Synthesis and Biological Evaluation of Quinazoline-4-thiones

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Abstract: Several 2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3H)-thiones and 2-methyl-3-phenylquinazoline-4(3H)-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-methyquinazoline-4(3H)-thione exhibited higher activity than the isoniazid standard against Mycobacterium avium and M. kansasi. Most of the compounds possessed photosynthesis-inhibiting activity. 6-Chloro-2,2-dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3H)-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(3H)-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(3H)-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(3H)-thiones, nor 2-methyl-3-phenylquinazoline-4(3H)-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(3H)-thiones (1) and 2-methyl-3-phenylquinazoline-4(3H)-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(3H)-thione can be prepared by condensation of 2-aminothiobenzamides with acetone under mild conditions [41]. Other way to obtain quinazoline-4(3H)-thiones is the ring closure of 2-acylaminothiobenzamides in basic medium [28,42].

2,2-Dimethyl-3-phenyl-1,2-dihydroquinazoline-4(3H)-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(3H)-thiones (2a-g) were prepared by thionation of the corresponding 2-methyl-3-phenylquinazolin-4(3H)-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.
### 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. IC₅₀ values are given in Table 4. The 6-chloro analog 1g was the most effective inhibitor of OER. Its IC₅₀ 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 photosynthesis-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. IC₅₀ values could be determined only for compounds 1a (IC₅₀ = 49.61 µmol dm⁻³), 1b (IC₅₀ = 40.91 µmol dm⁻³), 1c (IC₅₀ = 34.88 µmol dm⁻³), 1d (IC₅₀ = 155.09 µmol dm⁻³), and 1g (IC₅₀ = 74.4 µmol dm⁻³). IC₅₀ 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 EC₅₀ was 155.20 µmol dm⁻³ (EC₅₀ of MnCl₂ 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(3H)-thiones 1 and 2-methyl-3-phenylquinazoline-4(3H)-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 (EC₅₀ = 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 P₄O₁₀ 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. ¹H- and ¹³C-NMR spectra were recorded for DMSO-d₆ 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 ¹H and 39.7 for ¹³C). 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, ¹H- and ¹³C-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(3H)-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(3H)-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(3H)-thiones (1) and 2-methyl-3-phenylquinazoline-4(3H)-thiones (2). |
|---|---|---|---|---|---|
| Compd. | Formula M. w. | X | R | M.p. (°C) | Elemental analysis % Calc. / % Found |
| | | | | Yield (%) | C | H | N | S |
| 1a | C$_{16}$H$_{16}$N$_2$S | 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$_2$S | 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$_2$N$_2$ | 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$_2$S | 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$_{30}$N$_2$S | H | 4-C$_2$H$_5$ | 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$_2$S | H | 4-isoC$_3$H$_7$ | 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$_2$S | 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$_2$N$_2$S | 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$_3$N$_2$S | 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$_2$S | Cl | 4-isoC$_3$H$_7$ | 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$_2$S | Cl | 4-C$_4$H$_9$ | 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$_{15}$ClN$_2$S | 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$_{16}$H$_{10}$Cl$_2$N$_2$S | 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$_{16}$H$_{10}$Cl$_2$N$_2$S | Cl | 4-Cl | 202-204 | 56.09 | 3.14 | 8.72 | 9.98 |
| | 321.2 | | | 76 | 56.35 | 3.08 | 8.75 | 10.26 |
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, 129.4, 128.9, 121.7, 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) |
|--------|--------------------------|----------------------|
| lg     | 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, CH3) | 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 |
| h      | 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 AA’, BB’, 2H, H3’, H5’), 6.81 (d, 1H, J=8.79, H8), 1.37 (s, 6H, CH3) | 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 |
| i      | 8.09 (d, 1H, J=2.48, 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, CH3) | 189.6, 143.1, 141.5, 133.0, 130.1, 121.5, 121.4, 117.5, 73.3, 27.0 |
| j      | 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, CH3), 1.23 (d, 6H, J=6.87, CH3) | 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     | 8.13 (d, 1H, J=2.75, 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, CH2), 1.64-1.51 (m, 2H, CH2), 1.40-1.24 (m, 2H, CH2), 1.35 (s, overlapped, 6H, CH3), 0.90 (t, 3H, J=7.42, CH3) | 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, CH3) | 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=2.48, H5), 7.91 (dd, 1H, J=8.79, J=2.47, H7), 7.73 (d, 1H, J=8.79, H8), 7.66-7.57 (m, 3H, H2´, H5´, H6´), 7.46-7.40 (m, 1H, H4´), 2.19 (s, 3H, CH3) | 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, CH3) | 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, CH3) | 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, CH3), 2.17 (s, 3H, CH3) | 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.** | **H-NMR δ (ppm), J (Hz)** | **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, CH3), 1.26 (d, 6H, J=7.14, CH3) | 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, OCH3), 2.19 (s, 3H, CH3) | 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 °C 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 | M. tuberculosis CNCTC My 331/88 14d/21d | M. avium CNCTC My 330/88 14d/21d | M. kansasii CNCTC My 235/80 14d/21d | M. kansasii 6509/96 14d/21d |
|---|---|---|---|---|---|---|
| 1c | H | 3,4-Cl₂ | 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-isoC₃H₇ | >31/>125 | >62.5/>250 | 31/>62.5 | >62.5/>125 |
| 1k | Cl | 4-C₄H₉ | >62.5/>125 | >31/>125 | 31/>62.5 | 62.5/>62.5 |
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 Král'ova 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\(_2\)H\(_5\) | -\(^a\)                    |
| 1f     | H | 4-isoC\(_3\)H\(_7\) | -\(^a\)                    |
| 1g     | Cl| H      | 1.5                           |
| 1h     | Cl| 3-Cl   | 3.5                           |
| 1i     | Cl| 3,4-Cl\(_2\) | 3.0                        |
| 1j     | Cl| 4-isoC\(_3\)H\(_7\) | 251                        |
| 1k     | Cl| 4-C\(_4\)H\(_9\) | 280                        |
The green algae *Chlorella vulgaris* Beij. were cultivated statically at room temperature according to Král'ová *et al.* [47] (photoperiod 16 h light/8 h dark; irradiation: 90 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) 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 \(\mu\text{mol dm}^{-3}\)). Diuron was used as the standard.

**Artemia screening 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 \(\mu\text{L}\) 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.

| Compd. | X   | R     | IC\(_{50}\) (\(\mu\text{mol dm}^{-3}\)) |
|--------|-----|-------|---------------------------------------|
| 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\(_3\)H\(_7\) | 351                              |
| 2g     | Cl  | 4-OCH\(_3\) | 268                              |
| Diuron | -   | -     | 1.9                                   |

*a*The value could not be determined.
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