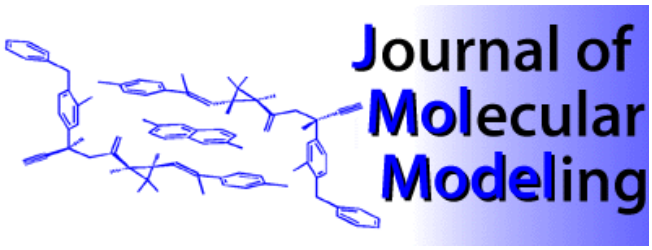
J. Mol. Model. **1997**, *3*, 332 – 337



Molecular Modeling Study on Dapsone and Sulfonamides Comparing Structures and Properties with Respect to Anti-Leprosy Activity[$]

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Received: 13 June 1997 / Accepted: 18 July 1997 / Published: 13 August 1997

Abstract

Despite the very close structural relationship between dapsone (4,4'-diaminodiphenyl sulfone, 4,4' sulphonyldianiline, diaphenyl sulphone, DDS) and sulfanilamide (p-aminobenzene sulfonamide), being the prototype of all other sulfonamides, only dapsone shows remarkable efficient pharmacological activity against Mycobacterium leprae. Cells of certain micro-organism need para-aminobenzoic acid (PABA), the latter playing the role of natural substrate to biosynthesis of folic acid. Sufones and sulfonamides show competitive antagonism as chemical analogs of PABA. It is most surprising that, despite of sharing this molecular mechanism, only dapsone shows anti-leprosy activity in vivo. The study was accomplished using molecular mechanics (SYBYL) and semiempirical methods (MOPAC). The calculations of aromaticity, charges, protonation by MOPAC, and of lipophilicity by our empirical program LIPOP(hilicity) give evidence that dapsone is more lipophilic (log P values 0.97) than sulfanilamide (-0.67). The extremely lipophilic cell wall of Mycobacterium leprae contributes to the surprising difference in anti-leprosy activity. Sulfonamides are more or less deprotonated (45 to 99 %) at physiological pH units, whereas dapsone is totally undissociated. This results in different permeability rates into the bacterial cells in vivo. Compared to other sulfones and sulfonamides, the unique combination of high lipophilicity and low ionic dissociation favors anti-leprotic potency in dapsone. On principle, amide groups do not hinder activity, but cause acidity and subsequently dissociation.

Keywords: Dapsone, Sulfonamides, Mycobacterium leprae, Lipophilicity, Molecular Modeling

Introduction

In 1873 Mycobacterium leprae was detected by G.H.Armauer Hansen. It can be found in the cells of liver, kidney, spleen, nerves, and skin of infected people. The typical mutilations and paralysis stem either from injuries of body parts which

[$] *Presented at the 11. Molecular Modeling Workshop, 6 -7 May 1997, Darmstadt, Germany*

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lost sensitivity or from progressive destruction of peripheral and motoric nerves. Over 10 million people still suffer from Leprosy worldwide. The spreading by direct body contact is an attribute of misery, poverty and insufficient sanitary conditions.

Dapsone is one of the extremely few pharmaceutical agents, which is effective against Mycobacterium leprae [1]. The activity is lower if dapsone is derivated. Acetosulfone has a N-acetylsulfamoyl substitution on one of the two benzene rings in ortho position. In thiazolsulfone one of the two aromatic rings is isosterically transformed into a thiazol ring (see Figure 1). As described in Principles of Medicinal Chemistry [2] : " ... the mode of action of the sulfones is apparently the same as for the sulfonamides. The reason for the particular effectiveness of dapsone and the comparative lack of utility of the other sulfonamides in treating Hansen's Disease (leprosy) is not understood."

Inside the cells, dapsone and the chemotherapeutic class of sulfonamides share the same mode of mechanism. They inhibit the biosynthesis of folic acid. The biomolecular mechanism is explained experimentally as competitive antagonism to p-aminobenzoic acid (PABA). In evidence the antimetabolites show a higher affinity than PABA to the binding site of dihydropteridin acid synthetase. Yet the inhibition of this enzyme itself is shown to be fully reversible if only PABA is directly administered in high concentration in vitro. During pharmacotherapy the administration of dapsone causes severe metabolic damages to sensitive micro- organism but not to man who does not biotransform PABA to folic acid, the latter being a vitamin for man. The antimicrobiotic effect of dapsone against Mycobacterium leprae is of bacteriostatic nature due to subsequent block of DNA and RNA synthesis. On the other hand the known sulfonamides do not act effectively against Mycobacterium leprae.

