Synthesis and Biological Evaluation of Nonylprodigiosin and Macrocyclic Prodigiosin Analogues

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*Nonylprodigiosin (**4**) and various of its analogues have been prepared by Suzuki cross-coupling reactions of a well accessible pyrrolyl triflate with (hetero)aryl boronic acid derivatives bearing alkenyl side chains. The resulting alkenes or dienes were subjected to metathesis dimerization or ring-closing metathesis (RCM) reactions, respectively, by using a ruthenium indenylidene complex as the catalyst. The biological activity of the products thus obtained was tested in two different assays monitoring i) the proliferation of murine spleen cells induced by lipopolysaccharides (LPS) and concanavalin A (Con A), and ii) the vacuolar acidification of baby hamster kidney (BHK) cells. Compounds **4** and **21** suppressed Con A-induced T-cell proliferation much more potently than LPS-induced B-cell proliferation. Furthermore, compounds **4** and **26** markedly inhibited vacuolar acidification, although other compounds exhibited no or only marginal effects. Thus, the immunosuppressive activity of prodigiosins toward T-cell proliferation seems to be mediated through cellular targets distinct from vacuolar acidification, and the prodigiosin analogues might be powerful tools to dissect these biological responses. The X-ray crystal structure of the macrocyclic product **25** has been determined, showing that the replacement of one pyrrole ring of the parent compound **4** by a phenyl group does not alter the overall electronic features of the remaining heterocyclic ring system of these alkaloids.*

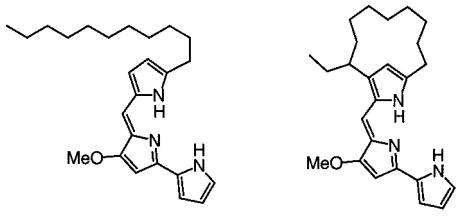
KEYWORDS:

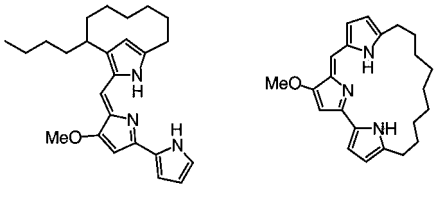
alkaloids · immunosuppression · metathesis · prodigiosins · Suzuki reaction

Introduction

Recently it has been shown that deeply red colored prodigiosin alkaloids produced by a restricted group of *Streptomyces* and *Serratia* strains exhibit significant immunosuppressive activities at doses that are not cytotoxic.[1–4] The molecular and cellular target of undecylprodigiosin (**1**), the most abundant member of this family, has yet to be determined, although it is known that this compound acts as H^+/Cl^- symporter that uncouples proton translocation and thereby leads to perturbation of vacuolar acidification. Moreover, the data presently available suggest that the mechanism of action of this compound and its closely related cyclic congeners such as **2** – **4** is distinctly different from that of cyclosporin or FK-506 which define the standards in the field of immunotherapy.[5, 6] As a consequence, a synergetic effect on the immune system is observed if, for example, **1** and FK-506 are administered simultaneously.[7]

Although the actual therapeutic window may be too narrow for direct clinical applications of **1** – **4**,[8] these alkaloids constitute important lead structures in the search for synthetic analogues with more favorable pharmacological properties.[9] All attempts to map the structure – activity relationship in prodigiosin alkaloids, however, must consider their specific propensity to exist in two isomeric forms, **I** and **II**, in solution (Scheme 1). The interconversion and equilibrium distribution strongly depends on the pH value of the medium, that is, on the degree of protonation of the basic nitrogen atom.[9a] Because it is unlikely





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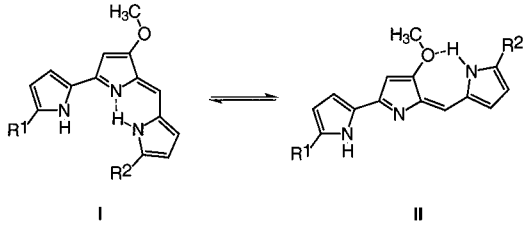
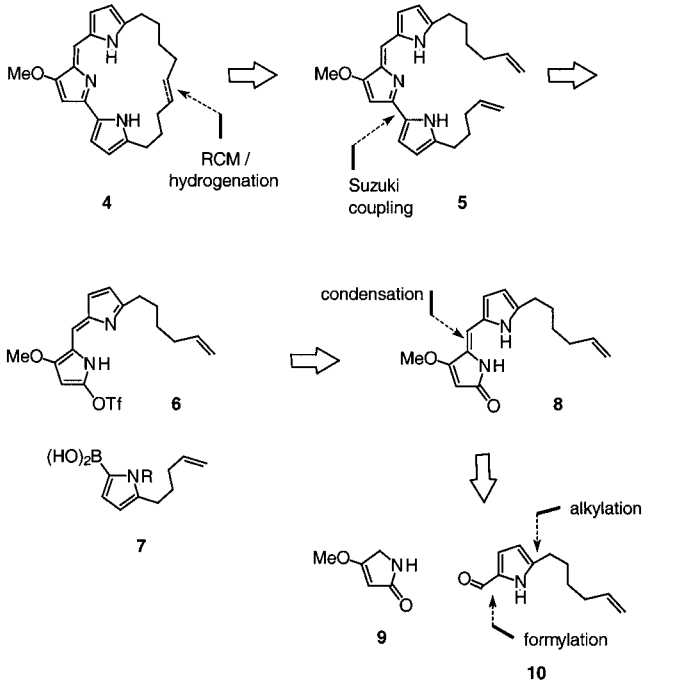


I II

Scheme 1. *Different isomers of the heterocyclic domain of prodigiosins stabilized by intramolecular hydrogen bonds.*

that both isomers show the same affinity to the yet unknown biological receptor, prodigiosin derivatives with a defined configuration may be of considerable interest for more detailed biochemical and pharmaceutical investigations.

Therefore, we reasoned that the naturally occurring macrocyclic nonylprodigiosin (**4**), isolated in 1970 by Gerber from *Actinomadura madurae*,[10] constitutes a particularly attractive target since its alkyl chain spans all three heterocyclic rings and thereby locks the *Z* configuration of the azafulvene entity. We have recently completed an efficient total synthesis of **4** based upon a Suzuki cross-coupling reaction for the formation of its heterocyclic domain and a ring-closing olefin metathesis (RCM) for the cyclization of the macrocyclic ring.[11, 12] Here we describe the preparation of various nonylprodigiosin analogues by exploiting the flexible design of our synthesis blueprint. Upon comparison with the biological activity of the acyclic parent compound **1**, these new derivatives provide insights into the links between the configuration of the azafulvene unit as well as the presence of an intact pyrrolylpyrromethene chromophore and the biological response to alkaloids of this type.



