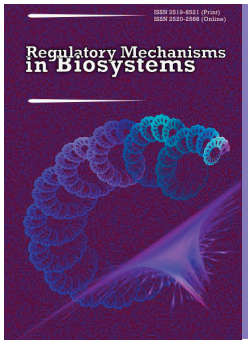

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Antifungal activity of 5-(2-nitrovinyl) imidazoles and their derivatives against the causative agents of vulvovaginal candidiasis

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Development of resistance of yeast-like fungi belonging to the *Candida* genus to the existing antifungal medicines as well as the high toxicity and low tolerance of these medicines in many cases stipulate the acute need for new antibiotic compounds. In this context, an extremely promising group of chemical substances is imidazole derivatives. Therefore, the search for more active and less toxic medical preparations based on imidazole is a relevant and important issue for medical practice. Considering the urgent demand for new antifungal preparations, four new nitro containing imidazole derivatives were synthesized – 5-(2-nitrovinyl) imidazoles and their derivatives. Then *in vitro* by means of double serial dilution in Saburo liquid nutritious medium we examined their antifungal action against eight clinical strains of yeast-like fungi belonging to the *Candida* genus: *C. albicans*, *C. guillermondii*, *C. krusei*, *C. glabrata*, *C. kefyr*, *C. tropicalis*, *C. inconspicua* and *C. zeylanoides*). They were isolated and identified from the vulvovaginal content of women suffering from vulvovaginal candidiasis. 3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole and 2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorophenyl)-1H-imidazole were found to be the most active compounds – mean values of minimal fungistatic concentrations of these compounds against all the eight examined strains were 34.9 and 39.7 μg/mL (with the ranges of these concentrations 5.2–83.3 μg/mL) respectively. 2,4-dichloro-3-methyl-4-{2-nitroethyl-1-[1-(4-fluorophenyl)-1H-imidazole-5-yl]}-1H-pyrazole-5-ole and 1-naphthyl-5-(2-nitrovinyl)-4-chloro-1H-imidazole were found to possess lower anti-*Candida* activity – mean values of their minimal fungistatic concentrations against all the eight clinical strains were 48.8 and 51.4 μg/mL (with the ranges of these concentrations 10.4–166.7 μg/mL) respectively. The highest antifungal activity of 5-(2-nitrovinyl) imidazoles and their derivatives was found to be against the clinical strains *C. krusei*, *C. kefyr* and *C. inconspicua* – mean values of minimal fungistatic concentrations concerning the indicated strains were 11.7–26.0 μg/mL. The examined nitro containing imidazole derivatives were found to manifest a slightly lower anti-*Candida* action against the clinical strains *C. tropicalis* and *C. guilliermondii* (mean values of minimal fungistatic concentrations 31.3 and 36.5 μg/mL). The lowest antifungal activity of the examined compounds was detected against the clinical strains *C. albicans* and *C. glabrata* – mean values of minimal fungistatic concentrations against these strains were 85.9 and 79.4 μg/mL. Comparison of anti-*Candida* activity of different 5-(2-nitrovinyl) imidazoles and their derivatives enabled us to select their most promising representatives and give recommendations for further synthesis of new nitro containing imidazole derivatives with pronounced antifungal properties.

Keywords: nitro containing imidazole derivatives; yeast-like fungi; *Candida*; clinical strains; antifungal means; anti-*Candida* action; antimycotic agents.

Introduction

Candidiasis is the most widespread fungal infection in the world. Causative agents of candidiasis – *Candida* spp. are the 4th most widespread microorganisms, being often recorded among patients who take broad-spectrum antibacterial drugs, after immunosuppressive therapy, parenteral nutrition, undergoing various invasive medical procedures, in HIV-infected newborns and AIDS patients, and most frequently in patients suffering from vulvovaginal candidiasis (Hani et al., 2015).

