V, 127103-15-5; VI, 127103-16-6; VII, 63495-82-9; VIII, 127103-17-7; IX, 127103-18-8; X, 127103-19-9; XI, 127129-86-6; XII, 127129-87-7; XIII, 127129-88-8; BocD(OBzl), 7536-58-5; K(Z)OBzl, 24458-14-8; BocD(OBzl)K(Z)OBzl, 33838-60-7; BocS(OBzl), 23680-31-1; BocS(OBzl)OSu, 13650-73-2; D(OBzl), 2177-63-1; BocS(OBzl)D(OBzl), 127103-20-2; D(OBzl)OBzl·TsOH, 2886-33-1; Bock(Z), 2389-45-9; P-OBzl·HCl, 16652-71-4; Bock(Z)P-OBzl, 68280-74-0; BocD(OBzl)OSu, 13798-75-9; K(Z), 1155-64-2; Bock(Z)OSu, 34404-36-9; P, 147-85-3; BocE(OBzl), 13574-13-5; BocE(OBzl)K(Z)P, 127103-21-3; BocS(OBzl)E(OBzl)K(Z)P, 127103-22-4; BocOrn(Z), 2480-93-5; BocOrn(Z)P-OBzl, 127103-23-5; BocD(OBzl)Orn(Z)P-OBzl, 127103-24-6; BocS(OBzl)D-(OBzl)Orn(Z)P-OBzl, 127129-89-9; BocA, 15761-38-3; BocAD-(OBzl)K(Z)P-OBzl, 127103-25-7; Boc-D-D(OBzl), 51186-58-4; Boc-D-D(OBzl)K(Z)P-OBzl, 127103-26-8; BocD-OBzl, 30925-18-9; BocDOBzlβK(Z)P-OBzl, 127103-27-9; BocS(OBzl)DOBzlβK(Z)-P-OBzl, 127103-28-0; BocS(OBzl)-D-D(OBzl)K(Z)P-OBzl, 127103-29-1.

Synthesis and Broad-Spectrum Antiviral Activity of 7,8-Dihydro-7-methyl-8-thioxoguanosine

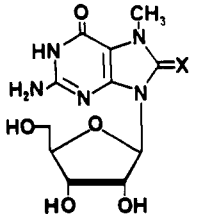
Elizabeth M. Henry,† Ganesh D. Kini,*,‡ Steven B. Larson, Roland K. Robins, Hassan A. Alaghamandan, and Donald F. Smee§

ICN Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, California 92626. *Received December 21, 1989*

2,6,8-Trichloro-7-methylpurine (3) was converted to 2-chloro-8,9-dihydro-7-methyl-8-thioxopurin-6(1*H*)-one (5) by utilizing the difference in reactivity of the 2-, 6-, and 8-positions in the trichloropurine ring system to nucleophilic displacement. Compound 5 was subsequently glycosylated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose according to the Vorbrüggen procedure to yield 2-chloro-8,9-dihydro-7-methyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (6). Removal of the benzoyl protecting groups, followed by amination of 7 with liquid ammonia at 150 °C, gave 7,8-dihydro-7-methyl-8-thioxoguanosine (2). The structure of compound 2 was confirmed by X-ray crystallographic analysis. Compounds 1 (7,8-dihydro-7-methyl-8-oxoguanosine) and 2 were evaluated for activity in various animal virus infection models. Against banzi, Semliki Forest, and San Angelo viruses in mice, 2 was highly active when administered before virus inoculation.

Introduction

Purine nucleosides are of considerable interest to the medicinal chemist as potential chemotherapeutic agents. Reviews on the antitumor[1,2] and antiviral[3] activity of purine nucleosides have appeared in the literature. Nucleoside derivatives such as 8-bromoguanosine,[4] 8-mercaptoguanosine,[4] and 7,8-dihydro-7-methyl-8-oxoguanosine (1)[5] have been shown to be stimulators of the humoral (B-cell) immune system.[6-9] Robins and coworkers[5] reported the synthesis of 7,8-dihydro-7-methyl-8-oxoguanosine in 1969. Subsequently, we reported a new and facile synthesis of this compound.[10] We have now found that compound 1 exhibits antiviral activity as well.[11] A report from our laboratory on the synthesis[11] and broad-spectrum antiviral activity[12] of another guanosine analogue, 7,8-dihydro-7-thia-8-oxoguanosine, has recently been published. As part of our program for the synthesis of purine nucleoside derivatives as potential antiviral and antitumor agents, the synthesis of 7,8-dihydro-7-methyl-8-thioxoguanosine (2) was pursued and accomplished. This synthesis and the in vivo antiviral activity of the purine nucleoside derivatives 1 and 2 are the subject of this paper.