Scheme 2. *Retrosynthetic analysis for nonylprodigiosin (**4**). OTf = triflate = trifluoromethanesulfonate.*

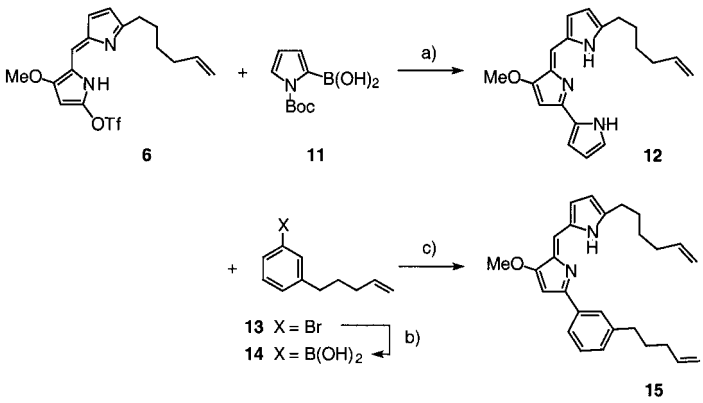
Results and Discussion

Chemistry

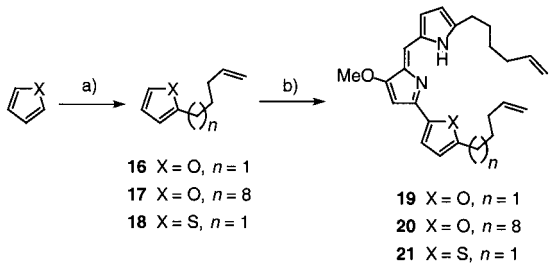
Our total synthesis of nonylprodigiosin (**4**) is based on the disconnections shown in Scheme 2.[11] Thus, RCM leads to the efficient formation of the macrocyclic ring from diene **5**,[13, 14] which was assembled by a Suzuki reaction of the boronic acid **7** with the heteroaryl triflate **6**.[15, 16] The latter is derived from the lactam **8** that can be prepared in a few routine steps on a multigram scale from commercially available precursors. Due to its stability and easy accessibility, we reasoned that this compound may serve as a convenient platform for the preparation of various analogues of **4** if synthon **7** is replaced by other suitably functionalized boronic acid derivatives.

This concept was put into practice as shown in Schemes 3 and 4. Thus, a palladium-catalyzed Suzuki reaction of triflate **6** with *N*-Boc-pyrrol-2-ylboronic acid (**11**)[17] affords product **12**, which differs from undecylprodigiosin (**1**) only in the length of the alkyl chain and the presence of a double bond that provides a handle for further functionalization. Likewise, cross-coupling of **6** with the readily accessible phenylboronic acid derivative **14** gave product **15** in good yield.[18]

Similar attempts to prepare prodigiosin analogues, however, in which one of the pyrrole units is replaced by another



Scheme 3. *Synthesis of dienes **12** and **15**. a) [Pd(PPh$_3$)$_4$] (cat.), aq Na$_2$CO$_3$, DME, 85 °C, 73 %; b) 1. nBuLi, Et$_2$O; 2. B(OMe)$_3$; 3. aq HCl; c) [Pd(PPh$_3$)$_4$] (cat.), aq Na$_2$CO$_3$, DME, 55 °C, 60 %. Boc = tert-butoxycarbonyl.*



Scheme 4. *Synthesis of dienes **19**–**21**. a) 1. nBuLi, Et$_2$O; 2. 1-bromo-4-pentene (→**16**, 73 %; →**18**, 36 %), or 1-bromo-11-dodecene (→**17**, 93 %); b) 1. nBuLi, THF; 2. B(OMe)$_3$; 3. triflate **6**, [Pd(PPh$_3$)$_4$] (cat.), aq Na$_2$CO$_3$, DME, (→**19**, 78 %; →**20**, 64 %; →**21**, 81 %).*

heteroaromatic ring, met with failure.[19] The problem was traced back to the high lability of the required furan- or thiophene boronic acid derivatives, which undergo rapid protodeborylation and could therefore not be obtained in sufficiently pure form. To circumvent this problem, we employed the "in situ" borylation/Suzuki cross-coupling method[20] summarized in Scheme 4. Thus, deprotonation of 2-(4-pentenyl)furan (**16**) with *n*BuLi followed by addition of $B(OMe)_3$ affords a borate complex which is transferred by a cannula into a solution of triflate **6** in 1,2-dimethoxyethane (DME). Addition of catalytic amounts of $[Pd(PPh_3)_4]$ and aqueous Na_2CO_3 effects a smooth cross-coupling of these components affording product **19** in 78% yield. Compound **20** bearing a longer side chain on the furan ring as well as the thiophene analogue **21** have been prepared analogously.

These alkene derivatives were then subjected to RCM or metathesis dimerization reactions by using the newly developed ruthenium indenylidene complex **22** (Cy=cyclohexyl) as the catalyst of choice (Table 1).[21] In its presence, alkene **12** undergoes a high-yielding dimerization with formation of product **26** containing two tethered pharmacophore units.[22] In line with our previous investigations on RCM-based macrocyclizations,[13, 14] the hydrochloride salts of dienes **15**, **19**, and **20** cyclize without incident to the corresponding nonylprodigiosin analogues **25**, **23**, and **24**, respectively, in good to excellent yields if the reactions are carried out under high-dilution conditions. Only the thiophene derivative **21** resisted ring closure; this failure, however, can be explained by the well precedented incompatibility of standard metathesis catalysts with sulfur-containing substrates.[23]

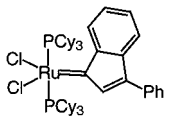


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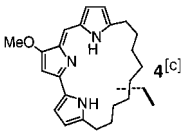
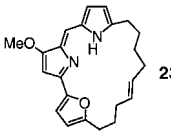
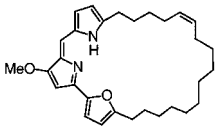
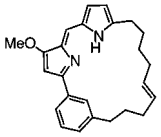
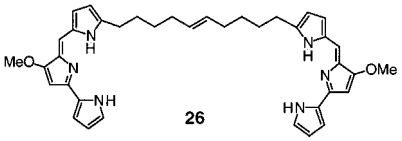
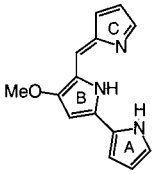
Table 1. *Preparation of nonylprodigiosin and its analogues by RCM or metathesis dimerization.*[a]

Substrate[b]	Product	Yield [%]
5	 **4**[c]	65
19	 **23**	64
20	 **24**	57
15	 **25**	86
12	 **26**	93

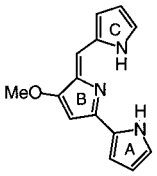
[a] All reactions were carried out in refluxing CH_2Cl_2 with 5–10 mol% of complex **22** as the catalyst. [b] HCl salts of the substrates were used for RCM. [c] Nonylprodigiosin (**4**) is obtained by hydrogenation of its unsaturated analogue formed by RCM, see ref. [11]; the site of ring closure is indicated by the dotted line.

Structure

The chemical behavior of prodigiosins can be inferred from the two tautomeric forms **III** and **IV**, which differ essentially in the localization of the basic site within the heterocyclic ring system.[24] Whereas recent NMR studies seem to indicate that protonation occurs preferentially at the nitrogen atom of the C ring thus favoring tautomer **III**,[9a] an X-ray crystallographic study of **4**·HCl showed beyond doubt that this compound in the solid state is best represented by tautomer **IV**.[11]



III



IV

To study how the replacement of one of the pyrrole rings by a different arene entity affects the electronic pattern within the π system of the pharmacophore, an X-ray diffraction study of compound **25** has been carried out (Figures 1 and 2).[25] The analysis of the relevant bond lengths shows that the central B ring constitutes the basic azafulvene entity, very much in line with the situation found in the parent compound **4**. Therefore it must be concluded that modifications of the A ring as described in this paper do not alter the overall electronic features of the remaining heterocyclic domain, thus rendering products **23**–**25** relevant mimics for nonylprodigiosin.

