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SEARCH FOR ANTIMICROBIAL COMPOUNDS AMONG MALEOPIMARIC-ACID DERIVATIVES

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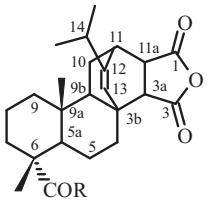
The antimicrobial and antifungal activities of maleopimaric acid (MPA) and several of its 2- and 6-derivatives were investigated. An analysis of the results showed pronounced antimicrobial activity for 2-allylmaleopimarimide and MPA N-*benzylcarboxamide against test strains of Gram-negative and Gram-positive microorganisms and antifungal activity of 2-allylmaleopimarimide against lower-fungus test strain* Candida albicans. *New methods for producing MPA* N-*benzylcarboxamide and* N-*hydroxymaleopimarimide that increased the yields of target products were proposed.*

Keywords: maleopimaric acid, antimicrobial activity, antifungal activity.

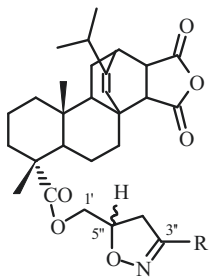
The rise in antibiotic-resistant microorganisms is responsible for the emergence of persistent nosocomial infections, among others [1]. Infectious vectors remain problematical for global medicine. The increase of nosocomial and opportunistic infections stimulated chemists to discover new antimicrobial agents. Immunodeficient patients (e.g., cancer chemotherapy, organ transplantation, HIV diseases) are at risk of disease and death caused by systematic fungal infections, so-called fungemias. Maleopimaric acid (MPA) is a diene adduct of the plant metabolite levopimaric acid and maleic anhydride and represents an available, inexpensive, and chiral matrix for synthesizing more complicated constructs [2]. It can be used for targeted drug delivery and new chemotherapy preparations.

MPA and its derivatives modified at the 2-position and the C-6 carboxylic acid were synthesized to discover compounds with antimicrobial and antifungal activity and to define the structure–activity relationship.

MPA (**1**) was synthesized by the literature method [3]. 4,5-Dihydroisoxazole derivatives of MPA **2**–**5** were produced via 1,3-dipolar cycloaddition of MPA allyl ester with benzaldehyde nitrile oxides under conditions generating the nitrile oxide from the oxime via oxidation by sodium hypochlorite [4]. Amide derivative **6** was obtained in quantitative yield by reacting MPA acid chloride with benzylamine in anhydrous CH_2Cl_2 in the presence of Et_3N. *N*-Hydroxymaleopimarimide **7** was produced in 87% yield by reacting **1** with hydroxylamine hydrochloride in refluxing anhydrous Py under Ar. 2-Allylmaleopimarimide (**8**) was synthesized by reacting **1** with allylamine in refluxing toluene in a flask with a Dean-Stark trap [4]. New methods for preparing compounds **6** and **7** were developed, increased the yields of the synthesized compounds, and refined several physicochemical data lacking in the original publications [5, 6].



1, 6



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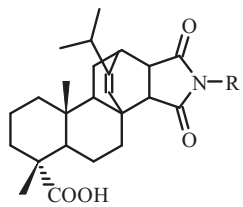

7, 8

1: R = OH; **2:** R = Ph; **3:** R = 3-IC_6H_4; **4:** R = 4-$MeOC_6H_4$; **5:** R = 2,3-$(OCH_2O)C_6H_3$; **6:** R = NHBn; **7:** R = OH; **8:** R = All

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TABLE 1. Antimicrobial and Antifungal Activity of **6** and **8**

Compound	Minimal inhibitory concentration (MIC), μg/mL								
	1	2	3	4	5	6	7	8	9
6	3.12	6.25	3.12	200	6.25	1.56	3.12	3.12	200
8	3.12	3.12	25	200	1.56	6.25	200	200	25
Ceftriaxone	0.5	–	0.5	0.05	0.05	0.05	0.05	0.05	0.05
Miconazole [8]	–	–	–	–	–	–	–	–	25

1 – *St. aureus*, 2 – *Str. pyogenes*, 3 – *E. coli*, 4 – *P. vulgaris*, 5 – *K. pneumoniae*, 6 – *Ent. aerogenes*, 7 – *Ps. aeruginosa*, 8 – *E. cloacae*, 9 – *C. albicans*

Antimicrobial and antifungal activity of **1**–**8** was studied using nonpathogenic test microorganisms *Escherichia coli*, *Enterobacter cloacae*, *E. aerogenes*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and lower fungus *Candida albicans*.

The screening results showed that MPA (**1**), esters **2**–**5**, and imide **7** inhibited metabolism in Gram-negative and Gram-positive bacteria and lower fungus *C. albicans* with MIC of 50 μg/mL.

Compounds **6** with benzylamine as the amide component and **8** with *N*-allylpyrrolidine possessed pronounced activity. Table 1 presents the antimicrobial and antifungal activities of MPA derivatives **6** and **8**. However, these compounds exhibited weak antimicrobial activity (MIC 200 μg/mL) against *P. vulgaris*. Compound **8** showed low antimicrobial activity against *P. aeruginosa* and *E. cloacae*; compound **6**, against yeast-like *Candida* fungi.

A structure–activity relationship of the MPA derivatives could be found from the results. Thus, introducing into MPA (3-aryl-4,5-dihydroisooxazol-5-yl)methanol groups did not enhance the antimicrobial and antifungal activity. However, an imide fragment in MPA produced pronounced antimicrobial activity against most test strains because of the modification of the anhydride ring or the amide component in the 6-position.

