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Chemistry of Ring-Substituted 4-(Benzothiazol-2-yl)phenylnitrenium Ions from Antitumor 2-(4-Aminophenyl)benzothiazoles

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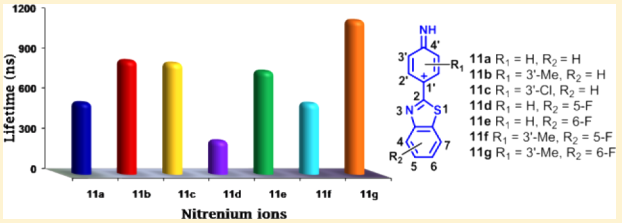
Supporting Information

ABSTRACT: Ring-substituted derivatives of 2-(4-aminophenyl)benzothiazole, **1a**, **1b–g**, are under development as antitumor agents. One derivative, **1f**, has reached phase 1 clinical trials as the prodrug **2f**, Phortress (NSC 710305). These amines are activated by CYP450 1A1, apparently into hydroxylamines **8a–g** that are likely metabolized into esters that ionize into nitrenium ions responsible for cellular damage. Previously we showed that **9a**, the acetic acid ester of **8a**, generates the long-lived (530 ns) nitrenium ion **11a** by hydrolysis or photolysis in water. In this study, azide trapping shows that **9b–g** generate **11b–g** via rate-limiting N–O heterolysis. Ion lifetimes, estimated from azide/solvent selectivities, range from 250 to 1150 ns with identical lifetimes for **11a** and **11f**. Differences in biological activity of the amines are likely not due to differences in the chemistry of the cations but to differences in metabolic activation/deactivation of individual amines. Unlike the nitrenium ions, lifetimes of the esters are strongly dependent on the 3′-Me substituent. Esters containing 3′-Me (**9b**, **9f**, **9g**) have lifetimes of 5–10 s compared to 400–800 s for esters without 3′-Me (**9a**, **9c**, **9d**, **9e**). This restricts 3′-Me esters to cells/tissues in which activation occurs, concentrating their effects in tumor cells if metabolism is restricted to those cells.

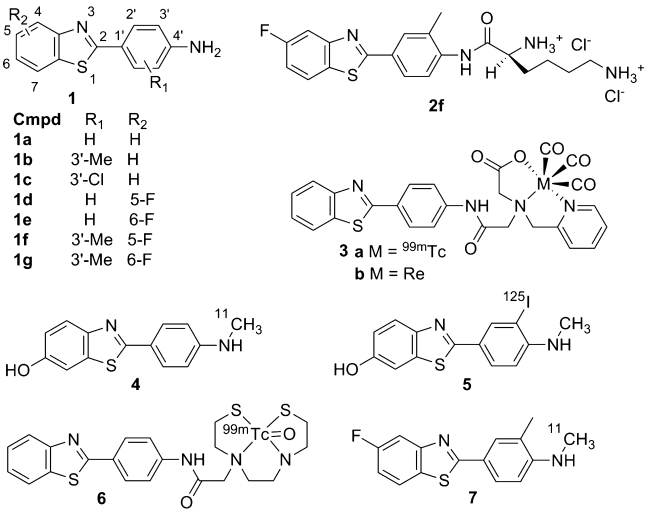


INTRODUCTION

Since 1996 a series of benzothiazole derivatives based on the lead compound 2-(4-aminophenyl)benzothiazole, **1a** (Chart 1), have been under development as antitumor agents.[1] Unsubstituted **1a** was found to have activity against the human breast cancer cell lines MCF-7 (IC_{50} = 0.3–0.8 nM) and MDA 468 (IC_{50} = 1.6 nM).[1] Substitution at the 3′-position by methyl (**1b**) or halogens, including Cl (**1c**), led to enhanced activity against the original cell lines and a broader spectrum of activity against certain colon, ovarian, and renal cancer cell lines, although other cell lines were insensitive to the drugs.[1−3] Studies with fluorinated analogues including **1d–g** showed that fluorination at C-5 or C-7 prevented metabolic deactivation of the drug without compromising antitumor activity.[4] The fluorinated water-soluble prodrug **2f**, Phortress (NSC 710305), was approved for phase 1 clinical trials in Britain in 2004.[5,6]

Initial reports from the preclinical and clinical trials on Phortress were favorable,[7] but very recently it was reported that a 50-patient study was terminated due to a combination of pulmonary and liver toxicity and marginal efficacy.[8] Nevertheless, **1f** and **2f** continue to be evaluated in preclinical studies,[9] and other drug candidates containing the 2-(4-aminophenyl)benzothiazole moiety continue to be investigated.[10−21] The complexes **3** are being developed as radiopharmaceuticals for imaging (**3a**) and targeted radiotherapy (**3b**, ^{186}Re, ^{188}Re) of breast cancer.[10,11] Other derivatives of **1a**, including **4–6** and **7** that has the same ring substitution pattern as **1f**, are being investigated as radiopharmaceuticals for binding and in vivo imaging of Aβ-plaques that are associated with the early stages of Alzheimer's disease.[12−16] Still other derivatives of **1a** are under investigation as antimicrobial and

Chart 1



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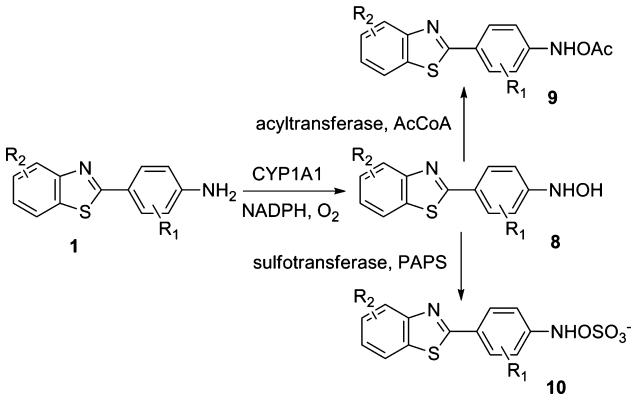
antifungal agents.[17−20] The 2-(4-aminophenyl)benzothiazole moiety is appearing in a significant number of drug candidates.[21]

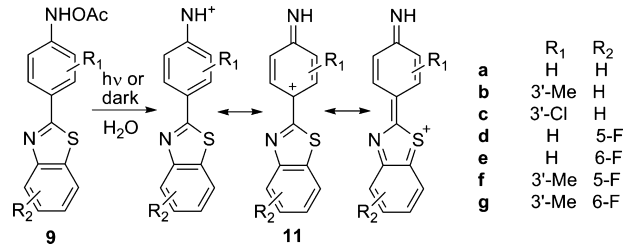
Metabolism of 2-(4-aminophenyl)benzothiazoles into the active metabolite in sensitive cell lines requires the constitutive presence of the 1A1 isoform of cytochrome P450, CYP1A1, that is also induced by the drug.[22] Activation also leads to detoxification because CYP1A1 hydroxylates these drugs, except those fluorinated at C-5 or C-7, leading to an inactive C-6-hydroxylated metabolite.[22,23] Metabolism is associated with translocation of the aryl hydrocarbon receptor (AhR) to the nucleus.[24,25] The mechanism of action of **1a** and its simple ring-substituted derivatives is thought to involve selective uptake into sensitive cells followed by AhR binding and translocation into the nucleus, induction of CYP1A1, oxidation and conversion of the drug into an electrophilic reactive intermediate, and formation of extensive DNA adducts resulting in cell death.[6,21] It is suspected that the active metabolite of **1** is the hydroxylamine **8** or, more likely, an ester derivative, **9** or **10** (Scheme 1).[6,26] It was proposed that the

Scheme 1. Probable Metabolism of 1



nitrenium ion **11** (Scheme 2) is responsible for the antitumor activity of **1**, but, until recently, no direct evidence supporting this proposal was available.[26−28]

