

J. Chem. Cryst. **2006**, 36(9), 557-561.

Crystal Structures of 3-Methyl-1,2,4-benzotriazine 1-oxide and 2-oxide

Junnotula, V.; Sarkar, U.; Barnes, C. L.; Thallapally, P. V.; Gates, K. S.

Crystal structures of 3-methyl-1,2,4-benzotriazine 1-oxide and 2-oxide

Venkatraman Junnotula,⁽¹⁾ Ujjal Sarkar,⁽¹⁾ Charles L. Barnes,⁽¹⁾
Praveen K. Thallapally,⁽¹⁾ and Kent S. Gates^{(1,2)*}

Received January 23, 2006; accepted April 6, 2006

The compound 3-methyl 1,2,4-benzotriazine 1,4-dioxide (**1**) belongs to a new class of clinically promising, bioreductively-activated antitumor drugs. Reductive metabolism of these triazine di-*N*-oxides typically produces mixtures of mono-*N*-oxide analogues. As part of our efforts toward characterization of the *in vitro* metabolism of **1**, we synthesized the 1-oxide (**2**) and 2-oxide (**3**) analogues and characterized these compounds using X-ray crystallography. Compounds **2** and **3** (C₈H₇N₃O) crystallized in the monoclinic space group *P2₁/c*. Unit cell parameters for **2**: *a* = 9.0466(7), *b* = 10.5959(8), *c* = 7.8981(6) Å, β = 98.4940(10), and *z* = 4. Unit cell parameters for **3**: *a* = 5.7193(4), *b* = 9.3774(7), *c* = 13.8427(11) Å, β = 101.6370(10), and *z* = 4.

KEY WORDS: Crystal structure; 3-methyl 1,2,4-benzotriazine 1-oxide; 3-methyl 1,2,4-benzotriazine 2-oxide; Metabolism.

Introduction

The compound 3-methyl-1,2,4-benzotriazine 1,4-dioxide (**1**) belongs to a new class of bioreductively-activated, hypoxia-selective anti-tumor drugs.¹ Enzymatic reduction of these drugs yields a radical intermediate that goes forward to cause DNA damage selectively under the hypoxic conditions that exist in tumor cells.^{2–9} As part of this DNA-damage process, the di-*N*-oxide drug is typically converted into a mixture of mono-*N*-oxide metabolites.¹⁰ It is difficult to conclu-

sively assign the site of oxygen attachment in these metabolites using NMR spectroscopy.^{10–12} Accordingly, as part of our efforts to characterize the products arising from *in vitro* metabolism of **1**, we synthesized authentic standards of the 1-oxide (**2**) and 2-oxide (**3**) analogues and characterized these compounds using X-ray crystallography.

Experimental

We prepared compounds **1–3** by using a slight modification of the synthetic route devised by Atallah and coworkers (Scheme 1).¹³ ¹H-NMR and mass spectroscopic data of these compounds match with literature data.¹³

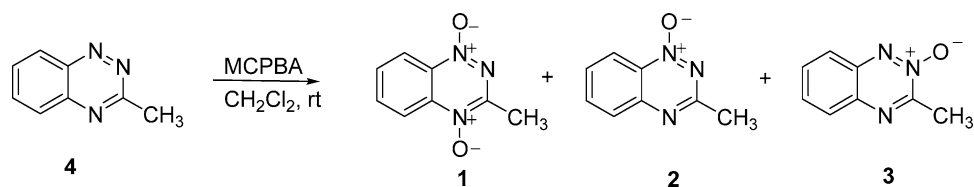
Crystallography

Upon slow evaporation, dilute solutions of compounds **2** and **3** in 1:1 ethyl acetate-hexane

⁽¹⁾Department of Chemistry, University of Missouri-Columbia, Columbia, MO 65211, USA.

⁽²⁾Department of Biochemistry, University of Missouri-Columbia, Columbia, MO 65211, USA.

* To whom correspondence should be addressed Department of Chemistry, 125 Chemistry Building, University of Missouri-Columbia, Columbia, MO 65211, USA; e-mail: gatesk@missouri.edu


Scheme 1. Synthesis of compounds **2** and **3**.

