Substituted *N*-phenylpyrazine-2-carboxamides, Their Synthesis, Hydro-lipophilic Properties and Evaluation of Their Antimycobacterial, Antifungal and Photosynthesis-inhibiting Activity

Martin Doležal[1]*, Zdeněk Osička[2], Jan Zitko[1], Jiří Kuneš[1], Josef Jampílek[3,4], Marcela Vejsová[1], Vladimír Buchta[5], and Katarína Kráľová[6]

[1] Faculty of Pharmacy in Hradec Králové, Charles University in Prague, 500 05 Hradec Králové, Czech Republic; e-mail: martin.dolezal@faf.cuni.cz, tel: +420-49-5067389, fax: +420-49-5512423

[2] Bioveta a.s., Komenského 212, 683 23 Ivanovice na Hané, Czech Republic

[3] Zentiva k.s., U Kabelovny 130, 102 37 Prague 10, Czech Republic

[4] Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 61242 Brno, Czech Republic

[5] Department of Clinical Microbiology, Charles University Medical School and Teaching Hospital, Sokolská 581, Hradec Králové 500 05, Czech Republic

[6] Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina Ch-2, 84215 Bratislava, Slovakia

* Authors to whom correspondence should be addressed.

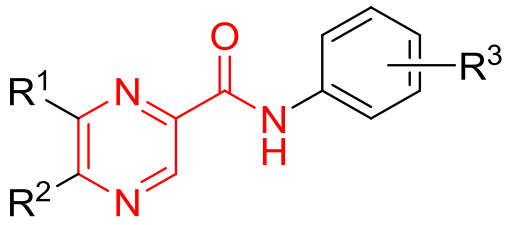
Abstract: A series of sixteen pyrazinamide analogues with the -CONH- linker connecting the pyrazine and benzene rings was synthesized by the condensation of chlorides of substituted pyrazinecarboxylic acids with ring-substituted (chlorine) anilines and characterized. The results of *in vitro* antimycobacterial screening indicated some interesting antimycobacterial activity. 6-Chloro-*N*-(4-chlorophenyl)pyrazine-2-carboxamide (**6**) has shown the highest activity against *Mycobacterium tuberculosis* strain H37Rv (65% inhibition at 6.25 μg mL^{-1}). The highest antifungal effect against *Trichophyton mentagrophytes*, the most susceptible fungal strain tested, was found for 6-chloro-5-*tert*-butyl-*N*-(3,4-dichlorophenyl)pyrazine-2-carboxamide (**16**) (MIC = 62.5 μmol mL^{-1}). 6-Chloro-5-*tert*-butyl-*N*-(4-chlorophenyl)pyrazine-2-carboxamide (**8**) was the most active in the inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts (IC_{50} = 43.0 μmol mL^{-1}). The compounds were analyzed using RP-HPLC to determine lipophilicity. Optimal log *P* value for studied series was not confirmed. For all the compounds, the relationships between the lipophilicity and the chemical structure of the studied compounds are discussed, as well as their structure-activity relationships (SAR).

Keywords: Pyrazinecarboxamides; Lipophilicity; *In vitro* antimycobacterial activity; *In vitro* antifungal activity; Spinach chloroplasts; PET inhibition; Structure-activity relationships.

INTRODUCTION

Compounds possessing -NHCO- moiety simulating a peptide bond in their molecule show a broad range of biological effects. Pyrazinamide with its simple structure gives a good opportunity for further modification regarding an increase of its antimycobacterial activity. All compounds were assayed *in vitro* against major Mycobacterium and various Fungi species [1-6]. Some compounds were found to exhibit photosynthesis-inhibiting activity [2,5,7,8]. Various *N*-substituted amides of pyrazinecarboxylic acid were prepared and evaluated as potential abiotic elicitors [9-12].

This is a follow-up paper to our previous articles [1-12] dealing with synthesis and biological activities of ring-substituted pyrazine derivatives. Primary *in vitro* screening of the synthesized compounds was performed against four mycobacterial strains and eight fungal strains. The compounds were also tested for their photosynthesis-inhibiting activity (the inhibition of photosynthetic electron transport in spinach chloroplasts (*Spinacia oleracea* L.). This paper describes preparation, biological evaluation and structure-activity relationship studies in series of pyrazinamide analogues. We synthesized in preference the compounds with the lipophilic and/or electron-withdrawing substituents on the benzene moiety (R^3, chlorine), and the compounds with the substitution on the pyrazine nucleus with R^1 (hydrogen, chlorine) and/or R^2 (hydrogen, *tert*-butyl) moiety (see Scheme 1).



