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Synthesis and Fungistatic Activity of Bicyclic Lactones and Lactams against *Botrytis cinerea*, *Penicillium citrinum*, and *Aspergillus glaucus*

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Supporting Information

ABSTRACT: Six analogues of natural *trans*-4-butyl-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3**) and three derivatives, **11**, **12**, and **13**, of Vince lactam (**10**) were synthesized and tested as fungistatic agents against *Botrytis cinerea* AM235, *Penicillium citrinum* AM354, and six strains of *Aspergillus*. Moreover, bioresolution carried out by means of whole cell microorganisms and commercially available enzymes afforded opposite enantiomerically enriched (−) and (+) isomers of Vince lactam (**10**), respectively. The effect of compound structures and stereogenic centers on biological activity has been discussed. The highest fungistatic activity was observed for four lactones: **3**, **4**, **7**, and **8** (IC_{50} = 104.6−115.2 μg/mL) toward *B. cinerea* AM235. *cis*-5,6-Epoxy-2-aza[2.2.1]heptan-3-one (**13**) indicated significant fungistatic activity (IC_{50} = 107.1 μg/mL) against *Aspergillus glaucus* AM211. *trans*-4-Butyl-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3**) and *trans*-4-butyl-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**7**) exhibited high fungistatic activity (IC_{50} = 143.2 and 110.2 μg/mL, respectively) against *P. citrinum* AM354 as well.

KEYWORDS: *lactones, Vince lactam, IC_{50}, Botrytis cinerea, Penicillium thomi*

INTRODUCTION

It has been assessed that pathogenic fungi, mainly filamentous ones, are the cause of at least 20% of losses in the food industry and crop production,[1] and also they are responsible for mycotoxin contamination of foods.[2,3] Filamentous fungi pose a serious health hazard for people with low immunoresistance, causing dermatomycosis and pulmonary mycosis during hospital treatment. Additionally, they are often the cause of allergies.[4−6]

Nowadays, there is growing interest in new compounds that inhibit growth of filamentous fungi.[7−9] The most attractive are the ones of low toxicity and high biodegradability or the ones of high selectivity of action, not affecting the growth of saprophytic fungi.

Such compounds may include natural lactones biosynthesized by plants. Plants have evolved many secondary metabolites involved in their defense against phytopathogens.[10−13] Compounds with a lactone moiety display a wide spectrum of biological activities, such as fungistatic, bacteriostatic, and cytostatic ones.[14−16] They are often found among insect pheromones.[17] Additionally, several lactones are present in vegetables, fruits, and food products, being responsible for their aromas (mainly fruity ones) and taste (sweet, coconut, or spicy).[18]

In this regard we have developed interest in bicyclic lactones produced by plants of the family Apiaceae Lindl. (*Ligusticum officinale* (loveroot, old English lovage), *Ligusticum chuanxiong*, *L. wallichii* (Chinese lovage), *Angelica sinensis* (Chinese angelica), *Apium graveolens* (celeriac), and *Petroselinum crispum* (parsley)). Some of them are described in the literature.[19−23] There is a need to perform a systematic study on the relationship between structure and biological activity of these compounds. For this purpose we have synthesized natural lactone, its derivatives, and lactam analogues.

In this paper we have obtained bicyclic lactones, compounds of selective growth inhibition activities toward filamentous fungi of the genera *Aspergillus*,[24,25] *Penicillium*,[26,27] and *Botrytis*,[28−30] and we analyzed them with respect to the structure−activity relationship, including the effect of a stereogenic center. The fact that (+)- and (−)-enantiomers may have different biological activities is the background of the modern approach to understanding the mechanisms of action of biologically active compounds.[31]

The biocatalytic potential of microorganisms and their enzymes is used in the production of many valuable chemical compounds.[32] Microbial systems are very profitable due to the short time in which they can double their biomass. Additionally, the methods of genetic manipulations of microorganisms are well-known and established.[33] Therefore, biotransformations using microorganisms and isolated enzymes have enormous potential in the production of pharmaceuticals and compounds useful in agriculture.

MATERIALS AND METHODS

Analytical Methods. Composition of extraction mixtures after biotransformations, syntheses, and purity of products were determined on the basis of complementary information received from gas chromatography (GC) and thin layer chromatography (TLC). TLC analyses were carried out on glass plates covered with silica gel (silica gel 60 F_{254} (Merck)) and developed in eluting systems containing hexane/acetone or methylene chloride/methanol. The same eluents were applied for separation of the products by column chromatography (silica gel 60, 230−400 mesh).

GC analyses were performed with an Agilent 6890N GC instrument, equipped with a flame ionization detector (FID) and

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capillary columns Carbowax (30 m, 0.53 mm, film = 0.88 μm) and HP-1 (25 m, 0.32 mm, film = 0.25 μm), using H_2 as carrier gas at 2 mL/min of flow. Enantiomeric excesses of the products were determined on chiral columns: Cyclosil-B (30 m × 0.25 mm × 0.25 μm). Specific optical rotations were measured with an Autopol IV automatic polarimeter (Rudolph), equipped with a thermostatic system.

The structures of compounds were determined by means of spectral analysis: ^{1}H NMR, ^{13}C NMR, IR. and GC-MS. NMR spectra were recorded with a Bruker Avance DRX-500 spectrometer in $CDCl_3$ solutions. IR spectra were measured with a Mattson FTIR-300 Thermo Nicolet spectrometer. Molecular weights of products were established on the basis of GC-MS spectra (ionization energy = 70 eV), recorded with a Varian Chrompack GC/MS CP-3800 Saturn 2000 instrument.

Chemicals. The chemicals used for the syntheses were purchased from Sigma-Aldrich and Fluka. Potato dextrose agar (PDA) for fungistatic tests was purchased from BTL in Poland. Didecyldimethylammonium chloride (DDAC; 50% solution in propan-2-ol/water 2:3) was purchased from Merck. The substrate for biotransformation and fungistatic tests, racemic 2-azabicyclo[2.2.1]hept-5-en-3-one (**10**), was purchased from Sigma-Aldrich.

Enzymes. The following hydrolases were used for bioresolution of racemic lactam **10**: isolated esterase immobilized on Eupergit C from hog liver and esterase from *Rhizomucor miehei* recombinant from *Aspergillus oryzae* were purchased from Fluka, and lipases from *Aspergillus niger* (Amano), *Burkholderia cepacia*, *Candida antarctica* (lipase B immobilized on Innobead 150, recombinant from *Aspergillus oryzae*), *Mucor javanicus*, *Mucor miehei*, *Penicillium camemberti*, *Rhizopus oryzae*, and Lipozymer from *Mucor miehei* were purchased from Sigma-Aldrich.

Microorganisms. In our study, in both biotransformation processes and fungistatic activity tests, the microorganisms from the collection of the Institute of Botany of Medical University of Wrocław were used.

