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Synthesis and Biological Evaluation of Pyrazinecarboxamides

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Abstract: Unsubstituted, halogenated and/or alkylated pyrazine derivatives connected *via* -CONH- bridge with substituted anilines were synthesized and tested against *Mycobacterium tuberculosis* strain $H_{37}Rv$. Condensation of chlorides of substituted pyrazine-2-carboxylic acids with ring-substituted amines yielded a series of amides of pyrazine-2-carboxylic acid, 6-chloropyrazine-2-carboxylic acid, 5-*tert*-butylpyrazine-2-carboxylic acid or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic acid, respectively. The synthetic approach, analytical, spectroscopic, lipophilicity and biological data of twelve newly synthesized compounds are presented. Structure-activity relationships among the chemical structure, the antimycobacterial, antifungal, photosynthesis-inhibiting and antialgal activity of the evaluated compounds are discussed. Pyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**2**) has shown the highest activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (99%

inhibition). The highest antifungal effect against *Trichophyton mentagrophytes*, the most susceptible fungal strain tested, was found for 5-*tert*-butylpyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**8**, MIC = 62.5 μmol mL^{-1}). The highest reduction of chlorophyll content in *Chlorella vulgaris* was found for 6-chloropyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**5**, IC_{50} = 12.1 μmol L^{-1}).

Keywords: Pyrazinecarboxamides; *In vitro* antimycobacterial, antifungal, photosynthesis inhibition and antialgal activity; Lipophilicity determination.

Introduction

In connection with our research into antimycobacterial active pyrazine derivatives [1] we are interested in binuclear analogues with -CONH- bridge [2-5]. Various compounds possessing -CONH- group were found to inhibit photosynthetic electron transport [6-9]. Amides of 2-alkylpyridine-4-carboxylic acid, 2-alkylsulfanylpyridine-4-carboxylic acid inhibited oxygen evolution rate in *Chlorella vulgaris* and their inhibitory activity depended on the lipophilicity of the compounds [2, 3]. Previous studies [3-5, 10] showed that alkylation, amidation, aroylation of the pyrazine ring or substitution of the pyrazine with chlorine increase antituberculotic and/or antifungal activity in series of functional derivatives of pyrazinecarboxylic acid. [11]. Some of those derivatives influenced production of flavonolignans in an *in vitro* culture [12].

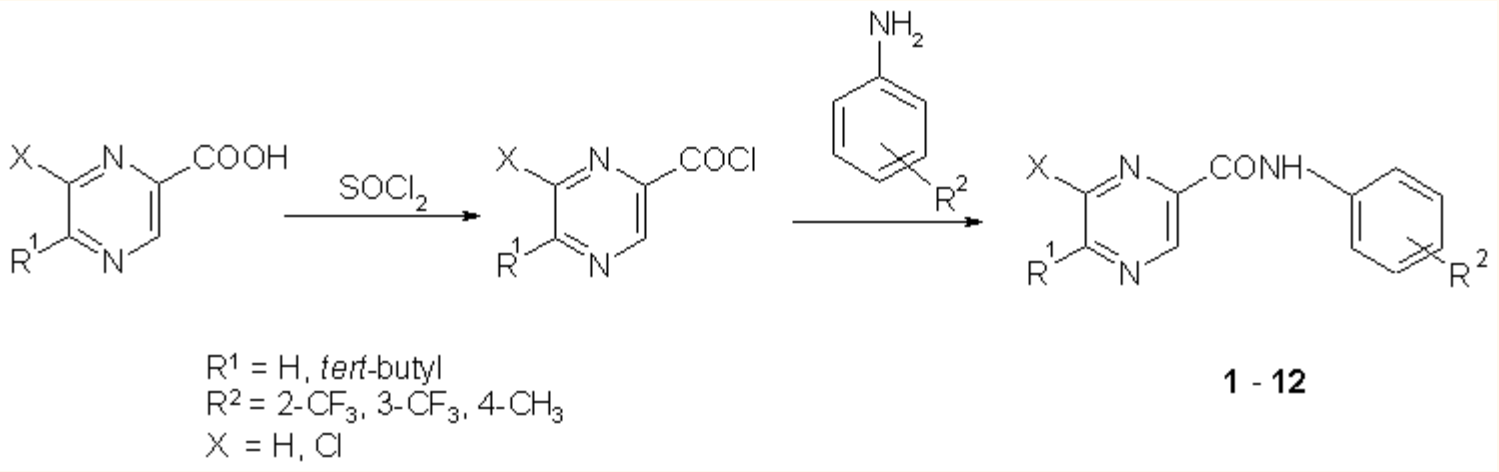
The presented study is concerned with the synthesis of the series of amides prepared from pyrazinecarboxylic acids and and 2-trifluoromethylaniline, 3-trifluoromethylaniline or 4-methylaniline, respectively.

