# New Antifolate 4,4′-Diaminodiphenyl Sulfone Substituted 2,4-Diamino-5-benzylpyrimidines. Proof of Their Dual Mode of Action and Autosynergism

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***Key Words:*** *autosynergism, benzylpyrimidines, dihydrofolate reductase, E. coli*

## Summary

New 4,4′-diaminodiphenylsulfone substituted 2,4-diamino-5-benzylpyrimidines were synthesized. These compounds are highly active inhibitors of both bacterial dihydrofolate reductase (DHFR) and dihydropteroic acid synthase (SYN). The simultaneous inhibition of both enzymes leads to autosynergism in whole cells in the same way as known for combinations of sulfonamides with trimethoprim. The inhibitory activity is demonstrated in cell-free systems of DHFR and SYN derived from various species *(M. lufu, E. coli, C. albicans)* and in whole cell systems of the mycobacterial strain *M. lufu*. The compounds are rare examples for the combination of two mechanisms of action in one molecule.

## Introduction

Inhibitors of folate biosynthesis are used in the therapy of infectious diseases. Predominant examples are sulfones, sulfonamides, and benzylpyrimidines. Sulfones (SU) and sulfonamides (SA) act as competitive inhibitors of the 7,8-dihydropteroate synthase (SYN), and 2,4-diamino-5-benzylpyrimidines are competitive inhibitors of the 7,8-dihydrofolate reductase (DHFR). The combination of SU or SA with benzylpyrimidines leads to strong synergism in antibacterial action[1]. The combination of 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (Trimethoprim, TMP) with sulfisoxazole (Co-trimoxazol) is marketed and successfully used for the treatment of various bacterial infections. The inhibitory activity against mycobacteria is, however, low.

Based on the crystal structure of *E. coli* DHFR Kuyper and colleagues[2,3] have designed TMP derivatives which could reach an additional binding site, the arginine-57. These derivatives possess a carboxylic acid group at a certain distance from the benzyl moiety. Indeed a strong increase in inhibitory activity against isolated *E. coli* DHFR was found. The interaction of the charged group with arg-57 was confirmed by X-ray and NMR analyses[3,4]. However, the activity against whole cell *E. coli* was disappointingly low.

These authors were also the first to try to structurally join inhibitors of SYN and DHFR. For this purpose they linked the 3′-position of TMP (I) not to long chain carboxylic acids but instead to sulfonamide residues[5] (Fig. 1).

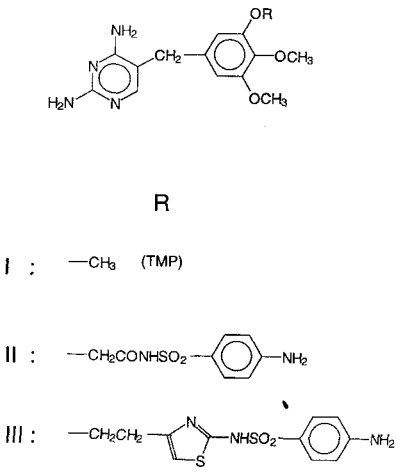


**Figure 1.** Structures of compounds combining features of DHFR- and SYN-inhibitors, from ref. [5].

Compound II showed a remarkable increase in inhibitory activity against DHFR compared to TMP with $K_i$ values of $0.008 \times 10^{-8}$ M and $0.13 \times 10^{-8}$ M, respectively. As expected both derivatives exerted also inhibitory activity against SYN isolated from *E. coli*. The affinity was, however, less than observed for sulfathiazole (STZ) used as a control ($K_i$ values: II = $61 \times 10^{-8}$ M, III = $19 \times 10^{-8}$ M, STZ = $3.5 \times 10^{-8}$ M). Despite the inhibitory activity against both enzymes the MIC values of the new derivatives were disappointingly low. TMP was, as already found in comparison with the derivatives bearing carboxylic acid substituents, a much more powerful inhibitor (100 to 500 times) of whole cell *E. coli*. Reversal experiments with exogenous *p*-aminobenzoic acid (PABA) indicated no significant contribution of inhibition of SYN to the antibacterial activity. The authors[5] explained this result by the predominance of DHFR related activity, i.e. by the much lower $K_i$ values of II and III for *E. coli* DHFR as compared to SYN.

This argument does, however, not explain the observed high MIC values as compared to TMP despite the 16 times lower $K_i$ values of II and III against DHFR. The reason for this is probably a restricted permeation of the bacterial cell wall due to the negative charge of the sulfonamide III. It is known from QSAR studies that for sulfonamides having a $pK_a$ value below ≈6.5 the permeation of the bacterial cell wall becomes the rate limiting step[6]. Also binding to the lipopolysaccharide in the outer membrane of *E. coli* could contribute to the high MIC value, as we have shown that structures of this type can bind to lipopolysaccharide of *E. coli* [7].

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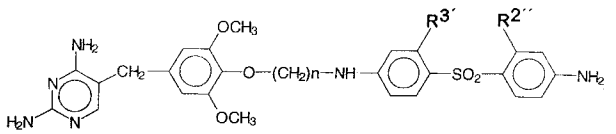
On the basis of this information new diaminobenzylpyrimidine derivatives have been designed with the following aims:

1) to extend the antibacterial spectrum to mycobacteria by increasing the lipophilicity of the derivatives, because mycobacteria possess a highly lipophilic cell wall.

2) to avoid the possibly negative influence of ionization on bacterial cell wall permeation. Therefore the COOH- or $SO_2NH$-group of the Kuyper or Hyde substances was replaced by a polarized $SO_2$-group in such a way that it could also interact with arg-57 as shown for the TMP derivatives synthesized by Hyde and Kuyper.

3) to combine two types of folate inhibitors, i.e. diaminobenzyl pyrimidines as inhibitors of DHFR with diaminodiphenyl sulfones as known powerful inhibitors of mycobacterial SYN[8]. The much stronger affinity of sulfones in comparison to sulfonamides towards SYN of mycobacteria could be an additional advantage, so that autosynergism could become feasible.

In this paper we report that these aims could be achieved for **K-107** and **K-130** (Fig. 2), trimethoprim-derivatives described in previous papers[9,10] and two other compounds **DRS-383** and **DRS-506** (Fig. 2) for which the synthesis is described in this paper (Scheme 1). The **DRS** compounds have been prepared to study the influence of a *ortho*-substitution in the sulfone moiety on the binding to SYN. According to the known mechanism of action one primary $NH_2$-group of the diaminodiphenylsulfone should be retained for SYN affinity[8]. Synthesis and inhibitory activity of the conjugates and precursors against enzyme extracts of various species and the corresponding whole cells will be described.



