ChemPubSoc Europe

DOI: 10.1002/cbic.201500446

CHEMBIOCHEM Communications

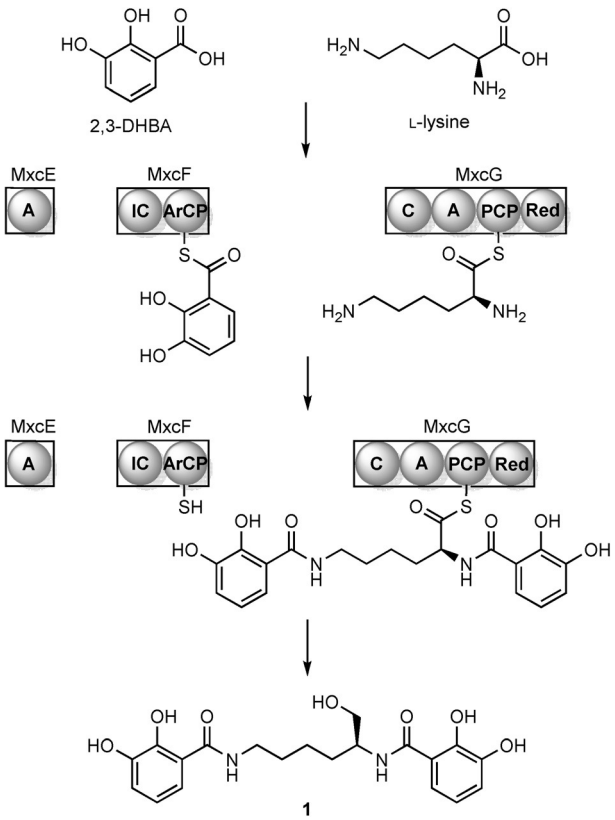
Harnessing Enzymatic Promiscuity in Myxochelin Biosynthesis for the Production of 5-Lipoxygenase Inhibitors

Juliane Korp,[a] Stefanie König,[b] Sebastian Schieferdecker,[a] Hans-Martin Dahse,[a] Gabriele M. König,[c] Oliver Werz,[b] and Markus Nett*[a]

The siderophore myxochelin A is a potent inhibitor of human 5-lipoxygenase (5-LO). To clarify whether the iron-chelating properties of myxochelin A are responsible for this activity, several analogues of this compound were generated in the native producer *Pyxidicoccus fallax* by precursor-directed biosynthesis. Testing in a cell-free assay unveiled three derivatives with bioactivity comparable with that of myxochelin A. Furthermore, it became evident that inhibition of 5-LO by myxochelins does not correlate with their iron affinities.

The Gram-negative myxobacteria are ubiquitous soil bacteria with a complex life cycle that involves the formation of multicellular fruiting bodies.[1] Another special feature of these microorganisms is their biosynthetic versatility.[2] The predatory myxobacterium *Pyxidicoccus fallax* HKI 727, for instance, was described as a producer of new macrolide antibiotics that were highly active against Gram-positive bacteria.[3] Crude extracts from the same strain also displayed inhibitory effects against leukemic cells, and after bioactivity-guided fractionation the active principle was identified as the catechol siderophore myxochelin A (**1**).[4,5] Pharmacological studies revealed that **1** is a potent inhibitor of human 5-lipoxygenase (5-LO).[4] This enzyme catalyzes two initial steps in the conversion of arachidonic acid into leukotrienes, these being known as mediators of inflammatory and allergic reactions.[6] Increasing evidence suggests that leukotrienes also play an important role in tumorigenesis.[7] Because the active site of 5-LO harbors a non-heme iron atom, which acts as a redox mediator during the catalytic reaction,[8] it seemed plausible to ascribe the 5-LO inhibitory effects of **1** to its iron-chelating properties. Experimental support for this assumption was provided by the analysis of two naturally occurring myxochelin derivatives in which the two catechol units of **1** are partially methoxylated. The corresponding compounds were shown to be weaker iron ligands than **1** and they also exhibited reduced 5-LO inhibition.[4]

To explore further the importance of free catechol residues for the biological activity, we set out to analyze additional myxochelins differing in their aromatic substitution profile. Precursor-directed biosynthesis (PDB) was considered a convenient method for preparing the desired compounds. Previous studies had established the molecular basis for myxochelin biosynthesis (Scheme 1),[9] and the involved enzymes were known to possess a certain degree of substrate flexibility,[10] which is an essential prerequisite for the application of PDB.[11–14] Already, the incorporation of 2- and 3-hydroxybenzoic acid into the



Scheme 1. Myxochelin biosynthesis involves the condensation of two molecules of 2,3-dihydroxybenzoic acid (2,3-DHBA) with L-lysine. The assembly of the lysine diamide intermediate is carried out by the nonribosomal peptide synthetase MxcG. The sequence of the two amidation reactions is not known. NADPH-dependent release from MxcG provides the lysinol moiety of **1**.

[a] *J. Korp, Dr. S. Schieferdecker, Dr. H.-M. Dahse, Dr. M. Nett*
Leibniz Institute for Natural Product Research and Infection Biology
Hans Knöll Institute
Adolf-Reichwein-Strasse 23, 07745 Jena (Germany)
E-mail: markus.nett@hki-jena.de

[b] *S. König, Prof. Dr. O. Werz*
Pharmaceutical and Medicinal Chemistry
Institute of Pharmacy, Friedrich-Schiller-Universität Jena
Philosophenweg 14, 07743 Jena (Germany)

[c] *Prof. Dr. G. M. König*
Institute for Pharmaceutical Biology
Rheinische Friedrich-Wilhelms-Universität Bonn
Nussallee 6, 53115 Bonn (Germany)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201500446.

myxochelin backbone had been demonstrated in in vitro reactions in the presence of the heterologously produced biosynthesis enzymes MxcE, MxcF, and MxcG.[10] Despite the limited number of substrates tested and the bypassing of cellular uptake issues, the successful replacement of the natural precursor 2,3-DHBA corroborated the feasibility of PDB for producing additional myxochelins.

Initial feeding studies were conducted in 100 mL cultures of *P. fallax* HKI 727 and involved a total of 34 aryl carboxylic acids (Table S1 in the Supporting Information). The test compounds included mainly halogen- and hydroxy-substituted benzoic acids. Moreover, the incorporation of several heteroaromatic carboxylic acids was also evaluated. All potential precursors were added as filter-sterilized solutions (75 mg L^{-1}) to the cultivation medium prior to inoculation with *P. fallax*. Except in the case of the feeding of 1*H*-imidazole-4-carboxylic acid, we did not observe any growth defects in the supplemented cultures. This suggests that substrate toxicity was not a problem. Unlike in our previous study,[4] cultivations were carried out in the presence of the polystyrene XAD7HP, because the adsorber resin was found to increase the myxochelin yield, possibly due to avoidance of feedback inhibition and improved compound recovery.[15] After seven days of incubation, the resin was separated from the fermentation broth and extracted with acetone. HPLC analysis confirmed that every extract contained **1**, which was identified by comparison with an authentic standard. Furthermore, eight extracts showed new peaks with distinctive UV profiles that were likely due to myxochelin analogues (Figure 1).