The aim of this work is to search for the structure-activity relationships in the mentioned series, *i.e.* to continue in studying of the substituent variability influence on the antimycobacterial, antifungal, photosynthesis-inhibiting or antialgal activities, and to determine the importance of increased hydro/lipophilic properties for biological effect of the newly prepared substituted pyrazinecarboxamides.

Results and Discussion

The synthesis of amides is shown in Scheme 1. Condensation of chlorides of pyrazine-2-carboxylic acid [13], 6-chloropyrazine-2-carboxylic acid [14], 5-*tert*-butylpyrazine-2-carboxylic acid [3] or 6-chloro-5-*tert*-butylpyrazine-2-carboxylic [3] acid with ring-substituted anilines yielded a series of amides of mentioned pyrazine-2-carboxylic acids **1**-**12**. The melting points, yields, elemental analyses, IR, ^{1}H and ^{13}C NMR spectral data for all the compounds prepared are given in Experimental. Purity (%), calculated the logarithm of capacity factor *K* (log *K*), and calculated lipophilicities (log *P*) of the compounds studied are shown in Table 2.

Scheme 1. Synthesis of some substituted pyrazine-2-carboxamides **1**-**12**.



All compounds prepared were evaluated on their *in vitro* antimycobacterial susceptibility. Some interesting results were obtained. The highest activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (99% inhibition) was found for pyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**2**). Other derivatives showed lower activities: pyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**3**, 86%), 6-chloropyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**5**, 77%), and 6-chloropyrazine-2-carboxylic acid (4-methylphenyl)amide (**6**, 71%). In the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) program compounds effecting <90% inhibition in this primary screen (*i.e.* MIC > 6.25 mg mL^{-1}) are not generally evaluated further [15]. The antimycobacterial evaluation of compounds **10**-**12** is not finished.

The evaluation of *in vitro* antifungal activity of the synthesized compounds was performed against eight fungal strains. The results showed no interesting activity against majority of fungal strains tested. Only compound 5-*tert*-butylpyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**8**) exhibited some moderate *in vitro* antifungal activity against *Trichophyton mentagrophytes*, the most susceptibility fungal strain evaluated, (MIC = 62.5 μmol mL^{-1}). This activity is only modest one in comparison with fluconazole, the standard (MIC = 3.91 μmol mL^{-1} after 120 h, see Table 1). The negative results of antifungal screening do not allow us to draw detailed conclusions on some structure–activity relationships.

Majority of all the compounds studied inhibited photosynthetic electron transport in spinach chloroplasts (see Table 1). Based on the obtained results it could be concluded that OER-inhibiting activity of investigated compounds depends beside lipophilicity also on the electron accepting or withdrawing power of the substituents. However, their inhibitory activities were very low. At higher applied concentrations the solubility of some compounds in the suspensions of spinach chloroplasts was limited. The most effective inhibitor was compound **7** (IC_{50} = **0.055** μmol L^{-1}). For group of compounds **1**-**3** (X = H; R^1 = H), **4**-**6** (X = Cl; R^1 = H) and **7**-**9** (X = H; R^1 = *tert*-butyl) the activity increased with increasing lipophilicity of the compound (with the exception of compound **8**). On the other hand, for group of compounds **10**-**12** the OER-inhibiting activity showed a decrease with increasing compound lipophilicity. Based on the obtained results it could be concluded that OER-inhibiting activity of investigated compounds depends beside lipophilicity also on electron-withdrawing properties of the substituents. Some of the compounds under study reduced the chlorophyll content in *Chlorella vulgaris*. The IC_{50} values related to reduction of chlorophyll content in *Chlorella vulgaris* could be determined only for four compounds (**3**, **5**, **6**, **7**)

and the highest effect was found for 6-chloropyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**5**, IC_{50} = 12.1 μmol L^{-1}).

