a comparison of delocalization energy differences among polycyclic hydrocarbon diol epoxides to account for relative carcinogenicities; however, their calculations cannot take into account the presence or relative configuration of the hydroxyl groups in the diol epoxides. In light of the success of the present calculations and recent experimental evidence for marked differences in the relative mutagenicity[22,23] and carcinogenicity[24,25] of such isomeric diol epoxides, a more elaborate calculational study—both of the monocyclic and polycyclic derivatives—is in progress.

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Synthesis and Quantitative Structure–Activity Relationships of Some Antibacterial 3-Formylrifamycin SV *N*-(4-Substituted phenyl)piperazinoacethydrazones

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A series of 14 3-formylrifamycin SV *N*-(4-substituted phenyl)piperazinoacethydrazones has been synthesized and evaluated for their antimicrobial activity. The compounds were found active against *Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium phlei*, and *Mycobacterium tuberculosis* but not as active as rifampin. The compounds also exhibited significant activity against *Clostridium perfringens* and in this bacterial system some were more active than rifampin. The QSAR showed that the activity against *B. subtilis* depended only on lipophilicity, and the regression equation was linear. A parabolic relationship between the antibacterial activity and lipophilicity of the compounds was found in *Staph. aureus*. Additionally, the activity was dependent upon the electronic and steric effects of the phenyl substituents. The sensitivity of *M. phlei* to the compounds was found to correlate well with a linear combination of hydrophobic, electronic, and steric parameters. No statistically significant correlation was possible between the physicochemical parameters studied and the activity of the compounds against *C. perfringens* and *M. tuberculosis*.

5-Nitro-2-furaldehyde *N*-(4-nitrophenyl)piperazinoacethydrazone (**1**) was reported in 1971 to be active against *Mycobacterium tuberculosis*.[1] Since nitrofurans, in general, do not have antitubercular properties, the activity

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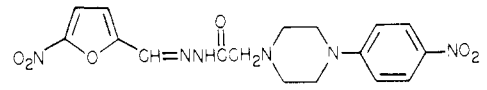

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may be attributed to the *N*-(4-nitrophenyl)piperazinoacethydrazone side chain, possibly by favorably altering the lipophilicity of the molecule. In the rifamycin antibiotic family, hydrazide–hydrazone derivatives of 3-formylrifamycin SV have been found to show a broad antibacterial spectrum.[2] This report deals with the results of antimicrobial evaluations of 14 previously unreported 3-formylrifamycin SV *N*-(4-substituted phenyl)piperazinoacethydrazones and the quantitative structure–activity relationships (QSAR) from the data.

Method. In order to ascertain that the physicochemical parameters of the compounds be noncollinear, cluster analysis as proposed by Hansch et al.[3] was utilized in this study for guiding the choice of aromatic substituents of the 3-formylrifamycin SV derivatives. Since it was initially intended to correlate the antibacterial activity with only hydrophobic (π and π^2) and electronic ($\mathcal{F}$ and $\mathcal{R}$) parameters, the 10 level of set 2 of Hansch's clusters was selected and from this 14 analogues resulted. Because of synthetic difficulties no substituents were chosen from clusters 9 and 10. Later during the course of regression analysis, MR and σ were added to the independent variable list. Table I is a squared correlation matrix of the phenyl substituents and their π, π^2, σ, $\mathcal{F}$, $\mathcal{R}$, and MR variables. The physicochemical constants of the aromatic substituents were taken from a literature compilation.[4]

Synthesis. The hydrazone derivatives, with the exception of the acetamido analogue **5n**, were prepared in a four-step reaction sequence, starting from the formation of the *N*-(4-substituted phenyl)piperazines (Table II). Treatment of the piperazines with ethyl chloroacetate afforded the ethyl *N*-(4-substituted phenyl)piperazinoacetates (Table III), which were allowed to react with hydrazine hydrate to form the *N*-(4-substituted phenyl)piperazinoacethydrazides (Table IV). The piperazinoacethydrazides condensed readily with 3-formylrifamycin SV yielding the final 3-formylrifamycin SV *N*-(4-substituted phenyl)piperazinoacethydrazones (Table V). Compound **5n** was similarly synthesized from *N*-(4-nitrophenyl)piperazine (**2h**). 3-Formylrifamycin SV was kindly supplied by Professor P. Sensi of Gruppo Lepetit spa, Milan, Italy.

Those *N*-(4-substituted phenyl)piperazines with electron-withdrawing substituents were obtained by the fusion of anhydrous piperazine in a fivefold excess and the corresponding substituted halobenzenes according to the procedure of Bent et al.[5] (method A), whereas those with electron-donating substituents were obtained by a ring-closure method, which involved the reaction of bis(2-chloroethyl)amine hydrochloride and the appropriate substituted anilines in the presence of a 1.5 mol excess of sodium carbonate (method B).

Antimicrobial Evaluations. The hydrazones were initially screened for in vitro antimicrobial activities at 50 μg/mL. Only those microorganisms that showed susceptibility at this concentration were further subjected to the MIC determinations. The antibacterial activity of the hydrazones is listed in Table VI, corresponding data for rifampin being included for comparison.

The hydrazones were inactive against the Gram-negative bacteria and fungi but were active against the Gram-positive bacteria, with the exception of *Streptococcus faecalis*, and the acid-fast bacteria tested. The antibacterial spectrum of the hydrazones is thus in line with those of the rifamycin antibiotic family. Only in the *Clostridium perfringens* system were some of the hydrazones as active as rifampin. None of the hydrazones showed any effect on the replication of vaccinia virus in mouse L-929 cells. However, varying degrees of toxicity to the L-929 cells were observed for the series.

