International Journal of Advanced Research and Development
www.advancedjournal.com
ISSN: 2455-4030
Received: 17-11-2022, Accepted: 04-12-2022, Published: 20-12-2022
Volume 7, Issue 6, 2022, Page No. 21-25

Development of the method to enhancing of biological activity of polyene antibiotics and its use for environmental improvement

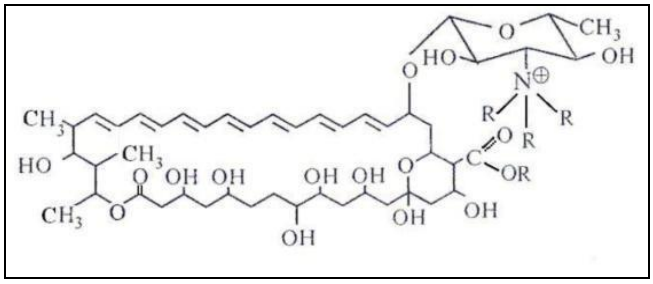
Vafa Kh. Qasimova
Baku State University, Baku, Azerbaijan

Abstract
It has been shown that in the complex use of polyene antibiotics with dimethyl sulfoxide, the biological activity of antibiotics sharply increases. Comparative physical and chemical characteristics of dimethyl sulfoxide and polyene antibiotics are presented. Effects of complex interaction of dimethyl sulfoxide and polyene antibiotics with bilayer lipid membranes are considered. By the method of bilayer lipid membranes parameters of biological activity of polyene antibiotics are determined. It is shown that amphotericin B and levorin were the most effective among all polyene antibiotics is studied. The results of the dependence of the ionic conductivity of lipid membranes on the concentration of amphotericin B and levorin and on the concentration of cholesterol in membranes are presented. On the basis of polyene antibiotics, effective membrane-active preparation against viral and fungal diseases of plants have been developed.

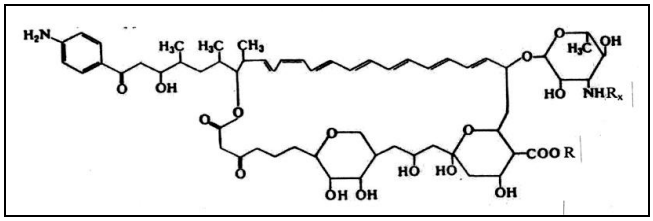
Keywords: polyene antibiotics, dimethyl sulfoxide, amphotericin b, Levorin, bilayer lipid membranes, membrane conductivity

Introduction

Polyene antibiotics (PA) are one of the most effective compounds in the fight against fungal infections (Gray-*et al*., 2012; Kovacic, Cooksy, 2012) [10, 15]. The main representatives of PA are amphotericin B, nystatin, mycoheptin and levorin, whose chemical structure is shown in Fig. 1. PA molecules contain in their composition a lactone ring, a conjugated system of double bonds and a hydrophilic chain consisting of hydroxyl and carbonyl groups. It is research that the presence of a certain number of double bonds in the PA chromophore is an important factor determining their biological activity. Amphotericin B and nystatin differ from each other in the number of double bonds in the structure of the chromophore polyene molecules (Fig. 1). Double bonds are less in nystatin than in amphotericin B and therefore the biological activity of nystatin is essentially lower. The choice of PA as an object of investigation was not by chance. Polyene is the only class of compounds that form in the cell and lipid membranes molecular channels that are selectively permeable to ions and organic compounds. The peculiarity of PA is that it is the only class of compounds that form in the cell and lipid membranes molecular channels that are selectively permeable to ions and organic compounds (Kasumov, 2009; Efimova *et al*., 2014) [14, 8]. Investigations of the molecular mechanism of PA-membranes interaction have shown that channels of a certain structure are created in a complex with sterols (Recamier *et al*., 2010; Cohen, 2010) [18, 6]. The biological activity of PA is dependent on the chemical structure of polyene molecule and at presence of sterol in the cell membranes (Samedova *et al*., 2018) [19]. PA more sensitive to ergosterol-containing membranes. Due to this essential properties amphotericin B successfully used in medicine for the treatment of systemic fungal infections (Cohen, 2010) [6]. A comparative analysis of the biological activity of amphotericin B and nystatin shows that amphotericin B is about 6 times more effective against most fungi than nystatin (Aszalos *et al*., 1985) [1]. It was showed that the conductivity of the amphotericin B channel inbilayer lipid membranes (BLM) is about 10 times higher than the nystatin channel (Kasumov, 2009) [14]. Amphotericin B and Manuscript Click here to download Manuscript renamed-2b25d.docx Click here to view linked References nystatin are very close to each other in their chemical structure, but the membranes with cholesterol are more than 100 times sensitive to amphotericin B, than to nystatin.



