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Article

Synthesis of Antibacterial Norlabdane Compounds with Rearranged Cycle B and Molecular Docking Studies

Alexandru Ciocarlan ¹, Lidia Lungu ¹, Sergiu Shova ², Nicoleta Vornicu ³ and Natalia Bolocan ¹, Veaceslav Kulcitki ¹ and Aculina Aricu ^{1,*}

¹ Moldova State University, Institute of Chemistry, 2028 Chisinau, Republic of Moldova

² "P. Poni" Institute of Macromolecular Chemistry, 700487 Iasi, Romania

³ Metropolitan Center of Research T.A.B.O.R., 700066 Iasi, Romania

* Correspondence: aculina.aricu@gmail.com (A.A.); Tel.: (+373) 691 86 602

Abstract: The synthesis of tetra- and pentanorlabdane compounds with rearranged cycle B based on commercially available (+)-sclareolide is reported. Desired compounds were prepared from intermediate ketones *via* Baeyer-Villiger oxidation. The structures of synthesized compounds were confirmed by spectral IR, 1D (¹H, ¹³C and ¹⁵N, DEPT) and 2D (H-COSY, H,C-HSQC, H,C-HMBC, H,N-HMBC, NOESY) NMR analyses, mass-spectrometry and single crystal X-rays diffraction. Two of the four obtained compounds showed high antifungal and antimicrobial activity, comparable and exceeding that of standards Caspofungin and Kanamycin. DFT calculations revealed the stability of compounds in gas, DCM, water and methanol. Molecular docking to four targets (1KZN, 1U1Z, 3QLS, 2Q85) showed good agreement with the results of *in vitro* evaluation and confirmed the biological activity of compounds **3** and **4**, with binding affinities comparable and for some targets exceeding that of Caspofungin and Kanamycin.

Keywords: (+)-sclareolide; methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate; Baeyer-Villiger oxidation; antimicrobial activity; DFT

1. Introduction

It is well known that natural pentanorlabdane lactones and some of the tetranorlabdane compounds possess a wide range of biological activity [1,2]. A large number of synthetic polyfunctional tetra- and pentanorlabdane biologically active compounds, including nitrogen containing compounds were prepared starting from (+)-sclareolide *via* related intermediate methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate (**1**) and drim-8-en-7-one (**2**) (drimenone) [3–11]. These series can be completed by recently reported hybrid tetra- and pentanorlabdane heterocyclic compounds [12–14]. All these structures were obtained with the involvement of functional groups from the side chain or from cycle B of the initial molecules, without modifying their carbon skeleton. Currently, only one synthesis of some lactams with the modified cycle B obtained by the Beckmann rearrangement of the oximes corresponding to compounds **1** and **2** was reported [15].

However, methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate (**1**) and drim-8-en-7-one (**2**) offer new synthetic opportunities and this research confirmed hypothesis by the synthesis of new tetra- and pentanorlabdane compounds with rearranged cycle B.

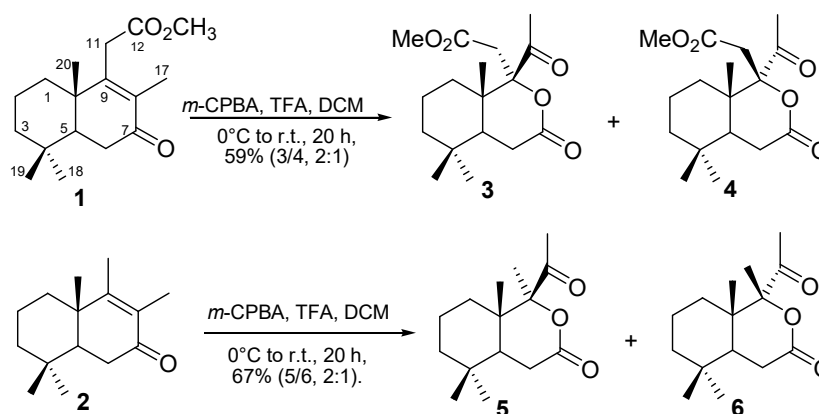
2. Results and Discussion

2.1. Synthesis

The starting materials, methyl 7-oxo-13,14,15,16-tetranorlabd-8-en-12-oate (**1**) and drim-8-en-7-one (**2**) (drimenone), traditionally, are prepared synthetically because only trace amounts of the last were detected in natural sources [26]. They can be prepared by earlier known procedures in two and three steps, respectively, from commercially available (-)-sclareolide, in 60% and 57% yields [3,27], or by recently improved methods from the same starting material in comparable yields [28].

