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Combination of molecular modeling and quantitative structure–activity relationship analysis in the study of antimycobacterial activity of pyridine derivatives

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Abstract

A set of 4-benzylsulfanyl derivatives of pyridine-2-carbonitriles and pyridine-2-carbothioamides, previously tested for their antimycobacterial activity, were analysed by quantitative structure–activity relationship (QSAR) techniques, using some physicochemical and quantum–chemical parameters. The resulting QSAR revealed that the activity increases with electron withdrawing substituents in the benzyl moiety of studied compounds. HOMO orbitals can play an important role in the description of the mechanism of interactions at the molecular level. Additionally, the results of multiple linear regression indicate the differences between *Mycobacterium tuberculosis* and *M. avium*. The hydrophobicity of studied compounds is important for activity against *M. avium*.

Keywords: 4-(benzylsulfanyl)pyridine-2-carbonitrile; 4-(benzylsulfanyl)pyridine-2-carbothioamide; Antimycobacterial activity; Quantum–chemical calculations; Structure–activity relationships

1. Introduction

The stark reality, largely overlooked, is that among infectious diseases, tuberculosis (TB) is the leading cause of the death. Each year, there are an estimated 8 million new cases of TB and ~ 3 million deaths from the disease. Approximately one-third of the world's population harbours *Mycobacterium tuberculosis* and is at risk for developing the disease (Rouhi, 1999). The serious problem with regard to treatment has become the multi-drug resistance strains of *M. tuberculosis* and the infections caused by atypical mycobacteria (nontuberculous mycobacteria), especially by *M. avium* complex (MAC), which is considered inherently resistant to the used antituberculous drugs (Bermudez and Young, 1995). Disseminated infection with *M. avium* complex is the most common systematic bacterial infection complicat-

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ing AIDS (Wolinsky, 1992). The basis for the resistance of MAC complex has been largely ascribed to the complex structure of the cell wall and the resulting impermeability (Inderlied et al., 1993).

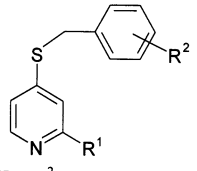
Recently, we have described the synthesis and the antimycobacterial activity of 4-benzylsulfanyl derivatives of pyridine-2-carbonitrile and -2-carbothioamide (Fig. 1). The determination of the in vitro activity of these compounds revealed the significant activity both against *M. tuberculosis* and *M. avium*. Whereas *M. avium* are moderately susceptible towards isoniazide (INH), the newly prepared compounds display practically the same activity against both tested strains, so that they reach the activity of INH against *M. tuberculosis* (MIC 4 μmol/l) and their activity against *M. avium* exceed that of INH (Klimešová et al., 1999a).

In the present paper, quantitative structure–activity relationship (QSAR) analysis of some active 4-benzylsulfanyl derivatives is reported. We tried to obtain the information of mechanism of action at the molecular level and to analysed the cause of differences of the activity of compounds against mentioned strains of mycobacteria by using multivariable linear regression analysis.

Physicochemical and quantum–chemical parameters taken into consideration in QSAR study are log P for the hydrophobic effects, σ, ε_{HOMO}, ε_{LUMO} as the electronic influences, MR (molar refractivity) for the steric interactions.

2. Materials and methods

The synthesis of 4-benzylsulfanyl derivatives of pyridine-2-carbonitrile and pyridine-2-carbothioamide have already been described (Klimešová et al., 1999a).



R^1= CN, $CSNH_2$; R^2= H, Cl, Br, F, CH_3, NO_2, OCH_3

Fig. 1. Structures of compounds **1–40.**

The antimycobacterial activity against *M. tuberculosis* CNCTC My 331/88 and *M. avium* CNCTC My 330/88 were determined as a minimum inhibitory concentration (MIC). Testing was carried out in Šula's semisynthetic medium (SEVAC Prague). After incubation at 37°C for 14 days, MIC was determined and expressed in mol/l (Klimešová et al., 1999a). MIC is the lowest concentration of a substance, at which the inhibition of the growth of mycobacteria occurred. For QSAR study the activities are given in log $1/C$ values, where C is the molar concentration of the observed MIC (Table 1).