Amphotericin B



Levorin

Fig 1: Chemical structure of polyene antibiotics

The research of PA-membranes interaction mechanism shows that antibiotics in a complex with sterols create ionic channels of a certain structure (Kasumov, 2009) [14]. However, despite the presence of a large amount of PA and their derivatives, none of them can be compared with amphotericin B and levorin for the treatment of systemic fungal infections. In recent years, the efforts of scientists is aimed to search of new PA forms and developing new ways to deliver them to affected organs and tissues (Caffrey *et al*.,

2008) [4]. Interest in antifungal drugs has increased even more because of the wide spread of HIV infection, which has proved to be sensitive to the presence of a fungal infection in the body (De Marie *et al*., 1994) [7]. There are data that about 90% of HIV-infected patients are affected by a fungal infection, due to a sharp weakening of the body's immune system (Mamidi *et al*., 2002; Mesa-Arango *et al*., 2012) [16, 17]. In addition, when the transplantation of various organs and bone marrow is performed, patients are prescribed immunosuppressive drugs. However, they create conditions for the appearance of HIV and fungal infection in patients (Sepkowitz, 2002) [20]. It has been stimulated the necessary for aneven deeper study of the mechanism of action of PA at the molecular level. This was largely facilitated by the decoding of the chemical structure of PA and the development of ways of modifying the polyene molecule (Volmer *et al*., 2010; Belakhov *et al*., 2016). [24, 3] The use of antibiotics with a known molecular structure makes it possible to conduct research at the molecular level. The main idea of this work is to determine the degree of enhancement of the biological activity of PAby studying the physicochemical properties of PA in complex with dimethyl sulfoxide (DMSO) on bilayer lipid membranes (BLM).

Materials and Methods

Polyene molecule have amphoteric properties, by ionizing they form a cation in an acidic environment and anion in an alkaline one. In combination with DMSO, the polyenes are a liquid of a dark yellow color, a bitter taste, with a specific odor. For preparing the active form of PA, first it is necessary to convert the antibiotic substance from the powdered form (crystal) into a molecular form. At the same time, the transfer of the antibiotic substance to the most effective form is achieved. After thoroughly mixing PA with DMSO solution is kept for 24 hours at room temperature. Then the liquid is filtered and stored in a dark, cool place. As a result, a PA mother solution is obtained ready to use. The use of PA in such a combination of components is highly effective. Biological activity of PA is determined by BLM method (Ibragimova *et al*., 2006) [12]. BLM was obtained from general phospholipids isolated from cells by applying a drop of phospholipids to the opening in a Teflon cell. The total phospholipids were purified from cholesterol and other neutral lipids by acetone washing and stored at 0°C at a concentration of 20 mg/ml in chloroform-methanol solution in a volume ratio (2:1). The integral conductivity of membranes was studied depending on the concentration of antibiotic in the regime of fixation of the potential. At a certain concentration of antibiotic, the maximum conductivity of membranes is reached, which is taken as an active component of PA. Basic information on the mechanism of PA functioning in membranes was obtained using the BLM method. The scheme of electric measurements of BLM is shown in Fig. 2.

