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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Polyhydroxyalkanoate-based 3-hydroxyoctanoic acid and its derivatives as a platform of bioactive compounds

Jelena Radivojevic[1,2] · Sanja Skaro[2] · Lidija Senerovic[1] · Branka Vasiljevic[1] · Maciej Guzik[3,5] · Shane T. Kenny[3] · Veselin Maslak[2] · Jasmina Nikodinovic-Runic[2] · Kevin E. O'Connor[3,4]

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Abstract A library of 18 different compounds was synthesized starting from (*R*)-3-hydroxyoctanoic acid which is derived from the bacterial polymer polyhydroxyalkanoate (PHA). Ten derivatives, including halo and unsaturated methyl and benzyl esters, were synthesized and characterized for the first time. Given that (*R*)-3-hydroxyalkanoic acids are known to have biological activity, the new compounds were evaluated for antimicrobial activity and in vitro antiproliferative effect with mammalian cell lines. The presence of the carboxylic group was essential for the antimicrobial activity, with minimal inhibitory concentrations against a panel of bacteria (Gram-positive and Gram-negative) and fungi (*Candida albicans* and *Microsporum gypseum*) in the range 2.8–7.0 mM and 0.1–6.3 mM, respectively. 3-Halogenated octanoic acids exhibited the ability to inhibit *C. albicans* hyphae formation. In addition, (*R*)-3-hydroxyoctanoic and (E)-oct-2-enoic acids inhibited quorum sensing-regulated pyocyanin production in the opportunistic pathogen *Pseudomonas aeruginosa* PAO1. Generally, derivatives did not inhibit mammalian cell proliferation even at 3-mM concentrations, while only (E)-oct-2-enoic and 3-oxooctanoic acid had IC_{50} values of 1.7 and 1.6 mM with the human lung fibroblast cell line.

Keywords Polyhydroxyalkanoate · (R)-3-Hydroxyalkanoic acids · Antimicrobial activity · Chemical derivatization

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✉ Jasmina Nikodinovic-Runic
jasmina.nikodinovic@imgge.bg.ac.rs

✉ Kevin E. O'Connor
Kevin.oconnor@ucd.ie

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, P.O. Box 23, Belgrade 11010, Serbia

[2] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade, Serbia

[3] Bioplastech, NovaUCD, Belfield Innovation Park, Belfield, Dublin 4, Ireland

[4] University College Dublin, Belfield, Dublin 4, Ireland

[5] Present address: Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239 Cracow, Poland

Introduction

Bio-based production of chemical building blocks from renewable resources is an attractive alternative to using finite petroleum-based resources. Polyhydroxyalkanoates (PHAs) are a family of natural polyesters, usually produced by prokaryotes as cytoplasmatic water-insoluble storage compounds of carbon and energy (Fig. 1a). They are biodegradable polymers consisting of usually chiral hydroxyalkanoic acids (Fig. 1b) (Chen 2009; Steinbüchel and Valentin 1995). PHAs can be made from renewable biological resources such as glucose, glycerol, and fatty acids (Follonier et al. 2014; Nikodinovic-Runic et al. 2013). PHAs have a number of uses ranging from compostable packaging materials or as materials for medical applications, such as drug delivery (Chen 2011; Chen and Wu 2005).

PHAs are classified into two major subdivisions by the carbon chain length of their monomeric units (Fig. 1b), i.e., short-chain-length-3-hydroxyalkanoates (scl-3HA) have 3–5 carbon atoms and medium-chain-length-3-hydroxyalkanoates (mcl-3HA) with 6–16 carbon atoms (Chen 2009). PHAs are optically active polymers containing

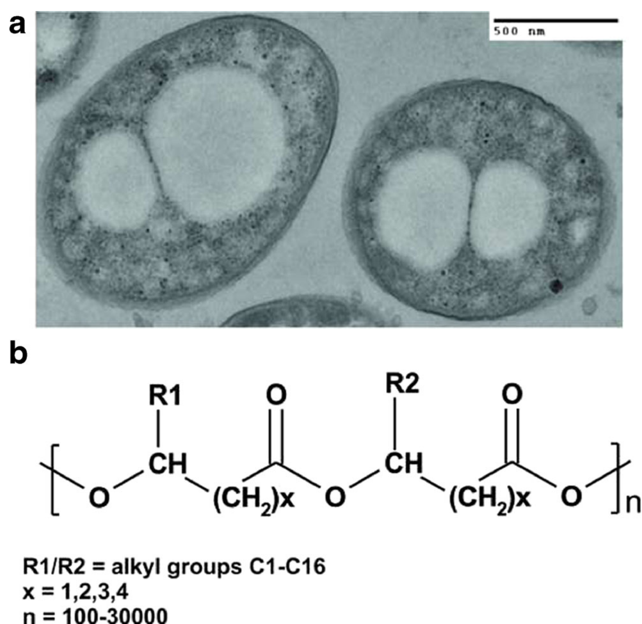

Fig. 1 Polyhydroxyalkanoates (PHA). **a** As intracellular bacterial carbon and energy storage material of *Pseudomonas putida*. **b** General structural formula

mostly the (*R*)-enantiomer of 3-hydroxyalkanoates. Various (*R*)-3-hydroxyalkanoic acids have been reported to constitute PHA polymers, with diverse functional side groups such as unsaturated bonds, ester, cyano, amino, halogen, and phenyl, which greatly expand their potential applications (Steinbüchel and Valentin 1995, Grage et al. 2009). Enantiomerically pure (*R*)-3-hydroxyalkanoic acids (3*R*-HAs) can be conveniently prepared by depolymerizing the biosynthesized PHA either enzymatically (Lee et al. 1999, Ren et al. 2005; Ren et al. 2007) or chemically (de Roo et al. 2002). After chemical degradation by acid methanolysis, the obtained (*R*)-3-hydroxyalkanoyl methyl esters are saponified to yield the corresponding (*R*)-3-hydroxyalkanoic acids (Ruth et al. 2007). Therefore, PHAs can be considered as a source of chiral compounds that have great potential as valuable synthons for the chemical and pharmaceutical industry (Ren et al. 2010; Tortajada et al. 2013).

There are several methods of chemical synthesis of hydroxylated alkanoic acids (*R*-HAs). General drawbacks of these reactions include limited amounts and scalability; the requirement of often expensive, chiral metal-complex catalysts; the contamination of the end product with catalysts; and the high price of pure substrates (Ren et al. 2010). They can be chemically synthesized by enantioselective reduction of the corresponding 3-keto acids (Noyori et al. 2004). For certain products, this process requires the synthesis of precursor molecules at an elevated pressure of 100 atm and in the presence of chiral catalysts, which makes the synthetic procedure difficult (Case-Green et al. 2008; Wang et al. 1999). Other synthetic approaches include stereoselective functionalization through Sharpless' asymmetric epoxidation and hydroxylation or through Brown's asymmetric allylboration (Brown and Ramachandran 1991). Products often have lower enantiomeric excesses than those obtained by biosynthetic processes (Lee et al. 1999).