The following microorganisms were used: *Aspergillus candidus* AM386, *Aspergillus glaucus* AM211, *Aspergillus nidulans* AM243 *Aspergillus ochraceus* AM456, *Aspergillus* sp. AM31, *Aspergillus wenthi* AM 413, *Botrytis cinerea* AM235, *Candida viswanathi* AM120, *Fusarium culmorum* AM282, *Fusarium oxysporum* AM13, *Inonotus radiatus* AM70, *Laetiporus sulphurens* AM514, *Penicillium camemberti* AM83, *Penicillium citrinum* AM354, *Penicillium notatum* AM904, *Penicillium thomi* AM91, *Pezicula cinnamomea* AM53, *Poria placenta* AM38, *Prosthemium* sp. AM52, *Rhodotorula marina* AM77, *Rhodotorula rubra* AM4, *Sclerophoma pythiophila* AM55, *Sparassis crispa* AM535, *Stemphylium botryosum* AM279, *Yarrowia lipolytica* AM77.

Synthesis of Lactones 2−4 and 6−8. *cis-3-Oxabicyclo[4.3.0]-nonan-2-one* (**2**) *and cis-3-Oxabicyclo[4.3.0]non-7-en-2-one* (**6**) were prepared in good yields according to the literature procedures[34] and confirmed by GC-EIMS: 141 (M + 1) for lactone **2**; 139 (M + 1) for lactone **6**.

trans-4-Butyl-cis-3-oxabicyclo[4.3.0]nonan-2-one (**3**) was obtained in a three-step synthesis: To 0.48 g (0.02 mol) of magnesium activated with iodine was added anhydrous diethyl ether (50 mL), and the mixture was stirred, followed by the slow addition of freshly distillated *n*-butyl bromide (2.88 g; 0.021 mol). The reaction was continued until all magnesium reacted, and then the reaction mixture was heated under reflux for 1 h. After the mixture had cooled to room temperature, anhydrous cadmium chloride (1.65 g; 0.009 mol) was added, and the reaction mixture was heated under reflux for 1 h. After the mixture had cooled to room temperature, a diethyl ether solution of *cis*-1,2,3,6-tetrahydrophthalic anhydride (**1**) (1.39 g; 0.009 mol) was added dropwise at room temperature and heated under reflux for 6 h. The reaction mixture was poured into 10% HCl and extracted with diethyl ether (5 × 50 mL). The extract was dried over $MgSO_4$, and then solvent was evaporated off.

Reduction. Crude product was diluted with 50 mL of methanol and reduced by $NaBH_4$ (0.342 g; 0.009 mol) with 200 μL of 0.1 M NaOH. The reaction mixture was stirred overnight at room temperature, and then methanol was evaporated off.

Lactonization. Crude product of reduction was diluted with a solution of 50 mL of THF/H_2O (2:1 v/v), and then 70% $HClO_4$ was dropped until the pH of the reaction mixture was acidic. The reaction mixture was heated under reflux for 3 h. THF was evaporated, and the product was extracted with diethyl ether (5 × 50 mL). The extract was dried over $MgSO_4$, then solvent was evaporated off, and finally crude product was purified by column chromatography using hexane/acetone (3:1) as eluent to give lactone 0.76 g (43% yield after three-step synthesis and purification).

The physical and spectral data of lactone **3** are a follows: mp 104 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (t, 3, J = 7.1 Hz, CH_3-13), 1.07−1.10 (m, 2, CH_2-12), 1.29−1.36 (m, 2, CH_2-9), 1.37−1.65 (m, 8, CH_2-6, CH_2-7, CH_2-8, CH_2-11), 1.70−1.72 (m, 1, one of CH_2-10), 2.15−2.18 (m, 1, one of CH_2-10), 2.29−2.31 (m, 1, CH-5), 2.69−2.72 (m, 1, CH-1), 4.20−4.24 (m, 1, CH-4); ^{13}C NMR (151 MHz) δ 13.91 (CH_3-13), 22.48 (CH_2-12, CH_2-11), 22.55 (CH_2-7), 22.88 (CH_2-10), 23.58 (CH_2-8, CH_2-6), 27.96 (CH_2-9), 38.95 (CH-5), 42.08 (CH-1), 82.03 (C-4), 178.10 (C-2); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 2253 (m), 1765 (s); GC-EIMS 197 (M + 1).

trans-4-Butyl-cis-3-oxabicyclo[4.3.0]non-7-en-2-one (**7**) was obtained by using the same procedure as for *trans*-4-butyl-*cis*-3-oxabicyclo[4.3.0]-nonan-2-one (**3**) from *cis*-4-cyclohexene-1,2-dicarboxylic anhydride (**5**) (1.37 g; 0.009 mol). Lactone **7** was obtained with 45% (0.79 g) yield. The physical and spectral data of lactone 7 are as follows: oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3, J = 7.1 Hz, CH_3-13), 1.28−1.39 (m, 3, one of CH_2-11, CH_2-12), 1.45−1.50 (m, 1, one of CH_2-11), 1.50−1.54 (m, 2, one of CH_2-11, one of CH_2-10), 1.68−1.74 (m, 1, one of CH_2-10), 1.77−1.82 (m, 1, one of CH_2-6), 1.94−1.97 (m, 1, one of CH_2-6), 2.30−2.32 (m, 1, one of CH_2-9), 2.38−2.41 (m, 1, one of CH_2-9), 2.49−2.51 (m, 1, CH-5), 2.78−2.81 (m, 1, CH-1), 4.29−4.32 (m, 1, CH-4), 5.63−5.65 (m, 2, CH-7, CH-8); ^{13}C NMR (151 MHz) δ 13.92 (CH_3-13), 19.63 (CH_2-12), 21.98 (CH_2-6), 22.56 (CH_2-9), 28.00 (CH-1), 28.91 (CH_2-11), 35.32 (CH_2-10), 39.95 (CH-5), 82.61 (CH-4), 124.35 (CH-7), 125.22 (CH-8), 178.65 (C-2); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1767 (s), 1521 (m); GC-EIMS 195 (M + 1).

4,4-Dibutyl-cis-3-oxabicyclo[4.3.0]nonan-2-one (**4**). To a stirred solution of 1.24 g (0.05 mol) of magnesium (activated with iodine) in anhydrous diethyl ether (80 mL) was slowly added *n*-butyl bromide (6.85 g; 0.05 mol). The reaction was continued until all of the magnesium was reacted, and then *cis*-1,2,3,6-tetrahydrophthalic anhydride (**1**) (2.62 g; 0.017 mol) was added dropwise at room temperature. After 12 h, the reaction mixture was poured into 10% HCl and extracted with diethyl ether (5 × 60 mL). The extract was dried over $MgSO_4$, the solvent was evaporated off, and the crude product was purified by column chromatography using hexane/acetone (3:1) as eluent to give 3.31 g of lactone **4** (yield = 77%). The physical and spectral data of lactone **4** are as follows: mp 43−45 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87−0.91 (m, 6, CH_3-13, CH_3-13′), 1.02−1.14 (m, 4, CH_2-12, CH_2-12′), 1.19−1.34 (m, 8, CH_2-7, CH_2-8, CH_2-11, CH_2-11′), 1.49−1.51 (m, 4, CH_2-6, CH_2-9), 1.70−1.75 (m, 3, CH_2-10′, one of CH_2-10), 2.11−2.18 (m, 2, CH-5, one of CH_2-10), 2.94−2.97 (m, 1, CH-1); ^{13}C NMR (151 MHz) δ 14.01 (CH_3-13, CH_3-13′), 22.79 (CH_2-12), 22.95 (CH_2-12′), 23.14 (CH_2-11′), 23.17 (CH_2-11), 31.04 (CH_2-7), 24.65 (CH_2-10′), 25.85 (CH_2-10), 26.16 (CH_2-8), 31.41 (CH_2-6), 34.04 (CH_2-9), 39.79 (CH-5), 41.74 (CH-1), 88.79 (C-4), 177.90 (C-2); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1765 (s); GC-EIMS 253 (M + 1).

