Strategies in the development of new drugs and drug combinations against leprosy, demonstrated on the example of folate and gyrase inhibitors

J K SEYDEL,* M ROSENFELD,* M SATHISH,*
M WIESE,* K-J SCHAPER,* G HACHTEL,†
R HALLER,† M KANSY† & A M DHOPLE‡
*Borstel Research Institute, D-2061 Borstel, FRG, †Pharmaceutical Institute, Kiel University, 2300 Kiel, FRG; ‡Florida Institute of Technology, Melbourne, USA

According to the reports of the pharmaceutical industry 5000–10,000 compounds have to be synthesized to get one compound introduced into therapy.

The aim of the modern approaches in drug design which can be summarized under the title 'Quantitative Structure–Activity Relationship (QSAR)' analysis is to change this uneconomic ratio, i.e. to reduce the number of derivatives which have to be synthesized, to decrease costs but also the number of experimental animal studies. This is especially important for the development of antileprotic drugs where drug development is mainly performed in research laboratories outside the pharmaceutical industry.

It is without doubt that the further development of suitable *in vitro* test systems, increased knowledge in molecular biology and the application of modern computerized techniques for the analysis of quantitative structure–activity relationships can serve to reach this aim.

The position of the modern medicinal chemist in comparison to his predecessor, who had to face these problems 20 years ago, is much more favourable. The optimization of drugs is much less dependent on chance and chance correlation. The reasons for this are better information about biological systems (metabolic pathways, enzyme kinetics, X-ray structure of receptors etc.), the meaning of structural molecular properties, their manipulation and the knowledge of the importance of various computerized methods for data analysis.

With these methods, which will be discussed in a relation to a few selected examples, it is possible to speed up the optimization of lead compounds within a congeneric set of derivatives, to detect and quantify outliers and to gain information on the mechanism of action.[1]
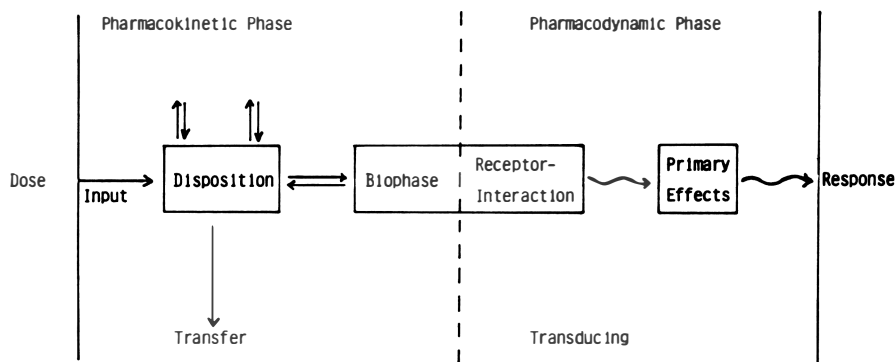
Figure 1. Schematic drawing of steps involved in drug action.[10]

The major problem in the application of these techniques is the complexity of the biological system and of the steps involved in drug action.

This is demonstrated in a simplified drawing in Figure 1. A dose of a drug is administered to a biological system, for example an experimental animal. The dose gets absorbed to a certain extent, is distributed, metabolized, bound to serum and tissue constituents and eliminated. These complex pharmacokinetic events determine the amount of drug which reaches the target in the biophase. A response is released which is measured as a pharmacodynamic effect. Even if we could succeed in relating the degree of the measured effect released by various derivatives to certain molecular properties, it is almost impossible to deduce the causal connection because of the complex nature of the system. In case of the development of antibacterial drugs we are in a somewhat better position. We can measure the inhibitory effect outside the host organism. But even then several steps can be rate limiting, i.e. become decisive for no, some or excellent effect. This is demonstrated in Figure 2. The rate limiting step can be the permeability of the bacterial cell wall, the binding of the drug to the target enzyme or its reactivity to form products, toxic to the bacterial cell.

In leprosy we have the special and unfortunate situation that *Mycobacterium leprae* does not multiply under *in vitro* conditions, i.e. in a test tube. So far for the determination of inhibitory effects of compounds only the mouse footpad technique has been used with the limitations just discussed. We have also to realize that so far neither new drugs have been found nor existing drugs have been developed by this technique. All antibacterials used have been developed against other infections, especially mycobacteriosis including tuberculosis. These drugs dapsone, rifampicin, clofazimine, prothionamide—to name some of them—are all active against a variety of mycobacterial strains; the ranking in activity is sometimes changed comparing results from different bacterial strains. As long as
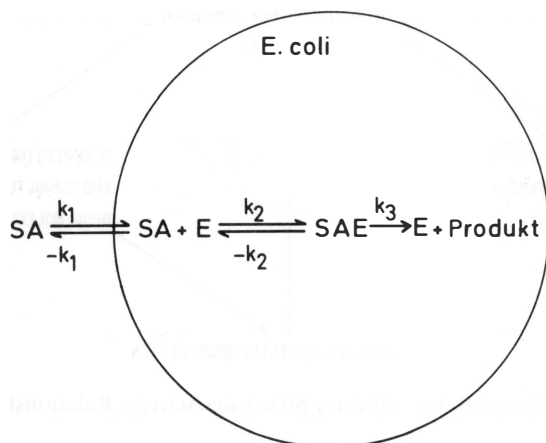
Figure 2. Possible rate limiting steps in antibacterial action.

no breakthrough in cultivation of *M. leprae* or in the transformation of genetic information from *M. leprae* to a cultivable bacterium is achieved the following procedures can be helpful for a systematic optimization of known drugs and also to generate new leads for therapeutics against leprosy:

1   Use of model mycobacterial strains as *in vitro* screening system.
2   Use of isolated target enzymes derived from mycobacteria, including armadillo derived *M. leprae*, for optimization of activity and to detect possible problems in cell-wall permeability.
3   Study of pharmacokinetics and serum activity tests in healthy volunteers after completion of toxicity studies in experimental animals.

