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Synthesis and Biological Evaluation of Quinazoline-4-thiones

Lenka Kubicová [1,*], Martin Šustr [1], Katarína Kráľová [2], Vladimír Chobot [1], Jitka Vytlačilová [1], Luděk Jahodář [1], Pia Vuorela [3], Miloš Macháček [1], Jarmila Kaustová [4]

[1] Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Heyrovského 1205, 500 05 Hradec Králové, Czech Republic. Tel. +420 49 5067339, Fax +420 49 5210002.

[2] Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-2, 842 15 Bratislava, Slovak Republic.

[3] Department of Pharmacy, Division of Pharmacognosy, University of Helsinki, P.O. Box 56, (Viikinkaari 5), Helsinki, Finland.

[4] National Reference Laboratory for Mycobacterium Kansasii, Institute of Hygiene, 728 92 Ostrava, Czech Republic.

[*]Author to whom correspondence should be addressed; e-mail: kubicova@faf.cuni.cz

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Abstract: Several 2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3*H*)-thiones and 2-methyl-3-phenylquinazoline-4(3*H*)-thiones were synthesized and tested for their antimycobacterial, photosynthesis-inhibiting, and antialgal activity. Antimycobacterially active compounds were found among the 6-chloro substituted compounds. 6-Chloro-3-(4-isopropylphenyl)-2-methylquinazoline-4(3*H*)-thione exhibited higher activity than the isoniazid standard against *Mycobacterium avium* and *M. kansasii*. Most of the compounds possessed photosynthesis-inhibiting activity. 6-Chloro-2,2-dimethyl-3-phenyl-1,2-dihydro-quinazoline-4(3*H*)-thione and its 3´-chloro- and 3´,4´-dichloro analogs were most effective in the inhibition of oxygen evolution rate in spinach chloroplasts. Of compounds selected for toxicological screening, 6-chloro-3-(4-isopropylphenyl)-2-methyl-quinazoline-4(3*H*)-thione was the only one active in the brine shrimp bioassay.

Keywords: Quinazoline-4-thiones, mycobacteria, photosynthesis-inhibiting activity, chloroplasts, alga, toxicological screening, *Artemia salina*.

Introduction

Tuberculosis continues to be a devastating disease worldwide and is believed to be present in about one third of the world's population [1]. The increasing incidence of multi-drug-resistant tuberculosis is emerging as a major infectious disease problem throughout the world [2]. Mycobacterial diseases caused by the *Mycobacterium avium - M. intracellulare* complex show a rising occurrence among children, the elderly, and HIV-infected patients, and they are frequently fatal [3]. The search for potential antimycobacterial drugs is consequently one of the primary tasks of present-day medicinal chemistry.

Various nitrogen containing heterocycles have been recently studied for their antibacterial or antimycobacterial effects, e.g. 3,5-dinaphthyl-2-pyrazolines [4], 2-phenyl-5,5-dialkylimidazolinones [5], 4-amino-5-aryl-1,2,4-triazoles [6], triazolo- or tetrazolopyrrolopyrimidines [7], 5-alkylsulfanyl-tetrazoles [8-11], benzimidazoles [12], 1,3-benzoxazinediones [13-15], quinazolines [14, 16-19], and quinoxalines [20, 21]. Reviews of antimycobacterially active derivatives containing one or more nitrogen atom in the five- or six-membered ring have been published [16, 22-24].

The derivatives of quinazolin-4-one are potential drugs which can possess hypnotic, analgesic, antiallergic, anticonvulsant, antimalarial, and other effects [25]. In our previous study we found that some 3-phenylquinazolin-4(3*H*)-ones were active against atypical strains of mycobacteria [18]. The conversion of the oxo group into the thioxo function leads, in general, to an increase in antimycobacterial activity [14, 15, 26, 27]. Although the antimicrobial activity of some substituted quinazoline-4-thiones is known [27, 28], the antimycobacterial activity of neither 2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3*H*)-thiones, nor 2-methyl-3-phenylquinazoline-4(3*H*)-thiones has been studied yet.

In this paper, we describe the synthesis of two series of quinazoline-4-thiones, 2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3*H*)-thiones (**1**) and 2-methyl-3-phenylquinazoline-4(3*H*)-thiones (**2**), and results of their testing for antimycobacterial activity against *Mycobacterium tuberculosis*, *M. avium*, and *M. kansasii*.

Because of previous experience that various compounds with carbamoyl or thiocarbamoyl group, e.g. acylanilides, thioacylanilides, and their cyclic analogs, can inhibit the photosynthetic electron transport in autotrophic organisms [29-35], the photosynthesis-inhibiting and antialgal activity of the compounds **1** and **2** was also determined.

Based on the results of the biological tests, four compounds, **1h**, **1i**, **2b**, and **2f,** were selected for a toxicological screening bioassay and tested using brine shrimp larvae (*Artemia salina* L.) as the sensitive organism [36].