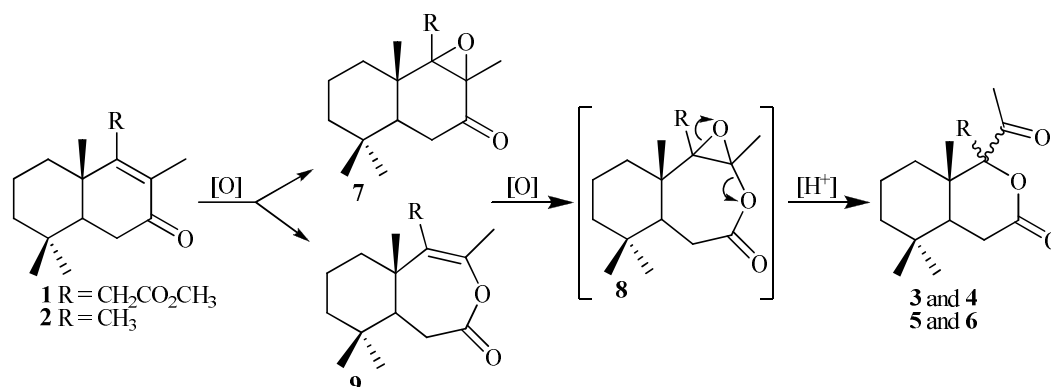
Baeyer-Villiger oxidation (BVO) is widely and efficiently used more than one century for the conversion of ketones into lactones or esters [29], some of these compounds being reported as biologically active. We tried to apply a modified procedure of BVO for terpenic ketones **1** and **2**, using 3-chloroperbenzoic acid (*m*-CPBA) combined with trifluoroacetic acid (TFA). Authors proved its efficiency for a series of cyclic, including α,β -unsaturated, and acyclic ketones which were converted into corresponding esters and lactones in shorter time and high yields [30].

According to TLC data the reaction mixture derived from ketoester **1** contains one major product and that derived from drimenone **2** two chromatographically separable products. The NMR analysis showed that unseparable mixture of compounds **3/4** and individual compounds **5** and **6** are not expected seven-membered enol lactones, as normal products of BVO. Surprisingly, in both cases after BVO reaction epimeric on C₉ six-membered ring lactones **3/4** and **5/6** were obtained in 57% and 88% overall yields, respectively (Scheme 1).



Scheme 1. Baeyer-Villiger oxidation of norlabdane lactones **1** and **2**.

The literature doesn't report such cases of rearrangement for compounds from norlabdane series during BVO, but an explanation of compounds **3-6** formation can be found. One of the ways of the ketones **1** and **2** conversion, probably, starts from epoxidation of the C₈₋₉ double bond with epoxyketone **7** formation, followed by BVO which leads to epoxylactone **8** (Scheme 2).



Scheme 2. The proposed mechanism of the Baeyer-Villiger oxidation of norlabdane ketones **1** and **2**.

Another way for the formation of compound **8**, starts with BVO of ketones **1** and **2** which gives enol lactone **9** followed by the epoxidation of the double bond. Such types of transformations have been described for cholestane-type compounds, when both processes occur as a result of treating α,β -unsaturated ketones with peroxyacid [31].

However, in our case, as a result of both oxidative processes and due to acidic medium, a rearrangement of intermediate seven-membered ring epoxilactone **8** into a mixture of enantiomeric more stable six-membered lactones **3/4** and **5/6** occurs (Scheme 2).

2.2. Single-Crystal X-Ray Diffraction Study

The structures of compounds **4**, **5** and **6** were definitely confirmed by single crystal X-rays diffraction. According to X-ray crystallography, they crystallize in the Sohnke $P2_12_12_1$, $P2_1$ and $P2_12_12_1$ space groups, respectively and exhibit a molecular crystal structure, where the asymmetric part is consisting from one neutral entity, as shown in Figure 1A–C, respectively. There are no co-crystallized solvate molecules in both crystals. The bond distances and angles are summarized in Table 1.

Table 1. Results of "in vitro" antimicrobial and antifungal activity of tested compounds.

Sample	MIC (µg/mL)				
	<i>Aspergillus niger</i>	<i>Penicillium frequentans</i>	<i>Alternaria alternata</i>	<i>Bacillus polymyxa</i>	<i>Pseudomonas aeruginosa</i>
1/2	>128	>128	>128	>128	>128
3/4	0.064 (±0.035)	0.064 (±0.035)	0.064 (±0.035)	2.0 (±0.060)	2.0 (±0.060)
Caspofungin*	0.38	0.38	0.38	-	-
Kanamycin*	-	-	-	2.0	2.0

*Standard compound.

