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Cationic amphiphilic meroterpenoids: synthesis, antibacterial, antifungal and mutagenic activity

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Abstract

In this research, using the thia-Michael reaction, cationic amphiphilic meroterpenoids containing fragments of mono- and sesquiterpenoids were synthesized. The bacteriostatic and fungistatic activity of synthesized meroterpenoids against the fungi *Saccharomyces cerevisiae* and *Candida* sp., Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Salmonella typhimurium*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*) bacteria was studied. The compound containing the farnesyl fragment was most active against *Saccharomyces cerevisiae* (MIC 0.039 mg/mL), *Candida* sp. (MIC 0.078 mg/mL), Gram-positive bacteria *Staphylococcus epidermidis* (MIC 0.02 mg/mL) and Gram-negative *Salmonella typhimurium* (MIC 0.078 mg/mL). Besides, the Ames test demonstrated the absence of direct mutagenic action in all the studied compounds.

Keywords

terpenoids
antifungal
antibacterial
thia-Michael addition
resazurin assay

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Key findings

- Synthesis of meroterpenoids based on click thia-Michael addition reactions.
- Evaluation of MIC/MBC of meroterpenoids on cell lines of pathogenic bacteria and fungi using the resazurin assay.
- Evaluation of the mutagenicity of meroterpenoids in the Ames test.

Supplementary materials

1. Introduction

Pathogenic microorganisms have been posing a great danger to human health for centuries [1, 2]. Over the past twenty years, the emergence of new dangerous, antimicrobial agent-resistant strains has been occurring with alarming regularity [3, 4]. At the moment, the task of finding and developing new drugs to fight bacteria and fungi is extremely urgent.

One of the targets of antimicrobial drugs is cell membranes [5]. The membrane components of Gram-negative and Gram-positive bacteria are negatively charged [6, 7]. The cell wall of fungi mainly consists of components containing a negative charge [8]. Compared to bacterial and fungal cell membranes, the plasma membranes of mammalian cells are less negatively charged [9]. These differences between the structure of bacterial, fungal and mammalian cell membranes provide a rational basis for the development of several types of positively charged drug candidates. Interest in the development of antimycotic and antimicrobial cationic drugs is gaining relevance every day [10, 11]. The most promising compounds from this point of view are cationic amphiphilic drugs, such as quaternary ammonium salts due to their high affinity for the lipid membrane [12]. Quaternary ammonium salts of amphiphilic nature are widely used in medicine [13, 14].

In recent years, more and more attention has been paid to the development of antimicrobial agents based on natural and semi-synthetic sources [15–19]. Terpenoids represent one of the most extensive and structurally diverse groups of natural compounds [20, 21]. The availability and diversity of natural terpene compounds exhibiting a wide

range of biological activity attracts the attention of researchers more and more every year [22–25]. Lately, antifungal and antibacterial activity of natural terpenoids and derivatives based on them has been shown [26–32]. Therefore, the introduction of quaternary ammonium groups into natural terpenoids having lipophilic fragments with high biological activity is of particular interest for the creation of therapeutic agents.

Thus, here we present the concept of creating cationic amphiphilic meroterpenoids with bacteriostatic and fungistatic effects against pathogenic strains of fungi and bacteria. The concept is based on the thia-Michael reaction between terpenyl acrylates and 2-(dimethylamino)ethanethiol.