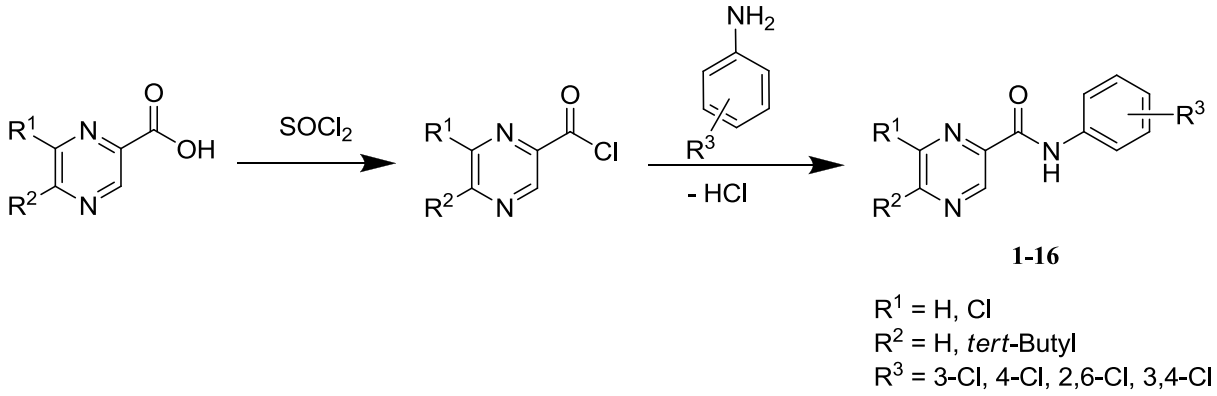
Scheme 1. Pyrazinamide (red colour) structure modification (black colour)

Lipophilicity is one of the most important physical properties of biologically active compounds. It influences the transport of a molecule through cellular membranes, because drugs cross biological barriers most frequently through passive transport, which strongly depends on their lipophilicity. Lipophilicity also characterizes drug solubility [13]. Lipophilicity of pyrazinamide is quite low (Log *P* = -1,31/CLogP = -0.67632), therefore in an effort to increase it we have chosen the hydrophobic electron-withdrawing (chlorine), and bulky substitutents on the pyrazine (*tert*-butyl), and the combination of substituents (chlorine) on the benzene part.

The aim of this work is to find the structure-activity relationship (SAR) in the mentioned series, *i.e.* to continue in the study of the substituent variability influence on the biological activity, and to determine the importance of increased lipophilic properties for biological effect of the newly prepared substituted pyrazinecarboxamides.

RESULTS AND DISCUSSION

The final compounds **1-16** were prepared by the anilinolysis of substituted pyrazinoylchlorides (see Scheme 2) in good yield. [2,3,5]



Scheme 2. Synthetic pathway and general formula of prepared amides **1-16**.

All compounds were assayed *in vitro* against *Mycobacterium tuberculosis* H37Rv. In the tuberculosis antimicrobial acquisition and coordinating facility (TAACF) program [14] the compounds >90% inhibition in this primary screen (*i.e.* MIC >6.25 mg/mL) were further evaluated to determine their actual minimum inhibitory concentration (MIC) in the MABA. Finally, no tested derivative overcame this limit (see Table 1).

The evaluation of in vitro antifungal activity of the synthesized compounds was performed against eight fungal strains. The results showed no interesting activity against the majority of fungal strains tested.

Over 50% of commercially available herbicides act by reversibly binding to photosystem II (PS II), a membrane-protein complex in the thylakoid membranes which catalyses the oxidation of water and the reduction of plastoquinone [15] and thereby inhibit photosynthesis [16-18]. Some organic compounds, e.g. substituted anilides of 2,6-disubstituted pyridine-4-thiocarboxamides [19] or pyrazinecarboxylic acids [5] were found to interact with tyrosine radicals Tyr_Z and Tyr_D which are situated in D_1 and D_2 proteins on the donor side of PS II and due to this interaction interruption of the photosynthetic electron transport occurred.

