(m, 1, H-3″), 5.64 (d, J = 3.46 Hz, 1, H-4″), 6.89 (m, 1, H-2′), 7.34 (m, 1, H-3′), 7.43 (m, 3, H-4′, H-6′, H-7′), 7.78 (m, 1, H-5′), 8.09 (m, 1, H-8′). Anal. ($C_{22}H_{26}NO_7Na$) C, H; N: calcd, 5.96; found, 6.46, 6.44.

Glusulase[21] hydrolysis and quantitative analysis (in duplicate) of the liberated propranolol by GC/MS[22] showed both **9a** and **9b** to contain 100.9% each of the theoretical amount of propranolol. Not surprisingly, **11** was only 14% hydrolized under identical conditions. In addition, the HPLC retention volumes for the glucuronides **9a** and **9b** are identical with the retention volumes of the propranolol glucuronides isolated from dog urine.

Acknowledgment. The authors thank U. K. Walle and M. Wilson for assistance in these studies. The glucuronide from dog urine was generously provided by S. Bai. Supported in part by NIH Grant GM-20387.

Registry No. 1, 90-15-3; (*R*)-**2**, 87144-72-7; (*R*)-**3**, 87102-64-5; **4**, 21085-72-3; **5a**, 87102-65-6; **5b**, 87102-66-7; **6a**, 87102-67-8; **6b**, 87102-68-9; **7**, 87102-69-0; **8a**, 58657-79-7; **8b**, 87144-74-9; **9a**, 87102-70-3; **9b**, 87144-73-8; **10**, 87102-72-5; **11**, 87102-71-4; methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosid)uronate, 7355-18-2; D-glucurono-3,6-lactone, 32449-92-6; epichlorohydrin, 106-89-8.

Synthesis and Biological Activity of Various 3′-Azido and 3′-Amino Analogues of 5-Substituted Pyrimidine Deoxyribonucleosides

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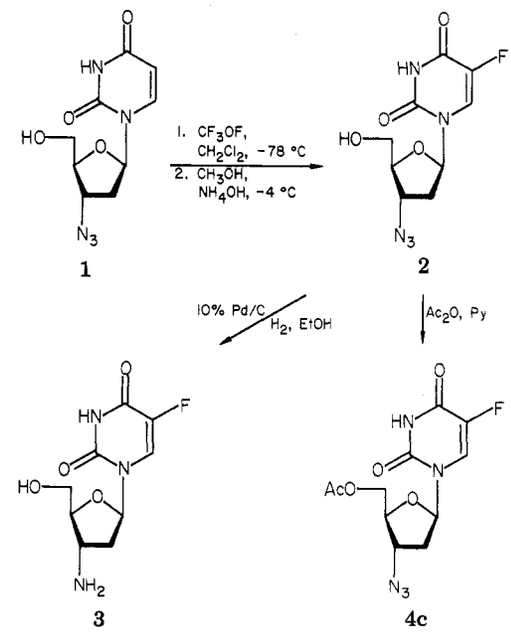
Various new 5-substituted 3′-azido- and 3′-amino derivatives of 2′-deoxyuridine and 2′-deoxycytidine have been synthesized and biologically evaluated. Among these compounds, 3′-amino-2′,3′-dideoxy-5-fluorouridine (**3**), 3′-amino-2′,3′-dideoxycytidine (**7a**), and 3′-amino-2′,3′-dideoxy-5-fluorocytidine (**7c**) were found to be the most active against murine L1210 and sarcoma 180 neoplastic cells in vitro, with an ED_{50} of 15 and 1 μM, 0.7 and 4 μM, and 10 and 1 μM, respectively. The 3′-azido derivatives, **2** and **6c**, were less active in comparison with their 3′-amino counterparts. In addition, the 5-fluoro-3′-amino nucleosides, **3** and **7c**, were tested against L1210 leukemia bearing CDF_1 mice. Our preliminary findings indicate that compound **7c** (6 × 200 mg/kg) was as active as the positive control, 5-fluorouracil (6 × 20 mg/kg), yielding a T/C × 100 of 146 and 129, respectively. However, **3** was found to be inactive in this experiment.

3′-Amino-3′-deoxythymidine was synthesized previously by Miller and Fox[2] and by Horwitz et al.[3] independently. This compound has been found to strongly inhibit the replication of L1210 murine leukemia both in vitro[4] and in mice.[5] The biochemical basis for its anticancer activity is under study, and the findings of Fischer et al.[6] suggest that interference with DNA synthesis is involved. Recently, 3′-amino-2′,3′-dideoxycytidine (**7a**), a new derivative of 2′-deoxycytidine, has been synthesized in this laboratory[7] and found to not only have excellent aqueous solubility but also to be a potent inhibitor of L1210 and P388 murine leukemias both in vitro and in mice.[7,8] These findings demonstrate that the structure modification of thymidine and 2′-deoxycytidine in the 3′-position with an amino group leads to compounds with significant antineoplastic activity. Furthermore, we have found that (**7a**) was resistant to deamination by partially purified cytidine–deoxycytidine deaminase from human KB cells.[8,9]

Scheme I



Based on these findings, various new 5-substituted 3′-azido and 3′-amino derivatives of 2′-deoxyuridine and 2′-deoxycytidine, including 3′-azido-2′,3′-dideoxy-5-fluorouridine (**2**), 3′-amino-2′,3′-dideoxy-5-fluorouridine (**3**), 3′-

(1) Visiting scientist from the Institute of Materia Medica, Chinese Academy of Medical Science, Beijing, The People's Republic of China.
(2) Miller, N.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 1772.
(3) Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076.
(4) Lin, T. S.; Prusoff, W. H. *J. Med. Chem* **1978**, *21*, 109.
(5) Lin, T. S.; Fischer, P. H.; Prusoff, W. H. *Biochem. Pharmacol.* **1981**, *31*, 125.
(6) Fischer, P. H.; Lin, T. S.; Prusoff, W. H. *Biochem. Pharmacol.* **1979**, *28*, 991.
(7) Lin, T. S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 544, and unpublished results.
(8) Mancini, W. R.; Lin, T. S. *Biochem. Pharmacol.* **1983**, *32*, 2427.
(9) Mancini, W. R.; Lin, T. S.; Prusoff, W. H., unpublished results.

