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Full Paper

Substituted Pyrazinecarboxamides: Synthesis and Biological Evaluation†

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Abstract: Condensation of the corresponding chlorides of some substituted pyrazine-2-carboxylic acids (pyrazine-2-carboxylic acid, 6-chloropyrazine-2-carboxylic acid, 5-*tert*-butylpyrazine-2-carboxylic acid or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic acid) with various ring-substituted aminothiazoles or anilines yielded a series of amides. The syntheses, analytical and spectroscopic data of thirty newly prepared compounds are presented. Structure-activity relationships between the chemical structures and the anti-mycobacterial, antifungal and photosynthesis-inhibiting activity of the evaluated compounds are discussed. 3,5-Bromo-4-hydroxyphenyl derivatives of substituted pyrazinecarboxylic acid, **16-18**, have shown the highest activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (54-72% inhibition). The highest antifungal effect against *Trichophyton mentagrophytes*, the most susceptible fungal strain tested, was found for 5-*tert*-butyl-6-chloro-*N*-(4-methyl-1,3-thiazol-2-yl)pyrazine-2-carboxamide (**8**, MIC = 31.25 μmol·mL^{-1}). The most active inhibitors of oxygen evolution rate in spinach

chloroplasts were the compounds 5-*tert*-butyl-6-chloro-*N*-(5-bromo-2-hydroxyphenyl)-pyrazine-2-carboxamide (**27**, IC_{50} = 41.9 μmol·L^{-1}) and 5-*tert*-butyl-6-chloro-*N*-(1,3-thiazol-2-yl)-pyrazine-2-carboxamide (**4**, IC_{50} = 49.5 μmol·L^{-1}).

Keywords: Pyrazinecarboxamides; *in vitro* antimycobacterial, antifungal and photosynthesis inhibition activity; lipophilicity determination.

Introduction

One third of the world's population is infected with tuberculosis (TB), therefore today TB still represents one of the major worldwide public health problems. The current recommended strategy is facing two problems: multidrug resistance and HIV/AIDS pandemic [1]. There is an urgent need for new antimycobacterial drugs, especially for treatment of multi-drug resistant tuberculosis (MDR-TB), a growing problem among HIV-infected patients [2]. Additionally, in patients with impaired cellular immunity, mycobacterial and fungal (*Aspergillus*, *Histoplasma*, etc.) infections predominate and may coexist [3]. Pyrazinamide (PZA) is an important sterilising tuberculosis drug that helps to shorten the duration of current chemotherapy regimens for tuberculosis. PZA enters *Mycobacterium tuberculosis* by passive diffusion, is converted to pyrazinoic acid by nicotinamidase (pyrazinamidase) and is then excreted by a weak efflux pump [4].

In connection with our research into antimycobacterial pyrazine derivatives [5] we were interested in binuclear analogues containing -CONH- bridges [6-9]. Various compounds possessing -CONH- groups were found to inhibit photosynthetic electron transport [10-13]. Amides of 2-alkylpyridine-4-carboxylic acid and 2-alkylsulfanylpyridine-4-carboxylic acid inhibited oxygen evolution rates in *Chlorella vulgaris* and their inhibitory activity depended on the lipophilicity of the compounds [6,7]. This paper is the continuation of our studies of antimycobacterial active pyrazinecarboxylic acid derivatives, especially binuclear compounds connected by -CONH- bridges [5]. Previous studies [7–9,14] showed that alkylation, amidation, aroylation of the pyrazine ring or substitution of the pyrazine with chlorine increased antituberculotic and/or antifungal activity in series of functional pyrazine-carboxylic acid derivatives. We have recently reported the synthesis of a series of amides prepared from the substituted pyrazinecarboxylic acids and some aminophenols, halogenated and alkylated anilines. All these amides possess some antimycobacterial, antifungal and antialgal properties [7-9,15].

The present study is concerned with the synthesis of the series of heterocyclic amides prepared from substituted pyrazine-2-carboxylic acids and 2-aminothiazole, 2-amino-4-methyl- or 2-amino-5-methylthiazole, and 2-bromoaniline, 2,6-dibromo-4-aminophenol, 3-methoxyaniline, 3,5-dimethoxy-aniline, 5-bromo-2-hydroxyaniline or 3,4-dichloroaniline, respectively. One of the derivatives synthesized, the *N*-thiazol-2-yl amide of pyrazine-2-carboxylic acid (**1**) was originally prepared by Kushner and its antimycobacterial activity was tested, too [16].

One of the major goals for the physico-chemical characterisation of drugs is the prediction and/or measurement of their lipophilicity. The logarithm of the octanol-water partition coefficient (log *P*) has become the most widely used parameter for defining lipophilicity and various *in silico* calculation software packages have made possible the use of log *P* values in predictive models for absorption, distribution, excretion and metabolism properties of drugs [17,18]. Reversed phase high-performance

liquid chromatography (RP-HPLC) provides an easy, reliable and accurate way to determine the concentration of a compound in solvents used for the measurement of partition coefficients. The chromatographic retention time directly relates to the compound's distribution between the mobile and the stationary phases. The retention factor (*K*) determined from the retention time (T_R) and death time (T_D) as (T_R - T_D) is equal to the *ratio* of the average number of analyte molecules in the stationary phase to the average number of molecules in the mobile phase (cf. Eq. 1) during the elution process. Log *K*, calculated from the capacity factor K, is used as the lipophilicity index converted to log *P* scale [19].

