Nucleic Acid Related Compounds. 51. Synthesis and Biological Properties of Sugar-Modified Analogues of the Nucleoside Antibiotics Tubercidin, Toyocamycin, Sangivamycin, and Formycin[1,§]

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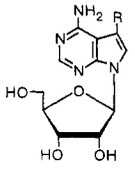
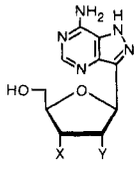
Treatment of 7-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (formycin) with α-acetoxyisobutyryl bromide followed by deprotection of the resulting trans-vicinal acetoxy bromides and hydrogenolysis of the separated bromohydrins gave 2′-deoxy- (23%) and 3′-deoxyformycin (32%) after complete deprotection and purification of their hydrochloride salts. An analogous sequence gave 3′-deoxytoyocamycin and/or 3′-deoxysangivamycin in ~80% yields from toyocamycin. Antiviral, antineoplastic, and antimetabolic effects were evaluated for the formycin compounds and 4-amino-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine (tubercidin), its 5-cyano- (toyocamycin), and 5-carbamoyl- (sangivamycin) antibiotic congeners in comparison with their 2′-deoxy, 3′-deoxy, and arabino analogues. In all cases, the modified-sugar compounds were less cytotoxic than the parent antibiotics. The majority also exhibited lower antiviral potency. However, the *xylo*-tubercidin analogue retained potent antiherpes 1 and 2 activity with decreased cytotoxicity. Labeled metabolite studies suggested that effects of these compounds on RNA and/or protein synthesis might be more significant than interference with DNA synthesis.

The broad spectrum of biological activity (antimetabolic, antiviral, antineoplastic, and antiparasitic)[2,3] of the pyrrolo[2,3-*d*]pyrimidine (i.e., tubercidin, **1**; toyocamycin, **2**; and sangivamycin, **3**) and pyrazolo[4,3-*d*]pyrimidine (e.g., formycin, **4**) nucleoside antibiotics has stimulated considerable interest in the synthesis and biological evaluation of modified analogues. A sizeable number of base-modified analogues have been prepared and tested,[2-8] whereas parallel investigations of the sugar-modified compounds have been less numerous.[2,3,5,9-11] The present study was aimed at comparative elvaluations of several sugar-modified derivatives of the antibiotics **1–4** as antiviral, antineoplastic, and antimetabolic agents in different cell systems.



Tubercidin (**1**, R=H)
Toyocamycin (**2**, R=CN)
Sangivamycin (**3**, R=$CONH_2$)

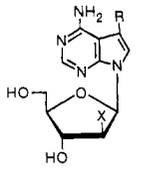


Formycin (**4**, X=Y=OH)
2′-dFor (**5**, X=OH, Y=H)
3′-dFor (**6**, X=H, Y=OH)

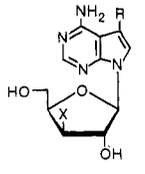
Chemistry

Chemical syntheses of 2′-deoxy- (**5**)[12,13] and 3′-deoxyformycin (**6**),[12,13] 2′-deoxy- (**7**)[14] and 3′-deoxytubercidin (**13**),[12,13,15] and the arabino (**10**)[16] and xylo (**16**)[16] diastereomers of tubercidin were reported some time ago. Our more recently developed four-stage conversion of ribonucleosides to their 2′-deoxy counterparts[17] provided **7**, 2′-deoxytoyocamycin (**8**), and 2′-deoxysangivamycin (**9**)[10] in 65–69% overall yields from the parent antibiotics.[18] Our four-stage oxidation–reduction sequence provided convenient access to 4-amino-7-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyrimidine (**10**) and its 5-cyano (*ara*-toyocamycin, **11**) and 5-carbamoyl (*ara*-sangivamycin, **12**) analogues in 35–60% overall yields.[19] Our recent work with α-acetoxyisobutyryl bromide[20] indicated that improvements to reported syntheses of 2′-deoxy- (**5**)[11-13] and 3′-deoxyformycin (**6**)[12,13] could be realized.

Treatment of 4-monohydrate (Scheme I) with excess α-acetoxyisobutyryl bromide in acetonitrile resulted in



	R	X
7	H	H
8	CN	H
9	$CONH_2$	H
10	H	OH
11	CN	OH
12	$CONH_2$	OH



	R	X
13	H	H
14	CN	H
15	$CONH_2$	H
16	H	OH

formation of a lipophilic product mixture (TLC, presumably[13] **17a** + **18a**, R = $COC(OCOCH_3)(CH_3)_2$, R′ =

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§This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University in March 1986.

