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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW DITHIOCARBAMATE DERIVATIVES

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Abstract: *Nowadays there are various antifungal agents. These included many substances, including derivatives of dithiocarbamates. The article aims to synthesize new derivatives of dithiocarbamate and study their antifungal activity. To analyze and identify synthesized substances and determine their structural formulas, the following spectral methods were used: IR (Shimadzu 8400 FTİR), 1H NMR (Bruker 500 MHz UltraShield) in DMSO-d6 (internal standard - tetramethylsilane), Mass spectroscopy (Agilent 1100 Series LC / MSD Trap VL & SL). Elemental analysis (C, H və N) was performed using a Perkin Elmer analyzer. The biological activity of synthesized derivatives of dithiocarbamates was explored and 100µg/ml dilution of compound* ***J2*** *(*2-((4-morpholinophenyl)amino)-2-oxoethyl 4-benzylpiperazine-1-carbodithioate) *showed identical effect against Candida albicans, Candida crusei, Candida parapsilosis as fluconazole. Compound* ***A4*** *(*2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-2-oxoethyl-4-methylpiperazine-1-carbodithioate) *showed the same activity against Candida parapsilosis and Candida glabrata as a standard drug.*

Keywords: *dithiocarbamate derivatives, antifungal activity, antibacterial activity*
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Introduction

Today there are many diseases caused by microorganisms. Some of these diseases are caused by the spread of pathogenic fungal infections. There are approximately 1.5 million different species of fungi on Earth, but just 300 of them are known to make people sick. Fungal diseases are often caused by fungi that are common in the environment [1].

A series of N-mono- and N, N-disubstituted dithiocarbamates were investigated as inhibitors of three b-carbonic anhydrases (CAs, EC 4.2.1.1) from fungal pathogens Cryptococcus neoformans, Candida albicans and Candida glabrata, that is, Can2, CaNce103 and CgNce103, respectively. The new class of b-CA inhibitors may have potential for developing antifungal agents with a diverse mechanism of effect as compared to clinically used drugs for which drug resistance was reported [2].

Brug resistance for establishing antifungals, such as azole derivatives (fluconazole and ketoconazole), is a driving force in global mortality due to fungal infection. Identification of new molecular entities structurally unrelated to the above may represent a valid strategy to overcome resistance to currently available drugs. In an effort to develop highly potent antifungal agents, we report herein a series of 27 compounds of dithiocarbamate and rhodanine molecules containing pyridine moiety and their antifungal activity. Among synthesized compounds, several analogues showed potent antifungal activity [3].

A new series of Schiff bases of 4-(4-aminophenyl)-morpholine were synthesized and described by IR, 1H-NMR, 13C-NMR, mass spectral and elemental analyses. The compounds were screened for antibacterial (*Staphylococcus aureus* (ATCC 9144), *Staphylococcus epidermidis* (ATCC 155), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 4678),

Escherichia coli (ATCC 25922)), antifungal (*Candida albicans* (ATCC 2091) and *Aspergillus niger* (ATCC 9029)) activities [4-7].

Antibacterial activity was tested against Staphylococcus aureus (MTCCB 737), Pseudomonas aeruginosa (MTCCB 741), Streptomyces epidermidis (MTCCB1824) and Escherichia coli (MTCCB1652), as well as antifungal activity against Aspergillus fumigatus nigra Aspergillus.

A series of substituted piperazine derivatives were synthesized and tested for antimicrobial activity. Also, the antibacterial activity was tested against *Staphylococcus aureus* (MTCCB 737), *Pseudomonas aeruginosa* (MTCCB 741), *Streptomyces epidermidis* (MTCCB1824) and *Escherichia coli* (MTCCB1652), and antifungal activity against *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. All synthesized compounds showed significant activity against bacterial strains but were found to be less active against tested fungi. In vitro toxicity tests revealed that some compounds showed lesser toxicity against human erythrocytes [5].