46 afforded crystals suitable for X-ray diffraction
 47 analysis. Data was collected on Bruker SMART
 48 system at 173 K. Crystal structures were solved
 49 by using SHELX programs.^{14,15} Conditions for
 50 crystal structures data collection and structure re-
 51 finement are given in Table 1.

52 Results and discussion

53 Compounds **2** and **3** crystallized in the mon-
 54 oclinic space group $P2_1/c$. Atomic coordinates
 55 and equivalent isotropic displacement parameters
 56 of the non-hydrogen atoms are given in
 57 Table 2. Bond lengths and bond angles are shown
 58 in Table 3 and Table 4, respectively. Fig. 1 shows
 59 the crystal structures of compounds **2** and **3**.
 60 Solid-state structures of **2** and **3** indicate that the
 61 molecules form layered structures, in which the
 62 molecules are arranged in an infinite zig-zag fash-

ion connected exclusively by C–H–O and C–H–N
 hydrogen bridges as shown in Figs. 2 and 3.¹⁶
 Hydrogen bridging parameters for compounds **2**
 and **3** are given in Table 5. The presumably weak
 intermolecular hydrogen bridging interactions in
 the crystals of **2** and **3** compared to the strong
 hydrogen bonds occurring in crystals of the
 3-amino-1,2,4-benzotriazine derivatives¹² may
 explain the higher solubility of 3-methyl benzotri-
 azine derivatives in water.¹ The inversion-related
 layers in the crystals of **2** and **3** are stacked

Table 1. Crystallographic Data for Compounds **2** and **3**

	Compound 2	Compound 3
Chemical formula	C ₈ H ₇ N ₃ O	C ₈ H ₇ N ₃ O
Formula weight	161	161
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Temperature (K)	173	173
Unit cell dimensions		
a (Å)	9.0466(7)	5.7193(4)
b (Å)	10.5959(8)	9.3774(7)
c (Å)	7.8981(6)	13.8427(11)
β (°)	98.4940(10)	101.6370(10)
Volume (Å ³)	748.78(10)	727.15(9)
Z	4	4
D_{calc} (mg/m ³)	1.430	1.472
R_1	0.0523	0.0455
WR_2	0.1162	0.1154

Table 2. Final Coordinates and Equivalent Isotropic Displacement Parameters of the Non-hydrogen Atoms for Compounds **2** and **3**

Atom	x	y	z	$U(\text{eq})$ (Å ²)
Compound 2				
O1	−0.02543	0.24251	−0.24317	0.0474
N1	0.11175	0.08706	−0.11676	0.0364
N2	0.08691	0.20896	−0.13999	0.0350
N3	0.33624	0.12926	0.07734	0.0355
C1	0.23743	0.05336	−0.00840	0.0340
C2	0.18174	0.30033	−0.05392	0.0333
C3	0.30748	0.25550	0.05552	0.0331
C4	0.40489	0.34501	0.14380	0.0399
C5	0.37497	0.47164	0.12225	0.0443
C6	0.24842	0.51336	0.01118	0.0453
C7	0.15206	0.42942	−0.07801	0.0391
C8	0.26011	−0.08627	0.00847	0.0434
Compound 3				
O1	0.34844	0.08772	0.35632	0.0394
N1	0.41637	0.21719	0.36546	0.0286
N2	0.26532	0.31828	0.33071	0.0301
N3	0.73820	0.37372	0.42566	0.0279
C1	0.65740	0.24552	0.41359	0.0273
C2	0.34967	0.45519	0.34384	0.0265
C3	0.58744	0.48459	0.39181	0.0260
C4	0.66679	0.62631	0.40516	0.0303
C5	0.51296	0.73541	0.37122	0.0338
C6	0.27551	0.70601	0.32228	0.0346
C7	0.19412	0.56887	0.30872	0.0319
C8	0.80443	0.11866	0.44881	0.0353

