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# Redox Properties and Thiol Reactivity of Geldanamycin and its Analogues in Aqueous Solutions

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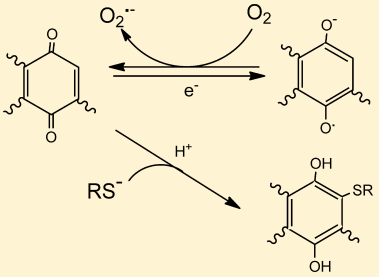
Supporting Information

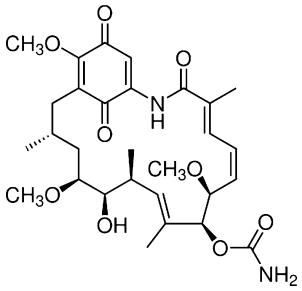
**ABSTRACT:** Geldanamycin (GM), a benzoquinone ansamycin antibiotic, is a natural product inhibitor of Hsp90 with potent and broad anticancer properties, but with unacceptable levels of hepatotoxicity. Less toxic C17-substituted analogues have been synthesized including 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) and the water-soluble 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG). Redox properties and thiol reactivity are central to the therapeutic and toxicologic effects of quinones, and the question arises as whether the extent of toxicity of GM, 17-AAG, and 17-DMAG is related to their redox potentials. Using pulse radiolysis, the one-electron redox potentials (vs NHE) at pH 7.0 of GM and 17-AAG have been determined to be −62 ± 7 mV and −273 ± 8 mV, respectively, whereas a value of −194 ± 6 mV has been previously published for 17-DMAG. The rate constants of the reaction of GM and its analogues with glutathione, cysteine, or dithiothreitol under anoxia at pH 7.4 followed the order GM > 17-DMAG > 17-AAG, which correlates with the order of the redox potential of the quinone/semiquinone couple. Thus, GM reacts much faster with thiols compared to the less toxic 17-DMAG and 17-AAG, and is also expected to be more readily reduced by reductases to the respective semiquinone radical, which either decomposes to yield the respective hydroquinone or reduces oxygen to superoxide. Because both redox cycling and thiol reactivity have been associated with quinone toxicity, it is concluded that the toxicity of benzoquinone ansamycins is directly related to the redox potential of the quinone/semiquinone couple.




## INTRODUCTION

Geldanamycin (GM, Scheme 1), a benzoquinone ansamycin antibiotic, is a natural product with potent and broad anticancer properties. GM interferes with the action of the heat shock protein 90 (Hsp90) leading to its degradation,[1,2] but it has also been suggested that its antitumor effect is mediated through the production of reactive oxygen species as is the case with other quinones.[3,4] The progression of GM to clinical trials was halted due to unacceptable levels of hepatotoxicity.[5] Less toxic structural analogues have been synthesized differing only in their 17-substituent including 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) and the water-soluble 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) (Scheme 1), which have entered clinical trials.[6−9]

**Scheme 1. Structures of GM (R = $CH_3O$), 17-AAG (R = $CH_2$=$CHCH_2NH$), and 17-DMAG (R = $(CH_3)_2NCH_2CH_2NH$)**




Cytotoxicity associated with exposure to quinones has generally been associated with one-electron redox cycling and interaction with cellular nucleophiles, such as protein and nonprotein sulfhydryls (RSH). Redox cycling of quinones may lead to oxidative stress due to superoxide formation (eq 1) while addition of $RS^-$ to quinone yields thioether−hydroquinone (Michael adduct) via reaction 2, which is also capable of redox cycling and might contribute to quinone-mediated carcinogenicity and neurotoxicity.[10−12] The role of Michael adduct formation in quinone toxicity is not well understood. It is difficult to separate the biological effects caused by Michael adduct formation from that of reactive oxygen generation since redox cycling is inherent to both quinones and thioether−hydroquinones, which form different quinones via autoxidation and cross oxidation.[10−12]

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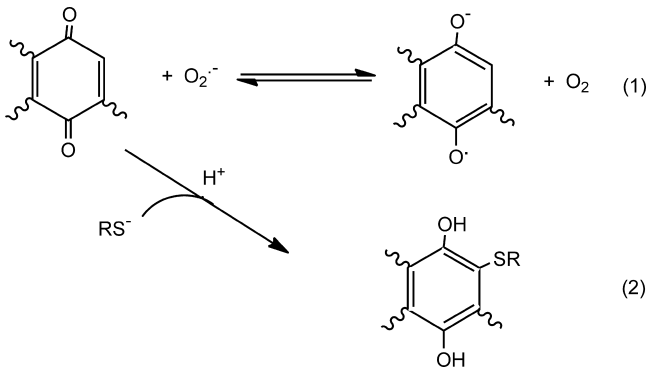


The toxicity of GM has been ascribed to its bioreduction to the respective semiquinone radical, which reduces oxygen to superoxide radical,[13−15] and also to its relatively fast reaction with GSH compared to 17-AAG and 17-DMAG.[16,17] *In vivo* toxicity studies demonstrated that GM was markedly more toxic than 17-AAG and 17-DMAG,[18−20] and the question arises as to whether the extent of the toxicity is related to the redox potentials of these benzoquinone ansamycins.

In contrast to aprotic organic solvents, direct determination of one-electron redox potentials of quinones in aqueous solutions using routine electrochemical techniques is almost impossible because of the instability of the semiquinone radicals and the limited solubility of quinones in water. Under these circumstances, the determination of the redox potential of quinones in aqueous solution requires much more complicated nondirect methods, mostly pulse radiolysis. Recently, the redox potential of the water-soluble 17-DMAG has been determined to be −194 ± 6 mV at pH 7.0 by pulse radiolysis.[21] In the present study, the redox potentials of the water insoluble GM and 17-AAG have been determined by pulse radiolysis, and the redox potentials of GM and its analogues have been correlated with their reactivity toward various thiols.

