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Synthetic prodigiosenes and the influence of C-ring substitution on DNA cleavage, transmembrane chloride transport and basicity†

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Analogues of the tripyrrolic natural product prodigiosin bearing an additional methyl and a carbonyl group at the C-ring were synthesised and evaluated. *In vitro* anticancer activity screening (NCI) and the study of modes of action (copper-mediated cleavage of double-stranded DNA and transmembrane transport of chloride anions) showed that the presence of the methyl group is not detrimental to activity. Furthermore, although the presence of an ester conjugated to the prodigiosene C-ring seems to decrease both pK_a and chloride transport efficiency compared to the natural product, these analogues still exhibit a high rate of chloride transport. All analogues exhibit good *in vitro* anticancer activity and reduced toxicity compared to the natural product: compare an acute systemic toxicity of 100 mg kg^{-1} in mice *vs.* 4 mg kg^{-1} for prodigiosin, pointing towards a larger therapeutic window than for the natural product.

Introduction

Prodigiosin is a red tripyrrolic pigment isolated from bacteria of the *Serratia* and *Streptomyces* genus (Fig. 1, **1**).[1–4] This natural product and some of its natural or synthetic analogues possess a wide range of biological properties such as antimalarial,[5] antifungal,[6] immunosuppressant[7–12] and antibiotic activities,[13] as well as the ability to induce apoptosis of malignant cells.[14–17] The anticancer activity has been attributed to various processes[18] such as phosphatase inhibition,[19] copper-mediated cleavage of double-stranded DNA,[20–23] and/or the transmembrane transport of H/Cl ions.[24–28] Considering these properties, considerable effort has been directed toward the synthesis of prodigiosin and analogues.[29–36] We have synthesised prodigiosin derivatives (named prodigiosenes),[37] including those with an extra methyl group on the C-ring (**2**) as well as analogues (**3**) that bear a C-ring conjugated carbonyl.[38–40] These extra groups have been introduced so as to confer increased stability of the synthetic intermediates and facile isolation of this genre of compounds compared to the natural product.[41] In this article we report the influence of these C-ring modifications upon the ability of the prodigiosenes to effect *in vitro* anticancer activity and copper-mediated double-stranded DNA cleavage, as well as their ability to catalyze transmembrane anion exchange. Of particular importance are our findings that: (i) the extra methyl group on the C-ring of **2** does not significantly diminish the *in vitro* anticancer activity, DNA cleavage or the anion transport activities of the synthetic analogue relative to prodigiosin **1**; (ii) the analogues generally retain the potent *in vitro* anticancer activities of the parent natural product; and (iii) the electron-withdrawing carbonyl functionality on the C-ring makes the synthetic esters **3** less basic than the natural product **1**, which helps to rationalise why the ester analogues are less effective than prodigiosin **1** as transmembrane anion transporters at pH 7.43. This last

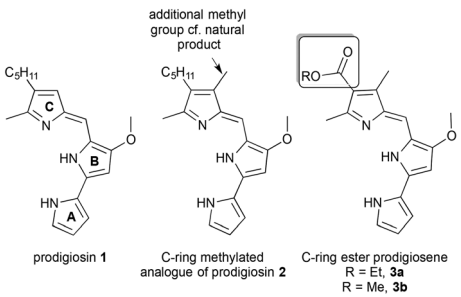

Fig. 1 Natural product prodigiosin (**1**) and C-ring analogues.

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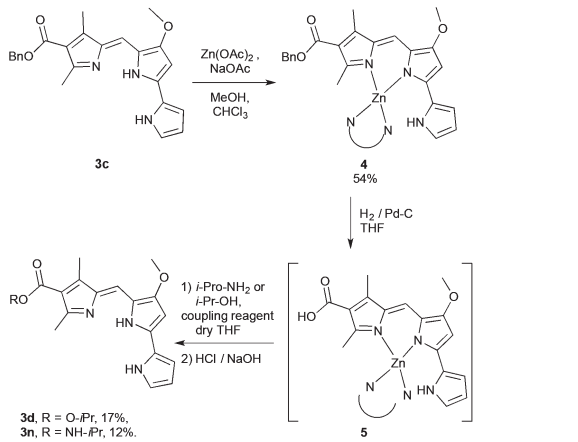
†Electronic supplementary information (ESI) available: Synthesis details; growth inhibition and cytotoxicity assays; anion exchange transport assays; NMR titrations; DNA cleavage assays; total growth inhibition and half maximal lethal concentration data; ^1H and ^{13}C NMR spectra for novel compounds. See DOI: 10.1039/c3ob40477c

finding, that prodigiosene basicity and anion transport rates can be modulated by C-ring functionalization, may be of potential biological relevance since one proposed mode of action for prodigiosenes[25,27] is the modification of the internal pH of cancerous cells by a transmembrane H/Cl co-transport process.[2] Many cancerous cells possess an intracellular pH that is more basic than normal cells – conditions that favor cell growth, transformation and metabolism.[42] In contrast, a decreased intracellular pH can lead to cell cycle arrest and apoptosis.[43,44] As such, the control of intracellular pH by modulating the pK_a of extremely potent anion transporters, such as those based on the prodigiosin scaffold, could be potentially exploited for cancer therapy.

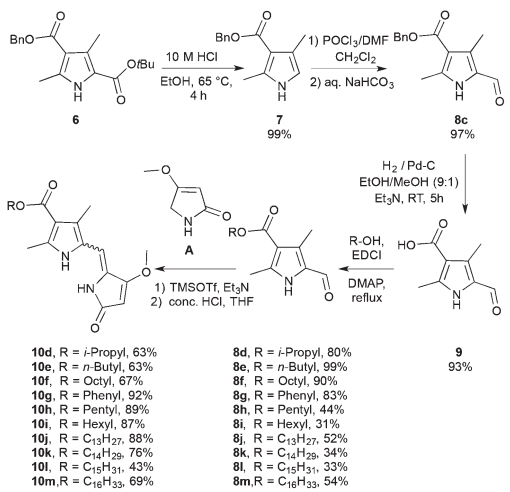
Results and discussion

C-ring amido- and ester-functionalised prodigiosenes

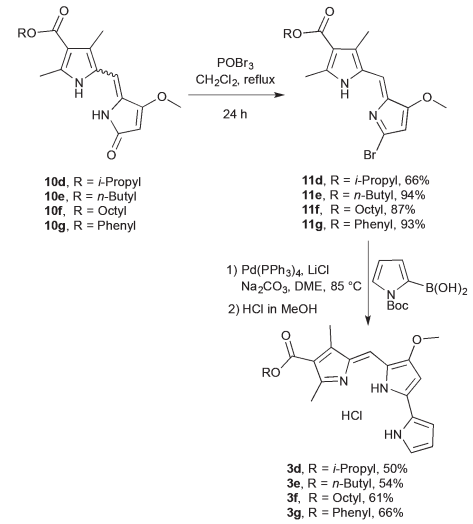
For this work a methylated analogue of prodigiosin, compound **2** (Fig. 1), was synthesised, as well as the ester and amido C-ring prodigiosenes **3b–n** (Schemes 1–5). The ester and amido linkages provided an opportunity to easily introduce a range of chain lengths and thus modify the lipophilicity of the C-ring, as well as an opportunity to evaluate the biological effects of the electron-withdrawing carbonyl substituent. In order to prepare new C-ring ester- (**3d–m**) and amido- (**3n**) prodigiosenes we first envisioned a convergent approach by modification of the known prodigiosene **3a**.[38] However, our initial attempts to hydrolyse/re-esterify or *trans*-esterify the ethyl ester functionality of **3a** (see Fig. 1) were largely unsuccessful, although we were able to prepare the methyl analogue **3b** *via* this general route.[40] Less drastic conditions involving hydrogenolysis of the benzyl ester **3c**[45] (Scheme 1) were also unsuccessful in that the corresponding carboxylic acid could not be isolated, due to its rapid decomposition, and *in situ* re-esterification was unsuccessful. As such, we hypothesised that the



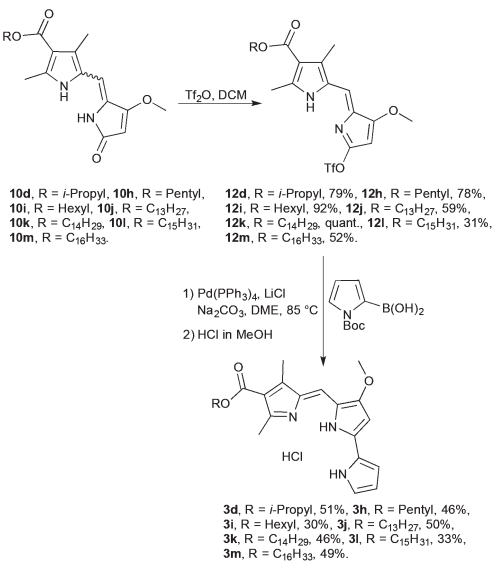
Scheme 1 Synthesis of functionalised prodigiosene **3d** and **3n**.



