Bioorganic & Medicinal Chemistry Letters, Vol.2, No.11, pp. 1427-1434, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

Rational Design of Purine Nucleoside Phosphorylase Inhibitors: Design of 2-(2'-Haloethyl) and 2-Ethenyl Substituted Quinazolinone Alkylating Agents.

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(*Received* 30 *June* 1992)

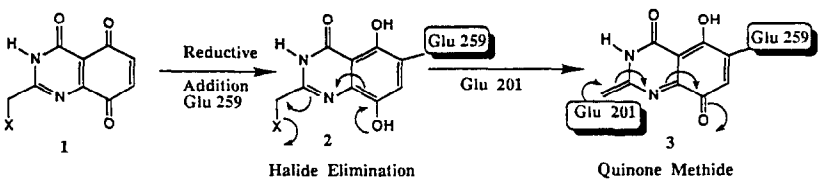
Summary: The synthesis, elimination/addition mechanism, and purine nucleoside phosphorylase inhibitory properties of 2-(2'-bromoethyl) and 2-ethenyl substituted quinazolinone-based quinones are described herein. Both 2-substituted quinazolinone derivatives were designed to act as reductive alkylating agents of glutamate residues in the purine nucleoside phosphorylase active site. Mechanistic studies provided evidence of a novel alkylation process involving a quinazolinone prototropic tautomer. The tautomer is the result of a prototropic shift of the C(1') proton of the 2-alkyl group to the N(1) position of the quinazolinone ring. Both 2-substituted quinazolinones rapidly and irreversibly inactivate purine nucleoside phosphorylase.

Introduction

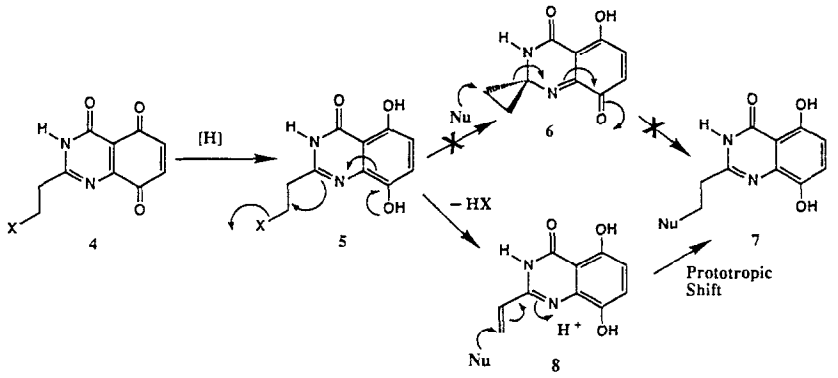
Purine nucleoside phosphorylase (PNPase) catalyses the reversible phosphorolysis of inosine and guanosine to the respective bases and ribose 1-phosphate. Inhibitors of PNPase could have utility in the treatment of organ rejection, malaria, gout, and cancer.[1] Consequently, there has been a great deal of effort devoted to the design of effective PNPase inhibitors, most of which are purine-based systems.[2] Work in this laboratory, however, has been devoted to the rational design of new PNPase inhibitors based on the quinazolinone ring system.[3,4] Our approach involves molecular modeling coupled with mechanistic organic chemistry. The latter is important in the design of an alkylating center with suitable reactivity. The choice of quinazolinone systems as inhibitors was prompted by the possibility of utilizing quinone and quinone methide[5] chemistry in alkylating agent design.

Shown in Scheme 1 is the quinazolinone system **1** which was designed to crosslink glutamate residues 201 and 259 in the PNPase active site.[4] Compound **1**, in fact, rapidly and irreversibly inactivates the enzyme. Molecular models of active site bound **1**[4] place the glutamate residues slightly less than two bond lengths away from the alkylating centers. The strategy for improving the potency of **1** was therefore to bring one or both alkylating centers closer to the glutamate residues. Shown in Scheme 2 is the haloethyl derivative **4** wherein the 2-alkylating center has been extended by one carbon. Reductive addition of glutamate to **4** in the PNPase active site would lead to formation of an alkylating spirocyclopropane species from the haloethyl group. The reductive formation of the spirocyclopane species is illustrated in Scheme 2, Path **5**→**6**→**7**.[6] Our mechanistic studies revealed that reductive activation of the haloethyl group as an alkylating center actually does occur, but by Path **5**→**8**→**7**. In this report, we present the results of our PNPase inhibition studies.

