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# Substituent Effects on Reactive Oxygen Species (ROS) Generation by Hydroquinones

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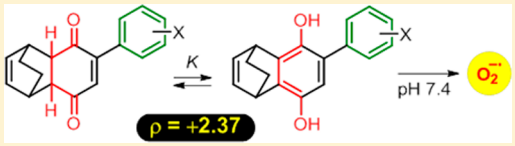
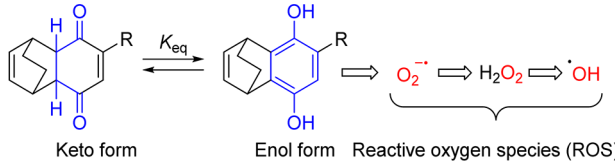
Supporting Information

**ABSTRACT:** In order to understand the structural aspects of stabilization of hydroquinones and their ability to generate reactive oxygen species (ROS), we designed and synthesized a series of 6-aryl-2,3-dihydro-1,4-benzoquinones. These compounds equilibrate with the corresponding 6-aryl-1,4-dihydroxybenzenes in an organic medium; a linear free energy relationship analysis gave $\rho$ = +2.37, suggesting that this equilibrium was sensitive to electronic effects. The propensity of the compound to enolize appears to determine ROS-generating capability, thus offering scope for tunable ROS generation.




During aerobic respiration, inadvertent 1e transfer to molecular $O_2$ produces the superoxide radical anion $O_2^{-\bullet}$.[1,2] $O_2^{-\bullet}$ is converted to hydrogen peroxide ($H_2O_2$), which in the presence of trace metal ions through Fenton chemistry can generate the highly reactive hydroxyl radical $^\bullet$OH.[1−3] Together, $O_2^{-\bullet}$, $H_2O_2$ and $^\bullet$OH are considered as reactive oxygen species (ROS) and are central to redox biology, immune response, an understanding of the pathology of numerous diseases, including cancer, and aging.[4−6] Recently, a number of studies provide evidence for the use of ROS as a therapeutic.[4] For example, cancers have been reported to have impaired capability to maintain redox homeostasis[4] and are sensitive to small molecules capable of generating ROS.[7,8] Drug resistance in bacteria can be overcome by enhancement of ROS,[9] suggesting possible applications for ROS generators as an adjuvant that can enhance the efficacy of existing drugs.[10−16] However, the precise roles of ROS are yet to be completely understood[17] and the rate of ROS generation plays an important role in the observed biological effects.[11] Although multiple strategies can be used for ROS generation with variable rates, a single scaffold with control over ROS generation rates is not available.[18−20] Such a tool would allow us to study the differences in biological effects of varying ROS generation. Furthermore, due to comparable physicochemical properties offered by a single scaffold, development of one with diverse ROS generation profiles would be useful. We considered 6-aryl-2,3-dihydro-1,4-benzoquinones as a possible candidate scaffold for tunable ROS generation (Scheme 1). A mechanism for ROS generation from these compounds involves enolization as the first step that produces an aromatic 1,4-diol, which reacts with oxygen to produce $O_2^{-\bullet}$.[12] Placing substituents on the position adjacent to the carbonyl functional group might affect the propensity of the keto form to enolize. Being able to systematically alter the position of this equilibrium might offer opportunities to tune ROS generation. In addition to ROS generation, hydroquinones find frequent use as a functional group capable of transferring electrons[21−23] and are components of numerous bioactive natural products.[24−26] Understanding substituent effects on stabilizing the enol would hence enable us to better characterize the reactivity of this important functional group.

**Scheme 1. Equilibration of 6-Aryl-2,3-dihydro-1,4-benzoquinones with Their Corresponding Diols, Which under Physiological Conditions React with Oxygen To Produce ROS**




In order to synthesize the 6-aryl-2,3-dihydrobenzoquinone scaffold, substituted *p*-benzoquinones **15**−**26** were first synthesized using a silver nitrate catalyzed arylation of 1,4-benzoquinone (**14**) with functionalized arylboronic acids (Table S1 (Supporting Information), entries 1−12).[27] Next, **14**−**26**, containing electron-withdrawing and electron-donating groups, were independently reacted with 1,3-cyclohexadiene to produce the corresponding Diels−Alder adducts in yields ranging from 67 to 95% (Table 1, entries 1−13). A $^1$H nuclear magnetic resonance ($^1$H NMR) spectroscopy study conducted on **2** showed that this compound exclusively existed in the keto form (**2a**), as evidenced by signals for the hydrogens of $\alpha$ and $\beta$ (bridgehead) to the C═O, which appeared in the range 3.0−3.4 ppm (Figure S1 (Supporting Information)). A similar result was recorded for the 4-methyl as well as 4-methoxyphenyl derivatives **3** and **4** (Figure S1), suggesting no major effect of introduction of an electron-donating group on the position of the equilibrium (Table 1). Next, the $^1$H NMR spectrum for the

