Short communication

Synthesis and antimycobacterial evaluation of substituted pyrazinecarboxamides

Martin Dolezal [a,*], Pavlina Cmedlova [a], Lukas Palek [a], Jarmila Vinsova [a], Jiri Kunes [a], Vladimir Buchta [b], Josef Jampilek [c], Katarina Kralova [d]

[a] *Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic*
[b] *Department of Clinical Microbiology, Faculty of Medicine and University Hospital, Charles University in Prague, Sokolska 581,*
500 05 Hradec Kralove, Czech Republic
[c] *Zentiva a.s., U Kabelovny 130, 102 37 Prague 10, Czech Republic*
[d] *Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynska Dolina, 842 15 Bratislava, Slovak Republic*

Abstract

Unsubstituted, halogenated and/or alkylated pyrazine-2-carboxylic acid amides connected *via* −CONH− bridge with substituted anilines were synthesized using currently known synthetic pathways. The synthetic approach, analytical, spectroscopic, lipophilicity and biological data of 20 newly synthesized compounds are presented. Structure−activity relationships among the chemical structures, the antimycobacterial, antifungal, photosynthesis inhibiting and antialgal activity of the evaluated substituted *N*-phenylpyrazine-2-carboxamides are discussed. 5-*tert*-Butyl-6-chloro-*N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**19**) has shown the highest activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MIC = 3.13 µg/mL). The highest antifungal effect against *Trichophyton mentagrophytes*, the most susceptible fungal strain tested, was found for *N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**14**, MIC = 62.5 µmol/mL). The highest reduction of chlorophyll content in *Chlorella vulgaris* was found for pyrazine-2-carboxylic acid (3-trifluoromethylphenyl)amide (**9**, IC_{50} = 12.1 µmol/L).
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1. Introduction

Tuberculosis is still a major global disease infecting one third of the world's population and killing almost two million people each year. Multidrug-resistant strains of *Mycobacterium tuberculosis* in conjunction with the spread of HIV infection, often lead to the treatment failure. Thus, much effort is being made to develop novel anti-tuberculosis drugs which are more safe and efficacious [1]. There are two basic strategies for antimycobacterial drug development. The first is the extraordinary molecular diversity. The second results from original compounds made more accessible by the design of new, or the modernisation of, synthetic transformations. The evaluation of pyrazinoic acid, the pharmacologically active principle of first line anti-tuberculosis drug pyrazinamide, is intensively focused on. The minimal structure of pyrazine ring with an acyl moiety is sufficient for fatty acid synthase type I (FAS I) and antimycobacterial activity of some pyrazinecarboxylic acid derivatives [2].

In connection with our research focused on antimycobacterial active pyrazinecarboxylic acid derivatives we are interested in binuclear analogues with the −CONH− bridge [3−5]. The amide function, as considered in this work, is based on the bivalent moiety −CONH−, which can form centrosymmetric dimer pairs with the peptidic carboxamido group of some peptides, needed for binding to the receptor

* Corresponding author. Tel.: +420495067389; fax: +420495067167.
 E-mail address: dolezalm@faf.cuni.cz (M. Dolezal).

site, possibly by forming a hydrogen bond. Various compounds possessing −CONH− group have been found to inhibit photosynthetic electron transport [6–9]. Previous studies [4,5,10] showed that alkylation, amidation or aroylation of the pyrazine ring or substitution of pyrazine with chlorine can increase the antituberculotic and/or antifungal activity in series of functional derivatives of pyrazinecarboxylic acid [11]. Some of these derivatives influenced production of flavonolignans in an *in vitro* culture [12].

This study is continuation of our previous articles dealing with the *N*-heterocyclic derivatives as potential drugs [3–5,10–14] and is concerned with the synthesis of a series of amides prepared from pyrazinecarboxylic acids and various ring-substituted anilines. The aim of this work is to find the structure–activity relationship in the mentioned series, *i.e.* to continue the study 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.

2. Result and discussion

2.1. Chemistry

The synthesis of the amides is shown in Scheme 1. Condensation of chlorides of pyrazine-2-carboxylic acid [15], 6-chloropyrazine-2-carboxylic acid [16], 5-*tert*-butylpyrazine-2-carboxylic acid [4] or 6-chloro-5-*tert*-butylpyrazine-2-carboxylic [4] acid with ring-substituted anilines yielded a series of the substituted *N*-phenylpyrazine-2-carboxamides **1–20**.

2.2. Lipophilicity

Hydrophobicity parameters of anilides **1–20** were calculated (log P values) and measured by means of RP-HPLC determination of capacity factor K and subsequently log K was evaluated. The values of calculated lipophilicity (log P) of compounds ranged from 1.60 to 5.30. The increasing lipophilicity of the substituents on the pyrazine ring (H < 6-Cl < 5-*tert*-butyl < 6-Cl, 5-*tert*-butyl) affects the resulting lipophilicity of the compounds within individual series. log K values evaluated from the capacity factor K specify lipophilicity within these individual series of compounds.

Experimentally determined lipophilicity values (log K values) were lower than the corresponding calculated log P values. The linear dependence of log K *vs.* log P is shown in Fig. 1. The statistical parameters of this correlation ($r = 0.969$; $P < 0.0001$; $n = 20$) confirmed accordance between experimental log K values with the corresponding calculated log P values. The lowest lipophilicity was shown by *N*-(4-fluorophenyl)pyrazine-2-carboxamide (**2**), whereas 5-*tert*-butyl-6-chloro-*N*-(2-trifluoromethylphenyl)pyrazine-2-carboxamide (**18**) possessed the highest lipophilicity.

