Arch. Pharm. Res. Vol.21, No.2, pp. 187-192, 1998

Synthesis of the 7,8-Dihydro-7-deazapurine Derivatives and Their Antibiotic Activity

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(Received November 10, 1997)

The *cis*- and *trans*-diastereomers of the 7,8-dihydro-7-deazapurine derivatives were synthesized from the corresponding diastereomers of 4-*trans*-cyano-2-methyl-3-phenyl-5-oxopyrrolidine (**5**), which were reduced from the 2-*cis*- and 2-*trans*-diastereomers of 4-*trans*-cyano-2-hydroxymethyl-3-phenyl-5-oxopyrrolidine (**2**) *via* tosylation, iodination and following elimination, respectively. The prepared *cis*- and *trans*-diastereomers of 6-amino-2-mercapto-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (**8**) were transferred to the corresponding 2-methylthio-diastereomers **9** and following desulfurization with Raney-nickel leaded to the *cis*- and *trans*-diastereomers of 6-amino-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (**10**), respectively. The synthesized 7-deazapurine derivatives were tested for their antibiotic activity by the serial two-fold dilution method.

Key words : Deoxygenation, 4-Cyano-2-methyl-3-phenyl-5-oxopyrrolidine

INTRODUCTION

The naturally occurring 7-deazapurine derivatives and their synthetic analogs belong to the purine antagonist and show the interesting biological activities. Toyocamycin (**11**) and tubercidine (**12**) having anticancer, antiviral, and antibacterial metabolites as 7-deazapurine analogs were isolated from the *Streptomyces* species (Nishimura, *et al.*, 1956, and Anzai, *et al.*, 1957). After their total synthesis (Tolman, *et al.*, 1968 and 1969), a lot of 7-deazapurine derivatives and their nucleosides were synthesized, and in addition to their antiviral and antibacterial activity, their *in vitro* cell growth inhibition against L1210 and P388 leukemia were reported (Girgis, *et al.*, 1985, Joergensen, *et al.*, 1985, Seela, *et al.*, 1980 and 1988, Sinambela, *et al.*, 1986, Pichler, *et al.*, 1986, and Cottam, *et al.*, 1985).

In previously paper, we have described the synthesis of the new series of the 7-deazahypoxanthine and 7-deazaadenine derivatives (Sin, *et al.*, 1993 and 1997). Subsequently, this paper is reported the preparation of the *cis*- and *trans*-7,8-dihydro-7-deazaadenine derivatives from the corresponding diastereomers of 4-*trans*-cyano-2-methyl-3-phenyl-5-oxopyrrolidine (**5**), which are reduced from the diastereomers of 4-*trans*-cyano-2-ethoxycarbonyl-3-phenyl-5-oxopyrrolidine (**1**) *via* 4-*trans*-cyano-2-hydroxymethyl-3-phenyl-5-oxopyrrolidine (**2**) by deoxygenation, respectively (Pachaly, *et al.*, 1991, Sin, *et al.*, 1993 and 1997, and Morita, *et al.*, 1981). The synthesized *cis*- and *trans*-diastereomers of the 7-deazapurine derivatives are tested against *Bacillus subtilis*, and *Staphylococcus aureus* by the serial two-fold dilution method (Ueno, *et al.*, 1995) and the results together with discussion of the inhibitory effects in terms of their structural variation are reported.

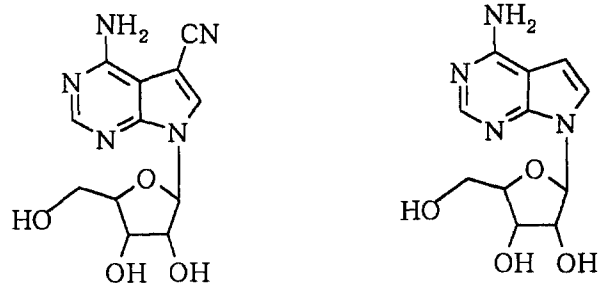

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Fig. 1. The chemical structures of toyocamycin (**11**) and tubercidine (**12**).

EXPERIMENTAL SECTION

Melting point (m.p.) were determined on Fisher-John melting point apparatus and are uncorrected. IR spectra were recorded with Perkin Elmer 783 spectrophotometer in cm^{-1}. NMR spectra were measured by Varian Gemini-200 for ^{1}H-NMR (200 MHz) and for ^{13}C-NMR (50 MHz). Thin-layer chromatography was performed on silica-gel 60 coated aluminum plate (Merk). All

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commercial chemicals were used as obtained and all solvent were purified by the standard procedures prior to use (Perrin, *et al.*, 1996 and Becker, *et al.*, 1990).