The information about the inhibitory activity obtained in these test systems is used to find those structural properties of the drug molecule, which are decisive for the inhibitory power *in vitro*. Various techniques of structure–activity relationship analysis are applied to detect such correlations. A general outline of the procedure is given in Figure 3.

A variety of descriptors can be used to describe certain structural or physicochemical properties of the drug molecules. Essential are often changes in lipophilic, electronic or steric properties. For detailed information one has to refer to the literature.[1–3]

Especially for the chemotherapy of leprosy another aspect is important in drug development, this is the combination of drugs. Again systems have to be developed which allow the quantification of possible synergistic or antagonistic effects of drug combination. Today it is generally agreed that multidrug therapy is a must in chemotherapy of leprosy.[4–8]

And last not least the inhibitory power of a certain derivative is not the only

SYNTHESIS OF NEW COMPOUNDS
(TRAINING SET)

DEVELOPMENT AND APPLICATION OF
BIOLOGICAL TEST SYSTEMS WITH
INCREASING COMPLEXITY

DESCRIPTION OF PHYSICOCHEMICAL
AND STRUCTURAL PROPERTIES OF
DRUG MOLECULES
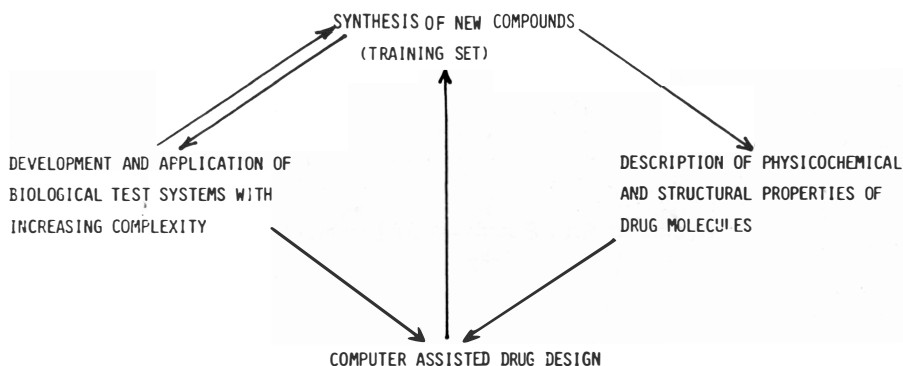
COMPUTER ASSISTED DRUG DESIGN

Figure 3. Diagram of steps in Quantitative Structure-Activity Relationship (QSAR) analysis.

parameter important for a compound to become a useful drug. Some other factors which have to be considered in the optimization procedure are listed in Table 1.

For the last points mentioned in this table, the analysis of the structural dependence of the pharmacokinetic properties is of the utmost importance.[9,10]

This general strategy is outlined on the example of folate inhibitors. The folate synthesizing enzyme system is an excellent target for drugs. The folate pathway is given in Figure 4. Dapsone (DDS) is a well known and powerful inhibitor of the pteroic acid synthetase of *M. leprae* and *M. lufu*. The question is if a further increase in activity can be achieved or if pharmacokinetic properties or tolerance can be optimized. The second suitable target in this enzymatic pathway is the dihydrofolate reductase (DHFR). A known powerful inhibitor is trimethoprim which is, however, restricted to gram negative bacteria only. The question is: is it possible to design a trimethoprim derivative with inhibitory activity against DHFR of mycobacteria. A combination of such a derivative with dapsone or a dapsone derivative could be of great interest for the therapy of leprosy.

Table 1. Possible aims in drug optimization.