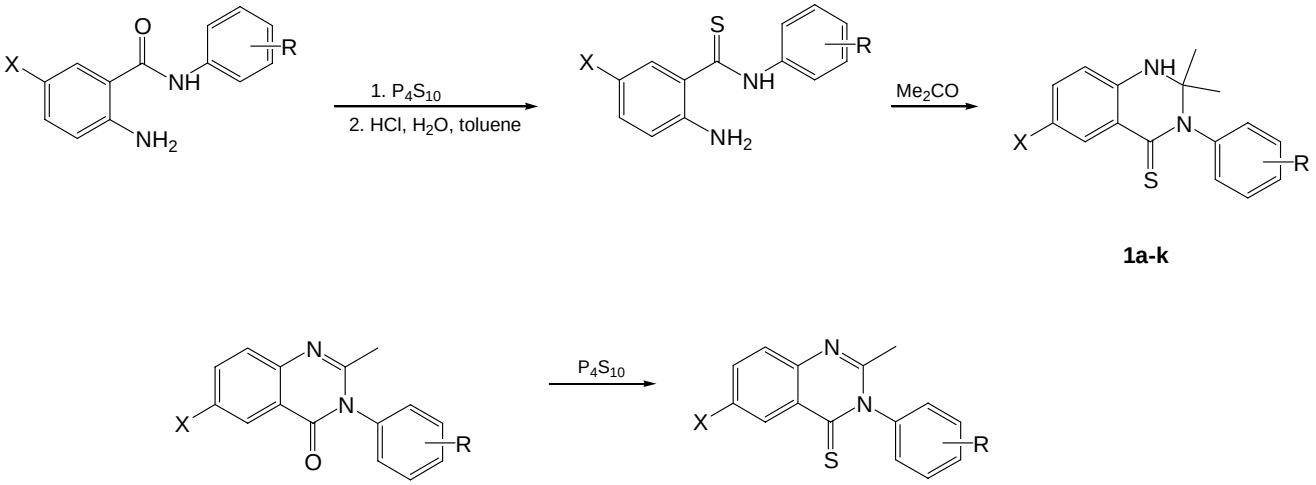
Results and Discussion

Chemistry

Routes for preparation of quinazoline-4-thione derivatives can involve cyclization of a convenient precursor, thionation of the corresponding oxo analogs, or condensation reactions [25, 28, 37, 38]. Efficient methods for synthesis of quinazolin-4-ones are e.g. acylation of 2-aminobenzamides with an appropriate acyl chloride followed by cyclization in basic medium [39], or one-pot synthesis under solvent-free conditions [40]. Derivatives of 2,2-dimethyl-1,2-dihydroquinazoline-4(3*H*)-thione can be prepared by condensation of 2-aminothiobenzamides with acetone under mild conditions [41]. Other way to obtain quinazoline-4(3*H*)-thiones is the ring closure of 2-acylaminothiobenzamides in basic medium [28,42].

2,2-Dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3*H*)-thiones (**1a-k**) were synthesized by condensation of the corresponding 2-amino-*N*-phenylthiobenzamides with acetone under the catalysis by silica gel. The reaction mixtures were allowed to stand at room temperature for 24 h, then concentrated *in vacuo*, and the products **1** were isolated by column chromatography on silica gel using petroleum ether with acetone as the mobile phase. The starting 2-amino-*N*-phenylthiobenzamides were prepared by a two-step process from 2-amino-*N*-phenylbenzamides. Treatment of 2-amino-*N*-phenylbenzamide with phosphorus decasulfide in pyridine afforded the corresponding pyridinium salt. Hydrolysis of the pyridinium salt in a toluene-water system gave 2-amino-*N*-phenylthiobenzamide [42]. 2-Methyl-3-phenylquinazoline-4(3*H*)-thiones (**2a-g**) were prepared by thionation of the corresponding 2-methyl-3-phenylquinazolin-4(3*H*)-ones with phosphorus decasulfide in pyridine. The syntheses are outlined in Scheme 1. The characteristic data of compounds **1a-k** and **2a-g** are given in Tables 1 and 2. Characteristic data of the intermediates were [43] or will be published elsewhere.