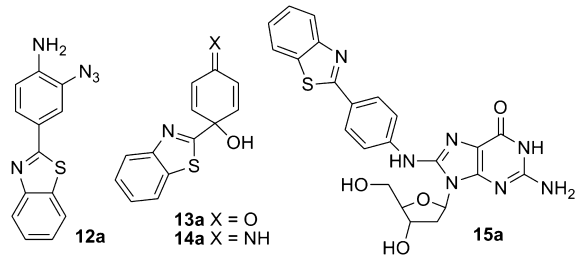
Scheme 2. Proposed Nitrenium Ion 11 Generated from 9



We recently reported the synthesis of **9a** and demonstrated that hydrolysis or photolysis of this material (Scheme 2) led to an N_3^--trappable reactive intermediate identified as **11a**.[29,30] Laser flash photolysis (lfp) of **9a** led to the direct detection of **11a** with λ_{max} 570 nm and an aqueous solution lifetime of 530 ns.[29] The effect of N_3^- on the kinetics of decay of **11a** monitored at 570 nm was consistent with N_3^- trapping data obtained during hydrolysis of **9a**, demonstrating that the same intermediate was generated during photolysis and hydrolysis.[29]

The structure of the major N_3^- adduct, **12a** (Chart 2), is consistent with that of N_3^- adducts derived from other *N*-

Chart 2



arylnitrenium ions.[30] The hydrolysis product formed in the absence of other nucleophiles, **13a**, was shown to arise from slow hydrolysis of the initially rapidly formed quinolimine, **14a**.[29,30] It was also shown that **11a** is efficiently trapped by 2′-deoxyguanosine (dG) to form a C-8 adduct, **15a**, analogous to the major adduct formed by the reaction of nitrenium ions derived from carcinogenic *N*-arylamines with dG and dG residues in DNA.[30] Reactions of **11a** with adenosine and cytosine were also demonstrated, but, consistent with the pattern previously observed for nitrenium ions derived from carcinogenic *N*-arylamines, these were much less efficient than reaction with dG.[31]

Because of continued interest in ring-substituted derivatives of **1a** in various fields of medicine we have investigated the chemistry of the ring-substituted esters **9b−g** and characterized the resulting nitrenium ions **11b−g**. The 3′-substituted esters were chosen because of the reported effects of 3′-substituents on the antitumor activities of the corresponding amines. The 5-F- and 6-F-substituted esters were chosen because substitution by F in that part of the ring system is a common tactic used to reduce metabolic inactivation of the drug. The choice of mono- and disubstituted esters allows both substituent effects to be examined independently and in combination. The ester **9f** was chosen because the ring-substitution pattern of that material is identical to that of Phortress, **2f**. The particular fluorinated compounds were also chosen because the 6-F substituent is in resonance contact with the positive charge of **11**, while the 5-F substituent is not. Results of our studies are reported herein.

RESULTS AND DISCUSSION

Synthesis and Product Characterization. Synthesis of the esters **9b−g** followed a path starting from the corresponding 2-(4-nitrophenyl)benzothiazoles **16b−g** that was previously published for **9a** (Scheme 3).[29] The nitro compounds **16b−g** are known materials generated by standard procedures.[4,23,32,33] Pd/C-catalyzed reduction of **16b−g** with hydrazine hydrate led to the hydroxylamines **8b−g** in good yield. These are stable materials that can be purified by chromatography. The esters were generated by the reaction of the hydroxylamines with pyruvonitrile in the presence of the tertiary amine catalyst *N*-ethylmorpholine. Pyruvonitrile reliably acetylates hydroxylamines, including the benzothiazole derivatives used here, at O rather than N.[29,34] The esters are much less stable than the hydroxylamines. Only **9c−e** could be isolated and characterized. All esters containing a 3′-Me substituent (**9b**, **9f**, **9g**) were too reactive to isolate. Instead, these compounds were generated in situ in dry CH_3CN solutions that were stable for several days at −40 °C. These compounds were characterized by their decomposition kinetics in aqueous solution, by UV−vis spectroscopy, and by their characteristic reaction products.

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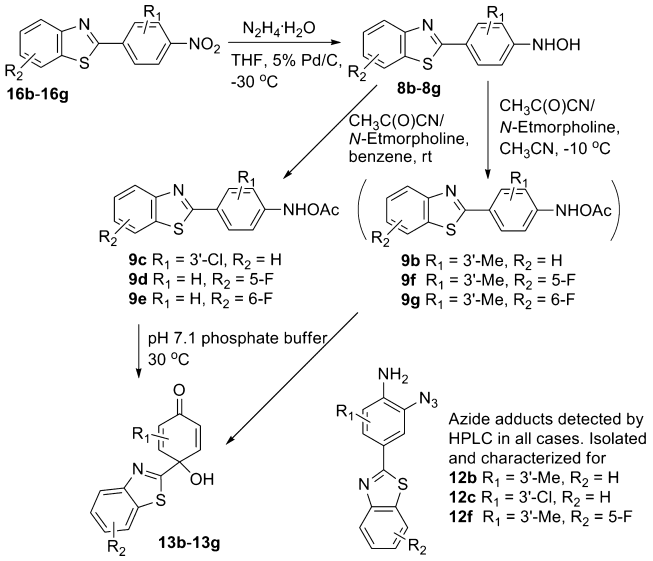
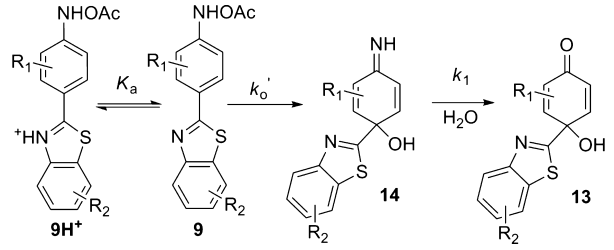
Scheme 3. Synthesis of 9b–g and Isolation of Characteristic Reaction Products



Scheme 4. General Kinetic Scheme for Decomposition of 9



The only isolated products of hydrolysis of all six esters in pH 7.0 phosphate buffer were the corresponding quinols **13b–g**. At the concentrations employed in kinetic and product analysis experiments (2.5×10^{-5} M), HPLC data indicate that the quinols are generated in 85–95% yield with no other significant products detected. Representative HPLC chromatograms are shown in Figures S1 and S2 in the Supporting Information. At the higher concentrations used in product isolation experiments (ca. 5×10^{-4} M) HPLC yields appear to be somewhat lower (60–70%), but no other significant products are detected by HPLC. Isolated yields of the pure quinols are in the range of 20–30% after several rounds of chromatography. The quinols are stable materials that are analogous to **13a** derived from hydrolysis of **9a**.[29,30] Similar quinols are the major hydrolysis products of esters of carcinogenic polycyclic *N*-arylhydroxylamines that decompose via a nitrenium ion intermediate.[35] Hydrolysis of all the esters at pH 7.0 in the presence of NaN_3 led to azide adducts, detected by HPLC as characteristically long retention time products, that were formed at the expense of the quinols. Representative HPLC chromatograms for the azide reactions are shown in Figures S1 and S2 in the Supporting Information. Three of these adducts (**12b**, **12c**, and **12f**) were isolated and fully characterized. These are completely analogous to **12a** formed by trapping of **11a** with N_3^- and with similar azide adducts obtained by trapping other nitrenium ions.[30,35] The formation of azide adducts predominately at the 2-position and water adducts predominately at the 4-position has been noted previously for 4-aryl- or 4-alkyl-substituted nitrenium ions and has been attributed to reversible formation of an unstable initial 4-azido adduct that rearranges to the observed adduct.[30,35,36]

Reaction Kinetics. Previously, hydrolysis of **9a** was shown to follow the kinetic pathway of Scheme 4.[30] At pH > pK_a (1.45 for **9aH**$^+$), hydrolysis proceeded at a pH-independent rate governed by k_o'. Because $k_o' > k_1$, it was possible to detect the formation and decay of the quinol imine **14a** during kinetics experiments.[29,30] Hydrolysis kinetics of **9b–g** were monitored by UV–vis spectroscopic methods in phosphate buffers (30 °C, 5 vol% CH_3CN/H_2O, 0.02 M total buffer, μ = 0.5 ($NaClO_4$)). For all compounds it was possible to detect two pseudo-first-order processes. Representative repetitive wavelength scans that show the biphasic transition from ester to quinol are shown in Figure S3 in the Supporting Information. Figure 1 shows a representative absorbance vs time plot for decay of **9d**. Depending on the wavelength chosen to monitor the reaction, the data fit either a standard first-order rate equation or a consecutive first-order rate equation governed by two rate constants. The larger rate constant, observed at all wavelengths, was pH independent within the pH range 5.6 to 7.4 for **9b–d** and **9f** (kinetics for decay of **9e** and **9g** were only monitored at pH 7.0). This rate constant has characteristics equivalent to k_o' previously observed for **9a** and was identified as such. The smaller rate constant, required for fitting of the data at some wavelengths, is moderately pH-dependent in these buffers as was previously observed for **9a**.[30] HPLC data taken during the hydrolysis of **9c** confirmed that the appearance of the peak for **13c** was governed by the slow decay of a transient HPLC peak identified as that of **14c**. This result is similar to that previously observed by HPLC analysis of the formation of **13a** during the decomposition of **9a**.[29] The smaller pH-dependent rate constant was identified as k_1.