Table I. Squared Correlation Coefficient Matrix of Physicochemical Parameters

	π	π^2	σ	$\mathcal{F}$	$\mathcal{R}$	MR
π	1.00					
π^2	0.62	1.00				
σ	0.15	0.12	1.00			
$\mathcal{F}$	0.19	0.18	0.64	1.00		
$\mathcal{R}$	0.02	0.03	0.55	0.03	1.00	
MR	0.35	0.85	0.11	0.04	0.10	1.00

Quantitative Structure–Activity Relationships. Regression equations were generated in the *Bacillus subtilis*, *Staphylococcus aureus*, *C. perfringens*, *Mycobacterium phlei*, and *M. tuberculosis* bacterial systems for all combinations of π, π^2, $\mathcal{F}$, $\mathcal{R}$, MR, and σ except those of σ, $\mathcal{F}$, and $\mathcal{R}$. Only those equations that are statistically significant at the 95% level or better are listed in Table VII. In these equations, n represents the number of data points upon which the equation is based, s the standard deviation, r the correlation coefficient, and S the significance level. The figures in parentheses are the 95% confidence limits. No significant and adequate quantitative correlation could be found in the bacterial systems of *C. perfringens* and *M. tuberculosis*.

Of the three equations (eq 1–3) generated for the activity of the compounds against *B. subtilis*, eq 3 had the highest correlation coefficient. However, the t test indicated that the σ and MR terms in eq 3 were significant only at the 93 and 91% levels, respectively. It would appear then that the addition of electronic and steric parameters to eq 2 was unjustified on statistical grounds and that the QSAR for the *B. subtilis* test system was adequately expressed by eq 2.

In 1975 Quinn and co-workers[6] reported eq 4 and 5 for

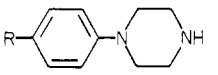
$$\log 1/C = 0.27(\pm 0.08)\log P - 0.62(\pm 0.17)\sigma^* - 0.15(\pm 0.10)E_s + 5.74(\pm 0.14) \quad (4)$$
$$n = 39;\ s = 0.24;\ r = 0.92$$

$$\log 1/C = -0.03(\pm 0.04)(\log P)^2 + 0.41(\pm 0.16)\log P + 0.76(\pm 0.20)D + 5.04(\pm 0.19) \quad (5)$$
$$n = 41;\ s = 0.27;\ r = 0.91$$

the correlation of some rifamycin B amides and their activity against *B. subtilis*. Thse workers suggested that eq 5, which was parabolic rather than linear in log P, was the better equation. However, upon closer examination of the 95% confidence interval of the regression coefficient for the $(\log P)^2$ term in eq 5, it can be argued that this coefficient is not significantly different from zero at the 95% level. Therefore, on a statistical basis, the better correlation equation is probably the linear eq 4. Equation 2 seemed to support this conclusion, although this equation was derived from a different type of rifamycin derivative.

Regression analysis provided four statistically significant equations (eq 6–9) in the *Staph. aureus* system. The hydrophobic parameter, π, was considered to be a better single parameter because eq 7 explained more data variance than eq 6. Both quadratic equations (eq 8 and 9) showed that the antibacterial activity of the hydrazones was a function of hydrophobic, electronic, and steric effects of the phenyl substituents and that a parabolic relationship existed between the activity and lipophilicity. The major difference between the two equations was that in eq 8 σ

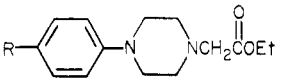
Table II. *N*-(4-Substituted phenyl)piperazines[a]



compd	R	method[b]	reflux time, h	yield, %	bp (mm) or mp, °C	formula	analyses
2b	Me	B	28	47	100-103 (0.3)[c]	$C_{11}H_{16}N_2$	
2c	Et	B	12	32	130-134.5 (1.3)	$C_{12}H_{18}N_2$	C, H, N
2d	*i*-Pr	B	51	87	167-168[d]	$C_{13}H_{20}N_2 \cdot 2C_6H_3N_3O_7$	C, H, N
2e	*t*-Bu	B	44	15	147-149 (1.2)[e]	$C_{14}H_{22}N_2$	
2g	OPh	B	45	79	113-114.5[d]	$C_{16}H_{18}N_2O$	H, N; C[f]
2h	NO_2	A	16	64	129-130[g]	$C_{10}H_{13}N_3O_2$	
2i	CF_3	A	45	81	86-88[h]	$C_{11}H_{13}F_3N_2$	C, H, N
2j	F	B	46	30	121-124 (0.9)[i]	$C_{10}H_{13}FN_2$	
2l	Br	B	23	32	250 dec	$C_{10}H_{13}BrN_2 \cdot HCl$	C, H, Br, Cl, N
2m	CN	A	28	55	193-193.5[j]	$C_{11}H_{13}N_3 \cdot C_6H_3N_3O_7$	H, N; C[k]

[a] *N*-Phenylpiperazine, *N*-(4-methoxy)piperazine, and *N*-(4-chlorophenyl)piperazine (2a, 2f, and 2k, respectively) were obtained commercially. [b] Method A is the piperazine fusion method; method B is the ring-closure method. [c] Lit.[9] bp 112 °C (1 mm). [d] Recrystallized from H_2O-EtOH. [e] Lit.[10] bp 180-185 °C (14 mm). [f] C: calcd, 75.56; found, 75.07. [g] Lit.[5] mp 129-130 °C. This compound was not recrystallized. [h] Recrystallized from petroleum ether. [i] Lit.[11] bp 118-123 °C (0.1 mm). [j] Recrystallized from EtOH. [k] C: calcd, 49.04; found, 49.69.