Scheme 1



Compound	X	R	Compound	X	R
1a	H	H	**1j**	Cl	4-isoC$_3$H$_7$
1b	H	4-Cl	**1k**	Cl	4-C$_4$H$_9$
1c	H	3,4-Cl$_2$	**2a**	Cl	H
1d	H	4-CH$_3$	**2b**	Cl	3-Cl
1e	H	4-C$_2$H$_5$	**2c**	Cl	4-Cl
1f	H	4-isoC$_3$H$_7$	**2d**	Cl	4-Br
1g	Cl	H	**2e**	Cl	4-CH$_3$
1h	Cl	3-Cl	**2f**	Cl	4-isoC$_3$H$_7$
1i	Cl	3,4-Cl$_2$	**2g**	Cl	4-OCH$_3$

Biological activity

Antimycobacterial activity

Antimycobacterial activity of the compounds was tested *in vitro* against *Mycobacterium tuberculosis*, *M. avium*, and *M. kansasii*, obtained from the Czech National Collection of Type Cultures (CNCTC), and a clinical isolate of *M. kansasii*, using the micromethod for the determination of the minimum inhibitory concentration (MIC). The MIC values of the compounds are given in Table 3. Antimycobacterially active compounds were found only among the 6-chloro derivatives (X = Cl). Derivatives **1h**, **2d**, **2f**, and **2g** demonstrated moderate activity against mycobacteria. The activity of derivative **2f** (X = Cl, R = 3-isopropyl), the most active compound, against *M. avium* and *M. kansasii* is worth mentioning. In some cases, the antimycobacterial activity observed after 14 days weared off after 21 days of incubation (**1c**, **1j**, **1k**). The other compounds showed no activity in the range of concentrations tested (data not given).

Photosynthesis-inhibiting activity in spinach chloroplasts

Most of the tested compounds inhibited the photosynthetic electron transport in spinach chloroplasts. The photosynthesis-inhibiting activity of the compounds was investigated as inhibition of oxygen evolution rate (OER) in spinach chloroplasts. IC_{50} values are given in Table 4. The 6-chloro analog **1g** was the most effective inhibitor of OER. Its IC_{50} value was comparable to that of the standard diuron (DCMU). 6-Unsubstituted compound **1a** was 60-fold less potent than **1g**. Substitution on the phenyl ring was unfavourable. Whereas mono- and dichloro derivatives **1h** and **1i** were approximately twice less potent than compound **1g**, alkyl derivatives **1j** and **1k** were more than 100-fold less potent. The relatively low photosyntesis-inhibiting activity of compounds **2** is probably a consequence of their low aqueous solubility, and hence their restricted passage through the hydrophilic regions of thylakoid membranes. A comparison of compounds **2a** and **2b** with their analogs **1g** and **1h**

indicates 75- to 100-fold decrease in activity. Photosynthesis-inhibiting activity of compounds **1d**, **1e**, and **1f** could not be determined due to their incomplete solubility.

Reduction of chlorophyll content in the green algae Chlorella vulgaris Beij.

Some of the compounds under study reduced the chlorophyll content in *Chlorella vulgaris* Beij. IC_{50} values could be determined only for compounds **1a** (IC_{50} = 49.61 μmol dm^{-3}), **1b** (IC_{50} = 40.91 μmol dm^{-3}), **1c** (IC_{50} = 34.88 μmol dm^{-3}), **1d** (IC_{50} = 155.09 μmol dm^{-3}), and **1g** (IC_{50} = 74.4 μmol dm^{-3}). IC_{50} value for the standard, a selective herbicide 1,1-dimethyl-3-(3,4-dichlorophenyl)urea (Diuron), was 7.3 μmol dm^{-3}. The other compounds were inactive (less than 5% reduction) or weakly active (27% (**1h**), 22% (**1k**), and 15% (**2b**) reduction of chlorophyll content) in the concentration range from 0.83 to 99.0 umol dm^{-3}. This could be due to their too low aqueous solubility.

Toxicological screening bioassay

Four compounds, **1h**, **1i**, **2b**, and **2f**, were selected according to their biological activity in antifungal [19], antimycobacterial, photosynthesis-inhibiting, and antialgal tests for toxicological screening bioassay using brine shrimp larvae (*Artemia salina* L.) as the sensitive organism. Only compound **2f** was found toxic. Its value of EC_{50} was 155.20 μmol dm^{-3} (EC_{50} of $MnCl_2$ was 41.44 mmol dm^{-3}). Other compounds tested demonstrated no significant toxicity in the range of used concentrations.

Conclusions

A series of novel 2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3*H*)-thiones **1** and 2-methyl-3-phenylquinazoline-4(3*H*)-thiones **2** was synthesized and tested for their antimycobacterial, photosynthesis-inhibiting, and antialgal activity. Compound **2f** (X = Cl, R = 3-isopropyl) exhibited better activity than isoniazid against *Mycobacterium avium* and *M. kansasii*. Unfortunately, it was found toxic in the brine shrimp bioassay (EC_{50} = 155.20 μmol dm^{-3}).

