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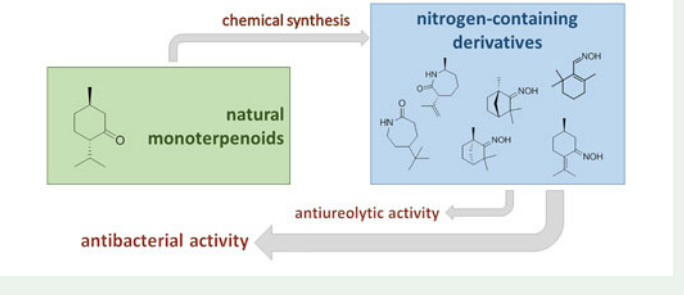
Synthesis of nitrogen-containing monoterpenoids with antibacterial activity

Agata Kozioł[a,b], Ewa Grela[a], Katarzyna Macegoniuk[a], Agnieszka Grabowiecka[a] and Stanisław Lochyński[a,b]

[a]Department of Bioorganic Chemistry, Faculty of Chemistry, Wroclaw University of Science and Technology, Wrocław, Poland; [b]Institute of Cosmetology, Wrocław College of Physiotherapy, Wrocław, Poland

ABSTRACT

Incorporation of the Beckmann rearrangement into the presented research resulted in the formation of nitrogen-containing terpenoid derivatives originating from naturally occurring compounds. Both starting monoterpenes and obtained derivatives were subjected to estimation of their antibacterial potential. In the presented study, *Staphylococcus aureus* was the most sensitive to examined compounds. The Minimal Inhibitory Concentration (MIC) experiments performed on *S. aureus* demonstrated that the (−)-menthone oxime **(−)-8** and (+)-pulegone oxime **(+)-13** had the best antibacterial activity among the tested derivatives and starting compounds. Their MIC_{90} value was 100 μg/mL. The obtained derivatives were also evaluated for their inhibitory activity against bacterial urease. Among the tested compounds, three active inhibitors were found – oxime **14** and lactams **(−)-15** and **16** limited the activity of *Sporosarcina pasteurii* urease with K_i values of 174.3 μM, 43.0 μM and 4.6 μM, respectively. To our knowledge, derivative **16** is the most active antiureolytic lactam described to date.



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1. Introduction

Terpenes are the largest class of secondary plant metabolites. This group contains approximately 30,000 compounds, including more than 400 monoterpenes. Their

CONTACT Stanisław Lochyński stanislaw.lochynski@pwr.edu.pl
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therapeutic use is associated with the antimicrobial activity. Microorganisms show differential susceptibility to compounds of plant origin. Gram-positive bacteria are usually more affected by monoterpenes than Gram-negative strains (Kozioł et al. 2014; Singh and Sharma 2015).

Further search for compounds with strong biological activity among monoterpene derivatives is a promising research goal. It is known that incorporation of the oxime group into naturally occurring compounds may enhance their antimicrobial activity (Kozłowska et al. 2017). Nitrogen-containing terpene derivatives (including lactams, oximes and amines) have also been described with diverse biological properties. To date, a limited but promising data have been published. Within the terpenoids containing an N-OH moiety, effective anticancer (Kahnt et al. 2018) and antimicrobial agents (Hertiani et al. 2010; Grishko et al. 2014) were found. Chemical modifications of monoterpenoids create a possibility of obtaining novel inhibitors of various enzymes (Gajcy et al. 2010). Librowski et al. (2001) described the strong local anaesthetic activity of hydroxyamino carane derivatives. Thus, both the increase in synthesis yield and the growing scientific knowledge of compounds substituted with nitrogen-containing groups provide great hope for the development of new therapeutic drugs in various medical fields (Adams and Robertson 1997).

Despite the long-established knowledge of natural monoterpenes biological activity, the properties of their nitrogen derivatives remain vastly unknown. In this study, we describe the chemistry of a group of nitrogen-containing monoterpene derivatives that includes 9 oximes and 3 lactams. The research is complemented by antibacterial studies against Gram-negative *Escherichia coli*, Gram-positive *Bacillus subtilis* and *S. aureus* strains. The antiureolytic characteristics against *Sporosarcina pasteurii* urease are also presented, as monoterpenoid derivatives constitute a novel group of inhibitors of this enzyme.

2. Results and discussion

2.1. Chemistry

The synthesis of oximes was carried out in an ethanolic solution of hydroxylamine hydrochloride in the presence of pyridine as a hydrogen chloride trapping agent. This method was chosen to synthesize crystalline oximes due to the ease of isolation of the post-reaction products after dilution with water. Oximes were the starting compounds used in the Beckmann rearrangement to create lactams (Table S1, Figure 1).