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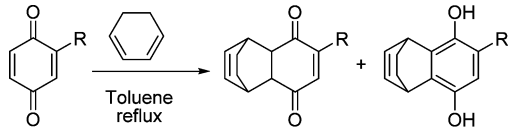
**Table 1. Synthesis of 1−13, Results of Keto−Enol Ratios Studied by $^1$H NMR, Percent Compound Remaining in Buffer Calculated from HPLC Studies, and $H_2O_2$ Generated from 1−13 in Buffer**




**14-26** **1a-13a** **1b-13b**

| entry | R | quinone | product | yield (%) | keto (%)[a] | enol (%)[a] | $K_{eq}$[b] | remaining (%)[c] | $H_2O_2$ ($\mu$M)[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | H | **14** | **1a** | 70 | 100 | n.d. | 0.0204[e] | 32 | 1.16 |
| 2 | Ph | **15** | **2a** | 73 | 100 | n.d. | 0.0204[e] | 74 | 1.66 |
| 3 | 4-MePh | **16** | **3a** | 83 | 100 | n.d. | 0.0204[e] | 60 | 0.75 |
| 4 | 4-MeOPh | **17** | **4a** | 74 | 100 | n.d. | 0.0204[e] | 86 | 1.07 |
| 5 | 4-BrPh | **18** | **5a + 5b** | 81 | 93 | 7[f] | 0.0753 | 25 | 0.85 |
| 6 | 4-ClPh | **19** | **6a + 6b** | 95 | 62[f] | 38 | 0.6129 | 52 | 3.67 |
| 7 | 4-FPh | **20** | **7a + 7b** | 66 | 89 | 11 | 0.1235 | 57 | 2.65 |
| 8 | 4-AcPh | **21** | **8a + 8b** | 84 | 74 | 26[f] | 0.3513 | 17 | 2.44 |
| 9 | 4-$NO_2$Ph | **22** | **9b** | 84 | n.d. | 100[f] | 49[e] | 9.6 | 1.59[g] |
| 10 | 4-CHOPh | **23** | **10b** | 81 | n.d. | 100[f] | 49[e] | 11.4 | 5.42 |
| 11 | 3-$NO_2$Ph | **24** | **11a + 11b** | 67 | 27 | 73 | 2.703 | 18 | 3.55 |
| 12 | 3-CHOPh | **25** | **12b** | 87 | n.d. | 100[f] | 49[e] | 40 | 3.55 |
| 13 | 2-MeOPh | **26** | **13a** | 70 | 100 | n.d. | 0.0204[e] | 70 | 0.45 |

[a]The percent keto and enol forms were estimated by $^1$H NMR spectroscopy in $CDCl_3$ unless otherwise indicated (spectra were recorded on a 400 MHz NMR spectrometer). n.d. = not detected. [b]$K_{eq}$ was estimated by the ratio of peaks correspnding to $\alpha$- and $\beta$-hydrogens to the C=O (for the keto form) and the bridgehead hydrogens (for the enol form). n.d. = not detected. [c]The compound (1 mM) was incubated in pH 7.4 phosphate buffer for 60 min at 37 °C under ambient aerobic conditions, and HPLC was used to determine percnet compound remaining. [d]The compound (10 $\mu$M) was incubated in pH 7.4 phosphate buffer under ambient aerobic conditions for 60 min, and $H_2O_2$ was assayed by an Amplex Red fluorescence assay. [e]Estimated by considering 98% of the major tautomer. [f]Experiment was conducted in DMSO-$d_6$. [g]HPLC analysis of this reaction mixture showed multiple unidentified products, suggesting possible side reactions or collateral consumption of $H_2O_2$ likely contributing to the diminished yield of $H_2O_2$.

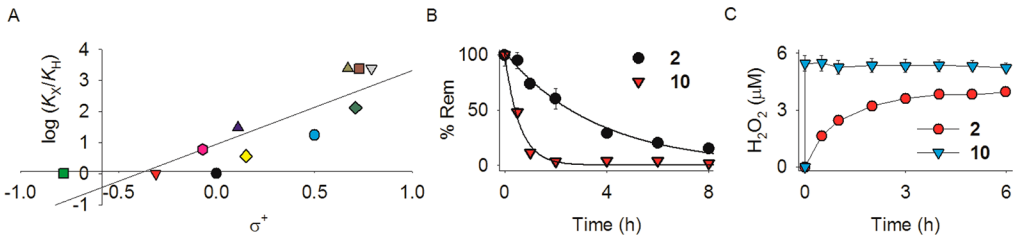



**Figure 1.** (a) Plot of log($K_X$/$K_H$) for keto−enol equilibria of 2,3-dihydrobenzoquinones versus the Hammett substitution constant $\sigma^+$ [28] (reaction constant $\rho$ = 2.37; $R^2$ = 0.7420). (b) Time course of decomposition of **2** (1 mM, $k_2$ = 0.28 $h^{-1}$, $R^2$ = 0.9875) and **10** (1 mM, $k_{10}$ = 1.48 $h^{-1}$, $R^2$ = 0.9546) in acetonitrile (pH 7.4 phosphate buffer (1/1, v/v)) based on HPLC analysis. (c) Time course of $H_2O_2$ generated from **2** (10 $\mu$M) and **10** (10 $\mu$M) over 6 h in pH 7.4 phosphate buffer measured using Amplex Red based fluorescence assay.

