Chemical Research in Toxicology

Article

pubs.acs.org/crt

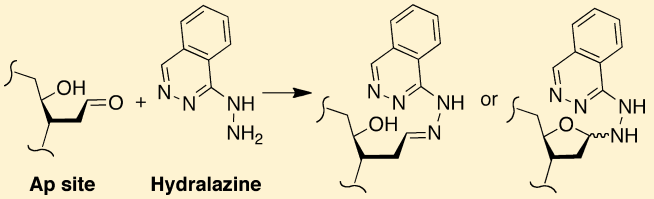
Covalent Adduct Formation between the Antihypertensive Drug Hydralazine and Abasic Sites in Double- and Single-Stranded DNA

Douglas Melton,[†] Calvin D. Lewis,[†] Nathan E. Price,[†] and Kent S. Gates*[,†,‡]

[†]Department of Chemistry, [‡]Department of Biochemistry, University of Missouri, 125 Chemistry Building, Columbia, Missouri 65211, United States

Supporting Information

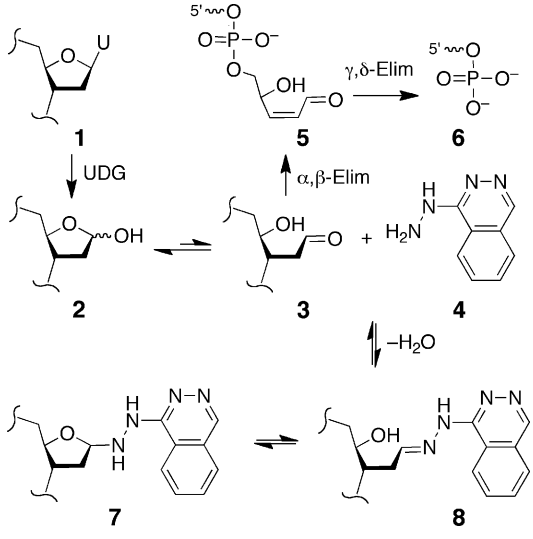
ABSTRACT: Hydralazine (**4**) is an antihypertensive agent that displays both mutagenic and epigenetic properties. Here, gel electrophoretic, mass spectroscopic, and chemical kinetics methods were used to provide evidence that medicinally relevant concentrations of **4** rapidly form covalent adducts with abasic sites in double- and single-stranded DNA under physiological conditions. These findings raise the intriguing possibility that the genotoxic properties of this clinically used drug arise via reactions with an endogenous DNA lesion rather than with the canonical structure of DNA.



INTRODUCTION

Hydralazine (1-hydrazinophthalazine, **4**, Scheme 1) is an antihypertensive agent that was introduced into the clinic in the early 1950s,[1,2] and this drug remains in use, primarily for the treatment of gestational hypertension.[3,4] In addition, **4** induces demethylation of cellular DNA,[5] a property that has given the compound a second life as a possible epigenetic drug.[6−8]

Scheme 1



Interestingly, a number of reports indicate that **4** is mutagenic in Ames assays.[9] Chemically induced mutagenesis typically involves covalent modification of the canonical nucleobases of the DNA in target cells.[10−12] Subsequent error-prone replication of the damaged DNA introduces mutations into the genetic code. Accordingly, a variety of DNA-damage mechanisms have been proposed to explain the mutagenic action of **4**, including oxidation of the drug to a DNA-damaging diazonium ion, diazene radical, or aryl radical, nucleophilic addition to pyrimidine residues in DNA, and oxidative conversion of a formaldehyde-derived hydrazone adduct into a DNA-alkylating species.[13−17] However, no consensus has emerged regarding a chemical mechanism for the damage of cellular DNA by **4**.

In the work described here, we explored the novel possibility that the mutagenic properties of the clinically used drug **4** arise via the drug's ability to covalently capture endogenous abasic (Ap) lesions in genomic DNA rather than by modification of canonical DNA bases. Ap sites are generated by spontaneous and enzymatic hydrolysis of the glycosidic bonds that hold the coding nucleobases to the 2-deoxyribose-phosphate backbone of DNA.[18−21] As a result, the DNA of normal mammalian tissue harbors between 50 000 and 200 000 Ap sites per cell.[22,23] Ap sites exist as an equilibrium mixture of the ring-closed hemiacetal **2** and ring-opened aldehyde **3** (Scheme 1).[24] The aryl hydrazine group of **4** has the potential to react with the Ap aldehyde residue to generate a hydrazone adduct (**7** or **8**, Scheme 1). Hydrazone formation is a well-known reaction that has found use in biochemistry and chemical biology for chemoselective ligations;[25−30] however, at the outset of our studies, it was by no means clear that medicinally relevant concentrations of **4** would be capable of forming adducts with Ap sites in DNA under physiological conditions. This is because hydrazone formation in neutral aqueous solution typically is rather slow.[26] As a result, hydrazone-forming reactions involving biomolecules usually employ high concentrations of at least one reaction partner, low pH (4−5), or an added organocatalyst.[25−27] To the best of our knowledge, the

