Partition Property of 5-Nitrothiopyrimidine Nucleoside

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Received February 26, 1997

5-Nitrouracil and 5-nitrouridine derivatives which C-2 and C-4 oxo-groups of the pyrimidine base were replaced by thio groups were synthesized. The lipophilicities of thiopyrimidine bases were enhanced significantly as indicated by P-values. Oxygen-sulfur exchange leading to 2-thiouracil (**2**) and 2,4-dithiouracil (**3**) were associated with 1.4- and 2.6-fold increase in P-value relative to that of uracil (**1**). The P-values of 5-nitro-2-thiouracil (**5**) and 5-nitro-2,4-dithiouracil (**6**) were increased 13.2- and 79.8-fold relative to that of 5-nitrouracil (**4**). Most of the 5-nitrothiopyrimidine bases and their nucleosides were found to be moderately active against *Staphylococcus aureus* and *Escherichia coli* except 5-nitrouracil (**4**).

Introduction

Nucleoside and nucleotide analogues are the most widely explored types of compounds in searching for potential chemotherapeutic agents. 5-Nitrouracil (**4**) has been known as an inhibitor of thymidine phosphorylase (thymidine-orthophosphate deoxyribosyl-transferase; EC 2.4.2.4) and 5-fluoro-2'-deoxyuridine phosphorylase from Walker 256 rat tumor.[1,2] Studies on the replacement reactions of the oxo-group by thio-group in nucleoside have attracted a great deal of interest after 6-thiopurines were found as effective antitumor agents.[3] The recent findings on enhancing the lipophilicity[4] and synthesizing the sulfur analogues of nucleosides have made the study on the synthesis and the biochemical characteristics of thionucleosides important. Therefore, we prepared 5-nitrouracil and 5-nitrouridine derivatives of which C-2 and C-4 oxygen atoms were substituted with sulfur atoms. Those compounds were *in vitro* evaluated for antibacterial activity against two bacteria; *Staphylococcus aureus* and *Escherichia coli* by broth dilution method.[5] The partition coefficient, a measure of lipophilicity,[6] in 1-octanol-sodium phosphate buffer, pH 7.0, was also determined for the thiopyrimidine bases to estimate the effects of lipophilicity on the antibacterial activity.

Method

Compounds. Uracil, phosphorus pentasulfide, 0.5 M nitronium tetrafluoroborate in sulfolane, sodium azide were purchased from Aldrich Co. and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose, stannic chloride anhydrous, hexamethyldisilazane, trimethylsilyl chloride were obtained from Fluka Co.

Synthesis. Thin layer chromatography was performed using Silica Gel 60 F_{254} (Merck) and TLC spots were detected by irradiation with a short wave UV (254 nm) and/or by charring after spraying with anisaldehyde.[8] All evaporations were carried out *in vacuo* with a rotary evaporator or by short-path distillation into a dryice-acetone cooling receiver under high vacuum. Column chromatography was performed using Silica Gel 60 (70-320 mesh, Merck). Melting points were determined with electrothermal capillary melting point apparatus. ^{1}H NMR spectra were obtained on Varian EM 360A with tetramethylsilane as an internal reference. Infrared spectra were recorded on a Mattson Polaris Fourier Transform IR spectrometer.

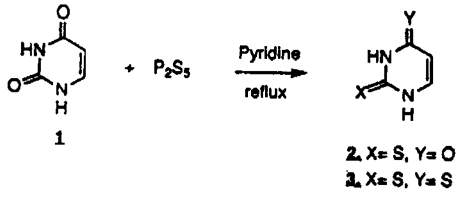
Antibacterial activity test. The modified pyrimidine bases and their nucleosides were *in vitro* evaluated for antibacterial activity by determining the MIC (with serial 2-fold broth dilution method) aganist two bacterial cells; *Staphylococcus aureus* and *Escherichia coli.*[5] Inoculum was standardized to equal a 0.5 McFarland turbidity standard (approximately 5×10^7 cells/mL) and then further diluted 1 : 200 with BSG to achieve 10^5-10^8 cells/mL. Begining with 2000 μg/mL of antibiotics, diluted them 10-fold serially with Muller Hinton Broth and incubated overnight at 37 °C. MICs were determined by the difference of transmittance between control and each antibiotic using Shimadzu Micro-Flow spectrophotometer CL-750.