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Experimental

General

The melting points were determined on a Kofler block and are uncorrected. The samples for elemental analyses and biological tests were dried over P_4O_{10} at 61 °C and 66 Pa for 24 h. Elemental analyses were performed on a C,H,N,S analyzer (FISONS AE 1110, Milano, Italy). The purity of the compounds was checked by TLC using petroleum ether-ethyl acetate (9:1) and petroleum ether-acetone (7:3) as the mobile phases. Column chromatography was performed on Silica gel Merck 60 with petroleum ether-acetone (9:1) or toluene. ^{1}H- and ^{13}C-NMR spectra were recorded for DMSO-d_6 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer (operating at 300 and 75 MHz, respectively). Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (2.49 for ^{1}H and 39.7 for ^{13}C). Multiplicities are given together with coupling constants (*J*, in Hz).

2-Amino-N-phenylthiobenzamides

A 100-mL flask was charged with the appropriate 2-amino-*N*-phenylbenzamide (0.05 mol), tetraphosphorus decasulfide (0.05 mol), and pyridine (35 mL). Reaction mixture was refluxed for 4-6 h and after cooling poured into ice water (250 mL). The obtained precipitate was placed in a 500-mL flask, toluene (150 mL), water (150 mL), and conc. hydrochloric acid (5 mL) were added and the mixture was refluxed for 8–18 h. After cooling to room temperature, the toluene layer was separated and the solvent evaporated *in vacuo*. The residue was chromatographed on silica gel (toluene) and the product recrystallized from aqueous ethanol (yield 25–45 %).

2,2-Dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3H)-thiones **1a-k**

2-Amino-*N*-phenylthiobenzamide (0.01 mol) was dissolved in acetone (50 mL) at room temperature and silica gel (4 g) was added to the solution under stirring. The reaction mixture was stirred for 24 h at room temperature and then concentrated *in vacuo*. The residue was chromatographed on silica gel using petroleum ether-acetone (9:1) as the mobile phase. The product was recrystallized from ethanol. The yields, melting points, ^{1}H- and ^{13}C-NMR spectral data as well as elemental analyses are summarized in Tables 1 and 2.

2-Methyl-3-phenylquinazoline-4(3H)-thiones **2a-g**

6-Chloro-2-methyl-3-phenylquinazolin-4(3*H*)-one (0.01 mol) was dissolved in pyridine (10 ml) and tetraphosphorus decasulfide (0.01 mol) was added. The reaction mixture was refluxed under stirring for 4 h. After cooling, the mixture was poured into ice water, the crude product was filtered off,

washed with water, and dried. 6-Chloro-2-methyl-3-phenylquinazoline-4(3*H*)-thione was isolated by column chromatography on silica gel using petroleum ether-acetone (9:1) as the mobile phase and recrystallized from ethanol. The yields, melting points, ^{1}H- and ^{13}C-NMR spectral data as well as elemental analyses are summarized in Tables 1 and 2.

Table 1. Analytical data of 2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3*H*)-thiones (**1**) and 2-methyl-3-phenylquinazoline-4(3*H*)-thiones (**2**).

Compd.	Formula M. w.	X	R	M.p. (°C) Yield (%)	Elemental analysis % Calc. / % Found			
					C	H	N	S
1a	$C_{16}H_{16}N_2S$	H	H	212-214[a]	71.61	6.01	10.44	11.95
	268.4			78	71.50	6.15	10.51	12.10
1b	$C_{16}H_{15}ClN_2S$	H	4-Cl	238-241	63.46	4.99	9.25	10.59
	302.8			82	63.54	5.10	9.15	10.70
1c	$C_{16}H_{14}Cl_2N_2$	H	3,4-Cl_2	199-201	56.98	4.18	8.31	9.51
	337.3			86	56.80	4.30	8.27	9.70
1d	$C_{17}H_{18}N_2S$	H	4-CH_3	229-231	72.30	6.42	9.92	11.35
	282.4			80	72.27	6.46	9.84	11.30
1e	$C_{18}H_{20}N_2S$	H	4-C_2H_5	187-188	72.93	6.80	9.45	10.82
	296.4			76	72.79	6.85	9.50	10.85
1f	$C_{19}H_{22}N_2S$	H	4-isoC_3H_7	199-200	73.51	7.14	9.02	10.33
	310.5			85	73.61	7.05	9.10	10.39
1g	$C_{16}H_{15}ClN_2S$	Cl	H	218-219	63.46	4.99	9.25	10.59
	337.3			83	63.58	4.83	9.14	10.70
1h	$C_{16}H_{14}Cl_2N_2S$	Cl	3-Cl	157-158	56.98	4.18	8.31	9.51
	337.3			77	56.82	4.25	8.45	9.53
1i	$C_{16}H_{13}Cl_3N_2S$	Cl	3,4-Cl_2	189-190	51.70	3.53	7.54	8.63
	371.7			81	51.72	3.57	7.44	8.71
1j	$C_{19}H_{21}ClN_2S$	Cl	4-isoC_3H_7	205-207	66.17	6.14	8.12	9.30
	344.9			79	66.35	6.01	8.10	9.47
1k	$C_{20}H_{23}ClN_2S$	Cl	4-C_4H_9	183-184	66.93	6.46	7.80	8.93
	358.9			82	66.90	6.41	7.87	8.79
2a	$C_{15}H_{11}ClN_2S$	Cl	H	153-154	62.82	3.87	9.77	11.18
	286.8			69	62.72	3.96	9.78	11.30
2b	$C_{15}H_{10}Cl_2N_2S$	Cl	3-Cl	172-173	56.09	3.14	8.72	9.98
	321.2			74	56.06	3.35	8.54	9.95
2c	$C_{15}H_{10}Cl_2N_2S$	Cl	4-Cl	202-204	56.09	3.14	8.72	9.98
	321.2			76	56.35	3.08	8.75	10.26

