

Purines. LII.¹⁾ Synthesis and Biological Evaluation of 8-Methylguanine 7-Oxide and Its 9-Arylmethyl Derivatives

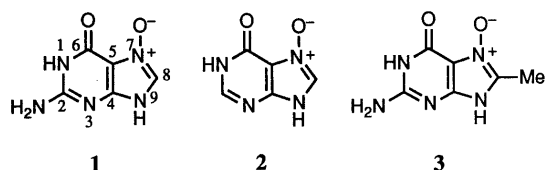
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The synthesis of 8-methylguanine 7-oxide (**3**) was accomplished *via* a "phenacylamine route", which started from condensation of α -(4-methoxybenzylamino)propiophenone (**6**), prepared by coupling of α -bromopropiophenone (**4**) and 4-methoxybenzylamine (**5**), with 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**7**) and proceeded through cyclization of the resulting phenacylaminopyrimidinone (**8**) and removal of the 4-methoxybenzyl group. The *N*-oxide **3** and its 9-arylmethyl derivatives **9** and **11** showed only very weak antileukemic activity and no antimicrobial activity.

Keywords phenacylation primary amine; condensation chloropyrimidinone-phenacylamine; cyclization nitro-phenacyl-amino; guanine 7-oxide 8,9-disubstituted; debenzilation benzyl carbenium ion; antitumor activity

Guanine 7-oxide (**1**) is an antitumor antibiotic isolated from the culture broths of certain *Streptomyces* species by three independent research groups, including some of us, in 1985.²⁻⁴⁾ The chemical synthesis of this *N*-oxide⁵⁾ and its 9-substituted derivatives has been achieved by us⁶⁾ *via* the multistep "phenacylamine route", which has also proved applicable to the synthesis of hypoxanthine 7-*N*-oxide (**2**).^{1,7)} As in the case of **2**,^{1,7)} **1** undergoes an apparent migration of the oxygen function from N(7) to C(8) in boiling AcOH.^{4,8)} Such instability is undesirable from the standpoint of drug design. This led us to design 8-methylguanine 7-oxide (**3**), a model for C(8)-blocked derivatives of **1**, for synthesis and biological evaluation in the present study.



The synthesis of the target **3** was so planned that it follows an α -methylphenacylamine version, as shown in Chart 1, of our recent "phenacylamine route" developed for the synthesis of **1**.⁶⁾ Condensation of α -bromopropiophenone (**4**) with 4-methoxybenzylamine (**5**) (2 molar eq) in boiling benzene for 8 h gave, after treatment with HCl, the amino

ketone hydrochloride **6**·HCl in 60% yield. The hydrochloride **6**·HCl was treated with 10% aqueous NaOH to generate the free base **6**, which was then allowed to react with the chloropyrimidinone **7**⁹⁾ (0.5 molar eq) in 50% (v/v) aqueous EtOH at 70–80 °C for 30 min, producing the phenacylaminopyrimidinone **8** in 73% yield. On treatment with 2 *N* aqueous NaOH in MeOH, **8** cyclized to afford the *N*-oxide **9** in 93% yield. Characterization of **9** as the 7-oxide was based on elemental analysis and similarities to the recently reported C(8)-H homologue (**12**)⁶⁾ in the mode of formation; in the ultraviolet (UV) spectra in acid, neutral, and basic media; and in the proton nuclear magnetic resonance (¹H-NMR) spectrum in 1 *N* D₂SO₄/D₂O except for the appearance of the C(8)-Me signal at δ 2.64 instead of the C(8)-H signal at δ 8.96. Removal of the 4-methoxybenzyl group from **9** was then effected by application of the previously reported, specific debenzilation method.^{1,6,7,10)} Thus, **9** was treated with 90% aqueous H₂SO₄ at 35 °C for 1.5 h in the presence of toluene to furnish the desired compound **3** in 63% yield. The use of conc. H₂SO₄ in this debenzilation reaction at room temperature for 2 h produced the sulfobenzyl analogue **11** as the major product (66% yield), and the yield of **3** was small (26%).