SCHEME 1



SCHEME 2



Synthesis

There are no reports of 2-haloethyl quinazolinone hydroquinones such as **5** in the literature. The preparation of **5** and related analogues was readily carried out as outlined in Scheme 3.

As depicted in Scheme 3, acylation of **9**[7] with β-bromopropionyl chloride afforded **10**, which was converted to the quinazolinone by treatment with 2% aq NaOH. These ring closure conditions resulted in HBr elimination to afford the vinylic quinazolinone **11**. Treatment of **11** with HBr resulted in *O*-demethylation, as well as anti-Markovnikov addition of HBr across the alkene, to afford the desired compound **5a**. The explanation for this mode of addition became apparent upon completion of the mechanistic study described herein. Finally, the chloro derivative **5b** was prepared by treating **5a** with refluxing concentrated HCl.

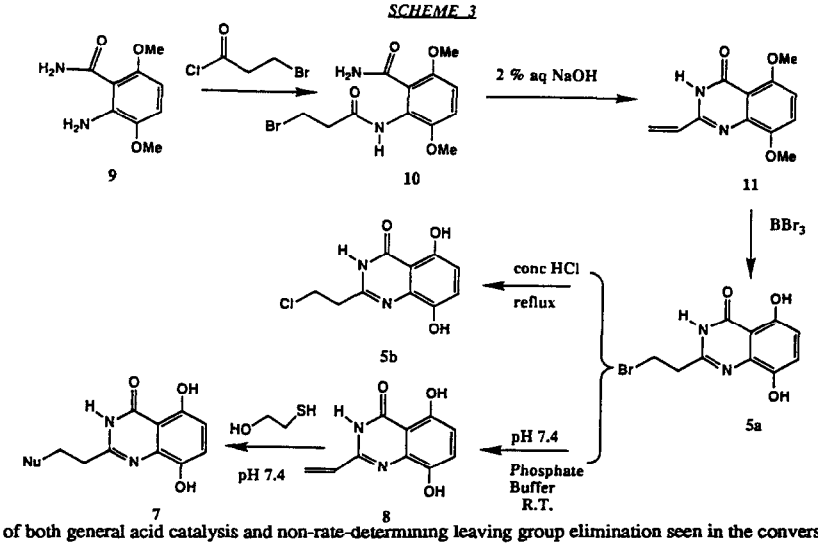
Hydrolysis of **5a** in pH 7.4 phosphate buffer afforded the alkene derivative **8** as the sole product. Monobasic phosphate catalyzes the elimination process to the extent that such conditions are useful for preparative scale reactions. Addition of hydroxyethyl mercaptan across the alkene group of **8** to afford **7** occurs with facility at pH 7.4. From the mechanistic studies of elimination presented herein and the Principle of Microreversibility, we were able to deduce the mechanism of this nucleophile addition reaction.

Mechanistic Studies of Elimination-Addition

Kinetic and Product Studies. In order to gain insights into haloethyl group reductive activation, we carried out a kinetic study of halide elimination from **5**. The mechanistic studies were carried out in aqueous buffers held at 30.0±0.2°C. Product studies in anaerobic aqueous buffer at pH 7.4, 8.5 and 9.5 revealed that **8** was the sole product. The rate of conversion of **5a** to **8** was followed at 368 nm with a UV-visible spectrophotometer. Absorbance vs. time plots were first order in character at all pH values and were independent of the leaving group present (Cl and Br in **5b** and **5a** respectively). Buffer catalysis was noted over the pH range of 6-8. The type of buffer catalysis (general base vs. general acid) was determined graphically as previously described.[8] There was no general base (dibasic phosphate) catalysis, but the reaction was catalyzed by general acid (monobasic phosphate) at $7.8 \times 10^{-4} M^{-1} s^{-1}$. From these findings it is apparent that the formation of **8** is a general-acid-catalyzed process which does not involve rate-determining leaving group elimination.

