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Synthesis of Some 2, 6-Disubstituted 4-Amidopyridines and -Thioamidopyridines, and Their Antimycobacterial and Photosynthesis-Inhibiting Activity

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Abstract: A group of 26 new 2-halogeno-6-alkylsulfanyl- and 2,6-bis-alkylsulfanyl-4-amidopyridines and corresponding thioamidopyridines was synthesised. Some of the amidopyridines and all thioamidopyridines were tested for their antimycobacterial activity against atypical mycobacterial strains. Promising photosynthesis-inhibiting activity was also found for some of the amidopyridines.

Keywords: Thioamidopyridines, antimycobacterial activity, photosynthesis-inhibiting activity.

Introduction

Some events during the past decade have dramatically changed the nature and magnitude of the problem of tuberculosis. The HIV epidemic and increasing resistance to antituberculous drugs dictate the need of development of new antituberculotics [1-3].

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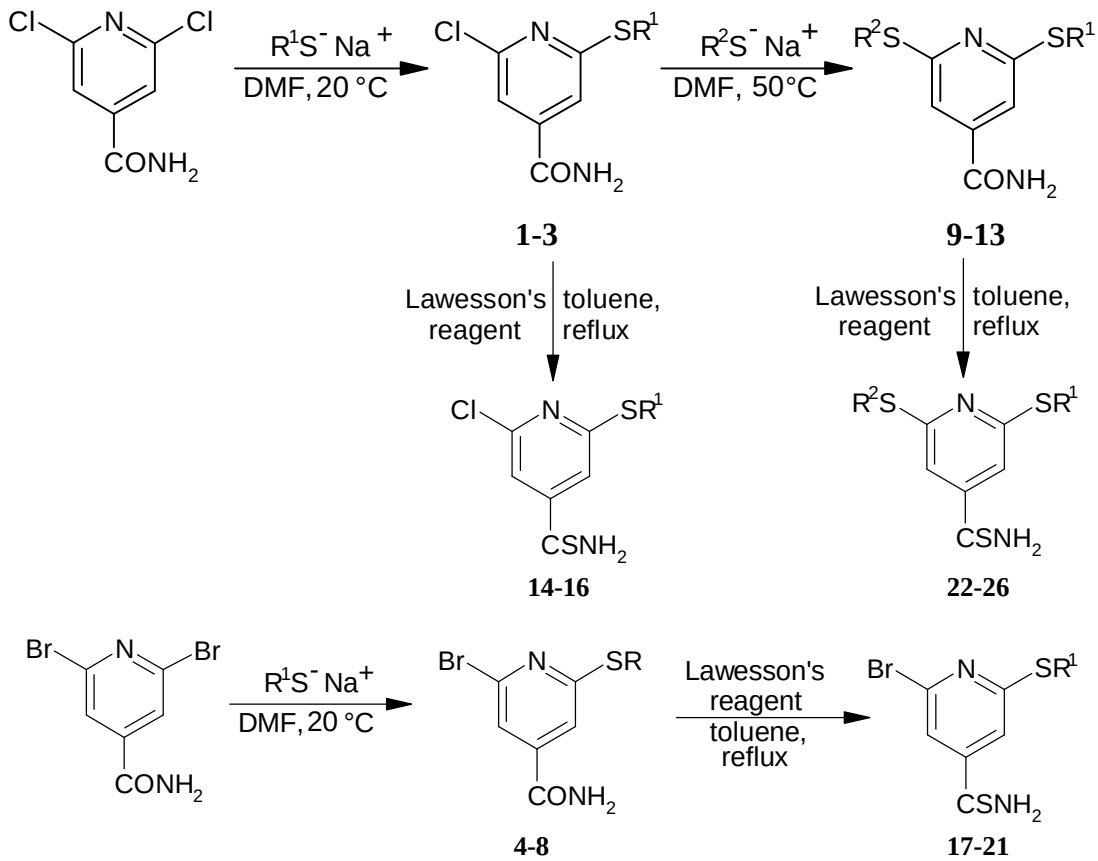
In our recent study [4], we modified the structure of therapeutically used antituberculous drugs ethionamide and prothionamide. Some of the more lipophilic derivatives showed promising activity against atypical mycobacterial strains. The present study extends the scope of lipophilic derivatives of 4-thioamidopyridines. Since it has been recently reported that 2-alkylsulfanyl-4-thioamidopyridines showing antimycobacterial [5] and antifungal activity [6] inhibit photosynthetic processes in algae and plant chloroplasts [7,8], the synthesised compounds were tested for their both antimycobacterial and photosynthesis-inhibiting activity.