It has been anticipated that chiral *R*-HAs are attractive compounds with high application potential (antibacterial, antiviral, synthons for organic synthesis, biofuels) (Ren et al. 2010). *R*-HAs have a similar structure to fatty acids which have been known as antimicrobial agents for more than 80 years (Desbois and Smith 2010). However, only few of these compounds are commercially available; thus, they have not yet been assessed for synthetic chemical modification and biological activity. Biotechnologically produced PHA can be tailored for their monomer composition by controlling the substrate feed which makes them an excellent target for further derivatizations. Therefore, we have used biotechnologically produced mcl-PHA consisting of predominantly (*R*)-3-hydroxyoctanoic acid monomers (Elbahloul and Steinbuchel 2009) and utilized it as a starting material for synthetic routes to generate a library of derivatives for biological activity evaluation.

Materials and methods

Materials, procedures for compound synthesis, and analytical methods

All chemical reagents were obtained from Sigma–Aldrich (Germany). Ethyl acetate, dichloromethane, and other solvents were of HPLC reagent grade and purchased from Fisher Scientific (Hampton, NH, USA).

The enantiomeric excess was determined by HPLC (Agilent Technologies, HP1100) with a Chiralpak ID column (Chiral Technologies Europe, Cedex, France) at 210 nm for all of the samples. NMR spectra were recorded on a Varian Gemini 200 (^{1}H NMR at 200 MHz, ^{13}C NMR at 50 MHz, for samples in deuterated chloroform) and on Bruker Avance III 500 (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz). Chemical shifts are expressed in ppm (δ) using tetramethylsilane as an internal standard, and coupling constants (*J*) are in hertz. IR spectra were recorded on a Nicolet 6700 FT instrument and are expressed in per centimeter. Mass spectra were obtained on Agilent Technologies 6210 TOF LC/MS instrument (LC: series 1200). Optical rotations were determined by a Rudolph Research Analytical AUTOPOL IV automatic polarimeter (10 cm, 1-ml cell).

Methyl 3-hydroxyoctanoate (1) (Hon et al. 2005) Poly-(*R*)-3-hydroxyoctanoic acid (500 mg, 3.12 mmol, 1 eq) was placed in methanol (20 ml), and sulfuric acid (1 ml, 18.8 mmol, 6 eq) was added. After 3 h of stirring at room

temperature, methanol was removed under reduced pressure and the residue was dissolved in dichloromethane, washed with saturated aqueous $NaHCO_3$ solution and then with brine, and dried over anhydrous $MgSO_4$. The solvent was removed in vacuum and the crude product was purified by dry flash chromatography (chloroform/ethyl acetate = 9/1 to 8/2) to yield a 450-mg (90 %) pure product, as colorless oil. $[\alpha]_D$ = −28 (c 1, $CHCl_3$); ee >99 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.15–3.89 (m, 1 H), 3.72 (s, 3 H), 2.90 (d, *J* = 4.0 Hz, 1 H), 2.58–2.34 (m, 2 H), 1.71–1.30 (m, 8 H), 0.89 (t, *J* = 6.3 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.5, 67.9, 51.7, 41.1, 36.4, 31.6, 25.1, 22.5, 13.9. IR (ATR): 3457, 2958, 2933, 2865, 1741, 1441, 1171.

Benzyl 3-hydroxyoctanoate (2) (Smith et al. 2011) To a solution of 3-hydroxyoctanoic acid (1.25 mmol, 1 eq) and Na_2CO_3 (3.75 mmol, 3 eq) in dimethylformamide (2 ml), benzyl-bromide (1.4 mmol, 1.1 eq) was added dropwise at room temperature. The resulting mixture was stirred for 24 h, and the reaction was monitored by thin-layer chromatography (TLC) (chloroform/ethyl acetate = 9/1) until full conversion of the acid was achieved. Water was added and the aqueous phase was extracted three times with diethyl ether, washed with brine, and dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was purified by dry flash chromatography on silica gel (chloroform/ethyl acetate = 9/1), and 188 mg (60 %) of product 2 as yellow oil was yielded. $[\alpha]_D$ = −20 (c 1, $CHCl_3$); ee 75 %; ^{1}H NMR (200 Hz, $CDCl_3$): δ 7.36 (s, 5 H), 5.15 (s, 2 H), 4.12–3.92 (m, 1 H), 2.87 (d, *J* = 4.1 Hz, 1 H), 2.66–2.34 (m, 2 H), 1.70–1.23 (m, 8 H), 0.88 (t, *J* = 6.4 Hz, 3 H). ^{13}C NMR (50 Hz, $CDCl_3$): 172.9, 135.6, 128.6, 128.4, 128.3, 68.0, 66.4, 41.3, 36.4, 31.6, 25.1, 22.5, 13.9. IR (ATR): 3448, 2957, 2932, 2860, 1737, 1459, 1168.

3-Hydroxyoctanoic acid (3) (Brandl et al. 1988; de Rijk et al. 2001) To a solution of methyl 3-hydroxyoctanoate (90 mg, 0.516 mmol) in THF (2.7 ml) and methanol (1.8 ml), a water solution of LiOH 2.5 M (0.9 ml) was carefully added. The reaction mixture was stirred for 24 h at room temperature. After 24 h, the pH was adjusted to 2 with 1 M HCl, and the resulting solution was washed three times with ethyl acetate (10 ml × 3) followed by washing with brine and drying over anhydrous $MgSO_4$. After removing the solvent under reduced pressure, pure compound 3 was obtained (79 mg, 96 %). $[\alpha]_D$ = −20 (c 1, $CHCl_3$); ee 99 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.57 (bs, 2 H), 4.04 (m, 1 H), 2.73–2.27 (m, 2 H), 1.75–1.11 (m, 8 H), 0.89 (t, *J* = 6.3 Hz, 3 H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 177.6, 68.1, 41.0, 36.4, 31.6, 25.1, 22.5, 13.9. IR (ATR): 3396, 2931, 2956, 2862, 1714, 1411, 1174.

Chemical synthesis of methyl 3-hydroxyoctanoate (1r) (Hon et al. 2005) A suspension of the activated zinc dust (2.28 g, 35 mmol) in 10 ml of anhydrous benzene was heated up to reflux for 10 min. To the refluxing suspension, a mixture of *n*-hexanal (4 ml, 33 mmol) and methyl bromoacetate (5.54 ml, 35 mmol) in 60 ml of benzene was slowly added over a period of 1 h. After 2 h, the reaction mixture was cooled to 0 °C. 1 M HCl was added to acidify the reaction mixture which was extracted with ether (40 ml × 3). The combined organic phase was dried (anhydrous $MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified on a silica gel column (petrol ether/ethyl acetate = 9/1) to give the desired β-hydroxy ester 1r (6.4 g) in 80 % yield as a colorless oil.

General procedure for 3-chloro derivative preparation (Takashi et al. 2003) To a solution of 3-hydroxyester (1 eq, 0.575 mmol) and triethylamine (0.15 eq, 0.086 mmol) in diisopropyl ether (4 ml), one half of a solution of $SOCl_2$ (1.175 eq, 0.675 mmol) in diisopropyl ether (5 ml) was added dropwise at −10 °C. The resulting mixture was then stirred for 10 min at −10 °C and then allowed to warm to room temperature. The second half of the $SOCl_2$ solution (5 ml) was then added dropwise to a reaction mixture and heated to 75 °C until full conversion of the alcohol. After cooling to room temperature, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with CH_2C1_2 (3 × 10 ml) and washed with brine and dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was purified by dry flash chromatography on silica gel (petrol ether/ethyl acetate = 95/5).

