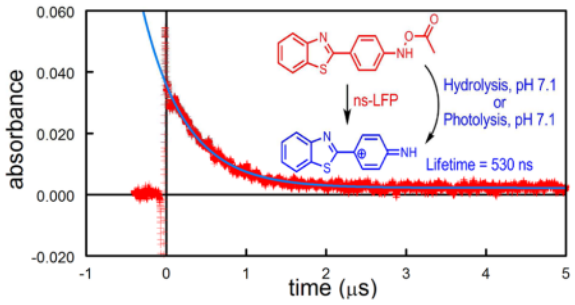
Published in final edited form as:
Org Lett. 2009 November 5; 11(21): 4862–4865. doi:10.1021/ol901959z.

Indirect and Direct Detection of the 4-(Benzothiazol-2-yl) phenylnitrenium Ion from a Putative Metabolite of a Model Anti-Tumor Drug

Mrinal Chakraborty[†], **Kyoung Joo Jin**[†], **Samuel C. Brewer III**[†], **Huo-Lei Peng**[§], **Matthew S. Platz**[§], and **Michael Novak**[*,†]
Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio 45056, and Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio 43210

Abstract



2-(4-Aminophenyl)benzothiazoles related to 1 are potentially important pharmaceuticals. Metabolism apparently involves oxidation and esterification to 3. In water, hydrolysis and photolysis of 3 generates the nitrenium ion 4 that can be detected indirectly by N_3^- trapping and directly by UV-vis spectroscopy following laser flash photolysis. The transient, with λ_{max} 570 nm, and a lifetime of 530 ns, reacts with N_3^- at a diffusion-controlled rate and generates the quinol 6 by reaction with water.

Benzothiazole derivatives such as 2-(4-aminophenyl)benzothiazole, **1**, are under investigation as anti-tumor, antifungal, and antibacterial agents,[1–3] and as radiopharmaceuticals for binding and *in vivo* imaging of Aβ-plaques, one of the earliest pathological processes in the development of Alzheimer's disease.[4] One anti-tumor derivative of **1** is currently in Phase 1 clinical trials in Great Britain.[5] The use of **1** and its derivatives as anti-tumor agents requires biological activation.[5,6] The proposed metabolism of **1** to form the active agent **3** is shown in Scheme 1, although neither **2** nor **3** had been isolated and characterized. It is presumed that **3** further decomposes into a reactive electrophile, but no direct evidence for this proposal has been presented.[7]

We have succeeded in synthesizing both **2** and **3** from 2-(4-nitrophenyl)benzothiazole using procedures we previously developed for making similar derivatives of carcinogenic aromatic amines (Scheme 2).[8] Reduction of the nitro compound[9] with hydrazine hydrate in the presence

novakm@muohio.edu.
[†]Miami University
[§]The Ohio State University

Supporting Information Available Experimental details, a Table of rate constants, Figure S1, synthesis of **2** and **3**, NMR spectra of **2** and **3**. This material is available free of charge via the internet at http://pubs.acs.org.

of 5% Pd/C catalyst generates **2** in moderate yield, while tratment of **2** with acetyl cyanide in the presence of *N*-ethylmorpholine provides **3** in satisfactory yield. We now report the indirect and direct detection of nitrenium ion **4** (Scheme 3) from hydrolysis and photolysis of **3**.

Kinetics of the decomposition of **3** (2.5×10^{-5} M) at pH 7.1 in phosphate buffer, and the formation of the major hydrolysis product **6** (Scheme 3, identified by HPLC and ^{1}H NMR comparison to an authentic sample[10]) monitored by UV spectroscopy, are described by two pseudo-first-order rate constants, k_o and k_1. HPLC studies (Figure 1A) show that the larger rate constant, k_o governs the decay of **3**, while the appearance of **6** is biphasic, and is fit well by a rate equation for two consecutive first-order reactions. The larger rate constant generated by the fit is equivalent in magnitude to k_o measured for the disappearance of **3**. The rate of appearance of **6** is limited by the smaller rate constant, k_1. Kinetics of the appearance of **6** are consistent with its formation from a long-lived intermediate (lifetime ca. 2 h at 10 °C) that is generated by hydrolysis of **3**. Steady-state photolysis of an identical aqueous solution of **3** for 30 s with UVB lamps leads to photo-decomposition of 96% of **3** (Figure 1B). Generation of **6** now occurs via a simple first-order process governed by k_1. Correction for the small amount of **3** remaining after photolysis shows that 92% of the observed yield of **6** under photolysis conditions is due to photo-decomposition of **3**.