4-bromo derivative **5** was recorded and this compound was found to exist predominantly in its keto form **5a**; however, a set of peaks at 4.2−4.5 ppm for the bridgehead hydrogens of the enol form **5b** was also observed (Figure S1). The major tautomer was the keto form **5a**, estimated as 93% (Table 1, entry 5). A similar result was recorded for **6**−**8** (Figure S1), whose equilibria were found to be 62−89% in favor of the keto tautomer (Table 1, entries 6−8).

Among the compounds with electron-withdrawing groups, the enol form was dominant and **9**, **10**, and **12** were found to exist nearly exclusively in the enol forms **9b**, **10b**, and **12b** while the 3-nitroaryl derivative **11** was 73% enol in DMSO-$d_6$ (Table 1, entries 9−12). The aryl derivative **13** with a 2-methoxy substituent was found to behave similarly to the 4-methoxy derivative **4**, and only the keto form **13a** could be detected (Table 1, entry 13).

On the basis of the ratios of the keto and enol forms, the equilibrium constant for enolization $K_{eq}$ was calculated (Table 1). In order to understand substituent effects on the position of this equilibrium, using these $K_{eq}$ values, a Hammett plot was constructed. A moderately linear correlation ($R^2$ = 0.6751) was observed with $\sigma$, and an overall reaction constant $\rho$ of +3.6 was obtained (see the Supporting Information, Figure S2). The resonance contribution to the position of the equilibrium was estimated using a similar plot that was constructed with $\sigma^+$, and a $\rho$ value of +2.37 was obtained, but with better linearity ($R^2$ = 0.7420, Figure 1a).[28] Previous reports of such equilibria in related $\beta$-diketones such as benzoylacetones and benzoylcyclohexanones indicate that the keto form is stabilized by electron-releasing groups.[29−31] Although the magnitude of the reaction constant $\rho$ derived from $\sigma^+$ and equilibrium constants in the aforementioned studies was significantly lower (i.e. 0.6−0.9[29−31]) than the $\rho$ value that we find in this study, the sign was positive, supporting similar trends in substituent effects on the stability of keto and enol forms.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Next, the electronic effect on the keto form's propensity to enolize was studied by exposing the compound to base, which would promote enolization. Compound **2**, which predominantly exists in the keto form **2a**, was exposed to NaOD (0.2 equiv) in DMSO-$d_6$, and the NMR spectrum was recorded after 15 min (see the Supporting Information, Figure S3). The formation of signals that were characteristic of the bridgehead hydrogens of the enolate was observed (indicated by arrows in Figure S3) with concomitant disappearance of the $\alpha$-keto hydrogens. A similar experiment conducted with **4a** showed that, in the same time period, 62% of **4a** remained and complete enolization was observed in 1 h, suggesting that an electron-donating group on the aryl ring significantly lowered the rate of enolization (see the Supporting Information, Figure S4). A similar experiment conducted with **5** (93/7; **5a**/**5b**) and **8** (77/23; **8a**/**8b**) showed nearly complete enolization in 15 min (see the Supporting Information, Figures S5 and S6). These observations are consistent with electron-donating groups significantly decreasing the propensity of the hydroquinone to enolize.

Our data suggest that the enolization of 6-aryl-2,3-dihydrobenzoquinones is dependent on electronic effects and the ratio of keto and enol tautomers can be modulated by simple structural modifications on the aryl ring. Accordingly, compounds with dominant enol forms are expected to be more reactive in ambient aerobic buffer and vice versa. When **1a** was dissolved in pH 7.4 phosphate buffer/acetonitrile (1/1, v/v), we found nearly 32% of the compound remaining after 60 min (Table 1, entry 1). When a similar experiment was conducted with **2a**, 74% of the compound remained after 1 h (Table 1, entry 2). Curve fitting of percent **2a** remaining to a first-order reaction gave a rate constant of 0.28 $h^{-1}$ ($R^2$ = 0.9875) and a half-life ($t_{1/2}$) of 2.49 h (Figure 1b). Compounds **3a** and **4a** with electron-donating groups were found to have comparable decomposition profiles after 1 h (Table 1, entries 3 and 4). In the cases of compounds **5**−**8** and **11**, which existed as mixtures of keto and enol forms in organic media, the percent remaining in these cases was calculated on the basis of the keto form and ranged from 18 to 57%, all lower in comparison with **2a** (Table 1, entries 5−8 and 11). Compounds **9b** and **10b** were 90% decomposed in 1 h, while 40% of the formyl derivative **12b** remained in the same time period (Table 1, entries 9, 10, and 12). Under these conditions, the first-order rate constant $k$ = 1.48 $h^{-1}$ ($R^2$ = 0.9546) was recorded for decomposition of **10b** (Figure 1b). The half-life of this compound was estimated as 0.47 h, which is lower in comparison with **2a** (Figure 1b).