A characteristic of myxochelin biosynthesis is the double incorporation of 2,3-DHBA. Supplementation of the producing bacterium with a structurally related compound led to randomized incorporation at the two possible positions, especially if the alternative substrate was fed at low concentrations. In this way, it was possible to generate up to three new analogues in a single feeding experiment, as shown in Figure 1 for the cultures that had been supplemented with benzoic acid and 2-fluoro- and 3-fluorobenzoic acid. In other cultures, however, only one or two additional peaks were detected during the UV-based metabolic profiling. Subsequent LC-MS analyses revealed that the corresponding extracts also featured the previously unobserved derivatives, albeit at very low concentrations (data not shown). Interestingly, the amounts of **1** produced were comparatively low relative to those of the abundant non-natural analogues. Because we did not observe significant differences in the yields of **1** between supplemented and unsupplemented *P. fallax* cultures, it seems likely that the cellular availability of 2,3-DHBA is a limiting factor for the production. With regard to substrate utilization, the myxochelin biosynthetic enzymes turned out to be highly permissive for fluorine substitution on the aromatic precursor, thus paralleling observations made in other natural product pathways.[16] In contrast, replacement with chlorine or bromine atoms was mostly not successful, possibly due to steric constraints. The only exception was 2-chlorobenzoic acid, which was converted into new derivatives. Of the hydroxy-substituted benzoic acids tested, 2- and 3-hydroxybenzoic acid were processed by the

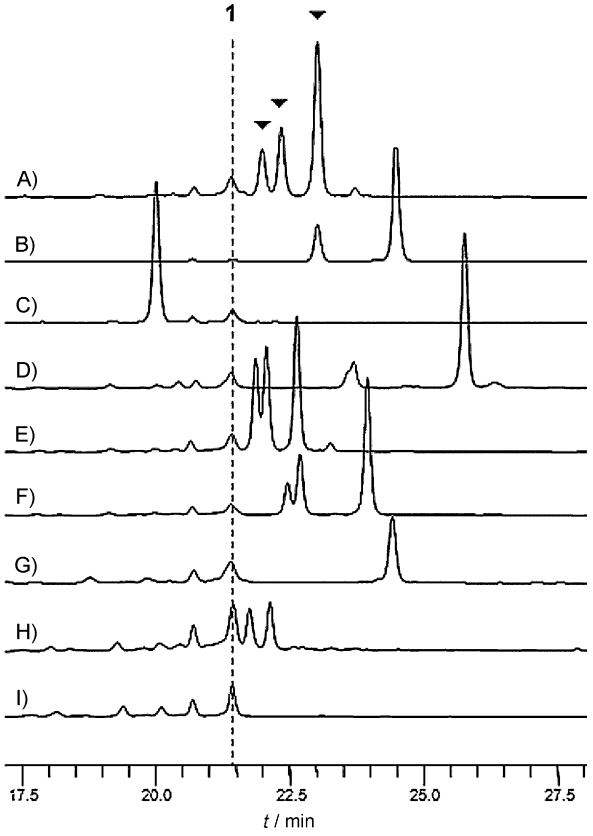


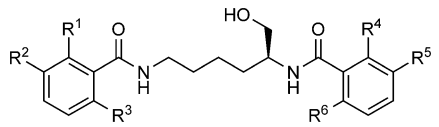
Figure 1. Metabolic analyses of *P. fallax* cultures grown in MD1 medium supplemented with A) benzoic acid, B) 2-hydroxy-, C) 3-hydroxy-, D) 2-fluoro-6-hydroxy-, E) 2-fluoro-, F) 3-fluoro-, G) 4-fluoro-, and H) 2-chlorobenzoic acid. I) Chromatogram of an unsupplemented culture. All chromatograms were recorded at 254 nm. New myxochelins were identified by their characteristic UV profiles. In profile (A), the metabolites are exemplarily indicated with triangles.

biosynthetic enzymes, whereas 4-hydroxybenzoic acid could not be incorporated. None of the administered heteroaromatic compounds led to the production of any novel myxochelin, thereby indicating the boundaries of the enzymatic flexibility.

In order to generate sufficient quantities for NMR-based structural characterization and subsequent biological testing, the feeding experiments of the positively tested substrates were repeated on a 3 L scale. Again, XAD7HP was added to the cultures to allow continuous harvesting of the secreted metabolites. Fermentations of *P. fallax* HKI 727 were typically run for seven days, after which no increase in cell density could be observed. After the removal of the culture broth by filtration, the metabolites were eluted from the adsorber resin with acetone. A preliminary fractionation of the resulting extracts was accomplished by flash column chromatography. Eventually, myxochelin A and 14 analogues were isolated by reversed-phase HPLC. The yields after purification ranged from $\approx$0.1 to $>$5 mg L^{-1} (Table S1). Derivatives that were only present at trace levels were not considered. Subsequently, all isolated myxochelins were fully characterized by NMR and MS analyses (Figures S1–S42).

To assess their 5-LO inhibitory activities, the compounds were tested in a cell-free assay against the recombinant human enzyme. After treatment, the 5-LO-mediated conver-

Table 1. Chemical structures and IC_{50} values of myxochelins for the inhibitory effects on 5-LO.



	R^1	R^2	R^3	R^4	R^5	R^6	5-LO IC_{50} [μM]	CAS activity
A	OH	OH	H	OH	OH	H	1.715 ± 0.269	++
J1	H	H	H	OH	OH	H	3.147 ± 0.307	n.d.
J2	OH	OH	H	H	H	H	2.390 ± 0.819	n.d.
J3	OH	H	H	OH	OH	H	1.522 ± 0.156	+
J4	OH	OH	H	OH	H	H	3.539 ± 0.029	+
J5	OH	H	H	OH	H	H	9.324 ± 0.305	–
J6	F	H	OH	OH	OH	H	1.442 ± 0.063	–
J7	OH	OH	H	F	H	OH	1.180 ± 0.300	–
J8	F	H	OH	F	H	OH	3.600 ± 0.733	–
J9	F	H	H	OH	OH	H	8.395 ± 0.676	n.d.
J10	OH	OH	H	F	H	H	8.094 ± 0.793	n.d.
J11	F	H	H	F	H	H	> 10	n.d.
J12	H	F	H	H	F	H	> 10	n.d.
J13	Cl	H	H	OH	OH	H	4.596 ± 1.690	n.d.
J14	OH	OH	H	Cl	H	H	3.640 ± 0.440	n.d.

The activity of the myxochelins in the CAS assay was ranked visually. ++: strong, +: weak, –: no iron affinity, n.d.: not determined.

sion of arachidonic acid into the corresponding 5-hydro-(pero)xyeicosatetraenoic acid was determined by HPLC.[17] In this assay myxochelins **J11** and **J12** were the only compounds that failed to inhibit 5-LO product formation, even at high concentrations (> 10 μM, Table 1). It is interesting that these are the only analogues devoid of aromatic hydroxy groups, thus suggesting a correlation of such moieties with 5-LO inhibition. Contrary to our original expectation, removal of either or both catechol units in **1** did not necessarily result in loss of bioactivity. Several derivatives that featured a single catechol unit turned out to be potent inhibitors, and three such molecules—that is, myxochelins **J3**, **J6**, and **J7**—showed activity comparable with that of **1** (IC_{50} = 1.72 μM), with IC_{50} values of 1.18 to 1.52 μM. In myxochelins **J5** and **J8**, the 2,3-DHBA moieties of **1** are replaced by salicylate and 6-fluorosalicylate residues. Whereas myxochelin **J5** is a relatively weak 5-LO inhibitor, myxochelin **J8** suppressed 5-LO activity with an IC_{50} value of 3.6 μM. It is hence obvious that the catechol units of **1** are not indispensable for the inhibition of 5-LO.

Next, we evaluated the iron affinities of the myxochelins in the well-established chrome azurol S (CAS) assay.[18] Several of the new derivatives gave only weak color changes relative to **1**, indicating that they barely removed the ferric iron from the dye complex, and so we are inclined to discount marked iron-chelating features of the compounds (Table 1, Figure S43). This finding was particularly noteworthy for myxochelins **J6** and **J7**, which were among the most potent 5-LO inhibitors. We therefore conclude that the 5-LO inhibitory effects of myxochelins do not parallel their iron affinities.