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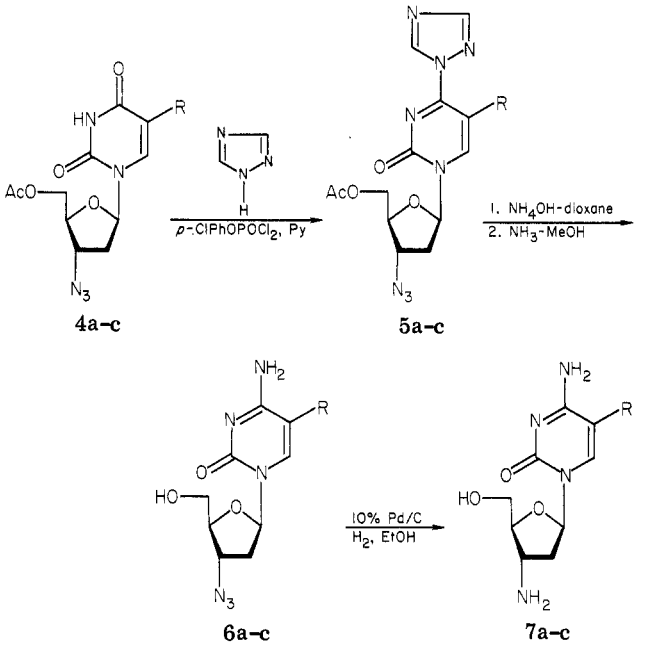
Scheme II[a]



[a] a, R = H; b, R = CH_3; c, R = F.

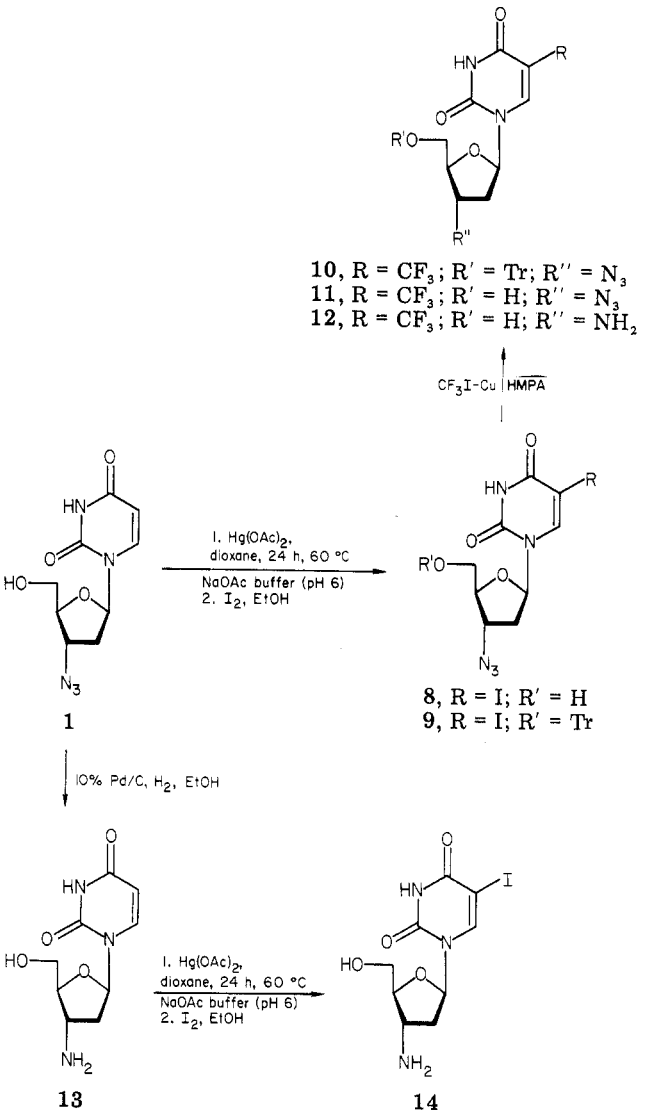
azido-2′,3′-dideoxy-5-methylcytidine (**6b**), 3′-azido-2′,3′-dideoxy-5-fluorocytidine (**6c**), 3′-amino-2′,3′-dideoxy-5-methylcytidine (**7b**), 3′-amino-2′,3′-dideoxy-5-fluorocytidine (**7c**), 3′-azido-2′,3′-dideoxy-5-(trifluoromethyl)-uridine (**11**), 3′-amino-2′,3′-dideoxy-5-(trifluoromethyl)-uridine (**12**), 3′-amino-2′,3′-dideoxy-5-iodouridine (**14**), 3′-azido-5′-fluoro-3′,5′-dideoxythymidine (**17**), and 3′-amino-5′-fluoro-3′,5′-dideoxythymidine (**18**), have been synthesized and biologically evaluated.

Chemistry. Treatment of 3′-azido-3′-deoxyuridine[7] (**1**) with trifluoromethyl hypofluorite (CF_3OF) in CH_2Cl_2 at −78 °C, followed by NH_4OH in CH_3OH at 4 °C,[10] gave the corresponding 5-fluoro-3′-azido derivative **2**. Catalytic (10% Pd/C) hydrogenation of **2** at 50 psi in EtOH afforded the 3′-amino analogue **3**. The 3′-azido-5′-*O*-acetyl derivative **4c** was obtained by acetylation of **2** with acetic anhydride in pyridine (Scheme I).

