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The Medicinal Chemistry of Anti-leprosy Drugs

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1 Introduction

This review presents:

(i) a summary of leprosy as a disease, its epidemiology, characteristics, and therapy. The important features of *Mycobacterium leprae*, the causative agent of leprosy, are summarized and drug testing systems briefly described.

(ii) the chemistry of the drugs used in therapy with special emphasis on the more recent developments and the use of qualitative (SAR) and quantitative (QSAR) methods for the analysis of structure–activity relationships. The anti-inflammatory drugs used to treat lepra reactions will not be considered.

(iii) recent developments in the identification of possible new targets for drug development particularly associated with the structure of the mycobacterial cell wall.

(iv) some brief ideas on the general importance of new antimycobacterial agents in the light of the growing incidence of infection by 'atypical' mycobacteria. This is of particular importance in patients with AIDS (acquired immune disease syndrome).

2 The Disease of Leprosy

A. Epidemiology and Clinical Features.—Leprosy is one of the oldest diseases known to man and it was the first disease for which an invading micro-organism was proposed as the causative agent.[1] It is presently one of six major world diseases which are the target of WHO special programmes. The causative agent is *Mycobacterium leprae*, a non-cultivatable but close relative of *M. tuberculosis*—the other major mycobacterial human pathogen. *M. leprae* is an obligate intracellular parasite which has a predilection for skin and peripheral nerves. It is described as an organism of high infectivity but low pathogenicity. This means that only a relatively small proportion of people infected by the bacillus go on to develop the disease. Figure 1 shows the current world distribution of leprosy, although small endemic foci of the disease in the southern United States and parts of Italy and Greece are not shown. Leprosy is known to occur in the USSR, within the Arctic Circle, and in China although there are no official figures for these countries. The disease itself is probably transmitted by mucosal discharge from the nose and mouth, spitting, nose blows *etc.* It has a long incubation period, generally put at 2–5 years, and is

[1] M. Hooper and M. G. Purohit, 'The Chemotherapy of Leprosy' in *Prog. Med. Chem.*, ed. G. P. Ellis and G. B. West, Vol. 20, Elsevier–North Holland, 1983, p. 1 and references cited therein.

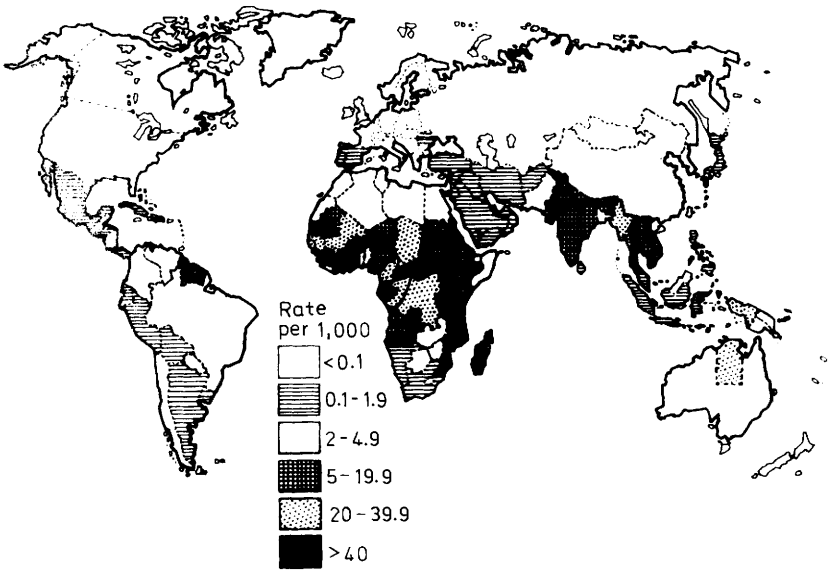

Figure 1 *World distribution of leprosy*
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described as a bi-polar disease. At one end of the bi-polar scale is lepromatous leprosy (LL) in which the patient has massive numbers of bacilli present and large numbers of antibodies to the bacilli but a compromised cell-mediated immune response (CMI) which cannot effectively kill and clear the parasite. At the other end of the scale is tuberculoid leprosy (TT) in which the patient has few bacilli and an efficient CMI. Between the two poles of the disease spectrum there exist various intermediate states which are characterized by bacteriological, immunological, and clinical features;[2] these are summarized in Figure 2. The bacteriological classification of the disease/organism includes the bacteriological index (BI), which is simply the number of bacilli (expressed on a logarithmic scale), and the morphological index (MI). The MI attempts to express the viability of the bacilli based on the staining characteristics: solid staining bacilli are regarded as viable whilst those with obvious fragmented staining are thought to be non-viable. The MI is expressed as the percentage of solid staining to non-staining bacilli. Such assessments obviously require skilled operators but even then the MI, which is often low or very low, varies enormously. This is important in both *in vitro* and *in vivo* drug testing systems as the viability of the innocula used is crucial to a successful test. More recently, the viability of bacilli has been assessed by alternative staining and counter staining techniques using fluorescein diacetate, which is

[2] J. T. Kvach, G. Munguia, and S. H. Strand, *Int. J. Lepr.*, 1984, **52**, 17; K. V. Harshan, H. K. Prasad, N. K. Chopra, R. S. Mishra, P. Gogiya, and I. Nath. *Int. J. Lepr.*, 1987, **55**, 316.

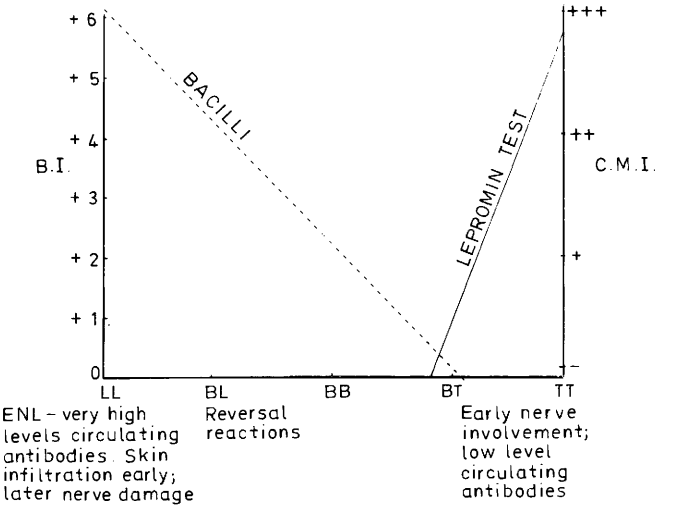


Figure 2 *A summary of the major immunological and bacteriological features of leprosy*

hydrolysed by esterases in live bacilli to give a green fluorescence, and ethidium bromide, which only penetrates dead bacilli and provides an orange background.[2] Rhodamine 123, a cationic dye, has been used to identify viable cells which have an intact transmembrane potential along which the dye passes and accumulates in the cell.[3]

Leprosy is known as a great 'mimic' of other diseases. Its diagnosis, particularly in the early stages, requires careful and perceptive clinical judgement. Unless early diagnosis is made and prompt therapy given the ensuing damage to peripheral sensory, motor, and secretion controlling nerves leads inexorably to extensive tissue damage and disfigurement, paralysis, blindness, and loss of fingers and toes (Figure 3).

B. The Organism.—*M. leprae* is a large cigar-shaped bacillus, 6—8 μm × 0.5 μm. Until the recent discovery of the nine-banded North American armadillo as a suitable alternative host, the only reliable source of the bacilli was the human leprosy patient. Even now the supply of *M. leprae* is very limited. The use of most of the laboratory supplies of *M. leprae* in vaccine preparation means that little is available for other studies. Nevertheless some studies of the biochemistry of the organism in both whole and broken cell systems have been carried out.[4,5] In line

[3] T. Matsuyama, *FEMS Microbiol. Lett.*, 1984, **21**, 153; O. Oddinsen, T. Nilson, and D. P. Humber, *Int. J. Lepr.*, 1986, **54**, 403; B. M. Kinsey, A. I. Kassis, F. Fahad, W. W. Layne, and S. J. Adelstein, *J. Med. Chem.*, 1987, **30**, 1757–1761.

[4] P. R. Wheeler, *Int. J. Lepr.*, 1984, **52**, 208.

[5] T. Mori, Y. Miyata, K. Kohsaka, and M. Makeno, *Int. J. Lepr.*, 1985, **53**, 600.

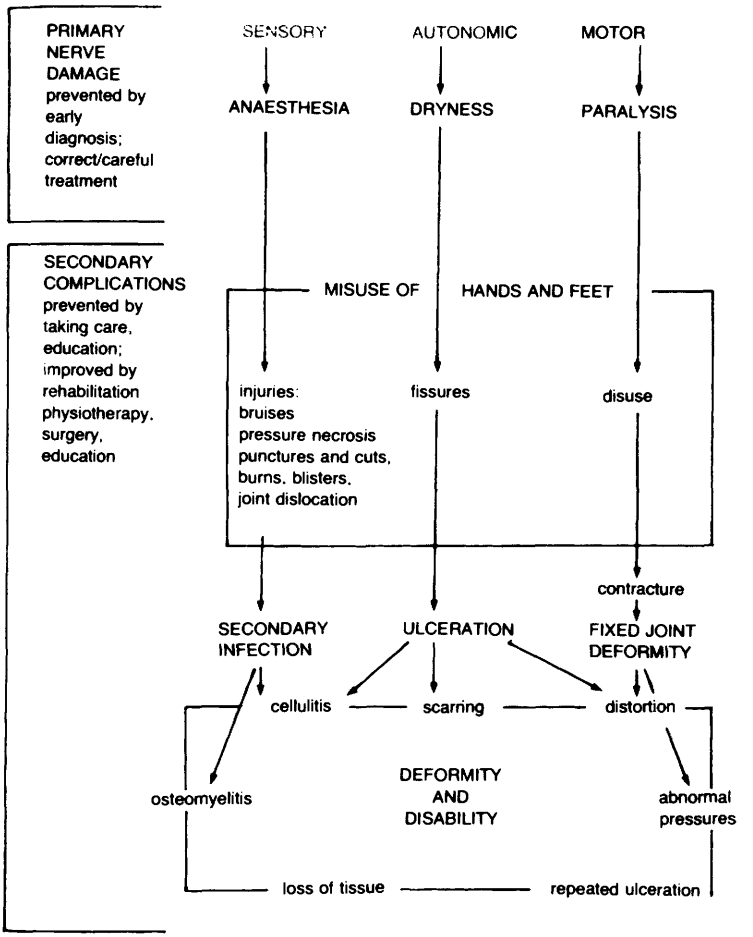


Figure 3 *The pathogenesis of leprosy following nerve damage*

with the variable viability of the bacilli, its slow growth, and the present inability to maintain the organism for any length of time (~14—20 days) in any culture medium, only quite low levels of metabolic activity have been observed. Some of the claims concerning unusual or unique metabolic activity in *M. leprae* remain highly contentious, particularly the presence of a unique dihydroxyphenylalanine (dopa) oxidase enzyme.[1,4–6] Such an uncertain biochemical base makes rational drug design at best a somewhat risky affair (see Section 5F). The structure and chemistry

[6] K. Prabhakaran, E. B. Harris, and W. F. Kirchheimer, *Microbios*, 1972, **5**, 273 and references cited therein.

of the distinctive cell wall[7,8] and the identification of a unique phenolic glycolipid with pronounced immunostimulant properties[9] provide somewhat more secure grounds for new drug development (see Section 5G).

