Articles

Synthesis and Anti-HIV Activity of Carbocyclic 2',3'-Didehydro-2',3'-dideoxy 2,6-Disubstituted Purine Nucleosides

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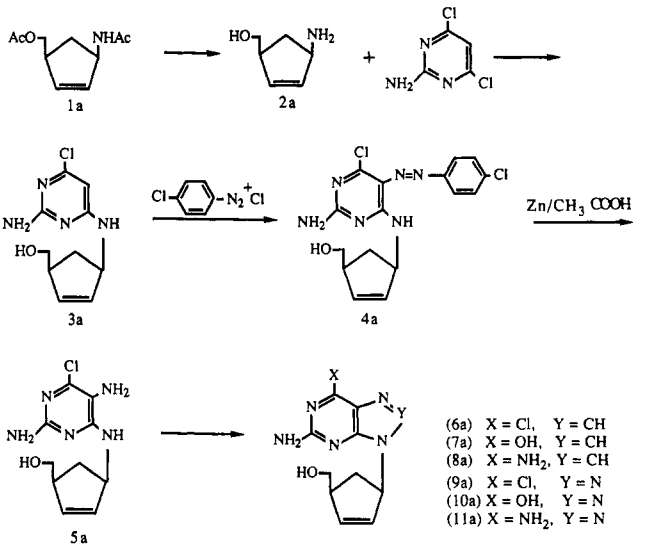
(±)-*cis*-[4-[(2,5-Diamino-6-chloropyrimidinyl)amino]-2-cyclopentenyl]carbinol (**5a**) was synthesized from 2-amino-4,6-dichloropyrimidine and *cis*-4-(hydroxymethyl)cyclopentenylamine (**2a**) by subsequent preparation of the 5-[(4-chlorophenyl)azo] derivative of the resulting pyrimidine (**3a**) and reduction of the azo moiety with zinc and acetic acid. The carbocyclic analogue of 2',3'-didehydro-2',3'-dideoxy 2-amino-6-chloropurine (**6a**) and the corresponding 8-azapurine (**9a**) were prepared from **5a**. The carbocyclic 2',3'-didehydro-2',3'-dideoxy analogues of guanine (**7a**) and 2,6-diaminopurine (**8a**), and 8-azaguanine (**10a**) and 8-aza-2,6-diaminopurine (**11a**) were prepared from **6a** and **9a**, respectively. The corresponding 2',3'-saturated series of 2-amino-6-substituted-purine carbocyclic nucleosides was prepared following the same scheme starting with *cis*-4-(hydroxymethyl)cyclopentylamine (**2b**). Carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine (carbovir, **7a**) emerged as a potent and selective anti-HIV agent. Its hydrolytic stability and its ability to inhibit the infectivity and replication of HIV in T-cells at concentrations of approximately 200–400-fold below toxic concentrations make carbovir an excellent candidate for development as a potential antiretroviral agent.

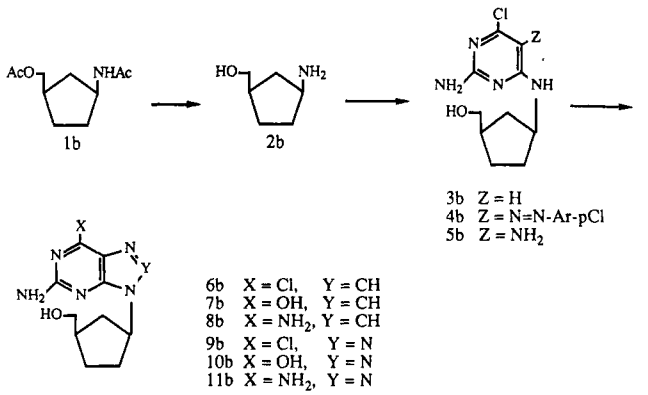
Despite intensive effort to discover drugs that may be of value in the systemic treatment of human viral infections, such infections have been singularly resistant to chemotherapy. The intracellular and intimate relationship between viral and host functions and metabolism makes it difficult to destroy a virus without irreparable damage to the host cell. Thus, there are few agents effective against viruses having an acceptable therapeutic index, i.e., the ratio of 50% cytotoxic dose, IC_{50}, to 50% antiviral dose, EC_{50}.

