Terpene-Based Ionic Liquids from Natural Renewable Sources As Selective Agents in Antifungal Therapy

Joanna Feder-Kubis,* Anita WNętrzak, and Anna Chachaj-Brekiesz

ABSTRACT: In this study, we present a new approach toward the design of ionic liquids with biological activity. Structural analysis of bioactive compounds was performed to design—in a technological and economic manner—salts with potential antifungal properties. The length of the alkyl chain as well as the task-specific component in the cation, the type of amine core, and the type of anion were considered as having an essential impact on achieving desired biological activity. Herein, we present the synthesis and characterization of ionic liquids based on monoterpene derivatives—namely, (1R,2S,5R)-(−)-menthol or bicyclic (1R)-endo-(+)-fenchol—from renewable sources. These new salts were synthesized with high yields (>96%) in mild conditions via a two-step procedure. Physicochemical properties (i.e., melting point, thermal stability, crystal shape, specific rotation, surfactant content, solubility, and surface activity) were analyzed in detail. The obtained results suggested the influence of the steric hindrance of the discussed salts on the reactivity, solubility, thermal stability, and surface properties of the studied compounds. Their potential selectivity in antifungal therapy was studied using Langmuir monolayer mimicking fungal (ergosterol) and mammalian (cholesterol) membranes. The model study confirmed the selective destabilizing activity of terpene-based ionic liquids on the fungus membrane.

KEYWORDS: (−)-menthol, (+)-fenchol, natural plant resources, antifungal agent, artificial membrane, Langmuir monolayers

INTRODUCTION

The estimation that there are more than five million fungal species worldwide is an appalling consideration. Also intimidating is the number—around 300—of fungal species known to cause disease in humans.1 The continued development and suitable use of both old and new antifungal drugs appear to be the most important factors in the effective and fully successful management of fungal infections.

The ever-increasing problem and growing resistance of organisms to treatment forces us to constantly search for new promising antifungal agents. Among very capable groups that are considered to be antimicrobial useful materials are ionic liquids (ILs).2 These specific compounds are composed entirely of ions3 and are considered to be compounds of a highly tunable nature due to their ability to choose dedicated cations and anions. This opens the door to designing compounds for specific applications, including antifungal therapy.4−6 The recent literature contains many examples of ionic liquids that exhibit antifungal activity, both for salts with a commonly known positively charged ion, having mostly the basic cation form (alkyl, aryl-substituted) of alkylimidazolium,7−9 alkylpyridinium ILs,7,8 alkylpyrrolidinium,10 alkylpiperidinium,11 and alkylquinolinium,12 and for those whose cations are more structurally complex or/and substituted with special, dedicated groups, such as quaternary ammonium salts with the (1R,2S,5R)-(−)-menthol moiety.11 Furthermore, antifungal activity is investigated for ionic liquids with a functional anion group—for instance, cholinium salts,3 quaternary ammonium salts with a lactate anion,8 or mandelic-acid-based ILs.12 In the vast majority of the aforementioned works, the tested ILs possessed noticeable activities against fungi;7,9−12 additionally, the efficiency of those activities was similar to, or even better than, that of commonly used microbiological standards.

Another aspect of the antifungal activities of ILs is microbial tests in terms of their application as wood preservation agents.13−15 A scientific paper describing “sweet” ionic liquids13 demonstrated that laboratory tests on their antifungal properties are very promising. The biological effectiveness of saccharinate-based chiral ionic liquids (CILs) with an alkyl substituent from pentyl to octyl was similar to, and even higher than, that noticed for the commercial material benzalkonium chloride.

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A noteworthy approach toward obtaining bioactive salts is the design and subsequent synthesis of ionic liquids that have multifunctional biological activities, in which one of the functions is antifungal activity.16−18 Interesting examples of such compounds are bifunctional benzo[1.2.3]thiadiazole-7-carboxylate salt-based terpene ionic liquids.16 Reported dual-function compounds might be successfully applied as antifungal and antibacterial agents. At the same time, discussed salts are known as plant resistance inducers. Such multifunctional compounds are extremely desirable and have a substantially increased applicability in various biotechnology areas.

