

## Indoloprodigiosins from the C-10 bipyrrolic precursor: New antiproliferative prodigiosin analogs

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**Abstract**—The condensation of the C-10 methoxybipyrrole precursor (**3**) of prodigiosin with indoles and a related pyrrole derivative yields novel analogs of prodigiosin. Biological evaluation of these products revealed compounds that inhibit cancer cell proliferation from 50 nM to 50  $\mu$ M.

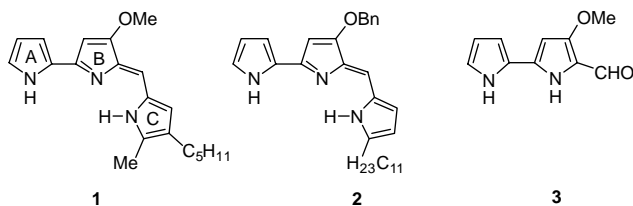
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There has recently been a resurgence of interest in prodigiosin (**1**), a major metabolite of *Serratia marcescens*.<sup>1</sup> The brilliant red pigment, known since the Middle Ages, has had a long dramatic history including its association with ‘the miracle of the bleeding host’.<sup>1a</sup> More recently, it has been used as an analytical marker in varied environmental applications such as the spread of infection in the British House of Commons and potential germ warfare by aerosols on the coast of California.<sup>1b</sup>

Early work on the structure of this pigment by Wrede led to a generally accepted tripyrrylmethene structure until 1960, when new studies by Wasserman et al. disclosed that pyrrole carboxamide was formed as a key degradation product.<sup>2a</sup> This finding showed unequivocally that a linear pyrrolylpyrromethene structure was correct<sup>2b</sup> and prompted the advancement of the methoxybipyrrole aldehyde (**3**) as the structure of the C-10 precursor, and the subsequent 1960 Rapoport and Holden synthesis of **1**.<sup>2c</sup>

Pyrrole alkaloids of this type exhibit a broad range of activity against bacteria, protozoa, and pathogenic fungi; however, dose-limiting systemic toxicities have precluded their clinical use as antibiotics.<sup>3</sup> Prodigiosin analogs have also been identified as immunosuppressants.<sup>4</sup> In fact, investigators at Pharmacia had advanced PNU-156804 (**2**) for the potential treatment of various autoimmune diseases including multiple sclerosis and rheumatoid arthritis.<sup>5</sup> More recently, prodigiosins have also been shown to possess proapoptotic properties.<sup>6</sup>

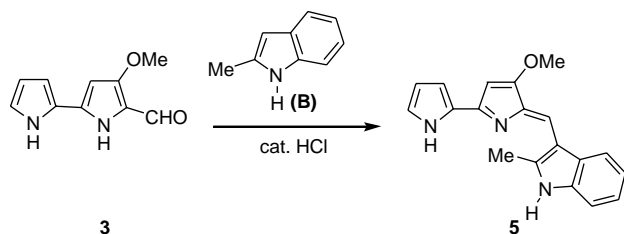
The characteristic cytotoxicity of the prodigiosins has been attributed to several qualities which have been ascribed to this family of alkaloids, from their propensity to intercalate into DNA,<sup>7</sup> to their ability to interfere with protein kinase C isozymes,<sup>8</sup> and/or to their capacity to modify the pH of cell organelles through their H<sup>+</sup>/Cl<sup>−</sup> symport activity across biological membranes.<sup>9</sup> However, substantial evidence to date links the cytotoxicity of the prodigiosins to their potent DNA-damaging properties under oxidative conditions, not unlike the clinically utilized bleomycins.<sup>10</sup> Prodigiosin (**1**) and related 4-methoxybipyrrolic natural products have been reported to bind DNA effectively<sup>11</sup> and facilitate oxidative DNA single strand and double strand cleavage in the presence of Cu(II) and other metal cations.<sup>12</sup> SAR studies have demonstrated that structural modifications, which mitigate the ability of the prodigiosins either to bind metal cations or to be oxidized, also diminish their cytotoxicity.<sup>13</sup> It has also been suggested recently that the cytotoxicity of some prodigiosin and roseophilin analogs is related to their activity as phosphatase inhibitors.<sup>14</sup>