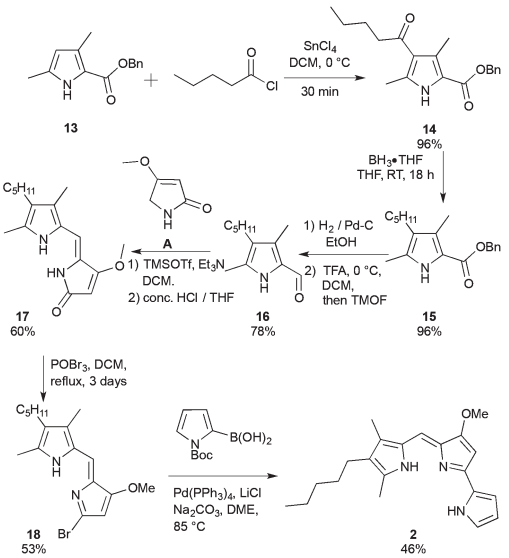
Scheme 2 Ester derivatization and synthesis of dipyrrinones.



Scheme 3 Synthesis of prodigiosenes *via* the bromo route.



Scheme 4 Synthesis of prodigiosenes *via* the triflate route.



Scheme 5 Synthesis of prodigiosene **2**.

formation of a metal-complex of the benzyl ester-containing prodigiosene **3c**, prior to hydrogenolysis, would generate a stable carboxylic acid derivative. For this purpose a Zn-complex[22] of prodigiosene **3c** was prepared (Scheme 1). Hydrogenolysis of this Zn complex **4** gave the desired carboxylic acid **5**, as confirmed using ^{1}H NMR spectroscopy. *In situ* functionalisation of the carboxylic acid was achieved *via* condensation with isopropyl amine in the presence of HBTU/DMAP, thus giving the amido-functionalised prodigiosene **3n** in very low yield (12%). Similarly, the isopropyl ester **3d** was also obtained in low yield (17%) from **5** and isopropyl alcohol using EDCI/DMAP, with competitive decomposition/polymerisation of the starting carboxylic acid substrate **5**.

Given the instability of the carboxylic acid **5** we decided to introduce diversity earlier in the synthesis. Thus to generate a new library of prodigiosenes, a series of 2-formyl pyrroles **8d–m** were synthesised and then used as starting materials for the preparation of several C-ring functionalised prodigiosenes (Scheme 2). First, the acid-promoted hydrolysis and decarboxylation of the Knorr pyrrole **6** provided the α-free pyrrole **7** in excellent yield.[40] Vilsmeier–Haack formylation gave the aldehyde **8c** in almost quantitative yields, and the carboxylic acid **9** was then obtained after hydrogenolysis over Pd/C. Re-esterification of **9** using a range of alcohols, and the reagents EDCI and DMAP, provided the required esters **8d–m**. Silylative Mukaiyama aldol condensation[46,47] (Scheme 2) followed by elimination of the OTMS functionality[48] gave the dipyrrinones **10d–m** in good-excellent yields as a mixture of *E*/*Z* isomers in ratios from 0/1 to 10/90 depending on the substituents.

The dipyrrinones **10d–m** were either brominated or triflylated, before Suzuki–Miyaura coupling with 1-Boc-pyrrole-2-boronic acid to furnish the required prodigiosenes **3d–m**. Indeed, bromination[40] with $POBr_3$ in DCM at 40 °C gave the brominated dipyrrins **11d–g** as pure *Z* isomers regardless of the original *E*/*Z* ratio of the dipyrrinones **10d–g** (Scheme 3). Suzuki–Miyaura coupling of **11d–g** and 1-Boc-pyrrole-2-boronic acid gave the prodigiosenes **3d–g**, isolated as their HCl salts.

To compare the reactivity of the bromo and triflyl dipyrrin intermediates we prepared the triflates **12d–m** (Scheme 4), again as *Z* isomers only. Although the triflates (**12**) were less stable than the corresponding bromides (**11**), and required storage under nitrogen at −18 °C, they were synthetically appealing as they were prepared in just a few hours as compared to reaction times of one day for the brominated analogs. Suzuki–Miyaura coupling gave similar yields, regardless of whether dipyrrinato triflates or bromides were used: for example, prodigiosene **3d** featuring an isopropyl ester was isolated as its HCl salt in 50% and 51% yield starting from the bromo and triflyl dipyrrins, respectively.

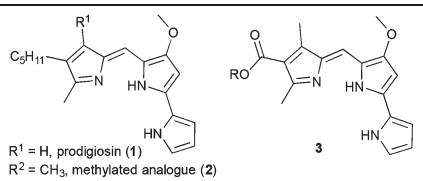
Synthesis of methylated analogue of prodigiosin

To evaluate the influence of the extra methyl group on the C-ring of the prodigiosene core, a methylated analogue (**2**) of prodigiosin (**1**) was prepared (Scheme 5). Friedel–Crafts acylation of **13**[49] using valeroyl chloride furnished the acylated pyrrole **14**, and subsequent reduction gave the desired pyrrole **15**. Hydrogenolysis of the benzyl ester, followed by decarboxylation and formylation gave the aldehyde **16**. Formation of the dipyrrinone **17** as a pure *Z* isomer and then bromination gave the brominated dipyrrin **18**. The free-base prodigiosene **2** was finally obtained using a Suzuki–Miyaura coupling.

In vitro anticancer activity

The *in vitro* activity of our synthetic prodigiosenes was evaluated at the National Cancer Institute (NCI) against a standard panel of 59 human cell lines, derived from nine cancer cell types. Table 1 reveals averaged concentrations for activity

Table 1 Mean *in vitro* activity of prodigiosenes over 59 cancer cell lines[a]



Entry	C-ring variation	GI_{50}[b] (nM mean)	TGI[c] (μM mean)	LC_{50}[d] (μM mean)
1	Natural product (**1**)	14	2.1	0.3
2	Methylated analogue (**2**)	15	0.1	1.0
3	R = *O*-methyl (**3b**)	347	6.0	49.0
4	R = *O*-benzyl (**3c**)	129	2.0	14.5
5	R = *O*-i-propyl (**3d**)	59	1.0	7.9
6	R = *O*-butyl (**3e**)	117	2.0	14.5
7	R = *O*-octyl (**3f**)	28	1.9	18.6
8	R = *O*-phenyl (**3g**)	214	2.0	14.5
9	R = *O*-pentyl (**3h**)	36	0.6	4.6
10	R = *O*-$C_{13}H_{27}$ (**3j**)	72	10.5	61.7
11	R = *O*-$C_{16}H_{33}$ (**3m**)	>1000	22.9	75.9
12	R = *N*H-i-propyl (**3n**)	>1000	12.6	42.7

[a] http://dtp.cancer.gov. [b] GI_{50} = half maximal growth inhibition concentration. [c] TGI = total growth inhibition concentration. [d] LC_{50} = half maximal lethal concentration.

across all lines for prodigiosenes **1–3**. Compared to the natural prodigiosin **1** (entry 1), the amido C-ring prodigiosene **3n** (entry 12) showed a dramatically decreased activity with significantly higher GI_{50} and TGI concentrations. On the other hand, prodigiosene **2** (merely bearing an extra methyl group on the C-ring *cf.* the natural product) exhibited a slightly improved anticancer activity, in terms of TGI values as compared to **1**, and a reduced toxicity (entry 2). Most of the prodigiosene esters (entries 3–10) exhibited anticancer activities that were similar in magnitude to those of the natural compound **1**, revealing that introduction of a C-ring carbonyl moiety is not detrimental to anticancer activity. The only ester studied that had poor anticancer activity was the decahexanoate **3m** (entry 11). Presumably **3m**, with its lipophilic hexadecane chain, aggregates in solution and is less efficient at penetrating the cell.

To enable an appreciation of the origin of the concentrations at which *in vitro* activity is incurred, Fig. 2 reveals GI_{50} concentrations for the natural product **1**, the methylated analogue **2** and the isopropyl ester **3d** across each of the 59 cell lines evaluated. With recognition of the nanomolar concentrations plotted, analysis of Fig. 2 reveals that prodigiosenes **2** and **3d** effect growth inhibition in a similar domain to the natural product **1**, for most strains. These screening activity results across a range of cancer cell types demonstrate that inclusion of neither the extra methyl group (compound **2**), nor a C-ring conjugated ester moiety (compound **3d**), is automatically detrimental to activity: this is important given the incorporation of the additional C-ring methyl group to facilitate synthesis of the prodigiosene core, and the incorporation of the ester moiety to enable different chain lengths and electronic effect to be probed. Fig. S15 and S16† reveal TGI and LC_{50} concentrations for **1**, **2** and **3d** across all cell lines evaluated, with similar results.