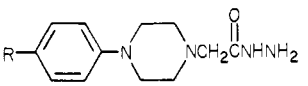
Table III. Ethyl *N*-(4-Substituted phenyl)piperazinoacetates



compd	R	reflux time, h	yield, %	bp (mm) or mp, °C	formula	analyses
3a	H	4	82	164-170 (0.7)[a]	$C_{14}H_{20}N_2O_2$	
3b	Me	18	97	55-55.6[b]	$C_{15}H_{22}N_2O_2$	C, H, N
3c	Et	7	99	180-184 (1.4)	$C_{16}H_{24}N_2O_2$	C, H, N
3d	*i*-Pr	19	64	56.5-57[c]	$C_{17}H_{26}N_2O_2$	C, H, N
3e	*t*-Bu	16	80	83.5-85[b]	$C_{18}H_{28}N_2O_2$	C, H, N
3f	OMe	20	81	40-40.5[d]	$C_{15}H_{22}N_2O_3$	
3g	OPh	23	36	66-67[e]	$C_{20}H_{24}N_2O_3$	C, H, N
3h	NO_2	19	91	122-123[f]	$C_{14}H_{19}N_3O_4$	
3i	CF_3	20	99	80.5-81.5[b]	$C_{15}H_{19}F_3N_2O_2$	C, H, N
3j	F	49	75	56-57[c]	$C_{14}H_{19}FN_2O_2$	C, H, N
3k	Cl	45	60	73-73.5[c]	$C_{14}H_{19}ClN_2O_2$	C, H, Cl, N
3l	Br	36	81	68.5-69[b]	$C_{14}H_{19}BrN_2O_2$	C, H, Br, N
3m	CN	26.5	87	87.5-88[b]	$C_{15}H_{19}N_3O_2$	C, H, N
3n	NHCOMe		74[g]	157-158[h]	$C_{16}H_{23}N_3O_3 \cdot 0.5H_2O$	C, H, N

[a] Lit.[12] bp 194-195 °C (7-8 mm). [b] Recrystallized from petroleum ether-Et_2O. [c] Recrystallized from petroleum ether. [d] Lit.[13] mp 41-42 °C. [e] Recrystallized from MeOH. [f] Lit.[1] mp 122-123 °C. [g] Yield is calculated on the basis of starting ethyl *N*-(4-nitrophenyl)piperazinoacetate. [h] Recrystallized from Me_2CO-H_2O.

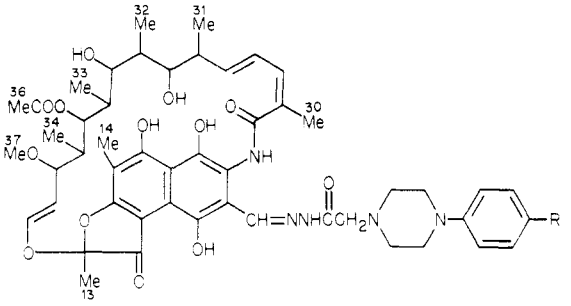
Table IV. *N*-(4-Substituted phenyl)piperazinoacethydrazides



compd	R	reflux time, h	yield, %	mp, °C (solvent)	formula	analyses
4a	H	13	92	93-94 (Et_2O-EtOH)	$C_{12}H_{18}N_4O$	C, H, N
4b	Me	19.5	54	131-132 (Et_2O-EtOH)	$C_{13}H_{20}N_4O$	C, H, N
4c	Et	13	70	115-115.5 (Et_2O-EtOH)	$C_{14}H_{22}N_4O$	C, H, N
4d	*i*-Pr	23	71	119.5-120 (Et_2O-EtOH)	$C_{15}H_{24}N_4O$	C, H, N
4e	*t*-Bu	23	87	143-144 (EtOH-H_2O)	$C_{16}H_{26}N_4O$	C, H, N
4f	OMe	12	35	121.5-122.5 (Et_2O-EtOH)	$C_{13}H_{20}N_4O_2$	C, H, N
4g	OPh	48	81	129-130 (MeOH)	$C_{18}H_{22}N_4O_2$	C, H; N[a]
4h	NO_2	13	92	166-167[b] (MeOH)	$C_{12}H_{17}N_5O_3$	
4i	CF_3	22.5	76	142.5-143 (Et_2O-EtOH)	$C_{13}H_{17}F_3N_4O$	C, H, N
4j	F	53	72	78.5-79.5 (Et_2O)	$C_{12}H_{17}FN_4O$	C, H, N
4k	Cl	24	84	137-137.5 (Et_2O-EtOH)	$C_{12}H_{17}ClN_4O$	C, H, Cl, N
4l	Br	18	77	151.5-152 (MeOH)	$C_{12}H_{17}BrN_4O$	C, H, Br, N
4m	CN	47	74	139.5-140.5 (H_2O)	$C_{13}H_{17}N_5O$	C, H, N
4n	NHCOMe	41	88	205.5-207 (Et_2O-EtOH)	$C_{14}H_{21}N_5O_2 \cdot H_2O$	C, H; N[c]

[a] N: calcd, 6.80; found, 7.22. [b] Lit.[1] mp 165-167 °C. [c] N: calcd, 22.64; found, 22.01.

