ANTIMYCOBACTERIAL 2-ARYLAMINO-2-THIOXOACETAMIDES*

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Dedicated to Professor Václav Horák on the occasion of his 70th birthday.

A series of 13 2-arylamino-2-thioxoacetamides was tested against *Mycobacterium tuberculosis* and *M. kansasii*. Structure–activity relationships were analyzed quantitatively using the Free–Wilson method. Evaluation of the antimycobacterial profile with regard to equipotency of both activities was accomplished by means of a recently proposed method for evaluation of activity profile with respect to intended therapeutical use.

Among other mechanisms of action of antituberculotic agents, chelation of the trace transient elements is often considered[1]. In vitro antimycobacterial activity has been found in a number of chelating analytical agents[2]. In our previous papers[3,4] the synthesis and the in vitro antimycobacterial activities of a novel group of potential antituberculotics, the thioxamides, were described. This present communication reports some results of antimycobacterial testing against *Mycobacterium tuberculosis* and *M. kansasii* obtained with a monothio analogues – 2-arylamino-2-thioxoacetamides, mostly 2-arylamino-2-thioxoacetanilides. In connection with the chelation hypothesis, the compounds studied include derivatives possessing thioanilide moiety substituted in position 2 by an amino or hydroxy group, i.e. a group which also is able to take part in chelation. As part of our research program, we studied quantitative relationships between chemical structure and antimycobacterial activity against both *Mycobacterium*

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species using the Free–Wilson method[5]. In addition, we evaluated the antimycobacterial profile in this series of compounds with the help of procedure developed recently[6,7]. For the sake of clarity, a brief outline of this procedure is provided too.

EXPERIMENTAL

Table I lists the compounds studied. Their synthesis is described elsewhere[8]. Here we present only the preparation of compound *VI* by an alternative method.

Antimycobacterial testing. The compounds studied were tested for their in vitro activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and *M. kansasii* PKG 8 in the form of dimethyl sulfoxide solution of the following concentrations: 1.3, 4.1, 12.3, 37, 111, 330, and 1 000 μmol/l. Minimum inhibitory concentrations (MIC) were taken after 15 day incubation at 37 °C on a liquid semisynthetic Sula medium (USOL Prague). In case of partial inhibition the respective value of concentration multiplied by two was considered. Table I summarizes the results in the form of logarithms of reciprocal MIC.

N-(4-Methylphenyl)-2-(4-methylphenylamino)-2-thioxoacetamide (*VI*)

A mixture of 4-methyloxalanilide (2.7 g, 10 mmol) and phosphorus pentasulfide (2.2 g, 10 mmol) in pyridine (50 ml) was refluxed for 15 min. After cooling, the mixture was poured on ice and the resulting precipitate was collected by filtration and purified by column chromatography on silica gel eluting with benzene–hexane (1 : 1). Crystallization from ethanol gave 1.2 (43%) of *VI* melting at 159 – 160 °C. For $C_{16}H_{16}N_2OS$ (284.4) calculated: 11.28% S; found 11.31% S.

TABLE I

Survey of the 2-arylamino-2-thioxoacetamides studied (R_1–NH–COCS–NH–R_2), antimycobacterial activities against *M. tuberculosis* A_1 and *M. kansasii* A_2 (A = –log MIC, MIC minimum inhibitory concentration in μmol/l), and values of complex criterion S_0, norm $g(\boldsymbol{A})$ and cosine coefficient $k(\boldsymbol{A}, \boldsymbol{U}_0)$ (see the text)