The correctness of structure **3** was supported by elemental analysis; the ¹H-NMR spectrum in 1 *N* D₂SO₄/D₂O [δ 2.63 (C(8)-Me)]; and the UV spectra in acid, neutral,

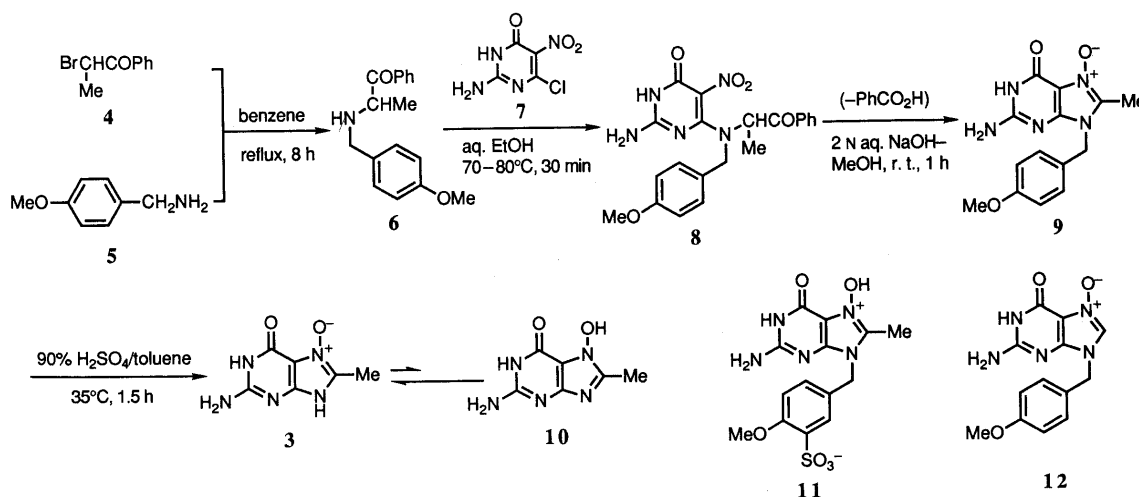


Chart 1

TABLE I. Antileukemic Activity of Guanine 7-Oxide Derivatives against Murine L5178Y Cells in Culture

N(7)-Oxide				
No.	Substituent		% inhibition (at 50 $\mu\text{g/ml}$)	IC ₅₀ ^{a)} ($\mu\text{g/ml}$)
	at N(9)	at C(8)		
1	H	H	— ^{b)}	1.10 ^{c)}
3	H	Me	12	— ^{b)}
12	4-Methoxybenzyl	H	80 ^{c)}	22.5 ^{c)}
9	4-Methoxybenzyl	Me	25	— ^{b)}
11	4-Methoxy-3-sulfobenzyl	Me	20	— ^{b)}

a) The IC₅₀ is defined as the concentration of a test compound required to inhibit cell growth by 50%. b) Not determined. c) Taken from ref. 6b.

and basic media that were similar to those^{6b)} of **1**. The UV spectrum of **3** in H₂O at pH 3.6 was also similar to the recently reported,^{6b)} neutral species spectrum of **1** at the same pH. Assuming that **3** has three pK_a values close to those (2.6, 5.8, and 9.5)⁴⁾ of the C(8)-H homologue **1**, such UV spectral similarity may suggest the preponderance of the N(7)-oxide form (**3**) over the N(7)-OH form (**10**) in H₂O for the neutral species of 8-methylguanine 7-*N*-oxide, as in the case^{6b)} of **1**.