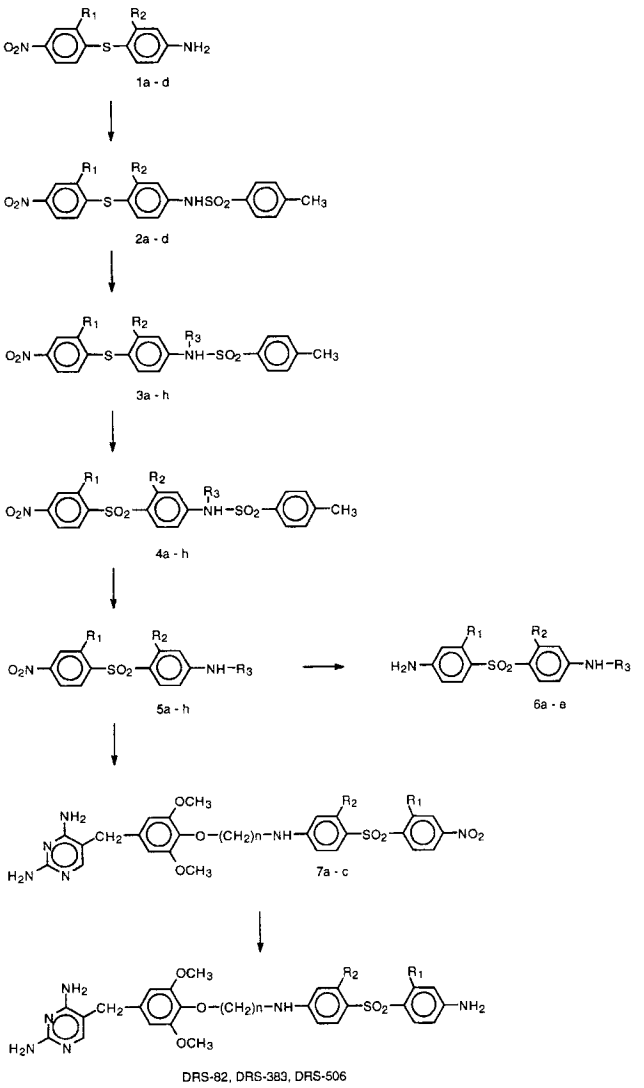
| compound | n | R3′ | R2″ |
|---|---|---|---|
| K-107 | 2 | H | H |
| K-130 | 3 | H | H |
| DRS-82 | 2 | H | $CH_3$ |
| DRS-383 | 3 | H | $CH_3$ |
| DRS-506 | 3 | $CH_3$ | H |

**Figure 2.** Investigated benzylpyrimidine derivatives bearing a sulfone moiety that combine stuctural features of SYN- and DHFR-inhibitors.

## Results and Discussion

### *Chemistry*

The reaction pathway used is shown in Scheme 1. The known 4,4′-diaminodiphenyl sulfones **6c–6e** were also synthesized using this method and had melting points identical to those reported in the literature (ref. [11] and references cited therein).




| comp. 1 - 6 | R1 | R2 | R3 |
|---|---|---|---|
| a | $CH_3$ | $CH_3$ | H |
| b | $CH_3$ | H | $C_3H_7$ |
| c | H | $CH_3$ | $C_3H_7$ |
| d | H | H | $C_3H_7$ |
| e | $CH_3$ | H | H |
| f | $CH_3$ | H | $C_3H_6Br$ |
| g | H | $CH_3$ | $C_3H_6Br$ |
| h | $CH_3$ | H | $C_2H_4Br$ |
| comp. 7 | R1 | R2 | n |
| a | $CH_3$ | H | 3 |
| b | H | $CH_3$ | 3 |
| c | $CH_3$ | H | 2 |

**Scheme 1**

The synthesis of **K-130** has been described in detail by Kansy et al.[10,21]. The new diaminodiphenyl sulfone substituted 2,4-diamino-5-benzylpyrimidines are synthesized under identical experimental conditions. Therefore only a brief description of the synthesis of the precursors and **DRS 383** and **DRS 506** is given. The diaminobenzylpyrimidine sulfone derivatives were obtained by etherification of the bromoalkyl sulfones **5f–h** with 2,4-diamino-5-(3,5-dimethoxy-4-hydroxybenzyl) pyrimidine. The reduction of the nitro-group of **7a–c** was carried out with excess hypophosphoric acid and palladium catalyst under nitrogen atmosphere. 2,4-Diamino-5-(3,5-dimethoxy-4-hydroxybenzyl) pyrimidine was synthesized according to Brossi[12].

The results of the etherification reaction of the bromosulfones with the phenolic group of the TMP derivative varied. DMF and potassium carbonate as mediator or methyl cellosolve and sodium were used. Small amounts of sodium iodide as catalyst were necessary.

## *Biology*

### *Inhibition of Isolated Enzyme Fractions of SYN and DHFR Derived from Various Species*

In contrast to the statement made in previous publications[9,10] we were now able to show that compound **K-130** does indeed inhibit both SYN and DHFR derived from *M. lufu*. The underestimated low solubility of **K-130** in buffer solution has led to the wrong observation. Assuming a low inhibitory activity of this compound against SYN due to the bulk effect of the diaminopyrimidinobenzyl substituent (see also the Kuyper derivatives) we had used highly concentrated (> 100 µM) test solutions in the *in vitro* inhibition assay. This led to our failure to observe crystallization of the inhibitor in the test. Using lower concentrations we found now strong inhibitory activity against SYN for **K-130**, **K-107** and additional new TMP derivatives.

**Table 1.** Inhibition ($IC_{50}$, $K_{iapp.}$ [µMol/l]) of SYN and DHFR derived from *Mycobacterium lufu*.

| Compound | SYN | | DHFR | |
|---|---|---|---|---|
| | $IC_{50}$ | $K_{iapp.}$ | $IC_{50}$ | $K_{iapp.}$ |
| **K-107** | 15.4 | 0.39 | 0.16 | 0.012 |
| **K-122** | no effect | – | 0.058 | 0.0043 |
| **K-130** | 9.0 | 0.23 | 0.040 | 0.0035 |
| **K-150** | no effect | – | 0.039 | 0.0029 |
| **DRS-82** | 21.8 | 0.55 | n.d.[a)] | – |
| **DRS-383** | 10.4 | 0.26 | 0.12 - | 0.0087 |
| **DRS-506** | 12.9 | 0.32 | 0.036 | 0.0027 |
| DDS | 1.2 | 0.03 | no effect | – |
| *N*-ethyl-DDS | 2.7 | 0.07 | n.d. | – |
| TMP | no effect | – | 0.31 | 0.023 |

[a)] n.d.: not determined

The mycobacterial strain *M. lufu* has been used as test organism for two reasons. First *M. lufu* is a suitable substitute for *M. leprae* which is not cultivable *in vitro*[13]. *M. lufu* shows a similar sensitivity pattern against various chemotherapeutics used in the treatment of leprosy and shows especially a very high sensitivity for 4,4′-diaminodiphenylsulfone (DDS), as does *M. leprae*. Secondly these bacteria, in contrast to *M. tuberculosis*, do not show significant cell adhesion and are therefore suitable for bacterial growth kinetic experiments using the Coulter counter technique[14].