Nowadays more and more publications are appearing which report the development of resistance of yeast-like fungi of the *Candida* genus to new and existing antifungal preparation (Whaley et al., 2016). Studies conducted in 98 laboratories in 34 countries have demonstrated that the sensitivity to 7 antifungal remedies known in therapeutic practice of clinical strains of yeast-like fungi depends on epidemiological value and geographic existence of isolates (Pfaller, 2012; Posteraro et al., 2015). Mostly, fungal diseases are treated with azoles (fluconazole, itraconazole, voriconazole, posaconazole) (Pfaller et al., 2013; Posteraro et al., 2015; Xiao et al., 2015), which in a number of cases demonstrate a high toxicity, have low tolerance and are characterized by a narrow spectrum of effect and cause formation of azole-resistant strains (Xiao et al., 2015; Jensen et al., 2016; Karabıçak & Alem, 2016; Whaley et al., 2016).

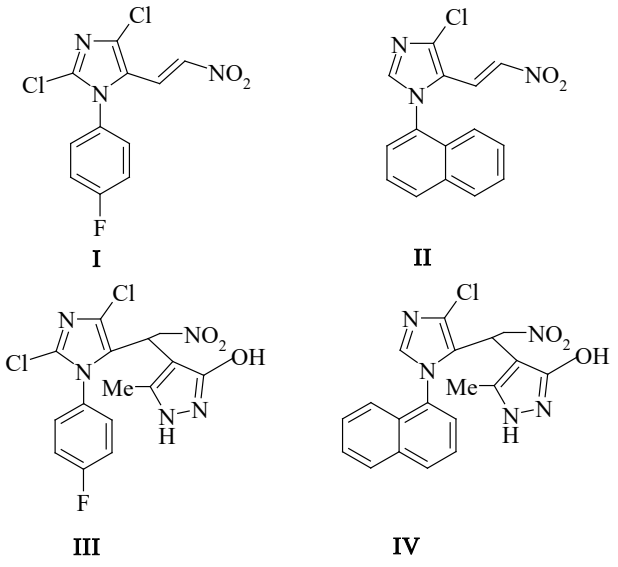
Therefore, the treatment of candidiasis was and remains a topical issue, especially considering the fact that due to administration of broad-spectrum antimycotic agents, a clear resistance of pathogens of fungal diseases is observed. Also, over many years, using antifungal imidazole derivatives has been relevant in medical practice. Apart from antifungal properties, these preparations have other pharmaceutical properties untypical for classic imidazole derivatives, including antiviral, anticancer, antituberculous and analgesic (Verma et al., 2013; Chen et al., 2014). Also, a relevant and important task is developing a methodology of structural modification of known imidazole derivatives by pharmacophore groups, which would provide a broad spectrum of structurally similar compounds – a basis for systemic investigation of "structure – activity" relations.

In this respect, an interesting preparation is a hybrid modification of the known imidazole derivatives by a fragment which plays an important part in the formation of antimicrobial properties of these compounds (Chornous et al., 2014).

The objective of our study was to investigate antifungal activity of new nitro-containing imidazole derivatives – 5-(2-nitrovinyl) imidazoles and their derivatives in relation to clinical strains of yeast-like fungi of the *Candida* genus isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis.

Materials and methods

During the determination of antifungal activity, we took into consideration the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the USA National Committee for Clinical Laboratory Standards. These recommendations were suggested by Clinical and Laboratory Standards Institute – document CLSI M27-A3, 2008, developed and recommended for examinations of antifungal activity of yeast-like fungi in liquid nutrient medium (Castanheira et al., 2014). We studied the antifungal action of four new nitro containing imidazole derivatives – two 5-(2-nitrovinyl) imidazoles (compounds I, II) and their two derivatives (compounds III, IV) synthesized at the Department of Medical and Pharmaceutical Chemistry, Bukovinian State Medical University with the following structural formulas:



Synthesis of the mentioned imidazole derivatives was done by the method (Chornous et al., 2014) in the following way.

1-aryl-4-chloro-5-(2-nitrovinyl)imidazoles I and II. 0.39 g (0.005 mole) of ammonium acetate was added to the solution of 0.01 mole of 2,4-dichloro-5-formylimidazole (for compound I) or 1-naphthyl-4-chloro-5-formylimidazole (for compound II) in 5 ml of nitromethane, and then boiled for 4 hours. The nitromethane excess was evaporated at reduced pressure, and the residue was crystallized from ethanol.