Methyl 3-chlorooctanoate (4) Yield 51 mg (46 %), yellow oil. $[\alpha]_D$ = −16 (c 1, $CHCl_3$); ee 22 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.3 (quint, *J* = 7 Hz, 1 H), 3.73 (s, 3 H), 2.75 (d, *J* = 6.8 Hz, 2 H), 1.84–1.21 (m, 8 H), 0.90 (t, *J* = 6.5 Hz, 3 H). ^{13}C NMR (50 Hz, $CDCl_3$): δ 171.1, 57.9, 51.9, 43.5, 37.9, 31.1, 25.9, 22.4, 13.9. IR (ATR): 2959, 2933, 2865, 1745, 1463, 1169. HRMS (ESI): calculated for $C_9H_{17}O_2Cl$ $(M + NH_4)^+$ 210.12553, found 210.12571.

Benzyl 3-chlorooctanoate (5) Yield 87 mg (56 %), yellow oil. $[\alpha]_D$ = −9 (c 1, $CHCl_3$); ee 17 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.37 (s, 5 H), 5.17 (s, 2 H), 4.32 (quint, *J* = 7 Hz, 1 H), 2.86–2.73 (m, 2 H), 1.85–1.10 (m, 8 H), 0.89 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.1, 135.8, 128.6, 128.4, 128.3, 66.6, 57.9, 43.6, 37.9, 31.1, 25.9, 22.4, 13.9. IR (ATR): 3066, 3034, 2956, 2931, 2861, 1741, 1458, 1159. HRMS (ESI): calculated for $C_{15}H_{21}O_2Cl$ $(M + NH_4)^+$ 286.15683, found 286.15665.

General procedure for 3-bromo derivatives (Anessian et al. 1987; Desmaris et al. 2003) To a solution of 3-hydroxy ester (1 eq, 0.2 mmol) and carbon tetrabromide (1.6 eq, 0.32 mmol) in dichloromethane (1 ml) at 0 °C, triphenylphosphine (1.13 eq, 0.225 mmol) was added. The mixture was stirred for 4 h at room temperature. After removal of the solvent under reduced pressure, the product was purified by dry flash chromatography on silica gel in gradient (petrol ether/ethyl acetate = 95/5 to 8/2).

Methyl 3-bromooctanoate (7) Yield 15 mg (32 %), yellow oil. $[\alpha]_D$ = +5 (c 1, $CHCl_3$); ee 16 %; ^{1}HNMR (200 Hz): ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.34 (quint, J = 7 Hz, 1 H), 3.72 (s, 3 H), 2.90 (d, J = 7 Hz, 2 H), 1.83–1.77 (m, 2 H), 1.63–1.16 (m, 6 H), 0.89 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (50 Hz, $CDCl_3$): δ 170.9, 51.9, 50.0, 44.1, 38.6, 30.9, 27.1, 22.4, 13.9. IR (ATR): 2957, 2932, 2860, 1746, 1438, 1157. HRMS (ESI): calculated for $C_9H_{17}O_2Br$ $(M + NH_4)^+$ 254.07502, found 254.07547.

Benzyl 3-bromooctanoate (8) Yield 60 mg (95 %), yellow oil. $[\alpha]_D$ = 0 (c 1, $CHCl_3$); ee 1 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36 (s, 5 H), 5.16 (s, 2 H), 4.36 (quint, 7 Hz, 1 H), 2.95 (d, J = 7 Hz, 2 H), 1.83 (dt, J = 8.7, 7.0 Hz, 2 H), 1.72–1.08 (m, 6 H), 0.89 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (50 Hz, $CDCl_3$): 170.2, 135.5, 128.6, 128.3, 66.7, 49.9, 44.3, 38.6, 30.9, 27.0, 22.4, 13.9. IR (ATR): 3729, 3065, 3034, 2956, 2930, 2861, 1740, 1457, 1151. HRMS (ESI): calculated for $C_{15}H_{21}O_2Br$ $(M + NH_4)^+$ 330.10632, found 330.10688.

General procedure for 3-fluoro derivatives (Delgado et al. 1991) To a cold solution (−70 °C) of 3-hydroxyester (1 eq, 0.24 mmol) in anhydrous CH_2Cl_2 (2 ml), diethylaminosulfur trifluoride (DAST) (1.1 eq, 0.264 mmol) was added under a stream of argon. After 20 min of stirring at −70 °C, the reaction was allowed to warm to room temperature and quenched with saturated solution of $NaHCO_3$ and water. The aqueous phase was extracted with dichloromethane (3 × 5 ml), washed with brine and dried over anhydrous $MgSO_4$. The residue was purified by dry flash chromatography (petrol ether/ethyl acetate = 95/5) to yield fluoro ester, containing olefinic by-products. These were efficiently removed by ozonolysis of the crude material in CH_2Cl_2 at −15 °C followed by treatment with 0.3 M periodic acid in THF for 1 h at room temperature. The solvent was removed under reduced pressure and the product was purified again by dry flash chromatography (petrol ether/ethyl acetate = 9/1).

Methyl 3-fluorooctanoate (10) Yield 17 mg, (40 %), colorless oil. $[\alpha]_D$ = −5 (c 1, $CHCl_3$); ee 19 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.12–4.99 (m, 1 H), 4.88–4.75 (m, 1 H), 3.72 (s, 3 H), 2.86–2.32 (m, 2 H), 1.88–1.14 (m, 8 H), 0.90 (t, J = 6.5 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.7 (d, J = 5.9 Hz), 90.4 (d, J = 158.1 Hz), 51.8 (d, J = 6.2 Hz), 40.2 (d, J = 24.2 Hz), 34.8 (d, J = 20.5 Hz), 31.4, 24.4 (d, J = 4.0 Hz), 22.4, 13.9. IR (ATR): 2956, 2865, 1744, 1439, 1172.

Benzyl 3-fluorooctanoate (11) Yield 40 mg (60 %), colorless oil. $[\alpha]_D$ = −3 (c 1, $CHCl_3$); ee 65 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36 (s, 5 H), 5.16 (s, 2 H), 5.08 (ddd, J = 12.5, 7.8, 4.7 Hz, 1 H), 4.83 (dt, J = 12.4, 4.2 Hz, 2 H), 2.95–2.27 (m, 2 H), 1.88–1.14 (m, 6 H), 0.89 (t, J = 6.5 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.2 (d, J = 5.6 Hz), 135.7, 128.6, 128.3, 128.2, 90.4 (d, J = 169.7 Hz), 66.6, 40.4 (d, J = 24.1 Hz), 34.8 (d, J = 20.5 Hz), 31.4, 24.4 (d, J = 4.3 Hz), 22.4, 13.9. IR (ATR): 3066, 3033, 2955, 2933, 2864, 1741, 1458, 1169. HRMS (ESI): calculated for $C_{15}H_{21}O_2F$ $(M + NH_4)^+$ 270.18638, found 270.18630.

Methyl (E)-oct-2-enoate (13) (El-Batta et al. 2007) Olefinic derivatives were obtained as by-product in 3-fluoro derivative synthesis. Yield 5 mg (15 %), colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.98 (dt, J = 15.6, 7.0 Hz, 1 H), 5.82 (dt, J = 15.6, 1.5 Hz, 1 H), 3.73 (s, 3 H), 2.20 (dq, J = 7, 1.5 Hz, 2 H), 1.46–1.26 (m, 6 H), 0.90 (t, J = 6 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.1, 149.8, 120.7, 51.2, 32.1, 31.2, 27.6, 22.3, 13.8. IR (ATR): 2930, 2956, 2860, 1727, 1437, 1271.