In summary, we have demonstrated that myxochelin analogues can be efficiently generated by PDB. Feeding experiments involving a single aromatic substrate usually give access to multiple derivatives, due to randomized incorporation at the two possible positions. Although untested, this peculiarity of myxochelin biosynthesis might be exploitable in a combinatorial fashion to introduce two different, non-natural building blocks at the same time. A total of 14 new myxochelin analogues were isolated and characterized in this study. All derivatives were produced in greater amounts than the parental molecule; this suggests that the cellular pool of 2,3-DHBA is a limiting factor for myxochelin biosynthesis in *P. fallax*. From a chemical perspective, the successful incorporation of 2-fluoro- and 2-chlorobenzoic acids has introduced handles that could enable further derivatization of the myxochelin skeleton.[19–21] Even more noteworthy are the new insights into the structure–activity relationships of the myxochelins. The catechol residues and, thus, the presence of iron-complexing bidentate ligand groups turned out to be non-essential for 5-LO inhibition. Furthermore, the bioactivity data for the PDB-derived myxochelins **J3**, **J6**, and **J7** represent promising starting points for further optimization studies.

Experimental Section

General experimental procedures: HPLC experiments were conducted with a Shimadzu HPLC system (LC-20AT, SPD-M20A). One- and two-dimensional NMR spectra were recorded at 300 K with Bruker Avance spectrometers and with [D_4]MeOH as solvent and internal standard. The solvent signals were referenced to δ_H = 3.31 ppm and δ_C = 49.0 ppm, respectively. High-resolution (HR) mass determination was performed with an Exactive Mass Spectrometer (Thermo-Scientific).

Preparation of 1-fluoro-3-(methoxymethoxy)benzene: Ethyldiisopropylamine (20 mmol) was added to a stirred solution of 3-fluorophenol (10 mmol) in dry dichloromethane (CH_2Cl_2, 10 mL). The mixture was cooled to 0 °C, after which chloro(methoxy)methane (15 mmol) was added. This reaction mixture was stirred at 0 °C for 10 min and subsequently at room temperature for 2 h. The reaction was quenched by the addition of water (10 mL). Organic and aqueous phase were separated in a separation funnel. The aqueous phase was exhaustively extracted with CH_2Cl_2. The reaction product was purified as a colorless liquid from the combined organic phases by silica gel column chromatography with use of a mixture of ethyl acetate (EtOAc) and dichloromethane as eluent (yield: 96.0 %).

1-Fluoro-3-(methoxymethoxy)benzene: ^{1}H NMR (500 MHz, [D_4]MeOH): δ = 7.24 (dt, $J_{H,H}$ = 8.4, $J_{H,F}$ = 6.9 Hz, 1 H; CH-5), 6.83 (ddd, $J_{H,H}$ = 8.4, 2.4, 0.8 Hz, 1 H; CH-4), 6.77 (dt, $J_{H,H}$ = 2.4, $J_{H,F}$ = 11.1 Hz, 1 H; CH-2), 6.70 (ddt, $J_{H,H}$ = 8.4, 2.4, 0.8, $J_{H,F}$ = 8.4 Hz, 1 H; CH-6), 5.16 (s, 2 H; CH_2-7), 3.43 ppm (s, 3 H; CH_3-8); ^{13}C NMR (125 MHz, [D_4]MeOH): δ = 164.9 (d, $J_{C,F}$ = 244.0 Hz; C-1), 160.1 (d, $J_{C,F}$ = 11.2 Hz; C-3), 131.4 (d, $J_{C,F}$ = 10.3 Hz; C-5), 113.1 (d, $J_{C,F}$ = 2.7 Hz; C-4), 109.3 (d, $J_{C,F}$ = 21.8 Hz; C-6), 104.7 (d, $J_{C,F}$ = 25.3 Hz; C-2), 95.5 (C-7), 56.3 ppm (C-8).

Preparation of 2-fluoro-6-hydroxybenzoic acid: 1-Fluoro-3-(methoxymethoxy)benzene (5.3 mmol) was dissolved in dry THF (5 mL) and dry *n*-hexane (2 mL). The solution was cooled to −78 °C in an acetone/dry ice bath, after which *n*-butyllithium (5.3 mmol) was added. This mixture was stirred at −78 °C for 2 h. Subsequently, the formed metalate was carboxylated by bubbling a stream of dry CO_2 through the cooled solution. The sample was allowed to warm to room temperature, and hydrochlorous acid (0.5 N, 10 mL) was

added. The mixture was stirred for 30 min and afterwards the organic solvents were distilled off. The aqueous phase was washed three times with EtOAc. After evaporation of the water, the crude product was purified by RP-HPLC. In total, 487 mg of 2-fluoro-6-hydroxybenzoic acid were isolated (yield: 58.8%).

2-Fluoro-6-hydroxybenzoic acid: ^{1}H NMR (300 MHz, [D$_4$]MeOH): δ = 7.40 (dt, $J_{H,H}$ = 8.4, $J_{H,F}$ = 6.0 Hz, 1H; CH-4), 6.74 ($J_{H,H}$ = 8.4, 1.0 Hz, 1H; CH-5), 6.62 ppm (ddd, $J_{H,H}$ = 8.4, 1.0, $J_{H,F}$ = 11.0 Hz, 1H; CH-3); ^{13}C NMR (75 MHz, [D$_4$]MeOH): δ = 171.9 (d, $J_{C,F}$ = 3.1 Hz; C-7), 64.4 (d, $J_{C,F}$ = 4.1 Hz; C-6), 163.9 (d, $J_{C,F}$ = 259.1 Hz; C-2), 136.2 (d, $J_{C,F}$ = 12.3 Hz; C-4), 113.9 (d, $J_{C,F}$ = 3.8 Hz; C-5), 107.6 (d, $J_{C,F}$ = 23.2 Hz; C-3), 104.3 ppm (d, $J_{C,F}$ = 14.5 Hz; C-1).

Incorporation studies: For the determination of substrate utilization, *P. fallax* HKI 727 was grown in MD1 medium [100 mL, casitone (0.3%), $CaCl_2 \cdot 2H_2O$ (0.07%), $MgSO_4 \cdot 7H_2O$ (0.2%), vitamin B_{12} (0.5 mg L^{-1}), trace elements solution SL-4 (1.0 mL L^{-1})] with Amberlite XAD7HP (2%). Substrates were added as filter-sterilized solutions (75 mg L^{-1}) prior to inoculation with *P. fallax*. The cultivation was carried out at 30 °C under oxic conditions and with gentle shaking (130 rpm). After seven days of incubation, the adsorber resin was separated from the culture broth by filtration and exhaustively extracted with acetone. The dried acetone extracts were resuspended in MeOH (0.5 mL) and analyzed by HPLC with use of an OTU LipoMare column (250×4.6 mm, 5 μm; AppliChrom). The solvents used were A) doubly distilled water + 0.1% trifluoroacetic acid, and B) MeOH. Separation of extract components was achieved under the following conditions: 10% B for 5 min, followed by a linear gradient to 100% B within 20 min, and 100% B for another 5 min. The flow rate was 1 mL min^{-1} and the elution of compounds was monitored with a diode array detector.

Production and isolation of myxochelin analogues: Production cultures of *P. fallax* HKI 727 were grown for seven days in MD1 medium (3 L) supplemented with resin (Amberlite XAD7HP, 2%) and the corresponding precursor (100 mg). The resin was recovered by filtration and extracted three times with excess acetone. After removal of the solvent, the residue was dissolved in aqueous MeOH (60%) and extracted three times with equal volumes of CH_2Cl_2. The CH_2Cl_2 layers were combined and subjected to flash open-column chromatography with silica gel C_{18} (30 g, Merck) as stationary phase. Elution started with CH_2Cl_2 (200 mL), followed by EtOAc, acetone, and MeOH (200 mL each). Fractions containing myxochelin analogues were identified by ^{1}H NMR and LC-MS measurements. The isolation of the myxochelin analogues required two successive HPLC purification steps. Initial separation was achieved by use of a gradient of MeOH and water + 0.1% trifluoroacetic acid on a Nucleodur C_{18} HTec column (250×10 mm, 5 μm; Macherey–Nagel) with a flow rate of 2 mL min^{-1}. For myxochelins **J9**, **J10**, **J11**, and **J12**, a gradient from 70 to 100% MeOH within 15 min was used, for myxochelins **J3** and **J5** from 70 to 100% within 60 min, for myxochelins **J1** and **J2** from 50 to 100% within 15 min, for myxochelins **J13** and **J14** from 60 to 100% within 15 min, and for myxochelins **J6**, **J7**, and **J8** from 10 to 100% within 10 min. Final purification of each analogue was achieved with isocratic 50% (myxochelins **J9**, **J10**, **J12**), 55% (myxochelins **J1**, **J2**, **J6**, **J7**, **J8**, **J13**, **J14**), 57.5% and 65% (myxochelin **J3**), and 75% (myxochelin **J11**) MeOH in water + 0.1% trifluoroacetic acid on a Nucleodur C_{18} Isis column (250×10 mm, 5 μm; Macherey–Nagel) with a flow rate of 2 mL min^{-1}. The elution of compounds was detected by wavelength monitoring at 210, 245, and 310 nm.