## MATERIALS AND METHODS

**Chemicals.** Water for solution preparation was purified using a Milli-Q system. All chemicals were of the highest available grade and were used as received. GM, 17-AAG, and 17-DMAG were purchased from LC Laboratories (Woburn, MA). Benzyl viologen ($BV^{2+}$), 1,4-naphthoquinone-2-sulfonate (NQS), glutathione (GSH), dithiothreitol (DTT), and L-cysteine (CysSH) were purchased from Sigma-Aldrich. Stock solutions of GM and its analogues were prepared in DMSO for studying their reactions with thiols. For pulse radiolysis study stock solutions of 17-AAG were prepared in 2-propanol since the latter was used as an $^{\bullet}OH$ scavenger forming the alcohol radical, which reduced the quinone moiety. The solubility of GM in alcohols is very low, and therefore, its stock solutions were prepared in DMF since its density and the rate constant of its reaction with $^{\bullet}OH$ are lower than those of DMSO.[22] The solubility of GM and 17-AAG in aqueous phosphate buffer (pH 7) containing 1−2 M 2-propanol was about 80 and 150 μM, respectively, which was sufficient for the pulse radiolysis experiments. Mass flow controllers (Tylan, Torrance, CA) were used for preparing gas mixtures of $N_2O$ and $O_2$. The concentration of oxygen-saturated solutions was taken as 1.2 mM at 22 °C and 690 mm Hg.

Kinetic measurements of thiol reactions with the drugs were carried out using an HP 8452A diode array spectrophotometer equipped with a thermostat (HP 89075C programmable multicell transport). All samples contained 50 μM DTPA to suppress possible catalysis by trace metal-ion contaminants. Oxygen was removed from the system by saturating all solutions with $N_2$ gas.

Pulse radiolysis experiments were carried out using a 5-MeV Varian 7715 linear accelerator (0.1−0.3 μs electron pulses, 200 mA current). A 200 W Xe lamp produced the analyzing light. A cutoff filter at 340 nm was used to minimize photochemistry. Measurements were done in 2 cm spectrosil cells with three light passes. The dose was determined using the thiocyanate dosimeter.[23] All experiments were carried out at room temperature.

## RESULTS

**Production of Radicals.** The delivery of an electron pulse to $N_2O$-saturated solutions ($[N_2O]$ = 24 mM) containing GM or 17-AAG (Q) and 1 − 2 M 2-propanol yields the respective $GM^{\cdot-}$ or $AAG^{\cdot-}(Q^{\cdot-})$ or their protonated forms through reactions 3 − 7.

$$H_2O \overset{\gamma}{\rightsquigarrow} e^-_{aq}\ (2.6),\ ^{\bullet}OH\ (2.7),\ H^{\bullet}\ (0.6),\ H_3O^+\ (2.6),\ H_2O_2\ (0.72) \quad (3)$$

In eq 3, the values in parentheses are *G*-values representing the yields of the species (in $10^{-7}$ M $Gy^{1-}$), which are lower by about 7% than those formed in the presence of high solute concentrations.

$$e^-_{aq} + N_2O + H_2O \rightarrow {}^{\bullet}OH + N_2 + OH^- \quad (4)$$

$$^{\bullet}OH/H^{\bullet} + (CH_3)_2CHOH \rightarrow (CH_3)_2C^{\bullet}OH + H_2O/H_2 \quad (5)$$

$$(CH_3)_2C^{\bullet}OH + Q \rightarrow Q^{\bullet-} + (CH_3)_2CO + H^+ \quad (6)$$

$$Q^{\bullet-} + H^+ \rightleftharpoons QH^{\bullet} \quad (7)$$

In some experiments $N_2O$ was replaced by 1 M acetone (reaction 8).

$$e^-_{aq} + (CH_3)_2CO + H^+ \rightarrow (CH_3)_2C^{\bullet}OH \quad (8)$$

The rate constant of reaction 6 was determined under limiting concentrations of $(CH_3)_2C^{\bullet}OH$ to be $(1.7 \pm 0.2) \times 10^9$ and $(2.2 \pm 0.1) \times 10^9$ $M^{-1}$ $s^{-1}$ for GM and 17-AAG, respectively (Figure 1S), similar to that previously reported for 17-DMAG.[21] The spectra of $GM^{\bullet-}$ and $AAG^{\bullet-}$ observed in the present study have maximum absorption around 450 nm while the absorption of the protonated radical at this region is significantly lower (Figure 1,

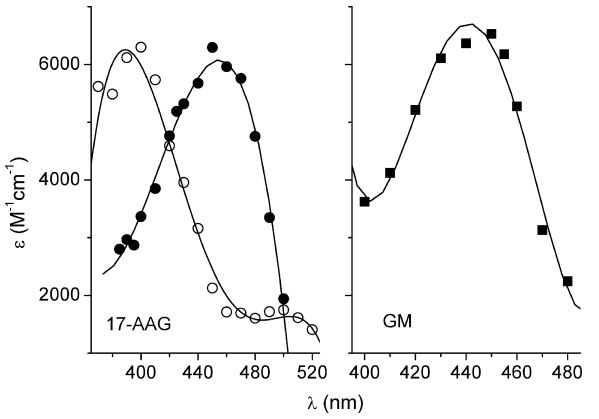


**Figure 1.** Absorption spectra of the semiquinone radicals. Solutions were saturated with $N_2O$ and contained 40 μM 17-AAG or GM and 1 M 2-propanol at pH 1.3 (○) or in 4 mM PB at pH 7.0 (●, ■). The spectra were corrected for the bleaching of GM and 17-AAG, which absorb at this region.


