Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 13, pp. 1607-1610, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00274-6

SYNTHESES AND ANTICANCER ACTIVITY OF RIBONUCLEOSIDE ANALOGUES CONTAINING THIO-SUBSTITUTED FIVE-MEMBERED HETEROCYCLIC BASE†

Yun Ding, Yun-Yan Zhang, Jiong Zhang[a], Yao-Quan Chen*

Laboratoty of Bio-organic & Natural Products Chemistry
Shanghai Institute of Organic Chemistry, The Chinese Academy of Sciences
354 Fenglin Road, Shanghai 200032, China

Abstract: Syntheses of two types of ribonucleoside analogues with thio-substituted five-membered heterocyclic base have been carried out. The one contains thio-substituted 1,3,4-triazole as its base (**2** to **7**) and the other consists of amino-thio-thiadiazoline β-D-ribofuranosides (**8** and **9**). Biological evaluation showed that one (**8**) among them exhibited IC_{50} of 3.94 x 10^{-8} mol/L against NCI CNS cancer cell line SNB19.

Nucleoside analogues are one important kind of antimetabolite agents which exhibit anticancer and antiviral activities. Their structural design and chemical synthesis have been extensively studied. Nucleoside analogues having five-membered heterocyclic base also cause wide attention. Among them, ribavirin, which contains a substituted triazole ring as its heterocyclic base, represents the most successful one.[1]

Due to its soft nucleophilicity and reversible redox properties, sulfur atom plays an important role in the life processes. Nucleoside analogues containing sulfur atom in the sugar moiety or in the heterocyclic moiety have been synthesized and some of them have been found to be active against cancer cells or viruses.[2] It is therefore of interest to synthesize nucleoside analogues with thio-substituted five-membered ring as a heterocyclic base and examine their biological activities. Here we wish to report our results.

Chemical Synthesis

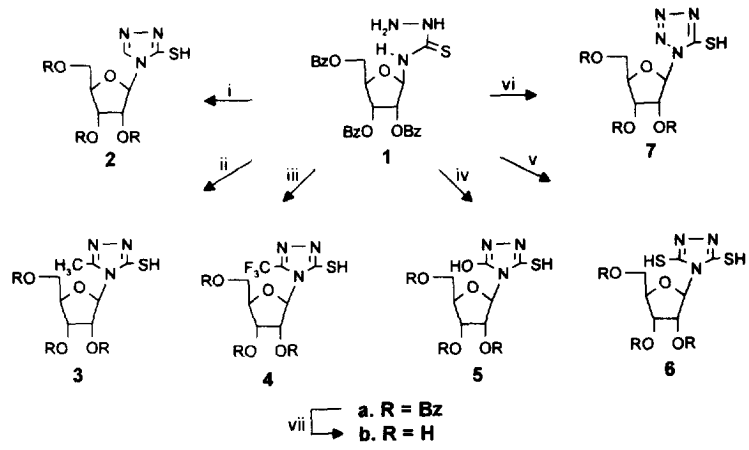
Syntheses of two types of the title compounds have been carried out. The first one includes ribonucleoside analogues containing thio-substituted 1,3,4-triazole as a heterocyclic base. The synthesis is shown in Scheme 1. Compound **1**, the central intermediate in this synthesis, was obtained by N-substitution of silver thiocyanate on 1-bromo-2,3,5-tri-O-benzoyl-β-D-ribofuranose followed by hydrazinolysis.[3] Condensation of **1** with various acids, esters, amides, or anhydrides in anhydrous solvent gave products **2a-7a**, which were debenzoylated by sodium methoxide to give the final products **2b- 7b**.[4-9]

---

†This paper is dedicated to the memory of Professor Yu Wang.

[a]Present address: Department of Chemistry, University of Iowa, Iowa City, IA 52242, USA.

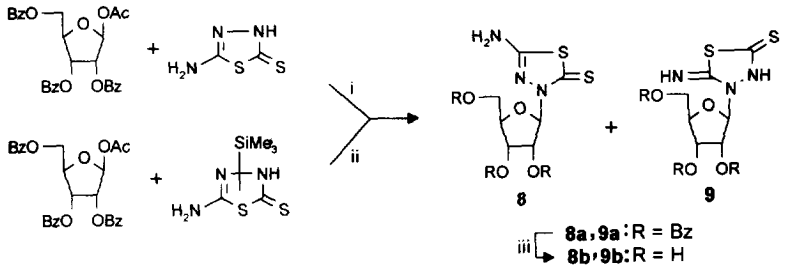
Scheme 1



i. $HCOOH/(CH_3CO)_2O$/DMAP, 50°C, 0.5h, 65%; ii. $CH_3C(OCH_3)_3/CH_2Cl_2$, refluxed,10h,65%; iii. $(CF_3CO)_2O/CH_2Cl_2$, r.t.,1h, 55%; iv. Im_2CO/THF,r.t. 4h, 75%; v. CS_2 refluxed,4h, 64%; vi. $NaNO_2/CH_3CO_2H/CH_2Cl_2$, r.t.,10min, 83%; vii. CH_3ONa/CH_3OH, r.t. 4h.