C. Drug Testing Systems.—(i) The classical and now universally accepted (but still debated) *in vivo* system is the mouse footpad test.[10] The mouse, a very poor host for *M. leprae*, will allow the bacilli to multiply following injection into the hind footpad. Growth is very slow. Drugs under test are administered at various dose levels, as a percentage by weight, in the mouse feed. After six months the first measurements of growth (by counting bacilli present) in control mice are made. Further counts are made at two—four week intervals over the next six months. The results are usually complete after twelve months. There are a number of variations in the procedures which allow drugs to be classified as bacteriostatic or bactericidal.[11,12] Both the armadillo and the severely immuno-compromised mouse (nude athymic mouse) will allow more extensive growth of *M. leprae*, but these are not generally used for drug testing.

(ii) The footpad test is both time consuming and expensive so other cultivatable mycobacteria have been used as *in vitro* model systems to test drug candidates; the organisms used include *M. tuberculosis*, *M. smegmatis*, and other mycobacteria, and the so called '*M. lufu*'. The appropriateness of such models has been the subject of quite fierce debate at times. Such models have some value but even *M. tuberculosis*, the most widely used, is sensitive to some drugs which do not affect *M. leprae* (*e.g.* isoniazid, pyrazinamide, ethambutol) and has only little or no susceptibility to drugs which are active against *M. leprae*, *e.g.* dapsone.

(iii) The inadequacies of both the footpad and other cultivatable mycobacteria to provide a rapid and accurate assessment of new candidate drugs has led to the use of a variety of alternative *in vitro* systems which utilize *M. leprae* either alone or after incorporation into human or mouse macrophage—a host cell occupied by the bacillus *in vivo*. These tests rely on the measurement of the uptake and/or utilization of radiolabelled substrates (*e.g.* dopa,[13] thymidine[14]) or changing levels of a key intracellular indicator, *e.g.* adenosine triphosphate,[15] or on changes in macrophage

[7] D. E. Minniking, 'Chemical Targets in the Cell Envelopes of the Leprosy Bacillus and related Mycobacteria' in 'Chemotherapy of Tropical Diseases', ed. M. Hooper, CRAC Critical Reports on Applied Chemistry, Vol. 21, John Wiley, 1987, p. 19.

[8] P. Draper, *Int. J. Lepr.*, 1984, **52**, 527.

[9] P. J. Brennan in 'The Mycobacteria: A Sourcebook, Part B', ed. G. P. Kubica and L. G. Wayne, Marcel Dekker, 1984, p. 991.

[10] C. C. Shepard, *J. Exp. Med.*, 1960, **112**, 445; *Int. J. Lepr.*, 1962, **30**, 291.

[11] C. C. Shepard, *Int. J. Lepr.*, 1969, **37**, 389.

[12] M. J. Colston, G. R. F. Hilson, and D. K. Banerjee, *Lepr. Rev.*, 1978, **49**, 7.

[13] E. J. Ambrose, N. H. Antia, and S. R. Khanolkar, *Nature (London)*, 1974, **269**, 853; S. R. Khanolkar, E. J. Ambrose, and P. R. Mahadevan, *J. Gen. Microbiol.*, 1981, **127**, 385.

[14] M. Sathish and I. Nath, *Int. J. Lepr.*, 1981, **49**, 187; A. Mittal, M. Sathish, P. S. Seshadri, and I. Nath, *J. Clin. Microbiol.*, 1983, **17**, 704.

[15] A. M. Dhople, *Int. J. Lepr.*, 1984, **52**, 183; S. G. Franzblau and R. C. Hastings, *Antimicrob. Agents Chemother.*, 1987, **31**, 780.

cell membrane expression[16] associated with the presence of live bacilli. These tests are currently under evaluation by the WHO but, at present, seem only capable of providing a very approximate identification of activity. Recently laser microbe mass analysis (LAMMA) has been used to study intracellular $^{23}Na^+/^{39}K^+$ ratios in single cells—untreated (1:10) and dapsone treated (10:4).[17] In addition the fingerprint mass spectra[17,18] arising from the organic material in the cells were also analysed by non-linear mapping techniques and could be divided into treated and untreated groups. The cation ratios correlated with ATP content measured on the same cells.

D. Chemotherapy.—The drugs presently used to treat leprosy can be divided into two main groups, first-line and second-line.

(i) *First-line drugs.* These are dapsone, rifampicin, and clofazimine. Until recently, extensive and often life-long monotherapy, particularly with dapsone, the first effective anti-leprosy drug, was commonplace. The emergence of resistant organisms as secondary (occurring during therapy) or primary (the original infection) resistance coupled with the recognition that shorter term therapy gains wider patient compliance has led to the current use of multi-drug treatment regimens. The proposed drug regimens vary somewhat with the clinical classification of the disease. Standard regimens proposed by the WHO THELEP panel[19] are now being evaluated worldwide, but other regimens are also being used.[20] Generally, for lepromatous leprosy, rifampicin (the most potent bactericidal drug) is used with dapsone and clofazimine or prothionamide over a five year period. For tuberculoid leprosy, two drugs, rifampicin and either dapsone or clofazimine, over two to three years are recommended. The interactions of these drugs *in vivo* is an imporant area of investigation.

(ii) *Second-line Drugs.* These include the thioamides prothionamide and ethionamide, the thiosemicarbazone thiacetazone, and the (little used) thiourea thiambutosine.

(iii) *Other Drugs.* The principal new compound is deoxyfructoserotonin which is discussed below. The activity of a number of known antimicrobial agents against *M. leprae* has been studied.

3 First-line Drugs

A. The Sulphones.—The first clearly effective anti-leprosy drug was the simple 4,4′-diaminodiphenylsulphone (DDS) dapsone (1, R = NH_2). It has been the mainstay of drug treatment ever since its introduction in 1947. It was discovered at the beginning of the modern era of chemotherapy and was first prepared in 1908.[1] *M. leprae* is exquisitely sensitive to dapsone and despite the widespread occurrence of

[16] M. V. Mankar, R. Jagannathan, and P. R. Mahadevan, *J. Bioscience*, 1984, **6**, 709.
[17] U. Seydel, B. Lindner, and A. M. Dhople, *Int. J. Lepr.*, 1985, **53**, 365.
[18] G. Wieten, J. Haverkamp, L. G. Berwald, D. G. Groothins, and P. Draper, *Ann. Microbiol.*, 1982, **133B**, 15.
[19] Chemotherapy of Leprosy for Control Programmes; Technical Report Series 675; WHO, Geneva, 1982.
[20] 'Questions and Answers on the Implementation of Multiple Drug Therapy (MDT) for Leprosy', Oxfam, Oxford, 1987.

resistant organisms it is frequently effective at increased dosages even in monotherapy.[1] The modern multi-drug regimens all include dapsone. Its mode of action has been clearly established in a series of elegant studies covering whole cell and broken cell systems involving *E. coli*, *M. lufu*, and *M. leprae*. Dapsone competes with 4-aminobenzoate the natural substrate for pteroate synthetase. It is not incorporated into false dihydrofolate molecules by *M. leprae* although *E. coli* does utilize sulphones in this way.[21,22] Despite the lack of activity of dihydrofolate inhibitors against *M. leprae* a synergistic effect with dapsone and brodimaprim [(34), p. 461] has been demonstrated both *in vitro* and *in vivo*.[23] In addition to this principal mode of action, dapsone also modulates the immune response[24] and acts as a scavenger of active oxygen species known to have an important role in inflammation.[25] It is the latter properties which make the drug useful in the treatment of immunological disorders such as dermatitis herpetiformis and rheumatoid arthritis.[26]

(i) *Synthesis.* The synthetic routes to the very large numbers of diaryl and heteroaryl sulphones are straightforward and have been described in a number of review articles.[1,27,28] A brief summary of the various synthetic routes is given in Figure 4. The drug has excellent pharmacokinetic properties and at low dosage has proved to be much less toxic than earlier studies indicated.

(ii) *Structure–Activity Relationships.* Two interesting QSAR studies involving a number of 4-substituted-4′-aminodiphenylsulphones (1) have been reported. The analysis of activity against *M. smegmatis*[29] and a number of independent variables gave equation 1 in Table 1 as the best equation.

Four compounds were dropped from the original twenty compounds: 4-NHCHO, 4-NHCOMe, 4-F, and 4-H. The first two gave higher than predicted activities, probably owing to some hydrolysis to dapsone. The equation identifies a mesomeric electron-releasing substituent (negative coefficient of *R*) which is hydrophilic (negative coefficient of *f*) as giving the highest activity. The substituent complying most closely with these criteria is the NH_2 group, *i.e.* dapsone is the best compound in this series. One possible criticism of this kind of analysis is the use of substituent constituents culled from the now extensive literature. The use of such data for substituents which are remote from other parts of the molecule has generally been found to be acceptable. The activity of the analogue [1, R =

[21] J. K. Seydel, M. Richter, and E. Wempe, *Int. J. Lepr.*, 1980, **48**, 18.
[22] J. K. Seydel and E. G. Wempe, *Int. J. Lepr.*, 1982, **50**, 20; V. M. Kulkarni and J. K. Seydel, *Chemotherapy*, 1983, **29**, 58.
[23] E. Freerksen and M. Rosenfeld, *Chemotherapy*, 1977, **23**, 356; E. Freerksen, M. Rosenfeld, E. Bonnici, G. de Pasquale, and M. Kruger-Theimer, *Chemotherapy*, 1978, **24**, 187.
[24] S. Tsutsumi and M. Gidoh, *Int. J. Lepr.*, 1985, **53**, 714.
[25] F. Rossi, P. Dri, P. Bellavite, G. Zabucchi, and G. Berton, 'Oxidative Metabolism of Inflammatory Cells' in 'Advances in Inflammation Research', ed. G. Weissmann, B. Samuelsson, and R. Paoletti, Vol. 1, Raven Press, 1979, p. 139.
[26] Martindale Extra Pharmacopoeia 28th Edition, ed. J. F. Reynolds, Pharmaceutical Society Press, 1982, p. 1492.
[27] C. E. Orzeeh, N. G. Nash, and R. D. Daley in 'Analytical Profiles of Drug Substances,' ed. K. Florey, Vol. 5. Academic Press, 1976, p. 87.
[28] L. Doub in 'Medicinal Chemistry', Vol. 5, ed. W. H. Hartung and E. E. Campaogne, Wiley, 1961, chapter 2, p. 350.
[29] D. Bawden and M. S. Tute, *Eur. J. Med. Chem.*, 1981, **16**, 299.