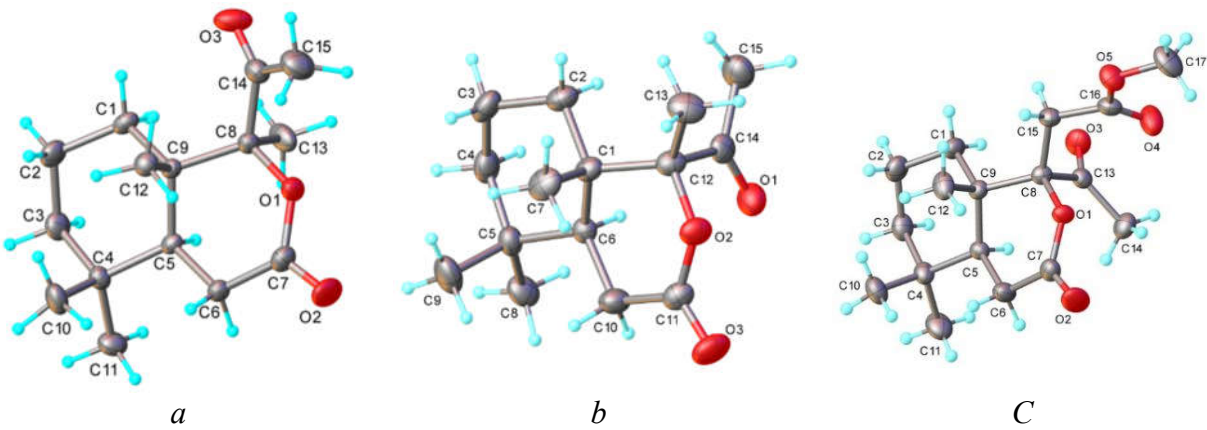


Figure 1. X-ray molecular structure of compounds **4** (a), **5** (b) and **6** (c) with atom labelling and thermal ellipsoids at 40% probability.

Further analysis has revealed that crystal structure of **4**, **5** and **6** is driven by C-H...O hydrogen bonding. The aggregation of the molecules in the crystal **4**, which occurs along hydrogen bonding, determines the formation of the discrete one-dimensional array having the form of zigzag chain running along *a* axis, as shown in Figure 2. In the crystals **5** and **6** C-H...O hydrogen bonds, being more numerous but weaker, dispose the crystal structure formed by the parallel packing of two-dimensional network. A fragment of 2D supramolecular layer is shown in Figures 3 and 4, respectively.

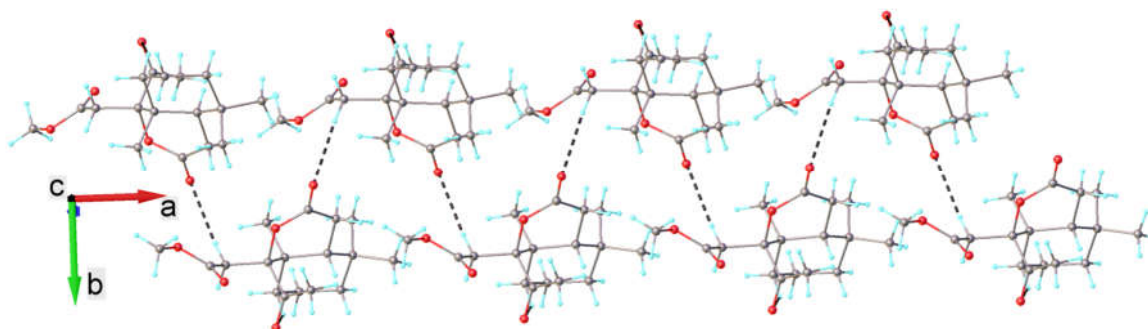


Figure 2. 1D supramolecular chain in the crystal structure 4.

H-bond parameters: C15-H $\cdots$ O2 [C15-H 0.97 Å, H $\cdots$ O2($x - 0.5, 0.5 - y, -z$) 2.565 Å, C15 $\cdots$ O2 3.525(4) Å, $\angle$ C15HO2 169.8°.