Synthesis of 4-Cyano-2-ethoxycarbonyl-3-phenyl-5-oxopyrrolidone (1)

By the Michael reaction with ethyl 2-cyano-3-phenylacrylate, N-(diphenylmethylene)glycine ethylester was transferred to the mixture of *erythro*- and *threo*-isomers of diethyl 4-cyano-N-(diphenylmethylene)-3-phenylglutamate (Pachaly, *et al.*, 1991, and Sin, *et al.*, 1993). Its *threo*-isomer was crystallized from MeOH and leaded *via* the acid-hydrolysis and then cyclization with potassium carbonate to the pyrrolidon-esters **1I**. The more soluble *erythro*-isomer was converted to the pyrrolidon-ester **1II**.

4-*trans*-Cyano-2-*trans*-ethoxycarbonyl-5-oxo-3-phenylpyrrolidine (1I): yield 22 %; m.p. 117°C (ethyl acetate/petroleum ether); IR (KBr) 3250, 2240, 1735 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ=7.38 (m, 5H, phenyl), 7.17 (s, 1H, NH), 4.40 (d, *J*=7.8 Hz, 1H, 2-H), 4.21 (m, 2H, $O\underline{CH_2}CH_3$), 3.92 (dd, *J*=10/7.8 Hz, 1H, 3-H), 3.73 (d, *J*=10 Hz, 1H, 4-H), 1.22 (t, *J*=7 Hz, 3H, $O\underline{CH_2}CH_3$); ^{13}C-NMR ($CDCl_3$,) δ=169.29 (s, C5), 167.14 (s, C=O), 136.59 (s, C1'), 129.58 (d, C3'), 128.98 (d, C4'), 127.34 (d, C2'), 115.53 (s, CN), 62.53 (t, $O\underline{CH_2}CH_3$), 60.55 (d, C2), 49.21 (d, C3), 41.85 (d, C4), 13.94 (q, $OCH_2\underline{CH_3}$).

4-*trans*-Cyano-2-*cis*-ethoxycarbonyl-5-oxo-3-phenylpyrrolidine (1II): yield 20%; m.p. 147°C (MeOH); IR (KBr) 3360, 2260, 1745, 1725 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ=7.37 (m, 4H, NH/phenyl), 7.23 (m, 2H, phenyl), 4.53 (d, *J*=7.4 Hz, 1H, 2-H), 4.23 (dd, *J*=11/7.4 Hz, 1H, 3-H), 4.17 (d, *J*=11 Hz, 1H, 4-H), 3.79 (m, 2H, $O\underline{CH_2}$-CH_3), 0.80 (t, 3H, $OCH^2\underline{CH_3}$); ^{13}C-NMR ($CDCl_3$) δ= 169.67 (s, C5), 169.45 (s, C=O), 132.94 (s, C1'), 129.19 (d, C3'), 129.06 (d, C4'), 127.51 (d, C2'), 115.79 (s, CN), 61.88 (t, OCH_2CH_3), 59.60 (d, C2), 48.51 (d, C3), 36.65 (d, C4), 13.47 (q, $OCH_2\underline{CH_3}$).

Synthesis of 4-cyano-2-hydroxymethyl-3-phenyl-5-oxopyrrolidine (2)

To a stirred solution of 2.5 g (10 mmol) pyrrolidone-ester **1** in 100 ml absolute ethanol were added 0.6 g (15 mmol) $NaBH_4$. After 5-8 hours at room temperature, the reaction mixture was neutralized by the ethanolic hydrochloride and evaporated completely, and water was added. The precipitated pyrrolidone-OH **2** was filtered, washed with water and crystallized from methanol.

4-*trans*-Cyano-2-*trans*-hydroxymethyl-3-phenyl-5-oxopyrrolidine (2I): yield 75%; m.p. 167°C; IR (KBr) 3420, 3210, 2250, 1710 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ= 8.53 (s, 1H, NH), 7.25-7.55 (m, 5H, phenyl), 4.99 (t, *J*=5 Hz, 1H, OH), 4.42 (d, *J*=11.6 Hz, 1H, 4-H), 3.63 (m, 2H, CH_2), 3.48 (m, 1H, 3-H), 3.3 (m, 1H, 2-H); ^{13}C-NMR (DMSO-d_6) δ=167.05 (s, C5), 137.18 (s, C1'), 128.76 (d, C3'), 128.05 (d, C2'), 127.80 (d, C4'), 117.89 (s, CN), 61.12 (t, CH2), 59.99 (d, C2), 46.77 (d, C3), 40.98 (d, C4).