Deuterium Labelling and Deuterium Kinetic Isotope Effect (KIE). The goals of these studies were to determine the following: the presence of tautomerism, the KIE associated with the conversion of **5a** to **8**, and the presence of a symmetric spirocyclopropane intermediate.

SCHEME 3

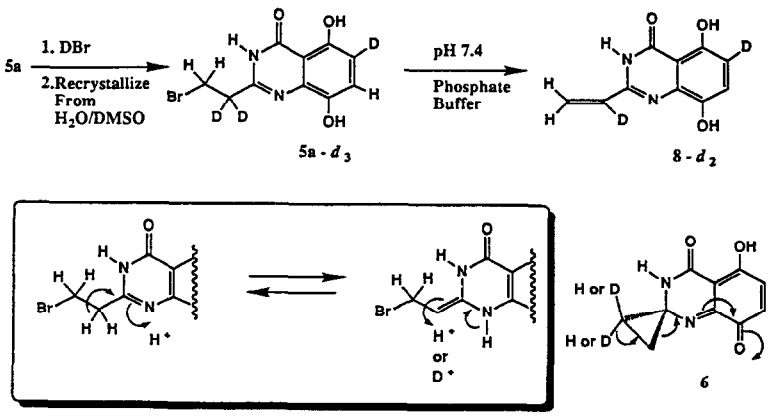


9 10 11 5b 5a 7 8

The presence of both general acid catalysis and non-rate-determining leaving group elimination seen in the conversion of **5a** to **8** suggests that this conversion must occur via an intermediate, perhaps a tautomeric species. The presence of exchangeable protons was assessed by treating **5a** with 48% DBr. The structure of the product **5a**-d_3 (Scheme 4) was determined with ^{1}H-NMR, see Experimental Section. Exchange of the C(1′)-protons of the bromoethyl group suggests that an acid catalyzed prototropic shift of the type shown in the inset of Scheme 4 can occur to afford the enamine tautomer. Exchange of the 6-proton is due to the electrophilic nature of the 6-position of the quinazolinone ring.[9]

The KIE associated with the formation of **8** was obtained by comparing rate constants for the disappearance of **5a** and **5a**-d_3. The KIE of 4.1 indicates that proton transfer from the C(1′)-position occurs in the rate determining step. This observation, and the absence of leaving group elimination in the rate determining step of **5a** to **8**, strongly suggested that rate-determining tautomerization is involved in alkene formation.

SCHEME 4

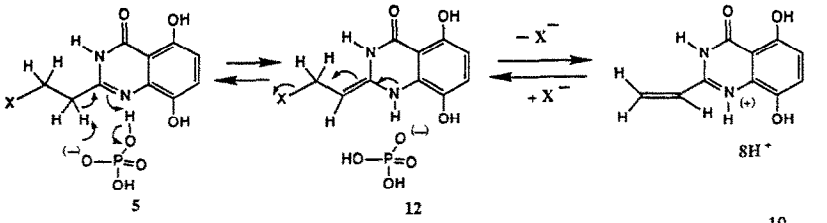


5a - d_3 8 - d_2 6

The product obtained from **5a**-d_3 confirmed that a symmetric spirocyclopropane intermediate (**6**) is not involved in alkene formation. If such an intermediate forms from **5a**-d_3, then the alkene **8**-d_2 would be a mixture of two deuterated alkenes.

The proposed mechanism for alkene (8) formation from 5a involves a rate-determining tautomerization step involving bifunctional catalysis followed by elimination of HBr, Scheme 5. Bifunctional catalysis, wherein monobasic phosphate transfers

SCHEME 5



and accepts a proton in a cyclic transition state, has been proposed by others but has never been proven to occur.[10] Consistent with a bifunctional catalysis mechanism, imidazole buffer did not catalyze this conversion. Tautomerism converts the bromoethyl group of **5a** to the allylic bromide **12**, which is in conjugation with the electron rich quinazolinone ring. The facile elimination of bromide is due to involvement of the nitrogen lone pair as shown in Scheme 5. According to the Principle of Microreversibility, the trapping of a nucleophile (such as hydroxymethyl mercaptide or a PNPase active site nucleophile) by **8** must involve addition to **8H**$^+$ to afford the tautomer **12**. The consequence of this nucleophile trapping mechanism in the formation of anti-Markovnikov addition products (see Synthesis Section).