2. Materials and methods

2.1. General experimental information

All reagents and solvents (Sigma-Aldrich, St. Louis, MO, USA) were used directly as purchased or purified according to the standard procedures. The ^{1}H and ^{13}C NMR spectra were recorded on an Avance 400 spectrometer (Bruker Corp., Billerica, MA, USA) (400 MHz for ^{1}H-atoms) for 5% solutions in $CDCl_3$ and DMSO-d_6. The residual solvent peaks were used as an internal standard. The FT-IR ATR spectra were recorded on a Spectrum 400 FT-IR spectrometer (Perkin Elmer, Seer Green, Lantrisant, UK) with a Diamond KRS-5 attenuated total internal reflectance attachment (resolution 0.5 cm^{-1}, accumulation of 64 scans, recording time 16 s in the wavelength range 400–4000 cm^{-1}). ESI-HRMS experiments of compounds were analyzed using an Impact II mass spectrometer with Elute UHPLC system («Bruker Daltonik GmbH», Germany). The column used was YMC-Triart C18 (50×2 mm; 3 μm). The temperature of the column thermostat was set at 40 °C and the temperature of the autosampler at 12 °C. The elution solvents used were 0.1% formic acid in Milli-Q water (A) and 0.1% formic acid in HPLC-grade acetonitrile (B), and the elution gradient was the following: 0 min at 5% B, 3 min at 95% B, 4 min at 95% B, 4.1 min at 95% B, 6 min at 95% B with flow rate of 0.3 mL/min. The injection volume was 2 μL. The analytes were ionized by electrospray in positive polarity. The ESI conditions were set with the capillary temperature of 220 °C, capillary voltage of 4.5 kV and sheath gas (N2) flow rate of 6 L/min. The measurements were made in the range *m/z* 300–3500. The solution of analyte (1 mg/mL, HPLC-grade methanol) was diluted in HPLC-grade acetonitrile to concentration 0.01 mg/mL. The solution of sodium iodide in Milli-Q water (0.2 mg/mL) was used as a calibrant. The relative error in determining the masses was no more than 5.0 ppm. For instrument control and data aquisition, the otofControl software (Bruker Daltonik GmbH, Version 5.2) was used. The data processing was performed by the DataAnalysis software (Bruker Daltonik GmbH, Version 5.3). The melting points were determined using the Boetius Block apparatus (VEB Kombinat Nagema, Radebeul, Germany).

2.2. Synthesis of compounds 2a–d

Compounds **2a–d** were synthesized according to published procedures [27].

2.3. General synthesis procedure 3a–d

A solution of 2.5 mmol of terpenyl acrylate (**2a–d**) in methanol was prepared in a 25 mL round-bottom flask. 2.1 mmol of 2-(dimethylamino)ethane-1-thiol hydrochloride was added to the resulting solution. Next, the mixture was refluxed for 56 h. The solvent was removed using a rotary evaporator. The crude product was recrystallized from hexane to remove the terpenyl acrylate. Then the crude product was dried on a rotary evaporator under reduced pressure. The products were obtained in the form of pale yellow powders.

2.3.1. 2-((3-(geranyloxy)-3-oxopropyl)thio)-*N*,*N*-dimethylethan-1-aminium chloride 3a

Yield: 0.7113 g (97%), pale yellow powder. *M*p = 66 °C. ^{1}H NMR ($CDCl_3$, δ, ppm, *J*/Hz): 1.59 (*s*, 3H, $-CH_3$), 1.67 (*s*, 3H, $-CH_3$), 1.70 (*s*, 3H, $-CH_3$), 2.03–2.10 (*m*, 4H, $-CH_2-CH_2-$), 2.65 (*t*, 2H, $-CH_2-C(O)-O$, $^{3}J_{HH}$ = 6.9 Hz), 2.83 (*d*, 6H, $-N^{+}(CH_3)_2$, $^{3}J_{HH}$ = 4.9 Hz), 2.84–2.86 (*m*, 2H, $-CH_2N^{+}-$), 3.02–3.06 (*m*, 2H, $-CH_2S-$), 3.18–3.24 (*m*, 2H, $-CH_2S-$), 4.61 (*d*, 2H, $-CH_2O-$, $^{3}J_{HH}$ = 7.1 Hz), 5.07 (*t*, 1H, =CH–, $^{3}J_{HH}$ = 6.5 Hz), 5.32 (*t*, 1H, =CH–, $^{3}J_{HH}$ = 6.6 Hz), 12.77 (*br.s.*, 1H, $-N^{+}H$). ^{13}C NMR (DMSO-d_6, δ, ppm): 16.16, 17.56, 24.60, 25.47, 25.77, 25.91, 30.94, 34.18, 41.84, 42.04, 55.26, 60.83, 118.39, 123.70, 131.06, 141.48, 171.28. FTIR (ν/cm^{-1}): 3016 (C=C–H); 2964 (CH_3); 2939 (CH_2); 2938 (CH_2–O); 2908 (CH_2–C(O)O); 2887 (CH_3); 2710–2376 (NH^{+}); 1725 (C=O); 1452 (CH_2); 1437 (CH_3); 1434 (CH_2–S); 1373 (CH_3); 1374 ($C(CH_3)_2$); 1362 ($C(CH_3)_2$); 1306 (CH_2)1243 (CH_2–S); 1228 (C–O–C); 1127 (C–O–C); 1044 (C–NH); 816 (C=C–H). HRMS: calculated $[M-Cl^{-}]^{+}$ *m/z* = 314.2148, found $[M-Cl^{-}]^{+}$ *m/z* = 314.2155.

