Chloride anion transport and copper-mediated DNA cleavage by C-ring functionalized prodigiosenes

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Supplementary information

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Figure S1

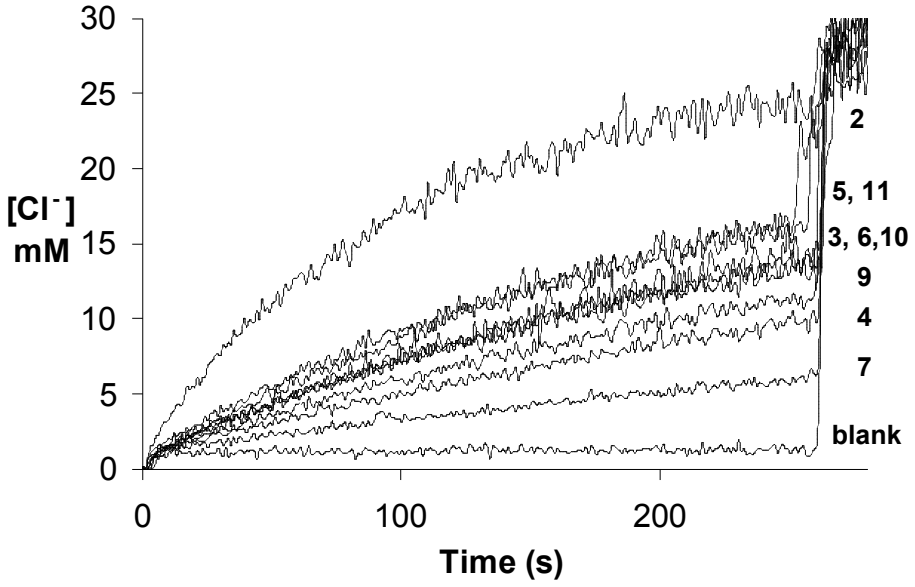

Fig. S1 Chloride transport across EYPC liposomes (25 °C) containing lucigenin in a 100 mM $NaNO_3$ – 10 mM sodium phosphate buffer (pH 6.4). Compounds **2-11** were added to give a 1:1000 ligand:lipid ratio. At t=0 s, NaCl was added to give an external Cl^- concentration of 25 mM. Lucigenin fluorescence was converted to $[Cl^-]$. The traces shown are the average of 3 trials.

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Materials and methods

The samples of all prodigiosenes were synthesized and characterized as previously reported.[1] All reagents, unless stated otherwise, were obtained commercially and used without further purification.

Chloride transport experiments

EYPC lipid (60 mg) was dissolved in 5 mL of a chloroform/methanol mixture (5 % MeOH). The resulting solution was evaporated under reduced pressure to produce a thin film that was dried *in vacuo* for 2 h. The lipid film was hydrated with 1 mL of a solution of 10 mM sodium phosphate (pH 6.4) containing 100 mM $NaNO_3$ and 1 mM lucigenin.[2] After 10 freeze/thaw cycles, the liposomes were extruded through a 100 nm polycarbonate membrane 21 times at room temperature. The liposome solution was passed through a Sephadex (G-25) column to remove excess dye (eluant = sodium phosphate buffer, pH 6.4, 100 mM $NaNO_3$). The isolated liposomes were diluted in 10 mM sodium phosphate (pH 6.4, 100 mM $NaNO_3$) to give a concentration of 25 mM in EYPC, assuming 100 % retention of lipid during the gel filtration process. In a typical experiment, 50 μL of the stock EYPC liposomes were diluted into 2 mL of 10 mM sodium phosphate (pH 6.4, 100 mM $NaNO_3$) to give a solution 0.5 mM in lipid. Compounds **1-11** were added to give a $9.0E^{-4}$:100 ligand:lipid ratio ($2.2x10^{-4}$ mM final concentration of **1-11**). To the cuvette containing the EYPC-transporter mixture was added 20 μL of 2.5 M NaCl solution through an injection port to give an external chloride concentration of ~25 mM. The fluorescence of the intravesicular chloride concentration was monitored at excitation 372 nm and emission at 504 nm for 500 s. After 470 s, 0.04 mL of 10 % Triton-X detergent was added to lyze the liposomes. The internal liposome chloride concentration was determined in accordance to previous literature reports.[3] All transport experiments were done in triplicate.