PNPase Inhibition Studies

The results of our mechanistic studies suggest that the bromoethyl group of **4** and the ethenyl group of **13** could act as alkylating centers. Both of these quinazolinone quinones were evaluated as inhibitors of human erythrocyte PNPase and both were found to inactivate the enzyme rapidly and irreversibly. Plots of velocity for the PNPase mediated phosphorolysis of guanosine vs. incubation time (min.) in the presence of inhibitor could be fit to a first order rate law for up to five halflives. The plot of the first order rate constant vs. the concentration of inhibitor provided the second order rate constant (M^{-1} min^{-1}) for inactivation as the slope. Found in Table I are the second order inactivation rate constants for **4**, **13**, and other derivatives.

Quinone **4** is substantially more active than the previously reported bromomethyl analogue **16**.[4] The ethenyl derivative **13**, on the other hand, is only as active as **16**. Currently, x-ray crystallographic studies are being carried out with enzyme-bound **4** in order to determine the site of alkylation. The factors which influence the magnitude of the second order inactivation constant are discussed below.

The second order inactivation of PNPase is thought to involve equilibrium binding of the inhibitor to the active site followed by first order alkylation, eq. 1

$$\text{I} + \text{PNPase} \overset{K_d}{\rightleftharpoons} \text{I} - \text{PNPase} \xrightarrow{k_{alk}} \text{Inactive Enzyme} \qquad (1)$$

Table I. Second Order Rate Constants for the Inactivation of PNPase by 2-Substituted Quinazolinone Quinones in 0.05 M Phosphate Buffer, pH 7.4 (μ = 0.1 KCl) at 30.0±0.2°C.

Number	R_2	k (M^{-1} min^{-1})	Ref.
4	$BrCH_2CH_2$	478	This study.
13	$CH{=}CH_2$	186	This study.
14	H	97	4
15	NH_2	284	This study.
16	$BrCH_2$	222	4
17	Ph	956	This study.

The second order rate constant for enzyme inactivation, at non-saturating concentrations of I, is therefore expressed as k_{alk}/K_d. Strategies for improving the potency of PNPase inhibitors include increasing k_{alk} and/or decreasing K_d. The value of k_{alk} could be increased by bringing the alkylating center closer to the active site nucleophile. The value of K_d could be decreased by improving inhibitor-active site binding interactions. The rationale behind designing quinones **4** and **13** is that k_{alk} will increase since the alkylating center is closer to Glu 201. The value of k_{alk} will not increase, however, if the reductive addition step is rate determining (Scheme 1). The amino acid composition surrounding the active site bound purine (or quinazolinone[4]) ring is largely hydrophobic with the exceptions of Glu-201 and Lys-205.[11] The 2-substituent could, therefore, be increasing potency by lowering K_d. The data in Table I suggest that this may actually be the case. Quinone **14** has no 2-substituent and inactivates the enzyme at 97 M^{-1} min^{-1}. Quinone **15** has a 2-amino substituent, which could form a salt with Glu 201, and inactivates the enzyme at 284 M^{-1} min^{-1}. Finally, quinone **17**, which bears a hydrophobic phenyl group, inactivates the enzyme at 956 M^{-1} min^{-1}.