Based on the log K data, the lipophilicity of the substituents in the aromatic part of the molecule increased in the following order: 4-F < 2-Cl-5-OH < 4-CH$_3$ < 3-CF$_3$ < 2-CF$_3$. This is in accordance with the values of corresponding π-parameters characterizing hydrophobicity of the substituents, namely 0.15 for F, 0.76 for 2-Cl, −0.50 for 5-OH, 0.60 for 4-CH$_3$ and 1.1 for 3-CF$_3$ [17]. For 2-CF$_3$ the value of the π-parameter is not published, from the comparison of π$^-$ values of 2-CF$_3$ (1.34) and 3-CF$_3$ (1.49) the lower lipophilicity of 2-CF$_3$ is evident.

The differences between the experimental (log K) and the calculated lipophilicity parameters (log P) were observed for 3-CF$_3$ and 2-CF$_3$ substituents. This could be connected with the fact that the calculating program for log P does not distinguish between different lipophilicities of substituents in *ortho*, *meta* and *para* positions. Anilides substituted by the 2-CF$_3$ moiety showed higher hydrophobicity than 3-CF$_3$ substituted compounds, probably due to the intramolecular interactions of the substituent in the C$_{(2)}'$ position of the anilide part of the molecule with the carboxamide (−CONH−) bridge in the individual compounds.

It can be assumed that capacity factor K/calculated log K values specify lipophilicity within the individual series of compounds.

2.3. Biological activity

2.3.1. In vitro antimycobacterial activities

All compounds prepared were evaluated on their *in vitro* antimycobacterial susceptibility. Some interesting results were obtained, as shown in Tables 1 and 2. The highest activity against *M. tuberculosis* H$_{37}$Rv was found for *N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**4**, 99% inhibition) and for 6-chloro-5-*tert*-butyl-*N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**19**, 95% inhibition). Other derivatives

R^1 = H, *tert*-butyl
R^2 = 4-CH$_3$, 3-CF$_3$, 2-CF$_3$, 4-F, 2-Cl-5-OH
X = H, Cl
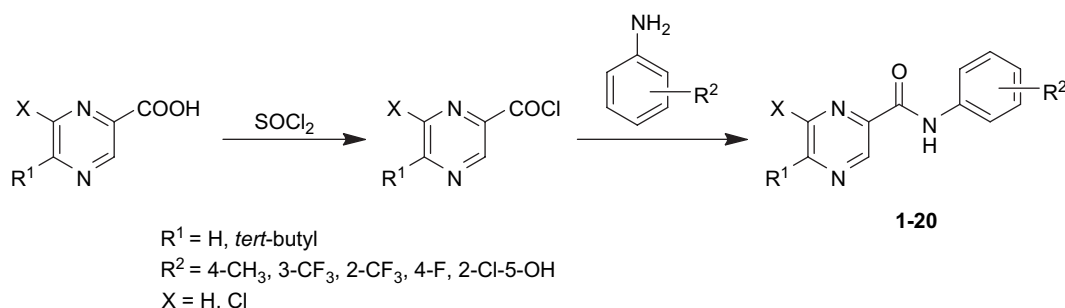
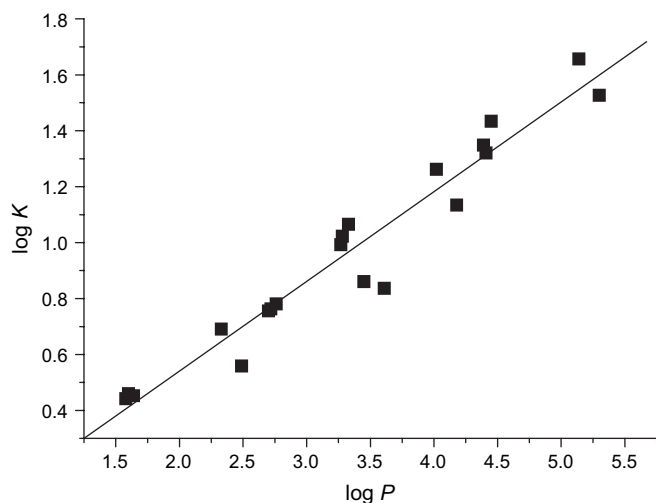Scheme 1. Synthesis of the discussed substituted pyrazine-2-carboxamides **1–20**.

Fig. 1. Dependence of experimentally determined log K values on calculated log P values of compounds **1–20**.

showing lower activities were N-p-tolylpyrazine-2-carboxamide (**5**, 86%), 6-chloro-N-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**9**, 77%), and 6-chloro-N-p-tolylpyrazine-2-carboxamide (**10**, 71%) (see Table 1).

In the tuberculosis antimicrobial acquisition and coordinating facility (TAACF) program compounds **4** and **19** effecting

>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. Simultaneously, the compounds were tested for their cytotoxicity, i.e. the determination of their 50% inhibitory concentrations (IC_{50}) in VERO cells. At the conclusion of secondary screening, the MIC and IC_{50} values are formed into a ratio termed selectivity index (SI). The SI is defined as the ratio of the measured IC_{50}, in VERO cells, to the MIC. Compound **19** showed a good MIC, but poor selectivity for mycobacteria (SI = >3.2). Finally, N-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**4**) with an MIC ≤ 6.25 μg/mL and an SI level of 10 or greater can be considered as an active compound at the second level of antimycobacterial evaluation (see Table 2) [18,19].