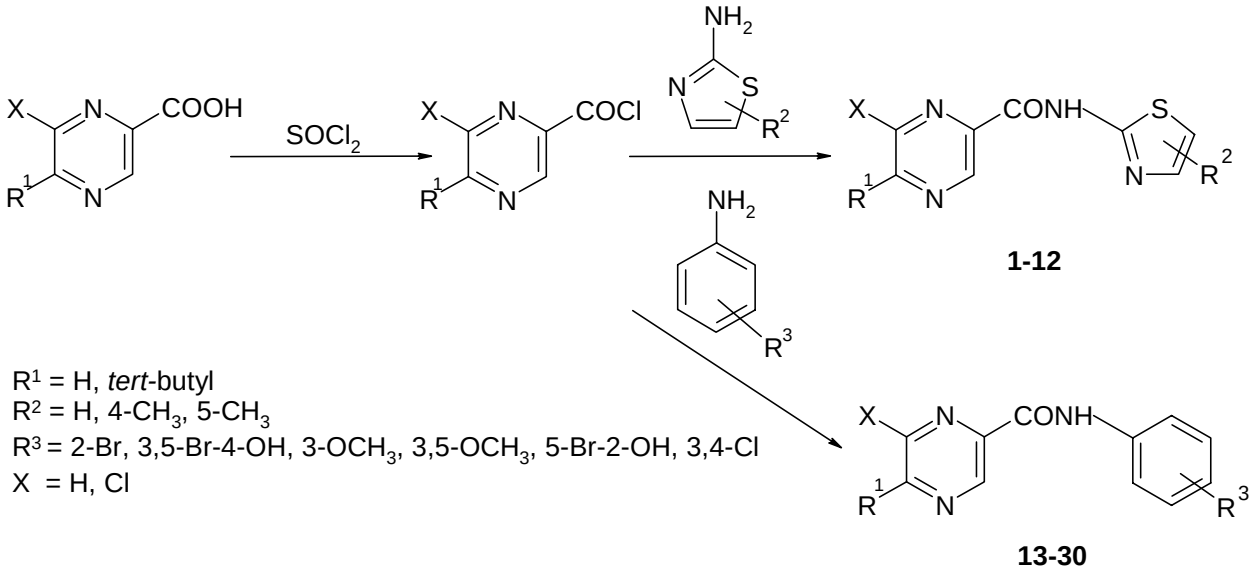
$$K = \frac{T_R - T_D}{T_D} \qquad \text{(Eq. 1)}$$

The aim of this work was to establish the structure-activity relationships in the mentioned series, *i.e.* to continue in studying of the substituent variability influence on the biological effect, and to determine the importance of increased hydrophobic properties for antimycobacterial, antifungal and photosynthesis-inhibiting activity of newly prepared substituted pyrazinecarboxamides.

Results and Discussion

The synthesis of amides is shown in Scheme 1. Condensation of chlorides of pyrazine-2-carboxylic acid [20], 6-chloropyrazine-2-carboxylic acid [21], 5-*tert*-butylpyrazine-2-carboxylic acid [7] or 6-chloro-5-*tert*-butylpyrazine-2-carboxylic [7] acid with 2-aminothiazoles and ring-substituted anilines yielded a series of amides of mentioned pyrazine-2-carboxylic acids **1-30**. The melting points, yields, elemental analyses, IR, ^{1}H- and ^{13}C-NMR spectral data for the all compounds prepared are given in the Experimental. Calculated log *P* values and measured log *K* values of all derivatives studied are shown in Table 1.

Scheme 1: Synthesis of some substituted pyrazine-2-carboxamides **1-30**.



All compounds prepared were evaluated for their *in vitro* antimycobacterial activity. Both the highest activity (72% inhibition) against *M. tuberculosis* and the highest lipophilicity (log *P* = 6.00) of

all compounds studied was found for 5-*tert*-butyl-6-chloro-*N*-(3,5-dibromo-4-hydroxyphenyl)-pyrazine-2-carboxamide (**18**). Two other compounds, **16**, **17**, with the identical substitution on the aromatic part of the molecule, exert a comparable activity. The majority of compounds exhibited only modest antimycobacterial activity (see Table 1 and Figure 1). 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 generally not evaluated further [22]. On the other hand, such "inactive" compounds may still have significant inhibitory activity and this data should not be ignored; analogues, derivatives, and alterations in physical properties may confer some positive changes in biological effects. Therefore synthesis and evaluation of other pyrazinecarboxylic acid derivatives is necessary to round out the structure-activity data.

Table 1: Calculated lipophilicity (log *P*), logarithm of capacity factors (log *K*), antimycobaterial evaluation (% of inhibition), antifungal susceptibility (MIC) and OER inhibition in spinach chloroplasts (IC_{50}) of compounds **1-30** in comparison with standards: pyrazinamide (PZA), fluconazole and atrazine.

