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Synthesis of new 2,4-diamino-5-benzylpyrimidines active against various bacterial species

M Kansy[1], JK Seydel[2], M Wiese[2], R Haller[3]

[1]*F Hoffmann-La Roche Ltd, PRTC, 4002 Basel, Switzerland;*
[2]*Borstel Research Institute, Institute for Experimental Biology and Medicine, D-2061 Borstel;*
[3]*Pharmaceutical Institute, University of Kiel, Gutenbergstr 76-78, D-2300 Kiel, Germany*

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Summary — New inhibitors of mycobacterial dihydrofolate reductase (DHFR) have been developed. These compounds show high inhibitory activities against Gram-negative and mycobacterial DHFR exceeding that for the commercially available DHFR blockers. Amongst these compounds **K-130** shows a 100-fold lower MIC against *M lufu* than the most active derivatives known so far (TMP, BDP). *Mycobacterium lufu* was used as a model for the non cultivable strain *M leprae*. **K-130** is also very active against other mycobacterial strains. Besides the higher affinity to the isolated enzyme, the increase in lipophilicity favours permeation of the mycobacterial cell wall and is responsible for the high inhibitory power of **K-130** against mycobacteria. The lower activity against Gram-negative bacteria (*E coli*), despite high affinity to the enzyme, is explained by the hydrophilic nature of the outer score of the cell-wall components.

chemotherapeutics / dihydrofolate reductase / 2,4-diamino-5-benzylpyrimidines / mycobacteria / *E coli* / cell wall interaction / lipophilicity

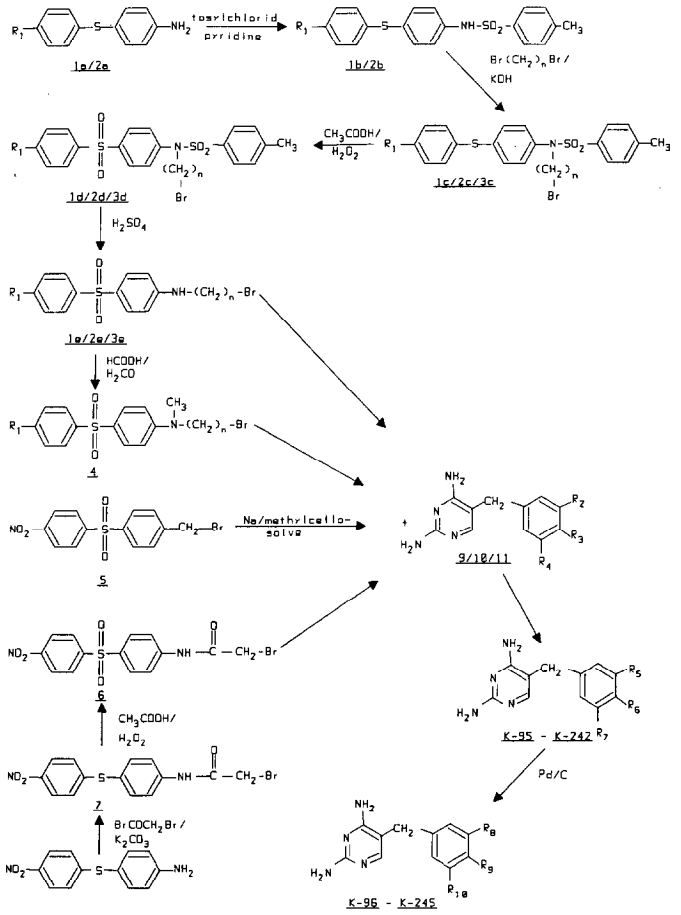
Introduction

2,4-Diamino-5-benzylpyrimidines play an important role in the chemotherapy of infectious diseases. As inhibitors of dihydrofolate reductase (DHFR, 5,6,7,8-tetrahydrofolate: $NADP^+$-oxidoreductase, EC 1.5.1.3) [2] they act as competitive antagonists of dihydrofolate. In combination with sulphonamides they are used especially in the treatment of Gram-negative bacterial infections. However, the treatment of mycobacterial infections, including leprosy, the known 2,4-diamino-5-benzylpyrimidines show no inhibitory activity at concentrations under therapeutic conditions.