Table I shows the cytotoxicities of **3** and its 9-arylmethyl derivatives (**9** and **11**) as found in a bioassay of antileukemic activity against murine L5178Y cells, together with those reported^{6b)} for the C(8)-H counterparts **1** and **12**. It may be seen that introduction of a methyl group into the antileukemic antibiotic guanine 7-oxide (**1**) at the 8-position causes its cytotoxicity to decrease significantly. A similar tendency is apparent for the 9-(4-methoxybenzyl) derivative (**9**). The sulfobenzyl derivative **11** is also very weakly cytotoxic.

In tests for antibacterial activity against *Staphylococcus aureus* 209P, *Escherichia coli* NIHJ, and *Pseudomonas aeruginosa* and for antifungal activity against *Candida albicans*, *Trychophyton mentagrophytes*, and *T. rubrum* using the conventional paper disk method, none of **3**, **9**, and **11** showed any activity even at a concentration as high as 1000 μ g/ml. These antimicrobial results are parallel to those obtained for **1** except that **1** possesses a weak anticandidal activity.⁴⁾

In conclusion, 8-methylguanine 7-oxide (**3**) has now become available as a result of the synthesis following the above four-step "phenacylamine route". Unfortunately, the *N*-oxide **3** and its 9-substituted derivatives **9** and **11** were found to show only very weak antileukemic activity and no antimicrobial activity.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Internal standards used for the measurements of ¹H-NMR spectra were Me₄Si (for Me₂SO-*d*₆ solutions), sodium 3-(trimethylsilyl)-1-propanesulfonate (for D₂O solutions), and sodium 3-(trimethylsilyl)propionate-2,2,3,3-*d*₄ (for solutions in 1N D₂SO₄/D₂O). See ref. 6b for details of instrumentation and measurements and of chromatographies. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br=broad, d=doublet, dd=doublet-of-doublets, m=multiplet, q=quartet, s=singlet, sh=shoulder.

2-[(4-Methoxybenzyl)amino]-1-phenyl-1-propanone Hydrochloride (6·HCl) A stirred solution of α -bromopropiophenone (**4**) (17.0 g,

79.8 mmol) and 4-methoxybenzylamine (**5**) (21.9 g, 160 mmol) in benzene (140 ml) was heated under reflux for 8 h and then allowed to cool. The colorless precipitate (**5**·HBr) that resulted was filtered off and washed with two 20-ml portions of benzene. The filtrate and washings were combined and concentrated to dryness *in vacuo* to leave a yellow oil. The oil was dissolved in ether (50 ml), and 10% (w/w) ethanolic HCl was added until the solution became sufficiently acidic. The resulting solution was concentrated *in vacuo* to leave a brownish solid. The solid was washed with acetone (2 $\times$ 10 ml) and recrystallized from EtOH (200 ml) to give a first crop (9.47 g, 39%) of **6**·HCl as colorless prisms, mp 183–204 °C (dec.). The usual work-up of the mother liquor from this recrystallization afforded a second crop (5.24 g, 21%) of **6**·HCl, mp 178–190 °C (dec.). The total yield of **6**·HCl was 14.71 g (60% from **4**). Recrystallization of the crude hydrochloride from EtOH yielded an analytical sample as colorless prisms, mp 185–204 °C (dec.); IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 2800–2400 (NH₂⁺), 1690 (COAr); ¹H-NMR (Me₂SO-*d*₆) δ : 1.53 (3H, d, *J*=7.3 Hz, CHMe), 3.76 (3H, s, OMe), 4.09 (2H, m, CH₂Ar), 5.24 (1H, br, CHMe), 6.96 [2H, d, *J*=8.8 Hz, C(3')-H and C(5')-H],¹¹⁾ 7.51 [2H, d, *J*=8.8 Hz, C(2')-H and C(6')-H],¹¹⁾ 7.4–8.2 (5H, m, C(6H)), 9.37 and 10.14 (1H each, br, NH₂⁺). Anal. Calcd for C₁₇H₁₉NO₂·HCl: C, 66.77; H, 6.59; N, 4.58. Found: C, 66.62; H, 6.56; N, 4.52.