Previously, through systematic research on various groups and homologous series of ILs that possess a naturally occurring moiety from plants (including the following salts: ammonium,19 pyridinium,20 alkylimidazolium,21 and alkoxymethylimidazolium22), we were able to conclude that most of the tested compounds are highly active in an antimicrobial sense. In turn, we selected those that showed particularly strong antibacterial and antifungal activity.23 In addition, our research has clearly shown that further targeted microbiological tests should be performed primarily on ammonium or imidazolium salts containing a long alkyl substituent.23 Also of significance is the optically active form of the tested salts, which significantly exceeds the microbiological effect in relation to its racemic counterparts, which we have shown in the example of menthol compounds.23 Importantly, in our next study, we determined the potential role of selected ammonium salts with the (1R,2S,5R)-(−)-menthol moiety as antifungal disinfectants and, furthermore, indicated their mechanism of action on cell envelopes, including the cell wall and plasma membrane.11 The chosen ionic liquids have great potential as disinfectants because they exhibit antifungal and antiadhesive activities and also because they do not cause hemolysis.11

The positive and very promising results encouraged us to engage in further research in the direction indicated by the described tests. In our current study, we present a new approach toward obtaining highly biological active ionic liquids, which might be extremely useful in developing powerful antimicrobial agents to treat different kinds of infections.

A good antifungal agent should differ between the host and fungi, acting only on the latter. The mechanism of this activity may be membrane-related; for that reason, the interactions of ILs with sterols that are characteristic of the mammalian and fungi membranes (cholesterol and ergosterol, respectively) are of great importance. We applied the Langmuir technique to investigate the affinity of ionic liquids to the cell membrane.24 In this technique, a one-molecule layer, formed at the aqueous solution−air interface from the membrane components, serves as a simple physical model of the natural biological membrane.25,26 The advantage of using the Langmuir monolayer technique is that it allows for the control of several parameters of the formed layer, including the degree of molecular packing, pressure, and composition. The thermodynamic analysis of the results enables the determination of the nature and strength of the interactions between the components of the investigated layer.27 Similar studies based on the Langmuir monolayer technique confirmed the selectivity of nystatin and monoamphiphilic pentacyclic triterpenes, as well as of amphotericin B and their derivatives, on the fungal membrane.28−32 Interestingly, the Langmuir monolayer technique was successfully used to investigate the influence of ILs on the rheological properties of the lipid membrane.33

EXPERIMENTAL SECTION

Synthesis of Terpene-Based Ionic Liquids. Materials for Synthesis and Purification. The chemicals for synthe-
The synthesis of quaternization agents—Monoterpene Moiety (\(\text{Chloride [C}_{14}\text{-Am-Men}[\text{Cl}]\))—analytically pure compounds with yields in the range of 96.5–30 mL). The resulting optically active chlorides were dried under stirring mixture of freshly distilled \(\text{N}_{2}\) at 0.88 mbar). The quaternization process took approximately 30 min (details are given in Table 1), but the reaction mixture was maintained at unchanged conditions for 4.5s and 5.00 (d, \(J = 6.8\ Hz, 2H, AB\) system, \(\text{O} - \text{CH}_2\)).

To measure the specific rotations of the obtained chiral salts, an Optical Activity Ltd. model AA-5 automatic polarimeter was applied. The tests were performed at a 578 nm sodium line, 25 °C. The measurement error parameters for the discussed apparatus, which provide four results for each measurement, are as follows: resolution ±0.01°, reproducibility ±0.01°, accuracy ±0.01°, and temperature probe measurement accuracy ±0.1 °C.

**Synthesis. General Procedure for the Synthesis and Purification of the Precursors of Quaternary Ammonium Salts with Monoterpene Moeity (2a and 2b).** Chloromethyl \(\text{(1R,2S,5R)-(−)-methyl ether (2a) and chloromethyl \(\text{(1R)-endo-(+)-fenchyl ether (2b) were obtained following the previously reported procedures, respectively. The synthesis of quaternization agents was carried out in toluene, maintaining anhydrous reaction environment conditions, by passing HCl through a mixture of formaldehyde and proper alcohol, \(\text{(1R,2S,5R)-(−)-methyl ether (1a) or \(\text{(1R)-endo-(+)-fenchyl ether (1b). The resulting chloromethyl terpeneyl ethers (2a and 2b) were purified by vacuum distillation. The yields of the obtained chiral esters and the distillation conditions were similar to those presented in the literature.**

The aliphatic amine, \(\text{N,N-dimethyltetradecylamine},\) was purified by vacuum distillation each time before use (bp = 148 °C; pressure 2–3 mbar).

