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] new derivatives caused inhibitory effect on *S. aureus* and *C. albicans*, with the MIC values ranging from 0.78 to 3.75 µg/mL and from 1.56 to 20.0 µg/mL, respectively. These compounds revealed no antibacterial effects against gram-negative bacteria, except for the **VII** and **IV** compounds, the MIC value for *E. coli* reaching to 12.5 µg/mL and 20.0 µg/mL, respectively (tab. 1, 2).

Table 1. Antimicrobial activity (MIC, µg/mL) of new 1-(2,4-(di-tert-butylphenoxy)-3- dialkylamino-2-propanol derivatives.

Compound	*S. aureus subsp. aureus* (ATCC® 25923™)	*E. coli* (ATCC® 25922™)	*P. aeruginosa* (ATCC® 27853™)	*C. albicans* NTCC 885/653
KVM-190 (**I**)	2.5	>20.0	>20.0	3.75
KVM-266 (**II**)	5.0	>20.0	>20.0	7.5
KVM-267 (**III**)	5.0	>20.0	>20.0	7.5
KVM-316 (**IV**)	5.0	20.0	>20.0	5.0
KVM-251 (**V**)	3.12	>20.0	>20.0	12.5
KVM-327 (**VI**)	1.56	>20.0	>20.0	6.25
KVM-219 (**VII**)	0.78	12.5	>20.0	1.56
KVM-220 (**VIII**)	2.5	>20.0	>20.0	3.75
KVM-269 (**IX**)	5.0	>20.0	>20.0	7.5
KVM-268 (**X**)	5.0	>20.0	>20.0	7.5
KVM-221 (**XI**)	7.5	>20.0	>20.0	5.0
KVM-222 (**XII**)	3.75	>20.0	>20.0	2.5
KVM-319 (**XIII**)	1.56	>20.0	>20.0	20.0
KVM-317 (**XIV**)	5.0	>20.0	>20.0	5.0
KVM-318 (**XV**)	1.56	>20.0	>20.0	5.0

Table 2. Structure of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives in correspondence with antibacterial and antifungal activity.

Compound	Substituents					MIC, μg/mL	
	R_1	R_2	R_3	R_4	Hal	*S. aureus*	*C. albicans*
I	2,4-[$(CH_3)_3C$]C_6H_3	CH_3	CH_3	$CH_2C_6H_5$	Cl-	2.5	3.75
II	2,4-[$(CH_3)_3C$]C_6H_3	CH_3	CH_3	$CH_2(C_6H_4)$-4-NO_2	Cl-	5.0	7.5
III	2,4-[$(CH_3)_3C$]C_6H_3	CH_3	CH_3	$CH_2(C_6H_4)$-4-OCH_3	Cl-	5.0	7.5
IV	2,4-[$(CH_3)_3C$]C_6H_3	CH_3	CH_3	$CH_2(C_6H_4)$-4-Cl	Cl-	5.0	5.0
V	2,4-[$(CH_3)_3C$]C_6H_3	CH_3	C_6H_{11}	$CH_2C_6H_5$	Cl-	3.12	12.5
VI	2,4-[$(CH_3)_3C$]C_6H_3	CH_3	C_6H_{11}	$CH_2(C_6H_4)$-4-Cl	Cl-	1.56	6.25
VII	2,4-[$(CH_3)_3C$]C_6H_3	(C_4H_8)		CH_3	I-	0.78	1.56
VIII	2,4-[$(CH_3)_3C$]C_6H_3	(C_4H_8)		$CH_2C_6H_5$	Cl-	2.5	3.75
IX	2,4-[$(CH_3)_3C$]C_6H_3	(C_4H_8)		$CH_2(C_6H_4)$-4-NO_2	Cl-	5.0	7.5
X	2,4-[$(CH_3)_3C$]C_6H_3	(C_4H_8)		$CH_2(C_6H_4)$-4-OCH_3	Cl-	5.0	7.5
XI	2,4-[$(CH_3)_3C$]C_6H_3	(C_6H_{12})		CH_3	I-	7.5	5.0
XII	2,4-[$(CH_3)_3C$]C_6H_3	(C_6H_{12})		$CH_2C_6H_5$	Cl-	3.75	2.5
XIII	2,4-[$(CH_3)_3C$]C_6H_3	[$(CH_2)_2CHCH_3(CH_2)_2$]		$CH_2(C_6H_4)$-4-NO_2	Cl-	1.56	20.0
XIV	2,4-[$(CH_3)_3C$]C_6H_3	[$(CH_2)_2CHCH_3(CH_2)_2$]		$CH_2(C_6H_4)$-4-CH_3	Cl-	5.0	5.0
XV	2,4-[$(CH_3)_3C$]C_6H_3	[$(CH_2)_2CHCH_3(CH_2)_2$]		$CH_2(C_6H_4)$-4-F	Cl-	1.56	5.0

2,4-[$(CH_3)_3C$]C_6H_3 –- 2,4-di-tert-butylphenyl radical.