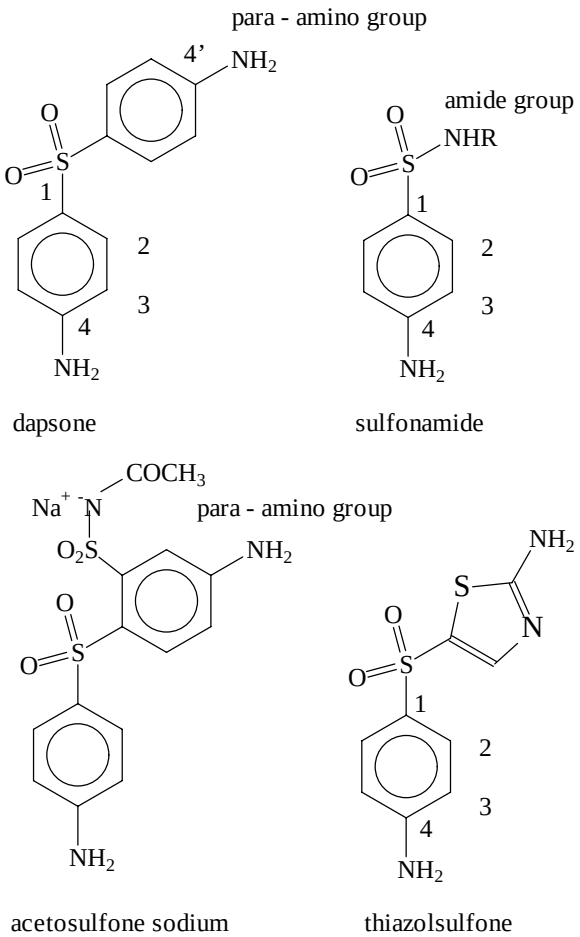


Figure 1. *Chemical structures and labeling of dapsone, derived sulfones and sulfonamides*

Computational methods

Structural differences of dapsone and sulfonamide prototype sulfanilamide are investigated theoretically. Application of Computer Aided Drug Design programs should provide evidence that changes in physicochemical molecular properties (ionization, aromaticity, hydrophilicity) between dapsone, other sulfones and related sulfanilamide account for different anti-leprosy activity.

1) In the first step the models of the sulfones and sulfonamides were built using molecular mechanics, SYBYL [3], and were compared to respective x-ray structures [4, 5] (SYBYL CRYSIN).

2) Upon geometrical refinement ESP charges were calculated by semiempirical molecular orbital package MOPAC (keywords AM1, PRECISE, LOCAL, BONDS, ESP) [6].

3) In the third step heat of formation of neutral and protonated forms, dipole moment, bond length, angles, bond order were interpreted in terms of ionization, aromaticity, and valences.

4) In the following step the torsion angles of lowest energy between the two benzene planes of dapsone were separately predicted by SYBYL GRIDSEARCH.

5) Then the obtained conformations were compared to the crystal structures.

6) The dissociation ratio between free base and protonated form at physiological pH value was computed based on experimental pKa values.

7) Finally, molecular lipophilicity was assessed and visualized using LIPOP [7]. The partition coefficient of octanol - water distribution, log P, was compared to ClogP [8] and experimental data.

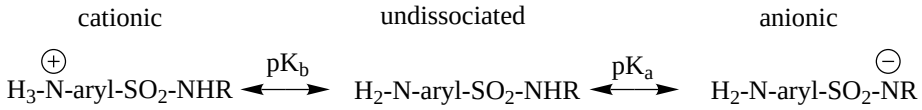
Molecular properties and biological data

Dapsone exists in different crystal forms. Its solubility in water is very low. Sulfonamides are of amphoteric character, stemming from the weak basic aromatic p-amino function (pKb

Table 1. *Lipophilicity, acidity, dissociation and amphoteric reactions of sulfonamides*

Sulfonamide	logP value calculated by LIPOP	pKa	dissociation in % of total concentration at pH 7.41
hypothetical anti-leprotic	>+0.2	>11	<0.01 %
sulfathiocarbamide	+0.9	4.8	99.8 %
sulfisomidine	+0.8	7.4	50.6 %
sulfacetamide	+0.7	5.4	99.0 %
sulfamethoxazole	+0.4	5.6	98.5 %
sulfamerazine	+0.3	7.0	72.0 %
sulfadoxine [a]	+0.2	5.8	97.6 %
sulfadiazine	-0.1	6.5	89.0 %
sulfaguanidine	-1.3	11.3	0.01 %
sulfacarbamide	-1.4	5.4	99.0 %

[a] clinically tested as weak anti-leprotic agent.



