A NEW PHARMACOPHORE OF POTENTIAL ANTIMYCOBACTERIAL ACTIVITY: THE DISULFIDE GROUP IN THE RING OF ALICYCLIC COMPOUNDS*

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Dedicated to Dr Miroslav Protiva on the occasion of his 75th birthday.

A series of thirty 2,3-dithiabicyclo[3.3.0]octa-4,8-diene derivatives was examined for antimycobacterial activity against *Mycobacterium tuberculosis*, *M. kansasii*, *M. avium*, and *M. fortuitum*. The structure–activity relationships were analysed by Free–Wilson method. The authors consider the disulfide moiety to be the pharmacophore of antimycobacterial activity. To verify the idea, several other monocyclic sulfides, 3,4-dihydro-1,2-dithiolane derivatives, were also evaluated.

Key words: 2,3-Dithiabicyclo[3.3.0]octa-4,8-dienes; Antimycobacterial activity; Structure–activity relationships; Free–Wilson analysis.

The search for new pharmacophores of antimycobacterial activity represents one of the contemporary trends in medicinal chemistry. At present, attempts are made to find broad-spectrum antimycobacterial substances possessing activity against atypical strains[2]. In our previous paper[3] we have demonstrated that an alkylthio group bound to an electron-deficient carbon atom is the pharmacophore of antimycobacterial activity. Another assumed pharmacophore group was a disulfidic moiety bound to an electron-deficient carbon atom. The first series of substances containing this pharmacophore were the derivatives of bis(1-aryl-5-tetrazolyl) disulfide[4]. The aim of the present paper is to extend the knowledge of this pharmacophore by introducing a disulfidic group into the ring of cyclic compounds.

* Part IX in the series Relationships Between the Chemical Structure of Substances and Their Antimycobacterial Activity to Atypical Strains; Part VIII: see ref.[1].

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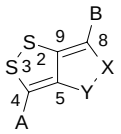
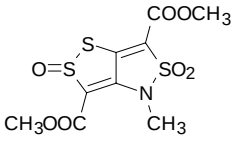


X–Y = moiety C

1-30

For A, B, and C see Table I



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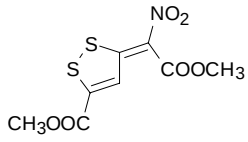

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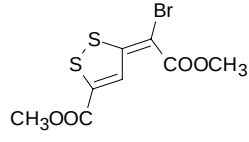

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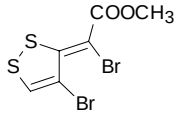

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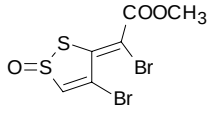

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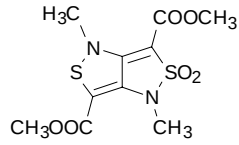

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EXPERIMENTAL AND CALCULATIONS

Chemicals. All compounds were prepared at the University of Munich in connection with other communications[5–7] or are ready for publication.

Microbiological evaluation. Antimycobacterial activity of the compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ (CNCTC), *M. kansasii* PKG 8 (Dr Runyon), *M. avium* 80/72 (CNCTC) and *M. fortuitum* 1021 (Prof. Hauduroy) was determined in Sula's semisynthetic medium (USOL, Prague, Czech Republic). The substances were added to the medium as solutions in dimethyl sulfoxide. The assays were performed at concentrations 4, 7, 15, 30, 60, 125, 250, and 500 μmol l^{-1}. The values of the minimum inhibitory activity (MIC) were read after 14 days of incubation at 37 °C and are given in Tables I and II.

Calculations. The programme Multireg and the Programme for linear correlation produced by Klemera for Microsoft Excel were used in the calculations; the values were calculated using the Free–Wilson method[8]. The results are summarized in Table III.

