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Synthesis and potent cytotoxic activity of 8-and 9-anilinophenanthridine-7, 10-diones

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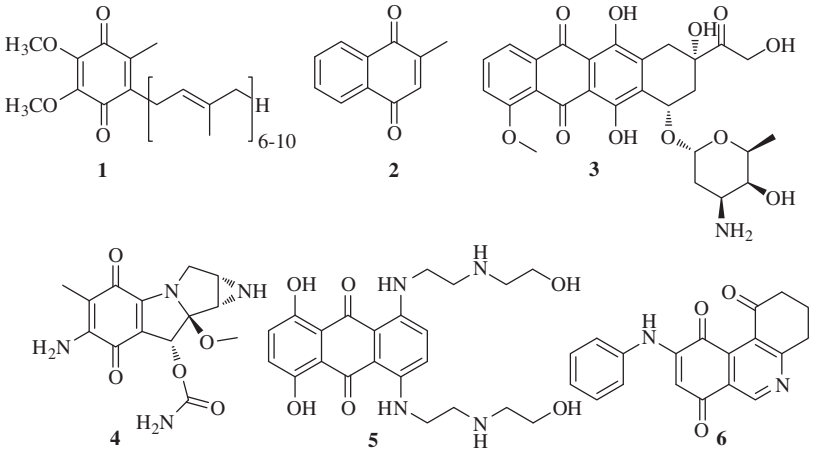
ABSTRACT

A series of 8-anilino and 9-anilinophenanthridine-7,10-diones was prepared and screened against various cancer cell lines to measure anti-proliferative activity. The compounds tested display potent cytotoxic activity in the micromolar and sub-micromolar range. These compounds are promising new leads for developing anticancer compounds.

Quinones are a valuable class of compounds with interesting physicochemical properties.[1–3] The redox activity of compounds such as ubiquinone (**1**) and menadione (**2**) play an important role in electron transport within living cells.[4] The redox activity of naturally-occurring and synthetic quinones is important in drug design. For instance, quinones such as adriamycin (**3**),[5–7] mitomycin C (**4**)[8,9], and mitoxantrone (**5**)[10,11] possess potent anticancer activity and are employed as chemotherapeutic drugs.



Recent reports have shown that anilinoquinones such as **6** display micromolar activity against a range of cancer cell lines.[12] In this work, we present a short synthesis of 8- and 9-anilinophenanthridine-7,10-diones starting from commercially available 2, 5-dimethoxybenzoic acid (**7**). These compounds displayed

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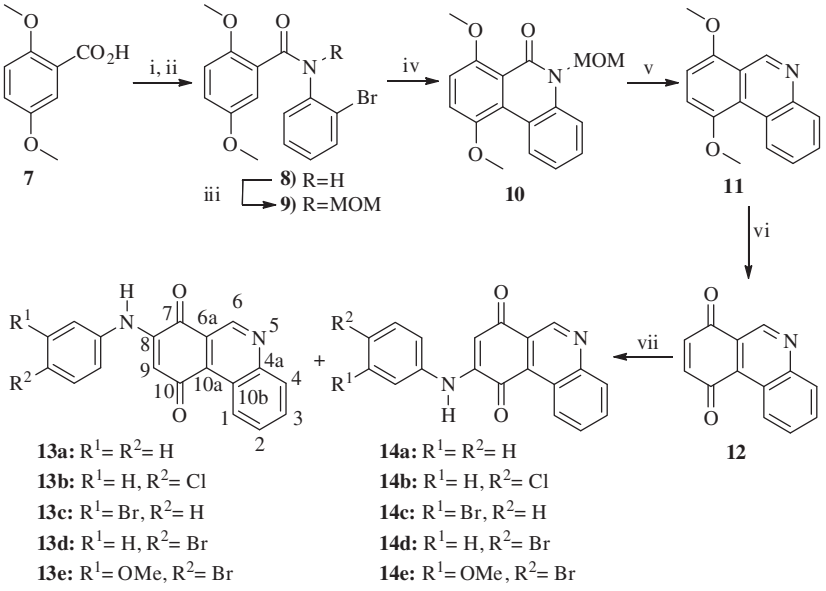


