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

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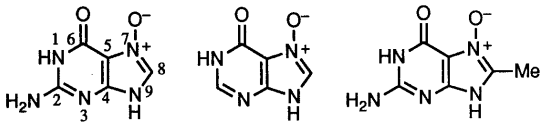
The synthesis of 8-methylguanine 7-oxide (3) was accomplished *via* a "phenacylamine route", which started from condensation of α-(4-methoxybenzylamino)propiophenone (6), prepared by coupling of α-bromopropiophenone (4) and 4-methoxybenzylamine (5), with 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (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–phenacylamino; guanine 7-oxide 8,9-disubstituted; debenzylation 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*-oxide[5] and its 9-substituted derivatives has been achieved by us[6] *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.



1 **2** **3**

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 (^{1}H-NMR) spectrum in 1 N D_2SO_4/D_2O 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 debenzylation method.[1,6,7,10] Thus, **9** was treated with 90% aqueous H_2SO_4 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. H_2SO_4 in this debenzylation 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 ^{1}H-NMR spectrum in 1 N D_2SO_4/D_2O [δ 2.63 (C(8)-Me)]; and the UV spectra in acid, neutral,

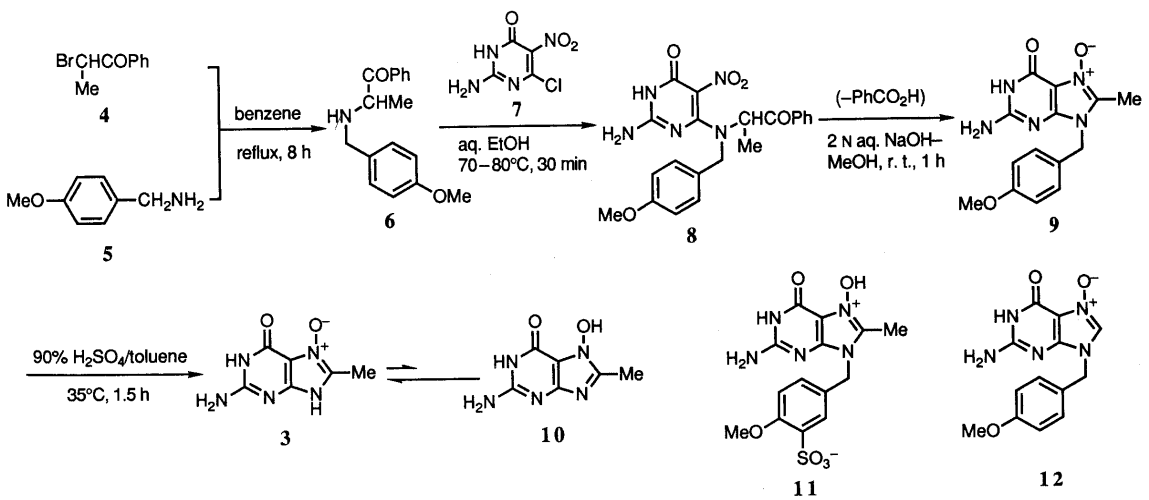


Chart 1

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

No.	N(7)-Oxide Substituent at N(9)	at C(8)	% inhibition (at 50 μg/ml)	IC_{50}[a] (μg/ml)
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 IC_{50} is defined as the concentration of a test compound required to inhibit cell growth by 50%. *b*) Not determined. *c*) Taken from ref. 6*b*.