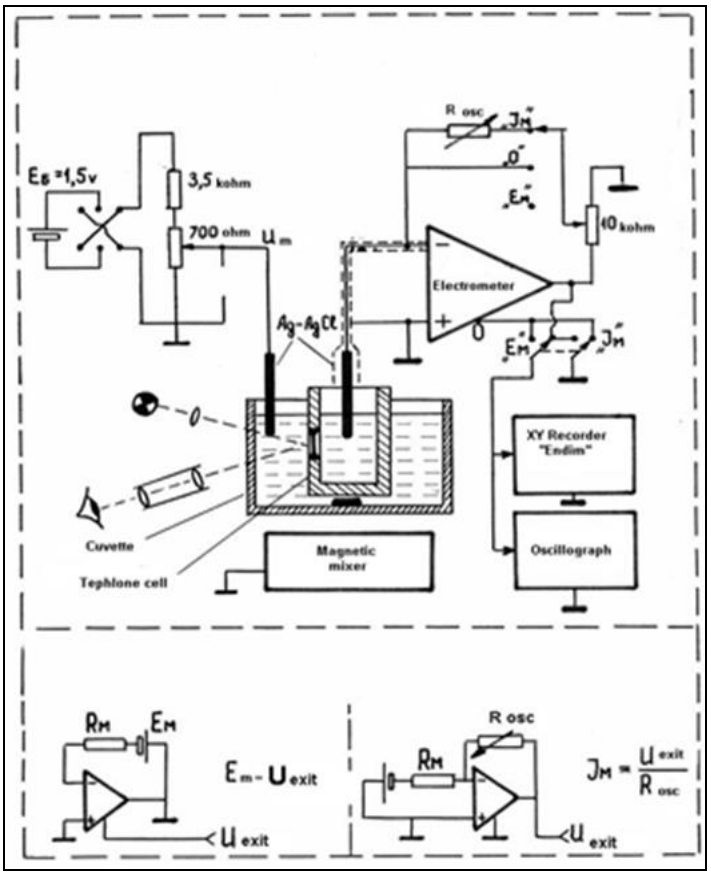


Fig 2: Scheme for measuring the electrical characteristics of lipid membranes in the mode of fixing the current and voltage of the membrane

On Fig.2 from below the left there is a diagram of measuring the potential of the membrane (Em) in the current fixation mode, and on the right it is measuring the current (Im) of the membrane in the voltage fixation mode. The aqueous solutions surrounding the membrane were mixed with a magnetic stirrer of lipid membranes for the corresponding ions by recording the change in the electrical conductivity of the membranes. Investigations of the integral conductivity and measurement of the membranepotentia were carried out in the mode of fixing the potential and current using a electro metric amplifier Keithley-301 (USA).

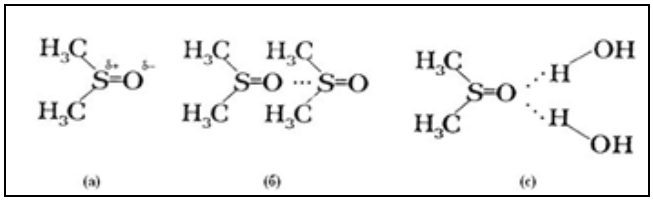


Fig 3: A schematic representation of the molecular structure of DMSO with a polar S=O bond:

a) – structure of DMSO; b) polymerically bond form of DMSO molecules; c) hydrogen bond between molecules of DMSO and water molecules (Vaisman, Berkowitz, 1992) [23].

