Gene and protein sequences:

Isopentenyl pyrophosphate isomerase (idi) from *Haematococcus lacustris* (GenBank: AAC32208.1)

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Geranylgeranyl pyrophosphate synthetase (crtE) from *Pantoea ananatis* (GenBank: ADD79325.1)

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RSLNFSRLADTFLKDDDGYTNPKSKVKDLIASLFVESVDI* (549 AS)

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Ribosomal binding site (RBS) sequences

(RBS-A): AGGAGGTTTGGA

(RBS-B): AACAAAATGAGGAGGTACTGAG

(RBS-C): AAGTTAAGAGGCAAGA

(RBS-D): TTCGCAGGGGGAAG

(RBS-E): TAAGCAGGACCGGCGGCG

Structure analysis and quantification

NMR spectra

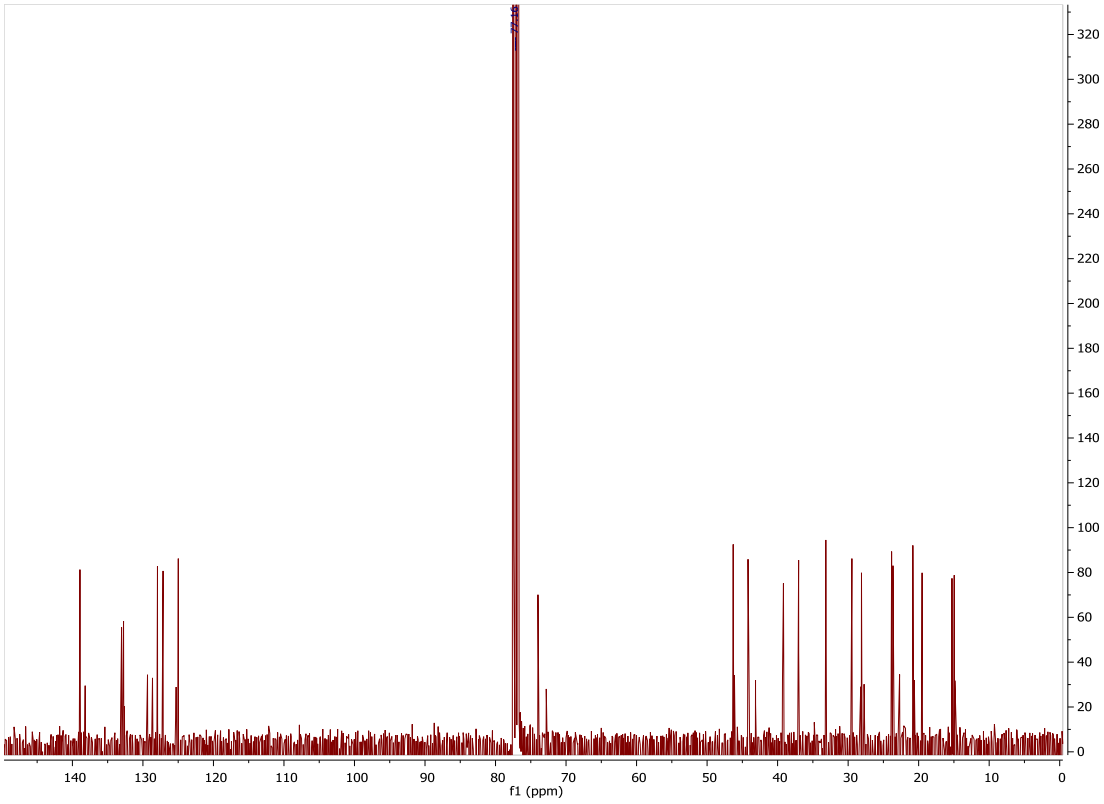