Following the identification of a retrovirus, referred to as human immunodeficiency virus (HIV), as the etiological agent of human acquired immunodeficiency syndrome (AIDS),[1-3] an intense effort has been made to identify drugs for the treatment or prevention of this debilitating, lethal disease. Although several nucleosides have been shown to have an in vitro anti-HIV activity commensurate with development to clinical trials,[4] only zidovudine (AZT, Retrovir) has received approval for the treatment of AIDS. While clinically useful in many settings, AZT is also associated with substantial toxicity, especially in myelosuppression.[5] Early clinical results with 2',3'-dideoxycytidine have indicated therapeutic activity, but also some undesirable side effects (e.g., peripheral neuropathy).[6]

Scheme I



Scheme II



Several other 2',3'-dideoxyribonucleosides of purine and pyrimidine nucleosides have been reported to exhibit

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Table I. Inhibitory Concentrations of Carbocyclic Nucleosides for P-388 Leukemia Cells in Culture[a]

compd	ED_{50}, μg/mL	compd	ED_{50}, μg/mL
6a	75	**6b**	>100
7a	>100	**7b**	>100
8a	>100	**8b**	80
9a	1.0	**9b**	18
10a	4.5	**10b**	51
11a	>100	**11b**	>100

[a] Cytotoxicity to P-388 cells in culture was determined by the protocol of the National Cancer Institute.[10]

anti-HIV activity and are currently being pursued as chemotherapeutic agents against AIDS.[7]

The present report provides an account of a synthetic route to a new class of carbocyclic nucleoside analogues that have been identified as potent and selective inhibitors of HIV in vitro. Thus, the syntheses of the (hydroxymethyl)cyclopentenyl compounds of formulas **6a–11a** and the (hydroxymethyl)cyclopentyl compounds of formulas **6b–11b**, from the versatile precursor *cis*-(4-acetamidocyclopent-2-enyl)methyl acetate (**1a**), were accomplished as outlined in Schemes I and II. Compound **1a**, prepared as previously described,[8] was hydrolyzed with aqueous barium hydroxide to obtain amino alcohol **2a**. Condensation of **2a** with 2-amino-4,6-dichloropyrimidine gave the corresponding pyrimidinylamino derivative **3a**. 5-[(*p*-Chlorophenyl)azo]pyrimidine **4a** was prepared with *p*-chlorobenzenediazonium chloride by the method of Shealy and Clayton.[9] Reduction of **4a** with zinc and acetic acid gave pyrimidine **5a**, which was subsequently converted to the 9-substituted 2-amino-6-chloropurine **6a** by ring closure with triethyl orthoformate. 2-Amino-6-chloropurine **6a** was converted to the carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine analogue **7a** with 0.3 N sodium hydroxide under reflux conditions, while treatment of **6a** with liquid ammonia gave 2,6-diaminopurine analogue **8a**.

Ring closure of **5a** with sodium nitrite and acetic acid gave (±)-(*cis*)-4-(5-amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-cyclopentenylcarbinol (**9a**) in good yield. Base hydrolysis of **9a** with sodium hydroxide gave 8-aza analogue **10a** of carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine, whereas treatment with liquid ammonia gave the corresponding 2,6-diaminopurine analogue **11a**.

The 2′,3′-saturated series was prepared as outlined in Scheme II. Thus, catalytic reduction of **1a** gave the appropriately substituted cyclopentane precursor **1b**. The remaining synthetic steps in the **b** series paralleled those described for the **a** series.

Results and Discussion

The ED_{50} cytotoxic concentrations of the carbocyclic dideoxynucleosides in P-388 mouse leukemia cell culture are listed in Table I. The 8-aza compounds **9a** and **10a** exhibited significant cytotoxicities in this assay. These results are consistent with our previous observations in other series;[11,12] i.e., when the carbocyclic purine was found to exhibit antitumor activity, the corresponding 8-aza analogue was more active.

Table II. Comparative Potency and Selectivity of Carbocyclic 2′,3′-Dideoxynucleoside Analogues as Inhibitors of HIV Replication in MT-2 Cells[a–c]

compd	EC_{50}, μg/mL	IC_{50}, μg/mL	TI
6a	0.40	6.97	17.3
6b	>100	>100	
7a	0.19	41.8	220
7b	>100	>100	
8a	4.7	>125.0	26.6
8b	16.7	>100	>5.9
DDC	0.29	44	152

[a] All data represent the means of several experiments. [b] Experimental details are described in ref 15. [c] The effective concentration, 50% (EC_{50}), represents the concentration of test agent that increases (protects) formazan production in infected cultures to 50% of untreated, uninfected cell controls. The inhibitory concentration, 50% (IC_{50}), represents the toxic concentration of drug that reduces formazan production in uninfected cultures to 50% of untreated, cell controls. Microcomputer-calculated EC_{50} is determined by simple linear interpolation from the data as described in ref 15. The therapeutic index (TI) is determined by dividing the IC_{50} by the EC_{50}.