4-*trans*-Cyano-2-*cis*-hydroxymethyl-3-phenyl-5-oxopyrrolidine (2II): yield 77%; m.p. 152°C; IR (KBr) 3365, 3215, 2250, 1700 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=8.54 (s, 1H, NH), 7.25-7.5 (m, 5H, phenyl), 4.84 (s (broad), 1H, OH), 4.63 (d, *J*=12.8 Hz, 1H, 4-H), 4.12 (dd, *J*= 12.8/8 Hz, 1H, 3-H), 3.81 (m, 1H, 2-H), 3.07 (m, 2H, CH_2); ^{13}C-NMR (DMSO-d_6) δ=168.21 (s, C5), 135.09 (s, C1'), 128.40 (d, C3'), 127.84 (d, C2'), 127.37 (d, C 4'), 118.24 (s, CN), 60.40 (t, CH2), 56.97 (d, C2), 47.37 (d, C3), 36.43 (d, C4).

Synthesis of 4-cyano-3-phenyl-2-(p-toluenesulfonyloxy) methyl-5-oxopyrrolidine (3)

A solution of 2.3 g (12 mmol) p-toluenesulfonylchloride in 30 ml absolute pyridine were added slowly to 2.16 g (10 mmol) pyrrolidone-OH **2** dissolved in 50 ml absolute pyridine at 0~5°C. After stirring 6~8 hours at room temperature, the solvent was removed completely in vacuum at 30°C. The oil residue was extracted with 100 ml $CHCl_3$, and washed with 19%-HCl, $NaHCO_3$-solution and water. The organic phase was dehydrated with Na_2SO_4 anhydrous, evaporated and the residue was dried with fine vacuum. The crude tosylate compound **3** was used immediately for the subsequent reaction.

4-*trans*-Cyano-3-phenyl-2-*trans*-(p-toluenesulfonyloxy)methyl-5-oxopyrrolidine (3I): ^{1}H-NMR ($CDCl_3$) δ= 7.78 (d, *J*=8.3 Hz, 2H, phenyl), 7.36 (m, 5H, phenyl), 7.23 (m, 2H, phenyl), 7.14 (s, 1H, NH), 4.03 (m, 3H, 2-H/CH_2), 3.76 (d, *J*=11 Hz, 1H, 4-H), 3.47 (dd, *J*=11/ 8.5 Hz, 1H, 3-H), 2.45 (s, 3H, CH3); ^{13}C-NMR ($CDCl_3$) δ=167.31 (s, C5), 145.89 (s, C1"), 135.11 (s, C1'), 132.04 (s, C4"), 130.36 (d, C2"), 129.73 (d, C3"), 129.17 (d, C4'), 128.16 (d, C3'), 127.58 (d, C2'), 115.86 (s, CN), 68.69 (t, CH_2), 58.39 (d, C2), 47.88 (d, C3), 41.93 (d, C4), 21.68 (q, CH_3).

4-*trans*-Cyano-3-phenyl-2-*cis*-(p-toluenesulfonyloxy)methyl-5-oxopyrrolidine (3II): ^{1}H-NMR ($CDCl_3$) δ=7.64 (d, *J*=8.2, 2H, phenyl), 7.35 (m, 5H, phenyl), 7.20 (m, 2H, phenyl), 6.67 (s, 1H, NH), 4.16 (m, 3H, 2-H/CH_2), 3.85 (d, *J*=10.7 Hz, 1H, 4-H), 3.60 (dd, *J*= 10.7/4.6 Hz, 1H, 3-H), 2.45 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=168.08 (s, C5), 145.78 (s, C1"), 132.49 (s, C 1'), 132.04 (s, C4"), 130.23 (d, C2"), 129.60 (d, C3"), 128.96 (d, C4'), 127.94 (d, C3'), 127.34 (d, C2'), 115. 80 (s, CN), 68.41 (t, CH_2), 55.13 (d, C2), 47.37 (d, C 3), 36.54 (d, C4), 21.66 (q, CH_3).

Synthesis of 4-cyano-2-methyl-3-phenyl-5-oxopyrrolidine (5)

The crude pyrrolidone-OTs **3** in 100 ml absolute a-

cetonitrile was reacted with 3 g (20 mmol) NaI at 80~85°C overnight to give the pyrrolidone-CH_2I **4**. To the cooled reaction mixture were added 0.5 ml acetic acid and 2.8 g (50 mmol) Zn-powder, and heated to 80~85°C for 2~3 hours. The excess Zn-powder was removed by filtration, the filtrate was evaporated, and the residue was extracted with chloroform. The organic phase was washed with water, dried over Na_2SO_4 anhydrous and concentrated. The residue was crystallized from ethyl acetate and petroleum ether.