The inhibitory activity of compounds **3**, **6** and **5** increased linearly with increasing lipophilicity of the compound (log *P* = 1.6, 2.72 and 3.61 respectively). However, further lipophilicity increase (compound **7**; log *P* = 4.02) led to activity decrease. The inhibition of chlorophyll content by other investigated compounds (**1**, **2**, **4**, **8**-**12**) was low in the whole studied concentration range (100-0.83 μmol L^{-1}) and for the majority of these compounds did not exceed 10% (*i.e.* 90% of the control). Results are shown in Table 1.

Hydrophobicity parameters of compounds **1**-**12** were calculated (log *P* values) and measured by means of RP-HPLC determination of capacity factor *K* and subsequently calculated log *K*. The values of calculated lipophilicity (log *P*) of compounds ranged from 1.60 to 5.30. It can be assumed, the computed log *P* values and the calculated log *K* values relatively correspond with expected lipophilicity increasing within individual series of compounds (pyrazine < 6-chloropyrazine < 5-*tert*-butylpyrazine < 6-chloro-5-*tert*-butylpyrazine derivatives). Capacity factor *K* / calculated log *K* values specify lipophilicity within individual series of compounds. Results are shown in Table 2.

6-Chloropyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**5**) was found as the most active compound in the three different biological assays. However, there is no general result about the SAR in compounds evaluated.

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Experimental

General

All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled under argon atmosphere. TLC was performed on Silufol UV 254 plates (Kavalier, Votice, Czech Republic) in the following solvent systems: acetone-toluene (1:1) and petroleum ether-ethyl acetate (9:1). The plates were detected in UV (254 nm). Melting points were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany). Infrared spectra were recorded in KBr pellets on an IR-spectrometer Nicolet Impact 400. ^{1}H and ^{13}C NMR Spectra were recorded on a Varian Mercury – Vx BB 300 (299.95 MHz for ^{1}H and 75.43 MHz for ^{13}C), Varian (Palo Alto CA, USA). Chemical shifts are given relative to internal $Si(CH_3)_4$. The purity of the compounds was checked by HPLC. Peaks in the chromatogram of the solvent (backgroud) were deducted from peaks in the chromatogram of the sample solution. A purity of the individual compounds was determined from area peaks in the chromatogram of the sample solution.

Lipophilicity HPLC determination (capacity factor K / calculated Log K)

The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used. The chromatographic column Symmetry® C_{18} 5 µm, 4.6 × 250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, U.S.A.) was used. The HPLC separation process was monitored by Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, U.S.A.). The mixture of MeOH p.a. (70.0%) and H_2O-HPLC – Mili-Q Grade (30.0%) was used as a mobile phase. The total flow of the column was 1.0 mL min^{-1}, injection 30 µL, column temperature 30 °C and sample temperature 10 °C. The detection wavelength 223 nm was chosen. The KI methanolic solution was used for the dead time (TD = 2.500 min) determination. Retention times (TR) were measured in minutes. The capacity factors *K* were calculated using the Millennium32® Chromatography Manager Software according to the formula $K = (TR - TD) / TD$. Log *K*, calculated from the capacity factor *K*, of the individual compounds are shown in Table 2.

Lipophilicity calculation

Log *P*, *i.e.* the logarithm of the partition coefficient for n-octanol/water, was calculated using the commercially available program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). The results are summarized in Table 2.

Synthesis of amides **1**-**12**

A mixture of acid, *i.e.* pyrazine-2-carboxylic [13], 6-chloropyrazine-2-carboxylic [14], 5-*tert*-butylpyrazine-2-carboxylic [3] or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic [3] acids, respectively, (50.0 mmol) and thionyl chloride (5.5 mL, 75.0 mmol) in dry toluene (20 mL) was refluxed for about 1 h. Excess of thionyl chloride was removed by repeated evaporation with dry toluene *in vacuo*. The crude acyl chloride dissolved in dry acetone (50 mL) was added drop wise to a stirred solution of the corresponding substituted amine (50.0 mmol) in 50 mL of dry pyridine keeping at the room temperature. After the addition was complete, stirring continued for another 30 min. The reaction mixture was then poured into 100 mL of cold water and the crude amide was collected and recrystallized from aqueous ethanol.

Pyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**1**).

Yield: 71%, m.p. 123 °C. For $C_{12}H_8F_3N_3O$ (267.2) calculated: 53.94% C, 3.02% H, 15.73% N; found: 54.29% C, 3.05% H, 15.53% N. $R_F = 0.77$.

IR spectrum (KBr), cm^{-1}: 3370 (N-H), 1704 (C=O), 1593 (phenyl), 1457 (N-H), 1319, 1298, and 1112 (pyrazine).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.26 (1H, bs, NH), 9.52 (1H, d, *J*=1.5 Hz, H3), 8.84 (1H, d, *J*=2.5 Hz, H6), 8.64 (1H, dd, *J*=2.5 Hz, *J*=1.5 Hz, H5), 8.54 (1H, d, *J*=7.4 Hz, H3'), 7.71-7.59 (2H, m, H5', H6'), and 7.33-7.24 (1H, m, H4').

^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.0, 147.8, 144.7, 144.0, 142.7, 134.9, 133.0, 126.2 (q, *J*=5.4 Hz), 124.6, 124.0 (q, *J*=272.9 Hz), 123.2, and 120.0 (q, *J*=29.7 Hz).

Pyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**2**).

Yield: 61%, m.p. 109 °C. For $C_{12}H_8F_3N_3O$ (267.2) calculated: 53.94% C, 3.02% H, 15.73% N; found: 53.94% C, 2.93% H, 15.62% N. R_F = 0.69.

IR spectrum (KBr), cm^{-1}: 3327 (N-H), 1680 (C=O), 1593 (phenyl), 1450 (N-H), 1337, and 1126 (pyrazine).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.80 (1H, bs, NH), 9.52 (1H, d, *J*=1.4 Hz, H3), 8.84 (1H, d, *J*=2.5 Hz, H6), 8.61 (1H, dd, *J*=2.5 Hz, *J*=1.4 Hz, H5), 8.08-8.05 (1H, m, H2'), 8.00-7.94 (1H, m, H4'), 7.52 (1H, t, *J*=8.0 Hz, H5'), and 7.46-7.40 (1H, m, H6').

^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.9, 147.9, 144.7, 143.8, 142.4, 137.7, 131.6 (q, *J*=32.7 Hz), 129.7, 123.8 (q, *J*=272.6 Hz), 122.8, 121.3 (q, *J*=4.0 Hz), and 116.5 (q, *J*=4.1 Hz).

Pyrazine-2-carboxylic acid (4-methyphenyl)amide (**3**).

Yield: 44%, m.p. 148 °C. For $C_{12}H_{11}N_3O$ (213.2) calculated: 67.59% C, 5.20% H, 19.71% N; found: 67.34% C, 5.57% H, 19.55% N. R_F = 0.70.

IR spectrum (KBr), cm^{-1}: 3348 (N-H), 1671 (C=O), 1593 (phenyl), 1523 (N-H), 1402, 1321, and 1023 (pyrazine).

^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.61(1H, bs, NH), 9.51 (1H, d, *J*=1.5 Hz, H3), 8.79 (1H, d, *J*=2.5 Hz, H6), 8.58 (1H, dd, *J*=2.5 Hz, *J*=1.5 Hz, H5), 7.68-7.60 (2H, m, AA', BB', H2', H6'), 7.24-7.16 (2H, m, AA', BB', H3', H5'), and 2.35 (3H, s, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 160.4, 147.4, 144.6, 144.5, 142.3, 134.6, 134.5, 129.6, 119.7, and 20.9.

6-Chloropyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**4**).

Yield: 47%, m.p. 148-150 °C. For $C_{12}H_7ClF_3N_3O$ (301.7) calculated: 47.78% C, 2.34% H, 13.93% N; found: 47.44% C, 2.31% H, 13.65% N. R_F = 0.84.

IR spectrum (KBr), cm^{-1}: 3384 (N-H), 1707 (C=O), 1594 (phenyl), 1543 (N-H), 1322, 1301, and 1109 (pyrazine).