In general, it can be concluded that with the exception of compounds **1**, **2** and **19** the increase of compound lipophilicity resulted in decreased antimycobacterial activity. On the other hand it is evident that the biological activity does not depend exclusively on the compound lipophilicity but it is probably affected also by electron accepting or withdrawing power of the substituents on the benzene ring. A large positive Hammett constant (σ-value) implies high electron-withdrawing power by inductive and/or resonance effect, relative to H; a large negative σ-value implies high electron-releasing power relative to H (the corresponding σ-values are 0.062 for 4-F, 0.430 for

Table 1

Antimycobaterial evaluation (% of inhibition), antifungal susceptibility (MIC), OER inhibition in spinach chloroplasts (IC_{50}), and reduction of chlorophyll content in C. vulgaris (IC_{50}) of compounds **1–20** in comparison with the standards: pyrazinamide (PZA), fluconazole (FLU) and diurone (DCMU)

Comp.	Structure			Antimycobacterial evaluation (% inhibition at 6.25 μg/mL)	Antifungal susceptibility (MIC[b], μmol/mL)	OER inhibition (IC_{50}, μmol/L)	Chlorophyll reduction	
	X	R^1	R^2				IC_{50} (μmol/L)	% of Control ± S.D.[d]
1	H	H	2-Cl-5-OH	0	>250/>250	722	–	97.1 ± 10.6
2	H	H	4-F	0	>500/>500	480	–	89.9 ± 5.7
3	H	H	2-CF$_3$	31	>250/>250	376	–	91.7 ± 4.9
4	H	H	3-CF$_3$	99	>500/>500	130	–	91.5 ± 2.7
5	H	H	4-CH$_3$	86	>500/>500	1475	70.9	–
6	Cl	H	2-Cl-5-OH	15	>250/>250	624	–	103.0 ± 8.3
7	Cl	H	4-F	44	500/>500	384	32.3	–
8	Cl	H	2-CF$_3$	26	500/>500	557	–	87.8 ± 4.3
9	Cl	H	3-CF$_3$	77	125/125	229	12.1	–
10	Cl	H	4-CH$_3$	71	250/250	1524	37.4	–
11	H	(CH$_3$)$_3$C	2-Cl-5-OH	0	>125/>125	[c]	–	122 ± 9.8
12	H	(CH$_3$)$_3$C	4-F	25	500/>500	524	–	94.5 ± 4.6
13	H	(CH$_3$)$_3$C	2-CF$_3$	7	250/250	55	32.9	–
14	H	(CH$_3$)$_3$C	3-CF$_3$	23	62.5/62.5	283	–	85.2 ± 5.1
15	H	(CH$_3$)$_3$C	4-CH$_3$	61	125/125	164	–	91.8 ± 2.2
16	Cl	(CH$_3$)$_3$C	2-Cl-5-OH	0	>125/>125	625	–	98.0 ± 6.7
17	Cl	(CH$_3$)$_3$C	4-F	15	>500/>500	103	–	101.6 ± 9.0
18	Cl	(CH$_3$)$_3$C	2-CF$_3$	6	250/250	205	–	94.4 ± 2.0
19	Cl	(CH$_3$)$_3$C	3-CF$_3$	95	250/250	173	–	90.1 ± 2.0
20	Cl	(CH$_3$)$_3$C	4-CH$_3$	0	>500/>500	73	–	92.9 ± 2.4
PZA	–	–	–	[a]	–	–	–	–
FLU	–	–	–	–	1.95/3.91	–	–	–
DCMU	–	–	–	–	–	1.9	7.3	–

[a] MIC = 12.5 μg/mL [10].

[b] Against T. mentagrophytes after 72 h/120 h.

[c] Not tested due to their low solubility in DMSO.

[d] IC_{50} was determined only for five compounds, for the other 15 compounds an average of Chl content decrease in the concentration range 0.83–100 μmol/L was determined.

Table 2
Primary antimycobaterial evaluation (% of inhibition), actual minimum inhibitory concentration (MIC), cytotoxicity (IC_{50}) and selectivity index (SI) of compounds **4** and **19**

Comp.	Structure			% Inhibition at 6.25 µg/mL	MIC (µg/mL)	IC_{50} (µg/mL)	SI
	X	R^1	R^2				
4	H	H	3-CF_3	99	6.25	>62.5	>10
19	Cl	$(CH_3)_3C$	3-CF_3	95	3.13	>10	>3.2

3-CF_3, and −0.170 for 4-CH_3) [20]. However, Hammett constants reflecting electron accepting or withdrawing power of *ortho* substituents are not used because steric effects can interfere with the purely electronic effects. Relatively low lipophilicity is typical also for some drugs with antimycobacterial activity, *e.g.* ethionamide (log $P = 1.22 \pm 0.29$), isoniazide (log $P = -0.89 \pm 0.24$), and pyrazinamide (log $P = -0.37 \pm 0.35$).

2.3.2. In vitro antifungal activities

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. Only compound 5-*tert*-butyl-*N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**14**) exhibited some moderate *in vitro* antifungal activity against *Trichophyton mentagrophytes*, the most susceptible fungal strain evaluated (MIC = 62.5 µmol/mL). This activity is the only modest one in comparison with fluconazole, the standard (MIC = 3.91 µmol/mL after 120 h, see Table 1). The negative results of antifungal screening do not allow us to draw detailed conclusions on any structure−activity relationships.

