oxazole and 10% Beckman BBS-3 solubilizer in toluene. Counting efficiency was 33%; control samples lacking the cofactor were found to have less than 5% of the respective sample counts. On the basis of specific activity, velocity is reported in the adjusted value of picomoles of ^3H_3O formed per minute in the assay.

Preincubation Studies. The enzyme (5×10^{-8} M) was preincubated at 30 °C in 50 μL of solution containing 5 mM 2-mercaptoethanol; 6 mM magnesium chloride; 0.24 mM EDTA; 12 mM Tris–acetate buffer, pH 6.8; and varying concentrations of inhibitor. After incubation for the indicated time period, the assay for the remaining active enzyme was started by the addition of 50 μL of a solution containing buffer and other components of the assay to give the same concentrations as noted in the enzyme assay. A high substrate concentration (40 μM) was used in these assays to afford reasonably high velocity and to competitively reduce any enzyme inactivation by the inhibitor during the assay. The assay was run for 30 s and treated as described in the enzyme assay section. Inactivation of the enzyme was measured by comparing the velocity at time zero to that at the indicated incubation times. Under the conditions of the assay, the uninhibited enzyme retained 95% of the initial activity after 20 min of incubation.

Antiviral and Antimetabolic Assays. The methodology for measuring the inhibition of virus-induced cytopathogenicity in primary rabbit kidney (PRK) cell cultures and the incorporation of 2′-deoxy[*methyl*-^{3}H]thymidine or 2′-deoxy[2-^{14}C]uridine into DNA of these cells has been described previously.[5,6]

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Quantitative Structure–Activity Correlations of Rifamycins as Inhibitors of Viral RNA-Directed DNA Polymerase and Mammalian α and β DNA Polymerases

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Twenty-two 3-substituted rifamycins were tested for inhibition of mammalian α and β DNA polymerase and viral RNA-dependent DNA polymerase ("reverse transcriptase"). Quantitative structure–activity relationships (QSAR) were formulated for the three systems. Inhibition is linearly dependent on the partition coefficient and is highly favored by the presence of bulky hydrazones or oximes. None of these agents proved to be a selective or specific inhibitor of reverse transcriptase. A correlation in terms of log P and $(\log P)^2$ was obtained from data on a more closely related set of analogues from a published study. For murine reverse transcriptase, $\log P_0 = 5.1$.

The rifamycins (Figure 1) represent a class of compounds obtained from chemical modification of some metabolic products produced by *Streptomyces mediterranei*.[2,3] Early studies showed that the rifamycins had good antimicrobial activity, which led to their clinical use as antibiotics for the treatment of tuberculosis and bacterial infections.[4] Their chief mechanism of action appears to be as inhibitors of bacterial RNA polymerase.[5] In addition, there have been reports that rifamycins are inhibitors of mammalian DNA polymerases and viral RNA-dependent DNA polymerase ("reverse transcriptase" or RT).[6] The finding of inhibitory effects on viral DNA polymerases suggested that systematic screening of several rifamycin analogues might produce a potent and specific inhibitor of the viral enzyme. Such an inhibitor would be of interest because it could potentially block the transformation of a cell by an RNA tumor virus.

In this paper, we present the results of our efforts to find a specific inhibitor of RNA-directed DNA polymerase by comparing the activities of several 3-substituted rifamycins (Table I) in inhibiting the viral enzyme as well as mammalian α and β DNA polymerases. In addition, we have attempted to test the hypothesis of whether increasing the size and the lipophilicity of the "tail" bound to rifamycin