In vitro DNA cleavage activity

In an attempt to correlate the anticancer activity of these synthetic compounds with some of their possible modes of action, several of the synthetic prodigiosenes were tested for their ability to cleave double-stranded DNA in the presence of Cu(II). For this purpose an electrophoretic gel assay was conducted using plasmid DNA and copper acetate with prodigiosenes **2**, **3b–g**, **3j** and **3k**.[26,50]

The EC_{50} values for DNA cleavage (effective concentration where 50% DNA nicking occurred) were established using densitometry analysis after 2 hours of incubation at 37 °C. These EC_{50} values are summarised in Table 2. No obvious trend between DNA cleavage propensity and prodigiosene structure emerged from these experiments. Note, however, that all of these synthetic derivatives maintained their potent DNA cleavage ability with EC_{50} values between 15 μM and 33 μM. An EC_{50} value of 9.2 μM for prodigiosin **1** itself was previously reported.[27]

Transmembrane anion transport activity

To further investigate possible mechanisms that might contribute toward the anticancer activities of prodigiosenes, the transmembrane anion transport properties of our synthetic prodigiosenes were evaluated using an EYPC (egg-yolk l-phosphatidylcholine) liposome model. For this assay the chloride-specific dye lucigenin was used as a probe to track chloride export from the liposome, since the fluorescence of the dye is selectively quenched by chloride but not by nitrate anions.[51] Thus, EYPC liposomes loaded with lucigenin and NaCl were

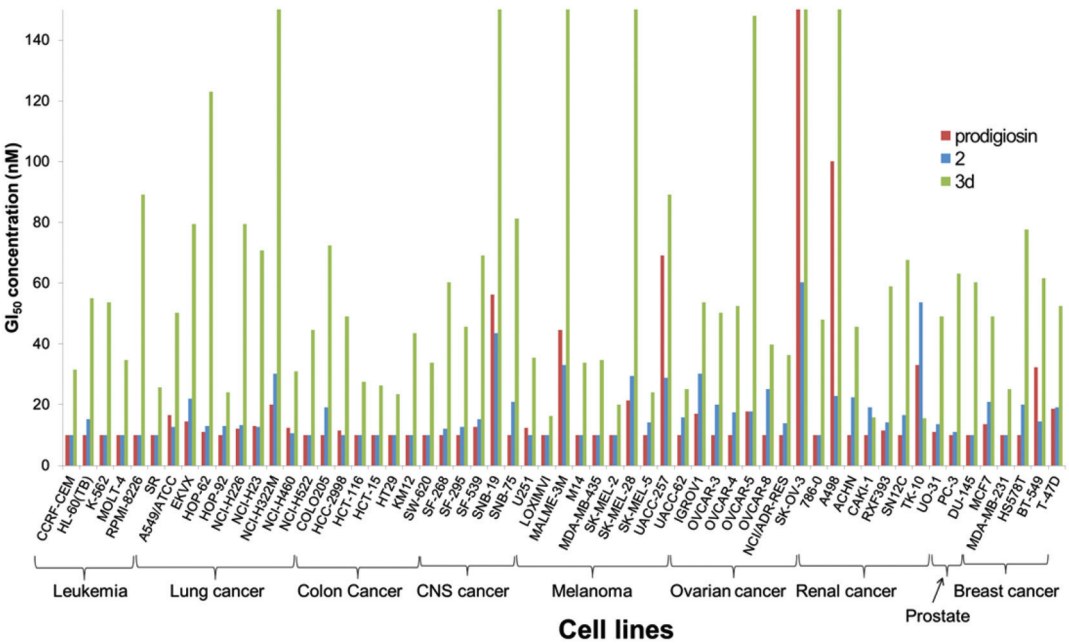
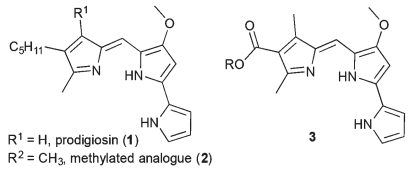


Fig. 2 GI_{50} concentrations (half maximal growth inhibition concentrations) of prodigiosin, **2** and **3d** against 59 human cancer cell lines representing 9 different cancer types; http://dtp.cancer.gov.

Table 2 Copper mediated DNA cleavage of prodigiosenes **2**, **3b–g**, **3j** and **3k**; EC_{50} values for 2 h incubation at 37 °C with 1 : 1 prodigiosene : Cu(II)



Entry	C-ring variation	Cu/DNA cleavage EC_{50} (μM, ±2%)
1	**2**	16
2	R = *O*-methyl (**3b**)	15
3	R = *O*-benzyl (**3c**)	33
4	R = *O*-i-propyl (**3d**)	21
5	R = *O*-butyl (**3e**)	31
6	R = *O*-octyl (**3f**)	33
7	R = *O*-phenyl (**3g**)	33
8	R = *O*-$C_{13}H_{27}$ (**3j**)	20
9	R = *O*-$C_{14}H_{29}$ (**3k**)	25

suspended in a buffered solution (pH 7.43) containing $NaNO_3$. Upon addition of prodigiosenes to the suspension of EYPC liposomes, the fluorescence of the intravesicular lucigenin increased, indicating that the added transporter was catalyzing an anion exchange process between the intravesicular chloride anion and the extravesicular nitrate anion. Fig. 3 shows representative fluorescence curves, plotted as a function of time, after addition of either the natural product prodigiosin **1**, the methylated analogue **2** or the pentyl-ester **3h** (in each case the prodigiosene was added at a concentration that was 0.04 mol% relative to total EYPC lipid). Under these conditions, the synthetic prodigiosene **2** had a similar Cl^-/NO_3^- anion transport

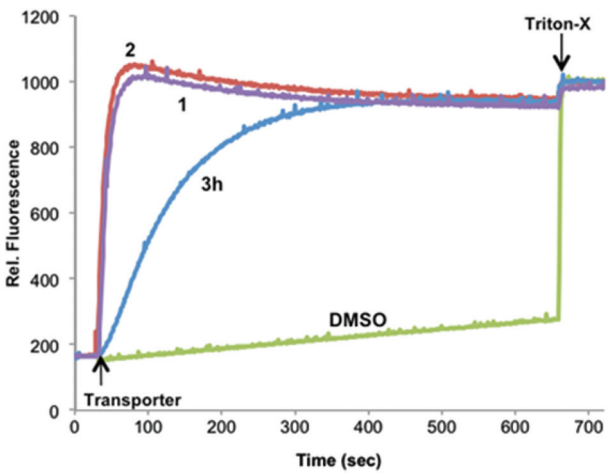


Fig. 3 Anion exchange assay for the natural product prodigiosin **1** relative to synthetic analogues **2** and **3h**. Anion exchange from EYPC liposomes was monitored by a change in the fluorescence of lucigenin at 25 °C after addition of 0.04 mol% of prodigiosene transporter relative to EYPC lipid. The EYPC liposomes, containing 1 mM lucigenin, 20 mM HEPES buffer (pH 7.43) and 100 mM NaCl were suspended in a solution of 20 mM HEPES buffer (pH 7.43) that contained 100 mM $NaNO_3$. At t = 30 s, the prodigiosene transporter was added and the fluorescence of lucigenin was monitored (λ_{ex} = 372 nm, λ_{em} = 504 nm). At t = 660 s, the addition of triton-X lysed the liposomes.

Table 3 EC_{50} values for the transmembrane anion transport abilities of **1**, **2** and **3h**

Prodigiosene	**1**	**2**	**3h**
EC_{50} [a] (nM)	3.2	1.3	18.7

[a] EC_{50} values indicate the concentration of transporter needed to achieve 50% of maximal chloride efflux at t = 150 s and 25 °C in 20 mM HEPES buffer (pH 7.43).

activity as prodigiosin: this indicates that addition of the extra methyl group to the C-ring does not diminish the anion transport activity. This is a significant result since it is easier to synthesise the methyl analogue **2** than it is the natural product prodigiosin **1**.