Quantum–chemical calculations were run on a PC computer using software HyperChem Suite for Windows (release 5.1). The semi-empirical AM1 (Austin Model 1) method was used for all molecular modeling calculations. The most stable conformations of the molecules were found by the molecular dynamics, by simulated heating to 700 K followed by the Monte Carlo method (293 K, 500 steps).

Table 1 indicates the highest occupied molecular orbital (ε_{HOMO}) and the lowest unoccupied molecular orbital energies (ε_{LUMO}) (eV) of compounds **1–40**. Calculations of log P values were carried out on the software HyperChem Suite for Windows (release 5.1), the module ChemPlus 1.6 using atomic parameters derived by Ghose et al. (1988), and later extended by Viswanadhan et al. (1989). The MR was estimated by the same method as log P. Atomic contributions for these calculations were published by Ghose and Crippen (1987), Viswanadhan et al. (1989). Parameters σ were taken from the table given by Kuchař and Rejholec (1987). The indicator variable I expresses the replacement of –CN group in position 2 of pyridine moiety by $-CSNH_2$ group. I is defined as 1 for CN derivatives and 0 for $CSNH_2$ derivatives.

Correlation and regression analyses of the QSAR study were run on a PC computer using the Microsoft Excel program. Multiple regression analysis which involves finding the best fit of dependent variable (antimycobacterial activities) to a linear combination of independent variables (descriptors) are used by the least squares method.

Table 1
Biological activity and physicochemical and quantum chemical parameter values of benzylsulfanyl derivatives of pyridine **1–40**

Compound	R^1	R^2	log $1/C$[a]	log $1/C$[b]	σ[c]	log P	MR (Å)	I[d]	HOMO (eV)	LUMO (eV)
1	CN	H	3.90	3.90	0.00	3.04	66.79	1	−8.8588	−0.7013
2	CN	2-Cl	–	4.21	–	3.55	71.59	1	−8.8679	−0.7037
3	CN	3-Cl	4.21	4.51	0.37	3.55	71.59	1	−8.9492	−0.7625
4	CN	4-Cl	4.51	4.80	0.23	3.55	71.59	1	−8.9552	−0.7681
5	CN	2-F	3.90	3.90	–	3.18	67.00	1	−8.8887	−0.7160
6	CN	3-F	4.21	4.21	0.34	3.18	67.00	1	−8.9648	−0.7742
7	CN	4-F	4.21	4.51	0.06	3.18	67.00	1	−8.9592	−0.7714
8	CN	3-Br	4.21	4.80	0.39	3.83	74.41	1	−8.9591	−0.7687
9	CN	4-Br	4.51	–	0.23	3.83	74.41	1	−8.9753	−0.7831
10	CN	3-CH_3	4.21	4.51	−0.07	3.50	71.83	1	−8.8387	−0.6895
11	CN	4-CH_3	4.21	4.51	−0.17	3.50	71.83	1	−8.8331	−0.6840
12	CN	3-NO_2	–	3.60	0.71	2.99	74.11	1	−9.1764	−1.3881
13	CN	4-NO_2	–	–	0.78	2.99	74.11	1	−9.2308	−1.5735
14	CN	4-OCH_3	–	–	–0.27	2.78	73.25	1	−8.8291	−0.6801
15	CN	3,4-Cl_2	4.21	4.51	0.60	4.07	76.40	1	−9.0254	−0.8866
16	CN	3,4-F_2	3.90	4.80	0.40	3.32	67.22	1	−9.0631	−0.9471
17	CN	2-Cl-6-F	–	–	–	3.69	71.81	1	−8.8639	−0.8559
18	CN	2-F-6-NO_2	–	3.90	–	3.13	74.33	1	−9.0481	−1.6005
19	CN	2,4-$(NO_2)_2$	4.21	4.51	–	2.94	81.44	1	−9.3927	−2.2682
20	CN	3,5-$(NO_2)_2$	4.80	4.51	1.42	2.94	81.44	1	−9.4452	−2.1272
21	$CSNH_2$	H	4.80	4.80	0.00	2.65	77.74	0	−8.5095	−0.8209
22	$CSNH_2$	2-Cl	–	–	–	3.17	82.55	0	−8.5126	−0.8226
23	$CSNH_2$	3-Cl	5.10	5.10	0.37	3.17	82.55	0	−8.5621	−0.8839
24	$CSNH_2$	4-Cl	5.10	5.10	0.23	3.17	82.55	0	−8.5584	−0.8777
25	$CSNH_2$	2-F	5.40	4.80	–	2.79	77.96	0	−8.5211	−0.8367
26	$CSNH_2$	3-F	5.10	4.80	0.34	2.79	77.96	0	−8.5630	−0.8821
27	$CSNH_2$	4-F	5.10	4.80	0.06	2.79	77.96	0	−8.5635	−0.8757
28	$CSNH_2$	3-Br	5.10	5.10	0.39	3.45	85.36	0	−8.5590	−0.8783
29	$CSNH_2$	4-Br	4.51	5.10	0.23	3.45	85.36	0	−8.5709	−0.8856
30	$CSNH_2$	3-CH_3	5.10	5.10	−0.07	3.12	82.78	0	−8.5000	−0.8098
31	$CSNH_2$	4-CH_3	4.80	4.80	−0.17	3.12	82.78	0	−8.4963	−0.8056
32	$CSNH_2$	3-NO_2	5.40	5.10	0.71	2.61	85.07	0	−8.6623	−1.2868
33	$CSNH_2$	4-NO_2	5.40	5.40	0.78	2.61	85.07	0	−8.6979	−1.4579
34	$CSNH_2$	4-OCH_3	–	4.80	−0.27	2.40	84.20	0	−8.4931	−0.8046
35	$CSNH_2$	3,4-Cl_2	5.10	5.40	0.60	3.69	87.35	0	−8.5912	−0.9191
36	$CSNH_2$	3,4-F_2	5.10	5.10	0.40	2.93	78.17	0	−8.6107	−0.9422
37	$CSNH_2$	2-Cl-6-F	5.10	–	–	3.31	82.76	0	−8.5059	−0.8285
38	$CSNH_2$	2-F-6-NO_2	–	–	–	2.75	85.28	0	−8.5577	−1.5065
39	$CSNH_2$	2,4-$(NO_2)_2$	5.10	4.80	–	2.56	92.39	0	−8.7340	−2.1602
40	$CSNH_2$	3,5-$(NO_2)_2$	5.40	5.10	1.42	2.56	92.39	0	−8.7724	−2.0254