Biological evaluation

The novel prodigiosin derivatives have been compared with undecylprodigiosin (**1**) in two different kinds of experiments. Specifically, this refers to i) proliferation of murine spleen cells induced by lipopolysaccharides (LPS) and concanavalin A (Con A), and ii) vacuolar acidification of baby hamster kidney (BHK) cells where prodigiosins were previously found to exhibit characteristic features at the cellular level.

LPS stimulation induces B-cell proliferation whereas Con A stimulation induces T-cell proliferation. Compound **1** suppressed the Con A-induced proliferation of T cells at an IC_{50} value of 0.6 nM which was a significantly lower concentration than the

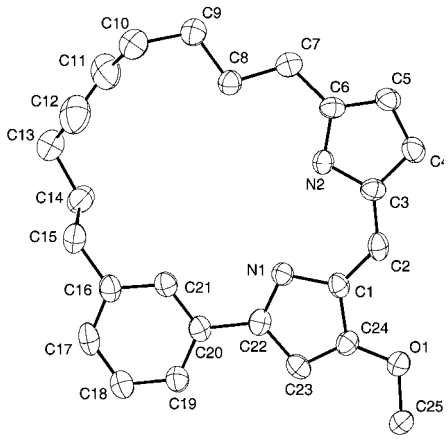


Figure 1. *Molecular structure of compound **25**; in the crystal the tautomer with the hydrogen atom attached to N2 is present. Anisotropic displacement parameters are shown at 50% probability and hydrogen atoms have been omitted for clarity. Selected bond lengths [Å] and angles [°]: N1-C1 1.407(4), C1-C24 1.450(5), C24-C23 1.340(4), C23-C22 1.448(5), C22-N1 1.325(4), C1-C2 1.364(4), C2-C3 1.404(5), N2-C6 1.353(4), C6-C5 1.379(4), C5-C4 1.383(5), C4-C3 1.394(5), C3-N2 1.392(4), C6-C7 1.495(4), C10-C11 1.481(5), C11-C12 1.296(6), C12-C13 1.467(5), C20-C22 1.491(4); C11-C10-C9 113.2(3), C12-C11-C10 127.3(4), C11-C12-C13 128.9(5), C12-C13-C14 109.7(3). The values for the intramolecular hydrogen bond (N2H···N1 2.27 Å, N2-H···N1 122°) are comparable to those for 384 examples of pyrrolylpyrromethene units (mean NH···N distance 2.4(2) Å, mean N-H···N angle 118(8)°) contained in the April 2000 version of the Cambridge Structural Database.*

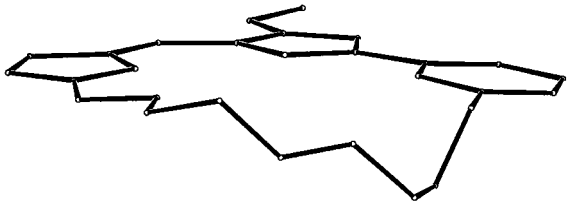


Figure 2. *Side-on view of compound **25** showing the planar arrangement of the two pyrrole rings and the phenyl ring. The alkyl chain deviates from this plane up to 1.28 Å. This is caused primarily by the torsion angle C14-C15-C16-C17 of −106.7° which forces the alkyl chain out of the molecular plane. In addition, there are two* gauche *conformations, located at C9-C10 and C13-C14. The C=C double bond forms a dihedral angle of only 18° with the ring plane, mainly due to the torsion angles around C10-C11 and C12-C13, which deviate by 47.8° and 74.3°, respectively, from the* anti-periplanar arrangement.

IC_{50} value toward the LPS-induced proliferation of B cells (15 nM). Although in these assays the activity of all new prodigiosin derivatives was substantially lower than that of **1**, compounds **4** and **21** exhibited an inhibitory profile similar to that of compound **1** in that these derivatives suppressed the Con A-induced T-cell proliferation more strongly than the LPS-induced B-cell proliferation (Figure 3, Table 2). The lower activity of the macrocyclic derivative **4** as compared with the parent compound **1** may be caused by the fact that one conformation of its pharmacophore is locked as discussed above (see Introduction); alternatively, one may speculate that the flexible undecyl side chain of **1** plays an important role for the insertion of this alkaloid into biological (e.g. lysosomal) membranes. Further structural variations are necessary in order to distinguish between these possibilities.

Prodigiosins were shown to act as H^+/Cl^- symporters that uncouple proton translocation resulting in perturbation of vacuolar acidification.[1, 26] This biological activity was assessed by staining BHK cells with a fluorescent dye (acridine orange) that is accumulated in the acidic granules. Fluorescent orange dots represent intracellular acidic granules such as lysosomes over the green background of cytoplasm and nuclei. Compound **1** significantly decreases the intensity and number of orange dots (Figure 4), which indicates the blockage of vacuolar acidification. Prodigiosin derivatives **4** and **26** also significantly prevent vacuolar acidification, whereas all other compounds exhibit no or only marginal effects. Thus, the data suggest that three

Table 2. *Summary of the biological effects of prodigiosin derivatives on the proliferation of murine spleen cells.*[a]

Compd	B-cell proliferation IC_{50} [nM]	T-cell proliferation IC_{50} [nM]	IC_{50} ratio (B cell/T cell)
1	15	0.6	24
4	360	26	14
21	1900	130	15
23	1500	2900	0.5
24	7900	2800	2.8
25	12000	4300	2.7
26	850	240	3.6

[a] IC_{50} values for inhibition of LPS-induced B-cell proliferation and Con A-induced T-cell proliferation were measured. The ratio of the two IC_{50} values (B-cell proliferation versus T-cell proliferation) was calculated.