The most pronounced effect was found in the **VII** compound. The MIC value against *S. aureus* was 0.78 μg/mL, *E. coli* – 12.5 μg/mL, and for *C. albicans* – 1.56 μg/mL.

Considering that biofilms are the main mode of microbial existence, the evaluation of their susceptibility to the tested compounds was of great scientific interest. Our clinical trials tested the following microbial strains: *S. aureus* 222, *E. coli* 311, *P. aeruginosa* 449, and *C. glabrata* 404.

The obtained results demonstrated that all the tested compounds, except for the **XIII** compound, prevented *S. aureus* biofilm formation. The use of 2.0×MIC decreased the mass of *MRSA* 222 biofilm by 82.5% up to 100 % compared to the untreated culture (tab. 3).

Table 3. Antibiofilm activity (%) of new 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives.

Compound	***S. aureus* 222**	***E. coli* 311**	***P. aeruginosa* 449**	***C. glabrata* 404**
	Control 100.0±3.6	Control 100.0±1.2	Control 100.0±9.6	Control 100.0±0.9
KVM-190 **(I)**	17.5±1.1*	36.1±0.8*	249.1±15.1*	261.3±6.6*
KVM-266 **(II)**	0.2±0.1*	31.6±5.4*	150.3±6.1*	119.5±6.6
KVM-267 **(III)**	0.7±0,4*	85.9±0,5*	129.9±3.1*	160.0±43.8*
KVM-316 **(IV)**	12.7±0.2*	42.8±2.1*	3.9±0.2*	83.2±2.6
KVM-251 **(V)**	1.2±0.2*	127.8±1.7*	164.1±9.6*	251.3±1.5*
KVM-327 **(VI)**	0.3±0.2*	77.6±1.7*	61.1±4.3*	3.0±2.4*
KVM-219 **(VII)**	88.8±1.5	88.0±1.5	37.2±0.2*	111.8±0.0
KVM-220 **(VIII)**	0.2±0.2*	77.7±0.4*	174.9±3.7*	77.5±2.5
KVM-269 **(IX)**	0.2±0.1*	91.1±1.7*	69.7±3.3*	9.6±1.9*
KVM-268 **(X)**	0.1±0,0*	92.8±0.4	145.5±1.6*	3.8±1.9*
KVM-221 **(XI)**	12.4±4.0*	111.4±0.7*	145.5±3.2*	187.5±7.5*
KVM-222 **(XII)**	0.0±0.0*	98.1±1.6	131.1±3.2*	161.9±13.0*
KVM-319 **(XIII)**	51.4±2.9*	93.7±1.1	106.2±3.1	17.3±3.3*
KVM-317 **(XIV)**	0.6±0.6*	106.9±1.8*	63.0±4.9*	1.3±0.9*
KVM-318 **(XV)**	0.4±0.3*	96.3±0.1	79.1±1.7*	11.5±5.1*

*$p<0.05$ in comparison with control.

At the same time *E. coli* biofilms were less susceptible to 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives. Among all tested derivatives, the **I**, **II** and **IV** compounds showed the most pronounced inhibitory effects (63.9%, 68.4% and 57.2%, respectively, $p<0.05$).

The 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives almost did not inhibit *P. aeruginosa* biofilm formation. Only two compounds, namely the **IV** (96.1%) and **VII** (62.8%) exhibited the most pronounced inhibitory effect, whereas the **VI** (38.9%), **IX** (30.3%), **XIV** (37.0%) and **XV** (20.9%) derivatives showed a lesser activity. Unlike these ones, the **I**, **II**, **III**, **V**, **VIII**, **X**, **XI**, **XII**, and **XIII** compounds even stimulated the *P. aeruginosa* biofilm formation.