The purpose of our work is to take into the consideration of the main directions of research in the creation of new active, antifungal substances - drugs of the future - including derivatives of dithiocarbamates.

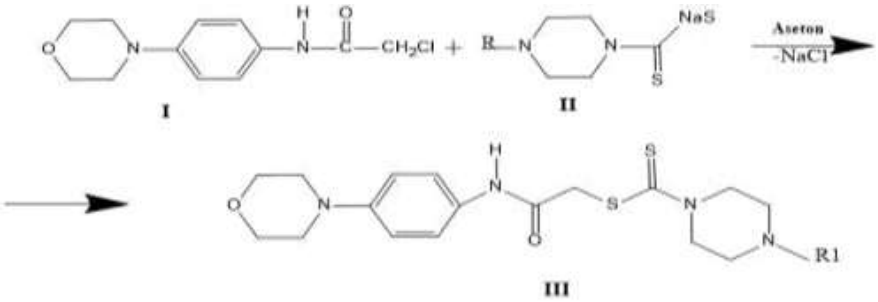
Materials and methods

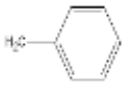
The substances cited were used as starting materials for synthesis of A4, A5, J1 and J2 compounds: N-methyl piperazine aniline (*Great Britain Maybridge*), 4-methyl piperazine, 4-ethyl piperazine, carbon disulfide, chloroacetyl chloride (*Sigma Aldrich*), sodium hydroxide, ethanol, 4-morpholinoaniline (*Great Britain Acros organics*), 4-methyl 4-benzyl piperazine and 4-benzyl piperazine, tetrahydrofuran (THF) as solvent and triethylamine (TEA) as catalyst.

To identify and analyze synthesized compounds and their structural formulas, spectral research methods were used: IR (Shimadzu 8400 FTİR), 1H NMR (Bruker 500MHz UltraShield) in DMSO-d6 (internal standart - tetramethylsilane) Mass Spectroscopy (Agilent 1100 Series LC / MSD Trap VL & SL). Elemental analysis (C, H və N) was performed using Perkin Elmer analyzer.

Results and discussion

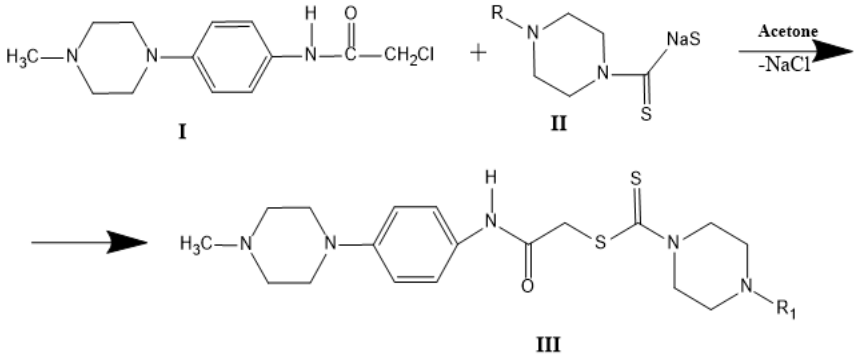
Synthesis of new derivatives of dithiocarbamates with morpholine moiety was performed in three steps: acetylation of 4-morpholineaniline with chloroacetyl chloride, synthesys of 4-methyl 4-piperazine-1-dithiocarbamate and 4-benzylpiperazine-1-dithiocarbamate sodium, final synthesis is reaction of acetylated 4-morpholineaniline with the synthesized 4-methyl 4-piperazine-1-dithiocarbamate and 4-benzylpiperazine-1-dithiocarbamate sodium salts through obtaining basic substances (Scheme 1).



R=R_1=  - substance J1; R=R_1=  – substance J2.

Scheme 1. Final step of the synthesis of dithiocarbamate dervatives (J1 and J2 compounds).

