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Advance of Seriniquinone Analogues as Melanoma Agents

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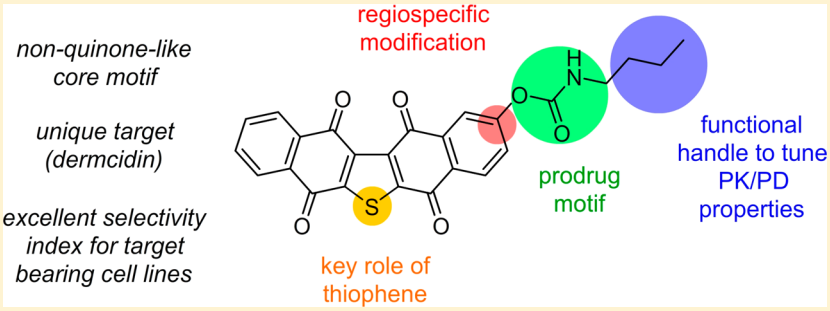
Supporting Information



ABSTRACT: Seriniquinone, a marine natural product, displayed potent cytotoxicity and selectivity against melanoma cancer cells. This selectivity, combined with a novel mode of action (MOA), prompted studies to translate a pharmacologically relevant lead. Herein, we report on structure−activity relationships (SARs), and provide a strategy to prepare analogues that retain activity and offer an improved water solubility and isomeric purity. From intermediates made on a gram-scale, derivatives were prepared and evaluated for their antiproliferation activity and melanoma selectivity. Overall these studies provide methods to install side chain motifs that demonstrate a common, and yet unique, biological profile.

KEYWORDS: *Drug discovery, melanoma, marine natural products, medicinal chemistry, seriniquinone, dermcidin*

Malignant melanoma, classified by genetic defects within pigment-producing melanocytes, is attributed to the largest number of skin-related cancer deaths.[1,2] Its incidence and mortality have been increasing steadily over the last 50 years. The unique epidemiological concerns for the generation of such tumors and the lack of curative treatment options place melanoma as one of the most dangerous cancers.[3,4] Once melanoma metastasizes, prognosis is poor, and the therapeutic options are limited. Although protective measures can be taken, the aggressive and rapid metastatic properties of melanoma-based tumors continue to challenge clinical treatments. The discovery of prevalent proto-oncogene protein B-raf (BRAF) mutations in at least 50% of melanoma tumors led to the development of BRAF inhibitors.[5] The recently approved BRAF inhibitors (Figure 1) for the treatment of metastatic melanoma have made a significant impact on patient survival, though the results were shadowed by the appearance of drug resistance.[6,7] Thus, selective antimelanoma leads with novel modes of action (MOAs) are still of great interest.[8−10]

Nature has provided a diverse cornucopia of bioactive substructures and molecular scaffolds for the development of new drugs, and these natural products continue to provide vital treatment options for cancer. Seriniquinone (**1**), a marine natural product isolated from a rare Gm+ (Gram-positive) bacterium, demonstrated potent activity and selectivity toward melanoma cell lines.[11] Preliminary cancer biology studies indicated that **1** (Figure 2) inhibits cell proliferation marked by autophagocytosis and induces cell death due to caspase-9 dependent apoptosis. Furthermore, mode of action studies suggested that **1** targets dermcidin (DCD),[11] a small protein whose role in cancer proliferation has been recently recognized.[12,13] To the best of our knowledge, no other small molecule has been demonstrated to bind this protein. Thus, the investigations into **1** would facilitate better understanding of the biological function and significance of dermcidin. Combined, these findings suggest **1** as a potential new pharmacophore against melanoma. However, the translation of **1** into a chemotherapeutic agent is complex. It requires significant medicinal chemistry efforts to generate a large number of derivatives to improve its "druglike"

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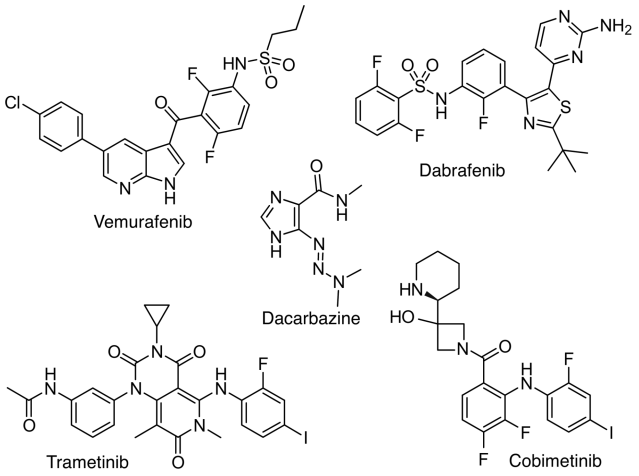