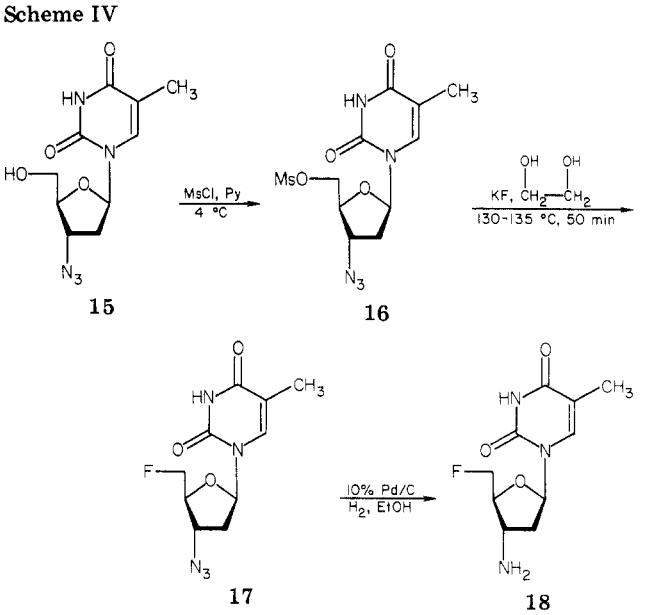
The 3′-azido-5′-*O*-acetyl derivatives, **4a–c**, were treated with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature for 3 days[11] to give the 4-triazolylpyrimidinone derivatives, **5a–c**. Subsequent treatment of **5a–c** with aqueous ammonia in dioxane (1:3, v/v) for 4 h and then saturated methanolic ammonia overnight at room temperature[11] yielded the 3′-azido analogues, **6a–c**. Compounds **6a–c** were then reduced under 50 psi of hydrogen in the presence of 10% palladium on charcoal at room temperature for 6 h to afford the desired **7a–c** (Scheme II).

Reaction **1** with mercuric acetate in dioxane and NaOAc buffer (pH 6) at 60 °C for 24 h, followed by iodination of the 5-mercuric nucleoside complex with iodine in EtOH,[12] yielded the 5-iodo-3′-azido derivative **8**. Tritylation of **8** by trityl chloride in pyridine at 100 °C for 2 h gave the 5′-*O*-trityl nucleoside **9**. Treatment of **9** with trifluoromethyl–copper complex[13,14] in hexamethylphosphoramide (HMPA) at 45–50 °C yielded the 5-(trifluoromethyl)-3′-azido derivative **10**. Detritylation of **10** with 80% acetic

Scheme III



Scheme IV



acid, followed by catalytic (10% Pd/C) hydrogenation in EtOH, gave the desired 3′-amino-2′,3′-dideoxy-5-(trifluoromethyl)uridine (**12**). 3′-Amino-3′-deoxy-5-iodouridine (**14**) was synthesized by iodination[12] of 3′-amino-

(10) Sharma, R. A.; Bobek, M.; Bloch, A. *J. Med. Chem.* **1974**, *17*, 466.
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Table I. ED_{50} Values of Various 3'-Azido and 3'-Amino Analogues of Pyrimidine Deoxyribonucleoside on the Replication of L1210 and Sarcoma 180 Cells in Vitro

compd	ED_{50},[a] μM	
	L1210	S-180
1	inactive	inactive
13	18	50
2	20	40
3	15	1
6a	inactive	inactive
7a	0.7	4
6b	inactive	inactive
7b	25	20
6c	25	
7c	10	1
11	400	100
12	150	100
14	400	
17	inactive	inactive
18	inactive	inactive

[a] The ED_{50} values were estimated from dose-response curves compiled from at least two independent experiments and represent the drug concentration required to inhibit replication of L1210 and S-180 by 50%.

2',3'-dideoxyuridine[7] (**13**). The synthesis of these compounds is depicted in Scheme III.

Treatment of 3'-azido-3'-deoxythymidine[3,4] (**15**) with methanesulfonyl chloride in pyridine at 4 °C generated the 5'-mesylate **16**. Displacement of the 5'-*O*-methanesulfonyl group with KF in ethylene glycol at 130–135 °C afforded the 5'-fluoro derivative **17**. Catalytic hydrogenation of **17** in the presence of palladium on charcoal in EtOH yielded the corresponding 3'-amino analogue **18** (Scheme IV).

The structures of these compounds have been characterized by proton NMR, UV, IR, and elemental analysis.

Biological Activity. The new 3'-azido and 3'-amino nucleoside derivatives were tested against murine L1210 and sarcoma 180 neoplastic cells in culture. The ED_{50} values summarized in Table I were estimated from dose–response curves and represent the drug concentration required to inhibit replication of L1210 and sarcoma 180 cells by 50%. Among these compounds, **3**, **7a**, and **7c** were found to be most active against both L1210 murine leukemia and sarcoma 180 neoplastic cells, with an ED_{50} of 15 and 1 μM, 0.7 and 4 μM, and 10 and 1 μM, respectively. Compound **7b** also inhibited the replication of L1210 and sarcoma 180 cells (ED_{50} = 25 and 20 μM, respectively), and **12** showed the least activity (ED_{50} = 150 and 100 μM). The 3'-azido derivatives, **2** and **11**, demonstrated less activity in comparison to the 3'-amino analogoues, whereas **6a** and **6b** were found to be totally inactive. The 3'-N_3-5'-F and 3'-NH_2-5'-F derivatives of thymidine, **17** and **18**, were not effective against either L1210 or sarcoma 180 neoplastic cells in vitro.

5-Fluoro-3'-amino nucleosides, **3** and **7c**, were screened against L1210 leukemia bearing CDF_1 female mice. Four groups of mice with six mice in each group were inoculated with 1×10^5 L1210 murine leukemia cells. Starting 24 h postinoculation, the first group of mice was injected with saline as the vehicle control, the second group with 20 mg/kg of 5-fluorouracil as positive control, the third group with 200 mg/kg of **3**, and the fourth group with 200 mg/kg of **7c** in saline, once a day, for 6 days. The average survival time of the vehicle control mice was 7.2 days. The 5-fluorouracil-treated mice survived an average of 9.3 days, giving a T/C × 100 value of 129. The **3**-treated mice died on an average time of 7.7 days, which was about the same as the untreated vehicle control. However, the mice treated with **7c** survived an average of 10.5 days, yielding a T/C × 100 of 146. These findings suggest that at the given dosage and schedule, compound **7c** was as active as 5-fluorouracil against L1210 leukemia in this experiment.