4-*trans*-Cyano-2-*trans*-iodomethyl-3-phenyl-5-oxopyrrolidine (4I): m.p. 217~218°C ($CHCl_3$); ^{1}H-NMR (DMSO-d_6) δ=8.69 (s, 1H, NH), 7.44 (m, 5H), 4.56 (d, J=10.7, 1H, 4-H), 3.40 (m, 2H, 2-/3-H), 3.40 (m, 1H, CH2), 3.20 (dd, J=9.3, 1H, CH_2); ^{13}C-NMR (DMSO-d_6): δ=166.43 (s, C5), 135.74 (s, C1'), 128.93 (d, C3'), 128.05 (d, C2'), 128.20 (d, C4'), 117.38 (s, CN), 57.85 (d, C2), 52.12 (d, C3), 40.90 (d, C4), 10.88 (t, CH_2).

4-*trans*-Cyano-2-*cis*-iodomethyl-3-phenyl-5-oxopyrrolidine (4II): m.p. 216~217°C ($CHCl_3$); ^{1}H-NMR (DMSO-d_6) δ=8.69 (s, 1H, NH), 7.35~7.55 (m, 5H), 4.56 (d, J=10.7 Hz, 1H, 4-H), 3.40 (m, 3H, 2-/3-H/CH_2), 3.20 (dd, J=10 Hz, 1H, CH_2); ^{13}C-NMR (DMSO-d_6) δ=166.43 (s, C5), 135.74 (s, C1'), 128.93 (d, C3'), 128.20 (d, C4'), 128.05 (d, C2'), 117.38 (s, CN), 57.85 (d, C2), 52.12 (d, C3), 40.90 (d, C4), 10.88 (t, CH_2).

4-*trans*-Cyano-2-*trans*-methyl-3-phenyl-5-oxopyrrolidine (5I): yield 58% from the *trans*-pyrrolidone-OH **2I**; m.p. 144~145°C; ^{1}H-NMR ($CDCl_3$) δ=7.26-7.59 (m, 5H), 6.74 (s, 1H, NH), 3.87 (dq, J=8.8/6.1 Hz, 1H, 2-H), 3.70 (d, J=11.6 Hz, 1H, 4-H), 3.33 (dd, J=11.6/8.8 Hz, 1H, 3-H), 1.30 (d, J=6.1 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=167.92 (s, C5), 135.75 (s, C1'), 129.51 (d, C3'), 128.80 (d, C4'), 127.51 (d, C2'), 116.39 (s, CN), 55.64 (d, C2), 54.97 (s, C3), 42.58 (d, C4), 19.27 (q, CH_3).

4-*trans*-Cyano-2-*cis*-methyl-3-phenyl-5-oxopyrrolidine (5II): yield 55% from the *cis*-pyrrolidone-OH **2II**; m.p. 126~127°C; ^{1}H-NMR ($CDCl_3$) δ=7.2 (m, 2H, phenyl), 7.4 (m, 3H, phenyl), 6.75 (s, 1H, NH), 4.13 (m, 2H, 2-/3-H), 3.89 (d, J=10.6 Hz, 1H, 4-H), 0.86 (d, J=6.2 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=168.58 (s, C5), 134.59 (s, C1'), 129.27 (d, C3'), 128.45 (d, C4'), 127.59 (d, C2'), 116.39 (s, CN), 52.68 (d, C2), 49.12 (s, C3), 36.53 (d, C4), 17.38 (q, CH_3)

Synthesis of 4-Cyano-5-ethoxy-2H-2-methyl-3-phenyl-3,4-dihydropyrrole (6)

1 g (5 mmol) pyrrolidone-CH_3 **5** in 100 ml dried dichloromethane was treated with 2.8 g (15 mmol) triethyloxonium tetrafluoroborate (TOTFB) and stirred at room temperature overnight. The reactant was mixed with 100 ml saturated $NaHCO_3$ solution at 0~5°C, and the organic phase was separated, washed with water and dried over Na_2SO_4 anhydrous. The solvent was removed, and the oil residue was used immediately for the subsequent reaction.