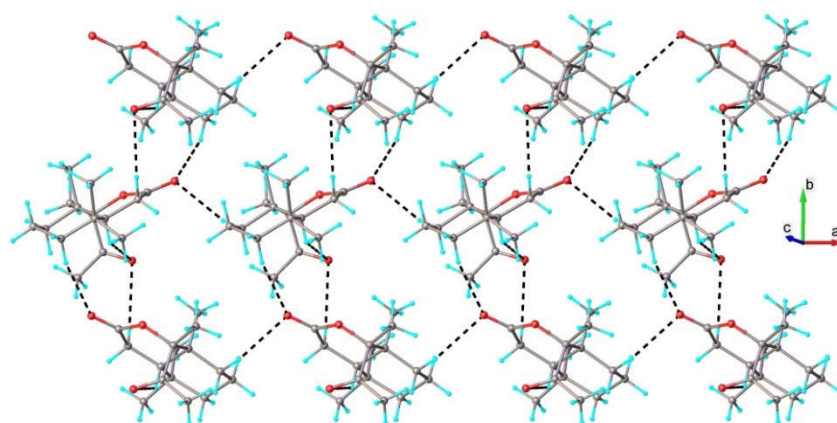


Figure 3. 1D supramolecular chain in the crystal structure 5.

H-bond parameters: C6-H $\cdots$ O3 [C6-H 0.99 Å, H $\cdots$ O3($1 - x, 0.5 + y, 1 - z$) 2.70 Å, C6 $\cdots$ O3 3.553(5) Å, $\angle$ C6HO3 144.1°; C15-H $\cdots$ O2 [C15-H 0.98 Å, H $\cdots$ O2($1 - x, y - 0.5, 1 - z$) 2.83 Å, C15 $\cdots$ O2 3.667(5) Å, $\angle$ C15HO2 143.5°.

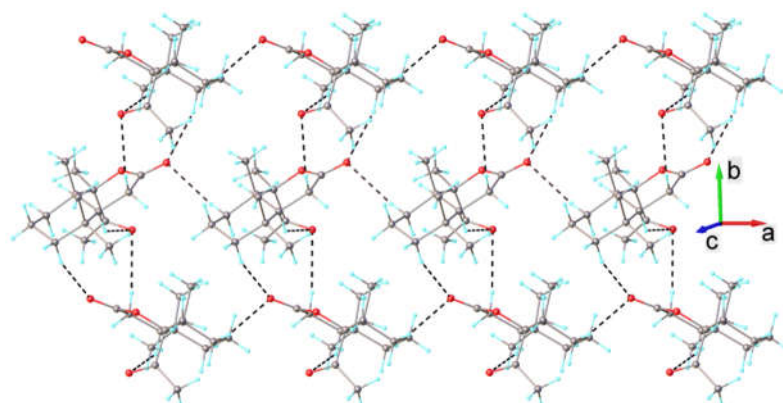


Figure 4. 2D supramolecular layer in the crystal structure 6.

H-bond parameters: C2-H $\cdots$ O3 [C2-H 0.90 Å, H $\cdots$ O3($x - 1, y, z$) 2.633 Å, C2 $\cdots$ O3 3.572(4) Å, $\angle$ C2HO3 158.4°; C4-H $\cdots$ O3 [C4-H 0.99 Å, H $\cdots$ O3($1 - x, y - 0.5, 1 - z$) 2.654 Å, C4 $\cdots$ O3 3.584(4) Å, $\angle$ C4HO3 156.5°; C10-H $\cdots$ O1 [C10-H 0.99 Å, H $\cdots$ O1($1 - x, 0.5 + y, 1 - z$) 2.704 Å, C10 $\cdots$ O1 3.553(5) Å, $\angle$ C10HO1 144.1°.

2.3. Antimicrobial Activity

The novel tetra- and pentanorlabdane compounds with rearranged cycle B **3-6** were preliminary screened for their *in vitro* antifungal and antibacterial activities against three pure cultures of fungi and two gram-positive and gram-negative bacteria strains (Table 1). Compounds **3** and **4** were inseparable from the racemic mixture, therefore were screened together. Compounds **5** and **6** did not show a significant biological activity in the test.

According to the antimicrobial activity evaluation data, compounds **3** and **4** showed higher antifungal activity than the reference compound Caspofungin at MIC= $6.4 \cdot 10^{-2}$ $\mu\text{g/mL}$, and comparable to the reference compound Kanamycin antibacterial activity at MIC= 2.0 $\mu\text{g/mL}$. The activity of compounds **3** and **4** is due to the co-existence of the lactone, $>\text{C}=\text{O}$ and ester groups.

2.4. DFT Study of the Structure of Compounds **3** and **4**

Considering that compounds **3** and **4** showed good antifungal and antimicrobial activity, and that epimerizable product distribution in a reaction is controlled by their thermodynamic stability, the study continued with computational methods, in order to reveal details regarding the stability of these epimers in various media.

The structures of the two epimers **3** and **4** were optimized to global minima, in gas, water, DCM and methanol, and the coordinates are presented in supplementary Tables SI–VIII. In gas, compound **4** is more stable than **3** with a difference in the Gibbs free energy of 23.3 kJ/mol. The relative energies of compounds **3** and **4** global minima in the studied solvents is shown in Figure 5.