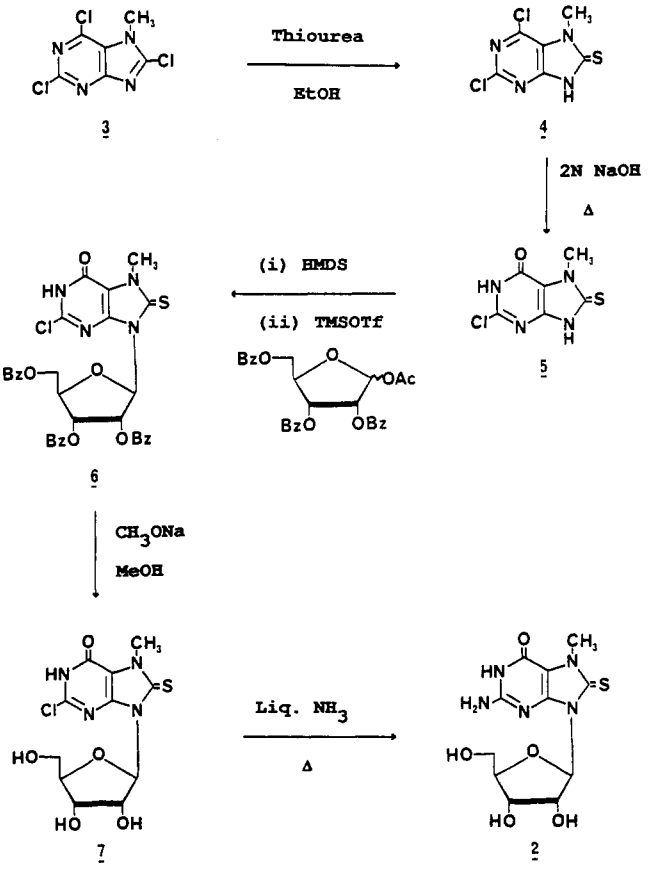


1: X = O
2: X = S

Chemistry

2,6,8-Trichloro-7-methylpurine (3), synthesized by a reported[13] procedure with slight modifications,[14] was the starting compound. The difference in reactivity of the 2-,

Scheme I



3 4 5 6 7 2

† Present address: Allergan Inc., 2525 Dupont Ave., Irvine, CA 92715.

‡ Present address: Brunswick Biotechnetics, 4116 Sorrento Valley Blvd., San Diego, CA 92126.

§ Present address: Utah State University, Logan, Utah 84322.

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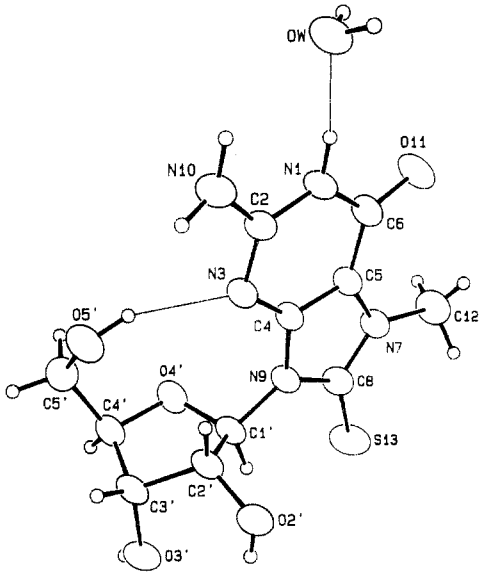

Figure 1. ORTEPII[27] drawing of 7,8-dihydro-7-methyl-8-thioxoguanosine (2) detailing the atom labeling. The thin lines represent hydrogen bonds. Thermal ellipsoids are drawn at the 50% probability level.

