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Influence of B-ring modifications on proton affinity, transmembrane anion transport and anti-cancer properties of synthetic prodigiosenes†

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Prodigiosin is the parent compound of the tripyrrolic natural products known as the prodigiosenes. Some of these natural products and their synthetic analogs show anti-cancer, immunosuppressive and anti-microbial actions, amongst other biological activities. One mechanism put forth to explain their biological activity is that since prodigiosenes are typically protonated at physiological pH they can alter intracellular pH *via* HCl co-transport (or Cl^-/OH^- exchange) across cell membranes. In this study we synthesized a series of prodigiosene analogs with different –*O*-aryl substituents attached to the B-ring of the tripyrrolic skeleton. NMR studies showed that these analogs can exist as a mixture of two stable α and β conformers in acidic solution, and that both conformers can bind anions in solution. We found that the electronic nature of the *O*-aryl substituent on the B-ring influences the rate at which these prodigiosenes catalyze transmembrane anion transport, *i.e.* the prodigiosenes with the higher pK_a had greater Cl^-/NO_3^- exchange rates. Four of the synthetic prodigiosenes were tested for their *in vitro* anti-cancer activities in the NCI60 human tumour panel. Despite their promising *in vitro* anti-cancer activity (GI_{50} values ranging from 18 to 74 nM), there was no evidence that this activity is influenced by the extent of protonation of these synthetic prodigiosenes.

Introduction

In the last decade various compounds that catalyze transmembrane transport of anions have been identified.[1] Much of this activity has been driven by supramolecular chemists interested in systems that work at the water-lipid interface.[2] Challenges in transmembrane anion transport are to extract an anion from water and then move it across a hydrophobic membrane. Such transport is facilitated by (1) amphiphilic "carriers" that bind anions and diffuse through the membrane or (2) compounds that self-assemble into channels that span the membrane and provide a low-energy pathway.[1,2] Efforts in this area have led to a better understanding of the factors that control anion transport. Thus, non-covalent interactions like hydrogen bonding, electrostatic effects, anion-π and halogen bonds have been used to effect anion transport.[3] Recent work has also clarified how ligand conformation,[4] flexibility,[5] and lipophilicity[6] can be used to catalyze transmembrane anion transport. Another important factor promoting this research is the possibility that anion transporters might be developed into therapeutics for diseases, like cystic fibrosis, that are caused by defective transport of Cl^-.[7]

Some synthetic transporters have been developed after considering how nature catalyzes the process. For example, peptides that contain domains of the Cl^- channel can transport anions across lipid membranes,[8] while modification of natural amphiphiles like cholic acid has provided potent anion carriers.[9] The tripyrrolic prodigiosenes are known to catalyze transmembrane anion transport.[10] The parent compound, prodigiosin (**1**), is a natural product isolated from *Serratia marcescens* (Fig. 1) that has much biological potential[11] as it is able to permeabilize cell membranes, alter intracellular pH and trigger apoptosis.[12] Early studies indicated that prodigiosin is too cytotoxic for healthy cells and, for this reason, it was not initially pursued as a drug candidate.[13] There has been a renewed interest in prodigiosenes since some synthetic analogs have promising anti-cancer,[14] immunosuppressive,[11b,15] anti-malarial,[16] and antimicrobial[17] activities at concentrations below where they are cytotoxic to healthy cells.

As depicted in Fig. 1 protonated prodigiosin **1**·H^+ has a binding pocket for anions, using hydrogen bonds and electrostatic interactions for anion coordination. The resulting amphiphilic neutral salt **1**·H^+Cl^- can then readily diffuse

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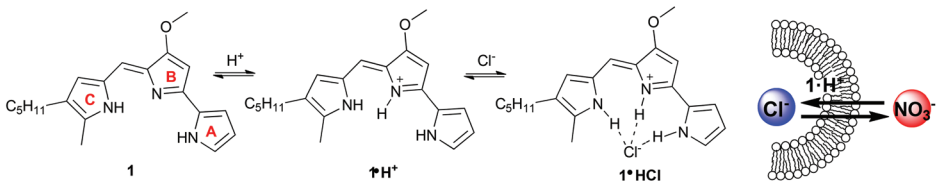


Fig. 1 Protonation of prodigiosin (**1**) and subsequent binding of chloride anion by **1**·H$^+$. The schematic at the right depicts the protonated prodigiosin **1**·H$^+$ catalyzing the exchange of anions across a lipid bilayer.