4,4-Dibutyl-cis-3-oxabicyclo[4.3.0]non-7-en-2-one (**8**) was obtained by using the same procedure as for 4,4-dibutyl-*cis*-3-oxabicyclo[4.3.0]-nonan-2-one (**4**) from *cis*-4-cyclohexene-1,2-dicarboxylic anhydride (**5**) (2.59 g; 0.017 mol). Lactone **8** was obtained with 75% (3.19 g) yield. The physical and spectral data of lactone **8** are as follows: oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87−0.91 (m, 6, CH_3-13, CH_3-13′), 1.22−1.34 (m, 8, CH_2-11, CH_2-12, CH_2-11′, CH_2-12′), 1.52−1.59 (m, 2, one of CH_2-10, one of CH_2-10′), 1.68−1.74 (m, 2, one of CH_2-10, one of CH_2-10′), 1.71−1.73 (m, 1, one of CH_2-6), 2.03−2.08 (m, 1, one of CH_2-6), 2.26−2.28 (m, 1, one of CH_2-9), 2.33−2.39 (m, 1, one of CH_2-9), 2.42−2.46 (m, 1, CH-5), 3.00−3.04 (m, 1, CH-1), 5.73−5.76 (m, 2, CH-7, CH-8); ^{13}C NMR (151 MHz) δ 13.95 (CH_3-13′),

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13.97 (CH_3-13), 21.83 (CH_2-12), 23.68 (CH_2-12′), 23.16 (CH_2-11), 23.17 (CH_2-11′), 25.86 (CH_2-9), 26.07 (CH-6), 32.42 (CH_2-10), 35.67 (CH-10′), 38.22 (CH-1), 39.67 (CH-5), 89.72 (C-4), 125.70 (CH-7), 126.01 (CH-8), 178.97 (C-2); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1757 (s), 1522 (m), 1425 (m); GC-EIMS 251 (M + 1).

Synthesis of Lactams 9 and 11−13. *cis-3-Azabicyclo[4.3.0]non-7-en-2-one (**9**).* Synthesis of lactam **9** was performed using a high-pressure autoclave Berghof HR equipped with a Teflon liner. To the solution of *cis*-3-oxabicyclo[4.3.0]nonan-2-one (**6**) (1.518 g, 0.011 mol) in 2.0 mL of DMF placed in the Teflon liner was added 60 mL of 25% ammonia. The Teflon liner was put in a tightly closed autoclave. The reaction mixture was stirred with heating at 160 °C for 15 h (pressure increases to 21 bar). Product was extracted with diethyl ether (5 × 50 mL). The extract was dried over $MgSO_4$, and the solvent was evaporated off. Crude product was purified by column chromatography methylene chloride/methanol (97:3). Lactam **9** was obtained with 65% (0.98 g) yield. The physical and spectral data of lactone **9** are as follows: mp 63−66 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80−1.85 (m, 1, one of CH_2-6), 2.11−2.17 (m, 1, one of CH_2-9), 2.23−2.26 (m, 1, one of CH_2-6), 2.38−2.41 (m, 1, CH-1), 2.48−2.50 (m, 2, one of CH_2-9, CH-5), 2.94−2.95 (m, 1, one of CH_2-4), 3.41−3.44 (m, 1, one of CH_2-4), 5.66−5.72 (m, 2, CH-7, CH-8), 6.14 (s, 1, NH); ^{13}C NMR (151 MHz) δ 21.82 (CH_2-6), 25.91 (CH_2-9), 31.71 (CH-5), 38.71 (CH-1), 47.65 (CH_2-4), 125.46 (CH-7), 126.07 (CH-8), 180.13 (C-2); IR (NaCl, cm^{-1}) 3154 (s), 2253 (s), 1695 (s); GC-EIMS 138 (M + 1).

*2-Butyric-2-azabicyclo[2.2.1]hept-5-en-3-one (**11**).* To 2-azabicyclo[2.2.1]hept-5-en-3-one (**10**) (0.3 g, 2.47 × 10^{-3} mol) was added anhydrous THF (5 mL), followed by the addition of NaH (8.3 × 10^{-3} mol). Then, butyric acid chloride at room temperature was added dropwise (5.64 × 10^{-3} mol). The reaction was continued until all of the substrate was reacted (checked by TLC, 48 h). The reaction mixture was filtered and purified by column chromatography using methylene chloride/methanol (98:2.5) as eluent to give 0.24 g of pure product **11** and 0.07 g of contaminated product **11** (yield = 63%, R_f = 0.27). The physical and spectral data of lactone **11** are as follows: oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, 3, J = 7.4 Hz, CH_3-11), 1.56−1.69 (m, 2, CH_2-10), 2.17 (dt, 1, J = 8.6, 1.7 Hz, one of CH_2-7), 2.27 (dt, 1, J = 8.6, 1.7 Hz, one of CH_2-7), 2.67−2.74 (m, 2, CH_2-9), 3.40−3.41 (m, 1, CH-4), 5.26−5.27 (m, 1, CH-1), 6.62−6.64 (m, 1, CH-5), 6.87−6.88 (m, 1, CH-6); ^{13}C NMR (151 MHz) δ 13.69 (CH_3-11), 17.81 (CH_2-10), 37.80 (CH_2-9), 54.69 (CH_2-7), 54.77 (CH-1), 60.24 (CH-4), 138.10 (CH-5), 140.50 (CH-6), 173.02 (C-8), 177.53 (C-3); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1700 (m), 1521 (m); GC-EIMS 180 (M + 1).

*2-Butyl-2-azabicyclo[2.2.1]hept-5-en-3-one (**12**).* To 2-azabicyclo[2.2.1]-hept-5-en-3-one (**10**) (0.3 g, 2.47 × 10^{-3} mol) was added anhydrous THF (5 mL), followed by the addition of NaH (8.3 × 10^{-3} mol). Then, butyl bromide at room temperature was added dropwise (4.96 × 10^{-3} mol). The reaction was continued until all of the substrate was reacted (checked by TLC, 48 h). The reaction mixture was filtered and purified by column chromatography using methylene chloride/methanol (98:2.5) as eluent to give 0.15 g of pure product **12** and 0.097 g of contaminated product **12** (yield = 54.4%; R_f = 0.33). The physical and spectral data of lactone **12** are as follows: oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (t, 3, J = 7.4 Hz, CH_3-11), 1.22−1.27 (m, 4, CH_2-9, CH_2-10), 2.11 (dt, 1, J = 7.5, 1.7 Hz, one of CH_2-7), 2.28 (dt, 1, J = 5.8, 1.7 Hz, one of CH_2-7), 2.84−2.88 (m, 1, one of CH_2-8), 3.09−3.14 (m, 1, one of CH_2-8), 3.30−3.31 (m, 1, CH-4), 4.14−4.15 (m, 1, CH-1), 6.62 (dt, 1, J = 7.3, 5.6 Hz, CH-5), 6.69 (dt, 1, J = 7.3, 4.8 Hz, CH-6); ^{13}C NMR (151 MHz) δ 13.81 (CH_3-11), 20.09 (CH_2-10), 29.92 (CH_2-9), 43.43 (CH_2-8), 54.00 (CH_2-7), 59.10 (CH-4), 63.14 (CH-1), 138.16 (CH-5), 139.66 (CH-6), 180.52 (C-3); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1695 (m), 1518 (m); GC-EIMS 165 (M + 1).

