


Cite this: *RSC Adv.*, 2024, 14, 35215

Received 16th August 2024
Accepted 28th October 2024

DOI: 10.1039/d4ra05941g

rsc.li/rsc-advances

Synthesis and antibiotic potential of myxocoumarin-inspired chromene dione analogs†

Antonia Behnsen,^{‡a} Gesa Hertrampf,^{‡a} Sandra Vojnović,^{*b} Jasmina Nikodinović-Runić^b and Tobias A. M. Gulder^{†*ac}

The rapid emergence of antibiotic resistance in recent years poses a substantial global health threat. Thus, the discovery of potent novel antibiotics is of utmost importance. One such compound class with promising antibiotic potential are the myxocoumarins from *Stigmatella aurantiaca* MYX-030, which exhibit exceptional antibiotic activities against several Gram-positive pathogens, including MRSA. Interestingly, the synthetic chromene dione precursors lacking the alkyl side chain also display promising antibiotic potential. Within this work, a focused library of chromene diones resembling the myxocoumarin A core structure was synthesized to explore structure–activity relationships. We were able to identify derivatives equipotent to the natural product but devoid of the alkyl chain and the nitro substituent to significantly facilitate synthetic access.

Introduction

Myxobacteria, Gram-negative bacteria belonging to the class of δ -proteobacteria, are valuable producers of biologically active secondary metabolites.¹ Myxobacterial natural products display a wide structural diversity and novel modes of action.^{1–4} Besides antineoplastic,^{1–4} antiparasitic,² antiviral,^{2–5} and antimalarial^{2–4} properties, many myxobacterial natural products exhibit antibacterial and antifungal activities.^{2–4,6} One clinically relevant example is ixabepilone (1; Fig. 1), an epothilone analog that is an FDA-approved anti-cancer chemotherapeutic for the treatment of metastatic breast cancer.^{7–9} Another prominent example is the polyketide soraphen A_{1α} (2). This broad-spectrum fungicide inhibits fungal acetyl-CoA carboxylase (ACC) in the biosynthesis of fatty acids.^{10–12} A myxobacterial metabolite with antibacterial potential is the macrolactone sorangicin (3), which inhibits the eubacterial RNA polymerase affecting RNA synthesis.^{13,14}

Myxocoumarins A (4) and B (5) were first isolated in 2013 from the strain *Stigmatella aurantiaca* MYX-030.¹⁵ Both natural

products contain a coumarin core structure, a nitro substitution at the aromatic system as well as an unusual, long alkyl side chain. The biological evaluation of myxocoumarin B (5) was enabled by the development of the first total synthesis in 2019.¹⁶ Comprehensive structure activity relationship studies revealed a high antibacterial potential as well as a lack of antifungal properties for 5.¹⁷ In the initial biological evaluation of myxocoumarin A (4), a strong broad range antifungal activity against agriculturally relevant pathogens, including *Blumeria graminis*, *Botrytis cinerea*, *Fusarium culmorum*, *Magnaporthe grisea* and

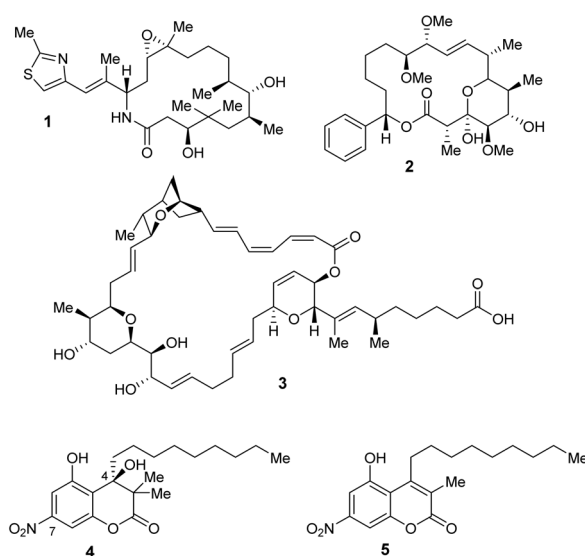


Fig. 1 Structures of the myxobacterial natural products ixabepilone (1), soraphen A_{1α} (2), sorangicin (3), and the myxocoumarins A (4) and B (5).