Synthesis of new derivatives of dithiocarbamates with piperazine moiety was performed in three steps: acetylation of N-methylpiperazineaniline with chloroacetyl chloride, synthesys of 4-methylpiperazine-1-dithiocarbamate and 4 ethylpiperazine-1-dithiocarbamate salts. Final synthesis was the reaction of acetylated 4-piperazineaniline with the synthesized 4-methylpiperazine-1-dithiocarbamate and 4 ethylpiperazine-1-dithiocarbamate salts by the obtaining of basic substances (Scheme 2).



R=R_1=CH_3- substance A4 ; R=R_1=C_2H_5- substance A5

Scheme 2. Final step of the synthesis of dithiocarbamate derivatives (A4 and A5)

Substance J1:
2-((4-morpholinophenyl)amino)-2-oxoethyl-4-(4-methylbenzyl)piperazine-1-carbodithioate:
Chemical formula: $C_{25}H_{32}N_4O_2S_2$. Yield: 79 %. Melting point 145.5°C. IR (KBr ν_{max} cm^{-1}): 3292 (N-H), 2852-2767 (Aliphatic C-H), 1666.5 (C=O), 1514-1473 (C=N və C=C), 813 (1,4-disubstituted benzene cycle). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2,3-4.63 (24H, m, Aliphatic protons), 6.89 (2H, d, Aromatic protons, J=9.04 Hz), 7.14 (2H, d, Aromatic protons, J=7.88 Hz), 7.20 (2H, d, Aromatic protons, J=7.92 Hz), 7.43 (2H, d, Aromatic protons, J=9.03 Hz), 10,04 (H, s, NH). MS(ES): M+1:422. Element analysis: Calculated (%); C, 56.97; H, 7.41; N, 16.61. Found (%); C, 56.78; H, 7.32; N, 16.54.

Substance J2:
2-((4-morpholinophenyl)amino)-2-oxoethyl-4-benzylpiperazine-1-carbodithioate: Chemical formula: $C_{24}H_{30}N_4O_2S_2$. Yield: 82 %. Melting point 148°C. IR (KBr ν_{max} cm^{-1}): 3292 (N-H), 2852-2767 (Aliphatic C-H), 1666.5 (C=O), 1514-1474 (C=N və C=C), 814 (1,4-disubstituted benzene cycle). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2,26-4.37 (24H, m, Aliphatic protons), 6.89 (2H, d, Aromatic protons, J=9.02 Hz), 7.15 (2H, d, Aromatic protons, J=7.88 Hz), 7.21 (2H, d, Aromatic protons, J=7.90 Hz), 7.43 (2H, d, Aromatic protons, J=9.07 Hz), 10,04 (H, s, NH). MS(ES): M+1:471. Element analysis: Calculated (%); C, 61.25; H, 6.42; N, 11.90. Found (%); C, 61.06; H, 6.35; N, 11.79.

Substance A4:
2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-2-oxoethyl-4-methylpiperazine-1-carbodithioate:
Chemical formula: $C_{19}H_{29}N_5OS_2$. Yield: 81 %. Melting point 196 °C. IR (KBr ν_{max} cm^{-1}): 3254 (N-H), 2918-2848 (Aliphatic C-H), 1657 (C=O),

1514-1415 (C=N və C=C), 820 (1,4-disubstituted benzene cycle). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2,23-4.21 (24H, m, Aliphatic protons), 6.88 (2H, d, Aromatic protons, J=8.99 Hz), 7.41 (2H, d, Aromatic protons, J=8.99 Hz), 10,03 (H, s, NH). MS(ES): M+1:408. Element analysis: calculated (%); C, 55.99; H, 7.17; N, 17.18. Found (%); C, 54.83; H, 7.02; N, 17.04.