Partition property. Thiopyrimidine bases were partitioned between the phases of mixture containing equal volumes of 1-octanol and 0.1 M sodium phosphate, pH 7.0.[6] The phases were separated by centrifugation (2,300 rpm × 20 min) and the concentration of pyrimidine base in each phase was determined by absorbance measurements with the wavelength determined from spectral studies[7] using Shimadzu UV-250 UV-Visible spectrophotometer.

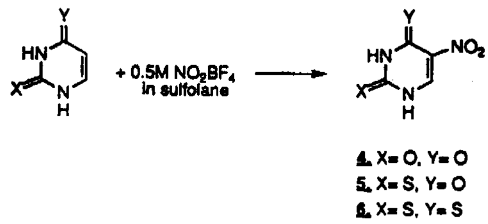
Experiment

Modified pyrimidine bases and their nucleoside derivatives were synthesized as shown in Scheme 1, 2, and 3.

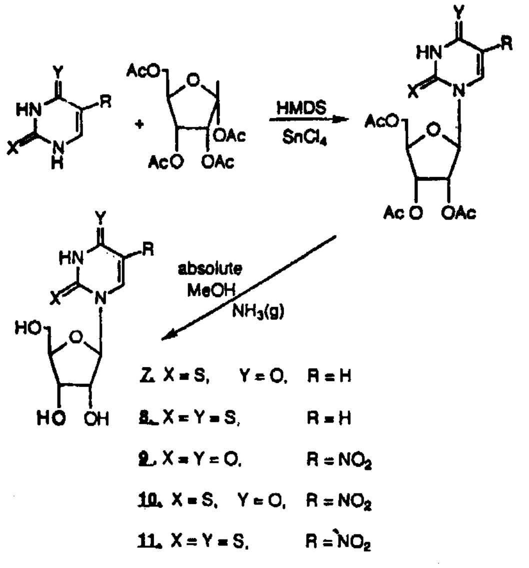
General procedure for thiation. A solution of uracil (0.224 g, 2 mmol, **1**) in 2 mL of boiling anhydrous pyridine was rapidly treated with a solution of phosphorus pentasulfide (0.267 g, 1.2 mmol) in 4 mL of boiling anhydrous



Scheme 1.



Scheme 2.



Scheme 3.

pyridine.[9] The resulting mixture was refluxed for 2 h at 130 °C. The solution was cooled and evaporated to dryness under diminished pressure. The crystalline residue was triturated with 10 mL of ether. The resulting mixture was separated on silica gel column (2×30 cm, $CHCl_3$: MeOH=49 : 1) and then recrystallized from EtOH : Et_2O (7 : 3), giving 0.063 g (47%) of white crystal (**2**) and 0.076 g (53%) of light yellow crystal (**3**).

2-Thiouracil (**2**): mp > 300 °C, ^1H NMR (DMSO-d_6) δ 7.8 (d, 2H, H-5, H-6), IR (KBr): 1660 cm^{-1} (C=O), 1550 cm^{-1} (C=S)

2,4-Dithiouracil (**3**): mp=293-295 °C, ^1H NMR (DMSO-d_6) δ 7.8 (d, 2H, H-5, H-6), IR (KBr): 1530, 1550 cm^{-1} (C=S)

General procedure for nitration. 2 mmol of uracil (0.224 g, **1**), 2-thiouracil (0.256 g, **2**), 2,4-dithiouracil (0.289 g, **3**) were dissolved respectively in 0.5 M nitronium tetrafluoroborate in sulfolane (total 8 mL or 4 mmol NO_2BF_4) and these solutions were left at room temperature for 21 h.[10] $CHCl_3$ (8 mL) was added to the reaction mixture, the resulting precipitate was filtered, washed with diethyl ether and then recrystallized from MeOH, giving 0.093 g (59%) of 5-nitrouracil (**4**), 0.061 g (35%) of 5-nitro-2-thiouracil (**5**), and 0.069 g (32%) of 5-nitro-2,4-dithiouracil (**6**). Nitration was confirmed with singlet in δ 8.7 of H-6 proton on ^1H NMR spectra.