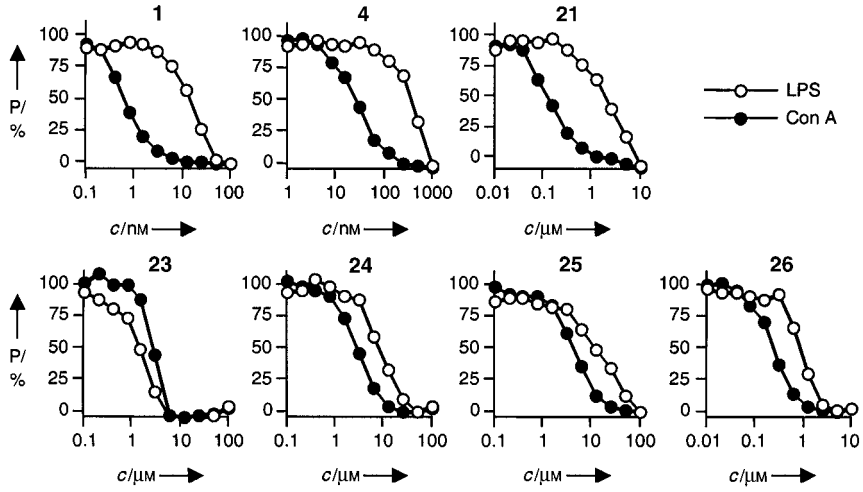
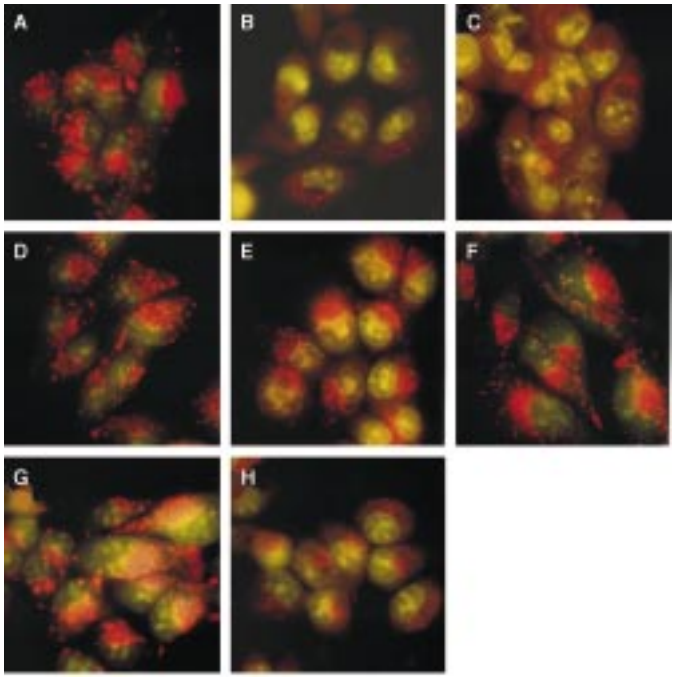


Figure 3. *Biological evaluation of the effects of prodigiosin derivatives on the proliferation of murine spleen cells. Mouse spleen cells were stimulated with 10 μg mL^{-1} of LPS (open circles) or 2 μg mL^{-1} of Con A (filled circles) in the presence of the compounds at serially diluted concentrations for 3 days. The proliferative response was assessed by an MTT assay. Data points represent the average of triplicate experiments; P = proliferation.*



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	Concentration (μM)				
	0.1	1	10	100	MIC
1	−	+	+		1
4		±	+		1-10
21				−	>100
23				±	>100
24				−	>100
25				−	>100
26		−	+		10

Figure 4. *Biological evaluation of the effects of prodigiosin derivatives on vacuolar acidification. BHK cells were pretreated with prodigiosin derivatives at the indicated concentrations for 30 min and further incubated in the presence of 5 μg mL^{-1} acridine orange for 30 min. The stained cells were examined by fluorescence microscopy. A) No compounds (negative control); B)* ***1*** *(10 μM); C)* ***4*** *(10 μM); D)* ***21*** *(100 μM); E)* ***23*** *(100 μM); F)* ***24*** *(100 μM); G)* ***25*** *(100 μM); H)* ***26*** *(10 μM). I) The table shows the concentrations at which the prodigiosin derivatives tested had (+) or did not have (−) an effect on vacuolar acidification; the minimum inhibitory concentration (MIC) for each compound is also given.*

pyrrole units are essential for the inhibitory activity of prodigiosins on vacuolar acidification.

Other types of inhibitors of vacuolar acidification such as concanamycins A and B (inhibitors of vacuolar-type H^+-ATPase) exhibit immunosuppressive activity in vitro and in vivo similar to prodigiosins, and also display preferential suppression of Con A-induced T -cell proliferation.[1, 26] We assumed that these agents augment the toxic effect of Con A by upregulation of Con A receptors on the cell surface through preventing the acidification of the Golgi apparatus and affecting glycoprotein processing. In this work, however, the immunosuppressive activity on Con A-induced T-cell proliferation and the inhibitory activity on vacuolar acidification were dissected especially with two prodigiosin derivatives, **21** and **26**, that essentially affect only one of these two biological responses. Thus, the immunosuppressive activity of prodigiosins is caused by mechanisms other than the inhibition of vacuolar acidification, and the analogues presented in this paper might be powerful tools to address the molecular targets specific for T-cell functions.

Experimental Section

General: All reactions were carried out under Ar in pre-dried glassware by using Schlenk techniques. The solvents were dried by distillation over the drying agents indicated and were stored and transferred under Ar: CH_2Cl_2 (P_4O_{10}), DME (Na/K), diethyl ether, 1,4-dioxane, THF (magnesium/anthracene). Flash chromatography: Merck silica gel (230 – 400 mesh) or activated aluminum oxide (Aldrich, neutral, Brockmann I, standard grade, ca. 150 mesh) with hexanes/ethyl acetate in various ratios as eluent. NMR spectroscopy: Spectra were recorded on a Bruker AMX 200 or DPX 300 spectrometer in the solvent indicated. Chemical shifts (δ) are given in ppm relative to TMS, coupling constants (J) in Hz. IR spectroscopy: Nicolet FT-7199, wavenumbers in cm^{-1}. MS: Varian CH-5 (70 eV); HR-MS: Finnigan MAT SSQ 7000 (70 eV). Melting-point determination: Gallenkamp apparatus (uncorrected). Elemental analyses: Dornis & Kolbe, Mülheim (Germany). Commercially available reagents (Aldrich, Fluka) were used as received.

Chemical syntheses

(1-*tert*-Butoxycarbonyl-2-pyrrolyl)boronic acid (11): *n*BuLi (1.6 M in hexanes, 3.90 mL, 6.24 mmol) is slowly added to a solution of 2,2,6,6-tetramethylpiperidine (765 mg, 5.41 mmol) in THF (11 mL) at −78 °C under Ar. After stirring for 10 min at this temperature, the mixture is allowed to warm to 0 °C within 30 min. After cooling again to −78 °C, a solution of *N*-Boc pyrrole (830 mg, 4.96 mmol)[27] in THF (20 mL) is added at such a rate that the temperature remains below −65 °C. The reaction mixture is stirred for 2 h at −78 °C prior to the addition of $B(OMe)_3$ (1.54 g, 14.82 mmol) in THF (40 mL). The reaction mixture is allowed to warm to ambient temperature overnight. For workup, aq HCl (0.25 M, 14 mL, 3.5 mmol) is added, the solvent is evaporated, the residue is extracted with Et_2O (3 × 15 mL), the combined organic phases are washed with water (2 × 6 mL) and dried (Na_2SO_4). The solution is slowly concentrated until a solid starts to precipitate. The mixture is then kept at 0 °C and the precipitated product is filtered off. Trituration with cold Et_2O and drying of the residue in vacuo affords the boronic acid **11** (660 mg, 63 %) as a colorless solid.[17] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42 (dd, 1 H, J = 3.2, 1.6 Hz), 7.28 (br. s, 2 H), 7.08 (dd, J = 3.3, 1.7 Hz, 1 H), 6.24 (t, J = 3.3 Hz, 1 H), 1.60 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.2, 128.7, 127.0, 112.0, 85.5, 27.9; ^{11}B NMR (64 MHz, $CDCl_3$): δ = 26.2; MS (EI): *m/z* (%): 211 (10) [*M*]$^+$, 155 (19), 138 (9), 111 (62), 110 (15), 93 (11), 57 (100), 41 (25), 29 (13).