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DNA cleavage experiments

Supercoiled plasmid pDesR3 DNA was prepared from Nova Blue cells using Qiagen QIA prep Spin Miniprep Kit (Qiagen, Hiden, Germany).[4] The concentration of DNA was determined by OD_{260}. Deionized water (18 Ω) was used for all aqueous solutions and manipulations. Agarose gel loading buffer = 40 mM Tris-OAc (pH 8.0), 5 mM EDTA, 40% glycerol, 0.3% bromophenol blue. Electrophoresis 1 x T.A.E. solution buffer = 40 mM Tris-acetic acid (pH 7.7), 2 mM EDTA.

Relaxation of supercoiled plasmid DNA by Cu-prodigiosene mixtures[5]

Reaction mixtures (20 μL total volume) contained 450 ng of supercoiled DNA, 10 mM MOPS (pH 7.4), 100 mM NaCl and the following concentrations of 1:1 $Cu(OAc)_2$:prodigiosene 0.5, 2, 5, 10, 15, 20, 25, 30 and 50 μM, generally in an acetonitrile/water (1/1) solution (exceptions are prodigiosene **3** in water/2-propanol (4/1), and prodigiosene **4** in methanol/water (1/1)). Fresh stock solutions of the Cu-prodigiosene mixture were prepared for each assay. Reaction mixtures were incubated for 30 min at 37 °C, and then 10 μL were quenched by the addition of 2 μL of loading buffer. The remaining reaction mixture (10 μL) was incubated for one more hour and after that quenched (again with 2 μL of loading buffer). Samples were loaded onto a 0.8 % agarose gel containing ethidium bromide (0.4 μg/mL). The gels were run in 1 x T.A.E. at 100 V for 30 min and photographed under UV light. DNA bands were quantified with image analysis software (Doc-itLS Version 5.5.5) and the EC_{50} values were obtained using the data analysis software (GraFit version 5.0.4).

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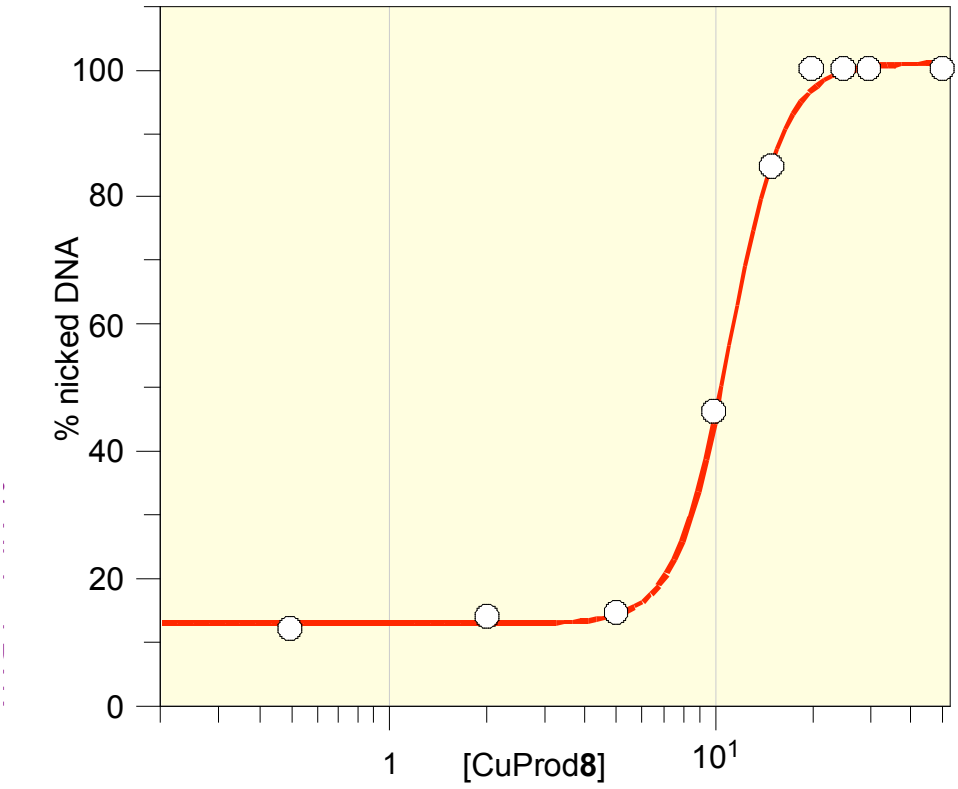
*EC_{50} curve for prodigiosene **8**:*