Myxochelin J1: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ = 7.79 (dd, $J_{H,H}$ = 8.0, 1.3 Hz, 1H; CH-2″), 7.79 (dd, $J_{H,H}$ = 8.0, 1.3 Hz, 1H; CH-6″), 7.50 (ddt, $J_{H,H}$ = 7.4, 1.3, 1.3 Hz, 1H; CH-4″), 7.42 (t, $J_{H,H}$ = 8.0, 7.4 Hz, 1H; CH-3″), 7.42 (t, $J_{H,H}$ = 8.0, 7.4 Hz, 1H; CH-5″), 7.18 (dd, $J_{H,H}$ = 8.0, 1.5 Hz, 1H; CH-6′), 6.91 (dd, $J_{H,H}$ = 8.0, 1.5 Hz, 1H; CH-4′), 6.68 (t, $J_{H,H}$ = 8.0 Hz, 1H; CH-5′), 4.13 (m, 1H; CH-2), 3.63 (dd, $J_{H,H}$ = 11.2, 5.8 Hz, 1H; CH-1a), 3.61 (dd, $J_{H,H}$ = 11.2, 5.5 Hz, 1H; CH-1b), 3.39 (t, $J_{H,H}$ = 6.9 Hz, 2H; CH_2-6), 1.74 (m, 1H; CH-3a), 1.68 (m, 2H; CH_2-5), 1.64 (m, 1H; CH-3b), 1.50 ppm (m, 2H; CH_2-4); ^{13}C NMR (150 MHz, [D$_4$]MeOH, 300 K): δ = 171.5 (C-7′), 170.6 (C-7″), 150.3 (C-2′), 147.3 (C-3′), 136.0 (C-1″), 132.5 (C-4″), 129.5 (C-3″), 129.5 (C-5″), 128.3 (C-2″), 128.3 (C-6″), 119.5 (C-4′), 119.5 (C-5′), 118.6 (C-6′), 116.8 (C-1′), 65.2 (C-1), 53.2 (C-2), 40.3 (C-6), 31.7 (C-3), 30.3 (C-5), 24.6 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{23}N_2O_5$: 371.1612 [*M*−H]$^-$; found: 371.1610.

Myxochelin J2: ^{1}H NMR (600 MHz, [D$_4$]MeOH, 300 K): δ = 7.75 (dd, $J_{H,H}$ = 8.1, 1.3 Hz, 1H; CH-2′), 7.75 (dd, $J_{H,H}$ = 8.1, 1.3 Hz, 1H; CH-6′), 7.49 (ddt, $J_{H,H}$ = 7.4, 1.3, 1.3 Hz, 1H; CH-4′), 7.41 (t, $J_{H,H}$ = 8.1, 7.4 Hz, 1H; CH-3′), 7.41 (t, $J_{H,H}$ = 8.1, 7.4 Hz, 1H; CH-5′), 7.27 (dd, $J_{H,H}$ = 8.1, 1.5 Hz, 1H; CH-6″), 6.92 (dd, $J_{H,H}$ = 7.9, 1.5 Hz, 1H; CH-4″), 6.69 (t, $J_{H,H}$ = 8.1, 7.9 Hz, 1H; CH-5″), 4.16 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$ = 11.2, 5.5 Hz, 1H; CH-1a), 3.61 (dd, $J_{H,H}$ = 11.2, 5.3 Hz, 1H; CH-1b), 3.39 (m, 2H; CH_2-6), 1.74 (m, 1H; CH-3a), 1.65 (m, 2H; CH_2-5), 1.63 (m, 1H; CH-3b), 1.50 ppm (m, 2H; CH_2-4); ^{13}C NMR (150 MHz, [D$_4$]MeOH, 300 K): δ = 171.4 (C-7″), 170.4 (C-7′), 150.0 (C-2″), 147.3 (C-3″), 135.9 (C-1′), 132.5 (C-4′), 129.5 (C-3′), 129.5 (C-5′), 128.2 (C-2′), 128.2 (C-6′), 119.5 (C-4″), 119.5 (C-5″), 119.0 (C-6″), 117.1 (C-1″), 65.1 (C-1), 52.6 (C-2), 40.7 (C-6), 31.7 (C-3), 30.3 (C-5), 24.6 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{25}N_2O_5$: 373.1758 [*M*+H]$^+$; found: 373.1752.

Myxochelin J3: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ = 7.70 (dd, $J_{H,H}$ = 8.1, 1.5 Hz, 1H; CH-6″), 7.34 (ddd, $J_{H,H}$ = 8.0, 7.1, 1.5 Hz, 1H; CH-4″), 7.27 (dd, $J_{H,H}$ = 8.0, 1.4 Hz, 1H; CH-6′), 6.91 (dd, $J_{H,H}$ = 8.0, 1.4 Hz, 1H; CH-4′), 6.91 (dd, $J_{H,H}$ = 8.0, 1.2 Hz, 1H; CH-3″), 6.84 (ddd, $J_{H,H}$ = 8.1, 7.1, 1.2 Hz, 1H; CH-5″), 6.69 (t, $J_{H,H}$ = 8.0 Hz, 1H; CH-5′), 4.16 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$ = 11.2, 5.5 Hz, 1H; CH-1a), 3.61 (dd, $J_{H,H}$ = 11.2, 5.3 Hz, 1H; CH-1b), 3.39 (t, $J_{H,H}$ = 7.1 Hz, 2H; CH_2-6), 1.75 (m, 1H; CH-3a), 1.68 (m, 2H; CH_2-5), 1.63 (m, 1H; CH-3b), 1.50 ppm (m, 2H; CH_2-4); ^{13}C NMR (150 MHz, [D$_4$]MeOH, 300 K): δ = 171.4 (C-7′), 171.0 (C-7″), 161.2 (C-2″), 150.0 (C-2′), 147.3 (C-3′),134.6 (C-4″), 128.7 (C-6″), 120.0 (C-5″), 119.6 (C-5′), 119.5 (C-4′), 119.0 (C-6′), 118.4 (C-3″), 117.1 (C-1′), 117.0 (C-1″), 65.0 (C-1), 52.6 (C-2), 40.3 (C-6), 31.7 (C-3), 30.3 (C-5), 24.6 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{23}N_2O_6$: 387.1562 [*M*−H]$^-$; found: 387.1564.