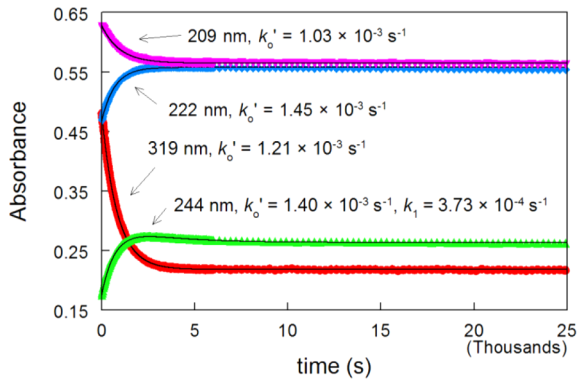


Figure 1. Absorbance vs time for hydrolysis of **9d** at pH 7.0 with fitted lines and rate constants.

The average values for k_o', as well as the value of k_1 at pH 7.0, for **9a–g** are provided in Table 1. Average values for rate constants were obtained at each pH from duplicate runs monitored at a single wavelength for **9b**, **9f**, and **9g** and single runs monitored at multiple wavelengths for **9c**, **9d**, and **9e**. The rate constant k_o' reported in Table 1 was then obtained by averaging the values for k_o' obtained at each pH except for **9e** and **9g** that were monitored only at pH 7.0. All individual rate constants obtained in this study are provided in Tables S1 through S6 in the Supporting Information. The most significant rate effect is caused by the 3′-Me substituent in **9b**, **9f**, and **9g** that causes a 60- to 100-fold increase in k_o' compared to the corresponding compounds without the 3′-Me substituent (**9a**,

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Table 1. Kinetic parameters for 9a−g and 11a−g

compound/cation	$10^3\ k_o'$ (s^{-1})	$10^4\ k_1$ (s^{-1}) at pH 7.0	$10^{-3}\ k_{az}/k_s$ (M^{-1})	$1/k_s$ (ns)
9a/11a	2.31 ± 0.05[a]	5.3 ± 0.1[a]	2.6 ± 0.3[b]	535 ± 20[b,c]
9b/11b	147 ± 26	0.92 ± 0.02	4.2 ± 0.4	850 ± 80[d]
9c/11c	2.67 ± 0.10	0.58 ± 0.01	4.1 ± 0.4	830 ± 80[d]
9d/11d	1.23 ± 0.21	3.7 ± 0.1	1.3 ± 0.1	250 ± 20[d]
9e/11e	2.19 ± 0.10	4.7 ± 0.1	3.8 ± 0.2	770 ± 40[d]
9f/11f	122 ± 2	0.27 ± 0.02	2.6 ± 0.4	530 ± 80[d]
9g/11g	155 ± 18	0.58 ± 0.01	5.7 ± 0.2	1150 ± 40[d]

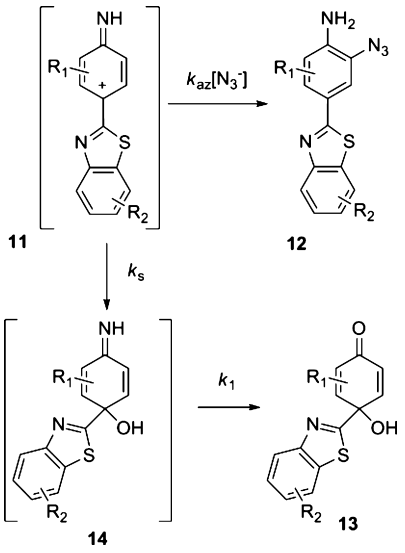
[a]See ref 30. [b]See ref 29. [c]Determined by direct measurement of k_s for **11a** generated by laser flash photolysis (ref 29). [d]Estimated assuming that k_{az} = $4.94 \times 10^9\ M^{-1}\ s^{-1}$ (ref 29).

9d, **9e**). Effects of the halogen substituents 3′-Cl, 5-F, and 6-F on k_o' are relatively small. The magnitude of k_o' is nearly equivalent within experimental error for **9a**, **9c**, and **9e**. The 5-F substituent of **9d** causes a ca. 2-fold decrease in k_o' compared to **9a**.

The rate of hydrolysis of **14a** into **13a** is dependent on relative rate constants for hydrolysis of diprotonated, monoprotonated, and neutral **14a** and the pK_as of the diprotonated and monoprotonated species.[30] Because the kinetics of hydrolysis of **14b−g** were not followed over a wide enough pH range to determine detailed kinetic parameters, it is not possible to ascertain substituent effects on individual ionization and rate constants for **14b−g**. It is clear from the data in Table 1 that the 3′-Me and 3′-Cl substituents cause about a 10-fold decrease in the rate of hydrolysis of the quinol imine at pH 7.0.

Azide Trapping. Hydrolysis of **9a** in buffers containing N_3^- leads to formation of an azide adduct without an increase in the rate of hydrolysis of **9a**, leading to the conclusion that ionization of **9a** generates the nitrenium ion **11a** (Scheme 5).[29]

Scheme 5. Kinetics of Azide Trapping of 11



The N_3^-/solvent selectivity, k_{az}/k_s, was determined by the $[N_3^-]$ dependence of the yields of **12a** and **13a** ("azide clock" method).[29] The selectivity ratio was confirmed and both rate constants were measured directly from kinetic studies on **11a** generated by lfp.[29] Laser flash photolysis cannot be implemented for **9b**, **9f**, and **9g** because of their short lifetimes in water, but rate constants determined by lfp and the "azide clock" method are in good agreement for nitrenium ions in the range of reactivity found in this study,[29,35,37] so the "azide clock" method was employed here. Hydrolysis of **9b−g** in pH 7.0 phosphate buffers containing N_3^- leads to an $[N_3^-]$-dependent decrease in the yield of **13b−g** with no increase in the hydrolysis rate constant. The rate constants k_o' measured in the presence and absence of N_3^- are summarized in Tables S1 through S6, and representative repetitive wavelength scans are shown in Figure S4 in the Supporting Information. The azide adduct is detected by HPLC as a long retention time product with a characteristically long wavelength λ_{max} at ca. 330−350 nm. The quinols **13a−g** have shorter HPLC retention times and λ_{max} at ca. 210−230 nm (Figures S1−S4). The product was isolated and fully characterized in three cases (**12b**, **12c**, and **12f**). Figure 2 shows a representative plot of HPLC peak area vs time for **12b** and **13b** as a function of $[N_3^-]$ determined from hydrolysis of **9b**.