4-[1-(1-aryl-4-chloro-1H-imidazole-5-yl)-2-nitrovinyl]-5-methyl-1H-pyrazole-3-oles III and IV. The mixture of 0.005 mole (2-nitrovinyl) imidazole I or II and 0.6 g (0.006 mole) 3-methyl-2-pyrazoline-5-one was boiled for 3 hours in 20 ml of water. The mixture was cooled, the formed sediment was filtered, dried and crystallized from acetic acid.

Compounds I–IV – crystallized substances of yellow or maize yellow colour, well soluble in high polar organic solvents. Their content was proven by the elemental analysis, and their structure – by the results of measuring of infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra. IR-spectra of the synthesized compounds were recorded on a UR-20 spectrophotometer in the table KBr. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Gemini spectrophotometer (300 MHz) in the solution of dimethylsulfoxide-d6 (DMSO)-d6, internal standard – tetramethylsilane. Chromate mass spectra were obtained on the example PE SCXAPI 150 EX, UV (250 nm) and ELSOJ detectors.

In the role of testing microorganisms, we used clinical strains of yeast-like fungi belonging to *Candida* genus isolated and identified from the vulvovaginal content of women suffering from vulvovaginal candidiasis including *C. albicans*, *C. guillermondii*, *C. krusei*, *C. glabrata*, *C. kefyr*, *C. tropicalis*, *C. inconspicua* and *C. zeylanoides* (Yakovychuk et al., 2017). To assess antibiotic sensitivity of microorganisms to new chemical compounds the following successive stages were performed. First of all, solutions of new chemical compounds were prepared for the serial dilution method (in the concentration 1000 μg/ml). Due to the fact that new chemical compounds differ considerably by the degree of solubility, dimethylsulfoxide (DMSO) was used as a solvent. Twofold serial dilutions of the examined compounds were prepared (from 500 μg/ml – 1st well of the microtiter plate to 0.2 μg/ml – 12th well of the microtiter plate).

The suspension of the examined yeast-like fungi (inoculum) was prepared from twenty-four-hour culture. Using an inoculation loop, we selected several one-type isolated colonies, transferred a small amount of the material into a test tube with sterile physiological solution, and using a DEN-1 Biosan densitometer, we obtained the suspension of microorganisms in the concentration of 1.5 x 10^{8} CFU/ml, which corresponded to the McFarland turbidity standard 0.5. Then, no later than 15 minutes, using a tenfold serial dilution in Saburo liquid nutrient medium, we obtained the appropriate working microbial suspension with yeast-like fungi in the concentration of 2.5 x 10^{3} CFU/ml. Antifungal activity was determined using the micromethod of twofold serial dilutions in disposable polystyrene 96-well microtiter plates with application of 8-channel sampler and incubated at the temperature of 35 °C during 24 hours.

At the final stage, we conducted visual analysis of the results, comparing the growth of the microorganism in the presence of new chemical compound with the growth of culture (control) without it. Minimal fungicidal concentration (MFcC) and fungistatic concentration (MFSC) were determined using the micromethod of serial dilutions within 1.9–500 μg/ml. The minimal fungistatic concentration was considered the concentration of the examined compound, in the presence of which, no growth of the culture was observed (by the level of microbial turbidity of the growth medium). MFcC was determined using inoculation on Petri dishes with sterile 2% dense Saburo growth medium without clearly observed growth of fungi which were transparent visually, and then incubated in the thermostat at the optimal temperature for growth of fungi. The results were analyzed for the test fungi after 48–72 hours, that is, the lack of growth of colonies was observed.

The control was the rows of microtiter plate wells with the used medium – control medium, and the lines of microtiter plate wells containing the test-strain in the medium with the solvent used to prepare the solution of the examined compound. In the growth controls of the test-strains and the solvent, the growth of microorganisms was observed. The medium control was transparent, indicating its sterility.