The second type of the title compounds consists of amino-thio-thiadiazoline β-D-ribofuranosides, which were synthesized via both condensation and fusion procedures[10] as shown in Scheme 2. Both of the procedures

Scheme 2



i.p-toluenesulfonic acid,120-130°C,20mmHg, 30min, 38% for **8a**, 15% for **9a**; ii.$SnCl_4/Cl(CH_2)_2Cl$, r.t.,8h,52% for **8a**, 29% for **9a**; iii.$NaOCH_3/CH_3OH$, r.t., 4h .

produced two positional isomers as proved by ^1H, ^{13}C NMR, mass spectra and elemental analysis data.[11,12] The one with higher R_f value was assigned to be 4-(β-D-ribofuranosyl)-2-amino-5-thio-1,3,4-thiadiazoline (**8b**) by X-ray crystallographic analysis,[13] therefore the other with lower R_f value was tentatively proposed to have the structure of 3-(β-D-ribofuranosyl)-2-imino-5-thio-1,2,4-thiadiazoline (**9b**). Further support came from the ^1H NMR signals of the amino and imino groups of the two compounds. Compound **8b** showed a symmetrical singlet (integrated 2H), which was due to two identical N-H protons; whereas **9b** gave a shouldered singlet (poorly resolved two singlet peaks, in total also integrated 2H), which was consistent with a ring NH and an imino NH.

Biological evaluation The *in vitro* biological evaluation performed by NCI NIH on NCI cancer cell lines showed that only **8b** exhibited IC_{50} of 3.94 x 10^{-8} mol/L against CNS cancer cell line SNB19. The other compounds did not display significant activies at the concentrations up to 10^{-4} mol/L.

Acknowledgements: The authors are grateful to The NCI NIH for the biological evaluation. We thank Professor C.K. Chu of College of Pharmacy, University of Georgia for his help in arrangement for the bio-assays.