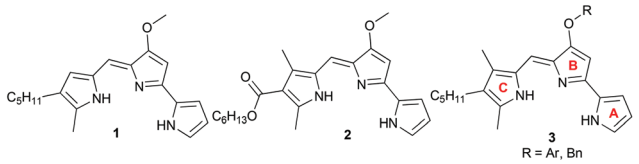


Fig. 2 Natural prodigiosin **1** and synthetic prodigiosenes **2** and **3**.

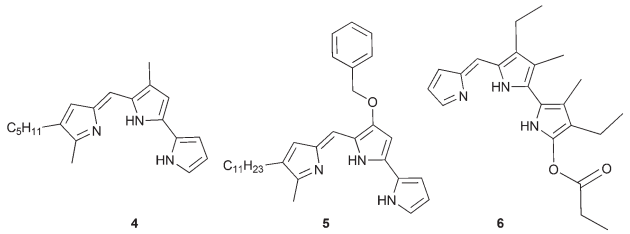


Fig. 3 Examples of B-ring modified prodigiosin analogs.

through the cell membrane. We have previously shown that prodigiosin **1**·H$^+$Cl$^-$ catalyzes the transmembrane exchange of anions such as Cl^-, NO_3^- and HCO_3^-.[10d–f] Prodigiosenes also alter intracellular pH, presumably through catalyzing co-transport of H$^+$Cl$^-$ or the exchange of Cl^-/OH^- across cell membranes. This ability to change intracellular pH is one potential mechanism by which prodigiosenes trigger apoptosis.[11a,12a,18] Other possible modes of actions include stranded-DNA cleavage in the presence of Cu^{2+},[19] as well as protein kinase[20] and Bcl-2 inhibition.[21] The effect of the electronic properties of the A-ring upon the proton affinity and the anti-cancer potency of some prodigiosenes has previously been reported, with no identified correlation between pK_a and the inhibition of cell proliferation.[22]

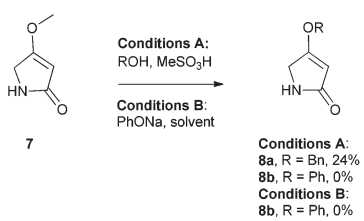
We recently found that ester **2** (Fig. 2), with an electron-withdrawing carbonyl group appended to the C-ring, was less basic by two orders of magnitude than the parent prodigiosin (**1**) (prodigiosin **1**·H$^+$, pK_a = 8.2; **2**·H$^+$, pK_a = 6.5) and also a much less effective anion transporter than **1**.[10f] We suggested that the ease of protonation of the carrier and, thus it's the consequent anion transport activity could be modulated by changing the electronic properties of the C-ring substituents. Herein, we describe a systematic study on some B-ring analogs that confirms transmembrane anion transport rates can be modulated by tuning the pK_a of the prodigiosene skeleton. We describe the synthesis, anion transport and *in vitro* anti-cancer properties of prodigiosenes **3** with different –OAr substituents attached to the tripyrrolic B-ring. The synthetic prodigiosenes (**3**, Fig. 2) used in this study all have an A-ring pyrrole, a B-ring pyrrole substituted with various aryl groups to modulate pK_a, and a C-ring pyrrole that differs from the natural compound **1** by an extra methyl group on the heterocyclic unit.

Synthesis of B-ring modified prodigiosenes

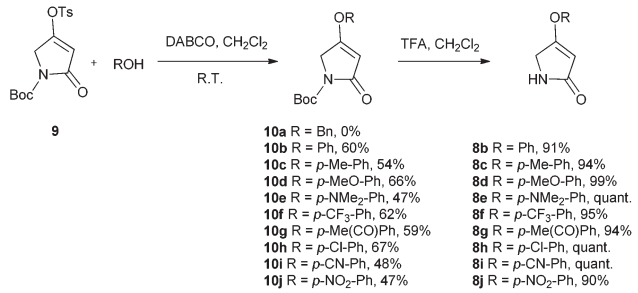
There are few reports regarding synthetic modification of the prodigiosene B-ring.[10a,15,23] Moreover, the impact of B-ring modifications on anti-cancer activity seems mixed. Indeed, the desmethoxy analog **4** (Fig. 3) was relatively inactive as an anti-cancer agent, as compared to the parent prodigiosin (**1**), suggesting that the –OMe group is essential for anti-cancer activity.[23a] However, some analogs without an –OMe group demonstrated reasonable anti-cancer activity. For example, PNU-156804 **5**, with an –OBn group, exhibited nM IC_{50} values against leukemia and melanoma cells,[15] and the synthetic analog **6** was active against lung cancer cell lines.[10a] In this study we prepared novel derivatives substituted with different –OAr groups on the B-ring, alongside common features at the A and C-ring, so as to probe the influence of electronic effects upon pK_a values, transmembrane anion exchange rates and anti-cancer activity. This is, to our knowledge, the first report of prodigiosenes substituted with –OAr groups on the B-ring.