Substance A5:

2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-2-oxoethyl-4-ethylpiperazine-1-carbodithioate:

Chemical formula: $C_{20}H_{31}N_5OS_2$. Yield: 84 %. Melting point 184°C. IR (KBr ν_{max} cm^{-1}): 3264 (N-H), 2974-2933 (Aliphatic C-H), 1658 (C=O), 1508-1429 (C=N və C=C), 825 (1,4-disubstituted benzene cycle). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2,29-4.21 (24H, m, Aliphatic protons), 6.88 (2H, d, Aromatic protons, J=9.05 Hz), 7.42 (2H, d, Aromatic protons, J=9.02 Hz), 10,03 (H, s, NH). MS(ES): M+1:422. Element analysis: Calculated (%); C, 56.97; H, 7.41; N, 16.61. Found (%); C, 56.78; H, 7.32; N, 16.54.

Biological activity screening

Anticandidal assay

It should be noted that synthesized products were tested for their in vitro growth inhibitory activity against human pathogenic, such as Candida albicans, Candida crusei, Candida parapsilosis and Candida glabrata. Fluconazole was used as a positive control factor. Anticandidal activity test was performed according to CLSI reference M27-A3 broth microdilution method [8]. Compounds were dissolved in DMSO. Further dilutions of the compounds and standard drug in test medium were prepared in the required quantities of 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent percent had no effect on yeast growth, a control test was also performed in calculated broth supplemented with only DMSO in the same dilutions used in our experiments and found inactive in culture medium. MIC_{50} and MIC parameters of synthesized compounds were calculated (**Table 1.**). Also, inhibition of new derivatives against Candida species was illustrated (**Diagrams 1 and 2**). Compound **J2** showed the same effect against *Candida albicans*, *Candida crusei*, *Candida parapsilosis* as fluconazole. Compound **A4** revealed the same effect against Candida parapsilosis and Candida glabrata as standard drug.

Table 1. MIC_{50} and MIC parameters of synthesized compounds.

Compound	Fungi species	Minimum inhibitory concentration 50 (MIC_{50})	Results of minimum inhibitory concentration
2-((4-morpholinophenyl)amino)-2-oxoethyl-4-benzylpiperazine-1-carbodithioate **(J2)**	Candida albicans	49.6 μq/ml	100 μq/ml
	Candida crusei	36.8 μq/ml	100 μq/ml
	Candida parapsilosis	37.57 μq/ml	100 μq/ml
	Candida glabrata	78.09 μq/ml	200 μq/ml
2-((4-morpholinophenyl)amino)-2-oxoethyl-4-(4-methylbenzyl)piperazine-1-carbodithioate **(J1)**	Candida albicans	78.78 μq/ml	200 μq/ml
	Candida crusei	77.15 μq/ml	200 μq/ml
	Candida parapsilosis	74.08 μq/ml	200 μq/ml
	Candida glabrata	77.16 μq/ml	200 μq/ml
2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-2-oxoethyl 4-methylpiperazine-1-carbodithioate **(A4)**	Candida albicans	76.71 μq/ml	200 μq/ml
	Candida crusei	75.58 μq/ml	200 μq/ml

	Candida parapsilosis	34.85 µq/ml	100 µq/ml
	Candida glabrata	37.48 µq/ml	100 µq/ml
2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-2-oxoethyl 4-ethylpiperazine-1-carbodithioate **(A5)**	Candida albicans	71.24 µq/ml	200 µq/ml
	Candida crusei	76.93 µq/ml	200 µq/ml
	Candida parapsilosis	35.44 µq/ml	100 µq/ml
	Candida glabrata	78.57 µq/ml	200 µq/ml