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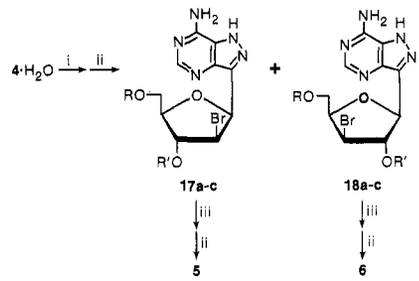
Table I. Antiviral Activity and Cytotoxicity of Pyrrolo- and Pyrazolopyrimidine Nucleosides in Primary Rabbit Kidney Cells

compound	MTC,[a] μg/mL	MIC_{50},[b] μg/mL			
		HSV-1 (KOS)	HSV-2 (G)	VV	VSV
tubercidin (1)	0.4	0.1	0.1	0.07	0.02
toyocamycin[c] (2)	0.04	>0.04	>0.04	≥0.04	>0.04
sangivamycin[c] (3)	1	0.07	0.07	0.02	0.02
2′-deoxytubericidin (7)	>400	50	40	10	125
2′-deoxytoyocamycin (8)	4	≥1	0.3	0.1	0.5
2′-deoxysangivamycin (9)	10	2	1	0.4	2
ara-tubercidin (10)	>400	125	40	40	>400
ara-toyocamycin (11)	10	7	2	1	≥10
ara-sangivamycin (12)	40	4	1	0.2	3
3′-deoxytubercidin (13)	10	>10	>10	1.5	>10
3′-deoxytoyocamycin (14)	1	≥0.4	≥0.4	0.1	>1
3′-deoxysangivamycin (15)	10	>10	>10	2	>10
xylo-tubercidin (16)	10	0.2	0.07	0.3	>10
formycin (4)	20	10	8	7	10
2′-deoxyformycin (5)	>400	>400	>400	>400	>400
3′-deoxyformycin (6)	>400	>400	>400	>400	>400
ribavirin	>400	>400	>400	20	300

[a] Minimum toxic concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50%. [c] The data for toyocamycin and sangivamycin are taken from ref 4. All data represent average values for two to three separate experiments.

Scheme I[a]



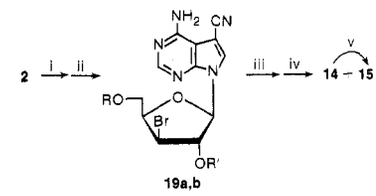
[a] (i) $(CH_3)_2C(OCOCH_3)COBr/CH_3CN$. (ii) $NH_3/MeOH$. (iii) $H_2/Pd\text{-}C/Et_3N/MeOH/(EtOAc)$.

$COCH_3$). Partial deprotection of this material by limited exposure to methanolic ammonia[13] and separation of the resulting mixture by medium-pressure chromatography on silica gel gave **17b** (17%) and **18b** (34%) (R = $COC(OCOCH_3)(CH_3)_2$, R′ = H) plus **17c** (12%) and **18c** (13%) (R = R′ = H).

Hydrogenolysis of **17b** + **17c** over 5% Pd–C followed by complete deprotection with methanolic ammonia and isolation of the crystalline hydrochloride salt gave 81% of 2′-deoxyformycin (**5**) hydrochloride (23% overall from **4**). Hydrogenolysis of **18b** + **18c** followed by deprotection, purification by silica column chromatography, and crystallization of the hydrochloride salt gave 68% of 3′-deoxyformycin (**6**) hydrochloride (32% overall from **4**).

Treatment of toyocamycin (**2**) (Scheme II) with excess α-acetoxyisobutyryl bromide in acetonitrile resulted in formation of the acylated 3′-bromo-3′-deoxy xylo mixture (TLC; **19a**; R = $COC(OCOCH_3)(CH_3)_2$, H; R′ = $COCH_3$). As noted previously,[13] treatment of such trans-vicinal acetoxy bromo nucleosides with $H_2/Pd\text{–}C$ resulted in formation of 2′,3′-dideoxy as well as 3′-deoxy products. Partial deprotection of **19a** with methanolic ammonia as described above for the formycin sequence resulted in

Scheme II[a]



[a] (i) $(CH_3)_2C(OCOCH_3)COBr/CH_3CN$. (ii) $Et_3N/MeOH$. (iii) $H_2/Pd\text{-}C/Et_3N/EtOH$. (iv) $NH_3/MeOH$. (v) Dowex 1×2 (OH^-).

formation of significant quantities of the 2′,3′-anhydro (ribo epoxide) byproduct. Treatment of **19a** with methanolic triethylamine at ~0 °C for an extended period effected deacetylation of O2′ with minimal deprotection of O5′ and internal cyclization to the epoxide. The resulting **19b** (R = $COC(OCOCH_3)(CH_3)_2$, H; R′ = H) was subjected to hydrogenolysis and deprotection with methanolic ammonia, and the 3′-deoxy product mixture was separated by silica column chromatography to give 63% of 3′-deoxytoyocamycin (**14**) and 7% of 3′-deoxysangivamycin (**15**).[10] A second sample of **2** that was subjected to this reaction sequence with more extended exposure to methanolic ammonia gave 65% of **14** and 18% of **15**. Slow passage of an aqueous methanolic solution of **14** (or mixed **14** + **15**) through a column of Dowex 1X2 (OH^-) resin resulted in quantitative conversion[18,19] to the 5-carbamoyl product (**15**).