Figure 1: *CBT-ol racemat ^{13}C NMR data.*

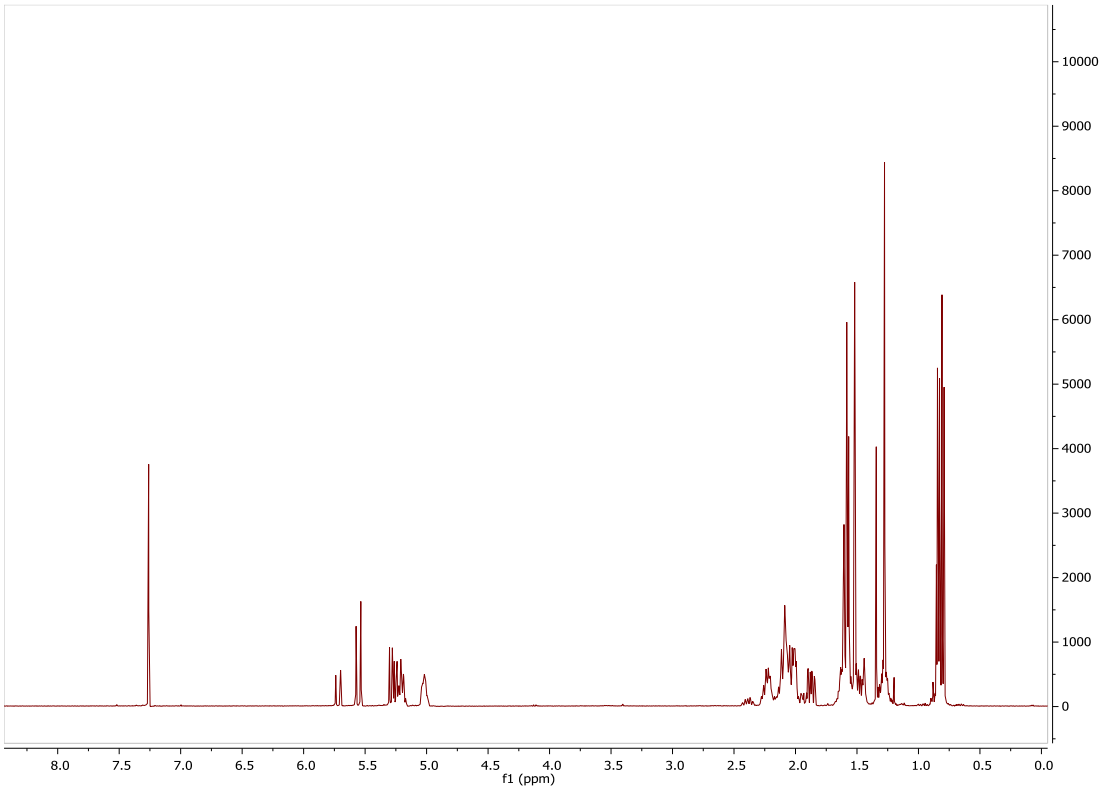

***Figure 2**: CBT-ol racemat ^{1}H NMR data.*

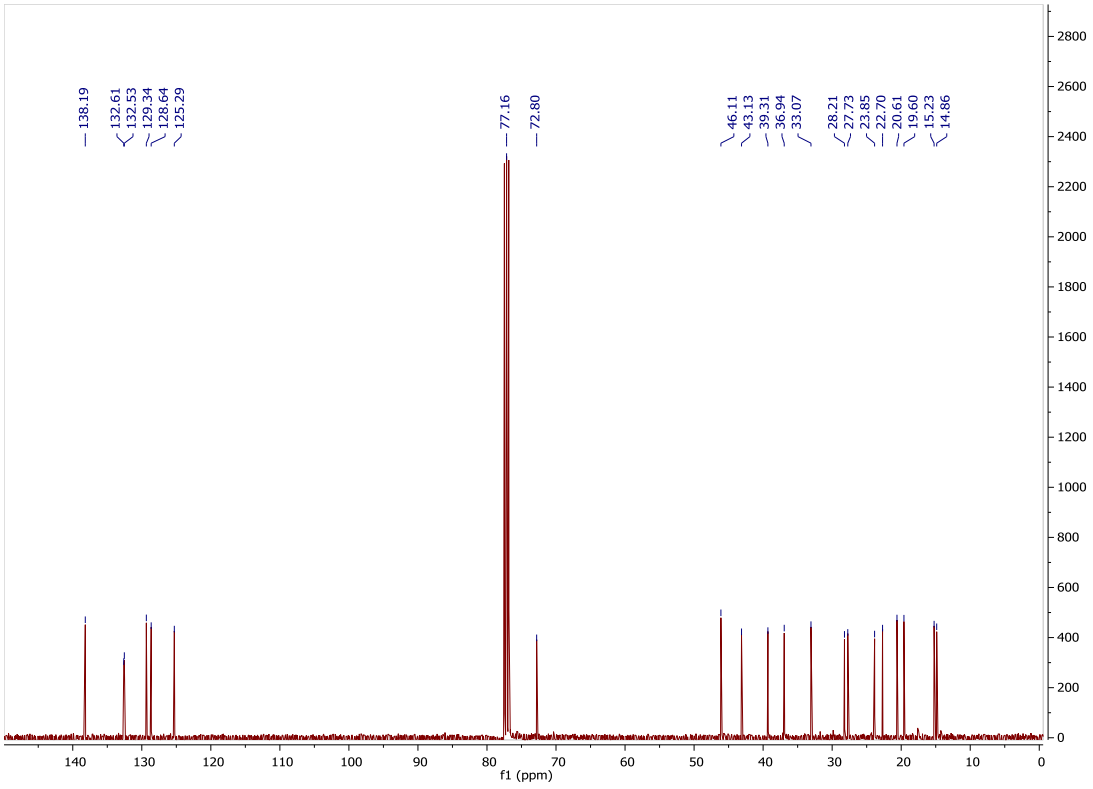

***Figure 3:** pure α-CBT-ol ^{13}C NMR data.*

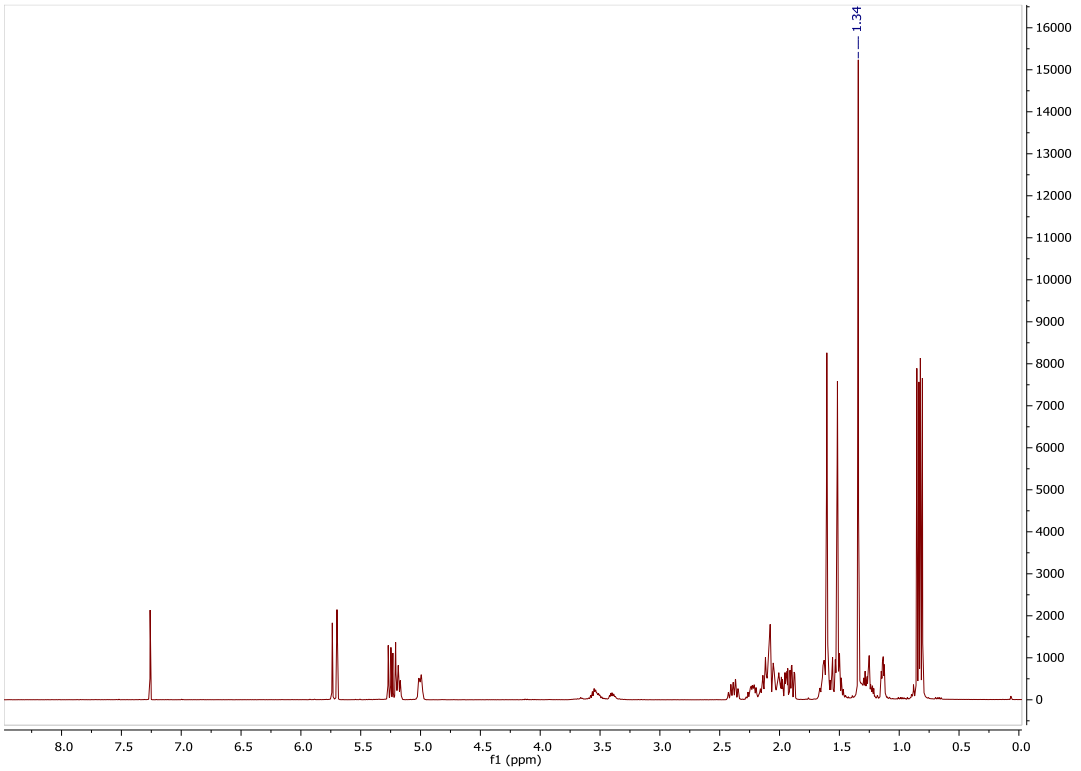

Figure 4: *pure α-CBT-ol ^{1}H NMR data*

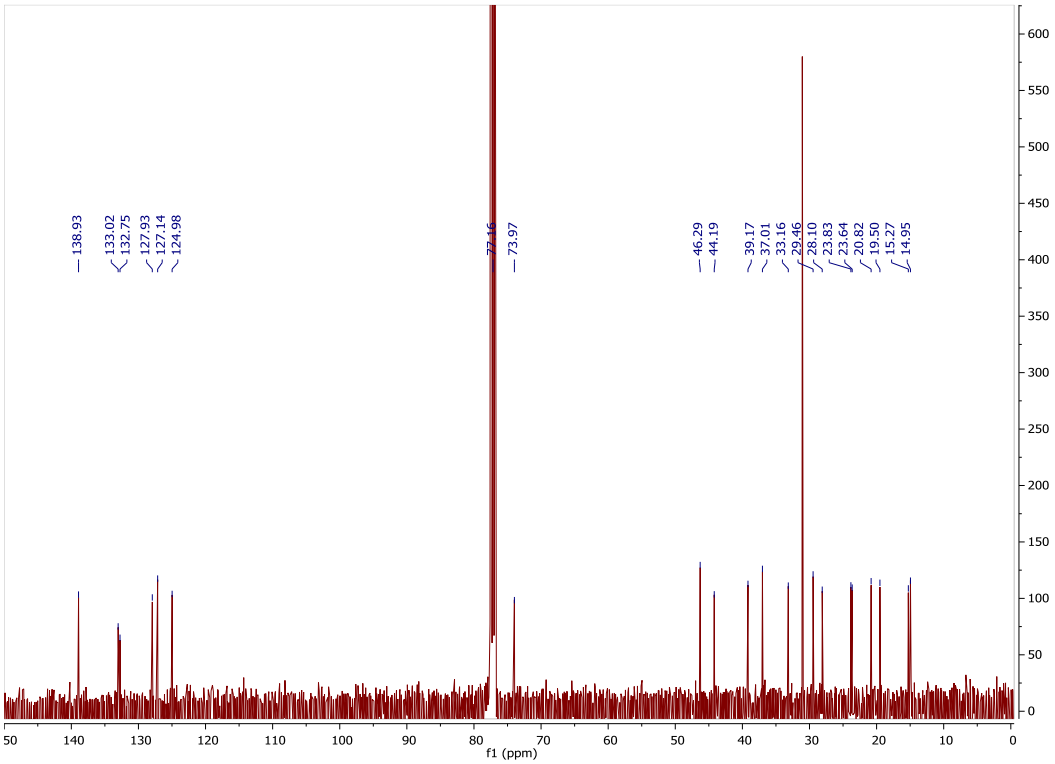