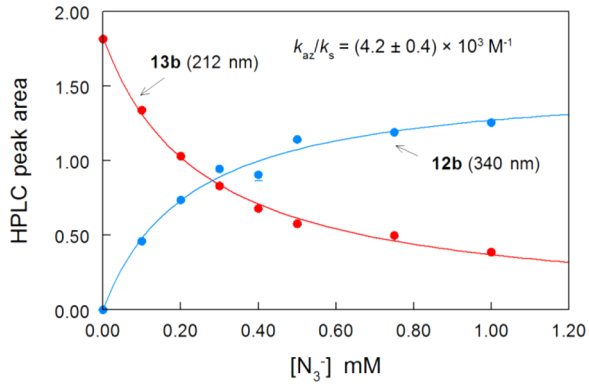


Figure 2. Determination of k_{az}/k_s for **11b** from the $[N_3^-]$-dependent yields of **12b** and **13b** monitored at the indicated wavelengths.

The selectivity ratio k_{az}/k_s is determined by application of the standard "azide clock" formulas to the product yields.[29,30,35,38] The azide/solvent selectivities for **11b−g** are summarized and compared to the value previously determined for **11a** in Table 1. The data show remarkably little dependence on the substituents within the ions that were under investigation. The ratios range over a factor of ca. 5 for all seven ions.

The rate constant k_{az} has been directly measured for **11a** at the apparently diffusion limited value of $(4.94 \pm 0.17) \times 10^9\ M^{-1}\ s^{-1}$.[29] If it is assumed that all the cations react with N_3^- at the same diffusion limited rate, the lifetime, $1/k_s$, of each cation can be estimated. The estimated lifetimes for **11b−g** are gathered in Table 1 with a comparison to the directly measured lifetime for **11a**. The lifetime of **11a** estimated from product yield data is identical to the directly measured value.[29] Lifetimes range from a low of 250 ns for **11d** to a high of 1150 ns for **11g**.

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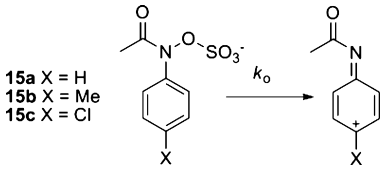
Both 3′-Me (**11b**) and 3′-Cl (**11c**) substituents kinetically stabilize the cation by a factor of about 1.6 compared to **11a**. The effect of 3′-Me is not surprising because Me is a weak EDG in aromatic linear free energy correlations and has been shown to kinetically stabilize arylnitrenium ions in aqueous solution when it is substituted ortho or para to the nitrenium center.[36,39,40] The effect of 3′-Cl is somewhat surprising, but there is precedent for moderate kinetic stabilization of arylnitrenium ions by Cl substituents that can interact with the positive charge via resonance.[36] Substituent effects for 5-F (**11d**) and 6-F (**11e**) differ. The 5-F substituent that is not in resonance contact with the positive charge moderately destabilizes the ion by a factor of ca. 2, while the 6-F substituent that can interact with the positive charge via resonance stabilizes the ion by a factor of ca. 1.4 compared to **11a**. It has previously been shown that the 4′-F substituent in a 4-biphenylylnitrenium ion kinetically stabilizes that cation by a factor of 2 in aqueous solution.[41] The effects of multiple substituents are approximately additive so that the lifetime of the 5-F, 3′-Me cation (**11f**) is approximately the same as **11a**, while the lifetime of the 6-F, 3′-Me cation (**11g**) is ca. 2-fold longer than that of **11a**. The cation that would be generated from Phortress, **2f**, is **11f**, so the lifetime of this ion is in the same range as the others and is remarkably similar to that of the unsubstituted ion.

Substituent Effects on Ionization of 9a−g. The most significant rate effect seen in these reactions is the large acceleration of ionization (k_o') of the 3′-Me-substituted esters **9b**, **9f**, and **9g** to form the corresponding cations. This appears to be predominately an electronic effect, although steric effects may also play a role in moderating the effect. It has previously been shown that the rate constants for hydrolysis of the *N*-sulfonatooxyacetanilides **15a−c** (Scheme 6) at 30 °C, the rate-determining step of which is ionization to form a nitrenium ion, occurs with rate constants, k_o, of 4.5×10^{-6} s^{-1} for **15a**, 1.3×10^{-3} s^{-1} for **15b**, and 9.5×10^{-6} for **15c**.[42] The 4-Me substituent stabilizes the transition state for ionization of these compounds much more than it stabilizes the nitrenium ion itself.[36] The 290-fold increase in k_o observed for **15b** compared to **15a** is 3- to 5-fold larger than the rate increase caused by the 3′-Me substituent observed in Table 1. Analysis of ortho-substituent effects for solvolysis of $ArC(Me)_2Cl$ in 90% aqueous acetone suggested that a modest 6-fold decrease in the solvolysis rate constant of the 2-Me-substituted compound compared to 4-Me is attributable to steric hindrance to formation of the coplanar carbocation.[43] The nitrenium ion **11** is also apparently planar[30] and would be expected to be subject to steric destabilization by ortho-substituents, although probably to a smaller extent than $ArC(Me)_2^+$. The expected combined magnitudes of the electronic acceleration and smaller steric destabilization would appear to explain the observed acceleration of hydrolysis of the 3′-Me-substituted esters **9b**, **9f**, and **9g**.

Scheme 6. Substituent Effects for Aqueous Solvolysis of 15a−c



Comparison of 4-(Benzothiazol-2-yl)phenylnitrenium Ions and Other Arylnitrenium Ions. We have previously noted that the aqueous solution lifetime and overall chemistry of **11a** is very similar to that of the 4-biphenylylnitrenium ion.[29−31] Experimental data and calculations agree that the 4-(benzothiazol-2-yl) substituent is as effective as a 4-phenyl substituent at stabilizing a fully formed arylnitrenium ion, but comparisons of ionization rate constants for the ester precursors show that this substituent destabilizes the transition state for formation of the cation in comparison to the 4-phenyl substituent.[29,30] The rate constant k_o' is ca. 100-fold smaller for **9a** than for the analogous ester precursor of the 4-biphenylylnitrenium ion, demonstrating that the inductive electron-withdrawing effect of this substituent dominates the electron-donating resonance effect in the transition state for ionization.[30] The substituted ions **11b−g** and their precursors **9b−9g** extend this comparison. These cations have lifetimes in water that are consistent with substituent effects on lifetimes observed for other arylnitrenium ions, and they generate similar reaction products with water and N_3^-. The magnitudes of the rate constants k_o' for ionization of **9b−g** are consistent with the inductive destabilization of the transition state for ionization that was previously observed for the parent ester, **9a**. Even the 60- to 100-fold increase in k_o' observed for the 3′-Me-substituted esters **9b**, **9f**, and **9g** in comparison to the corresponding esters without the 3′-Me substituent (**9a**, **9d**, **9e**) has quantitative precedent in the rate constants for the generation of other arylnitrenium ions in water.[36,42] The remarkable consistency in the properties of these ions in comparison to other arylnitrenium ions and the divergent inductive and resonance effects of the 4-(benzothiazol-2-yl) substituent both play a potential role in the biological activity of the parent amines as discussed below.

Implications for Antitumor Activity. None of the substituents have a major effect on the lifetimes of the nitrenium ion intermediates **11a−g**. Observed differences in the antitumor activity of the parent amines **1a−g** cannot be ascribed to differences in the stability of these ions. These differences are more readily attributable to differential metabolism of the amines. It has already been shown that 5-F (and 7-F) substituted amines such as **1d** and **1f** are less subject to metabolic deactivation through hydroxylation at C-6.[4] Suppression of metabolic deactivation coupled with a lack of significant change to the formation and reactivity of the nitrenium ion could account for the improved antitumor activity of these amines. It is important to note that the lifetimes of the nitrenium ions **11a−g** are in the same range (200−3000 ns) as those of nitrenium ions derived from well-known carcinogenic amines such as 2-aminofluorene and 4-aminobiphenyl.[35,37,41] The remarkable similarity of the lifetimes and chemistry of **11a** and the 4-biphenylylnitrenium ion has already been described.[29,30] Unless very carefully tuned for metabolic activation only in tumor cells, antitumor compounds that rely on nitrenium ion "warheads" are likely to cause unintended damage beyond the targeted tumor cells. The recent reports of pulmonary and liver toxicity encountered in clinical trials with Phortress suggest that the currently employed 2-(4-aminophenyl)benzothiazoles do not exhibit sufficiently selective metabolic activation.[8]