The compounds were compared for their inhibitory effects on HIV-induced cytopathogenicity in MT-2 cells (Table II). As a reference compound, 2′,3′-dideoxycytidine (DDC) was included, having been previously identified as among the most potent and selective inhibitors of HIV replication.[13,14] Four of the compounds in this series of 2′,3′-didehydro-2′,3′-dideoxy 2,6-disubstituted purine nucleosides were confirmed to have reproducible in vitro anti-HIV activity (defined as 50% or greater reduction of cytopathic effect in two or more independent experiments). The 2′,3′-unsaturated guanosine analogue (**7a**), subsequently named carbovir,[15] was the most selective anti-HIV compound. Its therapeutic index (ratio of 50% cytotoxic dose, IC_{50}, to 50% antiviral effective dose, EC_{50}) was 220. Although the corresponding 6-chloro derivative **6a** was active at only a 2-fold higher concentration than carbovir, its therapeutic index is 13 times lower. 2,6-Diaminopurine derivative **8a** exhibited a much higher EC_{50} than either **6a** or **7a**. However, the therapeutic index of **8a** was not obtained because no toxicity was observed at the highest concentration (due to solubility limitations) tested. Although **8a** required a higher concentration than carbovir to inhibit HIV replication, it represents an excellent candidate for further study because of its low toxicity. In addition, **8a** is readily converted to carbovir by adenosine deaminase (unpublished results) and may provide a slow-release form of active compound in vivo. The data in Table II indicate that saturation of the carbocyclic sugar moiety either decreased (**8b**) activity or abolished (**6b, 7b**) activity. The replacement of the C-8 carbon of the purine with nitrogen also abolished activity. Thus, the 8-aza

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Table III. Effect of Carbovir on HIV Replication When Measured by p24 gag Protein Expression in CEM Cells[a]

sample	p24 synthesis, ng/mL	% reduction in p24 expression
virus controls	>800	
carbovir (**7a**)	28	>99.97
AZT	32	>99.96
ddA	38	>99.95

[a] Suppression of p24 internal core antigen synthesis by test agents. Data obtained by antigen capture ELISA (Du Pont) of supernatants from treated or untreated (virus controls) microcultures prior to the addition of tetrazolium salt. For each drug, p24 determinations were performed at the drug concentration exhibiting maximum therapeutic effect.

analogues (**9**–**11**) were not included in Table II. The elimination of antiviral activity by substitution of a purine by an 8-azapurine in a series of active analogues is consistent with our previous observations.[11,15,16] Interestingly, this dramatic reversal of activity is the opposite of our observations on the antitumor properties of carbocyclic nucleosides.

Previous studies with related compounds prepared in our laboratory indicated that replacement of the guanine heterocycle by adenine decreased the sensitivity 10-fold.[15] Thus, structure–activity studies suggest that optimal anti-HIV activity is obtained when the heterocycle is a 2-amino-6-substituted purine (preferably guanine) and the carbocyclic moiety is 2′,3′-unsaturated.

The most effective compound from the present series, carbovir (**7a**), was evaluated for its inhibitory effects of the expression of viral antigen in HIV-infected CEM cells (Table III). Production of viral p24 core antigen at optimal inhibitory concentrations of the antiviral agents indicated comparable activities for AZT, 2′,3′-dideoxyadenosine, and carbovir.

Carbovir represents the most promising compound from this new class of potent and selective anti-HIV agents. Its hydrolytic stability and its ability to inhibit the infectivity and replication of HIV in T-cells at concentrations of approximately 200-fold (MT-2 cells) to 400-fold (CEM cells) below toxic concentrations make carbovir an excellent candidate for development as a potential antiretroviral agent in the treatment of AIDS patients.

Experimental Section

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra were obtained on JEOL TX 90QFT or Nicollet NT300 spectrometers and were recorded in Me_2SO-d_6. Chemical shifts are expressed in ppm downfield from Me_4Si. IR spectra were determined with KBr pellets on a Perkin-Elmer 281 spectrometer, and UV spectra were determined on a Beckman DU-8 spectrophotometer. Thin-layer chromatography (TLC) was performed on 0.25-mm layers of Merck silica gel 60F-254 and column chromatography was done on Merck 60 (230–400 mesh). All chemicals and solvents were reagent grade unless otherwise specified. Mass spectra were obtained with an AEI Scientific Apparatus Limited MS-30 mass spectrometer. High-resolution mass spectra were obtained for all compounds, and the molecular ion and fragmentation patterns were consistent with assigned structures.

(±)-*cis*-[4-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-2-cyclopentenyl]carbinol (3a). A mixture of **1a** (10.0 g, 50 mmol) and aqueous barium hydroxide (0.5 N, 400 mL) was refluxed overnight. After cooling, it was neutralized with dry ice. The precipitate was filtered out, and the aqueous solution was concentrated to dryness. The residue was extracted with absolute ethanol and concentrated again to yield **2a** as a coloroless syrup.