1   Extension of range of activity
2   Increase in biological activity
3   Increase in specificity (decrease in toxicity)
4   Change in pharmacokinetic properties: bioavailability; biological half-life; metabolism; distribution etc
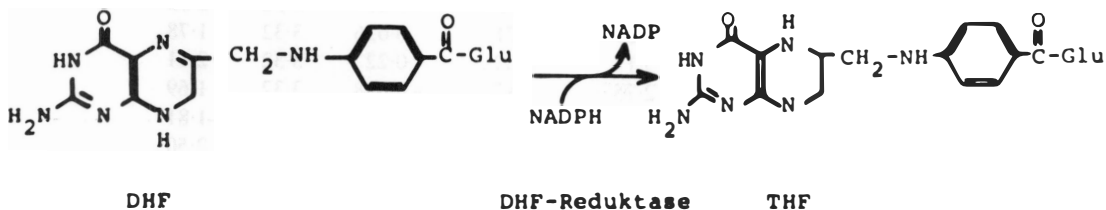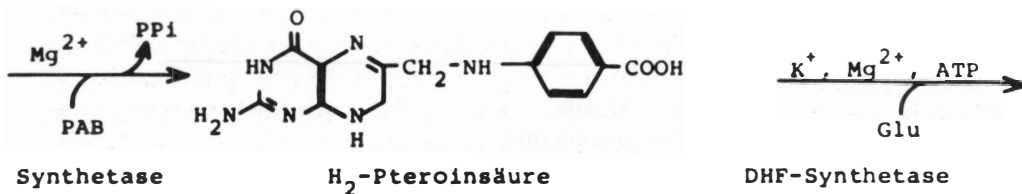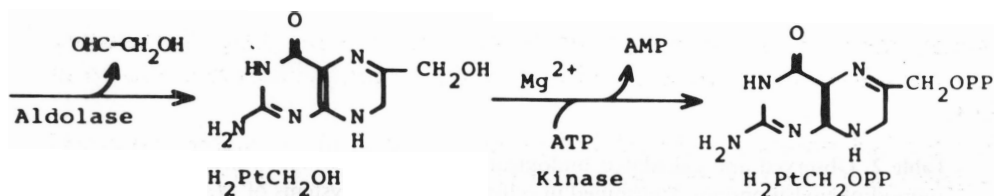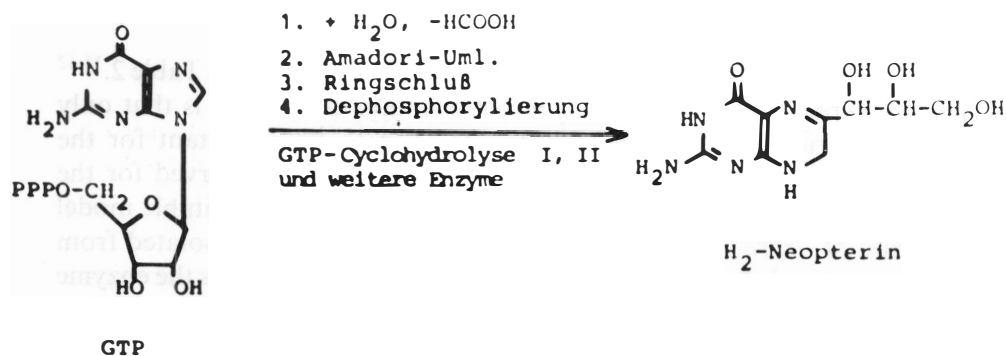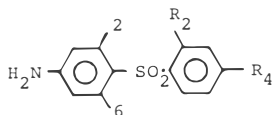5   Adaption of pharmacokinetics to other drugs used in combinations

Figure 4. Pathway of folate synthesis in bacterial cells.

Inhibitors of Dihydropteroic Acid Synthetase

The results of our optimization efforts for dapsone are summarized in Table 2.[11,12] The achieved increase in activity (eq 1) is not exciting. Of interest is that only electronic and steric effects of the substituents seem to be important for the observed differences in activity and that the same ranking is observed for the cultivable *M. lufu* and for *M. leprae* indicating that *M. lufu* is a suitable model strain to mimic *M. leprae* in this respect. In addition the enzyme isolated from both resistant strains shows the same sensitivity towards sulphones as the enzyme derived from sensitive strains (Table 3). This allows the conclusion that the resistance is not due to changes in the structure of the target enzyme.

Changes in the lipophilic properties of the derivatives are not responsible for the observed differences in their activity in cell-free systems. Therefore it is possible to change the lipophilicity without affecting the antibacterial activity

Table 2. Observed and calculated biological activity data of substituted 4′-aminodiphenylsulphones determined in cell-free (I_{50}, μM) systems of *M. lufu* and physicochemical descriptors used in QSAR analysis.

$$H_2N - \bigcirc\!\!\!\!{}^{2}_{6} - SO_2 - \bigcirc\!\!\!\!{}^{R_2} - R_4$$

R_2	R_4	$M.\ lufu_s$ cell-free enzyme $I_{50}\ [\mu M]$ obs./calc. (eq.1)		$\Delta ppm\ 2/6H$	V_{w2}	$\log k_r'$
H	NH_2(DDS)	1·20	1·59	−0·108	3·32	0·488
H	$NHCOCH_3$	4·05	4·20	−0·036	3·32	1·003
H	Br	9·69	6·83	0·00	3·32	2·38
H	$COOCH_3$	11·69	9·71	0·026	3·32	1·78
H	COOH	3·17	3·52	0·22	3·32	−2·11
H	NHC_2H_5	2·75	1·82	−0·098	3·32	1·69
H	$N(CH_3)_2$	2·39	2·05	−0·089	3·22	1·81
OCH_3	NHC_2H_5	1·72	1·24	−0·114	16·07	2·50
Cl	$NHCH(CH_3)_2$	0·86	1·17	−0·106	12·00	2·75
CH_3	NH_2	0·65	0·46	−0·178	13·67	0·94
CH_3	NHC_2H_5	1·09	0·47	−0·177	13·67	2·11

$$\log 1/I_{50} = -5·85\Delta ppm\ 2/6H + 0·066\ V_{w2} - 0·0031\ V_{w2}^2 + 0·42\ f_{ion.} - 1·06$$
(eq. 1) n = 50 r = 0·876 s = 0·21 F = 37.