Lipophilicity (log *k*) of the compounds was determined using RP-HPLC. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP column. Sixteen substituted *N*-phenylpyrazine-2-carboxamides were analysed using the RP-HPLC method for the lipophilicity measurement. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped non-polar C_{18} stationary RP column. In the present study the correlation between the RP-HPLC retention parameter log *k* and log *P* data calculated in various ways is shown, and the relationships between the lipophilicity and the chemical structure of the studied compounds are discussed. The distributive parameters π of various substituents are listed for the mentioned studied compounds. The determined π parameters of substituents can be used for describing

relationships between physico-chemical properties and biological activity of prepared ring-substituted pyrazine-based compounds. Although all the discussed compounds are relatively simple structures substituted within the series only by chlorine, interesting intramolecular interactions influencing lipophilicity were observed probably due to the presence of a pyrazine ring and a carboxamide moiety. Hydrophobicities (log *P*/Clog *P*) of the compounds **1**-**16** were calculated using two commercially available programs (ChemDraw Ultra 10.0 and ACD/LogP) and also measured by means of the RP-HPLC determination of capacity factors *k* with subsequent calculation of log *k*. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped non-polar C_{18} stationary RP column. The results are shown in Table 1. The program ChemOffice/log *P* does not resolve $C_{(2)}$–$C_{(4)}$ substitution in the benzene part of the molecule. Compound **1** shows the lowest lipophilicity, whereas compound **16** possesses the highest lipophilicity. The calculated log *P* data and the determined log *k* parameters correspond to the expected lipophilicity increasing within individual series of compounds (pyrazine < 6-chloropyrazine < 5-*tert*-butylpyrazine < 6-chloro-5-*tert*-butylpyrazine derivatives). This dependence is approximately linear. Hydrophobicity increases according to substitution in anilide part of the molecule this way: 3-Cl < 4-Cl < 2,6-Cl < 3,4-Cl. Anilides substituted by 3-Cl or 4-Cl moiety show similar log *k*. Distributive parameter π describes lipophilicity contribution of individual moieties substituted in some skeleton. Distributive constants π of individual substituents are dependent on the basic skeleton (aliphatic, aromatic, heteroaromatic), as well as on the character of the heteroaromatic system. A number of distributive parameters π for various substituents for all three substituent positions in the benzene ring have been described [20,21]. The determined π parameters of substituents can be used for describing relationships between the physico-chemical properties and activity of prepared ring substituted pyrazine based compounds. Due to similarity of the determined π phenyl parameters for compounds **1**-**4** (3-Cl) and **5**-**8** (4-Cl) it can be predicted that these individual/independent positions/substitutions do not show any intramolecular interactions between chlorine and the pyrazine core or carboxamide moiety contrary to disubstituted compounds **13**-**16** (3,4-Cl), where both chlorine atoms interact with each other. Results from Table 1 show quite different behaviour of both chlorine atoms in **9**-**12** (2,6-Cl).

Table 1. Structure of pyrazinecarboxamides **1-16**, their measured log *k* values, calculated lipophilicities (log *P*/Clog *P*), the determined distributive parameters π [20,21], the results of biological screening in comparison with the standards.