3-(4-Pentenyl)phenylboronic acid (14): *n*BuLi (1.6 M in hexanes, 5.00 mL, 8.00 mmol) is added to a solution of 1-bromo-3-(4-pentenyl)benzene (901 mg, 4.00 mmol) in Et_2O (10 mL) at 0 °C. After stirring for 90 min at this temperature, the mixture is allowed to warm to r.t. and stirred for another 3 h. After cooling to −78 °C, $B(OMe)_3$ (831 mg, 8.00 mmol) in Et_2O (2 mL) is added, the reaction mixture is warmed to 0 °C and quenched with 10 % aq HCl. The reaction mixture is extracted with Et_2O, the organic layer is extracted with 10 % aq NaOH (3 ×), the combined alkaline extracts are acidified with 10 % aq HCl until pH ≈ 1 – 2 is reached, and the precipitated solid is filtered off. The acidic filtrate is extracted with Et_2O, the extracts are dried (Na_2SO_4) and evaporated to give a second crop of the product. The crude boronic acid **14** (195 mg) thus obtained is used for the next step without further purification.

2-(4-Pentenyl)furan (16): Compound **16** (4.22 g, 73 %) is obtained as a colorless liquid according to ref. [28] by using furan (3.07 mL,

42.27 mmol) and 5-bromo-1-pentene (6.30 g, 42.27 mmol) as the starting materials. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.29$ (dd, $J = 1.8$, 0.7 Hz, 1H), 6.27 (dd, $J = 3.3$, 1.8 Hz, 1H), 5.97 (dd, $J = 3.3$, 0.7 Hz, 1H), 5.81 (ddt, $J = 16.9$, 10.3, 6.6 Hz, 1H), 5.27 – 4.95 (m, 2H), 2.63 (t, $J =$ 7.6 Hz, 2H), 2.16 – 2.06 (m, 2H), 1.78 – 1.68 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.1$, 140.7, 138.2, 114.9, 110.0, 104.8, 33.1, 27.3, 27.2; IR (neat): $\tilde{\nu} = 3115$, 3078, 2978, 2935, 2862, 1641, 1597, 1508, 1438, 1384, 1345, 1237, 1175, 1147, 1077, 1007, 914, 885, 798, 729, 599 cm^{-1}. MS (EI): *m/z* (%): 136 (19) $[M]^+$, 107 (7), 94 (76), 92 (13), 82 (20), 81 (100), 53 (29), 39 (17), 27 (15).

2-(11-Dodecenyl)furan (17): Compound **17** (1.954 g, 93%) is obtained as a colorless syrup according to ref. [28] by using furan (643 μL, 8.85 mmol) and 12-bromo-1-dodecene (2.187 g, 8.85 mmol) as the starting materials. ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 7.29$ (dd, $J =$ 1.9, 0.8 Hz, 1H), 6.28 (dd, $J = 3.1$, 1.9 Hz, 1H), 5.99 – 5.97 (m, 1H), 5.83 (ddt, $J = 16.9$, 13.3, 6.7 Hz, 1H), 5.03 – 4.90 (m, 2H), 2.61 (t, $J = 7.5$ Hz, 2H), 2.09 – 2.01 (m, 2H), 1.68 – 1.51 (m, 2H), 1.44 – 1.29 (m, 14H); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta = 156.9$, 140.7, 139.4, 113.9, 110.1, 104.6, 33.9, 29.7, 29.7, 29.6, 29.5, 29.3, 29.3, 29.1, 28.2, 28.0; IR (neat): $\tilde{\nu} =$ 3115, 3077, 2926, 2854, 1641, 1597, 1507, 1465, 1440, 1147, 1007, 994, 910, 885, 795, 726 cm^{-1}; MS (EI): *m/z* (%): 234 (16) $[M]^+$, 123 (10), 95 (44), 94 (16), 82 (35), 81 (100), 67 (10), 55 (17), 41 (24); HR-MS: *m/z*: calcd for $C_{16}H_{26}O$ 234.198365, found 234.198907.

2-(4-Pentenyl)thiophene (18): A solution of thiophene (2.84 g, 33.81 mmol) in Et_2O (20 mL) is added to *n*BuLi (1.6 M, 21.13 mL, 33.81 mmol) in Et_2O (20 mL) at 0 °C. The mixture is allowed to warm to r.t. and stirred for 90 min. After cooling to −15 °C, a solution of 5-bromo-1-pentene in Et_2O (20 mL) is added and the resulting mixture is refluxed for 30 h. The reaction mixture is cooled to r.t. and poured onto crushed ice. The aqueous layer is extracted with *tert*-butyl methyl ether, dried (Na_2SO_4), and concentrated. The crude product is purified by distillation (b.p. = 80 – 81 °C at 20 mbar) to give product **18** (1.84 g, 36%) as a colorless liquid. ^{1}H NMR (200 MHz, CD_2Cl_2): $\delta = 7.11$ (d, $J = 5.1$ Hz, 1H), 6.91 (dd, $J = 5.1$, 3.5 Hz, 1H), 6.79 (d, $J = 3.5$ Hz, 1H), 5.85 (ddt, $J = 17.1$, 10.4, 6.6 Hz, 1H), 5.08 – 4.95 (m, 2H), 2.84 (t, $J = 7.5$ Hz, 2H), 2.18 – 2.07 (m, 2H), 1.84 – 1.69 (m, 2H); ^{13}C NMR (50 MHz, CD_2Cl_2): $\delta = 145.5$, 138.4, 126.7, 124.2, 122.9, 114.7, 33.1, 31.1, 29.3; IR (neat): $\tilde{\nu} = 3076$, 2976, 2933, 2856, 1641, 1535, 1440, 1240, 1076, 991, 912, 851, 819, 693 cm^{-1}; MS (EI): *m/z* (%): 152 (14) $[M]^+$, 123 (7), 110 (67), 98 (29), 97 (100), 53 (10), 45 (14), 39 (12); elemental analysis calcd for $C_9H_{12}S$ (152.26): C 71.00, H 7.94; found: C 70.92, H 8.06.

12: A solution of triflate **6** (243 mg, 0.60 mmol),[11] LiCl (76 mg, 1.80 mmol), the boronic acid **11** (380 mg, 1.80 mmol), $[Pd(PPh_3)_4]$ (35 mg, 0.03 mmol), and aq Na_2CO_3 (2 M, 1.8 mL, 3.60 mmol) in DME (12 mL) is stirred at 90 °C for 20 h under Ar. A standard workup followed by flash chromatography on neutral alumina using hexanes/ethyl acetate (6:1 → 2:1) as the eluent provides compound **12** (141 mg, 73%) as a dark red solid. ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta =$ 6.88 (s, 1H), 6.73 (dd, $J = 3.6$, 1.3 Hz, 1H), 6.65 (dd, $J = 2.5$, 1.3 Hz, 1H), 6.52 (d, $J = 3.7$ Hz, 1H), 6.17 (dd, $J = 3.6$, 2.5 Hz, 1H), 6.14 (s, 1H), 5.88 (d, $J = 3.7$ Hz, 1H), 5.79 (ddt, $J = 16.9$, 10.3, 6.6 Hz, 1H), 5.02 – 4.91 (m, 2H), 3.98 (s, 3H), 2.11 (t, $J = 6.6$ Hz, 2H), 2.01 – 1.92 (m, 2H), 1.34 – 1.24 (m, 4H); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta = 169.5$, 160.6, 144.4, 139.0, 138.8, 128.4, 128.3, 122.8, 121.3, 116.0, 114.1, 113.2, 110.4, 108.8, 95.9, 58.6, 33.5, 28.9, 28.9, 27.1; MS (EI): *m/z* (%): 321 (100) $[M]^+$, 306 (12), 266 (10), 253 (31), 252 (69), 238 (26), 221 (7), 118 (11), 91 (33).