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Table 1), which is typical of benzosemiquinone radicals.[24,25] The absorption at 450 nm decreases upon decreasing the pH

**Table 1. Chemical Properties of the Semiquinone Radicals Derived from GM, 17-AAG, and 17-DMAG in Aqueous Solutions Containing 1−1.65 M 2-Propanol**

| | $\varepsilon_{450}(Q^{\bullet-})$, $M^{-1}$ $cm^{-1}$ | $pK_a(Q^{\bullet-})$ | $E_7(Q/Q^{\bullet-})$, mV |
|---|---|---|---|
| GM | 6600 | 4.0 ± 0.1 | −62 |
| 17-AAG | 6000 | 3.4 ± 0.1 | −273 |
| 17-DMAG[a] | 7200 | 3.2 ± 0.1 | −194 |

[a]Data from ref 21.

demonstrating $pK_a$ = 4.0 ± 0.1 and 3.4 ± 0.1 for GM and 17-AAG, respectively (Figure 2S).

**Redox Equilibria.** Upon the addition of a standard redox couple (S) at pH 7.0, reactions 9 and 10 may take place:

$$(CH_3)_2C^{\bullet}OH + S \rightarrow S^{\bullet-} + (CH_3)_2CO + H^+ \quad (9)$$

$$Q^{\bullet-} + S \rightleftharpoons S^{\bullet-} + Q \quad (10)$$

These reactions are followed by reduction of Q and/or S depending on the relative rate constants $k_6$ and $k_9$ and the relative concentrations of Q and S. If the rate of the decomposition of $Q^{\bullet-}$ (or $S^{\bullet-}$) is accelerated due to relaxation of reaction 10 (or −10) toward equilibrium while $[S]_o$ and $[Q]_o$ are in excess over $[(CH_3)_2C^{\bullet}OH]_0$, $K_{10}$ can be determined from the dependence of $k_{obs}$ (eq 11) or the residual absorption of $Q^{\bullet-}$ (or $S^{\bullet-}$) (eq 12) on $[S]_0$ and $[Q]_0$, and $E_7(Q/Q^{\bullet-})$ is calculated according to eq 13.

$$k_{obs} = k_{10}[S]_0 + k_{-10}[Q]_0 \quad (11)$$

$$K_{10} = \frac{[Q]_0[S^{\bullet-}]_{eq}}{[S]_0[Q^{\bullet-}]_{eq}} = \frac{[Q]_0}{[S]_0} \times \frac{[Q^{\bullet-}]_0 - [Q^{\bullet-}]_{eq}}{[Q^{\bullet-}]_{eq}} = \frac{[Q]_0}{[S]_0} \times \frac{[S^{\bullet-}]_{eq}}{[S]_0 - [S^{\bullet-}]_{eq}} \quad (12)$$

$$E_7(S/S^{\bullet-}) - E_7(Q/Q^{\bullet-}) = 0.06 \log K_{10} \quad (13)$$

**$O_2/O_2^{\bullet-}$ as a Standard Redox Couple.** $E(O_2/O_2^{\bullet-})$ was initially determined to be −160 mV (vs NHE, 1.0 M $O_2$),[26,27] but a careful re-examination of this redox couple indicates that a more accurate value is −180 mV.[28,29] $(CH_3)_2C^{\bullet}OH$ reaction with $O_2$ yields the respective peroxyl radical (reaction 14, from ref 22), which decomposes to yield $O_2^{\bullet-}$ in a process catalyzed by $HPO_4^{2-}$ (reactions 15, from ref 22).

$$(CH_3)_2C^{\bullet}OH + O_2 \rightarrow (CH_3)_2C(OH)OO^{\bullet} \quad k_{14} = 4 \times 10^9\ M^{-1}\ s^{-1} \quad (14)$$

$$(CH_3)_2C(OH)OO^{\bullet} + HPO_4^{2-} \rightarrow O_2^{\bullet-} + (CH_3)_2C{=}O + H_2PO_4^- \quad k_{15} = 1.1 \times 10^7\ M^{-1}\ s^{-1} \quad (15)$$

Pulse-irradiation of deaerated solutions containing GM or 17-AAG, 1−1.65 M 2-propanol, 1 M acetone, and 12 mM phosphate buffer (PB) at pH 7.0 exhibited different kinetic traces upon the addition of oxygen. The initial absorption of $AAG^{\bullet-}$ decreased upon increasing $[O_2]_0$ due to a competition between 17-AAG and $O_2$ for $(CH_3)_2C^{\bullet}OH$, and $AAG^{\bullet-}$ decayed via a first-order reaction (Figure 2, traces A and B), which was accelerated upon