Table 3. Comparison of biological activities of 4-substituted 4-aminodiphenylsulphones determined in cell-free enzyme systems of DDS-sensitive and DDS-resistant strains of *M. lufu* and *M. leprae* and QSAR-equations derived.

		i_{50} (cell-free enzyme system) μM				
R_2	R_4	*M. lufu*$_s$	*M. lufu*$_r$	*M. leprae*$_s$	*M. leprae*$_r$	*E. coli*
H	NH_2 (DDS)	1·20	1·18	0·42	0·28	35·3
H	NHC_2H_5	2·75	1·50	1·16	0·98	41·4
H	$NHCOCH_3$	7·0	4·05	1·88	1·26	81·6
H	OCH_3	6·0	9	—	2·87	115
H	$CONHNH_2$	12·7	9·6	4·58	3·45	164·3
H	Cl	13·0	18·7	7·16	5·33	—
H	NO_2	31	104	31·00	28·7	221

	n	r	s	F
eq. 2: $\log(1/i_{50\,M.\,leprae})^s = -7\cdot88(2\cdot56)\Delta ppm2/6H - 0\cdot739(0\cdot24)$	8	0·95	0·22	56·7
eq. 3: $\log(1/i_{50\,M.\,lufu})^s = -7\cdot92(1\cdot62)\Delta ppm2/6H - 1\cdot03(0\cdot10)$	14	0·95	0·15	112·0
eq. 4: $\log(1/i_{50\,M.\,leprae})^r = -9\cdot59(4\cdot5)\Delta ppm2/6H - 0\cdot65(0\cdot28)$	7	0·925	0·26	29·9
eq. 5: $\log(1/i_{50\,M.\,lufu})^r = -9\cdot21(2\cdot36\Delta ppm2/6H - 1\cdot15(0\cdot15)$	14	0·926	0·22	72·6

significantly. By these changes, however, pharmacokinetic properties can be altered. The result for a few derivatives is shown in Figure 5.[13] The increase in lipophilic properties of the drug decreases the clearance, i.e. increases the biological half-life of the compounds. This is important to adopt the pharmacokinetics of dapsone derivatives to the pharmacokinetics of other drugs which can be used in combination. A 'side effect' of this development was the decrease in toxicity. Some of the highly active new dapsone derivatives do not show the formation of methemoglobin in the cat even at high concentrations (Table 4).[14]

DHFR inhibitors

The second example of a 'rational' drug design is the development of a new DHFR inhibitor. Starting point was the observation that trimethoprim (TMP) shows no significant inhibitory activity against mycobacteria, however, to our surprise relatively strong inhibitory activity against the isolated target enzyme (Table 5). This seems to indicate limited permeability of the drug into the bacterial cell. The second consideration came from the knowledge of the X-ray structure of the DHFR.[15,16] It shows a positively charged arginine moiety in the cavity of the active centre of the enzyme. Other groups[16] had already shown that
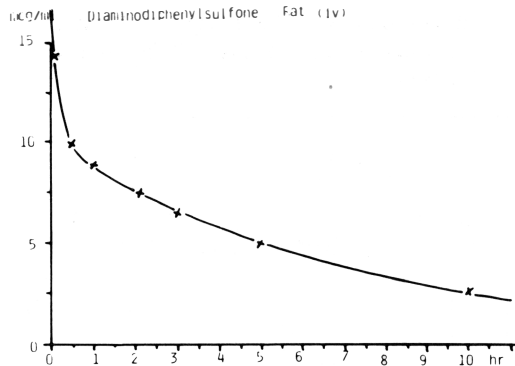
Dependence of renal clearance of sulfones on their
lipophilicity (rats, i.v. administration)

$$H_2N-\bigcirc-SO_2-\bigcirc-R$$

	log k_r'	Cl_{renal} [ml/min]
4-NH$_2$	0.488	0.919
4-OH	-1.067	3.35
4-COOH	-2.11	3.31
4-NHCH$_3$	1.288	0.789
18	0.09	1.96
19	1.339	0.72

$$\log Cl_{tot} = -0.21(\pm0.03)\log k_r' + 0.18(\pm0.04)$$

$$n = 6 \qquad r = 0.93 \qquad s = 0.12$$

Figure 5. Dependence of renal clearance (Cl$_{renal}$) of sulphones on their lipophilicity expressed as capacity factor, log k_r' determined by HPLC-analysis (rats, i.v. administration).

Table 4. Observed methemoglobin formation in cats after p.c. administration of 200 mg/kg of the indicated sulphones.[14]

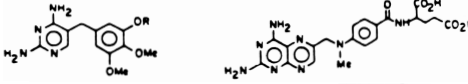|	5 hr	24 hr
Dapsone	6·09 %	4·4 %
H$_2$N-◯-SO$_2$-◯(CH$_3$)-NH$_2$	0·7 %	1·2 %
H$_2$N-◯-SO$_2$-◯(CH$_3$)-NHC$_3$H$_7$	0 %	0 %

Table 5. Observed biological activities of marketed and newly synthesized dihydrofolate reductase inhibitors against cell-free and whole cell systems of various mycobacteria.

	M. lufu $I_{50}[\mu M]$		M I C $[\mu M]$			
Compound	growth kinetics	cell-free enzyme*	*M. lufu*	*M. tub.* H37Rv	*M. marin.* SN1254	*E. coli*
Trimethoprim	95	0·07	>110	>110	28	1·4
Brodimoprim	45	0·06	80	94	18	1·4
Tetroxoprim	213	0·33	—	71	—	11·25
Diaveridin	—	—	>123	>123	46	8
Pyrimethamin	—	0·47	210	129	—	—
GH305	—	0·16	25·3	51	51	22·5
GH306	—	0·17	—	97	48	—
GH307	—	0·15	23	70	46	>45
GH308	—	0·81	22·3	33	5·6	22·5
GH310	—	1·11	31	10	2·6	4·0
K107	1·37	0·06	2·3	29	3·6	>45
K120	—	—	32·0	65	33	>90
K122	—	1·58	>58	3·4	13	11·25
K128	8·31	0·03	12·2	65	6·1	>45
K130	1·66	0·01	0·7	5·3	7·1	32
K132	—	0·26	11·0	7·3	16	11
K135	—	—	—	7·1	14	11
K137	—	0·04	1·9	2·8	30	>90
K138	—	0·08	3·8	7·7	>90	—