The results show that **6** is generated both by hydrolysis and photolysis of **3**, and suggest that a common pathway is involved in both processes.

Addition of N_3^- to the hydrolysis solution does not affect the rate of decomposition of **3**, but does significantly decrease the yield of **6** at very low [N_3^-] (Figure 2), demonstrating that N_3^- traps a reactive intermediate produced in a rate limiting step. As the yield of **6** decreases, the yields of two new products, not generated in the absence of N_3^-, increase. Application of the "azide clock" equations[11] to the yields of these three products generates the experimental k_{az}/k_s shown in Figure 2. Although the azide products have not yet been characterized, the structure of **6** and the trapping results show that N_3^- competes with the solvent for a selective cationic intermediate, **4**. The kinetics of the formation of **6** during hydrolysis of **3** implicates **5** as a precursor, although **5** has not yet been detected.

Laser flash photolysis (LFP) of **3** in O_2-saturated pH 7.1 phosphate buffer at 308 nm generates a transient UV spectrum with λ_{max} ca. 570 nm (Figure 3). The absorbance at 570 nm decays in a first-order manner (Supporting Information, Figure S1). The rate constant, k_{obs}, increases linearly with increasing [N_3^-] (Figure 4).

The slope of that plot is k_{az}, the second-order rate constant for reaction of N_3^- with the reactive intermediate, while the intercept is k_s, the pseudo-first-order rate constant for reaction of the intermediate with the aqueous solvent. The ratio k_{az}/k_s of $(2.64 \pm 0.13) \times 10^3$ M^{-1} is identical to that obtained from the azide-trapping experiments, demonstrating that both experiments detect the same intermediate, **4**, with a lifetime ($1/k_s$) of ca. 530 ns.

Scheme 3 summarizes the results of our experiments. This scheme is similar to that previously demonstrated for the decomposition of ester derivatives of carcinogenic aromatic hydroxylamines.[12] The cation **4** is about as selective as the 4-biphenylylnitrenium ion, **7** ($k_{az}/k_s = 2.9 \times 10^3$ M^{-1}) that also yields a quinol, **8**, as its major hydration product.[12] The intermediate detected after LFP is definitely **4**, not the imine **5** because the kinetics performed by UV spectroscopy and HPLC show that **5** has a lifetime of about 30 min at room temperature, while the transient generated during the LFP experiments has a lifetime of 530 ns.

An apparent imine intermediate can be detected by HPLC during the conversion of **7** into **8**.[12] This species has a lifetime of ca. 6 h at room temperature, while **7** has a lifetime of 560 ns

under the same conditions.[12] The quinol product **6** is also the hydration product of the related oxenium ion **9** (Scheme 4).[10]

The azide adduct identified in that study is **10**, and k_{az}/k_s for **9** at 80 °C is 310 M^{-1}.[10] The structure of **10** demonstrates that the charge in **9** is highly delocalized, and k_{az}/k_s comparisons to other oxenium ions show that the azide/solvent selectivity of **9** is similar to the 4-biphenylyloxenium ion, **11**.[10] Since **4**,**7**, **9**, and **11** react with N_3^- at or near the diffusion controlled limit, the aqueous solution lifetimes of **4** and **7** and also of **9** and **11** are very similar.[10,12,13] These results show that the 4-(benzothiazol-2-yl) group behaves as a significantly delocalizing and stabilizing substituent for both oxenium and nitrenium ions.