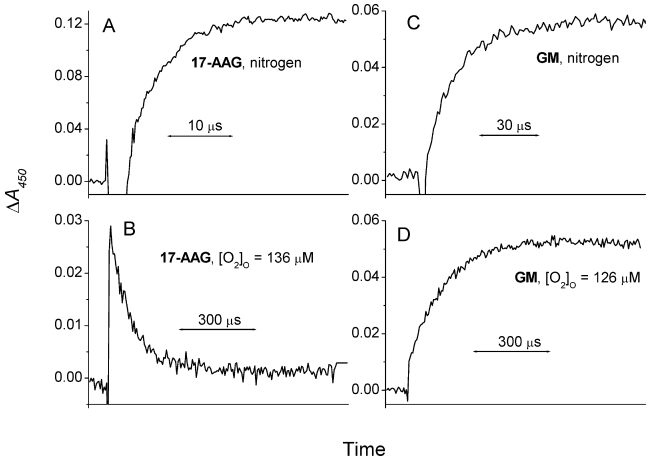


**Figure 2.** Effect of added oxygen on the formation and decay of $Q^{\bullet-}$. Kinetic traces A and B obtained upon pulse-irradiation (4.45 Gy/pulse) of solutions containing 100 μM 17-AAG in the absence and presence of 136 μM $O_2$. Kinetic traces C and D obtained upon pulse-irradiation (2.84 Gy/pulse) of solutions containing 40 μM GM in the absence and presence of 136 μM $O_2$. All solutions contained 1 or 1.65 M 2-propanol, 1 M acetone, and 12 mM PB (pH 7.0). The optical path length was 6.2 cm.

increasing $[O_2]_0$ or $[17\text{-AAG}]_0$. In contrast, GM was reduced by $O_2^{\bullet-}$ under the conditions where $(CH_3)_2C^{\bullet}OH$ reacts mainly with $O_2$ (Figure 2, traces C and D). These results demonstrate that eq 1 is shifted to the left in the case of 17-AAG and to the right in the case of GM, i.e., $E_7(\text{17-AAG/AAG}^{\bullet-}) < E(O_2/O_2^{\bullet-}) < E_7(\text{GM/GM}^{\bullet-})$.

In aerated solutions containing 30−80 μM GM, 1 M 2-propanol, and 1 M acetone, the yield of $GM^{\bullet-}$ was independent of $[GM]_0$ whereas $k_{obs}$ was linearly dependent on $[GM]_0$ resulting in a slope equal to $k_1 = (2.0 \pm 0.1) \times 10^8\ M^{-1}\ s^{-1}$ and an intercept close to zero (Figure 3S), i.e., $k_1 \gg k_{-1}$. In the presence of 30 μM GM and upon further increasing $[O_2]_0$ up to saturation, the yield of $GM^{\bullet-}$ decreased and $k_{obs}$ increased. Under our experimental conditions the half-life of $O_2^{\bullet-}$ formation via reaction 15 is about 10 μs. Therefore, the determination of $K_{-1}$ from the effect of $[O_2]$ on the residual yield of $Q^{\bullet-}$ is more accurate than its effect on the rate of the relaxation of reaction −1 toward equilibrium. Figure 3 demonstrates the linear relationship between $1/[GM^{\bullet-}]_{eq}$ and $[O_2]_0/[GM]_0$ yielding a slope/intercept equal to $K_{-1} = (9.6 \pm 1.5) \times 10^{-3}$. Hence, $E_7(\text{GM/GM}^{\bullet-}) = -59 \pm 4$ mV and $k_{-1} = (1.9 \pm 0.1) \times 10^6\ M^{-1}\ s^{-1}$.

In solutions containing 100 μM 17-AAG and 32−136 μM $O_2$, the decay of $AAG^{\bullet-}$ obeyed first-order kinetics (Figure 2B), and $k_{obs}$ was linearly dependent on $[O_2]_0$ (Figure 4). The slope and the intercept of the line in Figure 4 equal $k_{-1} = (8.6 \pm 0.7) \times 10^7\ M^{-1}\ s^{-1}$ and $k_1 = (1.0 \pm 0.6) \times 10^7\ M^{-1}\ s^{-1}$, respectively, where the latter value is inaccurate as is this kinetic approach for the determination of $K_{-1}$. The residual absorption of $AAG^{\bullet-}$ in the presence of 32 μM $O_2$ was 10.4% (close to zero at higher concentrations of $O_2$, e.g., Figure 2B) corresponding to $K_{-1} = 27$ and $E_7(\text{17-AAG/AAG}^{\bullet-}) = -266$ mV. These results require the use of other standard redox couples for accurate determination of $E_7(\text{17-AAG/AAG}^{\bullet-})$ and verification of $E_7(\text{GM/GM}^{\bullet-})$.


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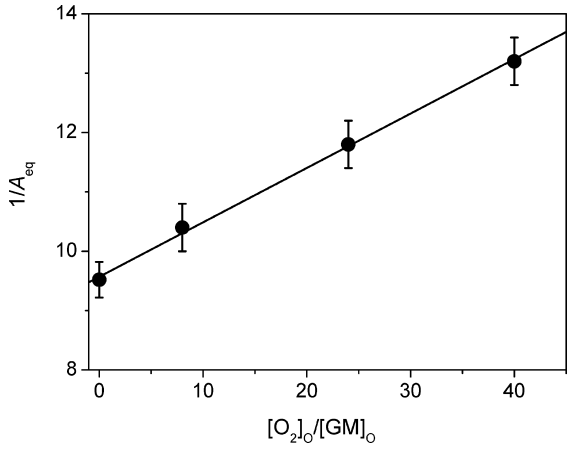


**Figure 3.** Effect of $[O_2]_0$ on the residual yield of $GM^{\bullet-}$. Solutions contained 30 $\mu$M GM, 1 M 2-propanol, 1 M acetone, 12 mM PB (pH 7.0), and 0, 0.24, 0.72, and 1.2 mM $O_2$. The absorption was monitored at 450 nm. The dose was 4.68 Gy/pulse and the optical path length 6.2 cm.