The experiment with every compound and every clinical strain of *Candida* yeast-like fungi was replicated three times. The analysis of the obtained digital results was conducted using Biostatistics (Biostat 3.0) program, developed for statistical processing of the results of medical and biological studies. We calculated mean values (x), standard deviations (± SD) and confidence intervals.

Results

During the examination of the antifungal activity of 5-(2-nitrovinyl) imidazoles and their derivatives in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering vulvovaginal candidiasis, the values of their minimal fungistatic concentrations were determined (Fig. 1–4). The data demonstrate that the highest anti-*Candida* activity in relation to the examined clinical strain of *C. albicans* was manifested by 3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole (compound IV) and 2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorovenyl)-1H-imidazole (compound I). Their mean values of minimal fungistatic concentrations (MFsC) were 31.3 and 41.7 ± 10.4 μg/ml respectively. Compounds III (2,4-dichloro-3-methyl-4-{2-nitroethyl-1-[1-(4-fluorophenyl)-1H-imidazole-5-yl]}-1H-

pyrazole-5-ole and compound II (1-naphthyl-5-(2-nitrovinyl)-4-chloro-1H-imidazole) demonstrated lower anti-candida activity – their mean values of minimal fungistatic concentrations (MFsC) were 104.2 ± 20.8 and 166.7 ± 1.6 μg/ml respectively.

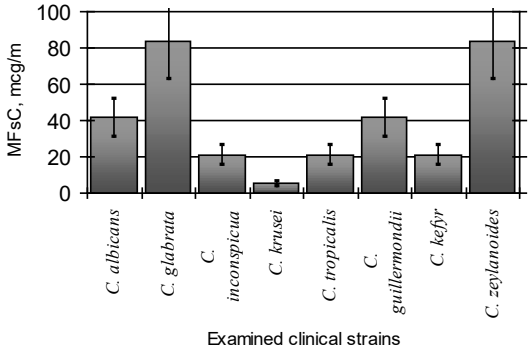


Fig. 1. Minimal fungistatic concentrations (MFsC) of 2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorophenyl)-1H-imidazole (compounds I) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

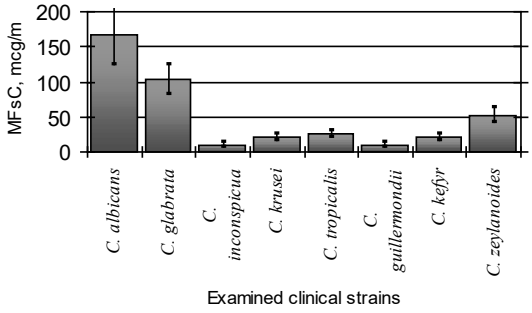


Fig. 2. Minimal fungistatic concentrations (MFsC) of 1-naphthyl-5-(2-nitrovinyl)-4-chloro-1H-imidazole (compound II) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

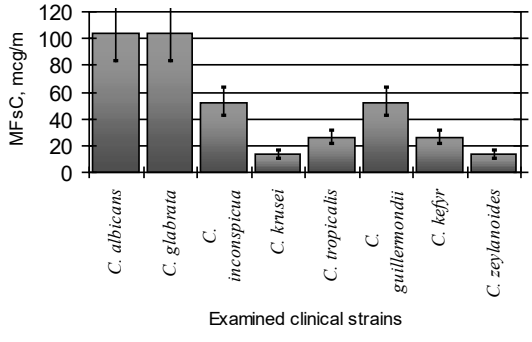


Fig. 3. Minimal fungistatic concentrations (MFsC) of 2,4-dichloro-3-methyl-4-{2-nitroethyl-1-[1-(4-fluorophenyl (compounds III) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

Minimal fungicidal concentrations (MFcC) of the examined compounds in relation to the abovementioned clinical strain of *C. albicans* were 2.0–3.3 times higher than their minimal fungistatic concentrations and were 104.2 ± 20.8 to 333.3 ± 3.2 μg/ml (Fig. 5–8).