= 11.7) and the N-H acidity of (sulfone) amide groups with varying pKa between 4.5 and 7.5 (see Table 1). Generally, solution is bad in organic lipophilic solvents [9].

The mycobacterial cell wall contains large amounts of unusual lipids [10]. The lower permeability of the cell wall to antibiotics and chemotherapeutic agents contributes to the drug resistance of mycobacteria. The low permeability of the mycobacterial cell wall, with its unusual structure, is now known to be a major factor in this resistance. Thus hydrophilic agents, i.g. sulfonamides, cross the cell wall slowly because the mycobacterial porin is inefficient in allowing the permeation of solutes and exists in low concentration. The agents are presumably slowed down by the lipid bilayer which is of unusually low fluidity and abnormal thickness. Besides the cell wall barrier there exist two further factors contributing synergistically to multiple drug resistance, the enzymatic inactivation of drugs, and carrier-mediated transport mechanism [11]. In some cases resistance phenomena are caused by bacterial overproduction of PABA. They are not considered in this paper.

On the other hand the different sensitivity levels of various mycobacterial species to lipophilic inhibitors are due to the differences in cell wall structure. More hydrophilic and polar agents in contrast, traverse the cell wall presumably through channels of porins [11] in both directions.

Results

Dapsone lacking the amide group of sulfanilamide possesses a symmetrical diphenyl sulfone substructure. The two phenyl planes in Dapsone are slightly distorted from what should be a symmetrical geometry. The torsion angle between the two benzene planes, C(1)C(2)-C(1')C(2'), corresponding to the most stable conformation of 140 , 144 , 150 results from either SYBYL, MOPAC, or X-ray data [4, 5].

Analyzing bond length, charge distribution, bond-order matrix and valence we find typical items of partial chinoid character instead of aromaticity: the bond length of exocyclic N(4) atom (the para- or 4- amino- nitrogen) and its adjacent C(4) atom (the ring carbon), is shortened (142 pm; against 147 pm), evidence for partial double bond type. Within the benzene ring there are four C-C bonds (1-2, 3-4, 4-5, 6-1 of 140 pm) having a bond order of 1.3, and two shorter C-C bonds (2-3 and 5-6 of 138 pm) with an bond order of 1.5 (see Figure 1).

The sulfur constitutes a polarizable atom, thanks to its soft d orbital and proximity to electronegative atoms (oxygen). The negative charges on both para-amino nitrogen are not significantly different in dapsone and sulfanilamide if calculated by the AM1 Coulson method [6] and compared to nitrogen in aniline: N(amine) in aniline -0.32; N(amine) in sulfanilamide -0.39; and N(amine) in dapsone -0.39. The

Table 2. *Listing of collected (*) and computed data; the results of the molecular modeling study are consistent with the experimental data.*

Molecular Property	**(Lead Compound) DAPSONE**	**(Prototype) Sulfanilamide**	**(Annotations) Sulfonamides**
acid strength:			
pKa (NH_4-aryl)	1	2.4	2.3 to 2.4
pKa (RNH-aryl)	not existent	10.43	4.5 to 7.5
electron density:			
charge N(amine)	-0.8 ESP	-0.9 ESP	(MOPAC-AM1)
base strength:			(the lower pKb
pKb (amine)	13	11.6	the stronger)
lipophilicity:			
log P by exper.	+ 0.97	- 0.67	(lipophilic: >0)
log P by Lipop	+ 0.8	- 0.4	+0.8 to -1.4
log P by ClogP	+ 1.1	- 0.8	(hydrophilic: <0)
dissociation: at pH = 7.41	< 0.01 %	0.2 %	99 % to 44 %
bio-reactivity:			
bacteriostatic concentration	5 [µg/ml] = 2E-5 [Mol/L]		ca. 50 [µg/ml] = 3E-4 [Mol/L]
anti-leprosic	yes	no	no (weakly sulfadoxine)

partial charge of N(amide) in sulfanilamide equals to - 0.95. Finally the charges were refined by the more sophisticated electrostatic potential (ESP) method as resumed in tab. 2. The amino group of dapsone possesses a weaker electron density which is consitent with a smaller amine base strength.