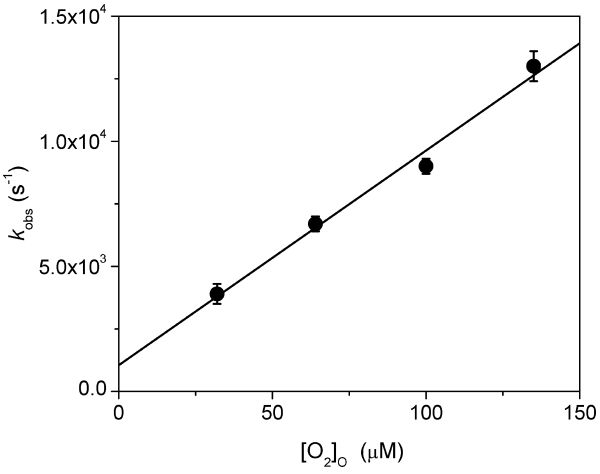


**Figure 4.** Effect of $[O_2]_0$ on the first-order decay of $AAG^{\bullet-}$. Solutions containing 100 $\mu$M 17-AAG, 1.5 M 2-propanol, and 1 M acetone in 12 mM PB (pH 7.0) were saturated with different mixtures of $N_2O$ and $O_2$, and the absorption was monitored at 450 nm. The dose was 4.45 Gy/pulse and the optical path length 6.2 cm.

**$BV^{2+}/BV^{\bullet+}$ as a Standard Redox Couple.** $E_7(BV^{2+}/BV^{\bullet+}) = -374$ mV, and $BV^{\bullet+}$ absorbs highly at 600 nm where the absorption of $Q^{\bullet-}$ is negligible.[28]

$$Q^{\bullet-} + BV^{2+} \rightleftharpoons BV^{\bullet+} + Q \quad (16)$$

As expected, GM was reduced by $BV^{\bullet+}$ in $N_2O$-saturated solutions containing 1 mM $BV^{2+}$, 20–80 $\mu$M GM, 1 M 2-propanol, and 4 mM PB (pH 7.0). The decay of $BV^{\bullet+}$ to zero obeyed first-order kinetics, and $k_{obs}$ was linearly dependent on $[GM]_0$ yielding a slope equal to $k_{-16} = (1.7 \pm 0.1) \times 10^9\ M^{-1}\ s^{-1}$ and an intercept close to zero, i.e., $k_{-16} \gg k_{16}$.

In contrast to GM, $[BV^{\bullet+}]$ did not decay to zero in the presence of 17-AAG. Typical kinetic traces are given in Figure 4S demonstrating the decrease in $[BV^{\bullet+}]_0$ due to the competition of 17-AAG with $BV^{2+}$ for $(CH_3)_2C^{\bullet}OH$ and the relaxation of reaction −16 toward equilibrium. Figure 5 demonstrates the linear relationship between $[BV^{2+}]_0/[BV^{\bullet+}]_{eq} - 1$ and $[17\text{-AAG}]_0/[BV^{2+}]_0$ yielding a slope equal to $K_{16} = 48 \pm 2$, and hence $E_7(17\text{-AAG}/AAG^{\bullet-})$ = −273 mV. The linear dependence of $k_{obs}/[BV^{2+}]_0$ on $[17\text{-AAG}]_0/[BV^{2+}]_0$ is given in Figure 5S,

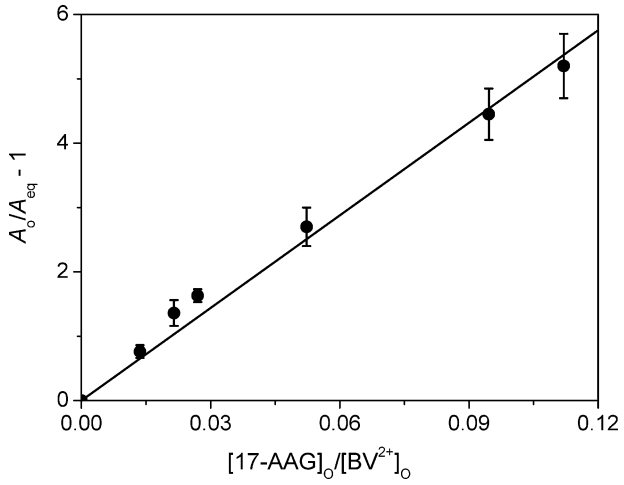


**Figure 5.** Effect of $[17\text{-AAG}]_0$ and $[BV^{2+}]_0$ on the residual absorption of $BV^{\bullet+}$. $N_2O$-saturated solutions containing 0.5–2 mM $BV^{2+}$, 15–90 $\mu$M 17-AAG, and 1–1.5 M 2-propanol in 4 mM PB (pH 7.0) were pulse-irradiated (2.4 Gy/pulse), and the absorption was monitored at 600 nm. The optical path length was 6.2 cm.

where the slope equals $k_{-16} = (9.0 \pm 0.6) \times 10^8\ M^{-1}\ s^{-1}$ and the intercept equals $k_{16} = (9.1 \pm 5.0) \times 10^6\ M^{-1}\ s^{-1}$, which is inaccurate. Using $K_{16} = 48 \pm 2$ derived from Figure 5 and $k_{-16} = (9.0 \pm 0.6) \times 10^8\ M^{-1}\ s^{-1}$ derived from Figure 5S, one calculates $k_{16} = (1.9 \pm 0.2) \times 10^7\ M^{-1}\ s^{-1}$, which is within the error limit of the value derived from the intercept of Figure 5S.