15: A solution of triflate **6** (110 mg, 0.27 mmol),[11] $[Pd(PPh_3)_4]$ (31 mg, 0.027 mmol), and the boronic acid **14** (195 mg of the crude product) in DME (15 mL) is treated with aq Na_2CO_3 (2.2 M, 0.9 mL, 1.98 mmol) and the resulting mixture is stirred for 2.5 h at 85 °C. A standard extractive workup followed by flash chromatography on neutral alumina using hexanes/ethyl acetate (50:1 → 10:1) as the eluent provides diene **15**. The fractions containing the product are combined and concentrated to a small volume, treated with a solution of HCl in Et_2O, and evaporated in vacuo. This affords **15**·HCl (71 mg, 60%) as a dark red solid. ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 14.58$ (br. s, 1H), 13.47 (br. s, 1H), 8.24 (s, 1H), 8.17 (d, $J = 7.7$ Hz, 1H), 7.41 (t, $J = 7.6$ Hz, 1H), 7.33 (d, $J = 7.7$ Hz, 1H), 7.24 (s, 1H), 7.05 – 7.03 (m, 1H), 6.34 – 6.32 (m, 2H), 5.95 – 5.77 (m, 2H), 5.09 – 4.92 (m, 4H), 4.03 (s, 3H), 3.01 (t, $J = 7.6$ Hz, 2H), 2.72 (t, $J = 7.7$ Hz, 2H), 2.17 – 2.09 (m, 4H), 1.91 – 1.76 (m, 4H), 1.56 – 1.46 (m, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta =$ 166.8, 157.7, 156.6, 143.6, 138.8, 138.7, 133.3, 132.1, 128.9, 128.8, 128.5, 127.2, 126.3, 120.9, 120.8, 114.7, 114.5, 114.3, 94.7, 59.1, 35.1, 33.5, 33.4, 30.5, 28.6, 28.5, 28.3; IR (KBr): $\tilde{\nu} = 3396$, 3334, 3073, 2996, 2927, 2856, 2752, 1622, 1572, 1545, 1525, 1484, 1459, 1362, 1269, 1239, 1183, 1133, 1048, 982, 949, 911, 815, 778, 694 cm^{-1}; HR-MS (FAB, positive mode): *m/z*: calcd for $C_{27}H_{32}N_2O$ 401.259288, found 401.258099.

19·HCl: A solution of the furan **16** (170 mg, 1.25 mmol) in THF (3 mL) at −78 °C under Ar is treated with *n*BuLi (1.6 M in hexanes, 0.78 mL, 1.25 mmol). The solution is allowed to warm to 0 °C and stirred for 1 h. After cooling again to −78 °C, $B(OMe)_3$ (167 μL, 1.50 mmol) is introduced and the solution is allowed to warm to 0 °C and stirred for 2 h. Triflate **6** (104 mg, 0.27 mmol),[11] $[Pd(PPh_3)_4]$ (25 mg, 0.022 mmol), and DME (5 mL) were placed into a second flask. The borate complex is transferred by syringe into the second flask at 40 °C and aq Na_2CO_3 (2 M, 1.10 mL, 2.20 mmol) is added to the reaction mixture. Stirring at 80 °C for 3 h followed by a standard extractive workup and flash chromatography on neutral alumina with hexanes/ethyl acetate (50:1 → 6:1) as the eluent provides diene **19**. Treatment of the free base thus formed with a solution of HCl in Et_2O and evaporation of the solvent affords **19**·HCl (86 mg, 78%) as a red solid. ^{1}H NMR (200 MHz, CD_2Cl_2): $\delta = 14.20$ (br. s, 1H), 13.73 (br. s, 1H), 8.50 (d, $J = 5.5$ Hz, 1H), 7.18 (s, 1H), 7.01 – 6.97 (m, 1H), 6.34 (d, $J = 5.5$ Hz, 1H), 6.33 – 6.27 (m, 2H), 5.92 – 5.75 (m, 2H), 5.10 – 4.91 (m, 4H), 4.00 (s, 3H), 2.93 (t, $J = 11.6$ Hz, 2H), 2.69 (t, $J = 11.6$ Hz, 2H), 2.12 – 2.01 (m, 4H), 1.79 – 1.68 (m, 4H), 1.48 – 1.39 (m, 2H); ^{13}C NMR (50 MHz, CD_2Cl_2): $\delta = 166.6$, 162.2, 155.7, 146.0, 143.6, 138.8, 138.0, 131.5, 127.0, 121.3, 120.3, 118.9, 115.1, 114.3, 113.9, 110.1, 93.3, 59.1, 33.5, 33.1, 28.6 (2 ×), 28.2, 27.8, 27.0; IR (KBr): $\tilde{\nu} = 3423$, 3088, 2927, 2856, 1640, 1611, 1547, 1411, 1286, 1233, 1184, 1136, 1047, 989, 964, 909, 783 cm^{-1}; MS (EI): *m/z* (%): 390 (100) $[M - HCl]^+$, 375 (14), 335 (25), 322 (24), 321 (32), 307 (15), 140 (9), 118 (15), 105 (16), 41 (14); HR-MS: *m/z*: calcd for $C_{25}H_{30}N_2O_2$ 390.230727, found 390.229664.

20·HCl: A solution of the furan **17** (465 mg, 1.98 mmol) in THF (5 mL) is treated with *n*BuLi (1.6 M in hexanes, 1.24 mL, 1.95 mmol) at −78 °C under Ar. The solution is allowed to warm to 0 °C and stirred for 1 h. After cooling again to −78 °C, $B(OMe)_3$ (264 μL, 2.38 mmol) is added and the solution is allowed to warm to 0 °C and stirred for 2 h. Triflate **6** (192 mg, 0.47 mmol),[11] $[Pd(PPh_3)_4]$ (52 mg, 0.045 mmol), and DME (15 mL) are added into a second flask. The borate complex is transferred by syringe into this second flask at 40 °C followed by addition of aq Na_2CO_3 (2.2 M, 1.80 mL, 3.96 mmol). The resulting mixture is stirred at 80 °C for 3 h. A standard extractive workup followed by flash chromatography on neutral alumina with hexanes/ethyl acetate (50:1 → 6:1) as the eluent provides diene **20**; treatment of the free base thus formed with a solution of HCl in Et_2O and evaporation of the solvent affords **20**·HCl (158 mg, 64%) as a dark red solid. ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 14.03$ (br. s, 1H), 13.58 (br. s, 1H), 8.45 (d, $J = 3.5$ Hz, 1H), 7.17 (s, 1H), 6.96 (t, $J = 3.0$ Hz, 1H), 6.32 (d, $J = 3.5$ Hz, 1H), 6.30 – 6.27 (m, 2H), 5.90 – 5.75 (m, 2H), 5.05 – 4.90 (m, 4H), 4.04 (s, 3H), 2.98 (t, $J = 7.6$ Hz, 2H), 2.73 (t, $J = 7.6$ Hz, 2H), 2.15 – 2.00 (m, 4H), 1.84 – 1.69 (m, 4H), 1.67 – 1.29 (m, 16H); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta = 166.6$, 162.8, 155.7, 146.2, 143.5, 139.4, 138.9,

131.4, 126.0, 121.3, 120.5, 118.9, 114.3, 113.9, 113.9, 110.0, 93.2, 59.1, 33.9, 33.5, 29.6, 29.6, 29.5, 29.4, 29.2 (2×), 29.0, 28.6 (2×), 28.4, 28.2, 27.8; IR (KBr): $\tilde{\nu}$ = 3439, 3081, 2996, 2927, 2854, 1631, 1611, 1554, 1544, 1495, 1441, 1360, 1288, 1226, 1181, 1039, 978, 969, 906, 781 cm^{-1}; HR-MS (FAB, positive mode): *m/z*: calcd for $C_{32}H_{44}N_2O_2$: 489.348102, found 489.347792.