4-*trans*-Cyano-5-ethoxy-2H-2-*trans*-methyl-3-phenyl-3,4-dihydropyrrole (6I): ^{1}H-NMR ($CDCl_3$) δ=7.35 (t, J=7.1 Hz, 3H, phenyl), 7.2~7.3 (m, 2H, phenyl), 4.32 (q, J=7.2 Hz, 2H, OCH_2CH_3), 3.97 (m, J=8 Hz, 1H, 2-H), 3.84 (d, J=10.4 Hz, 1H, 4-H), 3.31 (dd, J=10.4/8 Hz, 1H, 3-H), 1.39 (t, J=7.2 Hz, 3H, OCH_2CH_3), 1.32 (d, J=6.7, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=161.97 (s, C5), 138.08 (s, C1'), 129.29 (d, C3'), 128.16 (d, C4'), 127.45 (d, C2'), 117.11 (s, CN), 68.82 (t, OCH_2CH_3), 65.53 (d, C2), 58.81 (d, C3), 43.56 (d, C4), 21.19 (q, CH3), 14.15 (q, OCH_2CH_3).

4-*trans*-Cyano-5-ethoxy-2H-2-*cis*-methyl-3-phenyl-3,4-dihydropyrrole (6II): ^{1}H-NMR ($CDCl_3$) δ=7.33 (m, 3H, phenyl), 7.13 (d, J=8.5 Hz, 2H, phenyl), 4.33 (q, J=7.1 Hz, 2H, OCH_2CH_3), 4.33 (m, 1H, 2-H), 4.04 (d, J=3.7 Hz, 2H, 3-/4-H), 1.40 (t, J=7.1 Hz, 3H, OCH_2CH_3), 0.79 (d, J=7 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=163.11 (s, C5), 136.12 (s, C1'), 128.96 (d, C3'), 127.78 (d, C4'), 127.47 (d, C2'), 116.94 (s, CN), 65.53 (t, OCH_2CH_3), 64.76 (d, C2), 53.18 (d, C3), 38.37 (d, C4), 17.38 (q, CH_3), 14.13 (q, OCH_2CH_3).

Synthesis of 2,6-Diamino-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (7)

The crude ethoxy-dihydropyrrole **6** dissolved in 30 ml absolute ethanol was added to a solution of 0.48 g (5 mmol) guanidine-HCl and 0.12 g (5 mmol) sodium in 70 ml absolute ethanol and refluxed at 130~135°C for 12 hours. After cooling in ice bath, the reaction mixture was neutralized with ethanolic hydrochloride and evaporated completely. The residue was crystallized from methanol.

2,6-Diamino-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (7I): yield 58% from the *trans*-pyrrolidone-CH_3 **5I**; m.p. 245~250°C (dec.); ^{1}H-NMR (DMSO-d_6) δ=11.60 (s (broad), 1H, NH), 8.16 (s, 1H, NH), 7.58 (s, 2H, 2-NH_2), 7.30 (m, 3H, phenyl), 7.19 (d, J=7 Hz, 2H, phenyl), 6.80 (s, 2H, 6-NH_2), 3.88 (d, J=3 Hz, 1H, 7-H), 3.62 (m, 1H, 8-H), 1.27 (d, J=6.3 Hz, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ=167.35 (s, C6), 155.77 (s, C2), 148.75 (s, C4), 142.43 (s, C1'), 128.52 (d, C3'), 126.72 (d, C2'/C4'), 85.74 (s, C5), 62.04 (d, C8), 48.60 (d, C7), 22.06 (q, CH_3).

2,6-Diamino-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (7II): yield 50% from the *cis*-pyrrolidone-CH_3 **5II**; m.p. 250~255°C (dec.); ^{1}H-NMR (DMSO-d_6) δ=11.33 (s (broad), 1H, NH), 7.96 (s, 1H, NH), 7.53 (s, 2H, 2-NH_2), 7.31 (m, 3H, phenyl), 7.04 (d, J=6.2 Hz, 2H, phenyl), 6.74 (s, 2H, 6-NH_2), 4.30 (m, 2H, 7-/8-H), 0.69 (d, J=3 Hz, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ=168.80 (s, C6), 155.68 (s, C2), 148.03 (s, C4), 137.69 (s, C1'), 128.64 (d, C3'), 127.93 (d, C2'), 126.77 (d, C4'), 87.68 (s, C5), 58.16 (d, C8), 44.42 (d, C7), 16.52 (q, CH_3).

Synthesis of 6-Amino-2-mercapto-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (8)

According to the synthetic method for the 2-amino-7-deazaapurine **7**, the crude ethoxy-dihydropyrrole **6** was transferred with 0.38 g (5 mmol) thiourea to the 2-mercapto-7-deazapurine **8**.