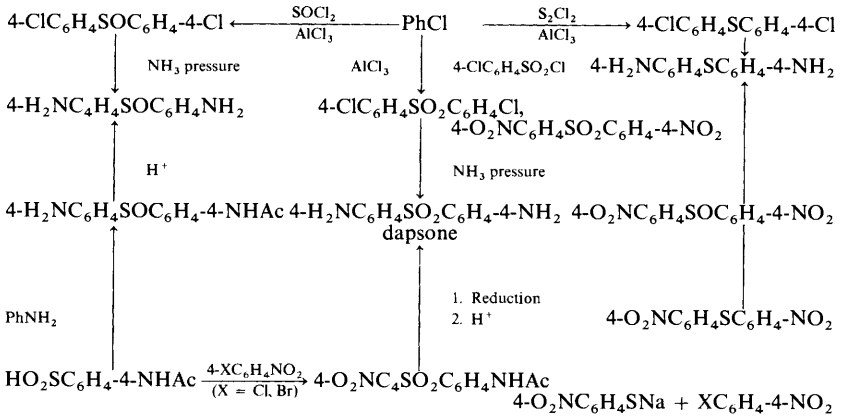
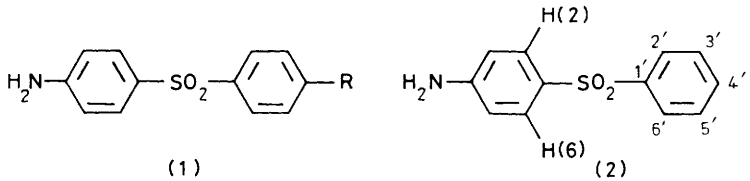


Figure 4 *A summary of synthetic routes to sulphones*



$NH(CH_2)_2OH$] was reasonably well predicted from this equation: MIC calc. 20.2 nmole ml^{-1}, observed 13.7 nmole ml^{-1}. The second analysis[30,31] using experimental parameters gave equations 2 and 3 against cell-free extracts of *M. smegmatis* and *M. lufu*. Δ p.p.m. H (2/6) correlates well with the Swain–Lupton R values (r = 0.924) or Hammett σ values (r = 0.956), and k' with the Hansch π values (r = 0.993). Hansch values are alternative substituent constants expressing the hydrophobicity of a substituent in a manner directly analogous to fragmental constants (f).[32] These equations are exactly analogous to equation 1 and can be interpreted in the same way. In equation 3 four compounds, which are highly ionized (R = CO_2H, $NHCH_2CO_2H$, OH), were excluded. However in this equation the k' term now becomes insignificant, and equation 4 incorporating only the electronic term is just as good. The authors proposed that the hydrophobic term k' was simply a measure of the ionization of the four excluded compounds. They replaced the k' term by an ionization term I_{ion}(I_{ion} = 1 when R = CO_2H,

[30] E. A. Coats, V. M. Kulkarny, A. K. Saxena, H. P. Cordes, and J. K. Seydel, 'Proceedings of XIIth International Leprosy Congress, New Delhi, 1984', Printaid, 1986, p. 666.

[31] J. K. Seydel, E. A. Coats, H. P. Cordes, M. Richter, H. Weise, and V. M. Kulkarni in 'Quantitative Approaches to Drug Design', ed. J. C. Deardon, Elsevier, 1983, p. 253.

[32] C. Hansch and A. Leo, 'Substituent Constants for Correlation Analysis in Chemistry and Biology', Wiley, 1979.

Table 1 *(A) QSAR equations describing the activity of sulphones against* M. smegmatis *and* M. lufu

Biological variable	*Coefficients and physico-chemical parameters*	*Constant*	*n*	*r*	*F*	*Equation No.*
log 1/MIC	$-1.22R - 0.21f$	−2.22	16	0.86	17.87	1
log $1/I_{50}$	-6.96Δ p.p.m. $- 0.16k'$	−0.68	17	0.86	20.4	2
log $1/I_{50}$	-7.50Δ p.p.m. $- 0.015k'$	−0.96	14	0.896	22.5	3
log $1/I_{50}$	-7.54Δ p.p.m.	−0.98	14	0.896	49.0	4
log $1/I_{50}$	-7.09Δ p.p.m. $- 0.69I_{ion}$	−0.95	17	0.899	29.8	5
log $1/I_{50}$	-6.89Δ p.p.m. $- 0.63I_{ion}$	−0.97	17	0.890	28.0	6
log $1/I_{50}$	-7.23Δ p.p.m. $- 0.57I_{ion}$	−1.02	17	0.966	99.5	7
log $1/I_{50}$	-9.36Δ p.p.m. $- 0.59I_{ion}$	−1.11	17	0.909	33.0	8
(B) Correlation of intramolecular conformational entropy (S) with biological and physico-chemical parameters						
p*C*	-5.91Δ p.p.m. $+ 0.507fi + 0.047MR(2)$	−5.06	36	0.90	47.6	9
p*C*	$-0.0075S$	+6.30	36	0.97	544.6	10
S	776Δ p.p.m. $- 59.8fi - 6.1\ MR(2)$	+167.8	36	0.91	52.2	11
(C) Correlations involving chemical shifts νSO_2*, and calculated charge densities,* Δq						
ap_E	$-0.29\ \ \Delta q(NH_2)$	+11.88	23	0.879	71.41	12
ap_E	$-0.121\ \Delta q(O)$	+13.57	23	0.942	166.13	13
ap_E	$-2.921\ \Delta\delta(NH_2)$	+2.00	27	0.884	89.38	14
ap_E	$-0.084\ \nu(SO_2)$	+95.58	28	0.852	68.96	15
ap_E	$-0.049\ \nu(SO_2)$	+58.49	48	0.810	87.92	16

Biological parameters: MIC = minimal inhibitory concentration (moles l^{-1}), I_{50} = concentration of drug (moles l^{-1}) causing 50% inhibition of the enzyme; p*C* = log $1/I_{50}$ (μ moles l^{-1}).

Physico-chemical parameters: *R* = Swain–Lupton resonance constant; *f* = Rekker's hydrophobic fragmental constant; Δ p.p.m. = difference in chemical shifts of H(2) and H(6) protons in the substituted compound and the reference compound (2; R(2) = R(3) = R(4) = H); *k'* = capacity factor determined by reverse-phase hplc.

Statistical terms: *n* = number of observations; *r* = correlation coefficient; *F* = *F* ratio, Fisher significance test. The usual significance level for these equations is 95%, from statistical tables the cut-off *F* values are: $F_{2,11} = 3.98$, $F_{2,13} = 3.80$, $F_{2,14} = 3.74$, $F_{1,21} = 4.32$, $F_{1,25} = 4.24$, $F_{1,26} = 4.22$, $F_{3,32} = 2.90$, $F_{1,34} = 4.13$, $F_{1,46} = 4.05$.

ap_E = log $1/EII_{50}$ = concentration of drug causing 50% inhibition of enzyme ÷ concentration of *p*-aminobenzoate; Δq = total net charge with respect of NH_2 protons of *p*-aminobenzoate; $\nu(SO_2)$ = symmetric stretching frequency of SO_2 group.

Reproduced by permission from C. Hansch and A. Leo, 'Substituent Constants for Correlation Analysis in Chemistry and Biology', Wiley, 1979; and R. F. Rekker, 'The Hydrophobic Fragmental Constant', Elsevier, 1977.

$NHCH_2CO_2H$, and 0.43 when R = OH) and obtained a slightly improved correlation, equation 5. The very similar form of equations 1 and 2 indicates that there is ready access to the target enzyme in whole cell systems, although perhaps the more significant hydrophobic term in equation 1 compared with equations 3 and 4 may indicate that a transport factor plays some part in the whole cell system.

A further interesting observation is expressed in equation 6 which correlates p.p.m. and k' with inhibition of the enzyme obtained from dapsone-resistant strains of *M. smegmatis*. There is no significant difference between equations 5 and 6, showing that the enzyme from resistant cells binds the drugs in the same way and to the same extent as sensitive cells. Resistance to dapsone must therefore be a result of altered permeability of the cell wall to dapsone or due to gene amplification giving rise to greater production of the target enzyme. However, since whole cell systems show a similar dependence on Δ p.p.m. H (2/6), resistance is due to increased enzyme production. This agrees with the clinical observation that dapsone resistance can sometimes be overcome by increasing the dose of the drug.

Using the same series of compounds against *M. lufu* generated similar equations for both sensitive (equation 7) and resistant (equation 8) strains. It also appears from these relationships that pteroate synthetase from *M. smegmatis* is to all intents and purposes just as good a model for sulphone activity as that obtained from *M. lufu*.

Recently a QSAR study of a more extended series of thirty-six sulphones (2), including all those in the previous studies, has been published.[33a] This used both linear free energy and molecular modelling methods. The linear free energy relationships were best expressed by equation 9 (Table 1B) which includes a molar refractivity term for the substituent R(2)′ [*M*R(2)′]. The molecular modelling programme identified a simple and comprehensive relationship between the intramolecular conformational entropy *S* (equation 10) which also correlated highly with the independent variables in equation 9 (see equation 11). This last equation indicates that as ionization and/or molar refraction (which measures both the volume and polarizability of the substituent) increase then *S* decreases. This would be expected since conformational flexibility would be decreased by either an increase in substituent size or an increase in charge which could possibly give rise to intramolecular bonding effects. The dependence on Δ p.p.m. is less clear. Molecular modelling indicates that there is more than one optimal conformation of these highly flexible molecules involving rotations round the S–C bonds of $\varphi_1 = 60°$, $\varphi_2 = 30°$ (Figure 5). The more densely populated are the active conformations the more active the compound. The authors suggested that interaction with the receptor may involve a two-step process in which an initial weak association is followed by a stronger interaction which occurs most readily when the molecule exists in the preferred 'active' conformation. It is of interest also that the most active compounds in this series are slightly more active than dapsone, the best being 2-chloro-4-amino-4′-aminodiphenyl sulphone. Another very recent paper[33b]

[33a] R. L. Lopez, R. A. Pearlstein, A. J. Hopfinger, and J. K. Seydel, *J. Med. Chem.*, 1987, **30**, 900.
[33b] P. G. De Benedetti, D. Iarossi, C. Menziani, V. Caiolfa, C. Frassineti, and C. Cennamo, *J. Med. Chem.*, 1987, **30**, 459—464.