The study of anti-*Candida* activity of new nitro containing imidazoles in relation to clinical strain of *C. glabrata* determined that the highest activity was demonstrated by the compound IV (MFsC is 26.0 ± 5.2), lower activity – by compound I (MFsC is 83.3 ± 20.8) (Fig. 1–4). Similarly to the case with the clinical strain of *C. albicans*, compounds II and III demonstrated the lowest anti-*Candida* activity, and against clinical strain of *C. glabrata*, their minimal fungistatic concentrations were 104.2 ± 20.8 μg/ml. It should be mentioned that fungicidal action of these compounds in relation to *C. glabrata* was rather low and equaled 208.3 ± 41.7 μg/ml for compounds I and IV and 500 μg/ml for compounds II and III (Fig. 5–8).

It was determined that 5-(2-nitrovinyl) imidazoles and their derivatives demonstrated higher antifungal activity against the examined clinical strain *C. inconspicua*. Thus, MFsC of the compound II in relation to this strain was 10.4 ± 2.6 μg/ml, compounds I and IV – 20.8 ± 5.2 μg/ml, and compound III – 52.1 ± 10.4 μg/ml (Fig. 1–4). Minimal fungicidal concentrations of these compounds against this clinical strain ranged significantly from 26.0 ± 5.2 to 208.3 ± 41.7 μg/ml (Fig. 5–8).

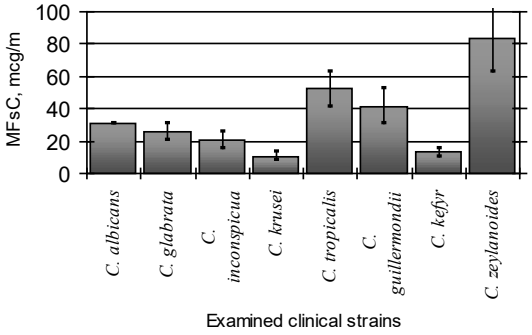


Fig. 4. Minimal fungistatic concentrations (MFsC) of 3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole (compounds IV) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

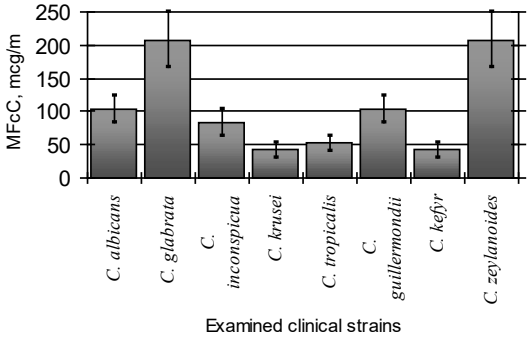


Fig. 5. Minimal fungicidal concentrations (MFcC) of 2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorovinyl)-1H-imidazole (compound I) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

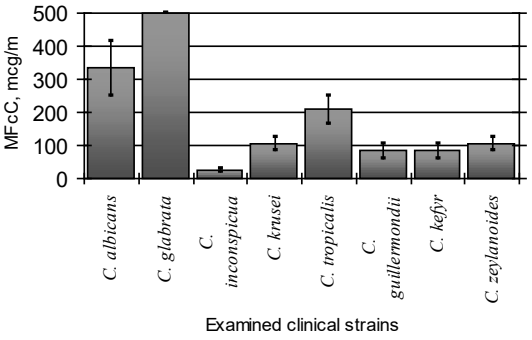


Fig. 6. Minimal fungicidal concentrations (MFcC) of 1-naphthyl-5-(2-nitrovinyl)-4-chloro-1H-imidazole (compound II) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

Similar patterns were found during the study of anti-*Candida* activity of the new nitro containing imidazole derivatives against strains *C. krusei* and *C. tropicalis*. Minimal fungistatic concentrations of the examined compounds in relation to clinical strain *C. krusei* ranged from 5.2 ± 1.3 μg/ml (compound I) to 20.8 ± 5.2 μg/ml (compound II), and minimal fungicidal concentrations – 20.8 ± 5.2 to 104.2 ± 20.8 μg/ml (Fig. 1–8).

