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RESIN ACIDS AS RAW MATERIAL FOR THE PREPARATION OF CYCLODEXTRIN COMPLEXES LOADED WITH DEHYDROABIETITOIC ACID AND CHROMENOL HYBRID

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Abstract. In this work new methods to obtain complexes from *β*-cyclodextrin and dehydroabietic acid with chromenol-triazol hybrid with the sizes limits of approximately 0.1-250 μm are reported. Kneading, co-evaporation and co-precipitation for the resolution of racemic 2-tert-butyl-3-(1*H*-1,2,4-triazol-1-yl)-2*H*-chromen-2-ol for obtaining micro- and nanoparticles have been optimized. *In vitro* dissolution studies of the synthesized compounds in phosphate buffer (pH 6.8) showed an improved dissolution rate of chromenol-triazol hybrid in the inclusion complexes compared to the free form. It has been found that *β*-complexes of *β*-cyclodextrin loaded with dehydroabietitoic acid and chromenol hybrid show good antibacterial activity with MIC and MBC values ranging from 0.72 to 44.45 μM. The evaluation results revealed that all compounds showed good antifungal activity with MIC values ranging from 0.02 to 0.4 mM and MFC from 0.07 to 0.52 mM better than the reference drugs ketoconazole (MIC and MFC values at 0.28-1.88 and 0.38 mM to 2.82 mM, respectively), bifonazole (MIC and MFC values at 0.32-0.64 and 0.64-0.81 mM) and nistatin (MIC and MFC values at 0.55-0.65 mM and 0.65-0.79 mM).

Keywords: *β*-cyclodextrin, dehydroabietic acid, 2-*tert*-butyl-3-(*1H*-1,2,4-triazol-1-yl)-2*H*-chromen-2-ol, chromenol-triazol hybrid, antimicrobial activity.

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Introduction

In recent years, interest in natural biologically active substances and their synthetic analogues has been growing steadily. However, most biologically active organic compounds are poorly soluble in water and, as a consequence, have low bioavailability. An important and well-designed approach to increase water solubility of certain organic moieties is the formation of host-guest inclusion complexes with specific molecules able to participate in this interaction. For example, *β*-cyclodextrin (*β*-CD) is a well-known host molecule able to form inclusion complexes in both solution and solid phase with a wide variety of solid and/or liquid hydrophobic compounds [1-4]. Moreover, crystalline bioactive ingredients are strongly preferred, since they are relatively easy to isolate, and the rejection of impurities inherent to the crystallization process and their physical-chemical stability, so most marketed pharmaceuticals consist of molecular crystals. In such crystalline assemblies the intermolecular interactions play a crucial role [5-8]. It is interesting to note that co-crystal co-formers are non-volatile molecules at ambient conditions. The existence of a space that can expand or contract to fit the solvents in the crystal structure appears to be important for the formation of pseudo-polymorphism with diverse solvents, regardless of whether the space is intramolecular or between molecular complexes. However, the most significant

problems involved in the application of the small molecules as bioactive compounds are their sometimes-low efficacy or high cytotoxicity. This can be solved by modification of bioactive ingredients involved in the complex formation, as well as through formulation of encapsulated compositions with the use of different natural or semisynthetic agents, like dehydroabietic acid (DHA) **1** (Figure 1) and cycloheptaamylose, as well [9-11]. The discovery that **1** itself possess fungicidal properties against *Aspergillus terreus* has stimulated interest in investigation of the acid **1** chemistry and has given impetus to search for other biological properties [12-14].

The aim of this work was to obtain complexes of *β*-cyclodextrin with water-insoluble biologically active compounds such as natural dihydroabietic acid and chromenol-triazole hybrid and to study their physicochemical properties and activity.

Experimental

Materials

β-Cyclodextrin (batch CYL-3190) was purchased from CycloLab R&D Ltd. (Hungary), anhydrous Na_2SO_4.

The solvents including, methanol (MeOH), methylene chloride and acetonitrile (MeCN) were of reagent grade and used without additional purification. Removal of all solvents was carried out under reduced pressure.

Methods

^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance DRX 400 spectrometer. Chemical shifts are given in ppm in the δ scale and referred to DMSO-d_6 (δ_H at 2.50 ppm) and to DMSO-d_6 (δ_C 39.52 ppm), respectively. The coupling constants (*J*) are reported in Hertz (Hz). The H, H-COSY, H, C-HSQC and 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 the DEPT pulse sequence.

The *IR spectra* were registered on a Spectrum-100 FT-IR spectrometer (Perkin-Elmer) by the ATR technique.

Elemental analyses for C, H, and N were carried out by using an Elementar Vario EL analyser.

Melting point values (uncorrected) were determined on a Boetius apparatus.

Thin-layer chromatography was carried out on Merck aluminum TLC plates, silica gel 60 coated with fluorescent indicator F254.

The morphology of the system was studied at VEGA TESCAN TS 5130 MM *scanning electron microscope* (SEM).

X-ray diffraction measurements were carried out on an Xcalibur E diffractometer equipped with a CCD area detector and a graphite monochromator utilizing MoK*α* radiation at room temperature. All calculations to solve and refine the structure were carried out with the programs SHELXS97 and SHELXL2014 [15,16]. Crystallographic data were deposited with the Cambridge Crystallographic Data Centre, CCDC 1985718.

For *dissolution studies*, 200 mg of substance and quantities of the inclusion complexes containing an equivalent amount of 1,2,4-triazole were used. The experiments were conducted under physiological conditions, at 37±0.5°C, using 900 mL of phosphate buffer (pH 6.8), with 24 hours stirring. Further, the suspensions were centrifuged and the absorbance of the supernatant was read at 319 nm after filtration through a 0.45 μm filter.