6-, and 8-positions in the trichloropurine ring system to nucleophilic displacement was the key element in the design and successful synthesis of 7,8-dihydro-7-methyl-8-thioxoguanosine, shown in Scheme I. Treatment of **3** with thiourea in refluxing ethanol resulted in the selective displacement of the 8-chloro group to yield 8,9-dihydro-2,6-dichloro-7-methylpurine-8-thione (**4**) in good yield. Hydrolysis of **4** with 2 N sodium hydroxide at reflux temperatures led to 2-chloro-8,9-dihydro-7-methyl-8-thioxopurin-6(1*H*)-one (**5**) in good yields as well. Compound **5** was silylated by refluxing with hexamethyldisilazane and subsequently glycosylated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose according to the procedure of Vorbrüggen et al.[15] to yield 2-chloro-8,9-dihydro-7-methyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (**6**). No *S*-glycosylated product was isolated under these conditions. Treatment of **6** with sodium methoxide in methanol resulted in removal of the benzoyl protecting groups to yield the deblocked nucleoside 2-chloro-8,9-dihydro-7-methyl-9-(β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (**7**). Amination of **7** with liquid ammonia at 150 °C resulted in 7,8-dihydro-7-methyl-8-thioxoguanosine (**2**). The structure of compound **2** was confirmed by X-ray crystallographic analysis.

Table I. Activities of Compounds 1 and 2 against Banzi, Semliki Forest and San Angelo Virus Infections in Mice

virus	compound	dose,[a] mg/kg	% survivors/ total	mean survival time,[b] days
Banzi	placebo		1/12 (8)	7.7 ± 1.8
	1	25	11/12 (92)[e]	8.0 ± 0.0
		50	12/12 (100)[e]	>21
	2	25	11/12 (92)[e]	9.0 ± 0.0
		50	10/12 (83)[d]	9.0 ± 0.0
San Angelo	placebo[c]		2/11 (18)	7.2 ± 1.6[d]
	1	25	11/12 (92)[e]	12.0 ± 0.0
		50	11/12 (92)[e]	10.0 ± 0.0
	2	25	9/12 (75)[e]	11.3 ± 2.3[f]
		50	10/12 (83)[e]	10.5 ± 0.7[f]
Semliki Forest	placebo[c]		2/12 (17)	6.2 ± 1.5
	1	25	8/12 (67)[e]	8.7 ± 3.8
		50	9/12 (75)[e]	9.7 ± 2.1[f]
	2	25	9/12 (75)[e]	8.0 ± 1.0[f]
		50	10/12 (83)[e]	9.0 ± 2.8

[a] Half-daily intraperitoneal doses were administered 24 and 18 h before virus inoculation. [b] Of mice that died. Survivors lived through 21 days. [c] A 2% sodium bicarbonate solution served as the placebo and as diluent for the compounds. [d] Standard deviation. [e] Statistically significant ($p < 0.05$), determined by the two-tailed Fisher exact test. [f] Statistically significant ($p < 0.05$), determined by two-tailed t test.

Table II. Activities of Compounds 1 and 2 against an Encephalomyocarditis (EMC) Virus Infection in Mice and a Rat Coronavirus Infection in Rats

virus	compound	dose,[a] mg/kg	% survivors/ total	mean survival time,[b] days
EMC	placebo[c]		1/12 (8)	4.0 ± 0.8[d]
	1	25	8/12 (67)[e]	8.3 ± 2.9[f]
		50	9/12 (75)[e]	8.0 ± 4.4[f]
	2	25	6/12 (50)	5.5 ± 1.0[f]
		50	9/12 (75)[e]	5.7 ± 0.6[f]
rat corona	placebo		1/11 (9)	7.4 ± 2.5
	1	100	9/12 (75)[e]	9.7 ± 0.6
	2	100	7/12 (58)[e]	9.4 ± 2.3

[a] Half-daily intraperitoneal doses were administered 24 and 18 h before virus inoculation. [b] Of mice or rats that died. Survivors lived through 21 days. [c] A 2% sodium bicarbonate solution served as the placebo and as diluent for the compounds. [d] Standard deviation. [e] Statistically significant ($p < 0.05$), determined by the two-tailed Fisher exact test. [f] Statistically significant ($p < 0.05$), determined by two-tailed t test.

X-ray Crystallography

Crystals of 7,8-dihydro-7-methyl-8-thioxoguanosine (**2**) were grown from an 18:1:1:1 ethyl acetate/methanol/acetone/water solution following chromatographic purification. The nucleoside crystallizes in the orthorhombic space group $P2_12_12_1$ as a monohydrate with cell parameters of $a = 6.9207$ (2) Å, $b = 10.5170$ (9) Å, $c = 20.315$ (2) Å and is isomorphous to the 8-oxo analogue (**1**).[16] The structure refined to an R value of 0.029 for 2874 reflections ($F \geq 4\sigma_F$). The molecular conformation is illustrated in Figure 1, which also details the atom labeling. The molecular conformation is syn with a glycosylic torsion angle, χ_{CN} = O4′–C1′–N9′C4, of 63.6 (2)°, which is stabilized by an