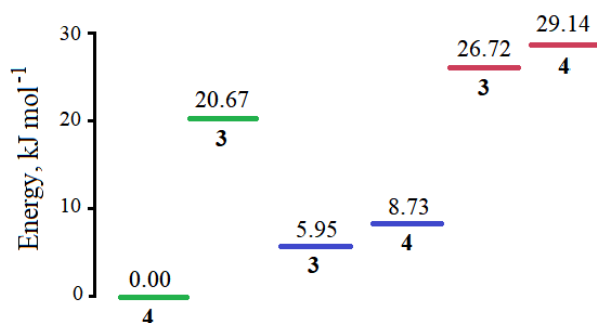


Figure 5. Relative energies of epimers **3** and **4** in DCM (green), methanol (blue) and water (red), calculated at the wB97X/def2-SVP level.

As seen in Figure 5, compound **4** is more stable than compound **3** in DCM, with a difference in the Gibbs free energy of 20.7 kJ/mol. In water and methanol, compound **3** is slightly more stable, by 2.4 kJ/mol and 2.78 kJ/mol, respectively.

2.5. Molecular Docking of Compounds **3** and **4**

In order to explain the mechanisms of inhibition of microorganisms through the intermolecular ligand-receptor interaction that define the antimicrobial activity, a comparative molecular docking study of epimers **3** and **4** was carried out on four protein models: DNA gyrase from *E. coli* (1KZN), Fabz from *P. aeruginosa* (1U1Z), dihydrofolate reductase from *C. albicans* (3QLS) and MurB from *E. coli* (2Q85).

The binding energies of the researched compounds (Table 2) are similar to those of the standards used, and in many cases they even exceed them, demonstrating the fact that they effectively bind to the target enzymes contributing to their inhibition. The results of computational calculations demonstrate the fact that most of the compounds have a higher binding affinity to 1U1Z, suggesting the idea that the interruption of fatty acid biosynthesis would be the most likely way of action of the investigated compounds on pathogens.

In the case of *E. coli* ADN girase (1KZN), Kanamycin has the highest binding affinity -6.8 kcal/mol, closely followed by compound **3**, (-6.6 kcal/mol), while compound **4** has a binding affinity very similar and a bit higher than Caspofungin, -6.2 kcal/mol and -6.1 kcal/mol, respectively. Both

epimers bind into the same pocket as the standards, Caspofungin and Kanamycin (supplementary Figure S1).

In binding to *P. aeruginosa* FabZ (1U1Z), compounds **3** and **4** have an activity of -7.7 kcal/mol, higher than Caspofungin (-7.3 kcal/mol), and close to that of Kanamycin (-8.0 kcal/mol). Both epimers bind into the same pocket as the standard Kanamycin (supplementary Figure S2).

In the case of *C. albicans* dihidrofolat reductase (3QLS), Caspofungin and Kanamycin have very similar values of binding affinity, of -7.7 kcal/mol and -7.6 kcal/mol, respectively, while compounds **3** and **4** have lower affinities, of -6.7 kcal/mol and -6.4 kcal/mol, respectively. Both epimers bind into the same pocket as the standard Caspofungin (supplementary Figure S3).

Table 2. The binding energy of the studied compounds to the target enzymes.

Compound	Calculated binding energy (kcal/mol)			
	<i>E. coli</i> DNA girase, 1KZN	<i>P. aeruginosa</i> FabZ, 1U1Z	<i>C. albicans</i> dihidrofolate reductase, 3QLS	<i>E. coli</i> MurB, 2Q85
3	-6.6	-7.7	-6.7	-7.1
4	-6.2	-7.7	-6.4	-6.4
Caspofungin	-6.1	-7.3	-7.7	-6.8
Kanamycin	-6.8	-8.0	-7.6	-8.1

Lastly, in the case of binding to *E. coli* MurB (2Q85), Kanamycin has an affinity of -8.1 kcal/mol, followed by compound **3** with a binding affinity of -7.1 kcal/mol, and then Caspofungin and compound **4** with binding affinities of -6.8 kcal/mol and 6.4 kcal/mol, respectively. Both epimers bind into one pocket, different from that of Caspofungin and Kanamycin (supplementary Figure S4).

3. Materials and Methodst

The following reagents and solvents were used in the research: dichloromethane (DCM), trifluoroacetic acid (TFA), *meta*-chloroperbenzoic acid (*m*-CPBA), petroleum ether (PE), ethyl acetate (EtOAc) and deuterated chloroform (CDCl₃). Reagents and solvents were purchased from Sigma-Aldrich.