Compound	R_1	R_2	A_1	A_2	S_0	$g(\boldsymbol{A})$	$k(\boldsymbol{A}, \boldsymbol{U}_0)$
I	cyclohexyl	2-HOC$_6$H$_4$	−1.392	−2.045	−2.430	2.474	−0.982
II	C$_6$H$_5$	4-H$_2$NC$_6$H$_4$	−2.346	−3.000	−3.780	3.808	−0.993
III	C$_6$H$_5$	2-H2NC$_6$H$_4$	−1.568	−2.045	−2.555	2.577	−0.991
IV	C$_6$H$_5$	2-HOC6H$_4$	−1.568	−2.045	−2.555	2.577	−0.991
V	4-CH$_3$C$_6$H$_4$	2-HOC$_6$H$_4$	−1.392	−2.045	−2.430	2.474	−0.982
VI	4-CH$_3$C$_6$H$_4$	4-CH3C$_6$H$_4$	−1.568	−2.522	−2.892	2.970	−0.974
VII	H	2-H$_2$NC$_6$H$_4$	−2.045	−2.522	−3.229	3.247	−0.995
VIII	H	4-H$_2$NC$_6$H$_4$	−3.000	−3.477	−4.580	4.592	−0.997
IX	1-naphthyl	2-H$_2$NC$_6$H$_4$	−2.045	−2.045	−2.892	2.892	−1.000
X	2-naphthyl	4-H$_2$NC$_6$H$_4$	−3.477	−2.522	−4.242	4.295	−0.988
XI	1-naphthyl	2-HOC$_6$H$_4$	−1.568	−2.045	−2.555	2.577	−0.991
XII	H	C$_6$H$_5$	−1.568	−2.522	−2.892	2.970	−0.974
XIII	2-naphthyl	2-H$_2$NC$_6$H$_4$	−2.522	−2.045	−3.229	3.247	−0.995

THEORETICAL

No drug produces only one effect: a drug is adequately described by means of the full profile of effects. As the measure of i-th biological activity, the logarithm of the reciprocal value of molar concentration C_i of the compound under study required to produce i-th effect in the predetermined intensity,

$$A_i = \log \frac{1}{C_i} \tag{1}$$

is used. Each compound is then represented by an ordered n-tuple of values A_i – the activity vector,

$$\boldsymbol{A} = (A_1, \ldots, A_n), \tag{2}$$

where n is the number of activities studied.

A general formal apparatus proposed for drug evaluation[6] with regard to considered therapeutical use is based on an idea of comparison with the drug of the intended profile of effects making full use of the vector representation. Accordingly, the "ideal" drug is characterized by vector $\boldsymbol{B}$

$$\boldsymbol{B} = (B_1, \ldots, B_n), \tag{3}$$

or $\boldsymbol{U}$

$$\boldsymbol{U} = \frac{\boldsymbol{B}}{g(\boldsymbol{B})} \quad \text{so that} \quad g(\boldsymbol{U}) = 1. \tag{4}$$

The Euclidean norm of vector $\boldsymbol{A}$

$$g(\boldsymbol{A}) = \left(\sum_{i=1}^{n} A_i^2 \right)^{1/2} \tag{5}$$

characterizes the overall potency of the compound studied.

We define so-called complex criterion S as the scalar (dot) product of vectors $\boldsymbol{A}$ and $\boldsymbol{U}$

$$S = (\boldsymbol{A},\boldsymbol{U}) = \sum_{i=1}^{n} A_i U_i . \tag{6}$$

As a consequence of Schwarz's inequality relating the scalar product of two vectors to the product of their Euclidean norms, a number $k(\boldsymbol{A},\boldsymbol{U})$,

$$k(\boldsymbol{A},\boldsymbol{U}) = \frac{(\boldsymbol{A},\boldsymbol{U})}{g(\boldsymbol{A})}, \tag{7}$$

which is called cosine coefficient (pertinent to vectors $\boldsymbol{A}$, $\boldsymbol{U}$), exists. The cosine coefficient – generally $k(\boldsymbol{P},\boldsymbol{Q})$ – complies with the axioms which are imposed on the measures of similarity $s(\boldsymbol{P},\boldsymbol{Q})$ of two objects represented by vectors $\boldsymbol{P}$, $\boldsymbol{Q}$:

$$\begin{aligned} a) \quad & s(\boldsymbol{P},\boldsymbol{Q}) = s(\boldsymbol{Q},\boldsymbol{P}) , \\ b) \quad & s(\boldsymbol{P},\boldsymbol{P}) = 1 , \\ c) \quad & s(\boldsymbol{P},\boldsymbol{Q}) \leq 1 . \end{aligned} \tag{8}$$

(The first axiom expresses the fact that two objects are similar to each other; the second in regard to the third that every object is most similar to itself.)

Following relation holds true for every $\beta > 0$

$$k(\beta\boldsymbol{P},\boldsymbol{Q}) = k(\boldsymbol{P},\boldsymbol{Q}) , \tag{9}$$

from which especially follows that

$$k(\boldsymbol{A},\boldsymbol{U}) = k(\boldsymbol{A},\boldsymbol{B}) . \tag{10}$$

In the special case of broad-spectrum compounds the "ideal" drug is represented by vector $\boldsymbol{B}_0$ or $\boldsymbol{U}_0$, their components are given by

$$B_{0i} = \text{const.} \qquad i = 1, \ldots, n \tag{11}$$

and

$$U_{0i} = \frac{1}{\sqrt{n}} \qquad i = 1, \ldots, n , \tag{12}$$

respectively.