The exclusive formation of 3′-halo-3′-deoxy xylo nucleosides of the pyrrolo[2,3-*d*]pyrimidine series in 2′,3′-acetoxonium ion-mediated reactions has been noted previously.[10,12–16] A general discussion of steric and electronic factors that contribute to variations in the ratios of 3′-halo-3′-deoxy xylo/2′-deoxy-2′-halo arabino isomers formed with tubercidin, adenosine, and formycin is available.[21]

Aqueous solutions of all samples of the 2′-deoxy (**5**, **7–9**) and 3′-deoxy (**6**, **13–15**) compounds used in the biological experiments were treated with excess sodium periodate for several hours followed by sodium borohydride and repurification. An analogous oxidation–reduction treatment under more carefully controlled conditions was applied to

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Table II. Antiviral Activity and Cytotoxicity of Pyrrolo- and Pyrazolopyrimidine Nucleosides in HeLa Cells

compound	MTC,[a] μg/mL	MIC_{50},[b] μg/mL		
		VSV	Coxsackie B-4	Coxsackie polio-1
tubercidin (1)	0.4	0.01	0.07	0.02
toyocamycin[c] (2)	0.4	0.07	≥0.4	≥0.4
sangivamycin[c] (3)	0.4	0.04	≥0.4	0.07
2′-deoxytubercidin (7)	>400	150	>400	150
2′-deoxytoyocamycin (8)	4	0.08	≥4	≥4
2′-deoxysangivamycin (9)	10	1	>10	>10
ara-tubercidin (10)	>400	≥350	>400	>400
ara-toyocamycin (11)	10	≥10	≥10	≥10
ara-sangivamycin (12)	10	1.5	>10	>10
3′-deoxytubercidin (13)	40	20	2	2
3′-deoxytoyocamycin (14)	1	≥1	0.7	≥1
3′-deoxysangivamycin (15)	40	≥40	≥40	≥40
xylo-tubercidin (16)	4	≥4	≥4	≥4
formycin (4)	1	≥1	>1	≥1
2′-deoxyformycin (5)	>400	>400	>400	>400
3′-deoxyformycin (6)	>400	>400	≥400	≥400
ribavirin	>400	4	40	20

[a-c] Footnotes as in Table I.

the arabino (10–12) and xylo (16) compounds to ensure the absence of trace quantities of the parent antibiotic ribonucleosides.[9]

Biological Evaluation

A. Antiviral Activity. The pyrrolo[2,3-*d*]pyrimidine and pyrazolo[4,3-*d*]pyrimidine derivatives were evaluated against herpes simplex virus type 1 (HSV-1, strain KOS), herpes simplex virus type 2 (HSV-2, strain G), vaccinia virus (VV), vesicular stomatitis virus (VSV), polio virus type 1, Coxsackie virus type B-4, reo virus type 1, parainfluenza virus type 3, sindbis virus, and measles virus in primary rabbit kidney (PRK) cells (Table I), HeLa cells (Table II), or Vero cells (Table III).

As shown previously,[4] tubercidin is a very potent but nonspecific antiviral agent; it is inhibitory to a wide variety of DNA and RNA viruses but only at a concentration slightly below the cytotoxic level. In all three cell cultures, tubercidin proved to be cytotoxic at a concentration of 0.4 μg/mL. Its minimum inhibitory concentration (MIC_{50}) for virus replication ranged from 0.01 to 0.3 μg/mL, depending on the assay system (Tables I–III). In comparison, ribavirin was only effective in the concentration range of 4–400 μg/mL.

Conversion of tubercidin to its 2′-deoxy, 3′-deoxy, ara, or xylo derivatives resulted in significant reductions in toxicity, and for the 2′-deoxy and ara derivatives, total loss of measured toxicity (Tables I–III). In most instances, there was a concomitant decrease in antiviral potency so that the specificity index was not improved. In some instances, however, the specificity index did show a marked increase: from 6 to >40 for 2′-deoxytubercidin against VV (Table I) and from 4 to 50 or 150 for *xylo*-tubercidin against HSV-1 and HSV-2, respectively (Table I). The specificity index of tubercidin for polio-1 (in HeLa cells) was not altered upon transition to 3′-deoxytubercidin (Table I).

As with tubercidin, substitution of "2-deoxyribose", "3-deoxyribose", or arabinose for the ribofuranosyl moiety of toyocamycin and sangivamycin brought about substantial decreases in cytotoxicity. Sangivamycin had a rather high specificity index (50) for VV and VSV in PRK cells (Table I) and of the sangivamycin derivatives, *ara*-sangivamycin proved the most specific (specificity index 200 against VV in PRK cells). In fact, *ara*-sangivamycin was the most active antiviral agent of the three arabinosyl derivatives (Tables I–III).

Toyocamycin was the most cytotoxic of the three pyrrolo[2,3-*d*]pyrimidine ribonucleosides: minimum toxic concentration (MTC) 0.04 μg/mL in PRK and Vero cells (Tables I and III). This high cytotoxicity precluded any specific antiviral activity. Substitution of the ribofuranosyl moiety of toyocamycin by 2- or 3-"deoxyribose" or arabinose decreased the cytotoxicity, and some of the sugar-modified toyocamycins gained a marked increase in specificity index: up to 50 for 2′-deoxytoyocamycin against VSV in HeLa cells (Table II).

