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Synthesis and Biological Evaluation of Chromium Bioorganometallics Based on the Antibiotic Platensimycin Lead Structure

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The recent discovery of the natural product platensimycin as a new antibiotic lead structure has triggered the synthesis of numerous organic derivatives for structure–activity relationship studies. Herein, we describe the synthesis, characterization and biological evaluation of the first organometallic antibiotic inspired by platensimycin. Two bioorganometallic compounds containing (η^6-pentamethylbenzene)$Cr(CO)_3$ (**2**) and (η^6-benzene)$Cr(CO)_3$ (**3**), linked by an amide bond to the aromatic part of platensimycin, were synthesized. Their antibiotic activities were tested against *B. subtilis* 168 (Gram positive) and *E. coli* W3110 (Gram negative) bacterial strains. Both compounds were found to be inactive against *E. coli* but derivative **2** inhibits *B. subtilis* growth at a moderate MIC value of 0.15 mM. To test the intrinsic toxicity of chromium, several chromium salts along with {η^6-(3-pentamethylphenyl propionic acid)}$Cr(CO)_3$ (**5**) and {η^6-(3-phenyl propionic acid)}$Cr(CO)_3$ (**6**) were tested against both bacterial strains. No activity was observed against *E. coli* for any of the compounds; *B. subtilis* growth was not inhibited by $Cr(NO_3)_3$ and only very weakly by **5**, $K_2Cr_2O_7$ and Na_2CrO_4 at MIC values of 0.5, 0.68 and 1.24 mM, respectively. Compounds **2**, **3**, **5** and **4** (the pure organic analogue of **2**) show similar cytotoxicity against HeLa, HepG2 and HT-29 mammalian cell lines. Furthermore, the cellular uptake and the intracellular distribution of compounds **2**, **3** and $Cr(NO_3)_3$ in *B. subtilis* were studied using atomic absorption spectroscopy to gain insight in to the possible cellular targets. Compound **2** was found to be readily taken up and distributed almost equally among cytosol, cell debris and cell membrane in *B. subtilis*.

Introduction

Emergence of bacterial resistance to existing antibiotics is a worldwide issue, particularly in hospitals, where antibiotics are heavily used.[1] As bacteria can share their resistance genes, antimicrobial resistance generated in patient care facilities ultimately spreads into surrounding communities. As a result, difficult-to-treat forms of infectious diseases are emerging, such as multidrug-resistant forms of tuberculosis. During the last two decades, the number of antibacterial agents that have been discovered and introduced into the market has steadily declined, and those agents that have been successfully launched failed to meet the challenges posed by emerging resistance to the existing antibiotics.[2] Therefore, new structural classes of antibiotics that exert their activity through novel mechanisms of action are urgently needed. In this context, the recent discovery of platensimycin (**1**), a natural product extracted from *Streptomyces platensis* by Wang et al., was an important contribution to antibiotic research.[3] The compound displays potent activity against Gram-positive bacterial strains (methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*) by selectively inhibiting the FabF enzyme in bacterial fatty acid biosynthesis. It has no effect on mammalian fatty acid biosynthesis. However, due to its low in vivo efficacy, the compound requires further improvement to succeed as a useful therapeutic agent.

Retrosynthetically, platensimycin can be synthesized by amidation of the carboxylic acid containing the tetracyclic cage with a suitably protected derivative of the aromatic amine.[4] The stereochemically complex tetracyclic entity contains six stereocenters and four quaternary carbon atoms; synthesis of this fragment is tedious compared to the straight forward synthesis of the protected aromatic amine.[5,6] Nicolaou et al. completed the first total synthesis of platensimycin in more than fifteen steps.[6] Several analogues of platensimycin were later synthesized and their antibacterial activities evaluated.[7–10] To date, modifications of the complicated tetracyclic cage were exclusively done by replacing it with different organic moieties, believed to fulfill the steric as well as hydrogen bonding re-

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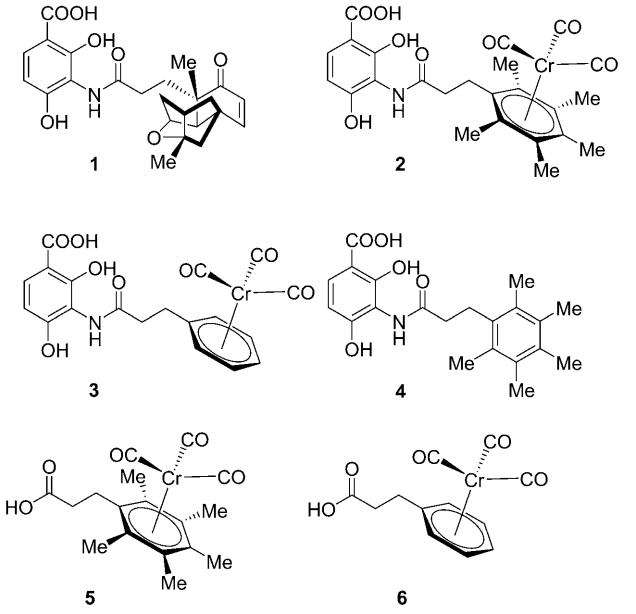


quirement of the tetracyclic cage, while at the same time being easier to synthesize. We were inspired by the possibility of replacing the tetracyclic cage by an organometallic core, and subsequently studying its influence on the bioactivity.

In recent years, organometallic compounds have attracted attention for medicinal applications, mainly in anticancer and antimalarial research.[11–18] First, organometallic agents may overcome resistance to established drugs via new and possibly metal-specific modes of action. Ferrocifens (ferrocene-modified tamoxifen), developed by Jaouen et al., exhibit activity against hormone-independent breast cancer, where hydroxytamoxifen and tamoxifens are inactive.[19,20] Ferroquine, a ferrocene derivative of chloroquine, revealed activity similar to that of the parent compound against a chloroquine-sensitive *P. falciparum* strain (HB3 5CQS), while comparison of activity of ferroquine and chloroquine against chloroquine-resistant *P. falciparum* (Dd2) shows the former is ten times more active than the latter.[21,22] In both cases, the metal fragment is introduced into the skeleton of an established drug with only moderate structural changes. Meggers et al. have described the use of Ru organometallics as potent kinase inhibitors and anticancer agents.[23,24] In their work, the metal compounds bear little resemblance to the original organic drug at first glance. The organometallic fragment rather serves as a versatile template to organize protein-interacting groups in 3D space.

In recent years, metal carbonyl compounds have attracted much attention because of their potential use as CO releasing molecules (CORM) for therapeutic purposes.[25–27] Two metal carbonyl derivatives of acetyl salicylic acid and Fe-nucleoside derivatives have also recently emerged as lead compounds with promising anticancer activity.[28–30] On the other hand, (η^6-arene)$Cr(CO)_3$ derivatives have been extensively described in the literature because of their use in organic synthesis,[31–33] but few biological applications were reported. Shrivastava et al. demonstrated the inhibitory effect of a chromium–Schiff base complex against the growth of *S. dysenteriae*, and its invasive and pathogenic potential.[34] For analytical purposes, the use of benzene-Cr-tricarbonyl derivatives of antiepileptic drugs in a carbonylmetalloimmunoassay (CMIA) has been proposed.[35] Other than that, organochromium compounds are rarely mentioned in a medicinal context. Conversely, chromium organometallics, especially chromium carbonyl derivatives, are highly stable and are among the best investigated classes of organometallic compounds, dating back to the investigations of Hieber and others at the beginning of the 20th century.