Conclusion

The mechanistic studies described herein indicate that the quinazolinone tautomer **12** readily forms in aqueous buffer at 30°C. This tautomer is the intermediate in both halide elimination from 2-(2′-haloethyl)quinazolinone and nucleophile additions to 2-ethenylquinazolinone. The results of these mechanistic studies and molecular modeling studies provided the rationale for studying 2-(2′-bromoethyl) and 2-ethenyl quinazolinone quinones (**4** and **13** respectively) as active site directed PNPase inhibitors. Quinones **4** and **13** rapidly inactivate the enzyme by second order processes at 478 M^{-1} s^{-1} and 186 M^{-1} s^{-1}, respectively. The factor which controls the magnitude of the second order rate constant appears to be the hydrophobicity of the 2-substituent. Thus, the 2 phenyl analogue **17** was the most active analogue tested, with a second order inactivation constant of 956 M^{-1} s^{-1}.

Experimental Section

Elemental analyses were performed by Desert Analytics Laboratory, Tucson, Arizona. All analytically pure compounds were dried under high vacuum in a heating pistol with refluxing methanol. All compounds had suitable microanalyses except hydroquinones **5a,b**, **7**, and **8**, which were difficult to obtain in an analytically pure form. ^{1}H-NMR spectra indicate pure material in all cases, however.

Uncorrected melting and decomposition points were characterized by color darkening without complete melting. All TLC was run with Merck silica gel 60 (F_{254}) plates, employing a variety of solvents. IR spectra were taken as KBr pellets; the strongest IR absorbances are reported. Routine ^{1}H NMR spectra were taken on a 300 MHz spectrometer; chemical shifts are reported relative to TMS. Mass measurements were carried out in the electron-impact mode.

Human erythrocyte purine nucleoside phosphorylase and guanosine were purchased from Sigma Chemical Company. The purine nucleoside phosphorylase has a specific activity of 21 units/mg of protein. PNPase activity (guanosine to guanine and ribose 1-phosphate) was followed by measuring the decrease in absorbance at 268 nm using a $\Delta\epsilon$ value of 2.38×10^3 M^{-1} cm^{-1} as previously described.[4]

The Preparation of New Compounds is outlined below in the order found in the text:

2-(2′-Bromopropionamido)-3,6-dimethoxybenzamide (10). To a cooled (0°C) solution of 2.0 g (10.2 mmol) of **9**[7] in 100 mL of dry methylene chloride was added 1.3 mL of pyridine followed by careful addition of 1.3 mL of 3-bromopropionyl chloride. The solution was stirred for 20 minutes with continued chilling. The crystals that formed were filtered off and rinsed with methylene chloride: 2.7 g (80%) yield; mp 165-166°C; TLC (ethyl acetate/acetic acid [9:1]) R_f = 0.40; IR (KBr) 3385, 3182, 3040, 1668, 1547, 1481, 1263, 1089, 1035, 630 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.23 (1 H, br s, propamido N-H), 7.32 and 7.10 (2 H, 2 br s, carbamyl protons), 7.00 and 6.94 (2 H, 2 d, J = 8.9 Hz, aromatic protons, no assignments made), 3.72 and 3.69 (6 H, 2 s, methoxy protons, no assignments made), 3.62 (2 H, br t, β-methylene protons), 2.84 (2 H, br t,

α-methylene protons). Addition of D_2O resulted in collapse of the propionamido and amide nitrogen protons. Mass Spectrum (EI mode) *m/z* 330(M^+, ^{79}Br), 332(M^+, ^{81}Br).

5,8-Dimethoxy-2-ethenylquinazolin-4(3*H*)-one (11). A suspension of 2.0 g (6.04 mmol) of **10** in 60 mL of 2% sodium hydroxide solution was gently warmed until the solid dissolved into solution. The solution was then stirred for 3 h at room temperature. The completed reaction was acidified to pH 7 with concentrated hydrochloric acid. The white crystals that formed were filtered off and rinsed with water: 1.33 g (95%) yield; mp > 195°C dec (slow darkening); TLC (ethyl acetate/acetic acid, [9:1]) R_f = 0.25; IR (KBr) 3439, 3107, 1680, 1562, 1481, 1327, 1265, 1099, 812 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.28 and 6.91 (2 H, 2 d, *J* = 9.0 Hz, C(6)-H and C(7)-H, no assignments made), 6.53 (2 H, m, C(1′)- and cis(2′)-H), 5.79 (1 H, dd, *J* = 3.8 and 8.15 Hz, trans-C(2′)-H proton), 3.84 and 3.80 (6 H, 2 s, methoxy protons, no assignments made); mass spectrum (EI mode), *m/z* 232 (M^+).