*Dehydroabietic acid (**1**)* was isolated from commercial disproportionate rosin as described before [17] in 85% yield, $[\alpha]_D^{20}$ +59.8 (*c* 0.01, MeOH), m.p. 168–170°C. IR (*v*, cm^{-1}): 2956 (carboxyl), 2928 (carboxyl), 2870, 2645, 1687 (C=O), 1458, 1458, 1388 (C-$(CH_3)_2$), 1279 (deformation vibrations OH), 1191, 1137, 951, 817, 719, 665. ^{1}H NMR: δ 12.2 (1H, s, CO_2H-19); 7.15 (1H, d, *J*= 8.2, H-11); 6.96 (1H, dd, *J*= 8.0, 1.7, H-12); 6.86 (1H, d, *J*= 1.7, H-14); 2.77 (1H, m, overlapping, H-16); 2.7–2.9 (2H, m, overlapping, H-7); 2.28 (1H, *J*= 12.0, H-1$_\alpha$); 2.0 (1H, dd, *J*= 12.3, 2.0, H-5$_\alpha$); 1.69–1.82 (1H, m, overlapping, H-6$_\beta$); 1.67 (1H, t, *J*= 12.0, H-3$_\alpha$); 1.6–1.73 (1H, *J*= 7.8, 1.4, H-2); 1.57 (1H, d, *J*= 12.0, H-3$_\beta$); 1.36–1.45 (1H, m, overlapping, H-6$_\alpha$); 1.26 (1H, tt, *J*= 12.0, 3.3, H-1$_\beta$); 1.15 (3H, s, CH_3-18); 1.15 (6H, d, *J*= 6.8, $(CH_3)_2$-15,17); 1.11 (3H, s, CH_3-20). ^{13}C NMR: δ 179.9 (C-19); 147.2 (C-9); 145.5 (C-13); 134.6 (C-8); 124.5 (C-11); 124.2 (C-12); 46.8 (C-4); 45.2 (C-5); 38.2 (C-1); 36.9 (C-10); 36.7 (C-3); 33.3 (C-15); 30.0 (C-7); 25.3 (C-20); 24.4 (C-16,17); 21.6 (C-6); 18.6 (C-2); 16.8 (C-18). Calculated (%) for $C_{20}H_{28}O_2$: C 79.96, H 9.39. Found (%): C 79.5, H 9.0.

2-*tert*-butyl-3-(1*H*-1,2,4-triazol-1-yl)-2*H*-chromen-2-ol (BTC) (**2**) and 3,3-dimethyl-1-(*1H*-1,2,4-triazol-1-yl)butan-2-one (**4**) were synthesized according to previously described procedures [18,19].

*The co-crystal (**6**)* was obtained by co-crystallisation after reflux of an equal amount of dehydroabietic acid (**1**) and chromenol-triazol hybrid (**2**) in MeCN. Yield 70%, white crystals, (MeCN), m.p. 136–138°C, $[\alpha]_D^{20}$ +33.8 (*c* 0.0072, MeOH). IR (*v*, cm^{-1}): 3318 (hydrogen bonds), 3314 (OH), 2956 (carboxyl), 2924 (carboxyl), 2868, 2573, 1698 (C=O), 1462 (C-CH_3), 1400, 1292, 1255, 1126 (C-O-C), 1055, 973, 893, 752 (=CH-), 673, 655. ^{1}H NMR: δ 12.2 (1H, CO_2H-19); 8.67 (1H, s); 8.18 (1H, s); 8.0 (1H, s, OH); 7.29 (1H, dd, *J*= 7.4, 0.8); 7.25 (1H, td, *J*= 7.8, 1.4); 7.13 (1H, s); 7,15 (1H, d, *J*= 8.2, H-11); 6.96 (1H, dd, *J*= 8.0, 1.7, H-12); 6.92 (1H, td, *J*= 7.8, 1.4); 6.89 (1H, d, *J*= 8.2); 6.86 (1H, d , *J*= 1.7, H-14); 2.77 (1H, m, overlapping, H-16); 2.7–2.9 (2H, m, overlapping, H-7); 2.28 (1H, *J*=12.0, H-1$_\alpha$); 2.0 (1H, dd, *J*= 12.3, 2.0, H-5$_\alpha$); 1.69–1.82 (1H, m, overlapping, H-6$_\beta$); 1.67 (1H, t, *J*= 12.0, H-3$_\alpha$); 1.6–1.73 (1H, *J*= 7.8. 1.4, H-2); 1.57 (1H, d, *J*= 12.0, H-3$_\beta$); 1.36–1.45 (1H, m, overlapping, H-6$_\alpha$); 1.26 (1H, tt, *J*= 12.0, 3.3, H-1$_\beta$); 1.15 (3H, s, CH_3-18); 1.15 (6H, d, *J*= 6.8, $(CH_3)_2$-15,17); 1.11 (3H, s, CH_3-20); 0.76 (9H, s).^{13}C NMR: δ 179.9 (C-19); 152.9; 151.8; 147.2 (C-9); 145.1; 145.5 (C-13); 134.6 (C-8); 131.1; 129.8; 128.1; 124.5 (C-11); 124.2 (C-12); 123.3; 121.4; 118.7; 114.8; 103.8; 46.8 (C-4); 45.2 (C-5); 42.9; 38.2 (C-1); 36.9 (C-10); 36.7 (C-3); 33.3 (C-15); 30.0 (C-7); 25.3 (C-20); 24.6; 24.4 (C-16,17); 21.6 (C-6); 18.6 (C-2); 16.8 (C-18). Calculated (%) for $C_{35}H_{45}N_3O_4$; C 73.52, H 7.35. Found (%) C 73.1, H 7.0.

*The system (**7**) w*as obtained using the kneading method, where the molar ratio of the components dehydroabietic acid (**1**) and chromenol-triazol hybrid (**2**) was 1:1, the working temperature being 20±2°C. A sufficient amount of distilled water was added to the mixture in order to form a paste. The paste was kneaded using a pestle for 90 minutes: the first 60 minutes by adding distilled water to compensate its losses by evaporation and maintain the appearance of pasta; next 30 minutes the mixture was milled to a fine powder. The obtained powder was stored in a parafilm sealed sample tube at room temperature (20±2°C). M.p. 131–134°C, IR (*v*, cm^{-1}): 3350, 3314 (hydrogen bonds), 3314 (OH), 2956 (carboxyl), 2925 (carboxyl), 2870, 2572, 1695 (C=O), 1460 (C-CH_3), 1400, 1286, 1256, 1127 (C-O-C), 1057, 969, 892, 752 (=CH-), 673, 655. ^{1}H NMR: δ 12.15 (1H, s, CO_2H-19); 8.67 (1H, s, H-3'); 8.18 (1H, s, H-4'); 8.0 (1H, s, OH); 7.29 (1H, dd, *J*= 7.4, 0.8); 7.25 (1H, td, *J*= 7.8, 1.4); 7.13 (1H, s); 7.15 (1H, d, *J*= 8.2, H-11); 6.96 (1H, dd, *J*= 8.0, 1.7, H-12); 6.92 (1H, td, *J*= 7.8, 1.4); 6.89 (1H, d, *J*= 8.2); 6.86 (1H, d, *J*= 1.7, H-14); 2.77 (1H, m, overlapping, H-16); 2.7–2.9 (2H, m, overlapping, H-7); 2,28 (1H, *J*= 12.0, H-1$_\alpha$); 2.0 (1H, dd, *J*= 12.3, 2.0, H-5$_\alpha$); 1.69–1.82 (1H, m, overlapping, H-6$_\beta$); 1.67 (1H, t, *J*= 12.0, H-3$_\alpha$); 1.6–1.73 (1H, *J*= 7.8, 1.4, H-2); 1.57 (1H, d, *J*= 12.0, H-3$_\beta$); 1.36–1.45 (1H, m, overlapping, H-6$_\alpha$); 1.26 (1H, tt, *J*= 12.0, 3.3, H-1$_\beta$); 1.15 (3H, s,CH_3-18); 1.15 (6H, d, *J*= 6.8, $(CH_3)_2$-15,17); 1.11 (3H, s, CH_3-20); 0.76 (9H, s).^{13}C NMR: δ 179.9 (C-19); 152.9; 151.8; 147.2 (C-9); 145.1; 145.5 (C-13); 134.6 (C-8); 131.1; 129.8; 128.1; 124.5 (C-11); 124.2 (C-12); 123.3; 121.4; 118.7; 114.8; 103.8; 46.8 (C-4); 45.2 (C-5); 42.9; 38.2 (C-1); 36.9 (C-10); 36.7 (C-3); 33.3 (C-15); 30.0 (C-7); 25.3 (C-20); 24.6; 24.4 (C-16,17); 21.6 (C-6); 18.6 (C-2);16.8 (C-18). Calculated (%) for $C_{35}H_{45}N_3O_4$; C 73.52, H 7.35. Found (%) C 73.2, H 7.1.