21: Substrate **18** (152 mg, 1.00 mmol) is added to *n*BuLi (1.6 M, 625 μL, 1.00 mmol) in Et_2O (5 mL) at 0 °C. The mixture is allowed to warm to r.t. and stirred for 90 min. The reaction mixture is cooled to −15 °C and $B(OMe)_3$ (139 μL, 1.25 mmol) is added. The mixture is warmed to 0 °C and stirred for 1 h, then allowed to warm to ambient temperature and stirred for another 30 min. The reaction mixture is concentrated to a small volume and transferred to a solution of triflate **6** (62 mg, 0.15 mmol)[11] and $[Pd(PPh_3)_4]$ (18 mg, 0.156 mmol) in DME (5 mL). Aq Na_2CO_3 (2 M, 1.00 mL, 2.00 mmol) is added and the resulting mixture is refluxed for 3 h. A standard extractive workup followed by flash chromatography on neutral alumina with hexanes/ethyl acetate (30:1 →6:1) as the eluent provides diene **21**; treatment of the free base thus formed with a solution of HCl in Et_2O and evaporation of the solvent affords **21**·HCl (55 mg, 0.124 mmol, 81 %) as a dark red solid. ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 14.19 (br. s, 1 H), 13.52 (br. s, 1 H), 8.83 (d, *J* = 3.9 Hz, 1 H), 7.14 (s, 1 H), 6.98 – 6.95 (m, 2 H), 6.29 (dd, *J* = 3.9, 1.5 Hz, 1 H), 6.09 (d, *J* = 1.9 Hz, 1 H), 5.91 – 5.77 (m, 2 H), 5.10 – 4.92 (m, 4 H), 4.00 (s, 3 H), 2.98 (t, *J* = 7.6 Hz, 2 H), 2.89 (t, *J* = 7.6 Hz, 2 H), 2.19 – 2.08 (m, 4 H), 1.86 – 1.75 (m, 4 H), 1.55 – 1.45 (m, 2 H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ = 166.7, 156.0, 153.8, 150.1, 138.8, 137.9, 134.1, 131.8, 129.42, 127.5, 126.9, 121.1, 119.0, 115.1, 114.3, 114.0, 94.6, 59.1, 33.5, 33.0, 30.6, 29.8, 28.6, 28.5, 28.2; IR (KBr): $\tilde{\nu}$ = 3413, 3169, 3052, 2926, 2856, 1723, 1639, 1583, 1548, 1505, 1447, 1409, 1383, 1286, 1185, 1045, 989, 906, 807, 771 cm^{-1}; MS (EI): *m/z* (%): 406 (100) $[M]^+$, 351 (18), 337 (33), 192 (5), 148 (8), 121 (17); HR-MS: *m/z*: calcd for $C_{25}H_{30}N_2OS$ 406.207885, found 406.208770.

23·HCl: A solution of diene **19**·HCl (26 mg, 0.06 mmol) in CH_2Cl_2 (25 mL) is slowly added to a solution of the ruthenium carbene complex **22** (5 mg, 0.006 mmol) in CH_2Cl_2 (50 mL) and the resulting mixture is refluxed for 40 h. For workup, the reaction mixture is washed with sat. aq Na_2CO_3, the organic layer is dried (Na_2SO_4), and the solvent is evaporated. The residue is subjected to flash chromatography on neutral alumina with hexanes/ethyl acetate (20:1 →6:1) as the eluent. The combined fractions containing the product are concentrated to a small volume, treated with a solution of HCl in Et_2O and evaporated in vacuo. This affords **23**·HCl (15.5 mg, 64 %) as a dark red solid. The ratio of isomers is *E*:*Z* = 4:1. ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 14.14 (br. s, 1 H), 13.22 (br. s, 1 H), 7.13 (s, 1 H), 7.10 (d, *J* = 3.4 Hz, 1 H), 6.27 – 6.22 (m, 2 H), 6.18 (s, 1 H), 5.93 – 5.84 (m, 1 H), 5.57 – 5.48 (m, 1 H), 4.00 (s, 3 H), 2.93 – 2.83 (m, 4 H), 2.18 – 1.77 (m, 8 H), 1.50 – 1.40 (m, 2 H); ^{13}C NMR (75 MHz, CD_2Cl_2): *E* isomer: δ = 167.2, 163.0, 155.5, 145.7, 142.7, 130.9, 130.7, 129.7, 128.1, 122.1, 119.6, 118.5, 114.4, 109.8, 92.8, 59.4, 31.7, 31.2, 29.3, 28.3, 28.2, 27.3, 26.9; *Z* isomer (resolved signals): δ = 131.1, 130.1, 130.0, 127.7, 119.7, 117.7, 109.7, 92.7, 59.3, 28.8, 28.7, 28.1 (2 C), 27.6, 27.5, 27.4; IR (KBr): $\tilde{\nu}$ = 3423, 3071, 2923, 2851, 1616, 1540, 1501, 1415, 1345, 1247, 1237, 1177, 1130, 1032, 968, 884, 799, 781, 703, 667 cm^{-1}; MS (EI): *m/z* (%): 362 (100) $[(M - HCl)]^+$, 347 (15), 280 (11), 105 (8); HR-MS: *m/z*: calcd for $C_{23}H_{26}N_2O_2$ 362.199427, found 362. 200162.