^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.98 (1H, bs, NH), 9.39 (1H, d, *J*=0.6 Hz, H3), 8.84 (1H, d, *J*=0.6 Hz, H5), 8.46 (1H, d, *J*=8.2 Hz, H3'), 7.73-7.59 (2H, m, H5', H6'), and 7.35-7.27 (1H, m, H4').

^{13}C NMR (75 MHz, $CDCl_3$), δ: 160.6, 159.8, 147.9, 147.7, 143.5, 142.1, 133.0, 126.3 (q, *J*=5.2 Hz), 125.0, 123.9 (q, *J*=272.9 Hz), 123.5, and 120.4 (q, *J*=32.7 Hz).

6-Chloropyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**5**).

Yield: 62%, m.p. 109 °C. For $C_{12}H_7ClF_3N_3O$ (301.7) calculated: 47.78% C, 2.34% H, 13.93% N; found: 48.09% C, 2.72% H, 13.97% N. R_F = 0.80.

IR spectrum (KBr), cm^{-1}: 3351 (N-H), 1680 (C=O), 1603 (phenyl), 1545 (N-H), 1330, 1169, and 1131 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.52 (1H, bs, NH), 9.40 (1H, s, H3), 8.84 (1H, s, H5), 8.07 (1H, bs, H2'), 7.99-7.94 (1H, m, H4'), 7.58-7.49 (1H, m, H5'), and 7.49-7.42 (1H, m, H6').

^{13}C NMR (75 MHz, $CDCl_3$), δ: 159.6, 147.9, 147.5, 143.4, 142.3, 137.4, 131.6 (q, *J*=32.7 Hz), 129.8, 123.7 (q, *J*=272.6 Hz), 123.0, 121.7, (q, *J*=3.7 Hz), and 116.7 (q, *J*=4.0 Hz).

6-Chloropyrazine-2-carboxylic acid (4-methylphenyl)amide (**6**).

Yield: 59%, m.p. 134 °C. For $C_{12}H_{10}ClN_3O$ (247.7) calculated: 58.19% C, 4.07% H, 16.97% N; found: 58.11% C, 4.45% H, 16. 32% N. R_F = 0.81.

IR spectrum (KBr), cm^{-1}: 3370 (N-H), 1694 (C=O), 1592 (phenyl), 1531 (N-H), 1320, 1168, and 1144 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.39 (1H, d, *J*=0.5 Hz, H3), 9.34 (1H, bs, NH), 8.79 (1H, s, H5), 7.67-7.59 (2H, m, AA', BB', H2', H6'), 7.24-7.17 (2H, m, AA', BB', H3', H5'), and 2.35 (3H, s, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 159.2, 147.4, 144.1, 142.2, 134.9, 134.3, 129.7, 119.9, and 20.9.

5-tert-Butylpyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**7**).

Yield: 47%, m.p. 82.5 °C. For $C_{16}H_{16}F_3N_3O$ (323.3) calculated: 59.44% C, 4.99% H, 13.00% N; found: 59.47% C, 5.10% H, 13.05% N. R_F = 0.88.

IR spectrum (KBr), cm^{-1}: 3356 (N-H), 2961, 2907, 2869 (*tert*-butyl), 1698 (C=O), 1593 (phenyl), 1539 (NH), 1322, 1171, and 1118 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 10.27 (1H, bs, NH), 9.40 (1H, d, *J*=1.4 Hz, H3), 8.70 (1H, d, *J*=1.4 Hz, H6), 8.57 (1H, d, *J*=8.2 Hz, H3'), 7.70-7.58 (2H, m, H5', H6'), 7.31-7.22 (1H, m, H4'), and 1.45 (9H, s, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 168.1, 161.5, 143.1, 141.0, 139.4, 135.1, 133.0, 126.2 (q, *J*=5.2 Hz), 124.3, 124.1 (q, *J*=272.8 Hz), 123.1, 119.8 (q, *J*=29.7 Hz), 37.1, and 29.7.

5-tert-Butylpyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**8**).

Yield: 38%, m.p. 84.5 °C. For $C_{16}H_{16}F_3N_3O$ (323.3) calculated: 59.44% C, 4.99% H, 13.00% N; found: 59.83% C, 5.09%

H, 13.17% N. R_F = 0.86.