*The binary system (**8**)* based on chromenol-triazol hybrid **2** and β-CD **3** has been prepared by the kneading method, the molar ratio of the components was 1:1, the working temperature being 20±2°C. In an agate mortar were added appropriate amounts of β-cyclodextrin and of chromenol-triazolhybrid, previously weighed on the electronic analytical balance.

A sufficient amount of distilled water was added to the mixture in order to form a paste. The paste was kneaded using a pestle for 90 minutes: the first 60 minutes by adding distilled water to compensate its losses by evaporation and maintain the appearance of pasta; next 30 minutes the mixture was milled to a fine powder. The obtained powder was stored in a parafilm sealed sample tube, at room temperature (20±2°C). M.p. 150–210°C. IR (*v,* cm^{-1}): 3290 (νOH), 2927 (δCCH), 1651, 1459, 1414, 1153, 1077, 1023, 998, 947, 855, 758 (δ CCO), 752. ^{1}H NMR: δ 8.67 (1H, s); 8.18 (1H, s); 8.0 (1H, s, OH); 7.28 (1H, dd, *J*= 7.4, 0.8); 7.25 (1H, td, *J*= 7.8, 1.4); 7.14 (1H, s); 6.90 (1H, td, *J*= 7.8, 1.4); 6.89 (1H, d, *J*= 8.2); 5.73 (2H, dd, OH-2,3$_{\beta\text{-CD}}$); 4.82 (1H, d, H-1$_{\beta\text{-CD}}$); 4.48 (1H, t, OH-6 $_{\beta\text{-CD}}$); 3.63 (3H, m, H-3,-5,-6 $_{\beta\text{-CD}}$); 3.29 (2H, m, H-2,-4 $_{\beta\text{-CD}}$); 0.76 (9H, s). ^{13}C NMR: δ 152.9 ; 151.8; 145.1; 131.1; 129.8; 128.1; 123.3; 121.4; 118.7; 114.8; 103.8; 102.4 (C-1$_{\beta\text{-CD}}$); 82.0 (C-4$_{\beta\text{-CD}}$); 73.5 (C-3$_{\beta\text{-CD}}$); 72.8 (C-2$_{\beta\text{-CD}}$); 72.5 (C-5$_{\beta\text{-CD}}$); 60.4 (C-6$_{\beta\text{-CD}}$) 42.9 (C-16'); 24.6 (C-17'-19'). Calculated (%) for $C_{57}H_{87}N_3O_{42}$; C 48.67, H 6.19. Found (%) C 48.2, H 5.8.

*The system (**9**)* based on acid **1**, chromenol-triazol hybrid **2** and β-CD **3** was obtained using the following procedure: to a stirred solution of β-CD in 50% aqueous MeOH was added in small

portions the mixture of dehydroabietic acid (**1**) and chromenol-triazol hybrid **2** in MeOH, and the reaction mixture was stirred for additional 24 hours. After evaporation of the solvents under vacuum and drying at heating (up to 60°C) for 3 hours, the remaining solid system (**9**) was stored in sealed with parafilm sample tube, at room temperature (20±2°C). M.p. 170–245°C. IR (ν, cm^{-1}): 3294 (νOH), 2925 (δ CCH), 1698, 1460, 1415, 1153, 1078, 1024, 999, 939, 820, 752. ^{1}H NMR: δ 8.68 (1H, s); 8.17 (1H, s); 8.0 (1H, s, OH); 7.30 (1H, dd, *J*= 7.4, 0.8); 7.25 (1H, td, *J*= 7.8, 1.4); 7.13 (1H, s); 7.15 (1H, d, *J*= 8.2, H-11); 6.96 (1H, dd, *J*= 8.0, 1.7, H-12); 6.92 (1H, td, *J*= 7.8, 1.4); 6.89 (1H, d, *J*= 8.2); 6.86 (1H, d, *J*=1.7, H-14); 5.71 (2H, dd, OH-2,3$_{\beta\text{-CD}}$); 4.83 (1H, d, H-1$_{\beta\text{-CD}}$); 4.47 (1H, t, OH-6$_{\beta\text{-CD}}$); 3.64 (3H, m, H-3,-5,-6$_{\beta\text{-CD}}$); 3.31 (2H, m, H-2,-4$_{\beta\text{-CD}}$); 2.77 (1H, m, overlapping, H-16); 2.7–2.9 (2H, m, overlapping, H-7); 2.28 (1H, *J*= 12.0, H-1$_{\alpha}$); 2.0 (1H, dd,*J*= 12.3, 2.0, H-5$_{\alpha}$); 1.69–1.82 (1H, m, overlapping, H-6$_{\beta}$); 1.67 (1H, t, *J*= 12.0, H-3$_{\alpha}$); 1.6–1.73 (1H, *J*= 7.8, 1.4, H-2); 1.57 (1H, d, *J*= 12.0, H-3$_{\beta}$); 1.36–1.45 (1H, m, overlapping, H-6$_{\alpha}$); 1.26 (1H, tt, *J*= 12.0, 3.3, H-1$_{\beta}$); 1.15 (3H, s, CH_3-18); 1.15 (6H, d, *J*= 6.8, $(CH_3)_2$-15,17); 1.11 (3H, s, CH_3-20); 0.76 (9H, s). The signal of the carboxyl group is not expressed due to the strong influence of intramolecular hydrogen bonds. ^{13}C NMR: δ 179.9 (C-19); 152.9; 151.8; 147.2 (C-9); 145.1; 145.5 (C-13); 134.6 (C-8); 131.1; 129.8; 128.1; 124.5 (C-11); 124.2 (C-12); 123.3; 121.4; 118.7; 114.8; 103.8; 102.4 (C-1$_{\beta\text{-CD}}$); 82.0 (C-4$_{\beta\text{-CD}}$); 73.5 (C-3$_{\beta\text{-CD}}$); 72.8 (C-2$_{\beta\text{-CD}}$); 72.5 (C-5$_{\beta\text{-CD}}$); 60.4 (C-6$_{\beta\text{-CD}}$), 46.8 (C-4); 45.2 (C-5); 42.9; 38.2 (C-1); 36.9 (C-10); 36.7 (C-3); 33.3 (C-15); 30.0 (C-7); 25.3 (C-20); 24.6; 24.4 (C-16,17); 21.6 (C-6); 18.6 (C-2); 16.8 (C-18). Calculated (%) for $C_{77}H_{115}O_{39}$; C 54.17, H 6.74. Found (%) C 53.7, H 6.3.