DISCUSSION

The results of antimycobacterial evaluation show that cyclic disulfides are potential antimycobacterial substances. Most of the 2,3-dithiabicyclo[3.3.0]octa-4,8-diene derivatives **1–30** were effective, nevertheless, their antimycobacterial activity depended on the structural modifications and the mycobacteria strains. The effects of individual modified molecular fragments (*i.e.* substituents A and B and moieties C) on the

TABLE I
Antimycobacterial activity of 2,3-dithiabicyclo[3.3.0]octa-4,8-diene derivatives (minimum inhibitory concentrations, MIC, μmol l^{-1})

Compound	Molecular fragment			MIC, μmol l^{-1}			
	A	B	C	*M. tuber.*	*M. kans.*	*M. avium*	*M. fortuit.*
1	$COOCH_3$	$NHCOCH_3$	–SCO–	60	30	500	–
2	$COOCH_3$	$COOC_2H_5$	–SCO–	30	30	30	–
3	$COOCH_3$	OC_2H_5	–SCO–	30	30	30	–
4	$COOCH_3$	$COOCH_3$	$–N(CH_3)SO_2–$	60	60	250	>500
5	H	$COOCH_3$	$–N(CH_3)SO_2–$	60	60	250	500
6	$COOCH_3$	$CONHNH_2$	$–N(CH_3)SO_2–$	500	500	500	>500
7	$COOCH_3$	$NHCOCH_3$	$–N(CH_3)SO_2–$	>500	>500	>500	>500
8	H	$NHCOCH_3$	$–N(CH_3)SO_2–$	250	500	500	250
9	H	OH	–SCO–	60	60	60	250
10	$COOCH_3$	H	$–N(CH_3)CO–$	30	30	60	60
11	$COOCH_3$	Br	$–N(CH_3)CO–$	30	30	125	125
12	$COOCH_3$	NO_2	$–N(CH_3)CO–$	250	250	500	500
13	$COOCH_3$	$COOCH_3$	$–N(CH_3)CO–$	125	125	125	>500
14	$COOCH_3$	OC_2H_5	$–N(CH_3)CO–$	125	125	60	500
15	$COOCH_3$	OCH_3	–NHCO–	60	60	500	>500
16	$COOCH_3$	SO_2NH_2	–NHCO–	>500	>500	>500	>500
17	$COOCH_3$	Br	–NHCO–	30	30	125	125
18	$COOCH_3$	$SCOOC_2H_5$	–NHCO–	60	60	500	500
19	$COOCH_3$	$NHCOCH_3$	–OCO–	250	250	250	>500
20	$COOCH_3$	Br	–OCO–	125	125	500	>500
21	$COOCH_3$	$COOC_2H_5$	–OCO–	60	60	125	125
22	$COOCH_3$	OC_2H_5	–OCO–	125	250	125	500
23	$COOCH_3$	NO_2	–OCO–	250	>500	>500	>500
24	H	NO_2	–SCO–	30	60	125	125
25	$COOCH_3$	$NHCH_2C_6H_5$	–SCO–	500	>500	>500	>500
26	$COOCH_3$	H	–SCO–	7	7	60	125
27	$COOCH_3$	OH	–SCO–	125	125	125	>500
28	$COOCH_3$	Br	–SCO–	60	60	60	250
29	$COOCH_3$	NO_2	–SCO–	60	60	30	250
30	H	$NHCOCH_3$	–SCO–	125	250	250	500
31[a]	$COOCH_3$	$COOCH_3$	$–N(CH_3)SO_2–$	500	500	>500	>500

[a] In position 3 *S*-oxide.