IR spectrum (KBr), cm^{-1}: 3350 (N-H), 2974, 2938, 2911, 2874 (*tert*-butyl), 1684 (C=O), 1604 (phenyl), 1543 (N-H), 1339, and 1133 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.78 (1H, bs, NH), 9.40 (1H, d, *J*=1.7 Hz, H3), 8.63 (1H, d, *J*=1.7 Hz, H6), 8.08 (1H, bs, H2'), 7.98-7.92 (1H, m, H4'), 7.51 (1H, t, *J*=8.0 Hz, H5'), 7.44-7.39 (1H, m, H6'), and 1.45 (9H, s CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 168.2, 161.4, 143.1, 140.9, 139.1, 138.0, 131.6 (q, *J*=32.4 Hz), 129.7, 123.8 (q, *J*=272.6 Hz), 122.7, 121.1 (q, *J*=4.0 Hz), 116.4 (q, *J*=4.0 Hz), 37.1, and 29.7.

5-tert-Butylpyrazine-2-carboxylic acid (4-methylphenyl)amide (**9**).

Yield: 83%, m.p. 143 °C. For $C_{16}H_{19}N_3O$ (269.4) calculated: 71.35% C, 7.11% H, 15.60% N; found: 71.58% C, 7.38% H, 15.62% N. R_F = 0.86.

IR spectrum (KBr), cm^{-1}: 3351 (N-H), 2977, 2957, 2922, 2906, 2872 (*tert*-butyl, methyl), 1676 (C=O), 1592 (phenyl), 1520 (NH), 1314, and 1147 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.60 (1H, bs, NH), 9.39 (1H, d, *J*=1.4 Hz, H3), 8.61 (1H, d, *J*=1.4 Hz, H6), 7.68-7.61 (2H, m, AA', BB', H2', H6'), 7.23-7.15 (2H, m, AA', BB', H3', H5'), 2.35 (3H, s, CH_3), and 1.44 (9H, s, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 167.6, 160.9, 142.9, 141.5, 138.9, 134.9, 134.2, 129.6, 119.7, 37.0, 29.7, and 20.9.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (2-trifluormethyphenyl)amide (**10**).

Yield: 26%, m.p. 81 °C. For $C_{16}H_{15}ClF_3N_3O$ (357.8) calculated: 53.72% C, 4.23% H, 11.75% N; found: 54.06% C, 3.89% H, 11.82% N. R_F = 0.89.

IR spectrum (KBr), cm^{-1}: 3366 (N-H), 2980, 2961, 2936, 2909, 2874 (*tert*-butyl, trifluormethyl), 1715 (C=O), 1592 (phenyl), 1532 (N-H), 1323, 1290, and 1120 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.99 (1H, bs, NH), 9.26 (1H, s, H3), 8.49 (1H, d, *J*=8.2 Hz, H3'), 7.72-7.57 (2H, m, H5', H6'), 7.33-7.24 (1H, m, H4'), and 1.56 (9H, s, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 165.0, 160.2, 146.0, 140.6, 140.2, 134.7, 133.0, 126.3 (q, *J*=5.2 Hz), 124.7, 124.0 (q, *J*=272.9 Hz), 123.3, 120.2 (q, *J*=30.0 Hz), 39.1, and 28.2.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (3-trifluormethyphenyl)amide (**11**).

Yield: 21%, m.p. 71.8 °C. For $C_{16}H_{15}ClF_3N_3O$ (357.8) calculated: 53.72% C, 4.23% H, 11.75% N; found: 53.49% C, 4.60% H, 12.03% N. R_F = 0.84.

IR spectrum (KBr), cm^{-1}: 3375 (N-H), 2985, 2960, 2940, 2910, 2870 (*tert*-butyl, trifluormethyl), 1720 (C=O), 1603 (phenyl), 1530 (N-H), 1325, 1250, and 1158 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.99 (1H, bs, NH), 9.04 (1H, s, H3), 8.63 (1H, d, *J*=1.7 Hz, H6), 8.08 (1H, bs, H2'), 7.98-7.92 (1H, m, H4'), 7.51 (1H, t, *J*=8.0 Hz, H5'), 7.44-7.39 (1H, m, H6'), 1.34 (9H, s and CH3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 168.2, 161.4, 144.9, 140.9, 139.1, 138.5, 131.6 (q, *J*=32.4 Hz), 129.7, 123.8 (q, *J*=272.6 Hz), 122.7, 120.9 (q, *J*=4.0 Hz), 119.4 (q, *J*=4.0 Hz), 37.1, 31.0, and 29.7.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (4-methylphenyl)amide (**12**).