To this syrup of crude **2a** were added 2-amino-4,6-dichloropyrimidine (99% pure, Aldrich, 12.3 g, 75 mmol), triethylamine (30 mL), and 1-butanol (200 mL), and the mixture was refluxed for 48 h. The volatile solvents were removed; the residue was absorbed onto silica gel (30 g) and it was packed into a column (5.0 × 15 cm) and eluted with $CHCl_3$-MeOH (40:1, 30:1, 20:1). The product fractions were collected and concentrated into a syrup. Acetone was added to precipitate the triethylamine salt. After filtration, the concentrated syrup was crystallized by addition of ethyl acetate to yield white, solid crystals: 9.15 g (76%); mp 132–134 °C; MS (30 eV, 200 °C) *m/e* 240 and 242 (M^+ and $M^+ + 2$), 209 ($M^+ - 31$), 144 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 238, 277, 302 nm in 0.1 N HCl. Anal. ($C_{10}H_{13}ClN_4O$) C, H, N.

(±)-*cis*-[3-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-cyclopentyl]carbinol (3b). Starting material **1a** (5 g, 25 mmol) was dissolved in ethanol (75 mL) and hydrogenated in the presence of 10% palladium–charcoal (50 mg). The catalyst was filtered out and the solvent was evaporated. The residual syrup (**1b**) was hydrolyzed by a barium hydroxide solution as described for **1a** above.

To **2b** (25 mmol) were added 2-amino-4,6-dichloropyrimidine (37.5 mmol), triethylamine (25 mL), and *n*-butanol (125 mL), and the mixture was refluxed for 48 h. It was processed as described in the procedure above to yield 2.7 g (44%) of crystalline **3b**, mp 122–124 °C. Recrystallization from ethyl acetate yielded **4b**: mp 124–126 °C; MS (30 eV, 200 °C) *m/e* 242 and 244 (M^+ and $M^+ + 2$), 211 ($M^+ - 31$), 144 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 238, 277, 302 nm in 0.1 N HCl. Anal. ($C_{10}H_{15}ClN_4O$) C, H, N.

(±)-*cis*-[4-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-2-cyclopentenyl]carbinol (4a). A cold diazonium salt solution was prepared from *p*-chloroaniline (1.47 g, 11.5 mmol) in 3 N HCl (25 mL) and sodium nitrite (870 mg, 12.5 mmol) in water (10 mL). This solution was added to a mixture of **3a** (2.40 g, 10 mmol), acetic acid (50 mL), water (50 mL), and sodium acetate trihydrate (20 g). The reaction mixture was stirred overnight at room temperature. The yellow precipitate was filtered and washed with cold water until neutral, then it was air-dried in a fumehood to yield 3.60 g (94%) of **4a**, mp 229 °C dec. The analytical sample was obtained from acetone–methanol (1:2): mp 241–243 °C dec; MS (30 eV, 260 °C) *m/e* 378 and 380 (M^+ and $M^+ + 2$), 282 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 279, 365 nm in 0.1 N HCl. Anal. ($C_{16}H_{16}Cl_2N_6O$) C, H, N.

(±)-*cis*-[3-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]cyclopentyl]carbinol (4b). A cold diazonium salt solution prepared as described above was added to a mixture of **3b** (2.42 g, 10 mmol), acetic acid (50 mL), water (50 mL), and sodium acetate trihydrate (20 g). Following the procedure above, compound **4b** was obtained as a yellow product, 3.69 g (94%), mp 260–262 °C dec. The crude product was recrystallized from acetone–methanol (3:1): MS (30 eV, 260 °C) *m/e* 380 and 382 (M^+ and $M^+ + 2$), 282 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1620, 1580 (C=C, C=N); UV λ_{max} 279, 365 in 0.1 N HCl. Anal. ($C_{16}H_{18}Cl_2N_6O$) C, H, N.

(±)-*cis*-[4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)-amino]-2-cyclopentenyl]carbinol (5a). A mixture of **4a** (379 mg, 1 mmol), zinc dust (0.65 g, 10 mmol), acetic acid (0.32 mL), water (15 mL), and ethanol (15 mL) was refluxed under nitrogen for 3 h. The zinc was removed, and the solvents were evaporated. The residue was absorbed onto silica gel (2 g) and it was packed into a column (2.0 × 18 cm) and eluted with $CHCl_3$–MeOH (15:1). A pink syrup was obtained. Further purification from methanol–ether yielded **5a** as pink crystals: 170 mg (66%); mp 168–170 °C; MS (30 eV, 220 °C) *m/e* 255 and 257 (M^+ and $M^+ + 2$), 224 ($M^+ - 31$), 159 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 238, 298 nm in 0.1 N HCl. Anal. ($C_{10}H_{14}ClN_5O$) C, H, N.