* 10 μM DHF

TMP derivatives bearing as substituents negatively charged ionized carboxy groups, led to an increased activity against the isolated enzyme (Figure 6); no activity, however, against the whole cells; this indicates problems in the permeation of the bacterial cell wall. We have therefore systematically synthesized a series of derivatives which possess higher lipophilicity and in addition a negatively charged but unionized group. The results are summarized in Table 5. The high inhibitory power—activity is increased by a factor of more than 100—is also shown by the bacterial growth kinetic experiment using *M. lufu* as model strain (Figure 7).

The results obtained[17,18] in *M. leprae* suspensions are encouraging where the ATP-level and ^{3}H-thymidine uptake is determined in the absence and presence of these derivatives. A strong inhibitory effect is observed (Table 6). An inhibitory

TMP analogs as inhibitors of E.coli DHFR

L.F.Kuyper et al., J.Med.Chem. 25,1120(1982)

R	$K_i \cdot 10^9$
$-CH_3$ (trimethoprim)	1.3
$-CH_2COOH$	2.6
$-(CH_2)_2COOH$	0.37
$-(CH_2)_3COOH$	0.035
$-(CH_2)_4COOH$	0.066
$-(CH_2)_5COOH$	0.024
$-(CH_2)_6COOH$	0.050
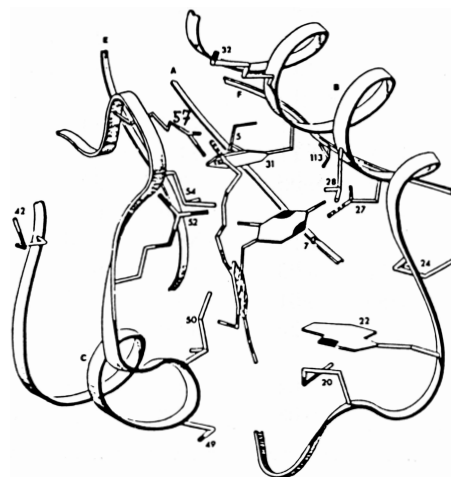
Figure 6. X-ray crystallography of *E. coli* derived DHFR and of its binary complex with a DHFR inhibitor (trimethoprim).[15]

effect can also be demonstrated in mice. The inhibitory effect is delayed despite the fact that the achieved blood levels of 3–4 μg/ml are well above the MIC of the derivative K-130 (Table 7). This is, however, not surprising because it is well documented that low inhibitory activities of DHFR inhibitors, as for example TMP, are found in mice despite the fact that this drug is an excellent chemotherapeutic in human infectious diseases caused by Gram negative bacteria.

This is leading back to the starting point of this paper. It seems not justified to rely only on mouse footpad experiments in developing new drugs, it may lead to unjustified decisions, the risk of producing false negatives cannot be excluded; K-130 seems to be a good example.
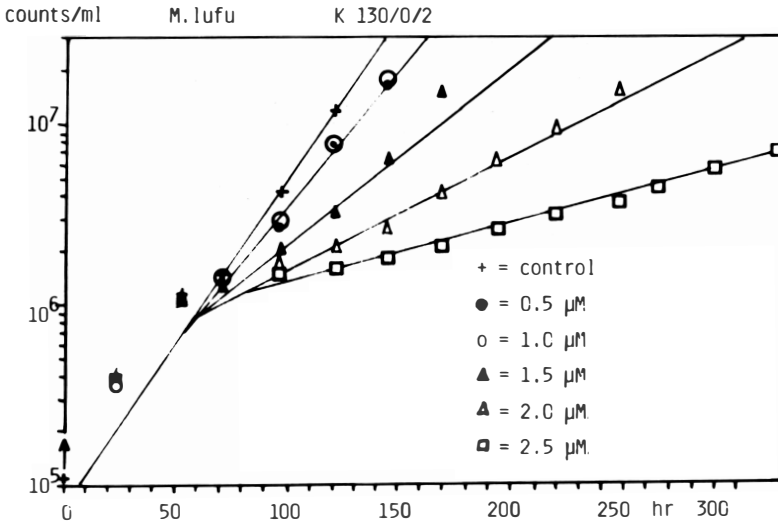
Figure 7. Typical generation rate curves of *M. lufu* at 30°C in the presence of concentrations of K-130 as indicated. +, control; ●, 0·5 μM; ○, 1·0 μM; ▲, 1·5 μM; △, 2·0 μM; □, 2·5 μM.