Results and Discussion

The synthesis of 2,6-disubstituted 4-amidopyridines and -thioamidopyridines is shown in Scheme 1. 2,6-Dichloro- or -dibromo-4-amidopyridine [9] was treated with an equimolar amount of the respective thiolate to give 2-halogeno-6-alkylsulfanyl-4-amidopyridines (**1-8**). 2-Alkylsulfanyl-6-hexylsulfanyl-4-amidopyridines (**9-13**) were prepared from 2-chloro-6-hexylsulfanyl-4-amidopyridine (**3**) in a similar fashion. Thionation of 4-amidopyridines (**1-13**) with the Lawesson's reagent afforded the 4-thioamidopyridines (**14-26**). The melting points, yields, and elemental analyses for compounds **1-26** are given in Table 1, and IR and ^{1}H NMR spectroscopic data in Table 2.



Scheme 1. Synthesis of 2,6-disubstituted 4-amidopyridines and thioamidopyridines.

As the amidopyridines were originally considered to be intermediates, only selected compounds were evaluated for their activity against *Mycobacterium tuberculosis*, *M. kansasii*, *M. avium*, and *M. fortuitum.* The minimum inhibitory concentrations (MIC) for the tested compounds are listed in Table 3, along with isoniazid as a reference standard. The tested compounds in the amidopyridine series (**1**, **4**, **7**, **9**, **12**) were inactive with the exception of **9** and, in part, **7**. Compound **9** showed greater activity against atypical strains, especially *M. kansasii* (MIC = 60 μmol.dm^{-3}), than isoniazid.

The thioamidopyridine series was more active in the antimycobacterial screening than the amidopyridine one. 2-Halogeno-6-alkylsulfanyl-4-thioamidopyridines (**14-21**) exhibited increasing antimycobacterial activity with prolonging the carbon chain in the alkylsulfanyl substituent up to seven carbons. Overall, 2-chloro substituted thioamidopyridines were more active than their 2-bromo analogues. Compounds **16** and **20** were the most promising, with MICs of 60 μmol.dm^{-3} against *M. tuberculosis* and *M. kansasii* (as well as *M. avium* for **16**).

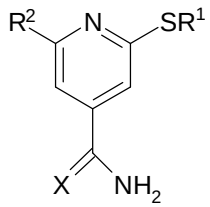
Among 2-alkylsulfanyl-6-hexylsulfanyl-4-thioamidopyridines (**22-26**), compound **22** was found to be the most active of the compounds studied. It showed, similar to compounds **23** and **24**, moderate activity against *M. tuberculosis* (MIC = 30 μmol.dm^{-3}). Additionally, it exhibited greater activity against *M. kansasiii* (MIC = 60 μmol.dm^{-3}), *M. avium* (MIC = 60 μmol.dm^{-3}), and *M. fortuitum* (MIC = 250 μmol.dm^{-3}) than isoniazid, as well as **16**. Increasing the total number of carbon atoms in both alkylsulfanyl side chains above ten caused a decrease in antimycobacterial activity, which is in agreement with our previous findings [4].

To better understand the structure-activity relationships, log P values were calculated (Table 3). We found that the lipophilicities of the most potent antimycobacterial compounds were different for all four strains employed. In the 4-thioamidopyridine series (**14-26**), the highest activities against *M. tuberculosis* were observed for compounds **22-24** with log P values between 5.75 and 7.34. The antimycobacterial activity of 4-thioamidopyridines against other three strains showed a sharp dependence on lipophilicity. In the case of *M. kansasii* and *M. avium*, the most active 4-thioamidopyridines **16** and **22** showed log P values ranging from 4.86 to 5.75, while the compounds with the highest activity against *M. fortuitum,* **23** and **24,** exhibited log P values 6.81 and 7.34.