*cis-5,6-Epoxy-2-azabicyclo[2.2.1]heptan-3-one (**13**).* To 2-azabicyclo[2.2.1]hept-5-en-3-one **10** (0.3 g, 2.47 × 10^{-3} mol) was added methylene chloride (15 mL), followed by the addition of *m*-CPBA (4.14 × 10^{-3} mol). The reaction was continued until all of the substrate was reacted (checked by TLC, 24 h). The reaction mixture was purified by column chromatography using methylene chloride/methanol (98:2.5) as eluent to give 0.265 g (yield = 77.3%; R_f = 0.29). The physical and spectral data of lactone **13** are as follows: mp 95−100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.62−1.64 (m, 1, one of CH_2-7), 1.80−1.82 (m, 1, one of CH_2-7), 2.84−2.90 (m, 1, CH-4), 3.56−3.58 (m, 1, CH-5), 3.64−3.69 (m, 1, CH-6), 3.87−3.90 (m, 1, CH-1), 6.24−6.26 (s, 1, NH); ^{13}C NMR (151 MHz) δ 31.12 (CH_2-7), 46.88 (CH-4), 51.46 (CH-1), 55.61 (CH-5), 55.63 (CH-6), 180.43 (C-3); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1720 (s), 1518 (m), 1422 (m); GC-EIMS 126 (M + 1).

Bioresolution of (±)-2-Azabicyclo[2.2.1]hept-5-en-3-one ((±)-10). *Microbial Transformations of (±)-2-Azabicyclo[2.2.1]hept-5-en-3-one ((±)-**10**).* (a) Screening Scale Biotransformation. Microorganisms were cultivated in Erlenmeyer flasks (300 mL) containing 75 mL of the cultivation medium S (a solution of peptone 1% and glucose 3%) or C (an aqueous solution of $NaNO_3$ 0.3%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4{\cdot}7H_2O$ 0.005%, $FeSO_4{\cdot}7H_2O$ 0.001 g, and saccharose 3%). After 3−5 days of growth, 0.02 g of substrate (±)-**10** in 0.5 mL of acetone was added to the shaken cultivation mixture. The transformation was continued for 24, 72, 120, and 144 h. The products were extracted with diethyl ether or chloroform and analyzed using TLC and GC techniques. Enantiomeric excesses were determined by GC using chiral column Cyclosil-B (30 m × 0.25 mm × 0.25 μm with temperature program 80 °C (1 min), 130 °C (1 °C/min), and 250 °C (40 °C/min). For selected microorganisms optimization of biotransformation conditions was performed with regard to increased enantioselectivity and efficiency of a biocatalyst.

(b) Preparative Scale Biotransformation in Bench-Bioreactor. Bench-scale batch runs were carried out in 7 L vessels of the bioreactor (Brunswick, USA) in the selected conditions established on the basis of screening experiments. Growth of *P. thomi* AM91 was performed with the following parameters under control: medium volume (3 L), aeration rate (1 v/m), stirring speed (200−300 rpm), temperature (26 °C), pH. After 4 days of growth on mineral medium ($NaNO_3$ 0.3%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4{\cdot}7H_2O$ 0.005%, $FeSO_4{\cdot}7H_2O$ 0.001 g, and saccharose 3%) (pH 4.2), 30 g of peptone was added (pH 8.3). After 4 h, 1.0 g of (±)-**10** in 2 mL of acetone was added. The biotransformation was followed by GC with chiral column, once the substrate (±)-**10** was not detectable; the reaction mixture was overnight extracted in the same manner as in the screening procedure. The crude product was purified by column chromatography (methylene chloride/methanol, 97:3). The yield of the biotransformation calculated only for lactam **10** was 70% and the enantiomeric excess, 93% (GC, chiral column); $[\alpha]_{589}^{20}$ = −565° (*c* 2.0, $CHCl_3$) ($[\alpha]_{589}^{20}$ = −525.9° (*c* 1.0, CH_3OH)).[35] The physical and spectral data of lactone **10** are as follows: mp 55 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.19−2.20 (m, 1, one of CH_2-7), 2.37 (dt, 1, J = 6.0, 1.7 Hz, one of CH_2-7), 3.19−3.23 (m, 1, CH-4), 4.31−4.35 (m, 1, CH-1), 5.85 (s, 1, NH), 6.64 (dt, 1, J = 7.4, 5.0 Hz, CH-5), 6.76 (dd, 1, J = 7.4, 5.0 Hz, CH-6); ^{13}C NMR (151 MHz) δ 53.21 (CH_2-7), 59.32 (CH-1), 60.29 (CH-4), 138.18 (CH-5), 141.12 (CH-6), 185.34 (C-3); IR (NaCl, cm^{-1}) 3019 (s), 2400 (m), 1709 (s), 1521 (m); GC-EIMS 110 (M + 1).

*Biotransformations of (±)-2-Azabicyclo[2.2.1]hept-5-en-3-one ((±)-**10**) with the Use of Isolated Enzymes.* (a) Screening Scale Biotransformation. Substrate (±)-**10** (5 mg) was added to enzyme (the amount equal to 20 enzyme units of activity), dissolved in 0.5 mL of the phosphate buffer of pH 7.2. The reaction was stirred at room temperature. At the end of the process the reaction mixture was filtered through Celite, and the biotransformation products were extracted with diethyl ether. The enantiomeric excess of substrate **10** was determined by means of GC with application of chiral columns.

(b) Preparative Scale Biotransformation. Substrate (±)-**10** (0.2 g) was added to enzyme (the amount equal to 800 enzyme units of activity of Lipase B from *Candida antarctica* immobilized on Innobead 150, recombinant from *Aspergillus oryzea*), dissolved in 40 mL of the phosphate buffer of pH 7.2. The reaction was stirred at room temperature. At the end of the process the reaction mixture was filtered through Celite, and the biotransformation products were extracted with diethyl ether. The enantiomeric excess of substrate **10**

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was determined by means of GC with application of chiral columns. The crude product was purified by column chromatography (methylene chloride/methanol, 97:3). The yield of the biotransformation was calculated only for lactam **10** and reached 74% with enantiomeric excess of (+)-**10**, ee = 99% (GC, chiral column); $[\alpha]_{589}^{20}$ = +565° (*c* 2.0, $CHCl_3$) ($[\alpha]_{589}^{20}$ = +555.9° (*c* 0.5, CH_3OH)).[35]

Antifungal Assays. All compounds dissolved in DMSO were mixed with sterilized PDA (0.01 mL of DMSO for 1 mL of agar) to achieve concentrations of 50, 100, 150, 200, 250, or 300 μg/mL, and the solution was poured into 6 cm Petri dishes. After inoculation by mycelium of a fungus, the tested dishes were incubated at 27 °C and 60−80% of relative humidity. The growth of mycelium was continued until the diameter of the growth zone in control sample dishes (nutrient with DMSO, without compound added) reached 4.5−5.0 cm. Then, the growth zone in experimental dishes was measured. All of the compounds were tested tripled at a concentration of 200 μg/mL as well as at three more concentrations, which were chosen individually. Fungistatic activity, expressed in IC_{50} (the half-maximal inhibitory concentration) values for each tested compound, was determined on the basis of four measured values, as a percent of growth inhibition. IC_{50} values were calculated according to the formula: $(1 - D_a/D_b) \times 100\%$, where D_a is the diameter of the growth zone in the experimental dish (cm) and D_b is the diameter of the growth zone in the control dish (cm). DDAC was used as reference compound. Statistical analysis was done using ANOVA ($p = 0.05$), and the means were compared by calculating the least significant difference (LSD, Tukey).