Table V. 3-Formylrifamycin SV *N*-(4-Substituted phenyl)piperazinoacethydrazones[a]



compd	R	reaction time, h	yield, %	approx decompn pt, °C	formula	analyses
5a	H	2	90	167	$C_{50}H_{63}N_5O_{13}$	C, H, N
5b	Me	2.5	99	162	$C_{51}H_{65}N_5O_{13}$	C; H, N[b]
5c	Et	2.5	85	172	$C_{52}H_{67}N_5O_{13}$	C, H, N
5d	*i*-Pr	1.5	91	170	$C_{53}H_{69}N_5O_{13}$	C, H, N
5e	*t*-Bu	2.5	84	179	$C_{54}H_{71}N_5O_{13}$	C, H, N
5f	OMe	2.5	99	159	$C_{51}H_{65}N_5O_{14}$	N; C, H[c]
5g	OPh	2	98	163	$C_{56}H_{67}N_5O_{14}$	N; C, H[d]
5h	NO_2	2.5	86	165	$C_{50}H_{62}N_6O_{15}$	H, N; C[e]
5i	CF_3	2.5	93	161	$C_{51}H_{62}F_3N_5O_{13}$	C, H, F, N
5j	F	1.5	95	170	$C_{50}H_{62}FN_5O_{13}$	C, H, F, N
5k	Cl	2.5	99	156	$C_{50}H_{62}ClN_5O_{13}$	C, H, Cl, N
5l	Br	2	96	174	$C_{50}H_{62}BrN_5O_{13}$	H, N; C, Br[f]
5m	CN	2.5	99	163	$C_{51}H_{62}N_6O_{13}$	C, N; H[g]
5n	NHCOMe	6.5[h]	29	183	$C_{52}H_{66}N_6O_{14}$	H; C, N[i]

[a] The hydrazones were purified by thorough washings with hexane. [b] H: calcd, 6.85; found, 7.38. N: calcd, 7.33; found, 6.81. [c] C: calcd, 63.01; found, 63.50. H: calcd, 6.74; found, 7.28. [d] C: calcd, 65.04; found, 65.77. H: calcd, 5.56; found, 6.87. [e] C: calcd, 60.84; found, 60.16. [f] C: calcd, 58.82; found, 59.28. Br: calcd, 7.83; found, 8.28. [g] H: calcd, 6.46; found, 7.00. [h] The reaction mixture was heated in a warm-water bath for the final 90 min. [i] C: calcd, 62.51; found, 61.22. N: calcd, 8.41; found, 9.03.

Table VI. Antibacterial Activity of 3-Formylrifamycin SV *N*-(4-Substituted phenyl)piperazinoacethydrazones and Rifampin[a]

	B. subtilis			*Staph. aureus*			*C. perfringens*		*M. phlei*			*M. tuberculosis*	
compd	MIC	obsd log 1/*C*	calcd[b] log 1/*C*	MIC	obsd log 1/*C*	calcd[c] log 1/*C*	MIC	obsd log 1/*C*	MIC	obsd log 1/*C*	calcd[d] log 1/*C*	MIC	obsd log 1/*C*
5a	5.4	5.24	5.06	0.057	7.22	7.17	0.007	8.13	7.13	5.12	5.01	8.7	5.03
5b	5.1	5.27	5.15	0.038 (±0.016)	7.40	7.43	0.007	8.14	7.00	5.14	5.22	10.5	4.96
5c	5.5	5.25	5.23	0.026	7.57	7.60	0.003	8.51	6.75	5.16	5.29	18.6	4.72
5d	4.9	5.30	5.31	0.027	7.56	7.61	0.003	8.52	3.25	5.48	5.32	9.0	5.04
5e	4.8	5.32	5.38	0.033 (±0.014)	7.49	7.49	0.002	8.70	3.63	5.44	5.31	7.5	5.12
5f	11.3 (±3.9)	4.93	5.06	0.054	7.26	7.27	0.007	8.14	5.25	5.27	5.17	15.0	4.81
5g	5.0	5.32	5.39	0.019 (±0.006)	7.74	7.71	0.008	8.11	6.00	5.24	5.37	15.0	4.84
5h	12.3	4.90	5.02	0.025	7.60	7.47	0.005	8.30	11.50	4.93	4.85	18.0	4.74
5i	9.8 (±3.4)	5.01	5.20	0.024	7.62	7.51	0.002	8.70	7.38	5.14	5.17	18.0	4.75
5j	4.8	5.30	5.09	0.084 (±0.036)	7.06	7.04	0.005	8.51	5.75	5.22	5.17	15.0	4.81
5k	4.8	5.31	5.18	0.036 (±0.010)	7.43	7.43	0.006	8.21	6.38	5.19	5.26	12.0	4.91
5l	5.0	5.31	5.20	0.035	7.46	7.54	0.006	8.23	6.13	5.22	5.29	8.4	5.08
5m	16.3 (±7.1)	4.77	4.97	0.082 (±0.035)	7.07	7.27	0.007	8.14	21.50	4.65	4.71	18.6	4.72
5n	12.0	4.92	4.91	0.060	7.22	7.17	0.010	8.00	31.00	4.59	4.64	18.0	4.74
rifampin	0.21	6.59		<0.007	<8.07		0.003	8.44	0.27	6.48		1.0	5.91

[a] Activity is expressed in both MIC (μg/mL) and log 1/*C*, where *C* is the minimal molar concentration. MIC determinations were carried out in triplicate in all bacterial systems except in *M. tuberculosis*. Antitubercular activity was determined in duplicate. The numbers in parentheses are standard deviations of three determinations. When no standard deviation is given, results are consistent in all three determinations. [b] Calculated by eq 2. [c] Calculated by eq 9. [d] Calculated by eq 15.

was used to describe the electronic effect of the substituents, whereas $\mathcal{R}$, the resonance component of σ, was used in eq 9. Since it has a larger correlation coefficient, eq 9 was considered to be the better equation of the two. According to eq 9, the optimum lipophilicity (π_o) of a substituent for activity against *Staph. aureus* was 0.52, and