Organic sulfoxides have a pyramidal structure with a sulfur atom at the apex. In sulfoxides (RR'SO) radicals R and R' differ from each other and exist in two optically active forms. The DMSO molecule is amphiphilic and highly polar. The negative pole of the dipole is on the oxygen atom. DMSO has an ordered structure that breaks down in the temperature range 40-60° C, which follows from the temperature dependence of the refractive index, density, viscosity and other characteristics. DMSO is a protophilic solvent, and therefore its associates are easily destroyed by the addition of substances that are proton donors. Research of the absorption spectra of DMSO in the wavelength range 350 nm to 2200 nm show that the DMSO spectrum in this interval is optically transparent. A high degree of solubility in DMSO of a number of organic substances is usually used to study their physical and chemical characteristics and molecular structure (Yu, Quinn, 1994). In tab. 1 shows data of some physical characteristics of DMSO and water. The relatively high boiling point and large latent heat of vaporization (53 J / M at 25 ° C) indicate that DMSO molecules are strongly associated (Vaisman, Berkowitz, 1992) [23]. DMSO has a dipole moment and due to the dipole-dipole interaction DMSO molecules orientate with relative to each other, forming a polymer chain due to oxygen bonds. When DMSO is mixed with water, an endothermic reaction takes place, at which the water temperature rises by 30°C. This effect indicates the formation of hydrogen bonds between the molecules of DMSO and water. DMSO has properties such as amphiphilicity, polarity and high resorption. Studies of biological activity of PA indicate that these compounds specifically interact with sterols of antibioticsensitive organisms, such as fungi and unicells (Gray *et al*., 2012) [10]. The investigation of molecular mechanism of PA - membranes interaction have shown that polyenes create channels in membranes through which ions and intracellular components that lead cell lysis can diffuse out of the cells into the external medium. There is an assumption that the biological activity of PA can depend on the nature of the intermolecular interactions between the charged groups of molecules of antibiotics and phospholipids. It is assumed that the incorporation of antibiotics into the membrane occurs as a result of the formation of a hydrogen bond between the PA and the phosphate groups of phospholipid molecules. A comparative analysis of the biological activity of amphotericin B and nystatin shows that amphotericin B is more effective against most fungi than nystatin (Aszalos *et al*., 1985; Ciesielski *et al*., 2016) [1, 5]. Comparative data show that the polyene chain in nystatin A1 and amphotericin A is the same and as experiments have shown, the antifungal activity of these two antibiotics is identical to each other. From these data it follows that the presence of a certain number of double bonds in the PA chromophore is an important factor determining their sensitivity to membranes. There is a direct relationship between the number of double bonds in the chromophore and the biological activity of antibiotics. At the higher number of double bonds in the PA chromophore, their biological activity increases. Levorin has a higher selectivity of action on membranes and differs from other PA by increased solubility in water. The structure of the lipid bilayer, as well as the structure of the penetrating molecules themselves, is an important factor causing permeability for water-soluble compounds. DMSO molecules have a high degree of resorption due to the fact that the magnitude of the dielectric constant of DMSO is between water and fats, Table 1. This indicates that DMSO enhances the permeability of a large number of drug compounds through biological membranes, and also promotes a sufficiently deep penetration into the cell. For the first time, the physical and chemical properties of amphotericin B and levorin in combination with DMSO and their mixed solutions in various ratios were studied (Ibragimova *et al*., 2002) [11]. The dependence of the conductivity of bimolecular membranes on the concentration of amphotericin B and levorin was studied. Amphotericin B sharply increases membrane permeability for ions, water, non-electrolytes and organic compounds. The dependence of membrane conductivity on the concentration of amphotericin B increases in proportion to the 8-10th degree and this degree depends on the structure of PA molecules. A sharp dependence of the membrane conductivity on concentration of amphotericin B allowed to assume that the ion permeability is associated with the formation in the membranes of polyene channels of an oligomeric structure. It seems that the system responsible for the selective permeability of membranes is localized in the hydrophilic chain of the amphotericin B molecule. When the concentration of DMSO in the aqueous solution is increased the efficiency of assembling polyene channels increases also. Amphotericin B at a concentration of 1 • 10-6M reduces the initial specific resistance of membranes (1-5·10-8 Ohm·sm2) prepared from total phospholipids by 105 -106 times. In Fig. 4. is showed the dependence of bimolecular membranes conductivity on concentration of amphotericin B at different concentrations of cholesterol in membranes, curves 1 and 2.