In ambient aerobic buffer, the hydroquinone is expected to rapidly react with oxygen to produce the corresponding 1,4-benzoquinone (see the Supporting Information, Table S3, compounds **27**−**29**). Its keto tautomer, on the other hand, would have to first tautomerize to generate hydroquinone enolate in buffer, and a sequential one-electron transfer from the enolate to molecular oxygen produces ROS such as $O_2^{-\bullet}$ and $H_2O_2$ (Scheme S2, Supporting Information).[12,13] We studied superoxide ($O_2^{-\bullet}$) generated from **1**−**13** using a reported chemiluminescence assay,[32] and results of this assay confirmed that **1**−**13** were capable of generating $O_2^{-\bullet}$ in buffer (see the Supporting Information, Figure S12). Addition of an electron to $O_2^{-\bullet}$ would lead to the formation of $H_2O_2$, which was assayed using a commercially available Amplex Red reagent.[33,14] All compounds tested were found to generate $H_2O_2$, and in a majority of the cases, we found that the presence of an electron-withdrawing substituent enhanced ROS generation (Table 1, entries 1−13). This result is consistent with enolization being the critical step in ROS production; once formed, the enolate reacts rapidly with $O_2$ to generate ROS (Scheme 1). Hence, the keto−enol ratio in organic media was a good indicator of the ability to generate ROS in buffer, suggesting that enolization is the key step to control ROS generation by these compounds.[37] Finally, the tunability offered by this scaffold is illustrated by time courses of $H_2O_2$ generated by **2a**, which is gradual, and **10b**, which rapidly dissociates to generate ROS (Figure 1c). Taken together, we provide evidence for predictably tuning keto−enol tautomerism in dihydrobenzoquinones by varying substituent electronics and the position of this equilibrium significantly affects ROS generation.

## EXPERIMENTAL SECTION

Compounds **1**,[34] **2**,[35] and **15**−**26**[27,36] have been previously reported, and analytical data that we recorded were consistent with the reported values.

**General Procedure for Synthesis of Compounds 1a−13a.** To a solution of 2-aryl-1,4-benzoquinone (1 mmol) in toluene (10 mL) was added freshly distilled 1,3-cyclohexadiene (2 mmol), and the mixture was refluxed. Upon complete consumption of the starting material (TLC analysis), the reaction mixture was evaporated to dryness under reduced pressure to obtain the crude product. The crude material was purified by silica gel column chromatography using an ethyl acetate (5 → 15%) and petroleum ether solvent system. This material was recrystallized in chloroform to obtain pure material.

**General Procedure for Synthesis of 27−29.** To a solution of 2,3-dihydro-1,4-benzoquinone (0.2 mmol) in tetrahydrofuran (THF, 5 mL) was added potassium carbonate (0.5 mmol, 2.5 equiv), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with 5 mL of deionized water and extracted with ethyl acetate (4 × 5 mL). The combined organic layer was dried (anhydrous $Na_2SO_4$, 5 g) and filtered, and the filtrate was evaporated under reduced pressure to obtain the crude product. The crude mixture was purified by a silica gel column by washing with ethyl acetate/petroleum ether (1/3 ratio, v/v). The organic solvent was evaporated under reduced pressure to obtain pure material.

**6-(4-Methylphenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (3a).** Starting from **16** (150 mg, 0.76 mmol), **3a** (174 mg, 66%) was isolated as a pale yellow solid: mp 116−118 °C; FT-IR ($\nu_{max}$, $cm^{-1}$) 2927, 2861, 1743, 1681, 1653, 1516, 1462, 1208, 1029; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.30 (d, $J$ = 8.2 Hz, 2H), 7.20 (d, $J$ = 8.0 Hz, 2H), 6.74 (s, 1H), 6.15−6.31 (m, 2H), 3.21−3.27 (m, 2H), 3.15 (dd, $J$ = 2.5, 9.3 Hz, 1H), 3.06 (dd, $J$ = 2.5, 9.3 Hz, 1H), 2.37 (s, 3H), 1.69−1.76 (m, 2H), 1.34−1. 69 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.6, 198.9, 151.7, 140.6, 137.7, 133.8, 133.4, 130.5, 129.3, 128.8, 50.7, 50.2, 35.6, 35.5, 24.8, 21.5; HRMS (ESI-TOF) for $[C_{19}H_{18}O_2 + Na]^+$ calcd 301.1204, found 301.1194. Anal. Calcd for $C_{19}H_{18}O_2$: C, 81.99; H, 6.52. Found: C, 81.71; H, 6.42.