Table II. Effect of 3'-Azido and 3'-Amino Analogues of Pyrimidine Deoxyribonucleoside on the Activity of Thymidine Kinase and Deoxycytidine Kinase Isolated from L1210 Cells

compd	activity, % control[a]			
	thymidine kinase		deoxycytidine kinase	
	5[b]	20[b]	5[b]	20[b]
UdR	93	65	88	99
1	98	68	92	92
13	105	71	97	94
2	78	71	94	89
3	92	82	93	93
3'-N_3-TdR	59	5	89	84
3'-NH_2-TdR	95	72	96	87
17	93	95	93	89
18	89	83	91	88
IUdR	26	0	87	97
14	95	71	87	91
11	79	19	91	85
12	81	61	101	87
6a	85	72	86	78
7a	77	84	89	85
6c	88	103	70	61
7c	81	70	82	73
6b	92	81	62	55
7b	93	80	90	82

[a] The enzymatic activity is expressed as the percent of the control activity, which was determined in the absence of analogue. [b] The numbers indicate the ratio of the concentration of analogue divided by the concentration of the natural nucleoside substrate for the enzyme, which was 0.1 mM for both [^{14}C]thymidine and [^{14}C]deoxycytidine.

Biochemical Evaluation. The effect of various nucleoside analogues on the activity of thymidine kinase and deoxycytidine kinase are shown in Table II. Three compounds that inhibited thymidine kinase most markedly are (in the order of decreasing potential) 2'-deoxy-5-iodouridine (IUdR), 3'-azido-3'-deoxythymidine, and 3'-azido-2',3'-dideoxy-5-(trifluoromethyl)uridine (**11**). All other modifications of thymidine had a reduced inhibitory effect on thymidine kinase activity. These include the presence of a fluorine atom substituted in either the 5'- or the 5-position of the 3'-azido and 3'-amino analogues of thymidine. Reduced activity was also found by replacement of the methyl group of thymidine with a hydrogen atom (UdR), as well as the presence of an azido or amino moiety in the 3'-position of UdR. The presence of an amino moiety in the 3'-position of thymidine, deoxyuridine, IUdR, or 2'-deoxy-5-(trifluoromethyl)uridine produced compounds with poor inhibitory activity against thymidine kinase.

None of the thymidine analogues had a significant inhibitory effect on deoxycytidine kinase activity. Of the various deoxycytidine analogues, only the 3'-azido analogues of 2'-deoxy-5-methylcytidine and 2'-deoxy-5-fluorocytidine, **6b** and **6c**, produced a modest inhibitory effect on deoxycytidine kinase activity. Little or no inhibition of thymidine kinase activity was produced by the deoxycytidine analogues.

(12) Lin, T. S.; Neenan, J. P.; Cheng, Y. C.; Prusoff, W. H.; Ward, D. C. *J. Med. Chem.* **1976**, *19*, 495.
(13) Kobayashi, Y.; Yamamoto, K.; Asai, T.; Nakano, M.; Kumadaki, I. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2755.
(14) Lin, T. S.; Gao, Y. S. *J. Med. Chem.* **1983**, *26*, 598.

Examination of these results with those of the ED_{50} values (Table I) reveal, with the exception of IUdR, a trend toward an inverse correlation. In other words, the best inhibitors of the deoxynucleoside kinases were among the least biologically active. This is exemplified by the fact that the poor enzyme inhibitors 3′-amino-3′-deoxythymidine[4] and 3′-amino-2′,3′-dideoxycytidine[7,8] are known potent cytotoxic agents against L1210 leukemia both in vitro and in vivo.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.2 mm). IR spectra were recorded on the Perkin-Elmer 21 spectrophotometer. The UV spectra were recorded on a Beckman 25 spectrophotometer, and the NMR spectra were taken on a Bruker 270 HX or a WM 500 spectrometer at 270 or 500 MHz using Me_4Si as internal reference. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

3′-Azido-2′,3′-dideoxy-5-fluorouridine (2). 3′-Azido-2′,3′-dideoxyuridine[7] (1; 30 g, 118 mmol) was dissolved in 183 mL of MeOH, and after the addition of 550 mL of CH_2Cl_2, the solution was cooled to −78 °C. A slow stream of CF_3OF was bubbled intermittently into the magnetically stirred solution, and the progress of the reaction was monitored by the disappearance of the UV-absorbing peak at 260 nm. The reaction was completed in approximately 3 h. The solvent and the excess reagent were removed in vacuo at room temperature, and the remaining adduct was dissolved in 500 mL of cold MeOH containing 31 mL of concentrated NH_4OH. The solution was then allowed to stand at 5 °C with stirring overnight. The solvent and NH_4OH were evaporated below 30 °C, under reduced pressure, to dryness, and the residue was purified by silica gel column (4 × 60 cm) chromatography ($CHCl_3$–EtOH, 4:1) to give 31 g (96%). The product was further purified by recrystallization from $CHCl_3$–EtOH (4:1): mp 148–149 °C; IR (KBr) 4.75 (azido) μm; UV (MeOH) λ_{max} 270 nm (ε 7857), λ_{min} 238 nm; UV (0.01 N HCl) λ_{max} 270 nm (ε 7142), λ_{min} 235 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ε 6857), λ_{min} 260 nm; NMR (Me_2SO-d_6) δ 2.32 (m, 2 H, 2′-H), 3.63 (m, 2 H, 5′-H), 3.83 (m, 1 H, 4′-H), 4.39 (m, 1 H, 3′-H), 5.35 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.05 (m, 1 H, 1′-H), 8.21 (d, 1 H, 6-H), 11.86 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{10}FN_5O_4$) C, H, N.

3′-Amino-2′,3′-dideoxy-5-fluorouridine (3). Compound **2** (0.5 g, 1.8 mmol) was dissolved in 30 mL of EtOH and hydrogenated at room temperature, under 50 psi of hydrogen pressure, in the presence of 100 mg of palladium on charcoal, for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness to give 0.42 g (93%). An analytical sample was obtained by recrystallization of **3** from EtOH: mp 103–106 °C; UV (MeOH) λ_{max} 271 nm (ε 13 230), λ_{min} 236 nm; UV (0.01 N HCl) λ_{max} 270 nm (ε 12 660), λ_{min} 235 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ε 11 177), λ_{min} 250; NMR (Me_2SO-d_6) δ 2.06 (m, 2 H, 2′-H), 3.42 (m, 2 H, 5′-H), 3.61 (m, 2 H, 3′-H and 4′-H), 4.60–5.42 (br s, 3 H, 3′-NH_2 and 5′-OH, D_2O exchangeable), 6.02 (m, 1 H, 1′-H), 8.32 (d, 1 H, 6-H). Anal. ($C_9H_{12}FN_3O_4$·EtOH) C, H, N.