2.3.3. In vitro herbicidal activities

The majority of all the compounds studied inhibited photosynthetic electron transport in spinach chloroplasts (see Table 2). Based on the obtained results it could be concluded that the activity of investigated compounds related to inhibition of oxygen evolution rate (OER) depends on lipophilicity and also on the electron accepting or withdrawing power of the substituents. However, the values of their inhibitory activities were rather low. At higher applied concentrations the solubility of some compounds in the suspensions of spinach chloroplasts was limited. The most effective inhibitor was compound **13** (5-*tert*-butyl-*N*-(2-trifluoromethylphenyl)pyrazine-2-carboxamide, $IC_{50} = 55$ µmol/L). Among the three most active compounds **13**, **20**, and **17** the optimal values of lipophilicity ranged from log $P = 4.02-4.41$. On the other hand, for the group of compounds **16**, **19**, and **18** with the highest lipophilicity the OER-inhibiting activity showed a decrease with increasing compound lipophilicity. The log P values of efficient inhibitors of photosynthetic electron transport with CONH group in their molecule are 2.78 ± 0.38 (diurone) and 3.15 ± 0.57 (linurone), corresponding with literature [21]. Some of the compounds under study reduced the chlorophyll content in *Chlorella vulgaris*. The IC_{50} values related to reduction of chlorophyll content in *C. vulgaris* could only be determined for five compounds (**5**, **7**, **9**, **10**, and **13**) and the highest effect was found for 6-chloro-*N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**9**, $IC_{50} = 12.1$ µmol/L).

The inhibitory activity of compounds **3**, **6** and **5** increased linearly with increasing lipophilicity of the compound (log $P = 1.60$, 2.72 and 3.61, respectively). However, further lipophilicity increases (compound **7**, log $P = 4.02$) led to reduced activity. The inhibition of chlorophyll content by other investigated compounds (**1**, **2**, **4**, and **8−12**) was low in the whole studied concentration range (0.83−100 µmol/L), and for the majority of these compounds did not exceed 10% (*i.e.* 90% of the control). The results are shown in Table 1.

3. Conclusion

The highest activity against *M. tuberculosis* H_{37}Rv was found for *N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**4**, MIC = 6.25 µg/mL) and for 6-chloro-5-*tert*-butyl-*N*-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (**19**, MIC = 3.13 µg/mL). In the TAACF program compounds effecting <90% inhibition in this primary screen (MIC > 6.25 µg/mL) are not generally evaluated further. On other side, inactive compounds may still have significant inhibitory activity, and this data should not be ignored; analogues, derivatives, and alterations in physical properties may confer drastic changes in biological effects. Therefore, synthesis and evaluation of other pyrazinecarboxylic acid derivatives are necessary to broaden the structure−activity data.

The more lipophilic substituents (CF_3 and CH_3) in positions 3 and 4 on the benzene part of molecule possess some positive influence on biological activity of the compounds studied. Based on the obtained results it could be assumed that the biological activity of the studied pyrazinecarboxamides did not depend exclusively on the compound lipophilicity but it was also affected by electron accepting or withdrawing power of the substituents on the benzene ring. In general it can be concluded that the most effective antimycobacterial compounds possess lower lipophilicity than the most active inhibitors of photosynthetic electron transport.

4. Experimental

4.1. Chemistry

4.1.1. Instrumentation and chemicals

All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled under argon atmosphere. The reactions were monitored and the purity of the products was checked by TLC (Silufol UV 254, Kavalier Votice, Czech Republic) using developing solvents petroleum ether/EtOAc (9:1). The plates were visualized using UV light (254 nm). Melting points (uncorrected) were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany). Elemental analyses were performed on an automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). UV spectra (λ, nm) were determined on a Waters Photodiode Array Detector 2996 (Waters Corp.,

Milford, MA, U.S.A.) in *ca.* 9×10^{-4} mol methanolic solution and log ε (the logarithm of molar absorption coefficient ε) was calculated for the absolute maximum λ_{max} of individual target compounds. Infrared spectra were recorded in Nicolet Impact 400 spectrometer in KBr pellets. ^{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) and Varian (Palo Alto CA, U.S.A.) in CDCl$_3$ or DMSO-d_6 solutions at ambient temperature. The chemical shifts δ are given in ppm related to tetramethylsilane (TMS) as internal standard. The coupling constants (J) are reported in Hz.

The purity of the final compounds was checked by HPLC, see Section 2.2. The detection wavelength 210 nm was chosen. Peaks in the chromatogram of the solvent (blank) were deducted from peaks in the chromatogram of the sample solution. The purity of the individual compounds was determined from area peaks in the chromatogram of the sample solution.

4.1.2. General procedure for pyrazinecarboxamide synthesis

A mixture of acid, *i.e.* pyrazine-2-carboxylic [15], 6-chloropyrazine-2-carboxylic [16], 5-*tert*-butylpyrazine-2-carboxylic [3] or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic [3] acids (50.0 mmol) and thionyl chloride (5.5 mL, 75.0 mmol) in dry toluene (20 mL) was refluxed for about 1 h. The 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 kept at room temperature. After the addition was complete, stirring was 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.

4.1.3. Data of prepared target compounds

4.1.3.1. N-(2-Chloro-5-hydroxyphenyl)pyrazine-2-carboxamide (1). Yield 47%. Anal. Calcd for $C_{11}H_8ClN_3O_2$ (249.5): 52.92% C, 3.23% H, 16.83% N. Found: 49.31% C, 3.85% H, 16.72% N. Mp 223–224 °C. HPLC purity 99.29%. log K: 0.4527. log P: 1.64 ± 0.41. TLC: $R_F = 0.78$. UV (nm) λ_{max}/log ε: 232.3/3.38. IR (KBr) cm^{-1}: 3348 (N–H), 1671 (C=O), 1593 (phenyl), 1529 (N–H), 1402, 1321, 1023 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.27 (br s, 1H, NH), 9.93 (br s, 1H, OH), 9.32 (d, 1H, $J = 1.40$ Hz, H3), 8.97 (d, 1H, $J = 2.50$ Hz, H6), 8.83–8.81 (m, 1H, H5), 7.87 (d, 1H, $J = 2.70$ Hz, H6′), 7.32 (d, 1H, $J = 8.80$ Hz, H3′), 6.61 (dd, 1H, $J = 8.80$ Hz, $J = 2.70$ Hz, H4′). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 160.9, 157.1, 148.7, 144.0, 143.9, 143.6, 134.5, 130.0, 113.5, 113.2, 109.3.