2.3.2. 2-((3-(*S*-perillyloxy)-3-oxopropyl)thio)-*N*,*N*-dimethylethan-1-aminium chloride 3b

Yield: 0.6197 g (85%), pale yellow powder. *M*p = 82 °C. ^{1}H NMR (CDCl3, δ, ppm, J/Hz): 1.42–1.53 (*m*, 1H, –CH–), 1.73 (*s*, 3H, $-CH_3$), 1.82–1.87 (*m*, 1H, $-CH_2-$), 1.93–2.00 (*m*, 1H, $-CH_2-$), 2.05–2.18 (*m*, 4H, $-CH_2-CH_2-$), 2.67 (*t*, 2H, $-CH_2-C(O)-O$, $^{3}J_{HH}$ = 6.9 Hz), 2.84 (*d*, 6H, $-N^{+}(CH_3)_2$, $^{3}J_{HH}$ = 4.8 Hz), 2.85–2.87 (*m*, 2H, $-CH_2N^{+}-$), 3.02–3.06 (*m*, 2H, $-CH_2S-$), 3.17–3.24 (*m*, 2H, $-CH_2S-$), 4.48 (*s,* 2H, $-CH_2O-$), 4.71 (*d*, 2H, $=CH_2$, $^{3}J_{HH}$ = 8.5 Hz), 5.75 (*m*, 1H, =CH–), 12.70 (*br.s.*, 1H, $-N^{+}H$). ^{13}C NMR (DMSO-d_6, δ, ppm): 20.60, 24.63, 25.74, 25.92, 26.78, 29.77, 30.91, 34.15, 41.91, 42.09, 55.63, 67.65, 109.02, 124.90, 132.39, 149.01, 171.22. FTIR (ν/cm^{-1}): 3012 (CH=C); 2961 (CH_3); 2938 (CH_2–C(O)O); 2937 (CH_2–O); 2921 (CH_2); 2888 (CH_3); 2835 (CH_2); 2709–2316 (NH^{+}); 1725 (C=O); 1644 (CH_2=C); 1473 (CH_2); 1464 (CH_3); 1457 (CH_2); 1436 (CH_2–S); 1427 (CH_2=C); 1374 (CH_3); 1242 (CH_2–S); 1230 (=C–O–C); 1145 (C–O–C); 1042 (C–NH); 888 (CH_2=C); 815 (H–C=C). HRMS-ESI: calculated $[M-Cl^{-}]^{+}$ *m/z* = 312.1991, found $[M-Cl^{-}]^{+}$ *m/z* = 312.1992.

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2.3.3. 2-((3-(*R*-myrtenyloxy)-3-oxopropyl)thio)-N,N-dimethylethan-1-aminium chloride 3c