Compd.	Formula M. w.	X	R	M.p. (°C) Yield (%)	Elemental analysis % Calc. / % Found			
					C	H	N	S
2d	$C_{15}H_{10}BrClN_2S$	Cl	4-Br	212-214	49.27	2.76	7.66	8.77
	365.7			73	49.39	2.74	7.59	8.90
2e	$C_{16}H_{13}ClN_2S$	Cl	4-CH_3	157-158	63.89	4.36	9.31	10.66
	(300.8)			70	63.71	4.48	9.25	10.60
2f	$C_{18}H_{17}ClN_2S$	Cl	4-isoC_3H_7	135-136	65.74	5.21	8.52	9.75
	328.9			68	65.85	5.18	8.47	9.68
2g	$C_{16}H_{13}ClN_2OS$	Cl	4-OCH_3	145-146	60.66	4.14	8.84	10.12
	316.8			59	60.70	4.12	8.91	10.07

[a]Reference [41] reports m.p. 212–214°C for compound **1a**.

Table 2. ^{1}H-NMR and ^{13}C-NMR spectral data

Compd.	**^{1}H-NMR δ (ppm), *J* (Hz)**	**^{13}C-NMR δ (ppm)**
1a	8.16 (dd, 1H, *J*=7.96, *J*=1.37, H5), 7.50-7.41 (m, 2H, H3′, H5′), 7.41-7.28 (m, 3H, NH, H7, H4′), 7.21-7.14 (m, 2H, H2′, H6′), 6.79-6.69 (m, 2H, H6, H8), 1.37 (s, 6H, CH_3)	190.4, 142.7, 142.6, 133.9, 132.6, 129.3, 129.2, 128.0, 121.0, 117.8, 115.2, 72.7, 27.1
1b	8.15 (dd, 1H, J=7.97, *J*=1.37, H5), 7.54-7.47 (m AA′, BB′, 2H, H2′, H6′), 7.38-7.29 (m, 2H, NH, H7), 7.26-7.19 (m AA′, BB′, 2H, H3′, H5′), 6.80-6.68 (m, 2H, H6, H8), 1.37 (s, 6H, CH_3)	190.8, 142.7, 141.4, 134.1, 132.6, 132.5, 131.3, 129.4, 120.9, 117.8, 115.3, 72.8, 27.0
1c	8.14 (d, 1H, *J*=7.97, H5), 7.72 (d, 1H, *J*=8.51, H5′), 7.56 (d, 1H, *J*=2.20, H2′), 7.42-7.30 (m, 2H, NH, H7), 7.28-7.22 (m, 1H, H6′), 6.81-6.69 (m, 2H, H6, H8), 1.40 (s, 6H, CH_3)	191.0, 142.8, 142.3, 134.3, 132.5, 131.7, 131.7, 131.2, 130.9, 130.2, 120.7, 117.9, 115.3, 73.0, 27.0
1d	8.19-8.12 (m, 1H, H5), 7.36-7.20 (m, 4H, NH, H7, H2′, H6′), 7.08-7.01 (m, 2H, H3′, H5′), 6.78-6.68 (m, 2H, H6, H8), 2.34 (s, 3H, CH_3) 1.35 (s, 6H, CH_3)	190.5, 142.7, 140.2, 137.2, 133.9, 132.7, 129.8, 128.9, 121.1, 117.7, 115.2, 72.7, 27.1, 20.9
1e	8.16 (d, 1H, *J*=7.96, H5), 7.36-7.24 (m, 4H, NH, H7, H2′, H6′), 7.11-7.04 (m, 2H, H3′, H5′), 6.74 (t, overlapped, 1H, *J*=7.83, H6), 6.71 (d, overlapped, 1H, *J*=7.83, H8), 2.64 (q, 2H, *J*=7.55, CH_2), 1.35 (s, 6H, CH_3), 1.21 (t, 3H, *J*=7.55, CH_3)	190.5, 143.3, 142.7, 140.3, 133.9, 132.7, 129.0, 128.6, 121.1, 117.7, 115.2, 72.7, 27.9, 27.1, 15.5
1f	8.19-8.13 (m, 1H, H5), 7.36-7.25 (m, 4H, NH, H7, H2′, H6′), 7.11-7.04 (m, 2H, H3′, H5′), 6.78-6.68 (m, 2H, H6, H8), 3.01-2.84 (m, 1H, CH), 1.34 (s, 6H, CH_3), 1.23 (d, 6H, *J*=6.87, CH_3)	190.5, 147.8, 142.7, 140.4, 133.9, 132.7, 128.9, 127.1, 121.1, 117.7, 115.1, 72.7, 33.2, 27.1, 24.0