Figure 5: *pure β-CBT-ol ^{13}C NMR data.*

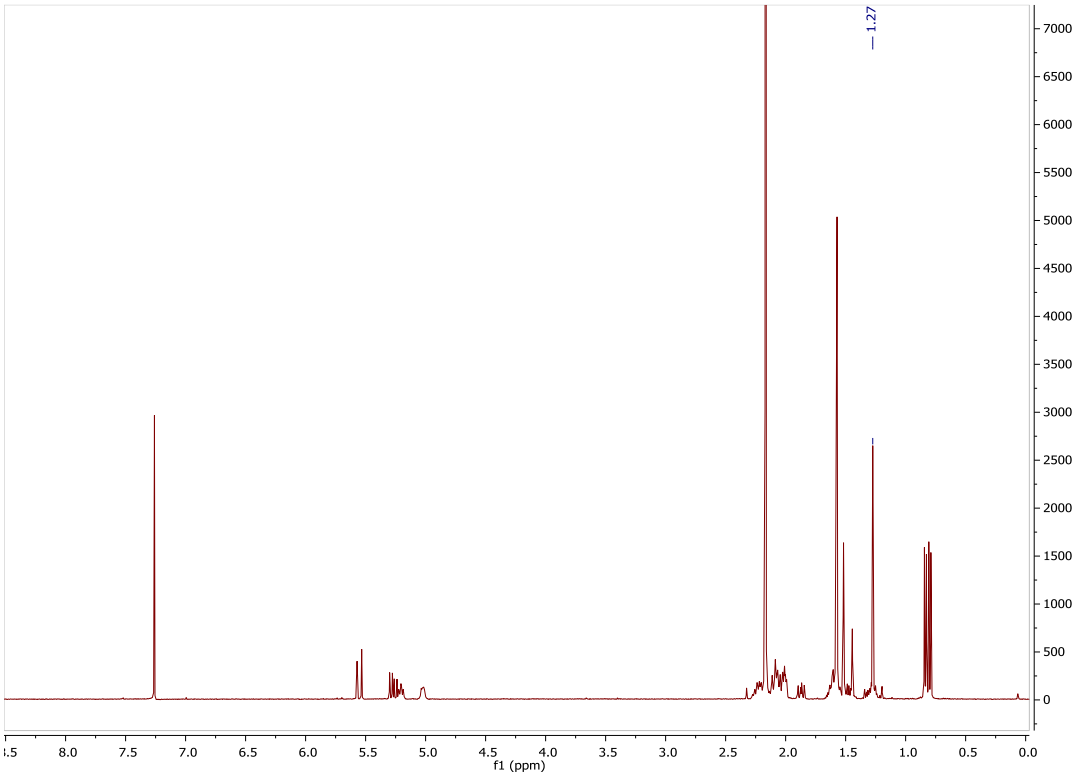

Figure 6: *pure β-CBT-ol ^{1}H NMR data.*

CBT-ol quantification

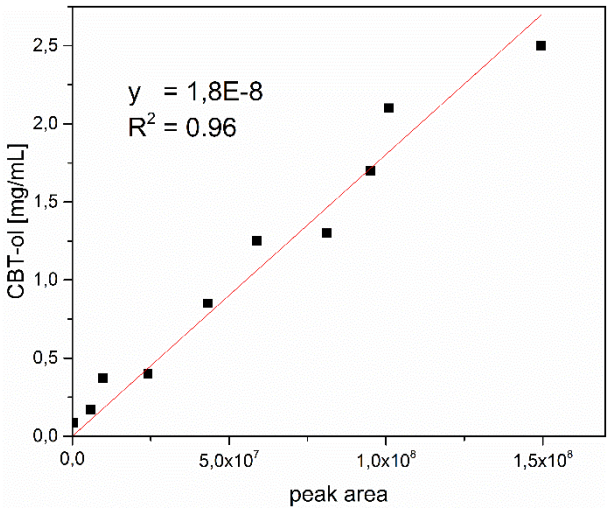

Figure 7: *calibration curve for GC-FID based CBT-ol quantification. Different concentrations of CBT-ol were used for peak-area evaluation via GC-FID.*