The pyrazolo[4,3-*d*]pyrimidine nucleoside formycin has previously been reported to exert some inhibitory activity against VV, HSV, and VSV in PRK cells.[5] However, as shown here, the specificity index of formycin is quite small: 5 at most (against parainfluenza-3 in Vero cells, Table III). The 2′-deoxy and 3′-deoxy derivatives of formycin were inactive in all of the antiviral assays (Tables I–III).

Thus, the antiviral data clearly indicate that the cytotoxicity, antiviral potency, and specificity of tubercidin, toyocamycin, sangivamycin, and formycin can be significantly altered by modifications in the sugar. For tubercidin, transformation of the ribose moiety to xylose results in a 25-fold decrease in cytotoxicity, whereas the inhibitory

Table III. Antiviral Activity and Cytotoxicity of Pyrrolo- and Pyrazolopyrimidine Nucleosides in Vero Cells

compound	MTC,[a] μg/mL	MIC_{50},[b] μg/mL				
		reo-1	parainfluenza-3	Sindbis	Coxsackie B-4	measles
tubercidin (1)	0.4	0.04	0.07	0.2	0.07	0.3
toyocamycin[c] (2)	0.04	≥0.04	≥0.04	>0.04	≥0.04	
sangivamycin[c] (3)	0.4	≥0.4	0.02	>0.4	0.02	
2′-deoxytubercidin (7)	>400	200	40	>400	40	>200
2′-deoxytoyocamycin (8)	1	>1	>1	>1	>1	0.5
2′-deoxysangivamycin (9)	10	>10	>10	>10	2	≥10
ara-tubercidin (10)	>400	>400	300	>400	>400	>400
ara-toyocamycin (11)	10	>10	≥10	>10	>10	≥10
ara-sangivamycin (12)	40	6	1	≥40	2	10
3′-deoxytubercidin (13)	100	10	20	≥100	60	≥100
3′-deoxytoyocamycin (14)	1	0.7	0.4	0.7	≥1	≥1
3′-deoxysangivamycin (15)	40	≥40	10	≥40	≥40	≥40
xylo-tubercidin (16)	40	≥40	≥40	≥40	≥40	≥40
formycin (4)	10	≥10	2	≥10	≥10	≥10
2′-deoxyformycin (5)	>400	>400	>400	>400	>400	≥400
3′-deoxyformycin (6)	>400	>400	>400	>400	>400	100
ribavirin	>400	75	50	65	70	30

[a-c] Footnotes as in Table I.

Table IV. Inhibitory Effects of Pyrrolo- and Pyrazolopyrimidine Nucleosides on the Growth and Metabolism of Murine Leukemia L1210 Cells

	MIC_{50},[a] μg/mL			
		incorporation into TCA insoluble material of		
compound	cell growth	[*methyl*-^{3}H]dThd	[5-^{3}H]Urd	[4,5-^{3}H]leucine
tubercidin (**1**)	0.040[b]	5.4	0.290	0.173
toyocamycin (**2**)	0.006[b]	38	0.071	0.039
sangivamycin (**3**)	0.019[b]	>100		0.014
2′-deoxytubercidin (**7**)	23	127	205	44
2′-deoxytoyocamycin (**8**)	0.109	10	0.340	0.265
2′-deoxysangivamycin (**9**)	1.21	16		
ara-tubercidin (**10**)	7.0	32	237	151
ara-toyocamycin (**11**)	2.0	14	10.3	4.80
ara-sangivamycin (**12**)	0.980	50	5.85	2.20
3′-deoxytubercidin (**13**)	0.38	90	3.20	2.08
3′-deoxytoyocamycin (**14**)	0.02	93	0.160	0.113
3′-deoxysangivamycin (**15**)	0.46	17		
xylo-tubercidin (**16**)	0.248	6.7		
formycin (**4**)	3.7	7.5	25.7	5.5
2′-deoxyformycin (**5**)	867	≥1000	>100	≥1000
3′-deoxyformycin (**6**)	>1000	>1000	>1000	≥1000

[a] Minimum inhibitory concentration required to reduce cell growth or [*methyl*-^{3}H]dThd, [5-^{3}H]Urd, and [4,5-^{3}H]leucine incorporation into TCA-insoluble material by 50%. [b] Data taken from ref 4. All data represent average values for at least three separate experiments.

effects on HSV-1 and HSV-2 remain unchanged. The MIC_{50} of *xylo*-tubercidin for HSV-1 (strain KOS) and HSV-2 (strain G) was 0.2 and 0.07 μg/mL, respectively, as shown in Table I and confirmed for several other strains of HSV-1 and HSV-2 (data not shown). The potency of *xylo*-tubercidin against HSV-2, combined with its relatively broad safety margin (specificity index: 150) makes *xylo*-tubercidin an attractive candidate for further studies on its efficacy against HSV-2 infections in animal models.

B. Antineoplastic Activity. Tubercidin, toyocamycin, sangivamycin, formycin, and their sugar-modified analogues were evaluated for their inhibitory effects on the growth and DNA, RNA, and protein synthesis of murine L1210 cells (Table IV). Their effect on cellular DNA synthesis was monitored by reduction of the incorporation of [*methyl*-^{3}H]dThd. Inhibition of cellular RNA synthesis and protein synthesis was monitored by reduction of the incorporation of [5-^{3}H]Urd and [4,5-^{3}H]Leu, respectively.