With this in mind, we embarked on a program to synthesize chromium-containing antibiotics based on the platensimycin lead structure. The initial concept was to replace the synthetically challenging tetracyclic entity by arene-chromium-tricarbonyl moieties (compounds **2** and **3**), which are sufficiently bulky. Furthermore, the three CO groups attached to the Cr ion may form hydrogen bonds with amino acid residues in the active site of FabF, mimicking the two oxygen atoms of the tetracyclic cage of platensimycin. This assumption was verified with a manual computer docking experiment. Interestingly, compound **2** fits well into the active site and the carbonyl groups of the $Cr(CO)_3$ moiety of **2** could interact via hydrogen bonding with three amino acid residues of the FabF enzyme—though not with the same amino acid residues to which the tetracyclic cage of platensimycin interacts (Figure 1). Encouraged by this initial result, we herein report the synthesis, antimicrobial activity and cytotoxicity studies of two chromium-containing bioorganometallic agents (**2** and **3**), which we be-

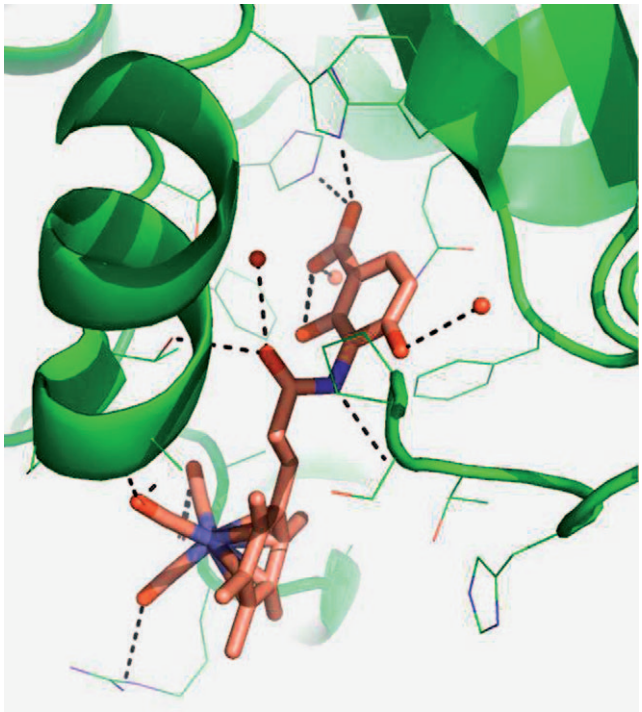


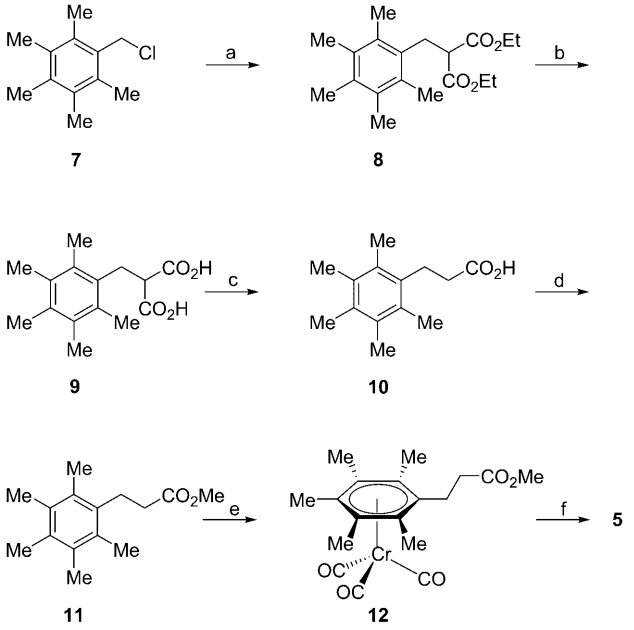
Figure 1. Manual docking of compound **2** in the binding site of FabF. Reasonable atomic coordinates for **2** were obtained from the structure of **14**, by manually replacing the protecting groups with hydrogen atoms. The model obtained was manually fitted into the reported X-ray crystal structure of FabF (PDB code 2fgx).[3]

lieve are the first chromium-based organometallic antibiotics reported thus far in the literature. The presence of the metal atoms allowed us to study the uptake and intracellular distribution of the chromium compounds using atomic absorption spectroscopy, thus gaining a first insight into possible cellular targets.

Results and Discussion

Synthesis and characterization

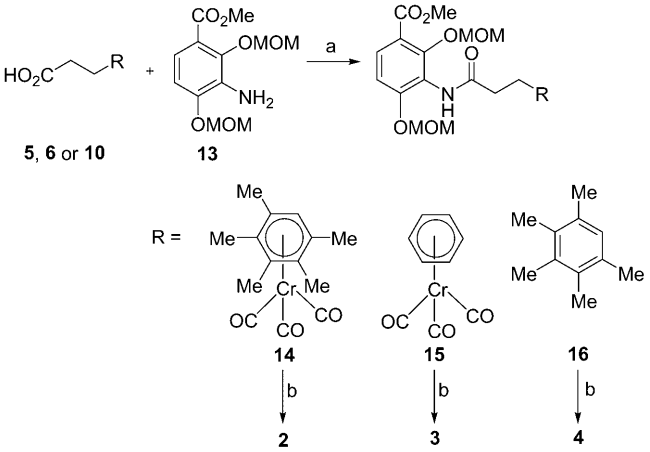
The protected aromatic amine **13** was synthesized from 2, 4-dihydroxymethyl benzoate following a known procedure.[5] {η^6-(3-Pentamethylphenyl propionic acid)}$Cr(CO)_3$ (**5**) was synthesized starting from pentamethylbenzyl chloride **7** via pentamethylphenyl propionate **11** in six steps (Scheme 1). Treatment of



Scheme 1. Synthesis of carboxylic acid **5**. *Reagents and conditions*: a) DEM (1.0 equiv), K_2CO_3 (25.0 equiv), CH_3CN, 75 °C, 50 h, 98%; b) NaOH (15.0 equiv), EtOH, 75 °C, 50 h, 90%; c) 185 °C, 1 h, 84%; d) MeOH, HCl, 65 °C, 12 h, 86%; e) $Cr(CO)_6$ (1.2 equiv), Bu_2O/THF (10:1), 140 °C, 50 h, 92%; f) KOH (21.0 equiv), MeOH/H_2O (1:2), 25 °C, 15 h, 95%.

7 with diethyl malonate (DEM) in the presence of K_2CO_3 gave **8** in 98% yield. The ^{1}H NMR spectrum of **8** showed a multiplet at 3.44 ppm for the α-H atom along with a triplet at 1.12 ppm and a quartet at 4.07 ppm corresponding to the protons of the ethyl ester groups. Ester hydrolysis with ethanolic NaOH solution provided **9** in good yield.[36] The absence of the proton signals of the ethyl ester groups in the ^{1}H NMR spectrum of **9** confirms its formation. Decarboxylation of **9** followed by esterification with MeOH/HCl gave intermediate **11**. The presence of the expected compound **11** was confirmed by ^{1}H NMR spectroscopy with the OMe protons appearing at 3.7 ppm as a singlet. Metalation proceeded smoothly by heating a mixture of **11** and chromium hexacarbonyl to obtain **12** in 92% yield following a procedure described by Smith et al.[37] Formation of **12** was confirmed by ^{13}C NMR signals at 238 ppm corresponding to Cr–*C*O and by IR spectroscopy with two strong stretching bands at 1929 and 1837 cm^{-1}, respectively. Compound **12** was saponified under basic condition conditions to provide carboxylic acid **5** (95% yield).[38] {η^6-(3-Phenyl propionic acid)}$Cr(CO)_3$ (**6**) was synthesized following the literature procedure.[39]

Carboxylic acids **5**, **6** and **10** were then coupled with amine **13** under similar reaction conditions as those described by Nicolaou et al.[6] to provide **14** (53% yield), **15** (63% yield) and **16** (80% yield), respectively (Scheme 2). ^{1}H NMR spectra of **14**, **15**



Scheme 2. HATU mediated amide bond formation followed by ester hydrolysis and MOM deprotection. *Reagents and conditions*: a) **5**, **6** or **10** (1.0 equiv), **13** (1.5 equiv), HATU (3.0 equiv), NEt_3 (3.0 equiv), DMF, 25 °C, 40 h, 53% (**14**), 63% (**15**) and 80% (**16**); b) LiOH·H_2O (50.0 equiv), THF/H_2O (4:1), 45 °C, 16 h; then 4 N HCl in dioxane, 25 °C, 0.5 h, 60% (**2**), 72% (**3**) and 87% (**4**).

and **16** showed two distinct doublets in the aromatic region and four singlets corresponding to the MOM protecting groups, along with five CH_3 signals arising due to the pentamethylbenzene ring (**14** & **16**), or five proton signals from the benzene ring containing the Cr tricarbonyl moiety (**15**). After hydrolyzing the methyl ester of **14**, treatment of the dry reaction mixture with HCl yielded the desired compound **2** in 60% yield. ^{1}H NMR spectroscopy confirmed unambiguously the presence of **2** with the absence of the signals from the MOM and OMe groups. A similar procedure was followed to obtain **3** (72% yield) and **4** (87% yield) from **15** and **16**, respectively.