Because dapsone (DDS), a folate synthase inhibitor, is the most used drug in the therapy of leprosy, it seemed useful to look for a dihydrofolate reductase inhibitor active against mycobacteria which could be used in combination therapy with dapsone. It was considered that such a combination should lead, in accordance with the observed synergism of sulphonamide/TMP combinations, to a synergistic effect.

During our attempts to design a new inhibitor of mycobacterial DHFR, we noticed that trimethoprim (TMP), which is inactive against mycobacteria whole cells, exerts a high inhibitory activity towards the isolated target enzyme (DHFR) [4]. Problems in permeating the lipid rich cell wall of these bacteria seem to be a major reason for the observed inactivity against mycobacteria. We therefore decided to increase the overall lipophilicity of the new compounds to be synthesized [5], and in addition tried to increase the affinity to the target enzyme through introduction of an appropriately positioned polarized SO_2-group in an attempt to reach the positively charged Arg-57 within the active center. This interaction has been shown for the α-carboxy group of methotrexate and the carboxy group of TMP-derivatives described by Kuyper [12]. Although the latter showed strongly increased affinity to the enzyme, the carboxy group ionized under physiological conditions led to compounds which were inactive against whole cells because of permeability problems. We therefore decided to combine diphenylsulphones and trimethoprim within one molecule [1, 4]. These compounds possess a polarized but not an ionized group at varying distances from the pharmacophoric diaminopyrimidine moiety.

The paper describes the synthesis of these new compounds and discusses the differences in inhibitory



Scheme 1. Reaction pathway for synthesis of new 2,4-diamino-5-benzylpyrimidines (for numbering see table I).

activity against Gram-negative bacteria and mycobacteria. *Mycobacterium lufu* [3] was used, serving as model for *Mycobacterium leprae* since no breakthrough had been made in cultivation of *M leprae* for MIC determinations.

Chemistry

The substituted 2,4-diaminobenzylpyrimidines were synthesized by etherification of the corresponding 2,4-diamino-5-(hydroxymethoxy)-benzylpyrimidines (**9**, **10**) described by Poe [8] according to the procedures described by Roth and Kompis [9–15] (scheme 1). The 2,4-diamino-5-(3,5-dimethoxy-4-hydroxy)benzylpyrimidine (**11**) was synthesized in accordance with the procedure of Brossi [16].

Preliminary trials using substituted benzaldehydes and the described anilino-morpholino procedure [17–19] for the synthesis of the substituted 2,4-diamino-5-benzylpyrimidines were not successful. The sulphides and sulphones **2a**, **2b**, **2c**, **2d**, **2e** were synthesized as described by Baker [23] and Anand [20, 21]. Compounds **1a** and **5** were produced as described by Wenner [22]. The sulfides **1b**, **1c**, **3c** and sulfones **1d**, **3d**, **1e**, **3e** were synthesized in accordance with these procedures (see *Experimental protocols*). Etherification of the 'hydroxy-benzylpyrimidines' was carried out using methylcellosolve as a solvent. The use of solvents such as DMSO and DMF for the etherification reactions resulted in very small yields. Table I and scheme 1 give an overview of the synthesized ethers and the reaction pathway taken.

Biological activity and discussion

Inhibition of DHFR enzymes and antibacterial activity

The newly developed compounds show high inhibitory activity against a cell-free enzyme extract derived from *M lufu*. The compound with the highest activity 2,4-diamino-5-{4-[3-(4''-aminophenyl-4'-sulphonyl-phenylamino)propoxy]-3,5-dimethoxybenzyl}pyrimidine **K-130** (table II) is about 10-fold more active than trimethoprim, which also shows remarkable inhibitory activity against the isolated enzyme but which is not active against *M lufu* whole cells.

The polarisation of the sulphone group is doubtlessly important for the observed activity of these compounds, as described in a previous paper [4].

K-130 is also about 5 times more active against the isolated *E coli* DHFR as compared to TMP, whereas whole cells of *E coli* are less sensitive towards **K-130**. The parallel increase in inhibitory activity against the 2 cell-free systems might be taken as support for the assumption of similarity of the binding site of *E coli* and *M lufu* derived DHFR with respect to these inhibitors.