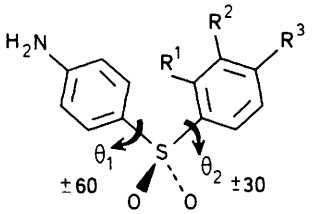
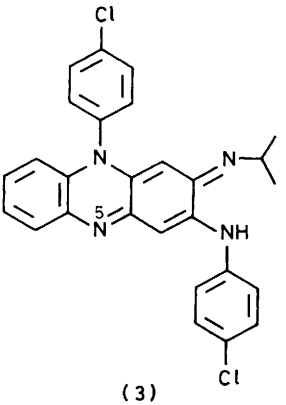


Figure 5 *Active conformations of diphenylsulphones*

describes a wide variety of SARs in a set of thirty-three 4′-2′,4′- and 2′,4′,6′-substituted 4-aminodiphenylsulphones with inhibitory activity against the dihydropteroate synthetase of *E. coli*. Both experimental n.m.r. (δ NH_2) and infrared stretching frequencies (ν, SO_2 symmetric) and calculated (CNDO/2) net total charge differences, Δq, at the amino N-atom, C(1),CH(2,6),CH(3,5),C(4) and sulphone O-atoms were used. The reference compound in this work was 4-aminobenzoate, the natural substrate of the enzyme. There were good correlations between many of the independent variables, in particular δNH_2 and $\nu(SO_2)$, $r = 0.84$, and $\Delta q(NH_2)$ and $\Delta q(O)$, $r = 0.90$. The best single parameter equations were between the biological activity, expressed as ap_E values, and the calculated charge densities on (i) the NH_2 group (equation 12), (ii) the oxygen atoms of the sulphone group (equation 13). Very similar correlations (equations 14 and 15) were found between the experimental variables $\Delta\delta NH_2$ and νSO_2 (sym) stretch. For various reasons *e.g.* ionization, lack of experimental parameters, the final number of compounds in each equation was less than the total number. This study was further extended to a combined group of twenty-eight sulphones and twenty sulphonamides. The best correlation was with $\nu(SO_2)$ (equation 16). The different correlations expressed in Table 1 are in fair agreement and support the general qualitative hypothesis, of Bell and Roblin,[33c] that the intensity of the negative charge on the sulphone oxygen atoms is the single key feature of these compounds. The identification of dapsone as the most active sulphone is also consistent with this data.

B. Iminophenazines.—Clofazimine (3), B663, was selected from a large number of deeply coloured, substituted iminophenazines. They are sometimes referred to as rimino (a contraction of R-imino) compounds or riminophenazines. The powerful anti-mycobacterial action of these compounds was first described in 1957. A recent review[1] summarizes their development up to 1983. Clofazimine is a very unusual compound. It is a highly hydrophobic molecule with an estimated log $P \sim 7.48$[34] (octan-1-ol/water), but measured in isoctane/buffer pH 5.15 as log $P = 5.01$.[35]

[33c] P. H. Bell and R. Roblin, Jr., *J. Am. Chem. Soc.*, 1942, **64**, 2405.
[34] N. E. Morrison and G. M. Marley, *Int. J. Lepr.*, 1976, **44**, 133.
[35] E. B. Canavan, A. G. Esmonde, J. P. Feely, J. M. Quigley, and R. F. Timoney, *Eur. J. Med. Chem.*, 1986, **21**, 199.



(3)

There have been reported p*K*a values of 8.35[36] and 8.37.[35] The discrepancies between the calculated and measured log *P* values are surprisingly large, but at pH 5.15 clofazimine would be totally ionized. The results from other workers suggest a somewhat lower ionization constant for clofazimine.[37] The preparation of clofazimine and its analogues involves precipitation with ammonia,[38] (p*K*a 9.25) which is a significantly stronger base. Clofazimine will therefore be substantially ionized at physiological pH 7.2. Furthermore it is known to accumulate in macrophages where the intracellular pH can be as low as 6.0. The most likely site of protonation is N(5). The unusual physio-chemical properties of clofazimine are reflected in its pharmacokinetics.[1,39] The drug is poorly absorbed from the gastro-intestinal tract unless presented in a micronized form. It accumulates in the body and appears in skin (causing a reddening and darkening). It is deposited in the white of the eye and crystals have been found in macrophages and the cells of the gastro-intestinal tract. A two-compartment pharmacokinetic model giving half lives ($t_{\frac{1}{2}}$) of 7 and 70 days has been proposed. Resistance to the drug has been reported and a need is now recognized for other compounds which will overcome this resistance. Laboratory strains of *M. smegmatis* which are resistant to the drug have been developed for screening purposes. The mechanism of action of clofazimine is not established. It binds externally to cytosine–guanine DNA base pairs *in vitro*.[34,36] The binding has been shown to be to guanine residues only. The DNA of *M. leprae* has a high guanine–cytosine content. The redox properties of clofazimine can divert up to 20% of cellular oxygen[40] and disrupt normal mitochondrial oxidation processes.[37] It has also been proposed that cytotoxic oxygen species, hydrogen peroxide and superoxide, are generated as a consequence

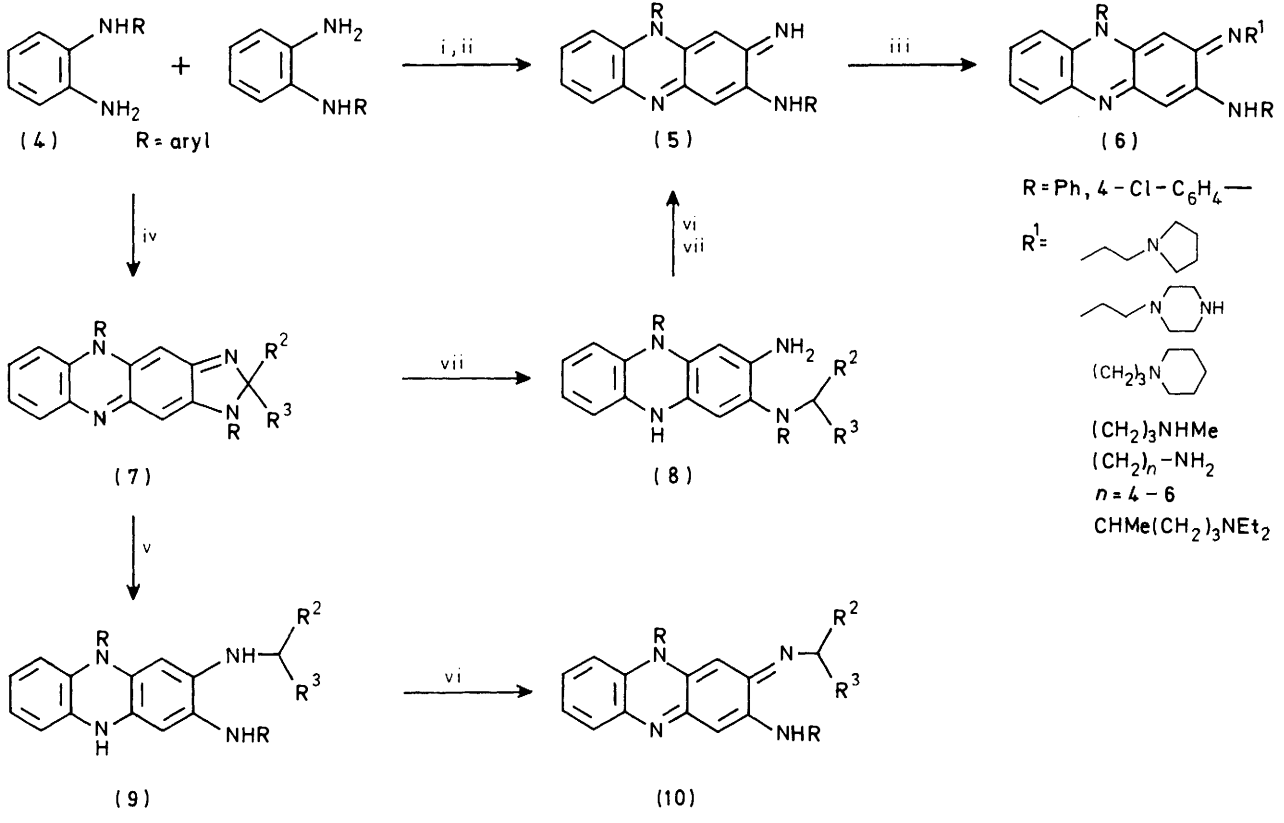
[36] N. E. Morrison and G. M. Marley, *Int. J. Lepr.*, 1976, **44**, 475.
[37] P. M. Rhodes and D. Wilkie, *Biochem. Pharmacol.*, 1973, **22**, 1047.
[38] J. F. O'Sullivan, *J. Chem. Res.* (*S*), 1984, 52.
[39] L. Levy, *Am. J. Trop. Med. Hyg.*, 1974, **23**, 1097.
[40] V. C. Barry, J. G. Belton, M. L. Conalty, J. M. Denneny, D. W. Edward, J. F. O'Sullivan, D. Twomey, and F. Winder, *Nature* (*London*), 1957, **179**, 1013.

of the drug's presence.[1,41] Such a process occurring inside the macrophage would enhance the killing of bacilli which are also found in macrophages. It has been reported that macrophages growing in the presence of clofazimine have greatly increased lysosomal granules, which are associated with more 'aggressive' destructive activity.[42] Clofazimine is an example of a lysosomotropic agent. Such compounds are actively transported into the lysosomes of macrophages possibly by a receptor-mediated endocytic process involving a carrier protein.[1,35] A variety of weak to moderate organic bases are also known to accumulate in lysosomes.[43] Some of these exert an anti-inflammatory action of value in the treatment of rheumatoid arthritis, possibly by inhibition of lysosomal enzymes *e.g.* chloroquine.[44] The useful anti-inflammatory activity of clofazimine may depend on the same kind of mechanism. Its anti-inflammatory activity is of value in leprosy since it suppresses erythema nodosum leprosum (ENL), an extensive inflammatory reaction occurring during treatment. A study in animal models of rheumatoid arthritis has shown it to be of potential value in this disease.[45]

(i) *Synthesis.* The synthetic routes to riminophenazines are outlined in Figure 6. The earlier syntheses[1] proved difficult to carry out and frequently gave variable yields of products which were difficult to handle. Recent modifications of these synthetic routes have been described which give reproducible and high yields.[38] Essentially, *N*-aryl *ortho*-phenylenediamines (4) undergo regiospecific oxidative dimerization to yield the parent iminophenazines (5) which react further with alkylamines to give substituted iminophenazines (6). Alternatively, oxidation with benzoquinone in the presence of a carbonyl compound gives an imidazolophenazine (7) which may be reduced with cleavage of the imino substituent (8) followed by subsequent aerial oxidation to the parent iminophenazine (5). A more selective reduction results in an alternative cleavage of the imidazoline ring (9) which after oxidation gives a substituted iminophenazine (10). The type of catalyst used in the reduction of these compounds is crucial and, rather surprisingly, allows full control of the reactions.

(ii) *Structure–Activity Relationships* (*SAR*). The earlier SAR studies have been reviewed.[1] Recently, *X*-ray crystallographic studies have been reported.[46] The critical importance of the bond angles round the trigonal N(3) atom in clofazimine and its analogues (Figure 7) form the basis of a simple SAR. However, only angle α can be precisely defined from the *X*-ray structures. In a series of *in vitro* active compounds it was found that α = 129.7—132.7° [av. 131.1(10)°] whilst in inactive compounds the values for α were 125.1—126.4° [av. 125.6(7)°]. The larger angle in

[41] C. E. J. van Rensberg, E. M. S. Gatner, F. M. J. H. Inkamp, and R. Anderson, *Antimicrob. Agents Chemother.*, 1982, **21**, 693; Y. Niwa and M. Ozaki, *J. Clin. Microbiol.*, 1984, **20**, 837; B. M. Zeis, R. Anderson, and J. F. O'Sullivan, *Antimicrob. Agensts Chemother.*, 1987, **31**, 789.
[42] J. Sarracent and C. M. Finlay, *Int. J. Lepr.*, 1984, **52**, 154.
[43] R. T. Dean, W. Jessup, and C. R. Roberts, *Biochem. J.*, 1984, **217**, 27.
[44] N. P. Hurst, J. K. French, A. L. Bell, G. Nuki, M. L. O'Donnell, W. H. Betts, and L. G. Cleland, *Biochem. Pharmacol.*, 1986, **35**, 3083.
[45] H. L. F. Currey and P. D. Fowler, *Br. J. Clin. Pharmacol.*, 1972, **45**, 676.
[46] U. Rychlewska, M. B. H. Broom, D. S. Eggleston, and D. J. Hodgson, *J. Am. Chem. Soc.*, 1985, **107**, 4768.