The tested compounds also inhibited photochemical activity of spinach chloroplasts. The IC_{50} values, *i. e.*, concentrations of the compounds causing 50% decrease of oxygen evolution rate in spinach chloroplasts with respect to the untreated control, are listed in Table 3. From the comparison of IC_{50} values of the 2-halogeno substituted 4-amido (**1-7**) and 4-thioamidopyridines (**14-21**) it can be concluded that amidopyridines exhibit greater inhibitory activity than the corresponding thioamidopyridines. For compounds **9-12,** a pronounced decrease in photosynthesis-inhibiting activity with the increasing lipophilicity of the compounds has been confirmed. This is in good agreement with the previously obtained results concerning photosynthesis-inhibiting activity of 2-alkylsulfanyl-4-thioamidopyridines in spinach chloroplasts and *Chlorella vulgaris* [7,8]. In the 4-amidopyridine series, the most active compounds **7**, **9**, **3**, and **6** showed log P in the range of 3.12-5.0, whereas the inhibitory activity of thioamidopyridines with log P > 3.27 showed a pronounced decrease.

Using EPR spectroscopy it was found that in the suspension of spinach chloroplasts the studied 4-thioamidopyridines interact with D^+ intermediate, i.e., with the radical of tyrosine 161 (Tyr_D) which is located in D_2 protein on the donor side of photosystem 2 [10], and due to this interaction the photosynthetic electron transport from the oxygen evolving complex to the core of photosystem 2 is impaired. The same site of action in the photosynthetic apparatus of spinach chloroplasts has also been confirmed for the structurally similar 2-alkylsulfanyl-4-thioamidopyridines [7].

Table 1. Analytical data of the prepared compounds**.**



Compd.	Formula M. w.	R^1, R^2	X	M. p. °C Yield %	Calculated / Found % C	% H	% N	% S	% Cl(Br)
1	$C_8H_9ClN_2OS$	C_2H_5,	O	162-163	44.34	4.19	12.93	14.80	16.36
	(216.7)	Cl		75	44.21	4.12	13.11	14.69	16.51
2	$C_9H_{11}ClN_2OS$	C_3H_7,	O	112-113	46.85	4.81	12.14	13.90	15.37
	(230.7)	Cl		76	46.65	4.73	12.35	13.62	15.50
3	$C_{12}H_{17}ClN_2OS$	C_6H_{13},	O	129-131	52.84	6.28	10.27	11.75	13.00
	(272.8)	Cl		72	52.76	6.21	10.39	11.64	13.14
4	$C_7H_7BrN_2OS$	CH_3,	O	178-180	34.02	2.86	11.34	12.97	32.34
	(247.1)	Br		70	33.96	2.78	11.46	12.89	32.48
5	$C_8H_9BrN_2OS$	C_2H_5,	O	160-162	36.80	3.47	10.73	12.28	30.60
	(261.1)	Br		73	36.66	3.35	10.85	12.18	30.76
6	$C_{10}H_{13}BrN_2OS$	C_4H_9,	O	113-115	41.53	4.53	9.69	11.09	27.63
	(289.2)	Br		71	41.31	4.41	9.90	10.81	27.81
7	$C_{13}H_{19}BrN_2OS$	C_7H_{15},	O	120-122	47.13	5.78	8.46	9.68	24.12
	(331.3)	Br		68	47.01	5.71	8.57	9.58	24.26
8	$C_{14}H_{21}BrN_2OS$	C_8H_{17},	O	123-125	48.70	6.13	8.11	9.28	23.14
	(345.3)	Br		65	48.43	6.01	8.31	9.11	23.26
9	$C_{14}H_{22}N_2OS_2$	C_6H_{13},	O	83-84	56.34	7.43	9.39	21.48	-
	(298.5)	SC_2H_5		75	56.29	7.41	9.45	21.43	
10	$C_{16}H_{26}N_2OS_2$	C_6H_{13},	O	86-88	58.86	8.03	8.58	19.64	-
	(326.5)	SC_4H_9		72	58.93	8.09	8.51	19.71	

Continuation of the Table 1.