**General Procedure for the Synthesis of Ionic Liquids (3a and 3b) with Monoterpene Moeity. Menschutkin Quaternization.** Freshly distilled chloromethyl \((1R,2S,5R)-(−)-methyl ether (2a) (12.489 g, 0.061 mol) or chloromethyl \((1R)-endo-(+)-fenchyl ether 2b (12.366 g, 0.061 mol) was added dropwise to a vigorously stirred mixture of freshly distilled \(\text{N,N-dimethyltetradecylamine (14.488 g, 0.060 mol) in dry hexane (45 mL). The quaternization process took approximately 30 min (details are given in Table 1)), but the reaction mixture was maintained at unchanged conditions for another 30 min. Both of the products (3a and 3b) were precipitated from the reaction mixture, filtered, and washed with dry hexane (3 × 30 mL). The resulting optically active chlorides were dried under reduced pressure (3–5 mbar) at 35 °C for 2 days to obtain analytically pure compounds with yields in the range of 96.5–99.5%.**

**Cationic Active Substance Content.** The active cationic content was analyzed for obtained ionic liquids through direct two-phase titration using the European Standard 2010 procedure for salts with a molecular mass lower than 500 g/mol.

**Surface Tension Measurements.** The surface tension (\(\gamma\) in mN/m) was measured using a Krüss surface tensiometer (Germany, model K107), by means of the ring detachment method. The temperature of the measurement cell was 25 °C and was controlled by a thermostat with an accuracy of ±0.1 °C. The calibration process was completed using double-distilled water before each measurement. All measurements were performed in triplicate. The critical micelle...
Table 2. Properties of Ionic Liquids with Terpene Moiety (3a and 3b)

| salt number | physical state | melting point (°C) | crystal shape | thermal stability Tc(m) (°C) | surfactant content (%) | specific rotation [α]D (°) | CMC (mol L−1) | γC(M−1) (mN m−1) |
|-------------|----------------|-------------------|---------------|----------------------------|------------------------|--------------------------|----------------|----------------|
| 3a          | solid (hygroscopic) | 51.8–52.4         | —             | 194.5                      | 99.1                   | −36.60 (± 0.998)         | 1.4 × 10−3  | 38.23          |
| 3b          | solid           | 55.9–56.3         | short, irregular needles | 170.1                      | 99.6                  | +7.01 (± 1.075)         | 5.0 × 10−3  | 40.40          |

"At 25 °C. "Accuracy ±0.1 °C. "Unmeasurable due to hygroscopicity. "Experiments carried out with a heating rate of 10 °C min−1. "Accuracy ±0.1%; x in methylene chloride. "Standard uncertainty for specific rotation a is u(α) = ±0.5°. "Standard uncertainty for concentration a is u(c) = ±0.002 g/100 mL.

concentration values (CMC) were obtained from the inflection in the curve of the Tc(M) vs the logarithm of salt concentration. The uncertainty of the method given by the manufacturer is ±0.1 mM−1. The accuracy of the Tc(M) measurements did not exceed the value of ±0.2 mM−1. The surface active properties—the critical micelle concentration (CMC) and the surface tension values at the CMC (γ[M])—are shown in Table 2.

**Langmuir and Langmuir–Blodgett Study. Materials.** Cholesterol (Chol) and ergosterol (Erg) of purity >99% were purchased from Sigma-Aldrich and used without any further purification. Cholesterol of spectroscopic grade (≥99.9%), stabilized with ethanol, was provided by Sigma-Aldrich. Cholesterol and methanol (POCH, Poland) of a standard purity of ≥99% were used to clean the Langmuir trough. In routine experiments, 0.1 M NaCl solution in ultrapure water (produced by the Millipore system) was used as a subphase.