4.1.3.2. N-(4-Fluorophenyl)pyrazine-2-carboxamide (2). Yield 83%. Anal. Calcd for $C_{11}H_8FN_3O$ (217.2): 60.83% C, 3.71% H, 19.35% N. Found: 60.40% C, 4.15% H, 19.45% N. Mp 154–155 °C. HPLC purity 99.21%. log K: 0.4416. log P: 1.58 ± 0.46. TLC: $R_F = 0.78$. UV (nm) λ_{max}/log ε: 221.7/3.48. IR (KBr) cm^{-1}: 3440 (N–H), 1665 (C=O), 1595 (phenyl), 1523 (N–H), 1409, 1318, 1027 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.84 (1H, br s, NH), 9.29 (1H, d, $J = 1.7$ Hz, H3), 8.92 (1H, d, $J = 2.5$ Hz, H6), 8.80 (1H, dd, $J = 2.5$ Hz, $J = 1.4$ Hz, H5), 7.98–7.87 (2H, m, H2′, H6′), and 7.26–7.15 (2H, m, H3′, H5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 161.9, 158.8 (d, $J = 240.8$ Hz), 148.0, 145.2, 144.3, 143.4, 134.8 (d, $J = 2.6$ Hz), 122.7 (d, $J = 7.8$ Hz), and 115.5 (d, $J = 22.0$ Hz).

4.1.3.3. N-(2-Trifluoromethylphenyl)pyrazine-2-carboxamide (3). Yield 71%. Anal. Calcd for $C_{12}H_8F_3N_3O$ (267.2): 53.94% C, 3.02% H, 15.73% N. Found: 54.29% C, 3.05% H, 15.53% N. Mp 123 °C. HPLC purity 99.66%. log K: 0.6911. log P: 2.33 ± 0.44. TLC: $R_F = 0.77$. UV (nm) λ_{max}/log ε: 221.7/3.29. IR (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, br s, 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).

4.1.3.4. N-(3-Trifluoromethylphenyl)pyrazine-2-carboxamide (4). Yield 61%. Anal. Calcd for $C_{12}H_8F_3N_3O$ (267.2): 53.94% C, 3.02% H, 15.73% N. Found: 53.94% C, 2.93% H, 15.62% N. Mp 109 °C. HPLC purity 98.81%. log K: 0.5585. log P: 2.49 ± 0.44. TLC: $R_F = 0.69$. UV (nm) λ_{max}/log ε: 221.7/3.44. IR (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, br s, 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).

4.1.3.5. N-p-tolylpyrazine-2-carboxamide (5). Yield 44%. Anal. Calcd for $C_{12}H_{11}N_3O$ (213.2): 67.59% C, 5.20% H, 19.71% N. Found: 67.34% C, 5.57% H, 19.55% N. Mp 148 °C. HPLC purity 99.56%. log K: 0.4595. log P: 1.60 ± 0.40. TLC: $R_F = 0.70$. UV (nm) λ_{max}/log ε: 218.2/3.47. IR (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, br s, 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.

4.1.3.6. 6-Chloro-N-(2-chloro-5-hydroxyphenyl)pyrazine-2-carboxamide (6). Yield 58%. Anal. Calcd for $C_{11}H_7Cl_2N_3O_2$ (284.1): 46.51% C, 2.48% H, 14.79% N. Found: 46.16% C, 3.42% H, 14.705% N. Mp 255 °C. HPLC purity 98.72%. log K: 0.7801. log P: 2.76 ± 0.43. TLC: $R_F = 0.72$. UV (nm) λ_{max}/log ε: 233.5/3.37. IR (KBr) cm^{-1}: 3365 (N–H), 1700 (C=O), 1593 (phenyl), 1528 (N–H), 1350, 1290, 1108 (pyrazine). ^{1}H NMR (300 MHz, DMSO) δ: 10.10 (1H, br s, NH), 9.93 (1H, br s, OH), 9.26 (1H, s, H3), 9.11 (1H, s, H5), 7.71 (1H, d, $J = 2.8$ Hz, H6′), 7.33 (1H, d, $J = 8.7$ Hz, H3′), and 6.64 (1H, dd, $J = 8.7$ Hz, $J = 2.8$ Hz, H4′). ^{13}C NMR (75 MHz, DMSO) δ: 159.9, 157.1, 148.3, 147.0, 143.9, 142.2, 134.4, 130.0, 114.4, 113.7, and 110.2.

4.1.3.7. 6-Chloro-N-(4-fluorophenyl)pyrazine-2-carboxamide (7). Yield 59%. Anal. Calcd for $C_{11}H_7ClFN_3O$ (251.7): 52.50% C, 2.80% H, 16.70% N. Found: 52.40% C, 3.205% H, 17.07% N. Mp 131–132 °C. HPLC purity 98.03%. log K: 0.7557. log P: 2.70 ± 0.48. TLC: $R_F = 0.82$. UV (nm) λ_{max}/log ε: 229.9/3.49. IR (KBr) cm^{-1}: 3375 (N–H), 1710 (C=O), 1594 (phenyl), 1533 (N–H), 1351, 1302, 1119 (pyrazine). ^{1}H NMR (300 MHz, DMSO) δ: 10.74 (1H, br s, NH), 9.22 (1H, d, $J = 0.6$ Hz, H3), 9.05 (1H, s, H5), 7.93–7.83 (2H, m, H2′, H6′), and 7.27–7.17 (2H, m, H3′, H5′). ^{13}C NMR (75 MHz, DMSO) δ: 160.8, 159.0 (d, $J = 241.0$ Hz), 147.7, 147.1, 145.3, 142.6, 134.5 (d, $J = 2.6$ Hz), 123.0 (d, $J = 8.1$ Hz), and 115.5 (d, $J = 22.3$ Hz).