Benzyl (E)-oct-2-enoate (14) Yield 6 mg (10 %), colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36 (s, 5 H), 7.02 (dt, J = 15.5, 6.9 Hz, 1 H), 5.86 (d, J = 15.6 Hz, 1 H), 5.14 (d, J = 11.8 Hz, 9 H), 2.55–2.06 (m, 1 H), 1.86–1.08 (m, 8 H), 0.88 (t, J = 6.4 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.6, 150.2, 136.2, 128.5, 128.2, 128.1, 120.8, 65.9, 32.2, 31.2, 27.6, 22.4, 13.9. IR (ATR): 2929, 2956, 3034, 3064, 2860, 1723, 1457, 1167. HRMS (ESI): calculated for $C_{15}H_{20}O_2$ $(M + NH_4)^+$ 250.18016, found 250.18019.

General procedure for 3-oxo derivatives (Collins et al. 1968) A solution of Collins reagent was prepared from chromium(VI) oxide (6 eq, 2 mmol) and pyridine (12 eq, 4 mmol) in dichloromethane. The mixture was stirred at room temperature under argon for 30 min; then, a solution of 3-hydroxyester compound (1 eq, 0.34 mmol) in dichloromethane was added and stirring was continued for 12 h at room temperature. The reaction was monitored by TLC (petrol ether/ethyl acetate = 9/1). Ether was added and the residue from the Collins reagent was washed three times with ether. The combined ether layers were filtered through a 1-cm column of silica gel, and the solvent was evaporated under reduced pressure and the residue purified by dry flash chromatography (petrol ether/ethyl acetate = 9/1).

Methyl 3-oxooctanoate (16) (Balducci et al. 2011; Mordant et al. 2004) Yield 20 mg (35 %), colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.74 (s, 3 H), 3.45 (s, 2 H), 2.53 (t, J = 7.3 Hz, 2 H), 1.68–0.95 (m, 6 H), 0.89 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 202.9, 167.7, 52.3, 48.9, 43.0, 31.1, 23.1, 22.3, 13.8. ^{1}H NMR: δ 12.03 (s, 1 H). IR (ATR): 2956, 2932, 2864, 1748, 1717, 1440, 1240, 1320, 1159.

Benzyl 3-oxooctanoate (17) (Balducci et al. 2011) Yield 67.5 mg (80 %), colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): 7.36 (s, 5 H), 5.18 (s, 2 H), 3.48 (s, 2 H), 2.50 (t, J = 7.3 Hz, 2 H), 1.58 (dt, J = 14.4, 7.3 Hz, 2 H), 1.43–1.07 (m, 4 H), 0.87 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 202.7, 167.1, 135.3, 128.6, 128.4, 128.3, 67.0, 49.2, 42.9, 31.1, 23.0, 22.3, 13.8. *enol*: ^{1}H NMR: δ 12.02 (s, 1 H). IR (ATR): 2957, 2931, 2866, 1744, 1717, 1646, 1458.

General procedure for deprotection of benzyl esters The benzyl ester was dissolved in MeOH, and a catalytic amount of 10 % Pd/C was added. The apparatus was evacuated and flushed with H_2. The mixture was stirred in an atmosphere of H_2 (50 psi) for 8 h. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to yield quantitatively carboxylic acid.

3-Chlorooctanoic acid (6) Yield 66 mg (100 %), yellow oil. $[\alpha]_D$ = −4.5 (c 1, $CHCl_3$); ee 27 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.53 (bs, 1 H), 4.35–4.08 (m, 1 H), 2.81 (d, J = 6.9 Hz, 2 H), 1.91–1.12 (m, 8 H), 0.90 (t, J = 6.5 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.5, 57.3, 43.4, 37.9, 31.1, 25.9, 22.4, 13.9. IR (ATR): 2932, 2958, 2861, 1716, 1286, 1433. HRMS (ESI): calculated for $C_8H_{15}O_2Cl$ $(M + NH_4)^+$ 196.10988, found 196.11039.

3-Bromooctanoic acid (9) Yield 71 mg (100 %), yellow oil. $[\alpha]_D$ = 0 (c 1, $CHCl_3$); ee 23 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.70 (bs, 1 H), 4.54–4.14 (m, 1 H), 2.96 (d, J = 6.9 Hz, 2 H), 1.95–1.15 (m, 8 H), 0.90 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.6, 49.1, 44.1, 38.6, 30.9, 27.1, 22.4, 13.9. IR (ATR): 2930, 2958, 2860, 1715, 1430, 1282. HRMS (ESI): calculated for $C_8H_{15}O_2Br$ $(M + NH_4)^+$ 240.05937, found 240.05985.

3-Fluorooctanoic acid (12) Yield 64 mg (100 %), white powder. $[\alpha]_D$ = −3 (c 1, $CHCl_3$); ee 66 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.05 (bs, 1 H), 5.00–4.85 (m, 1 H), 2.82–2.39 (m, 2 H), 1.81–1.18 (m, 8 H), 0.90 (t, J = 6.9 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.3 (d, J = 5.0 Hz), 90.2 (d, J = 169.5 Hz), 40.3 (d, J = 24.1 Hz), 34.8 (d, J = 20.4 Hz), 31.4, 24.5 (d, J = 4.3 Hz), 22.5, 13.9. IR (ATR): 2932, 2957, 2862, 1716, 1433, 1266. HRMS (ESI): calculated for $C_8H_{15}O_2F$ $(M + NH_4)^+$ 180.13943, found 180.13985.

(E)-Oct-2-enoic acid (15) (SDBS Web 2014) Yield 61 mg (100 %), colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 11.77–8.85 (bs, 1 H), 7.09 (dt, J = 15.6, 7.0 Hz, 1 H), 5.82 (dt, J = 15.6, 1.5 Hz, 1 H), 2.23 (dq, J = 7, 1.5 Hz, 2 H), 1.68–1.15 (m, 6 H), 0.90 (t, J = 6.5 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 152.6, 120.6, 34.1, 32.2, 31.3, 27.5, 22.4, 13.9. *IR (ATR): 2931, 2959, 2860, 2674, 1699, 1421, 1285. *cis isomer in trace.

3-Oxooctanoic acid (18) (Giddens et al. 2008) Yield 63 mg (99 %), white powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.97 (s, 1 H), 4.03 (s, 1 H), 2.72–2.23 (m, 2 H), 1.78–1.11 (m, 8 H), 0.89 (t, J = 6.3 Hz, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 177.5, 68.2, 41.3, 36.4, 31.6, 25.1, 22.5, 13.9. IR (ATR): 3536, 2955, 2926, 2857, 1771, 1681, 1440.

In vitro antimicrobial and antiproliferative assays

To test the antimicrobial properties of the synthesized derivative, a range of bacterial strains from the National Collection of Type Cultures (NCTC) and the American Type Culture Collection (ATCC) including *Escherichia coli* NCTC 9001, *Salmonella typhimurium* NCTC 12023, *Staphylococcus aureus* NCTC 6571, *Listeria monocytogenes* NCTC 11994, *Pseudomonas aeruginosa* PAO1 NCTC 10332, *Candida albicans* ATCC 10231, and *Micrococcus gypseum* ATCC24102 in previously described standard broth micro dilution assays (Casey et al. 2004) defined by the National Committee for Clinical Laboratory Standards (M07-A8) for bacteria and standards of the European Committee on Antimicrobial Susceptibility Testing (EDef7.1.).