**6-(4-Methoxyphenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (4a).** Starting from **17** (100 mg, 0.47 mmol), **4a** (101 mg, 74%) was isolated as an orange-yellow solid: mp 121−123 °C; FT-IR ($\nu_{max}$, $cm^{-1}$) 2928, 2859, 1743, 1678, 1652, 1515, 1462, 1258, 1023; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.39 (d, $J$ = 8.7 Hz, 2H), 6.90 (d, $J$ = 8.7 Hz, 2H), 6.72 (s, 1H), 6.18−6.33 (m, 2H), 3.82 (s, 3H), 3.23 (s, 2H), 3.13 (dd, $J$ = 2.1, 9.3 Hz, 1H), 3.05 (dd, $J$ = 2.1, 9.3 Hz, 1H), 1.72 (d, $J$ = 6.9 Hz, 2H), 1.38 (d, $J$ = 7.8 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.5, 199.2, 161.4, 151.0, 136.7, 133.8, 133.4, 130.5, 125.6, 114.1, 55.5, 50.8, 50.1, 35.5, 35.4, 24.9; HRMS (ESI-TOF) for $[C_{19}H_{18}O_3 + Na]^+$ calcd 317.1153, found 317.1149. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.64; H, 5.82.

**6-(4-Bromophenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (5a).** Starting from **18** (500 mg, 1.9 mmol), **5** (531 mg, 81%) was isolated as a pale yellow solid: mp 132−134 °C; FT-IR ($\nu_{max}$, $cm^{-1}$) 3531, 2920, 2873, 1741, 1651, 1460, 1339, 1201, 1073; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.50−7.54 (m, 2H), 7.24−7.27


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(m, 2H), 6.69−6.77 (m, 1H), 6.19−6.28 (m, 2H), 3.23 (dd, $J$ = 2.5, 5.0 Hz, 2H), 3.15 (d, $J$ = 2.5 Hz, 1H), 3.13 (d, $J$ = 2.5 Hz, 1H), 1.66−1.79 (m, 2H), 1.33−1.46 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.3, 198.4, 150.5, 138.4, 133.9, 133.4, 132.3, 132.1, 131.8, 130.9, 130.5, 124.8, 50.6, 50.2, 35.6, 35.5, 24.8; HRMS (ESI-TOF) for $[C_{18}H_{15}BrO_2 + Na]^+$ calcd 365.0152, found 365.0152. Anal. Calcd for $C_{18}H_{15}BrO_2$ calcd. C, 62.99; H, 4.41. Found, C, 63.21; H, 4.09. Note: NMR data presented here are for the major isomer.

**6-(4-Chlorophenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (6a).** Starting from **19** (300 mg, 1.4 mmol), **6** (391 mg, 95%) was isolated as a yellow solid: mp 149−151 °C; FT-IR ($\nu_{max}$, cm$^{-1}$) 3581, 3422, 3055, 2952, 2869, 1742, 1653, 1467, 1259, 1040; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.41−7.45 (m, 4H), 6.82 (s, 1H), 6.12−6.23 (m, 2H), 3.23 (dd, $J$ = 2.4, 9.5 Hz, 1H), 3.01−3.13 (m, 3H), 1.67 (d, $J$ = 8.2 Hz, 2H), 1.20−1.40 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 199.2, 198.4, 150.4, 138.4, 133.9, 133.4, 130.6, 130.2, 129.4, 128.7, 113.3, 63.8, 50.6, 50.2, 35.6, 35.5, 25.0, 24.8 (a mixture of keto (62%) and enol (38%) tautomers); HRMS (ESI-TOF) for $[C_{18}H_{15}ClO_2 + Na]^+$ calcd 321.0658, found 321.0656. Note: NMR data presented here are for the major isomer.

**6-(4-Fluorophenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (7a).** Starting from **20** (300 mg, 1.48 mmol), **7** (277 mg, 66%) was isolated as a yellow semisolid: FT-IR ($\nu_{max}$, cm$^{-1}$) 3567, 2961, 1742, 1695, 1652, 1513, 1462, 1220, 1157; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.36−7.41 (m, 2H), 7.03−7.12 (m, 2H), 6.72 (s, 1H), 6.24 (dd, $J$ = 3.4, 4.3 Hz, 2H), 3.22−3.27 (m, 2H), 3.14 (dd, $J$ = 2.5, 9.3 Hz, 1H), 3.06 (dd, $J$ = 2.5, 9.3 Hz, 1H), 1.67−1.78 (m, 2H), 1.32−1.43 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.3, 198.6, 165.2, 162.7, 150.4, 138.2, 133.9, 133.3, 131.0, 130.9, 115.8, 115.6, 113.5, 50.6, 50.2, 35.6, 35.5, 24.8; HRMS (ESI-TOF) for $[C_{18}H_{15}FO_2 + Na]^+$ calcd 305.0953, found 305.0957. Note: NMR data presented here are for the major isomer.