CBT-ol microbial toxicity

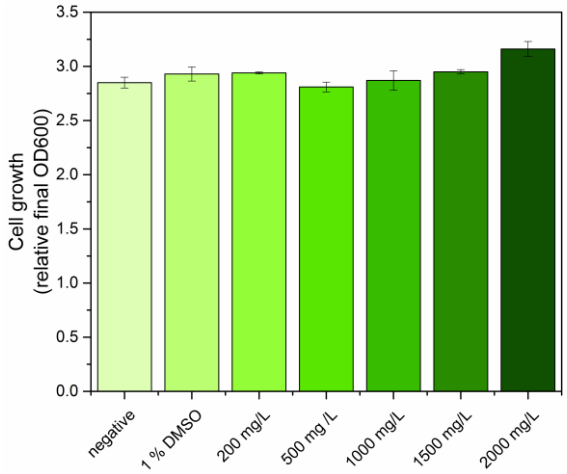

Figure 8: *toxicity of CBT-ol to E. coli. An overnight culture of E. coli HMS174 (DE3) transformed with pSB4K5-CBT was used for inoculation of 2 mL cultures containing up to 2 g/L CBT-ol at an initial OD_{600} of 0.05. The CBT-ol was therefore solved in DMSO. Final OD_{600} was measured after cultures were grown at 37 °C for 18 h. The experiments were carried out in triplicates. Shown is the mean final OD_{600} and the error bars represent the standard deviation from the mean. The solubility limit of CBT-ol is reached at around 200 µg/L. Higher concentrations result in a precipitation and a turbid solution. However, even high concentrations do not seem to affect bacterial growth.*

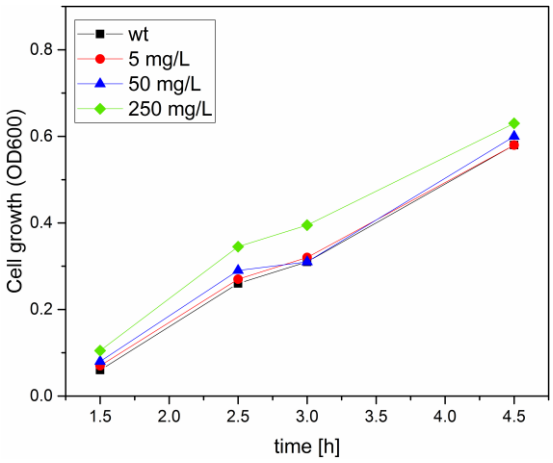

Figure 9: *influence of CBT-ol on early E. coli growth. An overnight culture of E. coli HMS174 (DE3) was used for inoculation of 2 mL cultures containing CBT-ol. Cultures were grown at 37 °C for 4.5 h.*

Bioassay protocol:

CBTol

50 mg of CBT-ol were dissolved in 1 ml of sterile DMSO (purity 99.5 %, p.a.). This stock solution was stored at -20 °C.

Cell Culture

In this study we used human cervix carcinoma cell line HeLa (ACC 57), human breast adenocarcinoma cell line MCF-7 (ACC 115) and the insect cell line *Spodoptera frugiperda* (Sf-21) from the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig Germany. Human cells were cultured in 25 cm^2 tissue culture flaks in RPMI 1640 medium supplemented with 10 % (v/v) fetal bovine serum (FBS) and 4 mM L-Glutamine at pH 7.4, 37 °C and 5 % CO_2. For passaging the spent media was removed and the cells were detached with 2 ml accutase solution and centrifuged at 180 *g* for 8 min. Cells were diluted with fresh media for a final volume of 7.5 ml with a cell concentration of 30,000 cells/cm^2. Insect cells were cultivated in ExCell 420 using shaker flaks at 27 °C and 50 rpm. For passaging the cell density was adjusted to $7*10^5$ cells/ml in 12ml of fresh medium. Cultures were used up to 3 months.

MTT-Assay

Human cells were seeded with 30,000 cells/cm^2, insect cells were seed with 80,000 cells/cm^2 in 96-well plates with 100 µl per well and cells were allowed to adhere for 24 h. The

supernatant was then replaced with fresh medium containing the appropriate amount of CBTol. Exponentially growing cells were then incubated for 24 and 48 h. As a vehicle control cells were exposed to 0.5 % (v/v) dimethylsulfoxide (DMSO). All experiments were performed in hexaplicates. For the evaluation of metabolic activity the MTT assay originally described by Mosmann 1983 was adapted to our purposes. This test is widely adopted und used in scientific research due to its applicability in high troughput screening (Riss *et al.* 2004). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) was purchased from Sigma-Aldrich (98 % purity). A stock solution (5 mg/ml) was prepared in 0.9 % (w/V) NaCl and stored at -20 °C in the dark. The supernatant of each well was replaced by 100 µl fresh RPMI 1640 culture medium containing 250 µl MTT stock solution. Cells were incubated for 4 h. Afterwards the plates were centrifuged at 3220 g followed by the careful removal of medium and MTT solution. Cell lysis was done using 20 µl 0.4 % (v/v) Igepal solution. Formazan crystals were dissolved by adding 180 µl DMSO and the absorbance measured at 570 nm in a Microplate Reader (EnSpire 2300, Perkin Elmer). As a blank a media control was carried and the mean was substracted from measured samples. For the determination of the dose-response curves, samples were normalized to the vehicle control (which was set to 100 %).