24·HCl: A solution of diene **20**·HCl (131 mg, 0.249 mmol) in CH_2Cl_2 (100 mL) is slowly added (over 4 h) at 40 °C to a solution of the ruthenium indenylidene complex **22** (23 mg, 0.025 mmol) in CH_2Cl_2 (250 mL). After stirring for 22 h at this temperature, the reaction mixture is cooled to r.t., washed with sat. aq Na_2CO_3 and the organic layer is dried (Na_2SO_4) and evaporated. Flash chromatography of the crude material on neutral alumina with hexanes/ethyl acetate (30:1 →2:1) as the eluent provides **24**, which after concentration to a small volume, treating with a solution of HCl in Et_2O, and evaporation of the solvent in vacuo is isolated as the deeply red solid hydrochloride salt **24**·HCl (71 mg, 57 %). The ratio of isomers is *E*:*Z* = 2.4:1. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 13.98 (br. s, 1 H), 13.47 (br. s, 1 H), 8.15 (d, *J* = 3.4 Hz, 1 H), 7.06 (s, 1 H), 6.87 – 6.85 (m, 1 H), 6.22 (d, *J* = 3.4 Hz, 1 H), 6.19 – 6.07 (m, 2 H), 5.39 – 5.19 (m, 2 H), 3.97 (s, 3 H), 3.01 – 2.95 (m, 2 H), 2.74 – 2.69 (m, 2 H), 2.06 – 1.69 (m, 8 H), 1.41 – 1.03 (m, 16 H); ^{13}C NMR (75 MHz, $CDCl_3$): *E* isomer: δ = 166.2, 162.4, 156.2, 145.6, 143.2, 131.3, 130.7, 129.4, 126.9, 121.1, 120.1, 118.9, 114.2, 110.7, 92.8, 58.9, 32.9, 31.7, 29.6, 29.2, 28.9, 28.8, 28.7, 28.5, 28.3, 28.2, 28.1, 28.0, 27.8, 26.9; *Z* isomer (resolved signals): δ = 166.0, 162.9, 156.1, 145.1, 143.1, 131.4, 130.3, 126.9, 120.8, 119.3, 114.0, 110.1, 92.7, 28.9, 28.6, 27.9, 27.0, 26.7; IR (KBr): $\tilde{\nu}$ = 3425, 3111, 2923, 2851, 1626, 1547, 1506, 1451, 1384, 1352, 1278, 1244, 1181, 1129, 1043, 966, 884, 838, 793, 776 cm^{-1}; HR-MS: *m/z*: calcd for $C_{30}H_{40}N_2O_2$ 460.308977, found 460.307802.

25:[25] A solution of diene **15**·HCl (67 mg, 0.153 mmol) in CH_2Cl_2 (100 mL) is slowly added to a solution of the ruthenium indenylidene complex **22** (5 mg, 0.006 mmol) in CH_2Cl_2 (100 mL) and the resulting mixture is refluxed for 24 h. For workup, the reaction mixture is washed with sat. aq Na_2CO_3 and the organic layer is dried (Na_2SO_4) and evaporated. Flash chromatography of the residue on neutral alumina with hexanes/ethyl acetate (20:1) as the eluent affords product **25** (49 mg, 86 %) as an orange crystalline solid. The ratio of isomers is *E*:*Z* = 10:1. ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 8.16 (s, 1 H), 7.55 (dd, *J* = 6.4, 1.2 Hz, 1 H), 7.37 (t, *J* = 7.6 Hz, 1 H), 7.28 (d, *J* = 7.6 Hz, 1 H), 6.88 (s, 1 H), 6.58 (d, *J* = 3.6 Hz, 1 H), 6.14 (s, 1 H), 6.03 (d, *J* = 3.6 Hz, 1 H), 5.52 – 5.49 (m, 2 H), 3.92 (s, 3 H), 2.79 – 2.73 (m, 4 H), 2.19 – 2.15 (m, 2 H), 2.02 – 1.98 (m, 2 H), 1.87 – 1.77 (m, 4 H), 1.64 – 1.55 (m, 2 H); ^{13}C NMR (75 MHz, CD_2Cl_2): *E* isomer: δ = 168.5, 165.4, 143.3, 142.9, 141.1, 134.5, 130.7, 130.7, 130.3, 130.0, 128.6, 126.6, 125.0, 119.9, 117.7, 110.2, 94.6, 58.4, 33.2, 32.3, 30.0, 29.6, 29.4, 28.6, 28.2; *Z* isomer (resolved signals): δ = 130.5, 130.1, 128.4, 125.8, 120.5, 117.9, 110.1, 94.8, 35.0, 31.0, 28.3, 28.0, 27.3, 26.7, 26.2; IR (KBr): $\tilde{\nu}$ = 3453, 3092, 3040, 3008, 2925, 2848, 1629, 1583, 1566, 1547, 1492, 1453, 1359, 1227, 1189, 1157, 1113, 1042, 939, 902, 776, 761, 695, 676, 654 cm^{-1}; MS (EI): *m/z* (%): 372 (100) $[M]^+$, 357 (22), 329 (7), 290 (18), 275 (5), 186 (3), 165 (3), 118 (7); HR-MS: *m/z*: calcd for $C_{25}H_{28}N_2O$ 372.220163, found 372.221209.

26·HCl: A solution of **12**·HCl (28 mg, 0.078 mmol; obtained by treatment of compound **12** with a solution of HCl in Et_2O and evaporation of the solvent) and ruthenium indenylidene complex **22** (3.6 mg, 0.004 mmol) in CH_2Cl_2 (4 mL) is refluxed for 30 h. For workup, the reaction mixture is washed with sat. aq Na_2CO_3, the organic layer is dried (Na_2SO_4) and evaporated, and the residue is subjected to flash chromatography on neutral alumina with hexanes/ethyl acetate (4:1 →1:1) as the solvent. The combined fractions containing the product are concentrated to a small volume, treated with a solution of HCl in Et_2O, and evaporated in vacuo. This affords **26**·HCl (25 mg, 93 %) as a dark red solid. The ratio of isomers is *E*:*Z* = 2.7:1. ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 12.92 (br. s, 2 H), 12.72 (br. s, 2 H), 12.69 (br. s, 2 H), 7.19 – 7.17 (m, 2 H), 6.94 (s, 2 H), 6.93 – 6.92 (m, 2 H), 6.81 – 6.79 (m, 2 H), 6.32 – 6.29 (m, 2 H), 6.15 (dd, *J* = 3.8, 1.8 Hz, 2 H), 6.07 (d, *J* = 1.9 Hz, 2 H), 5.39 – 5.36 (m, 2 H), 3.93 (s, 6 H), 2.84 (t, *J* = 7.5 Hz, 4 H), 2.05 – 1.97 (m, 4 H), 1.75 – 1.64 (m, 4 H), 1.43 – 1.33 (m, 4 H); ^{13}C NMR (75 MHz, CD_2Cl_2): *E* isomer: δ = 166.6, 152.7, 149.0, 130.3, 129.2, 127.3, 126.0, 122.3 121.6, 117.9, 116.3, 112.5, 112.0, 93.2, 59.0, 32.3, 29.2, 28.7, 28.1 ; *Z* isomer (resolved signals): δ = 132.0, 129.8, 110.3, 29.4, 28.8, 28.2, 27.0; HR-MS: *m/z*: calcd for $C_{38}H_{42}N_6O_2$ 615.344748, found 615.343202.

Biological evaluation: The bioassays were basically performed as previously described with a slight modification.[1, 26] *Proliferation of murine spleen cells*: Mouse spleen cells from C57BL/6 mice (female,

6 – 10 weeks old) were stimulated with 10 μg mL^{-1} of LPS or 2 μg mL^{-1} of Con A in the presence of indicated concentrations of prodigiosin derivatives for 3 d. The cells were then pulsed with 0.5 mg mL^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagents for 8 h. The dye was solubilized with 5% sodium dodecyl sulfate (SDS), and the absorbance at 595 nm was measured. *Visualization of vacuolar acidification*: Baby hamster kidney (BHK) cells were pretreated with indicated concentrations of prodigiosin derivatives for 30 min and then treated with 5 μg mL^{-1} of acridine orange for 30 min. The stained cells were washed with phosphate-buffered saline and examined by fluorescence microscopy.

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difference Fourier synthesis and was refined. Refinement of 254 parameters using all reflections converged at $R = 0.0617$, $wR = 0.1426$, highest residual electron density peak 0.399 Å^3. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-144263. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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