4.1.3.8. 6-Chloro-N-(2-trifluoromethylphenyl)pyrazine-2-carboxamide (8). Yield 47%. Anal. Calcd for $C_{12}H_7ClF_3N_3O$ (301.7): 47.78% C, 2.34% H, 13.93% N. Found: 47.44% C, 2.31% H, 13.65% N. Mp 148–150 °C. HPLC purity 99.69%. log K: 0.8602. log P: 3.45 ± 0.46. TLC: $R_F = 0.84$. UV (nm) λ_{max}/log ε: 220.5/3.26. IR (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, br s, 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).

4.1.3.9. 6-Chloro-N-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (9). Yield 62%. Anal. Calcd for $C_{12}H_7ClF_3N_3O$ (301.7): 47.78% C, 2.34% H, 13.93% N. Found: 48.09% C, 2.72% H, 13.97% N. Mp 109 °C. HPLC purity 98.71%. log K: 0.8362. log P: 3.61 ± 0.47. TLC: $R_F = 0.80$. UV (nm) λ_{max}/log ε: 219.8/3.35. UV (nm) λ_{max}/log ε: 232.3/3.38. IR (KBr) cm^{-1}: 3351 (N–H), 1680 (C=O), 1603 (phenyl), 1545 (N–H), 1330, 1169, and 1131 (pyrazine). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.52 (1H, br s, NH), 9.40 (1H, s, H3), 8.84 (1H, s, H5), 8.07 (1H, br s, 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).

4.1.3.10. 6-Chloro-N-p-tolylpyrazine-2-carboxamide (10). Yield 59%. Anal. Calcd for $C_{12}H_{10}ClN_3O$ (247.7): 58.19% C, 4.07% H, 16.97% N. Found: 58.11% C, 4.45% H, 16.32% N. Mp 134 °C. HPLC purity 99.39%. log K: 0.7627. log P: 2.72 ± 0.41. TLC: $R_F = 0.81$. UV (nm) λ_{max}/log ε: 222.7/3.37. IR (KBr) cm^{-1}: 3370 (N–H), 1694 (C=O), 1592 (phenyl), 1531 (N–H), 1320, 1168, and 1144 (pyrazine). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.39 (1H, d, $J = 0.5$ Hz, H3), 9.34 (1H, br s, 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.

4.1.3.11. 5-tert-Butyl-N-(2-chlor-5-hydroxyphenyl)pyrazine-2-carboxamide (11). Yield 35%. Anal. Calcd for $C_{15}H_{16}ClN_3O_2$ (305.8): 58.92% C, 5.27% H, 13.74% N. Found: 58.39% C, 6.335% H, 13.83% N. Mp 239–240 °C. HPLC purity 99.34%. log K: 1.0654. log P: 3.33 ± 0.42. TLC: $R_F = 0.87$. UV (nm) λ_{max}/log ε: 225.2/3.29. IR (KBr) cm^{-1}: 3376 (N–H), 2992, 2960, 2926, 2915 (*tert*-butyl), 1690 (C=O), 1595 (phenyl), 1528 (N–H), 1320, 1250, 11320 (pyrazine). ^{1}H NMR (300 MHz, DMSO) δ: 10.24 (1H, br s, NH), 9.93 (1H, br s, OH), 9.23 (1H, d, $J = 1.6$ Hz, H3), 8.93 (1H, d, $J = 1.6$ Hz, H6), 7.92 (1H, d, $J = 2.9$ Hz, H6′), 7.32 (1H, d, $J = 8.8$ Hz, H3′), 6.60 (1H, dd, $J = 8.8$ Hz, $J = 2.9$ Hz, H4′), and 1.39 (9H, s, CH$_3$). ^{13}C NMR (75 MHz, DMSO) δ: 167.8, 160.9, 157.2, 142.3, 141.2, 140.5, 134.6, 129.9, 113.1, 113.0, 108.9, 37.1, and 29.6.

4.1.3.12. 5-tert-Butyl-N-(4-fluorophenyl)pyrazine-2-carboxamide (12). Yield 47%. Anal. Calcd for $C_{11}H_{16}FN_3O$ (273.3): 65.92% C, 5.90% H, 15.37% N. Found: 64.92% C, 6.405% H, 15.525% N. Mp 178 °C. HPLC purity 99.58%. log K: 0.9928. log P: 3.27 ± 0.47. TLC: $R_F = 0.84$. UV (nm) λ_{max}/log ε: 222.9/3.34. IR (KBr) cm^{-1}: 3361 (N–H), 2984, 2961, 2920, 2906 (*tert*-butyl), 1680 (C=O), 1591 (phenyl), 1522 (N–H), 1318, 1150 (pyrazine). ^{1}H NMR (300 MHz, DMSO) δ: 10.45 (1H, br s, NH), 9.19 (1H, d, $J = 1.5$ Hz, H3), 8.84 (1H, d, $J = 1.5$ Hz, H6), 7.96–7.86 (2H, m, H2′, H6′), 7.25–7.15 (2H, m, H3′, H5′), and 1.39 (9H, s, CH$_3$). ^{13}C NMR (75 MHz, DMSO) δ: 166.9, 162.0, 158.7 (d, $J = 240.8$ Hz), 142.6, 142.6, 139.9, 134.9 (d, $J = 2.6$ Hz), 122.6 (d, $J = 8.0$ Hz), 115.5 (d, $J = 22.3$ Hz), 37.0, and 29.6.