Given the amphoteric reaction scheme for sulfanilamide discribed in Table 1, the first acid constant holds a pKa of 2.4 for the equilibrium between ammonium (H_3N-aryl) and neutral form (H_2N-aryl). To the second pKa a value of 10.43 is ascribed. Being by far the weakest acid of all sulfonamides, we replace this unsubstituted (R=H) sulfonamide (SO_2-NH-R) by sulfisomidine (R=aryl) having a more typical pKa of 7.4. The pKb of dapsone is reported as 13. (pKa = 1 of dapsonium cation) [9]. The pH value in aqueous tissue fluids is between 7.2 and 7.4 units. We adapted the program for pH calculations of weak acids from [12]. The parameters of dapsone; respectively sulfanilamide follow:

- the molar mass 248.3 u; resp. 172.2 u,
- the bacteriostatic blood concentrations 5 µg/mL = 2E–5 Mol/L; resp. 50 µg/mL = 3E–4 Mol/L. Simulating physiological pH values in water 99.9% of dapsone is undissociated, whereas dissociation varies between 99 % and 44 % among sulfonamides (pKa range from 4.5 to 7.5) [9].

The lipophilicity was assessed by our empirical program LIPOP(hilicity) [7] (see also Figure 2 and 3). There is conclusive evidence that dapsone is more lipophilic (log P values: Lipop +0.8, ClogP +1.1, exper. +0.97) than sulfanilamide (Lipop -0.4, ClogP -0.7, exper. -0.67). Many examined sulfonamides show negative log P values meaning that they are hydrophilic (see Table 1).

Moreover, the decrease of effectivity for other sulfones (roughly 0.003 mol daily dosis) than the lead compound dapsone (0.0004 mol daily dosis) can be correlated to the same parameters. Thiazolsulfone is almost as undissociated (99 %) as dapsone but far more hydrophilic (log P = –0.2). Acetosulfone sodium is even more hydrophilic (log P = -0.9) and less than 10 % of its equilibrium concentration is undissociated at pH of 7.

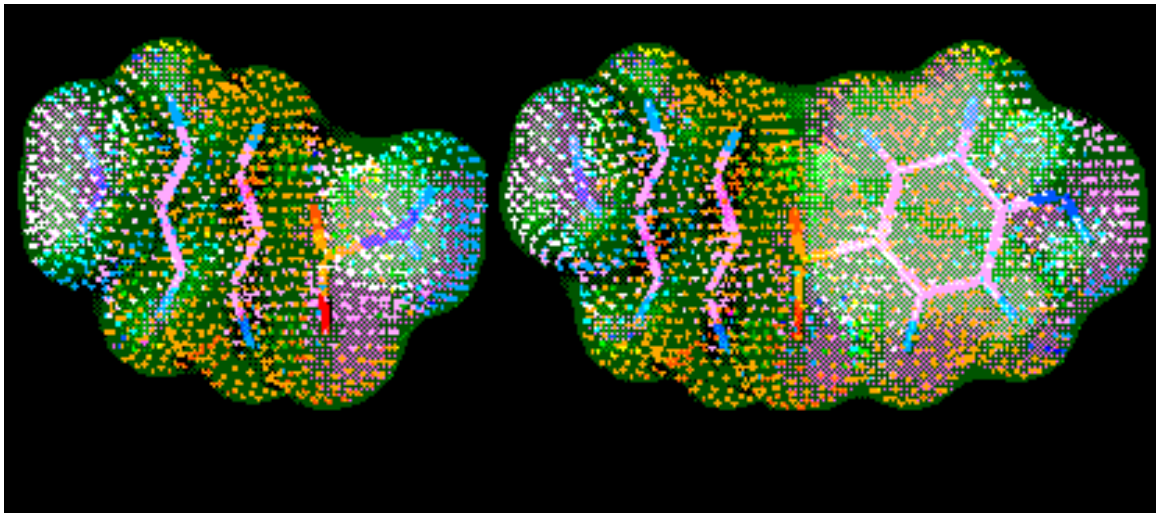


Figure 2. *Isolipophilicity plots visualize the heurestic lipophilic (green) and hydrophilic (blue) occupancy in space assessed by LIPOP. Only small hydrophilic areas are distinguished at the amino group and the sulfone substructure (-SO_2- yellow S, red O) of dapsone. The reminder is a large lipophilic overall region. In sulfanilamide (left) the hydrophobic area is restricted to the benzene ring itself, leading to predominantly hydrophilic (blue) environment, caused by SO_2-NH_2 group*