Parameter	Value	Std. Error
Y Range	88.1952	1.4514
EC_{50}	11.0631	0.1681
Slope factor	-5.2020	0.3928
Background	12.9417	0.9702

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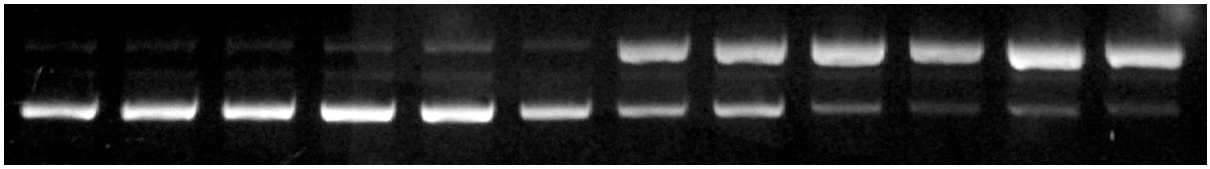
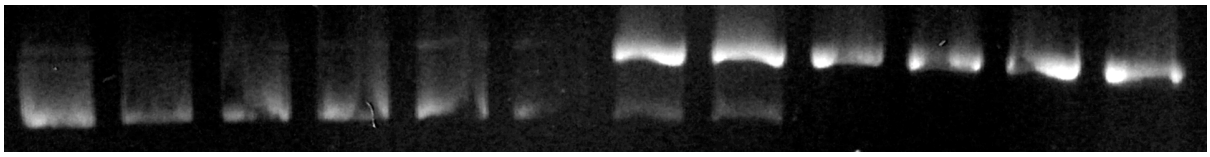
Electrophoresis gels after 30 min (A) and 90 min (B):

*Prodigiosin (**1**)*

(concentrations used for the assay = 0.5 μM, 1 μM, 2 μM, 5 μM, 10 μM, 15 μM, 25 μM, 30 μM and 50 μM)
A)



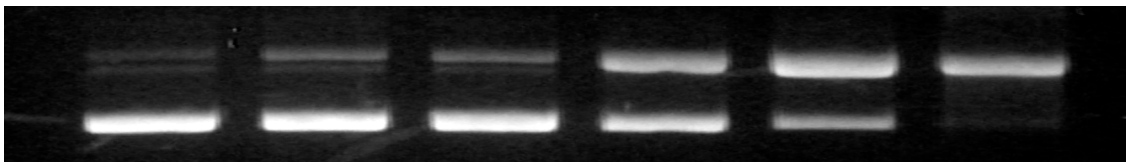
B)



*Prodigiosene **2***

(concentrations used for the assay = 0.5 μM, 2 μM, 5 μM, 10 μM, 15 μM and 20 μM)

A)



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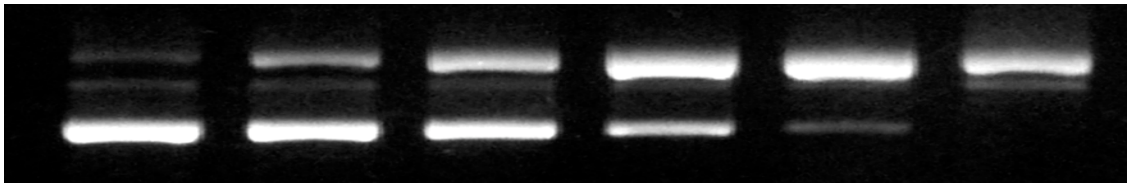

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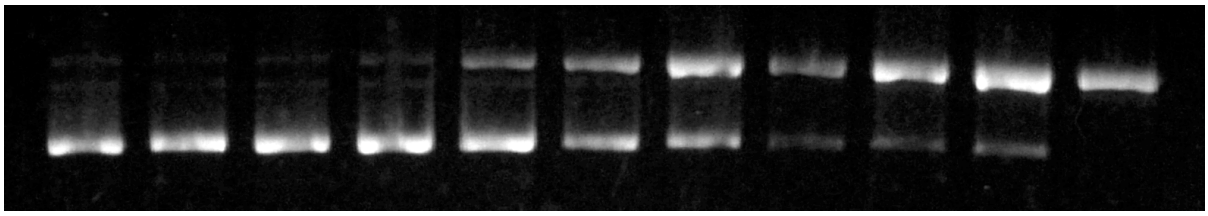
*Prodigiosene **3***