Compd.	Formula	R^1,	X	M. p. °C	Calculated / Found				
	M. w.	R^2		Yield %	% C	% H	% N	% S	% Cl(Br)
11	$C_{17}H_{28}N_2OS_2$	C_6H_{13},	O	109-111	59.96	8.29	8.23	18.83	-
	(340.6)	SC_5H_{11}		70	60.05	8.34	8.17	18.92	
12	$C_{19}H_{32}N_2OS_2$	C_6H_{13},	O	106-108	61.91	8.75	7.60	17.40	-
	(368.6)	SC_7H_{15}		67	61.93	8.72	7.55	17.43	
13	$C_{20}H_{34}N_2OS_2$	C_6H_{13},	O	96-98	62.78	8.96	7.32	16.76	-
	(382.6)	SC_8H_{17}		64	62.67	8.90	7.38	16.67	
14	$C_8H_9ClN_2S_2$	C_2H_5,	S	80-81	41.28	3.90	12.04	27.55	15.23
	(232.8)	Cl		89	41.16	3.81	12.17	27.41	15.38
15	$C_9H_{11}ClN_2S_2$	C_3H_7,	S	oil	43.81	4.49	11.35	25.98	14.37
	(246.8)	Cl		87	43.73	4.45	11.43	25.87	14.49
16	$C_{12}H_{17}ClN_2S_2$	C_6H_{13},	S	45-47	49.90	5.93	9.70	22.20	12.27
	(288.9)	Cl		91	49.69	5.78	9.81	22.01	12.42
17	$C_7H_7BrN_2S_2$	CH_3,	S	127-129	31.95	2.68	10.64	24.36	30.36
	(263.2)	Br		85	31.82	2.61	10.51	24.22	30.48
18	$C_8H_9BrN_2S_2$	C_2H_5,	S	107-108	34.66	3.27	10.11	23.13	28.83
	(277.2)	Br		92	34.58	3.22	10.03	22.96	28.98
19	$C_{10}H_{13}BrN_2S_2$	C_4H_9,	S	53-55	39.35	4.29	9.18	21.01	26.18
	(305.3)	Br		90	39.15	4.20	9.07	20.89	26.39
20	$C_{13}H_{19}BrN_2S_2$	C_7H_{15},	S	43-45	44.96	5.51	8.07	18.46	23.01
	(347.3)	Br		89	44.78	5.47	8.15	18.27	23.27
21	$C_{14}H_{21}BrN_2S_2$	C_8H_{17},	S	44-46	46.53	5.86	7.75	17.74	22.11
	(361.4)	Br		91	46.31	5.77	7.92	17.59	22.35
22	$C_{14}H_{22}N_2S_3$	C_6H_{13},	S	oil	53.46	7.05	8.91	30.58	-
	(314.5)	SC_2H_5		90	53.41	7.02	9.03	30.41	
23	$C_{16}H_{26}N_2S_3$	C_6H_{13},	S	58-60	56.10	7.65	8.18	28.08	-
	(342.6)	SC_4H_9		91	56.25	7.71	8.03	28.23	
24	$C_{17}H_{28}N_2S_3$	C_6H_{13},	S	62-63	57.26	7.91	7.86	26.97	-
	(356.6)	SC_5H_{11}		89	57.02	7.78	8.05	26.72	
25	$C_{19}H_{32}N_2S_3$	C_6H_{13},	S	71-73	59.33	8.39	7.28	25.00	-
	(384.7)	SC_7H_{15}		89	59.47	8.42	7.15	24.78	
26	$C_{20}H_{34}N_2S_3$	C_6H_{13},	S	62-64	60.25	8.60	7.03	24.12	-
	(398.7)	SC_8H_{17}		87	60.03	8.47	7.25	23.91	

Table 2. IR and ^{1}H NMR spectroscopic data of the prepared compounds.