Table 6. Inhibitory effect on ^{3}H-thymidine uptake and ATP-level on *M. leprae* suspensions.[17]

Drug (DDS derivative)			Percent inhibition of *M. leprae* multiplication at drug concentrations (ng/ml)								
R$_2$	R$_4$		0	5	10	15	20	25	30	40	50
H	NH$_2$	a	0	0	0	15	80	100	100	100	100
		b	0	0	0	13	79	100	100	100	100
H	NHC$_2$H$_5$	a	0	0	0	0	0	0	0	17	17
		b	0	0	0	0	0	0	0	17	19
H	Cl	a	0	0	0	0	0	0	0	0	0
		b	0	0	0	0	0	0	0	0	0
128-0-2-KA		a	0	0	0	0	18	21	48	48	47
		b	0	0	0	0	17	22	48	50	45
130-0-1		a	0	0	0	0	61	82	100	100	100
		b	0	0	0	0	59	79	100	100	100

a = ATP assays; b = [^{3}H] Thymidine uptake assay

Table 7. Footpad harvest of *M. leprae* infected mice after the indicated length of treatment with K 130.[18]

	× 10^6 *M. leprae*/foot pad			
	4 months	6 months	7 months	9 months
control (%)	0·826 ± 0·089	1·69 ± 0·10	2·47 ± 0·169	3·41 ± 0·113
DDS 0·0001	0·78 ± 0·083	1·45 ± 0·10	1·85 ± 0·193	2·35 ± 0·08
K-130 0·03	0·47 ± 0·063	0·41 ± 0·043	0·13 ± 0·025	0·0
K-130 0·03 + DDS 0·0001	0·40 ± 0·069	0·30 ± 0·095	0·07 ± 0·023	0·0

Development of drug combinations

The last aspect to be discussed is the analysis of the effects of drug combinations and how to quantify possible synergistic, additive or antagonistic effects of drug combinations. There is no doubt that it could be very dangerous to combine drugs only on the basis of the activity observed for the single drug.

Suitable 'test systems' for such an analysis are the bacterial growth kinetic[19,20] and the checkerboard technique.[21] An example for the strong synergistic action of the DHFR blocker, Brodimoprim® or the new derivative K-130, respectively, with dapsone is shown in Figure 8(a) and (b).

Identical results are obtained by the checkerboard technique (Table 8). Table 9(a) and (b) shows an example of a combination where the combined action is less compared to the inhibitory activity of the single drugs. Such a combination cannot be recommended. Synergistic and antagonistic effects are mechanistically based and will be similar for various types of bacteria showing sensitivity against the single drugs.

Gyrase inhibitors

Another class of compounds which seems to be of interest for the chemotherapy of leprosy are the newly developed quinolones. These compounds are supposed to inhibit the enzyme gyrase responsible for the coiling of DNA. Several derivatives have been synthesized by various pharmaceutical companies and have been tested against various bacterial strains. So far the most effective derivative against the majority of the sensitive strains seems to be ciprofloxacin. It has a broad antibacterial spectrum including mycobacteria (Table 10). The results of a growth kinetic experiment with *M. lufu* are shown in Figure 9.
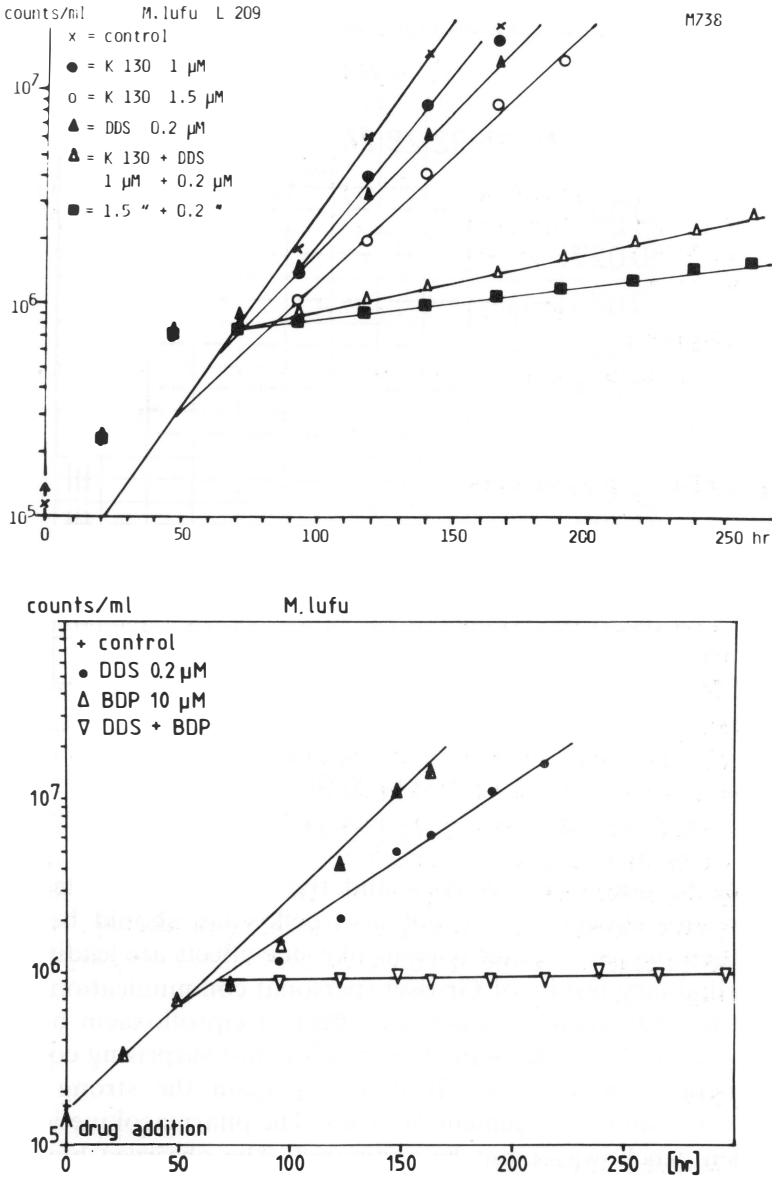
Figure 8. Bacterial generation rate of *M. lufu* at 31°C in the presence of (a) K 130 and dapsone (DDS) alone and in combination, x, control, ●, K130 1µM; ○, K130 1·5 µM; ▲, DDS 0·2 µM; △, K130+DDS 1µM+0·2 µM; ■, 1·5′ µM+0·2′ µM; and (b) brodimoprim (BDP) and dapsone (DDS) alone and in combination at the concentrations indicated. +, control; ●, DDS 0·2 µM; △, BDP 10 µM; ▽, DDS+BDP.