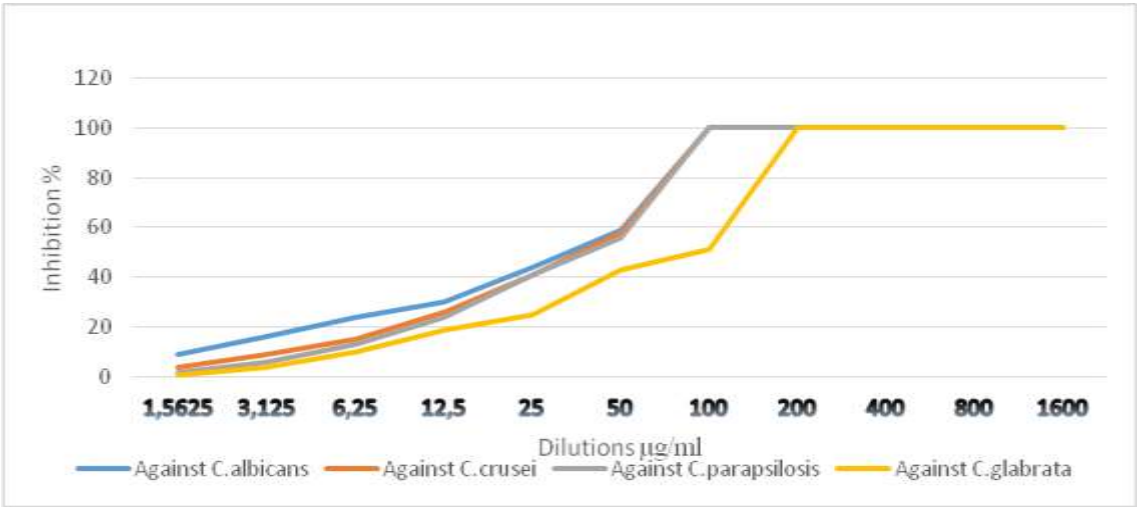


Diagram 1. Inhibition of Candida species by the compound 2-((4-morpholinophenyl) amino)-2-oxoethyl 4-benzylpiperazine-1-carbodithioate **(J2)**

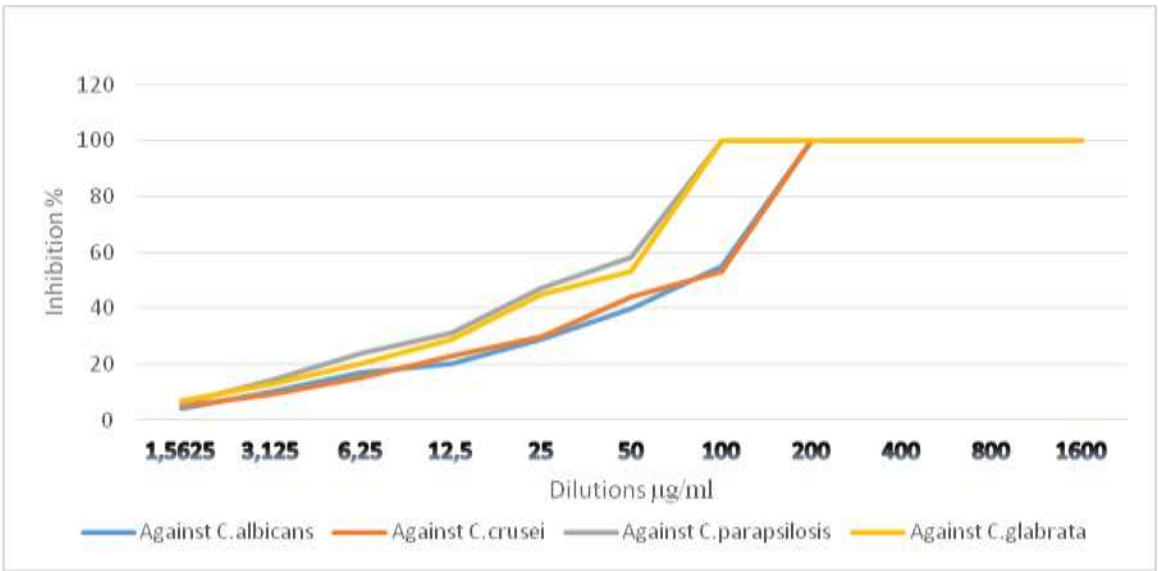


Diagram 2. Inhibition of Candida species by the compound 2-((4-(4-methylpiperazin-1-yl) phenyl) amino)-2-oxoethyl 4-methylpiperazine-1-carbodithioate **(A4)**