Against *C. tropicalis* clinical strain, minimal fungistatic concentrations of the studied compounds ranged from 20.8 ± 5.2 (compound I) to 52.1 ± 10.4 μg/ml (compound IV), and minimal fungicidal ones from 52.2 ± 10.3 (compound I) to 208.3 ± 41.7 μg/ml (compounds II and IV) (Fig. 1–8). It was determined that the examined new nitro-containing imidazole derivatives exhibit anti-*Candida* activity against the clinical strain of *C. guilliermondii*. The highest anti-*Candida* activity was demonstrated by the compound II (MFsC of 10.4 ± 2.6 μg/ml), and lower activity – by the compounds I and IV (MFsC of 41.7 ± 10.4 μg/ml. Fungistatic concentrations of the abovementioned compounds against the clinical strain of *C. guilliermondii* were twice as high as fungistatic ones (compound III), higher by 2.5 (compounds I and IV) and 8 times (compound II) (Fig. 1–8).

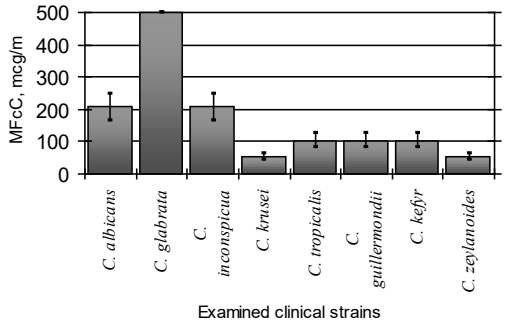


Fig. 7. Minimal fungicidal concentrations (MFcC) of 2,4-dichloro-3-methyl-4-{2-nitroethyl-1-[1-(4-fluorophenyl (compounds III) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

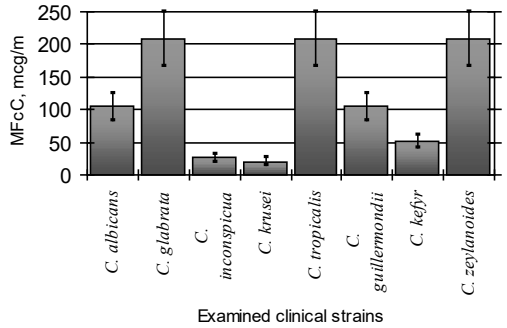


Fig. 8. Minimal fungicidal concentrations (MFcC) of 3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole (compounds IV) in relation to *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (n = 3)

Two of the four examined compounds (compounds I and II) demonstrated minimal fungistatic concentrations against the clinical strain of *C. kefyr*: at the level of 20.8 ± 5.2 μg/ml, and compounds IV and III – at the level of 13.0 ± 2.6 and 26.0 ± 5.2 μg/ml respectively (Fig. 1–4). Minimal fungicidal concentrations of these compounds against *C. kefyr* strain ranged from 41.7 ± 10.4 (compound I) to 104.2 ± 20.8 μg/ml (compound III).

Lower anti-*Candida* activity of the examined compounds was determined against the clinical strain of *C. zeylanoides*. Minimal fungistatic concentrations of the three of four examined compounds against *C. zeylanoides* clinical strain ranged from 52.1 ± 10.4 to 83.3 ± 20.8 μg/ml. Only the compound III demonstrated a slightly higher anti-*Candida* activity – its MFsC was 13.0 ± 2.6 μg/ml. Similar results were observed in the minimal fungicidal concentrations – for the three out of four examined compounds they ranged from 104.2 ± 20.8 to 208.3 ± 41.7 μg/ml, and compound III demonstrated lower MFcC – 52.1 ± 10.4 μg/ml (Fig. 5–8).

Discussion

Comparing anti-*Candida* activity of the examined 5-(2-nitrovinyl) imidazoles and their derivatives against the clinical strains of *Candida*, it should be mentioned that these compounds demonstrated the highest activity against the clinical strain of *C. krusei*. Their minimal fungistatic concentrations against this strain ranged from 5.2 ± 1.3 to 20.8 ± 5.2 μg/ml. Mean values of the minimal fungistatic concentrations of all four examined compounds against this equaled 12.5 μg/ml.