Received: September 8, 2014
Published: November 6, 2014

reaction of aryl hydrazines with Ap sites in DNA has not previously been examined under physiologically relevant conditions. In the work described here, we employed gel electrophoretic, mass spectroscopic, and chemical kinetics methods to provide evidence that medicinally relevant concentrations of **4** rapidly form covalent adducts with abasic sites in double- and single-stranded DNA under physiological conditions.

EXPERIMENTAL PROCEDURES

Materials. Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Hydralazine hydrochloride, 2-deoxy-D-ribose, sodium hydroxide, and other chemicals were purchased from Sigma-Aldrich (St. Louis, Mo) and used without further purification. The enzyme uracil DNA glycosylase (UDG) was purchased from New England Biolabs (Ipswich, MA). [γ-^{32}P]-ATP (6000 Ci/mmol) was purchased from PerkinElmer (Waltham, MA). C18 Sep-Pak cartridges were purchased from Waters (Milford, MA), and BS Polyprep columns were obtained from BioRad (Hercules, CA). Measurement of radioactivity in polyacrylamide gels was carried out using a Personal Molecular Imager (Bio-Rad) with Quantity One software (v.4.6.5).

Reaction of 4 with Double- and Single-Stranded DNA Oligonucleotides. The 2′-deoxyuridine-containing oligonucleotides used here were 5′-^{32}P-labeled, annealed with their complementary strand (in the case of duplex **B**), and treated with uracil DNA glycosylase (UDG) to form the Ap site at a defined location using standard procedures.[31−33] Subsequent reactions were carried out in HEPES buffer (50 mM, pH 7) containing NaCl (0.1 M) at 37 °C in a final volume of 10 μL unless otherwise specified. Efficient formation of the Ap site resulting from UDG treatment was confirmed by workup of the DNA with NaOH (3.3 μL of a 500 mM stock solution in water), followed by incubation for 2 h at 37 °C. Typically, the Ap site was generated in >90% yield. The same NaOH workup was used to deduce the amount of base-labile lesions (e.g., Ap site) remaining in the labeled oligonucleotide at end of incubation either with or without compound **4**. As described above, the samples were mixed with NaOH (3.3 μL of a 500 mM stock solution in water), followed by incubation for 2 h at 37 °C. Control experiments showed that the pH of samples after mixing with NaOH in the base workup step was the same regardless of whether compound **4** was present. The samples (13.3 μL), without further purification, were mixed with formamide loading buffer (75−115 μL)[33] containing bromophenol blue to achieve approximately 800 cpm/1.5 μL. Samples were loaded onto a 20% denaturing polyacrylamide gel, the gel was electrophoresed at 1000−1500 V for 2−4 h until the bromophenol blue marker dye had migrated approximately 10−15 cm from the wells, and the resolved labeled DNA fragments were visualized by phosphorimager analysis. In the gel shift experiments (Figures 2 and S4), duplex **B** was incubated with **4** (100 μM) for 2 h under the standard reaction conditions described above and then diluted with loading dye without any further treatment or purification. Duplex **A** was incubated with **4** under the same conditions to ensure that reaction of the compound with native nucleobases (as opposed to the Ap site in duplex **B**) does not induce a gel shift. These samples were analyzed on a 20% denaturing polyacrylamide gel as described above except that the bands were run at least 30−35 cm from the wells to allow a clear separation of the Ap-oligonucleotide from the adducted oligonucleotide. In the case of control reactions involving the incubation of phthalazine with duplex **B**, the reaction conditions were the same as those describe above, with the exception that stock solutions of phthalazine were prepared by dissolution of the compound in DMSO at a concentration 100 mM, followed by dilution with water to a final concentration of 1 mM. The final concentration of phthalazine in the assay was 100 μM, and DMSO concentration was 0.1% (v/v).

The amount of full-length labeled oligonucleotide product remaining in reactions of duplex **B** and oligonucleotide **C** with **4** following NaOH workup were corrected for the small amounts of full-length oligonucleotide remaining after NaOH workup in a control sample that was not treated with **4**. Generally, the amount of remaining full-length product was less than 5% of total radioactivity in the lane. The small amounts of full-length product in these control samples presumably correspond to 2′-deoxyuridine-containing oligonucleotide (in duplex **A**) that was not converted to the Ap-containing oligonucleotide (in duplex **B**) by UDG.