Reagents: i, $FeCl_3$, H^+; ii, NH_3; iii, R^1NH_2, alkylamines; iv, benzoquinone/carbonyl compound R^2COR^3; v, Pt O_2/H or Pt/C (10%)/H_2; vi, air; vii, Pd/C (10%)/H_2

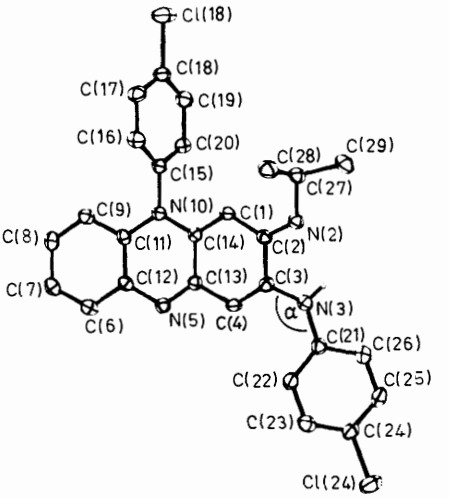
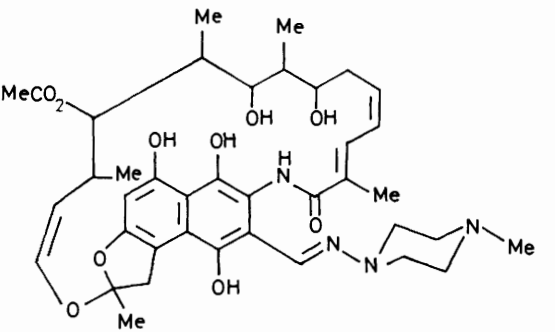


Figure 7 *Crystal structure of clofazinine* (3)
(Reproduced by permission from *J. Am. Chem. Soc.*, 1985, **107**, 4768)

the active compounds is thought to favour the formation of an intramolecular hydrogen bond between N(3)–H • • • N(2). The capacity to form an intramolecular hydrogen bond was interpreted as evidence of a capacity for intermolecular hydrogen bonding in solution. In particular, binding to DNA *via* O(6) and N(1)–H of guanine and N(3)–H and N(2) of clofazimine. An interesting feature of the crystal structure is the dihedral angle 80—90° between the phenyl ring at N(10) and the phenazine ring. This provides a sound explanation of the failure of clofazimine to intercalate between base pairs in DNA.



(11)

Very recently[47] new analogues of clofazimine designed (i) to be active against resistant organisms, (ii) not to accumulate in body fat and other tissues, (iii) to be evenly, rapidly, and comprehensively absorbed from the gastro-intestinal tract, and (iv) not to crystallize within cells have been reported. These compounds, representative examples are given in (6) (Figure 6) involve substitution at the imino nitrogen atom by an unbranched alkyl or branched alkyl chain bearing a primary, secondary, tertiary, or alicyclic amino group. Generally the p*K*a values of such amine-containing side chains are ~9.5—10.5 ensuring that these molecules will always be very substantially ionized under physiological conditions. To balance this increased hydrophilicity the aliphatic part of the substituents usually contains 6—8 hydrophobic methylene groups. It is interesting to note that two earlier compounds of similar structure had also shown good activity against *M. leprae* but were much less active against *M. tuberculosis*.[48] A recent QSAR study on a range of clofazimine analogues failed to find any relationship between lipophilicity or the steric features of the *N*-imino substituent and accumulation of these compounds in the spleen.[35] This is not surprising as a rather selective protein binding/transport mechanism appears to operate with these materials.

C. Rifamycins.—The development of rifampicin (11), a semi-synthetic compound, from the naturally occurring rifamycins produced by *Nocardia mediterranea* has been described in several reviews[1,49,50] which also summarize the very large number of other derivatives prepared and tested against a variety of gram positive and gram negative bacteria, mycobacteria, and viruses. Rifampicin is a broad spectrum antibiotic with a powerful bactericidal action against both *M. leprae* and *M. tuberculosis*. It was first introduced into the treatment of leprosy in 1970.[51] Rifampicin has a good pharmacokinetic profile except for a short half-life, $t_{\frac{1}{2}} \sim 3$ hours,[1] and has several undesirable side-effects, the most dangerous being hepatotoxicity. It is also a potent inducer of liver enzymes responsible for drug metabolism and can, theoretically at least, disrupt concurrent drug treatments, *e.g.* oral contraceptive cover. Because of these short-comings the drug has to be given and monitored under clinical supervision. Resistance to rifampicin emerges fairly quickly when it is used as the sole chemotherapeutic agent. Its use is now restricted to multi-drug regimens.[1,19,20,52] Rifampicin acts specifically by selectively binding to the subunit of bacterial DNA-dependent RNA polymerase leading to a massive

[47] M. L. Conalty, N. E. Morrison, and J. F. O'Sullivan, 'Proceedings of IVth European Leprosy Symposium, Genoa 1986', *Quaderni di Cooperazione sanitaria – Health Cooperation Papers*, 1987, in press.
[48] L. Levy, *Lepr. Rev.*, 1981, **52**, 23.
[49] M. Brufani 'The Ansamycins' in 'Topics in Antibiotic Chemistry', ed. P. Sammes, Vol. 1, Ellis-Horwood, 1977, p. 91.
[50] G. Lancini and W. Zanichelli in 'Structure–Activity Relationships among the Semi-synthetic Antibiotics', ed. D. Perlman, Academic Press, 1977, p. 531; W. Wehrli and M. Staehlin, 'Rifamycins and other Ansamycins,' in 'Mechanism of Action of Antimicrobial and Antitumour Agents, Antibiotics Volume III', ed. J. W. Corcoran and F. E. Hahn, Springer-Verlag, 1975, p. 252.
[51] R. J. W. Rees, J. M. H. Pearson, and M. F. R. Waters, *Br. Med. J.*, 1970, i, 89.
[52] C.-C. Guelpa-Lauras, J. H. Groeest, M. Constant-Desportes, and G. Brucker, *Int. J. Lepr.*, 1984, **52**, 101.

failure in protein synthesis.[49,50] Other DNA-dependent enzymes are also affected and with some compounds are the principal target.[53] This holds out the possibility of developing new derivatives which will be active against rifampicin-resistant strains of mycobacteria. There is renewed interest in rifamycin derivatives some of which, including rifabutine, Figure 8, are active against the HIV-1 virus[49,50,54] responsible for the destruction of some human T-cells and leading to AIDS. Whilst rifampicin is a very good anti-mycobacterial drug, it is thought that compounds with longer half-lives would be more suitable for the treatment of leprosy.

(i) *Synthesis.* The earlier reviews[1,49,50] provide a detailed coverage of the vast number of rifamycin derivatives which have been investigated. Figure 8 outlines the principal synthetic routes to the newer drugs which are being evaluated against *M. leprae.* The oxidation of the Mannich reaction product 3-diethylaminomethylrifamycin SV (12) affords 3-formylrifamycin SV (13). The aldehyde function can then be condensed with various amines, oximes, hydrazines, and other carbonyl reagents.[49,50,55] Rifampicin (11), rifapentine (14), also known as DL473, and FC 22,250 (15) are all prepared in this way. Oxidation of rifamycin SV (16, R = H) affords rifamycin S (17, R = H) which readily reacts with ammonia, aliphatic amines, and other nucleophiles to give the hydroquinones (16) including the piperazinyl compound R-76-1 (18) which is currently being evaluated in the treatment of leprosy (see ref. 61). The 3-bromo compound (19) is a useful intermediate. The hydroquinones (16) and the quinones (17) are readily interconverted. 3-Aminorifamycin S (17, R = NH_2) on further treatment with ammonia gives the quinoneimine (20). This reacts with cyclic ketones to yield spiroimidazolinerifamycin S derivatives (21) including rifabutin(e) (22). With aldehydes imidazolorifamycin SV derivatives (23) are formed. 3-Bromorifamycin S (19) reacts with 2-aminopyridine to give the zwitterionic pyridoimidazorifamycin SV compound (24). The total synthesis of rifampicin S has been achieved[56] and the synthetic challenge of the ansa chain with its eight continuous chiral centres is of current interest.[57] Certainly a fuller understanding of the geometry of the ansa chain and its structural consequences would be of interest. However, earlier studies involving modifications of this chain most commonly led to significant reductions in activity.[49]

(ii) *Structure–Activity Relationships.* Rifampicin (11) is a zwitterionic molecule with an isoelectric point of 4.8; in water $pKa_1 = 1.7$ (proton loss from the 8-hydroxy group) and $pKa_2 = 7.9$ (proton gain at the piperazinyl *N*-methyl atom).[1] The partition coefficient measured in a number of solvent systems gives log $P \sim 1.0$.

[53] D. Ungheri, C. Della Bruna, and A. Sanfilippo, *Drugs Exptl. Clin Res.*, 1984, **X**, 681.
[54] R. Anand, J. Moore, P. Feorino, J. Curran, and A. Srinivasan, *Lancet*, 1986, i, 97; A. M. Wuz and R. C. Gallo, *Biochem. Biophys. Acta*, 1974, **240**, 419.
[55] L. Marsili, C. R. Pasqualucci, A. Vigevani, B. Gioia, G. Schioppacassi, and G. Oronzo, *J. Antibiot.*, 1981, **XXXIV**, 1033.
[56] H. Nagaoka, W. Rutsch, G. Schmid, H. Iio, M. R. Johnson, and Y. Kishi, *J. Am. Chem. Soc.*, 1980, **102**, 7962; H. Iio, N. Nagaoka, and Y. Kishi, *J. Am. Chem. Soc.*, 1980, **102**, 7967.
[57] W. R. Rousch and A. D. Palkowitz, *J. Am. Chem. Soc.*, 1987, **109**, 953 and references cited therein.

Table 2 *Plasma* ($\mu g\ ml^{-1}$) *and tissue distribution* ($\mu g\ g^{-1}$) *of rifabutine and rifampicin*

	Plasma	*Liver*	*Lung*	*Spleen*	*Kidney*
Rifampicin	7	140	18	15	35
Rifabutine	2	60	85	48	40

However, the hydrophobic properties of most rifamycin derivatives are most frequently correlated with R_m values calculated from R_f values determined by reverse-phase thin layer chromatography. Rifapentine[58] (14) and FC 22,250 (15) are simple analogues of rifampicin and, like R-76-1 (18), are more hydrophobic than the parent compound. The spiro compound rifabutine, LM 427, (21) which is sometimes, confusingly, referred to as ansamycin[59] is of particular interest and also has increased hydrophobicity.[60,61] The *in vitro* biological activity of many rifamycins show a rather flat parabolic relationship with hydrophobicity measured by R_m values.[49] Such correlations allow the identification of optimal structures. However, *in vivo* pharmacokinetic factors such as plasma half-life, volume of distribution, and variations in tissue distribution can determine the selection of the best compound[60] (Table 2). These more hydrophobic compounds selectively accumulate in lung, liver, and spleen. The increased concentration in lung tissue is of obvious importance in treating pulmonary tuberculosis, whilst accumulation in the spleen may be advantageous in the treatment of leprosy. However, high concentrations of these drugs in the liver could indicate an increased hepatotoxicity.