5-Nitrouracil (**4**): mp > 320 °C, ^1H NMR (DMSO-d_6) δ 8.7 (d, 1H, H-6), IR (KBr): 1660 (C=O), 1420 cm^{-1} (-NO_2)

5-Nitro-2-thiouracil (**5**): mp > 320 °C, ^1H NMR (DMSO-d_6) δ 8.7 (d, 1H, H-6), IR (KBr): 1660 (C=O), 1540, 1550 (C=S), 1410 cm^{-1} (-NO_2)

5-Nitro-2,4-dithiouracil (**6**): mp > 320 °C, ^1H NMR (DMSO-d_6) δ 8.7 (d, 1H, H-6), IR (KBr): 1540, 1560 (C=S), 1450 cm^{-1} (-NO_2)

General procedure for the syntheses of nucleoside derivatives. Hexamethyl disilazane (HMDS), trimethylsilyl chloride, and stannic chloride were added to one mole of pyrimidine base and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in aqueous acetone.[11] The reaction mixture was stirred at room temperature for 12 h. The solution was poured into saturated $NaHCO_3$ and evaporated *in vacuo* to provide the solid residue of protected nucleoside derivatives; 2',3',5'-O-triacetyl-2-thiouridine (**7**, 60%), 2',3',5'-O-triacetyl-2,4-dithiouridine (**8**, 55%), 5-nitro-2',3',5'-O-triacetyl-uridine (**9**, 63%), 5-nitro-2',3',5'-O-triacetyl-2-thiouridine (**10**, 65%), and 5-nitro-2',3',5'-O-triacetyl-2,4-dithiouridine (**11**, 63%).

2',3',5'-O-Triacetyl-2-thiouridine (**7**): ^1H NMR ($CDCl_3$): δ 7.41 (d, 2H, H-5 and H-6), 5.95 (s, 1H, H-1'), 5.21 (s, 2H, H-5'), 4.23 (d, 2H, H-2', H-3'), 2.06 (s, 9H, $(CH_3)_3$), IR (KBr): 1660 (C=O), 1550 cm^{-1} (C=S)

2',3',5'-O-Triacetyl-2,4-dithiouridine (**8**): ^1H NMR ($CDCl_3$): δ 7.41 (d, 2H, H-5 and H-6), 5.95 (s, 1H, H-1'), 5.21 (s, 2H, H-5'), 4.23 (d, 2H, H-2', H-3'), 2.06 (s, 9H, $(CH_3)_3$), IR (KBr): 1650, 1700 amide (C=O), 1540 cm^{-1} (C=S)

2',3',5'-O-Triacetyl-5-nitrouridine (**9**): ^1H NMR ($CDCl_3$): δ 7.01 (s, 1H, H-6), 5.95 (s, 1H, H-1'), 5.21 (s, 2H, H-5'), 4.23 (d, 2H, H-2', H-3'), 2.06 (s, 9H, $(CH_3)_3$), IR (KBr): 1650, 1700 (amide C=O), 1400 cm^{-1} (-NO_2)

2',3',5'-O-Triacetyl-5-nitro-2-thiouridine (**10**): ^1H NMR ($CDCl_3$): δ 7.01 (s, 1H, H-6), 5.95 (s, 1H, H-1'), 5.21 (s, 2H, H-5'), 4.23 (d, 2H, H-2', H-3'), 2.06 (s, 9H, $(CH_3)_3$), IR (KBr): 1650, 1700 (amide C=O), 1550 (C=S), 1400 cm^{-1} (-NO_2)

2',3',5'-O-Triacetyl-5-nitro-2,4-dithiouridine (**11**): ^1H NMR ($CDCl_3$): δ 7.01 (s, 1H, H-6), 5.95 (s, 1H, H-1'), 5.21 (s, 2H, H-5'), 4.23 (d, 2H, H-2', H-3'), 2.06 (s, 9H, $(CH_3)_3$), IR (KBr): 1575, 1580 (C=S), 1430 cm^{-1} (-NO_2)

General procedure for deacetylation of protected nucleoside derivatives. One mmol of protected nucleoside was suspended in absolute MeOH (15 mL) and was saturated with dry ammonia gas by stirring at room temperature for 24 h.[12] The resulting solution was evaporated under diminished pressure at 35 °C and the residue was purified on a silica gel column packed in $CHCl_3$: MeOH : H_2O=65 : 25 : 4. Column was eluted with $CHCl_3$: MeOH : H_2O=65 : 25 : 4, solvent was removed *in vacuo* to provide solid residue. Residue was recrystallized from EtOH : Et_2O (8 : 2), deacetylated nucleoside derivatives of **7**, **8**, **9**, **10**, and **11** were obtained.