The antiproliferative activity of the compounds was assessed against the human lung fibroblast MRC5 cell line obtained from ATCC and melanoma cell A375 using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay (Hansen et al. 1989). The MTT assay was performed after 48 h of treatment with compounds three times in three replicates in comparison to control (DMSO-treated cells) that were arbitrarily set to 100 %.

Effects of PHA monomer derivative alkanoic acids on *Candida* yeast–hyphal transition

Morphological changes of *C. albicans* in the presence and absence of the alkanoic acids (3, 6, 9, 12, 15, and 18) were observed upon *C. albicans* growth on Spider medium as previously described (Shafreen et al. 2014). The plates were supplemented with sub-minimum inhibitory concentrations (MICs) of each derivative, as well as with C8, and incubated at 37 °C for 36 h. The morphology of *C. albicans* colony formed on the Spider medium was visualized using a light microscope (CKX41; Olympus, Tokyo, Japan).

Effects of PHA monomer derivative carboxylic acids on pyocyanin production by *P. aeruginosa* PAO1

To test the effect of derivatives 3, 6, 9, 12, 15, and 18 on pyocyanin production in *P. aeruginosa* PAO1, a slightly modified protocol of O'Loughlin et al. was used (O'Loughlin et al. 2013). *P. aeruginosa* PAO1 was grown in Kings A medium (1.5 % (*v*/*v*) glycerol, 20 g/l peptone, 1.64 g/l $MgCl_2$, 10 g/l K_2SO_4) at 37 °C for 24 h. An overnight *P. aeruginosa* PAO1 culture was diluted 1:1000 into 5 ml Kings A medium and, after addition of derivatives and C8 to a final concentration of 0.06 mM or an equivalent amount of DMSO as control, cultured for another 24 h at 37 °C with shaking. A culture aliquot (1 ml) was harvested at 14,000 rpm for 20 min, and the supernatant was analyzed for pyocyanin on a UV–vis spectrophotometer Ultrospec 3300pro (Amersham Biosciences, Piscataway, NJ, USA) at 695 nm. All experiments were performed in triplicate and repeated at least three times.

Results

Synthesis and characterization of the library of compounds based on hydroxyoctanoic acid

A library containing 18 compounds has been generated using (*R*)-3-hydroxyoctanoic acid monomers derived from a bacterial polyester PHA, as the starting material (Fig. 2). Predominantly halogen derivatives of (*R*)-3-hydroxyoctanoic acid methyl ester (1) obtained via acid methanolysis of biotechnologically produced mcl-PHA containing up to 97 % (*R*)-3-hydroxyoctanoic acid monomers (de Roo et al. 2002; Elbahloul and Steinbuchel 2009) were synthesized due to the importance of the halogen moiety in the currently used drugs. Spectral data of all compounds are given as supporting information (Supporting File S1).

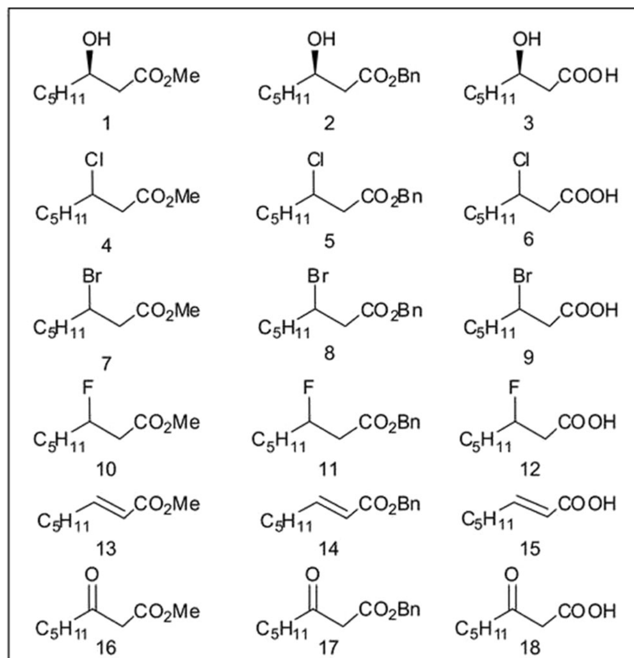

Fig. 2 Library of compounds (*1–18*) based on (*R*)-3-hydroxyoctanoic acid derived from bacterial polyester polyhydroxyoctanoate

Functionalization of the (*R*)-3-hydroxyoctanoic acid was not possible without protection of the free carboxylic group; therefore, all reactions were performed using methyl and benzyl esters. The methyl ester was hydrolyzed to generate 3-hydroxyoctanoic acid which was converted to the benzyl ester form which proved suitable for the preparation of derivatives as depicted in Fig. 3. In this way, the importance of the free carboxylic group for biological activity could be determined by direct comparison with biological activity of the generated esters. All chemical reactions used during this study were based on previously reported procedures (Albert et al. 2002; Anessian et al. 1987; Collins et al. 1968; Delgado et al. 1991; Takashi et al. 2003) with noted adaptations made to ensure successful reactions with 3-hydroxyoctanoyl esters derived from PHA as outlined in Fig. 4.

In order to compare the results that were obtained by derivatization of biological (*R*)-3-hydroxyoctanoate, we synthesized racemic methyl 3-hydroxyoctanoate (1r) by the Reformatsky reaction with a good product yield of 80 % using a previously described procedure (Hon et al. 2005). Recently, the use of this reaction for the related purpose of producing methyl and ethyl esters was published with similar reaction yields (Sailer et al. 2015). We used 1r to produce all other racemic derivatives from the library applying the same procedures applied on 1. In some instances such as 2r, 4r, 7r, and 11r, product yields were between 5 and 13 % better in comparison to yields when 1 was used as the starting material (Table 1).

Chloro derivatives (4 and 5) were made in a substitution reaction using thionyl chloride and triethylamine in diisopropyl ether with heating at 75 °C overnight. A significantly reduced ee was observed for all products synthesized under these conditions (ee from 17 to 27 %). In an attempt to improve enantioselectivity, we have performed reactions at lower temperatures (4 and 25 °C); however, reduction of the reaction temperature resulted in very poor product yields and no improvement of the enantioselectivity (results not shown).

Bromo derivatives (7 and 8) were made from 1 and 2 in reactions with carbon tetrabromide and triphenylphospine in dichloromethane (Fig. 4). Under these reaction conditions, all obtained bromo derivatives were racemic. According to the literature, this type of substitution results in an inversion of the configuration, which is probably the reason for complete racemization of the optically pure starting (*R*)-3-hydroxyoctanoic acid (Anessian et al. 1987; Desmaris et al. 2003).