Kinetics Analysis of the Reaction between 4 and DNA Oligonucleotides. The data for the reaction of duplex **B** with **4** (10 μM) or single-stranded oligonucleotide **D** with **4** (50 μM) shown in Figure 4 was fit to the equation for appearance of product via a first-order process: $Y_t = Y_\infty + (Y_0 - Y_\infty)\, e^{(-kt)}$, where Y_t is the reading for product at time t, Y_0 is the reading at time 0, and Y_∞ is the final reading when reaction is complete (see pp. 22−23 of ref 34). Both Y_∞ and Y_0 were floated in the fitting process. Fitting provided an observed pseudo-first-order rate constant for each reaction. The r^2 values for the resulting fits were 0.98 ± 0.01. Average values and standard deviations were obtained by fitting and averaging the resulting values from at least three separate experiments. The apparent second-order rate constants were obtained by dividing the observed first-order rate constants by the concentration of **4** in the reaction (10 μM **4** in the reaction with duplex **B** and 50 μM **4** in the reaction with oligo **D**). Alternatively, exploiting the equation $\ln[(Y_t - Y_\infty)/[(Y_0 - Y_\infty)] = -kt$, a plot of $\ln[(Y_t - Y_\infty)/[(Y_0 - Y_\infty)]$, or $\ln|Y_t - Y_\infty|$ versus time was generated for each reaction, and the data was fit to a line (Figure S7).[34] The slope of the resulting lines in these plots corresponds to $-k$. Again, the apparent second-order rate constant for each reaction was obtained by dividing the observed first-order rate constant by the pseudo-first-order concentration of **4** employed in the reaction. The values calculated for the second-order rate constants by this graphical method matched well with those obtained by the nonlinear curve-fitting method (Figure S7).

Static Nanospray QTOF-MS of Adduct-Containing DNA. The oligonucleotide sample was analyzed in a 40 mM dimethylbutylammonium acetate (pH 7.1) buffer. Negative ion MS spectra was taken for mass range of 280−3200 Da on an Agilent 6520A QTOF MS with Chip Cube source (G4240A). Monoisotopic neutral masses were calculated from the multiply charged ion spectra of signals present in the 500−2000 Da mass range. Sample introduction was done with New Objective Econo12-N uncoated borosilicate glass emitters. Negative ion spectrum was acquired at a capillary potential sufficient to initiate spray of the sample. The nitrogen gas was heated at 290 °C and introduced at a flow rate of 4 L/min. The fragmentor, skimmer, and octapole1 RF Vpp potentials were set to 200, 65, and 750 V, respectively. External calibration was done with the Agilent ESI-low calibration tuning mixture (cat. no. G1969-85000), and data analysis was performed with Agilent Mass Hunter Workstation qualitative analysis software v B.02.00, build 2.0.197.0, with Bioconfirm Software (2008). Peptide isotope model was assumed, and peak set height threshold for extraction was set to ≥500 counts. Deconvolution was carried out with a 0.1 Da step size with a result of 20 iterations of the algorithm calculation

RESULTS

Gel Electrophoretic Evidence for a Reaction between 4 and an Abasic Site in Duplex DNA. Here, we examined the reaction of **4** with Ap sites in synthetic DNA oligonucleotides. Toward this end, the Ap-containing DNA duplex **B** was generated by treatment of the corresponding 5′-^{32}P-labeled, 2′-deoxyuridine-containing duplex **A** with uracil DNA glycosylase (UDG).[31,32] Efficient formation of the Ap site was confirmed by treatment of the DNA with mild alkali to generate a mixture of the expected 3′-4-hydroxy-2-pentenal-5-phosphate (**5**) and 3′-phosphate (**6**) cleavage products (Figure 1, lane 4).[18,35,36] Our initial approach for detecting the reaction of **4** with the DNA abasic site capitalized on the expectation that formation of a hydrazone adduct **7**/**8** would render the Ap-containing oligonucleotide resistant to cleavage under mild alkaline conditions, analogous to the properties of the oxime adduct derived from reaction of methoxyamine with an Ap site