The strong inhibitory activity of aminodiphenyl sulfones against SYN derived from *M. lufu* and against whole cell cultures has been demonstrated previously[15]. Surprisingly substitution by the bulky diaminopyrimidine benzyl group leads only to a loss in activity by a factor of ≈10 as compared to the unsubstituted DDS. If, however, the remaining primary amino-group of the diphenylsulfone is replaced by a methyl- or nitro group (**K-150, K-122**), the inhibitory activity against SYN is lost – up to a concentration which could be tested (solubility) – whereas the inhibitory activity towards DHFR is not affected by the substitution of the primary amino group (Table 1). This clearly indicates that these compounds interact with the two different enzymes requiring different substructures. The finding is in agreement with the generally accepted mechanism of action that for sulfonamides and sulfones to be active, one primary amino group is a necessary precondition.

From QSAR analysis of the inhibitory activity of sulfones against SYN derived from *P. berghei* it has been found that substitution *ortho* to the sulfone group increases the activity against SYN up to a certain critical volume of the substituent, the methyl- and chloro derivatives being the most active ones[16]. Therefore precursor sulfones have been synthesized (Table 2, **6a–e**) bearing methyl-groups in ortho position to the sulfone group on one or both of the phenyl rings and an additional alkyl substituent at one of the primary amino groups. Only for **6e** was a slight increase in activity against *M. lufu* derived SYN observed compared to DDS and for **6d** compared to **6c**.

**Table 2.** $IC_{50}$ values [µMol/l] of 4,4′-diaminodiphenyl sulfones against SYN derived from *M. lufu and E. coli.*

| Compound | R1[a)] | R2[a)] | R3[a)] | $IC_{50}$ | |
|---|---|---|---|---|---|
| | | | | *M. lufu* | *E. coli* |
| **6a** | $CH_3$ | $CH_3$ | H | 1.5 | n.d. |
| **6b** | $CH_3$ | H | $C_3H_7$ | 4.2 | n.d. |
| **6c** | H | $CH_3$ | $C_3H_7$ | 1.6 | 22 |
| **6d** | H | H | $C_3H_7$ | 2.0 | n.d. |
| **6e** | $CH_3$ | H | H | 0.6 | n.d. |
| DDS | H | H | H | 1.2 | 34 |
| *N*-ethyl-DDS | H | H | $C_2H_5$ | 2.7 | 38 |

[a)] see scheme 1
[b)] not determined

The sulfones **6b** and **6c** with methyl groups in ortho position to the $SO_2$ group and with one primary amino group were selected as substituents for the synthesis of the new benzyl

pyrimidines **DRS-82, 383**, and **506**, as they are the correct precursor sulfones and tested against SYN and DHFR. No significant difference in inhibitory activity against SYN from *M. lufu* was found for the *o*-methyl substituted and unsubstituted derivatives. The *ortho* effect shown for the sulfones alone especially on *P. berghei* derived SYN was not detectable.

### *Inhibition of Whole Cell Mycobacteria*

The effect of simultaneous inhibition of the two enzymes can only be studied in whole cell cultures. Because of the demonstrated inhibition of both isolated enzymes synergism could be expected as documented for the combination of the single drugs sulfisoxazole and TMP (Co-trimoxazol) against gram negative bacteria[17] or DDS with brodimoprim against mycobacterial infections[18]. Two techniques have been used to study the biological activity of the described compounds in *M. lufu* cultures. To get information on the time dependency of inhibition, a bacterial growth kinetic technique has been used. An example is documented in Figure 3 and the derived $IC_{50}$ values are summarized in Table 3. From Figure 3 it can be seen that the onset of inhibition occurs approximately after two bacterial generation times and that this onset is independent of the drug concentration used. After onset of inhibition first order kinetics were observed. Therefore it can be concluded that the permeation process is not rate limiting.

The $IC_{50}$ values determined in whole cell cultures and the MIC values are, however, as observed usually much higher than the $IC_{50}$ values derived in the cell-free system. Reasons could be binding to cell wall components and/or differences in co-factor (NADPH) concentration used in the enzyme assay and present in bacterial cells.

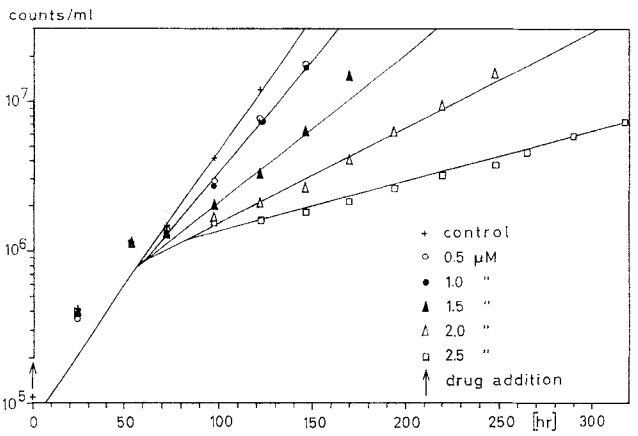



**Figure 3.** Bacterial growth kinetics of *M. lufu* in the presence of increasing concentrations of compound **K-130** as indicated.

For the new benzyl pyrimidines **K-107**, **K-130**, **DRS-383**, and **DRS-506** a ≈80 fold increase in activity against *M. lufu* cultures compared to TMP is observed. This can only partly be due to the increase in affinity towards DHFR (≈10 fold) as brodimoprim with similar lipophilicity to the new compounds reaches a twofold increase in affinity towards DHFR and a comparable increase in inhibitory activity against whole cell *M. lufu*[19] (Table 3). This leads to the assumption that simultaneous inhibition of SYN is responsible for the observed increase in activity. This is further supported by a related derivative, **K-150** that shows a much higher MIC. It possesses an identical $IC_{50}$ towards DHFR but lacks a aminodiphenylsulfone substructure with binding affinity to SYN. The ratios of the $IC_{50}$ values for the inhibition of *M. lufu* derived DHFR and the $IC_{50}$ values obtained in *M. lufu* cultures for a series of homologous benzyl pyrimidines also support these arguments (Table 4). The ratio is about 200 with increasing tendency with lipophilicity (log *k'*). The derivatives, however, capable to perform a dual mode of inhibition, show significantly smaller ratios despite similar lipophilicity. Most striking is the comparison of **K-130** and **K-150** differing only in the primary amino group.