The activity of rifabutine, LM427, against the 'atypical' *M. avium*[62] and *M. intracellulare*[59] and HIV-1[54] has also been reported. The importance of this for AIDS patients remains to be evaluated (see Section 6). A recent extension of the selective distribution of rifamycin derivatives is provided by the pyridoimidazo-rifamycin SV derivatives (24)[63] (Figure 8). These compounds are useful broad spectrum antibiotics which, because of their zwitterionic nature, are not absorbed from the gut. They are still, however, able to cross the membranes of bacterial cells and destroy them. Interestingly, the simple quaternary compounds (16, R = $\overset{+}{N}Et_3$) were unable to cross whole cell membranes and therefore had little *in vitro* activity. Nevertheless they strongly inhibit the target enzyme in broken cell systems.

[58] V. Arioli, M. Berti, G. Carniti, E. Randisi, E. Rossi, and R. Scotti, *J. Antibiot.*, 1981, **XXXIV**, 1026; J.-Y. You, *Kangshengsu*, 1985, **10**, 223; Ji Baohong, Chen Jiakun, Lu Xizhen, Wang Shiyu, Ni Guoxing, Hou Yuhong, Zhou Daohai, and Tang Quankui, *Int. J. Lepr.*, 1986, **54**, 563; S. R. Pattyn, *Antimicrob. Agents Chemother.*, 1987, **31**, 134.

[59] V. K. Perumal, P. R. J. Gangadharam, L. B. Heifets, and M. D. Iseman, *Am. Rev. Respir. Dis.*, 1985, **132**, 1278.

[60] A. Sanfilippo, C. Della Bruna, L. Marsili, E. Morvillo, C. R. Pasqualucci, G. Schioppacassi, and D. Ungheri, *J. Antibiot.*, 1980, **XXXIII**, 1193.

[61] WHO Report of the 5th meeting of the *ad hoc* drug development subgroup of the Scientific Working Group on the Chemotherapy of Leprosy TDR/THELEP/DRUG DEV/85.3 1985.

[62] L. B. Heifets, M. D. Iseman, P. J. Lindholm-Levy, and W. Kanes, *Antimicrob. Agents Chemother.*, 1985, **28**, 570.

[63] E. Marchi, L. Montecchi, A. P. Venturini, G. Mascellani, M. Brufani, and L. Cellai, *J. Med. Chem.*, 1985, **28**, 960.

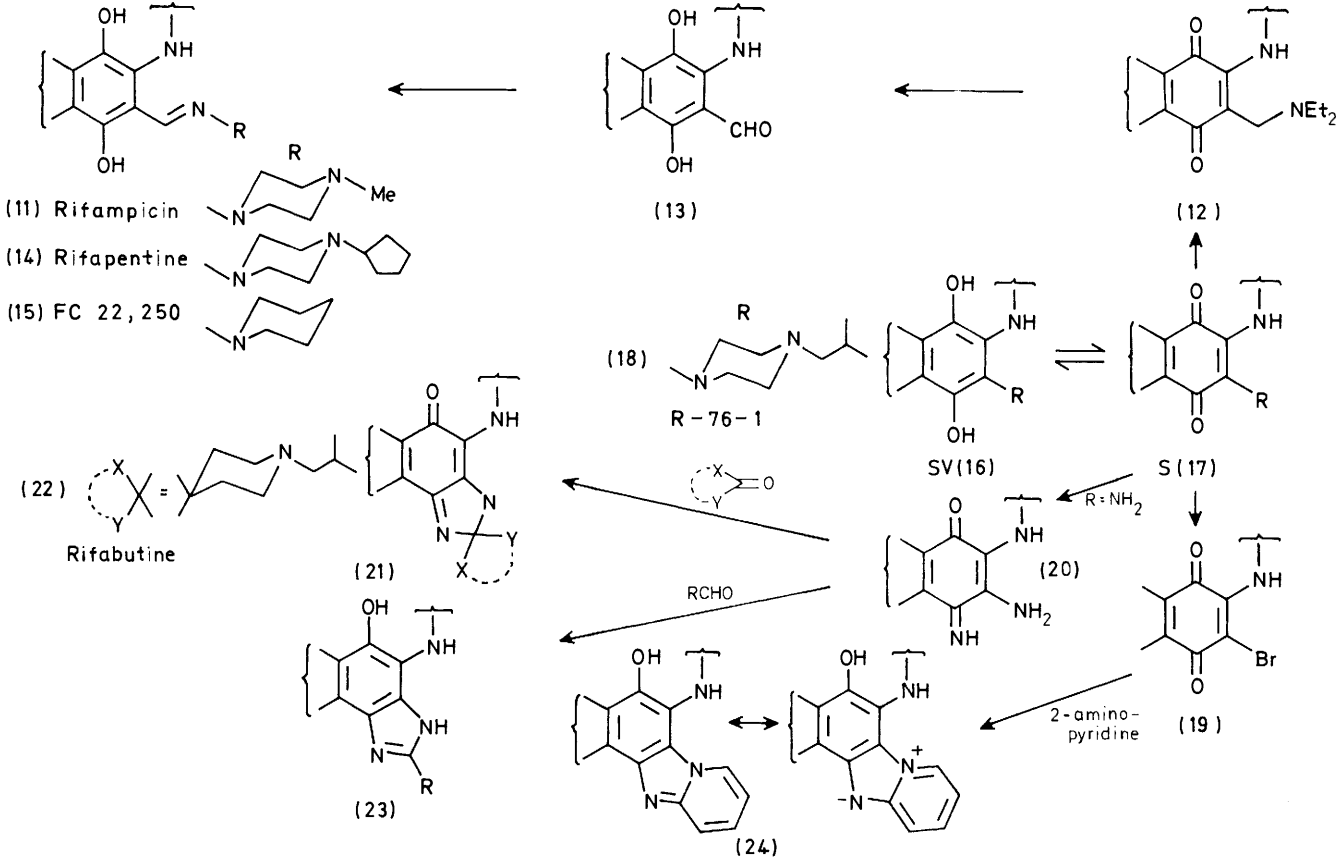
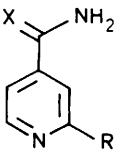


Figure 8 *Principal synthetic transformations of rifamycins*



(25)

	X	R
(a)	S	Et
(b)	S	Pr^n
(c)	S	H
(d)	S	Bu^t
(e)	S	NMe_2

(26)

	X	R
(a)	S→O	Et
(b)	S→O	Pr^n

(27)

	X	R
(a)	=N—CN	Et
(b)	=N—CN	Pr^n

4 Second-line Drugs

The three groups of drugs in this category are all old thiocarbonyl-containing compounds.[64] They all give rise to unpleasant and sometimes dangerous side-effects, particularly hepatotoxicity and blood dyscrasias. Only a small number of articles on these compounds have appeared since the quite recent review.[1]

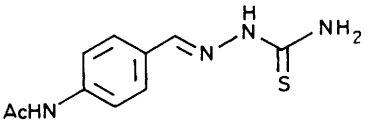
A. Thioamides.—Ethionamide (25a) and prothionamide (25b) are the most popular second-line drugs. They both have some bactericidal properties[65] and being cheap form part of multi-drug regimens in several parts of the world. Both drugs have short half-lives ~3 h and are metabolized to the slightly longer acting sulphoxide derivatives (26a,b).[66] Increasingly prothionamide (25b) is being preferred because of its lower systemic toxicity.[67]

(i) *Synthesis.* The preparation of these compounds is by long established and conventional methods.[68]

(ii) *Structure–Activity Relationships.* Only a very brief SAR study, in the mouse footpad, has been reported. This indicates that ethionamide and prothionamide (25a,b) are the best compounds in this series.[67] However, the unsubstituted compound (25c) was nearly as active but both the t-butyl and dimethylamino compounds (25d,e) showed very little activity. The replacement of the sulphur atom by the bioisosteric cyanoimino group[69] (27a,b) destroyed all activity[70,71] despite its established equivalence in other systems.[69]

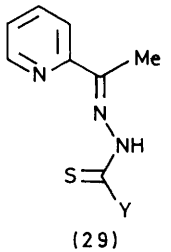
[64] 'Medicinal Chemistry', ed. A. Burger, 3rd edition, Part 1, Wiley, 1970.
[65] R. J. W. Rees, *Int. J. Lepr.*, 1987, **55**, 11.
[66] J. H. Peters, G. Ross, and J. F. Murray, Jnr., *Int. J. Lepr.*, 1983, **51**, 54.
[67] C. C. Shepard, P. J. Jenner, G. A. Ellard, and R. D. Lancaster, *Int. J. Lepr.*, 1985, **53**, 587.
[68] N. V. Sedgwick, 'The Organic Chemistry of Nitrogen', 3rd edition revised I. T. Millar and H. D. Springall, Clarendon Press, 1966, p. 248.
[69] C. A. Lipinski, 'Biososterism in Drug Design' in *Annu. Rep. Med. Chem.*, ed. D. M. Bailey, Vol. 21, Academic Press, 1986, p. 286.
[70] M. Hooper, B. W. Allen, and D. F. Self, XIIth International Leprosy Symposium, Delhi, 1984, Abstract 420.
[71] C. C. Shepard, R. M. van Landringham, and L. L. Walker, *Lepr. Rev.*, 1983, **54**, Special Issue, 235.

B. Thiosemicarbazones.—Thiacetazone (28) is cheap and readily available. It is still widely used in the treatment of leprosy, despite its lack of bactericidal properties.[1,65] A variety of thiosemicarbazones have been investigated for antibacterial and antiprotozoal activity.[1,72–74] Of particular interest are derivatives of 2-acetylpyridine[1,72–74] and 2-acetylpyridine-*N*-oxide.[75] Representative examples of these compounds are given in structures (29)—(31).



(28)

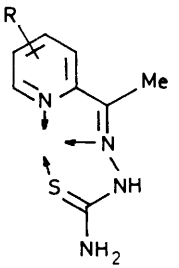
(i) *Synthesis.* Many compounds are available by the classic reaction involving a carbonyl compound and a thiosemicarbazide. Alternative routes involving carbothioates[72,76,77] can be used.