In the Beckmann method of lactam synthesis from monoterpene ketones modified by Zabża et al. (1972), a strongly acidic medium is not used. We introduced the *p*-toluenesulfonyl chloride reagent in this reaction, for the "second type" rearrangement.

Besides the starting monoterpenes **1**, **3–7** (Table S1), the compound *t*-butylcyclohexanone **2** was incorporated into the presented research. It was chosen as the analogue of monoterpenoids with a cyclohexane ring that contains 10 carbon atoms in its structure.

2.2. Biological activity

Monoterpene ketones chosen as the starting compounds in the presented study have already been described with negligible antibacterial activity (Gallucci et al. 2009;

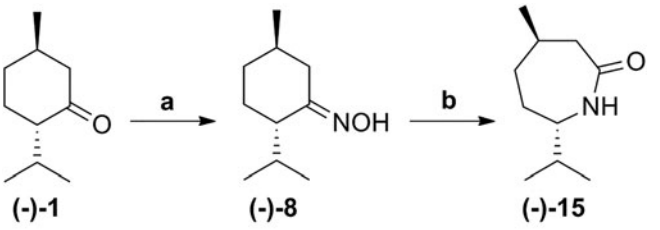

Figure 1. Synthesis of oxime **(−)-8** and lactam **(−)-15** from (−)-menthone **(−)-1**.

Rasoul et al. 2012; Kozioł et al. 2014). This characteristics was confirmed in our research group (Kozioł et al. 2018), we observed that nearly all monoterpene ketones had no bacteriostatic activity up to 250 μg/mL under standard, CLSI (2012) approved conditions. None of compounds **1–7** affected the growth of *Escherichia coli*. Only (−)-carvone **(−)-4** and (+)-dihydrocarvone **(+)-5** were characterised with a Minimal Inhibitory Concentration (MIC_{50}) of 250 μg/mL against *Staphylococcus aureus* and *Bacillus subtilis*.

Subsequently, all synthesized nitrogen-containing derivatives were evaluated for their antibacterial activity (Table S2, Figure 2). Some chemical modifications of monoterpene ketones resulted in a significant increase in antibacterial activity. Nearly all oximes with isopropyl and isopropene substitutions (**(−)-8**, **(−)-11**, **(+)-11**, **(+)-13** and **16**, with the exception of **(+)-12**) inhibited the growth of Gram-positive bacteria. **(−)-8**, **(−)-11** and **(+)-11**, namely (−)-menthone and carvone oximes, were effective against both *B. subtilis* and *S. aureus* with MIC_{90} values of 150 μg/mL or lower. The MIC_{90} value of **(−)-8** against *S. aureus* was calculated as 100 μg/mL. In the presented research, *S. aureus* was more susceptible to monoterpene oximes than the other two bacterial strains. Compounds ineffective against *B. subtilis* and *E. coli* – **(+)-13** and **16** – restricted the growth of *S. aureus*, with MIC_{90} values of 100 and 150 μg/mL, respectively. Additionally, compounds **(−)-8** and **(+)-13** were the most effective among tested nitrogen derivatives, both demonstrating a MIC_{50} = 25 μg/mL and a MIC_{90} = 100 μg/mL against *S. aureus*. On the other hand, carvone oximes (**(−)-11**, **(+)-11**) were the only compounds effective against all tested bacteria, with MIC values considerably higher for Gram-negative *E. coli* (250 μg/mL) than for both Gram-positive strains. A similar pattern of susceptibility to monoterpene derivatives was previously observed and described (Cristani et al. 2007).

In addition to the antibacterial characteristics presented above, the inhibitory properties of monoterpene ketones and obtained derivatives were evaluated against *Sporosarcina pasteurii* urease (Table S3, Figure 2). To date, only a limited group of monoterpenoid urease inhibitors has been described in the literature. First, both carvone stereoisomers were discovered to limit the activity of soil urease, consisting of a variety of enzyme isoforms (Efimia et al. 2014). Further experiments conducted in our research group confirmed the effectiveness of (−)-carvone (**(−)-4**) against bacterial urease (K_i = 418.6 μM). We characterized monoterpenoid ketones and their derivatives