Discussion

The theoretical calculations, presented in this paper, are in excellent agreement to the collected literature data. Mycobacteria, members of which cause leprosy, produce cell walls of unusually low permeability, which contribute to their resistance to therapeutic agents. Antibiotics and chemotherapeutic agents depend on their lipophilic and acid properties whether they penetrate or not by passive transmembrane diffusion. Sulfones and sulfonamides are either more hydrophilic and/or more acid (deprotonated) at physiological pH values. Of all compounds only dapsone combines synergistically high lipophilicity and low ionic dissociation necessary for cell wall permeation of Mycobacterium leprae. On principle, amide groups do not hinder activity, but give rise to subsequent deprotonation (dissociation). Anti-leprotic acetosulfone pos-

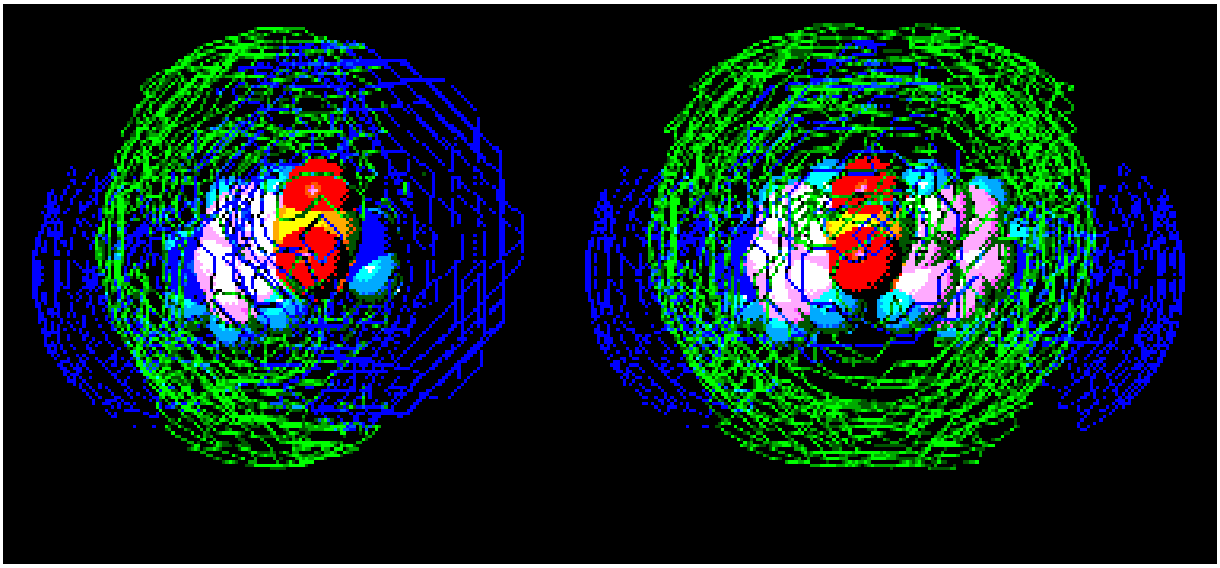


Figure 3. *The lipophilicity is quantitatively color coded; (hydrophilic =) blue - cyan - white (= neutral =) white - yellow - green (= lipophilic) and fitted to the molecules surface as dots to visualize their molecular property; sulfanilamide left, dapsone right; an option within LIPOP*

sesses both, a sulfone (R-SO_2-R) and a sulfonamide (R-SO_2-NH-R) substructure. Indeed, cross resistance with dapsone and weak anti-leprotic potency have been ascribed to long-acting sulfonamides such as sulfadoxine, (N-[5,6-dimethoxy pyrimidin-4-yl] sulfanilamide), sulfadimethoxine (N-[2,6-dimethoxy pyrimidin-4-yl] sulfanilamide), and sulfamethoxy-pyridazine (N-[6-methoxy pyridazin-3-yl] sulfanilamide).