Table VII. Regression Equations Generated in the *B. subtilis*, *Staph. aureus*, and *M. phlei* Bacterial Systems

equation	n	s	r	r^2	E	S	eq no.
B. subtilis							
$\log 1/C = -0.33(\pm 0.31)\sigma + 5.19(\pm 0.11)$	14	0.17	0.56	0.31	5.35	>90.0	1
$\log 1/C = 0.16(\pm 0.09)\pi + 5.06(\pm 0.10)$	14	0.14	0.73	0.53	13.69	>99.5	2
$\log 1/C = 0.18(\pm 0.10)\pi - 0.22(\pm 0.24)\sigma - 0.01(\pm 0.01)$MR $+ 5.18(\pm 0.14)$	14	0.12	0.85	0.72	8.62	>99.5	3
Staph. aureus							
$\log 1/C = 0.02(\pm 0.01)$MR $+ 7.25(\pm 0.18)$	14	0.18	0.56	0.31	5.44	>95.0	6
$\log 1/C = 0.16(\pm 0.10)\pi + 7.31(\pm 0.11)$	14	0.16	0.71	0.50	12.09	>99.0	7
$\log 1/C = -0.24(\pm 0.20)\pi^2 + 0.31(\pm 0.16)\pi + 0.26(\pm 0.23)\sigma + 0.04(\pm 0.03)$MR $+ 7.08(\pm 0.18)$	14	0.11	0.89	0.79	9.01	>99.5	8
$\log 1/C = -0.30(\pm 0.18)\pi^2 + 0.31(\pm 0.14)\pi + 0.50(\pm 0.32)\mathcal{R} + 0.05(\pm 0.03)$MR $+ 7.12(\pm 0.14)$	14	0.07	0.93	0.87	13.34	>99.5	9
M. phlei							
$\log 1/C = 0.22(\pm 0.10)\pi + 5.00(\pm 0.11)$	14	0.16	0.81	0.66	22.28	>99.9	11
$\log 1/C = 0.29(\pm 0.11)\pi - 0.02(\pm 0.01)$MR $+ 5.11(\pm 0.13)$	14	0.13	0.88	0.77	18.48	>99.9	12
$\log 1/C = -0.10(\pm 0.09)\pi^2 + 0.35(\pm 0.14)\pi + 5.04(\pm 0.10)$	14	0.13	0.88	0.77	19.00	>99.9	13
$\log 1/C = 0.27(\pm 0.10)\pi - 0.23(\pm 0.22)\sigma - 0.02(\pm 0.01)$MR $+ 5.16(\pm 0.13)$	14	0.11	0.92	0.85	18.99	>99.9	14
$\log 1/C = 0.30(\pm 0.09)\pi - 0.42(\pm 0.32)\mathcal{R} - 0.02(\pm 0.01)$MR $+ 5.09(\pm 0.10)$	14	0.10	0.94	0.88	23.50	>99.9	15

the electronic $\mathcal{R}$ was the most important variable, having the largest coefficient in the equation.

A QSAR between the antibacterial activity of 3-formylrifamycin SV oximes, rifamycin B amides, and rifamycin SV iminomethylpiperazines and the lipophilicity of these compounds has been reported by Pelizza et al.[7] In their study lipophilicity was expressed by the chromatographically determined R_m constants, which have been shown to correlate well with log P values and Hansch π constants.[8] Using only the R_m term in the regression analysis, Pelizza et al.[7] found that the antibacterial activity of the three types of rifamycin derivatives against *Staph. aureus* was parabolically related to the lipophilicity of the compounds as shown in the highly significant (99.9% level, $F_{2,73} = 104$) eq 10. When each series of rifamycin de-

$$\log 1/C = -0.84R_m^2 - 0.18R_m + 8.02 \quad (10)$$
$$n = 76;\ s = 0.39;\ r = 0.86$$

rivatives was examined individually, a parabolic relationship was also obtained. The parabolic eq 9 derived in this work supports the result reported by Pelizza and his co-workers.

Five highly significant correlation equations (eq 11–15) were obtained in the *M. phlei* bacterial system. Equation 11 with a single π term accounted for 66% of the variation in activity. Addition of a MR term to eq 11 gave eq 12, which was significant at the same level as eq 11 but which explained 77% of the variation in antibacterial activity against *M. phlei*. An equivalent improvement in the correlation coefficient was obtained when a term in π^2 was added to eq 11. The resulting eq 13 was also highly significant as determined by the F test. Because of the high collinearity of π^2 and MR, it is difficult to state whether eq 12 or 13 is the more accurate description of the relationship between the structure and activity. The addition of the electronic σ term to eq 12 resulted in a slight improvement in the correlation coefficient as shown in eq 14. This equation indicated a linear relationship between activity and hydrophobic, electronic, and steric effects. A similar linear equation, eq 15, in which the electronic effect of the substituents on activity was expressed by $\mathcal{R}$ was also significant. Since the correlation coefficient of eq 15 was slightly higher than that of eq 14 and the 95% confidence intervals for the regression coefficients of eq 15 were consistently more acceptable than those in eq 14, eq 15 was probably the best regression equation for the *M. phlei* system.