Figure 1. Exemplary structures of FDA approved drugs for melanoma including Dabrafenib (BRAF inhibitor),[14] Vemurafenib (BRAF inhibitor),[15] Trametinib (Mitogen-activated protein kinase (MEK) inhibitor),[16] Cobimetinib (MEK inhibitor),[17] and Dacarbazine (DNA alkylator).[18,19] Other agents (not shown) including Encorafenib (BRAF inhibitor) and Binimetinib (MEK inhibitor) have ended phase III, and NDA requests are filed.

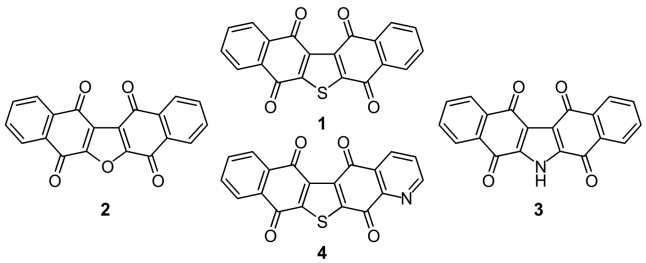

Figure 2. Structures of seriniquinone (**1**) and core analogues **2**–**4**.

properties. Herein, we report the design and synthesis of seriniquinone derivatives that aim to investigate their structure–activity relationship (SAR) profile and provide a solution to pharmacological optimization.

A preliminary SAR study was initiated to identify the pharmacophore of **1** (Figure 2). We began by preparing furanyl- and pyrrolyl-derivatives **2** and **3**, respectively, to understand the role of the central core (Figure 2). As illustrated in Scheme 1, the synthesis of **2** started from **5**,[20] which, upon treatment with aqueous NaOH solution, gave rise to dihydroxylated **6**. Compound **6** was then converted to **2** under acidic conditions. The preparation of **3** began with treatment of **7**[21] with ammonia followed by cyclization, affording pyrrolyl-derivative **3**. We also prepared quinoline analogue **4** (Scheme S3) to explore the functional tolerance within the aryl substituents.

Samples of **2**–**4** were screened across three cell lines (Table 1). Furan **2** was 3–22-fold less active than **1**. The pyrrolyl-analogue **3** proved to be even less active, while **4** displayed activity comparable to or better than **1** (Table 1). While **1** was readily accessed synthetically,[11] its low solubility in both aqueous and organic media directed our attention to explore analogues with modifications in the aryl rings. Here, our goal was to prepare agents with comparable activity profile to **1** but with an improved solubility and associated pharmacological properties.

Scheme 1. Synthesis of Core Analogues 2 and 3

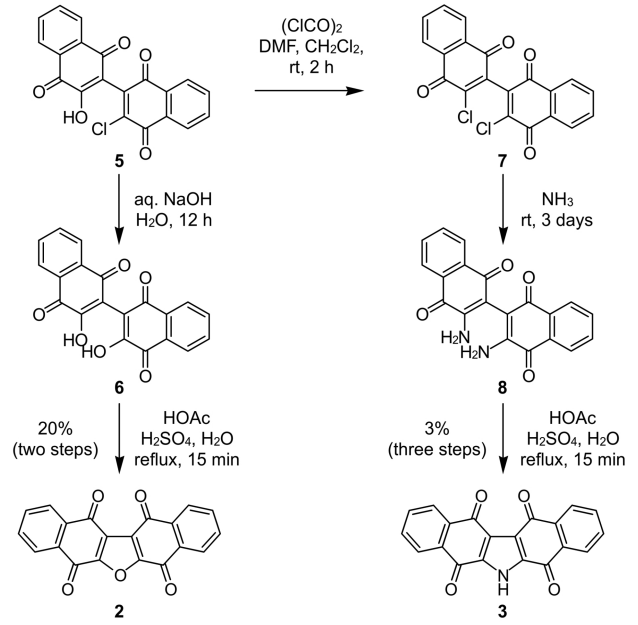

Table 1. Amorphous Solubility (*S*) and Activity Data (IC_{50} Values)[a]