**Keywords:** Prodigiosin; Antiproliferative agent; Indole; Pyrrole.

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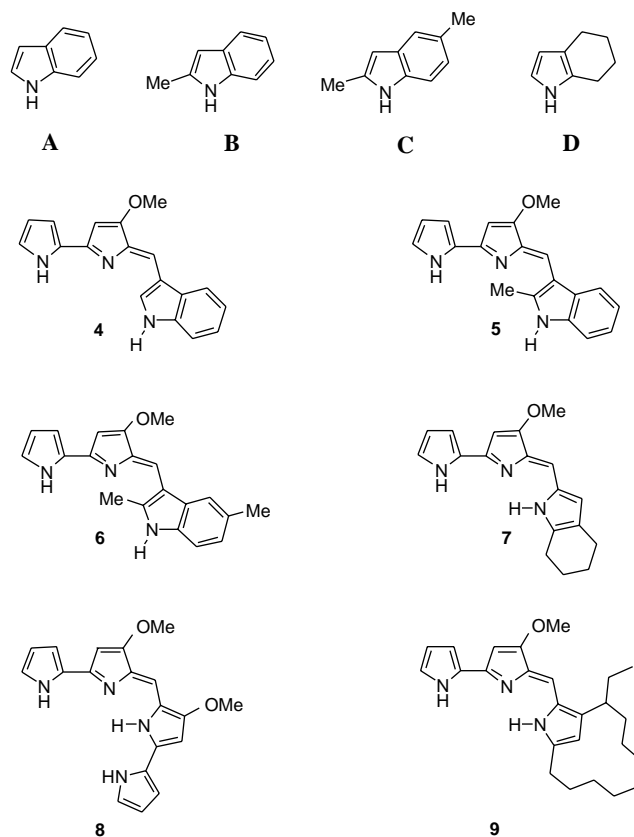
We now report studies on the preparation and biological evaluation of novel analogs of prodigiosin, formed by the condensation of the C-10 methoxybipyrrole aldehyde precursor **3** with indole derivatives.<sup>1c</sup> These analogs represent nonnatural prodigiosins for which their DNA damaging properties have been decoupled from their cytotoxicity in three cell lines, an unexpected and unusual observation for prodigiosin alkaloids.



Each of the indoles **A–C** and the tetrahydroindole derivative **D** underwent ready reaction with the methoxybipyrrole aldehyde. The work-up procedure yielded the condensation products in good yields (60–70%) as red solids.<sup>15</sup> The products of the reaction are assigned the structures **4–7** based on their HRMS, <sup>1</sup>H, and <sup>13</sup>C NMR characterization. The preparation works very much the same for all the substrates (**A–D**) and is a direct adaptation of the method used to prepare prodigiosin analogs from the bipyrrole precursor and various pyrroles. Structural assignments for the products **4–6** are in accord with typical patterns of indole reactivity involving substitution at the free  $\beta$ -position. In the case of the tetrahydro derivative **D**, a normal pyrrole-like activity would be expected at the  $\alpha$ -position. Reactions of **3** with other heterocycles including thiophene, furan or imidazole ring systems rapidly developed a deep blue color which we believe indicates deformylation of the bipyrrole **3** with concomitant self-condensation providing a blue pigment (**8**) isolated in earlier work.<sup>16</sup>

To characterize these compounds, as well as prodigiosin (**1**) and metacycloprodigiosin (**9**),<sup>17</sup> we utilized an MTS cell proliferation assay to assess cytotoxicity. Exponentially growing A549, DLD-1, HT29, MDA-MB-231, and NCI-H460 cancer cells were incubated with various concentrations of the compound for 72 h and we measured the concentration at which cell proliferation was 50% inhibited.

As shown in Table 1, we found the IC<sub>50</sub> for prodigiosin (**1**) to be 0.03–0.17  $\mu$ M, while the IC<sub>50</sub> for metacycloprodigiosin (**9**) was 0.3–1.7  $\mu$ M, approximately 10-fold less active. A 2- to 10-fold increase in IC<sub>50</sub> was observed for the unsubstituted indole of **4** versus the 2-methylindole derivative **5** and the dimethylindole derivative **6**. The analog whose inhibition of cellular proliferation was greatest was compound **7**, which demonstrated an IC<sub>50</sub> range between 0.2 and 0.8  $\mu$ M in the three cell lines, similar to metacycloprodigiosin (**9**). This is not a surprising result, given that ring C of **7** should react as a dialkyl pyrrole similar to compounds **1** and **9**. The aliphatically substituted C-ring pyrrole compounds (**1**, **7**, and **9**) possessed the greatest activity, while the incorporation of



the additional pyrrole ring and additional methoxy substituent in **8** appears to reduce cell proliferation inhibition of the chemotype by 100-fold, more akin to the indoloprodigiosin analogs (**4–6**). Thus, while the indoloprodigiosins described herein are not as cytotoxic as the naturally occurring methylamyl prodigiosin, they represent new prodigiosin manifolds that can be exploited in SAR efforts.

A high content screening assay was used to measure cells that stained positive for the DNA damage marker phospho-histone H2AX (Ser139).<sup>18</sup> We incubated various concentrations of the compounds with A549 cells for 18 h and then fixed, stained, and determined the concentration at which there is a 50% half-maximal increase in the cellular response relative to the DMSO controls.<sup>19</sup> Interestingly, we noted that there is no detectable DNA damage activity with all compounds tested (**1** and **4–9**) using this DNA damage marker (data not shown).

We serendipitously observed that prodigiosin (**1**) is fluorescent and can be visualized in live A549 cancer cells using *epi*-fluorescence with a standard rhodamine filter set (Fig. 1) after a 2 h exposure. Live cells were counter-stained with the DNA binding dye Hoechst 33258 (1  $\mu$ g/mL for 30 min) to identify nuclei, washed into PBS, and microscopically imaged. To our surprise, prodigiosin (**1**) was predominantly localized to the cytoplasm including areas near, but not within, the nucleus. Several observations indicated that the observed rhodamine channel staining is prodigiosin.

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