Compd.	IR, (cm^{-1})	^{1}H NMR, δ (ppm)
1	3019, 2972 (CH aliph.) 1690 (C=O)	7.38 d, J = 1, 1 H, arom.; 7.27 d, J = 1, 1 H, arom.; 6.09 bs, 1 H, NH; 5.90 bs, 1 H, NH; 3.18 q, J = 7, 2 H, SCH_2; 1.37 t, J = 7, 3 H, CH_3
2	3013, 2968 (CH aliph.) 1689 (C=O)	7.38 d, J = 1, 1 H, arom.; 7.27 d, J = 1, 1 H, arom.; 6.53 bs, 1 H, NH; 6.42 bs, 1 H, NH; 3.14 t, J = 7, 2 H, SCH_2; 1.73 sext, J = 7, 2 H, CH_2; 1.02 t, J = 7, 3 H, CH_3
3	3014, 2959, 2931 (CH aliph.) 1689 (C=O)	7.38 d, J = 1, 1 H, arom.; 7.27 d, J = 1, 1 H, arom.; 6.19 bs, 2 H, NH_2; 3.15 t, J = 7, 2 H, SCH_2; 1.69-1.26 m, 4 H, $(CH_2)_2$; 0.87 t, J = 7, 3 H, CH_3
4	3019, 2970 (CH aliph.) 1696 (C=O)	7.44-7.46 m, 2 H, arom.; 6.15 bs, 1 H, NH; 5.75 bs, 1 H, NH; 2.60 s, 3 H, CH_3
5	3018, 2969, 2936 (CH aliph.) 1695 (C=O)	7.40-7.42 m, 2 H, arom.; 6.06 bs, 1 H, NH; 5.88 bs, 1 H, NH; 3.18 q, J = 7, 2 H, SCH_2; 1.37 t, J = 7, 3 H, CH_3
6	3014, 2962, 2933 (CH aliph.) 1694 (C=O)	7.40-7.42 m, 2 H, arom.; 6.24 bs, 2 H, NH_2; 3.14 t, J = 7, 2 H, SCH_2; 1.73 m, 2 H, CH_2; 1.44 m, 2 H, CH_2; 0.92 dist. t, J = 5, 3 H, CH_3
7	3014, 2958, 2930 (CH aliph.) 1690 (C=O)	7.40-7.42 m, 2 H, arom.; 6.19 bs, 2 H, NH_2; 3.14 t, J = 7, 2 H, SCH_2; 1.70-1.25 m, 10 H, $(CH_2)_5$; 0.87 dist. t, J = 5, 3 H, CH_3
8	3013, 2957, 2929 (CH aliph.) 1689 (C=O)	7.39-7.41 m, 2 H, arom.; 6.14 bs, 2 H, NH_2; 3.14 t, J = 7, 2 H, SCH_2; 1.70-1.26 m, 12 H, $(CH_2)_6$; 0.87 dist. t, J = 5, 3 H, CH_3
9	3013, 2960, 2931 (CH aliph.) 1686 (C=O)	7.14 s, 2 H, arom.; 6.32 bs, 2 H, NH_2; 3.21 q overlapping with 3.17 t, 4 H both, 2 × SCH_2; 1.1 - 1.9 m, 8 H, 4 × CH_2; 1.38 t, J = 7, 3 H, SCH_2**CH_3**; 0.90 dist. t, J = 5, 3 H, CH_3
10	3010, 2961, 2932 (CH aliph.) 1686 (C=O)	7.14 s, 2 H, arom.; 6.03 bs, 2 H, NH_2; 3.20 t, J = 7, 4 H, 2 × SCH_2; 1.1 - 1.9 m, 12 H, 6 × CH_2; 0.95 t, J = 6, 3 H, $S(CH_2)_3$**CH_3**; 0.90 dist. t, J = 5, 3 H, CH_3
11	3010, 2960, 2931 (CH aliph.) 1686 (C=O)	7.14 s, 2 H, arom.; 6.14 bs, 2 H, NH_2; 3.19 t, J = 7, 4 H, 2 × SCH_2; 1.1 - 1.9 m, 14 H, 7 × CH_2; 0.90 dist. t, J = 5, 6 H, 2 × CH_3
12	3009, 2959, 2930 (CH aliph.) 1686 (C=O)	7.14 s, 2 H, arom.; 6.02 bs, 2 H, NH_2; 3.19 t, J = 7, 4 H, 2 × SCH_2; 1.1 - 1.9 m, 18 H, 9 × CH_2; 0.90 dist. t, J = 5, 6 H, 2 × CH_3
13	3010, 2959, 2929 (CH aliph.) 1686 (C=O)	7.14 s, 2 H, arom.; 6.20 bs, 2 H, NH_2; 3.19 t, J = 7, 4 H, 2 × SCH_2; 1.1 - 1.9 m, 20 H, 10 × CH_2; 0.90 dist. t, J = 5, 6 H, 2 × CH_3

Continuation of the Table 2.