^aChair of Technical Biochemistry, Technical University of Dresden, Bergstraße 66, 01069 Dresden, Germany. E-mail: tobias.gulder@helmholtz-hips.de

^bInstitute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvodete Stepe 444a, Belgrade, 11000, Serbia. E-mail: sandra.vojnovic@imgge.bg.ac.rs

^cDepartment of Natural Product Biotechnology, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Department of Pharmacy at Saarland University, Campus E8.1, 66123 Saarbrücken, Germany

† Electronic supplementary information (ESI) available: Biological Data, experimental procedures, analytical data and spectra. See DOI: <https://doi.org/10.1039/d4ra05941g>

‡ These authors contributed equally.



Phaeospora nodrum, was found.^{15,18} However, a lack of activity against the human fungal pathogens *Candida auris* and *C. albicans* was observed during the biological investigation of synthetic **4**. Interestingly, **4** was found to exhibit excellent activity against Gram-positive bacteria, including *Staphylococcus aureus*, methicillin-resistant *S. aureus* and *Bacillus subtilis*.¹⁸

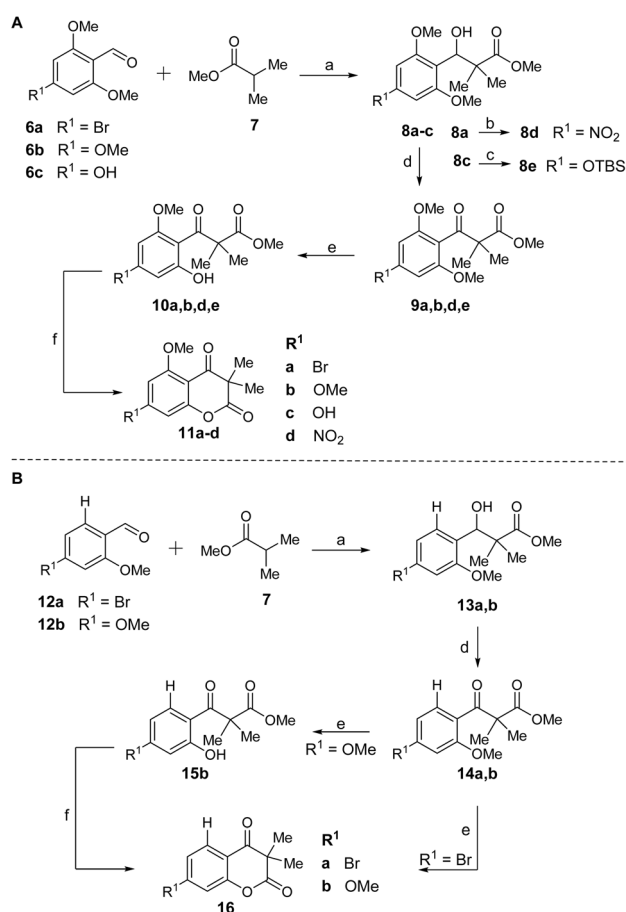
Besides **4** and first derivatives thereof, its direct synthetic chromene dione precursor, with the myxocoumarin substitution pattern in place only lacking the long alkyl side chain, was biologically evaluated as well and found to also have promising activity against Gram-positive bacteria. Thus, we aimed to explore the antibacterial potential of these chromene dione scaffolds more broadly within this work.