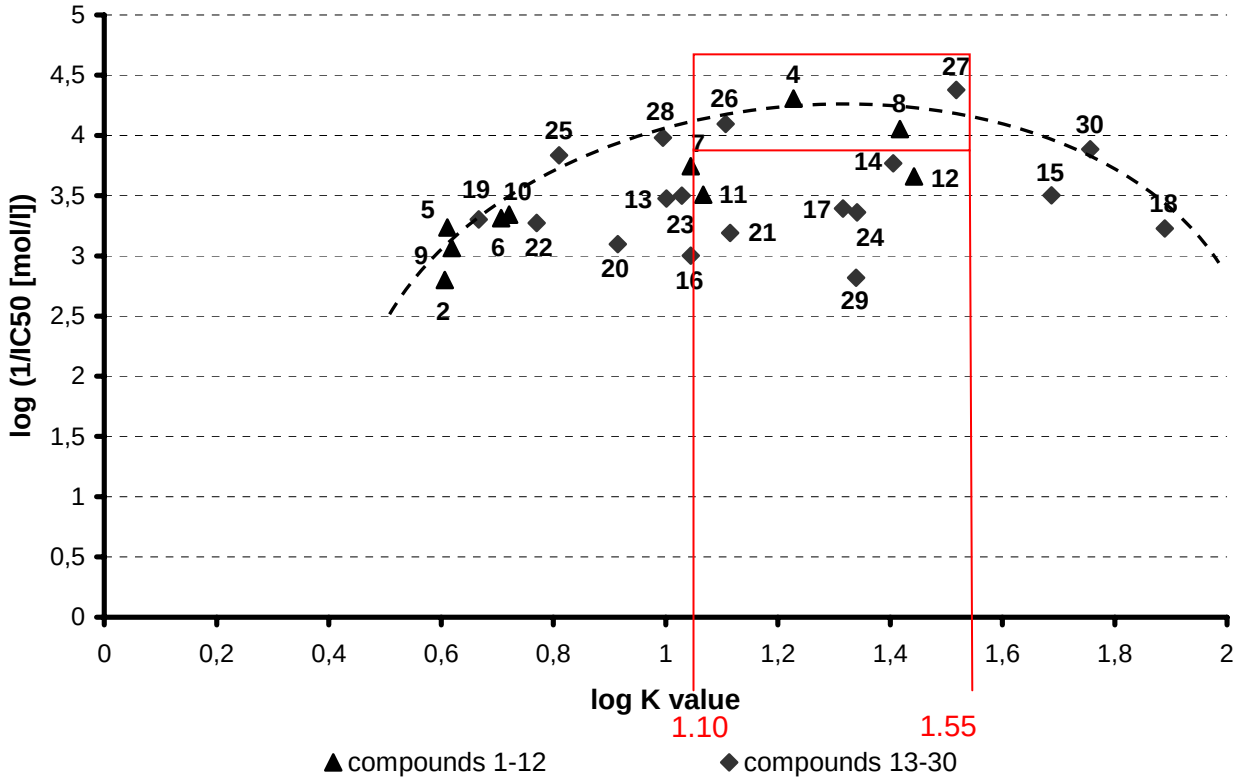
Compound	X	R^1	R^2 / R^3	log *P*	log *K*	% Inhibition at 6.25 μg mL^{-1}	MIC[b] (μmol mL^{-1})	IC_{50} (μmol.L^{-1})
1	H	H	H	0.31	0.5565	0	>500/>500	*b*
2	Cl	H	H	1.21	0.6064	32	>500/>500	1589.0
3	H	$(CH_3)_3C$	H	2.44	0.8987	47	125/125	*b*
4	Cl	$(CH_3)_3C$	H	3.34	1.2282	42	125/125	49.5
5	H	H	4-CH_3	1.01	0.6112	0	>500/>500	582.8
6	Cl	H	4-CH_3	1.91	0.7068	21	250/500	485.5
7	H	$(CH_3)_3C$	4-CH_3	3.14	1.0446	35	125/250	180.6
8	Cl	$(CH_3)_3C$	4-CH_3	4.04	1.4174	52	31.25/31.25	88.8
9	H	H	5-CH_3	0.65	0.6193	10	1000/1000	862.2
10	Cl	H	5-CH_3	1.55	0.7213	15	125/250	453.7
11	H	$(CH_3)_3C$	5-CH_3	2.77	1.0670	65	125/125	311.4
12	Cl	$(CH_3)_3C$	5-CH_3	3.67	1.4425	61	31.25/62.5	219.0
13	Cl	H	2-Br	2.94	1.0014	28	>500/>500	333.8
14	H	$(CH_3)_3C$	2-Br	3.51	1.4057	22	500/500	170.7
15	Cl	$(CH_3)_3C$	2-Br	4.63	1.6873	25	250/250	315.4
16	Cl	H	3,5-Br-4-OH	4.31	1.0450	69	>500/>500	995.2
17	H	$(CH_3)_3C$	3,5-Br-4-OH	4.88	1.3158	54	>500/>500	404.3
18	Cl	$(CH_3)_3C$	3,5-Br-4-OH	6.00	1.8895	72	125/125	590.3
19	Cl	H	3-OCH_3	2.42	0.6671	2	>500/>500	499.8
20	H	$(CH_3)_3C$	3-OCH_3	2.98	0.9146	53	>500/>500	799.5
21	Cl	$(CH_3)_3C$	3-OCH_3	4.10	1.1148	23	250/250	644.0
22	Cl	H	3,5-OCH_3	2.46	0.7701	11	1000/1000	533.0
23	H	$(CH_3)_3C$	3,5-OCH_3	3.02	1.0286	5	500/500	317.2

Table 1. Cont.

24	Cl	$(CH_3)_3C$	3,5-OCH_3	4.14	1.3407	0	125/250	435.1
25	Cl	H	5-Br-2-OH	3.34	0.8105	0	>500/>500	146.2
26	H	$(CH_3)_3C$	5-Br-2-OH	3.91	1.1070	0	>500/>500	80.3
27	Cl	$(CH_3)_3C$	5-Br-2-OH	5.03	1.5181	30	125/125	41.9
28	Cl	H	3,4-Cl	4.15	0.9950	61	125/250	104.8
29	H	$(CH_3)_3C$	3,4-Cl	4.72	1.3395	15	125/125	1525.1
30	Cl	$(CH_3)_3C$	3,4-Cl	5.84	1.7563	0	62.5/62.5	130.1
PZA	-	-	-	0.37	-	100[a]	-	-
fluconazole	-	-	-	0.99	-	-	1.95/3.91	-
atrazine	-	-	-	1.03	-	-	-	1.0

[a] MIC = 12.5 μg mL^{-1}, data from [27]; [b] against *T. mentagrophytes* after 72 h / 120 h; [c] not tested due to their low solubility in DMSO.

Figure 1: Quasi-parabolic dependence between logarithm of retention factor (log *K*) and photosynthesis-inhibiting activity {log ($1/IC_{50}$ [mol/L])} of studied compounds **1**-**30**.