*The system (**10**)* based on acid **1**, chromenol-triazole hybrid **2**, and β-CD **3**, was prepared by the kneading method, same as the system (**8**). Molar ratio of components 1:1:1, working temperature 20±2°C. M.p. 150–245°C. IR (ν, cm^{-1}): 3676 (H_2O), 3277, 2972, 2902, 1693, 1451, 1154, 1126, 1077, 1050, 1027, 758 (δ CCO), 751.^{1}H NMR: δ 8.68 (1H, s); 8.17 (1H, s); 8.0 (1H, s, OH); 7.29 (1H, dd, *J*= 7.4, 0.8); 7.25 (1H, td, *J*= 7.8, 1.4); 7.12 (1H, s, overlapping); 7.15 (1H, d, *J*= 8.2, H-11); 6.96 (1H, dd, *J*= 8.0, 1.7, H-12); 6.92 (1H, td, *J*= 7.8, 1.4); 6.89 (1H, d, *J*= 8.2); 6.86 (1H, d, *J*= 1.7, H-14); 5.72 (2H, dd, OH-2,3$_{\beta\text{-CD}}$); 4.83 (1H, d, H-1$_{\beta\text{-CD}}$); 4.48 (1H, t, OH-6$_{\beta\text{-CD}}$); 3.61 (3H, m, H-3,-5,-6$_{\beta\text{-CD}}$); 3.39 (2H, m, H-2,-4$_{\beta\text{-CD}}$); 2.77 (1H, m, overlapping, H-16); 2.7–2.9 (2H, m, overlapping, H-7); 2.28 (1H, *J*= 12.0, H-1$_{\alpha}$); 2.0 (1H, dd, *J*= 12.3, 2.0, H-5$_{\alpha}$); 1.69–1.82 (1H, m, overlapping, H-6$_{\beta}$); 1.67 (1H, t, *J*= 12.0, H-3$_{\alpha}$); 1.6–1.73 (1H, *J*= 7.8, 1.4, H-2); 1.57 (1H, d, *J*= 12.0, H-3$_{\beta}$); 1.36–1.45 (1H, m, overlapping, H-6$_{\alpha}$); 1.26 (1H, tt, *J*= 12.0, 3.3, H-1$_{\beta}$); 1.15 (3H, s, CH_3-18); 1.15 (6H, d, *J*= 6.8, $(CH_3)_2$-15,17); 1.11 (3H, s, CH_3-20); 0.76 (9H, s).The signal of the carboxyl group is not expressed due to the strong influence of intramolecular hydrogen bonds. ^{13}C NMR: δ 179.9 (C-19); 152.9; 151.8; 147.2 (C-9); 145.1; 145.5 (C-13); 134.6 (C-8); 131.1; 129.8; 128.1; 124.5 (C-11); 124.2 (C-12); 123.3; 121.4; 118.7; 114.8; 103.8; 102.4 (C-1$_{\beta\text{-CD}}$); 82.0 (C-4$_{\beta\text{-CD}}$); 73.5 (C-3$_{\beta\text{-CD}}$); 72.8 (C-2$_{\beta\text{-CD}}$); 72.5 (C-5$_{\beta\text{-CD}}$); 60.4 (C-6$_{\beta\text{-CD}}$); 46.8 (C-4); 45.2 (C-5); 42.9 (C-16'); 38.2 (C-1); 36.9 (C-10); 36.7 (C-3); 33.3 (C-15); 30.0 (C-7); 25.3 (C-20); 24.6; 24.4 (C-16,17); 21.6 (C-6); 18.6 (C-2); 16.8 (C-18). Calculated (%) for $C_{77}H_{115}O_{39}$; C 54.17, H 6.74. Found (%) C 53.8, H 6.4.

Antimicrobial activity

For the evaluation of the antimicrobial activity, the successive double dilution method was used. For this, at the initial stage, 1 mL of Sabouraud broth for test fungi and 1 mL of the Peptone broth for bacteria were introduced into a series of 10 tubes. Subsequently, 1 mL of the analysed compound was dropped into the first test tube, then, the obtained mixture was pipetted, after which 1 mL of it was transferred to the next tube, so the procedure was repeated until the tube no. 10 of the series. At the same time, 24 hour test-microbial cultures were prepared. On the second day, a preliminary analysis of the results was made. The last tube from the series in which no visible growth of microbial strains has been detected is considered to be the minimal inhibitory concentration (MIC) of the compound. For the estimation of the minimal bactericidal and fungicidal concentrations (MBC and MFC), the contents of the test tubes with MIC and with higher concentrations were seeded on Sabouraud and Peptone agar from Petri dishes. The concentration of the tested compound that does not allow the growth of any colony of microbial strains is considered to be the minimal fungicidal and bactericidal concentrations of the compound [19].

The antimicrobial activity of dehydroabietic acid (**1**), BTC **2** and microparticulate systems was evaluated against different bacterial species: *Bacillus subtilis*, *Pseudomonas fluorescens*, *Erwinia amylovora*,

Erwinia carotovora and *Xanthomonas campestris.* As well as fungi species*: Aspergillus fumigatus, Aspergillus versicolor, Aspergillus ochramensis, Aspergillus niger, Trichoderma viride, Penicillium funiculosum, Penicillium ochrochloron, Penicillium verrucosum var. cyclopium, Candida albicans, Saccharomyces cerevisiae, Plasmodium falciparum 3D7* and *Dd2 cultures*.