Crystal structures of 3-methyl-1,2,4-benzotriazine 1-oxide and 2-oxide

Table 3. Bond Distances (Å) for Compounds 2 and 3

	Compound 2	Compound 3
O1–N1	1.2564	1.2732
N1–N2	1.3192	1.3072
N1–C1	1.3657	1.4298
N2–C2	1.4011	1.3707
N3–C1	1.3136	1.2867
N3–C3	1.3687	1.3715
C1–C8	1.4969	1.4814
C2–C3	1.4058	1.4149
C2–C7	1.4015	1.4106
C3–C4	1.4074	1.4074
C4–C5	1.3744	1.3692
C5–C6	1.4068	1.4171
C6–C7	1.3659	1.3677
C4–H4	0.9500	0.9500
C5–H5	0.9500	0.9500
C6–H6	0.9500	0.9500
C7–H7	0.9500	0.9500
C8–H8A	0.9800	0.9800
C8–H8B	0.9800	0.9800
C8–H8C	0.9800	0.9800

74 (benzene-with-triazine) along the *c*-axis as shown
75 in Figs. 4 and 5.

76 Characterization of substituted 1,2,4-
77 benzotriazine-*N*-oxides by NMR spectroscopy
78 can be problematic.^{10–12} Thus, the X-ray
79 crystal structures here reported for compounds

Table 4. Bond Angles (°) for Compounds 2 and 3

	Compound 2	Compound 3
O1–N1–N2	118.17	119.29
O1–N1–C1		118.01
O1–N2–C2	119.86	
N2–N1–C1	116.88	122.70
N1–N2–C2	121.98	116.21
C1–N3–C3	115.54	118.64
N1–C1–N3	127.09	121.42
N1–C1–C8	113.87	115.73
N3–C1–C8	119.03	122.85
N2–C2–C3	116.53	121.60
N2–C2–C7	121.15	118.79
C3–C2–C7	122.32	119.61
N3–C3–C2	121.95	119.41
N3–C3–C4	120.18	120.54
C2–C3–C4	117.87	120.04
C3–C4–C5	119.94	119.58
C4–C5–C6	120.75	120.41
C5–C6–C7	121.04	121.02
C2–C7–C6	118.07	119.33

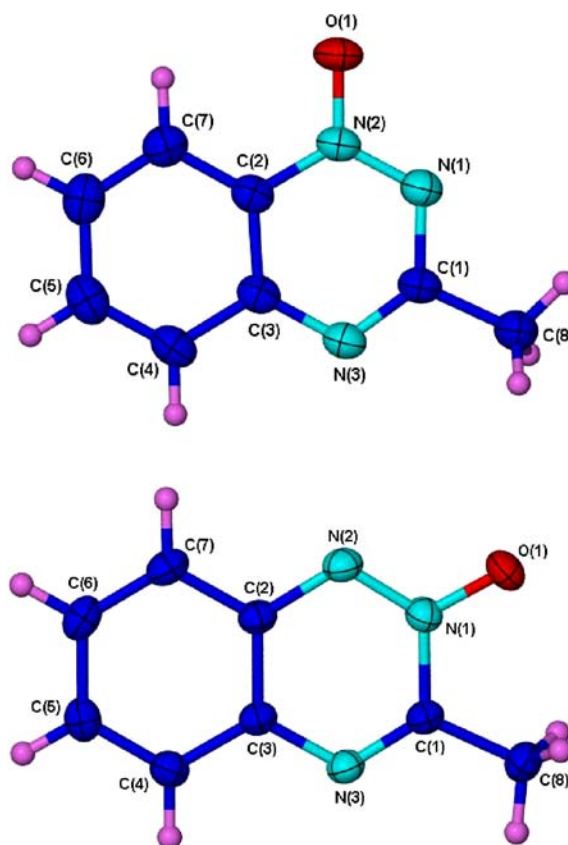


Fig. 1. ORTEP diagrams of compounds 2 and 3.

2 and 3 provide unambiguous assignment of
the *N*-oxide regiochemistry in these 3-methyl-
1,2,4-benzotriazine derivatives. Regiochemical
characterization of *N*-oxide metabolites is necessary
for understanding the chemical mechanisms

Table 5. Hydrogen bridging interactions in compounds 2 and 3

D–H–A	d(D–H) (Å)	d(H···A) (Å)	d(D···A) (Å)	DHA (°)
Compound 2				
C8–H8B···O1	0.980	2.340	3.298	164.00
C7–H7···N1	0.980	2.629	3.549	163.29
C5–H5···N3	0.980	2.757	3.662	159.59
Compound 3				
C7–H7···O1	0.950	2.580	3.467	156.00
C4–H4···N3	0.950	2.815	3.722	159.88
C6–H6···N2	0.950	2.783	3.538	137.09