**Table 3.** Whole cell activities [µMol/l] against *M. lufu and E. coli.*

| Compound | IC50 | | MIC | |
|---|---|---|---|---|
| | *M. lufu* | *E. coli* | *M. lufu* | *E. coli* |
| **K-107** | 1.4 | n.d.[a] | 1.8 | >45 |
| **K-130** | 1.7 | 3.2 | 1.0 | 32 |
| **DRS-383** | 2.6 | 4.6 | 3.5 | 16 |
| **DRS-506** | 4.4 | 2.6 | 6.1 | 16 |
| **K-150** | 36.9 | n.d. | 28.4 | >64 |
| Trimethoprim | 50.0 | 1.0 | 220 | 2 |
| Brodimoprim | 30.4 | 0.8 | 98 | 0.5 |
| DDS | 0.45 | 18.0 | 0.17 | 16 |

[a] not determined

**Table 4.** Ratios of inhibitory activities of benzyl pyrimidines against *M. lufu.*

| Compound | $IC_{50\_w.c}$ / $IC_{50\_DHFR}$ | $IC_{50\_w.c}$ / $IC_{50\_DHFR}$ | $K_{i\_SYN}$ / $K_{i\_DHFR}$ | log $k'_{Octanol}$ |
|---|---|---|---|---|
| Tetroxoprim | 187 | – | – | 0.42 |
| Trimethoprim | 180 | – | – | 0.55 |
| Methioprim | 183 | – | – | 1.03 |
| Brodimoprim | 200 | – | – | 1.62 |
| **K-150** | 946 | – | – | 3.88[a] |
| **DRS-383** | 22.3 | 86 | 30 | 2.65[a] |
| **K-107** | 23.2 | 102 | 33 | 1.64 |
| **K-130** | 41.5 | 264 | 66 | 2.09 |
| **DRS-506** | 121.0 | 358 | 119 | 2.65 |

[a] Calculated from the value of **K-130** using π-substituent constants

If additional derivatives are included a significant regression equation can be derived for the correlation of whole cell ($IC_{50\_w.c.}$) with cell-free ($IC_{50\_c.f.}$) activity and lipophilicity for those benzylpyrimidines which act solely on DHFR.

$\log (IC_{50w.c.}) = 0.85(\pm 0.21) \log(IC_{50c.f.}) - 0.26(\pm 0.14) \log k' + 2.43(\pm 0.23)$
$n = 13 \quad r^2 = 0.90 \quad s = 0.17 \quad F = 44.7 \quad r^2_{cv} = 0.83$

Compounds **K-107**, **K-130**, **DRS-383**, and **DRS-506** prove to be outliers. Much higher $IC_{50\_w.c.}$ values are calculated according to the regression equation. This underlines the unexpected high inhibitory power against *M. lufu* cultures compared to their $IC_{50}$ values against isolated DHFR.

More information can be derived from a comparison of these ratios with the ratio of $IC_{50}$ and $K_i$ values obtained for SYN and DHFR inhibition (Table 4). The synergism is strongest (small ratio $IC_{50\_w.c.}/IC_{50\_c.f.}$) if the ratio $K_{i\_SYN}/K_{i\_DHFR}$ is small, i.e., if the affinities to SYN and DHFR are not too different in magnitude. This result supports the reasoning of Kuyper et al.[3] for the missing autosynergism for their derivatives, where this ratio is much higher in case of derivative II.

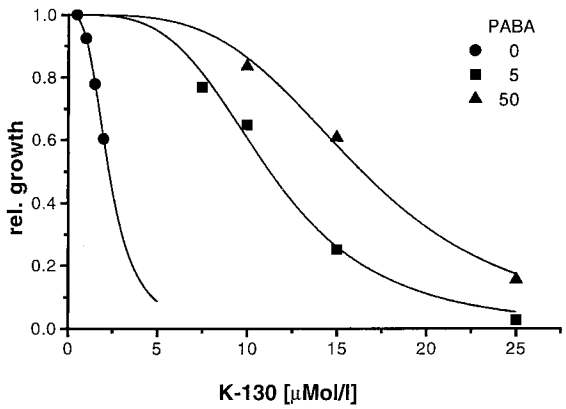



**Figure 4**. Reversal of growth inhibition of **K-130** against *M. lufu* by increasing PABA concentrations.

The best proof, however, that the inhibitory power of **K-107**, **K-130**, **DRS-383**, and **DRS-506** determined against *M. lufu* derived SYN is sufficiently high to contribute to the inhibition of whole cell bacteria multiplying under pseudo steady state conditions can be derived from an experiment, in which the inhibition of SYN is antagonized by the addition of PABA. The result of such an experiment is illustrated in Figure 4 for **K-130**. In agreement with the results presented in this paper, the $IC_{50}$ value of **K-130** is about 2 µMol/l in the absence of PABA. Upon addition of increasing amounts of PABA the dose response curves are shifted to the right. The obtained $IC_{50}$ values as function of PABA concentration can be fitted by a Michaelis Menten type equation. From this fit and the $IC_{50}$-value at 50 µMol/l PABA the final $IC_{50}$ value is estimated to be ≈16 µMol/l (Fig. 5). This remaining inhibitory activity ($IC_{50}$ ≈16 µM) is due to the inhibition of DHFR, as the activity against SYN has been completely antagonized. The $IC_{50}$ for the homologous benzyl pyrimidine **K-150** with an affinity directed solely towards DHFR is 36 µM. This shows that the strong increase in whole cell activity compared to that in cell free systems is due to the simultaneous inhibition of SYN, leading to autosynergism.

Whereas the inhibition of SYN by **K-130** in whole cells is documented by its reversal by PABA, the simultaneous inhibition of DHFR can be demonstrated by its reversal upon addition of thymine. The latter reverses the thymine less death of the cells produced by the blockade of DHFR. Table 5 documents the MIC values of **K-130** against *E. coli* in the absence and presence of thymine. For comparison the MIC values of brodimoprim are also given.

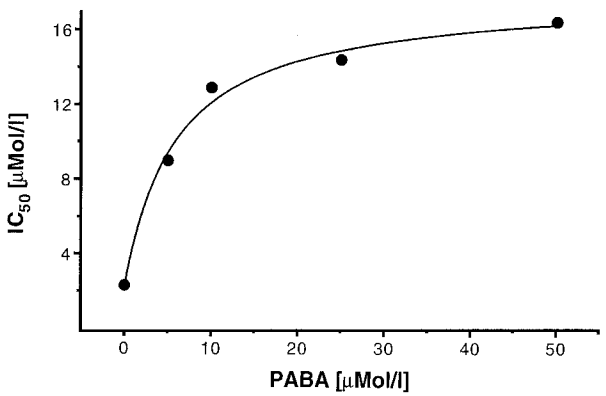



**Figure 5**. $IC_{50}$ values of **K-130** against *M. lufu* in the presence of various PABA concentrations.

**Table 5.** MIC-values [µg/ml] in the absence and presence of thymine.

| Compound | no thymine | 1 pM thymine |
|---|---|---|
| **K-130** | 1 | 8 |
| Brodimoprim | 40 | 160 |

The stronger effect on reversal of inhibition by thymine in case of **K-130** is a further support for the dual mode of action of K-130.

Therefore the observed lack of dual mode of action for the Wellcome derivatives despite their activity against both enzyme systems *in vitro* could be caused by the unfavourable ratio of affinities ($K_i$) against the two enzymes in case of derivative II and by problems in permeation of the bacterial cell wall in case of derivative III which is almost totally ionized under culture conditions

### *Inhibition of SYN and DHFR Derived from Other Species and Whole Cell Examinations*

The new diaminobenzyl pyrimidine sulfones have also been tested against SYN derived from *E. coli* and *C. albicans* as well as in whole cell cultures of these species. The results are summarized in Table 6. It can be shown that these derivatives also inhibit SYN derived from *E. coli*. The $IC_{50}$ values are almost identical with DDS.

The inhibitory activity of the new derivatives against *E. coli* and *C. albicans* derived DHFR is again improved as compared to TMP. Remarkable is the observed increase in inhibitory activity of **DRS-506** against *C. albicans* derived DHFR by a factor of 300 in comparison to TMP.