Compd.	^{1}H-NMR δ (ppm), *J* (Hz)	^{13}C-NMR δ (ppm)
1g	8.11 (d, 1H, *J*=2.48, H5), 7.58 (bs, 1H, NH), 7.54-7.48 (m AA′, BB′, 2H, H2′, H6′), 7.37 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.27-7.20 (m AA′, BB′, 2H, H3′, H5′), 6.81 (d, 1H, *J*=8.79, H8), 1.37 (s, 6H, CH_3)	189.0, 142.4, 141.5, 133.6, 131.3, 129.4, 129.1, 128.2, 121.9, 121.4, 117.4, 73.0, 27.0
1h	8.11 (d, 1H, *J*=2.48, H5), 7.60 (bs, 1H, NH), 7.51-7.46 (m, 2H, H2′, H6′), 7.38 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.34-7.31 (m, 1H, H5′), 7.23-7.18 (m, 1H, H4′), 6.81 (d, 1H, *J*=8.79, H8), 1.37 (s, 6H, CH_3)	189.3, 143.5, 141.5, 133.8, 133.5, 131.2, 131.0, 129.3, 128.4, 128.3, 121.6, 121.4, 117.5, 73.1, 27.0
1i	8.09 (d, 1H, *J*=2.47, H5), 7.73 (d, 1H, *J*=8.79, H5′), 7.63 (bs, 1H, NH), 7.59 (d, 1H, *J*=2.20, H2′), 7.38 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.26 (dd, 1H, *J*=8.51, *J*=2.47, H6′), 6.82 (d, 1H, *J*=8.51, H8), 1.40 (s, 6H, CH_3)	189.6, 142.1, 141.5, 134.0, 131.8, 131.6, 131.3, 131.1 130.1, 121.5, 121.4, 117.5, 73.3, 27.0
1j	8.13 (d, 1H, *J*=2.75, H5), 7.52 (bs, 1H, NH), 7.36 (dd, 1H, *J*=8.51, *J*=2.47, H7),7.34-7.29 (m AA′, BB′, 2H, H2′, H6′), 7.13-7.05 (m AA′, BB′, 2H, H3′, H5′), 6.80 (d, 1H, *J*=8.79, H8), 3.02-2.85 (m, 1H, CH), 1.35 (s, 6H, CH_3), 1.23 (d, 6H, *J*=6.87, CH_3)	189.0, 148.1, 141.5, 140.1, 133.5, 131.3, 128.8, 127.2, 121.9, 121.3, 117.3, 73.0, 33.2, 27.1, 24.0
1k	8.12 (d, 1H, *J*=2.47, H5), 7.52 (bs, 1H, NH), 7.36 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.29-7.23 (m AA′, BB′, 2H, H2′, H6′), 7.10-7.04 (m AA′, BB′, 2H, H3′, H5′), 6.80 (d, 1H, *J*=8.79, H8), 2.61 (t, 2H, *J*=7.41, CH_2), 1.64-1.51 (m, 2H, CH_2), 1.40-1.24 (m, 2H, CH_2), 1.35 (s, overlapped, 6H, CH_3), 0.90 (t, 3H, *J*=7.42, CH_3)	189.0, 142.2, 141.5, 140.1, 133.5, 131.3, 129.2, 128.8, 121.9, 121.3, 117.4, 73.0, 34.6, 33.1, 27.0, 22.0, 14.0
2a	8.49 (d, 1H, *J*=2.48, H5), 7.90 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.73 (d, 1H, *J*=8.79, H8), 7.63-7.48 (m, 3H, H3′, H4′, H5′), 7.44-7.37 (m, 2H, H2′, H6′), 2.17 (s, 3H, CH_3)	188.1, 154.5, 142.4, 141.5, 135.2, 132.4, 130.2, 130.0, 129.3, 129.1, 127.9, 25.3
2b	8.49 (d, 1H, *J*=2.47, H5), 7.95-7.89 (m, 1H, H7), 7.44 (d, 1H, J=8.52, H8), 7.66-7.57 (m, 3H, H2′, H5′, H6′), 7.46-7.40 (m, 1H, H4′), 2.19 (s, 3H, CH_3)	188.1, 154.2, 143.5, 141.5, 135.3, 134.2, 132.5, 131.8, 130.1, 129.5, 129.2, 129.1, 128.3, 127.1, 25.3
2c	8.48 (d, 1H, J=2.47, H5), 7.91 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.73 (d, 1H, *J*=8.79, H8), 7.70-7.62 (m AA′, BB′, 2H, H2′, H6′), 7.52-7.44 (m AA′, BB′, 2H, H3′, H5′), 2.18 (s, 3H, CH_3)	188.2, 154.3, 141.5, 141.2, 135.3, 133.9, 132.4, 130.3, 130.1, 129.8, 129.2, 129.1, 25.4
2d	8.49 (d, 1H, J=2.47, H5), 7.92 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.83-7.77 (m AA′, BB′, 2H, H2′, H6′), 7.74 (d, 1H, *J*=8.79, H8), 7.45-7.38 (m AA′, BB′, 2H, H3′, H5′), 2.18 (s, 3H, CH_3)	188.1, 154.3, 141.7, 141.5, 135.3, 133.3, 132.4, 130.4, 130.1, 129.2, 129.1, 122.5, 25.4
2e	8.50 (d, 1H, J=2.47, H5), 7.90 (dd, 1H, *J*=8.65, *J*=2.47, H7), 7.72 (d, 1H, *J*=8.65, H8), 7.41-7.34 (m AA′, BB′, 2H, H2′, H6′), 7.30-7.23 (m AA′, BB′, 2H, H3′, H5′), 2.39 (s, 3H, CH_3), 2.17 (s, 3H, CH_3)	188.2, 154.7, 141.5, 139.9, 138.7, 135.1, 132.3, 130.7, 130.1, 129.3, 129.2, 127.6, 25.3, 21.0