RESULTS AND DISCUSSION

On the one hand, the aim of our research was the synthesis of *trans*-4-butyl-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3**), one of the possible diastereoisomers of naturally occurring lactones. On the other hand, we planned to obtain a number of its derivatives from two groups of compounds: bicyclic lactones and bicyclic lactams. Our goal was to compare the fungistatic activities of natural lactone **3** and both groups of synthesized derivatives with those of lactone and lactam moiety. Moreover, we conducted enantioselective hydrolysis of lactam (±)-**10** by applying whole cells of fungi and isolated enzymes as well. The resulting compounds, including enantiomerically enriched isomers of lactam **10**, were tested for fungicidal activity against selected strains of filamentous fungi of the *Botrytis*, *Penicillium*, and *Aspergillus* genera.

Synthesis of Bicyclic Lactones 2−4 and 6−8. Taking into consideration the structure of natural lactone **3** (Figure 1), the effects of substituents at carbon atom C-4 and the C7−C8 double bond on fungistatic activity were investigated. Therefore, starting from two commercially available anhydrides, *cis*-1,2,3,6-tetrahydrophthalic anhydride (**1**) and *cis*-4-cyclohexene-1,2-dicarboxylic anhydride (**5**), the corresponding lactones without substituents ((±)-**2**, (±)-**6**), with *n*-butyl substituent ((±)-**3**, (±)-**7**), with two *n*-butyl substituents ((±)-**4**, (±)-**8**) at carbon atom C-4 were synthesized (Scheme 1). The influence of the double bond on the biological activity was also examined by comparing the results obtained from lactones (±)-**6**, (±)-**7**, and (±)-**8** possessing a C7−C8 double bond with their saturated derivatives (±)-**2**, (±)-**3**, and (±)-**4**.

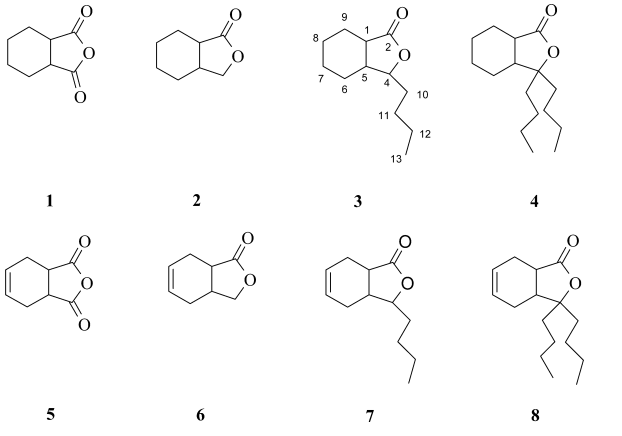
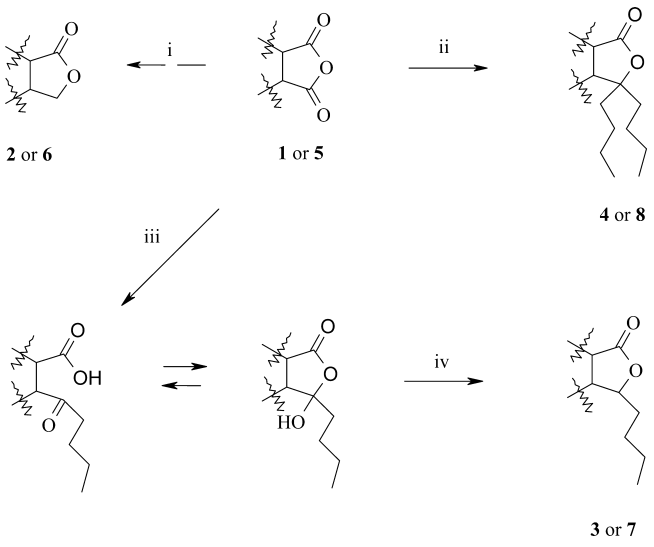


Figure 1. Structures of anhydrides (**1**, **5**) and bicyclic lactones (**2**−**4**, **6**−**8**) examined for fungistatic tests.

Scheme 1[a]



[a]i, $LiAlH_4$ Et_2O; ii, *n*-C_4H_9MgBr (3 equiv), Et_2O or THF; iii, (1) $Cd(n\text{-}C_4H_9)_2$, Et_2O or THF, (2) HCl; iv, (1) $NaBH_4$, MeOH, (2) $THF/H_2O/HClO_4$, reflux.

Lactones (±)-3-oxabicyclo[4.3.0]nonan-2-one ((±)-**2**) and (±)-3-oxabicyclo[4.3.0]non-7-en-2-one ((±)-**6**) were obtained with high yield in reduction of the respective anhydrides **1** and **5** with lithium aluminum hydride in anhydrous diethyl ether. Signals in the ^1H NMR spectrum from two protons at carbon atom C-4 from lactone **2** were observed at 3.92 ppm (d, 1, J = 8.8 Hz, one of CH_2-4) and 4.16 ppm (dd, 1, J = 8.8, 5.0 Hz, one of CH_2-4); and from lactone **6** at 4.00 ppm (dd, 1, J = 8.8, 2.0 Hz, one of CH_2-4) and 4.30 ppm (dd, 1, J = 8.8, 5.1 Hz, 1H, one of CH_2-4).

(±)-*trans*-4-Butyl-*cis*-3-oxabicyclo[4.3.0]nonan-2-one ((±)-**3**), of the structure of the natural lactone,[19−23] was obtained in a three-step synthesis carried out without purification of intermediate products. In the first step the anhydride **1** reacted with a cadmium−organic compound[36,37] obtained from the reaction of *n*-butylmagnesium bromide with cadmium chloride. The reaction product isolated from acidic condition was a mixture of ketoacid and its cyclic form. The purification process of mixtures of ketoacid and the product of its cyclization was inefficient. Therefore, after evaporation of solvents, reduction with $NaBH_4$ in methanol and subsequent product cyclization in a mixture of $THF/H_2O/HClO_4$ was performed. Finally, the purification of reaction mixture on column chromatography afforded only one diastereoisomer, (±)-*trans*-**3**. The structure of the expected lactone (±)-*trans*-**3** was confirmed by the presence of a multiplet at 4.20−4.24 ppm from one proton at C-4 in the ^1H NMR spectrum. Lactone (±)-*trans*-4-butyl-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one

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((±)-7) was prepared from the respective anhydride **5** in the same procedure. The signal from proton at carbon atom C-4 was observed as a multiplet at 4.29−4.32 ppm.

The other two lactones, (±)-4,4-dibutyl-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one ((±)-**4**) and (±)-4,4-dibutyl-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one ((±)-**8**), were obtained with good yields in Grignard reactions of 3 equiv of *n*-butylmagnesium bromide with 1 equiv of the corresponding anhydrides **1** and **5** (Scheme 1).