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(2) P. Sensi, R. Ballotta, and A. Greco, *Farmaco, Ed. Sci.*, **16**, 165 (1961).
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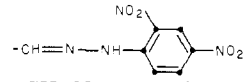
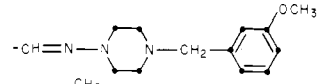
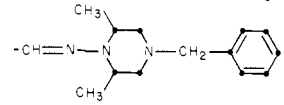
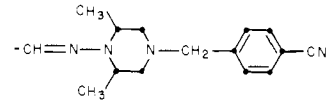
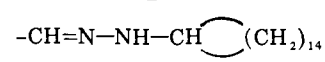
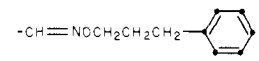
(4) S. Furesz, V. Arioli, and R. Pallanza, *Antimicrob. Agents Chemother.*, 770 (1965).
(5) (a) R. J. White, G. C. Lancini, and L. G. Silvestri, *J. Bacteriol.*, **97**, 761 (1960); (b) H. Bickel, F. Knusel, W. Kump, and L. Neipp, *Antimicrob. Agents Chemother.*, 352 (1966); (c) F. Kradulfer, L. Neipp, and W. Sackerman, *ibid.*, 359 (1966).
(6) (a) C. Gurgo, R. K. Ray, L. Thiry, and M. Green, *Nature (London), New Biol.*, **229**, 111 (1971); (b) M. Green, M. Rokutanda, K. Fujinaga, H. Rokutanda, C. Gurgo, R. K. Ray, and J. T. Parsons in "The Biology of Oncogenic Viruses", L. Silvestri, Ed., North-Holland, Amsterdam, 1971, pp 193–205; (c) R. C. Gallo, S. S. Yang, R. G. Smith, F. Herrera, R. C. Ting, S. N. Borrow, C. Davis, and S. Fujioka, *ibid.*, pp 210–220; (d) R. C. Gallo, S. S. Yang, and R. C. Ting, *Nature (London)*, **228**, 927 (1970).

Table I. Inhibition of DNA Polymerase α and β and Reverse Transcriptase

no.	R	concn, mol/L, yielding 50% inhibn ($\times 10^{-5}$) α polymerase	β polymerase	RT
1	H	76	110	37
2	-OH	23	37	1.3
3	-CHO	10.2	10.4	1.7
4	$-CH_2-N(C_2H_5)_2$	100	110	4.0
5	$-CH_2-c-N(CH_2CH_2)_2N$	300	420	145
6	$-CH=N-N[(CH_2)_2CH_3]_2$	6.5	4.5	1.35
7	$-CH=N-N[(CH_2)_4CH_3]_2$	4.3	4.8	0.44
8	$-CH=N-N[(CH_2)_7CH_3]_2$	2.4	0.74	0.32
9		4.5	5	0.54
10	$-CH=N-c-NC_4H_8$	24	24	1.7
11	$-CH=N-c-N(CH_2CH_2)_2N-CH_3$	34	15	4.2
12	$-CH=N-c-N(CH_2CH_2)_2N-NH_2$	16.5	1.6	2.5
13		14	12	1
14		3.8	2.1	1.25
15		4.7	2.2	1.6
16		3.1	2.2	0.66
17		2.4	1.9	0.33
18		2.5	2.2	0.65
19		1.15	1.5	0.085
20	$-CH=NO(CH_2)_7CH_3$	1.5	1.3	0.10
21	$-CH=NOCH(C_6H_5)_2$	2.4	1.0	0.25
22		3.4	0.66	0.56

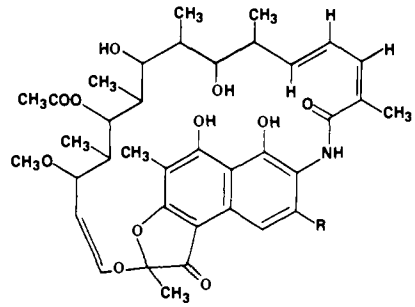


Figure 1. Basic structure of rifamycins. R is in the 3 position; R = H for rifamycin SV.

SV will lead to a more specific inhibitor of the viral enzyme, as was originally suggested by Tischler et al.[7]

Experimental Section

Biological. Nicked calf thymus DNA and poly[2′-*O*-methylcytidylic acid·d(pG)12–18] were purchased from Bethesda Research Laboratories, Inc., Rockville, MD.

DNA-dependent DNA polymerases α and β isolated from embryos of NIH-Swiss mice were purchased from Dr. V. S. Sethi, Anthem Research.[8] Simian sarcoma virus (SSV-1) derived from the tissue culture fluids of 71 API cell line was the source of viral RNA-dependent DNA polymerase. The SSV-1 RNA-dependent DNA polymerase was purified according to the procedure of Abrell and Gallo.[9]

The final reaction mixture (100 μL) for the DNA-dependent DNA polymerases α and β was composed of 20 μL of enzyme in 50% glycerol, 1 mM dithiothreitol, 0.5 M KCl, 50 mM Tris-HCl, pH 7.5; 5 μL of test compound at various concentrations in 100% Me_2SO; and 75 μL of cocktail. This cocktail was composed of 1 μL of 2 M KCl; 10 μL of 0.05 M $MgCl_2$; 2.5 μL of 0.2 M dithiothreitol; 12.5 μL of [^{3}H]TTP (1 mCi/μL); 2 μL each of 5 mM dATP, dGTP, and dCTP; 25 μL of activated calf thymus DNA (200 μg/mL in 5 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.7); and 18 μL of H_2O.