We first required a reliable method to obtain the O-substituted pyrrolinones **8** (Scheme 1), as this moiety would become the B-ring of the ultimate prodigiosenes. Following a patented procedure[24] the –OBn pyrrolinone **8a** was successfully obtained from the –OMe pyrrolinone **7** (Scheme 1). However, using phenol as a nucleophile instead of benzyl alcohol failed to give pyrrolinone **8b**.[25] Attempts using sodium phenoxide were also unsuccessful (Conditions B).

To accomplish the key substitution with phenolates, we decided to use the –OTs pyrrolinone **9**,[26] bearing a competent leaving group (Scheme 2). By varying the nature and amount of the base and solvent, as well as the concentration of the pyrrolinone, we found a suitable method for the formation of



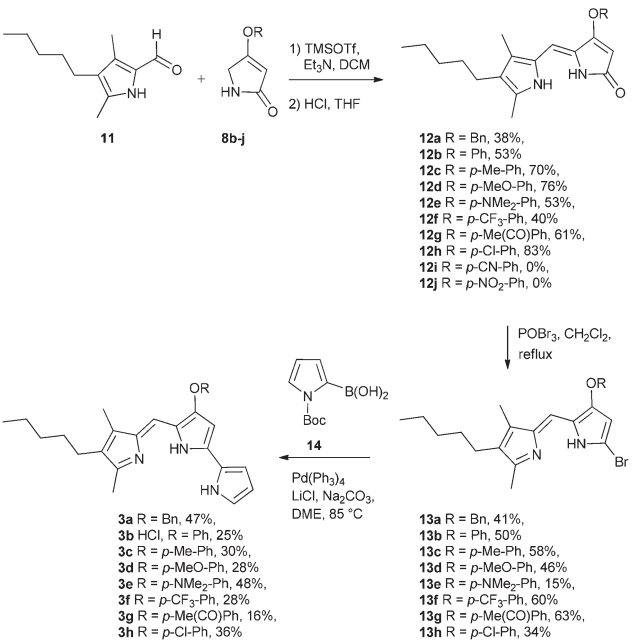
Scheme 1 Direct substitution of pyrrolinone **7**.



Scheme 2 Preparation of –OAr pyrrolinones **8**.

10b using 2.2 equivalents of DABCO in CH_2Cl_2 at room temperature with the tosylate **9** at a concentration of 0.02 M (see ESI Table S1†). Using these optimized conditions, we prepared nine pyrrolinones, **10b–j**, in moderate-good yields (47–62%), regardless of whether the phenol contained an electron-withdrawing or an electron-donating substituent. Acid-catalyzed deprotection of the *N*-Boc group then gave the pyrrolinones **8b–8j** in excellent yields.

With the B-ring precursors **8** in hand we then prepared dipyrrinones **12a–h** (intermediates that contain the B- and C-rings of the ultimate prodigiosene targets, Scheme 3). Aldol condensation of aldehyde **11**[10f] with pyrrolinones **8b–j**, promoted by TMSOTf and Et_3N, proceeded in good yield, except when the *O*-aryl group was substituted with strong electron-withdrawing groups (**8i** R = Ph-*p*CN and **8j** R = Ph-*p*NO_2). Apparently, the presence of a strong electron-withdrawing group in **8i** and **8j** makes the ether prone to hydrolysis or substitution, as we always detected the corresponding phenol in the crude mixture (TLC) after the reaction. Bromination of dipyrrinones **12a–h**, followed by Suzuki–Miyaura coupling using (1-Boc-1*H*-pyrrol-2-yl)boronic acid **14**,[27] gave the prodigiosenes **3a–h** in moderate yields. As described below, we next conducted mechanistic studies to investigate prodigiosene conformation in solution, proton affinity, transmembrane anion transport activity and anti-cancer properties for this class of B-ring adducts.

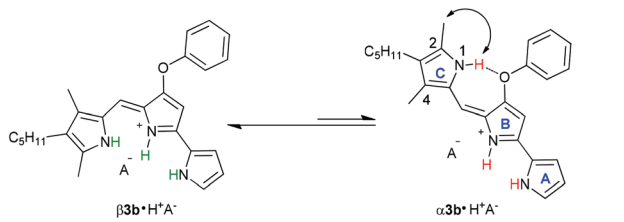


Scheme 3 Synthesis of prodigiosenes **3**.