**Langmuir–Blodgett Isotherms.** The investigated lipids and ionic liquids were dissolved in chloroform with a typical concentration of 0.3–0.4 mg/mL. Mixed solutions of IL/Erg and IL/Chol were obtained by mixing appropriate volumes of the respective stock solutions. Langmuir monolayers were formed through the spreading of an aliquot of the above-mentioned solutions onto the surface of a 0.1 M NaCl solution. The π–a isotherms were recorded using a single-barrier Langmuir–Blodgett trough (NIMA 301S, total area = 300 cm²) with a compression speed of 20 cm²/min. Surface pressure was measured using the Wilhelmy plate method (ashless chromatography paper) with a sensitivity of ±0.1 mN/m. The temperature of the aqueous subphase was kept constant at 20 °C ± 0.1 °C. Each measurement was repeated 2–3 times to ensure the high reproducibility of the obtained isotherms to ±1 Å²/molecule.

**Brewster Angle Microscopy.** The texture of monolayers was visualized using a Brewster angle microscope (ultraBAM instrument, Accurion GmbH, Goettingen, Germany) equipped with a 50 mW laser (emitting p-polarized light at a wavelength of 658 nm) integrated with a KSV 2000 700 cm² double-barrier Langmuir trough (KSV 2000, total area = 700 cm²). The monolayer formation procedure was identical to that described above. BAM images presented in this paper show monolayer fragments of 720 × 400 μm².

**Langmuir–Blodgett (LB) Transfer and Imaging of Transferred Monolayers with Atomic Force Microscopy (AFM).** Langmuir monolayers of ionic liquid/sterol (1:1) mixed systems were transferred using the double-barrier Langmuir–Blodgett trough (NIMA 612D, total area = 600 cm²). The monolayer was transferred onto a mica substrate at a surface pressure of 30 mN/m (a value corresponding to physiological conditions) using a dipper speed of 2 mm min−1. Transfer ratio values were close to 1. AFM topographies were collected in noncontact mode, in ambient with an AFM microscope (Park Scientific Instruments). Areas of 2.5 μm × 2.5 μm were scanned using a 0.2–0.5 Hz scan rate. NSG01 cantilevers (resonant frequency: 87 kHz–230 kHz, force constant 1.45 N/m–15.1 N/m, tip curvature radius: 6 nm–10 nm, purchased from NT-MDT) were applied. Collected AFM images were flattened (second-order polynomial), and then profiles were extracted using Gwyddion (ver. 2.30) software.

**RESULTS AND DISCUSSION**

**Detailed Structure Analysis for Choosing the Proper Ionic Liquids for Antifungal Therapy.** This study presented terpene-based ionic liquids, which contain natural renewable sources in their structures, as potential biological material in antifungal therapy. To understand the manner of choosing the proper compounds for such an investigation, a diagram allowing for the interpretation of crucial structural factors is shown (Scheme 1).

**Scheme 1. Approach for Designing Ionic Liquids with Biological Activities**

Presented here is a strategy for designing ionic liquids that are distinguished by four important elements of the structure, which might be essential when salts with biofunctional activities are needed. We postulate that, when one is designing the proper compounds for further biological research, the appropriate choice of each component is of key importance. In many cases, the synthesis of numerous sets of salts is not needed, as it is possible to carry out an analysis defining the elements of the ionic liquids’ structure that influence their biological activity. This approach provides an opportunity to select the proper structure elements for targeted use and is highly justified from both technological and economic points of view.

The length of the alkyl chain attached to the nitrogen atom in the cation is one of the most studied factors in the literature. Based on the data in the literature, as well as on our own results, it should be clearly stated that salts with longer alkyl chains show stronger biological properties when antimicrobiological activities are considered. Therefore, in our current project, only salts with a long alkyl chain were chosen—specifically, those with a tetradecyl chain. The selection of this particular length of alkyl substituent among other possible choices of long hydrophobic tail, such as decyl, hexadecyl, or even octadecyl, is determined by several factors: (i) Insoluble Langmuir monolayers are usually formed...
The next element that influences biological properties is the type of amine core.\textsuperscript{43} In this study, we have considered only salts with an aliphatic amine core, as our previous biological results\textsuperscript{20} clearly demonstrated that this kind of quaternary ammonium salt exhibits the broadest and strongest antimicrobial properties.