Microbial Cultivation

To evaluate antimicrobial potential of CBTol the following strains were used: *Escherichia coli* (DSM 498), *Bacillus subtilis* (DSM 347), *Micrococcus luteus* (DSM 1605), *Zygosaccharomyces rouxii* (DSM 7525), *Schizosaccharomyces pombe* (972h) and *Candida glabrata* (DSM 11226). All strains except for *S. pombe* were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig Germany.

E. coli, *B. subtilis* and *M. luteus* were cultured in Standard I medium containing 1 g/L Glucose, 3 g/L Yeast extract, 15 g/L Pepton from casein and 5.85 g/L sodium chloride. The pH was adjusted to 7.4. *Z. rouxii*, *S. pombe* and *C. glabrata* were cultured in YEPD medium containing 20 g/L Glucose, 3 g/L yeast extract and 20 g/L Pepton from casein. The pH was adjusted to 6.3. Media were made by dissolving the Glucose in 200 mL and the remaining substances in 800 ml of deionized water. They were then sterilized in separate bottles by autoclavation at 121 °C for 20 minutes to minimize maillard products. After cooling both parts were mixed under sterile conditions.

Yeast and bacteria were cultured in shaker flasks at 30 °C and 100 rpm. For the evaluation of the antimicrobial potential of CBTol cultures were adjusted to an optical density of 0.1 from an exponentially growing culture to avoid lag-phases. The CBTol stock solution was diluted with DMSO and added to the cultures for a final concentration of 0.5 % (v/v) of DMSO, which did not affect microbial growth and was used as a vehicle control. Cultures with appropriate amount of CBTol were then transferred to a 96 wellplate. The plate was then cultivated and analysed for at least 12 hours by an Microplate Reader (EnSpire 2300, Perkin Elmer) using the following protocol. Temperature was set to 30 °C and the plate was shaken biorbitally at 250 rpm every 5 minutes for 60 seconds. The absorption at 650 nm was read every 30 minutes. To determine the inhibition of microbial growth, the growth rate during exponential growth phase was determined and normalised to the vehicle control (which was set to 100 %). In addition the maximum optical density ($OD_{650,max}$) at the end of the cultivation was compared to the vehicle control.