NMR experiments reveal that both α and β conformations of 3b·H$^+$ bind and exchange anions

Transmembrane exchange of Cl^- and NO_3^- anions by prodigiosin (**1**) involves anion binding by the protonated transporter (**1**·H$^+$). This is followed by diffusion of this neutral complex **1**·H$^+$Cl$^-$ across the lipid membrane, and Cl^-/NO_3^- anion exchange at the lipid–water interface.[10d] The anion binding and transport processes are complicated by the fact that protonated prodigiosenes often exist as two isomers (Scheme 4). This α/β conformational equilibrium can vary as a function of ligand structure and environmental conditions.[23b,28] As depicted in Scheme 4, the prodigiosene α and β conformations should both bind anions.

We investigated qualitative aspects of the solution-state conformation of the –OPh derivative **3b**·H$^+$. Our goals were (1) to determine whether **3b**·H$^+$ exists in both α and β conformations in solution and (2) to obtain evidence that **3b**·H$^+$ undergoes anion exchange in solution. The complex **3b·HCl** (1 mM) showed one set of signals, consistent with the β conformation, when initially dissolved in $CDCl_3$ (see spectra on p. 56 of ESI†). However, after being in $CDCl_3$ solution over time the same sample showed two sets of ^{1}H NMR signals, in an approximate 3 : 1 ratio. Based on analogy with previous NMR studies of other prodigiosenes in $CDCl_3$, by Rizzo *et al.* and by Quesada *et al.*,[23b,28] we assigned the major set of NMR signals to the β isomer of **3b·HCl** and the minor set of signals to the α isomer. We next investigated whether both conformers of **3b**·H$^+$ undergo anion binding and exchange in solution. Thus, we titrated methanesulfonic acid (MsOH) into a solution of **3b·HCl** in $CDCl_3$ and followed changes in ^{1}H NMR signals for the pyrrolic N*H* protons (δ 12–13 ppm). We reasoned that changes in this region of the spectrum would reflect anion binding and exchange between chloride and methanesulfonate anions, since it is the pyrrole N*H* atoms that hydrogen bond with the anion. Fig. 4 shows representative spectra from the titration. The N*H* region of the ^{1}H NMR spectrum for the **3b·HCl** salt (Fig. 4a) indicates a mixture of isomers, but the NMR signals were poorly resolved. However, this overlap



Scheme 4 β and α conformational isomers of prodigiosene **3b**. Both the major β and minor α isomers should be able to bind anions.

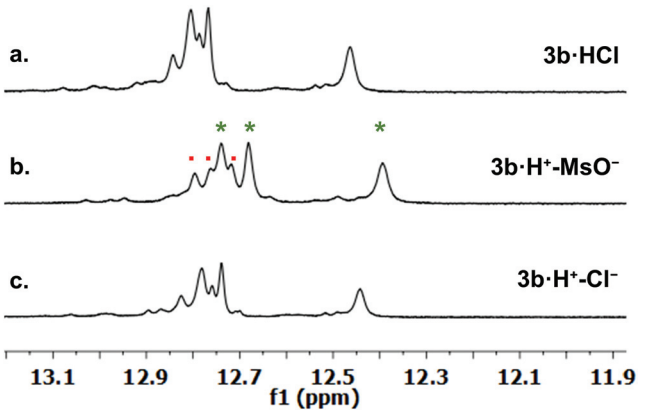


Fig. 4 ^{1}H NMR spectra in $CDCl_3$ for the pyrrolic N*H* region of: (a) **3b·HCl**; (b) after addition of 6 eq. of MsOH to sample **a**, two sets of N*H* signals were observed for each α (·) and β (*) conformer; (c) after addition of 1 eq. of tetrabutylammonium chloride (TBACl) to sample **b**.

for the pyrrolic N*H* protons was removed upon the addition of MsOH to a solution of **3b·HCl** (Fig. 4b). As shown in Fig. 4b, we observed two well-resolved sets of three pyrrolic N*H* signals for the **3b·HOMs** complex. There are two important findings here. First, the N*H* chemical shifts change upon addition of the MsOH, suggesting that both the α and β isomers bind anions. Thus, as shown in Fig. 4b chemical shifts for the six N*H* protons moved upfield when MsOH was added to a solution of **3b·HCl**. These changes in N*H* chemical shifts indicate anion exchange, as Cl^- is replaced by the softer methanesulfonate anion in the binding site of the prodigiosene. Second, the α/β isomer ratio for **3b·HCl** and **3b·HOMs** does not change significantly with a change in bound anion (Fig. 4c). A 2D NOESY NMR experiment of the **3b·HOMs** complex allowed us to assign signals for the three N*H* protons in the major β isomer and for the three N*H* protons in the α isomer. The NOE correlation that best supports our assignments can be observed between one of the minor pyrrole NH signals and the signal corresponding to the C2 methyl group of the α isomer (Fig. 5).