The final reaction mixture (50 μL) for the SSV-1 RNA-dependent DNA polymerase was composed of 20 μL of enzyme in 20% glycerol, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin. 50 mM Tris-HCl, pH 7.5; 5 μL of test compound at various concentrations in 100% Me_2SO; and 25 μL of cocktail. The cocktail was composed of 2.5 μL of 1 M Tris-HCl, pH 7.5; 0.75 μL of 4 M KCl; 2.5 μL of 10 mM $MnCl_2$; 1.25 μL of 0.2 M dithiothreitol; 0.25 μL of bovine serum albumin (1 mg/mL); 0.25 μL of 1 M NaF; 15 μL of [^{3}H]dGTP (1 mCi/mL); 2.5 μL of poly[2′-*O*-methylcytidylic acid·d(pG)12–18] in 10 mM Tris-HCl, pH 7.4, at 25 OD units/mL at 260 nm and 1 mM EDTA.

The order of addition of reactants into the test tubes was always as follows: test compound, enzyme, and cocktail. The drug and enzyme were thoroughly mixed and left on ice for 10 min before the addition of the cocktail. The final mixture was then mixed

(7) A. N. Tischler, F. M. Thompson, L. J. Libertini, and M. Calvin, *J. Med. Chem.*, 17, 948 (1974).
(8) V. S. Sethi and P. Okano, *Biochim. Biophys. Acta*, **454**, 230 (1976).
(9) J. W. Abrell and R. C. Gallo, *J. Virol.*, **12**, 431 (1973).

Table II. Physicochemical Parameters and Observed and Predicted Activities of Rifamycins

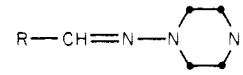
			log (1/C)[a]					
			α polymerase		β polymerase		RT	
no.	log P	I	obsd	pred (eq 1)	obsd	pred (eq 2)	obsd	pred (eq 3)
1	0.78	0.0	3.12	2.90	2.96	2.82	3.43	3.31
2	0.11	0.0	2.64	2.72	2.64	2.70	2.64	3.11
3	0.13	0.0	2.99	2.72	2.99	2.71	2.99	3.12
4	1.55	0.0	3.00	3.04	3.00	2.92	4.40	3.47
5	1.76	0.0	2.52	2.88	2.38	2.82	2.84	3.30
6	3.85	1.0	4.19	4.27	4.35	4.52	4.87	5.12
7	3.47	1.0	4.36	4.17	4.32	4.45	5.36	5.00
8	3.62	1.0	4.62	4.21	5.13	4.48	5.49	5.05
9	3.81	1.0	4.35	4.27	4.30	4.52	5.26	5.11
10	2.75	1.0	3.62	3.98	3.62	4.32	4.77	4.79
11	1.29	1.0	3.47	3.58	3.82	4.06	4.38	4.35
12[b]	0.02	1.0	3.78	3.78	4.80	4.18	4.60	4.56
13	3.34	1.0	3.85	4.13	3.92	4.42	5.00	4.96
14	4.28	1.0	4.42	4.38	4.68	4.60	4.90	5.25
15	3.71	1.0	4.33	4.23	4.66	4.49	4.80	5.08
16	4.05	1.0	4.51	4.32	4.65	4.55	6.60	5.18
17	4.75	1.0	4.62	4.51	4.72	4.68	5.48	5.39
18	6.05	1.0	4.60	4.86	4.66	4.91	5.19	5.78
19	6.78	1.0	4.94	5.05	4.82	5.04	6.07	6.00
20	5.20	1.0	4.94	4.80	4.89	4.87	6.00	5.70
21	4.96	1.0	4.62	4.60	5.00	4.74	5.60	5.48
22	4.83	1.0	4.46	4.53	5.18	4.69	5.25	5.41

[a] Log (1/C) = the logarithm of the reciprocal of the concentration which produces 50% inhibition. [b] Not included in eq 3.