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O5′→N3 intramolecular hydrogen bond [d(O5′···N3) = 3.011 (2) Å]. Thus, the C5′–O5′ side chain is in the gauche–gauche orientation. The sugar conformation is C_2-*endo* ($^2T_1$) with a pseudorotation angle of 156.3° and an amplitude of pucker of 38.7°.[17] These general conformational parameters are similar to other 8-substituted guanosines such as 8-bromo-,[18] 8-chloro-,[19] and 8-methylguanosine.[20] A syn conformation has been suggested as a necessary molecular conformation for B-cell activation,[21] an effect that is produced by all of the 8-substituted guanosines mentioned above. Details of the crystal and molecular structure are reported elsewhere.[22]

Antiviral Activity

Compounds **1** and **2** were evaluated for activity in various animal virus infection models. Against banzi, Semliki Forest, and San Angelo viruses in mice, **2** was highly active when administered before virus inoculation to decrease overall mortality (Table I). Its effect was similar to that produced by compound **1**. Against EMC virus, **1** and **2** protected against mortality (Table II). Rats treated with **2** were resistant to mortality induced by rat coronavirus, as were animals treated with the other nucleoside, 7,8-dihydro-7-methyl-8-oxoguanosine (**1**) (Table II). Overall, the efficacies of **1** and **2** were very similar.

Banzi, San Angelo, and Semliki Forest viruses represent classes of flavi-, bunya-, and alphaviruses that are transmitted by insect vectors. These types of viruses are of military importance and of interest to developing nations where insect-borne diseases are prevalent. The EMC virus is a picornavirus related to rhinoviruses which are some of the causative agents of the common cold. We use EMC virus as a model since rhinoviruses do not propagate in small animals. Rat coronavirus is probably the closest animal model to a coronavirus-induced common cold in man.[23] The results of these studies suggest that **2** and related compounds could be used on a prophylactic basis or possibly early in therapy against relevant human virus infections.

Experimental Section

Chemistry. ^{1}H NMR data were obtained at 300 MHz on an IBM NR-300 spectrometer in $(CD_3)_2SO$ or $CDCl_3$ solvents. The chemical shifts are expressed in δ values (parts per million) relative to the residual protons in the deuterated solvents. Melting points were obtained in open capillaries using a Haake-Buchler apparatus and are uncorrected. Combustion analyses, reported for certain select intermediates, were performed by Robertson Laboratories, Florham Park, NJ.

Virus Infection Models. Swiss Webster female mice or pregnant Fischer rats were purchased from Charles River Labs, Wilmington, MA. Several Encephalitis virus models have been developed with banzi,[24] encephalomyocarditis[25] (EMC), San Angelo,[26] and Semliki Forest[12] viruses. All of these viruses were inoculated intraperitoneally (ip) into 20-g mice. Rat coronavirus[23] was inoculated intranasally into suckling (3–4 days old) rats causing them to die of pneumonia. Viruses were pretitrated in the animals to identify doses which were 10 times the 50% lethal dose (LD_{50}). Each experiment was conducted with 10 LD_{50}.

Compounds were administered 0.2 mL/mouse or 0.1 mL per rat by ip injection 24 and 18 h before virus inoculation. This regimen was optimal for treatment against various RNA viruses.[24] All experiments ran for 21 days, at which time the animals were considered cured from the lethal phase of the infections. Statistical evaluations compared drug-treated groups to respective placebo controls. Increases in survival numbers were evaluated by the two-tailed Fisher exact test. Mean survival time increases were statistically analyzed by the two-tailed Student's t test.

2,6-Dichloro-8,9-dihydro-7-methylpurine-8-thione (4). To a suspension of 2,6,8-trichloro-7-methylpurine (**3**, 6.00 g, 25.2 mmol) in ethanol (200 mL) was added thiourea (2.12 g, 27.8 mmol), and the mixture was maintained at reflux for 2.0 h. The resulting clear yellow solution was adsorbed onto silica gel and chromatographed over silica gel (flash chromatography) with 10% acetone in hexane as eluent to yield **4** as a yellow solid (4.94 g, 83.4%): mp 240–242 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3 H, NCH_3), 14.31 (s, 1 H, N*H*). Anal. Calcd for $C_6H_4Cl_2N_4S$ (C, H, N, S, Cl).