and basic media that were similar to those[6b] of **1**. The UV spectrum of **3** in H_2O 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 H_2O 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 μg/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 ^{1}H-NMR spectra were Me_4Si (for Me_2SO-d_6 solutions), sodium 3-(trimethylsilyl)-1-propanesulfonate (for D_2O solutions), and sodium 3-(trimethylsilyl)propionate-*2,2,3,3*-d_4 (for solutions in 1 N D_2SO_4/D_2O). See ref. 6*b* 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 × 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 ν_{max}^{Nujol} cm^{-1}: 2800—2400 (NH_2^+), 1690 (COAr); ^{1}H-NMR (Me_2SO-d_6) δ: 1.53 (3H, d, J = 7.3 Hz, CHMe), 3.76 (3H, s, OMe), 4.09 (2H, m, CH_2Ar), 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, COPh), 9.37 and 10.14 (1H each, br, NH_2^+). *Anal.* Calcd for $C_{17}H_{19}NO_2$·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(3*H*)-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 CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried over anhydrous $MgSO_4$, 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 H_2O and EtOH, and dried to yield **8** (1.45 g, 73% from **7**) as a pale yellow solid, mp 183—185 °C (dec.); IR ν_{max}^{Nujol} cm^{-1}: 3320, 3230 (NH_2 and NH), 1683 (COAr and CONH); ^{1}H-NMR (Me_2SO-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, CH_2Ar), 5.53 (1H, q, J = 6.5 Hz, CHMe), 6.7—7.1 (2H, br, NH_2), 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, COPh), 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 2 N 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 H_2O (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 H_2O (2 × 1 ml), EtOH (1 ml), and ether (2 × 1 ml), and dried to give **9**·1/5H_2O (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 P_2O_5 at 2 mmHg and 100 °C for 8 h gave an analytical sample of **9**·1/5H_2O as colorless prisms, mp 263—267 °C (dec.); UV $\lambda_{max}^{95\%aq.EtOH}$ 231 nm (ε 21400),[12] 273 (8800),[12] 330 (1500)[12]; $\lambda_{max}^{H_2O}$ (pH 1) 256 (12900), 280 (sh) (9500), 334 (1600); $\lambda_{max}^{H_2O}$ (pH 7) 230 (28800), 269 (11200), 334 (1700); $\lambda_{max}^{H_2O}$ (pH 13) 226 (30100), 273 (11400), 345 (1800); ^{1}H-NMR (1 N D_2SO_4/D_2O) δ: 2.64 [3H, s, C(8)-Me], 3.82 (3H, s, OMe), 5.35 (2H, s, CH_2Ar), 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 $C_{14}H_{15}N_5O_3$·1/5H_2O: 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**·1/5H_2O (396 mg, 1.3 mmol) in toluene (4 ml) was added dropwise 90% aqueous H_2SO_4 (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 1 N aqueous NaOH and adding 1 N aqueous HCl until the resulting solution became pH 5. The precipitate that resulted was filtered off, washed successively with H_2O (2 × 1 ml) and MeOH (2 × 1 ml), and dried to furnish **3**·2/3H_2O (159 mg, 63%), mp > 300 °C. Further recrystallization in a similar manner and drying over P_2O_5 at 2 mmHg and room temperature for 48 h yielded an analytical sample as a microcrystalline

solid, mp >300 °C; UV $\lambda_{max}^{H_2O}$ (pH 1) 250 nm (ε 12400), 275 (8100); $\lambda_{max}^{H_2O}$ (pH 3.6) 229 (15200), 256 (8900), 276 (sh) (7600); $\lambda_{max}^{H_2O}$ (pH 7) 233 (19400), 283 (6900); $\lambda_{max}^{H_2O}$ (pH 13) 229 (19300), 282 (7200); ^{1}H-NMR (1 N D_2SO_4/D_2O) δ: 2.63 [s, C(8)-Me]. *Anal.* Calcd for $C_6H_7N_5O_2 \cdot 2/3H_2O$: 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**·1/5H_2O (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**·2/3H_2O (49.5 mg, 26%) as a colorless solid, mp >300 °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**·3/2H_2O (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**·3/2H_2O as colorless prisms, mp >300 °C; positive to a test for detection of sulfur by the sodium fusion method[13]; ^{1}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 $C_{14}H_{15}N_5O_6S \cdot 3/2H_2O$: 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.