Scheme 1. Reagents and conditions: (i) $SOCl_2$, 80 °C, 1 h; (ii) 2-bromoaniline, K_2CO_3 (2 equiv), THF, rt, 16 h, 97%; (iii) NaH, THF, MOMCl, 40 °C, 16 h, 86%; (iv) 10 mol % $Pd(PPh_3)_4$, K_2CO_3 (2 equiv), DMF, 140 °C, 92–96%; (v) $LiAlH_4$ in dry Et_2O for 1 h, then 1 N HCl, 90%; (vi) CAN, 1:1 CH_3CN–H_2O, 0 °C for 1 h, 78%; (vii) 0.5 equiv aniline, 1:1 CH_3CN–H_2O, 1% AcOH, rt, 16 h, 55–90%.

micromolar and sub-micromolar activities against various cancer cell lines in the sulforhodamine B (SRB) assay. To our knowledge, this is the first time such compounds have been reported in the literature, and several of the products presented herein are far more potent than related anilinoquinones.[12–14]

The synthesis of the anilinophenanthridine-7,10-diones is outlined in Scheme 1. Starting from 2,5-dimethoxybenzoic acid (**7**), the tertiary amide **9** was rapidly accessed in three steps and in high yield following the literature procedure.[15] Cyclization of the tertiary amide **9** with catalytic Pd(0) in the presence of PPh_3 gave the desired phenanthridinone **10** in 96% yield. Compound **10** was readily reduced to the corresponding phenanthridine **11** using $LiAlH_4$,[15–17] and subsequently oxidized with cerium(IV) ammonium nitrate at 0 °C to yield the quinone, phenanthridine-7,10-dione (**12**), in 78% yield.

The initial addition of aniline to the quinone **12** was carried out with a 1:1 ratio of reactants and resulted in 50% conversion of the quinone into the products **13** and **14**, and 50% conversion into the reduced quinone (Scheme 2). This is presumably due to the formation of the adducts **15** and **16**, which in turn rapidly react with **12** resulting in the formation of the anilinoquinones **13** and **14** and the quinol **17**. Upon work-up with aqueous $NaHCO_3$, the quinol **17** is oxidized by aerial oxygen and the quinone **12** is recovered. However, an attempt to push the conversion of **12** toward the desired

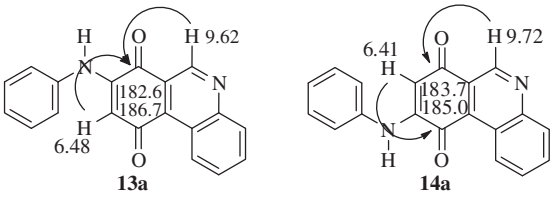
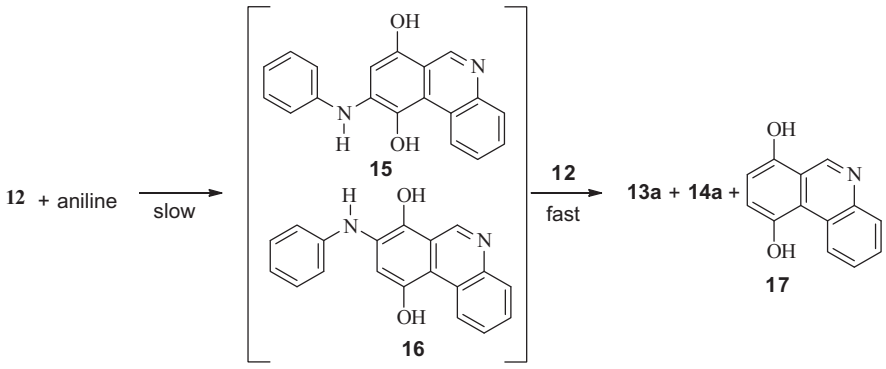


Figure 1. Key $^3J_{CH}$ correlations for **13a** and **14a** obtained by HMBC.

products by bubbling air through the reaction mixture was not successful. Thus for optimal yields, only 0.5 equiv of the aniline was required. Stirring the reaction mixture at room temperature for 16 h resulted in a mixture of 8-anilinophenanthridin-7,10-diones (**13a–e**) and the 9-anilino isomers (**14a–e**, Scheme 1). Comparison of the integrations of the singlet quinonic proton signals in the δ 6.3–6.5 range of the ^{1}H NMR spectra showed that roughly a 1:1 mixture was obtained in each reaction. The compounds were separated and purified using C18 reverse-phase preparatory HPLC. Assignment of the isomers was accomplished by HMBC (Fig. 1). Specifically, the 8-anilino compounds had $^3J_{CH}$ signals for H(6) and H(9) coupled to same carbonyl, C(7). On the other hand, the 9-anilino compounds had distinct non-overlapping $^3J_{CH}$ signals for H(6) to C(7) and H(8) to C(10). The $^2J_{CH}$ couplings were not



Scheme 2. Reaction of quinone **12** with aniline.