X-ray crystallography

Single crystals of **14** suitable for X-ray diffraction were grown from acetonitrile at 0 °C. Compound **14** crystallized in the monoclinic space group $P2_1/c$ with two independent molecules in the asymmetric unit; an ORTEP plot of one of the independent molecules is shown in Figure 2 (see figure legend for selected bond distances and angles).[40] The organometallic moiety does not contain any unusual structural features.[39] Hydrogen bonds are formed between neighboring molecules

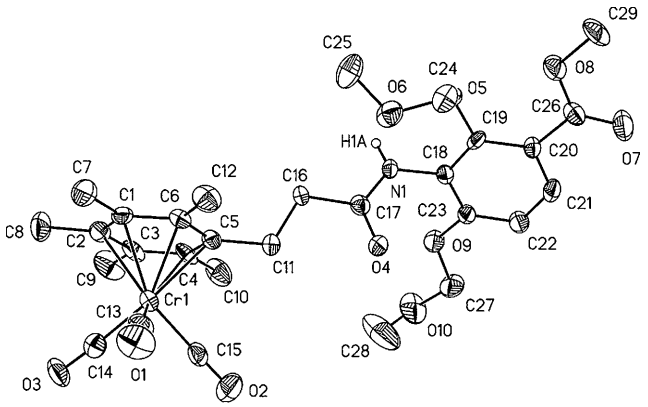


Figure 2. ORTEP plot of compound **14** in the asymmetric unit at 35% probability levels. Selected bond distances (Å) and angles (°): Cr1–C_6-(centroid) = 1.760; Cr1–C(of benzene ring) = 2.209(5)–55(6); Cr1–C (of CO, average) = 1.815(6); C≡O (average) = 1.173(6); C13–Cr1–C14 = 89.5(3); C13–Cr1–C15 = 86.7(3); C14–Cr1–C15 = 90.5(3).

using the amide bond NH and O atoms (N1···O14 = 2.79 Å, N1–H1a···O14 = 153°).

Antimicrobial activity

The antimicrobial activity of the new compounds was tested against *B. subtilis* 168 (Gram +) and *E. coli* W3110 (Gram −) bacterial strains. Minimum inhibitory concentration (MIC) values are listed in Table 1. No inhibition of *E. coli* growth was observed for any of the compounds up to 200 μg mL^{-1}.

Table 1. MIC values of platensimycin, **2**, **3**, **4**, **5**, **6** and some Cr salts against *B. subtilis* and *E. coli*.

Compounds	$MIC_{B.\ subtilis\ 168}$		$MIC_{E.\ coli\ W3110}$	
	[μg mL^{-1}]	[mM]	[μg mL^{-1}]	[mM]
Platensimycin	0.2	0.0005	>88	>0.20[9]
2	80	0.15	>200	>0.40
3	>200	>0.46	>200	>0.46
4	100	0.27	>200	>0.54
5	180	0.50	>200	>0.56
6	>200	>0.70	>200	>0.70
$Cr(NO_3)_3{\cdot}9H_2O$	>200	>0.84	>200	>0.84
$K_2Cr_2O_7$	200	0.68	>200	>0.68
Na_2CrO_4	200	1.24	>200	>1.24

However, the purely organic compound **4** and the chromium organometallic **2** were found to have activity against the Gram-positive bacterial strain selectively. Compounds **4** and **2** inhibit *B. subtilis* growth at a MIC value of 0.27 mM and 0.15 mM, respectively. Hence, putting the $Cr(CO)_3$ moiety into the organic framework of **4** increases the activity against *B. subtilis* by approximately twofold. Although the activity of platensimycin could not be reached by these compounds, the organometallic derivatization strategy led to an enhancement of the bioactivity over a purely organic analogue. A similar case was reported recently for the derivatization of an aspirin analogue as its hexacarbonyl dicobalt complex, which also triggered a significant modulation of the biological properties.[28,41]

Compound **3** is again totally inactive against both bacterial strains up to 200 μg mL^{-1}. To test for the intrinsic bacterial toxicity of chromium, several Cr salts in different oxidation states (+III and +VI), as well as the carboxylic acid **5** and **6** (with Cr in 0 oxidation state) were also tested (see Table 1). No activity was observed against *E. coli* for any of the compounds. *B. subtilis* growth was also not inhibited by $Cr(NO_3)_3$ and only very weakly inhibited at 0.68 and 1.24 mM of $K_2Cr_2O_7$ and Na_2CrO_4, respectively, excluding Cr toxicity as the sole or main mechanism of action.

The antibacterial activity of compounds **2**, **4**, and **5**, and the fact that these compounds were only active against Gram-positive bacteria, encouraged us to carry out further biological testing. To distinguish between bacteriocidal and bacteriostatic action, survival tests were carried out with the active compounds and the Cr salts. *B. subtilis* cells (10^5 cells mL^{-1} of LB medium) were incubated with the compounds at twice the MIC. After exposure for 10 min, cell survival was unaffected in $Cr(NO_3)_3$ medium (1.64 mM), while after 18 h the percentage of survival was reduced to 71% of untreated control cells (see table S1 in the Supporting Information). Interestingly, compounds **2** and **5** caused almost immediate cell death at twice the MIC value, with less than 1% survival after exposure for 10 min. This same feature was also found for compound **4**, indicating a strong bacteriocidal effect for all new compounds. This again proves that chromium toxicity is not the sole or even the main mechanism of action.

Cellular uptake and intracellular distribution in *B. subtilis*

Thanks to the presence of chromium in the compounds, we were able to gain further insight into their activity by determining the uptake and intracellular distribution of the organometallic compounds using atomic absorption spectroscopy (AAS).[42] *B. subtilis* cultures were incubated with the compounds and the Cr content was measured in the whole bacteria, as well as in different cellular components. The results of the cellular uptake study are summarized in Table 2. Overall, bacterial uptake of compound **3** and the salt $Cr(NO_3)_3{\cdot}9H_2O$ was very poor (3% and 2%, respectively) in comparison to compound **2** (30%). The differences in uptake of these compounds indicate that the Cr organometallic derivative **2** is

Table 2. Determination of the cellular uptake (%) by measuring the Cr content in *B. subtilis* cells and the culture medium using atomic absorption spectroscopy.

Compounds	Cr content [nM]			Uptake	Recovery
	culture medium[a]	cell pellet	supernatant	[%]	[%]
Control	2.5	0	2.8	–	–
2	2702.5	799.4	1868	30	99
3	2318.3	77.4	2004	3	90
$Cr(NO_3)_3{\cdot}9H_2O$	4121	62.7	2980	2	74

[a] Complete culture medium with cells.

highly stable. Indeed, if it was unstable and therefore destroyed in the bacterial culture medium, similar Cr content would have been found for compounds **2**, **3** and the Cr salt. On the other hand, lack of activity of compound **3** could be a reflection of its poor uptake.

The results of the intracellular distribution study are summarized in Table 3. Analysis of the cellular components revealed that compound **2** accumulated mainly in the cytosol (32%) and cell debris (34%). Furthermore, the chromium compound was present to a significant amount in the water soluble (20%) and water insoluble fractions (14%) of the membrane, although the cellular membrane fraction is relatively small. This could be indicative of a mode of action involving interference with the membrane structure or function. The fact that compound **2** accumulated both in the cytosolic and membrane fractions could also be indicative of multiple targets as recently reported by Dyson et al. for a dual effect of a Ru organometallic compound for enzyme inhibition purposes.[43] The intracellular distribution data of the compounds and the percentage survival tests indicate that the mechanism of action of these new compounds is somewhat different from platensimycin.

Table 3. Determination of the intracellular distribution of the compounds in *B. subtilis*.[a]

Compounds	Cell debris [%]	Membrane[b] [%]		Cytosol [%]
		insoluble	soluble	
2	34	15	19	32
3	22	2	16	60
$Cr(NO_3)_3{\cdot}9H_2O$	16	0	4	80

[a] Intracellular distribution of chromium in different cellular fractions was determined using atomic absorption spectroscopy. [b] Chromium content in the water soluble and water insoluble fractions of the cytoplasmic membrane fractions.