[a] The molar concentrations (mol/l) of the MIC values against *Mycobacterium tuberculosis*.
[b] The molar concentrations (mol/l) of the MIC values against *Mycobacterium avium*.
[c] Kuchař and Rejholec (1987).
[d] Indicator variable.

In the equations, the figures in the parentheses are the standard errors of the regression coefficients, n is the number of compounds, r is the multiple correlation coefficient, r^2 is the determination coefficient, F is the significance test (F-test) and s is the standard error of estimate. F-test values are for all equations statistically significant at the 1% level of probability.

3. Results and discussion

The values of physicochemical parameters and frontier molecular orbital energy levels for compounds **1–40** are given in Table 1. The antimycobacterial activities of studied compounds against *M. tuberculosis* and *M. avium* are represented with the log $1/C$ values. C is the molar concentration of the observed MIC given in mol/l. An increase in log $1/C$ value indicates the enhancement of the activity.

The distribution of the HOMO orbitals in the most active nitrile **20** and thioamide **33** are shown in the Figs. 2 and 3. Distribution of HOMO orbital is different in thioamide and nitrile derivatives. By thioamides HOMO orbital is mostly located on sulfur in the thioamide group. Whereas in nitriles, HOMO orbital is located on sulfur in the sulfanyl group and in the surrounding of the sulfanyl group of pyridine moiety. Atoms with the highest density of this orbital could be the place of the highest reactivity of the substance and the compound could react there as a nucleophilic reagent according to frontier molecular orbitals theory.