The low activity against *E coli* whole cells could be explained either by limitation in permeation of the *E coli* cell wall which possesses a very hydrophilic outer score, or by 'specific' interactions with cell-wall components, such as lipopolysaccharides (LPS), leading to inactivation of a certain fraction **K-130** and thus decreasing the amount of drug which can bind to DHFR. To further prove the hypothesis that the binding to cell-wall constituents might be responsible for the observed difference in inhibitory activity against whole cells, as compared to cell-free enzyme extracts, *E coli* mutants (*E coli* F 470–F583) have been used. To varying degrees, these mutants possess an altered and incomplete core characterized by decreased amounts of oligosaccarides and phosphorus. A detailed description of these mutants has been published [6].

Table I. Numbering of scheme 1.

No	R_1	R_2	R_3	R_4	R_5 [R_8 (compound **K-128–K245**)	*n* (**1a–4**)	R_6 R_9	R_7 R_{10}]
1a	CH_3	–	–	–	–	–	–	–
2a	NO_2	–	–	–	–	–	–	–
1b	CH_3	–	–	–	–	–	–	–
2b	NO_2	–	–	–	–	–	–	–
1c	CH_3	–	–	–	–	3	–	–
2c	NO_2	–	–	–	–	2	–	–
3c	NO_2	–	–	–	–	3	–	–
1d	CH_3	–	–	–	–	3	–	–
2d	NO_2	–	–	–	–	2	–	–
3d	NO_2	–	–	–	–	3	–	–
1e	CH_3	–	–	–	–	3	–	–
2e	NO_2	–	–	–	–	2	–	–
3e	NO_2	–	–	–	–	3	–	–
4	NO_2	–	–	–	–	3	–	–
9	–	OH	OCH_3	H	–		–	–
10	–	OCH_3	OH	H	–		–	–
11	–	OCH_3	OH	OCH_3	–		–	–
K-127	–	–	–	–	$OCH_2C_6H_4SO_2C_6H_4NO_2$		OCH_3	H
K-132	–	–	–	–	$O(CH_2)_2NHC_6H_4SO_2C_6H_4NO_2$		OCH_3	H
K-135	–	–	–	–	$O(CH_2)_3NCH_6H_4SO_2C_6H_4NO_2$		OCH_3	H
K-116	–	–	–	–	OCH_3		$OCH_2C_6H_4SO_2C_6H_4NO_2$	H
K-121	–	–	–	–	OCH_3		$O(CH_2)_2NHC_6H_4SO_2C_6H_4NO_2$	H
K-95	–	–	–	–	OCH_3		$OCH_2C_6H_4SO_2C_6H_4NO_2$	OCH_3
K-105	–	–	–	–	OCH_3		$O(CH_2)_2NHC_6H_4SO_2C_6H_4NO_2$	OCH_3
K-122	–	–	–	–	OCH_3		$O(CH_2)_3NHC_6H_4SO_2C_6H_4NO_2$	OCH_3
K-202	–	–	–	–	OCH_3		$OCH_2CONHC_6H_4SO_2C_6H_4NO_2$	OCH_3
K-242	–	–	–	–	OCH_3		$O(CH_2)_3NCH_3C_6H_4SO_2C_6H_4NO_2$	OCH_3
K-128	–	–	–	–	$OCH_2C_6H_4SO_2C_6H_4NH_2$		OCH_3	
K-138	–	–	–	–	$O(CH_2)_2NHC_6H_4SO_2C_6H_4NH_2$		OCH_3	H
K-137	–	–	–	–	$O(CH_2)_3NHC_6H_4SO_2C_6H_4NH_2$		OCH_3	H
K-120	–	–	–	–	OCH_3		$OCH_2C_6H_4SO_2C_6H_4NH_2$	H
K-220	–	–	–	–	OCH_3		$O(CH_2)_2NHC_6H_4SO_2C_6H_4NH_2$	H
K-96	–	–	–	–	OCH_3		$OCH_2C_6H_4SO_2C_6H_4NH_2$	OCH_3
K-107	–	–	–	–	OCH_3		$O(CH_2)_2NHC_6H_4SO_2C_6H_4NH_2$	OCH_3
K-130	–	–	–	–	OCH_3		$O(CH_2)_3NHC_6H_4SO_2C_6H_4NH_2$	OCH_3
K-150	–	–	–	–	OCH_3		$O(CH_2)_3NHC_6H_4SO_2C_6H_4CH_3$	OCH_3
K-203	–	–	–	–	OCH_3		$OCH_2CONHC_6H_4SO_2C_6H_4NH_2$	OCH_3
K-245	–	–	–	–	OCH_3		$O(CH_2)_3NCH_3C_6H_4SO_2C_6H_4NH_2$	OCH_3

Table II. Comparison of dihydrofolate reductase inhibition (I_{50}) and *in vitro* antibacterial activity (MIC) of dihydrofolate reductase inhibitors. log k' = capacity factor determined by HPLC [28].