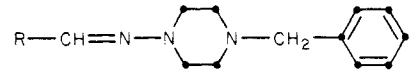
and incubated for 45 min at 37 °C. The amount of radioactivity in the products was determined according to the procedure of Weinstein, Bhardwaj, and Li.[10] Inhibition results were expressed as percent of control activity. The dose of the test compound that inhibited the enzyme activities by 50% was extrapolated from plots of percent inhibition vs. rifamycin derivative concentration. The dose–response curves were constructed as follows: five dose levels in triplicate between 10^{-3} and 10^{-7} M were first tested. Then additional tests were carried out at five dose levels in triplicate at 0.2 to 0.3 log intervals centered around the estimated concentrations that inhibited the respective enzyme activities by 50%. The mean values of the respective triplicate samples were used for calculations of enzyme activities. The mean of the experimental tubes for each dose minus the mean of background divided by the mean of control tubes minus the mean of background gave the percent inhibition at each dose level. All control experiments contained an equivalent amount of 100% Me_2SO. The kinetics of reaction for the control samples of the three respective enzymes were linear for at least 60 min under the described incubation conditions. The standard error of replicate analyses was less than 10%.

Chemical. Log P values for the rifamycins included in this study are given in Table II. Octanol/water partition coefficients were experimentally determined for compounds **1**, **5**–**8**, **11**, **16**, and **22**.[11] In addition, experimental values were determined for compounds **23**–**25** (R represents rifamycin SV substituted in the 3 position).

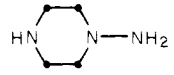
The method of calculation for the remaining compounds is given below. π and fragment values are from the compilation of Hansch and co-workers.[12] The fragment notations are those which appear in that reference. The numbers in brackets refer to the compound numbers in Tables I and II.



23
log P = 1.12



24
log P = 3.36



25
log P = −0.29

In calculating the partition coefficients of compounds with large aliphatic rings the following fragment factors were used:[13]

atoms in ring	f/CH_2
6	0.40
7–8	0.35
>9	0.30

(1) $\log P_{[2]} = \log P_{[1]} + \pi_{OH} = 0.78 - 0.67 = 0.11$

(2) $\log P_{[3]} = \log P_{[1]} + \pi_{CHO} = 0.78 - 0.65 = 0.13$

(3) $\pi_{CH_2N(Et)_2} = \log P_{N(CH_3)_3} - f_H + 2f_{CH_2}$[14] = −0.15 − 0.23 + 0.92 = 0.54

$\log P_{[4]} = \log P_{[1]} + \pi_{CH_2N(Et)_2} = 0.78 + 0.54 = 1.32$

(4) $\log P_{2,4\text{-dinitroaniline}} = 1.55$

This value was estimated from log P values for 2- and 4-nitroaniline.[15]

$\log P_{[9]} = \log P_{[23]} - \log P_{piperazine} + \log P_{2,4\text{-dinitroaniline}} = 1.12 + 1.17 + 1.55 = 3.84$

(5) $\log P_{[10]} = \log P_{[23]} - \log P_{piperazine} + \log P_{pyrrolidine} = 1.12 + 1.17 + 0.46 = 2.75$

(6) $\log P_{[12]} = \log P_{[23[} - \log P_{piperazine} + \log P_{[25[} = 1.12 + 1.17 - 0.29 = 2.00$

(7) $\log P_{[13]} = \log P_{[24]} + \pi_{CH_3O} = 3.36 - 0.02 = 3.34$

(8) $\log P_{[14]} = \log P_{[24]} + 2f_{CH_3} - 2f_H + 2F_{bYN} = 3.36 + 1.78 - 0.46 - 0.40 = 4.28$

(9) $\log P_{[15]} = \log P_{[14]} + \pi_{CN} = 4.28 - 0.57 = 3.71$

(10) $\log P_{[17]} = \log P_{[16]} + 2f_{CH_2} = 4.05 + 0.70 = 4.75$

(11) $\log P_{[18[} = \log P_{[16[} + 5f_{CH_2} = 4.05 + 2.00 = 6.05$

(12) $\log P_{NH_2CH\frown(CH_2)_{14}} = f_{NH_2} + f_{CH} + 14f_{(ring\ CH_2)} = -1.54 + 0.43 + (14 \times 0.40) = 4.49$

$\log P_{[19]} = \log P_{[23]} - \log P_{piperazine} + \log P_{NH_2CH\frown(CH_2)_{14}} = 1.12 + 1.17 + 4.49 = 6.78$

(13) $\log P_{[20]} = \log P_{[22[} - \log P_{C_6H_6} + \log P_{pentane} = 4.83 - 2.13 + 3.11 = 5.81$

(14) $\log P_{[21[} = \log P_{[22]} - f_{C_6H_5} + 2f_{CH_2} - f_H + 2f_{C_6H_5} + F_b + F_{GBr} = 4.83 - 1.90 - 1.32 - 0.23 + 3.80 + 0.12 - 0.22 = 5.08$