Table 1
Growth inhibition (GI_{50}) of test compounds against cancer cell lines. NT = Not tested due to poor solubility

	GI_{50} ± SEM (μM) determined via SRB assay					
Substrate	MCF-7	NCI-H460	SF268	A549	DU145	A8
13a	0.094 ± 0.008	0.062 ± 0.001	0.633 ± 0.043	0.184 ± 0.006	0.086 ± 0.000	0.070 ± 0.011
13b	0.697 ± 0.014	0.561 ± 0.017	6.283 ± 1.322	0.848 ± 0.026	0.680 ± 0.016	0.586 ± 0.048
13c	0.678 ± 0.029	0.265 ± 0.004	0.858 ± 0.020	0.642 ± 0.021	0.569 ± 0.035	0.498 ± 0.072
13d	0.676 ± 0.014	0.554 ± 0.012	1.289 ± 0.413	0.667 ± 0.034	0.671 ± 0.022	0.584 ± 0.05
13e	0.065 ± 0.002	0.060 ± 0.002	0.098 ± 0.015	0.077 ± 0.004	0.061 ± 0.007	0.061 ± 0.006
14a	0.338 ± 0.003	0.933 ± 0.021	0.557 ± 0.032	2.013 ± 0.776	2.430 ± 0.193	0.490 ± 0.107
14b	0.396 ± 0.003	0.633 ± 0.002	0.553 ± 0.024	0.910 ± 0.119	0.722 ± 0.025	0.546 ± 0.139
14c	NT	NT	NT	NT	NT	NT
14d	2.981 ± 0.029	4.477 ± 1.249	3.947 ± 0.588	5.139 ± 0.114	3.149 ± 0.728	1.905 ± 0.161
14e	0.400 ± 0.037	0.686 ± 0.79	0.552 ± 0.013	0.821 ± 0.006	0.691 ± 0.023	0.520 ± 0.074
Taxol	0.008 ± 0.000	0.007 ± 0.000	0.050 ± 0.010	0.041 ± 0.012	0.036 ± 0.012	0.058 ± 0.001

observed. Interestingly, the quinonic H(8) chemical shifts were 0.05–0.07 ppm lower than the H(9) chemical shifts.

The purified compounds **13a–e** and **14a–e** were screened against various cancer cells lines using the SRB assay to determine the growth inhibition.[18,19] The human cancer cell lines assayed were breast (MCF-7), lung (NCI-H460, A549), brain (SF268), prostate (DU145), and epothilone-resistant ovarian cancer cells (A8). All compounds were tested in triplicate. In this assay, the cells were incubated in a 96-well plate with varying concentrations of the test compounds (10 μM down to 0.001 μM) for 48 h, then fixed with 50% TFA. The cells were washed with distilled water and fixed with SRB. After washing away excess SRB, the remaining protein-bound SRB was solubilized with Tris base (100 μL, 10 mM) and the relative protein concentrations were determined spectrometrically at 515 nm. The results, reported as the concentration required for 50% growth inhibition (GI_{50}), are shown in Table 1.

The results show that most of the compounds were active at single-digit and sub-micromolar ranges against a variety of cancer cells. Compound **14c** could not be tested due to its poor solubility. Overall, compound **13e** was the most potent synthetic compound against all the cell lines, while compound **14d** was the least active. Compound **13a** was the second best inhibitor. Compounds **13a**[20] and **13e**[21] show similar activity against the A8 cell line as Taxol, but were ten-fold less effective against SF268. Within the 8-anilino series, product **13e** was the most active followed by **13a** while compounds **13b–d** shared similar activities. No such trend was observed with the 9-anilino series in which **14a**,[22] **14b,** and **14e**[23] all had very similar activities within the standard deviations observed. Only the bromo compound **14d** displayed strong attenuation of activity within the series.

In summary, we have outlined a brief synthesis of 8- and 9-anilinophenanthridine-7,10-diones starting from 2,5-dimethoxybenzoic acid and various anilines. These compounds display promising cytotoxic activity against several cancer cell lines and may be useful as leads for anticancer compounds. Further work is underway to determine the mechanism of action of these new quinones as well as to optimize desirable physicochemical properties.