5-(2-nitrovinyl) imidazoles and their derivatives demonstrated considerably lower anti-*Candida* activity against the clinical strains of *C. kefyr* and *C. inconspicua* (mean values of MFsC 20.2 and 26.0 μg/ml), and *C. tropicalis* and *C. guilliermondii* (mean values of MFsC 31.3 and 36.5 μg/ml). The studied compounds demonstrated the lowest antifungal activity against the clinical strains of *C. albicans* and *C. glabrata* – mean values of MFsC in relation to these strains were 85.9 and 79.4 μg/ml.

The analysis of anti-*Candida* effectiveness of certain examined compounds in relation to all eight studied *Candida* clinical strains revealed the following. Compound IV was the most active (3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole). The mean value of minimal fungistatic concentrations of this compound against all eight studied clinical strains of yeast-like *Candida* fungi was 34.9 μg/ml (with the range of MFsC against these clinical strains equaling 10.8 to 83.3 μg/ml). Compound I (2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorovinyl)-1H-imidazole) demonstrated a slightly lower mean value of minimal fungistatic concentrations against all eight studied clinical strains of yeast-like *Candida* fungi – 39.7 μg/ml (with the range of MFsC against these clinical strains equaling 5.2–83.3 μg/ml). Even lower anti-*Candida* activity was demonstrated by compound III (2,4-dichloro-3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ol) and II (1-naphthyl-5-(2-nitrovinyl)-4-chloro-1H-imidazole). Their mean values of minimal fungistatic concentrations against all eight studied clinical strains of *Candida* yeast-like fungi were 48.8 and 51.4 μg/ml respectively (with MFsC in relation to these clinical strains ranging 13.0–104.2 and 10.4–166.7 μg/ml).

Comparing anti-candida activity of the examined 5-(2-nitrovinyl)imidazoles and their derivatives with the results of other imidazole derivatives, described in the scientific literature, we determined the following.

A well-known first-line antifungal drug recommended by the World Health Organization (WHO), which has an exceptional therapeutic effect in treating infections caused by *Candida* yeast-like fungi is fluconazole. Its poor water solubility, absence of the effect in case of invasive aspergillosis and increase of fluconazole-resistant isolates of *C. albicans*, limit its clinical use and therefore its being an object of studies focused on modifying the structure of fluconazole. Synthesized fluconazole analogues demonstrated antifungal activity against *C. albicans*, *C. mycoderma* and *C. utilis* with minimal inhibiting values at the level of 32 μg/ml (Zhang, 2013).

The examined 3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole (Compound IV) demonstrated anti-*Candida* activity against clinical strain of *C. albicans* on the level of fluconazole analogues with the value of minimal fungistatic concentration equaling 31.3 μg/ml, and compound I (2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorovinyl)-1H-imidazole) demonstrated activity close to fluconazole analogues with minimal fungistatic concentration of 41.7 ± 10.4 μg/ml.

Minimal fungistatic concentration of 1-substituted 4-chloro-5-(2-nitrovinyl)imidazoles against antibiotic-resistant strain of *C. albicans* isolated from the patients was 62.5 μg/ml, and the minimal fungicidal concentration – 125 μg/ml (Chornous et al., 2014). The examined compounds IV and I exhibited higher anti-*Candida* activity compared with 1-substituted 4-chloro-5-(2-nitrovinyl)imidazoles – their minimal fungistatic concentrations were 31.3 and 41.7 μg/ml respectively, and minimal fungicidal concentrations – 104.2 μg/ml.

Examination of anti-*Candida* activity of 3-imidazole-1-aryl-2-propene-1-ones revealed their fungistatic activity ranging 62.5–125.0 μg/ml, and the fungicidal activity – 125–250 μg/ml (Melnyk, 2016). The presented results coincide with results which we obtained on the clinical strain of *C. albicans* yeast-like fungi, isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis (MFsC ranged 31.3–166.7 μg/ml, MFcC ranging 104.2–333.3 μg/ml).