The most significant difference in the chemistry of derivatives of **1a−g** is the discovery in this study that the 3′-Me substituent

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has a remarkable effect on the reactivity of the esters **9b**, **9f**, and **9g**. In general, the 4-(benzothiazol-2-yl) substituent inhibits ionization of the esters **9a**−**g** in comparison to other nitrenium ion precursors, but the 3′-Me substituent accelerates ionization. The 3′-Me-substituted esters have aqueous solution lifetimes at 30 °C in the range of 5 to 10 s compared to 400 to 800 s for their counterparts without the 3′-Me substituent. This would likely limit the mobility of the 3′-Me-substituted esters to the cells in which metabolic activation occurs and would concentrate their effects within such cells. Two-photon flash photolysis studies of photorelease and diffusion through cells of caged fluorescein, a dye about the same size as **9a**−**g** with a similar, largely planar aromatic structure, has shown that diffusion from one lens fiber cell to adjoining cells is isotropic within deep regions of the lens and has a time constant of ca. 10 s at 21 °C.[44] Fluorescien has a pK_a of 6.4 and would be largely present in its anionic carboxylate form at physiological pH. The esters **9a**−**g** are uncharged at that pH. It appears that fluorescein diffuses across cell membranes via an unassisted process through gap junction channels.[44] However, if a specific ion channel-assisted mechanism enhances the diffusion of fluorescein anion across membranes, that mechanism would not be available to **9a**−**g**, so the fluorescein result would be an upper limit for diffusion of these uncharged esters. Diffusion of an uncharged chemical species with a lifetime of ca. 5−10 s from a tumor cell to nonadjoining cells more than a few cells removed would be unlikely. The reduced lifetime of the 3′-Me esters may improve the selectivity of the antitumor drug as long as the metabolic activation is confined to tumor cells.

EXPERIMENTAL SECTION

Kinetics and Azide Trapping Experiments. Kinetics experiments were carried out by UV−vis spectroscopy at 30 °C in aqueous 0.02 M phosphate buffers, pH 5.6 to 7.4, (5 vol% CH_3CN−H_2O, μ = 0.5 ($NaClO_4$)). Solutions were incubated in the thermostatted cell holder for ca. 20 min before kinetic runs were initiated by injecting 15 μL of ca. 5.0 mM solutions of **9b**−**g** in CH_3CN into 3 mL of the buffer to obtain a final concentration of ca. 2.5×10^{-5} M. The 3′-Me esters **9b**, **9f**, and **9g** were generated in situ (see below) in dry CH_3CN at ca. 5.0 mM concentration and were used for kinetic and product studies without isolation. Absorbance vs time data were collected at 327 nm for **9b**, 319, 250, 220, and 209 nm for **9c**, 319, 244, 222, and 209 nm for **9d**, 322, 300, 245, and 224 nm for **9e**, 330 nm for **9f**, and 320 nm for **9g**. Absorbance vs time data at each wavelength were fit to either a first-order or consecutive first-order rate equation.

A series of NaN_3 solutions from ca. 0.1 mM to 10 mM in [N_3^-] (exact concentration range depended on the ester) were prepared in 0.02 M phosphate buffer, pH 7.04, (5 vol% CH_3CN-H_2O, μ = 0.5 ($NaClO_4$)). Solutions were incubated at 30 °C in a water bath for ca. 20 min in a Teflon capped round-bottom reaction vial before 15 μL of a 5.0 mM solution of **9b**−**g** in CH_3CN was added to 3 mL of the buffer. A control experiment in phosphate buffer in the absence of NaN_3 was also included in each run. Reaction mixtures were analyzed in duplicate after completion of the reaction by 20 μL injections of the reaction mixture from each vial onto an HPLC (C-18 reverse phase column, 70/30 MeOH/H_2O eluent, 1 mL/min, monitored by UV absorbance). Wavelengths monitored were 340 and 212 nm for **9b**, **9c**, **9f**, and **9g**, and 338 and 220 nm for **9d** and **9e**. HPLC peak areas were plotted against [N_3^-] and fit to standard azide clock equations.[29,30,35,38] Kinetics of the decomposition of **9b**−**9g** were monitored in N_3^- containing buffers (1 to 50 mM [N_3^-]) at 327 nm for **9b**, 345, 319, and 268 nm for **9c**, 350, 326, and 260 nm for **9d**, 350, 321, 290, and 260 nm for **9e**, 330 nm for **9f**, and 324 nm for **9g**.

Synthesis. *Synthesis of the Hydroxylamines **8b**−**g**.* The nitro starting materials **16b**−**g** are known compounds that were prepared by published procedures.[4,23,32,33] The appropriate nitro compound **16b**−**g** (1.0 mmol) was added to a three-neck 100 mL round-bottom flask. Dry, freshly distilled THF (50 mL) was added, and the mixture was stirred under nitrogen while 0.314 g of 5% Pd/C was added. The flask was then put into an ethylene glycol−dry ice bath at −30 °C. After the flask had cooled for several minutes, 50 μL of hydrazine monohydrate was added via syringe and the mixture was stirred for 15 min. The flask was removed from the bath and allowed to reach room temperature. Reaction progress was monitored by TLC (20/80 EtOAc/hexanes for **16c**, **16d**, and **16e**; 10/90 EtOAc/CH_2Cl_2 for **16b**, **16f**, and **16g**). An additional 50 μL of hydrazine monohydrate was added after the reaction flask was immersed into the −30 °C bath again. The reaction mixture was monitored periodically by TLC until there was no sign of starting material. Occasionally, additional small amounts of hydrazine hydrate (in 10 μL aliquots) were added to completely remove the nitro compound before quenching the reaction mixture. The reaction mixture was then filtered through Celite and rotary evaporated. If necessary, the product was purified by flash chromatography on silica gel (10/90 EtOAc/CH_2Cl_2). Isolated yields ranged from 60% to 90%.

*N-(4-(Benzo[d]thiazol-2-yl)-2-methylphenyl)hydroxylamine (**8b**).* 153 mg (60%); mp 158−160 °C (decomp); IR 3200, 1600, 1460, 1422, 1273 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (3H, s), 7.17 (1H, d, J = 8.5 Hz), 7.37 (1H, t, J = 8.0 Hz), 7.48 (1H, t, J =8.0 Hz), 7.72, (1H, d, J = 1.5 Hz), 7.83 (1H, dd, J = 8.5, 1.5 Hz), 7.94 (1H, d J = 8.0 Hz), 8.04 (1H, d, J = 8.0 Hz), 8.49 (1H, s), 8.61 (1H, s); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 16.9, 111.5, 121.9, 122.07, 122.11, 123.2, 124.8, 126.3, 126.4, 128.4, 134.0, 152.7, 153.8, 168.0; high-resolution MS (ES, positive) $C_{14}H_{13}N_2OS$ (M + H) calcd 257.0749, found 257.0746.

*N-(4-(Benzo[d]thiazol-2-yl)-2-chlorophenyl)hydroxylamine (**8c**).* 249 mg (90%); mp 164−167 °C (decomp); IR 3268, 1600, 1469, 1435, 1219 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.27 (1H, d, J = 8.3 Hz), 7.40 (1H, t, J = 7.5 Hz), 7.50 (1H, t, J = 7.5 Hz), 7.91−7.98 (3H, m), 8.07 (1H, d, J = 7.8 Hz), 8.89 (1H, s) 8.95 (1H, s); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 114.0, 117.2, 122.6, 122.8, 124.5, 125.5, 127.0, 127.6, 127.8, 134.5, 150.4, 154.0, 166.8; high-resolution MS (ES, positive) $C_{13}H_{10}ClN_2OS$ (M + H) calcd 277.0202, 279.0172, found 277.0196, 279.0182.