3′-Azido-3′-deoxy-5′-*O*-acetyl-5-fluorouridine (4c). Acetic anhydride (8.5 g, 83 mmol) was added dropwise to a stirred solution of 3′-azido-3′-deoxy-5-fluorouridine (**2**; 4.5 g, 16.6 mmol) in 50 mL of pyridine at 0 °C. The resulting solution was allowed to stand overnight at 4 °C. The reaction mixture was then dissolved in $CHCl_3$ (200 mL) and washed in a separatory funnel with 200-mL portions of water (2 times), saturated $NaHCO_3$ (2 times), and water again (2 times). The $CHCl_3$ layer was then clarified with Norit and filtered, and the filtrate was dried with anhydrous $MgSO_4$ and filtered again. The filtrate was then concentrated to a glassy residue and further dried in vacuo at 45 °C for 2 h to yield 2.4 g (48%). The product was used immediately without further purification for the next preparation: R_f 0.52 (acetone–CH_2Cl_2, 3:7).

4-Triazolylpyrimidine Intermediates (5a–c). 3′-Azido-2′,3′-dideoxy-5′-*O*-acetyluridine[7] (4a, 5.70 g, 1.93 mmol) was dissolved in 50 mL of pyridine. While stirring in a cold water bath, 4-chlorophenyl phosphorodichloridate (3.70 g, 2.90 mmol) was added dropwise, followed by the addition of 1,2,4-triazole (4.00 g, 5.79 mmol). This mixture was stirred at room temperature for 3 days, after which it was concentrated (~30 °C) to a glass. The residue was dissolved in 50 mL of CH_2Cl_2 and washed with 60 mL of water (2 times) and 50% $NaHCO_3$ solution. The CH_2Cl_2 layer was clarified with Norit and filtered, and the filtrate was dried ($MgSO_4$) and filtered again, and the filtrate was evaporated in vacuo to yield 4.17 g (62%) of a glassy syrup: R_f 0.48 (acetone–CH_2Cl_2, 3:7).

The 4-triazolylpyrimidine intermediates **5b** (R_f 0.36) and **5c** (Rf 0.30) were synthesized by the same methodology from 3′-azido-3′-deoxy-5′-*O*-acetylthymidine[4] (**4b**) and 3′-azido-2′,3′-dideoxy-5′-*O*-acetyl-5-fluorouridine (**4c**), respectively.

3′-Azido-2′,3′-dideoxycytidine (6a). Compound **5a** (4.17 g, 1.20 mmol) was dissolved in 80 mL of NH_4OH–dioxane (1:3), and the mixture was stirred for 4 h at room temperature in a Wheaton pressure bottle. This solution was then concentrated to a glass, and the resulting residue was stirred overnight in the pressure bottle at room temperature in 80 mL of saturated methanolic ammonia. The solution was then reduced to a small volume in vacuo and passed through a column (4 × 60 cm) packed with EM silica gel 60 (280 g) using MeOH–$CHCl_3$ (1:3) as eluting solvents. The desired fractions with an R_f of 0.32 were collected and concentrated to afford 2.57 g (86%). Compound **6a** effervesced around 143 °C (lit.[7] effervescence around 140 °C). The IR, UV, and NMR spectra of this compound were identical with the spectra obtained from a sample that was synthesized by a previous method.[7] The yield of product was also greatly improved over the old method[7] (86 vs. 31%).

3′-Azido-2′,3′-dideoxy-5-methylcytidine (6b). Compound **6b** was synthesized from **5b** (2.1 g, 5.8 mmol) by the same method as described above, yielding 1.3 g (85%): R_f 0.32 (MeOH–$CHCl_3$, 1:3); mp 131–132 °C; IR (KBr), 4.76 (azido) μm; NMR (Me_2SO-d_6) δ 1.83 (s, 3 H, 5-CH_3), 2.49 (m, 2 H, 2′-H), 3.61–3.65 (m, 2 H, 5′-H), 3.80 (m, 1 H, 4′-H), 4.34 (m, 1 H, 3′-H, 3′-H)8 5.17 (t, 1 H, 5′-OH, D_2O exchangeable), 6.08 (t, 1 H, 1′-H), 6.80 (br s, 1 H, 4-NH_a D_2O exchangeable), 7.56 (br s, 1 H, 4-NH_b, D_2O exchangeable), 7.59 (s, 1 H, 6-H). Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-fluorocytidine (6c). Compound **6c** was prepared from **5c** (1.5 g, 4.4 mmol) by the same procedure as depicted for **6a**. Except in this case an eluting solvent system of MeOH–$CHCl_3$ (1:6) was used for the silica gel column chromatography. The yield was 0.7 g (59%): R_f 0.24 (MeOH–$CHCl_3$, 1:6); mp 164–166 °C; IR (KBr), 4.75 (azido) μm; NMR (Me_2SO-d_6) δ 2.25–2.31 (m, 2 H, 2′-H), 3.58 (m, 1 H, 5′-H_a), 3.66 (m, 1 H, 5′-H_b), 3.83 (m, 1 H, 4′-H) 4.34 (m, 1 H, 3′-H), 5.24 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.02 (t, 1 H, 1′-H), 7.49 (br s, 1 H, 4-NH_a, D_2O exchangeable), 7.72 (br s, 1 H, 4-NH_b, D_2o exchangeable), 8.06 (d, 1 H, 6-H). Anal. ($C_9H_{11}FN_6O_3$) C, H, F, N.

3′-Amino-2′,3′-dideoxycytidine (7a). A solution of **6a** (5.38 g, 21.0 mmol) in 150 mL of EtOH was hydrogenated under 50 psi of hydrogen pressure for 5 h, in the presence of 10% palladium on charcoal (0.4 g). After filtration, the filtrate was concentrated until a white solid began to precipitate. The solution was allowed to stand overnight at 4 °C and then filtered to collect 3 g (65%) of product: ninhydrin test positive; mp 206–208 °C (lit.[7] mp 207 °C). The UV and NMR spectra of this compound were identical with that of a sample prepared previously.[7]

3′-Amino-2′,3′-dideoxy-5-methylcytidine (7b). A solution of **6b** (1.15 g, 4.30 mmol) in 60 mL of EtOH was hydrogenated by the procedure described for the preparation of **7a**, to afford 0.7 g (70%) of **7b**: ninhydrin test positive; mp 218 °C dec; NMR (Me_2SO-d_6) δ 1.68 (br s, 1 H, 3′-NH_2, D_2O exchangeable), 1.82 (s, 3 H, 5-CH_3), 1.92–2.00 (m, 2 H, 2′-H), 3.31–3.35 (m, 1 H, 4′-H), 3.49 (m, 1 H, 3′-H, 3.56 (d, 1 H, 5′-H_a), 3.65 (d, 1 H, 5′-H_b), 4.93 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.04 (t, 1 H, 1′-H), 6.69 (br s, 1 H, 4-NH_a, D_2O exchangeable), 7.17 (br s, 1 H, 4-NH_b, D_2O exchangeable), 7.69 (s, 1 H, 6-H). Anal. ($C_{10}H_{16}N_4O_3$) C, H, N.