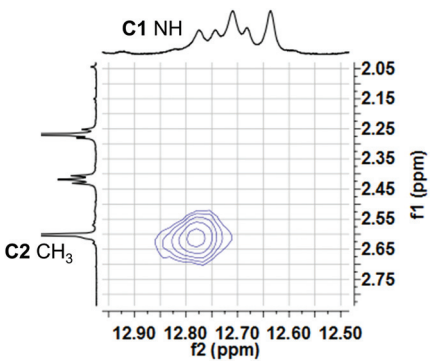


Fig. 5 A region of the ^{1}H–^{1}H NOESY spectrum of **3b·OMs** in $CDCl_3$ shows a NOE correlation between one of the "minor" pyrrole N*H* protons and C2 methyl hydrogens. This correlation is consistent with the pyrrolic N*H* of the C-ring being involved in an intramolecular H-bond with the –OPh group of the B-ring (see Scheme 4).

For this α isomer, the C-ring N*H* proton forms an intramolecular hydrogen bond with the –OPh group on the B-ring (Scheme 4). Presumably, this hydrogen-bonded N*H* proton exchanges much more slowly with residual water in the $CDCl_3$ solvent and, therefore, shows a NOE correlation with its neighbouring C2-methyl group.

These NMR titrations allowed us to conclude that (1) both the major β and minor α isomer of **3b·H$^+$** bind and exchange anions and (2) Cl^- interacts more strongly than OMs^- with **3b·H$^+$**. We next determined the apparent pK_a values for some *O*-aryl analogs with the intent to correlate their ease of protonation with their rates of transmembrane anion transport.

Determination of apparent pK_a values for synthetic prodigiosenes

We hypothesized that prodigiosene basicity would correlate with its efficiency as an anion transporter, since the protonated prodigiosene **3b·H$^+$** is the likely transporter. The concept that transporter efficiency can be tuned by modulating the acidity of hydrogen bond donors has been demonstrated with other systems.[29] To learn how B-ring substitution influences the acid-base properties of these synthetic prodigiosenes we used a spectrophotometric method, described by Manderville,[22] to measure the apparent pK_a values for five B-ring analogs: the –OPh derivative (**3b·H$^+$**), two analogs with electron-donating groups on the –OAr ring (**3d·H$^+$** R = Ph-*p*OMe, **3e·H$^+$** R = Ph-*p*NMe_2), and two analogs with electron-withdrawing groups on the B-ring (**3f·H$^+$** R = Ph-*p*CF_3, **3h·H$^+$** R = Ph-*p*Cl).

In solution, protonated prodigiosenes are pink with absorbance maxima above 500 nm. The free-base absorbs below 500 nm. Fig. 6 shows representative pH-dependent spectra for **3d** and **3f** in 1 : 1 acetonitrile–water at 25 °C. Absorption maxima for the free-base of the Ph-*p*OMe analog **3d** (λ_{max} = 484 nm) and its protonated form **3d·H$^+$** (λ_{max} = 541 nm) are well separated (Fig. 6a). The Ph-*p*CF_3 derivative showed similar properties, with λ_{max} = 450 nm for the free-base **3f** and λ_{max} = 545 nm for **3f·H$^+$** (Fig. 6b). Furthermore, the ionization states of **3d**/**3d·H$^+$** and **3f**/**3f·H$^+$** change smoothly as a function of pH, both revealing isosbestic points. The apparent pK_a values, which correspond to values for the equilibrium mixture of α and β isomers, for these B-ring prodigiosenes were determined from plots of the log(ionization ratio) *vs.* pH.[22] As seen in

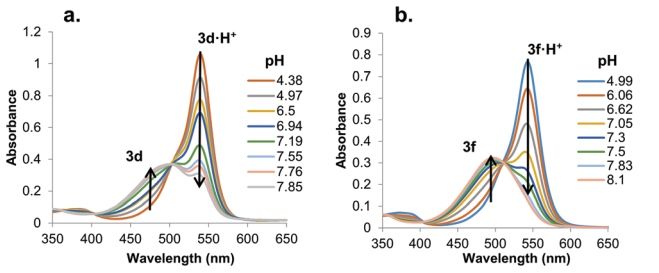


Fig. 6 UV-Vis absorbance spectra for a. **3d** and b. **3f** as a function of pH in 1 : 1 CH_3CN–H_2O (v/v) at 25 °C (0.1 M NaCl).