The formula for calculating the complex criterion S_0 for this special case can be written as

$$S_0 = \frac{1}{\sqrt{n}} \sum_{i=1}^{n} A_i \tag{13}$$

and for the cosine coefficient as

$$k(\boldsymbol{A},\boldsymbol{B}_0) = \frac{1}{g(\boldsymbol{A})\sqrt{n}} \sum_{i=1}^{n} A_i . \tag{14}$$

Application to QSAR Methods

Application of QSAR regression equations in connection with the above-mentioned results in the drug design is obvious:

If Hansch equations (*15*) exist for all activities studied, i.e.

$$A_i = f(\text{physico-chemical parameters}) \quad \text{for} \quad i = 1, \ldots, n, \tag{15}$$

then the analogous equation holds true for complex criterion S

$$S = f'(\text{physico-chemical parameters}). \tag{16}$$

If each activity studied can be expressed as a sum of fragment contributions (*17*) according to the Free–Wilson method[5], i.e.

$$A_i = \sum_{k=0}^{q} a_{ik} x_k \qquad \text{for} \quad i = 1, \ldots, n, \tag{17}$$

where $x_k = 1$ if k-th fragment is present in the compound under study and $x_k = 0$ if not, then

$$S = \sum_{k=0}^{q} s_k x_k, \tag{18}$$

where

$$s_k = (\boldsymbol{a}_k, \boldsymbol{U}) = g(\boldsymbol{a}_k)\, k(\boldsymbol{a}_k, \boldsymbol{U}), \tag{19}$$

by which analogous criteria for fragments are defined.

The calculations of the Free–Wilson analysis were carried out on a table computer using a Multireg-H program.

RESULTS AND DISCUSSION

The mathematical model developed by Free and Wilson[5] to study structure–activity relations quantitatively is applicable to analogous series of compounds with corresponding activity data. The Free–Wilson method (*17*) is based on the assumption that each molecular fragment makes additive and constant contribution to the activity studied. The fragment contributions for both *Mycobacterium* species are summarized in Table II. The results of statistical analysis showed that the assumption of the Free–Wilson method is fulfilled in both systems, i.e. the molecular fragments contribute to the total activity almost independently and additively. The results suggest that the most active compound against *M. tuberculosis* with regard to the selected fragments would be N-cyclohexyl- and N-(4-methylphenyl)-2-phenylamino-2-thioxoacetamide. N-(2-Naphthyl)-2-phenylamino-2-thioxoacetamide is predicted to be the most active derivative against *M. kansasii*.

Comparison of fragment contributions (Table II) shows that 2-hydroxy- and 2-aminophenyl groups are significant activity enhancement fragments, however they do

not increase the activity over an unsubstituted phenyl group. The chelation hypothesis is only partially supported by the fact that significant activity lowering fragment is 4-aminophenyl group.

For drug evaluation with regard to the considered therapeutical use a procedure was applied utilizing vector representation of compounds in the space of biological activities and comparison with the drug of the intended profile of effects. The activities studied as different qualities are measured in homogeneous units and are thus mutually comparable. A comparison of the evaluated compound with the ideal drug of a predefined profile of activities is reflected mathematically by means of scalar (dot) product of vectors representing both compounds. Out of formal reasons, the complex criterion S is proposed as scalar product $(\boldsymbol{A},\boldsymbol{U})$ (Eq. (6)), where the drug with which the comparison is made is represented by the unit vector $\boldsymbol{U}$. If we assume such a selection of concentration units which gives activity values of uniform signs after logarithmic transformation, then the most suitable drug according to this criterion is that to which the largest value of complex criterion S belongs. The term complex is motivated by the

TABLE II

Results of Free–Wilson analysis and characteristics of antimycobacterial profile for fragments: contributions to activity against *M. tuberculosis* a_1, against *M. kansasii* a_2, values of complex criterion s_0 (contribution to complex criterion S_0), norm $g(\boldsymbol{a})$ and cosine coefficient $k(\boldsymbol{a},\boldsymbol{U}_0)$ (see the text).