**6-(4-Acetylphenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (8a).** Starting from **21** (500 mg, 2.2 mmol), **8** (566 mg, 84%) was isolated as a yellow solid: mp 127−128 °C; FT-IR ($\nu_{max}$, cm$^{-1}$) 3563, 2980, 2862, 1742, 1659, 1519, 1461, 1354, 1170, 1044; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.95 (dd, $J$ = 2.1, 8.4 Hz, 2H), 7.46 (dd, $J$ = 1.8, 8.3 Hz, 2H), 6.76 (d, $J$ = 2.2 Hz, 1H), 6.25 (dd, $J$ = 2.5, 4.8 Hz, 2H), 3.04−3.30 (m, 4H), 2.59 (d, $J$ = 2.2 Hz, 3H), 1.73 (d, $J$ = 7.4 Hz, 2H), 1.38 (d, $J$ = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.3, 198.3, 197.6, 150.6, 144.4, 139.1, 134.0, 133.4, 129.2, 129.1, 128.4, 127.5, 50.6, 50.2, 35.7, 35.5, 26.8, 24.8; HRMS (ESI-TOF) for $[C_{20}H_{18}O_3 + Na]^+$ calcd 329.1153, found 329.1154. Anal. Calcd for $C_{20}H_{18}O_3$: C, 78.41; H, 5.92. Found: C, 78.09; H, 5.57. Note: NMR data presented here are for the major isomer.

**NMR Data of Enol Tautomer, 6-(4-Acetylphenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-diol (8b).** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.73 (s, 1H), 7.99 (s, 1H), 7.91 (d, $J$ = 8.3 Hz, 2H), 7.57 (d, $J$ = 7.8 Hz, 2H), 6.42−6.49 (m, 3H), 4.39 (s, 1H), 4.26 (s, 1H), 2.54 (s, 3H), 1.40 (d, $J$ = 8.1 Hz, 2H), 1.28 (d, $J$ = 9.6 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 198.05, 145.1, 145.0, 140.9, 135.8, 135.6, 135.0, 134.2, 132.0, 129.9, 128.4, 126.1, 113.7, 33.7, 33.3, 27.2, 25.4, 25.3; HRMS (ESI-TOF) for $[C_{20}H_{18}O_3 + H]^+$ calcd 307.1334, found 307.1330.

**6-(4-Nitrophenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-diol (9b).** Starting from **22** (100 mg, 0.41 mmol), **9b** (64 mg, 47%) was isolated as a pale yellow semisolid: FT-IR ($\nu_{max}$, cm$^{-1}$) 3517, 3452, 2936, 2869, 1741, 1653, 1517, 1460, 1196, 1021; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.79 (s, 1H), 8.17−8.20 (m, 3H), 7.69 (d, $J$ = 8.7 Hz, 2H), 6.49 (s, 1H), 6.44−6.46 (m, 2H), 4.40 (s, 1H), 4.26 (s, 1H), 1.40 (d, $J$ = 7.6 Hz, 2H), 1.27 (d, $J$ = 9.9 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 172.8, 166.1, 147.3, 146.1, 145.2, 140.9, 135.7, 135.6, 134.4, 132.9, 130.7, 125.0, 123.6, 113.6, 33.7, 33.4, 25.3, 25.1; HRMS (ESI-TOF) for $[C_{18}H_{15}NO_4 + H]^+$ calcd 310.1079, found 310.1078.

**6-(4-Formylphenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-diol (10b).** Starting from **23** (150 mg, 0.71 mmol), **10b** (168 mg, 81%) was isolated as a pale yellow solid: mp 193−195 °C; FT-IR ($\nu_{max}$, cm$^{-1}$) 3568, 2930, 2862, 1743, 1684, 1598, 1551, 1520, 1461, 1266, 1067, 1020; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.96 (s, 1H), 8.73 (s, 1H), 8.03 (s, 1H), 7.86 (d, $J$ = 8.2 Hz, 2H), 7.65 (d, $J$ = 8.1 Hz, 2H), 6.37−6.58 (m, 3H), 4.40 (s, 1H), 4.26 (s, 1H), 1.40 (d, $J$ = 7.9 Hz, 2H), 1.28 (d, $J$ = 8.9 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 193.2, 146.6, 145.1, 140.9, 135.7, 135.6, 134.5, 134.3, 132.3, 130.3, 129.7, 126.0, 113.7, 33.7, 33.3, 25.4, 25.3; HRMS (ESI-TOF) for $[C_{19}H_{16}O_3 + H]^+$ calcd 293.1177, found 293.1173.