Results and discussion

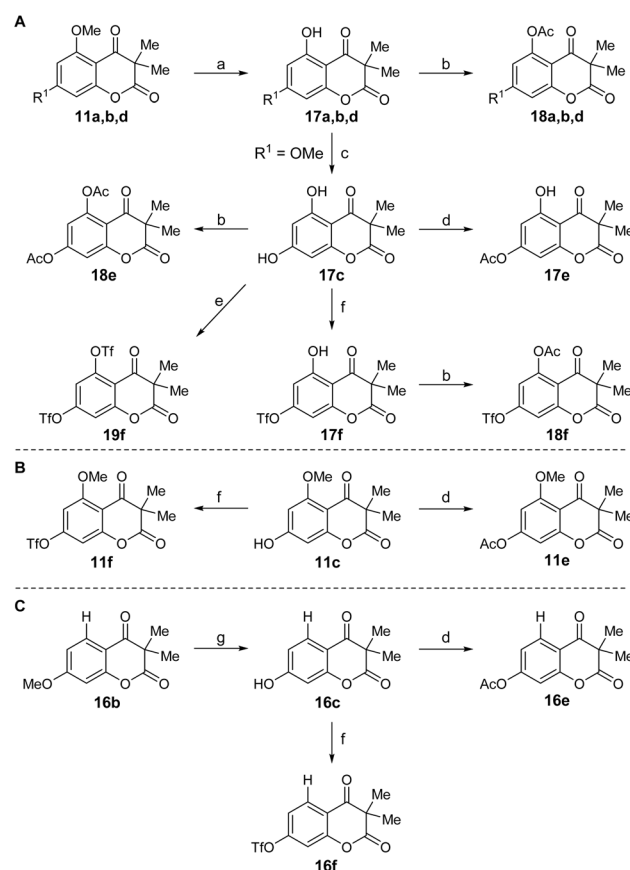
Synthesis of myxocoumarin-based chromene diones

The first step of the total synthesis of **4**, as developed by our group in 2023,¹⁸ employs an aldol addition using methyl

isobutyrate (**7**) and 4-bromo-2,6-dimethoxybenzaldehyde (**6a**) to synthesize β -hydroxy ester **8a** (Scheme 1). The substitution pattern at the aromatic portion can readily be altered by simple exchange of the aldehyde building blocks. Besides **6a**, we thus employed 2,4,6-trimethoxybenzaldehyde (**6b**), 4-hydroxy-2,6-dimethoxybenzaldehyde (**6c**), 4-bromo-2-methoxybenzaldehyde (**12a**) and 2,4-dimethoxybenzaldehyde (**12b**) within the current work to deliver benzylic alcohol **8a–c** and **13a,b**. Compound **8a** was then used to convert into the nitro-bearing derivative **8d** by Pd-catalyzed nitration reaction. The aldol addition was followed by an oxidation with DMP, resulting in the respective β -keto esters **9a,b,d,e** and **14a,b**. Phenolic precursor **8c** had to be TBS-protected for this oxidation step to generate intermediate β -hydroxy ester **8e**. The β -keto esters were converted into **10a,b,d,e** and **15b** by selective *O*-demethylation with BBr₃. Lastly, a FeCl₃-catalyzed *trans*-esterification of **10** and **15b** afforded the cyclized chromene diones **11a–d** and **16b**. For compound **14a**, a direct cyclization was observed in the *O*-demethylation step, resulting in **16a**. To test the influence of the C5 and C7 substituents of the



Scheme 1 Synthetic route to chromene dione derivatives. (A) Synthetic pathway to access derivatives **11a–d**. (B) Synthesis towards chromene diones **16a,b**. Reagents and conditions: (a) 1. *n*-BuLi (1.5 eq.), DIPA (1.5 eq.), THF, -78°C , 1 h; 2. **7** (1.0 eq.), -78°C , 1 h; 3. **6a–c/12a,b** (1.1 eq.), -78°C , 3.5 h. (b) Pd₂(dba)₃ (2.5 mol%), *t*-BuBrettPhos (6.0 mol%), TDA (5.0 mol%), NaNO₂ (2.0 eq.), *t*-BuOH, 100°C , 22 h. (c) Imidazole (1.05 eq.), TBSCl (1.05 eq.), DMF, 0°C to rt, 16 h. (d) DMP (1.1 eq.), DCM, rt, 3–5 h. (e) BBr₃ (1.1 eq.), DCM, -78°C to rt, 13–20 h. (f) FeCl₃·6H₂O (0.5 eq.), DCM, 40°C , 24–72 h.