The work presented here illustrates the versatility of the Hansch approach to QSAR. From a series of 14 carefully chosen derivatives of 3-formylrifamycin SV it was possible to derive significant relationships between their antibacterial activities and structures, as expressed by their physicochemical parameters. More importantly, the QSAR developed for the series of compounds in this report substantiated the QSAR obtained from other structurally dissimilar derivatives of rifamycin.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus using open capillaries and are uncorrected. Thin-layer chromatography was performed on Eastman chromagram sheets, 13181 silica gel with fluorescent indicator (No. 6060). Elemental analyses were performed by Robertson Laboratory, Florham Park, N.J., and Dr. F. B. Strauss, Oxford, England. Where elemental analyses are indicated by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

The structures of all compounds were confirmed by their IR spectra and of all new compounds by the NMR spectra as well. The IR spectra were taken on a Perkin-Elmer 237B grating infrared spectrophotometer as KBr disks in the cases of solid compounds or neat in the cases of liquid compounds. With the exception of the rifamycin hydrazones, the NMR spectra were recorded on a Varian T-60 instrument with $CDCl_3$ or Me_2SO-d_6 as solvent and Me_4Si as internal standard. The NMR spectra of the rifamycin hydrazones were recorded on a Varian CFT-20 instrument.

***N*-[4-(Trifluoromethyl)phenyl]piperazine (2i) (Method A).** A mixture of 22.5 g (0.10 mol) of 4-bromo-1-(trifluoromethyl)benzene and 43.1 g (0.50 mol) of anhydrous piperazine was heated in an oil bath at ca. 100 °C for 45 h. The hot melt was poured into a 250 mL of 10% NaOH solution to give 18.7 g (0.08 mol) of **2i** (81.2%). Recrystallizations from petroleum ether gave yellow crystals: mp 86–88 °C; IR (KBr) 3250 (NH, piperazine), 2955, 2835 cm^{-1} (CH_2, piperazine); NMR ($CDCl_3$) δ 1.67 (1 H, s, NH), 3.15 [8 H, m, $N(CH_2CH_2)_2N$], 7.25 (4 H, 2 d, Ph). Anal. ($C_{11}H_{13}N_2F_3$) C, H, N.

The above procedure was followed in preparing **2h** and **2m**.

***N*-(4-Isopropylphenyl)piperazine (2d) (Method B).** A mixture of 39.6 g (0.22 mol) of bis(2-chloroethyl)amine hydrochloride, 47.0 g (0.44 mol) of Na_2CO_3, and 30.0 g (0.22 mol) of *p*-isopropylaniline in 150 mL of EtOH was heated to reflux for

51 h. The solvent was concentrated and the residue redissolved in H_2O. The aqueous solution was extracted with C_6H_6. The dry ($MgSO_4$) extracts were removed under reduced pressure leaving a red oil that was distilled to give 19.4 g (0.10 mol) of **2d** (86.6%) as a colorless liquid: bp 118–119 °C (0.40 mm); IR (melt) 2945, 2805 cm^{-1} (CH_2, piperazine); NMR ($CDCl_3$) δ 1.20 [6 H, d, $(CH_3)_2CH$, J = 7 Hz], 1.75 (1 H, s, NH), 3.05 [8 H, s, N-$(CH_2CH_2)_2N$], 2.57–3.23 [1 H, m, $CH(CH_3)_2$], 6.97 (4 H, 2 d, Ph).

The above procedure was followed in preparing **2b,c,e,g,j,l**.

Ethyl *N*-(4-Chlorophenyl)piperazinoacetate (3k). The aqueous solution of 10.0 g (0.04 mol) of *N*-(4-chlorophenyl)-piperazine dihydrochloride was neutralized with alkali. The free base was dissolved in 50 mL of Me_2CO and to the solution was added 4.5 g (0.04 mol) of ethyl chloroacetate and 3.1 g (0.04 mol) of $NaHCO_3$. The mixture was heated to reflux until only one spot was visible on silica gel TLC [MeOH–C_6H_6 (95:5)] (ca. 45 h) and then filtered. The filtrate was concentrated to give 6.3 g (0.02 mol) of **3k** (60.8%). Recrystallizations from petroleum ether yielded colorless crystals: mp 73–73.5 °C; IR (KBr) 2975, 2830 (CH_2, piperazine), 1750 cm^{-1} (C=O, ester); NMR ($CDCl_3$) δ 1.31 (3 H, t, $COOCH_2CH_3$, J = 7 Hz), 2.64–3.37 [8 H, m, N-$(CH_2CH_2)_2N$], 3.28 (2 H, s, NCH_2CO), 4.27 (2 H, quartet, $COOCH_2CH_3$, J = 7 Hz), 7.10 (4 H, 2 d, Ph). Anal. ($C_{14}H_{19}ClN_2O_2$) C, H, Cl, N.

The above procedure was followed in preparing **3a–j**, **2l**, and **3m**.

Ethyl *N*-(4-Acetamidophenyl)piperazinoacetate (3n). To a boiling mixture of 7.0 g (0.13 g-atom) of iron filings, 100 mL of EtOH, 30 mL of H_2O, and 3 mL of concentrated HCl was added in small portions a solution of 7.0 g (0.024 mol) of ethyl *N*-(4-nitrophenyl)piperazinoacetate in 100 mL of EtOH. After the addition was complete, another 2 mL of concentrated HCl was added and the mixture was heated on the steam bath for 3 h. Approximately 25 g (0.3 mol) of $NaHCO_3$ was then added and the mixture was heated for another 10 min. The hot mixture was then filtered and the precipitate was washed with 50 mL of hot EtOH. The solvent was removed from the filtrate and the residue was extracted with $CHCl_3$. The dry ($MgSO_4$) extracts were concentrated to give an oil, to which acetic anhydride was added yielding 5.4 g (0.018 mol) of **3n** (73.8%). Recrystallizations from H_2O–Me_2CO and decolorization with activated charcoal gave crystals of the hemihydrate: mp 157.5–158 °C; IR (KBr) 3300–3250 (NH, amide), 2965, 2820 (CH_2, piperazine), 1745 (C=O, ester), 1650 cm^{-1} (C=O, amide); NMR ($CDCl_3$) δ 1.28 (3 H, t, CH_2CH_3, J = 7 Hz), 2.10 (3 H, s, CH_3CONH), 2.30 (s, H_2O of crystallization), 2.60–3.25 [8 H, m, $N(CH_2CH_2)_2N$], 3.25 (2 H, s, CH_2CO), 4.25 (2 H, quartet, $COOCH_2CH_3$, J = 7 Hz), 6.95 (4 H, 2 d, Ph). Anal. ($C_{16}H_{23}N_3O_3 \cdot 0.5H_2O$) C, H, N.