Results and discussion

Recently it was demonstrated that (*Z*)-4,4-dimethyl-1-(4-nitrophenyl)-2-(*1H*-1,2,4-triazol-1-yl)pent-1-en-3-one with anti-tuberculosis activity can be easily prepared from 3,3-dimethyl-1-(*1H*-1,2,4-triazol-1-yl)butan-2-one (**4**) [20]. Chromenes are widespread in natural products and attracted much attention by medicinal chemistry researchers [17,21]. From the point of view of bioactivity, the hybrid system of 1,2,4-triazol and chromenol is an interesting subject for study. In order to reduce duration of treatment, frequency and quantity of the administered doses of antibacterial agents, and to reduce the side effects, the recent discovery of a new drugs and different micro- and nanoparticle *β*-CD based systems have been proposed [10,11,17,22-26]. Recently attention has only been paid to the tandem reactions of salicylic aldehydes or salicylic imines with *α,β*-unsaturated compounds [21]. Some years ago an *α,β*-unsaturated ketone containing a 1,2,4-triazolyl and pivaloyl groups directly attached to the carbon-carbon double bond was synthesized by the interaction of **4** with a 4-nitrobenzaldehyde [20]. It was found that an applicable route to 1*H*-1,2,4-triazol functionalized chromene **2** would go through the coupling of substituted triazolylethanones with salicylic aldehyde [16].

Herein, it was proposed the preparation of *β*-CD **3** particles containing biological active compounds: DHA **1** and BTC **2** (Figure 1).

Thus, the synthesis of 1*H*-1,2,4-triazole-functionalized chromenol (BTC) **2** was carried out for the first time using tandem reactions of compound **4** with salicylic aldehyde (Scheme 1) [18].

Two different sets of conditions for the achieving of the co-precipitation were tried to obtain the co-crystal particles of the dehydroabietic acid **1** with chromenol-triazol hybrid **2**. This particular system was chosen, since the two epimers of racemic compound **5** have opposite configuration at stereogenic center (quaternary carbon atom on the hydroxyl group) and one of them may produce more stable systems with **1**.

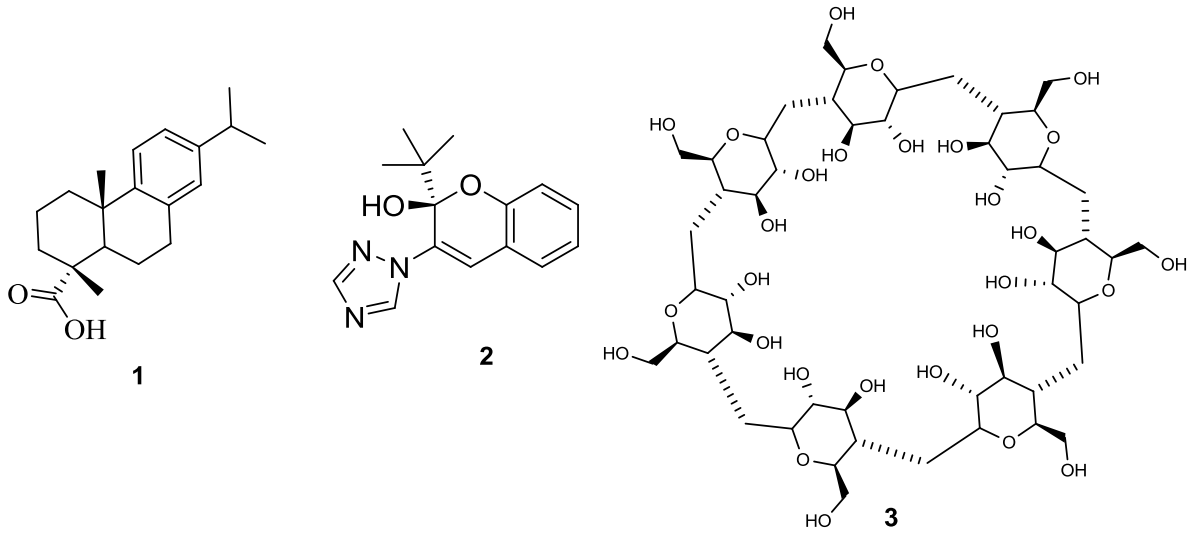
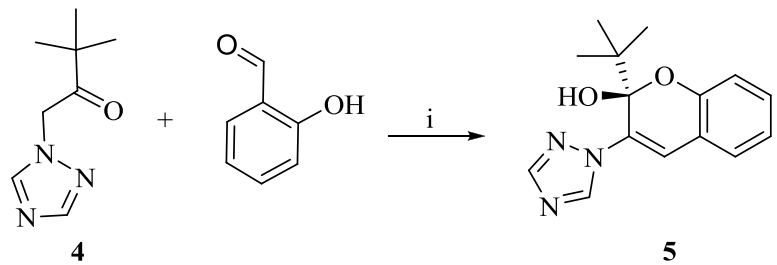


Figure 1. Chemical structures of dehydroabietic acid 1 and target chromene-triazol hybrid 2.



Reagents and conditions: (i) benzene, piperidine/AcOH (cat, reflux), 5 h, 70%.

Scheme1. Synthesis of *1H*-1,2,4-triazol functionalized chromenol.

X-ray diffraction results

The molecular structure and intermolecular interactions among ingredients of the co-crystal **6** were characterized by the single crystal X-ray diffraction method. The colourless needle-like crystals of **6** belong to the non-centrosymmetric orthorhombic space group $P2_12_12_1$, have the unit cell parameters a= 6.5059(6), b= 10.990(2), c= 44.484(7) Å, V= 3180.6(8) Å^3, and represent the co-crystals with 1:1 stoichiometry formed by natural acid **1** and hybrid **5** components, Figure 2*(a)*.

The structure of the co-crystal **6** was refined using 4893 [R(int)= 0.0681] independent reflections to $R1$ = 0.0899 and $wR2$= 0.0953 for 1750 reflections with $I > 2\sigma(I)$ and GOF= 1.006. The maximum and minimum residual electron densities in the difference synthesis were 0.150 and -0.154 e·Å^{-3}.

The interaction of dehydroabietic acid with compound **2** yielded a material that was a DHA-BTC co-crystallization product **6**. It has been found that the rate of formation of DHA-BTC systems is very sensitive to the composition of the medium. For example, more than 70% of the expected material is formed (TLC data) within 4 hours when the solvent is MeCN at 80°C. The product is then isolated by filtration.