A)



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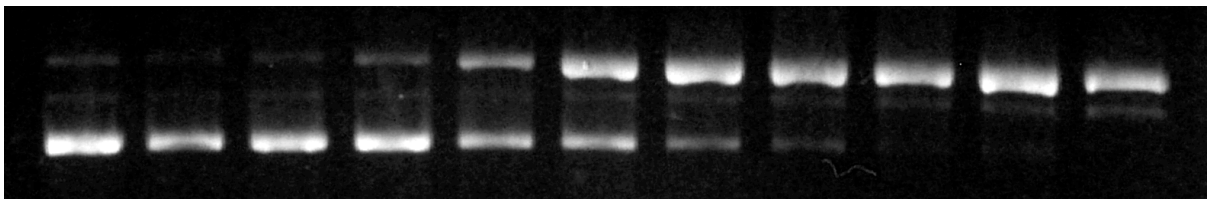
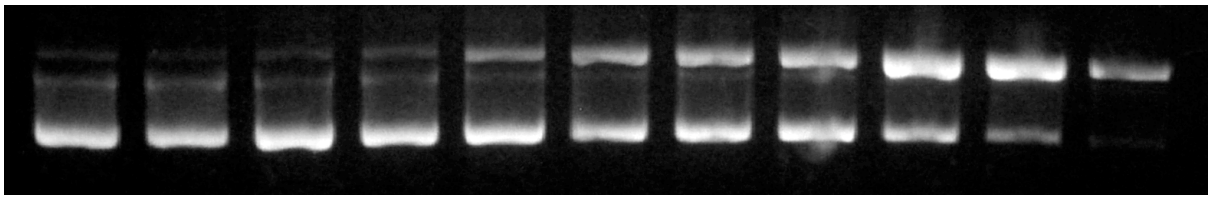


*Prodigiosene **4***

A)



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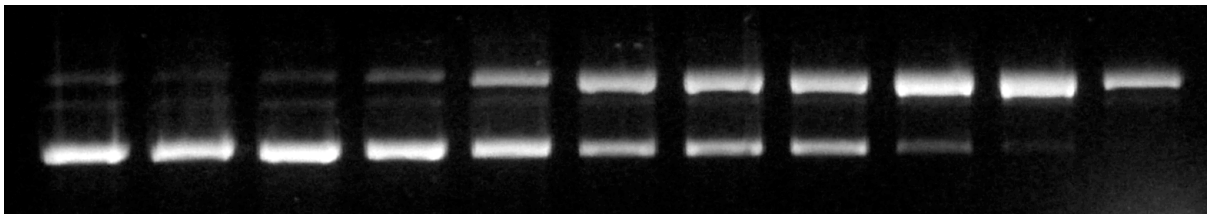

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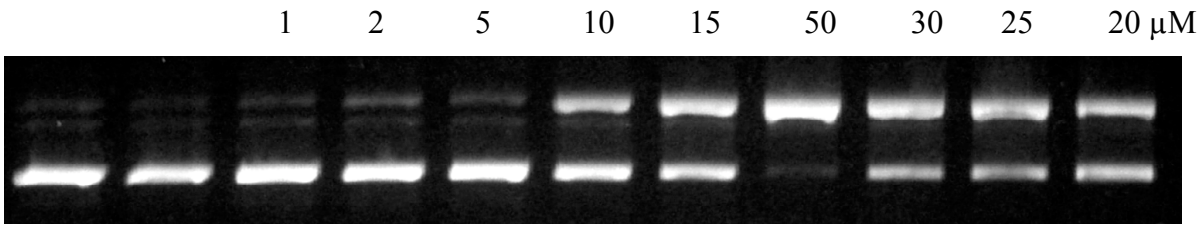
*Prodigiosene **5***

A)