Another important parameter is the task-specific component of the cation, which should strengthen the compound’s biological activity.\textsuperscript{44} We have already proven that ionic liquids with a (1R,2S,5R)-(-)-menthol moiety strongly influence antimicrobial properties. Additionally, salts with this monoterpene moiety act much better than do those without such a task-specific component, having incorporated a more typical component, like an alkyl or alkoxyalkyl chain.\textsuperscript{23} Therefore, we have selected (-)-menthol as a major functional substituent. For comparison, we also chose another monoterpene derivative, (+)-fenchol. These monoterpene alcohols are used in many biological and medical areas.\textsuperscript{45} On this basis, we assumed that these task-specific components should strengthen the biological activities of the designed salts.

The next important structural element that we took into account was the type of anion. This allowed us to control the physical properties of the obtained salts.\textsuperscript{46} In the current study, the excellent water solubility of the tested ionic liquids was desired. For that reason, the chloride anion was selected.

Detailed structure analysis enables us to select promising candidates for microbiological agents. Moreover, such an approach is in agreement with sustainable development because, at the stage of synthesis planning, only crucial elements of the structure are considered, which significantly reduces the amount of synthesized compounds.

**Synthesis of Ionic Liquids (3a and 3b) with the Monoterpene Moiety.** The naturally occurring plant origins, (1R,2S,5R)-(-)-menthol and (1R)-endo-(+)-fenchol are commercially available, and these compounds were used for the synthesis of new ionic liquids. These well-known natural sources belong to monoterpene alcohols as follows: (i) (1R,2S,5R)-(-)-menthol being a monocyclic compound and (ii) (1R)-endo-(+)-fenchol having a bicyclic ring structure. The synthetic pathway involving two steps was conducted in mild synthesis conditions and is presented in Scheme 2. The quaternary agents were prepared by a first stage—namely, chloromethyl (1R,2S,5R)-(-)-menthoxymethyl ether (2a) and chloromethyl (1R)-endo-(+)-fenchyl ether (2b). These chloromethyl terpenyl ethers were obtained through chloromethylation of the appropriate monoterpene alcohol. Such optical active ethers are attractive reagents for quaternization. The process of preparing these compounds should be carried out under strictly anhydrous conditions; otherwise, the obtained ethers readily undergo hydrolysis. Vacuum distillation was used for purification to create transparent liquid products with a satisfactory yield (higher than 93%).\textsuperscript{19,24}

In the next step, new quaternary ammonium chlorides having monoterpene moieties were synthesized in a Menschutkin reaction. Quaternization was performed under anhydrous conditions using distilled N,N-dimethyltetradecylamine and freshly distilled proper chloromethyl terpenyl ether. The reaction was performed at room temperature. Anhydrous hexane was used as a solvent, as the target product easily precipitated in such reaction media. In this context, special attention should be paid to the fact that the obtaining of [(1R,2S,5R)-(-)-menthoxymethyl]dimethyltetradecylammonium chloride (3a) took approximately half the time of the obtaining of [(1R)-endo-(+)-fenchoxymethyl]-dimethyltetradecylammonium chloride (3b). This confirmed that the ether derivative of (-)-menthol (2a) is more reactive,
the logarithm of the IL concentration. The minimum surface tension value is similar for both quaternary ammonium salts tested and ranges from 38.23 to 40.40 mN m\(^{-1}\) (Table 2). The concentration at which the surface tension reaches the plateau—defined as the critical micelle concentration (CMC)—is different for the ILs under discussion. It can be observed that the CMC value increased concurrently with the increase in the number of cyclic groups in the structure of the salts. For (−)-menthol derivative [C\(_{14}\)-Am-Men][Cl] (3a), which has one cycling monoterpene group, the CMC value is 1.4 \times 10^{-5} mol L\(^{-1}\). Meanwhile, the CMC value of [C\(_{14}\)-Am-Fen][Cl] (3b) is higher and reaches 5.0 \times 10^{-5} mol L\(^{-1}\). In our opinion, this is due to the steric hindrance of the salts, which was also observed in the context of other parameters, such as reactivity and solubility. Therefore, it should be emphasized that the IL having one cyclic ring (3a)—and, thus, being a smaller steric hindrance than its bicyclic counterpart (3b)—decreased the surface tension more efficiently, which means that it has better surface properties. Surface-active tests indicate that terpene-based ILs (3a and 3b) self-assemble very easily in aqueous solutions.