*N-(4-(5-Fluorobenzo[d]thiazol-2-yl)phenyl)hydroxylamine (**8d**).* 237 mg (91%); mp 184−186 °C (decomp); IR 3205, 3133, 1602, 1567, 1471, 1450, 1244 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.91 (2H, d, J = 8.5 Hz), 7.25 (1H, dt, J = 8.9, 2.3 Hz), 7.75 (1H, dd, J = 9.9, 2.2 Hz), 7.87 (2H, d, J = 8.6 Hz), 8.07 (1H, m), 8.64 (1H, s), 8.91 (1H, s); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 108.6 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 23.9 Hz), 112.7 (2C, s), 113.3 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 24.6 Hz), 123.5, 123.7 (1C, d, $^3J^{13}_{C\text{-}}{}^{19}_{F}$ = 10.1 Hz), 128.7 (2C, s), 130.1, 155.2 (1C, d, $^3J^{13}_{C\text{-}}{}^{19}_{F}$ = 12.4 Hz), 155.5, 161.8 (1C, d, $^1J^{13}_{C\text{-}}{}^{19}_{F}$ = 240.5 Hz), 171.0; high-resolution MS (ES, positive) $C_{13}H_{10}FN_2OS$ (M + H) calcd 261.0498, found 261.0492.

*N-(4-(6-Fluorobenzo[d]thiazol-2-yl)phenyl)hydroxylamine (**8e**).* 227 mg (87%); mp 161−163 °C (decomp); IR 3290, 3132, 1601, 1567, 1479, 1452, 1245 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.92 (2H, d, J = 8.3 Hz), 7.33 (1H, m), 7.87 (2H, d, J = 8.3 Hz), 7.93−7.99 (2H, m), 8.63 (1H, s), 8.88 (1H,s); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 108.9 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 27.2 Hz), 112.8 (1C, d, $^3J^{13}_{C\text{-}}{}^{19}_{F}$ = 16.4 Hz), 115.0 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 26.2 Hz), 123.6 (2C, s), 123.7, 128.6 (2C, s), 135.6 (1C, d, $^3J^{13}_{C\text{-}}{}^{19}_{F}$ = 12.3 Hz), 151.1, 155.3, 159.9 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 242.3 Hz), 168.3; high-resolution MS (ES, positive) $C_{13}H_{10}FN_2OS$ (M + H) calcd 261.0498, found 261.0500.

*N-(4-(5-Fluorobenzo[d]thiazol-2-yl)-2-methylphenyl)-hydroxylamine (**8f**).* 235 mg (86%); mp 155−158 °C (decomp); IR 3179, 2900, 1603, 1570, 1524 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.17 (3H, s), 7.17 (1H, d, J = 8.5 Hz), 7.28 (1H, t, J = 9.0 Hz), 7.73 (1H, s), 7.78 (1H, d, J = 10.0 Hz), 7.83 (1H, d, J = 8.0 Hz), 8.10 (1H, m), 8.56 (1H, s), 8.62 (1H, s); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 17.3, 108.5 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 22.5 Hz), 111.8, 113.3 (1C, d, $^2J^{13}_{C\text{-}}{}^{19}_{F}$ = 23.75 Hz), 122.3, 123.3, 123.7 (1C, d, $^3J^{13}_{C\text{-}}{}^{19}_{F}$ = 10.0 Hz), 126.8, 128.8, 130.1, 153.3, 155.2 (1C, d, $^3J^{13}_{C\text{-}}{}^{19}_{F}$ = 12.5 Hz), 161.8 (1C, d, $^1J^{13}_{C\text{-}}{}^{19}_{F}$ = 238.8 Hz), 171.2; high-resolution MS (ES, positive) $C_{14}H_{12}FN_2OS$ (M + H) calcd 275.0654, found 275.0656.

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*N-(4-(6-Fluorobenzo[d]thiazol-2-yl)-2-methylphenyl)-hydroxylamine (**8g**).* 203 mg (74%); mp 178−182 °C (decomp); IR 3288, 3200 (br), 1608, 1570, 1455, 1265 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.16 (3H, s), 7.18 (1H, d, J = 8.0 Hz), 7.35 (1H, t, J= 8.0 Hz), 7.71 (1H, s), 7.80 (1H, d, J= 8.5 Hz), 7.94−7.98 (2H, m), 8.51 (1H, s), 8.62 (1H, s). ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 17.3, 109.0 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 25.6 Hz) (7.98), 111.9 (7.18), 115.1 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 25.0 Hz) (7.35), 122.3, 123.4, 123.6 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 8.8 Hz) (7.94), 126.7 (7.80), 128.7 (7.71), 135.6 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 12.1 Hz), 151.1, 153.1, 159.8 (1C, d, $^1J^{13}_{C-}{}^{19}_{F}$ = 241.8 Hz), 168.5; high-resolution MS (ES, positive) $C_{14}H_{12}FN_2OS$ (M + H) calcd 275.0654, found 275.0652.

*Synthesis of the Esters **9b−g**.* The stable esters **9c**, **9d**, and **9e** were made as previously described for **9a**.[29] The appropriate hydroxylamine (0.21 mmol) was added to 30 mL of dry, freshly distilled benzene containing 29 μL of *N*-ethylmorpholine (0.26 mmol). The suspension was stirred at room temperature for 15 min to maximize dissolution of the hydroxylamine. Pyruvonitrile (16.5 μL, 0.23 mmol) was added via syringe in 3−4 μL portions over a period of 10 min. As the pyruvonitrile was added, the hydroxylamine dissolved completely. After the last addition, the reaction mixture was stirred for another 15 min. The reaction mixture was transferred to a small separatory funnel and washed consecutively with 2 × 7 mL of ice cold 0.5 M NaOH, 2 × 7 mL of ice cold 5% aq $NaHCO_3$, and 2 × 7 mL of ice cold distilled water. The solution was dried over Na_2SO_4 and concentrated by rotary evaporation to obtain the ester in 50−70% isolated yield. Esters were purified by column or radial chromatography on silica gel using 20/80 EtOAc/hexanes. Purified compounds were stored at −40 °C and periodically repurified if necessary.

*O-Acetyl-N-(4-(benzo[d]thiazol-2-yl)-2-chlorophenyl)-hydroxylamine (**9c**).* 47 mg (70%) mp 93−96 °C (decomp); IR 3227, 1751, 1602, 1472, 1218 cm^{-1}; ^1H NMR (500 MHz, CD_3CN) δ 2.23 (3H, s), 7.20 (1H, d, J = 8.0 Hz), 7.41 (1H, t, J = 7.5 Hz), 7.51 (1H, t, J = 7.5 Hz), 7.98 (3H, m), 8.07 (1H, s), 8.92 (1H, s); ^{13}C NMR (125.8 MHz, CD_3CN) δ 18.9, 116.9, 120.4, 122.9, 123.7, 126.4, 127.5, 128.1, 128.8, 129.5, 135.8, 146.8, 154.9, 166.9, 170.9; high-resolution MS (ES, positive) $C_{15}H_{11}ClNaN_2O_2S$ (M + Na) calcd 341.0127, 343.0097, found 341.0129, 343.0060.

*O-Acetyl-N-(4-(5-fluorobenzo[d]thiazol-2-yl)phenyl)-hydroxylamine (**9d**).* 39 mg (61%); mp 68−70 °C (decomp); IR 3248, 1739, 1608, 1453, 1246 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2) δ 2.23 (3H, s), 7.10 (2H, d, J = 8.7 Hz), 7.14 (1H, td J = 8.9, 2.4 Hz), 7.68 (1H, dd, J = 9.7, 2.4 Hz), 7.83 (1H, dd, J = 8.8, 5.2 Hz), 8.01 (2H, d, J = 8.6 Hz), 8.87 (1H, s); ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 18.8, 108.9 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 23.9 Hz), 113.4 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 25.1 Hz), 115.4 (2C, s), 122.4 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 10.0 Hz), 128.4, 128.5 (2C, s), 130.4, 149.6, 155.2 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 12.0 Hz), 162.0 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 242.4 Hz), 169.9, 170.2; high-resolution MS (ES, positive) $C_{15}H_{11}FNaN_2O_2S$ (M + Na) calcd 325.0423, found 325.0420.