Myxochelin J4: ^{1}H NMR (600 MHz, [D$_4$]MeOH, 300 K): δ = 7.80 (dd, $J_{H,H}$ = 7.9, 1.6 Hz, 1H; CH-6′), 7.35 (ddd, $J_{H,H}$ = 8.2, 7.2, 1.6 Hz, 1H; CH-4′), 7.17 (dd, $J_{H,H}$ = 7.9, 1.5 Hz, 1H; CH-6″), 6.90 (dd, $J_{H,H}$ = 7.9, 1.5 Hz, 1H; CH-4″), 6.89 (dd, $J_{H,H}$ = 8.2, 1.2 Hz, 1H; CH-3′), 6.86 (ddd, $J_{H,H}$ = 7.9, 7.2, 1.2 Hz, 1H; CH-5′), 6.68 (t, $J_{H,H}$ = 7.9 Hz, 1H; CH-5″), 4.16 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$ = 11.2, 5.3 Hz, 1H; CH-1a), 3.62 (dd, $J_{H,H}$ = 11.2, 5.2 Hz, 1H; CH-1b), 3.38 (t, $J_{H,H}$ = 7.1 Hz, 2H; CH_2-6), 1.77 (m, 1H; CH-3a), 1.69 (m, 2H; CH_2-5), 1.64 (m, 1H; CH-3b), 1.50 ppm (m, 2H; CH_2-4); ^{13}C NMR (150 MHz, [D$_4$]MeOH, 300 K): δ = 171.5 (C-7″), 170.8 (C-7′), 160.8 (C-2′), 150.2 (C-2″), 147.3 (C-3″), 134.6 (C-4′), 129.2 (C-6′), 120.1 (C-5′), 119.5 (C-4″), 119.5 (C-5″), 118.6 (C-6″), 118.3 (C-3′), 117.4 (C-1′), 116.8 (C-1″), 65.0 (C-1), 52.6 (C-2), 40.3 (C-6), 31.7 (C-3), 30.3 (C-5), 24.6 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{23}N_2O_6$: 387.1562 [*M*−H]$^-$; found: 387.1561.

Myxochelin J5: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ = 7.80 (dd, $J_{H,H}$ = 8.0, 1.6 Hz, 1H; CH-6′), 7.70 (dd, $J_{H,H}$ = 8.0, 1.6 Hz, 1H; CH-6″), 7.35 (ddd, $J_{H,H}$ = 8.3, 7.1, 1.6 Hz, 1H; CH-4′), 7.34 (ddd, $J_{H,H}$ = 8.4, 7.1, 1.6 Hz, 1H; CH-4″), 6.88 (dd, $J_{H,H}$ = 8.3, 1.2 Hz, 1H; CH-3′), 6.87 (dd, $J_{H,H}$ = 8.4, 1.2 Hz, 1H; CH-3″), 6.84 (ddd, $J_{H,H}$ = 8.0, 7.1 Hz, 1.2 Hz,

1H; CH-5″), 6.68 (ddd, $J_{H,H}$=8.0, 7.1, 1.2 Hz, 1H; CH-5′), 4.16 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$=11.2, 5.3 Hz, 1H; CH-1a), 3.62 (dd, $J_{H,H}$=11.2, 5.3 Hz, 1H; CH-1b), 3.39 (t, $J_{H,H}$=7.1 Hz, 2H; CH_2-6), 1.77 (m, 1H; CH-3a), 1.67 (m, 2H; CH_2-5), 1.64 (m, 1H; CH-3b), 1.49 ppm (m, 2H; CH_2-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ=171.0 (C-7″), 170.8 (C-7′), 161.2 (C-2″), 160.9 (C-2′), 134.6 (C-4′), 134.6 (C-4″), 129.2 (C-6′), 128.7 (C-6″), 120.1 (C-5″), 120.0 (C-5′), 118.4 (C-3″), 118.3 (C-3′), 117.4 (C-1″), 117.0 (C-1′), 65.0 (C-1), 52.6 (C-2), 40.3 (C-6), 31.7 (C-3), 30.3 (C-5), 24.6 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{25}N_2O_5$: 373.1758 $[M+H]^+$; found: 373.1754.

Myxochelin J6: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ=7.30 (dt, $J_{H,H}$=8.4, $J_{H,F}$=6.7 Hz, 1H; CH-4″), 7.27 (dd, $J_{H,H}$=8.1, 1.5 Hz, 1H; CH-6′), 6.91 (dd, $J_{H,H}$=7.9, 1.5 Hz, 1H; CH-4′), 6.70 (dd, $J_{H,H}$=8.4, 1.0 Hz, 1H; CH-5″), 6.69 (t, $J_{H,H}$=8.1, 7.9 Hz, 1H; CH-5′), 6.59 (ddd, $J_{H,H}$=8.4, 1.0, $J_{H,F}$=11.8 Hz, 1H; CH-3″), 4.16 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$=11.2, 5.5 Hz, 1H; CH-1a), 3.62 (dd, $J_{H,H}$=11.2, 5.4 Hz, 1H; CH-1b), 3.41 (t, $J_{H,H}$=7.1 Hz, 2H; CH_2-6), 1.76 (m, 1H; CH-3a), 1.69 (m, 2H; CH_2-5), 1.65 (m, 1H; CH-3b), 1.50 ppm (m, 2H; CH_2-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ=171.4 (C-7′), 168.4 (C-7″), 162.9 (d, $J_{C,F}$=5.2 Hz; C-6″), 162.4 (d, $J_{C,F}$=247.4 Hz; C-2″), 150.0 (C-2′), 147.2 (C-3′),134.2 (d, $J_{C,F}$=12.8 Hz; C-4″), 119.5 (C-4′), 119.5 (C-5′), 119.0 (C-6′), 117.1 (C-1′), 114.5 (d, $J_{C,F}$=2.6 Hz; C-5″), 107.2 (d, $J_{C,F}$=14.8 Hz; C-1″), 106.8 (d, $J_{C,F}$=24.6 Hz; C-3″), 65.0 (C-1), 52.6 (C-2), 40.4 (C-6), 31.6 (C-3), 30.1 (C-5), 24.5 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}FN_2O_6$: 407.1613 $[M+H]^+$; found: 407.1615.

Myxochelin J7: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ=7.31 (dt, $J_{H,H}$=8.4, $J_{H,F}$=6.7 Hz, 1H; CH-4′), 7.18 (dd, $J_{H,H}$=8.1, 1.5 Hz, 1H; CH-6″), 6.90 (dd, $J_{H,H}$=7.9, 1.5 Hz, 1H; CH-4″), 6.72 (dd, $J_{H,H}$=8.4, 1.1 Hz, 1H; CH-5′), 6.68 (t, $J_{H,H}$=8.1, 7.9 Hz, 1H; CH-5″), 6.61 (ddd, $J_{H,H}$=8.4, 1.1, $J_{H,F}$=11.9 Hz, 1H; CH-3′), 4.18 (m, 1H; CH-2), 3.66 (dd, $J_{H,H}$=11.2, 4.9 Hz, 1H; CH_2-1a), 3.62 (dd, $J_{H,H}$=11.2, 5.0 Hz, 1H; CH-1b), 3.40 (t, $J_{H,H}$=7.1 Hz, 2H; CH-6), 1.75 (m, 1H; CH-3a), 1.69 (m, 2H; CH_2-5), 1.65 (m, 1H; CH-3b), 1.51 ppm (m, 2H; CH_2-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ=171.5 (C-7″), 168.4 (C-7′), 162.8 (d, $J_{C,F}$=5.4 Hz; C-6′), 162.4 (d, $J_{C,F}$=247.0 Hz; C-2′), 150.3 (C-2″), 147.3 (C-3″), 134.2 (d, $J_{C,F}$=12.9 Hz; C-4′), 119.5 (C-4″), 119.5 (C-5″), 118.6 (C-6″), 116.8 (C-1″), 114.5 (d, $J_{C,F}$=2.7 Hz; C-5′), 107.3 (d, $J_{C,F}$=14.8 Hz; C-1′), 106.8 (d, $J_{C,F}$=24.7 Hz; C-3′), 64.5 (C-1), 52.6 (C-2), 40.3 (C-6), 31.7 (C-3), 30.2 (C-5), 24.4 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}FN_2O_6$: 407.1613 $[M+H]^+$; found: 407.1615.