	I_{50} *and MIC in* µmol/l						
Compound	*E coli*		*M lufu*		*M tuberc*	*M marin*	*log k'*
	DHFR	*MIC*	*DHFR*	*MIC*	*MIC*	*MIC*	
Trimethoprim	0.00123	1.4	0.312	> 110	110	28	–0.59
Brodimoprim	0.00164	1.4	0.152	> 80	94	18	0.82
Pyrimethamin	1.158	–	1.045	210	129	–	1.28
K-96	0.0247	> 90	0.266	30.7	> 61.4	> 61.4	
K-107	0.00102	> 45	0.158	1.8	32.7	15.8	
K-122	0.000438	11.3	0.231	58.2	3.6	14.6	
K-130	0.000556	32.0	0.04	1.0	4.1	6.1	1.28
K-128	0.114	> 45	0.091	12.2	61.1	6.1	
K-138	0.098	> 90	0.289	3.8	11.5	7.7	0.83
K-137	0.0819	> 90	0.113	2.3	2.1	26.6	
K-150	0.0019	> 64	0.039	28.4	28.4	–	
K-120	0.0119	–	0.275	–	–	–	
K-203	0.0973	–	0.034	–	–	–	

The results have been summarized in figure 1. There is indeed a correlation between increase in inhibitory power of **K-130** with increasing core alteration, leading finally to the deep rough mutant F583. The onset of inhibition occurs, however, for all species at the same time so that the inhibition does not seem to be diffusion-controlled.

The proposed interaction with cell wall constituents has been further confirmed by NMR binding measurements using whole cell bacteria and lipopolysaccharides (LPS) derived from a smooth mutant. In case of **K-130** a strong interaction was observed expressed as a change in $1/T_2$ relaxation rate. In contrast, only a very weak interaction with LPS was observed for TMP (fig 2).

This is also true for a mixture of 4'-*N*-propyl-4,4'-diaminodiphenyl-sulphone and 3,5-dimethoxy-4-hydroxybenzylpyrimidine, components of **K-130**. Binding measurements using a centrifugation technique and cell wall fragments of *E coli* resulted in 21% binding for trimethoprim as compared to 84% for **K-130**. These preliminary results could be interpreted in favour of a 'specific' interaction of **K-130** with LPS-components, affording a charge and hydrophobic substructures.

The increase in inhibitory power of **K-130** by a factor of 100 against mycobacteria as compared to trimethoprim is besides the increase in affinity probably due to the increase in lipophilicity of **K-130** [4, 7] (table II). The latter favours the permeation through the lipid-rich cell wall of mycobacteria, whereas the more hydrophilic trimethoprim molecules cannot easily diffuse through the lipid-rich cell wall. The relatively low MIC of compound **K-122** against *E coli* is probably due to an additional effect of the NO_2-group. This has also been observed for 4-amino-4'-nitrodiphenylsulphone.

Experimental protocols

General description

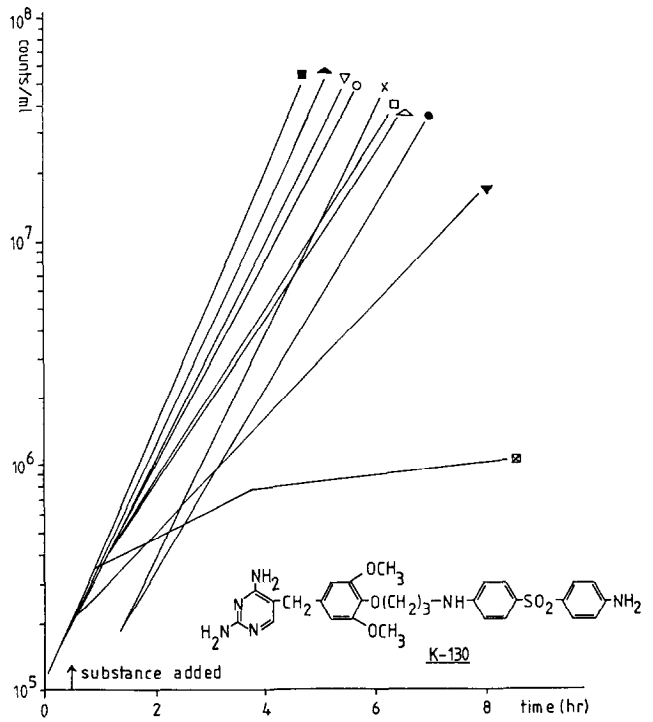
All melting points are uncorrected and were determined using a Dr Tottoli melting point apparatus (Büchi/Switzerland). The NMR spectra are in full accordance with the assumed struc-

tures; analytical values (C, H, N,) are within ± 0.4% of theoretical values.