2-Amino-6-[(4-methoxybenzyl)(1-methyl-2-oxo-2-phenylethyl)amino]-5-nitro-4(3H)-pyrimidinone (8) The hydrochloride **6**·HCl (2.90 g, 9.48 mmol) was added to an excess of 10% aqueous NaOH, and the aqueous mixture was extracted with three 10-ml portions of CH₂Cl₂. The CH₂Cl₂ extracts were combined, dried over anhydrous MgSO₄, and concentrated to dryness *in vacuo* to leave the free base **6** as a pale yellow oil, which was dissolved in 50% (v/v) aqueous EtOH (36 ml). After addition of the chloropyrimidinone **7**⁹⁾ (900 mg, 4.72 mmol), the solution of **6** was stirred at 70–80 °C for 30 min and then kept at room temperature overnight. The precipitate that resulted was collected by filtration washed successively with small amounts of H₂O and EtOH, and dried to yield **8** (1.45 g, 73% from **7**) as a pale yellow solid, mp 183–185 °C (dec.); IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3320, 3230 (NH₂ and NH), 1683 (COAr and CONH); ¹H-NMR (Me₂SO-*d*₆) δ : 1.43 (3H, d, *J*=6.5 Hz, CHMe), 3.71 (3H, s, OMe), 4.33 and 4.57 (1H each, d, *J*=16 Hz, CH₂Ar), 5.53 (1H, q, *J*=6.5 Hz, CHMe), 6.7–7.1 (2H, br, NH₂), 6.80 [2H, d, *J*=8.8 Hz, C(3')-H and C(5')-H],¹¹⁾ 7.08 [2H, d, *J*=8.8 Hz, C(2')-H and C(6')-H],¹¹⁾ 7.1–7.9 (5H, m, C(6H)), 10.76 (1H, br, NH). This sample was homogeneous on thin-layer chromatographic (TLC) analysis and was used in the next cyclization step without further purification.

9-(4-Methoxybenzyl)-8-methylguanine 7-Oxide (9) Compound **8** (340 mg, 0.803 mmol) was dissolved in a mixture of 2N aqueous NaOH (6.8 ml) and MeOH (3.4 ml), and the resulting mixture was stirred at room temperature for 1 h. The colorless crystals (presumed to be the Na salt of **9**) that resulted were collected by filtration and then dissolved in H₂O (15 ml). The aqueous solution was brought to pH ca. 5 by addition of 10% aqueous HCl, and the colorless crystals that deposited were filtered off, washed successively with H₂O (2 $\times$ 1 ml), EtOH (1 ml), and ether (2 $\times$ 1 ml), and dried to give **9**·1/5H₂O (229 mg, 93%), mp 195–215 °C (dec.), which exhibited a single spot on a TLC plate. Recrystallization from 50% (v/v) aqueous MeOH and drying over P₂O₅ at 2 mmHg and 100 °C for 8 h gave an analytical sample of **9**·1/5H₂O as colorless prisms, mp 263–267 °C (dec.); UV $\lambda_{\text{max}}^{95\% \text{ aq. EtOH}}$ 231 nm (ϵ 21400),¹²⁾ 273 (8800),¹²⁾ 330 (1500)¹²⁾; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 1) 256 (12900), 280 (sh) (9500), 334 (1600); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 7) 230 (28800), 269 (11200), 334 (1700); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 13) 226 (30100), 273 (11400), 345 (1800); ¹H-NMR (1N D₂SO₄/D₂O) δ : 2.64 [3H, s, C(8)-Me], 3.82 (3H, s, OMe), 5.35 (2H, s, CH₂Ar), 7.00 [2H, d, *J*=8.9 Hz, C(3')-H and C(5')-H],¹¹⁾ 7.33 [2H, d, *J*=8.9 Hz, C(2')-H and C(6')-H],¹¹⁾ Anal. Calcd for C₁₄H₁₅N₅O₃·1/5H₂O: C, 55.15; H, 5.09; N, 22.97. Found: C, 55.45; H, 5.07; N, 22.71.