References and notes

1. Witkowski, J.T; Robins, R.K.; Sidwell, R.W.; Simon, L.N. *J. Med. Chem.*, **1972**, *15*, 1150; *Ribavirin: A broad spectrum antiviral agent*, Smith, R.A. and Kirkpatrik, W., Ed.; Academic Press: New York, 1980.
2. For examples, see Hirota, K.; Tomishi, T.; Maki, Y. *Heterocycles* **1987**, *26*, 3089; Belleau, B.; Dixit, D.; Nguyen-Ga, N.; Kraus, J.L. *At 5th International Conference on AIDS*. Montreal , Canada, June 4-9, 1989; Palomino, E.; Meltsner, B.R.; Kessel, D.; Horwitz, J.P.*J. Med. Chem.* **1990**, *33*, 258; Kung, P.P.; Jones, R.A. *Tetrahedron Lett.* **1991**, *32*, 3919; Secrist III, J.A.; Tiwari, K.N.; Riordan, J.M.; Montgomery, J.A. *J. Med. Chem.* **1991**, *33*, 2361; Wigerinck, P.; Snoeck R.; Claes, P.; De Clercq, E.; Herdewijn, P. *ibid.***1991**, *34*, 1767; Dyson, M.R.; Coe, P.L.; Walker, R.T. *J. C. S. Chem. Commun.* **1991**, 741; Goodwin, J.T.; Glick, G.O. *Tetrahedron Lett.* **1993**, *34*, 5549; Jeong, L.S.; Nicklaus, M.C.; George, C.; Marquez, V.E. *ibid.* **1994**, *35*, 7569; 7573; Xiang, Y.; Teng, Q.; Chu, C.K. *ibid.* **1995**, *36*, 3781; Van Praanen, N.A.; Freeman, G.A; Short, S.A.; Harvey, R.; Jansen, R.; Szczech, G.; Koszalka, G.W. *J. Med. Chem.* **1996**, *39*, 538; Rahim, S.G.; Trivedi, N.; Bogunovic-Batchelor, M.V.; Hardy, G.W.; Mills, G.; Selway, J.W.T.; Snowden, W.; Littler, E.; Coe, P.L.; Basnak, I.; Walker, R.T. *ibid.* **1996**, *39*, 789; Saluja, S.; Zou, R.; Drach, J.C.; Townsend, L.B. *ibid.* **1996**, *39*, 881; Nishizono, N.; Koike, N.; Yamagata, Y.; Fujii, S.; Matsuda, A. *Tetrahedron Lett.* **1996**, *37*, 925.
3. Hohnjec, M.; Kobe, J.; Valcavi, U.; Japelj, M. *J. Heterocycl. Chem.*, **1975**, *12*, 909.
4. **2a**, colorless needles from ethyl acetate, yield 65%. mp 180-182.5°C (lit.[2] 179-180°C). ^{1}H NMR ($CDCl_3$), 8.12-7.37 (m, 16H, PhH, 5H), 6.85 (d, 1H, 1'H), 5.91 (m, 2H, 2',3'H), 4.78 (m, 3H, 4',5'H). MS (m/z), 546 (MH^+), 445 (M^+ - base), 105 (Bz). **2b**, yield 84%. mp 135-135.5°C (lit.[2] 135-137°C). $[\alpha]_D^{20}$ = -12.1° (c 1.0, CH_3OH). λ_{max} = 256nm (ε 3.5 x 10^4). ^{1}H NMR (DMSO-d_6), 8.70 (s, 1H, 2H), 5.81 (d, 1H, 1'H, *J*=4.47Hz), 5.52 (d, 1H, OH, *J*=5.63Hz), 5.17 (m, 2H, OH), 4.19, 4.10, 3.90 (m, 3H, 2',3',4'H), 3.65-3.55 (m, 2H, 5'H). MS(m/z), 234 (MH^+), 133 (M^+ - base), 102 (base).
5. **3a**: white powder, yield 65%. mp 217-218°C. ^{1}H NMR ($CDCl_3$), 8.10-7.34 (m, 15H, PhH), 5.92-5.76 (m, 3H, 1',2',3'H), 4.73-4.61 (m, 3H, 4',5'H), 2.56 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$), 166.154-164.924 (PhCO, CS), 133.892-128.235 (C-Ph), 88.312 (1'C), 86.180, 79.729, 74.232 (2',3',4'C), 70.759 (5'C), 15.75 (CH_3). Elemental anal. calcd. for $C_{29}H_{25}N_3O_7S$: C, 62.25; H, 4.50; N, 7.51. Found: C, 62.21; H, 4.40; N, 7.39. **3b**, yield 41%. m.p. 153.5-155 °C. $[\alpha]_D^{20}$ = -12.1° (c 0.7, CH_3OH). ^{1}H NMR (CD_3OD), 5.13 (d, 1H, 1'H), 4.24 (m, 1H, 2'H), 3.92-3.63 (m, 4H, 3',4',5'H), 2.75 (s, 3H, CH_3). MS (m/z), 248 (MH^+), 133 (M^+ -base), 116 (base). HRFABMS, calcd. for $C_8H_{13}N_3O_4S$: 247.0627. Found: 247.0586.
6. **4a**, white powder, yield 55%. mp 164-165°C. ^{1}H NMR ($CDCl_3$), 8.04-7.37 (m, 15H, PhH), 6.48 (br, NH), 6.16 (d, 1H, 1'H), 5.85 (m, 2H, 2',3'H), 4.68 (m, 3H, 4',5'H). ^{13}C NMR ($CDCl_3$), 166.405-165.641 (PhCO), 163.053 (CS), 149.0 (CF_3), 134.115-128.640 (CPh), 86.510 (1'C), 79.965, 74.160, 71.731, (2',3',4'C), 64.085 (5'C). ^{19}F NMR ($CDCl_3$),12.30. MS, m/z, 445 (M^+ - base), 105 (Bz), 69 (CF_3). Elemental anal. calcd. for $C_{29}H_{22}F_3N_3O_7S$: C, 56.77; H,3.61; N, 6.85. Found: C, 56.67; H, 3.44; N, 6.87. **4b**, yield 62%. mp 230°C (dec.). $[\alpha]_D^{20}$ = -16.6° (c 0.18, CH_3OH). UV (CH_3OH), λ_{max} = 270nm (ε 1.6 x 10^5), λ_{min} = 233. ^{1}H NMR (CD_3OD), 5.21(d, 1H, 1'H, *J* = 8.29Hz), 4.90 (m, 2H, 2',3'H), 4.29 (m, 1H, 4'H), 3.89 (m, 2H, 5'H). ^{13}C NMR (CD_3OD), 173.791 (CS), 126.700, 123.100, 119.514, 115.900 (q, CF_3, *J* = 3.59Hz), 84.750 (1'C), 72.229, 71.817, 66.892 (2',3',4'C), 65.797 (5'C). ^{19}F NMR (CD_3OD), 10.50. MS (m/z), 302 (MH^+), 170 (base + 2H), 133 (M^+ - base). HRFABMS, calcd. for $C_8H_{10}F_3N_3O_4S$: 301.0344. Found: 301.0355.
7. **5a**, white powder, yield 75%. mp 90.5-93°C. ^{1}H NMR ($CDCl_3$), 8.08-7.27 (m, 15H, PhH), 5.98-5.69 (m, 3H, 1',2',3'H), 4.73-4.57(m, 3H, 4'5'H). ^{13}C NMR ($CDCl_3$), 169.550, 152.237 (CS, CO), 166.312-164.945