*O-Acetyl-N-(4-(6-fluorobenzo[d]thiazol-2-yl)phenyl)-hydroxylamine (**9e**).* 35 mg (55%); mp 99−101 °C (decomp); IR 3239, 1766, 1609, 1455, 1223 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2-d_2) δ 2.29 (3H, s), 7.15 (2H, d, J = 8.5 Hz), 7.26 (1H, td, J = 9.0, 2.6 Hz), 7.64 (1H, d, J = 8.2, 2.6 Hz), 7.99 (1H, dd, J = 8.8, 4.8 Hz), 8.04 (2H, d, J = 8.6 Hz), 8.92 (1H, s); ^{13}C NMR (125.8 MHz, CD_2Cl_2-d_2) δ 18.8, 107.8 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 27.0 Hz), 114.7 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 24.6 Hz), 115.5 (2C,s), 123.8 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 9.3 Hz), 128.4 (2C,s), 128.5, 135.9 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 11.3 Hz), 149.4, 150.9, 160.4 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 244.6 Hz), 167.1, 170.2; high-resolution MS (ES, positive) $C_{15}H_{11}FNaN_2O_2S$ (M + Na) calcd 325.0423, found 325.0420.

The esters **9b**, **9f**, and **9g** were too reactive to isolate. Instead they were prepared in dry CH_3CN. A general procedure follows. The appropriate hydroxylamine (0.25 mmol) was dissolved in 50 mL of dry CH_3CN and stirred under N_2 at 0 to −10 °C for 10 min followed by addition of 33 μL (0.30 mmol) of *N*-ethylmorpholine. Pyruvonitrile (18.0 μL, 0.25 mmol) was added in 6.0 μL fractions every 10 min over a period of 20 min. The solution was left under N_2 at 0 °C for 1 h after the last addition of pyruvonitrile. Disappearance of the hydroxylamine and appearance of a new spot was detected by TLC of the reaction mixture on silica gel (20/80 EtOAc/hexanes). Solutions were stored at −40 °C for no more than one to two days for use in kinetics or product isolation studies.

Product Isolation. *Quinols **13b−g**.* For **13c**, the corresponding ester **9c** (0.25 mmol) was dissolved in 20 mL of CH_3CN. That solution was added in 1 mL aliquots at 4 h intervals to a flask containing 500 mL of pH 7.0 phosphate buffer, identical to that used in the kinetic studies, incubated at 30 °C in a constant temperature water bath. After the last addition, the reaction flask was incubated for an additional 12 h at 30 °C prior to extraction with 3 × 100 mL of CH_2Cl_2. The CH_2Cl_2 extract was dried over Na_2SO_4 and rotary evaporated to obtain a dark green solid that was purified by radial chromatography on silica gel (60/40 EtOAc/hexanes) to obtain 21 mg (30%) of **13c**.

For **13d**, the 20 mL solution of **9d** was added to the buffer in 1 mL aliquots every 15 min, and the mixture was incubated for 3 h before extraction. The dark yellow-brown solid obtained after rotary evaporation was purified by column chromatography on silica gel (20/80 EtOAc/hexanes), followed by radial chromatography on silica gel (15/85 EtOAc/CH_2Cl_2) to obtain 21 mg (33%) of **13d**.

For **13e**, the 20 mL solution of **9e** was added to the buffer in 1 mL aliquots every 30 min, and the mixture was incubated for 5 h before extraction. The dark yellow-brown solid was purified in the same way as **13d** to obtain 19 mg (30%) of **13e**.

For **13b**, **13f**, or **13g** the 50 mL CH_3CN solution of **9b**, **9f**, or **9g** described above was added in 3 mL aliquots at 10 min intervals to a flask containing 500 mL of pH 7.0 phosphate buffer incubated at 30 °C in a constant temperature water bath. After the last addition, the reaction flask was incubated for an additional 24 h at 30 °C. The reaction mixture was extracted with 3 × 100 mL of CH_2Cl_2. The CH_2Cl_2 extract was dried over Na_2SO_4 and rotary evaporated to obtain a yellowish solid that was purified by radial chromatography on silica gel (10/90 EtOAc/CH_2Cl_2) to obtain 15 mg (23%) of **13b**, 15 mg (22%) of **13f**, and 14 mg (20%) of **13g**.

*4-(Benzo[d]thiazol-2-yl)-4-hydroxy-2-methylcyclohexa-2,5-dienone (**13b**).* mp 175−176 °C; IR 3320 (br), 1672, 1641, 1503, 1436, 1052 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2) δ 1.93 (3H, d, J = 1.5 Hz), 4.24 (1H, s (br)), 6.30 (1H, d, J = 9.5 Hz), 6.77 (1H, m), 6.98 (1H, dd, J = 9.8, 3.1 Hz), 7.43 (1H, td, J = 7.1, 1.2 Hz), 7.52 (1H, td, J = 7.3, 1.2 Hz), 7.91 (1H, d, J = 7.7 Hz), 7.99 (1H, d, J = 8.2 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 15.3 (1.93), 71.3, 122.0 (7.91), 123.1 (7.99), 125.7 (7.43), 126.5 (7.52), 128.4 (6.30), 135.5, 135.8, 142.8 (6.77), 147.3 (6.98), 152.5, 171.4, 185.3; high-resolution MS (ES, positive) $C_{14}H_{11}NaNO_2S$ (M + Na) calcd 280.0408, found 280.0406.

*4-(Benzo[d]thiazol-2-yl)-2-chloro-4-hydroxycyclohexa-2,5-dienone (**13c**).* mp 139−141 °C; IR 3270 (br), 1678. 1620, 1471, 1436, 1286 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.43 (1H, s (br)), 6.45 (1H, d, J = 9.9 Hz), 7.07 (1H, dd, J = 9.9, 2.9 Hz), 7.20 (1H, d, J = 2.9 Hz), 7.46 (1H, td, J = 8.2, 1.1 Hz), 7.54 (1H, td, J = 8.3, 1.2 Hz), 7.91 (1H, d, J = 8.0 Hz), 8.04 (1H, d, J = 8.2 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 72.6, 122.1 (7.91), 123.3 (8.04), 126.2 (7.46), 126.8 (7.54), 127.7 (6.45), 133.3, 135.6, 143.4(7.20), 147.7 (7.07), 152.0, 169.5, 177.9; high-resolution MS (ES, positive) $C_{13}H_8ClNaNO_2S$ (M + Na) calcd 299.9862, 301.9832, found 299.9855, 301.9833.

*4-(5-Fluorobenzo[d]thiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (**13d**).* mp 128−130 °C; IR 3370 (br), 1663, 1617, 1453, 1140 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2-d_2) δ 4.2 (1H, s (br)), 6.32 (2H, d, J = 10.0 Hz), 6.99 (2H, d, J = 10.0 Hz), 7.23 (1H, td, 8.8, 2.5 Hz), 7.69 (1H, dd, J = 9.4, 2.4 Hz), 7.87 (1H, dd, 8.5, 5.0 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2-d_2) δ 71.0, 109.3 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 23.7 Hz), 114.6 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 25.2 Hz), 123.0 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 10.0 Hz), 128.7 (2C, s), 131.4, 147.1 (2C, s), 153.5 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 12.1 Hz), 161.9 (1C, d, $^1J^{13}_{C-}{}^{19}_{F}$ = 243.9 Hz), 173.4, 184.5; high-resolution MS (ES, positive) $C_{13}H_8FNaNO_2S$ (M + Na) calcd 284.0157, found 284.0157.

*4-(6-Fluorobenzo[d]thiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (**13e**).* mp 168−170 °C; IR 3333 (br), 1669, 1626, 1505, 1455, 1251, 1198 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2-d_2) δ 4.08 (1H, s), 6.31 (2H, d, J = 10.5 Hz), 6.98 (2H, d, J = 10.0 Hz), 7.26 (1H, td, J = 9.0, 2.5 Hz)), 7.60 (1H, dd, J = 8.2, 2.6 Hz), 7.95 (1H, dd, J = 9.0, 5.0 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2-d_2) δ 70.9, 108.2 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$

= 26.9 Hz), 115.3 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 24.8 Hz), 124.3 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 9.5 Hz), 128.7 (2C, s), 136.9 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 11.1 Hz), 147.1 (2C, s), 149.3, 160.7 (1C, d, $^1J^{13}_{C-}{}^{19}_{F}$ = 246.2 Hz), 170.3, 184.4; high-resolution MS (ES, positive) $C_{13}H_8FNaNO_2S$ (M + Na) calcd 284.0157, found 284.0147.