The inhibition of whole cell cultures of *E. coli* and *C. albicans* is, however, disappointingly low, probably due to problems in cell wall permeation as discussed before. In the

**Table 6.** Inhibition ($IC_{50}$ μM) of SYN and DHFR derived from various species.

| Compound | SYN *E. coli* | DHFR *E. coli* | DHFR *C. albicans* |
|---|---|---|---|
| **K-107** | 22 | 0.0010 | 8.20 |
| **K-122** | n.d.[a)] | 0.00047 | 0.55 |
| **K-130** | 24 | 0.00055 | 0.27 |
| **K-150** | n.d. | 0.00054 | 0.47 |
| **DRS-82** | 36 | n.d. | n.d. |
| **DRS-383** | 64 | 0.0010 | 0.47 |
| **DRS-506** | 30 | 0.0011 | 0.10 |
| DDS | 34 | no effect | no effect |
| *N*-ethyl-DDS | 42 | n.d. | n.d. |
| TMP | n.d. | 0.0022 | 30.4 |

[a)] n.d.: not determined

case of *E. coli* we were able to show that the high MIC values are due to interaction with cell wall components. If *E. coli* mutants with cell wall deficiencies were used as test organism a corresponding increase in inhibitory activity was observed with decreasing length of the sugar moieties in the LPS core region[10,20].

## Conclusion

The simultaneous consideration of factors responsible for high affinity to the target enzymes SYN and DHFR and necessary properties for permeation of mycobacterial cell walls has led to compounds which show autosynergism in inhibition of *M. lufu* folate synthesis.

## Acknowledgment

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## Experimental

*General methods.* All solvents and reagents for synthesis were of reagent-grade quality, purchased – if possible – anhydrous, and used without further purification. The $^1$H- and $^{13}$C-NMR spectra were in full accordance with the assumed structures. All NMR spectra were obtained in $D_6$-DMSO, recorded on a Bruker WH 90 or Bruker AM 360 L spectrometer, respectively 90 or 360 MHz for protons, at 20 °C with TMS as external standard. All melting points are uncorrected and were determined using an E. Leitz melting point microscope No. 535806. Elemental analyses were performed by Ilse Beetz. Mikroanalytisches Laboratorium, Kronach, Germany, and were within 0.4% of the theoretical values. Thin layer chromatography (TLC) was carried out on silica plates (Macherey-Nagel: Polygram SIL G/$UV_{254}$) using either 1:1 ethyl acetate/hexane (System A) or 5:1 ethyl acetate/methyl cellosolve (System B). For column chromatography Merck silica gel 60, 230–400 mesh was used. The whole cells were determined with a Coulter counter from Counter Electronics LTD, Dunstable, Beds, UK.

*Synthesis*

The synthesis of compound **K-130** and its precursors is described elsewhere[12f].

*4′-Nitro-4-N-tosylaminodiphenyl Sulfide Derivatives (**2a–d**)*

4-Amino-4′-nitrodiphenyl sulfide derivatives **Ia–d** were prepared in 40–50% yield according to Baker[8] and references therein. Equivalent amounts of **1a–d** (10–50 mmol) and 4-toluenesulfonyl chloride were dissolved in 10–50 mL dry pyridine and magnetically stirred for at least 3 h. Then a cold mixture of 30–150 mL water and 30–150 mL EtOH was added and the precipitate was collected on a Büchner funnel. The yields after recrystallization from EtOH/EtOAc were 89–95%.

*2-Methyl-4′-(N-propyl-N-tosyl)-amino-4-nitrodiphenyl Sulfide (3b), 2-Methyl-4-(N-propyl-N-tosyl)-amino-4′-nitrodiphenyl Sulfide (3c), and 4-(N-propyl-N-tosyl)-amino-4′-nitrodiphenyl Sulfide (**3d**)*

3.0 g (7.5 mmol) **2b, 2c** or **2d** 3 mL 1-bromopropane, 1.0 g sodium iodide and 0.1 g copper(I) iodide in 50 mL EtOH were heated. To the boiling solution 0.4 g (10.0 mmol) sodium hydroxide in 5 mL water was added. After refluxing for 17 h the solvent and unreacted 1-bromopropane were removed by distillation. The remainder was dissolved in 20 mL EtOAc, filtered, washed twice with 10 mL water and dried over $Na_2SO_4$. After filtering and distillation the precipitate was crystallized from EtOH/EtOAc. Yield **3b** 3.1 g (79%), TLC (A) $R_f$ 0.84, mp 118–119 °C. Yield **3c** 73%, TLC (A) $R_f$ 0.84, mp 83–84 °C. Yield **3d** 83%, TLC (A) $R_f$ 0.84, mp 126–127 °C.

*4-[N-(3-Bromopropyl)-N-tosyl]amino-2′-methyl-4′-nitrodiphenyl Sulfide (**3f**) and 4-[N-(3-bromopropyl)-N-tosyl]amino-2-methyl-4′-nitrodiphenyl Sulfide (**3g**)*

15.0 g (36.2 mmol) **2f**, 40 mL 1,3-dibromopropane, 10.0 g sodium iodide and 0.1 g copper(I) iodide in 50 mL EtOH were heated. To the boiling solution 2.24 g (40.0 mmol) potassium hydroxide in 5 mL water was slowly added. After refluxing for 17 h the solvent was removed under reduced pressure and the unreacted 1,3-dibromopropane by steam distillation. The dry remainder was dissolved in 250 mL hot EtOH and filtered. The nitrosulfone precipitated in yellow needles. Yield **3f** 15.3 g (79%), TLC (A) $R_f$ 0.88, mp 96–98 °C. Yield **3g** 74%, TLC (A) $R_f$ 0.88, mp 112–113 °C.

*4-[N-(3-Bromoethyl)-N-tosyl]amino-2′-methyl-4′-nitrodiphenyl Sulfide (**3h**)*

Compound **3h** was synthesized from **2b** according to the method for **3f** using 1,2-dibromoethane. The solvent and unreacted ethylene bromide were both distilled from the product under reduced pressure. Yield 95%, TLC (A) $R_f$ 0.87, mp 132–133 °C.

*2,2′-Dimethyl-4-N-tosylamino-4-nitrodiphenyl sulfone (**4a**)*

The suspension of 2.5 g (5.8 mmol) **3a** in 7 mL $CHCl_3$ and 2.3 g MCPBA (90%) was magnetically stirred for 24 h. After dilution with 30 mL EtOAc the benzoic acids were extracted 5 times with 30 mL portions of a saturated $NaHCO_3$ solution. The organic phase was dried over $Na_2CO_3/Na_2SO_4$. After filtration and removing solvents the product was crystallized from EtOAc. Yield 1.6 g (60%), TLC (A) $R_f$ 0.93, mp 160–161 °C.