Table 8. Antibacterial effects of brodimoprim (BDP) and dapsone (DDS) alone and in combinations on *M. lufu.* − = growth; + = no growth. Fractional inhibition index (FII) calculated. Strain, *M. lufu*; medium, Dubos + albumin; inoculum, 5×10^{-5} mg germs.

Brodimoprim

DDS \ BDP (mcg/ml)	40	36	32	28	24	20	16	12	8	4	0
0,03	—	—	—	—	—	—	—	—	—	—	—
0,027	—	—	—	—	—	—	—	—	—	—	+
0,024	—	—	—	—	—	—	—	—	—	—	+
0,021	—	—	—	—	—	—	—	—	—	—	++
0,018	—	—	—	—	—	—	—	—	—	—	+++
0,015	—	—	—	—	—	—	—	—	—	—	+++
0,012	—	—	—	—	—	—	—	—	—	—	+++
0,009	—	—	—	—	—	—	—	—	—	—	+++
0,006	—	—	—	—	—	—	—	—	—	(1)	+++
0,003	—	—	—	—	—	—	—	—	(2)	+	+++
0 (mcg/ml)	—	+	+	++	+++	+++	+++	+++	+++	+++	

F.I.I.

(1) 0,30

(2) 0,21

According to our results *in vitro* and the serum activity tests, not only ciprofloxacin and ofloxacin[17,18,24] but also pefloxacin should be suitable for treatment of leprosy, if no other reasons like side-effects are leading to another decision. Preliminary results of Grosset (personal communication) obtained in mouse footpad experiments indicate no effect of ciprofloxacin but significant antileprotic activity for pefloxacin. This result is not surprising considering the pharmacokinetic data available. It indicates again the strong influence of pharmacokinetics on the judgement of drugs. The pharmacokinetics of drugs in mice and men can however not be compared. The observed blood levels for ciprofloxacin in man should be sufficient for therapy (Figure 10), especially as ciprofloxacin shows an extremely good distribution into various tissues and also an accumulation in various cells. This observation is of great importance because mycobacteria are intracellularly growing organisms. First results on combinations of ciprofloxacin with other drugs are not conclusive. No significant synergism was observed for combinations with DDS, prothionamide or brodimoprim. The combination with rifampicin seems to tend to be slightly antagonistic.

Table 9 (a) and (b). Antibacterial effects of rifampicin (RMP) and lamprene (LP) alone and in combination on *M. lufu* and *M. marinum* showing antagonistic effects. LP, lamprene; RMP, rifampicin, (a) strain, *M. lufu*, (b) *M. marinum* SN1254.

(a)

RMP \ LP	0.05	0.045	0.04	0.035	0.03	0.025	0.02	0.015	0.01	0.005	0 µg/ml
0.05	−	−	−	−	−	−	−	−	−(4)	−	−
0.045	−	−	−	−(3)	(+)	+	+	+	+	+	+
0.04	−	−	−	+	+	+	+	+	+	+	+
0.035	−	−	−	+	+	+	+	+	+	+	+
0.03	−	−	−	+	+	+	+	+	+	+	+
0.025	−	−	−	+	+	+	+	+	+	+	+
0.02	−	−	−(2)	+	+	+	+	+	+	+	+
0.015	−	−	.+	++	++	++	+++	+++	+++	+++	+++
0.01	−	−(1)	+	++	+++	+++	+++	+++	+++	+++	+++
0.005	−	−	+	++	+++	+++	+++	+++	+++	+++	+++
0 µg/ml	−	+	++	+++	+++	+++	+++	+++	+++	+++	

FII

(1) = 1.1;   (2) = 1.2;   (3) = 1.6;   (4) = 1.2

(b)

RMP \ LP	0.4	0.36	0.32	0.28	0.24	0.20	0.16	0.12	0.08	0.04	0 µg/ml
2.0	−	−	−	−	−	−	−	−	−	−	−
1.8	−	−	−	−	−	−	−	−	−	−	−
1.6	−	−	−	−	−	−	−	−	−(3)	−	−
1.4	−	−	−	−	−	−(2)	(+)	+	+	+	
1.2	−	−	−	−	+	+	++	++	++	++	
1.0	−	−	−	−	+	+	++	++	++	++	
0.8	−	−	−	−	−(1)	++	++	+++	+++	+++	+++
0.6	−	−	.−	−	+	++	+++	+++	+++	+++	+++
0.4	−	−	−	−	+	++	+++	+++	+++	+++	+++
0.2	−	−	−	−	+	++	+++	+++	+++	+++	+++
0 µg/ml	−	−	−	−	+	++	+++	+++	+++	+++	

FII

(1) = 1.37;   (2) = 1.44;   (3) = 1.28

Table 10. Antibacterial activity of ciprofloxacin against various mycobacteria compared to some tuberculostatic drugs. (Range MIC, μg/ml)