As the stereo configuration of diterpenoid acid **1** is well established, the crystal structure unambiguously indicates the *S*-configuration of the chiral atom in BTC. In the crystal the acid **1** and chromenol-triazol hybrid **5** molecules form O1-H···N4'= 2.686(10) Å strong hydrogen bond between 1,2,4-triazole and carboxylic groups that results in supramolecular synthon was for the first time reported for co-crystals of a triazole drug with 1,4-dicarboxylic acids [27]. It should be noted however, that the above-mentioned separation was not observed with *β*-CD **3** as described for ibuprofen [12]. The crystal structure also shows that symmetry related by translation BTC molecules are interlinked in infinite chains along crystallographic axis *a* by O20'-H···N2'= 2.785(9) Å hydrogen bonds.

Dissolution studies

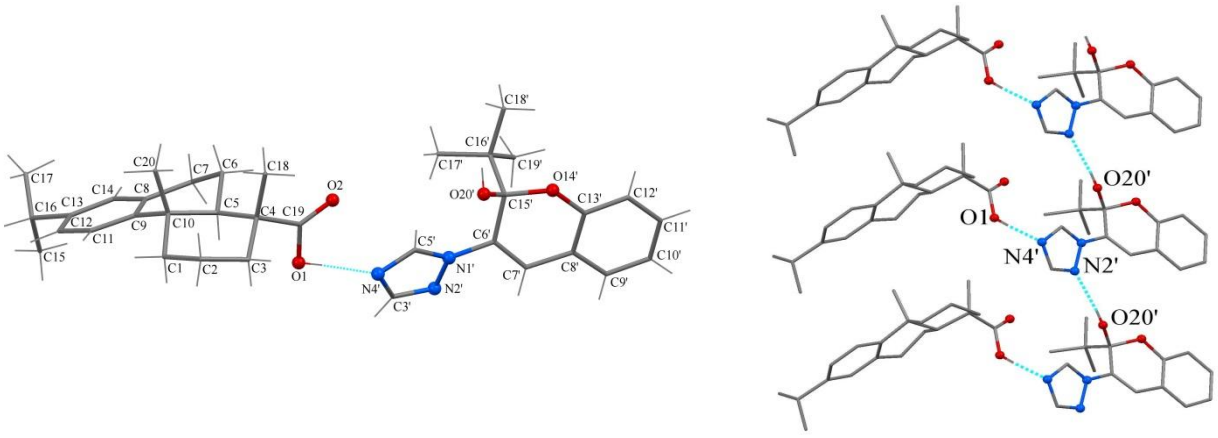
Previously it was found that ciprofloxacin interacts pharmacodynamically with antifungal agents by altering their growth inhibitory activity against *Candida. albicans* and *Aspergilus. fumigates* [28]. It has been hypothesized that the synergistic interactions may have beneficial implications for the outcome of antifungal therapy, for example system antifungal dehydroabietic acid **1** loaded with BTC **2** and *β*-cyclodextrin **3**.

In the present study, in an attempt to improve the solubility characteristics of micro- and nanoparticle diterpenoid acid **1**, BTC **2** and *β*-CD **3** based systems, were prepared their crystalline and amorphous forms with defined size and crystallinity.

The kneading method is a relatively simple one, which consists in the precise weighing of the acid **1** and hybrid **5**, mixing and shredding them in dry phase for a few minutes followed by addition of some amount of H_2O. The mixture **7** becomes a paste that has been triturated for 1.5 hours and dried to give the final product.

FTIR and NMR spectral results

The IR-spectra of the components **1**, **2** and **6** are similar to spectrum of **7** with the differences consisting in shifts of band characteristic for OH group: **1** at 1279 cm^{-1}; **2** at 1232 cm^{-1}, 3069 cm^{-1}; **6** at 1255 cm^{-1}, 3318 cm^{-1}; **7** at 1285 cm^{-1} and 3113 cm^{-1} (Figure S1).



(a) ***(b)***

Figure 2. View of the formula unit of 6 with numbering scheme illustrates the stereo configuration of 1 and 2 components and their interaction *via* O-H···N hydrogen bond (*a*). Hydrogen bonded chain in the crystal of 6 (*b*).

The structure of co-crystal **6** and system **7** was studied by NMR spectroscopy, which is a very sensitive method that allows to establish the nature of the intermolecular interaction. The presence of intermolecular interaction in the structure of co-crystal **6** was established by comparing the NMR spectra of the initial compounds **1** and **2**. The main difference was the shift of the signals of the aromatic part of the chromenol molecule, the vinyl proton, and the *tert*-butyl group to a lower field. The similarity of several aspects of the NMR spectrum of system **7** with that of co-crystal **6** led to a preliminary determination of the structure and to a tentative assignment of structure to this material (Figure S2).

The interaction of BTC **2** with *β*-CD **3**, which has not been studied previously, was found to yield system **8**. Analysis of the FTIR spectra shows that it is mainly the sum of the spectra of its components. It is worth noting that some of the absorption bands of the compounds disappear in the spectrum of the system **8**. These are, for instance, the bands at 755 cm^{-1} and 2926 cm^{-1} in the *β*-CD spectrum, which correspond to the vibrations (*δ*CCO + *δ*CCH) and ν_{as}CH, respectively, and the bands at 750 cm^{-1}, 1459 cm^{-1} (C=N, triazole ring) and 1509 cm^{-1} (benzene ring) in the 2*H*-chromenole spectrum. Also, one can observe a shift of characteristic bands at 755 cm^{-1} (*β*-CD) and 750 cm^{-1} (2*H*-chromenole) to 752 cm^{-1} and 758 cm^{-1}, decrease in the intensity of absorption bands of 2*H*-chromenole at 1459 cm^{-1} (C=N, triazole ring) and 1509 cm^{-1} (benzene ring). All these observations have prompted us to conclude that the 2*H*-chromenole molecules are incorporated into the *β*-cyclodextrin structure and that there are interaction forces between the compounds, as a result of the formation of the inclusion complex.