Yield: 0.5687 (78%), pale yellow powder. *M*p = 59 °C. ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.81–0.82 (*br.s.*, 3H, –CH_3–), 1.15–1.17 (*s*, 1H, –CH–), 1.28 (*s*, 3H, –CH_3–), 1.96–2.01 (*m*, 2H, –CH_2–), 2.09–2.11 (*m*, 1H, –CH–), 2.40 (*dt*, 2H, –CH–, $^3J_{HH}$ = 8.8, 5.6 Hz), 2.66 (*t*, 2H, –CH_2–C(O)–O, $^3J_{HH}$ = 6.9 Hz), 2.85 (*d*, 6H, –$N^+(CH_3)_2$, $^3J_{HH}$ = 5.0 Hz), 2.89–2.91 (*m*, 2H, –CH_2S–), 2.99–3.05 (*m*, 2H, –CH_2S–), 3.20–3.25 (*m*, 2H, –CH_2N^+–), 4.43–4.53 (*m*, 2H, –CH_2O–), 5.56 (*m*, 1H, =CH–), 12.72 (*br.s.*, 1H, –N^+H). ^{13}C NMR (DMSO–d_6, δ, ppm): 21.06, 28.55, 31.06, 31.37, 34.55, 37.81, 40.45, 41.89, 42.08, 44.77, 55.41, 66.85, 121.21, 129.39, 171.29. FTIR (ν/cm^{-1}): 3649–3150 (R_2N–H); 2946 (CH_2–C(O)O); 2943 (CH_2), 2938 (CH_3); 2937 (CH_2–O); 2914 (CH_2); 2831 (CH_2); 2762–2251 (NH^+); 1728 (C=O); 1471 (CH_2); 1429 (CH_2–S); 1383 ($C(CH_3)_2$); 1365 ($C(CH_3)_2$); 1248 (CH_2–S); 1170 (C–O–C); 1046 (=C–O–C); 809 (C=CH). HRMS-ESI: calculated $[M–Cl^-]^+$ *m/z* = 312.1991, found $[M–Cl^-]^+$ *m/z* = 312.1989.

2.3.4. 2-((3-(2*E*,6*Z*-farnesyloxy)-3-oxopropyl)thio)-N,N-dimethylethan-1-aminium chloride 3d

Yield: 0.7360 g (84%), pale yellow powder. *M*p = 94 °C. ^{1}H NMR ($CDCl_3$, δ, ppm., *J*/Hz): 1.60 (*s*, 3H, CH_3), 1.68 (*s*, 6H, CH_3), 1.70 (*s*, 3H, CH_3), 2.03–2.10 (*m*, 8H, –CH_2–CH_2–), 2.65 (*t*, 2H, –CH_2–C(O)–O, $^3J_{HH}$ = 6.8 Hz), 2.83 (*d*, 6H, –$N^+(CH_3)_2$, $^3J_{HH}$ = 4.8 Hz), 3.03–3.07 (*m*, 2H, –CH_2S–), 3.18–3.23 (*m*, 2H, –CH_2S–), 4.62 (*d*, 2H, –CH_2O–, $^3J_{HH}$ = 7.2 Hz), 5.09 (*t*, 2H, =CH–, $^3J_{HH}$ = 5.9 Hz), 5.33 (*t*, 1H, =CH–, $^3J_{HH}$ = 7.1 Hz), 12.87 (*br.s.*, 1H, –N^+H). ^{13}C NMR (DMSO–d_6, δ, ppm): 15.79, 16.15, 17.54, 23.14, 24.63, 25.47, 25.65, 25.93, 26.16, 31.06, 34.19, 41.83 42.08, 55.32, 60.82, 118.42, 123.51, 124.07, 130.66, 134,71, 141.45, 171.27. FTIR (ν/cm^{-1}): 3500–3200 (R_2N–H); 2966 (CH_2–C(O)O); 2962 (CH_3); 2920 (CH_2); 2854 (CH_2); 2804–2250 (NH^+); 1728 (C=O); 1669 (C=C); 1452 (CH_2); 1442 (CH_2–S); 1377 ($C(CH_3)_2$); 1356 ($C(CH_3)_2$); 1240 (CH_2–S); 1238 (=C–O–C); 1148 (C–O–C); 830 (=C–H). HRMS-ESI: calculated $[M–Cl^-]^+$ *m/z* = 382.2774, found $[M–Cl^-]^+$ *m/z* = 382.2776.