Melting points (mp) were determined on a Boetius hot-stage apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a 1-dm microcell, in CHCl₃. Infrared spectra (IR) were obtained on Bio-Rad-Win-IR and Spectrum-100FT-IR (Perkin-Elmer) spectrometers with ATR technique. ¹H, and ¹³C NMR spectra were recorded in CDCl₃ on Bruker Avance DRX 400 and Bruker Biospin Avance 500 spectrometers. Chemical shifts are given in parts per million values (ppm) in δ scale and referred to CHCl₃ (δ_H at 7.26 ppm) and to CDCl₃ (δ_C 77.00 ppm), respectively. Coupling constants (J) are reported in Hertz (Hz). Correlation spectroscopy (H,H-COSY), heteronuclear single quantum correlations (H,C-HSQC), and heteronuclear multiple-bond correlations (H,C-HMBC) experiments were recorded using standard pulse sequences, in the version with z-gradients, as delivered by Bruker Corporation. Carbon substitution degrees were established by distortionless enhancement by polarization transfer (DEPT) pulse sequence. GS-MS analysis were run on an Agilent-5975C model provided a DB-17 fused silica capillary column, 30 × 0.25 mm i.d., with helium as carrier gas, coupled with a Agilent-5975C spectrometer (EI, 70 eV). High-resolution mass spectra (MS) were run on Kratos MS80 spectrometer (EI, 70 eV) with home-built data system. The ration *m/z* and relative intensity (%) are indicated for the significant peaks. For analytical thin-layer chromatography (TLC), Sorbfil and Merck silica-gel plates 60 G in 0.25 mm layers were used. The chromatograms were sprayed with concentrated H₂SO₄ and heated at 80°C for 5 min. Column chromatography was carried out on Across (60-200 mesh) and Merck silica gel 60 (70-230 mesh) using mixture of petroleum ether (bp 40-60 °C) with EtOAc in gradient. All solvents were purified and dried by standard techniques before use. Organic extracts were dried over anhydrous Na₂SO₄, then filtered and evaporated under reduced pressure.

3.1. General Procedure of Baeyer-Villiger Oxidation of Ketones **1** and **2**

To a solution of α,β -unsaturated ketone (1 mmol) in DCM (4 mL) *m*-CPBA (2.2 mmol) was added. After cooling to 0 °C, freshly distilled TFA (0.076 mL) was added dropwise. In continuation, the reaction mixture was warmed to room temperature and stirred in the dark for 20 hours, then diluted with DCM (20 mL) and washed consecutively with aqueous Na₂SO₃ (10%) (15 mL), saturated K₂CO₃ solutions, water (10 mL), and dried over anhydrous Na₂SO₄. After solvent removal under reduced pressure, the crude reaction products were purified by column chromatography on silica gel (eluent PE/EtOAc gradient 90/10→70/30) to yield:

Mixture of methyl 2-((1*S*,8*aS*)-1-acetyl-5,5,8*a*-trimethyl-3-oxooctahydro-1*H*-isochromen-1-yl)acetate (**3**) and methyl 2-((1*R*,8*aS*)-1-acetyl-5,5,8*a*-trimethyl-3-oxooctahydro-1*H*-isochromen-1-yl)acetate (**4**). White crystals (177 mg, 57%, in ~2:1 ratio). IR (ν , CCl₄, cm⁻¹): 2954, 1742, 1715, 1438, 1353, 1180, 1046, 816.

Compound 3. ¹H NMR (400 MHz, CDCl₃, δ): 3.65 (s, 3H, CH₃COO), 3.15 (d, 1H, *J* = 16.5, H-11), 2.82 (d, 1H, *J* = 16.5, H-11), 2.76 (dd, 1H, *J*₁ = 18.7, *J*₂ = 7.0, H-6), 2.58 (dd, 1H, *J*₁ = 19.3, *J*₂ = 12.6, H-6), 2.40 (s, 3H, H₁₇), 1.09 (s, 3H, H-20), 0.93 (s, 3H, H-19), 0.83 (s, 3H, H-18). ¹³C NMR (100 MHz, CDCl₃, δ): 209.5 (C=O), 170.3 (C $\equiv$ O), 169.7 (C₁₂), 93.9 (C₉), 52.1 (CH₃COO), 44.1 (C₅), 40.8 (C₁₀), 40.5 (C₃), 38.7 (C₁₁), 33.0 (C₄), 32.3 (C₁₈), 32.1 (C₁), 30.6 (C₁₇), 29.6 (C₆), 21.3 (C₁₉), 17.9 (C₂), 15.7 (C₂₀).