antimycobacterial activity can be best evaluated after separation of the resultant activity into the effects of the individual modified molecular fragments by means of Free–Wilson method. In the case of *M. tuberculosis* and *M. kansasii*, the structure–antimycobacterial activity relationships of the compounds were proved on the level of statistical significance 0.5 whereas an analogous relationship for *M. avium* was less significant. The results show that no advantage is gained by introducing a substituent into the position 4 (substituent A). The influence on the activity, however, was not marked and in the evaluation against *M. kansasii* it was not virtually. Concerning the position 8 (substituent B), we can again expect that the unsubstituted substances will exhibit the highest antimycobacterial activity. Substitution with an ethoxy group has a greater effect only against *M. avium*. Another important substitution is with an alkoxycarbonyl (*i.e.* methoxycarbonyl or ethoxycarbonyl) group, or possibly also with bromine. The effect against *M. kansasii* could be favourably influenced also by a methoxy or an $SCOOC_2H_5$ group. It is interesting that an increase in the effect cannot be expected even in the case of substitution with a $CONHNH_2$ or an $SONH_2$ group. Of the modifications of the molecule in positions 6 and 7 inside the ring (moiety C), the moiety –SC(=O)– proved to be the best. The second best was the moiety –NHCO–, which, however, was not virtually effective against *M. kansasii*.

Although monocyclic disulfides **32–34** were also antimycobacterially effective, the number of the prepared compounds was too small to allow a reliable study of structure–activity relationships.

S-Oxidation of the disulfide moiety in both the bicyclic and monocyclic compounds brought about a strong decrease in activity (compare the activities of compounds **4** and **31** and also compounds **34** and **35**). Compound **36**, which does not contain the disul-

TABLE II
Antimycobacterial activity of 3,4-dihydro-1,2-dithiaolane derivatives and isonicotinoyl hydrazine (minimum inhibitory concentrations, MIC, μmol l^{-1})

Compound	MIC, μmol l^{-1}			
	M. tuberculosis	*M. kansasii*	*M. avium*	*M. fortuitum*
32	250	500	>500	>500
33	30	60	250	250
34	15	30	125	125
35	250	250	500	500
INH[a]	7	250	500	500

[a] Isonicotinylhydrazine.

fidic molecular fragment, exhibited no antimycobacterial activity. As follows from the present study, the disulfidic molecular fragment in cyclic compounds **1–35** can be considered to be the pharmacophore of the antimycobacterial activity. The most active compounds of the studied series approach isoniazide in their *in vitro* activity against *M. tuberculosis* but they are much more effective against *M. kansasii* and *M. avium*. On the other hand, their activity against *M. fortuitum* is not significant.

TABLE III
Free–Wilson structure–antimycobacterial activity analysis: contributions of individual fragments to log MIC values

Molecular fragment		Δ log MIC		
		M. tuberculosis[a]	*M. kansasii*[b]	*M. avium*[c]
A	$COOCH_3$	0.037	0.015	0.025
	H	–0.221	–0.074	–0.127
B	OC_2H_5	–0.185	–0.111	–0.532
	COOR[d]	–0.297	–0.409	–0.055
	OCH_3	–0.015	–0.311	0.468
	OH	0.276	0.222	–0.169
	Br	–0.285	–0.311	–0.132
	NO_2	0.089	0.318	0.319
	H	–0.856	–0.830	–0.431
	$NHCH_2C_6H_5$	0.915	1.228	0.805
	SO_2NH_2	1.215	0.889	0.768
	$SCOOC_2H_5$	0.015	–0.310	0.467
	$NHCOCH_3$	0.265	0.348	0.025
	$CONHNH_2$	0.323	0.300	–0.016
C	$–N(CH_3)SO_2–$	0.343	0.308	0.361
	$–N(CH_3)CO–$	0.193	0.098	–0.089
	–OCO–	0.2090	0.2815	0.1797
	–SCO–	–0.248	–0.319	–0.160
	–NHCO–	–0.249	0.020	–0.123
	μ_0	1.997	2.077	2.330

Statistical evidence: [a] $r = 0.914$, $s = 0.296$, $F = 4.14$, $t = 2.16$, $n = 30$; [b] $r = 0.911$, $s = 0.337$, $F = 3.97$, $t = 2.16$, $n = 30$; [c] $r = 0.819$, $s = 0.398$, $F = 1.85$, $t = 2.16$, $n = 30$; [d] R = CH_3 or C_2H_5.

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