Chemical structure/hydro-lipophilicity parameters									Biological data		
Comp.	**R[1]**	**R[2]**	**R[3]**	**log *k***	**log *P*/Clog *P*** ChemOffice	**log *P*** ACD/LogP	$\pi_{determined}$ Ph/Pyr	π Ph/Pyr	Antimycobacterial evaluation Inhibition [%]	Antifungal susceptibility MIC [μmol/mL]	PET inhibition in spinach chloroplasts IC_{50} [μmol/mL]
1	H	H	3-Cl	0.4914	1.15/2.2090	2.17±0.41	0.08/0.00	0.77/0	14	500/>500	290.1
2	Cl	H	3-Cl	0.7864	2.05/2.9347	3.29±0.42	0.10/0.30	0.77/0.77	14	125/125	262.0
3	H	$(CH_3)_3C$	3-Cl	1.0996	3.28/4.0350	3.85±0.41	0.26/0.61	0.77/1.88	0	>250/>250	**47.0**
4	Cl	$(CH_3)_3C$	3-Cl	1.4896	4.18/4.7607	4.98±0.43	0.25/1.00	0.77/2.65	0	>250/>250	103.0
5	H	H	4-Cl	0.4987	1.15/2.2090	2.13±0.41	0.09/0.00	0.73/0	4	>250	b
6	Cl	H	4-Cl	0.8185	2.05/2.9347	3.25±0.42	0.13/0.32	0.73/0.77	**65**	>500	486.0
7	H	$(CH_3)_3C$	4-Cl	1.1043	3.28/4.0350	3.81±0.41	0.16/0.61	0.73/1.88	0	>250	1502.0
8	Cl	$(CH_3)_3C$	4-Cl	1.5015	4.18/4.7607	4.91±0.43	0.26/1.00	0.73/2.65	24	>250	**43.0**
9	H	H	2,6-Cl	0.6656	1.71/1.2331	2.17±0.41	0.25/0.00	1.52/0	0	>500	b
10	Cl	H	2,6-Cl	0.9696	2.61/1.9531	3.29±0.43	0.28/0.30	1.52/0.77	0	>250	925.0
11	H	$(CH_3)_3C$	2,6-Cl	1.2802	3.83/3.0591	3.85±0.42	0.34/0.61	1.52/1.88	0	250/250	153.0
12	Cl	$(CH_3)_3C$	2,6-Cl	1.6631	4.73/3.7791	4.97±0.44	0.42/1.00	1.52/2.65	0	125/125	61.0
13	H	H	3,4-Cl	0.7162	1.71/2.8131	3.03±0.42	0.30/0.00	1.50/0	8	>250	b
14	Cl	H	3,4-Cl	0.9950	2.61/3.5331	4.15±0.44	0.31/0.28	1.50/0.77	**61**	125/250	104.8
15	H	$(CH_3)_3C$	3,4-Cl	1.3395	3.83/4.6391	4.72±0.43	0.40/0.62	1.50/1.88	15	125/125	1525.1
16	Cl	$(CH_3)_3C$	3,4-Cl	1.7563	4.73/5.3592	5.84±0.45	0.51/1.04	1.50/2.65	0	**62.5/62.5**	130.1
standards		pyrazinamid		-	-1,31/-0.67632	-	-	-	a	-	-
		fluconazol		-	0,99/-0.44	-	-	-	-	1.95/3.91	-
		atrazine		-	2,57/2.70318	-	-	-	-	-	1.0

a MIC = 12.5 mg/mL [22].

b Not tested due to their low solubility in DMSO.

Only two compounds **6** and **14** possess some interesting antimycobacterial activity (see Table 1 and Scheme 3), from the point of view of SAR it can be drawn the positive effect of chlorine atom for both pyrazine and both benzene ring, and the negative influence of alkyl introduction on the pyrazine nucleus. 6-Chloro-*N*-(4-chlorophenyl)pyrazine-2-carboxamide (**6**) exerted the activity against *Mycobacterium tuberculosis* strain H37Rv 65% inhibition at 6.25 μg mL^{-1}, 6-chloro-*N*-(3,4-dichlorophenyl)pyrazine-2-carboxamide (**14**) manifested 61%. However such low-active compounds (**6**,**14**) should not be ignored; their chemical analogues, and alterations in physico-chemical properties may confer some positive changes in biological effects. Therefore synthesis and evaluation of other derivatives is necessary to round out the structure-activity data in series of substituted *N*-phenylpyrazine-2-carboxamides.

The more lipophilic compound (log $P < 5$) with chlorine atoms in position 3 and 4 on the benzene part of molecule possess some weak antifungal activity, 6-chloro-5-*tert*-butyl-*N*-(3,4-dichlorophenyl)pyrazine-2-carboxamide (**16**) exhibited effect (MIC = 62.5 μmol mL^{-1}) against *Trichophyton mentagrophytes*, the most susceptible fungal strain tested. This activity is the only modest one in comparison with fluconazole, the standard (MIC = 3.91 mmol/mL after 120 h for fluconazole, see Table 1). The negative results of antifungal screening do not allow us to draw detailed conclusions on any structure-activity relationships.

Also introducing of chlorine atoms into aromatic ring as well as pyrazine moiety of the studied molecules enhanced the effectiveness of photosynthesis—inhibiting activity. The most effective inhibitor from the series were 6-chloro-5-*tert*-butyl-*N*-(4-chlorophenyl)pyrazine-2-carboxamide (**8,** IC_{50} = 43 μmol/mL) and 5-*tert*-butyl-*N*-(3-chlorophenyl)-pyrazine-2-carboxamide (**3,** IC_{50} = 47 μmol/mL), measured on photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts (see Table 1).