(29)

Y
(a) NH_2
(b) NHEt
(c) $NHPr^i$
(d) $NHPr^n$
(e) NHC_6H_{11}
(f) NH – 1 – adamantyl
(g) NBu^i_2
(h) $N(CH_2)_n$

$n = 3 - 6, 12$



(31)

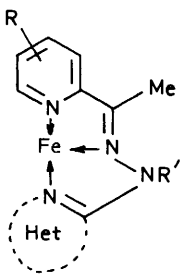
R
(a) 5 – Et
(b) 4 – Me
(c) 5 – Me
(d) 5 – OMe
(e) 6 – Me

(30) pyridine – *N* – oxides of (29)

(ii) *Structure–Activity Relationships.* The 2-acetylpyridine derivatives (29)—(31) are clearly outside the earlier SAR analyses.[1] It has been shown that the 2-acetyl but not the 3- and 4-isomers are active.[74] A study of some eighteen compounds in

[72] *Drugs of the Future*, 1982, **VII**, 38.
[73] F. M. Collins, D. L. Klayman, and N. E. Morrison, *J. Gen. Microbiol.*, 1982, **128**, 1349 and references cited therein.
[74] K.-J. Schaper, J. K. Seydel, M. Rosenfeld, and J. Kazda, *Lepr. Rev.*, 1986, **57**, Supplement 3, 254 and references cited therein.
[75] A. S. Dobek, D. L. Klayman, J. P. Scovill, and E. T. Dickson, Jnr., *Chemotherapy*, 1986, **32**, 25 and references cited therein.
[76] D. L. Klayman, J. F. Bartosevich, J. S. Griffin, C. J. Mason, and J. P. Scovill, *J. Med. Chem.*, 1979, **22**, 855.
[77] D. L. Klayman, J. P. Scovill, J. F. Bartosevich, and C. J. Mason, *J. Med. Chem.*, 1979, **22**, 1367.

the series represented by (29a—h) identified an optimal partition value, log $P \sim 3.0$, against the fast growing *M. smegmatis* but a log $P_0 \sim 4.0$ for optimal activity against a number of slow growing mycobacteria.[73] Paradoxically, the active compounds against *M. leprae* had optimal log *P* values ~ 2.0.[78] The use of calculated log *P* values in this study can be criticized. However, the general trends if not the absolute values should be valid. The azepine derivative (29h, $n = 6$) has been identified as an antimicrobial agent of the future.[72] Another interesting study including some different semicarbazones [(31) gives some representative compounds] combined an SAR and mode of action analysis. It correlated the antitumour activity of these thiosemicarbazones against ribonucleotide diphosphate reductase (RDR) with their antibacterial activity against *M. lufu*. The strong metal-chelating properties of the tridentate thiosemicarbazones (31) were thought to be responsible for their biological activities. The sterically hindered compound (31e) showed little activity in agreement with this proposal. A replacement of the thiocarbonyl group, thought to be the reason for the toxicity of these compounds, was then sought. The metal-chelating tridentate ligand of the heterocyclic hydrazones of general formula (32a) was identified and proved to be an acceptable replacement. The exact formulae of the best compounds in this series have not yet been disclosed. No relationship between biological activity and metal chelation was identified since all the compounds were powerful chelators of iron. Because a tyrosinyl radical was thought to play a key role in the mechanism of RDR it was proposed that the hydrazone N–H group might be involved in a crucial radical-forming step. In support of this suggestion replacement of this hydrogen atom by a methyl group (32b) caused a dramatic loss of activity.[74] Both the thiosemicarbazones (31a—d) and the hydrazones (32a) showed pronounced synergistic effects with dapsone and/or trimethoprim against *M. lufu* and with fluorouracil against DNA synthesis. The common actions of all these drugs is against DNA/RNA synthesis so the observed synergistic effects are not surprising.[74] The emergence of these new heterocyclic hydrazones as novel chemotherapeutic agents will depend on their having satisfactory pharmacokinetic profiles and low toxicity.



R as (31)
R′
(a) H
(b) Me

(32)

[78] N. E. Morrison and F. M. Collins, *Int. J. Lepr.*, 1981, **49**, 180.

C. Thioureas.—At present thiambutosine (33a), the established drug in this group, is not advised for the treatment of leprosy. It has a very poor pharmacokinetic profile, no bactericidal action, and a variety of toxic side-effects.[1] Nevertheless it continues to be used because it is cheap and readily available in many parts of the world.

(i) *Synthesis.* The classical methods involving amines and isothiocyanates usually provide access to most of these compounds, particularly *N,N'*-disubstituted thioureas.[79,80] An alternative method involving sequential replacement of the thioalkyl groups in dialkylthiocarbonates gives access to mixed *N,N'*-dialkyl and *N*-aryl-*N'*-alkyl thioureas.[80,81]

(ii) *Structure–Activity Studies.* There is an extensive literature on thioureas as chemotherapeutic agents but little systematic analysis of SAR has been carried out. A series of twenty-seven thioureas [(33b); representative compounds are given] active against *M. tuberculosis* have been examined and, of a wide variety of independent physio-chemical parameters examined, the Verloop steric constant L[82] gave a reasonable correlation which was significant at the 95% level (equation 17)[83]

$$\log 1/\text{MIC} = -0.06L^2 + 1.0L + 1.40 \qquad n = 27 \quad r = 0.88 \tag{17}$$

L = the Verloop steric constant which measures the length of the substituent
L_0 = 8.67 corresponding to an optimal chain length of O–n-C_4H_9 to O–n-C_6H_{13}

Thiambutosine (33a) and the related anti-tubercular drug thiocarlide (33c) are therefore close to the optimal structure for the diarylthioureas. An interesting aspect of the analysis is that only a single aryl ring bearing the substituent X (33) appears to be necessary. This is in agreement with many of the extensive earlier observations.[84] New compounds are currently being evaluated in which the following changes have been made:

(a) one arylamino group, Y–NH in (33) is replaced by a variety of other substituents,

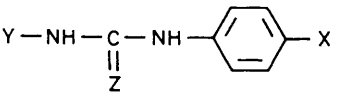
(b) the essential $-OC_6H_{13}$ side chain is replaced by a side chain of the same length but bearing more hydrophilic groups,

(c) the essential side chain is replaced by one possessing various functional groups associated with irreversible enzyme inhibitory activity.

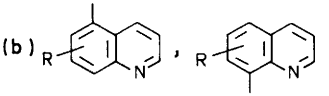
This last change was introduced because there is some early evidence that these compounds might act by inhibiting mycolic acid synthesis.[1,64]

The preliminary testing results[85] do show that the early analysis, equation 17, is still valid, and more hydrophilic groups have been introduced into these

[79] N. V. Sidgwick, ref. 68, p. 432.
[80] F. H. S. Curd, J. A. Hendry, T. S. Kenny, A. G. Murray, and F. L. Rose, *J. Chem. Soc.*, 1948, 1630.
[81] G. J. Durant, J. C. Emmett, C. R. Ganellin, P. D. Miles, M. E. Parsons, H. D. Prain, and G. R. White, *J. Med. Chem.*, 1977, **20**, 901.
[82] A. Verloop, J. Tipker, and W. Hoogenstraaten in 'Drug Design', Vol. 7, ed. E. J. Ariens, Academic Press, 1976, p. 165.
[83] M. Hooper and S. N. Kulkarni, *Br. J. Pharmacol.*, 1982, **77**, 574P.
[84] P. C. Eisman, E. A. Konopka, and R. L. Mayer, *Am. Rev. Tuber.*, 1954, **70**, 121; E. A. Konopka, T. Gisi, P. C. Eisman, and R. L. Mayer, *Proc. Soc. Exptl. Biol. Med.*, 1955, **89**, 388.
[85] M. Hooper, S. N. Kulkarni, and M. D. Yates, unpublished data.



(33)

	Y	X	Z
(a)	4-Me_2N-C_6H_4	O-n-C_4H_9	S
(b)		Me_2N, O-alkyl, Cl, Ph *etc.*	S
(c)	4-i-$C_5H_{11}O$-C_6H_4	O-i-C_5H_{11}	S
(d)	4-Me_2N-C_6H_4	OBu^n	=N—CN
(e)	4-Me_2N-C_6H_4	OBu^n	$=CHNO_2$
(f)	4-Me_2N-C_6H_4	OBu^n	$=C(CO_2Et)_2$

compounds (33) at both X and Y without significant changes in activity. Initial attempts to combine these features with a molecular fragment which replaces the toxicophoric thiourea moiety are also in hand.[85] However, the replacement of the sulphur atom by the *N*-cyano,[81,86] nitromethylene,[87] or malonylidene groups (33 d—f) led to loss of activity.[70]

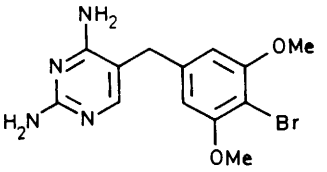
5 New Drugs and Drug Targets

Most of the new drugs have been selected from known antibacterial agents which have not previously been investigated for activity against *M. leprae* or have shown little activity in earlier tests.

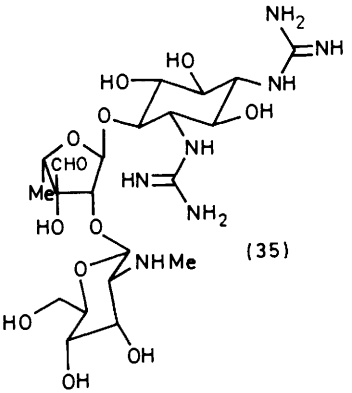
A. Dihydrofolate Reductase Inhibitors (DHFRIs).—Many early studies showed that DHFRIs had no significant effect on *M. leprae*. The synergism between dapsone and several DHFRIs was first demonstrated in cell-free systems.[1,21–23,88a] From the diaminopyrimidines available, brodimaprim (34)[88b] was selected and in combination with dapsone its clinical efficacy was demonstrated in Malta.[23,61,88a]

B. Aminoglycosides.—Streptomycin (35) was the first antituberculosis drug to be introduced into therapy. At various times it has been advocated as an effective anti-leprosy drug.[1,89] Other aminoglycosides, kanamycin, amikacin, gentamycin, and

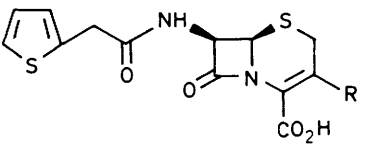
[86] A. Hantzch and M. Wolvekamp, *Justus Liebigs Ann. Chem.*, 1904, **331**, 265.
[87] R. Gomper and H. Schaefer, *Chem. Ber.*, 1967, **100**, 591.
[88a] J. K. Seydel, M. Rosenfeld, M. Sathish, M. Wiese, K.-J. Schaper, G. Hatchel, R. Haller, M. Kansy, and A. M. Dhople, *Int. J. Lepr.*, 1986, **57** Suppl. 3, 235.
[88b] *Drugs of the Future*, 1982, **7**, 93; 1985, **10**, 157.
[89] R. H. Gelber, *Int. J. Lepr.*, 1985, **55**, 78 and references cited therein.