Scheme 2 Derivatization of chromene diones by selective *O*-demethylation, *O*-acetylation and *O*-triflation starting from **11a,b,d** (A), **11c** (B), **16b** (C), respectively. Reagents and conditions: (a) BBr₃ (1.1–2.5 eq.), DCM, -78°C (**11d**) or 0°C (**11a,b**) to rt, 20 h. (b) Ac₂O (135–200 eq.), pyridine (160–235 eq.), DCM, rt, 21 h. (c) BBr₃ (5.0 eq.), DCM, 0 to 40°C , 3 d. (d) Ac₂O (1.05–1.1 eq.), pyridine (1.05–1.5 eq.), DCM, rt, 17–20 h. (e) Tf₂O (2.2 eq.), pyridine (2.2 eq.), DCM, 0°C to rt, 4.5 h. (f) Tf₂O (1.05–1.1 eq.), pyridine (1.05–1.5 eq.), DCM, 0°C to rt, 4.5 h. (g) BBr₃ (3.5 eq.), DCM, -78°C to rt, 7 d.



chromene diones on their biological properties, chromene diones **11a–d** were further derivatized (Fig. 2 and Scheme 2).

Compounds **17a,b,d** were obtained through selective *O*-demethylation of **11a,b,d** using BBr_3 . To furnish compound **17c**, another *O*-demethylation had to be carried out from derivative **17b**. Additionally, compound **16c** was afforded by selective *O*-demethylation of **16b**. Using hydroxy derivatives **11c**, **16c** and **17a–d**, a selection of *O*-acetylated and *O*-triflated chromene diones was prepared. While compounds **11e,f**, **16e,f** and **17e,f** were obtained by either selective *O*-acetylation or *O*-triflation of the C7 hydroxy group of **11c**, **16c** and **17c**, respectively, **18a,b,d** and **18f** were generated by selective *O*-acetylation of the C5 hydroxy group of **17a–d**. Compounds **18e** and **19f** were made available using **17c** and an excess amount of Ac_2O and Tf_2O , respectively (Scheme 2).

Biological activities

With 23 chromene dione derivatives in hand (Fig. 2), we next investigated their biological profile, particularly focusing on antibacterial activities. All derivatives were initially assessed against the Gram-positive bacterial strain *S. aureus* and the human fungal pathogen *C. auris* which was of interest due to its multidrug resistance to all established antifungal classes. Most derivatives were inactive against both the bacterial and the fungal pathogen at the maximum tested concentrations ($100 \mu\text{g mL}^{-1}$). Derivatives **17b** and **18b** showed weak MIC values of $50 \mu\text{g mL}^{-1}$ against *S. aureus*. However, a total of eight chromene

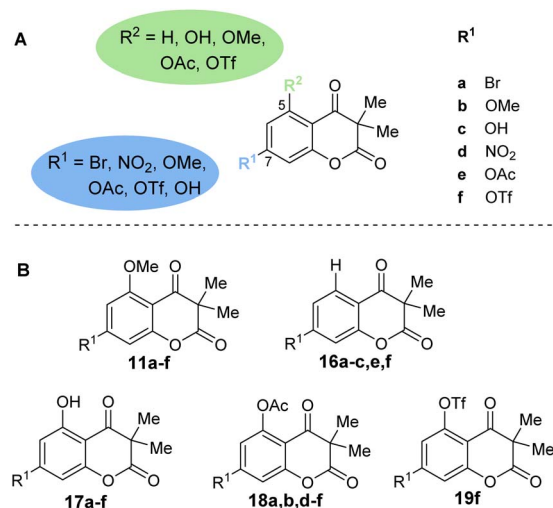
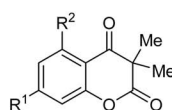