Within the tubercidin series, the L1210 cell growth inhibitory effects decreased in the order: tubercidin > *xylo*-tubercidin > 3′-deoxytubercidin > *ara*-tubercidin > 2′-deoxytubercidin. This order of antiproliferative activity agrees with that found by Cass et al.[9] except for inversion of the 3′-deoxy and ara compound activities.

Conversion of toyocamycin, the most potent inhibitor of tumor cell growth of the entire series (MIC_{50} = 0.006 μg/mL), to its 3′-deoxy- (3-fold less), 2′-deoxy- (18-fold less), or ara (300-fold less) derivatives resulted in significant reductions in cytotoxicity.

Substitution of the ribofuranosyl moiety of sangivamycin by "3-deoxyribose", arabinose, and "2-deoxyribose" decreased the cytotoxic effects as reflected by the increasing MIC_{50} concentrations from 0.019 μg/mL for sangivamycin to 0.46, 0.98, and 1.21 μg/mL, respectively. This ordering parallels that found by Maruyama et al.[10] for the deoxy derivatives relative to the parent antibiotic.

Formycin proved to be a rather weak inhibitor of L1210 cell proliferation (MIC_{50} = 3.7 μg/mL) in comparison with the pyrrolo[2,3-*d*]pyrimidine antibiotics (MIC_{50} = 0.6–4.0 × 10^{-2} μg/mL). Its conversion to 2′- or 3′-deoxyformycin resulted in annihilation of measured cytotoxicity in the L1210 system.

Rosowsky et al. have recently reported inhibition of the growth of S49 lymphoma cells by 2′-deoxyformycin. Interestingly, this activity was enhanced only slightly by inclusion of the adenosine deaminase inhibitor EHNA, whereas the activity of formycin was increased ~100-fold.[11] Substrate reactivities of 2′- and 3′-deoxyformycin with that enzyme were noted some time ago,[12] but no kinetic parameters were determined.

It is noteworthy that the MIC_{50} concentrations for inhibition of L1210 cell proliferation were lower than those required to suppress DNA synthesis with all the pyrrolo[2,3-*d*]pyrimidine compounds evaluated in this study. Lower concentrations give 50% inhibition of incorporation of leucine and/or uridine than were required for suppression of DNA synthesis with the compounds examined except *ara*-tubercidin (2′-deoxytubercidin is lower for leucine, but higher for uridine).

Our data clearly demonstrate that the in vitro antineoplastic effects of tubercidin, toyocamycin, sangivamycin, and formycin are markedly decreased by substitution of "2-deoxyribose", "3-deoxyribose", arabinose (or xylose) for the ribofuranosyl moiety. The weak inhibitory action of these compounds on the incorporation of thymidine into cellular DNA suggests that their cytotoxicity might result more directly from interference with RNA and/or protein synthesis rather than DNA synthesis.

Conclusions

A modified synthesis of 2′-deoxyformycin (23% overall from formycin) and 3′-deoxyformycin (32% overall) essentially doubles the yield of the 2′-deoxy isomer obtained in prior studies.[11-13] A four-stage sequence provides 3′-deoxytoyocamycin and/or 3′-deoxysangivamycin in ~80% yields from toyocamycin. Treatment of the 2′-deoxy-, 3′-deoxy-, arabino-, and xylo-modified compounds with sodium periodate and then sodium borohydride eliminates trace quantities of the toxic parent ribonucleoside antibiotics that otherwise can produce spurious biological effects.

Cytotoxicity was reduced in every case by modification of the sugar moiety of the antibiotics. Relative effects on the incorporation of [*methyl*-^{3}H]dThd, [5-^{3}H]Urd, and [4,5-^{3}H]Leu in murine L1210 leukemia cells suggested that the majority of these compounds affected RNA and protein synthesis to a greater extent than DNA synthesis.

Although the antiviral potency of most of the sugar-modified analogues was also diminished, certain compounds had increased selectivity indexes. The *xylo*-tubercidin analogue was significantly less cytotoxic, but retained potent antiherpes simplex type 1 and 2 activity.

Animal model studies with this xylo compound will be reported separately.

Experimental Section

Melting points were determined on a Reichert microstage block and are uncorrected. UV spectra were determined on a Hewlett-Packard 8450A instrument. ^{1}H NMR spectra were determined by the High-Field NMR Laboratory at the University of Alberta on Bruker WH-200 or WH-400 spectrometers with Me_2SO-d_6 as solvent and Me_4Si as internal standard. High-resolution EI mass spectra were determined by the Mass Spectrometry Laboratory at the University of Alberta with an AEI MS-50 instrument at 70 eV with direct sample introduction. Evaporations were conducted at <30 °C (bath temperature) with a Büchi rotary evaporator with a Dewar dry-ice condenser under water aspirator or mechanical oil pump vacuum. All solvents were distilled before use and all chemicals were reagent grade. The NH_3/MeOH was prepared by saturation of MeOH at 0 °C with NH_3 gas and stored at −18 °C. TLC was performed on Merck silica sheets (5735) with detection of products under a 2537-Å lamp and/or spraying with H_2SO_4 and charring. Column chromatography was performed on Merck silica gel (7734, 60–200 mesh) and medium-pressure chromatography on Merck Kieselgel 60H (7736) "wet-packed" at 150 psi in MeOH/$CHCl_3$ (5:95) and eluted at 30 psi. Elemental analyses were determined by the Microanalytical Laboratory at the University of Alberta and results agreed within ±0.4% of theory.