dx.doi.org/10.1021/tx5003657 | Chem. Res. Toxicol. 2014, 27, 2113−2118

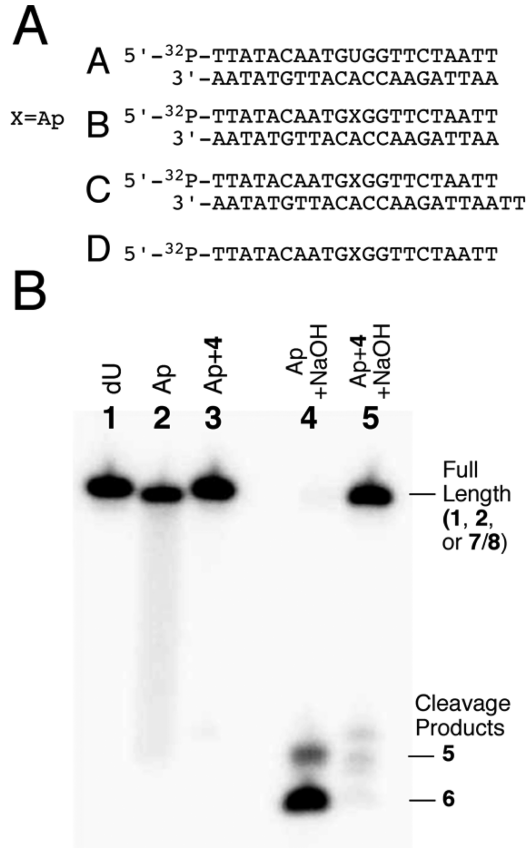


Figure 1. (A) DNA sequences used in this study. (B) Treatment with **4** blocks NaOH-mediated cleavage of the Ap-containing strand in duplex **B**. In a typical assay, 5′-^{32}P-labeled duplexes (30−50 000 cpm) were incubated in HEPES buffer (50 mM, pH 7) containing NaCl (100 mM) at 37 °C for 2 h (10 μL final volume). Alkaline workup involved addition of 3.3 μL of a 0.5 N NaOH stock solution, followed by incubation for 2 h at 37 °C. Following incubation, the samples were mixed with formamide gel loading buffer (typically, 80 μL into 13 μL of NaOH-treated DNA), loaded onto a 0.4 mm thick 20% denaturing polyacrylamide gel, and electrophoresed at 1500 V for approximately 2 h, and the labeled fragments were visualized by phosphorimager analysis. Lane 1: Uracil-containing ^{32}P-labeled 2′-deoxyoligonucleotide duplex **A**. Lane 2: Ap-containing duplex **B** (no alkaline workup). Lane 3: Ap-containing duplex **B** + **4** (no alkaline workup). Lane 4: Ap-containing duplex **B** (alkaline workup). Lane 5: Ap-containing duplex **B** + **4** with alkaline workup.

in DNA.[37] We found that incubation of duplex **B** with **4** (100 μM) in HEPES buffer (50 mM, pH 7, containing 100 mM NaCl) for 2 h at 37 °C rendered the ^{32}P-labeled, Ap-containing strand almost completely refractory to strand cleavage induced by NaOH workup (Figure 1, lane 5). This result was striking because an early study showed that, in unbuffered water, the interaction of phenylhydrazine hydrochloride with Ap-containing DNA fragments induced strand cleavage at the Ap site rather than formation of a phenylhydrazone adduct on the full-length strand.[38,39] Under our reaction conditions, incubation of **4** with duplex **B** generated little or no strand cleavage above background (Figure 1, lane 5). Identical results were obtained when **4** was incubated with a longer, 35 base pair, duplex containing a single Ap site (Figure S1). Medicinally relevant plasma concentrations of **4** are in the low micromolar range,[40] so we examined the reaction of duplex **B** with a 1 μM concentration of **4** in HEPES buffer (50 mM, pH 7) at 37 °C for 1 h. This resulted in 67 ± 5% inhibition of NaOH-mediated strand cleavage (Figure S2). Compound **4** is reported to undergo slow decomposition to phthalazine in aqueous solutions near neutral pH ($t_{1/2}$ ∼ 7 h).[41] A control experiment showed that phthalazine (10 μM) did not significantly inhibit NaOH-mediated strand cleavage of duplex **B** (Figure S3) and, thus, does not contribute to the action of **4** described here.

