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

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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 $A_{1\alpha}$ (**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*, *Magnaprthe grisea* and

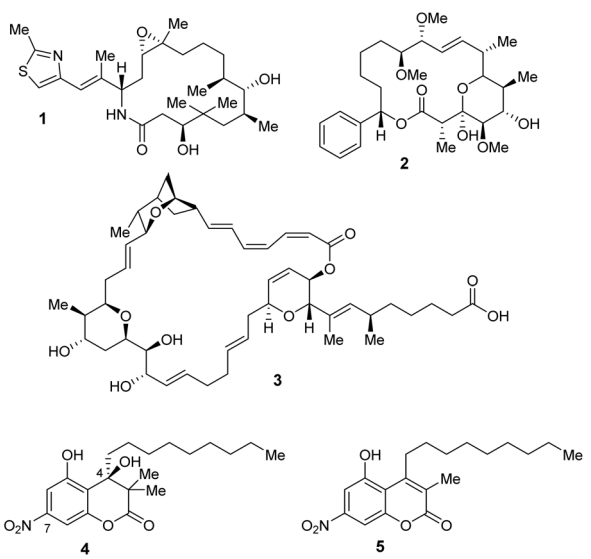

Fig. 1 Structures of the myxobacterial natural products ixabepilone (**1**), soraphen $A_{1\alpha}$ (**2**), sorangicin (**3**), and the myxocoumarins A (**4**) and B (**5**).

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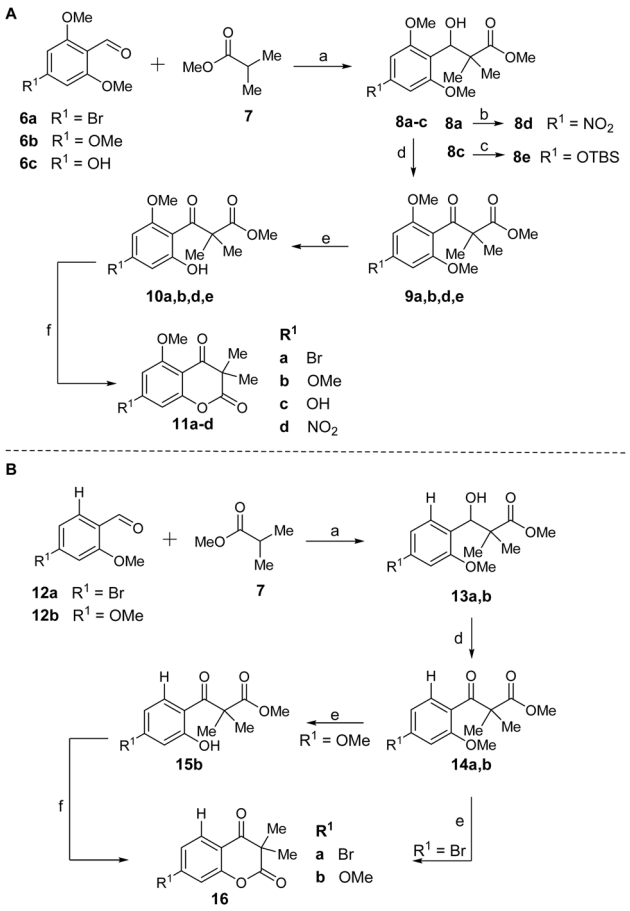
Phaeospaeria nodrum, was found.[15,18] However, a lack of activity against the human fungal pathogens *Candida auris* and *C. albicans* was observed during the biological investigation of synthetic **4**. Interestingly, **4** was found to exhibit excellent activity against Gram-positive bacteria, including *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.