It is now apparent that putative metabolites of anti-tumor benzothiazoles will give rise to selective, long lived nitrenium ions in aqueous solution. Although metabolites of carcinogenc aromatic amines have long been known to generate highly selective nitrenium ion intermediates in aqueous solution,[12] this is, to the best of our knowledge, the first demonstration that a putative metabolite of an anti-tumor drug also generates such an intermediate. We are continuing this study with an emphasis on the reaction of **4** and related nitrenium ions with biological nucleophiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Donors of the American Chemical Society Petroleum Research Fund (Grant # 43176-AC4) and the NIH/NIGMS (Grant # R15 GM088751-01) for support of this work. KJJ thanks Miami University for an HHMI Summer Undergraduate Research Fellowship, and SCB thanks the OCUR-REEL program supported by the Chemical Division of NSF for an undergraduate summer research fellowship. The support of the NSF in Columbus and the OSU Center for Chemical and Biophysical Dynamics is greatly appreciated.

References

1. Shi DF, Bradshaw TD, Wrigley S, McCall CJ, Lelieveld P, Fichtner I, Stevens MFG. J Med Chem 1996;39:3375–3384. [PubMed: 8765521]Bradshaw TD, Wrigley S, Shi DF, Schultz RJ, Paull KD, Stevens MFG. Brit J Cancer 1998;77:745–752. [PubMed: 9514053]Hutchinson I, Jennings SA, Vishnuvajjala BR, Westwell AD, Stevens MFG. J Med Chem 2002;45:744–747. [PubMed: 11806726]
2. Yildiz-Oren I, Yalcin I, Aki-Sener E, Ucarturk N. Eur J Med Chem 2004;39:291–298. [PubMed: 15051178]
3. Ra CS, Jung BY, Park G. Heterocycles 2004;62:793–802.
4. Tzanopoulou S, Pirmettis IC, Patsis G, Paravatou-Petsotas M, Livaniou E, Papadopoulos M, Pelecanou M. J Med Chem 2006;49:5408–5410. [PubMed: 16942013]
5. Bradshaw TD, Westwell AD. Curr Med Chem 2004;11:1009–1021. [PubMed: 15078163]Brantley E, Antony S, Kohlhagen G, Meng L, Agama K, Stinson SF, Sausville EA, Pommier Y. Cancer Chemoth Pharm 2006;58:62–72.
6. Chua MS, Kashiyama E, Bradshaw TD, Stinson SF, Brantley E, Sausville EA, Stevens MFG. Cancer Res 2000;60:5196–5203. [PubMed: 11016648]
7. O'Brien SE, Browne HL, Bradshaw TD, Westwell AD, Stevens MFG, Laughton CA. Org Biomol Chem 2003;1:493–497. [PubMed: 12926251]Hilal R, Khalek AAA, Elroby SAK. THEOCHEM 2005;731:115–121.
8. Kazerani S, Novak M. J Org Chem 1998;63:895–897. [PubMed: 11672091]Novak M, Kazerani S. J Am Chem Soc 2000;122:3606–3616.
9. Stevens MFG, Shi DF, Castro A. J Chem Soc, Perkin Trans 1;1996:83–93.
10. Wang YT, Jin KJ, Myers LR, Glover SA, Novak M. J Org Chem 2009;74:4463–4471. [PubMed: 19476314]

11. Richard JP, Jencks WP. J Am Chem Soc 1982;104:4689–4691.Richard JP, Jencks WP. J Am Chem Soc 1982;104:4691–4692.Richard JP, Jencks WP. J Am Chem Soc 1984;106:1383–1396.
12. Novak M, Kahley MJ, Eiger E, Helmick JS, Peters HE. J Am Chem Soc 1993;115:9453–9460.McClelland RA, Davidse PA, Hadzialic G. J Am Chem Soc 1995;117:4173–4174.
13. Novak M, Glover SA. J Am Chem Soc 2004;126:7748–7749. [PubMed: 15212501]Wang YT, Wang J, Platz MS, Novak M. J Am Chem Soc 2007;129:14566–14567. [PubMed: 17985908]