Compd.	IR, (cm^{-1})	^{1}H NMR, δ (ppm)
14	2991, 2931, 2874 (CH aliph.) 1603 (C=O)	7.64 bs, 1 H, NH; 7.37 d, J = 1, 1 H, arom.; 7.29 d, J = 1, 1 H, arom.; 7.3 bs, 1 H, NH; 3.18 q, J = 7, 2 H, SCH_2; 1.38 t, J = 7, 3 H, CH_3
15	2996, 2968, 2934 (CH aliph.) 1603 (C=O)	7.7 bs, 1 H, NH; 7.38 d, J = 1, 1 H, arom.; 7.29 d, J = 1, 1 H, arom.; 7.2 bs, 1 H, NH; 3.17 t, J = 7, 2 H, SCH_2; 1.74 sext, J = 7, 2 H, CH_2; 1.05 t, J = 7, 3 H, CH_3
16	2996, 2959, 2931 (CH aliph.) 1603 (C=O)	7.82 bs, 1 H, NH; 7.36 d, J = 1, 1 H, arom.; 7.28 d, J = 1, 1 H, arom.; 7.3 bs, 1 H, NH; 3.17 t, J = 7, 2 H, SCH_2; 1.69-1.26 m, 8 H, $(CH_2)_4$; 0.90 dist. t , J = 5, 3 H, CH_3
17	3001, 2932 (CH aliph.) 1603 (C=O)	7.76 bs, 1 H, NH; 7.40 d, J = 1, 1 H, arom.; 7.29 d, J = 1, 1 H, arom.; 7.26 bs, 1 H, NH; 2.59 s, 3 H, CH_3
18	2992, 2932 (CH aliph.) 1603 (C=O)	7.79 bs, 1 H, NH; 7.39 d, J = 1, 1 H, arom.; 7.29 d, J = 1, 1 H, arom.; 7.26 bs, 1 H, NH; 3.19 q, J = 7, 2 H, SCH_2; 1.38 t, J = 7, 3 H, CH_3
19	2999, 2962, 2933 (CH aliph.) 1603 (C=O)	7.88 bs, 1 H, NH; 7.37 d, J = 1, 1 H, arom.; 7.29 d, J = 1, 1 H, arom.; 7.3 bs, 1 H, NH; 3.18 t, J = 7, 2 H, SCH_2; 1.25-1.86, 4 H, $(CH_2)_2$; 0.95 t, J = 6, 3 H, CH_3
20	2997, 2958, 2929 (CH aliph.) 1603 (C=O)	7.85 bs, 1 H, NH; 7.38 d, J = 1, 1 H, arom.; 7.29 d, J = 1, 1 H, arom.; 7.3 bs, 1 H, NH; 3.17 t, J = 7, 2 H, SCH_2; 1.25-1.86, 10 H, $(CH_2)_5$; 0.89 dist. t, J = 5, 3 H, CH_3
21	2998, 2957, 2928 (CH aliph.) 1603 (C=O)	7.77 bs, 1 H, NH; 7.37 d, J = 1, 1 H, arom.; 7.28 d, J = 1, 1 H, arom.; 7.26 bs, 1 H, NH; 3.16 t, J = 7, 2 H, SCH_2; 1.2-1.8 m, 12 H, $(CH_2)_6$; 0.88 dist. t, J = 5, 3 H, CH_3
22	3004, 2960, 2930 (CH aliph.) 1601 (C=O)	7.13 s, 2H, arom.; 6.1 bs, 1 H, NH; 5.8 bs, 1 H, NH; 3.20 q overlapping with 3.19 t, 4 H both, 2 × SCH_2; 1.2 -1.8 m, 8 H, 4 × CH_2; 1.34 t, J = 7, 3 H, SCH_2**CH_3**; 0.90 dist. t, J = 5, 3 H, CH_3
23	2996, 2961, 2931 (CH aliph.) 1601 (C=O)	7.76 bs, 1 H, NH; 7.13 s, 2H, arom.; 7.2 bs, 1 H, NH; 3.19 t, J = 7, 4 H, 2 × SCH_2; 1.1-1.9 m, 12 H, 6 × CH_2; 0.95 t, J = 6, 3 H, $S(CH_2)_3$**CH_3**; 0.90 dist. t, J = 5, 3 H, CH_3
24	2995, 2960, 2931 (CH aliph.) 1601 (C=O)	7.76 bs, 1 H, NH; 7.13 s, 2H, arom.; 7.2 bs, 1 H, NH; 3.19 t, J = 7, 4 H, 2 × SCH_2; 1.1-1.9 m, 14 H, 7 × CH_2; 0.90 dist. t, J = 5, 6 H, 2 × CH_3
25	2996, 2959, 2930 (CH aliph.) 1601 (C=O)	7.61 bs, 1 H, NH; 7.13 s, 2H, arom.; 7.2 bs, 1 H, NH; 3.19 t, J = 7, 4 H, 2 × SCH_2; 1.1-1.9 m, 18 H, 9 × CH_2; 0.90 dist. t, J = 5, 6 H, 2 × CH_3
26	2995, 2958, 2929 (CH aliph.) 1601 (C=O)	7.66 bs, 1 H, NH; 7.13 s, 2H, arom.; 7.2 bs, 1 H, NH; 3.18 t, J = 7, 4 H, 2 × SCH_2; 1.1-1.9 m, 20 H, 10 × CH_2; 0.90 dist. t, J = 5, 6 H, 2 × CH_3