7-Amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrazolo[4,3-*d*]pyrimidine (2′-Deoxyformycin, 5) and 7-Amino-3-(3-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (3′-Deoxyformycin, 6). A 2.28-g (8 mmol) sample of formycin (4) monohydrate in 100 mL of CH_3CN was treated with 5.92 mL of α-acetoxyisobutyryl bromide and the mixture was stirred for 24 h at ambient temperature. TLC (MeOH/$CHCl_3$, 2:8) indicated disappearance of 4 and formation of an essentially homogeneous rapidly migrating product mixture. The solution was evaporated and the residue dissolved in $CHCl_3$. The organic phase was washed with saturated $NaHCO_3/H_2O$ and $NaCl/H_2O$, dried (Na_2SO_4), and evaporated. The residue was dissolved in a minimum volume of MeOH and treated with 100 mL of NH_3/MeOH for 2 h at ambient temperature. TLC (MeOH/$CHCl_3$, 15:85) indicated disappearance of the rapidly migrating mixture with formation of two pairs of slower migrating products. The solution was evaporated thoroughly and the residue dissolved in a minimum volume of $CHCl_3$ and applied to a column (4.5 × 50 cm) of silica gel. This "medium-pressure" column was developed with MeOH/$CHCl_3$ (5:95) at 30 psi. Four cleanly separated fractions were collected and solvents evaporated to give (a) 1.24 g (34%) of **18b**, (b) 0.62 g (17%) of **17b**, (c) 0.34 g (13%) of **18c**, and (d) 0.33 g (12%) of **17c**.

Fractions (b) and (d) were combined, dissolved in 150 mL of MeOH, 1.25 mL of Et_3N, and 312 mg of 5% Pd–C catalyst added, and the mixture was shaken for 48 h at ambient temperature under 10 psi of H_2. The mixture was filtered with a Celite pad, the filter cake washed with 3 × 200 mL of hot MeOH, and the combined filtrate was evaporated. The residue was stirred with 50 mL of NH_3/MeOH for 48 h at ambient temperature. The solution was evaporated and the residue treated with EtOH and evaporated several times. A dilute solution of HCl/EtOH was added and evaporated, and the crystal mass was recrystallized from absolute EtOH to give 0.55 g (81%) of **5**·HCl; mp 194–196 °C (lit. mp 200–202 °C,[12] 194–196 °C[13]); spectral data agreed with literature values.[12,13]

Combined fractions (a) and (c) were dissolved in 150 mL of MeOH and 75 mL of EtOAc, 1.5 mL of Et_3N and 463 mg of 5% Pd–C catalyst were added, and the mixture was hydrogenolyzed as described above. The residue obtained after deprotection with NH_3/MeOH was purified by silica column chromatography (MeOH/$CHCl_3$, 15:85) before conversion to the hydrochloride salt. This was recrystallized from EtOH to give 0.73 g (68%) of **6**·HCl; mp 204–207 °C (lit. mp 214–216 °C,[12] 207–209 °C[13]); spectral data agreed with literature values.[12,13]

4-Amino-5-cyano-7-(3-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3′-Deoxytoyocamycin, 14) and 4-Amino-5-carbamoyl-7-(3-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3′-Deoxysangivamycin, 15). A mixture of 583 mg (2 mmol) of toyocamycin (2), 1.23 mL of α-acetoxyisobutyryl bromide, and 25 mL of CH_3CN was stirred for 6.5 h at ambient temperature. The solution was evaporated and the residue dissolved in EtOAc. The organic phase was washed with saturated $NaHCO_3/H_2O$ and $NaCl/H_2O$, dried (Na_2SO_4), and evaporated. The resulting solid foam was dissolved in 12 mL of absolute MeOH, cooled to 0 °C, and treated with 1.67 mL (1.21 g, 12 mmol) of freshly distilled Et_3N. Stirring was continued for 24 h at ~0 °C. (Significant quantities of the 2′,3′-anhydro compound were formed during analogous partial deprotection reactions with NH_3/MeOH.) Evaporation of the solution gave a syrup that was dissolved in 80 mL of EtOH, treated with 8 mL of Et_3N and 500 mg of 5% Pd–C catalyst, and shaken for 48 h at ambient temperature under 10 psi of H_2. The mixture was filtered with a Celite pad, the filter cake washed with 2 × 250 mL of hot EtOH, and the combined filtrate evaporated. The residue was treated with 10 mL of NH_3/MeOH for 36 h at ambient temperature. Evaporation of this solution gave a residual mixture that was purified by silica column chromatography (MeOH/$CHCl_3$, 7:93).