2-(2′-Bromoethyl)-5,8-dihydroxyquinazolin-4(3*H*)-one (5a). To a suspension of 405 mg (1.75 mmol) of **11** in 15 mL of dry benzene was added 0.48 mL of 99% boron tribromide. The mixture was refluxed for 2 h and then cooled to room temperature. Methanol (5 mL) was added to decompose excess boron tribomide. The solvents were evaporated *in vacuo* and the residual oil was crystallized by adding 50:50 dimethyl sulfoxide - water: 341 mg (69%) yield; mp > 202°C (slow darkening); TLC 2% acetic acid in ethyl acetate) R_f = 0.67; IR (KBr) 3420, 3175, 3055, 2951, 1676, 1633, 1581, 1465, 1379, 1249, 1037, 848, 651, 538 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 12.58, 10.96, and 8.85 (3 H, 3 br s, N(3)-H, 5-OH, and 8-OH, no assignments made), 7.14 and 6.69 (2 H, 2 d, *J* = 8.9 Hz, C(6)-H and C(7)-H, no assignments made), 4.00 (2 H, t, *J* = 6.9 Hz, 2′-methylene protons), 3.24 (2 H, t, *J* = 6.8 Hz, 1′-methylene protons); mass spectrum (EI mode) *m/z* 284 (M^+, ^{79}Br), 286 (M^+, ^{81}Br).

2-(2′-Chloroethyl)-5,8-dihydroxyquinazolin-4(3*H*)-one (5b). A solution of 100 mg (0.49 mmol) of 5a in 6.0 mL in concentrated hydrochloric acid was refluxed for 12 h. The excess hydrochloric acid was evaporated *in vacuo* and the residue was recrystallized from water: 58 mg yield. ^1H NMR analysis of this solid showed approximately 2:1 ratio of chloroethyl and hydroxyethyl derivatives. Separation was achieved by silica gel preparative TLC with 5% acetic acid in ethyl acetate as eluant (the sample was applied to the plate dissolved in methanol). The band of highest R_f value was scrapped from the developed plate and the silica was extracted with 5% methanol in ethyl acetate. Evaporation of the extracts followed by precipitation of the residue from ethyl acetate-hexane afforded **5b**: 21 mg (18%) yield; mp 197-200°C dec; TLC (2% acetic acid in ethyl acetate) R_f = 0.58; IR (KBr) 3406, 3176, 3059, 1676, 1633, 1577, 1465, 1249, 1039, 852, 667 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) 7.07 and 6.61 (2 H, 2 d, *J* = 8.6 Hz, C(6)-H and C(7)-H, no assignments made), 4.13 (2 H, t, *J* = 7.0 Hz, C(2′)-methylene protons), 3.10 (2 H, t, *J* = 6.9 Hz, C(1′)-methylene protons); mass spectrum (EI mode) *m/z* 240 (M^+).

5,8-Dihydroxy-2-ethenylquinazolin-4(3*H*)-one (8). To a solution of 260 mg (0.91 mmol) of **5a** in 50 mL of deoxygenated dimethyl sulfoxide was added 200 mL of deoxygenated 0.2 M pH 7.4 potassium phosphate buffer (μ = 1.0 KCl). The solution was allowed to sit in a nitrogen atmosphere for 36 h. The solution was extracted with ethyl acetate (3 × 350 mL). The organic extracts were washed with water (3 × 200 mL), dried over sodium sulfate and evaporated. The residue was recrystallized from ethyl acetate - hexane: 136 mg (73%) yield; mp 201-203°C; TLC (2% acetic acid in ethyl acetate) R_f = 0.58; IR (KBr) 3410, 3178, 3140, 1662, 1574, 1460, 1242, 1035, 842, 808, 690, 538 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 12.55, 11.04 and 8.97 (3 H, 3 br s, N(3)-H and hydroxy protons, no assignments made), 7.14 and 6.70 (2 H, 2 d, *J* = 8.7 Hz, C(6)-H and C(7)-H, no assignments made), 6.77 (1 H, dd, *J* = 1.2 H and 18.0 Hz, cis-C(2′)-H), 6.58 (1 H, dd, *J* = 10.6 Hz and 18 Hz, C(1′)-H), 5.85 (1 H, dd, *J* = 1.5 Hz and 10.6 Hz, trans-C(2′)-H). Mass spectrum (EI mode), *m/z* 204 (M^+).