For quantitative evaluation of the structure–antimycobacterial activity relationships the multivariable regression analysis based on independent variables σ, log P, MR, ε_{HOMO}, ε_{LUMO} were used. The indicator variables I designating the nitriles and thioamides was introduced in all equations. Table 2 displays the QSAR equations for *M. tuberculosis*. Equations 1–2 indicate that hydrophobic effect expressed by log P has a little influence on the activity. Correlation results, shown in two variable equations 3–4, exhibit that electronic parameters σ and quantum–chemical parameters ε_{HOMO} are much more important for the activity.

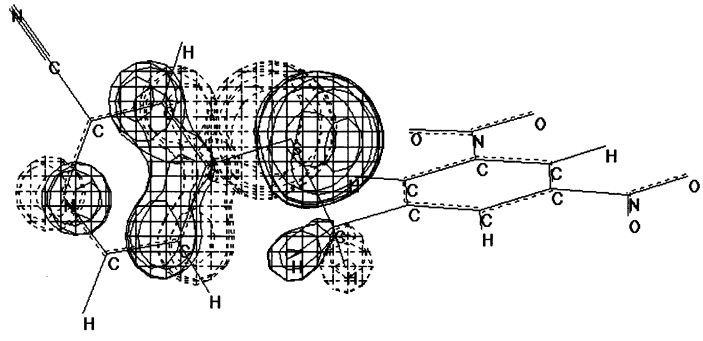


Fig. 2. HOMO orbital of the compound 20. Orbital contour value is 0.04.

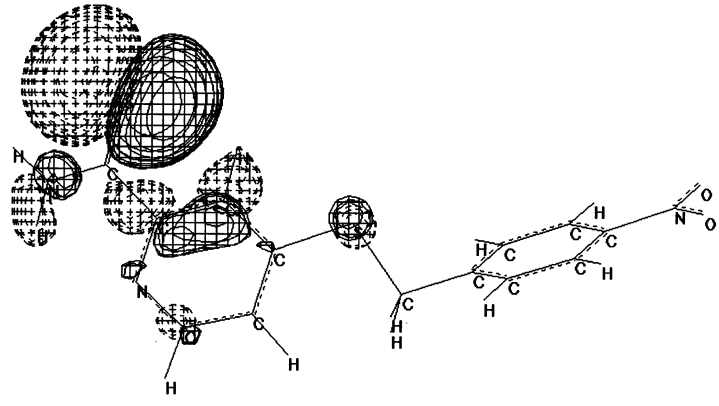


Fig. 3. HOMO orbital of the compound 33. Orbital contour value is 0.025.

Table 2
QSAR equations and statistical parameters for *Mycobacterium tuberculosis*

Equation number	Equation	N	r	r^2	s	F
1	$\log(1/C) = -0.781(\pm 0.326)\ \mathrm{HOMO} - 0.015(\pm 0.129)\ \log P - 1.193(\pm 0.185)\ I - 1.566(\pm 2.985)$	31	0.907	0.823	0.222	41.72
2	$\log(1/C) = 0.367(\pm 0.101)\ \sigma - 0.082(\pm 0.118)\ \log P - 0.761(\pm 0.093)\ I + 5.184(\pm 0.370)$	26	0.924	0.854	0.197	42.58
3	$\log(1/C) = -0.797(\pm 0.291)\ \mathrm{HOMO} - 1.206(\pm 0.145)\ I - 1.745(\pm 2.497)$	31	0.907	0.823	0.218	64.87
4	$\log(1/C) = 0.384(\pm 0.096)\ \sigma - 0.797(\pm 0.077)\ I + 4.931(\pm 0.064)$	26	0.922	0.850	0.195	65.09

Table 3
QSAR equations and statistical parameters for *Mycobacterium avium*

Equation number	Equation	N	r	r^2	s	F
5	$\log(1/C) = -0.723(\pm 0.348)\ \mathrm{HOMO} + 0.498(\pm 0.139)\ \log P - 1.166(\pm 0.199)\ I - 2.660(\pm 3.168)$	33	0.840	0.706	0.250	23.16
6	$\log(1/C) = 0.186(\pm 0.117)\ \sigma + 0.401(\pm 0.138)\ \log P - 0.780(\pm 0.113)\ I + 3.785(\pm 0.422)$	27	0.826	0.682	0.249	16.48
7	$\log(1/C) = -0.236(\pm 0.377)\ \mathrm{HOMO} - 0.755(\pm 0.192)\ I + 2.983(\pm 3.241)$	33	0.759	0.576	0.296	20.36
8	$\log(1/C) = 0.385(\pm 0.135)\ \log P - 0.811(\pm 0.107)\ I + 3.880(\pm 0.402)$	33	0.813	0.661	0.264	29.33