3′-Amino-2′,3′-dideoxy-5-fluorocytidine (7c). Compound **6c** (0.98 g, 3.62 mmol) in 100 mL of EtOH was hydrogenated by the same methodology as depicted previously, to yield 0.52 g (59%) of **7c**: ninhydrin test positive; mp 198–199 °C dec; NMR (Me_2SO-d_6) δ 1.97–2.06 (m, 4 H, 2′-H, and 3′-NH_2, D_2O ex-

changeable), 3.37 (m, 1 H, 4'-H), 3.53 (m, 1 H, 3'-H), 3.59 (m, 1 H, 5'-H_a), 3.67 (m, 1 H, 5'-H_b), 5.03 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.96 (m, 1 H, 1'-H), 7.39–7.59 (br d, 2 H, 4-NH_2, D_2O exchangeable), 8.19 (d, 1 H, 6-H). Anal. ($C_9H_{13}FN_4O_3$) C, H, F, N.

3'-Azido-2',3'-dideoxy-5-iodouridine (8). A mixture of **1** (4.0 g, 15.8 mmol) and mercuric acetate (16 g, 50 mmol) in 300 mL of 0.5 M NaOAc buffer (pH 6) and 160 mL of dioxane was heated at 60 °C with stirring for 24 h. The solution was cooled to room temperature, and a 0.2 M solution of iodine in EtOH (400 mL) was added. The reaction mixture was then stirred for an additional 90 min and filtered. The filtrate was concentrated to a smaller volume (~20 mL) and extracted with $CHCl_3$ (5 × 100 mL). The combined $CHCl_3$ solution was clarified with Norit, dried ($MgSO_4$), concentrated, and passed through a short silica gel column ($CHCl_3$–EtOH, 10:4). The solvent was then evaporated to dryness in vacuo, and the solid residue was crystallized from EtOH to give 2.1 g (35%): mp 125–127 °C; UV (EtOH) λ_{max} 284 nm, λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 2.38 (m, 2 H, 2'-H), 3.64 (m, 2 H, 5'-H), 3.84 (m, 1 H, 4'-H), 4.32 (m, 1 H, 3'-H), 5.67 (br s, 1 H, 5'-OH, D_2O exchangeable), 6.02 (t, 1 H, 1'-H), 8.38 (s, 1 H, 6-H), 11.3 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{10}IN_5O_4$) C, H, I, N.

3'-Azido-2',3'-dideoxy-5'-*O*-trityl-5-iodouridine (9). Triphenylmethyl chloride (3.18 g, 11.0 mmol) was added to a solution of **8** (3.6 g, 9.5 mmol) in 20 mL of pyridine. The reaction mixture was heated at 100 °C with stirring for 2 h. The resulting solution was added, at a slow flow rate, to a mixture of ice–water (2:3) in a blender blending for 5 min. The crude solid product was collected by filtration and further purified by silica gel column (4 × 60 cm) chromatography ($CHCl_3$–MeOH, 14:1) to give 3.3 g (56%): mp 101–103 °C; IR (KBr) 4.75 (azido) μm; UV (MeOH) λ_{max} 286 nm; NMR (Me_2SO-d_6) δ 2.50 (m, 2 H, 2'-H), 3.35 (br s, 2 H, 5'-H), 3.95 (m, 1 H, 4'-H), 4.50 (m, 1 H, 3'-H), 6.10 (m, 1 H, 1'-H) 7.35 (br s, 15 H, aromatic ring), 8.00 (s, 1 H, 6H), 11.65 (br s, 1 H, 3-NH, D_2O exchangeable).

3'-Azido-2',3'-dideoxy-5-(trifluoromethyl)uridine (11). Trifluoromethyl iodide (24.2 g, 123 mmol) and copper powder[15] (15 g, 236 mmol) in HMPA (80 mL) were stirred in a stainless-steel bomb at 110–120 °C for 3 h. The bomb was cooled to room temperature, and the excess copper powder was removed by filtration through Celite in a glove box under argon. The dark green trifluoromethyl–copper complex solution was added to the 5-iodo derivative **9** (3.3 g, 5.3 mmol), and the reaction mixture was stirred in a glass pressure bottle under argon at 45–50 °C overnight (~20 h). The resulting solution was then added to ice–H_2O (500 mL) and extracted with EtOAc (6 × 300 mL). The combined solution was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was dissolved in 80% HOAc (90 mL) and heated at 110 °C with stirring for 15 min. The solution was allowed to cool to room temperature, and 15 mL of H_2O was added. The solid material was removed by filtration, and the filtrate was concentrated to dryness under reduced pressure. The crude product was then purified by silica gel column (4 × 60 cm) chromatography (Et_2O–EtOAc, 2:1) to afford 0.43 g (25%): mp 116–117 °C; IR (KBr) 4.75 (azido) μm; UV (MeOH) λ_{max} 262 nm (ϵ 10 180), λ_{min} 228 nm; UV (0.1 N NaOH) λ_{max} 260 nm (ϵ 7270), λ_{min} 236 nm; NMR (Me_2SO-d_6) δ 2.44 (m, 2 H, 2'-H), 3.64 (q, 2 H, 5'-H), 3.87 (m, 1 H, 4'-H), 4.34 (q, 1 H, 3'-H), 5.43 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.99 (m, 1 H, 1'-H), 8.71 (s, 1 H, 6-H), 11.86 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{10}H_{10}F_3N_5O_4$) C, H, F, N.