(±)-*cis*-[3-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-cyclopentyl]carbinol (5b). A mixture of **4b** (3.08 g, 8.1 mmol), zinc dust (5.2 g, 80 mmol), acetic acid (2.6 mL), water (130 mL), and ethanol (130 mL) was refluxed under nitrogen for 3 h and worked up as described above. The mixture was absorbed onto silica gel (18 g) and it was packed into a column (4.0 × 8 cm) and

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eluted with $CHCl_3$–MeOH (20:1) to yield **5b** as yellow-pink crystals, 1.44 g (69%). The product was recrystallized from methanol–ether to yield **5b**: mp 143–145 °C; MS (30 eV, 200 °C); *m/e* 257 and 259 (M^+ and $M^+ + 2$), 226 ($M^+ - 31$), 159 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 238, 298 nm in 0.1 N HCl. Anal. ($C_{10}H_{16}ClN_5O$) C, H, N.

(±)-*cis*-[4-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-cyclopentenyl]carbinol (6a). A mixture of **5a** (1.41 g, 5.5 mmol), triethyl orthoformate (30 mL), and hydrochloric acid (12 N, 1.40 mL) was stirred overnight. The suspension was dried in vacuo. Diluted hydrochloric acid (0.5 N, 40 mL) was added and the mixture was reacted at room temperature for 1 h. The mixture was neutralized to pH 8 with 1 N sodium hydroxide and absorbed onto silica gel (7.5 g) and it was packed into a column (4.0 × 10 cm) and eluted by $CHCl_3$–MeOH (20:1) to yield off-white crystals of **6a**, 1.18 g (80%). The crude product was recrystallized from ethanol to yield **6a**: mp 145–147 °C; MS (30 eV, 220 °C) *m/e* 265 and 267 (M^+ and $M^+ + 2$), 235 ($M^+ - 30$), 169 (B_+); IR (KBr) 3600–2600 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 243, 309 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 8.01 (s, 1 H, H-8), 6.98–6.80 (s, 2 H, NH_2, D_2O exchangeable), 6.15–6.05 and 5.94–5.84 (dd, 2 H, vinyl CH=CH, J = 5.0 Hz), 5.50–5.32 (m, 1 H, H-1′), 4.79–4.60 (t, 1 H, CH_2OH, D_2O exchangeable), 3.51–3.39 (d, 2H, CH_2OH), 2.95–2.71 (m, 1 H, H-4′), 2.69–2.55 (m, 1 H, CHH′), 1.72–1.50 (m, 1 H, CHH′). Anal. ($C_{11}H_{12}N_5OCl \cdot {}^3/_4H_2O$) C, H, N.

(±)-*cis*-[3-(2-Amino-6-chloro-9*H*-purin-9-yl)cyclopentyl]carbinol (6b). A mixture of **5b** (1.3 g, 5 mmol), triethyl orthoformate (30 mL), and hydrochloric acid (12 N, 1.30 mL) was stirred overnight. The reaction mixture was processed as described above to yield off-white crystals of 6b: 1.00 g (73%); mp 151–153 °C; MS (30 eV, 230 °C) *m/e* 267 and 269 (M^+ and $M^+ + 2$), 236 ($M^+ - 31$), 169 (B^+); IR (KBr) 3600–2600 (NH_2, OH), 1620, 1580 cm^{-1} (C=C, C=N); UV λ_{max} 243, 309 in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 8.26 (s, 1 H, H-8), 6.91–6.88 (s, 2 H, NH_2, D_2O exchangeable), 4.75–4.65 (m, 1 H, H-1′), 4.65–4.60 (t, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.32–1.55 (m, 7 H, CH_2–CH_2, CHH′, H-4′). Anal. ($C_{11}H_{14}N_5OCl \cdot {}^1/_2H_2O$) C, H, N.

(±)-*cis*-2-Amino-1,9-dihydro-9-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-6*H*-purin-6-one (7a). A mixture of **6a** (266 mg, 1 mmol) and aqueous sodium hydroxide (0.33 N) was refluxed for 5 h and absorbed onto silica gel (2 g) that was then packed into a column (2.0 × 7.5 cm) and eluted with $CHCl_3$–MeOH (5:1). The crude product was recrystallized from methanol–water (1:4) to yield white crystals of **7a**: 152 mg (61%); mp 254–256 °C dec; MS (30 eV, 200 °C) *m/e* 247 (M^+), 217 ($M^+ - 30$), 151 (B^+); IR (KBr) 3600–2600 (NH_2, OH), 1700, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 278 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 10.57–10.50 (s, 1 H, 6-OH, D_2O exchangeable), 7.60–7.56 (s, 1 H, H-8), 6.50–6.35 (s, 2 H, NH_2, D_2O exchangeable), 6.14–6.06 and 5.89–5.81 (dd, 2 H, CH=CH vinyl, J = 5.0 Hz), 5.38–5.26 (m, 1 H, H-1′), 4.76–4.65 (t, 1 H, CH_2OH, D_2O exchangeable) 3.47–3.39 (d, 2 H, CH_2OH), 2.92–2.80 (m, 1 H, H-4′), 2.65–2.55 (m, 1 H, CHH′), 1.64–1.50 (m, 1 H, CHH′). Anal. ($C_{11}H_{13}N_5O_2 \cdot {}^3/_4H_2O$) C, H, N.