Fragment	a_1	a_2	s_0	$g(\boldsymbol{a})$	$k(\boldsymbol{a},\boldsymbol{U}_0)$
Skeleton	−2.005	−2.375	−3.097	3.108	−0.996
R_1 H	−0.192	−0.422	−0.434	0.464	−0.936
C_6H_5	0.301	0.066	0.259	0.308	0.842
4-$CH_3C_6H_4$	0.331	0.088	0.296	0.342	0.864
1-naphthyl	0.009	0.109	0.083	0.110	0.760
2-naphthyl	−0.669	0.294	−0.265	0.730	−0.363
cyclohexyl	0.331	0.088	0.296	0.342	0.864
R_2 4-$CH_3C_6H_4$	0.106	−0.234	−0.091	0.257	−0.353
2-HOC_6H_4	0.282	0.243	0.371	0.372	0.997
2-$H_2NC_6H_4$	0.097	0.199	0.210	0.222	0.945
4-$H_2NC_6H_4$	−0.750	−0.603	−0.957	0.963	−0.994
C_6H_5	0.628	0.275	0.639	0.686	0.932
	Statistical evaluation				
r	0.991	0.984	0.995		
s	0.172	0.161	0.139		
F	19.375	10.167	33.862		
n	13	13	13		

fact that the criterion can be decomposed into two agents, of which one concerns the given compound itself and only the second reflects the intention with which the compound is prepared and pharmacologically tested. The first is the Euclidean norm of the activity vector $\boldsymbol{A}$ (Eq. (*5*)) as a measure of its overall potency and the second is the cosine coefficient (Eq. (*7*)) as a measure of similarity to the ideal drug. It means that in evaluating according to the complex criterion S the overall potency can compensate a smaller relative agreement with the profile of ideal drug. Using the cosine coefficient only, this effect is, of course, eliminated. The more the value of the cosine coefficient approaches the value of ± 1, the more the profile of the compound under study relatively agrees with the profile of the "ideal" drug. We talk about a relative agreement since for the cosine coefficient the relation (*10*) holds true. This means that for the values of cosine coefficient relative magnitudes of individual activities are of importance. Thus the unambiguous determination of vector representing the "ideal" drug necessary for definition of the complex criterion S, does not influence the value of the cosine coefficient for the given choice of activity profile. In practical calculation of the cosine coefficient it must be taken into consideration that the relation between the magnitudes of activities expressed by both positive and negative numbers will not be reflected in the calculated norm of the activity vector in an appropriate manner, which thus distorts the calculated value of the cosine coefficient.

The results of complex antimycobacterial evaluation of the compounds studied are presented in Table I. On the basis of the convention accepted for this study, the components of vector $\boldsymbol{A}_j$ representing the j-th compound are logarithms of reciprocal minimum inhibitory concentration values (Eq. (*1*)). The values of complex criterion S_0 are all negative. The norm $g(\boldsymbol{A})$ is a non-negative number by definition; the smaller the value of the norm in this case, the larger the overall antimycobacterial activity. It is evident that the norm of activity vector in this case strongly correlates with the complex criterion S_0, which is interpreted as a measure of balance of both effects. On the basis of this criterion N-cyclohexyl-2-(2-hydroxyphenyl)-2-thioxoacetamide (*I*) and N-(4-methylphenyl)-2-(2-hydroxyphenyl)-2-thioxoacetamide (*V*) are evaluated best. Compounds *VI* and *IX* illustrate the significance of the norm of activity vector or the cosine coefficient for the evaluation. They do not differ in the value of complex criterion S_0, but the second of them (*IX*) possesses the maximal possible absolute value of the cosine coefficient. It is evident that larger value of activity against *M. tuberculosis* (compared with activity against *M. kansasii*) of compound *VI* reflected by the value of the norm $g(\boldsymbol{A})$ compensates smaller agreement with the ideal broad-spectrum profile as indicated by the value of the cosine coefficient.

The values of complex criterion S_0 are analyzable by the Free–Wilson method directly or the respective fragment contribution s_0 can be calculated from the contributions to the single activities (Eq. (*19*)). The results are shown in Table II. When interpreting the resulting decision criteria, the effect of different signs of fragment

contributions must be kept in mind. Comparison of fragments shows that the value of cosine coefficient of 2-hydroxyphenyl or 2-aminophenyl group is larger than that of unsubstituted phenyl group, however according to the value of complex criterion s_0 the phenyl group is preferred. With regard to the selected fragments, N-cyclohexyl- or N-(4-methylphenyl)-2-phenylamino-2-thioxoacetamide are predicted to have the best broad-spectrum antimycobacterial profile.

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