Compd.	^{1}H-NMR δ (ppm), *J* (Hz)	^{13}C-NMR δ (ppm)
2f	8.51 (d, 1H, J=2.48, H5), 7.90 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.73 (d, 1H, *J*=8.79, H8), 7.48-7.41 (m AA´, BB´, 2H, H2´, H6´), 7.33-7.27 (m AA´, BB´, 2H, H3´, H5´), 3.06-2.90 (m, 1H, CH), 2.16 (s, 3H, CH_3), 1.26 (d, 6H, *J*=7.14, CH_3)	188.1, 154.8, 149.3, 141.5, 140.1, 135.1, 132.3, 130.0, 129.3, 129.2, 128.0, 127.6, 33.3, 25.3, 24.0
2g	8.50 (d, 1H, *J*=2.47, H5), 7.90 (dd, 1H, *J*=8.79, *J*=2.47, H7), 7.72 (d, 1H, J=8.79, H8), 7.35-7.28 (m, AA´, BB´, 2H, H2´, H6´), 7.14-7.07 (m AA´, BB´, 2H, H3´, H5´), 3.83 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3)	188.5, 159.4, 155.1, 141.5, 135.1, 135.1, 132.3, 130.0, 129.3, 129.3, 129.0, 115.2, 55.6, 25.4

Biological assays

Antimycobacterial activity

For the *in vitro* evaluation of antimycobacterial activity of the substances, the following strains were used: *Mycobacterium tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80, and *M. avium* CNCTC My 330/88, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, and a clinic isolate of *M. kansasii* 6509/96. Antimycobacterial activity of the compounds against these strains was determined in Šula semisynthetic medium (SEVAPHARMA, Prague). Each strain was simultaneously inoculated into a Petri dish containing Löwenstein-Jensen medium for the control of sterility of the inoculum and its growth. The compounds were added to the medium in dimethyl sulfoxide (DMSO) solutions. The following concentrations were used: 250, 125, 62.5, 31, 16, 8, 4, 2, 1, and 0.5 μmol dm^{-3}. The minimum inhibitory concentrations (MICs) were determined after incubation at 37 ^oC for 14 and 21 days. MIC was the lowest concentration of a substance at which the inhibition of the growth occurred. The compound is considered active, when its MIC is lower than 1000 μmol dm^{-3}. Isoniazid was used as the standard.

Table 3. Antimycobacterial activity of compounds **1** and **2** expressed as MIC (μmol dm^{-3})

Compound	X	R	MIC (μmol dm^{-3})			
			M. tbc. **My 331/88 14d/21d**	***M. avium*** **My 330/88 14d/21d**	***M. kansasii*** **My 235/80 14d/21d**	***M. kansasii*** **6 509/96 14d/21d**
1c	H	3,4-Cl_2	62.5/>62.5	62.5/>62.5	>62.5/>62.5	>250/>250
1h	Cl	3-Cl	62.5/62.5	125/>250	62.5/125	>62.5/>62.5
1j	Cl	4-isoC_3H_7	>31/>125	>62.5/>250	31/>62.5	>62.5/>125
1k	Cl	4-C_4H_9	>62.5/>125	>31/>125	31/>62.5	62.5/>62.5