In contrast to the methylated analogue **2**, the pentyl-ester **3h** is a less efficient anion transporter than the natural product **1** (Fig. 3). To better quantify the transmembrane transport abilities of **1**, **2** and the ester-containing analogue **3h** we performed a series of concentration-dependent Hill analyses[52] for transmembrane Cl^-/NO_3^- exchange by these three analogues. This enabled us to calculate EC_{50} values for the transporters **1**, **2** and **3h**, defined as the concentration of transporter needed to achieve 50% of maximal chloride influx at t = 150 s at 25 °C (Table 3). The increased EC_{50} values for ester **3h**, relative to prodigiosin **1** and the methylated analogue **2**, indicates that substitution of the prodigiosene C-ring with an electron-withdrawing ester decreases the anion transport efficiency of the compound, since **3h** (EC_{50} = 18.7 nM) had an EC_{50} value that was approximately 10-fold higher than **2** (EC_{50} = 1.3 nM).

Even though the ester **3h** showed a decreased anion transport ability when compared to prodigiosin **1** and its methyl analogue **2**, it should be recognised that this synthetic prodigiosene remains an extremely potent anion transporter. For example, the experiments in Fig. 3 were conducted using a relatively low concentration of prodigiosene (just 0.04 mol% relative to EYPC lipid). For comparison, many synthetic transmembrane chloride transporters described in the literature are typically used at concentrations as high as 1–2 mol% relative to lipid to achieve similar rates of transmembrane chloride transport.[53]

Having demonstrated that ester **3h** was a formidable anion transporter we next investigated the influence of the alkoxy functionality of the ester upon the transmembrane anion transport in EYPC liposomes. Fig. 4 shows the increase in lucigenin fluorescence after the addition of various prodigiosene esters, all added at 0.04 mol% relative to the EYPC lipid. It is apparent from these results that the length of the alkoxy functionality influences the efficiency of anion transport under these conditions. For example, the best transporters were the "short-chain" prodigiosene esters (methyl, i-propyl, butyl and ethyl esters). Clearly, these short-chain alkyl esters were more effective anion transporters than esters that contained either a longer alkyl chain, such as the octanoate **3f**, or had aromatic substitution like the benzyl ester **3c** and the phenyl ester **3g**.

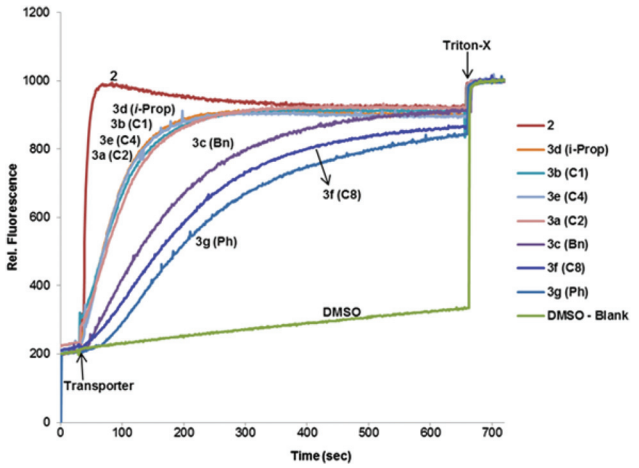


Fig. 4 Anion exchange assay for prodigiosenes **3a–g**, relative to **2**; change in fluorescence observed in EYPC liposomes using lucigenin assay at 25 °C; the data was collected using 0.04 mol% of prodigiosene transporter relative to EYPC lipid; liposomes containing 1 mM lucigenin, 20 mM HEPES buffer and 100 mM NaCl (pH 7.43) were suspend in 20 mM HEPES and 100 mM $NaNO_3$ solution (pH 7.43); at t = 30 s, prodigiosene transporter was added and the fluorescence monitored (λ_{ex} = 372 nm, λ_{em} = 504 nm); at t = 660 s, addition of triton-X; traces shown are an average of three trials.

To more clearly illustrate how the lipophilicity of the alkyl chain plays a significant role in the transmembrane anion transport ability of prodigiosene esters we carried out a set of comparative anion exchange assays using prodigiosenes **3i** (hexanoate), **3f** (octanoate) and **3k** (tetradecanoate). Fig. 5 shows that increasing the chain lengths of the C-ring ester alkoxy functionality results in slower rates of transmembrane anion exchange. This reduced activity with increasing chain length could be due to the more hydrophobic nature of the longer chain esters, as these longer alkyl chains would favor the formation of self-assembled aggregates such as micelles in water, thereby limiting the rate at which the compounds could partition into the bilayer lipid membrane. Indeed, Quesada and colleagues have recently shown such a trend for the anion transport properties of a series of synthetic tambjamines, dipyrrolic compounds that are analogous to prodigiosenes.[54]

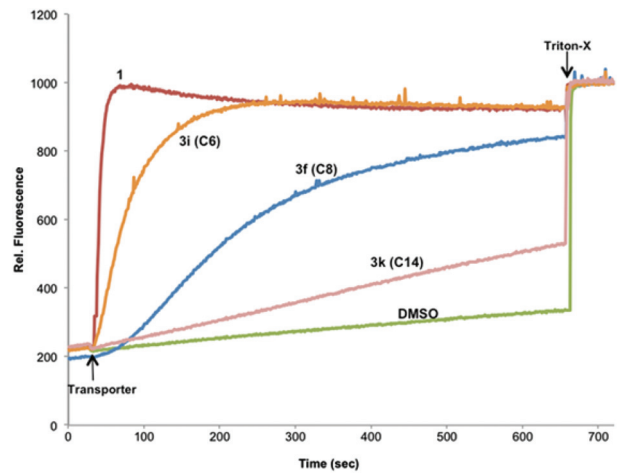
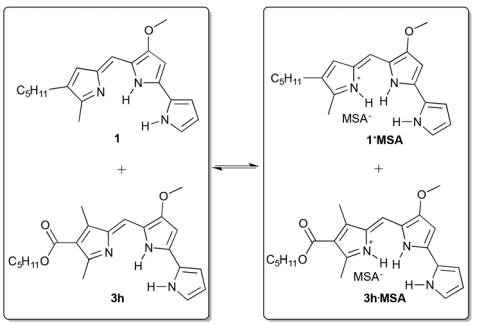


Fig. 5 Anion exchange assay for prodigiosenes **3i**, **3f**, **3k**, relative to **2**; change in fluorescence observed in EYPC liposomes using lucigenin assay at 25 °C; the data was collected using 0.04 mol% of prodigiosene transporter relative to EYPC lipid; liposomes containing 1 mM lucigenin, 20 mM HEPES buffer and 100 mM NaCl (pH 7.43) were suspended in 20 mM HEPES and 100 mM $NaNO_3$ solution (pH 7.43); at t = 30 s, prodigiosene transporter was added and the fluorescence monitored (λ_{ex} = 372 nm, λ_{em} = 504 nm); at t = 660 s, triton-X was added; traces represent averages of three trials.

^{1}H NMR titrations

As illustrated in Table 3 the Cl^-/NO_3^- anion transport data indicates that compounds featuring a C-ring conjugated ester (**3h**) exhibit transmembrane transport activity that is about an order of magnitude lower than the natural product **1** or the methylated analogue **2**. We hypothesised that the C-ring ester derivatives are less basic than **1**, and so the protonated form of the anionophore (the optimal species for binding an anion and transporting that anion across a hydrophobic barrier) would be less prevalent in the case of ester **3h**. To investigate the effect of the conjugated ester on the acid–base properties of the prodigiosenes we first carried out a competition NMR experiment in CD_3CN. Fig. 6 shows ^{1}H NMR spectra that report on the competition exhibited by the free-base forms of prodigiosin **1** and ester **3h** for a proton. Since prodigiosin **1** is more basic than the ester **3h** it was preferentially protonated as sub-stoichiometric amounts of acid were added to the mixture of compounds (Fig. 6, traces **b–g**). The preferential protonation is reflected by the selective change in chemical shift for the methoxy peak of prodigiosin **1**.

Trace **a** in Fig. 6 shows the ^{1}H NMR spectra after prodigiosin free-base **1** was mixed with one equivalent of the HCl salt of pentyl ester prodigiosene (**3h·HCl**). This trace showed that the mixture was converted to an equilibrium mixture of **1·HCl** (>90%) and the free-base of ester **3h**, demonstrating that prodigiosin **1** is more basic than its ester analogue **3h**. Next, we added one equiv. of TEAB (tetraethylammonium bicarbonate) so as to convert both compounds to their free-base forms;[55] the ^{1}H NMR chemical shift of the methoxy group of prodigiosin **1** appeared at δ 3.86 ppm and the methoxy peak of the free-base of ester **3h** at δ 3.88 ppm (trace **b**). After the addition of 0.4 equiv. of MSA (methanesulfonic acid), only the methoxy peak for prodigiosin **1** selectively broadened and decreased in relative intensity (trace **d**). The methoxy peak corresponding to the protonated species **1·H$^+$** began to appear at δ 4.01 ppm after addition of 0.6 equiv. of MSA (trace **e**). This peak grew and sharpened until 2 equiv. of acid had been added to the mixture (trace **i**). Simultaneously, the methoxy peak of ester **3h** at δ 3.88 ppm started to decrease after addition of 0.6 equiv. of MSA (trace **e**), and a new signal ultimately appeared for **3h·HCl** at δ 4.03 ppm. After the addition of 3 equiv. of MSA (trace **j**), both compounds were fully protonated. The results from this NMR competition experiment clearly indicate that prodigiosin **1** is more basic than the prodigiosene ester **3h**.