Compound 4. ¹H NMR (400 MHz, CDCl₃, δ): 3.67 (s, 3H, CH₃COO), 3.33 (d, 1H, *J* = 15.2, H-11), 2.82 (d, 1H, *J* = 15.8, H-11), 2.66 (dd, 1H, *J*₁ = 18.8, *J*₂ = 5.8, H-6), 2.45 (dd, 1H, *J*₁ = 18.4, *J*₂ = 13.1, H-6), 2.40 (s, 3H, H-17), 1.04 (s, 3H, H-20), 0.92 (s, 3H, H-19), 0.92 (s, 3H, H-18). ¹³C NMR (100 MHz, CDCl₃, δ): 209.3 (C₈), 169.4 (C₁₂), 169.3 (C $\equiv$ O), 93.8 (C₉), 52.2 (CH₃COO), 41.8 (C₅), 40.9 (C₃), 40.8 (C₁₀), 40.8 (C₁₁), 33.0 (C₄), 32.8 (C₁₈), 32.8 (C₁), 30.6 (C₁₇), 28.2 (C₆), 21.4 (C₁₉), 17.6 (C₂), 15.6 (C₂₀).

HR-EI-MS: Found 310.27802. C₁₇H₂₆O₅ Calcd. 310.3843. *m/z* 310 (M⁺, 1), 279 (29), 249 (36), 218 (4), 193 (19), 165 (10), 145 (9), 133 (11), 121 (6), 109 (24), 101 (8), 81 (15), 68 (4).

(1*S*,8*aS*)-1-acetyl-1,5,5,8*a*-tetramethylhexahydro-1*H*-isochromen-3(4*H*)-one (**5**). White crystals (83 mg, 33%), mp 133-134 °C (PE), [α]_D²⁰ -102.6 (c 2.6, CHCl₃). IR (ν , CCl₄, cm⁻¹): 2940, 1729, 1705, 1459, 1381, 1260, 1090, 825. ¹H NMR (400 MHz, CDCl₃, δ): 2.69 (dd, 1H, *J*₁ = 18.8, *J*₂ = 6.7, H-6), 2.47 (dd, 1H, *J*₁ = 18.9, *J*₂ = 12.4, H-6), 2.28 (s, 3H, H-17), 1.40 (s, 3H, H-20), 1.08 (s, 3H, H-11), 0.93 (s, 3H, H-19), 0.83 (s, 3H, H-18). ¹³C NMR (100 MHz, CDCl₃, δ): 209.7 (C=O), 171.3 (C $\equiv$ O), 94.6 (C₉), 44.1 (C₅), 40.9 (C₃), 40.0 (C₁₀), 33.0 (C₁), 32.9 (C₄), 32.3 (C₁₈), 29.6 (C₁₇), 29.3 (C₆), 21.2 (C₁₉), 18.7 (C₂₀), 18.1 (C₂), 14.7 (C₁₁). HR-EI-MS: Found 209.15444. C₁₅H₂₄O₃ Calcd. 253.2513. *m/z* 209 (M⁺, -44, 44), 181 (6), 123 (100), 69 (20), 65 (2), 54 (1), 42 (3).

(1*R*,8*aS*)-1-acetyl-1,5,5,8*a*-tetramethylhexahydro-1*H*-isochromen-3(4*H*)-one (**6**). White crystals (137 mg, 55%), mp 90-91 °C (PE), [α]_D²⁰ -73.6 (c 2.3, CHCl₃). IR (ν , CCl₄, cm⁻¹): 2937, 1733, 1714, 1455, 1354, 1279, 1101, 827. ¹H NMR (400 MHz, CDCl₃, δ): 2.61 (dd, 1H, *J*₁ = 18.8, *J*₂ = 6.2, H-6), 2.41 (dd, 1H, *J*₁ = 18.8, *J*₂ = 13.2, H-6), 2.31 (s, 3H, H-17), 1.44 (s, 3H, H-11), 1.02 (s, 3H, H-20), 0.91 (s, 3H, H-19), 0.89 (s, 3H, H-18). ¹³C NMR (100 MHz, CDCl₃, δ): 210.5 (C=O), 170.2 (C $\equiv$ O), 95.0 (C₉), 41.2 (C₅), 41.0 (C₃), 38.7 (C₁₀), 33.1 (C₁), 32.9 (C₄), 32.7 (C₁₈), 28.9 (C₁₇), 28.4 (C₆), 21.2 (C₂₀), 21.2 (C₁₁), 17.8 (C₂), 15.7 (C₁₉). HR-EI-MS: Found 209.15413. C₁₅H₂₄O₃ Calcd. 253.2513. *m/z* 209 (M⁺, -44, 47), 181 (6), 110 (3), 107 (7), 95 (13), 91 (2), 83 (8), 79 (4), 67 (11), 59 (4), 55 (15), 51 (1).