*2′-Methyl-4-[N-(3-bromopropyl)-N-tosyl]amino-4′-nitrodiphenyl Sulfone (**4f**) and Bromoalkyl-tosyl Sulfone Derivatives **4b–d** and **4g–h***

7.0 g (13.5 mmol) **3f**, 100 mL acetic acid and 100 mL hydrogen peroxide (30%) were stirred at 65 °C overnight. The solution was poured in 100 mL ice water, filtered, washed with 400 mL water, dried and recrystallized from EtOAc. Yield **4f** 7.0 g (94%), TLC (A) $R_f$ 0.95, mp 135–136 °C. Yield **4g** 92%, TLC (A) $R_f$ 0.95, mp 142–143 °C. Yield **4h** 94%, TLC (A) $R_f$ 0.95, mp 154–155 °C. Yield **4b** 91%, TLC (A) $R_f$ 0.93, mp 164–165 °C. Yield **4c** 91%, TLC (A) $R_f$ 0.93, mp 158–159 °C. Yield **4d** 91%, TLC (A) $R_f$ 0.93, mp 168–169 °C.

*4-(N-3-Bromopropyl)amino-2′-methyl-4′-nitrodiphenyl Sulfone (**5f**) and 4-(N-n-bromoalkyl)amino-4′-nitrodiphenyl Sulfone Derivatives (**5a–e** and **5g–h**)*

Dry, fine powdered 1.4 g (2,47 mmol) **4f** and 3 mL concentrated sulfuric acid were magnetically stirred for 1 h. The yellow liquid was slowly poured in 50 mL stirred ice water. The precipitate was separated and intensively washed with cold water. After drying the product was recrystallized from EtOH. Yield **5f** 0.88 g (86%), TLC (A) $R_f$ 0.65, mp 142–143 °C. Yield **5g**

89%, TLC (A) $R_f$ 0.65, mp 161–162 °C. Yield **5h** 90%, TLC (A) $R_f$ 0.67, mp 130–131 °C. Yield **5a** 92%, TLC (A) $R_f$ 0.58, mp 160–161 °C. Yield **5b** 90%, TLC (A) $R_f$ 0.65, mp 115–116 °C. Yield **5c** 92%, TLC (A) $R_f$ 0.63, mp 94–95 °C. Yield **5d** 85%, TLC (A) $R_f$ 0.65, mp 167–168 °C. Yield **5e** 85%, TLC (A) $R_f$ 0.59, mp 134–135 °C.

*4,4′-Diamino-2,2′-dimethyldiphenyl Sulfone (**6a**)*

0.81 g (2.6 mmol) **5a** was hydrogenated in 25 mL glacial acetic acid with 0.2 g Pd/C (10%) under normal pressure. After dilution with 100 mL $CH_2Cl_2$ the Pd-catalyst was filtered off. 2 M aqueous ammonia was used to neutralize the solution. The solvents were removed under reduced pressure and the remainder was recrystallized from methyl cellosolve/EtOH/water, to give 0.70 g (96%), TLC (A) $R_f$ 0.33, mp 262–263 °C. $^1$H-NMR: δ = 2.08 (s, 3H), 5.91 (s, 2H), 6.35 (d, $J$ = 2 Hz, 1H), 6.47 (dd, 1 H, $J$ = 2; 9 Hz), 7.65 (d, 1 H, $J$ = 9 Hz).

*4,4′-Diaminodiphenyl Sulfone Derivatives* **6b–e**

The nitro compounds **5b–e** were hydrogenated according to the method for **6a**.

*4-Amino-2-methyl-4′-N-propylaminodiphenyl Sulfone (**6b**)*

Yield 87%, TLC (A) $R_f$ 0.45, mp 170–171 °C, $^1$H-NMR: δ = 0.91 (t, $J$ = 7 Hz, 3H), 1.53 (m, 2H), 2.19 (s, 3H), 2.99 (m, 2H), 5.90 (s, 2H), 6.36 (d, $J$ = 2 Hz, 1H), 6.46 (dd, $J$ = 2; 8 Hz, 1H), 6.56 (t, $J$ = 5 Hz, 1H), 6.59 (AA′XX′, 2H), 7.41 (AA′XX′, 2H), 7.65 (d, $J$ = 8 Hz, 1H).

*4′-Amino-2-methyl-4-N-propylaminodiphenyl Sulfone (**6c**)*

Yield 84%, TLC (A) $R_f$ 0.46, mp 187–8 °C. $^1$H-NMR: δ = 0.91 (t, $J$ = 7 Hz, 3H), 1.53 (m, 2H), 2.19 (s, 3H), 2.99 (m, 2H), 5.90 (s, 2H), 6.34 (d, $J$ = 2 Hz, 1H), 6.47 (dd, $J$ = 2; 8 Hz, 1H), 6.56 (t, $J$ = 5 Hz, 1H), 6.59 (AA′XX′, 2H), 7.41 (AA′XX′, 2H), 7.65 (d, $J$ = 8 Hz, 1H).

*4′-Amino-4-N-propylaminodiphenyl Sulfone (**6d**)*

Yield 94%, TLC (A) $R_f$ 0.41, mp 221–2 °C. $^1$H-NMR: δ = 0.91 (t, $J$ = 7 Hz, 3H), 1.52 (m, 2H), 2.99 (m, 2H), 5.90 (s, 2H), 6.36 (d, $J$ = 2 Hz, 1H), 6.46 (dd, $J$ = 2; 9 Hz, 1H); 6.56 (AA′XX′, NH, 3H), 7.41 (AA′XX′, 2H), 7.65 (d, $J$ = 9 Hz, 1H).

*4,4′-Diamino-2-methyldiphenyl Sulfone (**6e**)*

Yield 94%, TLC (A) $R_f$ 0.24, mp 165–6 °C. $^1$H-NMR: δ = 2.19 (s, 3H), 5,89 (s, 2H), 5.99 (s, 2H), 6.36 (d, $J$ = 2 Hz, 1H), 6.46 (dd, $J$ = 2; 9 Hz, 1H), 6.60 (AA′XX′, 2H), 7.37 (AA′XX′, 2H), 7.65 (d, $J$ = 9 Hz, 1H).

*2,4-Diamino-5-{3,5-dimethoxy-4-[3-(4′-(2″-methyl-4″-nitrophenyl)-sulfonylanilino)propoxy]benzyl}pyrimidine (**7a**)*

2.0 g (4.86 mmol) **5f**, 1.29 g (4.86 mmol) 2,4-diamino-5-(3,5-dimethoxy-4-hydroxybenzyl) pyrimidine and 0.69 g potassium carbonate in 20 mL dry DMF were magnetically stirred under nitrogen atmosphere at room temperature for 7 d. The solution was poured in 150 mL ice water and after 15 min filtered. The crude precipitate (1.25 g) was dried, dissolved in methyl cellosolve and cleaned by column chromatography (column length: 25 cm, diameter: 1.5 cm), 1st solvent: 1:1 EtOAc/hexane until all unreacted strong yellow sulfone **5f** was eluted, 2nd solvent: 2:5 methyl cellosolve/EtOAc. The desired product **7a** is light yellow and was eluted first with the 2nd solvent, 50 mL fractions 4–7. Other dark yellow products, which were eluted afterwards, were not considered. Recrystallization from methyl cellosolve/water gave 0.82 g (28%), TLC (B) $R_f$ 0.67, mp 122–124 °C. $^1$H-NMR: δ = 1.84 (m, 2H), 2.09 (s, 3H), 3.24 (m, 2H), 3.52 (s, 2H), 3.68 (s, 6H), 3.90 (t, $J$ = 6 Hz, 2H), 5.70 (s, 2H), 6.10 (s, 2H), 6.55 (s, 2H), 6.69 (AA′XX′, 2H), 6.90 (t, $J$ = 5 Hz, 1H), 7.52 (s, 1H), 7.57 (AA′XX′, 2H), 8.20-8.22 (envelope, 3H).