Myxochelin J8: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ=7.31 (dt, $J_{H,H}$=8.4, $J_{H,F}$=6.7 Hz, 1H; CH-4′), 7.30 (dt, $J_{H,H}$=8.4, $J_{H,F}$=6.7 Hz, 1H; CH-4″), 6.71 (dd, $J_{H,H}$=8.4, 1.1 Hz, 1H; CH-5′), 6.70 (dd, $J_{H,H}$=8.4, 1.1 Hz, 1H; CH-5″), 6.61 (ddd, $J_{H,H}$=8.4, 1.1, $J_{H,F}$=11.8 Hz, 1H; CH-3′),), 6.59 (ddd, $J_{H,H}$=8.4, 1.1, $J_{H,F}$=11.8 Hz, 1H; CH-3″), 4.18 (m, 1H; CH-2), 3.66 (dd, $J_{H,H}$=11.2, 4.9 Hz, 1H; CH_2-1a), 3.62 (dd, $J_{H,H}$=11.2, 4.9 Hz, 1H; CH-1b), 3.42 (t, $J_{H,H}$=7.1 Hz, 2H; CH_2-6), 1.74 (m, 1H; CH-3a), 1.69 (m, 2H; CH_2-5), 1.64 (m, 1H; CH-3b), 1.51 ppm (m, 2H; CH_2-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ=168.4 (C-7′), 168.4 (C-7″), 163.0 (d, $J_{C,F}$=5.0 Hz; C-6′), 162.9 (d, $J_{C,F}$=5.2 Hz; C-6″), 162.5 (d, $J_{C,F}$=246.5 Hz; C-2′), 162.4 (d, $J_{C,F}$=247.3 Hz; C-2″), 134.3 (d, $J_{C,F}$=12.7 Hz; C-4′), 134.2 (d, $J_{C,F}$=12.5 Hz; C-4″), 114.6 (d, $J_{C,F}$=2.4 Hz; C-5′), 114.5 (d, $J_{C,F}$=2.6 Hz; C-5″), 107.2 (d, $J_{C,F}$=14.4 Hz; C-1′), 107.1 (d, $J_{C,F}$=14.8 Hz; C-1″), 106.8 (d, $J_{C,F}$=24.7 Hz; C-3′), 106.7 (d, $J_{C,F}$=24.6 Hz; C-3″), 64.5 (C-1), 52.6 (C-2), 40.4 (C-6), 31.7 (C-3), 30.1 (C-5), 24.3 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{23}F_2N_2O_5$: 409.1570 $[M+H]^+$; found: 409.1570.

Myxochelin J9: ^{1}H NMR (300 MHz, [D$_4$]MeOH, 300 K): δ=7.63 (dt, $J_{H,H}$=7.6, 1.9 Hz, 1H; CH-6″), 7.48 (m, 1H; CH-4″), 7.28 (dd, $J_{H,H}$=7.9, 1.6 Hz, 1H; CH-6′), 7.21 (dt, $J_{H,H}$=7.6, 1.1 Hz, 1H; CH-5″), 7.14 (ddd, $J_{H,H}$=8.3, 1.1, $J_{H,F}$=11.0 Hz, 1H; CH-3″), 6.92 (dd, $J_{H,H}$=7.9, 1.6 Hz, 1H; CH-4′), 6.69 (t, $J_{H,H}$=7.9 Hz, 1H; CH-5′), 4.16 (m, 1H; CH-2), 3.65 (dd, $J_{H,H}$=11.8, 5.5 Hz, 1H; CH-1a), 3.61 (dd, $J_{H,H}$=11.8, 5.5 Hz, 1H; CH-1b), 3.40 (t, $J_{H,H}$=6.9 Hz, 2H; CH_2-6), 1.72 (m, 1H; CH-3a), 1.67 (m, 2H; CH_2-5), 1.61 (m, 1H; CH-3b), 1.50 ppm (m, 2H; CH_2-4); ^{13}C NMR (75 MHz, [D$_4$]MeOH, 300 K): δ=171.4 (C-7′), 167.0 (C-7″), 161.2 (d, $J_{C,F}$=248.8 Hz; C-2″), 150.1 (C-2′), 147.3 (C-3′), 133.8 (d, $J_{C,F}$=8.8 Hz; C-4″), 131.2 (d, $J_{C,F}$=2.8 Hz; C-6″), 125.6 (d, $J_{C,F}$=3.4 Hz; C-5″), 124.6 (d, $J_{C,F}$=14.1 Hz; C-1″), 119.5 (C-4′), 119.5 (C-5′), 119.0 (C-6′), 117.1 (C-1′), 117.1 (d, $J_{C,F}$=22.8 Hz; C-3″), 65.1 (C-1), 52.6 (C-2), 40.7 (C-6), 31.6 (C-3), 30.2 (C-5), 24.5 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}FN_2O_5$: 391.1664 $[M+H]^+$; found: 391.1663.

Myxochelin J10: ^{1}H NMR (300 MHz, [D$_4$]MeOH, 300 K): δ=7.66 (dt, $J_{H,H}$=7.6, 1.8 Hz, 1H; CH-6′), 7.49 (m, 1H; CH-4′), 7.22 (dt, $J_{H,H}$=7.6, 0.9 Hz, 1H; CH-5′), 7.19 (dd, $J_{H,H}$=7.9, 1.4 Hz, 1H; CH-6″), 7.15 (ddd, $J_{H,H}$=8.3, 0.9, $J_{H,F}$=10.9 Hz, 1H; CH-3′), 6.91 (dd, $J_{H,H}$=7.9, 1.4 Hz, 1H; CH-4″), 6.68 (t, $J_{H,H}$=7.9 Hz, 1H; CH-5″), 4.13 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$=11.1, 5.3 Hz, 1H; CH-1a), 3.59 (dd, $J_{H,H}$=11.1, 5.4 Hz, 1H; CH-1b), 3.41 (t, $J_{H,H}$=6.9 Hz, 2H; CH_2-6), 1.74 (m, 1H; CH-3a), 1.71 (m, 2H; CH_2-5), 1.57 (m, 1H; CH-3b), 1.54 ppm (m, 2H; CH_2-4); ^{13}C NMR (75 MHz, [D$_4$]MeOH, 300 K): δ=171.5 (C-7″), 167.1 (C-7′), 161.2 (d, $J_{C,F}$=248.8 Hz; C-2′), 150.3 (C-2″), 147.3 (C-3″), 133.8 (d, $J_{C,F}$=8.5 Hz; C-4′), 131.3 (d, $J_{C,F}$=2.6 Hz; C-6′), 125.5 (d, $J_{C,F}$=3.8 Hz; C-5′), 124.7 (d, $J_{C,F}$=14.1 Hz; C-1′), 119.5 (C-4″), 119.5 (C-5″), 118.6 (C-6″), 117.1 (d, $J_{C,F}$=23.0 Hz; C-3′), 116.8 (C-1″), 64.9 (C-1), 53.1 (C-2), 40.3 (C-6), 31.7 (C-3), 30.2 (C-5), 24.4 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}FN_2O_5$: 391.1664 $[M+H]^+$; found: 391.1663.

Myxochelin J11: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ=7.69 (dt, $J_{H,H}$=7.6, 1.8 Hz, 1H; CH-6′), 7.66 (dt, $J_{H,H}$=7.6, 1.8 Hz, 1H; CH-6″), 7.50 (m, 1H; CH-4′), 7.50 (m, 1H; CH-4″), 7.24 (dt, $J_{H,H}$=7.6, 1.1 Hz, 1H; CH-5″), 7.24 (dt, $J_{H,H}$=7.6, 1.0 Hz, 1H; CH-5′), 7.17 (ddd, $J_{H,H}$=8.3, 1.0, $J_{H,F}$=10.9 Hz, 1H; CH-3′), 7.17 (ddd, $J_{H,H}$=8.3, 1.0, $J_{H,F}$=10.9 Hz, 1H; CH-3″), 4.13 (m, 1H; CH-2), 3.64 (dd, $J_{H,H}$=11.1, 5.4 Hz, 1H; CH-1a), 3.60 (dd, $J_{H,H}$=11.1, 5.4 Hz, 1H; CH-1b), 3.41 (t, $J_{H,H}$=6.9 Hz, 2H; CH_2-6), 1.75 (m, 1H; CH-3a), 1.69 (m, 2H; CH_2-5), 1.61 (m, 1H; CH-3b), 1.54 ppm (m, 2H; CH_2-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ=167.0 (C-7′), 166.9 (C-7″), 161.3 (d, $J_{C,F}$=248.5 Hz; C-2′), 161.3 (d, $J_{C,F}$=248.5 Hz; C-2″), 133.9 (d, $J_{C,F}$=8.6 Hz; C-4′), 133.9 (d, $J_{C,F}$=8.6 Hz; C-4″), 131.4 (d, $J_{C,F}$=2.6 Hz; C-6′), 131.3 (d, $J_{C,F}$=2.4 Hz; C-6″), 125.6 (d, $J_{C,F}$=3.3 Hz; C-5′), 125.6 (d, $J_{C,F}$=3.3 Hz; C-5″), 124.7 (d, $J_{C,F}$=13.1 Hz; C-1′), 124.6 (d, $J_{C,F}$=13.6 Hz; C-1″), 117.1 (d, $J_{C,F}$=23.0 Hz; C-3′), 117.1 (d, $J_{C,F}$=23.0 Hz; C-3″), 64.9 (C-1), 53.2 (C-2), 40.7 (C-6), 31.7 (C-3), 30.2 (C-5), 24.4 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{21}F_2N_2O_3$: 375.1562 $[M-H]^-$; found: 375.1531.