(10) B. I. Weinstein, N. Bhardwaj, and H. C. Li, *Anal. Biochem.*, **68**, 62 (1975).
(11) Partition coefficients for compounds 1 and 11 were determined at Midwest Research Institute, Contract N01-CM-33722. All others were measured at Pomona College, Contract N01-CM-67062.
(12) C. Hansch and A. J. Leo in "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley-Interscience, New York, 1979.
(13) A. J. Leo, personal communication.
(14) The fragment value of a methylene group attached to a tetrahedral nitrogen was reduced to 0.46.
(15) The partition coefficient of 2-nitroaniline is 1.83. In the 2,4-dinitro compound, the maximum steric and electronic effect was assumed to be that exerted by the 2-nitro substituent. With 1.83 as the base value, the 4-nitro group was given its usual aromatic π value (−0.28).

Table III. Development of Equations 1-3

intercept	log P	I	r	s	$F_{1,x}$[a]
		a. equation 1			
2.81	0.36		0.885	0.259	153.29
2.69	0.27	0.55	0.926	0.213	10.56
		b. equation 2			
2.93	0.37		0.842	0.476	48.82
2.68	0.18	1.14	0.918	0.360	15.35
		c. equation 3			
3.26	0.45		0.902	0.438	82.61
3.08	0.30	0.89	0.936	0.366	9.26

[a] $F_{1,18;\alpha=0.001} = 15.38$; $F_{1,19,\alpha=0.01} = 8.18$; $F_{1,20,\alpha=0.005} = 9.94$; $F_{1,20,\alpha=0.001} = 14.82$; $F_{1,21,\alpha=0.001} = 14.59$.

Results

The rifamycins included in this study fall into two general classes. The first is comprised of rifamycin SV derivatives in which the functional group is attached directly to the 3 position of the ansa ring (**1–5**). The second class of congeners are hydrazones and oximes of rifaldehyde (**3**). The biological data (Table I) indicate that the more active derivatives belong to this second class of compounds. To account for this difference in activity, an indicator variable I was employed. I assumes the value 1 when the derivative is an oxime or hydrazone of rifaldehyde and 0 for other congeners. Equations 1–3 were derived from the data in Tables I and II.

inhibition of α polymerase

$$\log (1/C) = 2.69(\pm 0.23) + 0.27(\pm 0.08)\log P + 0.55(\pm 0.35)I \quad (1)$$

$$n = 22;\ r = 0.962;\ s = 0.213$$

inhibition of β polymerase

$$\log (1/C) = 2.68(\pm 0.35) + 0.18(\pm 0.13)\log P + 1.14(\pm 0.60)I \quad (2)$$

$$n = 22;\ r = 0.918;\ s = 0.360$$

inhibition of reverse transcriptase

$$\log (1/C) = 3.08(\pm 0.35) + 0.30(\pm 0.13)\log P + 0.89(\pm 0.61)I \quad (3)$$

$$n = 21;\ r = 0.936;\ s = 0.366$$

$$\log (1/C) = 3.08(\pm 0.46) + 0.30(\pm 0.18)\log P + 0.98(\pm 0.79)I \quad (3a)$$

$$n = 22;\ r = 0.901;\ s = 0.477$$

Table III shows the development of eq 1–3. The use of log P alone as the independent variable produced a statistically significant equation. The addition of a term in $(\log P)^2$ resulted in minimal improvement and was shown to be unjustified by the F test. In each instance the use of the indicator variable I significantly improved the correlation.

Equations 1 and 2 contain all of the compounds in the study. Compound **12** was omitted in the development of eq 3. This omission might be reasonably attributed to unreliable test data resulting from the extremely poor solubility of **12**.

An attempt was made to explore the various components of I by seeing whether a correlation would result with the use of electronic and steric parameters in place of I. The electronic parameters σ_m and $\mathcal{F}$ and the molar refractivity (MR) were calculated for the series. The electronic parameters either alone or in combination with log P produced no improvement. However, the molar refractivity, scaled by 0.1, together with log P yielded equations for the inhibition of α and β polymerase and RT which were similar to eq 1–3.