*2,4-Diamino-5-{3,5-dimethoxy-4-[3-(3′-methyl-4′-(4″-nitrophenyl)-sulfonylanilino)propoxy]benzyl}pyrimidine (**7b**)*

**7b** was synthesized following the procedure for **7a** using 2.0 g (4.86 mmol) **5g**. Yield 24%, TLC (B) $R_f$ 0.67, mp 188–9°C. $^1$H-NMR: δ = 1.83 (m, 2H), 2.23 (s, 3H), 3.30 (m, 2H), 3.52 (s, 2H), 3.69 (s, 6H), 3.90 (t, $J$ = 7 Hz, 2H), 5.73 (s, 2H), 6.13 (s, 2H), 6.46 (d, $J$ = 1 Hz, 1H), 6.56 (s, 2H), 6.60 (dd, $J$ = 1; 8 Hz, 1H), 6.79 (t, $J$ = 5 Hz, 1H), 7.51 (s, 1H), 7.84 (d, $J$ = 9 Hz, 1H), 8.01 (AA′XX′, 2H), 8.36 (AA′XX′, 2H).

*2,4-Diamino-5-{3,5-dimethoxy-4-[3-(4′-(2″methyl-4″-nitrophenyl)-sulfonylanilino)ethoxy]benzyl}pyrimidine (**7c**)*

After 0.175 mg (7.61 mmol) sodium was dissolved in 35 mL methyl cellosolve all following reaction steps were carried out under dry nitrogen. With stirring 2.07 g (7.60 mmol) 2,4-diamino-5-(3,5-dimethoxy-4-hydroxybenzyl) pyrimidine was added. To the red solution 0.1 g sodium iodide and 3.0 g (7.30 mmol) **5h** were added. The mixture was magnetically stirred for 1 d and poured on ice. The dark yellow precipitate was dried and purified by with column chromatography as described for **7a**. Yield 0.73 g (17.0%), TLC (B) $R_f$ 0.67, mp 112–113 °C. $^1$H-NMR: δ = 2.53 (s, 3H), 3.3 (envelope, m, 2H), 3.51 (s, 2H), 3.67 (s, 6H), 3.93 (t, $J$ = 6 Hz, 2H), 5.70 (s, 2H), 6.09 (s, 2H), 6.73 (AA′XX′, 2H), 6.87 (t, $J$ = 5 Hz, 1H), 7.51 (s, 1H), 7.58 (AA′XX′, 2H), 8.21–8.23 (envelope, 3H).

*2,4-Diamino-5-{3,5-dimethoxy-4-[3-(4′-(4″-amino-2″-methylphenyl)-sulfonylanilino)propoxy]benzyl}pyrimidine (**DRS-383**)*

Under a slow stream of nitrogen 1.2 g (1.97 mmol) **7a** and 0.2 g Pd/C (10%) in 15 mL hypophosphorous acid were heated to 60 °C for 1.5 h. The yellow colour of the suspension turned to colourless. After cooling the catalyst was filtered off, the solution was diluted with 30 mL water and rendered alkaline with 2 M ammonium hydroxide. The precipitate was resolved in 2 M hydrochloric acid, cooled with ice, and cooled concentrated ammonia was slowly added. The first grey precipitates sedimented at low pH values were filtered off until the white product appeared. The white product was dissolved in 5 M HCl and cooled with ice water. Slowly addition of concentrated ammonia gave white flocks. Recrystallization from methyl cellosolve/water gave a yield of 0.51 g (45%), TLC (B) $R_f$ 0.35 (one spot), mp 108–109 °C, HPLC one signal (no *o*-methyl-DDS). $^1$H-NMR: δ= 1.84 (q, $J$ = 6 Hz, 2H, Pr-C$H_2$), 2.19 (s, 3H, DDS-C$H_3$), 3.24 (m, 2H, NH-C$H_2$), 3.52 (s, 2H, TMP-C$H_2$), 3.68 (s, 6H, OC$H_3$), 3.90 (t, $J$ = 6 Hz, 2H, O-C$H_2$), 5.77 (s, 1H, NH), 5.91 (s, 2H, $NH_2$), 6.16 (s, 2H, $NH_2$), 6.36 (s, 1H, DDS-H), 6.47 (dd, $J$ = 2; 8 Hz, 1H, DDS-H), 6.55 (s, 2H, TMP-H), 6.61 (AA′XX′, 2H, DDS-H), 7.42 (AA′XX′, 2H, DDS-H), 7.51 (s, 1H, TMP-H), 7.65 (d, $J$ = 9 Hz, 1H, DDS-H); Anal. ($C_{29}H_{34}N_6O_5S$) C, H, S.

*2,4-Diamino-5-{3,5-dimethoxy-4-[3-(3′-methyl-4′-(4″-anilino)sulfonyl-anilino)propoxy]benzyl}pyrimidine (**DRS-506**)*

**7b** was hydrogenated with hypophosphorous acid after the procedure for **DRS-383**. No recrystallization from methyl cellosolve/water was performed. Yield 42%, TLC (B) $R_f$ 0.35 (one spot), HPLC one signal (no *o*-methyl-DDS), mp 131–2 °C. $^1$H-NMR: δ = 1.84 (q, $J$ = 6 Hz, 2H, Pr-C$H_2$), 2.22 (s, 3H, DDS-C$H_3$), 3.24 (m, 2H, NH-C$H_2$), 3.53 (s, 2H, TMP-C$H_2$), 3.69 (s, 6H, OC$H_3$), 3.90 (t, $J$ = 6 Hz, 2H, OC$H_2$), 6.00 (s, 2H, $NH_2$), 6.05 (s, 2H, $NH_2$), 6.39 (s, 1H, DDS-H), 6.42-6.52 (envelope, 3H, DDS-H and NH), 6.56 (s, 2H, TMP-H), 6.58 (AA′XX′, 2H, DDS-H), 7.37 (AA′XX′, 2H, DDS-H), 7.50 (s, 1H, TMP-H), 7.70 (d, $J$ = 9 Hz, 1H, DDS-H), Anal. ($C_{29}H_{34}N_6O_5S \cdot 0.3$ HCl) C, H, N, S, Cl.