Synthesis of Bicyclic Lactams 9−13. Our studies concerned also bicyclic lactams, the second group of lactone **3** derivatives. We were interested in how the substitution of oxygen to nitrogen will influence the biological activity in the pair of lactone (±)-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**6**) and lactam (±)-*cis*-3-azabicyclo[4.3.0]non-7-en-2-one (**9**). The spectrum range of lactams applied in our research was increased by Vince lactam (2-azabicyclo[2.2.1]hept-5-en-3-one (**10**),[29−41] which we added to our studies (Figure 2). Both bicyclic lactams **9** and **10** have a double bond and γ-lactam moiety as well; however, they differ in the ring structure.

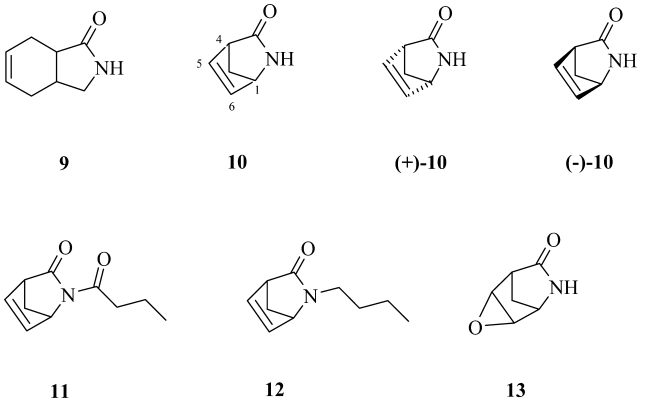
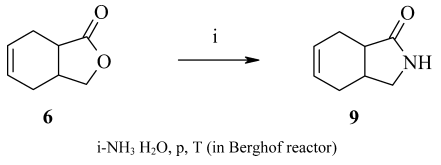


Figure 2. Structures of bicyclic lactams examined for fungistatic tests.

The obtained lactone (±)-**6** was used as the substrate for synthesis of lactam (±)-**9** conducted in a high-pressure autoclave (Berghof) using aqueous ammonia at high temperature and pressure as well (Scheme 2).

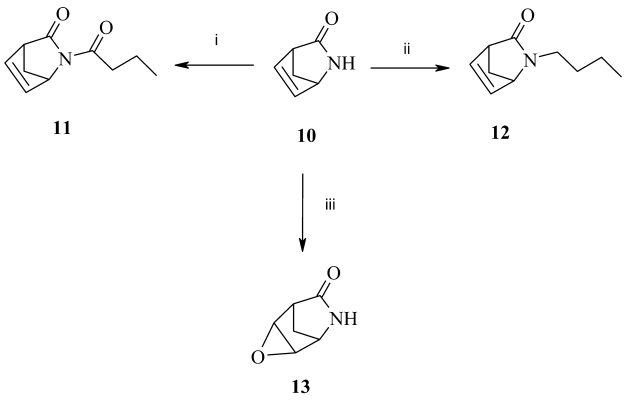
Scheme 2[a]



[a]i, NH_3 H_2O, p, T (in Berghof reactor).

Lactam (±)-**10**, with a structure similar to that of lactone **3** counterpart, was achieved by acylation of the nitrogen atom with butyric acid chloride, affording (±)-2-butyric-2-azabicyclo[2.2.1]hept-5-en-3-one ((±)-**11**) (Scheme 3). On the other hand, alkylation of lactam (±)-**10** with butyl bromide gave (±)-2-butyl-2-azabicyclo[2.2.1]hept-5-en-3-one ((±)-**12**) (Scheme 3). It was also worth checking the influence of the epoxy group on biological activity. Therefore, lactone (±)-**10** was oxidized to (±)-*cis*-5,6-epoxy-2-azabicyclo[2.2.1]heptan-3-one ((±)-**13**).

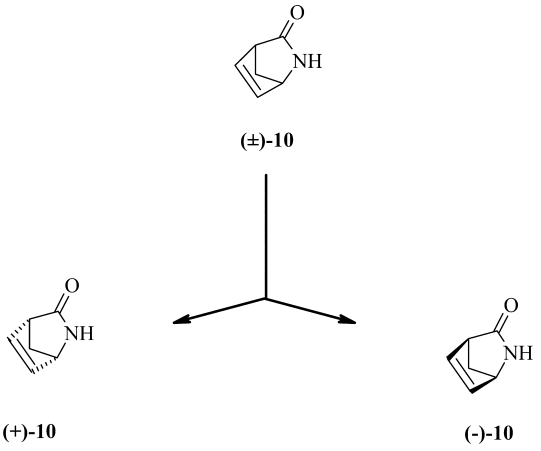
Scheme 3[a]



[a]i, NaH, THF, butyrate chloride; ii, NaH, THF butyl bromide; iii, *m*-CPBA, CH_2Cl_2.

Bioresolution of (±)-2-Azabicyclo[2.2.1]hept-5-en-3-one ((±)-10). Resolution of the racemic mixture of lactam (±)-**10** involved screening of nine commercially available enzymes (Scheme 4; Table 1). All enzymes (entries 1−9, Table 1)

Scheme 4



hydrolyzed more quickly enantiomer (−)-(1*R*,4*S*)-**10**, leaving in a reaction mixture unreacted optically active (+)-(1*S*,4*R*)-2-azabicyclo[2.2.1]hept-5-en-3-one ((+)-**10**) isomer. All enzymatic transformations were carried out in phosphate buffer solution. The best result was obtained after 7 days of biotranformation with *Candida antarctica* lipase B (recombinant in *Aspergillus oryzea*). The (−)-enantiomer of **10** was hydrolyzed preferentially, whereas the opposite isomer, (+)-**10** (ee = 99%), was extracted from the reaction mixture. The scale-up methodology (up to 200 mg of the substrate) was easily increased.

In parallel to enzymatic reactions, the lactam (±)-**10** was subjected to a microbial transformations with the following 18 strains: *Aspergillus ochraceus* AM456, *Candida viswanathi* AM120, *Fusarium culmorum* AM282, *Fusarium oxysporum* AM13, *Inonotus radiatus* AM70, *Laetiporus sulphurens* AM514, *Penicillium camemberti* AM83, *Penicillium notatum* AM904, *Penicillium thomi* AM91, *Pezicula cinnamomea* AM53, *Poria placenta* AM38, *Prosthemium* sp. AM52, *Rhodotorula marina* AM77, *Rhodotorula rubra* AM4, *Sclerophoma pythiophila* AM55,

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Table 1. Enantiomeric Excess of 2-Azabicyclo[2.2.1]hept-5-en-3-one 10 after 7 Days of Biotransformations

entry	bioreagent	enantiomer	ee (%)
1	esterase immobilized on Eupergit C from hog liver	(+)-1*S*,4*R*	42
2	esterase from *Rhizomucor miehei* recombined from *Aspergillus oryzae*	(+)-1*S*,4*R*	14
3	lipase A (Amano) from *Aspergillus niger*	(+)-1*S*,4*R*	40
4	lipase from *Burkholderia cepacia*	(+)-1*S*,4*R*	70
5	lipase B *Candida antarctica* recombined from *Aspergillus oryzae*	(+)-1*S*,4*R*	99
6	lipase from *Mucor javanicus*	(+)-1*S*,4*R*	75
7	lipase from *Mucor miehei*	(+)-1*S*,4*R*	30
8	lipase from *Penicillium camemberti*	(+)-1*S*,4*R*	93
9	Lipozymer, immobilized from *Mucor miehei*	(+)-1*S*,4*R*	6
10	*Candida viswanathi* AM120	(−)-1*R*,4*S*	57
11	*Penicillium camemberti* AM83	(−)-1*R*,4*S*	91
12	*Penicillium notatum* AM904	(−)-1*R*,4*S*	66
13	*Penicillium thomi* AM91	(−)-1*R*,4*S*	96
14	*Sclerophoma pythiophila* AM55	(−)-1*R*,4*S*	77

Sparassis crispa AM535, *Stemphylium botryosum* AM279, and *Yarrowia lipolytica* AM77.