Table IV. Squared Correlation Matrix for Equation 1

	log P	I	MR	σ_m	$\mathcal{F}$
log P	1.00	0.59	0.63	0.01	0.23
I		1.00	0.53	0.01	0.05
MR			1.00	0.06	0.24
σ_m				1.00	0.16
$\mathcal{F}$					1.00

Equation 4 is typical of the equations which were generated using MR. While eq 4 has an impressive correla-

inhibition of α polymerase

$$\log (1/C) = 2.74(\pm 0.25) + 0.31(\pm 0.11)\log P + 0.05(\pm 0.08)\mathrm{MR} \quad (4)$$

$$n = 22;\ r = 0.945;\ s = 0.255$$

tion coefficient (r) and standard deviation (s), the confidence interval on the MR term renders the coefficient of that term not substantially different from zero. In addition the F statistic ($F_{1,20} = 1.62$) does not justify the addition of the MR term. The principal value in examining these equations lay in the insight which they provided into the character of I. Equation 4 suggests that I is not merely a steric parameter, since MR substituted for I produces less satisfactory results. Further insight into the nature of I can be gained by examining the squared correlation matrix for eq 1 (Table IV). (Substantially identical coefficients were generated for eq 2 and 3.) Table IV shows the overlap between MR and log P (arccos $0.63 = 50.9^0$).[16] MR and those electronic parameters which measure inductive effects, σ_m and $\mathcal{F}$, are virtually orthogonal. I, therefore, has a fair amount of steric but no electronic character. It is conceivable that the hydrazones and oximes undergo hydrolysis in situ and that I in some measure reflects this.

Equations 1–3 are linear in log P and I and indicate that for these particular rifamycins increased inhibition of the enzymes lies in the direction of increased lipophilicity. Among the 3-substituted rifamycins, oximes and hydrazones of rifaldehyde are the more active members. However, the presence of these substituents in itself only adds about 1 log unit of activity ($I = 1$). The small slope associated with log P shows that large changes in lipophilicity are needed to affect the activity by an appreciable amount. Adding the hydrazone function and increasing the lipophilicity of the parent (**1**) by a factor of 10^5 in the case of **18** increase the activity by a factor of only 30 times for α polymerase and 50–60 times for β polymerase and RT. The highly lipophilic character of the most active members of this series may not be unusual for compounds which are involved in a one-step partitioning process.[17]

Tischler and co-workers examined the activities of a more closely related set of rifaldehyde hydrazones against murine RNA-directed DNA polymerase.[7] Some of these same compounds appear in the present study. Tischler used R_m values obtained from reverse-phase thin-layer chromatography as a measure of lipophilicity. For comparison, it was deemed worthwhile to attempt a correlation of these data. Table V gives the biological data and physicochemical constants for the compounds in Tischler's study.

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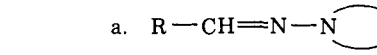
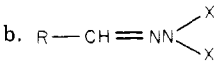
(17) (a) W. Schöpp and H. Aurich, *Acta Biol. Med. Ger.*, **31**, 19 (1973); (b) P. Desnuelle and P. Savary, *J. Lipid Res.*, **4**, 369 (1963).

Table V. Physicochemical and RT Inhibition Data for Rifamycins of Equations 5 and 6

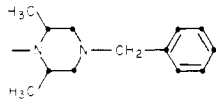
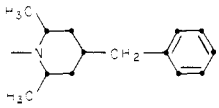
a. 

				log K_i		
no.	n	log P	R_m	obsd	pred (eq 5)	pred (eq 6)
16	10	4.05	1.20	0.69	0.65	0.67
26	5	2.35	0.56	1.76	1.82	1.64
27	6	2.75	0.68	1.51	1.45	1.41
28	7	3.10	0.80	1.23	1.17	1.20
29	8	3.45	0.92	0.95	0.94	1.01
30	12	4.65	1.49	0.48	0.48	0.46
31	15	5.55	2.06	0.30	0.47	0.44

b. 

				log K_i		
no.	X	log P	R_m	obsd	pred (eq 5)	pred (eq 6)
32	n-butyl	3.66	1.11	0.94	0.82	0.77
33	n-pentyl	3.47	1.43	0.61	0.93	0.49
34[a]	n-hexyl	3.51	1.80	0.40	0.91	0.38
35	n-octyl	3.60	2.61	0.97	0.86	0.94

c. DMB[b] analogues
R—CH=N—X

				log K_i		
no.	X	log P	R_m	obsd	pred (eq 5)	pred (eq 6)
14		4.28	0.85	0.63	0.57	1.12
36		6.30	1.15	0.77	0.68	0.72

[a] Not included in eq 5. [b] DMB = dimethylbenzylrifampicin.