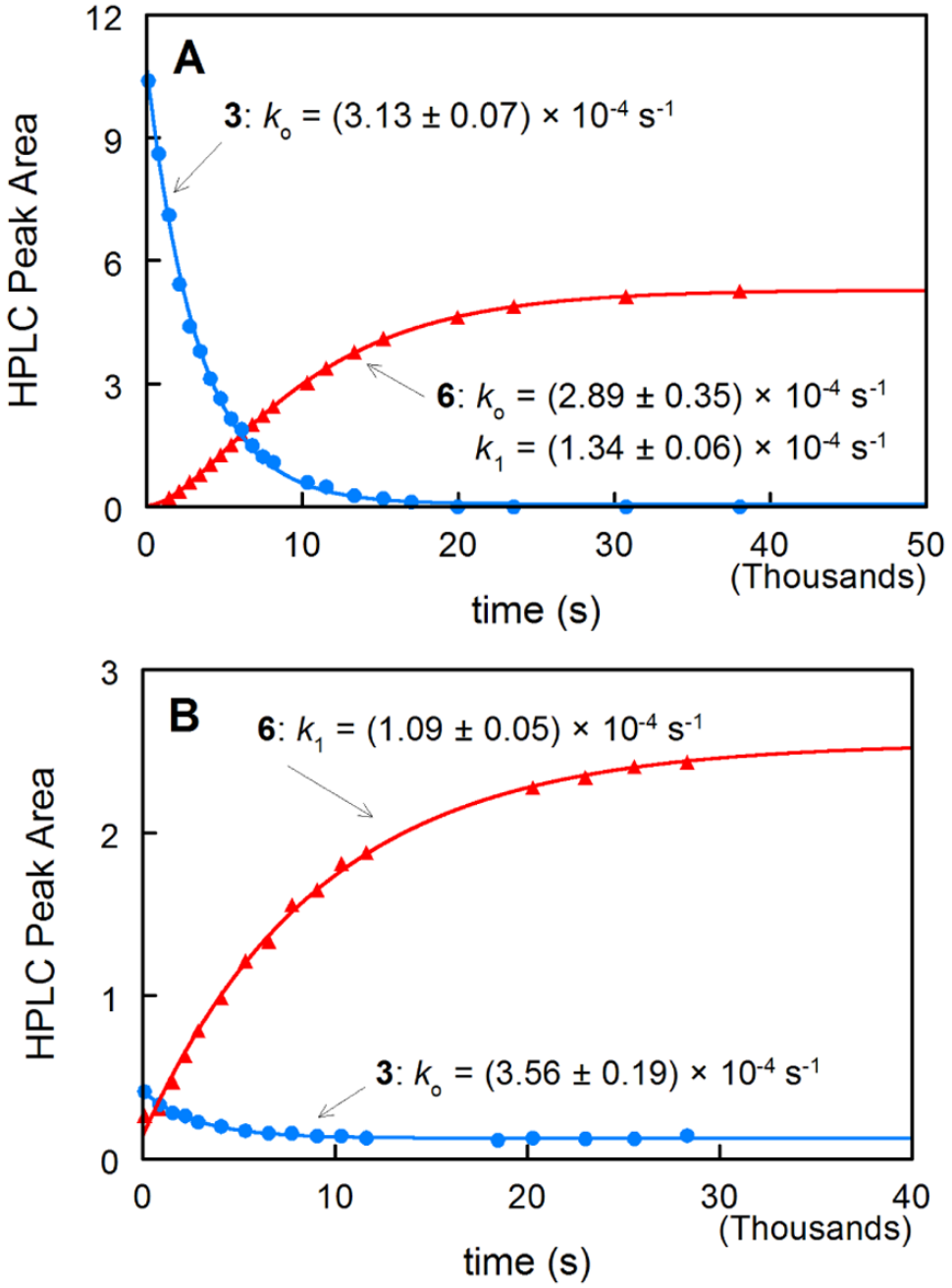


Figure 1.
Time course for the disappearance of **3** and formation of **6** at 10 °C in pH 7.1 phosphate buffer monitored by HPLC with UV detection at 212 nm: (A) hydrolysis reaction in the dark, (B) after steady-state photolysis for 30 s. Rate constants were obtained from fits to single or double exponential rate equations.