Chemistry

Synthesis of sulfides and sulphones

4-[N(3-Bromopropyl)-N-tosylamino]-4'-nitrodiphenylsulphide (3c)
40.05 g (0.1 mol) of 4-tosylamino-4'-nitrodiphenylsulphide (**2b**) [20–23] and 150 ml (1.5 mol) of 1,3-dibromopropane in 500 ml of ethanol were heated to boiling after adding 0.1 g of Cu(I)J. A solution of 14 g of potassium hydroxide in 30 ml of water was slowly added dropwise to the boiling solution over 8 h and boiling was continued for a further 2 h. The ethanol was removed under reduced pressure and excess dibromopropane by means of steam distillation. The remaining residue was cooled, dissolved accompanied by heating in 300 ml of ethylacetate, mixed with 4 g of carbon and filtered. The solution was mixed with 200 ml of ethanol, evaporated to approximately half the volume and kept cool. The precipitated crystals were filtered off and recrystallized in ethylacetate. Crystals, mp 112–114°C were obtained. Yield: 82%.



X = control
• = 5 µmol/l
□ = control
Δ = 5 µmol/l
▲ = control
○ = 5 µmol/l
∇ = control
▼ = 5 µmol/l
■ = control
□ = 5 µmol/l

E coli F470 — Increase 18% inhibition
E coli F612 — in 20% inhibition
E coli F516 — incomplete core 26% inhibition
E coli F588 — 46% inhibition
E coli F583 (deep rough) — 95% inhibition

Fig 1. Typical generation rate curves of *E coli* strains with increased deficiencies in the outer core at 37°C in the absence and presence of 5 µM **K-130**.

4-[N(3-Bromopropyl)-N-tosylamino]-4'-nitrodiphenylsulphone (3d)
36.5 g (0.07 mol) of 4-[*N*-(3-bromopropyl)-*N*-tosylamino]-4'-nitrodiphenylsulphide (**3c**) were stirred for 4 h at 65°C in a mixture of 80 ml concentrated hydrogen peroxide and 400 rnl of glacial acetic acid. The solution was diluted with 900 ml water and the product was completely precipitated by cooling in an ice-bath. Recrystallization took place in methanol, accompanied by the addtion of 5% dichloromethane. White crystals, mp 153–155°C were obtained. Yield: 94%.

4-[N(3-Bomopropyl)-amino]-4'-nitrodiphenylsulphone (3e)
10 g (0.018 mol) of 4-[*N*-bromopropyl)-*N*-tosylamino]-4'-nitrodiphenylsulphone (**3d**) were mixed with 20 ml of concentrated sulphuric acid and stirred for precisely 60 min at ambient temperature. The mixture was then poured on 500 ml of ice, filtered off, washed with water and dissolved in 150–200 ml of acetone accompanied by heating. The solution was then adjusted with concentrated aqueous ammonium to pH 9, filtered, concentrated by evaporation *in vacuo* to dryness and cooled. Recrystallization was performed in methanol/dichloromethane (90:10). Yellow crystals, mp 130–132°C were obtained. Yield: 97%.

4-[N(3-Bromopropyl)-N-(methyl)-amino]-4'-nitrodiphenylsulphone (4)
1.0 g (0.0025 mol) (**3e**) 0.025 mol formic acid and 0.01 mol formaldehyde were mixed under ice cooling. The suspension was heated for 5 h at 90°C. After cooling, 0.005 mol of concentrated hydrochloric acid were added. Formic acid and formaldehyde were evaporated under reduced pressure. The remaining substance was adjusted under cooling by 3 N NaOH to pH 7. The sulphone was extracted by dichloromethane and cleaned by column chromatography (solvent: dichloromethane column length: 25 cm diameter 3 cm, stat phase: silica gel (60) (Merck/particle size 65–200 µm). Yield: 65%.