(±)-*cis*-2-Amino-1,9-dihydro-9-[3-(hydroxymethyl)cyclopent-1-yl]-6*H*-purin-6-one (7b). Compound **6b** (280 mg, 1 mmol) was hydrolyzed with sodium hydroxide and purified as described above to yield white crystals of **7b**: 204 mg (77%); mp 274–276 °C dec; MS (30 eV, 320 °C) *m/e* 249 (M^+), 218 ($M^+ - 31$), 151 (B^+); IR (KBr) 3600–2600 (NH_2, OH), 1700, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 278 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 10.60–10.50 (s, 1 H, 6-OH, D_2O exchangeable), 7.80 (s, 1 H, H-8), 6.45–6.30 (s, 2 H, NH_2, D_2O exchangeable), 4.75–4.60 (m, 1 H, H-1′), 4.60–4.50 (t, 1 H, CH_2OH, D_2O exchangeable), 3.55–3.35 (d, 2 H, CH_2OH), 2.25–1.55 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{11}H_{15}N_5O_2 \cdot {}^3/_4H_2O$) C, H, N.

(±)-*cis*-[4-(2,6-Diamino-9*H*-purin-9-yl)-2-cyclopentenyl]carbinol (8a). Liquid ammonia was passed into a solution of **6a** (265 mg, 1 mmol) in methanol (10 mL) at −80 °C in a bomb. The bomb was sealed and heated at 75 °C for 48 h. Ammonia and methanol were evaporated. The residue was absorbed onto silica gel (2 g) and it was packed into a column (2.0 × 10 cm) and eluted with $CHCl_3$–MeOH (15:1). The crude product was recrystallized from ethanol to yield 196 mg (80%) of **8a**: mp 152–155 °C; MS (30 eV, 200 °C) *m/e* 246 (M^+), 229 ($M^+ - 17$), 216 ($M^+ - 30$), 150 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1700, 1650, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 290 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.60 (s, 1 H, H-8), 6.67–6.59 (s, 2 H, NH_2, D_2O exchangeable), 6.13–6.06 and 5.89–5.82 (dd, 2 H, CH=CH vinyl, J = 5.0 Hz), 5.77–5.70 (s, 2 H, NH_2, D_2O exchangeable), 5.42–5.32 (m, 1 H, H-1′), 4.77–4.69 (t, 1 H, CH_2OH, D_2O exchangeable), 3.47–3.40 (d, 2 H, CH_2OH), 2.92–2.79 (m, 1 H, H-4′), 2.66–2.52 (m, 1 H, CHH′), 1.64–1.51 (m, 1 H, CHH′). Anal. ($C_{11}H_{14}N_6dO$) C, H, N.

(±)-*cis*-[3-(2,6-Diamino-9*H*-purin-9-yl)cyclopentyl]carbinol (8b). Compound **6b** (280 mg, 1 mmol) reacted with ammonia as described above to yield white crystals of **8b**: 193 mg (77%); mp 208–211 °C; MS (30 eV, 220 °C) *m/e* 248 (M^+), 231 ($M^+ - 17$), 217 ($M^+ - 31$), 150 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1700, 1650, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 290 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.80 (s, 1 H, H-8), 6.72–6.60 (s, 2 H, NH_2, D_2O exchangeable), 5.80–5.68 (s, 2 H, NH_2, D_2O exchangeable), 4.74–4.60 (m, 1 H, H-1′), 4.60–4.54 (t, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.30–1.05 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{11}H_{16}N_6O$) C, H, N.

(±)-*cis*-[4-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-cyclopentenyl]carbinol (9a). To a cold solution of **5a** (225 mg, 1 mmol) in acetic acid (1.5 mL) and water (2.5 mL) was added sodium nitrite (83 mg, 1.2 mmol) in water (2 mL). The reaction was monitored by starch–potassium iodide paper. After stirring for 1 h at 0 °C, the precipitate was filtered and washed with cold water and then dried over phosphorus pentoxide in vacuo to yield **9a** as off-white crystals, 218 mg (81%). The crude **9a** was recrystallized from methanol: mp 153–155 °C dec; MS (30 eV, 220 °C) *m/e* 266 and 268 (M^+ and $M^+ + 2$), 236 ($M^+ - 30$), 170 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1650, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 248, 314 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.35–7.00 (br, 2 H, NH_2, D_2O exchangeable), 6.20–6.10 and 6.00–5.90 (dd, 2 H, CH=CH vinyl, J = 5.0 Hz), 5.62–5.55 (m, 1 H, H-1′), 4.45–4.38 (t, 1 H, CH_2OH, D_2O exchangeable), 3.60–3.50 (m, 2 H, CH_2OH), 2.95–2.92 (m, 1 H, H-4′), 2.80–2.60 (m, 1 H, CHH′), 2.00–1.80 (m, 1 H, CHH′). Anal. ($C_{10}H_{11}ClN_6O$) C, H, N.