Cytotoxicity

The notable differences in activity observed against different bacteria led us to test the compounds against mammalian cell lines also. Compounds **2**, **3**, **4**, and **5** showed similar cytotoxicity profiles against HeLa, HepG2 and HT-29 cell lines in both crystal violet and resazurin assays. The IC_{50} values were found to be in a similar range for all Cr compounds (0.01–0.04 mM), but much higher for derivative **4** (up to 0.165 mM, table S2 in the Supporting Information). Contrary to common belief, $Cr(NO_3)_3$ was found to be nontoxic towards all the mammalian cell lines ($IC_{50} > 1$ mM, see table S2 in the Supporting Information). Nonetheless, the fact remains that all chromium organometallics have similar IC_{50} values but differ greatly in their MIC values against *B. subtilis*. A slight change in the structure of the chromium organometallics, such as the replacement of a pentamethylbenzene (**2**) by benzene (**3**), has a dramatic effect on the MIC values.

Conclusions

In conclusion, the synthesis, characterization and biological evaluation of Cr tricarbonyl organometallic compounds, inspired by the platensimycin lead structure, has been described. Although Cr organometallic agents **2**, **3** and **5** were found to be cytotoxic against HeLa, HepG2 and HT-29 mammalian cell lines, only compound **2** had mentionable antimicrobial activity. Importantly, derivative **2** was found to be selective against Gram-positive bacteria, displaying activity against Gram-positive *B. subtilis* but not against Gram-negative *E. coli*. The cellular uptake and intracellular distribution of compounds **2**, **3** and $Cr(NO_3)_3$ in bacteria were studied using AAS. Compound **2** was found to be readily taken up by *B. subtilis* compared to analogue **3**. Apparently, the five methyl groups on the benzene ring help derivative **2** to cross the bacterial cell membrane, which is a preliminary structure–activity relationship (SAR) observation. Compound **2** was found to accumulate in almost equal amounts in the cytosol, cell debris and cell membrane, which might indicate a multicausal cell death mechanism in *B. subtilis*. Chromium accumulation has been studied in environmental toxicology; however, to the best of our knowledge, this is the first study that correlates antibacterial activity of metal compounds with their uptake in bacteria.

Chromate ions (with Cr in +VI oxidation state) are known to be toxic and mutagenic. For this reason, almost no chromium compounds have been considered for medicinal applications to date. The results described here show that there may indeed be opportunities for low-valent organometallic Cr compounds, for which we demonstrate a completely different activity profile. Activities against *B. subtilis* are moderate with MIC values of ~0.15 mM (80 $\mu g\,mL^{-1}$); this certainly needs to be further improved. Nicolaou et al. recently reported extensive SAR studies of platensimycin derivatives;[9] similarly, taking inspiration from their compounds and initial studies described herein, SAR can be performed by varying the metal cores and ligand combinations in order to improve the efficacy. Organometallic medicinal chemistry has so far focused mainly on antitumor agents. The results presented herein should encourage researchers to envision antibiotic activity as a worthwhile property as well. Synthesis of further derivatives and biological evaluation of new metal-containing antibiotics along those lines are under way in our laboratory.[44]

Experimental Section

Materials: All chemicals were of reagent grade quality or better, obtained from commercial suppliers, and used without further purification. Solvents were used as received or dried over 4 Å molecular sieves. All preparations were carried out using standard Schlenk techniques. Amine **13** was prepared following the previously reported procedure.[5] The analytical data match those previously reported.[5,6]

Instrumentation and methods: All reactions of Cr compounds were carried out in the absence of light. ^1H and ^{13}C NMR spectra were recorded in deuterated solvents on Bruker DRX 200, 250, 400 or 600 spectrometers at 30 °C. The chemical shifts (δ) are reported

in parts per million (ppm). The residual solvent peaks were used as an internal reference. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) and br (broad). Infrared spectra were recorded on an ATR unit using a Bruker Tensor 27 FTIR spectrophotometer at 4 cm^{-1} resolution. Signal intensity is abbreviated br (broad), s (strong), m (medium), and w (weak). ESI mass spectra were recorded on a Bruker Esquire 6000. The manual docking of compound **2** with FabF was done using PyMOL (PyMOL-1_2edu1-bin-win32). Crystallographic data for **14** were collected using a Bruker-axs SMART 1000 CCD diffractometer. The structure was solved by direct methods (SHELXS-97[45]) and refined against F^2 with all measured reflections (SHELXL-97,[46] Platon-Squeeze[47]).

Biology

MIC and bacterial survival tests: MIC values for *B. subtilis* 168 and *E. coli* W3110 were determined in test tubes containing Luria broth (LB) medium (2.5 mL) and appropriate compound concentrations up to 200 $\mu g\,mL^{-1}$. The tubes were inoculated with 10^5 cells mL^{-1} and incubated at 37 °C for 18 h. The MIC value was defined as the lowest concentration that inhibited visible growth. To distinguish between bacteriocidal and bacteriostatic action, test tubes containing compound at twice the MIC were inoculated with 10^5 exponentially growing cells mL^{-1}. Aliquots of these cultures were plated on LB agar plates ~10 min after inoculation, as well as after overnight incubation at 37 °C. Colony forming units (CFUs) were counted after incubation of the plates at 37 °C for 18 h.

Compound distribution in different cell fractions and culture medium: *B. subtilis* was grown to an OD_{600} value of 0.5 in LB medium. Cultures were then exposed to compounds **2** (1×MIC; 80 $\mu g\,mL^{-1}$), **3** (200 $\mu g\,mL^{-1}$) or $Cr(NO_3)_3{\cdot}9H_2O$ (200 $\mu g\,mL^{-1}$) for 45 min. A control culture was left untreated. To determine how much of each compound was taken up by cells and how much remained in the supernatant, cells from 10 mL culture medium were harvested by centrifugation (15 min, 16000 *g*, 4 °C). Cr content in the cell pellet and supernatant were compared to the total Cr content in 10 mL of the bacterial cultures. Uninoculated LB medium (10 mL) was used as a control. To investigate the intracellular distribution of Cr, cells were harvested by centrifugation (15 min, 3400 *g*, 4 °C). The pellet was resuspended in buffer (3 mL, 100 mM Tris, 1 mM EDTA, pH 7.5) and cells disrupted with a French pressure cell press (Heinemann, Schwäbisch Gmünd, Germany) at 900 psig by processing samples three times. The cell debris containing mainly the cell walls was harvested by centrifugation (20 min, 16000 *g*, 4 °C). The supernatant was separated into a soluble cytosolic fraction (supernatant) and a membrane fraction (pellet) by ultracentrifugation (2 h, 100000 *g*, 4 °C). The membrane pellet was incubated overnight in buffer (0.5 mL, 100 mM Tris, 1 mM EDTA, pH 7.5). In a second ultracentrifugation step (2 h, 100000 *g*, 4 °C), soluble and insoluble membrane components (supernatant and pellet, respectively) were separated. All fractions were autoclaved and dried to completion.

AAS measurements: For determination of the Cr content, the residues were resuspended in 10 mL distilled H_2O and diluted appropriately in case they afforded AAS signals out of the linear calibration range in the subsequent measurements. For AAS measurements, each 20 μL Triton X-100 (1%) and HNO_3 (13%) were added to 200 μL of the samples. Chromium measurements were taken using a ZEEnit 600 graphite furnace atomic absorption spectrometer (AnalytikJena AG) with Zeeman background correction. Probes were injected at a volume of 20 μL into regular graphite tubes. The heating procedure for the furnace is outlined in detail in table S3 in the Supporting Information. Chromium was detected at a wavelength of 357.9 nm with a bandpass of 0.8 nm. The mean area-under-curve (AUC) absorptions of duplicate injections were used throughout the study. Compounds **2**, **3** and a chromium atomic absorption spectroscopy standard solution (Acros) were used for calibration purposes.