The appropriately pooled earlier fractions were evaporated and the residue crystallized from H_2O to give 345 mg (63%) [experiment 2: 357 mg (65%)] of **14**: mp 160–162 °C; UV (H_2O) max 278, 230 nm (ε 13 900, 10 700), min 246, 221 nm (ε 4830, 9620); ^{1}H NMR δ 1.88 (ddd, $J_{3''-2'}$ = 2.5 Hz, $J_{3''-3'}$ = 13 Hz, $J_{3''-4'}$ = 6 Hz, 1, H3″), 2.19 ("septet", $J_{3'-2'}$ = 6 Hz, $J_{3'-4'}$ = 8.5 Hz, 1, H3′), 3.56 (m, $J_{5''-5'}$ = 12 Hz, 1, H5″), 3.75 (m, 1, H5′), 4.38 (m, 2, H2′, H4′), 5.14 ("t", J = 5.3 Hz, 1, OH5′), 5.70 (d, J = 4 Hz, 1, OH2′), 6.06 (d, $J_{1'-2'}$ = 1.5 Hz, 1, H1′), 6.87 (br s, 2, NH_2), 8.24 (s, 1, H2), 8.47 (s, 1, H6); ^{13}C NMR δ 156.91 (C4), 153.39 (C2), 149.49 (C7a), 131.77 (C6), 116.01 (CN), 101.21 (C4a), 90.90 (C1′), 82.35 (C5), 80.96 (C4′), 75.22 (C2′), 62.05 (C5′), 33.57 (C3′); MS, m/z 275.1018 (calcd for M^+, 275.1018). Anal. ($C_{12}H_{13}N_5O_3$) C, H, N.

Pooled later fractions were evaporated, and the residue was crystallized from H_2O to give 42 mg (7%) [experiment 2: 105 mg (18%)] of **15**: mp 279–280 °C (lit.[10] mp 280–281 °C); UV (H_2O) max 280, 231 nm (ε 13 900, 9670) min 256, 223 nm (ε 6940, 9190); ^{1}H NMR δ 2.01 (ddd, $J_{3''-2'}$ = 3.5 Hz, $J_{3''-3'}$ = 13.5 Hz, $J_{3''-4'}$ = 6.3 Hz, 1, H3″), 2.18 (ddd, $J_{3'-2'}$ = 6.5 Hz, $J_{3'-4'}$ = 9 Hz, 1, H3′), 3.65 (m, 2, H5′,5″), 4.34 (m, 1, H4′), 4.43 (m, 1, H2′), 4.96 (t, J = 6 Hz, 1, OH5′), 5.65 (d, J = 4 Hz, 1, OH2′), 6.09 (d, $J_{1'-2'}$ = 2 Hz, 1, H1′), 7.37 (s, 2, NH_2), 7.92 (s, 2, $CONH_2$), 8.06 (s, 1, H2), 8.10 (s, 1, H6); ^{13}C NMR δ 166.81 ($CONH_2$), 158.48 (C4), 153.26 (C2), 150.86 (C7a), 125.31 (C6), 111.24 (C5), 101.32 (C4a), 90.66 (C1′), 80.85 (C4′), 75.33 (C2′), 63.71 (C5′), 35.60 (C3′); MS, m/z 293.1129 (calcd for M^+, 293.1121). Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

An aqueous solution of 91 mg (0.31 mmol) of **14** hydrate was applied to a column (1 × 15 cm, ~10 mL) of Dowex 1X2(OH^-) resin packed in H_2O. The column was washed with ~500 mL of H_2O and allowed to stand overnight at ambient temperature. The column was washed with 200 mL of MeOH/H_2O (1:9) and the product was eluted with MeOH/H_2O (2:10). Evaporation of the eluate and crystallization of the residue from H_2O gave 82 mg (90%) of **15**. Analogous treatment of the crude reaction mixture of **14** and **15** gave a similar yield of crystallized **15**.

Biological Evaluations. For the methodology used to measure antiviral activity and cytotoxicity, see ref 22; for the procedures used to monitor L1210 cell growth and DNA, RNA, and protein synthesis, see ref 23.

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Registry No. 1, 69-33-0; 2, 606-58-6; 3, 18417-89-5; 4, 6742-12-7; 5, 40627-14-3; 5·HCl, 40627-15-4; 6, 40725-90-4; 6·HCl, 40627-13-2; 7, 60129-59-1; 8, 15676-19-4; 9, 83379-28-6; 10, 64526-34-7; 11, 90813-71-1; 12, 90813-74-4; 13, 40725-89-1; 14, 105582-76-1; 15, 83379-31-1; 16, 64526-29-0; 17a, 42867-63-0; 17b, 40627-37-0; 17c, 40627-38-1; 18a, 40627-32-5; 18b, 105661-43-6; 18c, 40627-36-9; 19a, 105582-77-2; 19b, 105582-78-3; α-acetoxyisobutyryl bromide, 40635-67-4.