4-Amino-4'-methyldiphenylsulphide (9)
19.6 g (0.08 mol) of 4-nitro-4'-methyldiphenylsulphide (**1a**) [22] were hydrogenated with 4 g (wet weight/stored under methanol) Raney nickel (W2) in 275 ml methanol for 6 h at 45°C and 4 bar. The solution was adjusted to pH 8 with concentrated aqueous ammonium and filtered. Recrystallization from methanol or reprecipitation in 3 N hydrochloric acid yielded white crystals, mp 182°C . Yield: 92%.

4-Tosylamino-4'-methyldiphenylsulphide (1b)
15 g (0.07 mol) of 4-amino-4'-methyldiphenylsulphide were mixed in 75 ml of pyridine with 14.7 g (0.077 mol) of toluene sulphochloride and stirred for 2 h at room temperature. The solution was then mixed with 100 ml of ethanol and 80 ml of water and cooled in an ice-bath. The precipitated substance was filtered off and washed with a large amount of water. Recrys-

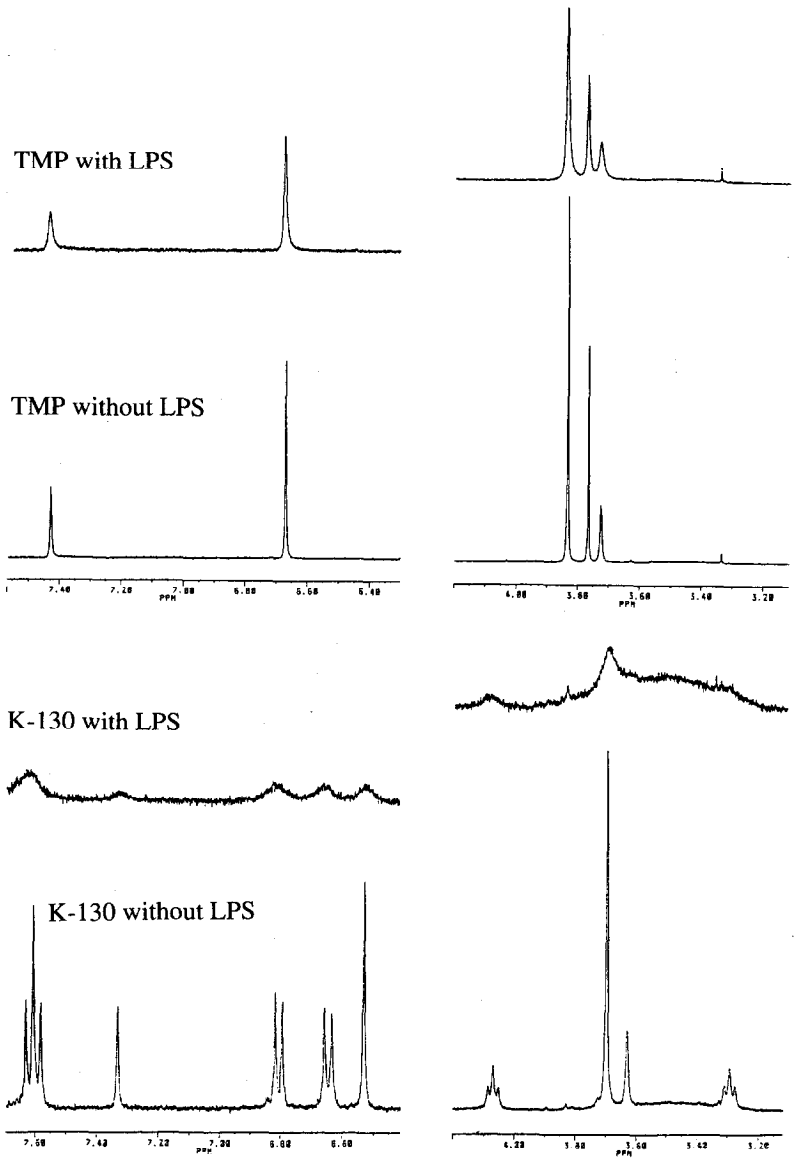


Fig 2. Parts of NMR spectra of TMP and **K-130** in the absence and presence of LPS. Concentrations are: TMP and **K-130** 1 mM, solvent 0.03 M phosphate buffer pH 6.0, pH 5.6 + 10% DMSO–d_6.

tallization with ethanol yielded white crystals, mp 147–149°C. Yield: 91%.