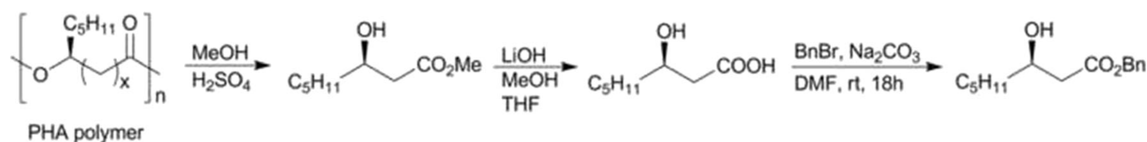

Fig. 3 PHA monomer synthesis and esterification

To produce fluorinated compounds, we used DAST at −70 °C in dichloromethane (Fig. 4). In this conversion, the yield of methyl ester (~40 %) was obtained, whereas the yield of benzyl ester was higher (60 %), which has previously been described for similar compounds. We also obtained an olefine as a by-product in fluorination reaction (13 and 14) which was isolated and characterized. Compounds 11 and 12 (Table 1, entries XI and XII) had higher enantiomeric excess than other halo derivatives (ee 65 %) which may be due to the reaction conditions that included a low temperature of −70 °C (Table 1).

Collins oxidation with chromium(VI) oxide in dichloromethane (Collins et al. 1968) was used for the synthesis of derivatives 16 and 17 which are important organic compounds with a wide range of applications. All alkanoic acids from the library (3, 6, 9, 12, 15, and 18) were produced from the corresponding benzyl esters in the reaction of deprotection using a catalytic amount of Pd/C in an atmosphere of hydrogen (50 psi). The reactions provided mild conditions, stoichiometric conversion, and no by-product generation.

We have generated 17 derivatives in a few steps with various yields (32–100 %). While the structures of the 3-hydroxyoctanoic acid-based derivatives are simple, there are ten derivatives, in the current study, which were completely characterized for the first time (4–12 and 14). Derivative 12 was previously reported but not characterized (Watanabe et al. 1989; Watanabe et al. 1983).

Biological activity of the library of compounds based on hydroxyoctanoic acid

The library of 3-substituted alkanoic acids and esters generated from PHA-derived (*R*)-3-hydroxyoctanoic acid (Fig. 2) was assessed for antimicrobial and antiproliferative properties.

Antimicrobial properties All of the synthesized compounds were evaluated for their activity against five bacterial strains, including *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *P. aeruginosa* PAO1, and two fungal species, *C. albicans* and *Microsporum gypseum.* Their antimicrobial activity was compared with the antibacterial and antifungal properties of octanoic acid (C8). MIC values for alkanoic acids were between 2.7 and 7.0 mM for bacterial strains, while a higher activity was detected against fungi with MIC values between 0.15 and 6.32 mM (Table 2). Generally, methyl and benzyl esters did not exhibit strong antimicrobial properties, with MIC values between 10 and 14 mM (results not shown).

Antibacterial activity was test strain dependant, and all halogen derivatives were up to twofold more active than the (*R*)-3-hydroxyoctanoic acid (3) but exhibit the same or lower activity in comparison to fatty acid C8. Chloro derivative 6 was the most active against *S. aureus* and *L. monocytogenes* (Table 2). Derivative 18 is structurally very similar to the starting monomer and it exhibited almost identical antimicrobial activity (Fig. 2, Table 2).

In general, the compounds investigated showed higher antimicrobial potential towards fungal than bacterial strains (Table 2). In particular, all halogenated acids (6, 9, and 12) showed promising antifungal activity against *C. albicans* and *M. gypseum* (Table 2). MIC values for planktonic growth were determined in standard Sabouraud dextrose broth. Compound 12 inhibited fungal growth at concentrations tenfold lower than the starting (*R*)-3-hydroxyoctanoic acid (3) and approximately fivefold lower in comparison to octanoic acid. Derivatives 6 and 9 showed sevenfold and ninefold lower MIC values in comparison to derivative 3, respectively. In addition, derivatives 6, 9, and 12 and octanoic acid (C8), at sub-MICs (75 % of the MIC value determined for the planktonic growth) showed complete inhibition

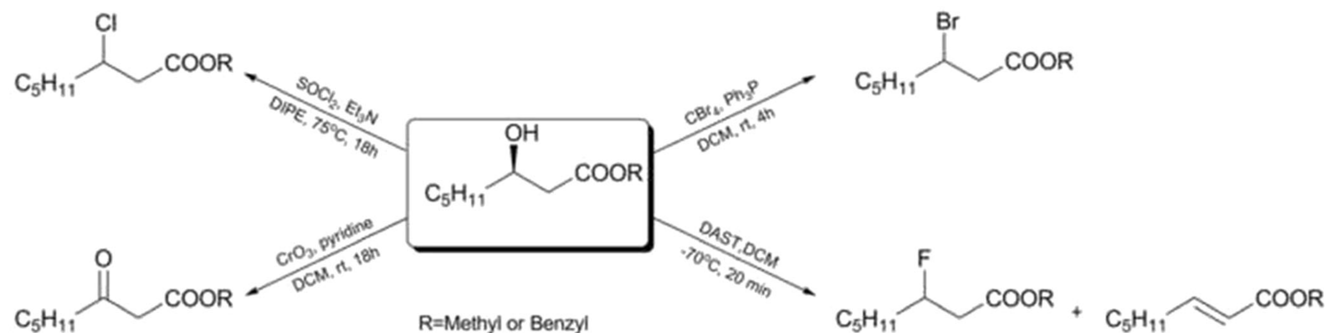

Fig. 4 Chemical synthesis of halogenated, keto, and unsaturated derivatives of octanoic acid

Table 1 Comparative synthesis of β-hydroxyoctanoic acid derivatives (1–18)

Entry	Compound	Reaction time (h)	Isolated yield (%)	ee (%)
I	1	3.0	90	>99
I′	*1r*	*3.5*	*80*	*6*
II	2	24	60	75
II′	*2r*	*24*	*65*	*1*
III	3	24	96	99
III′	*3r*	*24*	*96*	*9*
IV	4	0.5	46	22
IV′	*4r*	*0.5*	*56*	*35*
V	5	20	56	17
V′	*5r*	*20*	*64*	*13*
VI	6	8.0	100	27
VI′	*6r*	*8.0*	*100*	*12*
VII	7	4.0	32	16
VII′	*7r*	*4.0*	*45*	*2*
VIII	8	4.0	95	1
VIII′	*8r*	*4.0*	*97*	*1*
IX	9	8.0	100	23
IX′	*9r*	*8.0*	*100*	*5*
X	10	0.5	40	19
X′	*10r*	*0.5*	*46*	*8*
XI	11	0.5	60	65
XI′	*11r*	*0.5*	*71*	*9*
XII	12	8.0	100	66
XII′	*12r*	*8.0*	*100*	*8*
XIII	13	0.5	15	–
XIV	14	0.5	10	–
XV	15	8.0	100	–
XVI	16	20	35	–
XVII	17	20	80	–
XVIII	18	8.0	80	–