Compound	X	R	MIC (μmol dm^{-3})			
			M. tbc. **My 331/88 14d/21d**	***M. avium*** **My 330/88 14d/21d**	***M. kansasii*** **My 235/80 14d/21d**	***M. kansasii*** **6 509/96 14d/21d**
2d	Cl	4-Br	31/31	>31/>31	>31/>31	62.5/62.5
2f	Cl	4-isoC_3H_7	31/31	31/31	31/31	62.5/62.5
2g	Cl	4-OCH_3	31/>62.5	62.5/125	31/62.5	31/62.5
Isoniazid	-	-	0.5/1	>250/>250	>250/>250	4/4

Photosynthesis-inhibiting activity in spinach chloroplasts

Spinach chloroplasts were prepared by the procedure described by Walker [45]. The effect of the compounds studied on oxygen evolution rate (OER) in spinach chloroplasts was investigated spectrophotometrically in the presence of the electron acceptor 2,6-dichlorophenol-indophenol (DCPIP) according to Kráľova *et al*. [46]. The rate of photosynthetic electron transport was monitored as a photoreduction of DCPIP. The chlorophyll (Chl) content was 30 mg dm^{-3}. Samples were irradiated from the distance of 1 dm with a halogen lamp (250 W) through a 4-cm water filter to prevent overheating of the samples. The activity of compounds **1** and **2** was expressed as IC_{50} values, *i. e.* molar concentration causing a 50% decrease of OER with respect to the untreated control. For low solubility of the studied compounds in water, these were dissolved in DMSO. The applied solvent content (up to 4 v/v %) did not affect the photochemical activity in spinach chloroplasts. Diuron was used as the standard.

Table 4. Inhibition of oxygen evolution rate in spinach chloroplasts by compounds **1** and **2** expressed as IC_{50} (μmol dm^{-3})

Compd.	X	R	IC_{50} (μmol dm^{-3})
1a	H	H	93.4
1b	H	4-Cl	72.2
1c	H	3,4-Cl_2	29.8
1d	H	4-CH_3	-[a]
1e	H	4-C_2H_5	-[a]
1f	H	4-isoC_3H_7	-[a]
1g	Cl	H	1.5
1h	Cl	3-Cl	3.5
1i	Cl	3,4-Cl_2	3.0
1j	Cl	4-isoC_3H_7	251
1k	Cl	4-C_4H_9	280

Compd.	X	R	IC_{50} (μmol dm^{-3})
2a	Cl	H	140
2b	Cl	3-Cl	267
2c	Cl	4-Cl	146
2d	Cl	4-Br	295
2e	Cl	4-CH_3	260
2f	Cl	4-isoC_3H_7	351
2g	Cl	4-OCH_3	268
Diuron	-	-	1.9

[a]The value could not be determined.

Reduction of chlorophyll content in the green algae Chlorella vulgaris Beij.

The green algae *Chlorella vulgaris* Beij. were cultivated statically at room temperature according to Kráľová *et al.* [47] (photoperiod 16 h light/8 h dark; irradiation: 90 μmol m^{-2} s^{-1} PAR; pH 7.2). The effect of the compounds on algal chlorophyll (Chl) content was determined after 7-day cultivation in the presence of the compounds tested. The Chl content in the algal suspension was determined spectrophotometrically after extraction into methanol according to Wellburn [48]. The Chl content in the suspensions at the beginning of the cultivation was 0.1 mg dm^{-3}. Because of their low water solubility, the tested compounds were dissolved in dimethyl sulfoxide (DMSO). DMSO concentration in the algal suspensions did not exceed 0.25 v/v % and the control samples contained the same DMSO amount as the suspensions treated with the tested compounds. The antialgal activity of compounds was expressed as IC_{50} (the concentration of the inhibitor causing a 50% decrease in content of chlorophyll as compared with the control sample) or by the percentage of reduction in the investigated concentration range (0.89 – 99.0 μmol dm^{-3}). Diuron was used as the standard.

Artemia screeing bioassay

Artemia salina L. eggs were obtained from JBL NovoTermia (Germany). The method of Eppley [49] was applied for *A. salina* larvae hatching. The test was arranged according to Kiviranta *et al*. [50]. 24-h old larvae were pipetted into 96-well plates (15-20 larvae per a well). The microcrystalline suspensions of tested substances were prepared by sonication for 1 h in an ultrasonic bath. The solvent was 1% DMSO in artificial seawater (pH 8.0 ± 0.1). The substances were tested in 11 concentrations with 8 repetitions. The final volume was always 150 μL per well. Every experiment was repeated twice at least. The negative control was 1% DMSO solution. The sensitivity of the organism was specified by a solution of $MnCl_2$. The mortality was determined after 24 h.

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