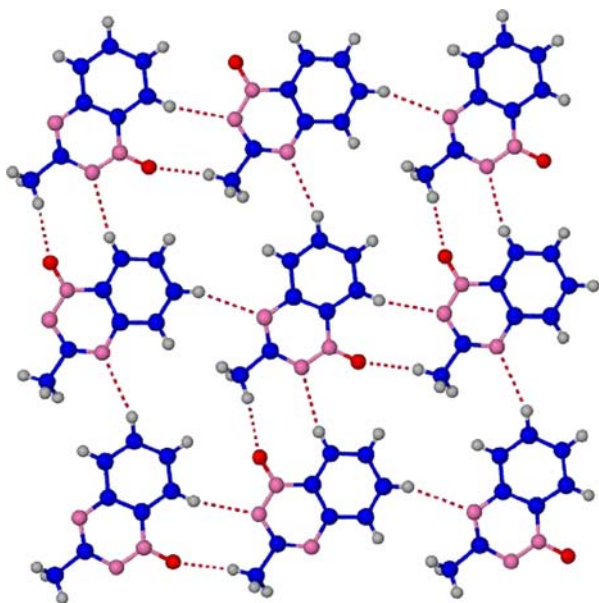


Fig. 2. Hydrogen bridging in 2.

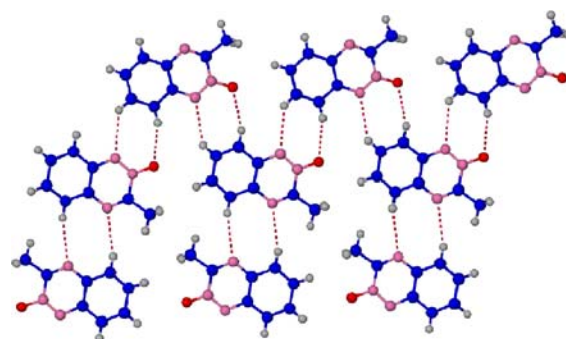


Fig. 3. Hydrogen bridging in 3.

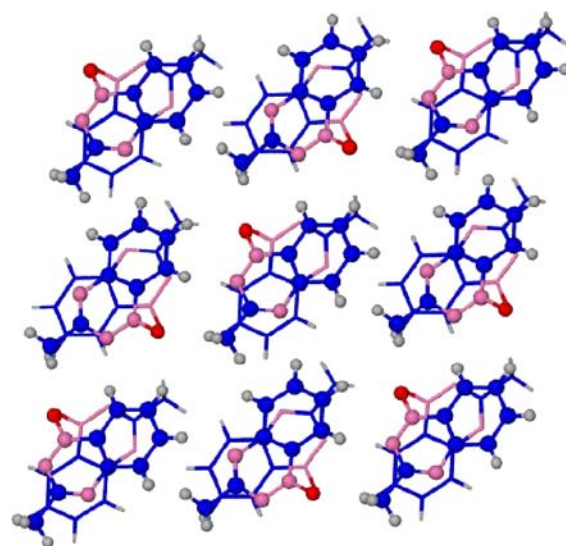


Fig. 4. Packing diagram of 2.

85 by which this class of bioreductively-activated
86 anticancer drugs derive their biological activity.

87 **Supplementary material** X-ray crystallographic data re-
88 ported in this paper is deposited with the Cambridge
89 Crystallographic Data Center as supplementary publication num-
90 bers CCDC 294738 (compound 2) and CCDC 294739 (compound
91 3). Copies of available material can be obtained, free of charge,
92 on application to the Director, CCDC, 12 Union Road, Cambridge
93 CB21EZ, UK.