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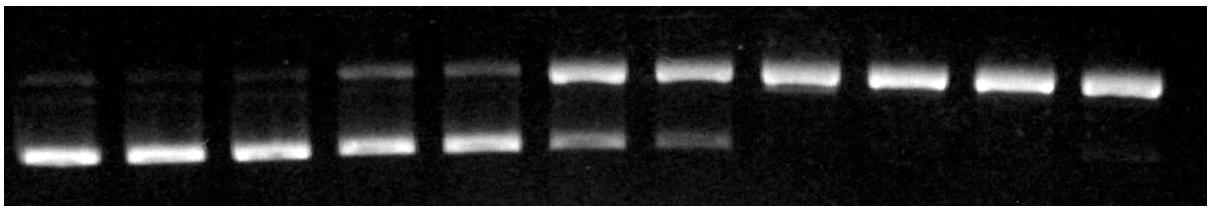
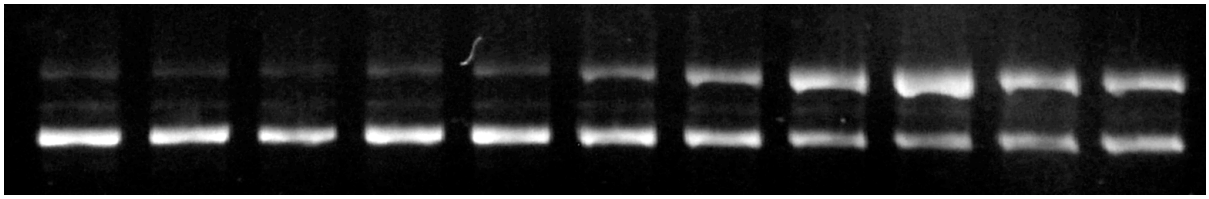
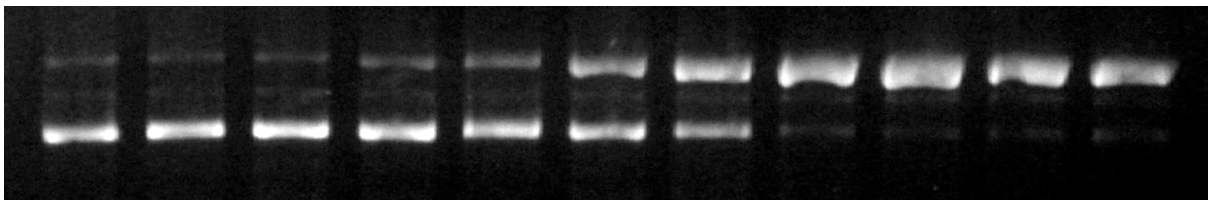


*Prodigiosene **6***

A)



B)



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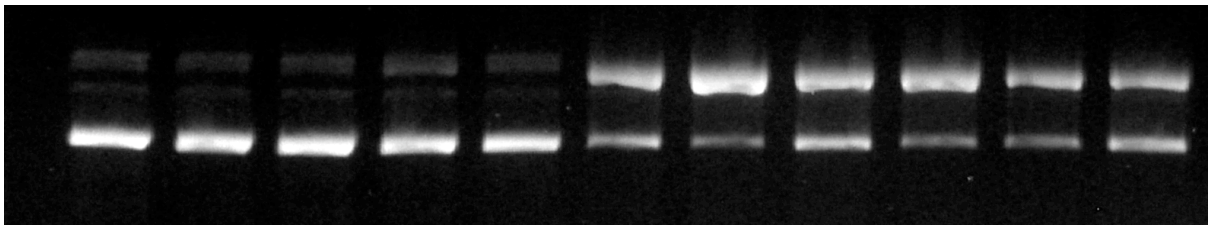
*Prodigiosene **7***

A)