Italicized entries denote chemically synthesized racemic derivatives

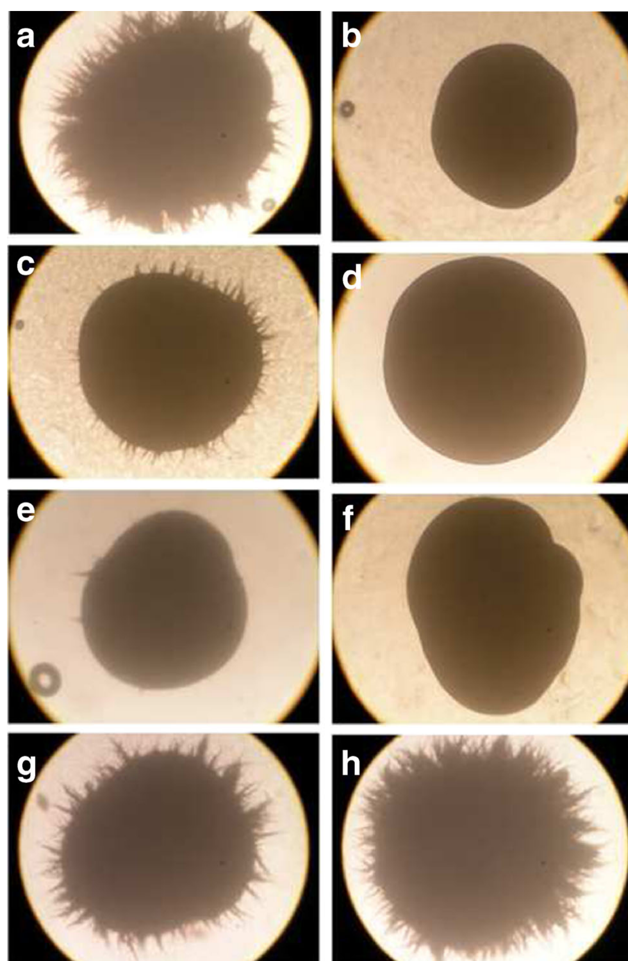

Fig. 5 *C. albicans* spread on Spider medium in the presence of derivatives: **a** DMSO control, **b** octanoic acid (C8), **c** 3, **d** 6, **e** 9, **f** 12, **g** 15, and **h** 18

of *C. albicans* filamentous growth on the hyphal-inducing Spider medium (Fig. 5). (*R*)-3-hydroxyoctanoic acid (3) reduced hyphae formation to a lesser extent in comparison to octanoic acid, while 15 and 18 appeared to have a positive effect on the hyphae formation at the tested concentrations. The transition from yeast to hyphal growth is important for *Candida* biofilm formation, so molecules that interfere with this process are highly

Table 2 Antimicrobial activity of carboxylic acids generated in this study expressed as minimal inhibitory concentration (MIC) in mM

Compound	*E. coli* NCTC 9001	*S. typhimurium* NCTC 120023	*S. aureus* NCTC 6571	*L. monocytogenes* NCTC 11994	*C. albicans* ATCC10231	*M. gypseum* ATCC24102
3	6.24	6.24	6.24	6.24	1.55	1.56
6	n.a.	5.60	2.79	2.79	0.22	1.12
9	4.48	4.48	4.48	4.48	0.18	0.90
12	6.16	6.16	6.16	n.a.	0.15	0.60
15	7.02	7.02	7.02	7.02	3.52	3.52
18	6.32	6.32	6.32	n.a.	6.32	3.16
C8	3.46	3.46	3.46	3.46	0.69	1.73

n.a. no activity under conditions tested

sought after as possible treatment options according to Shafreen et al. (2014).

Pyocyanin production As none of the compounds showed significant activity against *P. aeruginosa* PAO1, except for C8 with a relatively high MIC of 6.9 mM, we decided to assess the effect of acids generated in this study on pyocyanin production (Fig. 6). Pyocyanin is a blue green redox-active secondary metabolite that is produced by *P. aeruginosa* under quorum sensing regulation and one of the factors contributing to virulence of this strain (Lau et al. 2004; Whooley and McLoughlin 1982). It was evident that even at concentrations as low as 0.06 mM (between 5 and 10 μg mL^{-1}), 3 and 15 significantly inhibited pyocyanin production in *P. aeruginosa* PAO1, while 9 and C8 stimulated pyocyanin production in the same strain (Fig. 6).

In vitro antiproliferative effect of 3-substituted alkanoic acids The cytotoxicity of 3-substituted alkanoic acids 3, 6, 9, 12, 15, 18, and C8 which showed antimicrobial activity (Table 2) was carried out by determining antiproliferative activity with human fibroblasts in a concentration range 0.5–9 mM. No observable antiproliferative effect was detected against human fibroblasts after 48 h of treatment using all compounds at 1.5 mM concentration (Fig. 7). Compounds 3, 6, and 9 showed no cytotoxicity at 1.5 or 3 mM, nor at 9 mM (results not shown). Compounds 15 and 18 caused about 50 % inhibition of cell proliferation at 1.5 mM while 12 was toxic at 3 mM, inhibiting proliferation of 40 % of human fibroblasts (Fig. 7). The low cytotoxicity of 3 is in accordance with our previous study where IC_{50} values of (*R*)-3-hydroxydecanoic acid, as well as decanoic acid (C10), against several cell lines were determined to be above 3 mM (500 μg ml^{-1}) (O'Connor et al. 2013).

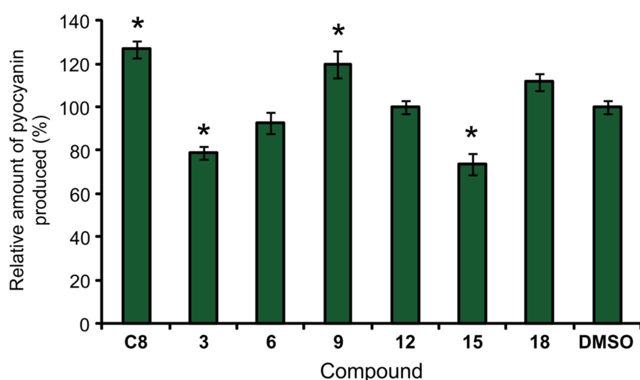

Fig. 6 Pyocyanin production by *Pseudomonas aeruginosa* PAO1 in response to carboxylic acids generated in this study. *Star* denotes statistically significant value ($p < 0.05$). Values are given relative to a control where cells are grown on Kings A medium with DMSO

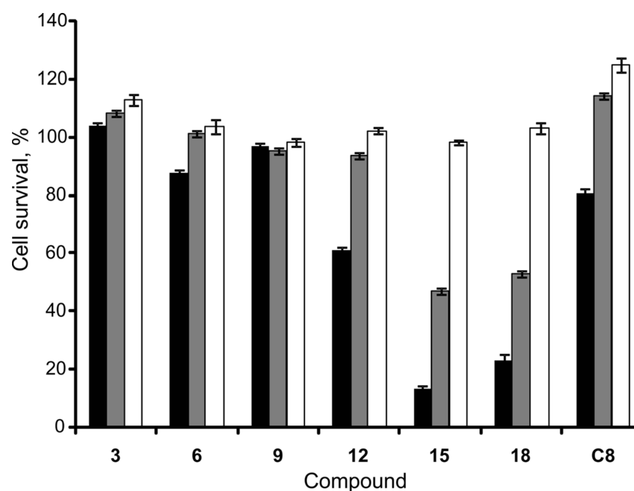

Fig. 7 In vitro antiproliferative effect of carboxylic acids on human fibroblasts following 48 h of exposure at a range of concentrations (*white bar* 0.3 mM, *gray bar* 1.5 mM, and *black bar* 3 mM)

Discussion

Various free fatty acids are attracting attention as potential therapeutic antimicrobial agents due to their potency, broad spectrum of activity, non-specific mode of action, and lack of classical resistance mechanisms against the actions of these compounds. Their antibacterial properties have been known for a while (Desbois and Lawlor 2013; Desbois and Smith 2010). Hydroxylated alkanoic acids are also gaining increased attention in terms of potential industrial applications (Ren et al. 2010).