Only strains of the *Penicillium* genus and *C. viswanathi* AM120 possess enzymes capable of the enantioselective hydrolysis of the γ-lactam ring. It needs to be emphasize that selected strains were not previously described as biocatalysts useful in racemic resolution of (±)-**10**. In our opinion the aforementioned strains are a valuable source of lactam hydrolases, and in this regard they can be considered for further investigation.

It should be noted that lipase isolated from *P. camemberti* catalyzed hydrolysis of the opposite enantiomer of (±)-**10** than whole cell culture of *P. camemberti* AM83. We assume that other enzymes from a broad range of lipases were involved in biotransformation.

In preparative biotransformation of (±)-**10** *P. thomi* AM91 was applied. This strain enantioselectively hydrolyzed (+)-**10** isomer, leaving enantiomerically enriched (−)-**10** isomer (ee = 93%) (structures given in Figure 2). During condition optimization of the biotransformation, it was noted that the enzymes involved in amide bond hydrolization were biosynthesized in the presence of peptone growth medium or in a similar source of nitrogen. In contrast, in the mineral medium supplemented with $NaNO_3$, hydrolysis did not occur. Therefore, in the preparative biotransformation, *P. thomi* AM91 initially grew on a mineral medium until peptone induction. After 4 days, 1% of peptone was added, and the pH of the medium increased to 8.3. After another 4 h, 1 g of (±)-**10** was added. Finally, (−)-**10** isomer with an enantiomeric excess of 93% was formed after 2 days of biotransformation.

Fungistatic Activity. The fungistatic activity of racemic mixtures of lactones **2**−**4** and **6**−**8** (Figure 1), lactams **9**−**13**, and enantiomerically enriched (+)-**10** and (−)-**10** isomers (Figure 2) against three phytopathogenic strains (*P. citrinum* AM354, *B. cinerea* AM235, *A. glaucus* AM211) was estimated. Screening tests were conducted at a concentration of 200 μg/mL. Then, IC_{50} in the range from 0 to 350 for each connection was determined. Compounds of IC_{50} above 350 were considered as not active, and the IC_{50} value was not specified.

Among the studied compounds, six of them ((±)-**3**, (±)-**4**, (±)-**7**, (±)-**8**, (±)-**9**, (±)-**10**) indicated high activity with IC_{50} from 104.6 to 130.0 μg/mL (entries 2, 3, and 5−8, Table 2) against *B. cinerea* AM235, the pathogen of grapes. Promising high fungistatic activity of lactones **3** (IC_{50} = 143.2 μg/mL) and **7** (IC_{50} = 110.2 μg/mL) against *P. citrinum* AM354, in contrast to low fungistatic activity against *A. glaucus* AM211 (epoxylactam **13** IC_{50} = 107.2 μg/mL), was observed. Our interests focused on combating fungi of the *Aspergillus* genus; therefore, we examined the activity of racemic lactones **2**−**4** and **6**−**8** and lactams **9** and **10** against the other five strains belonging to species of *Aspergillus*. Figure 3 shows the percentage of mycelium growth inhibition at a concentration of 200 μg/mL.

Table 2. Half-Maximal Inhibitory Concentration (IC_{50}) for Compounds 2−4 and 6−13 against *Aspergillus glaucus* AM211, *Botrytis cinerea* AM235, and *Penicillium citrinum* AM354[a]

entry	compound no.	*A. glaucus* AM211 (μg/mL)	*B. cinerea* AM235 (μg/mL)	*P. citrinum* AM354 (μg/mL)
1	**2**	142.9	>350	>350
2	**3**	246.3	104.6	143.2
3	**4**	238.6	115.2	>350
4	**6**	>350	>350	>350
5	**7**	>350	107.7	110.2
6	**8**	>350	103.3	>350
7	**9**	250.0	127.4	220.0
8	**10**	262.6	130.4	219.0
9	(+)-**10**	245.0	125.3	220.6
10	(−)-**10**	282.4	147.5	256.2
11	**11**	203.8	>350	>350
12	**12**	226.9	>350	>350
13	**13**	107.1	>350	289.0
14	DDAC	57.1	30.4	41.3

[a]Statistical analysis was done using ANOVA (p = 0.05), and the means were compared by calculating the least significant difference (LSD, Tukey). IC_{50} was calculated on the basis of standard curve with standard deviation ±1.745−2.050.

Fungistatic Acticity of Bicyclic Lactones 2−4 and 6−8. The tests were performed with six racemic bicyclic lactones **2**−**4** and **6**−**8** (Scheme 1) of the structure allowing determination of the effect of substituents on carbon atom C-4 and the influence of the C7−C8 double bond on fungistatic activity

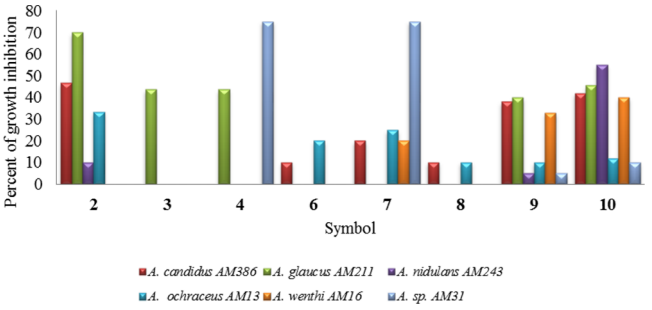

Figure 3. Percentage of growth inhibition of *Aspergillus* genus mycelium at a concentration of 200 μg/mL for lactones **2**−**4** and **6**−**8** and lactams **9** and **10**. Statistical analysis was done using ANOVA (p = 0.05), and the means were compared by calculating the least significant difference (LSD, Tukey). IC_{50} was calculated on the basis of standard curve with standard deviation ±1.745−2.050.

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against *A. glaucus* AM211, *B. cinerea* AM235, and *P. citrinum* AM354.

Effect of Substituents on Carbon Atom C-4 on Fungistatic Activity. Lactones (±)-**2** and (±)-**6**, without a substituent at carbon atom C-4, exhibited low fungistatic activity against tested strains (Table 2). Introducing one or two butyl groups caused a significant increase in fungistatic activity against *B. cinerea* AM 235 for (±)-**3** (IC_{50} = 104.6 μg/mL (entry 2, Table 2)), (±)-**7** (IC_{50} = 107.7 μg/mL (entry 5, Table 2)), (±)-**4** (IC_{50} = 115.2 μg/mL (entry 3, Table 2)), and (±)-**8** (IC_{50} = 103.3 μg/mL (entry 6, Table 2)), respectively.