*4-(5-Fluorobenzo[d]thiazol-2-yl)-4-hydroxy-2-methylcyclohexa-2,5-dienone (**13f**).* mp 99−100 °C; IR: 3276 (br), 1670, 1626, 1602, 1455, 1147, 1125 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.98 (3H, s), 4.00 (1H, s(br)), 6.35 (1H, d, J = 10.0 Hz), 6.79 (1H, s), 7.00 (dd, J = 9.5, 3.0 Hz), 7.30 (1H, m), 7.64 (1H, m), 8.00 (1H, dd, J = 9.0, 5.0 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 15.4, 71.3, 109.2 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ =23.9 Hz), 114.4 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ =25.2 Hz), 122.9 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 10.0 Hz), 128.6, 135.7, 142.4, 150.6, 153.5 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 12.6 Hz), 161.9 (1C, d, $^1J^{13}_{C-}{}^{19}_{F}$ =244.1 Hz), 174.1, 185.2; high-resolution MS (ES, positive) $C_{14}H_{10}FNaNO_2S$ (M + Na) calcd 298.0314, found 298.0312.

*4-(6-Fluorobenzo[d]thiazol-2-yl)-4-hydroxy-2-methylcyclohexa-2,5-dienone (**13g**).* mp 120−122 °C; IR 3240 (br), 1671, 1627, 1603, 1456, 1148, 1125 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.97 (3H, s), 3.99 (1H, s(br)), 6.34 (1H, d, J = 9.5 Hz), 6.79 (1H, s), 6.99 (1H, m), 7.29 (1H, t, J = 7.8 Hz), 7.64 (1H, d, J = 6.0 Hz), 7.99 (1H, m); ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 15.4, 71.3, 108.1 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 26.5 Hz), 115.1 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 25.3 Hz), 124.2 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 9.9 Hz), 128.6, 135.7, 136.9 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ =11.5 Hz), 142.4, 147.0, 149.3, 160.6 (1C, d, $^1J^{13}_{C-}{}^{19}_{F}$ =245.0 Hz), 171.2, 185.1; high-resolution MS (ES, positive) $C_{14}H_{10}FNaNO_2S$ (M + Na) calcd 298.0314, found 298.0311.

*Azide Adducts **12b**, **12c**, and **12f**. 2-Azido-4-(benzo[d]thiazol-2-yl)-6-methylaniline (**12b**).* The 50 mL CH_3CN solution of **9b** described above was injected in 1 mL aliquots at 5 s intervals into 500 mL of pH 7.0 phosphate buffer containing 10 mM NaN_3, identical to that used in the kinetics, incubated at 30 °C in a constant temperature water bath. After the last addition, the reaction mixture was kept at 30 °C for 3 h prior to extraction with 3 × 100 mL of CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4 and then rotary evaporated to get crude solid which was purified by radial chromatography on silica gel (20/80 EtOAc/hexanes) to obtain 22 mg (31%) of **12b**: mp 86−89 °C; IR 3390, 2111, 1620, 1475, 1436, 1420, 1328 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 2.23 (3H, s), 4.19 (2H, s (br)), 7.35 (1H, td, J = 7.3, 1.2 Hz), 7.46 (1H, td, J = 7.3, 1.2 Hz), 7.57 (1H, m), 7.73 (1H, d, J = 1.8 Hz), 7.89 (1H, dt, J = 8.0, 0.5 Hz), 7.97 (1H, dt, J = 8.0, 0.5 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 17.5 (2.23), 115.3 (7.73), 121.9 (7.89), 122.8 (7.97), 123.4, 123.9, 125.0 (7.35), 125.4, 126.5 (7.46), 126.8 (7.57), 135.1, 139.9, 154.6, 167.9; high-resolution MS (ES, positive) $C_{14}H_{11}NaN_5S$ (M + Na) calcd 304.0633, found 304.0624.

*2-Azido-4-(benzo[d]thiazol-2-yl)-6-chloroaniline (**12c**).* The ester **9c** (79 mg, 0.25 mmol) was dissolved in 20 mL of CH_3CN. That solution was added in 1 mL aliquots at 10 min intervals to 500 mL of a pH 7.0 phosphate buffer containing 50 mM NaN_3 incubated at 30 °C in a constant temperature water bath. After the last addition, the reaction mixture was kept at 30 °C for 2 h prior to extraction with 3 × 100 mL of CH_2Cl_2. The CH_2Cl_2 extract was dried over Na_2SO_4 and rotary evaporated to obtain a solid that was purified by radial chromatography on silica gel (20/80 EtOAc/hexanes) to obtain 25 mg (33%) of **12c**: mp 79−80 °C; IR 3382, 2108, 1613, 1471, 1221 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 4.65 (2H, s (br)), 7.41 (1H, t, J = 7.5 Hz), 7.52 (1H, t, J = 7.8 Hz), 7.82 (1H, s), 7.82 (1H, s), 7.94 (1H, d, J = 8.0 Hz), 8.02 (1H, d, J = 8.0 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2) 115.4, 119.1, 121.6, 122.7, 123.8,124.9, 125.0, 126.33, 126.36, 134.8, 137.8, 154.1, 166.0; high-resolution MS (ES, positive) $C_{13}H_8ClNaN_5S$ (M + Na) calcd 324.0087, 326.0057, found 324.0078, 326.0075.

*2-Azido-4-(5-fluorobenzo[d]thiazol-2-yl)-6-methylaniline (**12f**).* The 50 mL CH_3CN solution of **9b** described above was added in 2.5 mL aliquots at 15 min intervals to 500 mL of a pH 7.0 phosphate buffer containing 50 mM NaN_3 incubated at 30 °C in a constant temperature water bath. After the last addition, the reaction mixture was kept at 30 °C for 72 h prior to extraction with 3 × 100 mL of CH_2Cl_2. The CH_2Cl_2 extract was dried over $MgSO_4$ and rotary evaporated to obtain a yellow solid that was purified by radial chromatography (30/70 hexanes/CH_2Cl_2) to obtain 14 mg (18%) of **12f**: mp 86−88 °C; IR 3385, 2111, 1602, 1567, 1453, 1122 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.27 (3H, s), 4.26 (2H, s(br)), 7.15 (1H, td, J = 9.0, 2.5 Hz), 7.60 (1H, s), 7.67 (1H, dd, J = 10.0, 2.5 Hz), 7.75 (1H, d, J=2.0 Hz), 7.85 (1H, dd, J=8.5, 5.0 Hz); ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 17.1, 108.5 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 26.4 Hz), 112.9 (1C, d, $^2J^{13}_{C-}{}^{19}_{F}$ = 26.4 Hz), 114.9, 122.2 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 9.9 Hz), 123.0, 123.2, 125.0, 126.5, 130.2, 139.8, 155.2 (1C, d, $^3J^{13}_{C-}{}^{19}_{F}$ = 13.2 Hz), 161.9 (1C, d, $^1J^{13}_{C-}{}^{19}_{F}$ = 241.5 Hz), 170.1; high-resolution MS (ES, positive) $C_{14}H_{10}FNaN_5S$ (M + Na) calcd 322.0539, found 322.0531.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables S1 through S6, containing a summary of individual rate constants obtained in this study; Figures S1 and S2, HPLC chromatograms of the kinetic reaction mixtures for **9c** and **9g**; Figure S3, repetitive wavelength scans for the hydrolysis of **9d** and **9e**; Figure S4, repetitive wavelength scans for the decomposition of **9d** and **9e** in the presence of N_3^-; ^{1}H and ^{13}C NMR data for **8b−g**, **9c−e**, **12b**, **12c**, **12f**, and **13b−g**. 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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