2-Chloro-8,9-dihydro-7-methyl-8-thioxopurin-6(1*H*)-one (5). A suspension of 2,6,8-trichloro-7-methylpurine (**3**, 4.00 g, 16.8 mmol) and thiourea (1.40 g, 18.5 mmol) in ethanol (130 mL) was refluxed for 1 h. The solvent was removed in vacuo, and the residue was dissolved in 2 N aqueous sodium hydroxide (84 mL, 168 mmol). The resulting solution was refluxed overnight. After cooling, the solution was neutralized to pH 7.0 with concentrated HCl. The mixture was filtered, and the clear filtrate was acidified (pH 1–3) with concentrated HCl. The resultant yellow suspension was boiled for 10 min, cooled, and filtered. The filtered solid **5** was washed with water, acetone, and then dried (3.28 g, 90.6%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3 H, NCH_3), 13.56 (s, 1 H, N*H*), 13.90 (s, 1 H, N*H*).

2-Chloro-8,9-dihydro-7-methyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (6). A solution of 2-chloro-8,9-dihydro-7-methyl-8-thioxopurin-6(1*H*)-one (**5**, 12.3 g, 57.1 mmol) in hexamethyldisilazane (24.1 mL, 114 mmol), trimethylsilyl triflate (22.1 mL, 114 mmol), trimethylsilyl chloride (14.5 mL, 114 mmol), and dry acetonitrile (600 mL) was refluxed for 3 h. A solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (31.6 g, 62.8 mmol) in dry acetonitrile (300 mL) was added in one portion and refluxed for an additional 0.5 h. The clear yellow solution was cooled and poured slowly into a stirring 2% aqueous solution of $NaHCO_3$. The resulting mixture was extracted with ethyl acetate (1 L). The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residual oil was chromatographed over silica gel using chloroform as eluent until all nonpolar contaminants were eluted, followed by elution with acetone to yield **6** as a foam (28.58 g, 75.7%): ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3 H, NCH_3), 6.84 (d, 1 H, C_1H), 7.38–7.93 (m, 15 H, benzoyl protons), and other sugar protons. Anal. Calcd for $C_{32}H_{25}N_4O_8SCl$ (C, H, N, S, Cl).

2-Chloro-8,9-dihydro-7-methyl-9-(β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (7). To a suspension of 2-chloro-8,9-dihydro-7-methyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (**6**, 3.88 g, 5.88 mmol) in methanol (120 mL) was added freshly prepared 1 M sodium methoxide (19.4 mL, 19.4 mmol). The mixture was stirred for 3 h at room temperature, and the resulting solution was neutralized with H^+ resin (Dowex-50W X 8). Solvent was removed in vacuo, and the residue was triturated with ether, upon which a solid separated. The solid was filtered, washed well with ether, and dried to yield **7** (1.78 g, 86.8%). An analytical sample was prepared by recrystallization from methanol: ^{1}H NMR (DMSO-d_6) δ 3.51 (m, 2 H, C_5H), 3.78

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(s, 3 H, NCH_3), 3.84 (m, 1 H, C_4H), 4.20, 4.82 (2 t, 2 H, C_3H and C_4H), 6.36 (d, 1 H, J = 5.47 Hz, C_1H). Anal. Calcd for $C_{11}H_{13}N_4SCl$ (C, H, N, S, Cl).

7,8-Dihydro-7-methyl-8-thioxoguanosine (2). A suspension of 2-chloro-8,9-dihydro-7-methyl-9-(β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (**7**, 0.5 g, 1.4 mmol) in methanol (10 mL) was placed in a 200-mL stainless steel bomb and cooled in a dry ice/ethanol bath. Liquid ammonia (50 mL) was added; the bomb was sealed and heated in an oil bath (bath temperature 150 °C) overnight. The bomb was cooled in a dry ice/ethanol bath and opened. The ammonia was allowed to evaporate and the residue was chromatographed over silica gel (flash chromatography) with a mixture of ethyl acetate/acetone/water/methanol 15:1:1:1 as eluent to yield **2** as an amorphous solid (0.3 g, 60%): ^{1}H NMR (DMSO-d_6) δ 3.56 (m, 2 H, C_5H), 3.71 (s, 3 H, NCH_3), 3.78 (m, 1 H, C_4H), 4.21, 4.96 (2 dd, 2 H, C_3H and C_4H), 4.74 (t, 1 H, O*H*), 4.91 (d, 1 H, O*H*), 5.28 (d, 1 H, O*H*), 6.29 (d, 1 H, J = 5.41 Hz, C_1H), 6.62 (s, 2 H, NH_2), 11.15 (s, 1 H, N*H*); ^{13}C NMR (DMSO-d_6) δ 32.60, 62.24, 70.28, 70.42, 85.09, 89.40, 104.74, 148.75, 151.53, 153.48, 165.81. Anal. Calcd for $C_{11}H_{15}N_5O_5S$ (C, H, N, S).