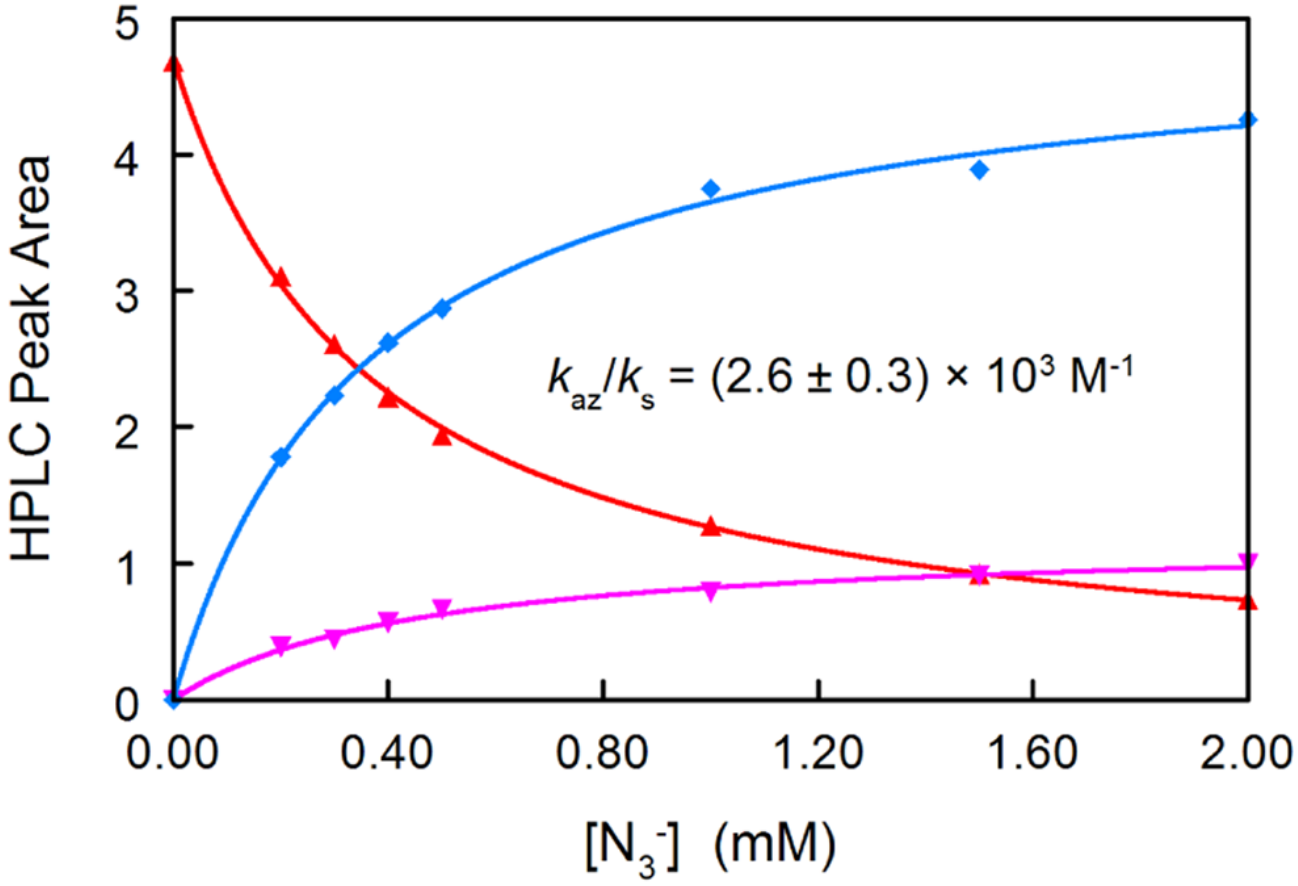


Figure 2.
Results of azide trapping experiment in pH 7.1 phosphate buffer at 30 °C. Key: **6**(▲, 212 nm), apparent major azide adduct (♦, 330 nm), apparent minor azide adduct (▼, 212 nm). The k_{az}/k_s is the average of the fit of all three materials to the standard "azide clock" formulae.