		IC_{50} values (μM)		
	S, μM	Malme-3M	SK-MEL-28	HCT-116
1	0.06	0.05	0.36	1.29
2	0.09	1.11	3.47	4.08
3	0.4	2.03	4.30	4.24
4	0.5	0.10	2.15	0.54
9a/9b	5.5	0.11	0.25	1.18
11a/11b	5.1	0.28	0.74	0.73
12a/12b	2.7	0.001	0.15	0.06
13a/13b	3.3	0.009	0.34	0.48
14a/14b	2.1	0.001	0.46	0.53
23	0.4	0.22	0.33	1.45
24	24.6	0.06	1.80	0.75
25	8.2	0.06	7.00	8.16
doxorubicin		0.50	3.0	0.48

[a]The data for **9**–**12** was collected with a 2:1 mixture of regioisomers a:b (Figure 3). *S* was determined at pH 6.5 for **1**–**4**, pH 6.5 for **9a/9b**–**14a/14b**, or pH 7.2 for **23**–**25**. IC_{50} values were reported as averages and were within 5% deviation over two repetitions via the MTT assay (see Table S1). Compound **9a/9b** and **10a/10b** would be the same in buffer, and hence only one was screened.

Beginning with α-myrcene, we were able to access acid **9a** as a mixture with regioisomer **9b** in gram-scale through a nine-step sequence.[11] Derivatization of this acid as a sodium salt **10a/10b** or amides **11a/11b**–**14a/14b** provided materials that allowed us to confirm that the activity was maintained in terms of both its potency and melanoma selectivity (Table 1). While these materials provided improved solubility, the lack of an effective procedure to separate isomers **a** and **b** (right, Figure 3) proved problematic. This was further complicated by the inability to deliver a regioselective approach to either regioisomer in **11**–**14**. Based on these issues, we turned our attention to explore an alternate route that would enable preparation of a single regioisomer (**a** or **b**, Figure 3).

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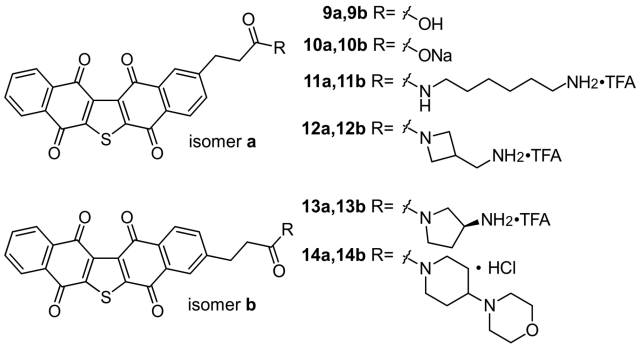
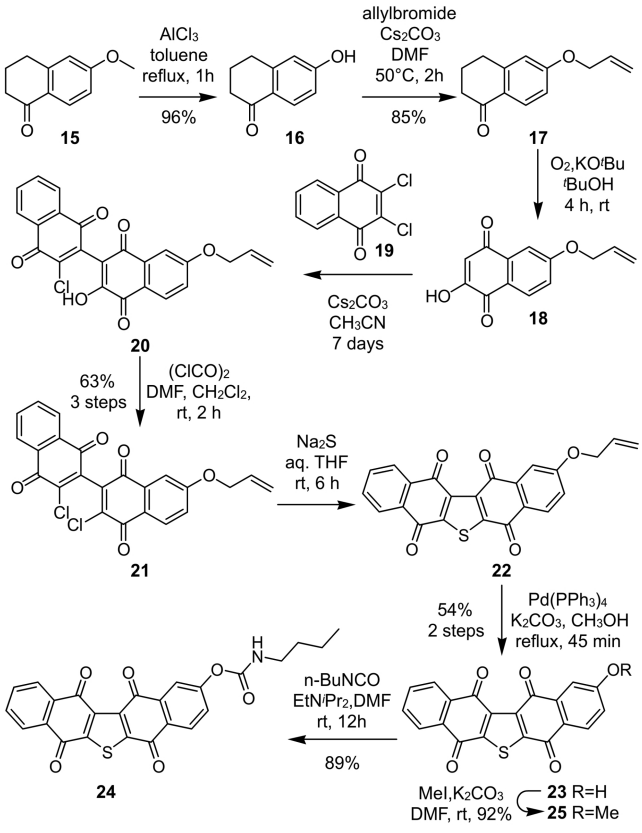


Figure 3. Structures of the synthetic analogues **9a**/**9b**, acid salt **10a**/**10b**, and amides **11a**/**11b**−**14a**/**14b**. These materials, prepared at milligram-scale for testing, were prepared as inseparable mixtures of regioisomers **a** and **b**.