(PhCO), 133.955-128.610 (CPh), 86.832 (1'C), 83.656, 74.223, 70.892 (2',3',4'C), 64.186 (5'C). MS (m/z), 561 (M^+), 445 (M^+ - base), 117 (base), 105 (Bz). HREIMS, calcd. for $C_{28}H_{23}N_3O_8S$: 561.1206. Found: 561.1200. **5b**, yield 60%. mp 220 (dec.). $[\alpha]_D^{20}$ = -2.6 (c 1.0, H_2O). UV (H_2O), λ_{max} = 256nm (ε 4.8 x 10^5), λ_{min} = 204nm. ^{1}H NMR (D_2O), 4.95 (s, 1H, 1'H), 4.16 (m, 1H, 2'H), 3.91-3.81 (m, 2H, 3',4'H), 3.57 (m, 2H,5'H). MS(m/z), 272 (M + Na^+), 250 (MH^+),115 (base). HRFABMS, calcd. for $C_7H_{12}N_3O_5S$: 250.0498. Found: 250.0502.

8. **6a**,white powder. yield 64%. mp 89-90°C. ^{1}H NMR ($CDCl_3$), 8.08-7.26 (m, 15H, PhH), 5.90-5.52 (m, 3H, 1',2',3'H), 4.73-4.58 (m, 3H, 4',5'H). ^{13}C NMR ($CDCl_3$), 183.82 (CS), 166.207-164.850 (PhCO), 160.14 (CSH), 133.974-128.207 (CPh), 87.522 (1'C), 84.338,74.089,70.749 (2',3',4'C), 63.81 (5'C). MS(m/z), 579 (MH^+), 445 (M-base), 134 (base), 105 (Bz). Elemental anal. calcd. for $C_{28}H_{23}N_3O_7S_2$: C, 58.22; H, 4.01; N, 7.27. Found: C, 58.60; H, 4.18; N, 6.91. **6b**, yield 71%. mp 202-204°C. $[\alpha]_D^{20}$ = 18.3° (c 0.6, CH_3OH). UV(CH_3OH), λ_{max} =300nm (ε 0.6 x 10^4), λ_{min} = 252nm. ^{1}H NMR (CD_3OD), 4.83 (s, 1H, 1'H), 4.69 (m, 1H, 2'H), 3.89 (m, 1H, 3'H), 3.64 (m, 1H, 4'H), 3.49 (m,2H, 5'H). MS(m/z), 265 (M^+), 133 (base + H^+ or ribosyl). HRFABMS, calcd. for $C_7H_{11}N_3O_4S_2$: 265.0191. Found: 265.0185.
9. **7a**,colorless needles from ethyl acetate, yield 83%. mp 112-114°C. ^{1}H NMR ($CDCl_3$), 8.10-7.33 (m, 15H, PhH), 5.93-5.75 (m,3H, 1',2',3'H), 4.81-4.57 (m, 3H, 4',5'H). MS(m/z), 547 (M^+ + 1), 445 (M^+ - base), 105 (Bz). Elemental anal. calcd. for $C_{27}H_{22}N_4O_7S$: C,59.33; H, 4.06; N, 10.25. Found: C, 59.01; H, 3.89; N, 9.97. **7b**, yield 45%. mp 220-221°C. $[\alpha]_D^{20}$ = -47.6° (c0.82, CH_3OH). UV(CH_3OH), λ_{max} = 220nm (ε 3.4 x 10^5), λ_{min} = 250nm. ^{1}HNMR (CD_3OD), 6.39 (d, 1H, 1'H, J = 3.05Hz), 4.76 (m, 1H,2'H), 4.60 (m, 1H, 3'H), 4.29 (m, 1H, 4'H),3.98-3.79 (m, 2H, 5'H). MS(m/z), 133 (M^+ - base), 102 (base). HRMS calcd. for CH_2N_4S (base), 102.0000. Found, 102.0000.
10. Ueda, T. In *Chemistry of Nucleosides and Nucleotides*, Townsend, L.B. Ed.; Plenum Press: New York and London, 1988, Vol. 1, pp. 2-5; Srivastava, P.C.; Robins, R.K.; Meyer, Jr., R.B. *ibid.*, pp. 130-136.