The evaluation of *in vitro* antifungal activity of the synthesized compounds was performed against eight fungal strains. The results revealed no interesting activity against the majority of strains tested. Only the compounds 5-*tert*-butyl-6-chloro-*N*-(5-methyl-1,3-thiazol-2-yl)pyrazine-2-carboxamide (**12**) and especially 5-*tert*-butyl-6-chloro-*N*-(4-methyl-1,3-thiazol-2-yl)pyrazine-2-carboxamide (**8**) showed some promising *in vitro* antifungal activity against *Trichophyton mentagrophytes*, the most susceptible fungal strain evaluated, (MIC = 31.25 – 62.5 μmol·mL^{-1}), although this activity is only modest in

comparison with fluconazole, the standard (MIC = 3.91 μmol·mL^{-1} after 120 h, see Table 1). The negative antifungal screening results do not allow us to draw detailed conclusions on potential structure–activity relationships. On the other hand, the influence of an increasing lipophilicity parameter on the increasing *in vitro* antifungal activity in the series of compounds evaluated is remarkable.

The majority of the thirty compounds studied inhibited photosynthetic electron transport in spinach chloroplasts (see Table 1 and Figure 1; compounds **1** and **3** were not tested for their photosynthesis-inhibition activity due to their low solubility in DMSO). The IC_{50} values varied in the range 41.9 to 1589 μmol·L^{-1}. The inhibitory activity of the studied compounds was relatively low, the most efficient inhibitors were compounds **8** (IC_{50} = 88.8 μmol·L^{-1}), **4** (IC_{50} = 49.5 μmol·L^{-1}), and mainly 5-*tert*-butyl-6-chloro-*N*-(5-bromo-2-hydroxyphenyl)pyrazine-2-carboxamide (**27**, IC_{50} = 41.9 μmol·L^{-1}). For the series of compounds **5-8** and **9-12** the biological activity showed a linear increase with increasing lipophilicity of the compounds within these series. In both series of anilides **13-15** and **16-18**, in the case of the lipophilic compounds **15** (log *P* = 4.63) and/or **18** (log *P* = 5.28) a significant activity decrease was observed. Results from previous observations have exposed the importance of the phenolic moiety for the photosynthesis-inhibiting activity in the previously studied series of substituted pyrazine-2-carboxamides [7, 8]. However, the biological activity of compounds **16-18** was lower than that of compounds **13-15**. We assume that this activity decrease was connected with the increased lipophilicity of the compounds due to the presence of two bromine atoms.

Hydrophobicity parameters (log *P* values) of compounds **1-30** were calculated 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 0.31 to 6.00. It can be assumed that the computed log *P* values and the calculated log *K* values correspond relatively with expected lipophilicity increases within individual series of compounds (pyrazine < 6-chloropyrazine < 5-*tert*-butylpyrazine < 6-chloro-5-*tert*-butylpyrazine). Capacity factor *K*/calculated log *K* values specify lipophilicity within individual series of compounds. Results are shown in Table 1.

The lower antimycobacterial activities of the compounds presented do not allow us to draw final conclusions on structure–activity relationships (SAR). Better SAR results are expressed in Figure 1, where the quasi-parabolic dependence between the logarithm of the retention factors (log *K*) and photosynthesis-inhibiting activity {log (1/IC_{50} [mol/L])} of all studied compounds is shown. Lipophilicity expressed as log *K* values ranged from 1.10 to 1.55.

From the point of view of the chemical structure, all compounds can be divided into two groups: (*i*) compounds with an aminothiazole moiety (**1-12**, triangles in Figure 1) and (*ii*) compounds with an aniline moiety (**13-30**, lozenges in Figure 1). The optimal substitution for the first group of compounds was found to be the methyl group on $C_{(5)}$ of the thiazole ring. The optimal substitution in the second group was found, in agreement with our previous results [7], to be the phenol and halogen (bromine) moieties. The compound 5-*tert*-butyl-6-chloro-*N*-(4-methyl-1,3-thiazol-2-yl)pyrazine-2-carboxamide (**8**) was identified as the most active one in the three different biological assays. However, there is no general trend in the SAR of the compounds evaluated.

Conclusions

In summary, the synthesis and biological evaluation of thirty new substituted amides of pyrazinecarboxylic acid are described. In the first series, among the compounds with substituted 2-aminothiazoles, the highest antifungal effect was found for 5-*tert*-butyl-6-chloro-*N*-(4-methyl-1,3-thiazol-2-yl)pyrazine-2-carboxamide (**8**). In the second series, among the compounds bearing ring-substituted anilines, the bromohydroxyphenyl derivatives of substituted pyrazinecarboxylic acid (**16-18**, **27**) have shown the highest biological activity, *i.e.* against *Mycobacterium tuberculosis* H_{37}Rv and inhibition of oxygen evolution rate in spinach chloroplasts, respectively.

Acknowledgements

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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 using KBr pellets on an Nicolet Impact 400 IR-spectrometer. ^{1}H and ^{13}C-NMR Spectra were recorded on a Varian Mercury – Vx BB 300 (Varian, Palo Alto, CA, USA; 299.95 MHz for ^{1}H and 75.43 MHz for ^{13}C). Chemical shifts are given relative to the internal $Si(CH_3)_4$. Log *P* values were computed using the CS ChemOffice Ultra ver. 7.0 program (CambridgeSoft, Cambridge, MA, USA) and are summarized in Table 1.

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 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 223 nm was chosen. The retention time (dead time) of the KI methanol solution was T_D = 2.382 min. Retention times (T_R) was measured in minutes, capacity factors were calculated (*K)*. The HPLC separation process was monitored using Millennium^{32}®

Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, U.S.A.). The calculated log *K* values of all compounds are shown in Table 1.