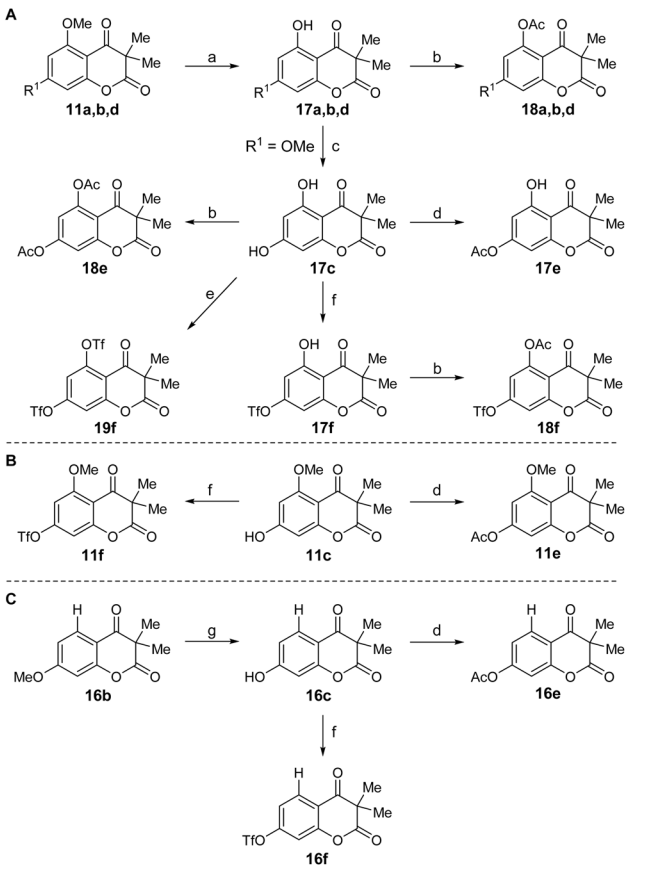
Results and discussion

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 BBr_3. Lastly, a $FeCl_3$-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) $Pd_2(dba)_3$ (2.5 mol%), *t*-BuBrettPhos (6.0 mol%), TDA (5.0 mol%), $NaNO_2$ (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) BBr_3 (1.1 eq.), DCM, −78 °C to rt, 13–20 h. (f) $FeCl_3 \cdot 6H_2O$ (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) BBr_3 (1.1–2.5 eq.), DCM, −78 °C (**11d**) or 0 °C (**11a,b**) to rt, 20 h. (b) Ac_2O (135–200 eq.), pyridine (160–235 eq.), DCM, rt, 21 h. (c) BBr_3 (5.0 eq.), DCM, 0 to 40 °C, 3 d. (d) Ac_2O (1.05–1.1 eq.), pyridine (1.05–1.5 eq.), DCM, rt, 17–20 h. (e) Tf_2O (2.2 eq.), pyridine (2.2 eq.), DCM, 0 °C to rt, 4.5 h. (f) Tf_2O (1.05–1.1 eq.), pyridine (1.05–1.5 eq.), DCM, 0 °C to rt, 4.5 h. (g) BBr_3 (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).

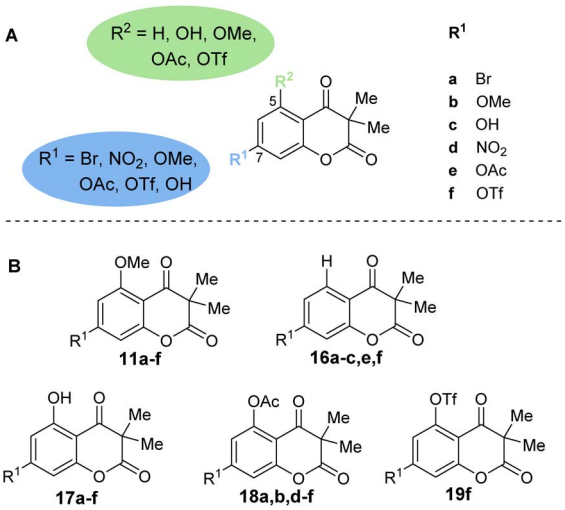
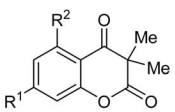


Fig. 2 (A) Structural changes introduced into the chromene dione scaffold. (B) Chemical structures of *O*-methylated (**11**), not substituted (**16**), phenolic (**17**), *O*-acetylated (**18**) and *O*-triflated (**19**) chromene dione analogs.

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 μg mL^{-1}). Derivatives **17b** and **18b** showed weak MIC values of 50 μg mL^{-1} against *S. aureus*. However, a total of eight chromene 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 *Actinerobacter baumannii*. Further,

Table 1 Heat map of the antibacterial and antifungal activity profile (in μg 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	—
S. aureus	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
B. subtilis	12.5	1.563	3.125	0.312	3.125	6.25	0.625	0.625	0.3
E. faecalis	6.25	12.5	50	6.25	12.5	50	12.5	12.5	—
L. monocytogenes	>100	12.5	6.25	25	12.5	25	25	>50	—
K. pneumoniae	6.25	12.5	25	6.25	12.5	50	12.5	12.5	—
A. baumannii	>100	6.25	12.5	6.25	6.25	12.5	12.5	>50	—
C. auris	100	3.125	25	12.5	3.125	25	50	>100	200
C. albicans	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 antibacterial potential with MIC values as low as 0.195 μg mL^{-1} against *S. aureus* and 0.098 μg 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 μg 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 μg mL^{-1} and 3.125 μg 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 R^1 = 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 (R^2 = OH, OAc). The only exception is the C7 triflate derivative, which also possess antibiotic potential with R^2 = 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.

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