Table 1 pK_a values and transmembrane anion exchange rates for some synthetic prodigiosenes with different –OAr groups on the B-ring. Transport experiments were repeated in triplicate and all traces reported in Fig. 7 are the average of those 3 trials

$3{\cdot}H^+$	pK_a	Initial rate for Cl^-/NO_3^- exchange (s^{-1})	$t_{1/2}$ for Cl^- efflux (s)
3d (Ph-*p*OMe)	7.3 ± 0.1	15.1 ± 0.2	65 ± 2
3e (Ph-*p*NMe_2)	7.4 ± 0.1	10.8 ± 0.3	87 ± 3
3b (Ph)	7.2 ± 0.1	10.8 ± 0.3	90 ± 5
3h (Ph-*p*Cl)	6.8 ± 0.1	3.4 ± 0.1	168 ± 3
3f (Ph-*p*CF_3)	6.7 ± 0.1	1.0 ± 0.1	287 ± 2

Table 1, analogs with electron-donating groups on the B-ring, namely $\mathbf{3d{\cdot}H^+}$ (R = Ph-*p*OMe, pK_a = 7.3) and $\mathbf{3e{\cdot}H^+}$ (R = Ph-*p*NMe_2, pK_a = 7.4) are weaker acids than are analogs with electron-withdrawing groups, $\mathbf{3f{\cdot}H^+}$ (R = Ph-*p*CF_3, pK_a = 6.7) and $\mathbf{3h{\cdot}H^+}$ (R = Ph-*p*Cl, pK_a = 6.8). This means that the free-bases of **3d** (R = Ph-*p*OMe) and **3e** (R = Ph-*p*NMe_2) are more basic than **3f** (R = Ph-*p*CF_3) and **3h** (R = Ph-*p*Cl). The increased basicity of **3d** and **3e** means that a greater percentage of these analogs would be protonated at physiological pH, which should enhance their ability to extract anions from water and transport these bound anions as neutral complexes **3·HA** across lipid membranes.

Correlation between anion transport rates and transporter basicity

Having established that B-ring substitution influences the acid-base properties of –OAr analogs **3b**, **3d–f** and **3h**, we next compared the ability of these synthetic prodigiosenes to catalyze the exchange of anions across phospholipid vesicles. Transmembrane transport was evaluated with a liposome model that uses the chloride-sensitive dye, lucigenin (LG), to monitor anion exchange.[30] This assay works because LG fluorescence is quenched by Cl^- but not by NO_3^-. Thus, egg-yolk phosphatidylcholine (EYPC) liposomes loaded with 1.0 mM LG and NaCl were suspended in a solution (pH 7.4) containing extravesicular $NaNO_3$. Upon the addition of our synthetic prodigiosenes **3** the fluorescence of the intravesicular LG increases, indicating Cl^- efflux and NO_3^- influx. Fig. 7 shows representative fluorescence curves, plotted as a function of time, after addition of analogs **3b**, **3d–f** and **3h**. We quantified transport rates by (a) determining the initial rate of change of the relative fluorescence of the intravesicular LG and (b) estimating the half-life of the exchange reaction (Table 1). The data in Fig. 7 and Table 1 contain important findings. First, these synthetic prodigiosenes, especially **3d** and **3e**, are potent anion transporters and relatively low transporter loadings are needed to observe significant Cl^- efflux from the liposomes. For example, we used only 0.0008 mol% of transporter relative to EYPC lipid (1 transporter for 125 000 lipid molecules or about 2.5 transporters per liposome) to generate the data in Fig. 7. This is one of the lowest transporter loadings reported to date.[5] Second, substitution of the B-ring with an –OAr group does not perturb the ability of these analogs to catalyze anion exchange. Indeed, the electron-rich $\mathbf{3d{\cdot}H^+}$ (R = Ph-*p*OMe) is qualitatively similar to the natural product prodigiosin $\mathbf{1{\cdot}H^+}$ in terms of the efficiency at which it exchanges Cl^- and NO_3^- anions across the EYPC bilayer (see ESI Fig. S6†) Most importantly, compounds substituted with electron-donating groups (**3d** and **3e**) are better anion transporters than analogs containing electron-withdrawing groups (**3f** and **3h**). These results indicate that the catalytic efficiency of transmembrane anion exchange correlates with how much of the prodigiosene is in its protonated form $\mathbf{3{\cdot}H^+}$.[31]