Otherwise, the aforementioned lactones affected the growth of *P. citrinum* AM354. Lactones with only one butyl group at C-4 were active: lactone (±)-**3** with the structure of natural lactone (IC_{50} = 143.2 μg/mL (entry 2, Table 2)) and its analogue (±)-**7** with the double bond at position C-7 (IC_{50} = 110.2 μg/mL (entry 5, Table 2)).

Effect of the C7−C8 Double Bond on Fungistatic Activity. The presence of the C7−C8 double bond in the racemic lactones **6**−**8** indicated negligible or no effect on fungistatic activity against *P. citrinum* AM354 and *B. cinerea* AM235. In contrast, significant differences exist while for the inhibition of the growth of mycelium of *A. glaucus* AM211. Saturated racemic lactones **2**−**4** (IC_{50} between 142.2 and 246.3 μg/mL, entries 1−3, Table 2) were more active than their analogues **6**−**8** (IC_{50} > 350 μg/mL, entries 4−6, Table 2) with a double bond.

Fungistatic Activity of Bicyclic Lactams 9−13. *Effect of Heteroatom in the Five-Membered Ring on Fungistatic Activity.* By comparing the fungistatic activities against *B. cinerea* AM235 of lactone (±)-**6** and its analogue, lactam (±)-**9** (entries 4 and 7, Table 2), a significant increase in activity (from IC_{50} > 350 μg/mL for lactone **6** to IC_{50} = 127.4 μg/mL for lactam **9**) was observed.

Effect of Ring Structure on Fungistatic Activity. Both bicyclic lactams (±)-**9** and (±)-**10** possessed a γ-lactam ring and a double bond; however, they differed in the space structure (Scheme 3). These compounds inhibited the growth of all tested fungi to a comparable extent (entries 7 and 8, Table 2); therefore, we assumed that the γ-lactam ring and the nitrogen proton N−H significantly affect the biological activity.

Effect of Substituents on the Nitrogen Atom of γ-Lactams on Fungistatic Activity. The fungistatic activity of substituted lactams (±)-**11** and (±)-**12**, especially against *B. cinerea* AM235 and *P. citrinum* AM354, considerably decreased in comparison to that of unsubstituted lactam (±)-**10** from IC_{50} = 130.0 μg/mL and 219.0 μg/mL (entry 8, Table 2) to IC_{50} > 350 μg/mL (entries 11 and 12, Table 2).

Effect of Double-Bond Oxidation on Fungistatic Activity. Lactam (±)-**13**, the epoxy derivative of lactam (±)-**10**, was active (IC_{50} = 107.1 μg/mL) against *A. glaucus* AM211 (entry 13, Table 2), but did not inhibit the growth of the other two strains tested.

Effect of the Stereogenic Center on Fungistatic Activity. Isomer (+)-(1*S*,4*R*)-**10** (ee = 91%) (entry 9, Table 2) inhibited more strongly the mycelium growth of all three strains (*A. glaucus* AM211, *B. cinerea* AM235, *P. citrinum* AM354), with an average of 15−20%, than its mirror image, (−)-(1*R*,4*S*)-**10** (ee = 93%) (entry 10, Table 2). Moreover, the (+)-**10** isomer was more active than the racemic mixture of 2-azabicyclo[2.2.1]hept-5-en-3-one ((±)-**10**).

Fungistatic Activity against the *Aspergillus* Genus. In general, synthesized lactones **2**−**4** and **6**−**8** and lactams **9** and **10** indicated low activity against *A. candidus* AM386, *A. nidulans* AM243, and *A. ochraceus* AM456 (Figure 3). Only compounds without an *n*-butyl substituent, (±)-3-oxabicyclo[4.3.0]nonan-2-one ((±)-**2**), (±)-3-azabicyclo[4.3.0]non-7-en-2-one ((±)-**9**), and (±)-2-aza-bicyclo[2.2.1]hept-5-en-3-one ((±)-**10**), inhibited more strongly the growth of mycelium of tested *Aspergillus* genus. Moreover, the growth of *A. glaucus* AM211 was inhibited by lactone (±)-**2**. Lactone (±)-4,4-dibutyl-3-oxabicyclo[4.3.0]nonan-2-one ((±)-**4**) and its analogue with a double bond ((±)-**7**) inhibited more strongly the growth of *Aspergillus* sp. AM31.

Analogues of natural *trans*-4-butyl-*cis*-3-oxabicyclo[4.3.0]-nonan-2-one (**3**) with a double bond at C-7 (**7**) or an additional butyl group at C-4 (lactones **4** and **8**) indicated the highest fungistatic activity (IC_{50} = 104.6−115.2 μg/mL) toward *B. cinerea* AM235. However, only *trans*-4-butyl-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3**) and *trans*-4-butyl-*cis*-3-oxabicyclo-[4.3.0]non-7-en-2-one (**7**) exhibited high fungistatic activity (IC_{50} = 143.2 and 110.2 μg/mL, respectively) against *P. citrinum* AM354, whereas lactones **3**, **4**, and **6**−**8** slightly inhibited the growth of mycelium of *A. glaucus* AM211.

The effect of a heteroatom in the five-membered ring on fungistatic activity was checked by comparing *cis*-3-oxabicyclo-[4.3.0]non-7-en-2-one (**6**) and its analogue, lactam *cis*-3-azabicyclo[4.3.0]non-7-en-2-one ((±)-**9**). A significant increase in activity (from IC_{50} > 350 μg/mL for lactone **6** to IC_{50} = 127.4 μg/mL for lactam **9**) against *B. cinerea* AM235 was observed. Also, (+)- and (−)-enantiomers of Vince lactam (**10**) and its three derivatives **11**−**13** were tested as fungistatic agents against *B. cinerea* AM235, *P. citrinum* AM354, and *A. gluacus* AM211. Isomer (+)-(1*S*,4*R*)-**10** with an average of 15−20% strongly inhibited mycelium growth of all tested strains.

The fungistatic activity of substituted nitrogen atom γ-lactams (±)-**11** and (±)-**12** considerably decreased in comparison to that of unsubstituted lactam (±)-**10** against *B. cinerea* AM235 and *P. citrinum* AM354. In contrast, oxidation of the double bond of lactam (±)-**10** increased the activity of epoxy derivative (**13**) (IC_{50} = 107.1 μg/mL) against *A. glaucus*. As we indicated low activity of tested compounds against *A. glaucus* AM211, we have checked other *Aspergillus* genus species. Only compounds without an *n*-butyl substituent ((±)-3-oxabicyclo[4.3.0]nonan-2-one ((±)-**2**), (±)-3-azabicyclo[4.3.0]non-7-en-2-one ((±)-**9**), and (±)-2-aza-bicyclo[2.2.1]hept-5-en-3-one ((±)-**10**)) strongly inhibited growth of mycelium of tested *Aspergillus* genus species. Lactone (±)-4,4-dibutyl-3-oxabicyclo[4.3.0]nonan-2-one ((±)-**4**) and its analogue with a double bond ((±)-**8**) strongly inhibited growth of *Aspergillus* sp. AM31.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR and ^{13}C NMR spectra of lactones 3, 4, 7, 8 and lactams 9−13. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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