(±)-*cis*-[3-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentyl]carbinol (9b). Compound **5b** (773 mg, 3 mmol) was reacted with sodium nitrite as described above to yield 666 mg (82%) of **9b**, which was recrystallized from methanol: mp 170–172 °C dec; MS (20 eV, 300 °C) *m/e* 268 and 270 (M^+ and $M^+ + 2$), 237 ($M^+ - 31$), 170 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1650, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 248, 314 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.75–7.46 (br, 2 H, NH_2, D_2O exchangeable), 5.15–4.95 (m, 1 H, H-1′), 4.10–3.65 (br, 1 H, CH_2OH, D_2O exchangeable), 3.55–3.25 (d, 2 H, CH_2OH), 2.40–1.60 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{10}H_{13}ClN_6O$) C, H, N.

(±)-*cis*-5-Amino-3,6-dihydro-3-[4-(hydroxymethyl)-2-cyclopentenyl]-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (10a). A mixture of **9a** (218 mg, 0.8 mmol) and aqueous sodium hydroxide (0.25 N, 10 mL) was refluxed for 3 h; and then it was adjusted to pH 3 with 6 N hydrochloric acid. The gelatinous precipitate was filtered and washed with cold water. It was dried over phosphorus pentoxide in vacuo to yield **10a** as an off-white solid, 181 mg (90%); mp 222–224 °C dec. After recrystallization from water, the melting point was 223–225 °C dec: MS (20 eV, 300 °C) *m/e* 248 (M^+), 217 ($M^+ - 31$), 152 (B^+); IR (KBr) 3600–3000 (NH_2, OH), 1750, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 253 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 10.95–10.90 (s, 1 H, 7-OH, D_2O exchangeable), 7.00–6.75 (br, 2 H, NH_2, D_2O exchangeable), 6.14–6.07 and 5.92–5.83 (dd, 2 H, CH=CH, J = 5.0 Hz), 5.61–5.46 (m, 1 H, H-1′), 4.76–4.67 (t, 1 H, CH_2OH, D_2O exchangeable), 3.60–3.40 (m, 2 H, CH_2OH), 2.97–2.81 (m, 1 H, H-4′), 2.67–2.52 (m, 1 H, CHH′), 1.89–1.75 (m, 1 H, CHH′). Anal. ($C_{10}H_{12}N_6O_2 \cdot {}^1/_2H_2O$) C, H, N.

(±)-*cis*-5-Amino-3,6-dihydro-3-[3-(hydroxymethyl)cyclopentyl]-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (10b). Compound **9b** (268 mg, 1 mmol) was hydrolyzed and processed as described above to yield **10b** as an off-white product, 203 mg (81%). The crude product was recrystallized from water to yield **10b**: mp 228–231 °C; MS (eV, 320 °C) *m/e* 250 (M^+), 219 ($M^+ - 31$), 152

(B^+); IR (KBr) 3600–3000 (NH_2, OH), 1750, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 253 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 11.05–10.95 (s, 1 H, 7-OH, D_2O exchangeable), 7.10–6.90 (br, 2 H, NH_2, D_2O exchangeable), 4.95–4.80 (m, 1 H, H-1'), 4.70–4.50 (br, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.32–1.55 (m, 7 H, H-4', CH_2CH_2, CHH'). Anal. ($C_{10}H_{14}N_6O_2 \cdot 1.25H_2O$) C, H, N.

(±)-*cis*-[4-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-cyclopentenyl]carbinol (11a). Compound **9a** (267 mg, 1 mmol) was processed as described for compound **6a** with a reaction time of 20 h at 60 °C. The residual mixture was absorbed onto silica gel (2 g); it was packed into a column (2.0 × 10 cm) and eluted by $CHCl_3$–MeOH (15:1) to yield **11a** as white crystals, 204 mg (83%). The crude product was recrystallized from ethanol–water (2:1) to yield **11a**: mp 240–242 °C dec; MS (30 eV, 240 °C) *m/e* 247 (M^+), 229 ($M^+ - 18$), 217 ($M^+ - 30$), 151 (B^+); IR (KBr) 3600–3100 (NH_2, OH), 1700, 1650, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 283 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.80–7.20 (br, 2 H, NH_2, D_2O exchangeable), 6.50–6.30 (s, 2 H, NH_2, D_2O exchangeable), 6.15–6.10 and 5.95–5.90 (dd, 2 H, CH=CH vinyl, J = 5.0 Hz), 5.65–5.55 (m, 1 H, H-1'), 4.75–4.65 (t, 1 H, CH_2OH, D_2O exchangeable), 3.55–3.40 (m, 2 H, CH_2OH), 2.95–2.85 (m, 1 H, H-4'), 2.65–2.55 (m, 1 H, CHH'), 1.90–1.80 (m, 1 H, CHH'). Anal. ($C_{10}H_{13}N_7O \cdot H_2O$) C, H, N.