Table VI. Development of Equations 5 and 6

a. equation 5

intercept	log P	$(\log P)^2$	r	s	$F_{1,x}$[a]
2.01	−0.28		0.752	0.293	13.05
5.12	−1.82	0.18	0.953	0.142	33.70

b. equation 6

intercept	R_m	R_m^2	r	s	$F_{1,x}$[b]
1.41	−0.42		0.585	0.364	5.74
3.04	−2.97	0.83	0.921	0.183	33.38

[a] $F_{1,11,\alpha=0.001} = 12.97$; $F_{1,10,\alpha=0.001} = 21.04$.
[b] $F_{1,12,\alpha=0.001} = 3.18$; $F_{1,11,\alpha=0.001} = 19.69$.

The R_m values in Table V are those reported by Tischler. The octanol/water partition coefficients of the cyclic hydrazones were calculated from the measured value for **16**.

Partition coefficients for the dialkylhydrazones were derived from measured values for the dipropyl-, dibutyl-, and dioctylhydrazones (**6**–**8**). The measured log P values for these three compounds were anomalous in that the expected increase in log P with increasing chain length does not occur. In fact, the log P values for the dipropyl-, dipentyl-, and dioctylhydrazones are remarkably close, possibly due to the folding of the side chain over the macrolide ring. For this reason, the log P of the dibutylhydrazone was arbitrarily assigned a value approximating the average of the values for the dipropyl and dipentyl derivatives (3.66). Similarly the dihexylhydrazone was assigned a log P close to the average of the values of the dipentyl- and dioctylhydrazones (3.51). The partition coefficient of **36** was calculated as follows:

$$\log P_{[36]} = \log P_{[14]} - \log P_{\text{piperazine}} + \log P_{\text{piperidine}} = 4.28 + 2.02 = 6.30$$

Equations 5 and 6 were derived from the data in Table V.

$$\log K_i = 5.12(\pm 1.26) - 1.82(\pm 0.61)\log P + 0.18(\pm 0.07)(\log P)^2 \quad (5)$$

$$n = 12;\ r = 0.953;\ s = 0.142$$

$$\log P_0 = 5.13(4.82 - 5.72)$$

$$\log K_i = 3.04(\pm 0.69) - 2.97(\pm 1.00)R_m + 0.83(\pm 0.32)R_m^2 \quad (6)$$

$$n = 13;\ r = 0.921;\ s = 0.183$$

$$R_m^0 = 1.79(1.66 - 2.01)$$

Table VI gives the development of eq 5 and 6. Only one compound (**34**) was omitted from eq 5. This omission could reasonably be attributed to a large deviation from the calculated log P for this dialkylhydrazone. As pointed out above, these dihydrazones tended to show anomalous behavior in those partition coefficients which were experimentally determined. Equation 6 contains all of the compounds. These equations express the same relationship and differ only in the lipophilic parameters. Equations 5 and 6 are parabolic in log P and R_m, respectively, and permit an estimate of the ideal lipophilicity. For inhibition of murine RNA-instructed DNA polymerase, log P_0 is close to 5.

Discussion

Our results show that among a series of 22 3-substituted rifamycins which were selected to cover a wide range of molecular sizes and lipophilicity as determined by log P values, none could be termed a "selective and specific" inhibitor of reverse transcriptase (RT). Every compound inhibited the viral enzyme as well as the mammalian α and β DNA polymerases. The most potent inhibitors of RT were also the most potent inhibitors of one or both mammalian DNA polymerases. In general, RT appeared to be the most sensitive to inhibition, followed by β and then α polymerase. The extrapolated concentrations required to inhibit 50% of the activity of α and β DNA polymerases were generally comparable to those reported by DiCloccio and Srivastava for compounds **6**, **9** and **14**.[18] The concentrations required to inhibit 50% of the RT activity, however, were approximately fivefold lower than those reported by them. This difference may reflect differences in our assay conditions, the major differences being the type of template used and the order of addition of assay mixtures. According to the review published by Gurgo,[19] the extent of inhibition of RT by rifamycins is not dependent on the type of template used[20-22] but is dependent on the order of addition of assay mixture components.[23] The elongation of chains initiated before drug addition is

(18) R. A. DiCloccio and B. I. S. Srivastava, *J. Natl. Cancer Inst.*, **61**, 1187 (1978).
(19) C. Gurgo, *Pharmacol. Ther., Part A*, **2**, 139 (1977).
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(21) C. Gurgo, D. P. Grandgenett, and M. Green in "Tumor Virus–Host Cell Interactions", A. Kolker, Ed., NATO Advanced Study Institute, Monte Carlo, Plenum Press, New York, 1975, pp 273–291.
(22) C. Gurgo and D. P. Grandgenett, *Biochemistry*, **16**, 786 (1977).
(23) A. M. Wu and R. C. Gallo, *Biochim. Biophys. Acta*, **340**, 419 (1974).