Cell culture, cytotoxicity and determination of inhibitory concentration at 50% growth (IC_{50}): HeLa, HepG2 and HT-29 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, penicillin (100 U mL^{-1}) and streptomycin (100 $\mu g\,mL^{-1}$) in a 5% CO_2 atmosphere. In vitro cytotoxicity of the antimicrobial compounds was studied on HeLa, HT-29 and HepG2 cells. Cell viability, which correlates with the metabolic activity of a cell, was determined by the resazurin assay. In addition to the cell viability, absolute cell numbers were determined by the crystal violet assay, which can be applied after elution of resazurin. Cells were seeded in 96-well cell-culture treated microtiter plates (MTP). After seeding, the cells were grown for 24 h under standard conditions. The compounds were dissolved in culture medium with 0.5% DMSO and applied to the cells in 10, 100, 1000 μM concentrations for 48 h. Every concentration was tested eightfold. Before resazurin was added to the cells, they were washed three times with phenol red-free RPMI 1640 medium. A 10% solution of resazurin (*v*/*v*) in phenol-red-free RPMI 1640 medium was added. Absorbance at 600 nm was directly measured on a Tecan Sapphire 2 microplate reader (Tecan, Germany) at 37 °C. After 2 h of incubation at 37 °C and 5% CO_2, the measurement was repeated. The decrease in absorbance gave the viability. Resazurin was removed and the cells were fixed with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for 15 min at room temperature. PFA was eluted two times with PBS and membranes were permeabilized by Triton X-100 (0.1%) in PBS for 10 min. Afterwards, aq crystal violet solution (0.04%) was added to the cells and the MTP was mechanically shaken for 1 h. The cells were washed with H_2O (×7), and crystal violet was eluted with 70% EtOH for 3.5 h. The absorbance was determined at 570 nm, cell biomass could be calculated after subtraction of 24 h pre-substance incubation absorbance values. The inhibitory concentration at 50% growth (IC_{50}) for both assays was also determined. Therefore, a series of concentrations suitable for the estimated IC_{50} value was applied to the cells according to the procedure described above. The obtained viability or cytotoxicity data were plotted against the concentration on a half-logarithmical scale and a sigmoidal function fit was performed with Origin 7 (Originlab, Northampton, USA) until the fit converged. IC_{50} values were directly calculated from the fit function.

Synthesis

Diester 8: The procedure for the preparation of this compound was reported by R. R. Aitken et al. but no spectroscopic characterization data are available.[36] The procedure was modified to improve the final yield as follows:

A solution of 1-chloromethyl-2,3,4,5,6-pentamethylbenzene (0.98 g, 5 mmol) in dry CH_3CN (15 mL) was added to a solution of diethyl malonate (0.76 mL, 5 mmol) and K_2CO_3 (1.65 g, 12.5 mmol) in dry CH_3CN (40 mL) under Ar and refluxed for 55 h. The reaction mixture was cooled to RT and evaporated to dryness. The solid residue was dissolved in EtOAc, filtered and the filtrate was concentrated in vacuo to give **7** as white powder (1.58 g, 98%), which was used without further purification: $R_f = 0.55$ (silica gel, hexanes/EtOAc,

2:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.12$ (t, 6H, $^3J = 7.13$ Hz, 2CH_3 of the ester), 2.12 (s, 6H, 2× *m*-CH_3), 2.14 (s, 3H, *p*-CH_3), 2.17 (s, 6H, 2× *o*-CH_3), 3.33 (m, 2H, Ph-CH_2), 3.44 (m, 1H, CH), 4.07 ppm (q, 4H, $^3J = 7.11$ Hz, 2CH_2 of the ester); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta =$ 13.9, 16.8, 16.9 (2CH_3 attached to the benzene ring), 29.1, 52.4, 61.3, 132.3, 132.6, 133.1, 133.7, 169.8 ppm (C=O); IR (KBr): $\tilde{\nu} = 3020$ (w), 2936 (w), 1742 (s, CO), 1727 (s, CO), 1464 (m), 1407 (m), 1391 (m), 1366 (m), 1344 (m), 1325 (m), 1301 (m), 1284 (m), 1220 (s), 1174 (s), 1150 (s), 1095 (m), 1063 (m), 1025 (m), 1004 (s), 859 (m), 777 cm^{-1} (m); MS (EI): *m/z* (%): 320.2 (25) $[M]^+$, 302.2 (20) $[M-H_2O]^+$, 161.1 (100) $[M-C_7H_{11}O_4]^+$.

Dicarboxylic acid 9: The procedure for the preparation of this compound was previously reported but no spectroscopic characterization data are available.[36] The procedure was modified to improve the final yield as follows:

A solution of ester (3.79 g, 11.84 mmol) in EtOH (150 mL) was treated with solid NaOH (7.10 g, 177.6 mmol) and refluxed for 20 h under Ar. The resulting suspension was concentrated in vacuo to obtain a white solid, which was redissolved in H_2O (150 mL) and washed with EtOAc to remove the organic impurities. The resulting solution was acidified to pH ~2 with HCl (1 N) and extracted with EtOAc (3×30 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated to give pure **8** as a white solid (2.81 g, 90%): $R_f = 0.21$ (silica gel, EtOAc/MeOH, 10:1); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 2.12$ (s, 9H, 3CH_3), 2.18 (s, 6H, 2CH_3), 3.11 (d, 2H, CH_2), 3.41–3.47 ppm (m, 1H, CH); ^{13}C NMR (250 MHz, CD_3OD): $\delta = 18.5$, 18.5, 18.6, 31.9, 55.2, 134.5, 134.6, 134.9, 135.4, 174.6 ppm (C=O); IR (KBr): $\tilde{\nu} = 2917$ (w), 1702 (s), 1426 (m), 1381 (w), 1330 (w), 1291 (m), 1281 (m), 1174 (m), 1661 (w), 1025 (w), 934 (m), 795 (w), 680 cm^{-1} (w); MS (EI): *m/z* (%): 264 (3.5) $[M]^+$, 220 (32) $[M-CO_2]^+$, 202 (14) $[M-H_2O-CO_2]^+$, 161 (100) $[M-C_3H_3O_4]^+$.

Carboxylic acid 10: β-Pentamethylphenylpropionic acid was obtained following the literature procedure.[36] As the spectroscopic characterization data are not available for this compound, it is given here: $R_f = 0.5$ (silica gel, hexanes/EtOAc, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.16$ (s, 6H, 2 ortho CH_3), 2.20 (s, 9H, 2× *m*- & 1× *p*-CH_3), 2.38–2.47 (m, 2H, CH_2), 2.95–3.04 ppm (m, 2H, CH_2); ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 16.7$, 17.2, 17.3, 26.2, 34.5, 132.1, 133.2, 133.7, 134.2, 180.1 ppm (C=O); IR (KBr): $\tilde{\nu} = 2920$ (m), 2702 (w), 2550 (w), 2359 (w), 2342 (w), 2323 (w), 2272 (w), 2162 (w), 2112 (w), 2049 (w), 1979 (w), 1698 (s, C=O), 1428 (m),1355 (m), 1309 (m), 1246 (w), 1210 (s), 1059 (w), 1045 (w), 997 (w), 945 (m), 820 (w), 742 (w), 690 (w), 617 cm^{-1} (w); MS (EI): *m/z* (%): 220 (46) $[M]^+$, 161 (100) $[M-C_2H_3O_2]^+$, 147 (42) $[M-C_3H_5O_2]^+$.

Methyl ester 11: A solution of **10** (0.794 g, 2.87 mmol) in anhydrous MeOH saturated with HCl (150 mL) was refluxed for 48 h. The reaction was followed by TLC. After consumption of starting material, the reaction was concentrated in vacuo and the residue was redissolved in H_2O (150 mL). The aqueous phase was extracted with EtOAc (3×30 mL), and the combined organic extracts were washed with saturated aq K_2CO_3 and distilled H_2O, then dried over Na_2SO_4, filtered and concentrated to give **11** as light brown powder (0.727 g, 86%): $R_f = 0.65$ (silica gel, hexanes/EtOAc, 2:1); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.19$ (s, 6H, 2× *o*-CH_3), 2.15 (s, 9H, 2× *m*- & 1× *p*-CH_3), 2.41–2.33 (m, 2H, CH_2), 3.01–2.92 (m, 2H, CH_2), 3.70 ppm (s, 3H, OMe); ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 16.7$, 17.2, 17.3, 26.5, 34.4, 52.0, 132.1, 133.1, 133.5, 134.5, 174.0 ppm (C=O); IR (KBr): $\tilde{\nu} = 3004$ (m), 2958 (w), 2856 (w), 2728 (w), 2599 (w), 2162 (w), 2111 (w), 2021 (w), 1725 (s, C=O), 1444 (m), 1373 (w), 1337 (m), 1286 (s), 1264 (s), 1157 (m), 1090 (m), 1050 (s), 1013 (s), 865 (m), 800 (s), 748 (m), 661 (w), 640 (w), 608 cm^{-1} (m); MS (EI): *m/z* (%): 233.8 (10) $[M]^+$, 219.8 (35) $[M-CH_2]^+$, 160.9 (100) $[M-C_3H_5O_2]^+$.