4.1.3.13. 5-tert-Butyl-N-(2-trifluoromethylphenyl)pyrazine-2-carboxamide (13). Yield 47%. Anal. Calcd for $C_{16}H_{16}F_3N_3O$ (323.3): 59.44% C, 4.99% H, 13.00% N. Found: 59.47% C, 5.10% H, 13.05% N. Mp 82.5 °C. HPLC purity 99.89%. log K: 1.2622. log P: 4.02 ± 0.46. TLC: $R_F = 0.88$. UV (nm) λ_{max}/log ε: 224.0/3.27. IR (KBr) cm^{-1}: 3356 (N–H), 2961, 2907, 2869 (*tert*-butyl), 1698 (C=O), 1593 (phenyl), 1539 (NH), 1322, 1171, and 1118 (pyrazine). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 10.27 (1H, br s, 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.

4.1.3.14. 5-tert-Butyl-N-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (14). Yield 38%. Anal. Calcd for C$_{16}$H$_{16}$F$_3$N$_3$O (323.3): 59.44% C, 4.99% H, 13.00% N. Found: 59.83% C, 5.09% H, 13.17% N. Mp 84.5 °C. HPLC purity 99.81%. log K: 1.1338. log P: 4.18 ± 0.46. TLC: $R_F = 0.86$. UV (nm) λ_{max}/log ε: 217.0/3.35. IR (KBr) cm^{-1}: 3350 (N–H), 2974, 2938, 2911, 2874 (*tert*-butyl), 1684 (C=O), 1604 (phenyl), 1543 (N–H), 1339, and 1133 (pyrazine). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.78 (1H, br s, NH), 9.40 (1H, d, $J = 1.7$ Hz, H3), 8.63 (1H, d, $J = 1.7$ Hz, H6), 8.08 (1H, br s, 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.

4.1.3.15. 5-tert-Butyl-N-p-tolylpyrazine-2-carboxamide (15). Yield 83%. Anal. Calcd for C$_{16}$H$_{19}$N$_3$O (269.4): 71.35% C, 7.11% H, 15.60% N. Found: 71.58% C, 7.38% H, 15.62% N. Mp 143 °C. HPLC purity 99.08%. log K: 1.0222. log P: 3.28 ± 0.40. TLC: $R_F = 0.86$. UV (nm) λ_{max}/log ε: 222.9/3.38. IR (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). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.60 (1H, br s, 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.

4.1.3.16. 5-tert-Butyl-6-chloro-N-(2-chloro-5-hydroxyphenyl)-pyrazine-2-carboxamide (16). Yield 69%. Anal. Calcd for C$_{15}$H$_{15}$Cl$_2$N$_3$O$_2$ (340.2): 52.96 % C, 4.44% H, 12.35% N. Found: 52.525% C, 5.765% H, 12.12% N. Mp 253 °C. HPLC purity 99.29%. log K: 1.4335. log P: 4.45 ± 0.44. TLC: $R_F = 0.92$. UV (nm) λ_{max}/log ε: 227.6/3.28. IR (KBr) cm^{-1}: 3386 (N–H), 2990, 2960, 2939 (*tert*-butyl), 1705 (C=O), 1591 (phenyl), 1529 (N–H), 1340, 1260, 1049 (pyrazine). ^{1}H NMR (300 MHz, DMSO) δ: 10.03 (1H, br s, NH), 9.93 (1H, br s, OH), 9.16 (1H, s, H3), 7.76 (1H, d, $J = 2.8$ Hz, H6′), 7.33 (1H, d, $J = 8.7$ Hz, H3′), 6.63 (1H, dd, $J = 8.7$ Hz, $J = 2.8$ Hz, H4′), and 1.50 (9H, s, CH$_3$). ^{13}C NMR (75 MHz, DMSO) δ: 163.8, 159.9, 157.1, 145.3, 141.4, 140.3, 134.4, 130.0, 114.0, 113.5, 109.8, 38.8, and 28.2.

4.1.3.17. 5-tert-Butyl-6-chloro-N-(4-fluorophenyl)pyrazine-2-carboxamide (17). Yield 61%. Anal. Calcd for C$_{15}$H$_{15}$ClFN$_3$O (307.8): 58.54% C, 4.91% H, 13.65% N. Found: 58.265% C, 5.40% H, 13.98% N. Mp 171–172 °C. HPLC purity 99.53%. log K: 1.3488. log P: 4.39 ± 0.49. TLC: $R_F = 0.93$.

UV (nm) λ_{max}/log ε: 228.7/3.33. IR (KBr) cm^{-1}: 3370 (N–H), 2978, 2956, 2929 (*tert*-butyl), 1700 (C=O), 1594 (phenyl), 1532 (N–H), 1327, 1262, 1148 (pyrazine). ^{1}H NMR (300 MHz, DMSO) δ: 10.62 (1H, br s, NH), 9.12 (1H, s, H3), 7.91–7.81 (2H, m, H2′, H6′), 7.26–7.16 (2H, m, H3′, H5′), and 1.50 (9H, s, CH$_3$). ^{13}C NMR (75 MHz, DMSO) δ: 163.0, 160.9, 158.9 (d, $J = 241.4$ Hz), 145.4, 142.8, 140.6, 134.6 (d, $J = 2.6$ Hz), 122.9 (d, $J = 8.0$ Hz), 115.5 (d, $J = 22.3$ Hz), 38.7, and 28.2.