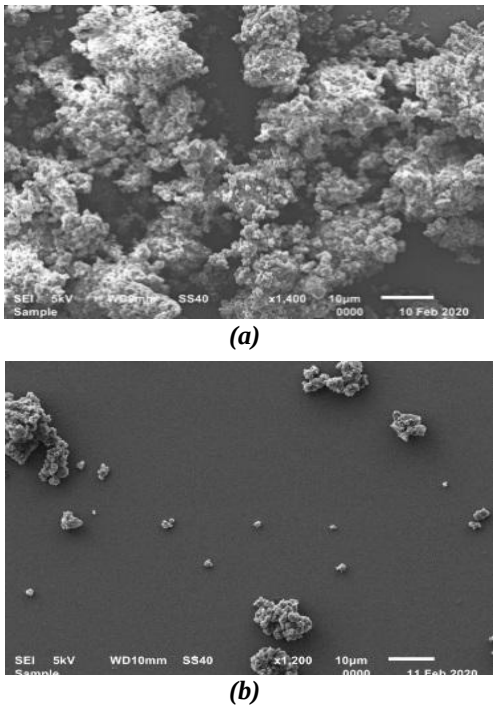
A ternary dehydroabietic acid **1**: chromenol-triazol hybrid **6**: *β*-CD **3** system by using two techniques was obtained. The co-evaporation method (the first one) involved the use of 50% aqueous MeOH solution of *β*-CD mixed with dehydroabietic acid **1**: chromenol-triazol hybrid **6** in MeOH, stirring for 24 hours followed by evaporation under vacuum and drying at heating (up to 60ºC) for 3 hours. Analysis of IR spectra of system **9** showed that the characteristic absorption bands of chromenol at 1459 cm^{-1} and 1509 cm^{-1} and dehydroabietic acid at 1686 cm^{-1} and 1279 cm^{-1} (C-C stretching vibrations) disappeared and the absorption band of the cyclodextrin shifted from 3289 cm^{-1} to 3294 cm^{-1}. The kneading method (the second one), as already mentioned, consisted in precise weighing the "host" and the "guest" ingredients followed by additional mixing with an amount of water. The resulting system has been dried and then crushed. In the second method, the same pattern is observed, and the absorption band at 3674 cm^{-1} indicates the presence of water molecules in system **10** (Figure S3).

The ^{1}H NMR spectra of the systems showed interaction between aromatic protons and the internal CD protons, when the isopropyl-1,2,3,4-tetrahydronaphthalene fragment enters into the CD cavities, as well as when the chromene ring is inside the CD cavities. On the other hand, in the case of triazole ring protons, their chemical shifts cannot be confidently attributed to the formation of the inclusion complex (Figure S4).

In vitro dissolution studies of the synthesized compounds compared to the pure in water phosphate buffer (pH= 6.8) showed an improved dissolution rate of chromenol-triazol hybrid in the inclusion complexes compared to the free form in the following order: DHA **1**: BTC **2** : *β*-CD **3**> DHA **1**: BTC **2**: *β*-CD **3**: H_2O> BTC **2**: *β*-CD **3**> co-cristal **6**> DHA **1**: BTC **2**.

SEM results

The scanning electron microscopy (SEM) image Figure 4*(a)* showed that the diameter of particles from system **7** was approximately 50–100 μm and such data implicated with optimal uniformity of the prepared system.



(a)

(b)

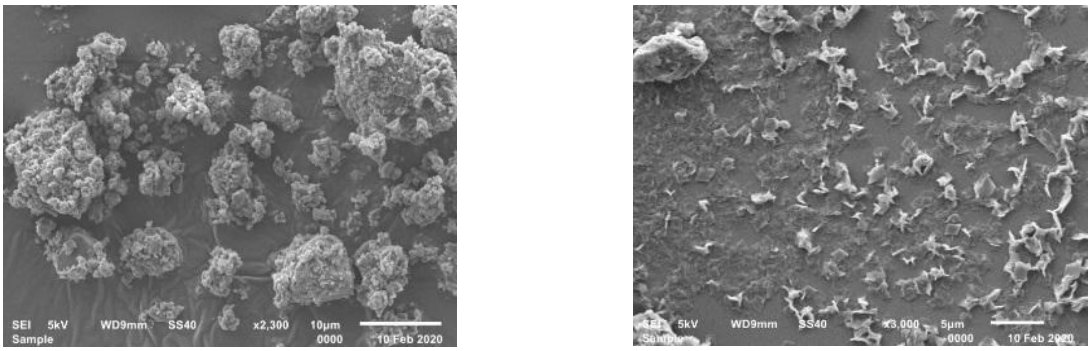
Figure 4. SEM micrographs for dry *(a)* and wet *(b)* particles of system 7.

The solubility of system **7** in water increased compared to the initial compounds - DHA (**1**) and BTC **2**. The morphology of the system after dissolution studies is characterized by the formation of a solid clot with main size range of the obtained particles falling in the limits of approximately 0.1–50 μm Figure 4*(b)*.

The system **8** consists of a mixture of irregularly shaped micro- and nanoparticles with predominance of the first. Wet particles of the system **8** are characterized by the formation of a firm gel and solid plates. The main size ranges of the obtained needle-like particles are approximately 0.1–250 μm. *β*-CD is a solubilizer and increases the solubility of the BTC 2.

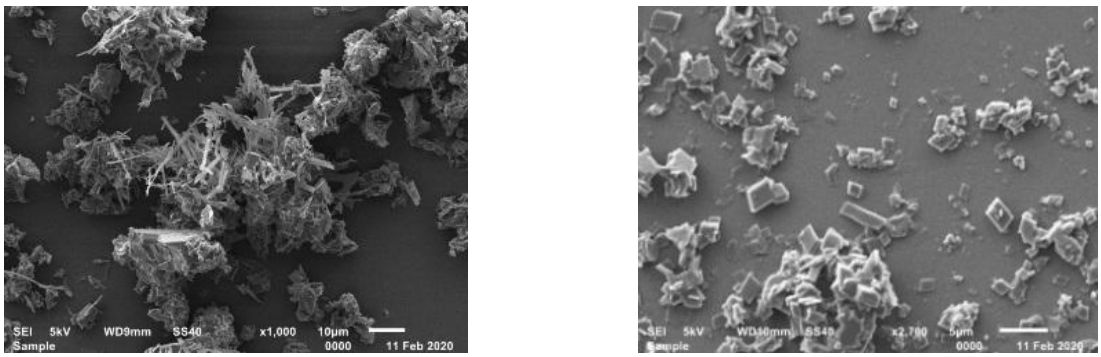
The particles surface of system **9** is smooth, in comparison with those of systems **7-8**, which have many rough edges. SEM analysis shows that dehydroabietic acid **1**, BTC **2**, and *β*-CD **3** are in close contact with each other and sufficiently dispersed, indicating that system **9** was successfully obtained, Figure 5. The visualized particle sizes showed that the wet particles of system **9** had homogenous morphology and did not produce aggregated structures, Figure 6*(b)*.

SEM investigation showed that the average sizes of the dehydroabietic acid **1**: BTC **2**: *β*-CD **3** particles were from 0.4 μm up to 3.9 μm, respectively. In case of the system dehydroabietic acid **1**: BTC **2** coated with *β*-CD and H_2O the formation of a firm gel has not been observed. Wet particles of system **10** are characterized by the formation of solids in the sizes limits of approximately 0.1–250 μm, Figure 7(*b)*.