Synthesis of amides **2a**-**r**

A mixture of acid, *i.e.* pyrazine-2-carboxylic [20], 6-chloropyrazine-2-carboxylic [21], 5-*tert*-butylpyrazine-2-carboxylic [7] or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic [7] acids, respectively, (50.0 mmol) and thionyl chloride (5.5 mL, 75.0 mmol) was refluxed in dry toluene (20 mL) for about 1 h. Excess thionyl chloride was removed by repeated evaporation *in vacuo* with fresh dry toluene. 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) in dry pyridine (50 mL) kept at room temperature. After the addition was complete, stirring continued for another 30 min. The reaction mixture was then poured into cold water (100 mL) and the crude amide was collected and recrystallized from aqueous ethanol.

Pyrazine-2-carboxylic acid thiazol-2-ylamide (**1**). Yield: 88%; m.p. 187-188 °C (Ref. [16]: m.p. 187-189 °C); For $C_8H_6N_4OS$ (206.2) calculated: 46.59% C, 2.93% H, 27.17% N; found: 46.55% C, 2.91% H, 26.98% N; R_F = 0.43; IR cm^{-1}: 3432 (N-H), 1668 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 11.14 (bs, 1H, NH), 9.52 (d, 1H, *J*=1.79 Hz, H3), 8.86 (d, 1H, *J*=1.79 Hz, H6), 8.65-8.63 (m, 1H, H5), 7.55 (d, 1H, *J*=3.57 Hz, H4´), and 7.09 (d, 1H, *J*=3.57 Hz, H5´); ^{13}C-NMR ($CDCl_3$), δ: 160.7, 157.3, 148.3, 144.9, 143.0, 142.7, 138.2, and 114.3.

6-Chloropyrazine-2-carboxylic acid thiazol-2-ylamide (**2**). Yield: 98%; m.p. 153-155 °C; For $C_8H_5ClN_4OS$ (240.7) calculated: 39.92% C, 2.09% H, 23.28% N; found: 40.03% C, 1.92% H, 23.33% N; R_F = 0.65; IR cm^{-1}: 3435 (N-H), 1675 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 10.91 (bs, 1H, NH), 9.17 (s, 1H, H3), 7.59 (d, 1H, *J*=3.57 Hz, H4´), 7.36 (d, 1H, *J*=3.57 Hz, H5´), and 1.50 (s, 9H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 163.6, 161.5, 158.2, 145.8, 141.7, 140.9, 137.7, 114.8, 38.8, and 28.2.

5-tert-Butylpyrazine-2-carboxylic acid thiazol-2-ylamide (**3**). Yield: 45%; m.p. 131-132 °C; For $C_{12}H_{14}N_4OS$ (262.3) calculated: 54.94% C, 5.38% H, 21.36% N; found: 55.06% C, 5.43% H, 21.38% N. R_F = 0.63; IR cm^{-1}: 3432 (N-H), 1676 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 11.02 (bs, 1H, NH), 9.39 (d, 1H, *J*=1.37 Hz, H3), 8.66 (d, 1H, *J*=1.38 Hz, H6), 7.54 (d, 1H, *J*=3.58 Hz, H4´), 7.07 (d, 1H, *J*=3.57 Hz, H5´), and 1.45 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$), δ: 168.8, 161.0, 157.4, 143.2, 139.7, 139.7, 138.1, 114.2, 37.2, and 29.7.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid thiazol-2-ylamide (**4**). Yield: 97%; m.p. 148-150 °C; For $C_{12}H_{13}ClN_4OS$ (296.8) calculated: 48.56% C, 4.42% H, 18.88% N; found: 48.46% C, 4.65% H, 18.80% N; R_F = 0.88; IR cm^{-1}: 3448 (N-H), 1675 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.49 (bs, 1H, NH), 9.17 (s, 1H, H3), 7.59 (d, 1H, *J*=3.6 Hz, H4´), 7.36 (d, 1H, *J*=3.6 Hz, H5´), and 1.50 (s, 9H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 163.6, 161.5, 158.2, 145.8, 141.7, 140.9, 137.7, 114.8, 38.8, and 28.2.

Pyrazine-2-carboxylic acid (4-methylthiazol-2-yl)amide (**5**). Yield: 67%; m.p. 144-145 °C; For $C_9H_8N_4OS$ (220.3) calculated: 49.08% C, 3.66% H, 25.44% N; found: 48.93% C, 3.78% H, 25.63% N;

R_F = 0.50; IR cm^{-1}: 3433 (N-H), 1670 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.27 (bs, 1H, NH), 9.20 (d, 1H, *J*=1.0 Hz, H3), 8.95 (d, 1H, *J*=1.0 Hz, H6), 7.88 (m, 1H, H5), 6.90 (d, 1H, *J*=1.0 Hz, H5´), and 2.40 (s, 3H, *J*=1.0 Hz, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 164.0, 163.6, 148.0, 147.8, 147.5, 145.6, 139.7, 111.2, and 17.7.

6-Chloropyrazine-2-carboxylic acid (4-methylthiazol-2-yl)amide (**6**). Yield: 97%; m.p. 192-194 °C; For $C_9H_7ClN_4OS$ (254.7) calculated: 42.44% C, 2.77% H, 22.00% N; found: 42.37% C, 2.70% H, 22.13% N; R_F = 0.74; IR cm^{-1}: 3434 (N-H), 1675 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.54 (bs, 1H, NH), 9.23 (s, 1H, H3), 9.04 (s, 1H, H5), 6.90 (d, 1H, *J*=1.0 Hz, H5´), and 2.30 (d, 3H, *J*=1.0 Hz, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 162.0, 158.3, 147.9, 147.5, 145.9, 144.5, 142.8, 109.0, and 16.7.