**NQS/$NQS^{\bullet-}$ as a Standard Redox Couple.** $E_7$(NQS/$NQS^{\bullet-}$) has been previously determined to be −60 mV against $O_2$ using $E(O_2/O_2^{\bullet-}) = -160$ mV,[30] and is now corrected to −80 mV for $E(O_2/O_2^{\bullet-}) = -180$ mV. The absorption changes were monitored at 390 nm where the absorption of $NQS^{\bullet-}$ ($\varepsilon_{390}$ ~12 000 $M^{-1}\ cm^{-1}$)[24,26,31] is significantly higher than that of $GM^{\bullet-}$ (Figure 1). Under the conditions where both $NQS^{\bullet-}$ and $GM^{\bullet-}$ are produced by the radiation, the decay of the absorption at 390 nm obeyed first-order kinetics and $k_{obs}$ increased upon increasing $[GM]_0$ or $[NSQ]_0$, which is attributed to relaxation of reaction −17 toward equilibrium.

$$GM^{\bullet-} + NQS \rightleftharpoons NQS^{\bullet-} + GM \quad (17)$$

Figure 6 shows the linear dependence of $k_{obs}/[NQS]_0$ on $[GM]_0/[NQS]_0$ resulting in a slope equal to $k_{17} = (1.5 \pm 0.3) \times 10^7\ M^{-1}\ s^{-1}$ and an intercept equal to $k_{-17} = (2.8 \pm 0.3) \times 10^7\ M^{-1}\ s^{-1}$. Hence, $K_{17} = 1.87 \pm 0.71$ and $E_7(GM/GM^{\bullet-}) = -64 \pm 9$ mV, which is in good agreement with the value determined against $O_2$.

**Thiol Reactions with GM and Its Analogues.** The rate constants of the reactions of GM, 17-DMAG, and 17-AAG with the tested thiols have been determined at pH 7.4 and 37 °C under anaerobic conditions to avoid complications due to autoxidation of the hydroquinones formed in this system.[10,32] Significant changes in the UV absorption spectrum of the quinones were observed upon addition of an excess of GSH, CysSH, or DTT. A typical result is shown in Figure 7 for the reaction of 40 $\mu$M GM with 0.5 mM CysSH. The decay of the absorption obeyed pseudo-first-order kinetics, and $k_{obs}$ was linearly dependent on $[RSH]_0$ (Table 2). The apparent rate constants derived from the slopes of the lines of $k_{obs}$ versus $[RSH]_0$ at pH 7.4, and the $k_2$-values calculated using $pK_a = 8.18$ and 8.75 for CysSH and GSH, respectively,[33] and 9.2 for the first dissociation of DTT,[34] are summarized in Table 2.


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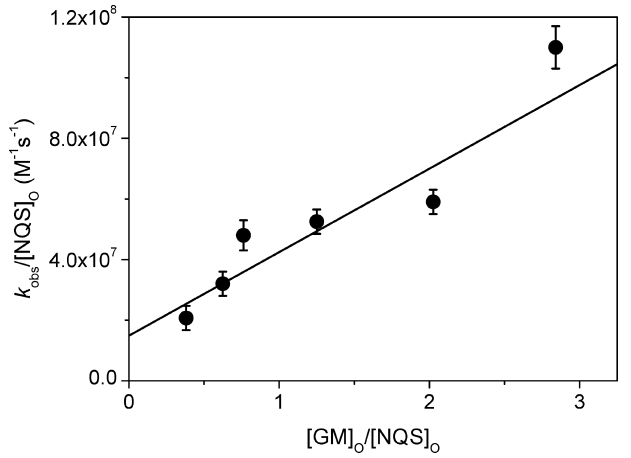


**Figure 6.** Dependence of the rate of approach to equilibrium on $[GM]_0$ and $[NQS]_0$. $N_2O$-saturated solutions containing 1 M 2-propanol in 4 mM PB (pH 7.0) were pulse-irradiated (4.84 Gy/pulse), and the absorption was monitored at 390 nm.

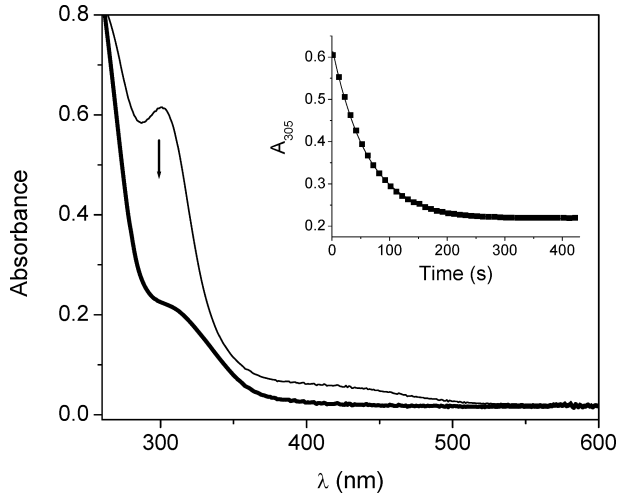


**Figure 7.** Reaction of GM with CysSH. The solid line corresponds to absorption spectrum of 40 $\mu$M GM and 50 $\mu$M DTPA in 20 mM PB (pH 7.4), and the bold line is the spectrum monitored 400 s after the addition of 0.5 mM CysSH, when spectral changes were complete. The inset contains the kinetic trace at 305 nm, and the solid line is the fit of the experimental data to a first-order reaction, $k_{obs} = 0.016\ s^{-1}$.