*2,4-Diamino-5-{3,5-dimethoxy-4-[2-(4′-(4″-amino-2″-methylphenyl)-sulfonylanilino)ethoxy]benzyl}pyrimidine (**DRS-82**)*

Prepared according to the method for **DRS-506**. Yield 68%, TLC (B) $R_f$ 0.37 (one spot), HPLC one signal (no *o*-methyl-DDS), mp 134–135 °C. $^1$H-NMR: δ = 1.91 (s, 3H, DDS-C$H_3$), 3.33 (m, 2H, NH-C$H_2$), 3.54 (s, 2H, TMP-C$H_2$), 3.68 (s, 6H, OC$H_3$), 3.94 (t, $J$ = 6 Hz, 2H, OC$H_2$), 5.92 (s, 2H, $NH_2$), 6.21 (s, 2H, $NH_2$), 6.35 (d, $J$ = 2 Hz, 1H, TMP-H), 6.46 (dd, $J$ = 2; 9 Hz, 1H, DDS-H), 6.51 (t, $J$ = 5 Hz, 1H, NH), 6.62-6.69 (envelope, AA′XX′, 3H, DDS-H and $NH_2$), 7.43 (AA′XX′, 2H, DDS-H), 7.0 (s, 1H, TMP-H),

7.66 (d, 1H, $J = 9$ Hz, DDS-H), Anal. ($C_{28}H_{32}N_6O_5S \cdot 0.6$ HCl) C, H, N, S, Cl.

*Biological Experiments*

The partially purified enzymes SYN and DHFR were derived from the test strains: *Mycobacterium lufu* L 209, *Escherichia coli* ATCC 11775 [22]and *Candida albicans* ATCC 11651[23].

*MIC test.* MIC determinations (serial dilutions tests) were performed in accordance with standard procedures[24]. For *M. lufu* a culture medium of Dubos with 0.5% albumin was used and the cultivation was performed at 32 °C.

*Bacterial growth kinetics.* To 50 mL of the diluted suspension containing $\approx 10^5$ cells/mL of *M. lufu* the inhibitor was added and the cultures were kept at 31 °C. Before taking an aliquot for measurement, the cultures were vigorously stirred for 1 min. The sample were diluted with particle free saline (0.875%)-formaldehyde (0.5%) solution to stop multiplication and to get a suspension with 500–90,000 organisms. Counting was performed on a Coulter counter equipped with a 30 µm orifice.

*Inhibition of SYN.* For the determination of folate production in cell free SYN extract, *Streptococcus faecalis* ATCC 8043 were used. A standard reaction mixture containing inhibitor, partially purified SYN, kinase, PABA, ATP, $MgCl_2$, and 6-hydroxymethyl-7,8-dihydropterine was incubated at 32 °C for 3 h. Different amounts were added to folate free growth medium and inoculated with *S. faecalis*. After 24 h the turbidity caused by grown *S. faecalis* was measured[8].

*Inhibition of DHFR.* The DHFR activity was determined spectrophotometrically by monitoring the decrease in absorbance at 340 nm as a function of time[9]. The assay reaction mixture contained 100 mM Tris buffer pH 7.2, 0.1 mM NADPH, isolated DHFR and varying amounts of inhibitor. After preincubation for 5 min at 25 °C the reaction was started by adding 0.03 mM $H_2$ folate.

$IC_{50}$. The inhibitory activity of SU and TMP derivatives which causes 50% inhibition of the synthesis of SYN or DHFR was calculated by nonlinear regression analysis using locally written software.

## References


[1] S.Q.M. Bushly, G.H. Hitchings, *J. Pharmacol. Chemother.* **1968**, *33*, 72–90.

[2] J.T. Bolin, D.J. Filman, D.A. Matthews, R.C. Hamlin, J. Kraut, *J. Biol. Chem.* **1982**, *257*, 13650–13662.

[3] L.F. Kuyper, B. Roth, D.P. Baccanari, R. Ferone, C.R. Beddell, J.N. Champness, D.K. Stammers, J.G. Dann, F.E.A. Norrington, D.J. Baker, P.J. Goodford, *J. Med. Chem.* **1982**, *25*, 1120–1122.

[4] G.C.K. Roberts, *Nato ASI Ser., Ser. A* **1986**, *107*, 73–86.

[5] R.M. Hyde, R.A. Paterson, C.R. Beddell, J.N. Champness, D.K. Stammers, D.J. Baker, P.J. Goodford, L.F. Kuyper, R. Ferrone, B. Roth, L.P. Elwell in *Chemistry and Biology of Pteridines* (Ed.: J.A. Blair); Walter de Gruyter & Co., Berlin, **1983**, pp. 505–509.

[6] G.H. Miller, P.H. Doukas, J.K. Seydel, *J. Med. Chem.* **1972**, *15*, 700–706.

[7] J.K. Seydel, M. Albores Velasco, E.A. Coats, H.-P. Cordes, B. Kunz, M. Wiese, *Quant. Struct.-Act. Relat.* **1992**, *11*, 205–210.

[8] V.M. Kulkarni, J.K. Seydel, *Chemotherapy* **1983**, *29*, 58–67.

[9] K.-H. Czaplinsky, M. Kansy, J.K. Seydel, R. Haller, *Quant. Struct.-Act. Relat.* **1987**, *6*, 70–72.

[10] M. Kansy, J.K. Seydel, M. Wiese, R. Haller, *Eur. J. Med. Chem.* **1992**, *27*, 237–244.

[11] H. Pieper, G. Krüger, K.R. Noll, J. Keck, J.K. Seydel, M. Wiese, *Arzneim.-Forsch./Drug Res.* **1989**, *39*, 1073–1080.

[12] A. Brossi, E. Grunberg, M. Hoffer, S. Teitel, *J. Med. Chem.* **1971**, *14*, 58–59.

[13] F. Portaels, *Ann. Soc. Belge. Med. Trop.* **1980**, *60*, 381–386.

[14] J.K. Seydel, E. Wempe, *Int. J. Leprosy* **1982**, *50*, 20–30.

[15] E.A. Coats, H.-P. Cordes, V.M. Kulkarni, M. Richter, K.-J. Schaper, M. Wiese, J.K. Seydel, *Quant.Struct.-Act.Relat.* **1985**, *4*, 99–109.

[16] M. Wiese, J.K. Seydel, G. Krüger, H. Pieper, K.R. Noll, J. Keck, *Quant.Struct.-Act.Relat.* **1987**, *6*, 164–172.

[17] J.K. Seydel, E. Wempe, G.H. Miller, *Chemotherapy* **1972**, *17*, 217–258.

[18] J.K. Seydel, E. Wempe, M. Rosenfeld, R. Jaganathan, P.R. Mahadevan, A.M. Dhople, *Arzneim.-Forsch./Drug Res.* **1990**, *40*, 69–75.

[19] J.K. Seydel, *J. Chemotherapy* **1993**, *5*, 422–429.

[20] M. Schop, Ph.D. Thesis, University of Kiel, **1993**.

[21] M. Kansy, Ph. D. thesis, University of Kiel, **1986**.

[22] R. Bartels, L. Bock, *J. Chromatogr.* **1990**, *523*, 53–60.

[23] R. Bartels, L. Bock, poster presented at 3rd ICBS, Sopron, Hungary, Sept. **1991**.

[24] E. Krüger-Thiemer, E. Wempe, M. Töpfer, *Arzneimittel-Forsch.* **1965**, *15*, 1309–1317.


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