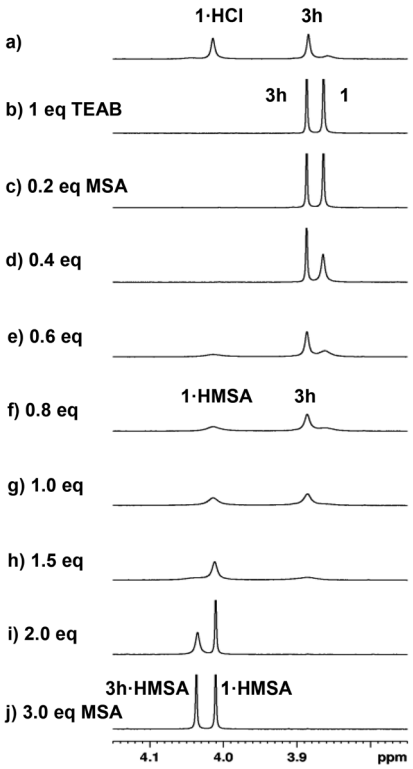


Fig. 6 ^{1}H-NMR spectra representing B-ring OCH_3 signals for titration of a 1 : 1 mixture of prodigiosin **1** and ester **3h** at 1 mmol L^{-1} in CD_3CN at 3 °C with MSA (methanesulfonic acid); 1 equiv. of TEAB (tetraethylammonium bicarbonate) was first added to the mixture to convert both compounds into their free-base forms; increments of MSA were sequentially added to the equimolar mixture of the two prodigiosenes.

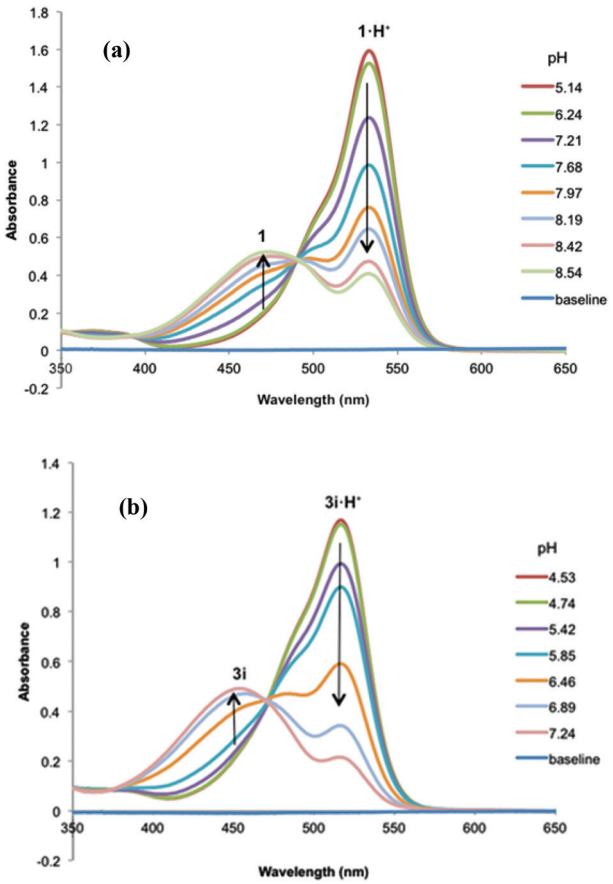


Fig. 7 UV-vis absorbance spectra for (a) prodigiosin **1** and (b) ester **3i** as a function of pH in 1 : 1 CH_3CN–H_2O (v/v) at 25 °C (0.1 M NaCl).

UV-vis titrations

Having obtained qualitative ^{1}H NMR evidence that the C-ring ester in **3** influences the proton affinity of the tripyrrolic unit we next determined the apparent pK_a values of prodigiosin **1** and a C-ring ester analogue **3i**. To measure these apparent pK_a values we used a spectrophotometric procedure described by Manderville and colleagues.[24] Fig. 7 shows UV-vis titrations for the natural prodigiosin **1** (Fig. 7a) and the synthetic ester **3i** (Fig. 7b) (in 1 : 1 acetonitrile–H_2O). The pK_a values were determined from a plot of log(ionization ratio) *vs.* pH. As can be seen in Fig. 7a the λ_{max} for the protonated form of prodigiosin **1**·H^+ at 533 nm and the λ_{max} for the free-base **1** at 460 nm are well separated. From this titration data we determined that the natural product **1**·H^+ has an apparent pK_a of 8.2. An apparent pK_a value of 7.98 in aqueous acetonitrile was previously reported.[22] For the hexyl ester derivative **3i**, the λ_{max} for the protonated form **3i**·H^+ was at 517 nm and λ_{max} for the free-base **3i** was at 450 nm (Fig. 7b). Based on the UV-vis spectra for the corresponding titration, we determined that the synthetic ester **3i**·H^+ has an apparent pK_a of 6.5. These UV-vis titrations clearly demonstrate that the electron-withdrawing carbonyl group on the prodigiosene C-ring of **3i** makes the ester significantly less basic than the natural product **1**. Relative to prodigiosin **1**, the reduced basicity of ester **3i** would make it more difficult to protonate this analogue near neutral pH, which would then decrease the ability of the esters to bind and transport anions, as we demonstrated experimentally.

Transmembrane anion transport as a function of pH

Having shown that the differing basicities of the natural product **1** and an ester-bearing analogue influence the transmembrane anion transport activity, we next investigated the pH dependence of Cl^-/NO_3^- exchange. For these experiments (Fig. 8) we prepared EYPC liposomes of 200 nm diameter that were filled with $NaNO_3$ (100 mM, pH 7.5 with 10 mM

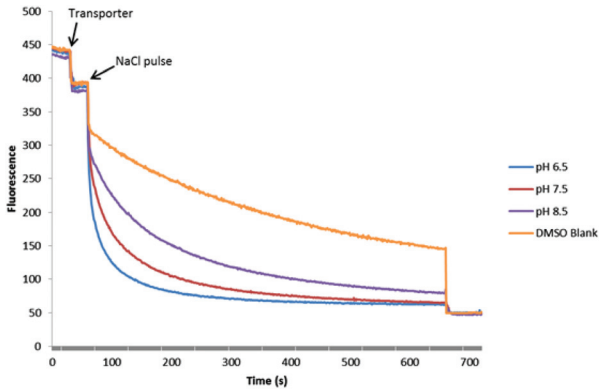


Fig. 8 Chloride influx promoted by 0.05 mol% of ester **3f** as a function of pH. At t = 20 s a solution of **3f** was added to a solution of EYPC vesicles (d = 200 nm), loaded with 100 mM $NaNO_3$ and suspended in 10 mM phosphate buffer (pH 6.5, 7.5 or 8.5). At t = 60 s NaCl solution was added to give a final extravesicular Cl^- concentration of 100 mM. At the end of the experiment, Triton detergent was added to lyse the vesicles. The faster fluorescence decay indicates faster anion exchange, as mediated by ester **3f**.

phosphate) and 1 mM of LG, the chloride-selective dye. At t = 30 s, the octyl-ester prodigiosene **3f** (0.05 mol% relative to EYPC lipid) was added to the liposome solution. Then, at t = 60 s, a pulse of 10 mM phosphate buffer (at pH 6.5, 7.5 or 8.5) containing 1 M NaCl and 100 mM $NaNO_3$ was added to give an extravesicular Cl^- concentration of 100 mM. Fig. 8 shows this transporter-mediated anion exchange as a function of extravesicular pH. The observed decrease in the LG fluorescence is due to influx of Cl^-, as catalyzed by the prodigiosene ester **3f**, and so faster decay indicates faster anion exchange. Chloride transport by **3f** clearly increased as the pH was decreased from 8.5 to 6.5. This systematic increase in transport activity with decreasing pH is consistent with better ion pairing between the target anions and the protonated tripyrrolic head-group of the carrier prodigiosene, whose pK_a we determined to be 6.5. These experiments clearly show that transmembrane transport of anions by the prodigiosene ester **3f** can be modulated by controlling the external pH.