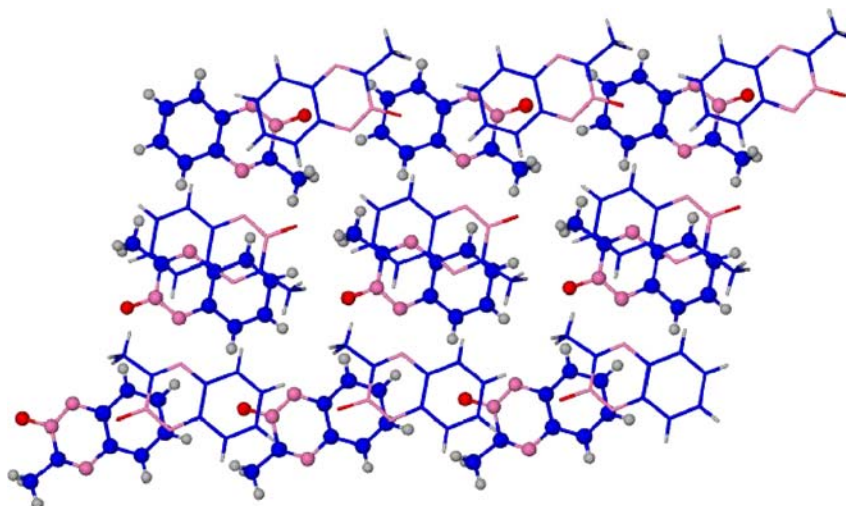


Fig. 5. Packing diagram of 3.

Crystal structures of 3-methyl-1,2,4-benzotriazine 1-oxide and 2-oxide

94 Acknowledgements

95 We thank National Institutes of Health (CA
96 100757) for support of this work.

97 References

- 98 1. Kelson, A.B.; McNamara, J.P.; Ryan, K.J.; Dorie, M.J.; McAfee,
99 P.A.; Menke, D.R.; Brown, J.M.; Tracy, M. *Anti-Cancer Drug*
100 *Design*. **1998**, *13*, 575–592.
- 101 2. Zeman, E.M.; Brown, J.M.; Lemmon, M.J.; Hirst, V.K.; Lee,
102 W.W. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1239–1242.
- 103 3. Brown, J.M. *Cancer Res.* **1999**, *59*, 5863–5870.
- 104 4. Denny, W.A.; Wilson, W.R. *Expert Opin. Invest. Drugs* **2000**, *9*,
105 2889–2901.
- 106 5. Laderoute, K.; Wardman, P.; Rauth, A.M. *Biochem. Pharmacol.*
107 **1988**, *37*(8), 1487–1495.
- 108 6. Lloyd, R.V.; Duling, D.R.; Romyantseva, G.V.; Mason, R.P.;
109 Bridson, P.K. *Mol. Pharmacol.* **1991**, *40*, 440–445.
7. Wardman, P.; Priyadarsini, K.I.; Dennis, M.F.; Everett, S.A.;
Naylor, M.A.; Patel, K.B.; Stratford, I.J.; Stratford, M.R.L.;
Tracy, M. *Br. J. Cancer* **1996**, *74*, S70–S74. 110
111
8. Daniels, J.S.; Gates, K.S. *J. Am. Chem. Soc.* **1996**, *118*, 3380–
3385. 112
113
9. Birincioglu, M.; Jaruga, P.; Chowdhury, G.; Rodriguez, H.; Di-
daroglu, M.; Gates, K.S. *J. Am. Chem. Soc.* **2003**, *125*, 11607–
11615. 114
115
116
10. Fuchs, T.; Chowdhary, G.; Barnes, C.L.; Gates, K.S. *J. Org.*
Chem. **2001**, *66*, 107–114. 117
118
119
11. Mason, J.C.; Tennant, G. *J. Chem. Soc. B.* **1970**, 911–916. 120
12. Fuchs, T.; Barnes, C.L.; Gates, K.S. *J. Chem. Crystallogr.* **2002**,
37(7/8), 387–392. 121
122
13. Atallah, R.H.; Nazar, M.J. *Tetrahedron.* **1982**, *38* (12), 1793–
1796. 123
124
14. Sheldrick, G.M., SHELXS-97, *Program for the Solution*
of Crystal Structures; University of Gottingen: Germany,
1997. 125
126
127
15. Sheldrick, G.M., SHELXS-97, *Program for the Refinement*
of Crystal Structures; University of Gottingen: Germany,
1997. 128
129
130
16. Desiraju, G.R. *Acc. Chem. Res.* **2002**, *35*, 565–573. 131