2.3.5. Resazurin assay

MIC of **3a–d** for *Staphylococcus aureus* ATCC® 29213™, *Staphylococcus epidermidis* clinical isolate, *Klebsiella pneumoniae* clinical isolate, *Pseudomonas aeruginosa* clinical isolate, *Salmonella typhimurium* TA 98, *Saccharomyces cerevisiae* and *Candida* sp. clinical isolate were determined with resazurine assay according to the published method [33]. Briefly, sterile 96 well plates were prepared and labelled under aseptic conditions, a volume of 180 μL of *S. cerevisiae* and *Candida* sp. suspensions ($5×10^5$ cells/mL) in Sabouraud broth and 180 μL of *S. aureus* ATCC® 29213™, *S. epidermidis, K. pneumoniae, P. aeruginosa*, *S. typhimurium TA 98* in LB-broth were pipetted into the first row of the plates. To all other wells 100 μL of broth was added. Then a volume of 20 μL of tested agent solutions in H_2O was added into the first row and serial dilutions were performed using a multichannel pipette. Each well had 100 μL of the medium with the test materials in sequentially descending concentrations. Each plate was wrapped loosely in cling film to ensure that the culture did not become dehydrated. The variants were prepared in triplicate and placed in an incubator set at 37 °C for 24 h. After incubation 10 μL of resazurin indicator solution (0.1%) was added into each well. The colour change was then assessed visually after 4 h incubation at 37 °C with resazurin. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which the colour change did not take place was taken as the MIC value. The MIC determination was carried out in three independent experiments.

2.3.6. Ames test

A histidine-dependent strain of *Salmonella typhimurium* TA 98, was used in the experiment. This strain carries mutations in the genes of the histidine operon, and exposure to mutagens can increase the frequency of spontaneous reversions from the initially histidine auxotrophic state to prototrophy [34]. *S. typhimurium* TA 98 was incubated overnight in L-broth with ampicillin (25 μg/mL). 5 mL of the overnight culture was transferred to 20 mL of fresh LB broth with ampicillin and incubated for 2 h at 37 °C with aeration. The cell pellet was resuspended in 0.02 M sodium phosphate buffer (pH = 7.2). Glucose minimal agar was preprepared and poured into Petri plates and 0.6% top agar into tubes. 0.1 mL of the bacterial suspension and 0.1 mL of the test agent solution were added to the melted and precooled to ~45 °C. The top agar was then stirred and poured onto the glucose minimal agar. After complete solidification of the agar, the plates were transferred to the thermostat and incubated at 37 °C.

Water was used as the solvent in the negative control of the Ames test. 4-Nitro-O-phenylenediamine (2.5 μg per plate) was used as a positive control. The results were recorded by counting revertant colonies after 48 h of incubation. The number of revertant colonies grown under the influence of terpenoids was compared with that in the negative control. A compound was considered to be non-mutagenic if the number of induced revertants was less than twice the number of spontaneous revertants as recommended in [35].

3. Results and Discussion

3.1. Synthesis of the meroterpenoids

The starting compound for designing the cationic amphiphilic meroterpenoids was 2-(dimethylamino)ethanethiol hydrochloride. We selected it because it contains an ammonium moiety and a reactive –SH group. Conjugated nucleophilic addition to electron-deficient alkenes and alkynes is one of the most frequently used methods for creating a new carbon-heteroatom bond [36–38]. The thia-Michael reaction is one of the convenient methods for creating C–S bonds. Therefore, terpene acrylates **2a–d** (Scheme 1)

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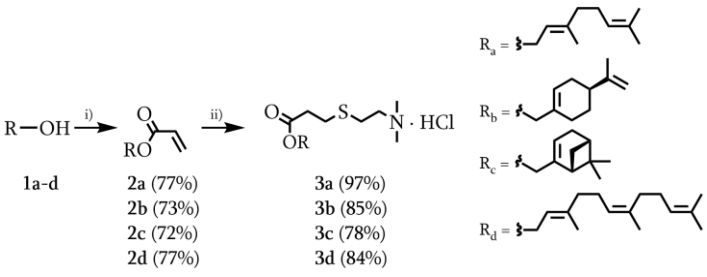
based on geraniol, *S*-perillyl alcohol, *R*-myrtenol and farnesol were selected as the initial terpene-containing compounds. Previously, farnesol activity was shown against such cell strains as *S. aureus* ATCC 43300 (MIC 0.128 mg/mL), *S. epidermidis* ATCC 12228 (0.032 mg/mL); however, farnesol does not show activity against most strains and clinical isolates [39]. Some geraniol derivatives have also been shown to have activity against *Candida albicans* (MIC 0.025 mg/mL) [40]. Terpene acrylates were obtained according to a previously published method [27]. The interaction of terpene acrylates with 2-(dimethylamino)ethanethiol hydrochloride was studied. Several possible approaches were considered. The solvent of the synthesis was varied: methanol, acetonitrile, DMF. The form of 2-(dimethylamino)ethanethiol used was either an amine or a hydrochloride. The best results were received when the reaction was carried out at the boiling point of methanol with 2-(dimethylamino)ethanethiol hydrochloride. As a result, target products **3a–d** were obtained in yields of 79–97%. Admixture of the initial acrylate was removed by recrystallization from hexane.