Conclusions

A series of amido and ester C-ring prodigiosenes were synthesised and their *in vitro* anticancer activities were determined using the NCI panel of 60 cancer cell lines. While the amido prodigiosene **3n** showed a significant decrease in its anticancer activity, the synthetic esters **3b–3g** had similar anticancer potency as the natural product prodigiosin **1**. The methylated analogue **2** exhibited very good anticancer activity, as compared to the natural product **1**, with a decreased TGI value and lower toxicity. These results show that the extra methyl group added to the C-ring is not detrimental to the anticancer activity of the synthetic prodigiosenes. While the presence of an ester on the C ring decreases the pK_a and the anion transport rate of prodigiosene ester **3**, these compounds are still good transporter as the experiments were run using a low concentration of prodigiosenes.[53] The prodigiosenes also maintained their ability to cleave DNA in the presence of copper. It seems difficult from this study to observe a trend between the structure and activities of prodigiosenes. However, we identified the isopropyl ester **3d** as an analogue to study further, as this prodigiosene exhibits better anticancer activity than the natural compound **1**, along with a decreased toxicity and a relatively significant anion transport ability. In view of these results, prodigiosene **3d** was selected for *in vivo* experiments and showed an acute systemic cytotoxic on mice of 100 mg kg^{-1} which is a great improvement compared to the parent prodigiosin (4 mg kg^{-1}) (http://dtp.cancer.gov/branches/btb/acute_tox.html). This may suggest a larger therapeutic window for compounds of this type, rather than for prodigiosin (*i.e.* effective dose much lower than systemic toxicity).

Experimental

General procedures

All chemicals were purchased and used as received unless otherwise indicated. Moisture sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon. Air- and moisture-sensitive compounds were introduced *via* syringe or cannula through a rubber septum. Flash chromatography was performed using Silicycle ultra pure silica (230–400 mm) or 150 mesh Brockmann III activated neutral alumina oxide as indicated. The NMR spectra were recorded using a 500 MHz spectrometer instrument using $CDCl_3$, DMSO-d_6 or MeOD as solvent and are reported in part per million (δ) using the solvent signals at 7.26 ppm for ^1H and at 71.16 ppm for ^{13}C while $CDCl_3$ was used, at 2.50 ppm for ^1H and at 39.52 ppm for ^{13}C while DMSO-d_6 was used and at 3.31 ppm for ^1H and at 49.00 ppm for ^{13}C while MeOD was used, as an internal reference with J values given in Hertz. Mass spectra were obtained using TOF and LCQ Duo ion trap instruments operating in ESI+ mode. The purity of all tested compounds was ≥95%, as determined by analytical HPLC. Melting points were determined using a Fisher-Johns melting point apparatus. Compounds **6**,[40] **7**,[40] **8c**,[40] **9**,[40] **3a–c**[40] and 4-methoxy-3-pyrrolin-2-one[49] were prepared using literature procedures.

General procedure 1 for the synthesis of esters 8d–i. To a stirred solution of 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid **9** (1.5 g, 8.97 mmol) in dry dichloromethane (50 mL) was added DMAP (1.2 g, 9.82 mmol) and EDCI (1.9 g, 9.91 mmol) followed by the alcohol (desired amount, as indicated for each compound) and the resulting solution was heated at reflux temperature for 2 days. The reaction mixture was then cooled to room temperature, washed twice with water and then with brine, dried (Na_2SO_4) and concentrated *in vacuo*. Purification using chromatography over silica (ethyl acetate–hexanes, 20/80) gave the desired products.

Isopropyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8d. According to general procedure 1 and using isopropanol (28 eq.), this compound was obtained as an off-white solid

(2.0 g, 80%). ^{1}H NMR ($CDCl_3$, 500 MHz) 1.34 (d, J = 6.0 Hz, 6 H), 2.54 (s, 3 H), 2.57 (s, 3 H), 5.19 (sept, J = 6.0 Hz, 1 H), 9.60 (s, 1H), 10.27 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.9, 14.6, 22.4, 67.3, 114.7, 128.4, 136.5, 143.8, 164.7, 177.6. HR-MS (ESI): $[M + Na]^+$ calcd for $C_{11}H_{15}N_1O_3Na$: 232.0944; found 232.0956.

General procedure 2 for the synthesis of dipyrrinone 10d–m. To a solution of 4-methoxy-3-pyrrolin-2-one (340 mg, 3.02 mmol, 2.2 eq.) in dry DCM (45 mL) was added triethylamine (1.14 mL, 8.22 mmol, 6.0 eq.) at 0 °C. Then TMSOTf (750 μL, 4.11 mmol, 3.0 eq.) was added drop-wise. After 20 min the aldehyde **8** (1 eq.) was added in dry DCM (45 mL). The reaction was stirred at this temperature for three hours, then TMSOTf (150 μL, 4.11 mmol, 0.6 eq.) was added. After one hour stirring at 0 °C the reaction was quenched by the addition of phosphate buffer (pH = 7, 100 mL). The solution was extracted with DCM (3 × 100 mL), washed with brine and then dried (Na_2SO_4). After evaporation of the solvent the resulting brown oil was dissolved in THF (90 mL) and concentrated aqueous HCl (300 μL) was added. After a few minutes the reaction was quenched *via* addition of saturated aqueous $NaHCO_3$ (200 mL), then extracted with DCM (3 × 100 mL) and washed with water (2 × 100 mL). After concentration of the combined organic fractions under vacuum, the resulting suspension was washed with water and hexane, using filtration, to give the product as a yellow solid.

(*Z*)-Isopropyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)-methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10d. According to general procedure 2 using aldehyde **8d** (1.4 g, 6.7 mmol), this compound was obtained as a yellow solid (1.84 g, 63% of a 5/95 *E*/*Z* mixture (determined using NMR)). ^{1}H NMR (DMSO-d_6, 500 MHz) 1.27 (d, J = 6.5 Hz, 6 H), 2.21 (s, 3 H), 2.45 (s, 3 H), 3.85 (s, 3 H), 5.03 (sept, J = 6.5 Hz, 1 H), 5.26 (s, 1 H), 6.02 (s, 1 H), 9.63 (s, 1 H), 10.88 (s, 1 H). ^{13}C NMR (DMSO-d_6, 125 MHz) 10.9, 13.6, 22.0, 58.4, 65.9, 91.4, 94.5, 111.8, 122.0, 124.0, 125.2, 139.0, 164.3, 166.9, 170.8. HR-MS (ESI): $[M + Na]^+$ calcd for $C_{16}H_{20}N_2O_4Na$: 327.1315; found 325.1313.

General procedure 3 for the synthesis of bromodipyrrins 11d–g. To a stirred suspension of **10** (11.7 mmol) in dry CH_2Cl_2 (250 mL) was added $POBr_3$ (6.70 g, 23.37 mmol, 2.0 eq.). The resulting solution was heated at reflux temperature under nitrogen for 17 h. After the reaction mixture was cooled to room temperature, sat. aqueous $NaHCO_3$ (500 mL) was added at 0 °C and the organic layer was separated, washed with brine and water, then dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The crude product was purified by passing a solution in EtOAc through a pad of silica gel eluting with EtOAc–hexane 80/20 to give a solid.

(*Z*)-Isopropyl 5-((5-bromo-3-methoxy-2*H*-pyrrol-2-ylidene)-methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 11d. According to general procedure 3 using dipyrrinone **10d** (1.0 g, 3.29 mmol), this compound was obtained as a green-yellow solid (0.80 g, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) 1.34 (d, J = 4.5 Hz, 6 H), 2.39 (s, 3 H), 2.58 (s, 3 H), 3.85 (s, 3 H), 5.19 (sept, J = 4.5 Hz, 1 H), 5.59 (s, 1 H), 6.93 (s, 1 H), 11.17 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 15.2, 22.4, 58.7, 67.0, 100.1, 114.4, 115.9, 126.4, 134.2, 139.2, 144.1, 147.1, 164.9, 167.4. HR-MS (ESI): $[M + H]^+$ calcd for $C_{16}H_{20}BrN_2O_3$: 367.0652; found 367.0622.

General procedure 4 for the synthesis of triflyl dipyrrins 12d, 12h–m. To a suspension of the dipyrrinone **10** (0.85 mmol, 1 eq.), in dry DCM (60 mL) at 0 °C was slowly added Tf_2O (400 μL, 2.38 mmol, 2.8 eq.). After 4 h stirring at this temperature, the reaction was quenched with sat. aqueous $NaHCO_3$ (70 mL), then extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, and then dried (Na_2SO_4). After evaporation of the solvents under reduced pressure, the crude material was purified using flash column chromatography (SiO_2, EtOAc–hexane 1/9).