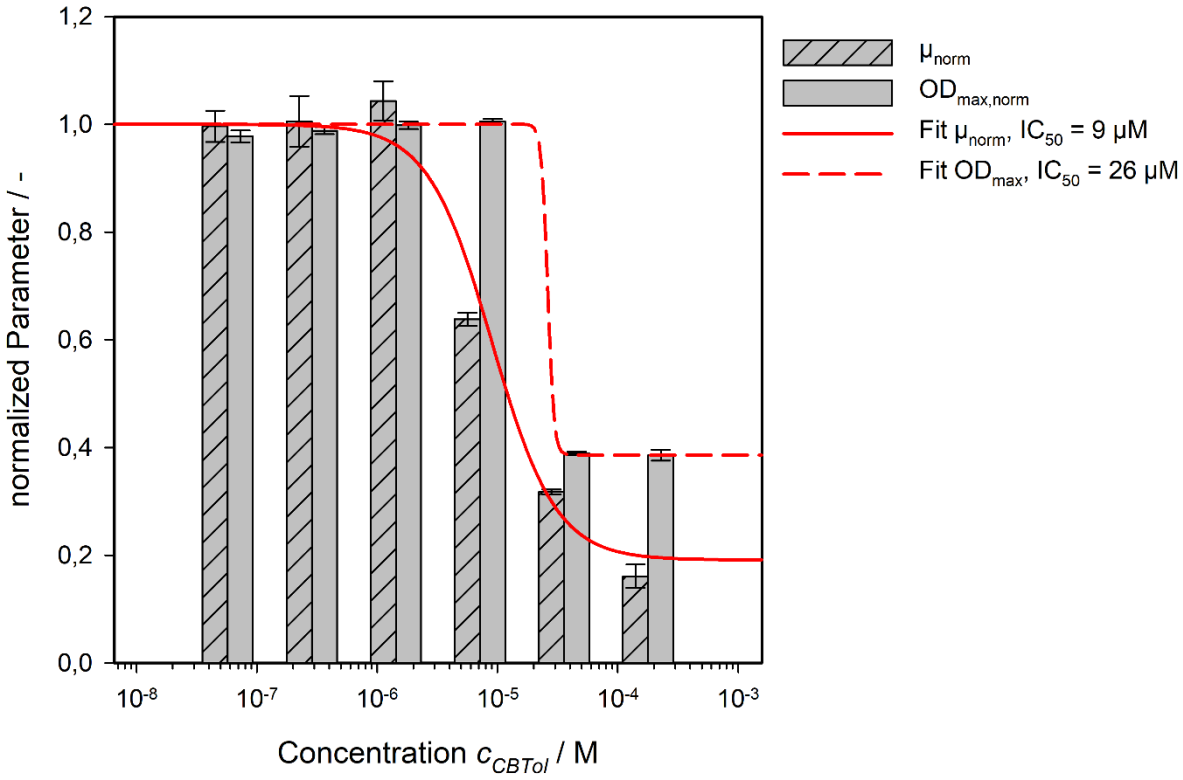

Figure 10: *B. subtilis response to CBT-ol treatment.*

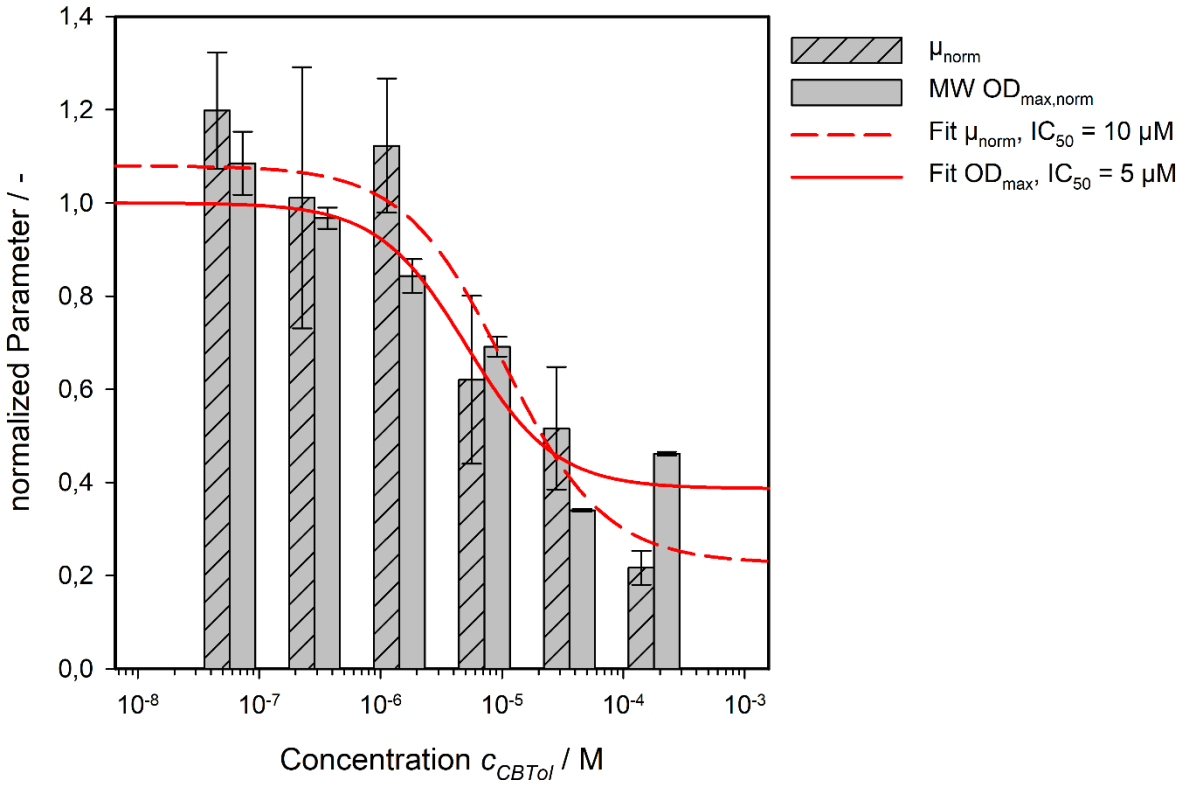

Figure 11: *M. luteus response to CBT-ol treatment.*