Yield: 31%, m.p. 162 °C. For $C_{16}H_{18}ClN_3O$ (303.8) calculated: 63.26% C, 5.97% H, 13.83% N; found: 63.17% C, 5.87% H, 13.69% N. $R_F = 0.83$.

IR spectrum (KBr), cm^{-1}: 3376 (N-H), 2978, 2956, 2929, 2867 (*tert*-butyl, methyl), 1701 (C=O), 1596 (phenyl), 1538 (N-H), 1317, 1260, and 1148 (pyrazine).

^1H NMR (300 MHz, $CDCl_3$), δ: 9.31 (1H, bs, NH), 9.26 (1H, s, H3), 7.67-7.60 (2H, m, AA', BB', H2', H6'), 7.23-7.16 (2H, m, AA', BB', H3', H5'), 2.35 (3H, s, CH_3), and 1.55 (9H, s, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ: 164.4, 159.6, 141.2, 140.2, 134.6, 134.5, 129.6, 119.9, 38.9, 28.3, and 20.9.

Antimycobacterial assay

Antimycobacterial evaluation was carried out in the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL, USA, which is a part of the National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.25 μg mL^{-1} against *Mycobacterium tuberculosis* strain $H_{37}Rv$ in BACTEC 12B medium using the BACTEC 460 radiometric system [15, 16]. The results are presented in Table 1.

In vitro antifungal susceptibility testing

The broth microdilution test [10, 17] was used for the assessment of *in vitro* antifungal activity of the synthesized compounds against *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon beigelii* 1188 (TB), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *Trichophyton mentagrophytes* 445 (TM). Fluconazole was used as a reference drug. The procedure was performed with twofold dilution of the compounds in RPMI 1640 medium (Sevapharma) buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulfonic acid. The final concentrations of the compounds ranged from 500 to 0.975 μmol L^{-1}. Drug–free controls were included. The minimal inhibitory concentrations (MICs) were determined after 24 h and

48 h of static incubation at 35 °C. With *T. mentagrophytes*, the final MICs were determined after 72 h and 120 h of incubation. The results of all compounds *in vitro* tested against *T. mentagrophytes*, the most susceptible fungal strain, are summarized in Table 1.

Study of inhibition of oxygen evolution rate in spinach chloroplasts

Chloroplasts were prepared by the procedure of Walker [18] from spinach (*Spinacia oleracea* L.). The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Kontron Uvikon 800, Kontron, Munich, Germany) using an artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kralova et al. [19] and the rate of photosynthetic electron transport was monitored as a photoreduction of DCPIP. The measurements were carried out in phosphate buffer (0.02 mol L^{-1}, pH 7.2) containing sucrose (0.4 mol L^{-1}), $MgCl_2$ (0.005 mol L^{-1}) and NaCl (0.015 mol L^{-1}). The chlorophyll content was 30 mg/l in these experiments and the samples were irradiated (~100 $W.m^{-2}$) from 10 cm distance with a halogen lamp (250 W) using a 4 cm water filter to prevent warming of the samples (suspension temperature 22 °C). The studied compounds were dissolved in DMSO due to their limited water solubility. The applied DMSO concentration (up to 4%) did not affect the photochemical activity in spinach chloroplasts (PET). The inhibitory efficiency (concentration) of the studied compounds has been expressed by IC_{50} values, *i.e.* by molar concentration of the compounds causing 50% decrease in the oxygen evolution relative to the untreated control. Comparable IC_{50} value for a selective herbicide diurone, 3-(3,4-dichlorophenyl)-1,1-dimethylurea was about 0.032 μmol L^{-1} [20]. The results are summarized in Table 1.