6-Amino-2-mercapto-8-*trans*-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (8I): yield 58% from *trans*-pyrrolidone-CH_3 **5I**; m.p. 255~260°C (dec.) (MeOH); ^{1}H-NMR (DMSO-d_6) δ=10.99 (s, 1H, SH), 7.80 (s, 1H, NH), 7.31 (m, 3H, phenyl), 7.18 (d, J=8.1 Hz, 2H, phenyl), 5.99 (s, 2H, NH_2), 3.83 (d, J=3.3 Hz, 1H, 7-H), 3.59 (qd, J=6/3.3 Hz, 1H, 8-H), 1.26 (d, J=6, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ=179.68 (s, C2), 166.89 (s, C6), 149.34 (s, C4), 142.66 (s, C1'), 128.51 (d, C3'), 126.72 (d, C2'), 126.63 (d, C4'), 87.20 (s, C5), 61.62 (d, C8), 48.90 (d, C7), 22.15 (q, CH_3).

6-Amino-2-mercapto-8-*cis*-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (8II): yield 50% from the *cis*-pyrrolidone-CH_3 **5II**; m.p. 255~260°C (dec.); ^{1}H-NMR (DMSO-d_6) δ=11.10 (s, 1H, SH), 7.53 (s, 1H, NH), 7.30 (m, 3H, phenyl), 7.04 (d, J=6.6 Hz, 2H, phenyl), 6.06 (s, 2H, NH_2), 4.25 (m, 2H, 7-/8-H), 0.70 (d, J=5.3 Hz, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ=179.15 (s, C2), 167.33 (s, C6), 149.37 (s, C4), 137.93 (s, C1'), 128.59 (d, C3'), 127.90 (d, C2'), 126.67 (d, C4'), 89.21 (s, C5), 55.95 (d, C8), 44.69 (d, C7), 16.68 (q, CH_3).

Synthesis of 6-Amino-2-methylthio-7-phenyl-8-methyl-7,8-dihydro-7(9H)-deazapurine (9)

To a stirred solution of 1.3 g (5 mmol) 2-mercapto-7-deazapurine **8** in 10 ml of 0.5 N-NaOH solution was added 0.5 ml (5 mmol) $(CH_3)_2SO_4$ at room temperature (Seela, *et al.*, 1980). After stirring for 1~2 h, the white precipitate was filtered, washed with water, and dried. The rest compound in filtrate was extracted with chloroform. The organic phase was dried over Na_2SO_4 anhydrous, and evaporated in vacuum. The residue was crystallized from methanol and water.

6-Amino-2-methylmercapto-8-methyl-7-*trans*-phenyl-7,8-dihydro-7(9H)-deazapurine (9I): yield 95%; m.p. 180~183°C (MeOH/H_2O); ^{1}H-NMR ($CDCl_3$) δ=7.29 (m, 5H, phenyl), 6.26 (s, 1H, NH), 4.30 (s, 2H, NH_2), 3.82 (s, 2H, 7-/8-H), 2.41 (s, 3H, SCH_3), 1.39 (d, J=5.5 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=170.51 (s, C2), 168.17 (s, C6), 157.28 (s, C4), 141.16 (s, C1'), 129.25 (d, C3'), 128.03 (d, C2'), 127.62 (d, C4'), 94.08 (s, C5), 62.77 (d, C8), 52.44 (d, C7), 21.59 (q, CH_3), 13.90 (q, SCH_3).

6-Amino-2-methylmercapto-8-methyl-7-*cis*-phenyl-7,8-dihydro-7(9H)-deazapurine (9II): yield 97%, m.p. 180~182°C (MeOH/H_2O); ^{1}H-NMR ($CDCl_3$) δ=7.27 (m, 3H, phenyl), 7.14 (m, 2H, phenyl), 5.32 (s, 1H, NH), 4.41 (d, J=9.8 Hz, 1H, 7-H), 4.33 (m, 1H, 8-H), 4.25 (s, 2H, NH_2), 2.46 (s, 3H, SCH_3), 0.82 (d, J=6.3, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ=170.47 (s, C2), 168.51 (s, C6), 157.40 (s, C4), 136.70 (s, C1'), 129.27 (d, C3'), 128.77 (d, C2'), 127.65 (d, C4'), 94.05 (s, C5), 56.28 (d, C8), 47.16 (d, C7), 18.32 (q, CH_3), 13.93 (q, SCH_3).

Synthesis of 6-Amino-8-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (10)

To a solution of 1.4 g (5 mmol) 2-methylthio-7-deazapurine **9** in 30 ml methanol and 15 ml NH_4OH was added 5 g Raney-nickel and refluxed for 5~6 hours. The catalyst was filtered off, and sufficiently washed with warm methanol. The solvent was evaporated completely and the residue was crystallized from methanol.