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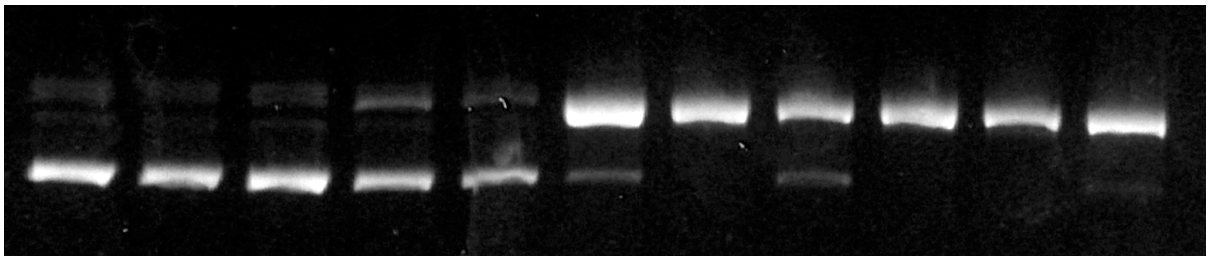
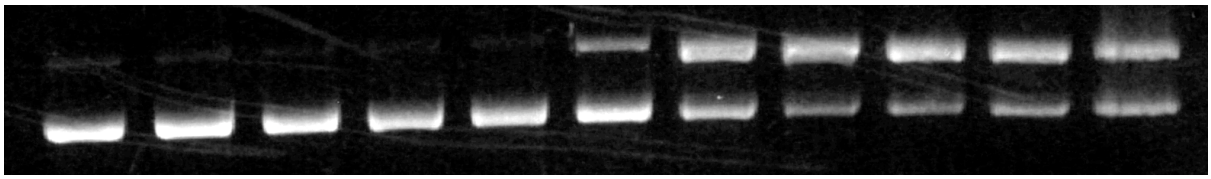


*Prodigiosene **8***

A)



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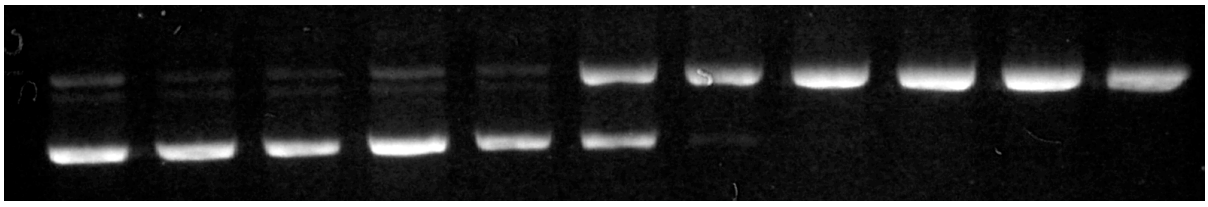

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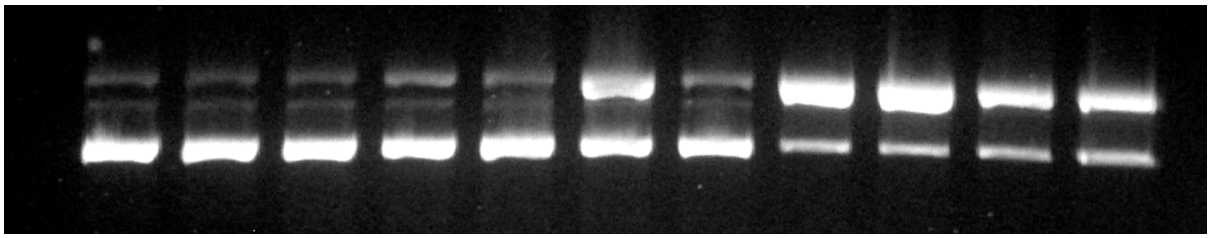
*Prodigiosene **9***

A)



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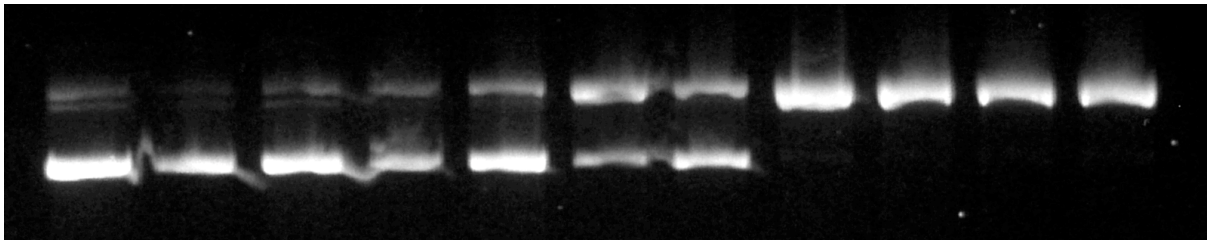
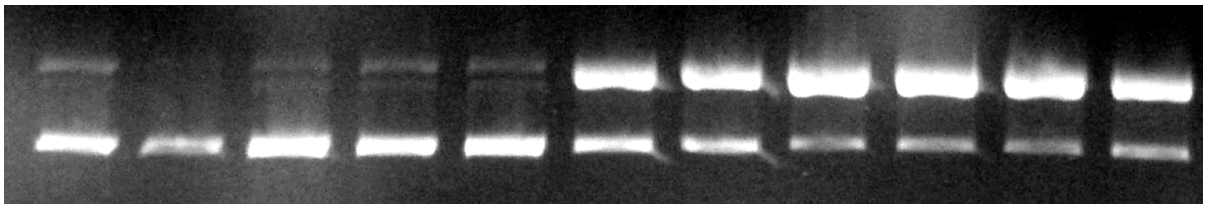