Our attention was drawn to commercially available 7-methoxy-1-tetralone (**15**) as a vehicle to address the issue associated with regioselectivity (Scheme 2). We began by converting 6-methoxy-1-tetralone (**15**) to **17** by a two-step sequence in 82% yield. Samples of **17** were then oxidized with O_2 in a mixture of *t*BuOK/*t*BuOH,[22] and the resulting product **18** was coupled directly thereafter with 2,3-dichloronaphthalene-1,4-dione (**19**). Pure **21** was then obtained by chlorination of crude coupling product **20** followed by flash chromatographic purification. In one batch, we were able to convert 16.3 g (0.10 mol) of **17** to 27.7 g (63%) of **21** with **19** as the major impurity.

Scheme 2. Gram-Scale Synthesis of Analogues 23−25



Dichloride **21** was then treated with Na_2S in aq THF at room temperature (rt) to yield **22**, which, after deprotection with $Pd(PPh_3)_4$ and K_2CO_3 in warm MeOH, afforded 12.2 g of **23** in 54% yield from **21** as a light brown solid. This material was then converted to carbamate **24** by treatment with *n*-butylisocyanate in DMF containing EtN^iPr_2 and methyl ether **25** by methylation with MeI in DMF containing K_2CO_3. Overall, this route provided effective access to the single regioisomeric products **23**−**25**.

We then assessed the activity of **23**−**25** and observed comparable activity of **23** and **24** to compound **1** (Table 1; Figure 4 for hydrolysis of **24** to **23**) across three cell lines.

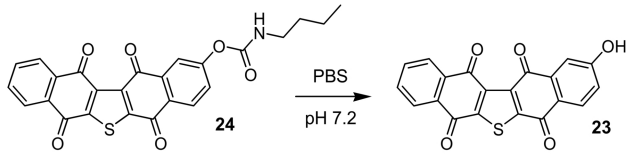


Figure 4. Hydrolytic release. Carbamate **24** hydrolyses to phenol **23** in aqueous media. Samples from DMSO stocks at 1.0 mg/mL (A, B) or 0.5 mg/mL (C, D) of **24** in PBS pH 7.2 undergo hydrolysis to purple salt **23** when diluted 20-fold and stored for 24 h at 23 °C.

While **25** was slightly less active to HCT-116 and SK-MEL-28, all three compounds, **23**, **24**, and **25**, shared similar enhanced activity in the melanoma cell line Malme-3M. The solubility displayed by **25** was comparable to that observed for **9a**/**9b**−**14a**/**14b**, while **24** was 5−10 times less soluble (Table 1). Phenol **23**, however, was insoluble in most media, similar to the parent seriniquinone (**1**), indicating that side chain derivatization was vital to compound solubility.

Next, we evaluated if **1**, **9a**/**9b**, and **23** displayed the DNA binding and redox activity typically ascribed to quinones.[22−24] Using the known DNA intercalator, doxorubicin, as a control (Figure 5), the fluorescence from **1** did not attenuate upon addition of ct-DNA, whereas doxorubicin demonstrated a potent quench in fluorescence upon binding. While **9a**/**9b** and **24** were less fluorescent than **1**, they also did not interact with DNA, as evident by the lack of attenuation (Figure 5).

Analogues **23**−**25** demonstrated remarkable stability in the solid state, with >99% purity retained at 23 °C for 3 months. In

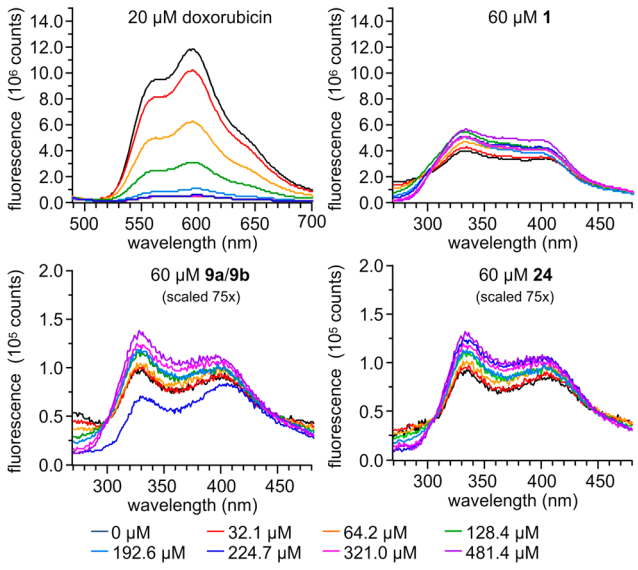


Figure 5. Fluorescence emission spectra upon titration of ct-DNA into 20 μM doxorubicin, 60 μM **1**, 60 μM **9a**/**9b**, or 60 μM **24**.