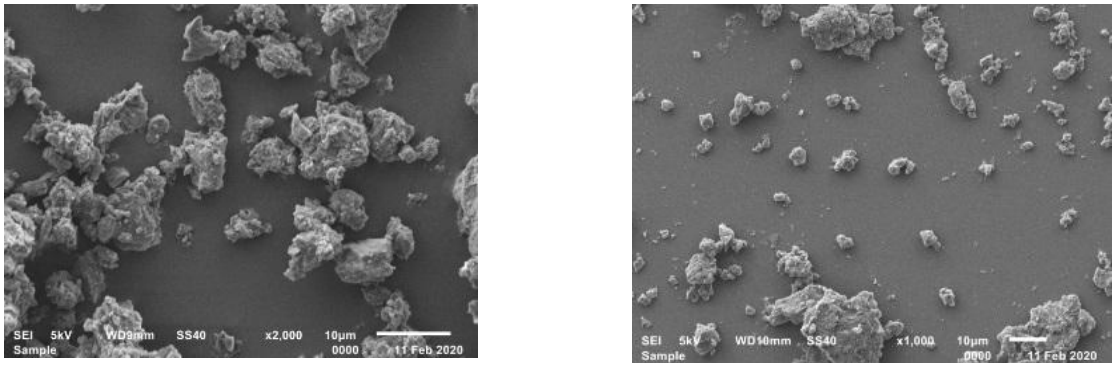
(a) ***(b)***

Figure 5. SEM micrographs for dry *(a)* and wet *(b)* particles of system 8.



(a) ***(b)***

Figure 6. SEM micrographs for dry *(a)* and wet *(b)* particles of system 9.



(a) ***(b)***

Figure 7. SEM micrographs for dry *(a)* and wet *(b)* particles of system 10.

The surface of the particles of systems **10** is almost similar to that of the particles of dry systems **8-9**, which have many rough edges, probably due to the presence of *β*-cyclodextrin, which adheres to the surface of the matrix.

Antimicrobial activity

All tested compounds showed good antibacterial activity with MIC and MBC values ranging from 0.72 to 44.45 mM. Systems **9** and **10** appeared to be the most active among all tested compounds. In contrast, low efficiency of the BTC **2** against the *Plasmodium falciparum* remained essentially unchanged in both *3D7* and *Dd2* cultures over the entire duration of the experiment, although fluctuating within 10% variation has been noted. The evaluation results revealed that all compounds showed good antifungal activity against *A. fumigatus, T. viride, C. albicans, S. cerevisiae, A. versicolor, A. ochraceus*, with MIC values ranging from 0.02 mM to 0.4 mM and MFC from 0.07 mM to 0.52 mM better than reference drugs ketoconazole (MIC and MFC values at 0.28–1.88 mM and 0.38 mM to 2.82 mM respectively), bifonazole (MIC and MFC values at 0.32-0.64 mM and 0.64-0.81 mM) and nistatin (MIC and MFC values at 0.55-0.65 mM and 0.65–0.79 mM) (Table S1).

The order of compounds can be presented as follows: **6**>**7**>**10**>**8**>**9**>**2**>**1**. The best antifungal activity as in case of antibacterial is displayed by systems **9**, **10** with MIC at 0.07 mM and MFC at 0.08–0.09 mM, while compound **1** showed the lowest activity. Thus, the antifungal potency of compounds against *Candida albicans* can be presented as follows: **6**>**7**>**9**>**10**>**8**>**2**>**1**, while against *Aspergillus fum* and the *Trichoderma viride.* : **9**>**6**>**10**>**8**>**2**>**1**. The most sensitive fungal appeared to be *Candida albicans*, while *Trichoderma viride* was the most resistant one. The results reveal that all prepared systems display a higher antifungal activity as compared to nystatin, which is used to treat *Candida* infections of the skin including oesophageal candidiasis, thrush, vaginal vast infections and diaper rash (Table S2).

Some differences in antifungal activity according to the method of synthesis could be noticed. Thus, the antifungal activity decreases in the following order of the preparation methods: co-evaporation, co-crystallisation, kneading.

This may be attributed to the differences in the enhanced solubility of the prepared systems, which should lead to different level of disruption of the fungal and bacteria cell membrane by extraction of the sterols and lipids, interaction of the positively charged cyclodextrins with negatively charged lipids or anionic phospholipids, or reduction of total blood cholesterol content by cyclodextrins. These effects have been well described in different examples [8,22]. The morphology of the obtained particles varies from spherical to irregularly-shaped with size range from 0.001 to 0.25 mM. The FTIR as well as NMR spectra analysis has revealed molecular interactions between active compounds and *β*-cyclodextrin-polymeric matter of the particles.

Conclusions

Complexes of *β*-cyclodextrin and dehydroabietic acid with chromenol-triazol hybrid were synthesized. In summary, kneading, co-evaporation and co-precipitation as example of the resolution technology of racemic 2-*tert*-butyl-3-(1*H*-1,2,4-triazol-1-yl)-2*H*-chromen-2-ol for obtaining micro- and nanoparticles have been optimized.

The morphology of the obtained particles varied from spherical to irregular shape with sizes from 0.001 to 0.25 mM. The particle diameter of system **7** (DHA-BTC) decreased from 50–100 μm to 0.1–50 μm. Based on the increase in solubility of systems **8-10**, molecular interactions between active compounds: dehydroabietic acid, chromenol-triazole hybrid, and *β*-cyclodextrin according to FTIR and NMR spectra indicate complex formation.

The obtained *β*-cyclodextrin: dehydroabietic acid : chromenol-triazole hybrid systems showed good activity against *Aspergillus fumigatus, Trichoderma viride, Penicillium funiculosum, Penicillium ochrochloron, Penicillium verrucosum var. Cyclopium, Candida albicans* and *Saccharomyces cerevisiae.* The antibacterial activity of the obtained systems was higher compared to the initial components: 5.75-fold for dehydroabietic acid and 2-fold for the chromenol-triazole hybrid. The complexes from *β*-cyclodextrin and dehydroabietic acid with chromenol-triazol hybrid were 2 and 1.5 times more active against the genus *Pseudomonas fluorescens* compared to the reference preparations ampicillin and chloramphenicol, respectively. It was shown that the solubility of the obtained complexes increased in comparison with the solubility of the reference compounds.

The use of cyclodextrins to obtain inclusion complexes of biologically active substances

seems to be a promising direction, which leads to physicochemical properties and bioavailability of biologically active compounds.

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Supplementary information

Supplementary data are available free of charge at http://cjm.asm.md as PDF file.

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