Seeking direct evidence of a covalent adduct between **4** and the Ap site in duplex **B**, we conducted experiments designed to detect altered gel mobility of the ^{32}P-labeled oligonucleotide in duplex **B** following treatment with **4**. When the DNA fragments were run at least 30 cm from the origin of a 20% denaturing polyacrylamide gel, we observed a clear shift in the gel electrophoretic mobility of the Ap-containing oligonucleotide upon treatment with **4** (Figure 2). The retardation in gel

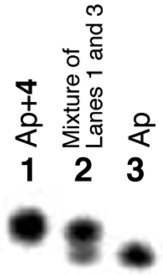


Figure 2. Treatment with **4** alters the gel mobility of the Ap-containing strand in duplex **B**. Lane 1: Ap-containing duplex **B** incubated with **4** (100 μM) in HEPES buffer (50 mM, pH 7) containing NaCl (100 mM) at 37 °C for 2 h. Lane 3: Ap-containing duplex **B** incubated in HEPES buffer (50 mM, pH 7) containing NaCl (100 mM) at 37 °C for 2 h. Lane 2 is a mixture of lanes 1 and 3 to ensure that the observed gel shift is due to the generation of a distinct species rather than a salt effect on gel migration. Following incubation, samples were mixed with formamide gel loading buffer containing bromophenol blue tracking dye. The resulting mixture was loaded onto a 0.4 mm thick 20% denaturing polyacrylamide gel and electrophoresed at 300 V for 1 h and then 700 V for 18 h. The bromophenol blue tracking dye migrated off the bottom of the gel after approximately 13−15 h.

mobility observed here was consistent with formation of a covalent drug−DNA adduct. Control experiments showed that incubation of **4** with the labeled dU-containing duplex **A** did not generate a gel-shifted product (Figure S4). This provided evidence that the gel shift shown in Figure 2 was due to reaction of **4** with the Ap site in duplex **B** rather than with native nucleobases in the labeled oligonucleotide.

Mass Spectrometric Analysis of the Adduct Generated in the Reaction of Hydralazine (4) with Duplex DNA. Mass spectrometric experiments provided further insight regarding the adduct formed in the reaction of **4** with the Ap-site in duplex DNA. In this experiment, duplex **C** was incubated with **4** (100 μM) in HEPES buffer (50 mM, pH 7, containing 100 mM NaCl) for 2 h at 37 °C, followed by desalting and ESI(−)-TOF-MS analysis. The deconvoluted mass spectrum revealed strong signals that closely matched the expected isotope envelope for the hydrazone adducts **7** or **8** (Figure 3). Duplex **C**, containing a truncated complementary strand, was used in these experiments instead of duplex **B** because signals arising from a potassium adduct of the complementary strand in duplex **B** fortuitously overlapped with those of the hydralazine-adducted strand.

Kinetics of the Reaction of Hydralazine (4) with Double- and Single-Stranded DNA. Finally, we explored

dx.doi.org/10.1021/tx5003657 | Chem. Res. Toxicol. 2014, 27, 2113−2118

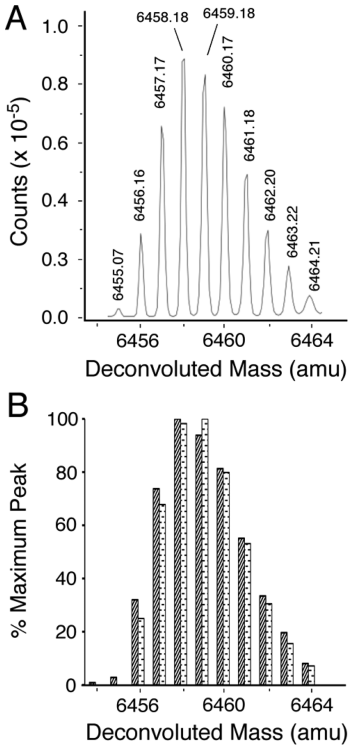


Figure 3. ESI(−)-TOF-MS analysis of duplex **C** containing the hydralazine adduct. (A) Deconvoluted neutral masses for duplex **C** treated with hydralazine (**4**). (B) Comparison of experimentally measured peak intensities (bars on the left side of each pair) and calculated results (bars on the right side of each pair) for the isobaric hydrazone adducts **7** and **8**. The average deviation between calculated and experimental percent-of-maximum peak intensities was 3.6%, and the average deviation between calculated and experimental masses was 6.3 ppm.

the rate at which **4** (10 μM) reacted with the Ap site in duplex **B** (Figure 4, upper trace). Nonlinear curve-fitting analysis of the

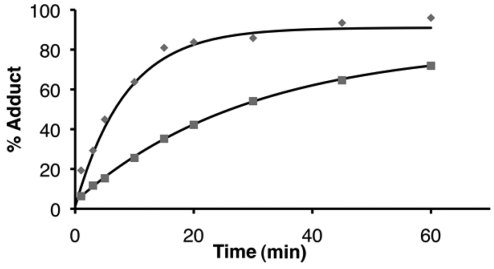


Figure 4. Time course for the formation of the hydralazine−DNA adduct in duplex and single-stranded DNA. The reaction was carried out in HEPES buffer (50 mM, pH 7) containing NaCl (0.1 M) at 37 °C, and hydralazine adduct formation was measured using the NaOH workup described in the text. The upper curve (diamonds) depicts results for the reaction of **4** (10 μM) with double-stranded DNA duplex **B**. The lower curve (squares) depicts the reaction of **4** (50 μM) with single-stranded DNA **D**.