In general, the negative results of biological screening do not allow us to draw detailed conclusions on some structure–activity relationships.

GENERAL EXPERIMENTAL PROCEDURE

Instrumentation and chemicals

All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled under argon atmosphere. Melting points were determined using a Melting Point Apparatus SMP 3 (BIBBY Stuart Scientific, UK) and are uncorrected. The reactions were monitored and the purity of the products was checked by TLC (Merck UV 254 TLC plates, Darmstadt, Germany) using developing solvents petroleum ether / EtOAc (9 : 1). Purification of compounds was made using Flash Master Personal chromatography system from Argonaut Chromatography (Argonaut Technologies, Redwood City, CA, USA). As sorbent, Merck Silica Gel 60 (0.040–0.063 mm) was used (Merck). Elemental analyses were performed on an automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). Infrared spectra were recorded in Nicolet Impact 400 spectrometer in KBr

pellets. 1H and 13C NMR Spectra were recorded on a Varian Mercury — Vx BB 300 (300 MHz for 1H and 75 MHz for 13C), Varian (Palo Alto CA, USA) in DMSO-d6 solutions at ambient temperature. The chemical shifts were recorded as δ values in ppm and were indirectly referenced to tetramethylsilane (TMS) *via* the solvent signal (2.49 for ^{1}H and 39.7 for ^{13}C in DMSO-d6).

General procedure for the synthesis of the title compounds

A mixture of acid, *i.e.* pyrazinecarboxylic, 6-chloropyrazine-2-carboxylic [2], 5-*tert*-butylpyrazine-2-carboxylic [3] or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic [3] acid, 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 dropwise to a stirred solution of the corresponding substituted amine (50.0 mmol) and pyridine (50.0 mmol) in 50 mL of dry acetone keeping at the room temperature. After the addition was complete, stirring continued for the next 30 min. Then the reaction mixture was poured into 100 mL of cold water and the crude amide was collected and purified by the column chromatography.

The studied compounds **1-16** are presented in the Table 1. The synthesis, physico-chemical data and analytical parameters of several compounds were described elsewhere, derivatives **1-4** [23], **5-8** [3] and **13-16** [7].

***N*-(2,6-dichlorophenyl)pyrazine-2-carboxamide** (**9**). Yield 66%. Anal. Calcd for $C_{11}H_7Cl_2N_3O$ (268.1): 49.28% C, 2.63% H, 15.67% N; Found: 49.51% C, 2.68% H, 15.21% N. Mp 151.0–152.0 °C. IR (KBr) cm^{-1}: 3377 (NH), 1685 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9,51 (bs, 1H, NH), 9.41 (s, 1H, H3), 8.85 (s, 1H, H5), 8.75 (s, 1H, H6), and 7.10-7.61 (m, 3H, H3′, H4′, H5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8, 147.8, 144.8, 142.7, 134.2, 133.5, 132.4, 129.8, 128.8, 128.5, and 123.5.

6-chloro-*N*-(2,6-dichlorophenyl)pyrazine-2-carboxamide (**10**). Yield 78%. Anal. Calcd for $C_{11}H_6Cl_3N_3O$ (302.6): 43.67% C, 2.00% H, 13.89% N; Found: 43.51% C, 1.98% H, 13.91% N. Mp 178.0–179.2 °C. IR (KBr) cm^{-1}: 3370 (NH), 1690 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.41 (bs, 1H, NH), 9.38 (s, 1H, H3), 8.83 (s, 1H, H5), 7.12-7.52 (m, 3H, H3′, H4′, H5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3, 147.8, 147.4, 143.2, 142.1, 136.1, 132.9, 130.7, 130.6, 128.3, and 121.5.