**6-(3-Nitrophenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-diol (11b).** Starting from **24** (100 mg, 0.46 mmol), **11** (96 mg, 67%) was isolated as a yellow solid: mp 163−165 °C; FT-IR ($\nu_{max}$, cm$^{-1}$) 3615, 2928, 1741, 1688, 1517, 1462, 1339, 1272, 1154; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.78 (s, 1H), 8.27−8.32 (m, 1H), 8.17 (s, 1H), 8.07 (dd, $J$ = 1.8, 8.1 Hz, 1H), 7.82−7.88 (m, 1H), 7.62 (t, $J$ = 8.0 Hz, 1H), 6.46 (dd, $J$ = 9.1, 12.9 Hz, 3H), 4.39 (s, 1H), 4.26 (s, 1H), 1.40 (d, $J$ = 8.0 Hz, 2H), 1.22−1.31 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 199.2, 198.4, 148.8, 148.0, 145.2, 141.6, 140.8, 139.2, 136.1, 135.9, 135.7, 135.6, 135.4, 134.3, 134.1, 132.4, 130.4, 130.0, 124.9, 124.7, 124.3, 124.2, 121.4, 113.5, 50.4, 50.1, 34.8, 33.7, 33.3, 25.4, 25.3, 24.7, 24.6 (a mixture of keto/enol (27/73) tautomers); HRMS (ESI-TOF) for $[C_{18}H_{15}NO_4 + Na]^+$ calcd 332.0899, found 332.0898. Note: NMR data presented here are for the major isomer.

**6-(3-Formylphenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-diol (12b).** Starting from **25** (400 mg, 1.88 mmol), **12b** (477 mg, 87%) was isolated as a yellow solid: mp 158−160 °C; FT-IR ($\nu_{max}$, cm$^{-1}$) 3455, 3323, 2913, 2865, 1833, 1742, 1675, 1514, 1459, 1288, 1146, 1071; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.00 (s, 1H), 8.72 (s, 1H), 8.00 (s, 1H), 7.97 (s, 1H), 7.76 (dd, $J$ = 1.4, 7.6 Hz, 2H), 7.56 (t, $J$ = 7.6 Hz, 1H), 6.45−6.48 (m, 3H), 4.40 (s, 1H), 4.27 (s, 1H), 1.40 (d, $J$ = 7.8 Hz, 2H), 1.29 (d, $J$ = 9.4 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 193.9, 145.1, 141.0, 140.7, 136.5, 135.8, 135.7, 135.7, 134.2, 131.7, 130.8, 129.3, 127.8, 125.9, 113.7, 33.7, 33.3, 25.4, 25.3; HRMS (ESI-TOF) for $[C_{19}H_{16}O_3 + H]^+$ calcd 293.1177, found 293.1170. Anal. Calcd for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52. Found: C, 77.66; H, 5.31.

**6-(2-Methoxyphenyl)-1,4,4a,8a-tetrahydro-1,4-ethanonaphthalene-5,8-dione (13a).** Starting from **26** (200 mg, 0.93 mmol), **13a** (193 mg, 70%) was isolated as an orange-yellow solid: mp 143−145 °C; FT-IR ($\nu_{max}$, cm$^{-1}$) 2927, 2856, 1743, 1655, 1518, 1462, 1256, 1167, 1019; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.37 (ddd, $J$ = 1.8, 7.3, 8.3 Hz, 1H), 7.08 (dd, $J$ = 1.8, 7.4 Hz, 1H), 6.94−6.99 (m, 1H), 6.90 (d, $J$ = 8.3 Hz, 1H), 6.65 (s, 1H), 6.16−6.39 (m, 2H), 3.76 (s, 3H), 3.13−3.26 (m, 3H), 3.07 (dd, $J$ = 2.7, 9.2 Hz, 1H), 1.66−1.78 (m, 2H), 1.34−1. 46 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 198.3, 196.8, 155.8, 151.5, 138.0, 133.1, 131.8, 130.2, 128.8, 122.9, 119.9, 110.2, 54.5, 49.4, 49.1, 34.1, 33.8, 24.1, 23.4; HRMS (ESI-TOF) for $[C_{19}H_{18}O_3 + Na]^+$ calcd 317.1153, found 317.1149. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.19; H, 6.04.

**6-Phenyl-1,4-dihydro-1,4-ethanonaphthalene-5,8-dione (27).** Starting from **2a** (50 mg, 0.19 mmol), **27** was isolated as a yellow semisolid (41 mg, 83%): FT-IR ($\nu_{max}$, cm$^{-1}$) 2928, 2817, 1648, 1585, 1488, 1337, 1011; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.33−7.48 (m, 5H), 6.68 (s, 1H), 6.33−6.50 (m, 2H), 4.39 (dd, $J$ = 1.7, 18.5 Hz, 2H), 1.50 (dd, $J$ = 4.8, 6.0 Hz, 2H), 1.35−1.44 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 183.7, 182.8, 148.6, 148.5, 144.4, 134.0, 133.2, 132.8, 132.1, 131.7, 130.9, 124.5, 34.2, 33.8, 24.8, 24.7; HRMS (ESI-TOF) for $[C_{18}H_{14}O_2 + H]^+$ calcd 263.1072, found 263.1063.