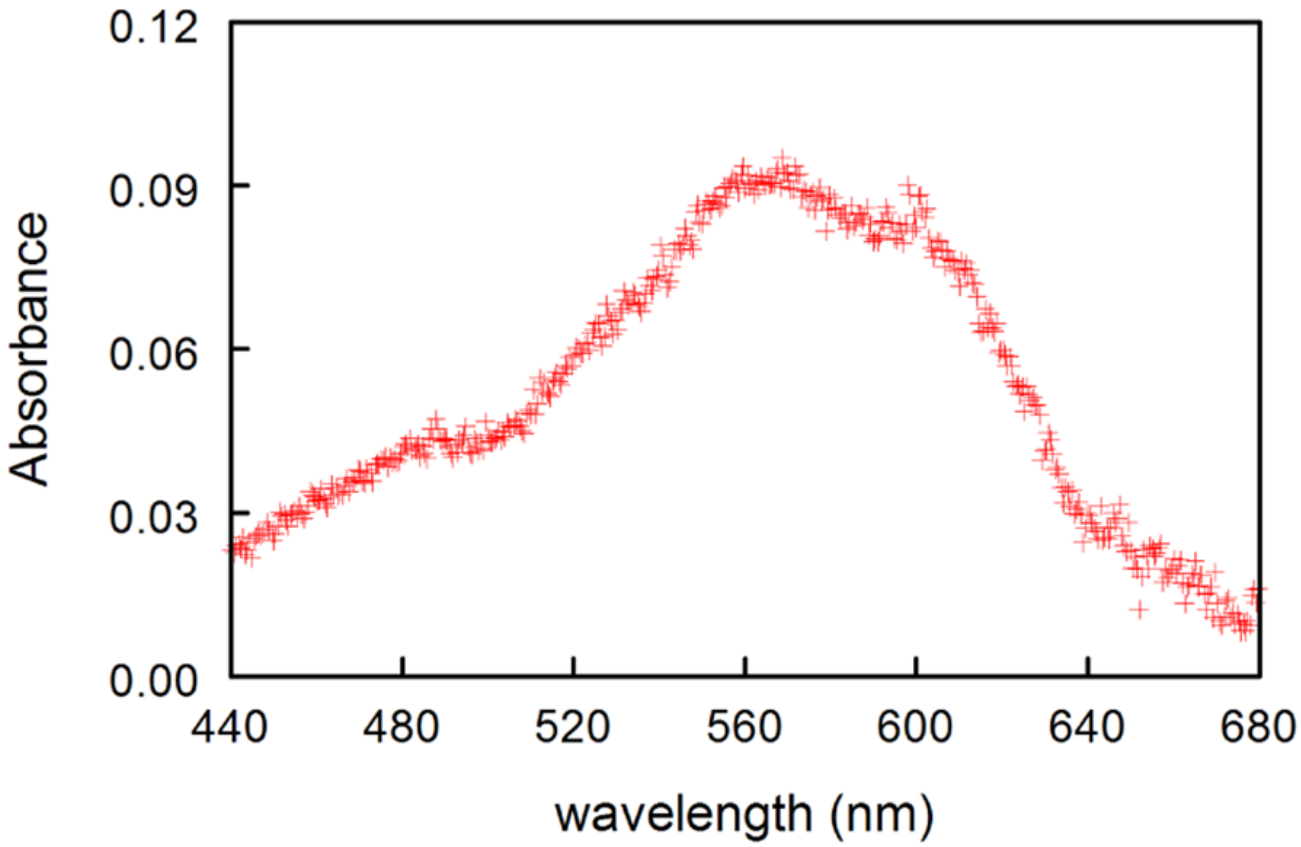


Figure 3.
Transient absorbance spectrum obtained 20 ns after 308 nm excitation of **3** in O_2-saturated pH 7.1 phosphate