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20. *Compound* **13a**: mp 211–212 °C. R_f 0.41 (1:3 EtOAc/light petroleum). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.48 (s, 1H, C–H), 7.30 (m, 3H, Ar–H), 7.46 (m, 2H, Ar–H), 7.52 (s, 1H, N–H), 7.75 (app. t, *J* = 7 Hz, 1H, Ar–H), 7.87 (app. t, *J* = 8 Hz, 1H, Ar–H), 8.18 (d, *J* = 8 Hz, 1H, Ar–H), 9.61 (d, *J* = 6 Hz, 1H, Ar–H), 9.62 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 105.1, 122.0, 122.6, 123.0, 125.9, 129.1, 129.8, 129.9, 130.1, 132.3, 133.7, 137.1, 142.9, 147.0, 152.8, 182.6 (CO), 186.7 (CO). ESI-MS *m*/*z* 301 ([M+H]$^+$, 100). HRMS calcd for $C_{19}H_{13}N_2O_2$: 301.0972 [M+H]$^+$; found: 301.0982.
21. *Compound* **13e**: mp: 230–232 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.94 (s, 3H, CH_3), 6.45 (s, 1H, C–H), 6.81 (m, 2H, Ar–H), 7.49 (s, 1H, N–H), 7.59 (d, *J* = 8 Hz, 1H, Ar–H), 7.75 (app. t, *J* = 8 Hz, 1H, Ar–H), 7.88 (app. t, *J* = 8 Hz, 1H, Ar–H), 8.17 (d, *J* = 8 Hz, 1H, Ar–H), 9.59 (d, *J* = 8 Hz, 1H, Ar–H), 9.61 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.4, 105.8, 106.5, 108.4, 115.6, 120.2, 121.9, 122.9, 129.0, 130.0, 130.1, 132.4, 134.1, 137.6, 142.5, 147.0, 152.8, 156.8, 182.3 (CO), 186.7 (CO). ESI-MS *m*/*z* 409 ([M+H]$^+$, ^{79}Br, 100), 411 ([M+H]$^+$, ^{81}Br, 98). HRMS calcd for $C_{20}H_{14}{}^{79}BrN_2O_3$: 409.0182 [M+H]$^+$; found: 409.0186.
22. *Compound* **14a**: mp 243–244 °C. R_f 0.41 (1:3 EtOAc/light petroleum). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.41 (s, 1H, C–H), 7.31 (m, 3H, Ar–H), 7.47 (m, 2H, Ar–H), 7.64 (s, 1H, N–H), 7.82 (app. t, *J* = 9 Hz, 1H, Ar–H), 7.88 (app. t, *J* = 8 Hz, 1H, Ar–H), 8.23 (d, *J* = 8 Hz, 1H, Ar–H), 9.45 (d, *J* = 9 Hz, 1H, Ar–H), 9.72 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 101.4, 122.0, 123.0, 123.6, 126.1, 126.8, 129.8, 129.9, 130.6, 130.7, 131.4, 137.1, 145.3, 148.0, 151.4, 183.7 (CO), 185.0 (CO). ESI-MS *m*/*z* 301 ([M+H]$^+$, 100). HRMS calcd for $C_{19}H_{13}N_2O_2$: 301.0972 [M+H]$^+$; found: 301.0983.
23. *Compound* **14e**: mp: 240–241 °C. R_f 0.33 (1:3 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.94 (s, 3H, CH_3), 6.40 (s, 1H, C–H), 6.84 (m, 2H, Ar–H), 7.60 (m, 2H, Ar–H, N–H), 7.83 (app. t, *J* = 8 Hz, 1H, Ar–H), 7.89 (app. t, *J* = 8 Hz, 1H, Ar–H), 8.24 (d, *J* = 8 Hz, 1H, Ar–H), 9.43 (d, *J* = 8 Hz, 1H, Ar–H), 9.72 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.5, 102.1, 106.8, 108.7, 116.0, 122.0, 123.4, 126.8, 129.8, 130.6, 130.8, 131.5, 134.1, 137.6, 144.9, 148.0, 151.5, 156.9, 183.7 (CO), 184.7 (CO). ESI-MS *m*/*z* 409 ([M+H]$^+$, ^{79}Br, 100), 411 ([M+H]$^+$, ^{81}Br, 100). HRMS calcd for $C_{20}H_{14}{}^{79}BrN_2O_3$: 409.0182 [M+H]$^+$; found: 409.0192.