5-*tert*-butyl-*N*-(2,6-dichlorophenyl)pyrazine-2-carboxamide (**11**). Yield 43%. Anal. Calcd for $C_{15}H_{15}Cl_2N_3O$ (324.2): 55.57% C, 4.66% H, 12. 69% N; Found: 55.63% C, 4.71% H, 13.08% N. Mp 53.5–55.0 °C. IR (KBr) cm^{-1}: 3365 (NH), 1685 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.67 (bs, 1H, NH), 9.37 (d, 1H, *J* = 1.37 Hz, H3), 8.61 (d, 1H, *J* = 1.37 Hz, H6), 7.12-7.48 (m, 3H, H3′, H4′, H5′), and 1.45 (s, 9H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ

168.2, 161.2, 143.2, 143.0, 142.1, 140.7, 139.0, 136.9, 133.0, 130.6, 127.7, 121.3, 118.9, 37.1, and 29.7.

5-*tert*-butyl-6-chloro-*N*-(2,6-dichlorophenyl)pyrazine-2-carboxamide (**12**). Yield 77%. Anal. Calcd for $C_{15}H_{14}Cl_3N_3O$ (358.7): 50.23% C, 3.93% H, 11.72% N; Found: 50.33% C, 3.71% H, 12.08% N. Mp 130.1–131.0 °C. IR (KBr) cm^{-1}: 3390 (NH), 1685 (CO). ^1H NMR (300 MHz, $CDCl_3$) δ 9.38 (bs, 1H, NH), 9.25 (s, 1H, H3), 7,12-7,48 (m, 3H, H3´, H4´, H5´), and 1.55 (s, 9H, CH3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 159.9, 145.8, 143.2, 142.1, 140.5, 140.3, 136.5, 133.0, 130.7, 128.2, 121.6, 119.1, 39.1, and 28.2.

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 the 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, injection 30 μL, column temperature 30 °C and sample temperature 10 °C. The detection wavelength of 210 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes.

The capacity factors *k* were calculated using the Millennium32® Chromatography Manager Software according to the formula $k = (t_R - t_D) / t_D$, where t_R is the retention time of the solute, whereas t_D denotes the dead time obtained via an unretained analyte. Log *k*, calculated from the capacity factor *k*, is used as the lipophilicity index converted to the log *P* scale [14]. The log *k* values of the individual compounds are shown in Table 1.

Lipophilicity calculations

Log *P*, *i.e.* the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programmes CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/LogP ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). Clog *P* values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of the CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, U.S.A.) software. The results are shown in Table 1.

In vitro antimycobacterial evaluation

Antimycobacterial evaluation was carried out in the tuberculosis antimicrobial acquisition and coordinating facility (TAACF), Southern Research Institute, Birmingham, AL, U.S.A., which is a part of the National Institutes of Health (NIH). Primary screening of all compounds

was conducted at 6.25 mg/mL against M. tuberculosis H37Rv (ATCC27294) in BACTEC 12B medium using both BACTEC 460 radiometric system and the Microplate Almar Blue Assay (MABA). Compounds demonstrating at least 90% inhibition in the primary screen were tested at lower concentration against M. tuberculosis H37Rv to determine the MIC testing by MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. [14,24] For the results see Table 1.

In vitro antifungal evaluation

Dept. of Medical and Biological Sciences at the Faculty of Pharmacy, Charles University performs antifungal susceptibility assays. Used method is microdilution panel bouillon method with RPMI medium with glutamine as a growth medium. Incubation takes 28 – 48 hours at atmospheric pressure, temperature 35 °C and darkness [25]. Tested strains: *Candida albicans* ATCC 44859, *C. tropicalis* 156, *C. krusei* E28, *C. glabrata* 20/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigantus* 231 and *Absidia corymbifera* 272. For the results of the most sensitive fungal strain *T. mentagrophytes*, see Table 1.

Study of inhibition photosynthetic electron transport (PET) in spinach chloroplasts

Chloroplasts were prepared from spinach (*Spinacia oleracea* L.) according to Masarovičová and Kráľová [26]. The inhibition of oxygen evolution rate (inhibition of photosynthetic electron transport, PET) in spinach chloroplasts was determined spectrophotometrically (Genesys 6, Thermo Scientific, U.S.A.) using the artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kráľová et al. [27] 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, pH 7.2) containing sucrose (0.4 mol/L), $MgCl_2$ (0.005 mol/L) and NaCl (0.015 mol/L). 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. The inhibitory efficiency of the studied compounds was expressed by IC_{50} values, *i.e.* by molar concentration of the compounds causing 50% decrease in the oxygen evolution rate relative to the untreated control. The comparable IC_{50} value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (Diurone®) was about 1.9 μmol/L [28]. The results are summarized in Table 2.

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