Thus, based on the thia-Michael reaction, an approach to the design of cationic amphiphilic meroterpenoids **3a–d** was developed. Optimal conditions for the synthesis between the starting terpene acrylates and a compound containing an ammonium group and a thiol fragment (2-(dimethylamino)ethanethiol hydrochloride) were selected. The target cationic amphiphilic meroterpenoids were obtained in high yields of 79–97%. The structure and composition of the obtained compounds were confirmed by a number of physical methods such as ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy, HRMS ESI.

3.2. Study of antifungal, antibacterial activity and the mutagenicity of the compounds 3a–d

The antifungal activity of compounds **3a–d** was studied using the yeast *Saccharomyces cerevisiae*, one of the best studied and easily grown model organisms. Experiments were also performed on a clinical isolate of *Candida* sp. The antibacterial activity of compounds **3a–d** was studied on Gram-positive (*Staphylococcus aureus*, clinical isolate of *Staphylococcus epidermidis*) and Gram-negative (*Salmonella typhimurium*, clinical isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria. Using the resazurin assay, it was shown that meroterpenoids **3a–d** (Table 1) have inhibitory properties against bacteria and fungi. The sesquiterpene farnesol derivative **3d** (0.039 mg/mL) showed the greatest activity against *Saccharomyces cerevisiae*. The monoterpene derivative **3a** (0.156 mg/mL), containing a geranyl moiety, also had moderate activity against the *Saccharomyces cerevisiae* cell line (Table 1). A study of the effectiveness of terpenoids **3a–d** against *Candida* cells was carried out on a clinical isolate of *Candida* sp. This fungal cell line turned out to be more resistant to the studied compounds than *Saccharomyces cerevisiae*. However, the trend remains: the derivatives of farnesol **3d** (0.078 mg/mL) and the derivatives of geraniol **3a** (0.625 mg/mL) showed the greatest activity. The bacteria *Staphylococcus aureus* turned out to be less sensitive to the action of meroterpenoids. The derivatives of geraniol **3a** and farnesol **3d** showed the greatest activity with the same MIC value of 0.3125 mg/mL. Clinical isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* turned out to be the most sensitive to the action of the myrtenol derivative **3c** – 0.625 and 0.156 mg/mL, respectively. Since the resazurin assay was carried out at pH 7.0–7.4, it can be argued that the studied compounds **3a–d** during the experiment were in the form of quaternized ammonium salts.

Mutagenicity is one of the many adverse properties of a compound, preventing it from becoming a commercial drug. Screening drug candidates for mutagenicity is a regulatory requirement for drug approval since mutagenic compounds pose a toxic hazard to humans. The Ames test is a short-term *in vitro* assay designed to detect genetic damage caused by chemicals. It has become the standard test for mutagenicity because it is relatively simple, rapid, and inexpensive.



Scheme 1 Reagents and conditions: i) CH_2=CHC(O)Cl, DIPEA, $CHCl_3$, −5 °C, ii) 2-(Dimethylamino)ethanethiol hydrochloride, CH_3OH, reflux.

Table 1 Antifungal and antibacterial activity of compounds **3a–d**.