2-[2′-(2″-Hydroxyethylthio)ethyl]quinazolin-4(3*H*)-one (7). To a solution of 30 mL of degassed 0.2 M pH 7.4 potassium phosphate buffer (μ = 1.0 KCl) containing 0.90 mL (12.7 mmol) of 2-mercaptoethanol was added 26 mg (0.127 mmol) of **8** in 1.0 mL of degassed dimethyl sulfoxide. The reaction was stirred for 1 h at room temperature with exclusion of oxygen (an argon atmosphere was employed). The completed reaction was extracted with 2 × 75 mL portions of ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate and evaporated. The residue was recrystallized from water: 21 mg (59%) yield; mp 160-165°C dec; TLC (10% acetic acid in ethyl acetate) R_f = 0.50; IR (KBr) 3431, 3140, 3055, 2928, 1668, 1628, 1581, 1465, 1248, 1037, 688 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) 11.01 and 8.80 (2 H, 2 br s, phenol protons), 7.12 and 6.67 (2 H, 2 d, J = 8.9 Hz, C(6)-H and C(7)-H, no assignments made), 4.81 (1 H, br s, C(5′)-hydroxide proton), 3.56 (2 H, t, J = 6.8 Hz, hydroxy methylene protons), 2.63, 3.01, and 2.90 (6 H, 3 t, J = 6.8 Hz, methylene protons, no assignments made). Addition of D_2O resulted in collapse of the phenol and hydroxy protons. Mass spectrum (EI mode) *m/z* 282 (M^+).

Deuterated 2-(2′-bromoethyl)-5,8-dihydroxyquinazolin-4(3*H*)-one (5a-d_3). A solution of 50 mg (0.22 mmol) of **5a** in 3.0 mL of 48% DBr in D_2O was refluxed for 12 h. The excess DBr solution was evaporated *in vacuo* and the residue was recrystallized from dimethyl sulfoxide-water: 41 mg (66%) yield; mp > 200°C; TLC (2% acetic acid in ethyl acetate) R_f = 0.70; IR (KBr) 3435, 3173, 2926, 1676, 1629, 1577, 1450, 1365, 1309, 1221, 1033 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) 12.56, 10.94, and 8.85 (3 H, 3 s, N(3)-H, 5-OH and 8-OH, no assignments made), 7.14 (1 H, s, C(7)-H), 3.99 (2 H, s, bromomethylene protons); mass spectrum (EI mode) *m/z* 287 (M^+, ^{79}Br), 289 (M^+, ^{81}Br).

Identification of 8-d_2. The trideuterated bromoethyl hydroquinone **5a-d_3** (50 mg, 0.17 mmol) was dissolved in 5.0 mL of dimethyl sulfoxide and 30 mL of 0.2 M pH 10.50 borate buffer, μ = 1.0 KCl was added. The solution was allowed to sit under a nitrogen atmosphere for 3.5 h. The solvents were degassed (argon purged) before use. The reaction solution was acidified to pH 6 with concentrated hydrochloric acid. The aqueous solution was extracted with 3 × 75 mL portions of ethyl acetate. The combined extracts were dryed over sodium sulfate and evaporated to dryness *in vacuo*. The residue was recrystallized from dimethyl sulfoxide/water: 33 mg yield. The product was analyzed by ^1H NMR (dimethyl-d_6 sulfoxide) and determined to consist of a 2:1 ratio of starting material and vinylic product. The ^1H NMR assignments for the deuterated vinylic product are: δ 11.01 and 8.95 (2 H, 2 s, hydroxy protons, no assignments made), 7.14 (1 H, s, C(7)-H), 6.76 (1 H, s, cis-C(2′)-H), 5.85 (1 H, s, trans-C(2′)-H). If scrambling occurred, a total of three vinylic protons would be observed. Addition of D_2O resulted in collapse of the hydroxy protons.