8-Methylguanine 7-Oxide (3) To a stirred suspension of **9**·1/5H₂O (396 mg, 1.3 mmol) in toluene (4 ml) was added dropwise 90% aqueous H₂SO₄ (2.12 g, ca. 19.5 mmol), and the mixture was stirred vigorously at 35 °C for 1.5 h, during which time a reddish purple color was produced. The toluene layer was then removed by decantation, and the reddish brown, oily residue was treated with 10% aqueous NaOH under ice-cooling in order to bring the resulting aqueous mixture to pH ca. 6. The colorless crystals that resulted were filtered off and recrystallized by dissolving them in 1N aqueous NaOH and adding 1N aqueous HCl until the resulting solution became pH 5. The precipitate that resulted was filtered off, washed successively with H₂O (2 $\times$ 1 ml) and MeOH (2 $\times$ 1 ml), and dried to furnish **3**·2/3H₂O (159 mg, 63%), mp >300 °C. Further recrystallization in a similar manner and drying over P₂O₅ at 2 mmHg and room temperature for 48 h yielded an analytical sample as a microcrystalline

solid, mp $>300^{\circ}\text{C}$; UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 1) 250 nm (ϵ 12400), 275 (8100); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 3.6) 229 (15200), 256 (8900), 276 (sh) (7600); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 7) 233 (19400), 283 (6900); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 13) 229 (19300), 282 (7200); $^1\text{H-NMR}$ (1 N $\text{D}_2\text{SO}_4/\text{D}_2\text{O}$) δ : 2.63 [s, C(8)-Me]. *Anal.* Calcd for $\text{C}_6\text{H}_7\text{N}_5\text{O}_2 \cdot 2/3\text{H}_2\text{O}$: C, 37.31; H, 4.35; N, 36.26. Found: C, 37.36; H, 4.32; N, 36.05.

9-(4-Methoxy-3-sulfobenzyl)-8-methylguanine 7-Oxide (11) To a stirred suspension of **9** ($1/5\text{H}_2\text{O}$) (300 mg, 0.984 mmol) in toluene (3 ml) was added dropwise conc. H_2SO_4 (0.81 ml, ca. 15 mmol), and the mixture was stirred vigorously at room temperature for 2 h, during which time a dark, reddish purple color was produced. The toluene layer was then decanted, and the oily residue was mixed with H_2O (3 ml) under ice-cooling. The aqueous mixture was washed with benzene (2×2 ml), brought to pH 5 by addition of 10% aqueous NaOH, and concentrated *in vacuo* to ca. one-third of its original volume. The precipitate that resulted was filtered off, washed with a little H_2O , and dried to give the debenzylated product **3** ($2/3\text{H}_2\text{O}$) (49.5 mg, 26%) as a colorless solid, mp $>300^{\circ}\text{C}$. The aqueous filtrate and washings were combined and concentrated to dryness *in vacuo*. The residue was extracted with MeOH (2×20 ml), and the methanolic extracts were concentrated *in vacuo* to leave **11** ($3/2\text{H}_2\text{O}$) (265 mg, 66%) as a slightly pink solid. Recrystallization of the solid from MeOH and drying over P_2O_5 at 2 mmHg and 50°C for 12 h afforded **11** ($3/2\text{H}_2\text{O}$) as colorless prisms, mp $>300^{\circ}\text{C}$; positive to a test for detection of sulfur by the sodium fusion method¹³⁾; $^1\text{H-NMR}$ (D_2O) δ : 2.53 [3H, br s, C(8)-Me], 3.90 (3H, s, OMe), 5.27 (2H, s, CH_2Ar), 7.12 [1H, d, $J=8.5$ Hz, C(5')-H],¹¹⁾ 7.41 [1H, dd, $J=8.5$ and 2.2 Hz, C(6')-H],¹¹⁾ 7.72 [1H, d, $J=2.2$ Hz, C(2')-H].¹¹⁾ *Anal.* Calcd for $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O}_6\text{S} \cdot 3/2\text{H}_2\text{O}$: C, 41.18; H, 4.44; N, 17.15. Found: C, 41.36; H, 4.25; N, 17.07.