Results and Discussion

5-Nitrouracil and 5-nitrouridine derivatives of which C-2 and C-4 oxygen atoms were replaced by sulfur atoms were designed and synthesized in order to know the correlationship between the modified structure and the antibacterial activity and the lipophilicity, and then to search the possibility of developing nucleoside antibiotic in this study.

The modified pyrimidine bases and their nucleosides were *in vitro* evaluated for antibacterial activity against two

Table 1. MIC[a] (μg/mL) of Pyrimidine Base and Its Nucleoside

Compound	Strain	
	S. aureus	*E. coli*
2-Thiouracil(**2**)	1.56	50.00
2,4-Dithiouracil(**3**)	1.56	100.00
5-Nitrouracil(**4**)	0.20	6.25
5-Nitro-2-thiouracil(**5**)	12.50	50.00
5-Nitro-2,4-dithiouracil(**6**)	6.25	100.00
5-Nitrouridine(**7**)	12.50	6.25
5-Nitro-2-thiouridine(**10**)	50.00	50.00
5-Nitro-2,4-dithiouridine(**11**)	12.50	50.00
Tetracycline[b]	0.5-20.00	2.5-50.00
Erythromycine[b]	0.01-3.00	2.5-20.00

[a] MIC, minimum inhibitory concentration, determined by 2-fold broth dilution method[5]. [b] For the purpose of comparison, the MICs of tetracycline and erythromycine are included[13]

Table 2. Partition Coefficient(P)[a] of Pyrimidine Base

Compounds	P-value	P ratio[b]
Uracil (**1**)	0.0527[c]	
2-Thiouracil (**2**)	0.0738	1.4
2,4-Dithiouracil (**3**)	0.137	2.6
5-Nitrouracil (**4**)	0.0417	
5-Nitro-2-thiouracil (**5**)	0.550	13.2
5-Nitro-2,4-dithiouracil (**6**)	3.326	79.8

[a] Measured in 1-octanol-0.1 M Na_3PO_4, pH 7.0[6,7]. [b] $P_{ratio}=P_A/P$ (A= **2**, **3**, **5** or **6**).

[c] $$P=\frac{[\mathbf{1}\text{ or }\mathbf{4}(1\text{-octanol})]}{[\mathbf{1}\text{ or }\mathbf{4}(0.1\text{ M }Na_3PO_4,\text{ pH }7.0)]}$$

bacterial cells; *S. aureus* and *E. coli* by broth dilution method. Most of the 5-nitrothiopyrimidine bases and their nucleosides were found to be moderately active against *S. aureus* and *E. coli* (Table 1) except 5-nitrouracil (**4**) (MIC= 0.2 μg/mL against *S. aureus*). The lipophilicities of sulfur-substituted pyrimidine bases were enhanced significantly, as indicated by increase in corresponding P-values (1-octanol-0.1 M sodium phosphate) (Table 2). Thus, oxygen-sulfur exchange leading to 2-thiouracil (**2**) and 2,4-dithiouracil (**3**) were associated with a 1.4- and 2.6-fold increase in P-value relative to that of uracil (**1**). The P-values of 5-nitro-2-thiouracil (**5**) and 5-nitro-2,4-dithiouracil (**6**) were increased 13.2- and 79.8-fold relative to that of 5-nitrouracil (**4**).

Low molecular weight compounds such as nucleosides permeate the cell membrane by one of several transport mechanisms depending on their chemical properties. Hydrophobic molecule is dissolved easily in the lipid layer of membrane and can permeate through it by passive diffusion. In this case the diffusion velocity is direct function of the oil-water partition coefficient of nucleoside compound. Now it is recognized that the oxygen-sulfur exchange is sufficiently good method to increase the lipophilicity for the easy permeation through the cell membrane. Above information could be used to modify the structure of nucleoside compound which had better biological activity. However, we could not find a certain correlationship between the synthesized nucleoside compounds in this study and the antibacterial activity and the degree of lipophilicity. Even though 2,4-dithiouracil (**3**) is more lipophilic than 2-thiouracil (**2**), antibacterial activities of those compounds are same (MICs are 1.56 μg/mL) against *S. aureus* or a difference (100 : 50 μg/mL) against *E. coli*. The P-values of 5-nitro-2-thiouracil (**5**) and 5-nitro-2,4-dithiouracil (**6**) were much larger than that of 5-nitrouracil (**4**), but their antibacterial activities were weak. These results may be useful in the design of potent and selective nucleoside derivatives against neoplastic tissues.

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