*Prodigiosene **10***

A)



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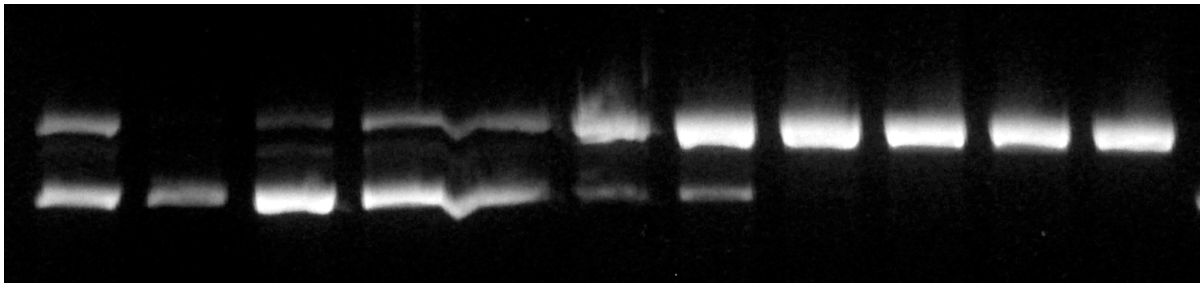

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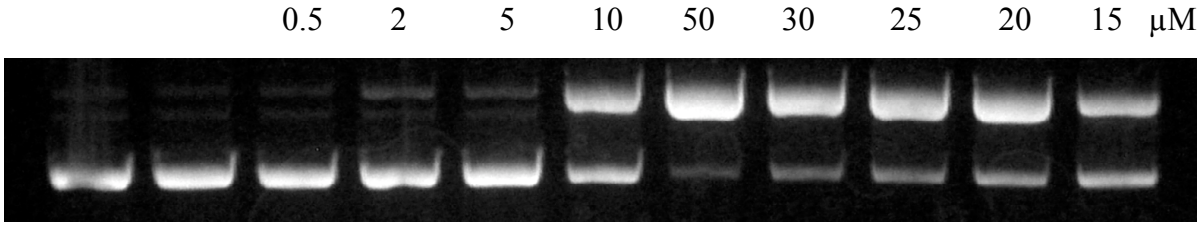
*Prodigiosene **11***

A)



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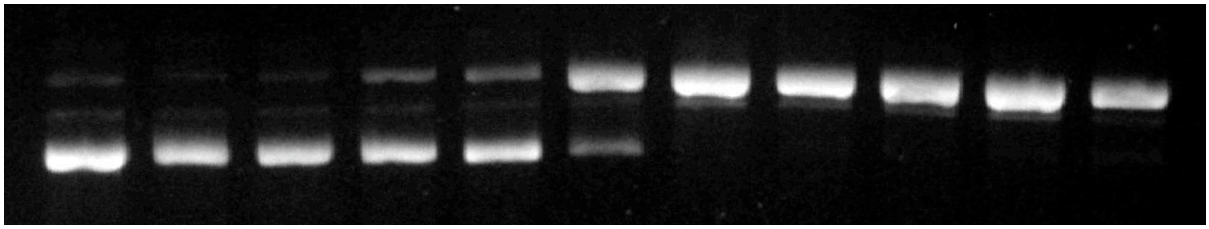

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(1) Regourd, J.; Al-Sheikh-Ali, A.; Thompson, A. *J. Med. Chem.* **2007**, in press.

(2) McNally, B. A.; Koulov, A. V.; Smith, B. D.; Joos, J. B.; Davis, A. P. *Chem. Commun.* **2005**, 1087-1089.

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(5) Melvin, M. S.; Tomlinson, J. T.; Saluta, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333-6334.