5-tert-Butylpyrazine-2-carboxylic acid (4-methylthiazol-2-yl)amide (**7**). Yield: 33%; m.p. 84-85 °C; For $C_{13}H_{16}N_4OS$ (276.4) calculated: 56.50% C, 5.84% H, 20.27% N; found: 56.44% C, 5.96% H, 20.18% N; R_F = 0.69; IR cm^{-1}: 3434 (N-H), 1676 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.22 (bs, 1H, NH), 9.20 (d, 1H, *J*=1.5 Hz, H3), 8.89 (d, 1H, *J*=1.5 Hz, H6), 6.90 (d, 1H, *J*=1.0 Hz, H5´), 2.30 (d, 3H, *J*=1.0 Hz, CH_3), and 1.39 (s, 9H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 167.5, 162.3, 157.0, 147.1, 142.9, 141.4, 140.7, 109.0, 37.1, 29.6, and 17.0.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (4-methylthiazol-2-yl)amide (**8**). Yield: 97%; m.p. 118-120 °C; For $C_{13}H_{15}ClN_4OS$ (310.8) calculated: 50.24% C, 4.86% H, 18.03% N; found: 50.17% C, 4.99% H, 18.09% N; R_F = 0.91; IR cm^{-1}: 3451 (N-H), 1675 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.47 (bs, 1H, NH), 9.15 (s, 1H, H3), 6.89 (d, 1H, *J*=1.0 Hz, H5´), 2.30 (d, 3H, *J*=1.0 Hz, CH_3), and 1.49 (s, 9H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 163.5, 161.7, 146.2, 145.8, 142.1, 141.8, 140.8, 108.9, 38.7, 28.2, and 16.8.

Pyrazine-2-carboxylic acid (5-methylthiazol-2-yl)amide (**9**). Yield: 66%; m.p. 247-248 °C; For $C_9H_8N_4OS$ (220.3) calculated: 49.08% C, 3.66% H, 25.44% N; found: 49.03% C, 3.51% H, 25.32% N; R_F = 0.42; IR cm^{-1}: 3435 (N-H), 1672 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.46 (bs, 1H, NH), 9.20 (d, 1H, *J*=1.65 Hz, H3), 8.91 (d, 1H, *J*=1.65 Hz, H6), 8.73-8.70 (m, 1H, H5), 7.28 (s, 1H, H4´), and 2.38 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 163.0, 158.3, 148.0, 147.8, 147.5, 143.9, 136.8, 127.4, and 11.5.

6-Chloropyrazine-2-carboxylic acid (5-methylthiazol-2-yl)amide (**10**). Yield: 98%; m.p. 214-215 °C; For $C_9H_7ClN_4OS$ (254.7) calculated: 42.44% C, 2.77% H, 22.00% N; found: 42.53% C, 2.70% H, 21.95% N; R_F = 0.79; IR cm^{-1}: 3436 (N-H), 1672 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.56 (bs, 1H, NH), 9.24 (s, 1H, H3), 9.05 (s, 1H, H5), 7.27 (s, 1H, H4´), and 2.39 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 161.7, 157.1, 147.9, 147.5, 144.5, 142.8, 134.1, 127.4, and 11.5.

5-tert-Butylpyrazine-2-carboxylic acid (5-methylthiazol-2-yl)amide (**11**). Yield: 33%; m.p. 114-116 °C; For $C_{13}H_{16}N_4OS$ (276.4) calculated: 56.50% C, 5.84% H, 20.27% N; found: 56.59% C, 5.80% H, 20.36% N; R_F = 0.80; IR cm^{-1}: 3435 (N-H), 1677 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.12 (bs, 1H, NH), 9.20 (s, 1H, H3), 8.88 (s, 1H, H6), 7.24 (s, 1H, H4´), 2.38 (s, 3H, *J*=1.0 Hz, CH_3), and 1.39 (s, 9H,

CH_3); ^{13}C-NMR (DMSO-d_6), δ: 167.5, 162.0, 155.8, 142.9, 141.4, 140.6, 135.2, 127.6, 37.1, 29.6, and 11.4.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (5-methylthiazol-2-yl)amide (**12**). Yield: 98%; m.p. 152-153 °C; For $C_{13}H_{15}ClN_4OS$ (310.8) calculated: 50.24% C, 4.86% H, 18.03% N; found: 50.37% C, 4.69% H, 17.79% N; R_F = 0.85; IR cm^{-1}: 3453 (N-H), 1678 (C=O); ^{1}H-NMR (DMSO-d_6), δ: 12.45 (bs, 1H, NH), 9.15 (d, 1H, *J*=0.5 Hz, H3), 7.27-7.24 (m, 1H, H4´), and 2.38 (d, 3H, *J*=0.5 Hz, CH_3), 1.49 (s, 9H, CH_3); ^{13}C-NMR (DMSO-d_6), δ: 163.4, 161.4, 156.7, 145.8, 141.8, 140.8, 134.4, 127.4, 38.7, 28.2, and 11.4.

6-Chloropyrazine-2-carboxylic acid (2-bromophenyl)amide (**13**). Yield: 24%; m.p. 118-119 °C; For $C_{11}H_7BrClN_3O$ (312.6) calculated: 42.27% C, 2.26% H, 13.44% N; found: 42.31% C, 2.17% H, 13.28% N; R_F = 0.86; IR cm^{-1}: 3436 (N-H), 1701 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 10.11 (bs, 1H, NH), 9.39 (d, 1H, *J*=0.55 Hz, H3), 8.83 (d, 1H, *J*=0.55 Hz, H5), 8.55 (dd, 1H, *J*=1.65 Hz, H6´), 7.61 (dd, 1H, *J*=7.97 Hz, *J*=1.37 Hz, H3´), 7.43-7.35 (m,1H, H5´); ^{13}C-NMR ($CDCl_3$), δ: 159.5, 147.8, 147.6, 143.8, 142.1, 135.0, 132.6, 128.5, 126.0, 121.6, and 114.1.