2-(2′-Bromoethyl)quinazoline-4,5,8(3*H*)-trione (4). To a chilled 0°C suspension of 100 mg (0.35 mmol) of **5a** in 2.0 mL dry methanol was added 88 mg (0.38 mmol) of dichlorodicyanobenzoquinone (DDQ). The reaction was stirred for 30 min with continued chilling. The crystals were filtered and rinsed with ethyl acetate: 56 mg (56%) yield; mp > 120°C dec (darkening); TLC (butanol-acetic acid-water [5:3:2]) R_f = 0.60; IR (KBr) 3441, 2922, 1707, 1680, 1575, 1483, 1317, 1105, 856 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.00 and 6.86 (2 H, 2 d, J = 10.4 Hz, C(6)-H and C(7)-H, no assignments made); mass spectrum (EI mode) *m/z* 282 (M^+ ^{79}Br), 284 (M^+ ^{81}Br).

2-Ethenylquinazoline-4,5,8(3*H*)-trione (13). To a cooled (0°C) suspension of 50 mg (0.245 mmol) of **8** in 1.0 mL of dry methanol was added 61 mg (0.268 mmol) of dichlorodicyanobenzoquinone (DDQ). The reaction was stirred for 20 min with continued chilling. The crystals that formed were filtered and rinsed with ethyl acetate: 33 mg (67%) yield; mp > 210°C dec (slow darkening); TLC (*n*-butanol-acetic acid-water) R_f = 0.54; IR (KBr) 3103, 3053, 1701, 1683, 1631, 1539, 1481, 1321, 1111, 860, 439 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.01 and 6.86 (2 H, 2 d, J = 10.5 Hz, C(6)-H and C(7)-H, no assignments made), 6.77 (1 H, d, J = 17.2 Hz, cis-C(2′)-ethenyl proton), 6.62 (1 H, dd, J = 17.2 and 10.3 Hz, C(1′)-ethenyl proton), 6.09 (1 H, d, J = 10.3 Hz, trans-C(2′)-vinylic proton); mass spectrum (EI mode), *m/z* 202 (M^+).

2-Aminoquinazolin-4,5,8(3*H*)-trione (15) was prepared by the three-step synthesis starting with 2-nitro-3,6-dimethoxybenzoic acid.[12] Steps included reduction of the starting material with H_2 Pd on carbon, cyanamide treatments, and finally DDQ oxidation: mp > 190°C dec; IR (KBr) 3610, 3480, 3140, 1508, 1371, 1346, 1246, 1070, 868, 713 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 11.47 (1 H, br s, N(3)-H), 8.60 (2 H, br s, 2-amino protons), 6.83 and 6.70 (2 H, 2 d, J = 10.3 Hz, C(6)-H and C(7)-H, no assignments made); mass spectrum (EI mode) *m/z* 191 (M^+).

2-Phenylquinazoline-4,5,8(3*H*)-trione (17) was prepared by the three-step synthesis starting with **9**. Steps included acylation of **9** with benzoyl chloride, ring closure and demethylation in HBr, and finally DDQ oxidation: mp 260°C; TLC (10% acetic acid in ethyl acetate) R_f = 0.41; IR (KBr) 3078, 2980, 1683, 1610, 1535, 1504, 1471, 1448, 1307, 1178, 1105, 1035, 854, 707, 437 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 8.27 and 7.64 (5 H, d, and m respectively, J = 7.5 Hz for d, phenyl protons), 7.05 and 6.91 (2 H, 2 d, J = 10.6 Hz, C(6)-H and C(7)-H, no assignments made); mass spectrum (EI mode), *m/z* 252 (M^+).

References

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Acknowledgment. The financial support of the National Science Foundation and a research career development award (to E.B.S.) from the National Institutes of Health are gratefully acknowledged.