6-Amino-8-*trans*-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (10I): yield 85%; m.p. 230~235°C (dec.); ^{1}H-NMR (DMSO-d_6) δ=7.87 (s, 1H, NH), 7.27 (m, 5H, phenyl), 6.84 (s, 1H, 2-H), 5.57 (s, 2H, NH_2), 3.85 (d, J=4.3 Hz, 1H, 7-H), 3.57 (dq, J=6/4.3 Hz, 1H, 8-H), 1.23 (d, J=6 Hz, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ=167.07 (s, C6), 158.01 (s, C4), 157.61 (s, C2), 142.84 (s, C1'), 128.42 (d, C3'), 127.09 (d, C2'), 126.51 (d, C4'), 96.11 (s, C5), 60.73 (d, C8), 50.41 (d, C7), 21.99 (q, CH_3).

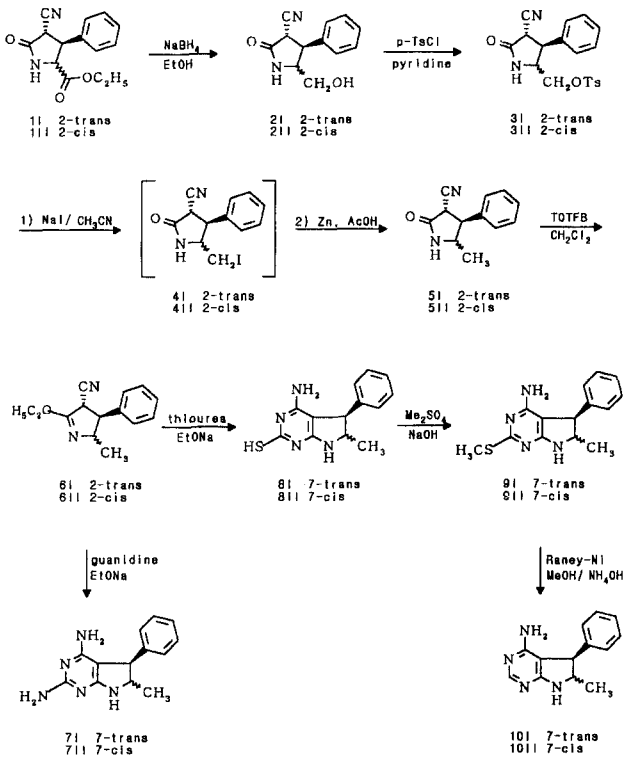
6-Amino-8-*cis*-methyl-7-phenyl-7,8-dihydro-7(9H)-deazapurine (10II): yield 80%; m.p. 220~225°C (dec.); ^{1}H-NMR (DMSO-d_6) δ=7.89 (s, 1H, NH), 7.28 (m, 3H, phenyl), 7.02 (d, J=7.3, 2H, phenyl), 6.71 (s, 1H, 2-H), 5.69 (s, 2H, NH_2), 4.26 (d, J=9.3 Hz, 1H, 7-H), 4.18 (m, 1H, 8-H), 0.74 (d, J=5.7 Hz, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ=168.36 (s, C6), 157.49 (s, C4), 157.25 (s, C2), 138.09 (s, C1'), 128.76 (d, C3'), 127.78 (d, C2'), 126.48 (d, C4'), 98.36 (s, C5), 55.36 (d, C8), 45.93 (d, C7), 16.76 (q, CH_3).

Biological assay

The MIC values against bacteria including *Bacillus subtilis* BD170, *Stapylococcus aureus* ACTT 6538 and *Escherichia coli* MV 1190 were determined by the serial two-fold dilution method. The bacteria were pre-cultured in 10 ml of nutrient-broth medium for 12 hours at 27°C on a shaker, and then diluted 100-fold with the same medium. Liquid culture containing the test compound were placed in the wells of a 96-well microplate at 27°C for 24 hours. The growth of the bacteria was evaluated by the degree of turbidity of the culture.

RESULTS AND DISCUSSION

The *cis*- and *trans*-diastereomers of the 7,8-dihydro-7-deazapurine derivatives was synthesized according to Scheme 1. Preparing the *cis*- and *trans*-diastereomers of the pyrrolidone-ester **1** was carried out from ethyl 2-cyano-3-phenylacrylate, which involved Michael reaction with N-(diphenylmethylene)glycine ethylester, the acid-hydrolysis, and finally cyclization (Pachaly, *et al.*, 1991, and Sin, *et al.*, 1993). Their respective yields



Scheme 1. The synthesis of the *cis*- and *trans*-7,8-dihydro-7-deazapurine derivatives

were deceived by the ratio of the *erythro*- and *threo*-isomers, which were produced by Michael reaction depending on the reaction temperature and simply isolated *via* the crystallization.