Myxochelin J12: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ=7.63 (ddd, $J_{H,H}$=8.0, 1.6, 0.9 Hz, 1H; CH-6′), 7.59 (ddd, $J_{H,H}$=8.0, 1.6, 0.9 Hz, 1H; CH-6″), 7.55 (ddd, $J_{H,H}$=2.7, 1.6, $J_{H,F}$=9.8 Hz, 1H; CH-2′), 7.50 (ddd, $J_{H,H}$=2.7, 1.6, $J_{H,F}$=9.8 Hz, 1H; CH-2″), 7.45 (dt, $J_{H,H}$=8.0, 2.5 Hz, 1H; CH-5′), 7.43 (dt, $J_{H,H}$=8.0, 2.5 Hz, 1H; CH-5″), 7.26 (m, 1H; CH-4′), 7.24 (m, 1H; CH-4″), 4.13 (m, 1H; CH-2), 3.62 (d, $J_{H,H}$=5.6 Hz, 2H; CH_2-1), 3.39 (m, 2H; CH_2-6), 1.74 (m, 1H; CH-3a), 1.68 (m, 2H; CH_2-5), 1.61 (m, 1H; CH-3b), 1.48 ppm (m, 2H; CH_2-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ=169.0 (d, $J_{C,F}$=2.2 Hz; C-7′), 168.7 (d, $J_{C,F}$=2.2 Hz; C-7″), 164.1 (d, $J_{C,F}$=245.6 Hz; C-3′), 164.1 (d, $J_{C,F}$=245.6 Hz; C-3″), 138.3 (d, $J_{C,F}$=6.6 Hz; C-1′), 138.2 (d, $J_{C,F}$=6.6 Hz; C-1″), 131.5 (d, $J_{C,F}$=7.2 Hz; C-5′), 131.4 (d, $J_{C,F}$=7.2 Hz; C-5″), 124.2 (d, $J_{C,F}$=2.5 Hz; C-6′), 124.0 (d, $J_{C,F}$=3.0 Hz; C-6″), 119.2 (d, $J_{C,F}$=21.7 Hz; C-4′), 119.2 (d, $J_{C,F}$=21.7 Hz; C-4″), 115.3 (d, $J_{C,F}$=23.5 Hz; C-2′), 115.2 (d, $J_{C,F}$=23.4 Hz; C-2″), 65.1 (C-1), 53.3 (C-2), 40.8 (C-6), 31.6 (C-3), 30.3 (C-5), 24.5 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{21}F_2N_2O_3$: 376.1526 $[M-H]^-$; found: 375.1530.

Myxochelin J13: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ = 7.41 (dd, $J_{H,H}$ = 8.0, 1.4 Hz, 1H; CH-3″), 7.38 (m, 1H; CH-4″), 7.35 (dd, $J_{H,H}$ = 7.6, 1.8 Hz, 1H; CH-6″), 7.29 (dd, $J_{H,H}$ = 8.1, 1.4 Hz, 1H; CH-6′), 7.29 (m, 1H; CH-5″), 6.93 (dd, $J_{H,H}$ = 7.9, 1.4 Hz, 1H; CH-4′), 6.70 (t, $J_{H,H}$ = 8.1, 7.9 Hz, 1H; CH-5′), 4.17 (m, 1H; CH-2), 3.65 (dd, $J_{H,H}$ = 11.1, 5.4 Hz, 1H; CH-1a), 3.62 (dd, $J_{H,H}$ = 11.1, 5.3 Hz, 1H; CH-1b), 3.38 (m, 2H; CH$_2$-6), 1.74 (m, 1H; CH-3a), 1.67 (m, 2H; CH$_2$-5), 1.64 (m, 1H; CH-3b), 1.53 ppm (m, 2H; CH$_2$-4); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ = 171.3 (C-7′), 170.1 (C-7″), 150.1 (C-2′), 147.3 (C-3′), 137.8 (C-1″), 132.0 (C-4″), 131.8 (C-2″), 130.9 (C-3″), 129.8 (C-6″), 128.1 (C-5″), 119.5 (C-4′), 119.5 (C-5′), 119.0 (C-6′), 117.1 (C-1′), 65.1 (C-1), 52.7 (C-2), 40.6 (C-6), 31.6 (C-3), 30.1 (C-5), 24.5 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}ClN_2O_5$: 407.1368 $[M+H]^+$; found: 407.1371.

Myxochelin J14: ^{1}H NMR (500 MHz, [D$_4$]MeOH, 300 K): δ = 7.42 (dd, $J_{H,H}$ = 7.4, 1.5 Hz, 1H; CH-3′), 7.41 (dd, $J_{H,H}$ = 7.4, 1.8 Hz, 1H; CH-6′), 7.38 (dt, $J_{H,H}$ = 7.4, 1.8 Hz, 1H; CH-4′), 7.30 (dt, $J_{H,H}$ = 7.4, 1.5 Hz, 1H; CH-5′), 7.21 (dd, $J_{H,H}$ = 8.1, 1.5 Hz, 1H; CH-6″), 6.92 (dd, $J_{H,H}$ = 7.9, 1.5 Hz, 1H; CH-4″), 6.70 (t, $J_{H,H}$ = 8.1, 7.9 Hz, 1H; CH-5″), 4.09 (m, 1H; CH-2), 3.63 (dd, $J_{H,H}$ = 11.1, 5.6 Hz, 1H; CH-1a), 3.58 (dd, $J_{H,H}$ = 11.1, 5.8 Hz, 1H; CH-1b), 3.42 (m, 2H; CH$_2$-6), 1.76 (m, 1H; CH-3a), 1.67 (m, 2H; CH$_2$-5), 1.57 (m, 2H; CH$_2$-4), 1.55 ppm (m, 1H; CH-3b); ^{13}C NMR (125 MHz, [D$_4$]MeOH, 300 K): δ = 171.5 (C-7″), 170.2 (C-7′), 150.3 (C-2″), 147.3 (C-3″), 138.0 (C-1′), 131.9 (C-4′), 131.8 (C-2′), 130.9 (C-3′), 129.8 (C-6′), 128.0 (C-5′), 119.5 (C-4″), 119.5 (C-5″), 118.6 (C-6″), 116.8 (C-1″), 65.1 (C-1), 53.1 (C-2), 40.3 (C-6), 31.6 (C-3), 30.3 (C-5), 24.5 ppm (C-4); HRMS (ESI): *m/z* calcd for $C_{20}H_{24}ClN_2O_5$: 407.1368 $[M+H]^+$; found: 407.1369.