Table 3. MIC of the tested compounds against used mycobacterial strains, IC_{50} values concerning inhibition of oxygen evolution rate in spinach chloroplasts by the tested compounds and calculated logP values of the prepared compounds.

Compd.	MIC ($\mu mol.dm^{-3}$)				IC_{50} ($\mu mol.dm^{-3}$)	calculated logP
	tuberculosis $H_{37}Rv$	*M. kansasii* *PKG8*	*M. avium* *80/72*	*M. fortuitum* *1021*	spinach chloroplasts	
1	500	1000	1000	1000	101.5	1.96 ± 0.38
2	-	-	-	-	58.4	2.49 ± 0.38
3	-	-	-	-	10.2	4.08 ± 0.38
4	>1000	>1000	>1000	>1000	76.7	1.52 ± 0.42
5	-	-	-	-	34.2	2.06 ± 0.42
6	-	-	-	-	10.6	3.12 ± 0.42
7	250	250	>1000	>1000	5.9	4.71 ± 0.42
8	-	-	-	-	-	5.24 ± 0.42
9	60	60	250	250	9.1	5.00 ± 0.41
10	-	-	-	-	203.5	6.06 ± 0.41
11	-	-	-	-	249.3	6.59 ± 0.41
12	1000	>1000	>1000	>1000	543.6	7.66 ± 0.41
13	-	-	-	-	258.8	8.19 ± 0.41
14	125	250	250	500	104.8	2.73 ± 0.41
15	125	125	250	500	9.3	3.27 ± 0.41
16	60	60	60	250	29.8	4.86 ± 0.41
17	500	500	500	1000	187.7	2.34 ± 0.48
18	250	250	500	500	19.6	2.87 ± 0.48
19	125	250	250	500	20.9	3.93 ± 0.48
20	60	60	125	500	61.0	5.52 ± 0.48
21	125	125	250	500	105.1	6.06 ± 0.48
22	30	60	60	250	-	5.75 ± 0.47
23	30	125	125	125	99.7	6.81 ± 0.47
24	30	250	125	125	157.5	7.34 ± 0.47
25	60	250	250	500	-	8.41 ± 0.47
26	125	250	250	500	-	8.94 ± 0.47
isoniazid	7	250	1000	1000	-	-0.89 ± 0.24

Experimental

General

Melting points were determined on a Kofler block, and are uncorrected. IR spectra were recorded on a Nicolet Impact 400 spectrometer in chloroform. ^{1}H NMR spectra were determined for solutions in $CDCl_3$ with TMS as the internal standard with a BS 587 (Tesla, Brno) 80 MHz apparatus. Column chromatography was performed on silica gel (Silpearl, Kavalier Votice). Elemental analyses were performed on a EA 1110 CHNS-O CE INSTRUMENTS elemental analyser.