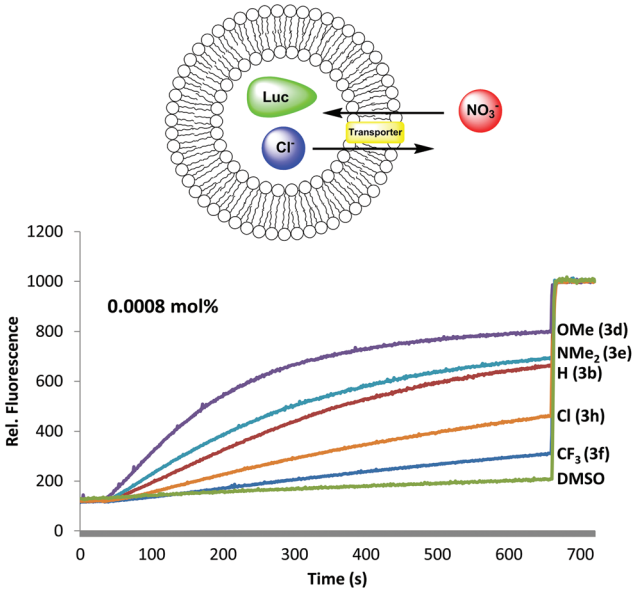


Fig. 7 Anion exchange assay for analogs **3b**, **3d–f** and **3h**. Change in fluorescence of lucigenin (LG) observed in EYPC liposomes at 25 °C. The data was collected using 0.0008 mol% of transporter compounds relative to EYPC lipid. Liposomes containing 1 mM lucigenin, 20 mM HEPES buffer and 100 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.

In vitro anti-cancer properties

The *in vitro* activity of some of the B-ring analogs was evaluated at the National Cancer Institute (NCI, http://dtp.cancer.gov) against the standard NCI60 panel of 60 human cell lines.[32] Prodigiosenes selected for testing were the benzyl (**3a**) and phenyl (**3b**) analogs and derivatives with an electron-donating (**3d** R = Ph-*p*OMe) and an electron-withdrawing group (**3h** R = Ph-*p*Cl). Activities against the NCI60 panel are reported in Table 2 as mean values averaged over all 60 cell lines; these activities are (1) the half maximal growth inhibition concentration, GI_{50}, (2) the total growth inhibition concentration, TGI, and (3) the half maximal lethal concentration, LC_{50}. All four synthetic prodigiosenes exhibited GI_{50} values in the nM range, similar in magnitude to **1**, indicating that replacement of the B-ring OMe by an *O*-aryl group is not universally detrimental to anti-cancer activity. They also show

Table 2 *In vitro* activity of some prodigiosenes over the NCI60 cancer cell lines. Values are a mean of two experiments (http://dtp.cancer.gov)

	1	**3a** (Bn)	**3b** (Ph)	**3d** (Ph-*p*OCH_3)	**3h** (Ph-*p*Cl)
GI_{50} (nM)[a]	14	44	18	74	62
TGI (μM)[b]	2.1	0.6	0.2	0.8	1.9
LC_{50} (μM)[c]	0.3	4.8	2.5	7.2	14.4

[a] GI_{50} = half maximal growth inhibition concentration. [b] TGI = total growth inhibition concentration. [c] LC_{50} = half maximal lethal concentration.

LC_{50} values in the μM range that could indicate a lower toxicity compared to the natural compound **1** (LC_{50} = 300 nM). The phenyl analog **3b** was the most active of the derivatives with a mean GI_{50} value of 18 nM. A more detailed analysis in Fig. 8 shows that **3b** was also typically the most active of the four synthetic prodigiosenes against all 60 cell lines.

Two questions that we sought to answer were would the prodigiosene basicity (1) correlate with its ability to catalyze transmembrane anion exchange and (2) correlate with its anti-cancer activity. The data presented herein suggest that the ease of protonation controls the transmembrane anion exchange. However, despite their significant *in vitro* anti-cancer activity, there is no evidence that those anti-cancer properties universally correlate with, or are caused by, the acid-base properties of the prodigiosene. Thus, the most basic prodigiosene **3d** and the least basic prodigiosene **3h** had similar mean GI_{50} values, 74 and 62 nM respectively, and both analogs were less potent than the phenyl analog **3b** (GI_{50} = 18 nM).