**Solubility of Ionic Liquids (3a and 3b) with Monoterpene Moiety.** Obtained ionic liquids with monoterpene moiety (3a and 3b) were tested for their dissolution ability according to the rules presented by Vogel et al. This test is often used to determine the solubility of ILs. The analysis was carried out by utilizing well-known polar and nonpolar solvents; the results are given in Table 3. The general tendency noticeable here is the excellent solubility of [C\(_{14}\)-Am-Men][Cl] (3a) at 25 °C in mostly tested solvents. The only exception concerns two nonpolar solvents—namely, hexane and diethyl ether—in which the discussed (−)-menthol derivative (3a) is completely insoluble even at 50 °C. Meanwhile, the solubility of [C\(_{14}\)-Am-Fen][Cl] (3b) is slightly different from that of monocyclic salt (3a). Except for the lack of solubility in hexane and diethyl ether, even at elevated temperatures, the (+)-fenchol derivative (3b) is completely insoluble at an ambient temperature in some nonpolar aprotic solvents: in tetrahydrofuran (THF) and ethyl acetate and in dimethyl sulfoxide (DMSO), which is a polar aprotic solvent. The solubility of [C\(_{14}\)-Am-Fen][Cl] in another polar aprotic solvent—namely, in N,N-dimethylformamide (DMF)—is very limited. The proper solubility, which can be described as “completely soluble” according to the characterization given in Vogel’s Textbook of Practical Organic Chemistry, appears when the temperature of the tests is increased to 50 °C. The excellent solubility of (+)-fenchol salt (3b) at 25 °C is observed when polar protic solvents are considered. Through an interpretation of the results, it is easy to determine that, in the given examples, the solubilities of the discussed salts depend on the functional group. As anticipated, ionic liquid with a (−)-menthol derivative (3a) is much more soluble in commonly used solvents, which is in agreement with less steric hindrance, as this salt has one cyclic ring, in contrast to the

**Surface Activity of Ionic Liquids (3a and 3b) with Monoterpene Moiety.** The surface-active parameters of the obtained terpene-based ILs are given in Table 2. Surface tension measurements were performed on a series of aqueous solutions of (−)-menthol and (+)-fenchol derivatives (3a and 3b). For both samples, an increase in IL concentration entails a decrease in surface tension until a minimum value is reached, after which the surface tension remains constant. Figures S1 and S2, given in the SI, show the data for surface tension versus

| SALT NO. | WATER | METHANOL | 1-Propanol | 2-Propanol | DMSO | DMF | THF | TOLUENE | ACETONITRILE | DIETHYL ETHER | HEXANE | CHLOROFORM | METHYLENE CHLORIDE | ETHYL ACETATE | ACETONE |
|---------|-------|----------|------------|------------|------|-----|-----|---------|-------------|-------------|--------|----------|----------------|--------------|---------|
| 3a      |       |          |            |            |      |     |     |         |              |             |        |          |                 |              |         |
| 3b      |       |          |            |            |      |     |     |         |              |             |        |          |                 |              |         |

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**Table 3. Solubilities of Ionic Liquids with Terpene Moiety (3a and 3b) at 25 °C and 50 °C**

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![Image](https://dx.doi.org/10.1021/acsbiomaterials.0c00447)
second salt based on (+)-fenchol (3b), which is bicyclic. Synthesized ILs with monoterpene moiety (3a and 3b) are stable in air, in aqueous solutions, and in commonly used organic solvents.

Langmuir Monolayer Study. The mechanism of ionic liquids’ biological activity may be membrane-related. For that reason, the interactions of these compounds with sterols characteristic of mammalian and fungi membranes (cholesterol and ergosterol, respectively) are of great importance. To investigate the affinity of ionic liquids to the cell membrane, we have applied the Langmuir technique. In our study, the monolayer formed by cholesterol molecules serves as a model of a mammalian membrane, while ergosterol film mimics a fungi membrane.