data gave an observed pseudo-first-order rate constant of 2.0 ± 0.2 × 10^{-3} s^{-1}, corresponding to an apparent second-order rate constant of 2.0 ± 0.2 × 10^{2} M^{-1} s^{-1}, for the reaction of **4** with duplex **B**. The reaction of **4** (1 μM) with a 35 base pair DNA duplex progressed with a comparable rate constant (Figure S6). Graphical analysis of the data shown in Figure 4 gave similar values for the rate constants (Figure S7). This rate constant is remarkably high compared to other hydrazone-forming reactions reported in the literature,[26] but it meshes with our observation that low micromolar concentrations of **4** capture the Ap site in duplex DNA with half-times inside of 1 h. For comparison, we monitored the reaction of **4** (50 μM) with the single-stranded oligonucleotide **D** (Figure 4, lower trace). Nonlinear curve-fitting analysis of this data afforded an observed pseudo-first-order rate constant of 6 ± 2 × 10^{-4} s^{-1} for this process, from which an apparent second-order rate constant of 11 ± 4 M^{-1} s^{-1} was calculated for the reaction of **4** with the Ap site in the single-stranded oligonucleotide **D**. These results showed the reaction of **4** with an Ap site in double-stranded DNA to be approximately 15-fold faster than that in single-stranded DNA. Noncovalent association of **4** at the Ap site in the double helix may drive formation of the hydrazone adduct. In addition, various DNA functional groups have the potential to catalyze hydrazone formation.[42]

CONCLUSIONS

In summary, we find that medicinally relevant concentrations of **4** rapidly capture Ap sites in double-stranded and single-stranded DNA under physiological conditions. We anticipate that formation of this hydralazine−DNA adduct will block the repair of Ap sites normally initiated by the enzyme apurinic endonuclease (APE). Supporting this supposition, the oxime formed by reaction of methoxyamine with Ap sites is refractory to processing by APE.[37,43] We further expect that polymerase bypass of the hydralazine−Ap adduct will be error-prone (mutagenic). Our findings raise the intriguing possibility that the genotoxic properties of the clinically used drug **4** arise via reactions with endogenous Ap lesions in the genome rather than with the canonical nucleobases of DNA. Furthermore, removal of hydralazine−DNA adducts by nucleotide excision repair (NER) processes could contribute to the loss of 5-methylcytosine residues from cellular DNA that characterizes the epigenetic properties of this drug.[5,44] From a general perspective, the mechanism described here involving reaction of a nitrogen nucleophile with the Ap-aldehyde group could be relevant to the mutagenic action and toxicity of various hydrazines, hydrazides, and anilines.[45,46] Finally, the rapid and high-yielding reactions described here suggest that **4** could serve as a platform for the development of new reagents that efficiently label Ap sites in DNA.

ASSOCIATED CONTENT

Supporting Information

Data pertaining to the reaction of **4** (1 μM) with duplex **B** and a 35 base pair Ap-containing duplex, control reaction showing that phthalazine does not react with duplex **B**, and graphical analysis of the kinetic data for reaction of **4** with **B** and **D**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: gatesk@missouri.edu; Phone: (573) 882-6763; Fax: (573) 882-2754.

Funding

We are grateful to the National Institutes of Health for support of this work (ES021007).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

MALDI mass spectrometry was conducted at the Charles W. Gehrke Proteomics Center at the University of Missouri, and we thank Beverly DaGue for assistance with these experiments. We thank Professor Phillip Wilmarth (Oregon State University) for assistance fitting the observed data to the expected results in the mass spectrometric experiments.

■ ABBREVIATIONS

Ap site, abasic site; UDG, uracil DNA glycosylase; APE, Ap endonuclease; NER, nucleotide excision repair

■ REFERENCES

(1) Hilker, R. R., Rhoads, P. S., and Billings, C. E. (1953) Clinical use of hydralazine and hexamethonium in treatment of hypertension. *J. Am. Med. Assoc. 153*, 5−9.

(2) Gross, F., Druey, J., and Meier, R. (1950) Eine neue gruppe blutdrucksenkender substanzen von besonderem wirkungscharakter. *Experientia 15*, 19−21.

(3) Magee, L. A., Abalos, E., von Dadelszen, P., Sibai, B., Easterling, T., and Walkinshaw, S. (2011) How to manage hypertension in pregnancy effectively. *Br. J. Clin. Pharmacol. 72*, 394−401.