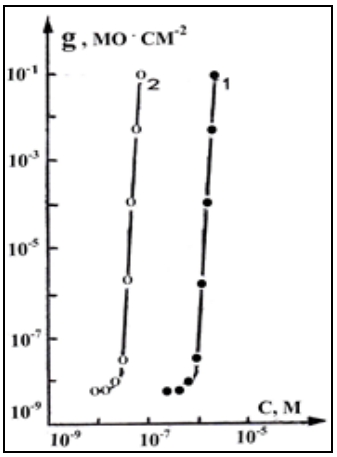


Fig 4: The dependence of BLM conductivity on the concentration of amphotericin B

Curve 1 was obtained on membranes from phospholipids with cholesterol in a weight ratio of 20:1, curve 2-2:1. Lipid membranes were formed in a solution of 10 mM KCI, pH=6,5, t=22^{0}C. The addition of cholesterol to phospholipids increases the effeciency of the antibiotic action (Fig.4, curve2). Membranes in the presence of amphotericin B are selectively permeable to monovalent anions. However, in the study of aromatic antibiotics, it was found that unlike amphotericin B, levorin causes selective permeability not for anions, but for cations of alkali metals. This antibiotic differs from nystatin, amphotericin B and mycoheptin by the presence in the molecules of an additional aromatic moiety-p-aminoacetophenone, which contains positively charged nitrogen. In Fig.5 shows the dependence of the conductivity of membranes on the concentration of levorin, Fig. 5, curve 1. An increase in the concentration of cholesterol in the membrane increases the efficiency of levorin, Fig. 5, curve 2. With increasing in the concentration of antibiotic, the conductivity of membranes increases proportionally to the fourth degree of levorin concentration. Research of the dependence of the membrane conductivity on the concentration of levorin led to the assumption of the presence in membranes of molecular size channels. It can be assumed that the selective permeability for cations is associated with the formation of negatively charged pores in the membranes. Rather, the transfer of cations across the membrane boundary is carried out through the hydrophilic parts of the channel. Essential information about the mechanism of membrane permeability in the presence of aromatic antibiotics can be taken from data on the transport through the membrane of small ions, such as guanidine and hydrazine (Kasumov, 2009) [14]. In the presence of levorin, these ions penetrate through the membrane much better than K + and Na + ions. The presence of the same number of double bonds in the chromophore of amphotericin B and levorin is an important factor determining their high sensitivity to membranes. The most effective of studied PA are amphotericin B and levorin.

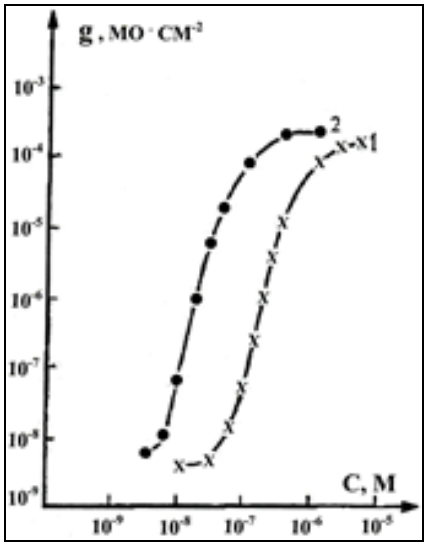


Fig 5: The dependence of lipid membranes conductivity on the concentration of Levorin

Curve 1 was obtained on membranes of phospholipids with cholesterol in a weight ratio of 20:1. Curve 2 was obtained on membranes of phospholipids with cholesterol in a weight ratio of 2:1. Solution:10 mMKCl, pH=6.5, t=22^{o}C.