Fig. 2 (A) Structural changes introduced into the chromene dione scaffold. (B) Chemical structures of *O*-methylated (**11**), not substituted (**16**), phenolic (**17**), *O*-acetylated (**18**) and *O*-triflated (**19**) chromene dione analogs.

diones showed interesting antibacterial activity against *S. aureus* and were thus evaluated against a broader range of Gram-positive and Gram-negative bacteria (Table 1), including MRSA, *B. subtilis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Listeria monocytogenes* and *Actinobacter baumannii*. Further,

Table 1 Heat map of the antibacterial and antifungal activity profile (in $\mu\text{g mL}^{-1}$) of chromene diones **11f**, **17a,d,f**, **18a,d,f** and **19f** against *S. aureus* NCTC 6571, *S. aureus* MRSA ATCC 43300, *B. subtilis* NCTC 5398, *E. faecalis* ATCC 29212, *L. monocytogenes* NCTC 1194, *K. pneumoniae* ATCC BAA 2146, *A. baumannii* ATCC 19606, *C. auris* ATCC 21092 and *C. albicans* ATCC 10231 compared to toxicity against MRC5 human fibroblast cells. The activity profile of myxocoumarin A (**4**) is given for comparison^a



Compound	11f	17a	17d	17f	18a	18d	18f	19f	4 ¹⁸
R^1	OTf	Br	NO_2	OTf	Br	NO_2	OTf	OTf	—
R^2	OMe	OH	OH	OH	OAc	OAc	OAc	OTf	—
<i>S. aureus</i>	6.25	3.125	3.125	0.195	3.125	3.125	0.195	0.195	0.6
MRSA	3.125	1.563	3.125	0.098	1.563	3.125	0.195	0.195	0.3
<i>B. subtilis</i>	12.5	1.563	3.125	0.312	3.125	6.25	0.625	0.625	0.3
<i>E. faecalis</i>	6.25	12.5	50	6.25	12.5	50	12.5	12.5	—
<i>L. monocytogenes</i>	>100	12.5	6.25	25	12.5	25	25	>50	—
<i>K. pneumoniae</i>	6.25	12.5	25	6.25	12.5	50	12.5	12.5	—
<i>A. baumannii</i>	>100	6.25	12.5	6.25	6.25	12.5	12.5	>50	—
<i>C. auris</i>	100	3.125	25	12.5	3.125	25	50	>100	200
<i>C. albicans</i>	25	12.5	50	3.125	12.5	50	25	>100	200
MRC5 IC_{50}	20.0	13.5	11.0	6.5	13.5	17.0	8.0	8.5	6.2
Selectivity index	6.4	8.6	3.5	66	8.6	5.4	41	43	20.7

^a NCTC = National Collection of Type Cultures (NCTC, Culture Collection of Public Health, Salisbury, UK). ATCC = American Type Culture Collection (Manassas, Virginia, USA). Green color indicates biological activity (darker green equals higher activity), orange color toxicity (darker orange equals higher toxicity).



the most common opportunistic human fungal pathogen *C. albicans* was also included in the screening.