continuous, and the elongation of primer molecules added after the drug does not occur.[22] The order of addition described in the DiCloccio and Srivastava paper[18] was template, drug, substrates, and enzyme. For this experimental design there is a potential for the formation of a stable enzyme–substrate–template complex before the inhibitory action of rifamycin becomes fully active. In our experiments the enzymes are always mixed with the drug and left in ice for 10 min before the addition of substrates and templates. There is no possibility for the formation of a stable enzyme–substrate–template complex and the synthesis or elongation of nascent chains. This small difference in experimental design may account for the observed fivefold difference in the concentration values required to inhibit 50% of the RT activity.

The most potent inhibitors were the hydrazones and oximes of rifaldehyde (**6-22**). Some of these compounds inhibited the RT activity by 50% at concentrations below 0.3×10^{-5} M. The compound showing the greatest selectivity was **4**, the (diethylamino)methyl derivative. This analogue had a 50% RT inhibition concentration of 4.0×10^{-5} and a ratio of inhibition as compared to α and β polymerase of 25 and 25.5, respectively.

Although the 3-substituted rifamycins appear to be the most active inhibitors of RT which have been reported to date, their potency still does not compare favorably with known inhibitors of other enzymes, such as methotrexate for dihydrofolate reductase ($K_i = 2.5 \times 10^{-10}$)[24] or deoxycoformycin for adenosine deaminase ($K_i = 2 \times 10^{-11}$).[25] In addition, the inhibition of RNA polymerase by rifampicin ($K_i = 1.2 \times 10^{-9}$ M) is much stronger and correlates well with the sensitivity of microorganisms to the cytotoxic effects of that agent.[26]

All attempts to correlate the size and the lipophilicity of the substitution at position 3 with the potency of inhibition of RT tended to support the earlier hypothesis of Tischler et al.[7] that increases in both factors increase the degree of inhibition observed; however, no increase in selectivity was noted. The data were analyzed using several factors, but the best correlations were obtained (r = 0.962 for α polymerase, 0.918 for β polymerase, and 0.936 for RT) when an indicator variable I was employed. The use of electronic and steric parameters in place of I failed to improve the correlation; however, a molar refractivity parameter yielded similar equations for the three enzyme systems which were, nevertheless, unacceptable on the basis of F statistics. It is curious that the best correlations obtained were linear rather than parabolic in nature and suggest that the ideal log P for inhibition of these enzymes has not yet been reached. By contrast, a similar analysis of data obtained by Tischler and co-workers does give a parabolic curve and thereby permits an estimate of the ideal lipophilicity (log P_0 = 5.13). Some differences in experimental techniques may have accounted for these apparent discrepancies. First, Tischler et al. determined enzyme inhibition constants against partially purified murine RNA-directed polymerase rather than against the purified simian sarcoma virus as was used in the present study. Second, Tischler used R_m values obtained from reverse-phase thin-layer chromatography as a measure of lipophilicity rather than the measured or calculated octanol/water partition coefficients used in the present study.

Another important conclusion from our study is that the log P of the most active members of this series of congeners may be well beyond the range of practical clinical usage. A previous study of rifamycin β-amides acting against four bacterial systems showed a similar tendency for the more active materials to be the most hydrophobic; e.g., for the inhibition of *M. aureus* and *S. faecalis*, log P_0 was found to be about 4.[27] The highly lipophilic nature of the active materials causes extreme difficulties in formulating acceptable products for administration to patients. Hansch has tabulated the relative hydrophobicities (log P's) of most of the antitumor drugs of established clinical value.[12] The majority of these agents have log P values in the −2.00 to +2.00 range. Only one drug, vinblastine, has a partition coefficient much above 3 (log P = 3.72). Drugs with higher log P values would have a tendency to bind indiscriminately to a variety of macromolecules in the cell, particularly lipids and proteins. As a consequence, the ability of these compounds to reach the target site is affected. The high degree of lipophilicity required for maximal inhibition of viral RT and the nonpreferential and nonspecific nature of action render the present family of available rifamycins ineffective against neoplastic diseases and processes of transformation in vivo. Those rifamycins tested against murine in vivo cancers have shown no activity.[28]

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(28) Drug Evaluation Branch, National Cancer Institute.