One-Component Monolayers. In the first stage of our Langmuir study, we checked the ability of ionic liquids differing in terms of the substituent (menthol vs fenchol) to form a stable monolayer at the air/water interface. Initially, experiments were carried out on a pure water subphase; however, investigated compounds were found to undergo dissolution during compression. Therefore, it was impossible to obtain full-range and repetitive isotherms. When we changed the subphase to 0.1 M aq. NaCl, we eliminated this problem.

Figure 1 confirms that the studied ionic liquids are capable of forming monomolecular layers at the surface of the saline subphase. They form liquid-type films, as proven by the compression moduli values (eq 1), shown in the inset.

\[
C_s^{-1} = -A\left(\frac{dz}{dA}\right)
\]  

This is also confirmed by the homogeneity of the BAM images throughout the entire compression process (domains appear only around the film collapse). A comparison of maximum \(C_s^{-1}\) values reveals that the changing of the menthol to fenchol substituent causes the monolayer to become more fluid.

Mixed Monolayers. Because it was proved that the investigated ionic liquids form floating monolayers, the Langmuir monolayer technique can be applied to the study of their interactions with the main lipids of the mammalian and fungi membranes, containing cholesterol and ergosterol, respectively. Figure 2 presents surface pressure (\(\Pi\))–mean molecular area (\(A\)) isotherms for the above-mentioned systems.

In looking at the isotherms, it can be seen that in all cases isotherms of mixed monolayers are situated between those obtained for the respective one-component films. Both [C\(_{14}\)-Am-Men][Cl] and [C\(_{14}\)-Am-Fen][Cl] exert a loosening effect on the sterol’s monolayers (more pronounced for mixtures with ergosterol). All mixed isotherms are similar in shape to the respective ionic liquid; however, they are shifted toward smaller areas with respect to pure compounds. For mixtures with cholesterol, the values of the collapse pressures of pure components and their mixtures are very similar. On the other hand, monolayers with ergosterol collapse at lower surface pressures, which may indicate that these films have lower stability.

Interactions between molecules can be analyzed qualitatively (based on the mean molecular area \(A_{12}\), see SI Materials, Figure S.3) and qualitatively (based on the excess free enthalpy of mixing \(\Delta G^{exc}\) defined as (eq 2)):

\[
N_A \int (A_{12} - (A_1 X_1 + A_2 X_2))d\Pi
\]

Negative values of \(\Delta G^{exc}\) mean that the interactions between two different molecules (sterol–ionic liquid) in the binary mixture are more attractive and more energetically beneficial than are the interactions between the same molecules (sterol–sterol and ionic liquid–ionic liquid) in a one-component monolayer.

From Figure 3, it is evident that ionic liquids affect both membranes, though in opposite ways. The interactions between ionic liquids and Erg are more repulsive (less attractive) than are those between molecules in their pure films. This effect is more pronounced for [C\(_{14}\)-Am-Men][Cl] (\(\Delta G^{exc} \approx 1500\) kJ/mol), while in a system mixed with [C\(_{14}\)-Am-Fen][Cl] \(\Delta G^{exc} \approx 800\) kJ/mol). Such behavior may lead to phase separation and can be responsible for the antifungal activity of the studied ionic liquid. On the other hand, favorable but rather weak interactions between ionic liquids and Chol are observed. The low strength of these interactions can be a huge advantage of the studied ionic liquids (3a and 3b) in terms of their antifungal activity. Namely, [C\(_{14}\)-Am-Men][Cl] and [C\(_{14}\)-Am-Fen][Cl] appear to have no effect on the cholesterol-containing layers and, therefore, on membranes not infected with fungi.

Similar conclusions were obtained based on the analysis of the mean molecular areas (\(A_{12}\), see SI Materials, Figure S.1). For mixtures with cholesterol (SI Materials, Figure S.1a and Figure S.1c), negative deviations from ideal behavior are observed (with the exception of the lowest pressure for the
system mixed with 3a, [C14-Am-Men][Cl]), indicating more attractive (or less repulsive) interactions occurring between ionic liquids and cholesterol. Meanwhile, for mixtures with ergosterol, positive deviations are observed, indicating...
repulsive (or less attractive) interactions. Those results confirm immiscibility and phase separation between molecules in ionic liquids/Erg monolayers.