The deoxygenation of the *cis*- and *trans*-pyrrolidone-CH_2OH **2** leaded *via* tosylation, iodination, and finally elimination to the *cis*- and *trans*-pyrrolidone-CH_3 **5**. In the tosylation of the *cis*- and *trans*-diastereomers **2**, the punctual reaction time was an indispensable condition to avoid producing the unidentified side compound. Their yields also depended on optimized reaction conditions including strict drying and temperature. The *cis*- and *trans*-diastereomers of the pyrrolidone-OTs **3** were transferred to the corresponding pyrrolidone-CH_2I **4** as intermediates and then eliminated with Zn (Morita, *et al.*, 1981). The yields of **5** were found > 55% from **2**, respectively. Their stereochemistry could be identified by the ^{1}H- and ^{13}C-NMR-data; compared with the *trans*-diastereomer **5I**, the 2-, 3-, and 4-proton signals of the *cis*-diastereomer **5II** were shifted in downfield between 0.2 and 0.8 ppm except the 2-methyl group, which was recorded at 0.86 ppm because of the inductive effect of the 3-phenyl group. Together with the carbon signal of the methyl group, the 2-, 3-, and 4-carbon signals of **5II** showed upfield shifts between 2 and 6 ppm when compared with **5I**.

The *cis*- and *trans*-ethoxy-dihydropyrrole **6** were achieved from the corresponding diastereomers **5** by treatment with TOTFB (Sin, *et al.*, 1993 and 1997) and following condensation with guanidine or thiourea gave the *cis*- and *trans*-diastereomers of the 7,8-dihydro-7-deazapurines **7** and **8**. This each step was carried out under strict drying condition to avoid decomposition of the unstable **6**. Additionally, the reaction temperature must be over 130°C to improve the yield (Sin, *et al.*, 1993).

The *cis*- and *trans*-2-hydro-7-deazapurine **10** could be prepared not only from the 2-methylthio-7-deazapurines **9** but also from the 2-mercapto-7-deazapurines **8** by the desufurization with raney-nickel (Seela, *et al.*, 1980), respectively. However, their yields from **9** were better than those from **8**, because of their distinct solubility.

The *cis*- and trans-diastereomers of the 7,8-dihydro-7-deazapurine derivatives **7, 8, 9**, and **10** were tested against *B. subtilis, S. aureus,* and *E. coli* and their antibiotic activities were shown in Table 1. The antibiotic activity against *B. subtilis* and *S. aureus* was identical except the *cis*-diastereomer **7II**. The *trans*-2-amino-7-deazapurine **7I** showed the highest antibiotic activity with MIC values > 125 μg/ml, and its *cis*-diastereomer **7II** was more active against *B. subtilis* (> 125 μg/ml) than against *S. aureus* (250 μg/ml). The activity of the *trans*-2-methylthio-7-deazapurine **9I** was better than the other diastereomer **9II**, but the diastereomers of the 2-hydro-7-deazapurine **10** were found with MIC values of 500 μg/ml without stereodifferentiation. The 2-mercapto-7-deazapurines **8** were inactive.

In all case no activity was observed against the gram negative organism *E. coli.*

A discussion about the antibiotic activity of the *cis*- and *trans*-2-substituted 7,8-dihydro-7-deazapurine derivatives in terms of the structure-activity-relationship could be summarized as follows; the activity against *B. subtilis* and *S. aureus* depending on the functional groups at 2 position of the 7,8-dihydro-7-deazapurine derivatives was found with decreasing potency in the order of 2-amino- > 2-hydro- > 2-methylthio > 2-mercapto-7-deazapurine derivatives. Concerning the stereochemistry of the compounds, the *trans*-configuration was essential for the antibiotic ac-

Table I. The MIC (μg/ml) against *Bacillus subtilis* and *Staphylococcus aureus* of the 7,8-dihydro-7-deazapurine derivatives

compound	7I	7II	8I	8II	9I	9II	10I	10II
B. subtilis BD170	>125	>125	>1000	>1000	250	>1000	500	500
S. aureus ACTT 6538	>125	250	>1000	>1000	250	>1000	>500	>500

tivity against all the tested bacteria except *E. coli*. As a effect of the increased lipophilia, the *trans*-2-mercapto-7-deazapurine **8I**, an inactive compound even for the concentration of 1000 μg/ml against *B. subtilis* and *S. aureus*, gained its activity after the methylation to the *trans*-2-methylthio-7-deazapurine **9I**. However, this change was not found between the *cis*-diastereomers of **8II** and **9II**.

ACKNOWLEDGMENT

This paper was supported in part by Korea Science and Engineering Foundation (KOSEF 9514005) and in part by Korea Research Foundation.

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