3'-Amino-2',3'-dideoxy-5-(trifluoromethyl)uridine (12). The 3'-azido derivative **11** (0.3 g, 0.9 mmol) was dissolved in 30 mL of EtOH and hydrogenated at room temperature for 2 h at 30 psi of hydrogen pressure in the presence of 10% palladium on charcoal (80 mg). The catalyst was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The solid residue was then crystallized from EtOH to yield 0.2 g (72%) of product: mp 184–185 °C dec; UV (MeOH) λ_{max} 262 nm (ϵ 9270), λ_{min} 230 nm; UV (0.01 N HCl) λ_{max} 261 nm (ϵ 9480), λ_{min} 226 nm; UV (0.01 N NaOH) λ_{max} 260 nm (ϵ 6590), λ_{min} 234 nm; NMR (Me_2SO-d_6) δ 2.14 (m, 2 H, 2'-H), 3.39 (m, 2 H, 5'-H), 3.64 (m, 2 H, 3'-H and 4'-H), 4.70–5.40 (br s, 3 H, 3'-NH_2 and 5'-OH, D_2O exchangeable), 5.96 (m, 1 H, 1'-H), 8.86 (s, 1 H, 6-H). Anal. ($C_{10}H_{12}F_3N_3O_4$) C, H, F, N.

3'-Amino-2',3'-dideoxy-5-iodouridine (14). Mercuric acetate (5.28 g, 16.6 mmol) was added to a solution of 3'-amino-2',3'-dideoxyuridine[7] (**13**; 1.50 g, 6.61 mmol) in 170 mL of 0.5 M NaOAc buffer (pH 6). The reaction mixture was heated at 50–55 °C for 21 h, cooled to room temperature, and diluted to 350 mL with water. A solution of iodine in EtOH (0.2 M, 150 mL) was then added, and the mixture was stirred at room temperature for an additional 90 min. The HgI_2 generated from the reaction was separated by filtration, and the solution was extracted with $CHCl_3$ (5 × 250 mL) to remove the excess iodine. The aqueous solution was then applied directly to a column (2 × 300 cm) packed with AG50W-X8 (H^+) ion-exchange resin and washed with 4 L of water. The product was eluted with 1 N NH_4OH solution, and 120 mL of elute was collected. The solvent was evaporated in vacuo to dryness, and the residue was crystallized from EtOH. The crystalline product was collected by filtration, washed with EtOH and ether, and dried to afford 1.32 g (57%). The compound started to darken around 175 °C and decomposed at 179–180 °C: NMR (Me_2SO-d_6) δ 2.04 (m, 1 H, 2'-H_a), 2.15 (m, 1 H, 2'-H_b), 3.45 (br m, 4 H, 5'-H and 3'-NH_2, D_2O exchangeable), 3.57 (m, 1 H, 4'-H), 3.67 (m, 1 H, 3'-H), 6.00 (q, 1 H, 1'-H), 8.50 (s, 1 H, 6-H). Anal. ($C_9H_{12}IN_3O_4$) C, H, N.

3'-Azido-3'-deoxy-5'-*O*-methanesulfonylthymidine (16). Methanesulfonyl chloride (6.4 g 56.0 mmol) was added dropwise to a solution of 3'-azido-3'-deoxythymidine[3,4] (**15**) in 40 mL of pyridine while stirring in an ice–water bath. After the addition, the solution was allowed to stand overnight at 4 °C. The reaction mixture was then added to 120 mL of ice–water with vigorous stirring. The resulting precipitate was collected by filtration and partially crystallized from EtOH to yield 5.6 g (90%): mp 165–166 °C dec; R_f 0.64 (EtOH–$CHCl_3$, 1:6); NMR (Me_2SO-d_6) δ 1.80 (s, 3 H, 5-CH_3), 2.41 (t, 2 H, 2'-H), 3.20 (s, 3 H, CH_3SO_3), 4.08 (m, 1 H, 4'-H), 4.42 (m, 3 H, 3'-H and 5'-H), 6.07 (t, 1 H, 1'-H), 7.32 (s, 1 H, 6-H), 11.2 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_{15}N_5O_6S$) C, H, N, S.

3'-Azido-3'-deoxy-5'-fluorothymidine (17). A mixture of **16** (5.17 g, 15.0 mmol) and potassium fluoride (4.35 g, 74.9 mmol) in 25 mL of ethylene glycol was stirred at 130–135 °C. After the mixture became a clear solution, this temperature was maintained for an additional 30 min. The solution was allowed to cool to room temperature, and acetone (500 mL) was added to precipitate the potassium mesylate. The salt was removed by filtration, and the solvent was evaporated under reduced pressure. The glassy residue was dissolved in 5 mL of a mixture of $CHCl_3$–EtOH (6:1), and the solution was applied directly to silica gel column (4 × 60 cm) using $CHCl_3$–EtOH (6:1) as the eluting solution. The desired fraction (R_f 0.66) was combined and concentrated to yield 0.96 g (24%) of product: mp 120–121 °C; IR (KBr) 4.76 (azido) μm; NMR (Me_2SO-d_6) δ 1.82 (s, 3 H, 5-CH_3), 2.35 (m, 1 H, 2'-H_a), 2.43 (m, 1 H, 2'-H_b), 3.98–4.06 (m, 2 H, 5'-CH_2F), 4.65 (m, 1 H, 4'-H), 4.71 (m, 1 H, 3'-H), 6.14 (t, 1 H, 1'-H), 7.41 (s, 1 H, 6-H), 11.32 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{10}H_{11}FN_5O_3$) C, H, F, N.

3'-Amino-3'-deoxy-5'-fluorothymidine (18). A solution of **17** (0.65 g, 2.42 mmol) in 50 mL of EtOH was reduced under 50 psi of hydrogen in the presence of 10% palladium on charcoal (0.15 g) at room temperature for 4 h. The catalyst was removed by filtration. The filtrate was concentrated to a glassy residue in vacuo. The residue was crystallized from EtOH to give 0.25 g (44%): ninhydrin test positive; mp 178–180 °C dec; NMR (Me_2SO-d_6) δ 1.82 (s, 3 H, 5-CH_3), 2.05 (m, 1 H, 2'-H_a), 2.16 (m, 1 H, 2'-H_b), 2.29 (br s, 2 H, 3'-NH_2, D_2O exchangeable), 3.31–3.45 (m, 2 H, 5'-CH_2F), 4.51–4.72 (m, 2 H, 3'-H and 4'-H), 6.16 (t, 1 H, 1'-H), 7.39 (s, 1 H, 6-H). Anal. ($C_{10}H_{13}FN_3O_3$) C, H, F, N.