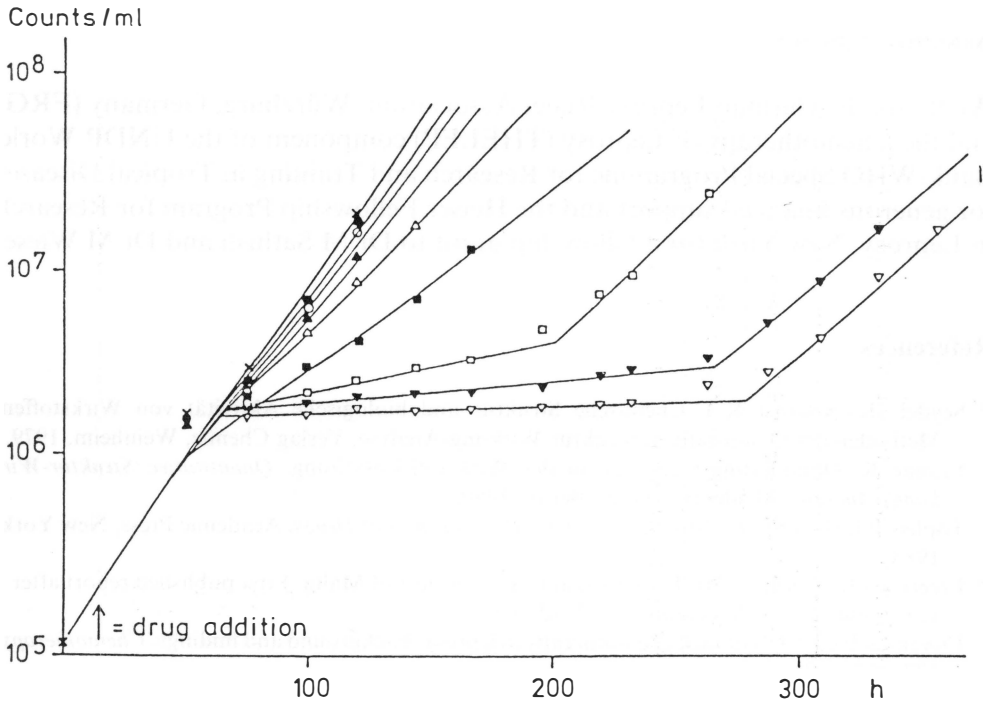| Strain	Medium Dubos/albumin	Medium Lockemann +0·5 % albumin							
	Ciprofloxacin	Ciprofloxacin	RMP	INH	PTH	EMB	SM	KM	CM
M. tub. H37Rv	0·125	0·25–1	0·06–0·125	0·015–0·03	1–2	4–8	2–4	2–4	2–4
M. lufu	0·06–0·2								
M. lufu DDS res.	0·06								
M. smegmatis ATCC607	0·06–1	0·125–0·5							
M. smegmatis DDS res.		0·25–0·5							
M. marinum SN 1254	0·125	0·5–1							
M. avium SN 304	0·5–1	2							
M. avium SN 403	0·125	0·125–0·25							
M. smegmatis SN 46	0·25–0·5	0·25–1							
Patient-Strain									
M. tub. Ro.		0·25	32						
M. tub. Pa.		0·125–0·5	> 32						
M. tub. Br.		0·25–0·5	32						
M. tub. Fi.		0·25	> 32						
M. africanum Ho.		0·25	> 32						
M. shimoidei Schi.		0·5	32						
M. avium Ri.	0·5	1–2	1–4						
M. avium Ra.		16	0·5–1						

Figure 9. Typical generation rate curves of the mycobacterial strain *M. lufu* at 31°C in the presence of various concentrations of ciprofloxacin (electronic, total counts). The curves, generation rate constants k_{app} ($s^{-1} \times 10^{-5}$) and the concentrations (μM) were as follows for the first inhibited phase: x, control 1·17, 0·0; ●, 1·26, 0·06; ○, 1·18, 0·08; ▲, 1·00, 0·10; △, 0·82, 0·12; ■, 0·56, 0·14; □, 0·22, 0·16; ▼, 0·09, 0·18; ▽, 0·06, 0·20. From (24).
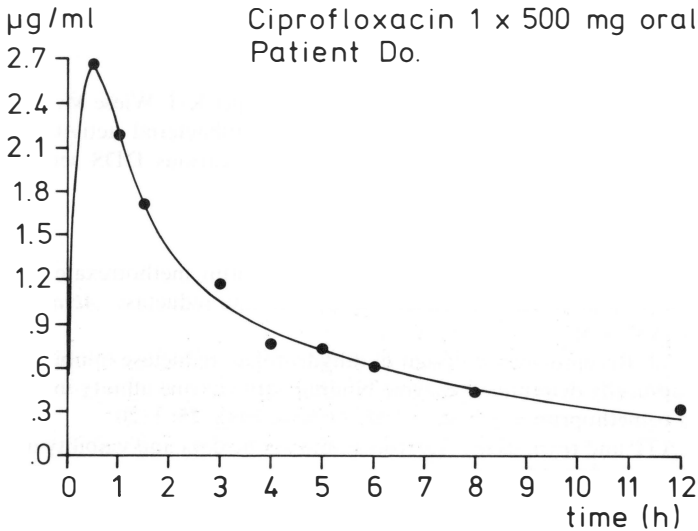

Figure 10. Plasma concentration profile (HPLC) after a single oral dose of 500 mg ciprofloxacin (patient Do.) and serum activity determination after 1 and 4 hr using bacterial strains as indicated.[24]

Acknowledgments

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