Compound	MIC (mg/mL)						
	Fungi		Bacteria				
	Saccharomyces cerevisiae	*Candida* sp.	*S. aureus*	*S. epidermidis*	*S. typhimurium*	*P. aeruginosa*	*K. pneumoniae*
3a	0.156	0.625	0.3125	0.156	0.156	5	1.25
3b	0.3125	1.25	0.625	0.3125	0.156	2.5	0.625
3c	0.3125	2.5	2.5	1.25	0.625	0.625	0.156
3d	0.039	0.078	0.3125	0.02	0.078	1.25	2.5
Fluconazole	0.016 [41]	0.032 [42]					

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In this research, the direct mutagenicity of terpenoids **3a–d** was determined using the Ames test in the variant without metabolic activation. The excess of the number of revertant colonies to the histidine prototrophy of the *S. typhimurium* TA 98 strain when treated with test agents **3a–d** over that in the variant without treatment (negative control) was determined. To validate the results obtained, we used a variant with the addition of a known mutagen, 4-Nitro-*O*-phenylenediamine (positive control) to the incubation medium at a concentration of 2.5 μg/plate. The results of the Ames test are presented in Tables 2–3. A tenfold excess of the number of revertants in the positive control over the negative control indicates the validity of the test system used. In none of the experimental variants studied we recorded the formation of a number of revertants that would exceed that in the negative control variant by more than 2.5 times. This could indicate the presence of mutagenic activity in the tested compounds. Thus, water-soluble terpenoid derivatives **3a–d** at the studied concentrations did not exhibit mutagenic activity in the Ames test without metabolic activation.

It was shown that the application of the thia-Michael reaction between terpenyl acrylates and 2-(dimethylamino)ethanethiol is valid for the design of cationic amphiphilic meroterpenoids with bacteriostatic and fungistatic effects against pathogenic strains of fungi and bacteria. Compound **3d** containing a farnesyl fragment was the most active against the fungi *Saccharomyces cerevisiae* (MIC 0.039 mg/mL) *Candida* sp. (MIC 0.078 mg/mL) and Gram-positive bacteria clinical isolate *Staphylococcus epidermidis* (MIC 0.02 mg/mL) and Gram-negative *Salmonella typhimurium* (MIC 0.078 mg/mL). Despite the fact that the activity of compound **3d** is slightly lower than that of the known drug fluconazole (Table 1), compound **3d** is not mutagenic, while fluconazole induces cytotoxic and geno-toxic alterations [43]. The Ames test showed the absence of direct mutagenic activity of all tested compounds.

Table 2 Results of control crops.

	Positive control	Negative control
Number of revertants ± SD	101.0±7.9	9.3±3.8

Table 3 Results of the analysis of mutagenic activity of compounds **3a–d**.

Compound	*C*, mg/mL	Number of revertants ± SD
3a	0.04	9.0±4.4
	0.08	9.7±5.8
3b	0.04	10.0±3.5
	0.08	6.0±7.1
3c	0.2	7.0±2.0
	0.4	8.5±4.9
3d	0.02	6.0±2.0
	0.04	12.6±9.6

4. Limitations

This article describes an approach to the design of cationic amphiphilic meroterpenoids with bacteriostatic and fungistatic effects. Despite the apparent simplicity of the synthesis of the target compounds, the use of this approach with other terpene acrylates or other Michael acceptors will require the search of optimal synthesis conditions, which is the subject of further research. Among other things, the cytotoxicity of the synthesized compounds remains to be studied.

5. Conclusions

Thus, based on the thia-Michael reaction, an approach to the design of cationic amphiphilic meroterpenoids containing geranyl, *S*-perillyl, *R*-myrtenyl or 2*E*,6*Z*-farnesyl fragments was developed. The target compounds were obtained in high yields of 79–97%. The bacteriostatic and fungistatic activity of the synthesized meroterpenoids against pathogenic strains of fungi and bacteria was studied. The compound containing the farnesyl fragment was the most active against fungi *Saccharomyces cerevisiae* (MIC 0.039 mg/mL), *Candida* sp (MIC 0.078 mg/mL), Gram-positive bacteria of the clinical isolate *Staphylococcus epidermidis* (MIC 0.02 mg/mL) and Gram-negative *Salmonella typhimurium* (MIC 0.078 mg/mL). Also, the Ames test showed the absence of direct mutagenic activity of all the studied compounds. The compounds developed in this research may find their use as safe antimicrobial or antiseptic agents.

• Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

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