**6-(4-Methoxyphenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-dione (28).** Starting from **4a** (50 mg, 0.17 mmol), **28** was isolated as an orange semisolid (19 mg, 38%): FT-IR ($\nu_{max}$, cm$^{-1}$) 2924, 1649, 1563, 1511, 1460, 1340, 1240, 1181, 1028; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.32−7.54 (m, 2H), 6.84−7.02 (m, 2H), 6.64 (s, 1H), 6.31−6.50 (m, 2H), 4.27−4.47 (m, 2H), 3.83 (s, 3H), 1.42−1.53 (m, 2H), 1.31−1.41 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 184.1, 183.6, 161.1, 148.5, 148.3, 144.8, 134.0, 133.9, 131.0, 130.6, 125.7, 114.0, 55.4, 34.2, 33.7, 24.8, 24.7; HRMS (ESI-TOF) for $[C_{19}H_{16}O_3 + H]^+$ calcd 293.1177, found 293.1174.

**6-(4-Acetylphenyl)-1,4-dihydro-1,4-ethanonaphthalene-5,8-dione (29).** Starting from **8a** (50 mg, 0.16 mmol), **29** (31 mg, 62%) was isolated as a yellow semisolid: FT-IR ($\nu_{max}$, cm$^{-1}$) 2929, 2872, 1682, 1650, 1602, 1460, 1359, 1268, 1142, 1041; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.91−7.99 (m, 2H), 7.51−7.56 (m, 2H), 6.72 (s, 1H), 6.40−6.44 (m, 2H), 4.35−4.43 (m, 2H), 2.61 (s, 3H), 1.49−1.54 (m,


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2H), 1.37−1.41 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 197.7, 183.6, 182.6, 148.6, 144.5, 137.8, 137.6, 134.0, 133.8, 132.9, 129.7, 128.5, 128.4, 128.3, 34.2, 33.8, 26.8, 24.8, 24.7; HRMS (ESI-TOF) for $[C_{20}H_{16}O_3 + H]^+$ calcd 305.1177, found 305.1172.

**$^1$H NMR Experiment for Studying Enolization Potential.** A stock solution of sodium deuteroxide (NaOD) in DMSO-$d_6$ was prepared by mixing 10 $\mu$L of 30 wt % NaOD in $D_2O$ with 490 $\mu$L of DMSO-$d_6$. The $^1$H NMR spectrum was recorded for **2** (10 mg) in DMSO-$d_6$ (0.6 mL) at 25 °C. To a solution of **2** in DMSO-$d_6$ (0.6 mL) was added 0.2 equiv of NaOD (10 $\mu$L from the aforementioned stock solution) at room temperature (25 °C), and after 15 min the $^1$H NMR spectrum was recorded on a 400 MHz NMR spectrometer. By following similar experimental conditions the $^1$H NMR experiments for **4**, **5**, **8**, and **10** were carried out.

**Stability Studies using HPLC.** A stock solution of compound (10 mM) was diluted to 1 mM in pH 7.4 phosphate buffer (100 mM)/acetonitrile ACN (1/1 ratio, v/v) and incubated at 37 °C over a period of 2−8 h. The reaction mixture was filtered (0.22 $\mu$m) and injected (25 $\mu$L) in a high-performance liquid chromatograph (HPLC) attached with a diode-array detector (the detection wavelength was 254 nm) and a Zorbax SB C-18 reversed-phase column (250 mm × 4.6 mm, 5 $\mu$m). A mobile phase of water/acetonitrile was used with a run time of 25 min: multistep gradient technology with a flow rate of 1 mL/min starting with 50/50 for 0−5 min, 40/60 for 5−10 min, 30/70 for 10−15 min, 20/80 for 15−20 min, 50.50 for 20−23 min, and 50/50 for 23−25 min.

**Superoxide Detection by Luminol Assay.**[32] 5-Amino-2,3-dihydro-1,4-phthalazinedione solution (Luminol, 4 mM) was prepared in 30 mM aqueous sodium hydroxide and stored under ice. To a microwell plate was added a stock solution of the compound (2 $\mu$L of 1 mM) to phosphate buffer (100 mM pH 8.0, 193 $\mu$L) followed by Luminol (5 $\mu$L, final 100 $\mu$M in 200 $\mu$L) in six repeats. The resulting mixture was incubated at 37 °C for 25 min, and chemiluminescence was measured using a microtiter plate reader.

**Estimation of Hydrogen Peroxide.**[33] A solution of the test compound (1 $\mu$L, 1 mM, 10 $\mu$M final concentration) was added to 25 mM pH 7.4 phosphate buffer (45 $\mu$L), and the mixture was incubated at 37 °C for 60 min. To the incubated reaction mixture was added 50 $\mu$L of a premixed solution of 10-acetyl-3,7- dihydroxyphenoxazine or Amplex Red (prepared by following the manufacturer's protocol from Invitrogen), and this mixture was incubated at room temperature for 25 min before measuring the fluorescence using a microtiter plate reader (excitation 550 nm; emission 590 nm).

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Tables and figures giving NMR spectra, HPLC traces, and yield data for **15**−**29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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