*4-N-(3-Bromopropyl)-N-tosylamino-4'-methyldiphenylsulphide (**1c**)*
Reaction conditions were as in the preparation of **2c** [20] using 1,3-dibrompropan for alkylation. Recrystallization was performed in methanol and white crystals, mp 113–115°C were obtained. Yield: 75%.

*4-[N-(3-Bromopropyl)-N-tosylamino]-4'-methyldiphenylsulphone (**1d**)*
Reaction conditions were as as in the preparation of **2d** [20]. Recrystallization was performed in methanol and white crystals, mp 137–138°C were obtained. Yield: 94%.

*4-[N-(3-Bromopropyl)-amino]-4'-methyldiphenylsulphone (**1e**)*
The reaction was as described in the synthesis of **2e** [20]. Recrystallization was performed in methanol and white crystals, mp 125–127°C were obtained. Yield: 93%.

4-Bromoacetylamino-4'-nitrodiphenylsulphide (7)
10 g (0.025 mol) 4-amino-4'nitrodophenylsulfide [24] were solved in 100 ml CH_2 Cl_2. A solution of 6.0 g K_2CO_3 in 100 ml water was added. Under ice cooling at 5–6°C and intense stiring 3.6 ml bromoacetylchloride were added over a 5-min period. After an additional 5 min of stiring the precipitated substance was filtered and washed with water and 3 N HCl. Yield: 80%.

4-Bromoacetylamino-4'-nitrodiphenylsulphone (6)
19 g (0.052 mol) 4-bromoacetylamino-4'-nitrodiphenylsulphide (7) were heated at 65°C for 4 h in a mixture of 40 ml hydrogen peroxide and 270 ml acetic acid. After cooling the solvent was poured into 400 ml of cold water and filtered. Yield: 75%.

Etherification

Method A. 1.2 g (0.052 mol) of sodium were dissolved in 250 ml of methylcellosolve, 0.05 mol of the phenol were added and stirred until the phenol was completely dissolved. The sulphone was added and stirred for 8 h at 60°C. The mixture was mixed with 700 ml ice, filtered, washed with water, dissolved in 300–400 ml of acetone accompanied by heating, filtered again and evaporated *in vacuo* to 75 ml. This solution was kept overnight at 5°C and the precipitated substance filtered off. Recrystallization in methanol/dichloromethane (90:10).

Method B. 0.03 mol of the corresponding phenol was dissolved in 30 ml of methylcellosolve accompanied by the addition of 0.03 mol of sodium methoxide. The solution was added dropwise under stirring over 5 h to a suspension of 0.03 mol of the sulphone at ambient temperature. The mixture was allowed to stand overnight, the precipitated substance was filtered off and washed in a small amount of ethanol. Recrystallization from methylcellosolve. Table III gives an overview of the yields of etherification and reduction.

Reduction
Method A. 0.006 mol nitro compound were hydrogenated in a mixture of 40 ml of methylcellosolve and 160 ml of methanol for 80 min, at 50°C and 5 bar with 1.5 g (wet weight) Raney nickel (W2). The solution was filtered, mixed with 1 l of water, acidified with dilute hydrochloric acid, filtered again and adjusted to pH 8 with 3 N aqueous ammonium under ice cooling . The precipitated substance was filtered off and well dried. It was recrystallized from methylcellosolve/water.

Method B. 0.00168 mol of the nitro compound was dissolved in 15 ml of phosphonic acid (50%) under nitrogen atmosphere. 0.1 wet Pd/C (10%) catalyst was carefully added and the suspension stirred at 50°C for 60 min. The catalyst was filtered and the filtrate was mixed with 75 ml of water. The solution was alkalized with 3 N aqueous ammonium. The precipitated substance was filtered off and washed with water. The substance could be cleaned by reprecipitation with 3 N aqueous ammonium after dissolving in HCl (3 N) and recrystallisation in 3 N HCl.

Method C. 1.0 g (0.0018 mol) of the nitro compound was hydrogenated in a mixture of 20 ml acetic acid 10 ml methanol with 0.5 g Pd/C under normal pressure. The catalyst was separated and 200 ml of water were added to the solution which was then neutralized with 3 N aqueous ammonium. The precipitated substance was filtered and crystallized in an acetone/water mixture.