(4) Slim, H. B., Black, H. R., and Thompson, P. D. (2011) Older blood pressure medications—do they still have a place? *Am. J. Cardiol. 108*, 308−316.

(5) Cornacchia, E., Golbus, J., Maybaum, J., Strahler, J., Hanash, S., and Richardson, B. (1988) Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. *J. Immunol. 140*, 2197−2200.

(6) Arce, C., Segura-Pacheco, B., Perez-Cardenas, E., Taja-Chayeb, L., Candelaria, M., and Duennas-Gonzalez, A. (2006) Hydralazine target: from blood vessels to the epigenome. *J. Transl. Med. 4*, 1−16.

(7) Ren, J., Singh, B. N., Huang, Q., Li, Z., Gao, Y., Mishra, P., Hwa, Y. L., Li, J., Dowdy, S. C., and Jiang, S.-W. (2011) DNA hypermethylation as a chemotherapy target. *Cell. Signalling 23*, 1082−1093.

(8) Burridge, S. (2013) Drugging the epigenome. *Nat. Rev. Drug. Discovery 12*, 92−93.

(9) Blanco, M., Martínez, A., Urios, A., Herrera, G., and O'Connor, J. E. (1998) Detection of oxidative mutagenesis by isoniazid and other hydrazine derivatives in *Escherichia coli* WP2 tester strain IC203, deficient in OxyR: strong protective effects of rat liver S9. *Mutat. Res. 417*, 39−46.

(10) Dipple, A. (1995) DNA adducts of chemical carcinogens. *Carcinogenesis 16*, 437−441.

(11) Shrivastav, N., Li, D., and Essigmann, J. M. (2010) Chemical biology of mutagenesis and DNA repair: cellular responses to DNA alkylation. *Carcinogenesis 31*, 59−70.

(12) Hemminki, K. (1993) DNA adducts, mutations and cancer. *Carcinogenesis 14*, 2007−2012.

(13) Sinha, B. K. (1983) Enzymatic activation of hydrazine derivatives. A spin-trapping study. *J. Biol. Chem. 258*, 796−801.

(14) Yamamoto, K., and Kawanishi, S. (1991) Free radical production and site-specific DNA damage induced by hydralazine in the presence of metal ions or peroxidase/hydrogen peroxide. *Biochem. Pharmacol. 41*, 905−914.

(15) Reilly, C. A., and Aust, S. D. (1997) Peroxidase substrates stimulate the oxidation of hydralazine to metabolites which cause single-strand breaks in DNA. *Chem. Res. Toxicol. 10*, 328−334.

(16) Dubroff, L. M., and Reid, R. J. J. (1980) Hydralazine−pyrimidine interactions may explain hydralazine-induced lupus erythematosus. *Science 208*, 404−406.

(17) Mathison, B. H., Murphy, S. E., and Shank, R. C. (1994) Hydralazine and other hydrazine derivatives and the formation of DNA adducts. *Toxicol. Appl. Pharmacol. 127*, 91−98.

(18) Gates, K. S. (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. *Chem. Res. Toxicol. 22*, 1747−1760.

(19) Guillet, M., and Bioteux, S. (2003) Origin of endogenous DNA abasic sites in *Saccaromyces cerevisiae*. *Mol. Cell. Biol. 23*, 8386−8394.

(20) Gates, K. S., Nooner, T., and Dutta, S. (2004) Biologically relevant chemical reactions of N7-alkyl-2′-deoxyguanosine adducts in DNA. *Chem. Res. Toxicol. 17*, 839−856.

(21) David, S. S., and Williams, S. D. (1998) Chemistry of glycosylases and endonucleases involved in base-excision repair. *Chem. Rev. 98*, 1221−1261.

(22) De Bont, R., and van Larebeke, N. (2004) Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis 19*, 169−185.

(23) Nakamura, J., and Swenberg, J. A. (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res. 59*, 2522−2526.

(24) Wilde, J. A., Bolton, P. H., Mazumdar, A., Manoharan, M., and Gerlt, J. A. (1989) Characterization of the equilibrating forms of the abasic site in duplex DNA using ^{17}O-NMR. *J. Am. Chem. Soc. 111*, 1894−1896.

(25) Mahal, L. K., Yarema, K. J., and Bertozzi, C. R. (1997) Engineering chemical reactivity on cell surfaces through oligosaccharide biosynthesis. *Science 276*, 1125−1128.

(26) Kool, E. T., Park, D.-H., and Crisalli, P. (2013) Fast hydrazone reactants: electronic and acid/base effects strongly influence rate at biological pH. *J. Am. Chem. Soc. 135*, 17663−17666.