Biochemical Procedures. The preparation of the thymidine affinity column and the procedure for initial extraction of the enzymes have been described.[8] After the enzyme sample was loaded onto the affinity column, thymidylate kinase was removed by the method of Chen and Prusoff.[16] Deoxycytidine kinase was eluted from the column by increasing the ionic strength to 0.2

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M Tris-HCl, pH 7.5, and the thymidine kinase isozymes were separated essentially as described by Lee and Cheng,[17] except that the cytoplasmic thymidine kinase (which was used in this study) was eluted in a buffer containing 10% glycerol, 2 mM dithiothreitol, 0.3 M Tris-HCl, pH 7.5, and 200 μM thymidine. The deoxycytidine kinase and cytoplasmic thymidine kinase were separately passed through a G-25 Sephadex column equilibrated with buffer (25 mM Hepes, pH 7.5, 10% glycerol, and 2 mM dithiothreitol) at 4 °C. These enzyme preparations were stored at −70 °C.

The assay procedures used were similar to those described for thymidine kinase[17] and deoxycytidine kinase.[18] Both assay mixtures contained 40 mM Hepes, pH 7.5 (37 °C), 5.6 mM phosphocreatine, 0.5 unit of phosphocreatine kinase, 75 μg of bovine serum albumin, 2 mM dithiothreitol, 2 mM ATP (all of which were obtained from Sigma Chemical Co.), and 2 mM $MgCl_2$. In addition, the deoxycytidine kinase and thymidine kinase mixtures respectively contained 0.1 mM [2-^{14}C]CdR (9.4 mCi/mmol) and 0.1 mM [2-^{14}C]TdR (14 mCi/mmol) with the appropriate enzyme preparation. The labeled nucleosides were obtained from Moravek Biochemicals, Inc.

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Registry No. 1, 84472-85-5; **2**, 87190-74-7; **3**, 85236-95-9; **4a**, 84472-87-7; **4b**, 66323-42-0; **4c**, 87190-75-8; **5a**, 87190-76-9; **5b**, 87190-77-0; **5c**, 87190-78-1; **6a**, 84472-89-9; **6b**, 87190-79-2; **6c**, 87190-80-5; **7a**, 84472-90-2; **7b**, 87190-81-6; **7c**, 87190-82-7; **8**, 85236-92-6; **9**, 87190-83-8; **10**, 87190-89-4; **11**, 87190-84-9; **12**, 87190-85-0; **13**, 84472-86-6; **14**, 85236-89-1; **15**, 30516-87-1; **16**, 87190-86-1; **17**, 87190-87-2; **18**, 87190-88-3; thymidine kinase, 9002-06-6; deoxycytidine kinase, 9039-45-6; 1,2,4-triazole, 288-88-0.

Pyridinylpiperazines, a New Class of Selective α_2-Adrenoceptor Antagonists

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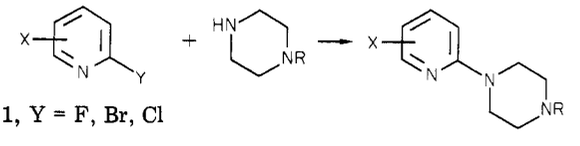
A series of 1-(2-pyridinyl)piperazine derivatives was synthesized and evaluated for adrenergic activity. In vitro activity was assessed through the antagonism of clonidine's effect in the rat, isolated, field-stimulated vas deferens and by the displacement of [^{3}H]clonidine from membrane binding sites of calf cerebral cortex. Antagonism of clonidine-induced mydriasis in the rat was used as an in vivo assay. Several members of the series proved to be potent, selective α_2-adrenoceptor antagonists. 1-(3-Fluoro-2-pyridinyl)piperazine was more potent than either yohimbine or rauwolscine in displacement of [^{3}H]clonidine and had a higher affinity for this binding site (α_2) than for the [^{3}H]prazosin site (α_1). In vivo, the 3-F derivative was more potent than the reference standards in reversing clonidine-induced mydriasis. None of the members of this series was more selective or potent than rauwolscine in antagonizing clonidine in the rat vas deferens.

In addition to the α_1-adrenoceptors of effector cells that mediate postjunctional responses to the neurotransmitter norepinephrine, other adrenoceptors are now known to be present at both pre- and postjunctional sites. These latter receptors, α_2-adrenoceptors, can be characterized and distinguished from α_1-adrenoceptors by their relative activities toward agonists and antagonists. The recently recognized potential of selective agonists and antagonists of α_2-adrenoceptors as therapeutic agents has stimulated the search for novel agents that will interact with these receptors.

Previous work[1] from these laboratories reported on the affinities of some piperazinylimidazo[1,2-*a*]pyrazines for α_1- and α_2-adrenoceptors. In this paper we describe the syntheses of some pyridinylpiperazine derivatives and their evaluation as selective α_2-adrenoceptor antagonists.

Chemistry. Most of the pyridinylpiperazines of Table I were synthesized by reaction of the appropriate 2-halopyridine **1** with either *N*-methylpiperazine or piperazine (Scheme I). In the latter case, the use of excess piperazine was preferred in order to circumvent formation of bis(pyridinyl)piperazines. The *N*-benzyl analogue **2h** was prepared by alkylation of **2c** with benzyl bromide, and the 3-amino derivative **2m** was obtained through catalytic reduction of the corresponding nitro compound **21**. All of the intermediate 2-halopyridines have either been reported previously in the literature or were prepared by established procedures.

Scheme I



1, Y = F, Br, Cl

2

Results and Discussion

Relative affinities of the pyridinylpiperazine derivatives of Table I for central α-adrenergic binding sites were determined by measurement of radioligand displacement from membrane binding sites of calf cerebral cortex. Displacement of [^{3}H]clonidine was used as a measure of interaction with α_2-adrenoceptor binding sites, while [^{3}H]prazosin displacement served as an assay for α_1-adrenoceptor affinity.

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