Expression, purification, and activity assay of human recombinant 5-LO: Expression of human recombinant 5-LO was accomplished by use of *E. coli* BL21(DE3) harboring plasmid pT3–5-LO.[17a] Lysis of the cells was achieved with triethanolamine (50 mM)/HCl (pH 8.0), EDTA (5 mM), soybean trypsin inhibitor (60 μg mL^{-1}), phenylmethanesulfonyl fluoride (1 mM), dithiothreitol (1 mM), and lysozyme (1 mg mL^{-1}), followed by sonification (3×15 s). After centrifugation of the homogenate (10 000 *g*, 15 min, 4 °C), the supernatant was centrifuged again (40 000 *g*, 70 min, 4 °C). The remaining supernatant was subjected to affinity chromatography with an ATP-agarose column. Purified 5-LO was diluted in PBS containing EDTA (1 mM) and freshly added ATP (1 mM) and incubated with the myxochelin test substances for 10 min at 4 °C, followed by 30 s at 37 °C. Addition of $CaCl_2$ (2 mM) and arachidonic acid (20 μM) initiated the 5-LO product formation. After 10 min, the reaction was terminated by adding ice-old MeOH (1 mL), and the production of 5-LO metabolites was analyzed by RP-HPLC.[17]

Chrome azurol S (CAS) assay: CAS agar plates were prepared as previously reported by use of dye agar solution (20 mL).[18] Five holes were punched in every agar and filled with the test solutions [50 μL; the myxochelin in question (200 μg) dissolved in MeOH (50 μL)]. The emergence of red halos due to the presence of iron(III)-chelating compounds was evaluated 5 min after spotting.

Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft (DFG) within SFB 1127 (Chemical Mediators in Complex Biosystems) and by the Excellence Graduate School "Jena School for Microbial Communication" (JSMC) is gratefully acknowledged. We thank Andrea Perner and Heike Heinecke for conducting MS and NMR measurements.

Keywords: biosynthesis · lipoxygenases · myxochelin · siderophores · substrate tolerance

[1] a) W. Dawid, *FEMS Microbiol. Rev.* **2000**, *24*, 403–427; b) D. Kaiser, M. Robinson, L. Kroos, *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a000380.
[2] a) M. Nett, G. M. König, *Nat. Prod. Rep.* **2007**, *24*, 1245–1261; b) S. C. Wenzel, R. Müller, *Mol. Biosyst.* **2009**, *5*, 567–574; c) T. F. Schäberle, F. Lohr, A. Schmitz, G. M. König, *Nat. Prod. Rep.* **2014**, *31*, 953–972.
[3] S. Schieferdecker, S. König, C. Weigel, H.-M. Dahse, O. Werz, M. Nett, *Chem. Eur. J.* **2014**, *20*, 15933–15940.
[4] S. Schieferdecker, S. König, A. Koeberle, H.-M. Dahse, O. Werz, M. Nett, *J. Nat. Prod.* **2015**, *78*, 335–338.
[5] B. Kunze, N. Bedorf, W. Kohl, G. Höfle, H. Reichenbach, *J. Antibiot.* **1989**, *42*, 14–17.
[6] O. Rådmark, O. Werz, D. Steinhilber, B. Samuelsson, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2015**, *1851*, 331–339.
[7] a) O. Rådmark, B. Samuelsson, *J. Int. Med. Res.* **2010**, *268*, 5–14; b) Y. Chen, Y. Hu, H. Zhang, C. Peng, S. Li, *Nat. Genet.* **2009**, *41*, 783–792; c) N. Guriec, C. Le Jossic-Corcos, B. Simon, J.-C. Ianotto, A. Tempescul, Y. Dréano, J.-P. Salaün, C. Berthou, L. Corcos, *Biochim. Biophys. Acta Mol. Basis Dis.* **2014**, *1842*, 2096–2105; d) J. Roos, C. Oancea, M. Heinssmann, D. Khan, H. Held, A. S. Kahnt, R. Capelo, E. la Buscató, E. Proschak, E. Pucetti, D. Steinhilber, I. Fleming, T. J. Maier, M. Ruthardt, *Cancer Res.* **2014**, *74*, 5244–5255.
[8] a) E. I. Solomon, J. Zhou, F. Neese, E. G. Pavel, *Chem. Biol.* **1997**, *4*, 795–808; b) A. W. Ford-Hutchinson, M. Gresser, R. N. Young, *Annu. Rev. Biochem.* **1994**, *63*, 383–417.
[9] a) B. Silakowski, B. Kunze, G. Nordsiek, H. Blöcker, G. Höfle, R. Müller, *Eur. J. Biochem.* **2000**, *267*, 6476–6485; b) N. Gaitatzis, B. Kunze, R. Müller, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11136–11141; c) Y. Li, K. J. Weissman, R. Müller, *J. Am. Chem. Soc.* **2008**, *130*, 7554–7555.
[10] N. Gaitatzis, B. Kunze, R. Müller, *ChemBioChem* **2005**, *6*, 365–374.
[11] a) R. J. M. Goss, S. Shankar, A. A. Fayad, *Nat. Prod. Rep.* **2012**, *29*, 870–889; b) H. Sun, Z. Liu, H. Zhao, E. L. Ang, *Drug Des. Dev. Ther.* **2015**, *9*, 823–833; c) S. Weist, R. D. Süssmuth, *Appl. Microbiol. Biotechnol.* **2005**, *68*, 141–150.
[12] a) C. N. Borissow, C. L. Graham, R. T. Syvitski, T. R. Reid, J. Blay, D. L. Jakeman, *ChemBioChem* **2007**, *8*, 1198–1203; b) S. N. Dupuis, T. Veinot, S. M. A. Monro, S. E. Douglas, R. T. Syvitski, K. B. Goralski, S. A. McFarland, D. L. Jakeman, *J. Nat. Prod.* **2011**, *74*, 2420–2424.
[13] S. Grüschow, E. J. Rackham, B. Elkins, P. L. A. Newill, L. M. Hill, R. J. M. Goss, *ChemBioChem* **2009**, *10*, 355–360.
[14] M. F. Kreutzer, H. Kage, J. Herrmann, J. Pauly, R. Hermenau, R. Müller, D. Hoffmeister, M. Nett, *Org. Biomol. Chem.* **2014**, *12*, 113–118.
[15] H. Reichenbach, G. Höfle, *Biotechnol. Adv.* **1993**, *11*, 219–277.
[16] a) A. M. Hill, B. L. Thompson, *Chem. Commun.* **2003**, 1360–1361; b) E. S. Adams, K. L. Rinehart, *J. Antibiot.* **1994**, *47*, 1456–1465; c) T. S. Chen, B. Petuch, J. MacConnell, R. White, G. Dezeny, B. Arison, J. D. Bergstrom, L. Colwell, L. Huang, R. L. Monaghan, *J. Antibiot.* **1994**, *47*, 1290–1294; d) R. J. P. Cannell, M. J. Dawson, R. S. Hale, R. M. Hall, D. Noble, S. Lynn, N. L. Taylor, *J. Antibiot.* **1993**, *46*, 1381–1389.
[17] a) L. Fischer, D. Szellas, O. Rådmark, D. Steinhilber, O. Werz, *FASEB J.* **2003**, *17*, 949–951; b) O. Werz, E. Bürkert, B. Samuelsson, O. Rådmark, D. Steinhilber, *Blood* **2002**, *99*, 1044–1052.
[18] B. Schwyn, J. B. Neilands, *Anal. Biochem.* **1987**, *160*, 47–56.
[19] A. Deb Roy, S. Grüschow, N. Cairns, R. J. M. Goss, *J. Am. Chem. Soc.* **2010**, *132*, 12243–12245.
[20] W. Runguphan, S. E. O'Connor, *Org. Lett.* **2013**, *15*, 2850–2853.
[21] C. F. Martinez-Farina, A. W. Robertson, H. Yin, S. Monro, S. A. McFarland, R. T. Syvitski, D. L. Jakeman, *J. Nat. Prod.* **2015**, *78*, 1208–1214.

Manuscript received: August 31, 2015
Accepted article published: September 29, 2015
Final article published: October 13, 2015