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

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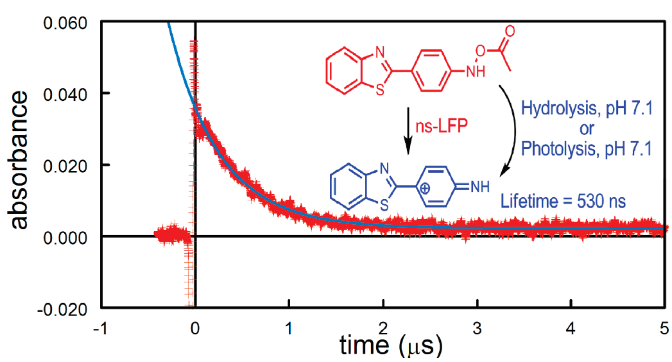
ABSTRACT



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 antitumor, antifungal, and antibacterial agents,[1−3] and as radiopharmaceuticals for binding and in vivo imaging of A*β*-plaques, one of the earliest pathological processes in the development of Alzheimer's disease.[4] One antitumor derivative of **1** is currently in phase 1 clinical trials in Great Britain.[5] The use of **1** and its derivatives as antitumor 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

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(1) Shi, D.-F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. *J. Med. Chem.* **1996**, *39*, 3375–3384. Bradshaw, T. D.; Wrigley, S.; Shi, D.-F.; Schultz, R. J.; Paull, K. D.; Stevens, M. F. G. *Br. J. Cancer* **1998**, *77*, 745–752. Hutchinson, I.; Jennings, S. A.; Vishnuvajjala, B. R.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2002**, *45*, 744–747.
(2) Yildiz-Oren, I.; Yalcin, I.; Aki-Sener, E.; Ucarturk, N. *Eur. J. Med. Chem.* **2004**, *39*, 291–298.
(3) Ra, C. S.; Jung, B. Y.; Park, G. *Heterocycles* **2004**, *62*, 793–802.
(4) Tzanopoulou, S.; Pirmettis, I. C.; Patsis, G.; Paravatou-Petsotas, M.; Livaniou, E.; Papadopoulos, M.; Pelecanou, M. *J. Med. Chem.* **2006**, *49*, 5408–5410.
(5) Bradshaw, T. D.; Westwell, A. D. *Curr. Med. Chem* **2004**, *11*, 1009–1021. Brantley, E.; Antony, S.; Kohlhagen, G.; Meng, L.; Agama, K.; Stinson, S. F.; Sausville, E. A.; Pommier, Y. *Cancer Chemother. Pharmacol.* **2006**, *58*, 62–72.
(6) Chua, M.-S.; Kashiyama, E.; Bradshaw, T. D.; Stinson, S. F.; Brantley, E.; Sausville, E. A.; Stevens, M. F. G. *Cancer Res.* **2000**, *60*, 5196–5203.

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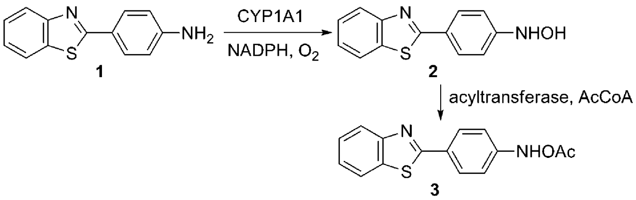
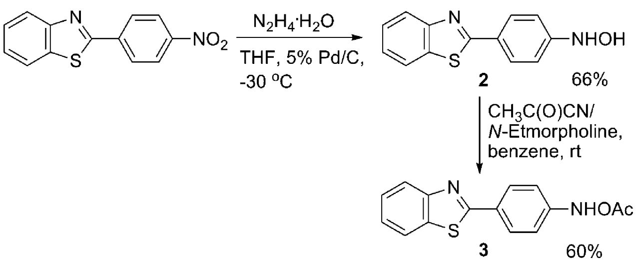
Scheme 1. Proposed Metabolism of **1**



decomposes into a reactive electrophile, but no direct evidence for this proposal has been presented.[7]