3.2. Crystallographic Studies

Single-crystal X-ray diffraction data were collected on an Oxford-Diffraction XCALIBUR Eos CCD diffractometer with graphite-monochromated MoK α radiation. The unit cell determination and data integration were carried out using the CrysAlisPro package from Oxford Diffraction [16]. A multi-scan correction for absorption was applied. The structures were solved with the SHELXT using the intrinsic phasing method and refined by the full-matrix least-squares method on *F*² with SHELXL [17,18]. Olex2 was used as an interface to the SHELX programs [19]. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were added in idealized positions and refined using a riding model. In the absence of a significant anomalous scattering, the absolute configuration of the structures could not be reliably determined, and therefore, Friedel pairs were merged and any references to the Flack parameter were removed. The molecular plots were obtained using the Olex2

program. Selected crystallographic data and structure refinement details for compounds **4**, **5** and **6** are provided in supplementary Table S9.

3.3. Antifungal and Antibacterial Activity Assay

The synthesized compounds were tested *in vitro* for antimicrobial activity on three species of fungi: *Aspergillus niger* (ATCC 53346), *Penicillium frequentans* (ATCC 10110) and *Alternaria alternata* (ATCC 8741), and both Gram-positive *Bacillus polymyxa* (ATCC 15970) and Gram-negative *Pseudomonas aeruginosa* (ATCC 27813) bacteria strains, provided by the American Type Culture Collection (ATCC, USA). Compounds Caspofungin and Kanamycin, both from Liofilchem (Roseto degli Abruzzi, Italy), were used as standards for antifungal and antibacterial activity testing at (MIC= 0.38 µg/mL) and (MIC= 2.0 µg/mL), respectively. Microorganism's suspensions were prepared using the method of successive agar dilutions according to the standard MIC [20], and their cultivation was carried out according to standard procedures [21].

3.4. DFT Calculations

The ORCA program was used to optimize and properly characterize the structures [22]. All the calculations were performed using the wB97X functional with def2-SVP basis set, and the empirical dispersion correction of Grimme's D3 in the DFT method [23]. Frequency analyses were done at the same level of theory. All the calculations in solvents (water, dichloromethane, methanol) were performed by employing the Solvation Model Density calculation model (SMD).

3.5. Molecular Docking

Four protein models were used in the study: DNA gyrase from *Escherichia coli* (PDB code: 1KZN), FabZ from *P. aeruginosa* (PDB code: 1U1Z), dihydrofolate reductase from *Candida albicans* (PDB code: 3QLS) and MurB from *E. coli* (PDB code: 2Q85).

The structures were prepared according to the protocol, using the AutoDockTools 1.5.7 program: water molecules and native ligands were removed from the protein structures, polar hydrogens and Gasteiger charges were added [24].

In the docking protocol, the ligands were treated as flexible molecules and the docking software was allowed to rotate all rotatable bonds of the ligands to obtain the ligand conformer with the optimal position in the active site of the enzyme. In each case, the grid was constructed and properly centered to cover the entire enzyme. Docking was performed using the routine procedure and default parameters of the molecular docking program Vina [25].

From the set of ligand conformations obtained by the docking procedure, the conformation with the lowest binding energy was selected for further analysis. The Discovery Studio Visualizer program was used to investigate the hydrophobic interactions and hydrogen bonds between the ligands and the investigated enzymes. The 3D structures of the Kanamycin and Caspofungin standards were retrieved from The Human Metabolome Database (hmdb.ca).

4. Conclusions

A series of novel tetra- and pentanorlabdane six-membered lactones with rearranged cycle B were synthesized in good yields by the Baeyer-Villiger oxidation with m-CPBA and the mechanism of their formation was proposed.

Computational calculations revealed that epimer **4** is more stable in gas and DCM, by 23.3 kJ/mol and 20.7 kJ/mol, respectively, while epimer **3** is more stable in water and methanol, by 2.4 kJ/mol and 2.78 kJ/mol, respectively.

Molecular docking results are in good agreement with experimental findings, showing that compounds **3** and **4** have a biological activity comparable, and in some cases exceeding that of the standards used, Caspofungin and Kanamycin. According to the values of calculated binding affinities, epimer **3** has a higher biological activity than epimer **4**.

Due to their original structure and biological activity reported compounds are of real scientific interest.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

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