Multisubstrate Inhibitors of Dopamine β-Hydroxylase. 2.[1] Structure–Activity Relationships at the Phenethylamine Binding Site

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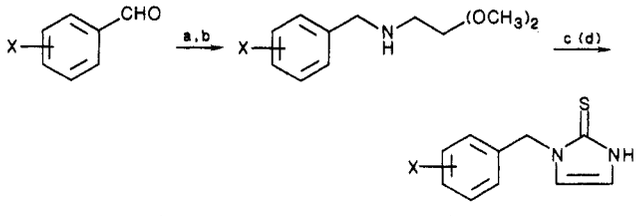
1-Aralkylimidazole-2-thiones have been shown to be potent multisubstrate inhibitors of dopamine β-hydroxylase (DBH; EC 1.14.17.1). In the present study, a series of 1-benzylimidazole-2-thiones was prepared to explore the effects of substitution in the benzyl ring on the inhibition of DBH. A detailed structure–activity relationship for in vitro activity was discovered and this was shown by a modified Hansch analysis to correlate ($r = 0.91$) with four key structural features of the benzyl ring: (1) the presence of a hydroxyl at the 4-position, (2) molar refractivity at the 3-, 4-, and 5-positions, (3) inductive effects of the substituents at the 3-, 4-, and 5-positions, and (4) π-electron density. The affinity (K_{is}) of eight substituted inhibitors for DBH was shown to correlate ($r = 0.75$) with the affinity (K_D) of comparably substituted tyramines for the ternary DBH–oxygen–tyramine complex. This correlate is used to support the hypothesis that binding of inhibitor to DBH occurs in a fashion that mimics the binding of tyramine substrates. The most potent inhibitors were selected for study in vivo in the spontaneously hypertensive rat model of hypertension. The changes in vascular dopamine and norepinephrine levels that resulted from oral administration of the inhibitors corresponded to the observed reduction in mean arterial blood pressure. A divergence between in vitro potency and in vivo efficacy upon oral dosing was noted and is suggested to result from an in vivo metabolic conjugation of the phenolic group of inhibitor.

Dopamine β-hydroxylase (DBH; EC 1.14.17.1), a copper-containing mixed-function oxidase that catalyzes the conversion of dopamine to norepinephrine, is an appealing target for the rational design of new agents of potential efficacy in the treatment of cardiovascular disorders.[2,3] We recently reported some 1-phenyl- and 1-phenyl-bridged imidazole-2-thiones to be potent inhibitors of DBH that appeared to act as multisubstrate mimics of the binding of oxygen and phenethylamine substrates to the reduced, catalytically active Cu^{1+} species of enzyme (Figure 1).[1] This study delineated a stringent set of structural requirements for maximal inhibitory activity, led to the optimization of chain length for the intersubstrate bridge between the portions of inhibitor that mimic oxygen and tyramine substrates, and identified 1-(4-hydroxybenzyl)- and 1-[(4-hydroxyphenyl)propyl]imidazole-2-thiones as optimal and equipotent DBH inhibitors.

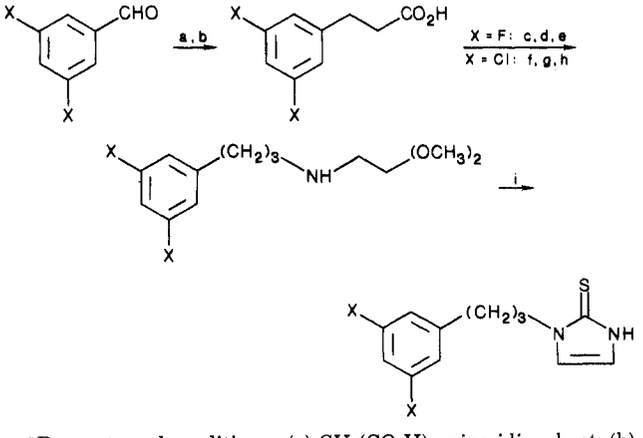
We report here the results of structure–activity relationship (SAR) studies on 1-benzylimidazole-2-thiones in which DBH inhibitory potency is compared to aryl substitution of the benzyl moiety, i.e., that portion of the inhibitor that binds the enzymatic phenethylamine site. The goals of this study were threefold. First, to generate SAR that would provide a basis for the design of multisubstrate inhibitors more potent than the parent compounds. Second, to identify a set of inhibitors of comparable in vitro potency but with differing lipophilicity and metabolic liability. It was anticipated that these would be useful in identifying the probable causes for the divergence between in vitro and in vivo potencies previously noted for several inhibitors.[3] Third, to compare the affinity of aryl-substituted inhibitors for DBH with the reported dissociation constants of identically substituted phen-

Scheme I[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$; (b) $NaBH_4$, EtOH; (c) H_2O, HCl, KSCN, EtOH, reflux; (d) BBr_3, CH_2Cl_2.

Scheme II[a]



[a] Reagents and conditions: (a) $CH_2(CO_2H)_2$, piperidine, heat; (b) 10% Pd/carbon, THF, 50 psig hydrogen; (c) $SOCl_2$; DMF, 60 °C; (d) $NH_2CH_2CH(OCH_3)_2$, CH_2Cl_2, 0 °C; (e) $LiAlH_4$, Et_2O, 22 °C; (f) BH_3·THF, 0 °C; (g) $(COCl)_2$, Me_2SO, CH_2Cl_2, NEt_3, −78 °C; (h) $NH_2CH_2CH(OCH_3)_2$, hexane, EtOH, $NaBH_4$; (i) H_2O, HCl, KSCN, EtOH, reflux.

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