Synthesis and Central Nervous System Actions of Thyrotropin-Releasing Hormone Analogues Containing a Dihydroorotic Acid Moiety†

Mamoru Suzuki,*‡ Hiroshi Sugano,‡ Kazuo Matsumoto,‡ Michio Yamamura,§ and Ryuichi Ishida§

Research Laboratory of Applied Biochemistry and Safety Research Laboratory, Tanabe Seiyaku Company, Ltd., 16-89, Kashima 3-chome, Yodogawa-ku, Osaka 532, Japan. *Received November 16, 1989*

A series of thyrotropin-releasing hormone (TRH) analogues in which the pyroglutamic acid residue was replaced by (*S*)-4,5-dihydroorotic acid (Dio-OH) and the related derivatives were prepared. Their central nervous system actions based on spontaneous locomotor activity, antagonistic effect on reserpine-induced hypothermia, and antagonistic effect on pentobarbital anesthesia were evaluated and the structure–activity relationships are discussed. Of these, (1-methyl-(*S*)-4,5-dihydroorotyl)-L-histidyl-L-prolinamide (**14b**) showed the most potent activities, which were 30–90 times greater than those of TRH. Moreover, the thyrotropin-releasing activity of **14b** was about 50 times weaker than that of TRH, and compound **14b** (TA-0910) was selected as a potent candidate.

Thyrotropin releasing hormone (TRH, **1**, L-pyroglutamyl-L-histidyl-L-prolinamide; Chart I) is known to cause a stimulating action on the central nervous system (CNS)[1] in addition to its endocrine action as a thyrotropin (TSH) releasing agent.[2] Recently, some chemical modifications of TRH to enhance the CNS actions and/or to decrease the endocrine activity have often been reported.[3-6]

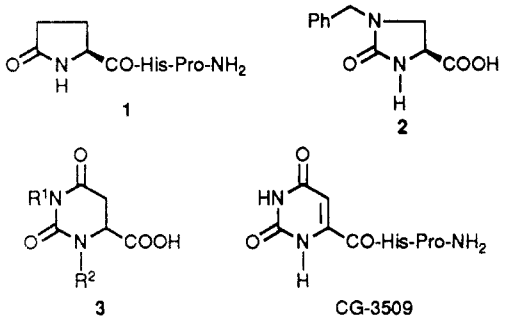
In this context, we have also studied the synthesis of TRH analogues to separate the CNS actions from the TSH-releasing activity, and through these studies we have focused on the lipophilic character in the TRH structure.[7,8] In one of our previous papers, we reported that the TRH analogue containing (*S*)-1-benzyl-2-oxoimidazolidine-4-carboxylic acid (**2**) in place of the pyroglutamic acid residue of TRH had 1.5–8 times greater CNS actions and about 16 times weaker hormonal activity than those of TRH itself.[8]

On the other hand, orotyl-His-Pro-NH_2 (CG-3509), while being clinically evaluated as a potent antidepressant, showed very weak endocrine activity (about $^1/_{13}$ times of TRH), but CNS actions were reported to be 2–3 times greater than those of TRH.[6]

Furthermore, it was reported that the analogues with six-membered rings such as L-piperidonecarboxylic acid (MK-771)[4b] and (*R*,*R*)-6-methyl-5-oxo-3-thiomorpholine-3-carboxylic acid (CG-3703)[6] in place of the pyroglutamic acid of TRH remarkably increased the CNS actions (40–66 times) on account of their complete resistance to degradation by the TRH-degrading serum enzyme and pyroglutamate aminopeptidase.[6b]

This information prompted us to attempt the enlargement of the 2-oxoimidazolidine moiety (**2**) to the related six-membered ring, and we chose the optically active dihydroorotic acid moiety, which is a hydrogenated skeleton of orotic acid (CG-3509). In this paper, we describe the syntheses and pharmacological activities of a series of TRH analogues containing various 4,5-dihydroorotic acid

Chart I



† Amino acids and their derivatives are L. Abbreviations follow the recommendations of the IUPAC-IUB Commission on Biological Nomenclature as given in *Eur. J. Biochem.* **1984**, *138*, 9–37.

‡ Research Laboratory of Applied Biochemistry.

§ Safety Research Laboratory.

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