5-tert-Butylpyrazine-2-carboxylic acid (2-bromophenyl)amide (**14**). Yield: 20%; m.p. 83-84 °C. For $C_{15}H_{16}BrN_3O$ (334.2) calculated: 53.91% C, 4.83% H, 12.57% N; found: 54.13% C, 4.91% H, 12.68% N; R_F = 0.94; IR cm^{-1}: 3439 (N-H), 1693 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 10.35 (bs, 1H, NH), 9.39 (d, 1H, *J*=1.37 Hz, H3), 8.71 (d, 1H, *J*=1.65 Hz, H6), 8.62 (dd, 1H, *J*=8.24 Hz, *J*=1.65 Hz, H6´), 7.59 (dd, 1H, *J*=8.25 Hz, *J*=1.65 Hz, H3´), 7.42-7.34 (m, 1H, H4´), 7.03 (dd, 1H, *J*=7.42 Hz, *J*=1.65 Hz, H5´), and 1.45 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$), δ: 168.0, 161.3, 143.0, 141.3, 139.4, 135.5, 132.5, 128.4, 125.4, 121.4, 113.8, 37.1, and 29.7.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (2-bromophenyl)amide (**15**). Yield: 17%; m.p. 116-117 °C; For $C_{15}H_{15}BrClN_3O$ (368.7) calculated: 48.87% C, 4.10% H, 11.40% N; found: 48.56% C, 4.21% H, 11.28% N; R_F = 0.92; IR cm^{-1}: 3435 (N-H), 1701 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 10.11 (bs, 1H, NH), 9.39 (s, 1H, H3), 8.83 (s, 1H, H5), 8.55 (dd, 1H, *J*=8.24 Hz, *J*=1.37 Hz, H6´), 7.61 (dd, 1H, *J*=8.24 Hz, *J*=1.37 Hz, H3´), 7.43-7.35 (m, 1H, H4´), 7.09-7.03 (m, 1H, H5´); ^{13}C-NMR ($CDCl_3$), δ: 159.8, 148.0, 147.9, 144.1, 142.3, 135.2, 132.8, 128.7, 126.2, 121.8, 114.4, 37.0, and 29.7.

6-Chloropyrazine-2-carboxylic acid (3,5-dibromo-4-hydroxyphenyl)amide (**16**). Yield: 14%; m.p. 191-193 °C; For $C_{11}H_6Br_2ClN_3O_2$ (407.5) calculated: 32.43% C, 1.48% H, 10.31% N; found: 32.33% C, 1.41% H, 10.27% N; R_F = 0.89; IR cm^{-1}: 3432 (N-H), 1685 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 10.74 (bs, 1H, NH), 9.86 (bs, 1H, OH), 9.20 (d, 1H, *J*=0.55 Hz, H3), 9.05 (d, 1H, *J*=0.5 Hz, H5), and 8.13 (s, 2H, H2´, H6´); ^{13}C-NMR ($CDCl_3$), δ: 160.8, 147.8, 147.8, 147.1, 144.8, 142.5, 132.4, 124.7, and 111.8.

5-tert-Butylpyrazine-2-carboxylic acid (3,5-dibromo-4-hydroxyphenyl)amide (**17**). Yield: 24%; m.p. 206-208 °C; For $C_{15}H_{15}Br_2N_3O_2$ (429.1) calculated: 41.99% C, 3.52% H, 9.79% N; found: 42.11% C, 3.41% H, 10.02% N; R_F = 0.95; IR cm^{-1}: 3432 (N-H), 1695 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 9.38 (d, 1H, *J*=0.55 Hz, H3), 9.29 (bs, 1H, NH), 8.83 (d, 1H, *J*=0.55 Hz, H5), 7.96 (s, 2H, H2´, H6´), 5.84 (s, 1H,

OH), and 1.48 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$), δ: 168.7, 161.5, 161.1, 143.3, 139.3, 139.1, 137.5, 123.4, 117.8, 37.4, and 29.9.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (3,5-dibromo-4-hydroxyphenyl)amide (**18**). Yield: 20%; m.p. 216-217 °C; For $C_{15}H_{14}Br_2ClN_3O_2$ (463.6) calculated: 38.87% C, 3.04% H, 9.06% N; found: 38.63% C, 2.97% H, 9.28% N; R_F = 0.89; IR cm^{-1}: 3432 (N-H), 1685 (C=O); ^{1}H-NMR ($CDCl_3$), δ: 9.31 (bs, 1H, NH), 9.24 (d, 1H, *J*=0.55 Hz, H3), 8.03 (s, 2H, H2´, H6´), 5.71 (s, 1H, OH), and 1.49 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$), δ: 161.2, 159.0, 147.8, 144.8, 142.7, 139.8, 132.2, 124.7, 112.1, 31.7, and 29.7.

6-Chloropyrazine-2-carboxylic acid (3-methoxyphenyl)amide (**19**). Yield: 74%; m.p. 139-140 °C; For $C_{12}H_{10}ClN_3O_2$ (263.7) calculated: 54.66% C, 3.62% H, 15.94% N; found: 54.72% C, 3.59% H, 16.09% N; R_F = 0.88; IR cm^{-1}: 3355 (NH), 2838 (OCH_3), 1681 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.39-9.37 (m, 2H, H3, NH), 8.80 (d, 1H, *J*=0.55 Hz, H5), 7.50 (t, 1H, *J*=2.20 Hz, H2´), 7.30 (d, 1H, *J*=7.96 Hz, H4´), 7.24-7.19 (m, 1H, H5´), 6.74 (ddd, 1H, *J*=7.96 Hz, *J*=2.47 Hz, *J*=1.10 Hz, H6´), and 3.84 (s, 3H, OCH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 160.2, 159.3, 147.5, 147.4, 143.9, 142.2, 138.0, 129.9, 112.2, 111.1, 105.5, and 55.4.