## DISCUSSION

The one-electron redox potentials of GM, 17-DMAG, and 17-AAG have been determined to be $-62 \pm 7$, $-194 \pm 6$, and $-273 \pm 8$ mV, respectively, by pulse radiolysis using several known redox couples in aqueous buffer at pH 7.0. The redox potentials of quinones are affected by the substituents, dissociation constants, and solvents.[32] Substitution into the ring by electron-donating groups reduces the one-electron redox potential of the quinone and raises the p$K_a$ of the semiquinone radical, whereas the opposite is expected for electron-withdrawing groups.[32] In the case of p-benzoquinone ($E_7$(BQ/BQ$^{\bullet-}$) = 0.09 V),[35] the redox potential of substituted p-benzoquinone can be predicted according to eq 18,[36] where $\sigma_p$ is the Hammett *para* substituent constant, which reflects the substituent as an electron-donating or -withdrawing group.[37]

$$E_7(\mathrm{Q/Q^{\bullet-}})(\mathrm{V\ vs\ NHE}) = E_7(\mathrm{BQ/BQ^{\bullet-}}) + 0.61 \sum \sigma_p \quad (18)$$

Accordingly, the different redox potentials of GM, 17-AAG, and 17-DMAG are due to the difference between $\sigma_p(OCH_3)$ = −0.27,[37] $\sigma_p(NHCH_2CH{=}CH_2)$, and $\sigma_p(NH(CH_2)_2N(CH_3)_2)$, respectively (Scheme 1). The value of $\sigma_p(NHCH_2CH{=}CH_2)$ has not been determined, but it is assumed to be −0.61 on the basis of $\sigma_p(NHCH_2CH_3)$ = −0.61.[37] Thus, $E_7$(GM/GM$^{\bullet-}$) − $E_7$(17-AAG/AAG$^{\bullet-}$) ≈ 0.21 V, which is in good agreement with the experimental values. The redox potential of 17-DMAG is higher than that of 17-AAG due to protonation of the terminal dimethylamine function at pH 7. This could raise the $\sigma_p$(NH-$(CH_2)_2N^+H(CH_3)_2$) above −0.61 in spite of the two carbon "insulation" between the strongly electron-withdrawing protonated terminal amine moiety and the electron-donating ring amino substituent.

The redox potential of other analogues of GM can be calculated using eq 18 and $E_7$(GM/GM$^{\bullet-}$) = −0.062 V. For example, 17-AAG is metabolized to 17-aminogeldanamycin (17-AG), which retains Hsp90 inhibitory activity.[38] Thus, $E_7$(GM/GM$^{\bullet-}$) − $E_7$(17-AG/AG$^{\bullet-}$) = 0.61($\sigma_p(OCH_3) - \sigma_p(NH_2)$), and using $\sigma_p(NH_2)$ = −0.66,[37] one calculates $E_7$(17-AG/AG$^{\bullet-}$) = −0.30 V.

According to the correlation reported between the p$K_a$ values of semiquinone radicals and the one-electron redox potentials of the respective quinones, the p$K_a$ values of DMAG$^{\bullet-}$ and AAG$^{\bullet-}$ should have been higher than that of GM$^{\bullet-}$ and around 5.[24,25] The relatively low p$K_a$ values of 3.2 for DMAG$^{\bullet-}$ and 3.4 for AAG$^{\bullet-}$ might be attributed to stabilization of the radical by an intramolecular hydrogen-bonding of the amino-linkage.

A good correlation has also been observed between the one-electron redox potential of many quinones in aqueous buffer and in aprotic organic solvents excluding those with hydroxyl-substituents.[39,40] The order of the redox potential of GM and its analogues in aqueous buffer at pH 7.0 (GM > 17-DMAG > 17-AAG) is different than that obtained previously in DMSO (17-DMAG > 17-AAG > GM),[15] which can be also attributed to internal hydrogen-bonding as is the case with hydroxyl-substituents.[39−41]

The addition of sulfur nucleophiles to quinones yields thioether−hydroquinone adducts as primary molecular products (reaction 2). The subsequent chemistry of these products involves autoxidation, cross-oxidation, disproportionation, and free radical interactions,[32] and is beyond the scope of this study. The rate constant for the reductive addition of RS$^-$ to quinone depends on the nucleophilicity of the thiol and on the electrophilicity of the quinone ring, which increases with the increase in the redox potential of the quinone. Therefore, it is expected that a quinone with a more positive redox potential will react faster with a thiol as previously demonstrated for GSH reaction with p-benzoqinone and methylated p-benzoquinones.[42] The present results (Table 2) demonstrate that the rates of the reaction with the tested thiols follow the order GM > 17-DMAG > 17-AAG and are in agreement with the order of $E_7$(Q/Q$^{\bullet-}$) of these drugs. The same order has been previously reported for the reaction in aerated solutions and presence of 5 mM[16,43] and 10 mM GSH,[16,43] suggesting that *order* of the reactivity of ansamycins toward GSH is unaffected by oxygen. A linear correlation is found between log($k_2$) and $E_7$(Q/Q$^{\bullet-}$) for all tested thiols demonstrating that only the absolute rates vary with the thiol (Figure 8). The $k_2$-value for the reaction of GSH with GM is about 4 orders of magnitude lower than that derived from the linear correlation between log($k_2$) and $E_7$(Q/Q$^{\bullet-}$) of p-benzoqinone and methylated p-benzoquinones[42] suggesting that the rate of reaction 2 is also influenced by steric factors.