Bioassay Procedure Compounds **3**, **9**, and **11** were subjected to *in vitro* bioassay of antileukemic activity against murine L5178Y cells in a manner similar to that described recently^{6b)} for **1** and related compounds. The results are given in Table I.

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References and Notes

- 1) Paper LI in this series, K. Ogawa, M. Nishii, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, **40**, 612 (1992).
- 2) a) D. L. Kern, G. C. Hokanson, J. C. French, and N. K. Dalley, *J. Antibiot.*, **38**, 572 (1985); b) R. C. Jackson, T. J. Boritzki, J. A. Besserer, K. L. Hamelehle, J. L. Shillis, W. R. Leopold, and D. W. Fry, *Adv. Enzyme Regul.*, **26**, 301 (1987).
- 3) a) M. Kitahara, K. Ishii, Y. Kumada, T. Shiraishi, T. Furuta, T. Miwa, H. Kawaharada, and K. Watanabe, *J. Antibiot.*, **38**, 972 (1985); b) M. Kitahara, K. Ishii, H. Kawaharada, K. Watanabe, T. Suga, T. Hirata, and S. Nakamura, *ibid.*, **38**, 977 (1985).
- 4) M. Nishii, J. Inagaki, F. Nohara, K. Isono, H. Kusakabe, K. Kobayashi, T. Sakurai, S. Koshimura, S. K. Sethi, and J. A. McCloskey, *J. Antibiot.*, **38**, 1440 (1985).
- 5) The designation "*N*-oxide" used in this paper follows that adopted in the literature: J. C. Parham, T. G. Winn, and G. B. Brown, *J. Org. Chem.*, **36**, 2639 (1971) and footnote 15 cited therein.
- 6) a) F. Nohara, M. Nishii, K. Ogawa, K. Isono, M. Ubukata, T. Fujii, T. Itaya, and T. Saito, *Tetrahedron Lett.*, **28**, 1287 (1987); b) K. Ogawa, M. Nishii, J. Inagaki, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, **40**, 343 (1992).
- 7) K. Ogawa, T. Saito, F. Nohara, M. Nishii, T. Itaya, and T. Fujii, *Heterocycles*, **27**, 885 (1988).
- 8) The resulting, crude sample of 8-hydroxyguanine was inactive in a preliminary test for cytotoxicity against murine L5178Y leukemia cell line *in vitro* or for antifungal activity against *Candida albicans*, which was carried out as described previously.^{1,6b)}
- 9) a) A. Stuart and H. C. S. Wood, *J. Chem. Soc.*, **1963**, 4186; b) D. T. Browne, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, ed. by W. W. Zorbach and R. S. Tipson, Interscience Publishers, New York, 1968, pp. 94–95; c) C. Temple, Jr., B. H. Smith, and J. A. Montgomery, "Nucleic Acid Chemistry," Part 1, ed. by L. B. Townsend and R. S. Tipson, Wiley-Interscience, New York, 1978, pp. 47–52.
- 10) a) L. M. Weinstock, R. J. Tull, A. W. Douglas, and I. Shinkai, *J. Org. Chem.*, **45**, 5419 (1980); b) N. J. Leonard, T. Fujii, and T. Saito, *Chem. Pharm. Bull.*, **34**, 2037 (1986).
- 11) For convenience, each aromatic carbon in the benzyl moiety is indicated by a primed number.
- 12) The ϵ values may not be accurate because of the poor solubility of the sample in 95% (v/v) aqueous EtOH.
- 13) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed., D. C. Heath and Co., Boston, 1955, pp. 226–228.