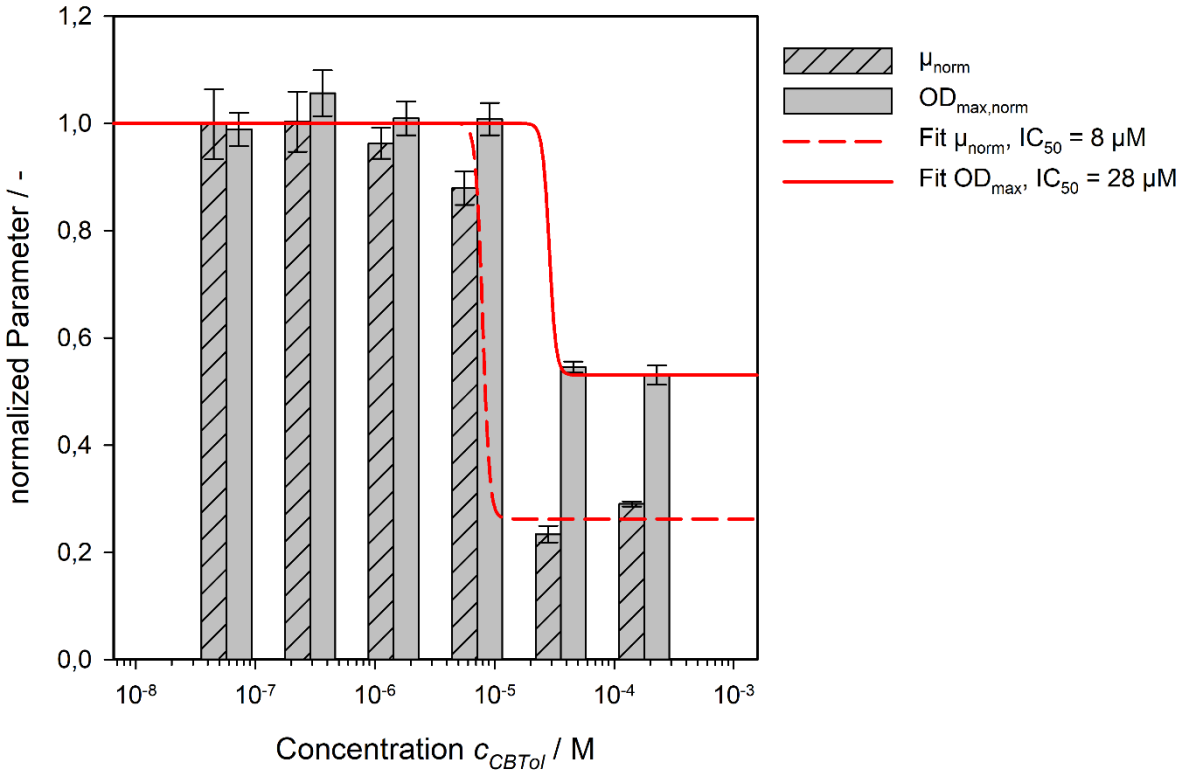

Figure 12: *S. pombe response to CBT-ol treatment.*

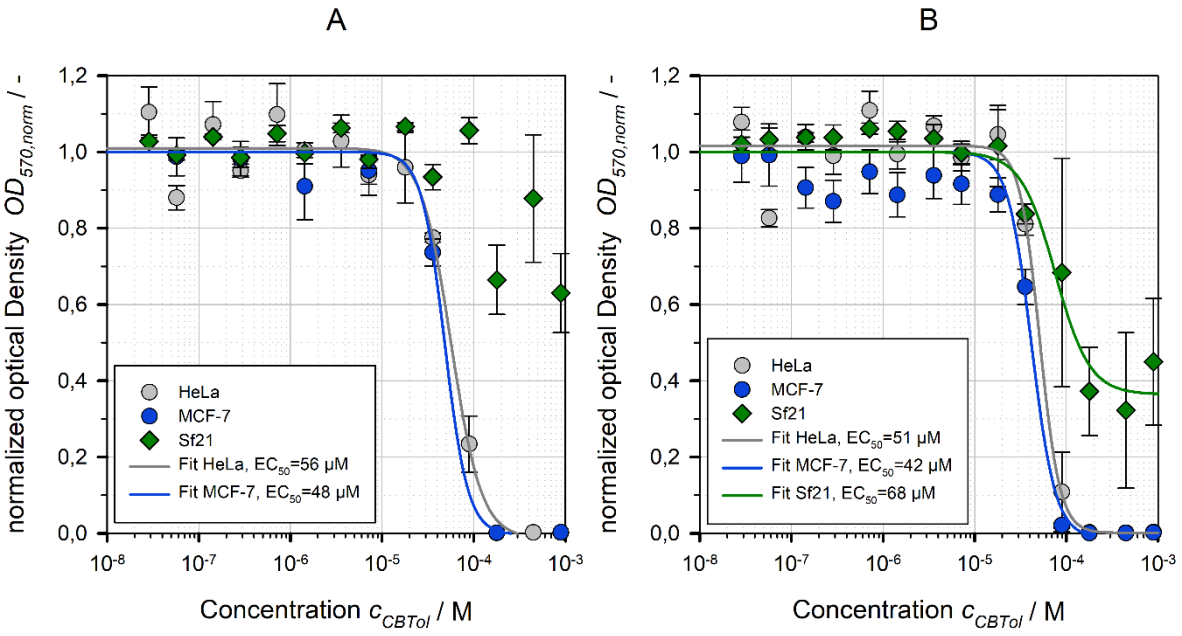

Figure 13: *HeLa, MCF-7 and Sf21 cell response to CBT-ol treatment after 24 h (A) and 48 h (B).*