(27) Dirksen, A., and Dawson, P. E. (2008) Rapid oxime and hydrazone ligations with aromatic aldehydes for biomolecular labeling. *Bioconjugate Chem. 19*, 2543−2548.

(28) Pfander, S., Fiammengo, R., Kirin, S. I., Metlzer-Nolte, N., and Jäschke, A. (2007) Reversible site-specific tagging of enzymatically synthethesized RNAs using aldehyde−hydrazine chemistry and protease-cleavable linkers. *Nucleic Acids Res. 35*, e25.

(29) Raindlová, V., Pohl, R., Sanda, M., and Hocek, M. (2010) Direct polymerase synthesis of reactive aldehyde-functionalized DNA and its conjugation and staining with hydrazines. *Angew. Chem., Int. Ed. 49*, 1064−1066.

(30) Wang, X., and Canary, J. W. (2012) Rapid catalyst-free hydrazone ligation: protein−pyridoxal phosphoramides. *Bioconjugate Chem. 23*, 2329−2334.

(31) Lindahl, T., Ljunquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977) DNA N-glycosidases: properties of uracil-DNA glycosidase from *Escherichia coli*. *J. Biol. Chem. 252*, 3286−3294.

(32) Varshney, U., and van de Sande, J. H. (1991) Specificities and kinetics of uracil excision from uracil-containing DNA oligomers by *Escherichia coli* uracil DNA glycosylase. *Biochemistry 30*, 4055−4061.

(33) Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Lab Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY.

(34) Espenson, J. H. (1995) *Chemical Kinetics and Reaction Mechanisms*, 2nd ed., pp 22−23, McGraw-Hill, Inc., New York.

(35) Bailly, V., and Verly, W. G. (1988) Possible roles of beta-elimination and gamma-elimination reactions in the repair of DNA containing AP (apurinic/apyrimidinic) sites in mammalian cells. *Biochem. J. 253*, 553−559.

(36) Bayley, C. R., Brammer, K. W., and Jones, A. S. (1961) The nucleotide sequence in deoxyribonucleic acids. Part V. The alkaline degradation of apurinic sites. *J. Chem. Soc.*, 1903−1907.

(37) Fortini, P., Bignami, M., and Dogliotti, E. (1990) Evidence for AP site formation related to DNA-oxygen alkylation in CHO cells treated with ethylating agents. *Mutat. Res. 236*, 129−137.

(38) Coombs, M. M., and Livingston, D. C. (1969) Reaction of apurinic acid with aldehyde reagents. *Biochim. Biophys. Acta 174*, 161−173.

(39) Vasseur, J.-J., Rayner, B., and Imbach, J.-L. (1988) Apurinic DNA reactivity: modelisation of apurinic DNA breakage by phenylhydrazine and formation of pyrazole adduct. *J. Heterocycl. Chem. 25*, 389−392.
(40) Shepherd, A. M., McNay, J. L., Ludden, T. M., Lin, M. S., and Musgrave, G. E. (1981) Plasma concentration and acetylator phenotype determine response to oral hydralazine. *Hypertension 3*, 580−585.
(41) Halasi, S., and Nairn, J. G. (1990) Stability studies of hydralazine hydrochloride in aqueous solutions. *J. Parenter. Sci. Technol. 44*, 30−34.
(42) Crisalli, P., and Kool, E. T. (2013) Important of ortho proton donors in the catalysis of hydrazone formation. *Org. Lett. 15*, 1646−1649.
(43) Liu, L., Yan, L., Donze, J. R., and Gerson, S. L. (2003) Blockage of abasic site repair enhances antitumor efficacy of 1,3-bis-(2-chloroethyl)-1-nitrosourea in colon tumor xenografts. *Mol. Cancer. Ther. 2*, 1061−1066.
(44) Wu, S. C., and Zhang, Y. (2010) Active DNA demethylation: many roads lead to Rome. *Nat. Rev. Mol. Cell Biol. 11*, 607−620.
(45) Stepan, A. F., Walker, D. P., Bauman, J., Price, D. A., Baille, T. A., Kalgutkar, A. S., and Aleo, M. D. (2011) Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: a perspective based on the critical examination of trends in the top 200 drugs marketed in the United States. *Chem. Res. Toxicol. 24*, 1345−1410.
(46) Parodi, S., De Flora, S., Cavanna, M., Pino, A., Robbiano, L., Bennicelli, C., and Brambilla, G. (1981) DNA-damaging activity in vivo and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. *Cancer Res. 41*, 1469−1482.