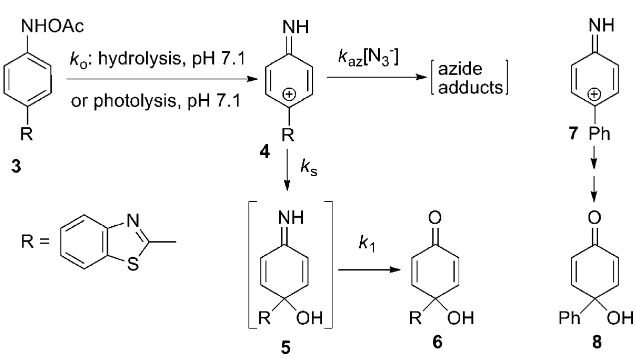
We have succeeded in synthesizing both **2** and **3** from 2-(4-nitrophenyl)benzothiazole using procedures we previously developed for making similar derivatives of carcinogenic aromatic amines (Scheme 2).[8] Reduction of the nitro

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



compound[9] with hydrazine hydrate in the presence 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**.

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



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_0 and k_1. HPLC studies (Figure 1A) show

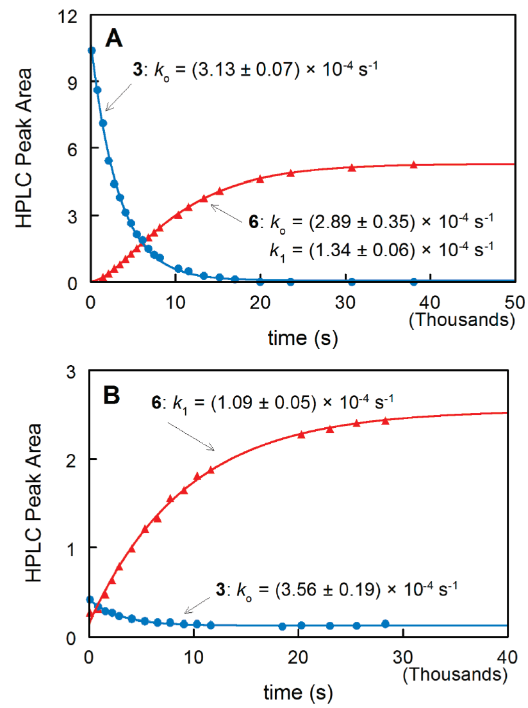


Figure 1. Time course for the disappearance of **3** and formation of **6** at 10 °C in pH 7.1 phosphate buffer monitored by HPLC with UV detection at 212 nm: (A) hydrolysis reaction in the dark and (B) after steady-state photolysis for 30 s. Rate constants were obtained from fits to single or double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

that the larger rate constant, k_0 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_0 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 photodecomposition 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 photodecomposition of **3**.

(7) O'Brien, S. E.; Browne, H. L.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G.; Laughton, C. A. *Org. Biomol. Chem* **2003**, *1*, 493–497. Hilal, R.; Khalek, A. A. A.; Elroby, S. A. K. *THEOCHEM* **2005**, *731*, 115–121.

(8) Kazerani, S.; Novak, M. *J. Org. Chem.* **1998**, *63*, 895–897. Novak, M.; Kazerani, S. *J. Am. Chem. Soc.* **2000**, *122*, 3606–3616.

(9) Stevens, M. F. G.; Shi, D.-F.; Castro, A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 83–93.

(10) Wang, Y.-T.; Jin, K. J.; Myers, L. R.; Glover, S. A.; Novak, M. *J. Org. Chem.* **2009**, *74*, 4463–4471.

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

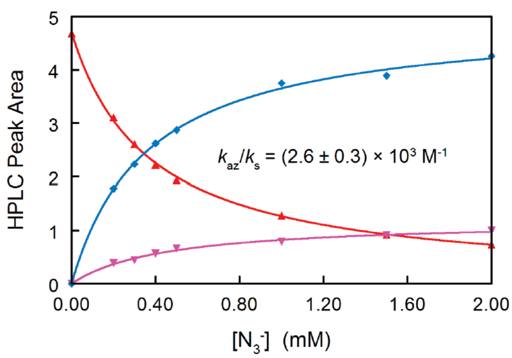


Figure 2. Results of azide trapping experiment in pH 7.1 phosphate buffer at 30 °C. Key: **6** (▲, 212 nm), apparent major azide adduct (♦, 330 nm), and apparent minor azide adduct (▼, 212 nm). The k_{az}/k_s is the average of the fit of all three materials to the standard "azide clock" formulas.