The regression analyses are statistically significant. The simple correlation between parameters σ and $\varepsilon_{\mathrm{HOMO}}$ exhibits the collinearity of these parameters. Statistical parameters of these correlations for the group of thioamide derivatives are $r = 0.937$, $r^2 = 0.878$, $s = 0.027$, $F = 43.5$ and $r = 0.961$, $r^2 = 0.923$, $s = 0.049$, $F = 77.7$ for the group of nitrile derivatives, respectively, therefore they are used in correlation equations separately. It is concluded that HOMO orbital can play an important role for the interaction of the molecule with the receptor at the molecular level (Eq. 3), the negativity of the $\varepsilon_{\mathrm{HOMO}}$ values is proportional to the increase of the activity. Extending the Eq. 3 by LUMO orbital as a further variable lead to the deterioration of statistic parameters ($F = 41.70$). In addition to that, the coefficient of above mentioned variable is insignificant ($0.003(\pm 0.222)$ LUMO). In accordance with the Equation 4, the activity is related with the electronic parameters of substituents in the benzyl moiety. The activity increases with electron withdrawing substituents in the benzyl moiety of studied compounds. The QSAR analysis reveals that the MR considered as the steric factor is not significant for the potency of studied compounds.

QSAR analysis for *M. avium* are summed up in Table 3. The results of correlation equations revealed some differences between strain *M. tuberculosis* and *M. avium*. Equations 5–6 indicate that both the electronic and the hydrophobic parameters participate in the activity, the hydrophobic effect being predominantly responsible for the activity determination (see Eqs. 7–8). Moreover, the correlation between the activities and Hammett constants σ as an electronic parameter is less statistically significant ($r = 0.752$, $F = 15.65$). The differences of the influence of hydrophobicity on the activity of studied compounds against both tested strains exhibited the following statistically significant equation

$$\log (1/C)_{\text{avium}} = 0.740(\pm 0.093)\ \log (1/C)_{\text{tbc}} + 0.392(\pm 0.119)\log P + 0.057(\pm 0.721)$$

$$n = 29;\ r = 0.846;\ r^2 = 0.716;\ s = 0.208;\ F = 32.62$$

This conclusion should be connected to the differences of the structure of the cell wall of *M. avium*.

The observed theoretical quantum–chemical calculations show that the HOMO orbital is related to the activity against both strains. On the basis of the localization of HOMO orbital (Figs. 2 and 3), the lone pair electrons of sulfur atom either on $CSNH_2$ group in thioamide derivatives or sulfanyl group in nitrile derivatives can interact with the receptor site. These conclusions confirm our earlier pronounced hypothesis that pharmacophores of antimycobacterial activity are the alkylsulfanyl group (Waisser et al. 1995) and the thioamide group (Klimešová et al. 1999b). HOMO orbitals are located on both pharmocophores. In the cases of nitriles derivatives, the benzylsulfanyl group should be responsible for the activity. Both groups can participate in the activity of thioamides on the basis of localization of HOMO orbital, the thioamide group being predominantly responsible.

4. Conclusions

This study revealed that the antimycobacterial activity of benzylsulfanyl derivatives of pyridine is related with the substituents on benzyl moiety. Electron withdrawing substituents cause the increases of the activity. According to the found influence of HOMO orbitals on the antimycobacterial activity, it can be concluded, that the receptor site seems to have an electron accepting property. The thioamide group or sulfanyl group of benzylsulfanyl moiety in studied compounds is the pharmacoforic site of the molecule. Additionally, the observed quantum–chemical calculations revealed the differences between the *M. tuberculosis* and *M. avium*. The activity of studied compounds against *M. avium* is also related to their hydrophobicity.

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