(*Z*)-Isopropyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)-oxy)-1*H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12d. According to general procedure 4 using dipyrrinone **10d** (800 mg, 2.63 mmol), this compound was obtained as a bright yellow solid (900 mg, 79%). ^{1}H NMR ($CDCl_3$, 500 MHz) 1.34 (s, 3 H), 1.35 (s, 3 H), 2.41 (s, 3 H), 2.56 (s, 3 H), 3.89 (s, 3 H), 5.19 (sept., J = 6.5 Hz, 1 H), 5.43 (s, 1 H), 7.12 (s, 1 H), 10.97 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.6, 15.2, 22.3, 59.0, 67.1, 87.6, 114.6, 118.8 (q, J = 319 Hz), 119.2, 126.0, 133.2, 135.4, 144.7, 161.8, 164.7, 168.2. HR-MS (ESI): $[M + H]^+$ calcd for $C_{17}H_{20}F_3N_2O_6S$: 437.0989; found 437.0976.

General procedure 5 for the synthesis of prodigiosenes 3d–m. Compound **11** or **12** (0.48 mmol, 1 eq.) was dissolved in DME (9 mL) then LiCl (60 mg, 1.44 mmol, 3 eq.) and boronic acid (121 mg, 0.57 mmol, 1.2 eq.) were added. The solution was degassed by bubbling with N_2, and then tetrakis(triphenylphosphine)palladium (56 mg, 10 mol%) was added. Then a degassed 2 M solution of Na_2CO_3 was added (1.0 mL, 1.92 mmol, 4 eq.) and the suspension was stirred at 85 °C for 18 h. After cooling the solution was poured into water (100 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine (100 mL), and then dried (Na_2SO_4). Purification using chromatography (Al_2O_3 neutral type III, EtOAc–hexane 1/9 then 2/8) gave a red film. It was dissolved in a mixture of MeOH–$CHCl_3$ (20 : 1) then a 0.1 M solution of HCl in MeOH (1.5 eq.) was added. After 15 min stirring the solvents were removed under reduced pressure. The obtained solid was filtrated and washed with water and hexane or methanol, to give a dark brown-red solid.

(*Z*)-Isopropyl 2-((4-methoxy-1*H*,1′*H*-2,2′-bipyrrol-5-yl)methylene)-3,5-dimethyl- 2*H*-pyrrole-4-carboxylate hydrochloride 3d. According to general procedure 5 using bromodipyrrinone **11d** (650 mg, 1.77 mmol), this compound was obtained as a red HCl salt (342 mg, 50%). According to general procedure 5 using dipyrrinone **12d** (900 mg, 2.06 mmol), this compound was obtained as a red HCl salt (410 mg, 51%). ^{1}H NMR ($CDCl_3$, 500 MHz) 1.35 (d, J = 6.0 Hz, 6 H), 2.50 (s, 3 H), 2.80 (s, 3 H), 4.03 (s, 3 H), 5.19 (sept, J = 6.0 Hz, 1 H), 6.08 (s, 1 H), 6.36–6.37 (m, 1 H), 6.98 (s, 1 H), 7.08 (s, 1 H), 7.27 (s, 1 H), 12.64 (bs, 1 H), 12.68 (bs, 1 H), 12.90 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 15.0, 22.3, 59.1, 67.5, 93.4, 112.5, 113.0, 116.2, 119.0, 122.1, 122.5, 123.5, 128.5, 140.6, 150.0, 150.4, 164.2, 166.7. HR-MS (ESI): $[M - HCl + H]^+$ calcd for

$C_{20}H_{24}N_3O_3$: 354.1812; found 354.1795. UV ($CHCl_3$) λ_{max} (ε): 500 (52 700), 528 (111 500). HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH 100%, 0.75 mL min^{-1}, λ = 451 nm, t_R = 38.7 min.

Benzyl 3,5-dimethyl-4-pentanoyl-1*H*-pyrrole-2-carboxylate 14. To a stirred solution of pyrrole **13**[49] (5.0 g, 21.8 mmol) in dry DCM (250 mL) at 0 °C was added drop-wise $SnCl_4$ (2.6 mL, 21.8 mmol) followed by valeroyl chloride (2.7 mL, 22.9 mmol) drop-wise and the resultant solution was stirred at 0 °C for 30 minutes. The reaction mixture was then poured slowly into water. 5% aqueous HCl (5 mL) was then added and the mixture was stirred for 5 minutes. The organic layers were separated and the aqueous layer was extracted into DCM. The combined organic layers were washed with brine and water, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification using chromatography over silica using 20% (v/v) ethyl acetate in hexanes as the eluent gave the desired product as a bright white solid (6.56 g, 96%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.93 (t, J = 7.5 Hz, 3 H), 1.41–1.34 (m, 2 H), 1.69–1.63 (m, 2 H), 2.49 (s, 3 H), 2.60 (s, 3 H), 2.71 (t, J = 7.5 Hz, 2 H), 5.32 (s, 2 H), 7.34 (tt, J = 7.0, 1.5 Hz, 1 H), 7.38–7.36 (m, 2 H), 7.42–7.40 (m, 2 H), 9.11 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.9, 14.1, 15.3, 22.7, 26.5, 42.7, 66.2, 117.6, 123.8, 128.3, 128.8, 129.8, 136.1, 138.1, 161.4, 198.6. HR-MS (ESI): $[M + H]^+$ calcd For: $C_{19}H_{23}N_1O_3$: 336.1570; found: 336.1575.

Benzyl 3,5-dimethyl-4-pentyl-1*H*-pyrrole-2-carboxylate 15. To a stirred solution of **14** (2.3 g, 7.34 mmol) in dry THF (50 mL) under nitrogen at 0 °C was added drop-wise BH_3·THF (1 M solution in THF, 14.7 mL, 14.7 mmol) *via* syringe. The resultant reaction mixture was stirred under nitrogen at room temperature for 18 hours. The reaction mixture was then quenched by the addition of water (effervescence) (5 mL) and 5% aqueous HCl (30 mL), with stirring at room temperature for 30 minutes. Brine (25 mL) was added and the reaction mixture was extracted into EtOAc (3 × 30 mL). The combined organic fractions were washed with saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried (Na_2SO_4), then concentrated *in vacuo*. Purification *via* chromatography over silica using 20% (v/v) ethyl acetate in hexanes as the eluent gave the desired product as a white solid which turned to light-pink color upon standing (2.10 g, 96%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.89 (t, J = 7.5 Hz, 3 H), 1.36–1.24 (m, 4 H), 1.44–1.38 (m, 2 H), 2.18 (s, 3 H), 2.28 (s, 3 H), 2.34 (t, J = 7.5 Hz, 2 H), 5.29 (s, 2 H), 7.32 (tt, J = 7.0, 1.5 Hz, 1 H), 7.39–7.35 (m, 2 H), 7.43–7.41 (m, 2 H), 8.58 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.9, 11.7, 14.2, 22.7, 24.1, 30.7, 31.8, 65.5, 116.4, 116.4, 122.8, 128.1, 128.2, 128.6, 129.9, 136.8, 161.4. HR-MS (ESI): $[M + Na]^+$ calcd for $C_{19}H_{25}N_1O_2Na$: 322.1778; found: 322.1767.

3,5-Dimethyl-4-pentyl-1*H*-pyrrole-2-carbaldehyde 16. To a mixture of benzyl 3,5-dimethyl-4-pentyl-1*H*-pyrrole-2-carboxylate **15** (1.5 g, 5.0 mmol) and a catalytic amount of palladium on activated carbon (10 mol%) in 500 mL round-bottom flask was added ethanol (125 mL) followed by 20 drops of triethylamine. Hydrogenolysis of the benzyl ester was achieved *via* stirring the reaction mixture for 2 h under 1 atmosphere of hydrogen. The mixture was then filtered through a plug of Celite® to remove the catalyst and the solid phase rinsed with methanol (3 × 50 mL). Removal of the solvent *in vacuo* gave the crude carboxylic acid as pale brown viscous syrup (1.05 g) that was immediately subjected to the next step. To a stirred solution of solution of the crude carboxylic acid (1.05 g, 5.02 mmol) in dry DCM (100 mL) at 0 °C was added drop-wise TFA (5.0 mL, 67.3 mmol) and the mixture was then stirred for 10 minutes. Trimethylorthoformate (3.75 mL, 34.3 mmol) was then added drop-wise to the reaction mixture and stirring was continued at room temperature for 10 minutes (complete conversion according to analysis using TLC). The reaction mixture was quenched by adding aqueous $NaHCO_3$ (50 mL). The organic layer was separated and the aqueous layer was extracted with DCM. The organic fraction was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. Purification *via* chromatography over silica using 20% (v/v) ethyl acetate in hexanes as the eluent gave the desired product as an off-white solid which turned to deep-green color upon standing (1.08 g, 78% over 2 steps). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.89 (t, J = 7.5 Hz, 3 H), 1.37–1.24 (m, 4 H), 1.45–1.39 (m, 2 H), 2.25 (s, 3 H), 2.26 (s, 3 H), 2.35 (t, J = 7.5 Hz, 2 H), 9.42 (s, 1 H), 10.65 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 8.9, 11.7, 14.1, 22.7, 23.8, 30.4, 31.7, 123.5, 127.9, 132.9, 137.0, 175.5. HR-MS (ESI): $[M + Na]^+$ calcd for: $C_{12}H_{19}N_1O_1Na$: 216.1359; found: 216.1350.