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

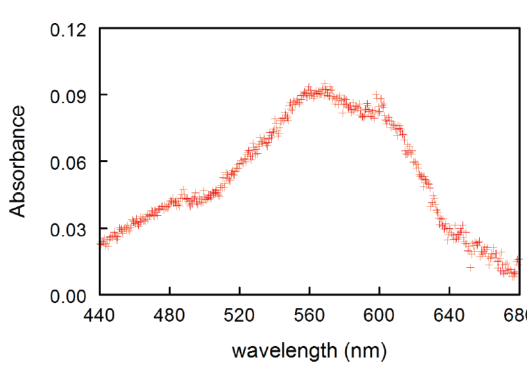


Figure 3. Transient absorbance spectrum obtained 20 ns after 308 nm excitation of **3** in O_2-saturated pH 7.1 phosphate buffer. The spectrum was recorded with a 20 ns window.

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).

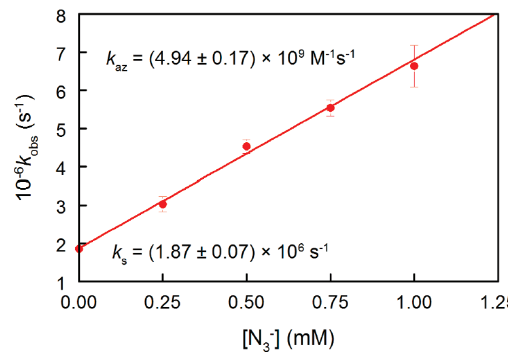


Figure 4. Plot of k_{obs} from LFP experiments vs. $[N_3^-]$. Data were fit by a weighted least-squares procedure to obtain k_s and k_{az}. The adjusted $r^2 = 0.9967$.

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 with 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.

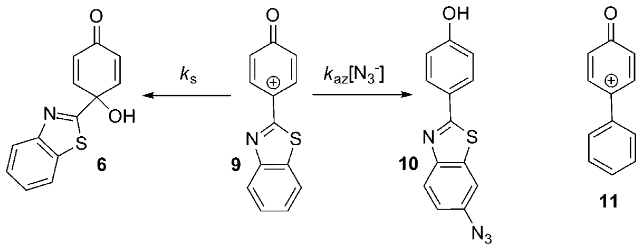
An apparent imine intermediate can be detected by HPLC during the conversion of **7** into **8**.[12] This species has a lifetime of ca. 6 h at room temperature, while **7** has a lifetime of 560 ns under the same conditions.[12] The quinol product **6** is also the hydration product of the related oxenium 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 that of the 4-bipheny-

(11) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4689–4691. Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4691–4692. Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1383–1396.

(12) Novak, M.; Kahley, M. J.; Eiger, E.; Helmick, J. S.; Peters, H. E. *J. Am. Chem. Soc.* **1993**, *115*, 9453–9460. McClelland, R. A.; Davidse, P. A.; Hadzialic, G. *J. Am. Chem. Soc.* **1995**, *117*, 4173–4174.

Scheme 4. Chemistry of the Related Oxenium Ion **9**



lyloxenium 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 antitumor benzothiazoles will give rise to selective, long-lived nitrenium ions in aqueous solution. Although metabolites of carcinogenic 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 antitumor 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.

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Supporting Information Available: Experimental details, a table of rate constants, Figure S1 showing the decay of the absorbance in O_2-saturated phosphate buffer, synthesis of **2** and **3**, and NMR spectra of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Novak, M.; Glover, S. A. *J. Am. Chem. Soc.* **2004**, *126*, 7748–7749. Wang, Y.-T.; Wang, J.; Platz, M. S.; Novak, M. *J. Am. Chem. Soc.* **2007**, *129*, 14566–14567.