***N*-(4-Chlorophenyl)piperazinoacethydrazide (4k).** A solution of 4.6 g (0.016 mol) of ethyl *N*-(4-chlorophenyl)-piperazinoacetate and 1.6 g (0.032 mol) of hydrazine hydrate in 25 mL of EtOH was heated to reflux until only one spot of the hydrazide was detected on silica gel TLC [MeOH–C_6H_6 (95:5)] (ca. 24 h). The solvent was then removed and the residue was washed with anhydrous Et_2O to yield 3.7 g (0.014 mol) of **4k** (84.8%). Recrystallizations from Et_2O–EtOH gave colorless crystals: mp 137–137.5 °C; IR (KBr) 3280 (NH, hydrazide), 2920, 2810 (CH_2, piperazine), 1660 cm^{-1} (C=O, hydrazide); NMR ($CDCl_3$) δ 2.58–3.23 [8 H, m, $N(CH_2CH_2)_2N$], 3.15 (2 H, s, CH_2CO), 3.81 (2 H, br s, $NHNH_2$), 7.08 (4 H, 2 d, Ph), 8.15 (1 H, br s, $NHNH_2$). Anal. ($C_{12}H_{17}ClN_4O$) C, H, Cl, N.

The above procedure was followed in preparing **4a–j** and **4l–n**.

3-Formylrifamycin SV *N*-(4-Chlorophenyl)piperazino-acethydrazone (5k). To a suspension of 1.00 g (0.001 mol) of 3-formylrifamycin SV in 25 mL of THF was added 0.37 g (0.001 mol) of *N*-(4-chlorophenyl)piperazinoacethydrazide. The course of the reaction was followed by TLC [$CHCl_3$–MeOH (9:1)]. After 2.5 h, when only the orange spot of the product was visible, the THF was evaporated under reduced pressure and the residue was washed well with hexane. The red solid was filtered to yield 1.3 g (0.001 mol) of **5k** (100%): decomposition point ≃156 °C; NMR[14] ($CDCl_3$) δ −0.24 (d, C-34), 0.59 (d, C-33), 0.76 (d, C-31), 1.00 (d, C-32), 1.80 (s, C-13), 2.04 (s, C-36), 2.16 (s, C-30), 2.22 (s, C-14), 2.75–3.18 [m, $N(CH_2CH_2)_2N$], 3.03 (s, C-37), 3.18 (s, $COCH_2N$), 6.75–7.21 (2 d, Ph). The numbering system of the molecule is given in Table IV. Anal. ($C_{50}H_{62}ClN_5O_{13}$) C, H, Cl, N.

The above procedure was followed in preparing **5a–j** and **5l–n**. However, some warming of the reaction mixture on a water bath was found necessary in obtaining **5n**.

Antimicrobial Evaluations. The hydrazones were tested for antimicrobial activity against three Gram-positive bacteria, *B. subtilis* 104, *Staph. aureus* 11, and *Strep. faecalis* 107; one anaerobic Gram-positive bacteria, *C. perfringens* 2; six Gram-negative bacteria, *Alcaligenes faecalis* 44, *Escherichia coli* 8, *Proteus vulgaris* 74, *Pseudomonas aeruginosa* 58, *Salmonella thompson* 140, and *Serratia marcescens* 25; two acid-fast bacteria, *M. phlei* 111 and *M. tuberculosis* H37RV; and two fungi, *Candida albicans* 48 and *Saccharomyces cerevisiae* 206. The microorganisms studied were from the stock culture collections of the Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, or the Division of Bacteriology, Pathology Institute, Halifax, Nova Scotia. The previously reported serial dilution method[15] was used to determine the MIC values of the hydrazones in all the bacterial systems with the exception of *M. tuberculosis*. The MIC of the compounds against this bacteria was determined by an agar dilution method in which the test hydrazone was dissolved in a volume of warm Middlebrook agar. After several dilutions, the varying concentrations of the hydrazone were dispensed into two quadrants of a quartered petri dish and two quadrants were filled with media containing no hydrazone. The media was allowed to cool and the quadrants were then inoculated with 0.1 mL of *M. tuberculosis* suspension, which had been adjusted to a MacFarland no. 1 standard spectrophotometrically. All petri dishes were then incubated under 5% CO_2 at 37 °C for 14 days. After this incubation period, the plates were examined for bacterial colonies.

Antiviral Evaluation. The method of plaque replication was used. A series of 1-day-old monolayer cultures of L-929 cells prepared in multiwells (Costar Product) was exposed to varying concentrations of the test compound using three well cultures per concentration. Within 1 h of exposure at 37 °C, vaccinia virus, diluted to contain a countable number of plaque-forming units, was added to all the well cultures in 0.1-mL volumes. To ensure the reliability of the test method, iododeoxyuridine was included in each test. After 3–4 days of incubation, all cultures were examined microscopically for a cytotoxic effect. The number of plaques that developed was enumerated after fixing the cultures with formal saline and staining with crystal violet. Vaccinia plaques appeared as small circumscribed unstained holes.