Biological experiments

Enzyme assay
DHFR activity was determined spectrophotometrically by monitoring the decrease in absorbance at 340 nm as a function of time. The assay reaction (1.0 ml) contains 100 mM Tris pH 7.2, 0.1 mM NADPH, 0.03 mM H_2 folate, partially purified enzyme derived from *Mycobacterium lufu* (*E coli*) (0.2 units) and varying amounts of inhibitor. Bovine and rat DHFR were obtained by Sigma and used under the same conditions. All components except H_2 folate were incubated at 25°C for 5 min. The reaction was started with H_2 folate. The activity was calculated by using E_{340} of 12300 M^{-1} cm^{-1}. One unit of the enzyme activity was defined as the amount of enzyme that produces 1 nmol of product/min. The measured activities were corrected for nonspecific background by subtracting the 'activity' of an assay to which 1 µmol/l methotrexate was added. I_{50} values were determined using a nonlinear least-squares fitting program.

Table III. Results of etherification and reduction.

Compound	*Yield/method etherification*	*Yield/method reduction*	*Melting point (°C)*
			(reduct)
K-127/K-128	91%/B	88%/A	215–218
K-132/K-138	21%/B	63%/A	144–146
K-135/K-137	61%/B	95%/A	210–211
K-116/K-120	47%/B	65%A	219–220
K-121/K-220	15%A	63%/A	–
K-95/K-96	60%/A	44%/A	243–246
K-105/K-107	20%/A + column chromatog[1]	76%/A	227
K122/K-130	51%/A	78%/A/B	203–205
K-202/K-203	51%/A	63%/C	–
K-242/K-245	54%/A	47%/B	–
K-150	48%/A	–	157

[1]Solvent: dichloromethane/methanol column length: 25 cm, diameter 3 cm, stat phase: sillica gel (60) (Merck/particle size 65–200 µm).

Bacterial strains
The following bacterial strains were used: *M tuberculosis* H32 Rv*; *M marinum* SN1254*; *M lufu* L209 [3]; *E coli* ATCC 11775.
E coli mutants F470, F612, F516, F583 were donated by Dr G Schmidt of Borstel Research Institute.

MIC test
The minimum inhibitory concentrations (MIC) were determined in accordance with standard methods. The culture medium used for mycobacteria with the exception of *M lufu* was a Lockemann medium with 0.5% bovine serum albumin. *M lufu* was cultivated on Dubos–Davis medium with 0.5% bovine serum albumin.

*Laboratory strains from the collection of the Borstel Research Institute.

Bacterial growth kinetics (E coli strains)
Escherichia coli mutants (F470–F612) were maintained on agar slants. The culture broth was dextrose–salts–casamino acids (vitamin-free) as described by Anton [25]. Samples of the experimental cultures were diluted with a particle-free saline (0.85%)-formaldehyde(0.2%) solution, so that a count of 500–20 000 organisms was obtained. Diluted samples were counted with a Coulter Counter model ZB equipped with a 30-μm opening. Counts per 50 μl were obtained. Instrument settings were: 1/aperture current: 1; 1/amplification: 1/2; matching switch: 40 K; gain: 10; lower threshold: 7, and upper threshold: maximum [26]. A similar procedure has been used for growth kinetic studies using *M lufu* as test organism [27].

NMR-binding measurements
All NMR spectra were recorded in deuterated phosphate buffer pH 6.0, pH 5.6 on a Bruker WH90 or Bruker AM 360 L NMR spectrometer, respectively, at 22°C with tetramethylsilane as external and acetone, as internal standard. The phosphate buffer was 0.03 M. Relaxation rates $1/T_2$ were calculated using the equation:

$$1/T_{2obs} = \pi \nu_{1/2}$$

Lipopolysaccharide serotype 055:35, smooth mutant *E coli* used for binding measurements was purchased from Sigma. Acetone was used to control influence of field changes. Broadening was determined with at least 2 different lipopolysaccharide concentrations at constant trimethoprim or **K-130** concentrations. For binding measurements of **K-130** and trimethoprim with cell wall fragments 20 μM or 40 μM solutions of **K-130** (trimethoprim) in 0.1 M phosphate buffer containing 4% (wet weight) sonificated *E coli* were used. After equilibrium these solutions were centrifuged for 1 h at 10 000 rpm and the concentration of the compounds in the supernatant was measured by HPLC.

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