Methyl ester 12: This compound was prepared following the procedure described by Smith et al.[37] with some modifications (1.2 equiv $Cr(CO)_6$ was used instead of 1.09 equiv). The product was obtained as a yellow solid (yield: 92%): $R_f = 0.73$ (silica gel, hexanes/EtOAc 1:1); ^{1}H NMR(250 MHz, $CDCl_3$): $\delta = 2.13$ (s, 6H, 2 ortho CH_3), 2.18 (s, 9H, 2 meta and 1 para CH_3), 2.36–2.45 (m, 2H, CH_2), 3.79–2.92 (m, 2H, CH_2), 3.65 ppm (s, 3H, OCH_3); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta = 16.4$, 16.9, 17.08, 26.8, 34.6, 51.9, 105.6, 106.6, 107.7, 107.8, 175.1 (COOMe), 237.8 ppm (Cr-C=O); IR (KBr): $\tilde{\nu} = 2926$ (w), 2360 (w), 1929 (s)/1837 (s) (C=O attach to Cr), 1733 (s, C=O of ester), 1420 (m), 1383 (m), 1364 (m), 1298 (m), 1257 (m), 1186 (m), 1165 (m), 1071 (w), 981 (m), 892 (m), 819 (w), 803 (m), 761 (w), 727 (w), 674 (s), 637 (s), 609 cm^{-1} (w); MS (ESI +): *m/z* (%): 393.02 (100) $[M+Na]^+$, 408.99 (86) $[M+K]^+$, 381.26 (60) $[M+K-CO]^+$.

Carboxylic acid 5: Ester hydrolysis was carried out following the procedure described by Lavastre et al.[38] with some modifications: H_2O and MeOH were degassed thoroughly before use. Workup involved the removal of MeOH after completion of the reaction and subsequent addition of H_2O. The mixture was acidified to pH 2 with HCl (1 N) forming a yellow precipitate, and extracted using EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated to give **5** as a yellow solid (95%): $R_f = 0.45$ (silica gel, hexanes/EtOAc, 1:1); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 2.13$ (s, 6H, 2× *o*-CH_3), 2.18 (s, 9H, 2× *m*- & 1× *p*-CH_3), 2.34–2.42 (m, 2H, CH_2), 2.78–2.86 ppm (m, 2H, CH_2); ^{13}C NMR (250 MHz, [D_6]acetone): $\delta = 18.6$, 19.1, 19.2, 28.8, 36.5, 109.5, 110.3, 111.1, 112.0, 175.7 (COOH), 238.3 ppm (Cr-C=O); IR (KBr): $\tilde{\nu} = 2961$ (w), 2923 (m), 2854 (w), 1936 (s)/1853 (s) (C=O attach to Cr), 1702 (s, C=O of acid), 1432 (w), 1414 (w), 1356 (w), 1308 (s), 1259 (m), 1211 (w), 1089 (s), 1015 (w), 754 (s), 651 (w), 621 (m), 610 cm^{-1} (m); MS (ESI −): *m/z* (%): 355.02 (100) $[M-H]^-$.

Amide 14: A stirred solution of the carboxylic acid **5** (534 mg, 1.5 mmol) in deoxygenated DMF (25 mL) was treated with HATU (1.70 g, 4.5 mmol) and NEt_3 (454 mg, 4.5 mmol) and the mixture was allowed to stir for 30 min under Ar. Amine **13** (612 mg, 2.25 mmol) was then added and the solution was stirred for a further 40 h at RT. The volume of DMF was then reduced to ~10 mL by in vacuo concentration. Saturated NaCl (100 mL) was added to the mixture, and the product was extracted using EtOAc (3× 30 mL). The organic layer was washed with distilled H_2O (3× 40 mL), saturated NaCl (1×50 mL) and dried over Na_2SO_4 to give a yellow semi-solid after removal of the solvent. Flash column chromatography (silica gel, hexanes/EtOAc, 1:1.5) afforded a yellow solid, which was washed with ice-cold Et_2O (2×30 mL) to give pure **14** as a yellow powder (484.2 mg, 53%) in two rotameric forms (minor and major): $R_f = 0.23$ (silica gel, hexanes/EtOAc, 1:1.5); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.10$, 2.14 (min), 2.23, 2.29, 2.32 (maj) (15H, 3CH_3), 2.63 and 3.02 (maj), 2.85 (min), (br s, 2H, 2CH_2), 3.55 and 3.57 (maj), 3.50 and 3.62 (min) (s, 3H, 2OCH_3), 3.89 (s, 3H, OCH_3), 5.09, 5.29 (s, 2H, 2OCH_2), 7.07 (d, 1H, ring proton), 7.84 (maj), 7.90 (min) (d, 1H, ring proton), 7.60 (maj), 6.86 (min) (s, 1H, NH); ^{13}C NMR (600 MHz, $CDCl_3$): $\delta = 16.3$ (min), 16.8, 17.0, 17.2 (maj), (CH_3), 25.9 (min), 26.3 (maj) (CH_2), 33.6 (min), 37.7 (maj) (CH_2), 52.2 (maj), 52.3 (min) (CO–OMe), 56.6 (maj), 56.9 (min) (CH_2–OMe), 57.2 (maj), 57.7 (min) (CH_2–OMe), 94.7 (OCH_2–OMe), 101.8 (maj), 102.0 (min) (OCH_2–OMe), 105.3, 107.2, 108.0, 108.9 (aromatic C containing $Cr(CO)_3$), 110.5 (min), 111.0 (maj), 117.5 (maj), 117.8 (min), 121.4 (maj), 121.7 (min), 130.7 (maj), 132.2 (min), 154.3 (maj), 155.9 (min), 156.6 (maj), 157.0 (min) (6 aromatic C), 165.0 (min), 165.3 (maj) (NH–CO), 169.2 (maj), 174.0 (min) (CO–OMe),

235.2 ppm (Cr–C=O); IR (KBr): $\tilde{\nu}$ = 1937 (s)/1850 (s) (Cr-C=O),1706 (m, CO-OMe), 1646 (m, NH-CO), 1599 (s), 1553 (s), 1433 (s),1391 (s), 1291 (m), 1289 (s), 1200 (m), 1090 (s), 1050 (m), 893 (s), 785 (s),732 (s), 675 (m), 636 cm^{-1} (m); MS (ESI +): *m/z* (%): 632.13 (100) $[M+Na]^+$, 496.21 (70) $[M+Na-Cr-3CO]^+$.

Amide 15: Prepared as described for **14**. Flash column chromatography (silica gel, hexanes/EtOAc, 1:1.5) gave **15** as a yellow powder (594 mg, 63%): R_f = 0.31 (silica gel, hexanes/EtOAc, 1:1.5); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.72 (br s, 2H, CH_2), 2.82 (br s, 2H, CH_2), 3.48 (s, 3H, OCH_3), 3.51 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.03 (br s, 2H, benzene ring proton), 5.22 (br s, 3H, benzene ring proton), 5.31 (s, 2H, O-CH_2), 5.36 (s, 2H, O-CH_2), 7.01 (d, 1H, ring proton), 7.56 (s, 1H, NH), 7.78 ppm (d, 1H, ring proton); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 30.1, 37.1, 52.2, 56.6, 57.2, 91.0, 93.1, 93.6, 94.6, 101.7, 110.8, 112.1, 117.6, 121.2, 130.8, 154.3, 157.5, 165.3, 168.9, 233.1 ppm; IR (KBr): $\tilde{\nu}$ = 3272 (w), 2954 (w), 1956 (s), 1866 (s), 1720 (s), 1656 (m), 1599 (m), 1522 (m), 1495 (w), 1431 (w), 1308 (m), 1275 (m), 1203 (m), 1107 (m), 1006 (m), 964 (m), 912 (w), 790 (m), 744 (m), 665 (s) cm^{-1}; MS (ESI +): *m/z* (%): 562.04 (100) $[M+Na]^+$.