Lipophilicity of the compounds was computed using a program ACD/LogP version 1.0 (Advanced Chemistry Development Inc., Toronto).

Synthesis of 2-halogeno-6-alkysulfanyl-4-amidopyridines **1-8**

2,6-Dichloro- or 2,6-dibromo-4-amidopyridine [9] (10 mmol) and the appropriate thiol (10 mmol) were dissolved in anhydrous *N,N*-dimethylformamide (10 mL). To the stirred solution sodium methoxide (10 mmol) in methanol (5 ml) was added dropwise. (Preparing 2-bromo-6-methylsulfanyl-4-amidopyridine, sodium methanethiolate (10 mmol) was added in several portions to the stirred solution of 2,6-dibromo-4-amidopyridine (10 mmol) in anhydrous *N,N*-dimethylformamide.) The reaction mixture was stirred at room temperature until TLC indicated a complete reaction. TLC was performed using petroleum ether : ethyl acetate (2:1) as the mobile phase. The mixture was poured into cold water. The crude product was filtered off, purified by column chromatography (petroleum ether : ethyl acetate, 2:1), and recrystallised from aqueous ethanol. The yields and melting points are given in Table 1, and the IR and NMR spectroscopic data in Table 2.

Synthesis of 2-alkylsulfanyl-6-hexylsulfanyl-4-amidopyridines **9-13**

To a stirred solution of 2-chloro-6-hexylsulfanyl-4-amidopyridine (**3**) (10 mmol) and the appropriate thiol (10 mmol) in anhydrous *N,N*-dimethylformamide (10 mL) sodium methoxide (10 mmol) in methanol (5 ml) was added dropwise. The reaction mixture was heated to about 50°C, stirred and maintained at this temperature until TLC indicated a complete reaction. TLC was performed using petroleum ether : ethyl acetate (2:1) as the mobile phase. The mixture was poured into cold water. The crude product was filtered off, purified by column chromatography (petroleum ether : ethyl acetate, 2:1) and recrystallised from aqueous ethanol. The yields and melting points are given in Table 1, and the IR and NMR spectroscopic data in Table 2.

Synthesis of 2,6-disubstituted 4-thioamidopyridines **14-26**

To a stirred solution of 2,6-disubstituted 4-amidopyridine (10 mmol) in anhydrous toluene (10 ml) Lawesson's reagent (5 mmol) was added and the reaction mixture was heated at reflux until TLC indi-

cated a complete reaction. TLC was performed using petroleum ether : ethyl acetate (4:1) as the mobile phase. The mixture was then evaporated under reduced pressure, the crude product was purified by column chromatography (petroleum ether : ethyl acetate, 4:1), and recrystallised from aqueous ethanol. The melting points and yields are given in Table 1, and the IR and NMR spectroscopic data in Table 2.

Biological assays

Antimycobacterial evaluation was carried out on a semisynthetic liquid protein-containing Šula medium (IMUNA, Šarišské Michal'any) buffered to pH 7.2. The following mycobacterial strains were used: *Mycobacterium tuberculosis* $H_{37}Rv$, *M. kansasii* PKG8, *M. avium* 80/72 *and M. fortuitum* 1021. The concentrations of the compounds in the medium were 1000, 500, 250, 125, 60 and 30 μmol.dm^{-3}. The MIC values were determined after 14 days of incubation at 37°C.

The oxygen evolution rate (OER) in spinach chloroplasts was determined spectrophotometrically (Specord UV VIS Zeiss Jena, Germany) by the Hill reaction. The measurements were carried out in phosphate buffer (20 mmol, pH = 7.2) containing sucrose (0.4 mol.dm^{-3}), $MgCl_2$ (5 mmol.dm^{-3}) and NaCl (15 mmol.dm^{-3}) using 2,6-dichlorophenol-indophenol as electron acceptor. Chlorophyll content in the samples was 30 mg.dm^{-3} and the samples were irradiated (~100 W.m^{-2}) from 10cm distance with a halogen lamp (250 W) using a water filter to prevent warming of the samples (suspension temperature 22 ^{o}C). The compounds were dissolved in dimethyl sulfoxide (DMSO) because of their limited water solubility. The applied DMSO concentration (up to 5%) did not affect OER.

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Samples Availability: Available from the authors.

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