The triflate chromene dione **17f** exhibited very strong anti-bacterial potential with MIC values as low as $0.195 \mu\text{g mL}^{-1}$ against *S. aureus* and $0.098 \mu\text{g mL}^{-1}$ against MRSA combined with a 33 to 66-fold higher IC_{50} value in MRC5 human fibroblast cells. The triflate derivatives **18f** and **19f** displayed equally strong antibacterial activities with MIC values of $0.195 \mu\text{g mL}^{-1}$ against *S. aureus* and MRSA combined with an up to 43-fold higher IC_{50} value against MRC5. Bromide derivatives **17a** and **18a** as well as nitro-substituted analogs **17d** and **18d** also exhibited strong activities against the Gram-positive strains *S. aureus* and MRSA with MIC values between $1.563 \mu\text{g mL}^{-1}$ and $3.125 \mu\text{g mL}^{-1}$. However, the MRC5 IC_{50} values only were between 3.5 and 8.6-fold higher than the respective MIC values. In relation to their MRC5 IC_{50} values, none of the eight compounds showed promising activities against the Gram-negative strains *K. pneumoniae* and *E. faecalis* nor against the Gram-positive bacterial strain *A. baumannii*.

The observed antibacterial activities make a clear structure activity relationship obvious, with bioactivities only being observed for chromene diones with $\text{R}^1 = \text{Br}$, NO_2 or OTf at C7. The introduction of other functional groups at this position, such as hydroxy, methoxy or acetyl, leads to the loss of antibacterial activity. Similarly, most compounds with antibacterial potential only tolerate hydroxy and acetyl groups at C5 ($\text{R}^2 = \text{OH}$, OAc). The only exception is the C7 triflate derivative, which also possess antibiotic potential with $\text{R}^2 = \text{OTf}$. Surprisingly, the triflate chromene diones tolerate a methoxy group at C5 (**11f**), whilst still displaying good biological activity. Overall, the triflate analogs **17f**, **18f** and **19f** display the strongest antibiotic potential against *S. aureus* and MRSA. In addition, these three chromene diones show stronger antibacterial potential and similar *in vitro* toxicity compared to the natural product myxocoumarin A (**4**) and known derivatives.

Conclusions

In conclusion, our work provides first structure activity data on a novel class of chromene dione antibiotics inspired by the myxocoumarin scaffold. The most potent derivatives possess remarkable sub-nanomolar antibacterial potential, even against MRSA, comparable to the structurally more complex parent natural product myxocoumarin A (**4**). The synthesis of many of the most active analogs is significantly more efficient, with overall reduced step-count and the omission of the relatively low-yielding Pd-catalyzed nitration step required for the synthesis of the natural product. Therefore, these potent chromene dione analogs pose a valuable alternative to the myxo-coumarins, not only as they are more readily accessible by synthesis, but also due to their antifungal activity against *Candida* species, which is not found in the natural product.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

A. B. and G. H. performed all synthetic and analytic work. A. B., S. V., and J. N. R. evaluated the biological activity of the compounds. J. N. R., S. V., and T. A. M. G. conceptualized and supervised the project. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was generously funded by the German Research Foundation (TAMG DFG GU 1233/1-1 and INST 269/971-1), the Ministry of Education, Science and Technological Development of the Republic of Serbia (JNR; agreement no. 451-03-47/2023-01/ 200042) and the DAAD (TAMG, NJR; Deutscher Akademischer Austauschdienst, Bilateral Project of Germany with the Republic of Serbia to SV and TAMG – 2020/2021). We thank Dr T. Lübken and his team (TU Dresden, Organic Chemistry I) for recording NMR spectra.