A special role in the formation of conducting amphotericin and levorin channels inside the membrane belongs to DMSO. DMSO has the ability sharply enhance the biological activity of PA and induce in membranes selective permeability for ions and organic compounds. The results of these experiments suggest that the mechanism of selective action is based on the specific interaction of antibiotic molecules with membranes. It has been shown that chromophores of PA molecules interacting with phospholipids and form channels in a stoichiometric ratio of 1:1. The stoichiometric coefficient of assembly of single channels for different PA can differ greatly from each other and can be from 3 to 17. It should be noted that the molecular structure of the hydrophilic part of the channel has not yet been established due to the lack of appropriate methods. for determining the exact localization of molecular groups lining the internal cavity of the channel. According to the conducted studies, the internal diameter of the channel is 7-10Ä (Ibragimova *et al*., 2006) [12]. Computer analysis showed that ionizing the formation of an ion channel in the presence of an amide derivative of amphotericin B, ionizing groups of molecules can be directed both inside and outside the channel, i.e. polar groups can be in two conformational forms, due to the rotation of mycosamine around the glycosidic bond (Baginski, Czub, 2009) [2]. There is an assumption that the biological activity of PA can depend on the nature of the intermolecular interactions between charged groups of molecules of antibiotics and phospholipids. The incorporation of antibiotics into the membrane occurs as a result of the formation of a hydrogen bond between the PA and the phosphate groups of the phospholipid molecules. Discussion Use of PA for improvement of environmental conditions. Widespread use of antibiotics in animal husbandry and plant growing was obtained after the adverse effects of the use of drugs, which along with the phytopathogenic microflora suppression, poison useful species of birds and animals feeding on pollinated plants. Antibiotics have a number of valuable advantages in the fight against phytopathogenic microorganisms in comparison with other substances. Antibiotics have a selective effect and, by inhibiting the development of phytopathogenic bacteria and fungi, are practically harmless to plants and animals (Shishido *et al*., 2005; Te Welscher *et al*., 2012) [21, 22]. When choosing an antibiotic, a necessary condition is the absence of toxicity. For example, at using of PA in low concentrations (10-6 to I0-4 M) are nontoxic to plants and animals (Ibragimova *et al*., 2014) [13]. Studies have shown that most of the antibiotics used are well penetrated and absorbed into tissues of animals and plants. The concentration of antibiotic that necessary for the suppression of pathogenic microflora in the tissues of animals and plants depends on the properties of the antibiotic and on external conditions. PA was used as a basis for the development of effective antiviral, antibacterial and antifungal preparation. Based on the data obtained, the minimum concentration of antibiotic, which corresponds to its maximum biological activity, is calculated. The studies made it possible to identify new Infanvir preparation from the PA group, which have the ability to effectively and selectively suppress viral and fungal infections of plants (Ibragimova *et al*., 2014) [13]. Treatment of plants affected by viral and fungal infection, by spraying preparation solution leads to an effective destruction of plant infections.

Result

As a result of laboratory studies of soil samples on which vegetable crops were grown, it was found that this soil contains a small amount of nitrogen, a high amount of phosphorus and a small amount of potassium, and the pH of the soil sample is weakly alkaline. Despite the missing mineral elements in the soil, where vegetable crops were grown, conducted studies showed high effectiveness of the Infanvir preparation on pathogenic microorganisms of vegetable crops (Ibragimova *et al*., 2014) [13]. It should be noted that the preparation has the ability to completely suppress the growth of Tobacco mosaic virus (Ibragimova *et al*., 2012) [11]. Infected plants after treatment by Infanvir preparation are not only cured, but regeneration of fading plants also occurs. It is assumed that the antiviral and antifungal effect of preparation is result by binding the antibiotic to the membranes and then a channel formation of molecular structure, which is expressed in the inhibitory effect of the preparation on the reproduction of viruses and fungal cells (Gasimova, 2015) [9]. The proposed remedy is non-toxic, harmless, which makes it possible to use the drug against environmental pollution and promote its rational use in agriculture in the cultivation of vegetable and horticultural crops.