(±)-*cis*-[3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentyl]carbinol (11b). Compound **9b** (268 mg, 1 mmol) was processed as described for **9a** to yield 220 mg of **11b** (88%), which was recrystallized from ethanol–water (1:2) to afford pink-white crystals: mp 223–225 °C; MS (30 eV, 250 °C) *m/e* 249 (M^+), 218 ($M^+ - 31$), 151 (B^+); IR (KBr) 3600–3100 (NH_2, OH), 1700, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 253, 283 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.85–7.25 (br, 2 H, NH_2, D_2O exchangeable), 6.50–6.30 (s, 2 H, NH_2, D_2O exchangeable), 4.95–4.85 (m, 1 H, H-1'), 4.65–4.60 (t, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.35–1.60 (m, 7 H, H-4', CH_2CH_2, CHH'). Anal. ($C_{10}H_{15}N_7O$) C, H, N.

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Registry No. **1a**, 61865-50-7; **1b**, 65898-98-8; **2a**, 122624-72-0; **2b**, 78795-20-7; **3a**, 122624-73-1; **3b**, 122624-74-2; **4a**, 122624-75-3; **4b**, 122624-76-4; **5a**, 122624-77-5; **5b**, 122624-78-6; **6a**, 118237-87-9; **6b**, 118237-86-8; **7a**, 118353-05-2; **7b**, 112915-00-1; **8a**, 118237-88-0; **8b**, 120330-36-1; **9a**, 122624-79-7; **9b**, 122624-80-0; **10a**, 122624-81-1; **10b**, 122624-82-2; **11a**, 122624-83-3; **11b**, 122624-71-9; 2-amino-4,6-dichloropyrimidine, 56-05-3; *p*-chloroaniline, 106-47-8.

Inhibitors of Cholesterol Biosynthesis. 1. *trans*-6-(2-Pyrrol-1-ylethyl)-4-hydroxypyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors. 1. Effects of Structural Modifications at the 2- and 5-Positions of the Pyrrole Nucleus

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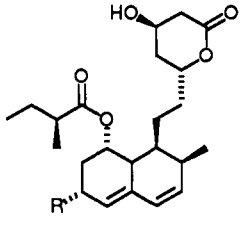
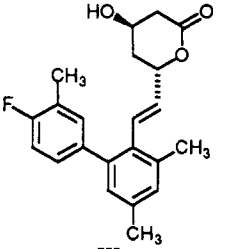
A novel series of *trans*-6-(2-pyrrol-1-ylethyl)-4-hydroxypyran-2-ones and their dihydroxy acid derivatives were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. A systematic study of substitution at the 2- and 5-positions of the pyrrole ring revealed that optimum potency was realized with the 2-(4-fluorophenyl)-5-isopropyl derivative **8x** (Table III), which possessed 30% of the in vitro activity of the potent fungal metabolite compactin (I). A molecular modeling analysis led to the description of a pharmacophore model characterized by (A) length limits of 5.9 and 3.3 Å for the 2- and 5-substituents, respectively, as well as an overall width limit of 10.6 Å across the pyrrole ring from the 2- to the 5-substituent and (B) an orientation of the ethyl(ene) bridge to the 4-hydroxypyran-2-one ring nearly perpendicular to the planes of the parent pyrrole, hexahydronaphthalene, and phenyl rings of the structures examined (Figure 3, θ = 80–110°). Attempts to more closely mimic compactin's polar isobutyric ester side chain with the synthesis of 2-phenylpyrroles containing polar phenyl substituents resulted in analogues (Table III, **8m–p**) with equal or slightly reduced potencies when compared to the 2-[(unsubstituted or 4-fluoro)phenyl]pyrroles, supporting the hypothesis that inhibitory potency is relatively insensitive to side-chain polarity or charge distribution in this area.

The discovery that the fungal metabolites compactin (I)[1] and mevinolin (II)[2] are not only potent inhibitors of the enzyme HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, but are also effective hypocholesterolemic agents in man[3] has led to a plethora of publications describing synthetic and biological studies of close structural analogues.[4]



I: R = H (compactin)
II: R = CH_3 (mevinolin)



III

The disclosure of a series of very potent 6-(*o*-biphenylyl)-substituted 4-hydroxypyran-2-ones (III) by Willard et al.[5] led us to hypothesize that the key structural

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