The worldwide increase in lepra, the additional problem of increasing multiple drug resistance, and the primary resistance of mycobacteria require new strategies in drug development and in therapy. The reason for resistance is manifold. One important factor is the change in cell wall construction which limits the permeability of the drugs to the target receptor. It is reported that within a class of tuberculostatic drugs, showing identical mode of action against mycobacteria tubercolosis, the more lipophilic derivative is more effective. In addition, it has been observed that mycobacteria are able to synthesize additional multilamellar cell wall components for protection (resistance).

Among several drug development strategies to overcome multiple drug resistance is improving the permeation properties of drugs. The different effectivity in treatment of leprosy is caused by pharmacokinetic differences of the agents [13]. As can be seen from the results, ionization and lipophilicity are the critical molecular properties. The passive diffusion rate through bacterial cell walls is directly proportional to the concentration gradient between the biological compartments i.e. extra- and intracellular aqueous vs. lipid barrier for nonionized molecules. Therefore lipid solubilty, approximately parametrized as log P, is crucial for anti-leprosy potency. The NH-acid strength implies dissociation rate lowering the concentration of bioactive nonionized sulfonamides. Since acidity effects hydrogen-bonding to water and lipid solubility reversely at the same time, both aspects should be considered.

We suggest to augment the lipophilicity and reduce the dissociation of sulfonamide groups while preserving the (pharmacodynamical) PABA-antimetabolite substructure.

Although octanol-water partitioning do not weight proportionally solute interaction with apolar (cell wall) - polar (intra-, extracellular liquid) phases and hydrogen bonding, because of 2 molar water content in saturated octanol phase, this traditional approach became a standard molecular parameter to describe pharmacological aspects.

Valuable information (Figure 2 and 3) about log P, molecular lipophilic potentials in space and on the molecular surface is easily obtained through LIPOP, a macro written for the molecular modeling program SYBYL.

Acknowledgments: We thank Mr. G. Helms, for photographing and Dr. W. Zimmermann for administrative support, both Pharmaceutical Institute, University Tübingen; Prof. Dr. H.-D. Höltje, Pharmaceutical Institute, University of Düsseldorf, Germany for discussion; and Fatol-Arzneimittel GmbH, D-66573 Schiffweiler, Germany, for information on dapsone; Tripos Associates, Inc. for Sybyl Programming Language support.

Supplementary material: LIPOP can be obtained from the authors electronically.

References

1. Wozel, G. *Dapson*; Georg Thieme: Stuttgart, New York, 1996; pp 2-26.
2. Foye, W. O.; Lemke, Th. L.; Williams, D. A. *Principles of Medicinal Chemistry*; Williams & Wilkins: Media PA, 1995; pp 766-767.
3. *SYBYL*; molecular modeling software, Tripos Ass. Inc.: St. Louis, MO; 1996.
4. Lin, H.O.; Baenziger, N.C.; Guillory, J.K. *J. Pharm. Sci.* **1974**, 63, 145-146.
5. Tiwari, R.K.; Singh, T.P. *Indian J. Phys.* **1982**, 56A, 420-426.
6. *MOPAC 6.0*; Quantum-Chemical Program Exchange; semiempirical CADD software; Indiana University; Bloomington; IN; 1990.
7. Scior, T.; Winiwarter, S.; Wermuth, C.G. Presented at the 9th Molecular Modeling Workshop; Thechnische Hochschule Darmstadt, Germany; May 1995.
8. *ClogP 3.42*; Medicinal Chemistry Project; Claremont, CA, 1986.
9. Roth, H.J.; Eger, K.; Troschütz R. *Arzneistoffanalyse : Reaktivität, Stabilität, Analytik*; Georg Thieme: Stuttgart, New York, 1985, pp 432.
10. Liu, J.; Barry, C.E.; Besra, G.S.; Nikaido H. *J. Biol. Chem.* **1996,** *271*, 29545-29551.
11. Jarlier, V.; Nikaido H. *FEMS-Microbiol-Lett.* **1994**, *123*, 11-18.
12. Ebert, K.; Ederer, H.; Isenhour, T.L. *Computer Applications in Chemistry*; VCH: Weinheim, 1989; pp 180-182.
13. Kaul, S.; Ritschel, W. A.; *Eur. J. Drug Metab. Pharmacokinet.* **1990,** 15, 211-217.
CAS-online, MEDLINE, BIOSIS and CC were searched for the following keywords: mycobacterium leprae, antilepro*, dapsone, sulfon*. sulfonamid*, lipophil*, diffusion, dos*, concentr*, model*