References

1. Aszalos A, Bax A, Burlinson N, Roller P, McNeal C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A. J Antibiot (Tokyo),1985:38:1699-1713.
2. Baginski M, Czub J. Amphotericin B and its new derivatives - mode of action. Curr Drug Metab,2009:10:459-69.
3. Belakhov VV, Kolodyaznaya VA, Garabadzhiu AV, Chistyakova TB, Smirnov IA. Application of the Todd-Atherton synthetic approach for chemical modification of tetraene macrolide antibiotic Lucensomycin. Russ J Gen Chem,2016:86:570-578.
4. Caffrey P, Aparicio JF, Malpartida F, Zotchev SB. Biosynthetic engineering of polyene macrolides: towards generation of improved antifungal and antiparasitic agents.Current Topics in Medicinal Chemistry,2008:8:639-653.
5. Ciesielski F, Griffin DC, Loraine J, Rittig M, Delves-Broughton J, Bonev BB. Recognition of membrane sterols by polyene antibiotics amphotericin B and natamycin, A 13C MAS NMR study.Frontiers in Cell and Developmental Biologyl4, Article,2016:57:1-12.
6. Cohen BE. Amphotericin B Membrane Action: role for two types of ion channels in eliciting cell survival and lethal effects. J Membrane Biol,2010:238:1-20.
7. De Marie S, Janknegt R, Bakker-Woudenberg IAJ. Clinical use of liposomal and lipid-complexed amphotericin B. J Antimicrob Chemother,1994:33:907-916.
8. Efimova SS, Schagina LV, Ostroumova OS. Investigation of channel-forming activity of polyene macrolide antibiotics in planar lipid bilayers in the presence of dipole modifiers. Acta Naturae,2014:6:67-79
9. Gasimova VKh. Use of polyene macrolide antibiotics in combination with dimethil sulfoxide as antiviral and antifungal agent at plant treatment. The “Proceedings of the Azerbaijan National Academy of Sciences (Biological and Medical Sciences),2015:70:109-113.
10. Gray KC, Palacios DS, DaileyI, Endo MM, Uno BE, Wilcock BC, Burke MD. Amphotericin primarily kills yeast by simply binding ergosterol. Proc Natl Acad Sci USA,2012:109:2234-2239.
11. Ibragimova VKh, Aliev DI, Alieva IN. Biophysical and medicobiological aspects of application of polyene antibiotics in combination with dimethyl sulfoxide. Biophysics,2002:47:774-781.
12. Ibragimova V, Alieva I, Kasumov Kh, Khutorsky V. Transient permeability induced by alkyl derivatives of amphotericin B in lipid membranes. Biochim Biophys Acta,2006:1758:29-37.
13. Ibragimova VKh, Samedova AA, Sultanova GG, Gasimov KhM. The antiviral and antifungal action of INFANVIR antibiotic at the vegetable crops. The First European Conference on Biology. Section 2. Physico-chemical Biology and Medical Sciences, Austria, Vienna, 2014, 45-50.
14. Kasumov KhM. Structure and membrane function of polyene macrolide antibiotics. Monograph, Moscow «Nauka», 2009, 1-512.
15. Kovacic P, Cooksy A. Novel, unifying mechanism for amphotericin B and other polyene drugs: electron affinity, radicals, electron transfer, autoxidation, toxicity and antifungal action.Med. ChemCommun,2012:3:274-280.
16. Mamidi A, DeSimone JA, Pomerantz RJ. Central nervous system infections in individuals with HIV-1 infection. J Neurovirol,2002:8:158-167.
17. Mesa-Arango AC, Scorzoni L, Zaragoza O. It only takes one to do many jobs: amphotericin B as antifungal and immunomodulatory drug. Front Microbiol,2012:3:286.
18. Récamier KS, Hernández-Gómez A, González-Damián J,Ortega-Blake I. Effect of membrane structure on the action of polyenes: I. Nystatin action in cholesterol- and ergosterol-containing membranes.Journal of Membrane Biology,2010:237:31-40.
19. Samedova AA, Tagizade TP, Kasumov KhM. Dependence of ion channel properties formed by polyene antibiotics molecules on the lactone ring structure. J Bioorganic Chemistry (in press), 2018.
20. Sepkowitz KA. Opportunistic infections in patients with and patients without Acquired Immunodeficiency Syndrome. Clin Infect Dis,2002:34:1098-1107.
21. Shishido M, Miwa C, Usami T, Amemiya Y, Johnson KB. Biological control efficiency of fusarium wilt of tomato by nonpathogenic F.oxysporum Fo-B2 in different environments. Phytopathology,2005:95:1072-1080.
22. Te Welscher YM, Van Leeuwen MR, De Kruijff B, Dijksterhuis J, Breukink E. Polyene antibiotic that inhibits membrane transport proteins. Proc Natl Acad Sci U.S.A,2012:109:11156-11159.
23. Vaisman II, Berkowitz ML. Local structural order and molecular associations in water-DMSO mixtures. Molecular dynamics study. Am Chem Soc,1992:114:7889-7896.
24. Volmer AA, Szpilman AM, Carreira EM. Synthesis and biological evaluation of amphotericin B derivatives. Nat Prod Rep,2010:27:1329-1349.
25. Yu Z, Quinn P. Dimethyl sulphoxide: a review of its applications in cell biology. Bioscience Reports,1994:14:259-281.