The results showed that continued searching for new antimicrobial and antifungal drugs among MPA derivatives with structures different in principle from known antibacterial drugs is promising.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in deuterated solvents with TMS internal standard on a Bruker Avance-III 500 MHz pulsed spectrometer at operating frequency 500.13 MHz for ^{1}H and 125.47 MHz for ^{13}C. Elemental analyses used a Euro EA 3000 analyzer. Melting points were determined on a Boetius apparatus and are uncorrected. NMR and IR spectra were recorded on equipment at the Khimiya CUC, UfIC, UFRC, RAS. Elemental analyses of all synthesized compounds agreed with those calculated.

The course of reactions was monitored by TLC on Sorbfil PTSKh-AF-A plates. Compounds were detected by spraying with H_2SO_4 solution (5%) followed by heating to 100–120°C. Column chromatography used silica gel 60 (0.063–0.2 mm, 70–230 mesh; Macherey-Nagel, Germany).

Antimicrobial and antifungal activities of **1**–**8** were determined by agar diffusion and double serial dilution in meat-peptone broth (MPB) at pH 7.2–7.4 [7]. The test cultures were microorganism strains deposited at the Tarasevich State Scientific-Research Institute for Standardization and Inspection of Medical and Biological Preparations, Ministry of Health of Russia, Department of Microbiology and Virology, Bashkir State Medical Institute: *E. coli*, *E. cloacae*, *Ent. aerogenes*, *P. vulgaris*, *K. pneumoniae*, *Ps. aeruginosa*, *St. aureus*, *Str. pyogenes*, and lower fungus *C. albicans*. The initial dilution was prepared by dissolving a compound (8 mg) in DMSO (1 mL) followed by dilution with MPB to the working concentration of 0.8 mg/mL. The microbe load was $2.0 \cdot 10^6$ microbes/mL of growth medium. Inoculations were incubated at 37°C for 24 h. Then, the presence or absence of test-culture growth was visually assessed. Antimicrobial activity was estimated from the minimal inhibitory concentration (MIC). The reference drugs were ceftriaxone (Krasfarma, Russia) and miconazole [8].

The physical and spectral characteristics of starting MPA (**1**), esters **2**–**5**, and 2-allylmaleopimarimide (**8**) agreed with literature data.

(6*R*,9a*R*)-*N*-Benzyl-12-isopropyl-6,9a-dimethyl-1,3-dioxotetradecahydro-1*H*-3b,11-ethenophenanthro[1,2-*c*]furan-6-carboxamide (6). A solution of MPA (**1**) acid chloride (0.6 g, 1.4 mmol) [9] in anhydrous CH_2Cl_2 (50 mL) was stirred and treated dropwise with benzylamine (0.15 mL, 1.4 mmol) and Et_3N (0.2 mL, 1.4 mmol). When the reaction was finished, the reaction mixture was treated with HCl solution (10%, 10 mL) and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated to afford **6** (0.7 g, 99%). $C_{31}H_{39}NO_4$. ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 15.57 (CH_3, C-6), 16.71 (CH_3, C-9a), 17.01 (CH_2, C-8), 19.88 (CH_3, C-14), 20.47 (CH_3, C-14), 21.05 (CH_2, C-5), 27.09 (CH_2, C-10), 32.66 (CH, C-14), 34.55 (CH_2, C-4), 35.61 (CH, C-11), 36.85 (CH_2, C-7), 37.62 (C, C-9a), 37.67 (CH_2, C-9), 40.31 (C, C-3b), 43.67 (CH_2, C-1′), 45.58 (CH, C-11a), 46.66 (C, C-6), 49.52 (CH, C-5a), 52.97 (CH, C-3a), 52.98 (CH, C-9b), 125.13, 127.55, 128.63 (CH, Ph), 127.37 (CH, C-13), 138.48 (C, Ph), 147.91 (C, C-12), 171.23 (C, C-1), 173.05 (C, C-3), 178.71 (C, CONH).

(6*R*,9a*R*)-2-Hydroxy-12-isopropyl-6,9a-dimethyl-1,3-dioxohexadecahydro-3b,11-ethenonaphtho[2,1-*e*]isoindole-6-carboxylic Acid (7). A mixture of MPA (**1**, 1.19 g, 2.97 mmol) and hydroxylamine hydrochloride (0.21 g, 6.36 mmol) in anhydrous Py (7.5 mL) was stirred and refluxed under Ar for 17 h. When the reaction was finished (TLC monitoring), the mixture was cooled to room temperature. The precipitate was filtered off. The filtrate was evaporated *in vacuo*. The residue was dissolved in Et_2O, washed with H_2O, dried over Na_2SO_4, and evaporated. The solid was dried *in vacuo* and ground to afford **7** (1.08 g, 87%), $C_{24}H_{33}NO_5$, mp 255–257°C, $[\alpha]_D^{20}$ –27.6° (*c* 1.0, $CHCl_3$ + MeOH, 3:2). IR spectrum (ν, cm^{-1}): 3433, 3317, 1750, 1709, 1695, 1688, 1464, 1377, 1234, 1180, 1159, 1150, 1141, 1076, 764. ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 15.45 (CH_3, C-6), 16.52 (CH_3, C-9a), 16.95 (CH_2, C-8), 19.82 (CH_3, C-14), 20.50 (CH_3, C-14), 21.58 (CH_2, C-5), 27.50 (CH_2, C-10), 32.61 (CH, C-14), 35.06 (CH_2, C-4), 35.26 (CH, C-11), 36.65 (CH_2, C-7), 37.50 (C, C-9a), 38.01 (CH_2, C-9), 40.61 (C, C-3b), 42.26 (CH, C-11a), 46.60 (C, C-6), 49.15 (CH, C-5a), 49.63 (CH, C-3a), 53.94 (CH, C-9b), 124.27 (CH, C-13), 146.99 (C, C-12), 172.99 (C, C-1), 174.03 (C, C-3), 181.70 (C, COO).

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