4.1.3.18. 5-tert-Butyl-6-chloro-N-(2-trifluoromethylphenyl)pyrazine-2-carboxamide (18). Yield 26%. Anal. Calcd for C$_{16}$H$_{15}$ClF$_3$N$_3$O (357.8): 53.72% C, 4.23% H, 11.75% N. Found: 54.06% C, 3.89% H, 11.82% N. Mp 81 °C. HPLC purity 99.75%. log K: 1.6560. log P: 5.14 ± 0.48. TLC: $R_F = 0.89$. UV (nm) λ_{max}/log ε: 229.9/3.26. IR (KBr) cm^{-1}: 3366 (N–H), 2980, 2961, 2936, 2909, 2874 (*tert*-butyl, trifluoromethyl), 1715 (C=O), 1592 (phenyl), 1532 (N–H), 1323, 1290, and 1120 (pyrazine). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.99 (1H, br s, 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.

4.1.3.19. 5-tert-Butyl-6-chloro-N-(3-trifluoromethylphenyl)pyrazine-2-carboxamide (19). Yield 21%. Anal. Calcd for C$_{16}$H$_{15}$ClF$_3$N$_3$O (357.8): 53.72% C, 4.23% H, 11.75% N. Found: 53.49% C, 4.60% H, 12.03% N. Mp 71.8 °C. HPLC purity 98.37%. log K: 1.5271. log P: 5.30 ± 0.48. TLC: $R_F = 0.84$. UV (nm) λ_{max}/log ε: 232.3/3.28. IR (KBr) cm^{-1}: 3375 (N–H), 2985, 2960, 2940, 2910, 2870 (*tert*-butyl, trifluoromethyl), 1720 (C=O), 1603 (phenyl), 1530 (N–H), 1325, 1250, and 1158 (pyrazine). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.99 (1H, br s, NH), 9.04 (1H, s, H3), 8.63 (1H, d, $J = 1.7$ Hz, H6), 8.08 (1H, br s, 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 CH$_3$). ^{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.

4.1.3.20. 5-tert-Butyl-6-chloro-N-p-tolylpyrazine-2-carboxamide (20). Yield 31%. Anal. Calcd for C$_{16}$H$_{18}$ClN$_3$O (303.8): 63.26% C, 5.97% H, 13.83% N. Found: 63.17% C, 5.87% H, 13.69% N. Mp 162 °C. HPLC purity 99.18%. log K: 1.3205. log P: 4.41 ± 0.42. TLC: $R_F = 0.83$. UV (nm) λ_{max}/log ε: 227.9/3.39. IR (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). ^{1}H NMR (300 MHz, CDCl$_3$) δ: 9.31 (1H, br s, 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.

4.2. 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 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 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 log P scale. The log K values of the individual compounds are summarized in Table 1 and Section 4.1.3.

4.3. Lipophilicity calculations

log P, *i.e.* the logarithm of the partition coefficient P for *n*-octanol/water, was calculated using the commercially available program ACD/log P version 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). The results are summarized in Table 1 and Section 4.1.3.

4.4. Biological methods

4.4.1. Antimycobacterial assay

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 μg/mL against *M. tuberculosis* $H_{37}Rv$ (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* $H_{37}Rv$ 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. Concurrent with the determination of MICs, the compounds were tested for their cytotoxicity (determination of 50% inhibitory concentrations, IC_{50}) in VERO cells at concentrations less than or equal to 62.5 μg/ml or 10 times the MIC for *M. tuberculosis* $H_{37}Rv$. After 72 h exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. At the conclusion of secondary screening, the MIC and IC_{50} values are formed into a ratio termed selectivity index (SI). At an SI level of 10 or greater, a compound is considered active at the second level [18,19]. The results are presented in Tables 1 and 2.

4.4.2. In vitro antifungal susceptibility testing

The broth microdilution test [10,22] 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 asahii* 1188 (TB), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *T. 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, Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulfonic acid. The final concentrations of the compounds ranged from 0.975 μmol/L to 500 μmol/L. 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.

4.4.3. Herbicidal activities

4.4.3.1. Study of inhibition of oxygen evolution rate in spinach chloroplasts.
Chloroplasts were prepared by the procedure of Walker from spinach (*Spinacia oleracea* L.) [23]. The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Kontron Uvikon 800, Kontron, Muenchen, Germany) using an artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kralova et al. [24] 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 ($\sim$100 W/m^2) from a 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. The comparable IC_{50} value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diurone, DCMU) was about 1.9 μmol/L [25]. The results are summarized in Table 1.

4.4.3.2. Reduction of chlorophyll content in the green algae C. vulgaris Beij. The green algae *C. vulgaris* Beij. were cultivated statically at room temperature according to Kralova et al. [26] (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 seven-day cultivation in the presence of the tested compounds. The Chl content in the algal suspension was determined spectrophotometrically (Kontron Uvikon 800, Kontron, Munchen, Germany) after extraction into methanol according to Wellburn [27]. The Chl content in the suspensions at the beginning of the cultivation was 0.01 mg/L. The applied compound concentrations were as follows: 0.83 μmol/L, 4.2 μmol/L, 8.3 μmol/L, 25 μmol/L, 50 μmol/L, 75 μmol/L, and 100 μmol/L. 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 the 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 as percentage of the control determined for the studied concentration range (0.83−100 μmol/L) with the corresponding standard deviation (S.D.). The comparable IC$_{50}$ value for a selective herbicide DCMU was about 7.3 μmol/L [23]. The results are summarized in Table 1.

Acknowledgments

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