Taking an advantage of the controllability of monomer composition during biotechnological production of mcl-PHA, we have decided to gain access to valuable (*R*)-3-hydroxyoctanoic acid monomers starting with a biological polymer that contains 97 % (*R*)-3-hydroxyoctanoic acid monomers (Elbahloul and Steinbuchel 2009). Due to the fact that 20 % of all pharmaceutical small-molecule drugs are halogenated (Marris 2006), we decided to synthesize halogen derivatives of a (*R*)-3-hydroxyoctanoic monomer (**1**) (Fig. 2). Halogen derivatives were obtained in good yields in single-step reactions (Figs. 3 and 4). Previous attempts to introduce chlorine into the structure of PHA meant feeding bacteria with 1-chlorooctane to obtain copolymers with a small amount of this halogen (Doi and Abe 1990). Arkin et al. described chlorination of PHA by using an unsaturated polymer and passing by chlorine gas through their solutions (Arkin and Hazer 2002). We have recently made 3-chlorodecanoic acid from (*R*)-3-hydroxydecanoate derived from the PHA applying procedure similar to one described herein (Szwej et al. 2015). On the other side, production of methyl 3-fluorohexadecanoate was described through a three-step synthesis by acylation of Meldrum's acid as the starting molecule (Delgado

et al. 1991). In the procedure described here, we obtained **12** in a one-step process using (*R*)-3-hydroxyoctanoate.

β-Keto esters, such as derivatives **16** and **17**, have been one of the most important intermediates in organic synthesis and have been used in the synthesis of naturally occurring enantiopure bioactive compounds (Case-Green et al. 2008; Giddens et al. 2008). Synthesis of β-keto esters via acetoacetic esters, via mixed malonic esters, from Meldrum's acid was reported that included a three-step synthesis using fatty acids as the starting material (Brinkerhoff et al. 2014). Using our described approach (Fig. 4), we have obtained products in just one step with excellent yields (up to 80 %), indicating a convenient way of obtaining these important compounds. Overall, using known chemical transformations, we have shown that mcl-PHA was a suitable starting material for the straightforward generation of different small molecules with good yields (Table 1).

The antibacterial properties of the compounds generated were not high in comparison to commercially available antibiotics, such as gentamicin which has MIC values in a range of 4 μM concentration; it was evident that the free carboxylic group was essential for the antimicrobial activity of the compounds (Table 2). This was in an agreement with the study of Kabara et al. that showed the free carboxyl group was necessary for the activity of lauric acid (Kabara et al. 1972). It has been proposed that the prime target of free fatty acid (FFA) action is the cell membrane, where FFAs disrupt the electron transport chain and oxidative phosphorylation. Besides interfering with cellular energy production, FFA may also inhibit other metabolic enzyme activities (such as enoyl-ACP reductase), impair nutrient uptake, be involved in the generation of peroxidation and auto-oxidation degradation products, or cause direct lysis of bacterial cells (Desbois and Lawlor 2013; Desbois and Smith 2010).

There was no observable difference in MIC values when (*R*,*S*)-3-hydroxyoctanoic acid (3r) was used in comparison to (*R*)-3-hydroxyoctanoic acid (data not shown). This is in slight disagreement with the previous study of Ruth et al. when commercially available (*R*,*S*)-3-hydroxyoctanoic acid exhibited slightly lower antibacterial activity to (*R*)-enantiomer against a range of *Listeria* species (Ruth et al. 2007). However, in the same study, no activity of C8 was reported, which is in disagreement with our current study and other works reporting the antibacterial properties of free fatty acids including octanoic acid (Marounek et al. 2003; Skrivanova et al. 2004). Previously, *R*-3-hydroxyoctanoic acid (3), (*R*)-3-hydroxy-8-nonenoic acid, and (*R*)-3-hydroxy-10-undecenoic acid were shown to inhibit the growth of *Listeria* species and *S. aureus* with a MIC of 1–5 mM while (*R*)-3-hydroxyphenylalkanoates also inhibited bacterial growth with MICs of 3–6 mM (Ruth et al. 2007; Sandoval et al. 2005). According to Allen et al., a mixture of 3-HAs exhibited antimicrobial activity against *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* with MICs ranging from 12.5 to 25 mg/ml (Allen et al. 2012), which was much higher than MICs determined in the current study for individual 3-substituted compounds (Table 2).

The introduction of a *trans* double bond (15) resulted in decreased antimicrobial activity twofold in comparison to octanoic acid. This is in agreement with the previous studies that the geometry of the double bond is important for the activity of unsaturated fatty acids. It has been reported that the addition of a *cis* double bond increased the activity of all fatty acids tested (C14, C16, and C18), where the *trans* isomer showed no activity (Kabara et al. 1972).

Due to the fact that the MIC values were not high against bacterial strains, we decided to test their potential to interfere with cell-to-cell signaling communication systems that control virulence in many pathogenic bacterial strains. Small molecules with the ability to interfere with pathogen communication (i.e., quorum sensing) are coming into focus as promising new treatment options, especially as pathogens become resistant to antibiotics and other drugs (Kalia and Purohit 2011). Little is known about the effect of C8 or similar fatty acids on quorum sensing in *P. aeruginosa*; however, it has been reported that *cis*-2-dodecenoic acid could modulate *P. aeruginosa* virulence through interference with quorum sensing and type III secretion system (Deng et al. 2013). On the other hand, longer chain fatty acids such as linoleic, oleic, and palmitic have been shown to inhibit quorum sensing in *Vibrio harveyi* BB170 and as such offer a means to control foodborne pathogens and reduce microbial spoilage (Widmer et al. 2007). Therefore, derivative 3, as well as 15, that showed significant inhibition of pyocyanin production (Fig. 6) could be further examined for quorum quenching or inhibitory ability. On the other side, an important link has been made between pyocyanin produced by *P. aeruginosa* and its activity on *Candida* morphology in mixed infections. At high concentrations, pyocyanin and its metabolites are fungicidal, while at subinhibitory concentrations, it inhibits filamentation and biofilm formation (Gibson et al. 2009; Morales et al. 2013). Taken together, derivative 9, as well as octanoic acid, may be suitable for further development in mixed *Pseudomonas*–*Candida* infections (Table 2, Fig. 6).

Given that antibacterial MIC values of 3-substituted alkanoic acids were between 2.8 and 7 mM, the generally low antiproliferative activity does not make them suitable targets for direct antibacterial applications. The MIC value for 6, 9, and 12 was between 0.15 and 0.22 mM with *C. albicans* ATCC10231 (Table 2), which is an order of magnitude lower than the concentration at which no cytotoxicity is observed with human fibroblasts for 6 and 9 and some cytotoxicy for 12 (Fig. 7) which is positive for the development of these compounds as antifungal agents.

We have shown that PHA can be a suitable source of valuable synthons that could be further derivatized using simple chemical transformations. Derivatives containing a carboxylic group exhibited low antibacterial and higher antifungal properties that could be further developed, considering the absence of in vitro toxicity. Furthermore, these compounds appear to be affecting bacterial and fungal signaling molecules.

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Conflict of interest The authors declare that they have no competing interests.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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