(*Z*)-5-(3,5-Dimethyl-4-pentyl-1*H*-pyrrol-2-ylmethylene)-4-methoxy-1,5-dihydro-pyrrol-2-one 17. To a stirred solution of 4-methoxy-3-pyrrolin-2-one (1.3 g, 11.4 mmol) and Et_3N (4.3 mL, 31.0 mmol) in dry DCM (100 mL) was added drop-wise TMSOTf (2.8 mL, 15.5 mmol) at 0 °C. After stirring for 15 min, a solution of the aldehyde **16** (1.0 g, 5.2 mmol) in dry DCM (50 mL) was added drop-wise at 0 °C. The reaction mixture was stirred for 1 h at the same temperature and the mixture was then poured into pH 7 phosphate buffer (325 mL). The organic phase was separated, dried using anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was diluted with THF (100 mL) and conc. aqueous HCl (1.2 mL) was added to the reaction mixture following by stirring at room temperature for 2 h. The reaction mixture was diluted with DCM (150 mL), poured into sat. aqueous $NaHCO_3$ (500 mL) and the layers separated. The aqueous phase was extracted with DCM and the combined organic extracts were dried (Na_2SO_4), then concentrated *in vacuo*. The crude deep brown solid was washed with cold hexane and cold water (1.5 L) to give the title compound as a brown solid (897 mg, 60%) which was subjected to the next step without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.89 (t, J = 7.5 Hz, 3 H), 1.35–1.26 (m, 4 H), 1.46–1.40 (m, 2 H), 2.11 (s, 3 H), 2.34 (s, 3 H), 2.36 (t, J = 7.5 Hz, 2 H), 3.89 (s, 3 H), 5.09 (s, 1 H), 6.37 (s, 1H), 10.19 (s, 1 H), 10.90 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 9.7, 11.6, 14.3, 22.8, 24.3, 30.7, 31.9, 58.2, 89.7, 100.9, 121.5, 121.7, 121.8, 126.0, 132.3, 167.9, 173.1. HR-MS (ESI): $[M + H]^+$ calcd for: $C_{17}H_{25}N_2O_2$: 289.1911; found: 289.1914.

(*Z*)-2-(5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-3,5-dimethyl-4-pentyl-1*H*-pyrrole 18. To a stirred suspension of **17** (318 mg, 1.1 mmol) in dry CH_2Cl_2 (30 mL) was added $POBr_3$ (1.6 g, 5.6 mmol). The resulting solution was heated at reflux

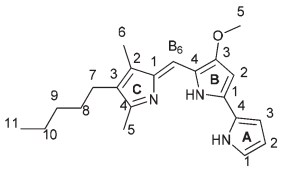


Fig. 9 Prodigiosene **2**.

temperature under nitrogen for 6 days (incomplete conversion). After the reaction mixture was cooled to room temperature, sat. aqueous $NaHCO_3$ (50 mL) was added at 0 °C and the organic layer was separated, then washed with brine (25 mL) and water. The organic fraction was dried (Na_2SO_4), and the solvent was evaporated *in vacuo*. The crude product was purified using silica gel column eluting with 20% EtOAc in hexane to give the title compound as a bright orange brown solid (203 mg, 53%); ^{1}H NMR ($CDCl_3$, 500 MHz) 0.89 (t, J = 7.5 Hz, 3 H), 1.35–1.24 (m, 4 H), 1.45–1.39 (m, 2 H), 2.13 (s, 3 H), 2.28 (s, 3 H), 2.34 (t, J = 7.5 Hz, 2 H), 3.82 (s, 3 H), 5.58 (s, 1 H), 6.88 (s, 1 H), 10.11 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 9.6, 12.4, 14.2, 22.7, 24.1, 30.3, 31.7, 58.4, 99.0, 116.4, 124.1, 126.4, 131.8, 136.4, 137.8, 143.1, 166.6. HR-MS (ESI) $[M + H]^+$ calcd for: $C_{17}H_{24}Br_1N_2O_1$: 351.1067; found: 351.1066.

(*Z*)-5-(3,5-Dimethyl-4-pentyl-pyrrol-2-ylidenemethyl)-4-methoxy-1*H*,1′*H*-[2,2′]bipyrrolyl hydrochloride 2 (Fig. 9). This compound was obtained according to general procedure 5 using bromodipyrrinone **18** (500 mg, 1.42 mmol) as a brown solid (220 mg, 46%). Free-base data (signal assignments based on NOE, COSY, HMBC, HMQC experiments): ^{1}H NMR (CD_2Cl_2, 3.6 × 10^{-2} M, 500 MHz) 0.85 (t, J = 7.5 Hz, 3 H, H^{C11}), 1.34–1.26 (m, 6 H, H^{C8}, H^{C9} and H^{C10}), 1.80 (s, 3 H, H^{C5}), 2.12 (s, 3 H, H^{C6}), 2.24 (t, J = 7.5 Hz, 2 H, H^{C7}), 3.96 (s, 3 H, H^{B5}), 6.10 (s, 1 H, H^{B2}), 6.16 (bs, 1 H, H^{A2}), 6.67 (bs, 2 H, H^{A1} and H^{A3}), 6.91 (s, 1 H, H^{B6}). ^{13}C NMR ($CDCl_3$, 125 MHz) 9.7, 10.5, 14.2, 22.7, 24.3, 30.5, 31.9, 58.4, 95.2, 109.7, 111.7, 113.5, 122.3, 123.2, 125.7, 128.9, 129.8, 137.0, 158.4, 158.5, 168.6. Then a solution of HCl in MeOH (0.75 M, 1.5 equiv.) was added to a solution of the free-base in MeOH (20 mL) to give the HCl salt as deep pink solid. (Signal assignments based on NOE, COSY, HMBC, HMQC experiments). ^{1}H NMR (CD_2Cl_2, 3.6 × 10^{-2} M, 500 MHz) 0.88 (t, J = 7.5 Hz, 3 H, H^{C11}), 1.35–1.25 (m, 4 H, H^{C10} and H^{C9}), 1.47–1.41 (m, 2 H, H^{C8}), 2.22 (s, 3 H, H^{C6}), 2.39 (t, J = 7.5 Hz, 2 H, H^{C7}), 2.50 (s, 3 H, H^{C5}), 3.99 (s, 3 H, H^{B5}), 6.14 (s, 1 H, H^{B2}), 6.35–6.33 (m, 1 H, H^{A2}), 6.92–6.91 (m, 1 H, H^{A3}), 7.07 (s, 1 H, H^{B6}), 7.18–7.17 (m, 1 H, H^{A1}), 12.60 (bs, 1 H), 12.65 (bs, 1 H), 12.74 (bs, 1 H). ^{13}C NMR (CD_2Cl_2, 3.6 10^{-2} M, 125 MHz) 10.1 (C^{C6}), 12.6 (C^{C5}), 14.2 (C^{11}), 22.9, 24.2 (C^{C7}), 30.2 (C^{C8}), 31.9, 59.1 (C^{B5}), 93.0 (C^{B2}), 111.7(C^{A2}), 113.4 (C^{B6}), 116.3 (C^{A3}), 119.9 (C^{A4}), 122.9, 124.6 (C^{C1}), 126 (C^{A1}), 127.5 (C^{C3}), 138.5 (C^{C2}), 146.8, 148.1 (C^{C4}), 165.7 (C^{B3}). UV ($CHCl_3$) λ_{max} (ε) 543 (127 000), 514 (40 000). HR-MS (ESI): $[M - HCl + H]^+$ calcd for: $C_{21}H_{28}N_3O_1$: 338.2227; found: 338.2208. HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH/(H_2O–NH_4OH, 500/1) 90/10, 0.75 mL min^{-1}, λ = 451 nm, ^tR = 41.3 min.

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