Amide 16: Prepared as described for **14** with some modifications (CH_2Cl_2 was used for extraction). Flash column chromatography (silica gel, hexanes/EtOAc, 2:1→1:1) gave **16** as a white solid (693 mg, 80%): R_f = 0.46 (silica gel, hexanes/EtOAc, 1:1.5); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.10–2.18 (br m, 15H, 5CH_3), 2.12–2.48 (br m, 2H, CH_2), 2.90–3.21 (br m, 2H, CH_2), 3.39 (s, 3H, O-CH_3), 3.45 (s, 3H, O-CH_3), 3.85 (s, 3H, CO-CH_3), 5.01 (s, 2H, O-CH_2), 5.25 (s, 2H, O-CH_2), 6.89 (d, 1H, benzene ring proton), 7.30–7.45 (br s, 1H, NH), 7.85 ppm (d, 1H, benzene ring proton); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 16.6, 17.1, 17.3, 52.4, 57.1, 57.7, 95.36, 102.0, 111.1, 118.7, 122.3, 130.9, 132.1, 133.1, 133.4, 135.0, 154.7, 157.3, 165.6 (NHCO), 171.0 ppm (COOMe); IR (KBr): $\tilde{\nu}$ = 3248 (w), 2906 (w), 1728 (s), 1661 (s), 1599 (m), 1531 (m), 1479 (m), 1485 (w), 1432 (m), 1397 (m), 1272 (s), 1190 (m),1143 (s), 1083 (w), 1055 (s), 980 (m), 962 (w), 921 (w), 827 (w), 721 cm^{-1} (w); MS (ESI +): *m/z* (%): 496.18 (100) $[M+Na]^+$.

Compound 2: A stirred solution of **14** (950 mg, 1.56 mmol) in degassed THF/H_2O (4:1, 40 mL) was treated with LiOH·H_2O (3.2 g, 78 mmol) under Ar and heated at 45 °C for 16 h. The reaction mixture was dried in vacuo, and degassed HCl (4 N) in dioxane was added (pH ~ 0). The mixture was stirred for 30 min, then diluted with saturated NaCl (100 mL) and extracted with EtOAc (3×30 mL). The combined organic extracts were washed with distilled H_2O (5× 50 mL) and saturated NaCl (2×25 mL), then dried over Na_2SO_4, filtered and concentrated to give a yellow solid. Flash column chromatography (silica gel, EtOAc/MeOH/AcOH, 100:0:0→20:1:0.1) yielded **2** (474 mg, 60%): R_f = 0.37 (silica gel, hexanes/EtOAc/MeOH, 20:1:0.5); ^{1}H NMR (400 MHz, [D_5]pyridine): δ = 1.97 (s, 6H, 2CH_3), 2.04 (s, 3H, 1CH_3), 2.15 (s, 6H, 2CH_3), 2.91–3.05 (br m, 2H, CH_2), 3.16–3.29 (br m, 2H, CH_2), 6.84 (d, 1H, benzene ring proton), 8.09 (d, 1H, benzene ring proton), 9.86 (br s, 1H, NH), 10.74 ppm (s, 1H, OH); ^{13}C NMR (250 MHz, [D_5]pyridine): δ = 19.0, 19.3, 19.5, 30.0, 39.5, 109.3, 109.9, 110.6, 111.0, 112.0, 112.5, 117.3, 132.5, 161.6, 161.8, 175.0 (NHCO), 177.4 (COOH), 238.8 ppm (Cr-C=O); IR (KBr): $\tilde{\nu}$ = 3370 (w), 2928 (br w), 2361 (w), 1860 (s), 1920 (s), 1665 (m), 1632 (m), 1605 (m), 1548 (m), 1382 (m), 1244 (m), 1152 (m), 1063 (m), 783 cm^{-1} (s); MS (ESI +): *m/z* (%): 506.05 (100) $[M-H]^-$.

Compound 3: Prepared as described for **2**. Flash column chromatography (silica gel, EtOAc/MeOH/AcOH, 100:0:0→20:1:0.4) yielded compound **3** as a yellow powder (523 mg, 72%): R_f = 0.62 (silica gel, EtOAc/MeOH/AcOH, 20:1:0.5); ^{1}H NMR (400 MHz, CD_3CN): δ = 2.78–2.83 (m, 2H, CH_2), 2.86–2.91 (m, 2H, CH_2), 5.43 (m, 1H, benzene ring proton), 5.53 (d, 2H, benzene ring proton), 5.61 (m, 2H, benzene ring proton), 6.49 (d, 1H, benzene ring proton), 7.32 (s, 1H, NH), 7.64 (d, 1H, ring proton), 8.36 ppm (s, 1H, OH); ^{13}C NMR (250 MHz, [D_5]pyridine): δ = 23.9, 39.2, 94.1, 96.4, 97.1, 111.1, 115.5, 116.4, 130.7, 131.2, 160.6, 161.8, 174.2, 176.9, 236.7 ppm; IR (KBr): $\tilde{\nu}$ = 3328 (w), 1961 (w), 1561 (w), 1971 (s), 1883 (s), 1638 (s), 1594 (s), 1539 (s), 1468 (m), 1521 (w), 1438 (w), 1332 (m), 1291 (m), 1258 (s), 1185 (w), 1061 (m), 1016 (m), 906 (w), 784 cm^{-1} (s); MS (ESI +): *m/z* (%): 435.98 (100) $[M-H]^-$.

Compound 4: Prepared as described for **2**. Flash column chromatography (silica gel, EtOAc/MeOH/AcOH, 100:0:0→20:1:0.2) yielded compound **4** as a white powder (614 mg, 87%): R_f = 0.28 (silica gel, hexanes/EtOAc/MeOH, 20:1:0.2); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 2.15(s, 9H, 3CH_3), 2.23 (s, 6H, 2CH_3), 2.42 (t, 2H, ^3J = 7.92, CH_2), 2.93 (t, 2H, ^3J = 7.92 CH_2), 6.47 (d, 1H, benzene ring proton), 7.59 (d, 1H, benzene ring proton), 9.11 (s, 1H, NH), 10.20 ppm (br s, 1H, OH); ^{13}C NMR (400 MHz, $(CD_3)_2SO$): δ = 16.6, 17.4 (3CH_3), 27.0, 35.9, 105.4, 108.5, 113.8, 129.7, 131.7, 132.5, 132.7, 135.3, 159.6, 159.8, 172.2 (NHCO), 172.8 ppm (COOH); IR (KBr): $\tilde{\nu}$ = 3322 (w), 2914 (w), 1650 (s), 1632 (s), 1588 (m), 1538 (s), 1456 (w), 1424 (m), 1373 (w), 1314 (m), 1257 (s), 1229m, 1189 (w), 1158 (w), 1064 (w), 982 (w), 824 (w), 985 (m), 683 cm^{-1} (m); MS (ESI +): *m/z* (%): 370.01 (100) $[M-H]^-$.

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[1] D. Häbich, F. von Nussbaum, *ChemMedChem* **2006**, *1*, 951.
[2] M. Leeb, *Nature* **2004**, *431*, 892.
[3] J. Wang, S. M. Soisson, K. Young, W. Shoop, S. Kodali, A. Galgoci, R. Painter, G. Parthasarathy, Y. S. Tang, R. Cummings, S. Ha, K. Dorso, M. Motyl, H. Jayasuriya, J. Ondeyka, K. Herath, C. Zhang, L. Hernandez, J. Allocco, A. Basilio, J. R. Tormo, O. Genilloud, F. Vicente, F. Pelaez, L. Colwell, S. H. Lee, B. Michael, T. Felcetto, C. Gill, L. L. Silver, J. D. Hermes, K. Bartizal, J. Barrett, D. Schmatz, J. W. Becker, D. Cully, S. B. Singh, *Nature* **2006**, *441*, 358.
[4] K. Tiefenbacher, J. Mulzer, *Angew. Chem.* **2008**, *120*, 2582; *Angew. Chem. Int. Ed.* **2008**, *47*, 2548.
[5] P. Heretsch, A. Giannis, *Synthesis* **2007**, *17*, 2614.
[6] K. C. Nicolaou, A. Li, D. J. Edmonds, *Angew. Chem.* **2006**, *118*, 7244; *Angew. Chem. Int. Ed.* **2006**, *45*, 7086.
[7] K. C. Nicolaou, T. Lister, R. M. Denton, A. Montero, D. J. Edmonds, *Angew. Chem.* **2007**, *119*, 4796; *Angew. Chem. Int. Ed.* **2007**, *46*, 4712.