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**Table 2. Rate Constants for the Reactions of Q with DTT, CysSH, and GSH (pH 7.4) and with their Deprotonated Forms under Anoxic Conditions and 37 °C**

| | [DTT], mM | $k_{obs}$, $s^{-1}$ | [CysSH], mM | $k_{obs}$, $s^{-1}$ | [GSH], mM | $k_{obs}$, $s^{-1}$ |
|---|---|---|---|---|---|---|
| 17-AAG | 2 | $2.4 \times 10^{-4}$ | 2.5 | $6.5 \times 10^{-5}$ | 12.3 | $\approx 3.5 \times 10^{-5}$ |
| | 4 | $3.9 \times 10^{-4}$ | 5 | $1.4 \times 10^{-4}$ | | |
| | 8 | $7.8 \times 10^{-4}$ | 10 | $2.75 \times 10^{-4}$ | | |
| $k_{app}$, $M^{-1}$ $s^{-1}$ | $0.098 \pm 0.005$ $M^{-1}$ $s^{-1}$ | | $0.0275 \pm 0.0013$ $M^{-1}$ $s^{-1}$ | | $\approx 2.8 \times 10^{-3}$ $M^{-1}$ $s^{-1}$ | |
| $k_2$ | $6.3 \pm 0.3$ $M^{-1}$ $s^{-1}$ | | $0.19 \pm 0.01$ $M^{-1}$ $s^{-1}$ | | $\approx 0.065$ $M^{-1}$ $s^{-1}$ | |
| 17-DMAG | 0.5 | $5.6 \times 10^{-4}$ | 1.83 | $4.4 \times 10^{-4}$ | 3.06 | $1.14 \times 10^{-4}$ |
| | 1 | $1.2 \times 10^{-3}$ | 3.67 | $7.9 \times 10^{-4}$ | 6.12 | $2.24 \times 10^{-4}$ |
| | 2 | $2.3 \times 10^{-3}$ | 7.32 | $1.47 \times 10^{-3}$ | 12.26 | $4.3 \times 10^{-4}$ |
| $k_{app}$ (pH 7.4) | $1.16 \pm 0.06$ | | $0.205 \pm 0.009$ | | $0.036 \pm 0.002$ | |
| $k_2$, $M^{-1}$ $s^{-1}$ | $74.4 \pm 3.7$ | | $1.44 \pm 0.07$ | | $0.84 \pm 0.04$ | |
| GM | 0.5 | 0.021 | 0.5 | 0.016 | 4 | 0.0093 |
| | 1 | 0.04 | 1 | 0.031 | 6 | 0.0135 |
| | 2 | 0.084 | 2 | 0.051 | 8 | 0.0179 |
| | 4 | 0.165 | | | 12 | 0.026 |
| $k_{app}$, $M^{-1}$ $s^{-1}$ | $41.3 \pm 2.0$ | | $26.9 \pm 1.4$ | | $2.2 \pm 0.1$ | |
| $k_2$,[a] $M^{-1}$ $s^{-1}$ | $(2.65 \pm 0.13) \times 10^3$ | | $189 \pm 10$ | | $51.5 \pm 2.3$ | |

[a]Calculated using $k_{app}$ and p$K_a$ = 9.2, 8.18, and 8.75 for DTT, CysSH, and GSH, respectively.

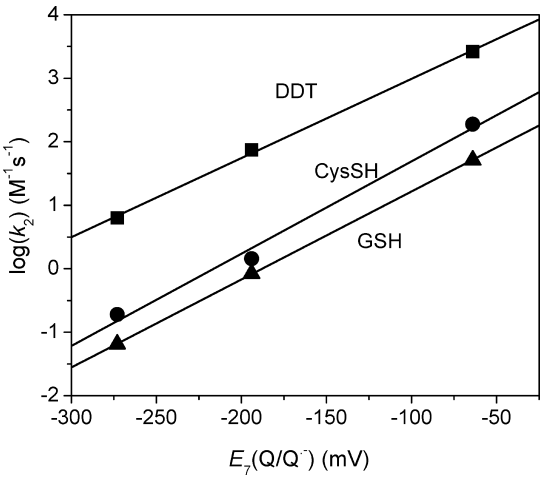


**Figure 8.** Rate constants of the addition of $RS^-$ to Q as a function of $E_7(Q/Q^{\bullet-})$.

## CONCLUSIONS

The one-electron redox potentials of GM and its C17-substituted analogues follow the order GM > 17-DMAG > 17-AAG, which correlates with the rate constants of the reaction of these ansamycins with various thiols. Thermodynamic considerations imply that the rate of one-electron bioreduction of the quinone increases upon increasing its redox potential. Thus, bioreduction of GM is more efficient compared to 17-DMAG and 17-AAG, where the respective semiquinone radicals either decompose to yield the respective hydroquinones or reduce oxygen to superoxide radical. The bioreduction is expected to be the rate-determining step, and therefore, the extent of superoxide formation should be the highest in the case of GM when superoxide is efficiently scavenged by biological targets. Because both one-electron redox cycling and thiol reactivity are associated with quinone toxicity and GM has been previously demonstrated to be more toxic relative to 17-DMAG and 17-AAG, it is concluded that the toxicity of benzoquinone ansamycins is directly related to the redox potential of the quinone/semiquinone couple.

## ASSOCIATED CONTENT

### Supporting Information

Dependence of $k_{obs}$ of the formation of the absorption at 450 nm on $[17\text{-AAG}]_0$ and $[GM]_0$, effect of pH on the absorption of the semiquinone radicals, dependence of $k_{obs}$ of GM reduction by superoxide radical on $[GM]_0$, kinetic traces of the formation and decay of $BV^{\bullet+}$ in the absence and presence of 17-AAG, effect of $[17\text{-AAG}]_0$ and $[BV^{2+}]_0$ on the rate of $BV^{\bullet+}$ decay. 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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## ABBREVIATIONS

17-AAG, 17-(allylamino)-17-demethoxygeldanamycin; $BV^{2+}$, benzyl viologen; CysSH, L-cisteine; 17-DMAG, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DTPA, diethylene triamine pentaacetic acid; DTT, dithiothreitol; GM, geldanamycin; GSH, glutathione; NQS, 1,4-naphthoquinone-2-sulfonate; PB, phosphate buffer; Q, quinone; RSH, thiol

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