(34)



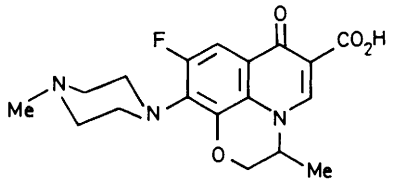
(35)



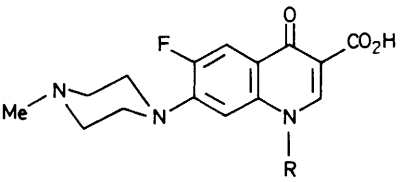
R

(36a) $-CH_2OCONH_2$

(36b) $-CH_2-N^+$



(37)



R

(38a) Et

(38b) 

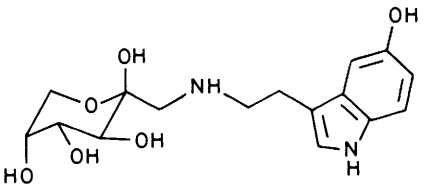
tobramycin do not appear to offer any advantage over the older drug.[89] The well known toxicity of streptomycin and the need for it to be given by injection, limit the possibilities for its use in the field.

C. β-Lactam Antibiotics.—Mycobacteria, because of the nature of their cell wall, are generally regarded as being insensitive to penicillins and other β-lactam

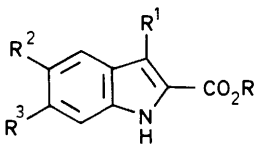
antibiotics. Recently a study of the activity of some new compounds in this class, in the mouse footpad, has been reported.[90] A number of cephalosporins were found to cause growth-delay in excess of 100 days: the best ones were cefoxitin (36a) and cephaloridine (36b).[61,90]

D. Quinolones.—There has recently been published a number of papers investigating the anti-mycobacterial activity of the third-generation quinolone derivatives.[91] The most active of these against *M. leprae* were ofloxacin (37) and pefloxacin (38a).[92–94] The latter has the better pharmacokinetic properties.[88] A study using *M. lufu* as a model organism has shown ciprofloxacin (38b) to have significant activity.[88]

E. Deoxyfructoserotonin.—This compound (39) which is a natural metabolite of serotonin (5-hydroxytryptamine), was originally prepared to study receptor pharmacology.[1] Subsequently it was shown to be active against *M. leprae*, *in vitro*, in the mouse footpad, and in man.[95,96] It has a completely novel structure for an

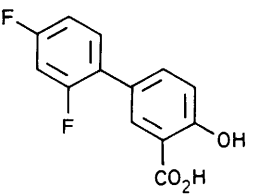


(39)



(40)

(a) $R = R^1 = R^2 = R^3 = H$



(41)

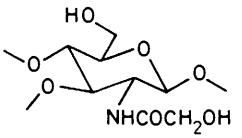
[90] C. C. Shepard, R. M. van Landringham, L. L. Walker, and R. C. Good, *Int. J. Lepr.*, 1985, **55**, 322.
[91] C. H. Collins and H. C. Uttlry, *J. Antimicrob. Chemother.*, 1985, **16**, 575; S. Davies, P. D. Sparham, and R. C. Spencer, *J. Antimicrob. Chemother.*, 1987, **19**, 605; O. J. W. Berlin, L. S. Young, and D. A. Bruckner, *J. Antimicrob. Chemother.*, 1987, **19**, 611; H. Gaya and M. V. Chadwick, *Eur. J. Clin. Microbiol.*, 1985, **4**, 345; C. Easmon and L. Verity, *Eur. J. Microbiol.*, 1987, **6**, 165.
[92] S. R. Pattyn, *Antimicrob. Agents Chemother.*, 1987, **31**, 671.
[93] C.-C. Guelph-Lauras, E. G. Perani, A.-M. Giroir, and J. H. Grosset, *Int. J. Lepr.*, 1985, **55**, 70.
[94] *Drugs of the Future*, 1985, **10**, 431; 1985, **10**, 787; 1986, **11**, 215; *Annu. Rep. Med. Chem.*, 1985, **20**, 145; 1986, **11**, 215; *Annu. Rep. Med. Chem.*, 1985, **20**, 145; 1986, **21**, 139 and references cited therein.
[95] L. Mester, L. Szabados, and M. Mester, ACS Symposium Series 215, ed. G. R. Walker and M. S. Feather, American Chemical Society, 1983, p. 451.
[96] E. J. Ambrose, N. H. Antia, T. J. Birdi, P. R. Mahadevan, L. Mester, N. F. Mistry, R. Mukherjee, and V. Shetty, *Lepr. Rev.*, 1985, **56**, 199.

anti-leprosy compound. It also inhibits mushroom tyrosinase and its structure is consistent with the hypothesis in the following section.

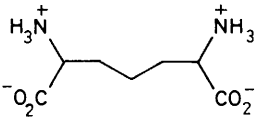
F. Tyrosinase Inhibitors.—The identification of a tyrosinase enzyme or a selective dopa uptake-oxidation system in *M. leprae* is contentious. Nonetheless this enzyme was selected as a possible target for drug development[1,97] (see Section 2B). Accordingly a series of indole analogues of general structure (40) were designed as possible inhibitors of the putative enzyme.[98a,b] These were prepared and tested *in vitro* against *M. leprae*. A number were active[98b,99] and data for indole-2-carboxylic acid (40a) have been reported.[99] A second series of compounds was developed using mushroom tyrosinase as a model system.[100] A SAR study identified substituted aromatic and heterocyclic carboxylic acids as enzyme inhibitors and from these the known anti-inflammatory drug diflunisal (41) was selected and tested. It was active *in vitro*.[101] Studies of both compounds in the mouse footpad gave contradictory results.[102] Further investigations are continuing.

G. Inhibitors of Cell Wall Development.—The cell walls of mycobacteria are distinctively different from those of other bacteria.[1,7,8] They possess *N*-glycollylmuramic acid (42), rather than *N*-acetylmuramic acid, as an alternating moiety in the peptidoglycan cell-wall polymers. The amino acid composition of the peptidoglycan also has unusual features. *meso*-Diaminopimelic acid (43) plays a distinctive role in cross-linking the peptidoglycan chains in the sacculus. It has been proposed as a target for drug design.[1] *M. leprae* also has glycine rather than alanine in the pendant pentapeptide chain of the peptidoglycan. *M. leprae* produces large quantities of highly antigenic glycolipids, (44) is a major type, which owe their antigenicity particularly to the two terminal sugar residues. These glycolipids are secreted by the bacillus to form an electron-transparent zone around it. These lipids may play an important role in protecting the organism, within the normally destructive environment of the macrophage, by inhibiting phagolysosomal fusion.[103] If production of these protective materials could be selectively inhibited it might render the bacillus more vulnerable to destruction by the normal host defence mechanisms. Chaulmoogric acid (45), an ancient bacteriostatic anti-leprosy drug, is incorporated into the cell wall lipids.[1] It provides another possible starting place for drug design.

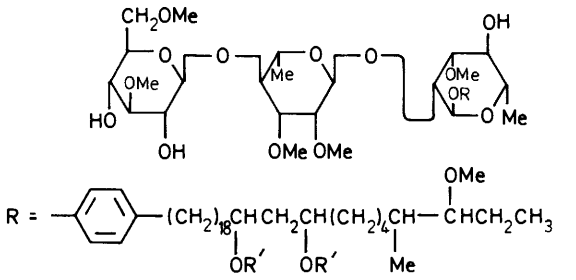
[97] M. Hooper, *Lepr. Rev.*, 1985, **56**, 57.
[98a] S. K. Yeap, Ph.D. Thesis, CNAA, 1987.
[98b] M. Hooper, P. R. Mahadevan, and S. P. Hiremath, unpublished data.
[99] P. R. Mahadevan, R. Jagannathan, A. Bhagria, S. Vejare, and S. Agarwal, *Lepr. Rev.*, 1986, **56** Suppl. C, 182.
[100] S. K. Yeap, M. Hooper, and E. G. Beveridge, *J. Pharm. Pharmacol.*, 1985, **37** Suppl. 1, 149P.
[101] E. G. Beveridge, A. Bhagria, M. Hooper, R. Jagannathan, P. R. Mahadevan, and S. K. Yeap, IVth European Leprosy Symposium Genoa, October 1986, *Quaderni di Cooperazione Sanitaria - Health Cooperation Papers*, 1987, in press.
[102] J. M. Colston, P. R. Mahadevan, and M. Hooper, unpublished data.
[103] S. W. Hunter, T. Fujiwara, and P. J. Brennan, *J. Biol. Chem.*, 1982, **257**, 15072; P. J. Brennan, *Int. J. Lepr.*, 1983, **51**, 387.



(42)

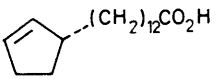


(43)



$R' = 2,4,6,8$-tetramethylhexacosan- and octacosanoate

(44)



(45)

6 Mycobacteria and AIDS

There is growing concern over the increased incidence of the so-called atypical mycobacterial infections. These infections which can mimic pulmonary tuberculosis are also frequently localized. They are due to environmental mycobacteria which are commonly non-pathogenic in man. The major organisms involved are *Mycobacterium avium*, *M. xenopi*, *M. fortuitum*, *M. chelonei*, *M. kansasii*, *M. malmoense*, *M. marinum*, and *M. intracellulare*.[104,105] These opportunistic organisms have different patterns of susceptibility to the different anti-mycobacterial agents but are frequently effectively treated by combination therapy with current drugs.[104,105] However in AIDS patients, who have severely compromised immune systems, generalized systemic mycobacterial infections

[104] E. Wolinsky, *Am. Rev. Respir. Dis.*, 1979, **119**, 107; M. D. Yates, J. M. Grange, and C. H. Collins, *J. Epidemiology and Community Health*, 1986, **40**, 295; J. M. Grange and M. D. Yates, *London Medicine*, 1987, 4; C. H. Collins, J. M. Grange, and M. D. Yates, *Medical Laboratory Sciences*, 1986, **43**, 262; A. J. France, D. T. McCleod, M. A. Calder, and A. Seaton, *Thorax*, 1987, **42**, 593; P. A. Jenkins, *Br. Med. J.*, 1987, **295**, 331.

[105] J. M. Grange and M. D. Yates, *J. Roy. Soc. Med.*, 1986, **79**, 226; L. S. Young, C. B. Inderlied, O. G. Berli, and M. S. Gottlieb, *Rev. Infect. Dis.*, 1986, **8**, 1024; Anon, *Morbidity and Mortality Weekly Review*, 1986, **35**, 448; H. Masur, C. Tuazon, V. Gill, G. Grimes, B. Baird, A. S. Fauci, and H. C. Lane, *J. Infect. Dis.*, 1987, **155**, 127.

commonly occur. It is necessary, therefore, to depend entirely on drugs to destroy all the opportunistic organisms. This is an impossible goal since at present all populations of mycobacteria contain mutants with resistance to every known drug. A figure of 1 per 10^{8-10} organisms is widely accepted for rifampicin.[106] Treatment, therefore, requires multi-drug regimens with potent bactericidal agents. Currently only rifampicin meets this criterion. There is a need for new, potent, bactericidal drugs which can be given in combination regimens to immuno-compromised patients. The understanding of drug action and the identification of target systems within mycobacteria in general, and *M. leprae* in particular, described in this review provide a number of effective starting points for the development of new chemotherapeutic agents for the treatment of mycobacterial diseases.

[106] P. R. J. Gangaharem, 'Drug Resistance in Mycobacteria', CRC Press, 1984, p. 9.