[8] K. C. Nicolaou, Y. Tang, J. Wang, A. F. Stepan, A. Li, A. Montero, *J. Am. Chem. Soc.* **2007**, *129*, 14850.
[9] K. C. Nicolaou, A. F. Stepan, T. Lister, A. Li, A. Montero, G. S. Tria, C. I. Turner, Y. Tang, J. Wang, R. M. Denton, D. J. Edmonds, *J. Am. Chem. Soc.* **2008**, *130*, 13110.
[10] J. Krauss, V. Knorr, V. Manhardt, S. Scheffels, F. Bracher, *Arch. Pharm.* **2008**, *341*, 386.
[11] C. G. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, *38*, 391.
[12] *Bioorganometallics: Biomolecules, Labelling, Medicine*, (Ed.: G. Jaouen), Wiley-VCH, Weinheim, **2006**.
[13] K. Strohfeldt, M. Tacke, *Chem. Soc. Rev.* **2008**, *37*, 1174.
[14] S. S. David, E. Meggers, *Curr. Opin. Chem. Biol.* **2008**, *12*, 194.
[15] N. Metzler-Nolte, *Angew. Chem.* **2001**, *113*, 1072; *Angew. Chem. Int. Ed.* **2001**, *40*, 1040.
[16] N. Metzler-Nolte, *Chimia* **2007**, *61*, 736.
[17] *Concepts and Models in Bioinorganic Chemistry*, (Eds.: H.-B. Kraatz, N. Metzler-Nolte), Wiley-VCH, Weinheim, **2006**.
[18] P. James, J. Neudörfl, M. Eissmann, P. Jesse, A. Prokop, H.-G. Schmalz, *Org. Lett.* **2006**, *8*, 2763.
[19] E. Hillard, A. Vessieres, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem.* **2006**, *118*, 291; *Angew. Chem. Int. Ed.* **2006**, *45*, 285.
[20] A. Nguyen, A. Vessieres, E. A. Hillard, S. Top, P. Pigeon, G. Jaouen, *Chimia* **2007**, *61*, 716.
[21] L. Delhaes, C. Biot, L. Berry, P. Delcourt, L. A. Maciejewski, D. Camus, J. S. Brocard, D. Dive, *ChemBioChem* **2002**, *3*, 418.
[22] C. Biot, G. Glorian, L. A. Maciejewski, J. S. Brocard, O. Domarle, G. Blampain, P. Millet, A. J. Georges, H. Abessolo, D. Dive, J. Lebibi, *J. Med. Chem.* **1997**, *40*, 3715.
[23] J. Maksimoska, L. Feng, K. Harms, C. Yi, J. Kissil, R. Marmorstein, E. Meggers, *J. Am. Chem. Soc.* **2008**, *130*, 15764.
[24] J. Maksimoska, D. S. Williams, G. E. Atilla-Gokcumen, K. S. M. Smalley, P. J. Carroll, R. D. Webster, P. Filippakopoulos, S. Knapp, M. Herlyn, E. Meggers, *Chem. Eur. J.* **2008**, *14*, 4816.
[25] P. Sawle, J. Hammad, I. J. S. Fairlamb, B. Moulton, C. T. O'Brien, J. M. Lynam, A. K. Duhme-Klair, R. Foresti, R. Motterlini, *J. Pharmacol. Exp. Ther.* **2006**, *318*, 403.
[26] A. J. Atkin, S. Williams, P. Sawle, R. Motterlini, J. M. Lynama, I. J. S. Fairlamb, *Dalton Trans.* **2009**, 3653.
[27] I. J. S. Fairlamb, J. M. Lynam, B. E. Moulton, I. E. Taylor, A. K. Duhme-Klair, P. Sawleb, R. Motterlini, *Dalton Trans.* **2007**, 3603.
[28] I. Ott, B. Kircher, C. P. Bagowski, D. H. W. Vlecken, E. B. Ott, J. Will, K. Bensdorf, W. S. Sheldrick, R. Gust, *Angew. Chem.* **2009**, *121*, 1180; *Angew. Chem. Int. Ed.* **2009**, *48*, 1160.
[29] I. Ott, K. Schmidt, B. Kircher, P. Schumacher, T. Wiglenda, R. Gust, *J. Med. Chem.* **2005**, *48*, 622.
[30] D. Schlawe, A. Majdalani, J. Velcicky, E. Heßler, T. Wieder, A. Prokop, H.-G. Schmalz, *Angew. Chem.* **2004**, *116*, 1763; *Angew. Chem. Int. Ed.* **2004**, *43*, 1731.
[31] M. Rosillo, G. Domínguez, J. Pérez-Castells, *Chem. Soc. Rev.* **2007**, *36*, 1589.
[32] A. Berger, J.-P. Djukic, C. Michon, *Coord. Chem. Rev.* **2002**, *225*, 215.
[33] A. Salzer, *Coord. Chem. Rev.* **2003**, *242*, 59.
[34] H. Yamini Shrivastava, S. Niranjali Devaraj, B. Unni Nair, *J. Inorg. Biochem.* **2004**, *98*, 387.
[35] M. Salmain, A. Vessieres, A. Varenne, P. Brossier, G. Jaouen, *J. Organomet. Chem.* **1999**, *589*, 92.
[36] R. R. Aitken, G. M. Badger, J. W. Cook, *J. Chem. Soc.* **1950**, 331.
[37] S. E. Gibson, M. H. Smith, *Org. Biomol. Chem.* **2003**, *1*, 676.
[38] I. Lavastre, J. Besancon, P. Brossiert, C. Moise, *Appl. Organomet. Chem.* **1990**, *4*, 9.
[39] R. Dabard, G. Jougen, *Bull. Soc. Chim. Fr.* **1974**, *7–8*, 1639.
[40] Crystallographic data of **14**: $C_{29}H_{35}CrNO_{10}$, M = 609.58; monoclinic, $P2_1/c$, $a = 8.342(2)$, $b = 57.10(1)$, $c = 12.676(4)$, $b = 95.662(9)$, $V = 6010(3)$ Å3, $Z = 8$, $R_1 = 0.0918$ for 6829 observed ($I > 2s$) reflections and $wR2 = 0.2309$ for all 10 205 reflections, $GOF = 1.038$, 740 parameters. Bruker-axs-SMART 1000 CCD, structure solution with direct methods (SHELXS97), and refined against F^2 with all measured reflections (SHELXL97). Due to the long b axis the R1-value of structure refinement converged by 0.0918. CCDC 722266 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[41] At this stage, it should be noted that determination of a binding constant of **2** or **4** with the FabF enzyme was not possible, as, to the best of our knowledge no enzyme assay is currently commercially available or even published.
[42] Compound **5** could not be analyzed due to precipitation when *B. subtilis* was incubated with the compound at twice its MIC.
[43] W. H. Ang, L. J. Parker, A. D. Luca, L. Juillerat-Jeanneret, C. J. Morton, M. L. Bello, M. W. Parker, P. J. Dyson, *Angew. Chem.* **2009**, *121*, 3912; *Angew. Chem. Int. Ed.* **2009**, *48*, 3854.
[44] For new metal-containing Platensimycin analogues, a patent is pending (EP09154125).
[45] G. M. Sheldrick in *SHELXS-97, Program for Solution of Crystal Structures*, University of Göttingen (Germany), **1997**.
[46] G. M. Sheldrick in *SHELXL-97, Program for Refinement of Crystal Structures*, University of Göttingen (Germany), **1997**.
[47] A. L. Spek, *Acta Crystallogr. Sect. A* **1990**, *46*, C34.

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