Thus, according to the study results the most pronounced inhibitory effect on biofilms formation were found for the **IV** compound, with a 96.1% decrease in *S. aureus* biofilm mass, 57.2% – *E. coli* and 96.1% – *P. aeruginosa.*

As regarding the *C. glabrata* biofilm formation, a pronounced inhibitory effect was demonstrated by the **VI**, **IX**, **X**, **XIII, XIV** and **XV** compounds (82.7%-98.7% biofilm mass decrease).

DISCUSSIONS

The comparative study of the antibacterial and antifungal activity of 1-[(2,4-(di-tert-buty-lphenoxy))-3-dialkylamino-2-propanol] derivatives with various substituents in the molecular structure showed that substitutes close to the amino fragment might affect the antimicrobial activity of the tested compounds (tab. 2).

By introducing the N-benzyl dimethylamine (**I**); N-benzyl pyrrolidine (**VIII**) and N-benzyl hexamethyleneamine (**XII**) groups to the structure of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] at the amino fragment, an inhibitory effect towards *S. aureus* and *C. albicans* ranging between 2.5-3.75 μg/mL was registered.

The addition of the substituents on the benzylic radical in the 4-position, namely the nitro group (**II, IX**), methoxy group (**III** and **X**) and chlorine group (**IV**) exhibited an increased inhibitory activity of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives. Their MIC values for *S. aureus* and *C. albicans* ranged between 5.0-7.5 μg/mL.

Another heterocyclic substituent (4-methylpiperidine) inserted to the 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] molecular derivatives, viz. **XIV** and **XV,** did not significantly affect their antifungal activities compared with **II** compound**.** At the same time, the antibacterial activity of **XV** compound (MIC 1.56 μg/mL) towards *S. aureus* increased, compared to the **II** compound (MIC 5.0 μg/mL).

Upon the insertion of a nitro group at the 4-position to the benzylic radical, viz. the **XIII** compound**,** it showed a reduced antifungal activity (MIC 20.0 μg/mL) in comparison with **XII** compound (2.5 μg/mL).

The replacement of one of the methyl groups for cyclohexyl in the molecule of the **V** compound led to a decrease of its antifungal activity (MIC 12.5 μg/mL), while its inhibitory effect towards *S. aureus* remained nearly unchanged (MIC 3.12 μg/mL) compared with the **I** compound (MIC 2.5 μg/mL).

The introduction of the chlorine at the 4-position led to completely different consequences: the MIC value of the **VI** compound showed a two-fold decrease in comparison with the MIC values of the **I** and **V** compounds (*S. aureus* – 1.56-3.12 μg/mL; *C. albicans* – 25.0-12.5 μg/mL). However, this decrease was not statistically significant.

The addition of N-methylpyrrolidine fragment to the amino group allowed to increase the antibacterial and antifungal activity of the **VII** derivative by 3.2 and 2.4 times, respectively (compared with **I** and **VIII** compounds). The MIC values towards *S. aureus* and *C. albicans* decreased to 0.78 μg/mL and 1.56 μg/mL, respectively.

The replacement of hexamethylenamine pyrrolidine radical in the **XI** derivative was accompanied by a decreased inhibitory activity (compared to the **VII** compound), whereas the MIC values towards *S. aureus* and *C. albicans* were 5.0-7.5 μg/mL.

This present study demonstrated that both tested derivatives 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] were able to suppress the gram-positive and gram-negative bacteria in fungal (*C. glabrata*) planktonic microorganisms and biofilms formation processes. The comparative structure-activity analysis showed that the inhibitory effect depended not only on the molecular structure and position of the substituents of

1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives, but also on the microbial strain used.

Thus, the trials carried out on planktonic and biofilm cultures demonstrated that the newly synthesized derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] exhibited an inhibitory effect against bacteria and fungi.

CONCLUSIONS

1. The studies on planktonic microorganisms demonstrated that the newly synthesized derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] have antibacterial and antifungal effects. This research showed that the anti-biofilm effects of the most evaluated compounds could specifically reduce the biofilm formation ability of *S. aureus* 222, *E. coli* 311, *P. aeruginosa* 449 and *C. glabrata* 404 by at least 50%, depending on the nature of the substituents used in their molecules.
2. The newly synthesized derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] represent a promising class of chemical compounds, which might lead to the development of novel antimicrobial agents intended for the treatment of many infectious diseases. Further researches are required to study the broad activity spectrum of compounds with the most pronounced antimicrobial action, as well as their antibacterial and antifungal mechanisms, acute toxicity and efficacy in vivo.

CONFLICT OF INTERESTS

Authors have no conflict of interests to declare.

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