For a deeper analysis of the effect of ionic liquids on model fungi and mammalian membranes, $\Delta G_{\text{tot}}$ values were calculated using the formula given below (eq 3)

$$\Delta G_{\text{exc}} + RT(X_1 \ln X_1 + X_2 \ln X_2)$$

and compiled with each other at a surface pressure corresponding to physiological conditions (30 mN/m). The greater the negative values of $\Delta G_{\text{tot}}$, the more stable the mixed system is (Figure 4).27

Positive values of $\Delta G_{\text{exc}}$ as well as significantly negative values of $\Delta G_{\text{tot}}$ for a system mixed with ergosterol indicate that those mixed films are thermodynamically less stable than are cholesterol/ionic liquid systems. Such a destabilizing effect is particularly visible in the case of [C14-Am-Men][Cl] and depends on the ionic liquid content.

In summary, it may be concluded that the incorporation of the studied ionic liquids into a fungi membrane destabilizes it, while their incorporation into a mammalian membrane is thermodynamically favorable and does not cause damage. The selective activity of ionic liquids can, therefore, be related to the destabilization of membranes containing ergosterol. It should be noted that we are aware that results from Langmuir surface activity analysis confirmed that tested terpene-based ILs transferred using the Langmuir—Blodgett (LB) technique onto a solid support (mica), and the topographies of the samples were examined at the nanoscale using atomic force microscopy (AFM).

As can be seen in Figure 5, the incorporation of ionic liquid into the ergosterol monolayer causes a clear phase separation. The addition of [C14-Am-Men][Cl] causes the appearance of domains, while the layer with [C14-Am-Fen][Cl] is more structured with hole-like pits. In both cases the studied compounds create disturbances in the homogeneity of membranes, which may be attributed to their cytotoxic, antifungal activity. Analogical samples with cholesterol were also examined, but AFM images do not show any specific features.

### CONCLUSIONS

Novel terpene-based ionic liquids that have (in the cation part of their structures) naturally occurring and commercially available fragments—[(1R,25,SR)-(−)-menthol or (1R)-endo-(+)-fenchol]—were synthesized with an excellent yield that exceeded 96% and were characterized by 1H NMR, 13C NMR, and HRMS spectroscopies and elemental analysis. The synthesis procedure consisted of two stages conducted under mild process conditions and turned out to be fast and highly effective. The thermal analyses confirmed that solid final products exhibited melting points below 100 °C. The salts differed in solubilities, which were evaluated in relation to various polar and nonpolar solvents. The ionic liquids with the (−)-menthol moiety (3a) were assessed as being much more soluble in tested solvents, which is associated with a single cyclic ring in their structure and, thus, had less steric hindrance in comparison to the salt with (+)-fenchol substituent (3b).

Produced salts should be classified as surface-active ionic liquids (SAILs) because their structure consists of a long hydrophobic chain, tetradecyl, and a hydrophilic headgroup. Because of the amphiphilic nature of these compounds, the surface properties were also investigated. The results of the surface activity analysis confirm that tested terpene-based ILs...
are able to decrease the surface tension of an aqueous solution by approximately 38–40 mN m−1.

The synthesis and detailed characterization aspects of the work were preceded by thorough structural analyses aimed at the selection of appropriate compounds for the planned research. The presented detailed structure analysis enabled us to choose promising candidates as antifungal agents at the design level, which is in agreement with sustainable development features. The authors declare no competing financial interest.

In this paper, we focus on the physicochemical aspects of IL affinity to mammalian and fungi membranes. We used an artificial membrane model (Langmuir monolayer) to confirm the antifungal activity of the studied compounds. Our model enables a preliminary verification of which of the membrane components (chol vs. erg) is important in the targeting of a drug to the interior of the cell. To better understand the ILs’ mode of action, our future work will focus on more complex (multicomponent) mixed systems mimicking membrane as well as biological samples.

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