buffer. Spectrum recorded with a 20 ns window.

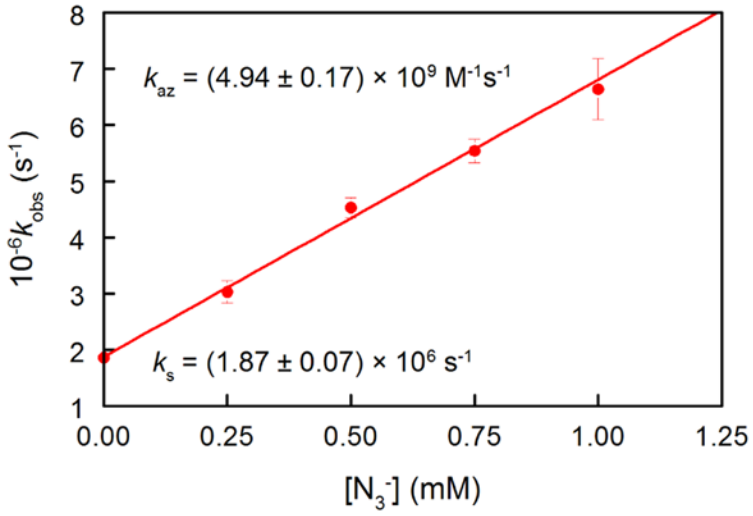
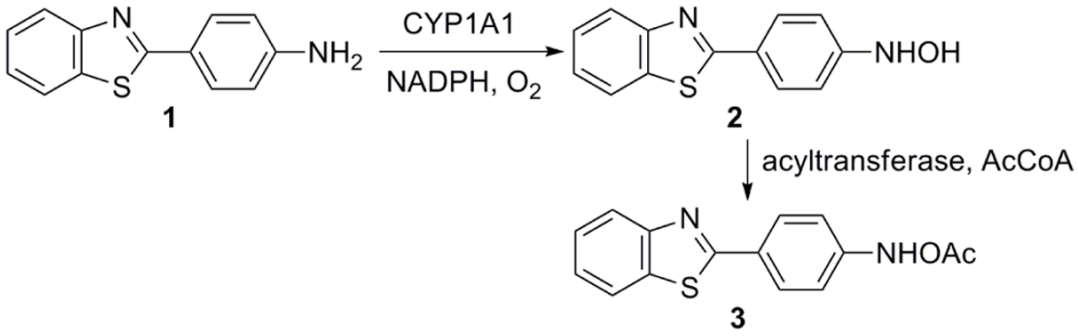
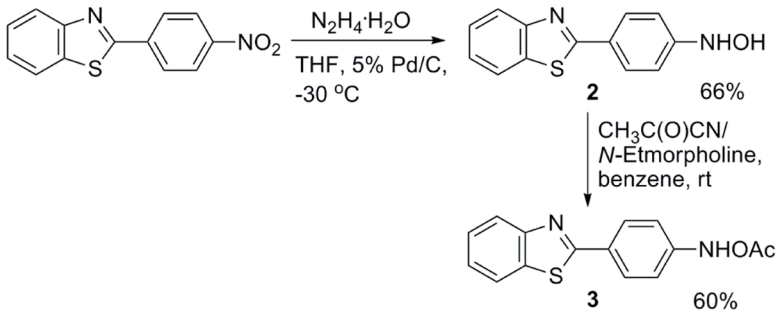