References

- 1 S. C. Wenzel, and R. Müller, Myxobacteria - unique microbial secondary metabolite factories, *Comprehensive Natural Products II: Chemistry and Biology*, ed. L. Mander, and H.-W. Liu, Elsevier, Amsterdam, 2010, pp. 189–222.
- 2 J. Herrmann, A. Abou Fayad and R. Müller, Natural products from myxobacteria: novel metabolites and bioactivities, *Nat. Prod. Rep.*, 2017, **34**, 135.
- 3 K. J. Weissman and R. Müller, Myxobacterial secondary metabolites: bioactivities and modes-of-action, *Nat. Prod. Rep.*, 2010, **27**, 1276.
- 4 M. A. Bhat, A. K. Mishra, M. A. Bhat, M. I. Banday, O. Bashir, I. A. Rather, S. Rahman, A. A. Shah and A. T. Jan, Myxobacteria as a Source of New Bioactive Compounds: A Perspective Study, *Pharmaceutics*, 2021, **13**, 1265.
- 5 J. P. Martinez, B. Hinkelmann, E. Fleta-Soriano, H. Steinmetz, R. Jansen, J. Diez, R. Frank, F. Sasse and A. Meyerhans, Identification of myxobacteria-derived HIV inhibitors by a high-throughput two-step infectivity assay, *Microb. Cell Factories*, 2013, **12**, 85.
- 6 T. F. Schäberle, F. Lohr, A. Schmitz and G. M. König, Antibiotics from myxobacteria, *Nat. Prod. Rep.*, 2014, **31**, 953.
- 7 K. L. Cheng, T. Bradley and D. R. Budman, Novel microtubule-targeting agents—the epothilones, *Biol. Targets Ther.*, 2008, **2**, 789.
- 8 N. Egerton, Ixabepilone (Ixempra), a Therapeutic Option for Locally Advanced or Metastatic Breast Cancer, *P&T*, 2008, **33**, 523.
- 9 R. H. Alvarez, V. Valero and G. N. Hortobagyi, Ixabepilone for the treatment of breast cancer, *Ann. Med.*, 2011, **43**, 477.



- 10 K. Gerth, N. Bedorf, H. Irschik, G. Höfle and H. Reichenbach, The soraphens: A family of novel antifungal compounds from *Sorangium cellulosum* (Myxobacteria), *J. Antibiot.*, 1994, **47**, 23.
- 11 Y. Shen, S. L. Volrath, S. C. Weatherly, T. D. Elich and L. Tong, A Mechanism for the Potent Inhibition of Eukaryotic Acetyl-Coenzyme A Carboxylase by Soraphen A, a Macrocyclic Polyketide Natural Product, *Mol. Cell*, 2004, **16**, 881.
- 12 A. Naini, F. Sasse and M. Brönstrup, The intriguing chemistry and biology of soraphens, *Nat. Prod. Rep.*, 2019, **36**, 1394.
- 13 H. Irschik, R. Jansen, K. Gerth, G. Höfle and H. Reichenbach, The mechanism of action of myxovalargin A, a peptide antibiotic from *Myxococcus fulvus*, *J. Antibiot.*, 1985, **38**, 1237.
- 14 E. A. Campbell, O. Pavlova, N. Zenkin, F. Leon, H. Irschik, R. Jansen, K. Severinov and S. A. Darst, Structural, Functional, and Genetic Analysis of Sorangicin Inhibition of Bacterial RNA Polymerase, *EMBO J.*, 2005, **24**, 674.
- 15 T. A. M. Gulder, S. Neff, T. Schütz, T. Winkler, R. Gees and B. Böhlendorf, The Myxocoumarins A and B from *Stigmatella aurantiaca* strain MYX-030, *Beilstein J. Org. Chem.*, 2013, **9**, 2579.
- 16 J. I. Müller, K. Kusserow, G. Hertrampf, A. Pavic, J. Nikodinovic-Runic and T. A. M. Gulder, Synthesis and initial biological evaluation of myxocoumarin B, *Org. Biomol. Chem.*, 2019, **17**, 1966.
- 17 G. Hertrampf, K. Kusserow, S. Vojnovic, A. Pavic, J. I. Müller, J. Nikodinovic-Runic and T. A. M. Gulder, Strong Antibiotic Activity of the Myxocoumarin Scaffold *in vitro* and *in vivo*, *Chem.–Eur. J.*, 2022, **28**, 14184.
- 18 G. Hertrampf, S. Vojnovic, J. I. Müller, C. Merten, J. Nikodinovic-Runic and T. A. M. Gulder, Synthesis, Stereochemical Determination, and Antimicrobial Evaluation of Myxocoumarin A, *J. Org. Chem.*, 2023, **88**, 14184.