11. **8a**, ^{1}H NMR (DMSO-d_6), 7.40-7.99 (m, 17H, PhH, NH), 6.73 (d, 1H, 1'H, J = 1.2Hz), 5.99 (m, 2H, 2',3'H), 4.84 (m, 1H, 4'H), 4.55, 4.66 (m, 2H, 5'H). ^{13}C NMR (DMSO-d_6), 182.410, 158.260 (heterocyclic C), 166.230, 165.380, 164.470 (PhCO), 134.000-128.310 (PhC), 87.260, 79.100, 78.590,74.390,70.750 (sugar C). MS (m/z), 577 (M^+), 445 (M - base). Elemental anal. calcd. for $C_{28}H_{23}N_3O_7S_2$: C, 58.23; H, 4.01; N, 7.28. Found: C, 58.10; H, 3.55; N, 6.84. **8b**, prismatic crystals from methanol. mp 195-196°C. ^{1}H NMR (DMSO-d_6), 7.30 (s, 2H, NH, D_2O exchangeable), 6.24 (d, 1H, 1'H, J = 3.7Hz), 5.38, 5.06 (d, 2H, 2',3'-OH, J = 5.9, 5.6Hz), 4.71 (t, 1H, 5'-OH, J = 5.8Hz), 4.21 (m, 1H, 2'H), 4.05 (m, 1H, 3'H), 3.83 (m, 1H, 4'H), 3.43-3.53 (m, 2H, 5'H). ^{13}C NMR (DMSO-d_6), 181.740, 157.630 (heterocyclic C), 89.150, 84.910, 73.500, 70.560, 62.310 (sugar C).MS (m/z), 265 (M^+),133 (base). Elemental anal. calcd. for $C_7H_{11}N_3O_4S_2$: C, 31.70; H, 4.18; N, 15.85. Found: C, 31.57; H, 4.03; N, 15.75.
12. **9a**, ^{1}H NMR (DMSO-d_6), 7.42-8.03 (17H, PhH, NH), 5.91 (d, 1H, 1'H,J = 3.6Hz), 5.79 (m, 2H, 2',3'H), 4.80 (m,1H, 4'H), 4.63-4.53 (m, 2H, 5'H).^{13}C NMR (DMSO-d_6), 171.67,144.23 (heterocyclic C), 166.370, 164.620, 164.380 (PhCO), 133.800-128.750 (PhC), 87.220, 80.080, 74.640, 71.350, 63.630 (sugar C). MS (m/z), 600 (M + Na^+), 445 (M-base). Elemental anal. calcd. for $C_{28}H_{23}N_3O_7S_2$: C, 58.23; H, 4.01; N, 7.28. Found: C,57.98; H, 3.84; N, 6.74. **9b**, foam-like soild, ^{1}H NMR (DMSO-d_6), 7.37 (s with shoulder, 2H, NH, D_2O exchangeable), 5.19 (d, 1H, 1'H, J = 4Hz), 5.45, 5.04 (d, 2H, 2',3'-OH, J = 4, 5.5Hz), 4.76 (t, 1H, 5'H, J = 3.7Hz), 3.97 (m,1H, 2'H), 3.90 (m, 1H, 3'H), 3.81 (m, 1H, 4'H), 3.40-3.45 (m, 2H, 5'H). ^{13}C NMH (DMSO-d_6), 170.960, 147.190 (heterocyclic C), 90.380, 85.790, 75.050, 70.630, 61.790 (sugar C). MS(m/z), 288 (M + Na^+), 266 (MH^+).
13. Measured on Rigaku AFC7R diffractometer with graphite monochromated Mo-Kα radiation and a 12kW rotating anode generator. Monoclinic crystal system with space group of $P2_1$ (#4), a=6.809(2), b=8.985(2), c= 9.130(1), β=103.93(1)°, Z=2, V=542.2(2)Å^3, D_c=1.600g/cm^3.

(*Received in Japan* 7 *April* 1997; *accepted* 19 *May* 1997)