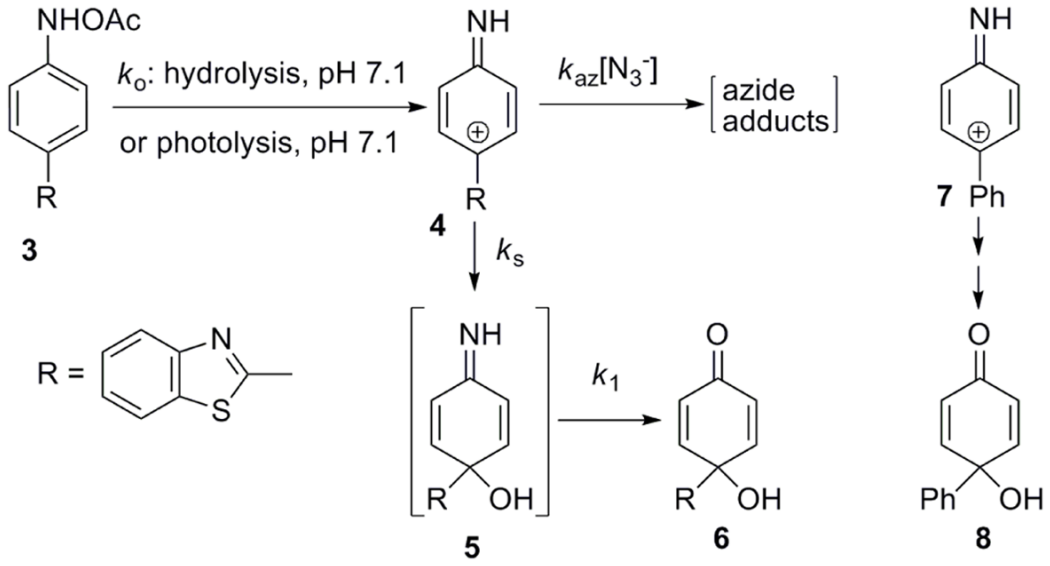
Figure 4.
Plot of k_{obs} from LFP experiments vs. [N_3^-]. Data were fit by a weighted least-squares procedure to obtain k_s and k_{az}. Adjusted r^2 = 0.9967.



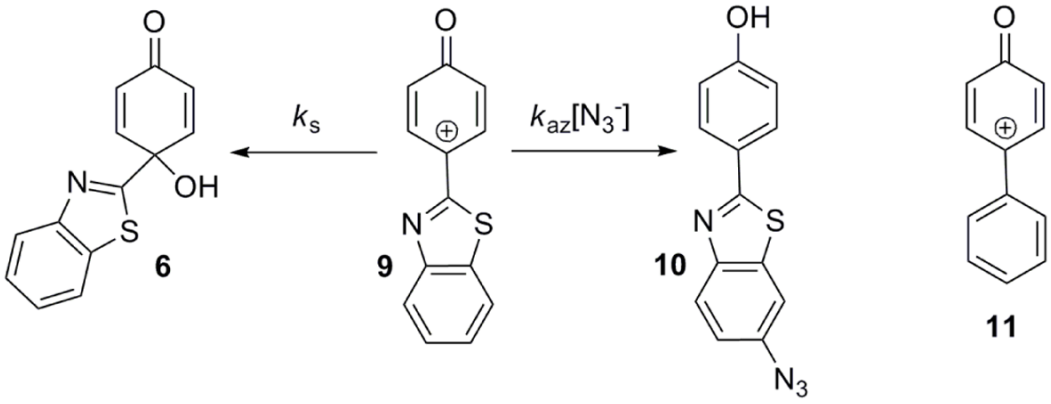
Scheme 1.
Proposed metabolism of **1**.



Scheme 2.
Synthesis of **2** and **3**.



Scheme 3.
Kinetic Scheme for **3** and **4**.



Scheme 4.
Chemistry of the related oxenium ion **9**.