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PBS at pH 7.2, solutions of **23** and **25** remained at >98% purity for 1 month at 23 °C and 48 h (limit of testing) at 37 °C in cell culture media as determined by monitoring by NMR. On the other hand, carbamate **24** underwent hydrolysis in aqueous media, converting to a purple phenolate of **23**, suggesting its potential to provide a means to improve the pharmacological properties of **24** through pro-drug optimization and activation strategies.

Next, we explored the effects of **1**, **23**, and **24** on DCD expression to validate that **23** and **24** target the same pathway as **1**.[11] As shown in Figure 6, we observed a comparable

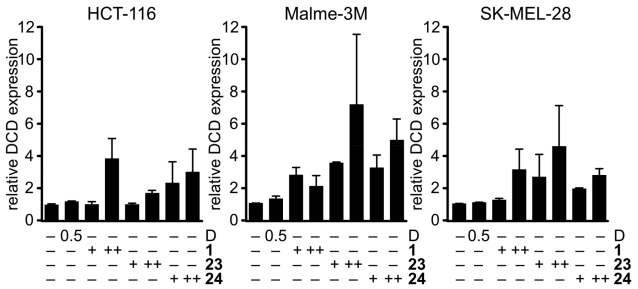

Figure 6. Relative DCD mRNA expression in HCT-116, Malme-3M, and SK-MEL-28 cells treated with **1**, **23**, and **24**. Concentrations were given in HCT-116 by 1 μM (+) and 10 μM (++), and in Malme-3M or SK-MEL-28 by 30 nM (+) and 100 nM (++). Doxorubicin (**D**; 0.5 μM) and DMSO (0.5%) were used as positive and negative controls, respectively. Total RNA was extracted and subjected to quantitative PCR analysis by a one-step RT-PCR system to detect the mRNA expression of DCD and RPLPO (large ribosomal protein).

increase in DCD expression in cells treated with **1** and phenol **23**. Across all three cell lines, phenol **23** and carbamate **24** demonstrated comparable or improved induction of DCD expression when compared to **1**. This increase in DCD expression (Figure 6) combined with activity data (Table 1) indicates that **23** and **24** share similar modes of action as **1**.[11]

Finally, to evaluate for "off-target" redox activity, we resorted to the CellROX assay[25,26] using HCT-116 cells. We did not detect oxidative stress elicited by these materials, as indicated by the lack of green fluorescent mitochondria due to the generation of reactive oxygen species (Figure 7, Figure S6). This, along with the lack of DNA binding (Figure 5), confirms that **9a**/**9b** and **24** do not act like quinones[24] but, rather, represent a unique DCD-targeting pharmacophore.

In summary, we identified the SAR within the seriniquinone core and further validated it as a unique pharmacophore. These studies provided single-isomeric analogues of **1** with improved

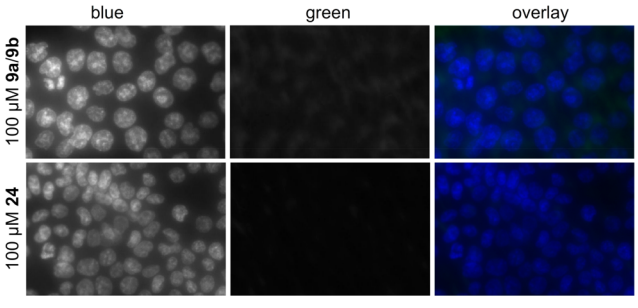

Figure 7. Confocal microscopic images of HCT-116 cells depicting the effects of **9a**/**9b** and **24** on oxidative stress from CellROX Green (green) and nuclear counterstaining from NucBlue (blue).

solubility and comparable activity. Analogues with improved solubility, while retaining the cytotoxicity and similar biological profiles, were synthesized. Finally, a new route was presented that provides a solution to the regioselectivity issue. Overall, these studies suggest that it is feasible to design melanoma-specific seriniquinone derivatives with druglike properties. While carbamate **24** suggests the possibility of pro-drug design, referring to it as a pro-drug would be premature without in depth *in vivo* studies. Efforts are now underway to complete pharmacokinetic/pharmacodynamic optimization and translate a lead molecule with viable *in vivo* activity against melanoma.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00391.

Experimental methods, spectroscopic data, and copies of select ^{1}H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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