Conclusions

Prodigiosenes, both naturally occurring and synthetic analogs, are outstanding catalysts for transport of anions across lipid membranes. Being able to control the efficiency of this transmembrane transport of anions would be valuable for various reasons, such as the potential development of therapeutics or anion sensors. In this study we synthesized some unique prodigiosene analogs with different –OAr substituents attached to the B-ring of the tripyrrolic skeleton. We measured the apparent pK_a values of five B-ring analogs, namely the *O*-phenyl derivative (**3b·H+**), two *O*-aryl analogs with electron-donating groups and two *O*-aryl analogs with electron-withdrawing groups. These five derivatives were then tested for their ability to catalyze transmembrane exchange of Cl^- and NO_3^- anions in EYPC liposomes. The data indicate that the efficiency of anion transport can indeed be modulated by the electronic nature of the *O*-aryl substituent on the B-ring. Thus, more basic analogs **3d** (R = Ph-*p*OMe, pK_a = 7.3) and **3e** (R = Ph-*p*NMe_2, pK_a = 7.2) showed significantly greater Cl^-/NO_3^- exchange rates than did the less basic analogs **3h** (R = Ph-*p*Cl, pK_a = 6.8) and **3f** (R = Ph-*p*CF_3, pK_a = 6.7). This suggests that the key factor controlling anion transport for this family of synthetic analogs, and presumably for the natural product prodigiosin, is the protonation of the prodigiosene to give an amphiphilic cation **3·H$^+$**. This cation extracts anions from water to give a neutral complex **3·HA** that then diffuses rapidly across the lipid membranes. In addition to the synthetic and mechanistic work we also evaluated the anti-cancer activity of four synthetic prodigiosenes (NCI60 human tumour panel).

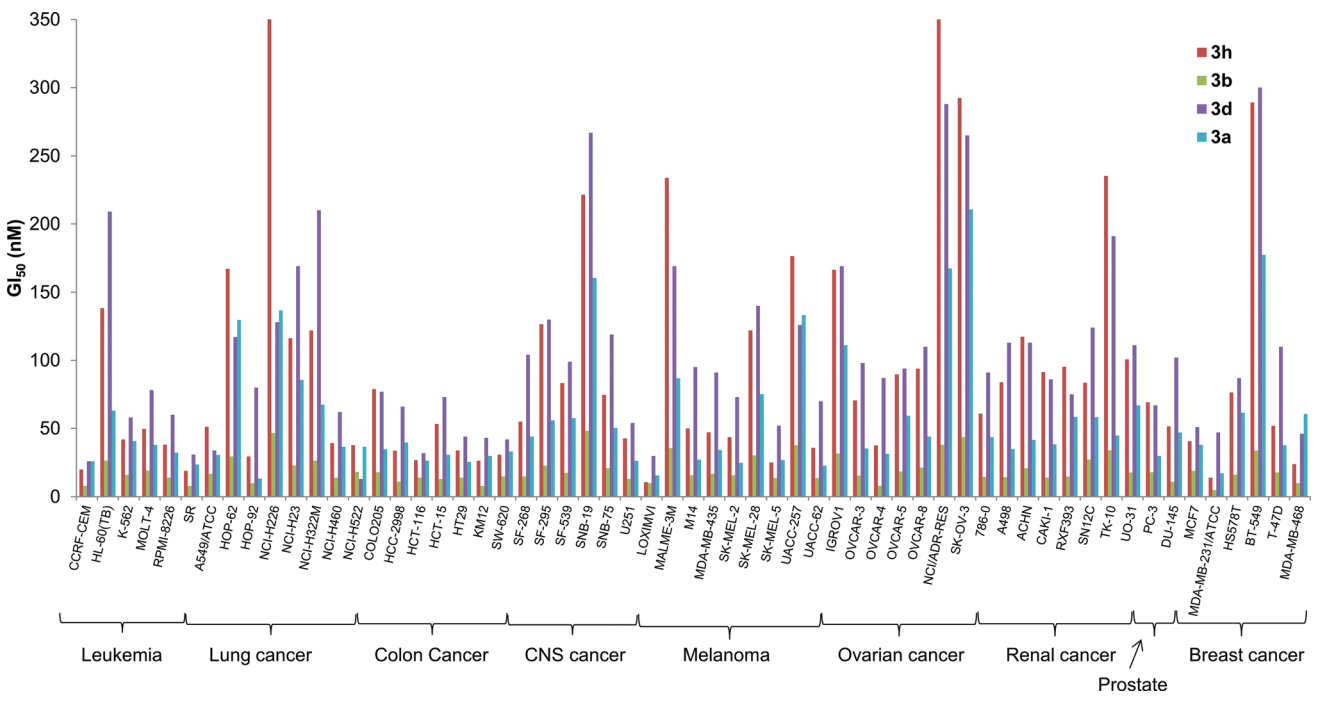


Fig. 8 GI_{50} concentrations (half maximal growth inhibition concentrations) of some synthetic prodigiosenes **3a**, **3b**, **3d** and **3h** against 60 human cancer cell lines representing 9 different cancer types; see http://http:dtp.cancer.gov.

All four analogs showed potent anti-cancer activities, with mean GI_{50} values in the nM range, indicating that substitution of the B-ring with OAr groups can give potent anti-cancer agents. Despite their promising *in vitro* anti-cancer activity there was no evidence that this activity is due to the ease of protonation of these synthetic prodigiosenes.

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