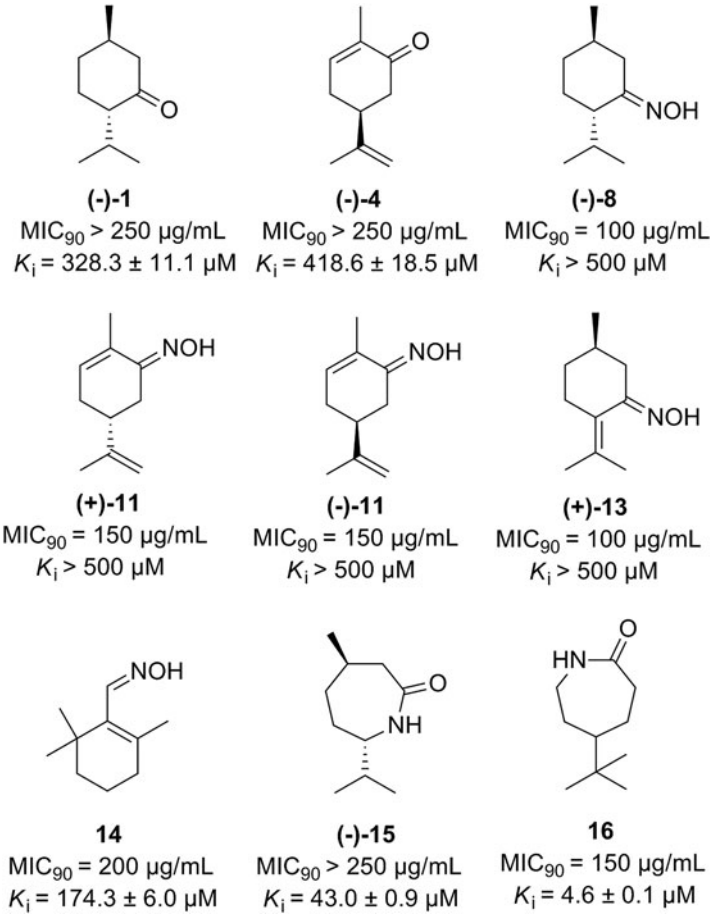

Figure 2. Structures and bioactivity of the most potent compounds. Presented Minimal Inhibitory Concentration (MIC_{90}) was evaluated against *Staphylococcus aureus* cells and inhibitory constant K_i calculated for purified *Sporosarcina pasteurii* urease. The characteristics of remaining compounds is presented in the Supplementary file in the Tables S1–S3.

as reversible inhibitors with a non-competitive mechanism of activity (Kozioł et al. 2018). In the presented article, three novel urease inhibitors were found among the tested nitrogen-containing derivatives. Compounds **14**, **(−)-15** and **16** effectively limited the activity of purified *S. pasteurii* urease. Their K_i values were calculated to be 174.3 µM, 43.0 µM and 4.6 µM, respectively. They were noticeably lower than the affinity constants obtained for the monoterpene ketone substrates. The inhibitory potency of the most active compound **16** was comparable to the standard, FDA-approved urease inhibitor – acetohydroxamic acid (AHA) characterized with K_i = 3.4 µM (Macegoniuk et al. 2016). Moreover, two of the most active derivatives share a lactam moiety and are, to our knowledge, the first group of lactams that actively inhibit bacterial urease at micromolar concentrations. Limited information on lactam-containing urease inhibitors can be found in the literature. In general, such compounds are described as inactive or weak urease inhibitors. A recently published paper (Mermer et al. 2017) describes the weak antiureolytic characteristics of a group of highly

substituted piperazine-azole hybrids against jack bean urease. Antiureolytic properties of the examined compounds may be a promising factor for treating a variety of infections caused by pathogenic, ureolytically active microorganisms.

3. Experimental section

The general chemistry of presented research and methodology of antibacterial experiments were presented in the Supplementary Material.

4. Conclusion

In the first stage of the presented reaction, nine pure oximes with a yield of 88–98% were synthesized. In the subsequent Beckmann rearrangement, three lactams with a yield of 96% were obtained from the selected oximes.

Incorporation of nitrogen-containing groups into the structures of monoterpenoids allowed achieving a significant improvement in their antibacterial activity. The microbiological tests demonstrated that **(−)-8** and **(+)-13** were the best antibacterial compounds among the tested derivatives. Both compounds were characterized with a MIC_{50} = 25 μg/mL and a MIC_{90} = 100 μg/mL against *S. aureus*.

Obtained derivatives were also screened for their antiureolytic characteristics against *S. pasteurii* urease. Compound **16** was found to be the most active of the examined derivatives, with a K_i = 4.6 μM. To our knowledge, this is the first lactam reported to inhibit the activity of bacterial urease with high effectiveness.

Disclosure statement

No potential conflict of interest was reported by the authors.

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