Regression Analysis of Antibacterial Activity Data. This was carried out by the method of least squares using the Statistical Package for the Social Sciences (SPSS), Version 6.5, and the CDC 6400 computer at the Dalhousie University Computer Centre.

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Notes

Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 7. Role of the Ribosyl Moiety in Enzymatic Binding of *S*-Adenosyl-L-homocysteine and *S*-Adenosyl-L-methionine

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A series of 2′,3′-acyclic analogues of *S*-adenosyl-L-homocysteine were synthesized and evaluated as inhibitors of *S*-adenosyl-L-methionine-dependent methyltransferases. The 2′,3′-acyclic analogues were prepared by periodate oxidation of the corresponding ribonucleosides, followed by reduction of the intermediate dialdehydes with sodium borohydride. These 2′,3′-acyclic ribonucleosides were inactive as inhibitors of histamine *N*-methyltransferase, catechol *O*-methyltransferase, phenylethanolamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase. These results suggest that the rigidity of the ribosyl ring of *S*-adenosyl-L-homocysteine is crucial to its enzymatic binding.

A general feature of *S*-adenosylmethionine (L-SAM)[2] dependent methyltransferases is the inhibition produced by the product, *S*-adenosyl-L-homocysteine (L-SAH).[3] In an effort to develop inhibitors of methyltransferases, several laboratories have reported the syntheses and the in vitro and in vivo biological activities of base or amino acid modified analogues of SAH.[3-7] Various sugar-modified analogues of SAH have also been synthesized and their inhibitory activities toward methyltransferases examined.[4,8-11] For example, the ribosyl group of SAH has been replaced by 2′-deoxyribosyl,[10] 3′-deoxyribosyl,[10] arabinofuranosyl,[10] 2′,3′-dihydroxycyclopentyl,[9,11] and cyclopentyl[8] groups. Coward and Sweet[8] have also reported the synthesis of a series of five carbon acyclic SAH analogues in which the 1′,4′-oxygen bridge of the ribosyl moiety was removed.

In an effort to further characterize the role of the ribosyl group of SAH in enzymatic binding, we have synthesized an acyclic analogue of SAH in which the 1′,4′-oxygen bridge has been retained but the 2′,3′-carbon bond cleaved, e.g., 2′-[*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]]-3′-[*S*-(*R*)-homocysteinyl]-3′-deoxy-(*S*)-glycerol (**1**, 2′,3′-acyclic L-SAH). Several related SAH analogues were also converted to their 2′,3′-acyclic derivatives (Chart I). The SAH analogues which were chosen for this study have well-recognized inhibitory activity toward specific methyltransferases [e.g., D-SAH,[12] histamine *N*-methyltransferase (HMT); L-SAHO,[12] catechol *O*-methyltransferase (COMT); 2-aza-SAH,[13] phenylethanolamine *N*-methyltransferase (PNMT); and 8-aza-SAH,[14] hydroxyindole *O*-methyltransferase (HIOMT)]. By converting these SAH analogues to their corresponding 2′,3′-acyclic derivatives, we could then evaluate the importance of the intact ribofuranosyl ring in binding to several methyltransferases. If these acyclic analogues exhibit inhibitory activity similar to the parent ribonucleoside, then the 2′,3′-acyclic ribosyl moiety might have general utility in the design of methyltransferase inhibitors.

Experimental Section

Melting points (uncorrected) were obtained on a calibrated Thomas-Hoover Uni-melt apparatus. Unless otherwise stated, the IR, NMR, and UV data were consistent with the assigned structures. IR data were recorded on a Beckman IR-33 spectrophotometer, NMR data on a Perkin-Elmer R-24B spectrophotometer (Me_4Si), and UV data on a Cary Model 14 spectrophotometer. Scintillation counting was done on a Beckman LS-150 scintillation counter. TLC were run on Analtech silica gel GF (250 μm) and Avicel F (250 μm). Spots were detected by visual examination under UV light and/or ninhydrin for compounds containing amine moieties.

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/mL and stored at −20 °F. SAM chloride (Sigma) was stored as a 10-mM aqueous stock solution. The following compounds were commercially available from the indicated sources: 3,4-dihydroxybenzoate (Aldrich), DL-β-phenylethanolamine, histamine hydrochloride, *N*-acetylserotonin, and L-SAH (Sigma). The SAH analogues were synthesized according to published procedures as cited below: D-SAH,[12] L-SAHO,[12] L-SAC,[15] 2-aza-SAH,[13] and 8-aza-SAH.[15]

General Procedure for Reduction of Ribonucleoside 2′,3′-Dialdehydes to 2′,3′-Acyclic Ribonucleosides. The ribonucleosides (e.g., L-SAH, D-SAH, SAM, etc.) were oxidized to the corresponding 2′,3′-dialdehydes using paraperiodic acid as previously described.[16] To a stirred solution of the nucleoside 2′,3′-dialdehyde (0.25 mmol) in 0.1 M phosphate buffer, pH 8.4 (6 mL), at ambient temperature was added slowly $NaBH_4$ (0.60 mmol) over a 30-min period. After 5 h the solution was adjusted to pH 5 with 5% HCl and then readjusted to pH 7 with 0.2 N NaOH. The product was purified by thick-layer chromatography on cellulose eluting with H_2O or EtOH–H_2O mixtures. The desired 2′,3′-acyclic ribonucleoside was recovered from the cellulose by extraction with H_2O, followed by lyophilization. The 2′,3′-acyclic ribonucleosides were not obtained in crystalline form, since their instability prohibited crystallization by classical techniques. The 2′,3′-acyclic ribonucleosides were characterized by their chromatographic properties (see Table I for the chromatographic systems used and the R_f values observed) and their spectral

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