Conclusion

New derivatives of piperazine-1-dithiocarbamate with morpholineaniline (**J1** and **J2**) and with N-methyl piperazineaniline (**A4** and **A5**) were synthesized and their structures identified. The biological activity of synthesized derivatives of dithiocarbamates were examined, MIC_{50} and MIC parameters of synthesized compounds were calculated and reveal antifungal activity of the compounds **J2** and **A4**.

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YENİ DİTİOKARBAMAT TÖRƏMƏLƏRİNİN SİNTEZİ VƏ BİOLOJİ FƏALLIĞI

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Hal-hazırda bir çox müxtəlif göbələk əleyhinə vasitələr vardır. Bunların arasında ditiokarbamatlar törəmələri də var. Bu işin məqsədi yeni ditiokarbamat törəmələrinin sintezi və antifunqal fəallığının öyrənilməsidir. Sintez olunmuş maddələrin tədqiqi və strukturlarının müəyyənləşdirmək üçün aşağıdakı spektral metodlardan istifadə edilmişdir: DMSO-d6-da IR (Shimadzu 8400 FTİR), 1H NMR (Bruker

*500 MHz UltraShield) (daxili standart - tetrametilsilan), Mass spektroskopiya (Agilent 1100 Series LC) / MSD Trap VL & SL). Element analizi (C, H və N) Perkin Elmer analizatoru vasitəsi ilə aparılmışdır. Sintez olunmuş ditiokarbamat törəmələrinin bioloji fəallığı tədqiq edilmiş, **J2** birləşməsinin 100 mkq / ml konsentrasiyada durulaşmada Candida albicans, Candida crusei, Candida parapsilosis ştamlarına qarşı flukonazol ilə müqayisədə eyni fəallıq nümayiş etdirmişdir. **A4** maddəsi, standart nümunə ilə (flükonazol) müqayisədə Candida parapsilozu və Candida glabrata ştamlarına eyni fəallığı göstərmişdir.*
***Açar sözlər:** ditiokarbamatlar törəmələri, antifunqal fəallığı*

СИНТЕЗ И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ НОВЫХ ПРОИЗВОДНЫХ ДИТИОКАРБАМАТОВ

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В настоящее время существует множество различных противогрибковых средств, среди которых есть и производные дитиокарбаматов. Целью данной работы является синтез новых производных дитиокарбаматов и изучение их противогрибковой активности. Для анализа и идентификации синтезированных веществ и определения их структурных формул использовались следующие спектральные методы: ИК (Shimadzu 8400 FTİR), 1H ЯМР (Bruker 500 MHz UltraShield) в ДМСО-d6 (внутренний стандарт - тетраметилсилан), Масс-спектроскопия (Agilent 1100 Series LC / MSD Trap VL & SL). Элементный анализ (C, H и N) проводили с использованием анализатора Perkin-Elmer. Была исследована биологическая активность синтезированных производных дитиокарбаматов и соединение J2 (2 - ((4-морфолинофенил) амино) -2-оксоэтил 4-бензилпиперазин-1-карбодитиоат) при концентрации 100 мкг / мл показало такую же активность в отношении Candida albicans, Candida crusei, Candida parapsilosis, что и флуконазол. Соединение A4 (2 - ((4-(4-метилпиперазин-1-ил) фенил) амино) -2-оксоэтил 4-метилпиперазин-1-карбодитиоат) проявило активность в отношении Candida parapsilosis и Candida glabrata, что и стандартное лекарственное средство.
***Ключевые слова:** противогрибковые средства, производные дитиокарбаматов*