Imidazoles on the basis of chromen demonstrated anti-*C. albicans* activity with minimal inhibiting concentrations of 12.5 μg/ml, similarly to the minimal inhibiting concentration (12.5 μg/ml) of ketoconazole

(Thareja, 2010). Compound IV (3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole) demonstrated anti-*Candida* activity against clinical strain of *C. albicans* with minimal fungistatic concentration of 31.3 μg/ml, which is 2.5 times less than the corresponding values of imidazoles on the base of chromen and ketoconazole. The examined 5-(2-nitrovinyl)imidazoles and their derivatives which we studied also exhibited lower anti-candida activity compared to clotrimazole, minimal inhibiting concentration of which was 5 μg/ml (Padmavathi, 2010), and fluconazole with minimal inhibiting concentration equaling 1 μg/ml (Gupta, 2012).

Minimal inhibiting concentrations of a number of new synthesized imidazole derivatives against *C. albicans* (ATCC 10231) and *C. albicans* (ATCC 24433) were found to be 16–32 μg/ml (Gupta, 2012). These results are also close to the data we obtained for anti-*Candida* activity of compound IV (3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ole) against the clinical strain of *C. albicans* with minimal fungistatic concentration of 31.3 μg/ml.

4-[1-(4-chloro-1H-imidazole-5-iл)-2-nitroalkyl]-5-methyl-1H-pyrazole-3-oles demonstrated fungistatic activity against an antibiotic-resistant strain of *C. albicans* isolated from the patients ranging 62.5–250 μg/ml, and fungicidal activity ranging 125–500 μg/ml (Chomous et al., 2014), which is lower compared to the antifungal activity of the compounds we studied – their fungistatic activity ranged 31.3–166.7 μg/ml, and the fungicidal activity – 104.2–333.3 μg/ml.

Similar results were obtained during comparing the anti-*Candida* activity of the examined compounds and 2-amino-4(1-aryl-4-chloro-1H-imidazole-5-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-3-carbonitryls, minimal fungistatic concentrations of which ranged 31.3–62.5 μg/ml, and minimal fungicidal concentrations 62.5–125 μg/ml (Melnyk, 2016).

Therefore, the comparison of the data we obtained with the literature data demonstrated that the examined 5-(2-nitrovinyl)imidazoles and their derivatives exhibit anti-candida activity which is higher or similar to that of the new synthetic imidazole derivatives. However, in certain cases, they demonstrate lower activity compared to the new synthetic compounds and preparations used in clinical work. On the one hand, this indicates a perspective of using 5-(2-nitrovinyl)imidazoles and their derivatives as antimicrobial agents (first of all anti-*Candida* compounds), and on the other hand, it indicates the need for the search for new representtatives of 5-(2-nitrovinyl)imidazoles and their derivatives with stronger antimicrobial properties.

Conclusions

Of the nitro-containing imadozole derivatives, the highest anti-*Candida* activity against the *Candida* clinical strains isolated from the vulvovaginal content of women suffering from vulvovaginal candidiasis was exhibited by 3-methyl-4-[1-(1-naphthyl-4-chloro-1H-imidazole-5-yl)-2-nitroethyl-]-1H-pyrazole-5-ol and 2,4-dichloro-5-(2-nitrovinyl)-1-(4-fluorophenyl)-1H-imidazole – mean values of their minimal fungistatic concentrations against the eight examined clinical strains of *Candida* yeast-like fungi were 34.9 and 39.7 μg/ml respectively.

It was determined that 5-(2-nitrovinyl)imidazoles and their derivatives demonstrated the highest activity against clinical strain of *C. krusei*, the minimal fungistatic concentrations of the examined compounds ranged from 5.2 ± 1.3 to 20.8 ± 5.2 μg/ml. New nitro containing imidazole derivatives exhibited lower anti-*Candida* activity against clinical strains of *C. kefyr*, *C. inconspicua*, *C. tropicalis* and *C. guilliermondii* (mean values of minimal fungistatic concentrations ranged 20.2–36.5 μg/ml).

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