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

The green algae *C. vulgaris* Beij. was cultivated statically at room temperature according to Kralova et al. [21] (photoperiod 16 h light/8 h dark; photosynthetic active radiation 80 μmol m^2 s; pH 7.2). The effect of the compounds on algal chlorophyll (Chl) content was determined after 4-day cultivation in the presence of the tested compounds. The Chl content in the algal suspension was determined spectrophotometrically (Kontron Uvikon 800, Kontron, Munich, Germany) after extraction into methanol according to Wellburn [22]. The Chl content in the suspensions at the beginning of the cultivation was 0.1 mg L^{-1}. The applied compound concentrations were as follows: 100, 75. 50, 25, 8.3, 4.2 and 0.83 μmol L^{-1}. Because of the low solubility of the studied compounds in water, these were dissolved in DMSO. DMSO concentration in the algal suspensions did not exceed 0.25% 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 IC50 (the concentration of the inhibitor causing a 50% decrease in content of chlorophyll as compared with the control sample) or as percentage of the control determined for the studied concentration range (100-0.83 μmol L^{-1}) with the corresponding standard deviation (S.D.). Comparable IC_{50} value for a selective herbicide diurone was about 7.3 μmol L^{-1} [20] The results are summarized in Table 1.

Table 1. Antimycobaterial evaluation (% of inhibition), antifungal susceptibility (MIC), OER inhibition in spinach chloroplasts (IC_{50}), and reduction of chlorophyll content (IC_{50}) of compounds **1**-**12** in comparison with standards: pyrazinamide (PZA), fluconazole (FLU) and diurone (DCMU).

Comp.	Structure			Antimycobaterial evaluation	Antifungal susceptibility	OER inhibition in spinach chloroplasts	Chlorophyll reduction	
	X	R^1	R^2	% Inhibition at 6.25 μg.mL^{-1}	MIC [c] (μmol mL^{-1})	IC_{50} (mmol L^{-1})	IC_{50} (μmol L^{-1})	% of control ± S.D. [d]
1	H	H	2-CF_3	31	>250/>250	0.376	-	91.7 ± 4.9
2	H	H	3-CF_3	**99**	>500/>500	0.130	-	91.5 ± 2.7
3	H	H	4-CH_3	**86**	>500/>500	1.4 75	70.9	70.9
4	Cl	H	2-CF_3	26	500/>500	0.557	-	87.8 ± 4.3
5	Cl	H	3-CF_3	**77**	**125/125**	0.229	**12.1**	**12.1**
6	Cl	H	4-CH_3	71	250/250	1.5 24	37.4	37.4
7	H	$(CH_3)_3C$	2-CF_3	7	250/250	**0.055**	32.9	32.9
8	H	$(CH_3)_3C$	3-CF_3	23	**62.5/62.5**	0.283	-	85.2 ± 5.1
9	H	$(CH_3)_3C$	4-CH_3	61	**125/125**	0.164	-	91.8 ± 2.2
10	Cl	$(CH_3)_3C$	2-CF_3	*a*	250/250	0.205	-	94.4 ± 2.0
11	Cl	$(CH_3)_3C$	3-CF_3	*a*	250/250	0.173	-	90.1 ± 2.0
12	Cl	$(CH_3)_3C$	4-CH_3	*a*	>500/>500	0.073	-	92.9 ± 2.4
PZA	-	-	-	*b*	-	-	-	-
FLU	-	-	-	-	1.95/3.91	-	-	-
DCMU	-	-	-	-	-	0.000032	7.3	-

[a] no results, [b] MIC = 12.5 μg mL^{-1}, data from [10], [c] against *T. mentagrophytes* after 72 h / 120 h, [d] IC_{50} was determined only for four compounds, an average decrease of Chl content was determined only in interval 0.83–100 μmol L^{-1} for other eight compounds.

Table 2. Purity, determined log *K* and comparison of calculated lipophilicity (log *P*) of compounds **1**-**12**.

Compound	Purity (%)	Log *K*	Log *P*
1	99.66	0.2078	2.33 ± 0.44
2	98.81	0.1873	2.49 ± 0.44
3	99.56	0.0965	1.60 ± 0.40
4	99.69	0.4604	3.45 ± 0.46
5	98.71	0.4129	3.61 ± 0.47
6	99.39	0.3336	2.72 ± 0.41

7	99.89	0.9161	4.02 ± 0.46
8	99.81	0.7543	4.18± 0.46
9	99.08	0.6530	3.28 ± 0.40
10	99.75	1.2859	5.14 ± 0.48
11	98.37	1.1354	5.30 ± 0.48
12	99.18	1.0574	4.41 ± 0.42

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