5-tert-Butylpyrazine-2-carboxylic acid (3-methoxyphenyl)amide (**20**). Yield: 81%; m.p. 79-80 °C; For $C_{16}H_{19}N_3O_2$ (285.3) calculated: 67.35% C, 6.71% H, 14.73% N; found: 67.48% C, 6.69% H, 14.95% N; R_F = 0.90; IR cm^{-1}: 3360 (NH), 2841 (OCH_3), 1677 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.65 (bs, 1H, NH), 9.39 (d, 1H, *J*=1.37 Hz, H3), 8.62 (d, 1H, *J*=1.37 Hz, H6), 7.55 (t, 1H, *J*=2.20 Hz, H2´), 7.28 (t, 1H, *J*=7.97 Hz, H5´), 7.22-7.17 (m, 1H, H4´), 6.72 (ddd, 1H, *J*=7.97 Hz, *J*=2.47 Hz, *J*=1.10 Hz, H6´), 3.85 (s, 3H, OCH_3), and 1.45 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 167.8, 161.1, 160.2, 142.9, 141.3, 1389.0, 138.6, 129.8, 111.9, 110.7, 105.2, 55.3, 37.0, and 29.7.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (3-methoxyphenyl)amide (**21**). Yield: 78%; m.p. 128-129 °C; For $C_{16}H_{18}ClN_3O_2$ (319.8) calculated: 60.09% C, 5.67% H, 13.14% N; found: 59.88% C, 5.62% H, 13.18% N; R_F = 0.86; IR cm^{-1}: 3380 (NH), 2840 (OCH_3), 1686 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.65 (bs, 1H, NH), 9.04 (d, 1H, H3), 7.50 (t, 1H, *J*=2.20 Hz, H2´), 7.30 (d, 1H, *J*=7.96 Hz, H4´), 7.24-7.19 (m, 1H, H5´), 6.71 (ddd, 1H, *J*=7.96 Hz, *J*=2.47 Hz, *J*=1.10 Hz, H6´), 3.74 (s, 3H, OCH_3 , and 1.34 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 165.2, 162.2, 160.0, 144.0, 142.7, 141.8, 139.0, 129.8, 112.7, 109.1, 105.5, 31.2, and 25.4.

6-Chloropyrazine-2-carboxylic acid (3,5-dimethoxyphenyl)amide (**22**). Yield: 64%; m.p. 211-212 °C; For $C_{13}H_{12}ClN_3O_3$ (293.7) calculated: 53.16% C, 4.12% H, 14.31% N; found: 52.81% C, 4.29% H, 14.02% N; R_F = 0.88; IR cm^{-1}: 3370 (NH), 2964, 2838 (OCH_3), 1685 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.38 (s, 1H, H3), 9.34 (bs, 1H, NH), 8.81 (s, 1H, H5), 6.98 (d, 2H, *J*=2.19 Hz, H2´, H6´), 6.33-6.30 (m, 1H, H4´), and 3.82 (s, 6H, OCH_3); ^{13}C-NMR ($CDCl_3$) δ 161.2, 159.3, 147.6, 147.4, 143.9, 142.2, 138.5, 98.2, 97.7, and 55.5.

5-tert-Butylpyrazine-2-carboxylic acid (3,5-dimethoxyphenyl)amide (**23**). Yield: 82%; m.p. 135-136 °C; For $C_{17}H_{21}N_3O_3$ (315.4) calculated: 64.74% C, 6.71% H, 13.32% N; found: 63.85% C, 6.71% H,

13.23% N; R_F = 0.90; IR cm^{-1}: 3360 (NH), 2961, 2838 (OCH_3), 1690 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.62 (bs, 1H, NH), 9.38 (d, 1H, *J*=1.37 Hz, H3), 8.62 (d, 1H, *J*=1.38 Hz, H6), 7.00 (d, 2H, *J*=2.20 Hz, H2´, H6´), 6.29 (t, 1H, *J*=2.20 Hz, H4´), 3.82 (s, 6H, OCH_3), and 1.44 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 167.8, 161.1, 161.1, 142.9, 141.3, 139.1, 139.0, 97.9, 97.2, 55.4, 37.1, and 29.7.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (3,5-dimethoxyphenyl)amide (**24**). Yield: 49%; m.p. 123-124 °C; For $C_{17}H_{20}ClN_3O_3$ (349.8) calculated: 58.37% C, 5.76% H, 12.01% N; found: 58.57% C, 5.91% H, 12.05% N; R_F = 0.92; IR cm^{-1}: 3376 (NH), 2960, 2839 (OCH_3), 1698 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.31 (bs, 1H, NH), 9.25 (s, 1H, H3), 6.99 (d, 2H, *J*=2.20 Hz, H2´, H6´), 6.30 (t, 1H, *J*=2.20 Hz, H4´), 3.82 (s, 6H, OCH_3), and 1.55 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 164.6, 161.1, 159.8, 145.7, 141.0, 140.2, 138.7, 98.1, 97.5, 55.4, 39.0, and 28.3.

6-Chloropyrazine-2-carboxylic acid (5-bromo-2-hydroxyphenyl)-amide (**25**). Yield: 71%; m.p. 154-155 °C; For $C_{11}H_7BrClN_3O_2$ (328.6) calculated: 40.21% C, 2.15% H, 12.79% N; found: 40.51% C, 1.93% H, 13.05% N; R_F = 0.85; IR cm^{-1}: 3370 (NH), 1682 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.27 (bs, 1H, NH), 9.22 (d, 1H, *J*=1.1 Hz, H3), 8.98 (d, 1H, *J*=1.1 Hz, H5), 7.74 (d, 1H, *J*=2.47 Hz, H2´), 7.02 (dd, 1H, H4´), 6.62 (d, 1H, H5´), and 5.06 (bs, 1H, OH); ^{13}C-NMR ($CDCl_3$) δ 165.2, 149.4, 142.9, 141.1, 139.0, 131.2, 128.5, 123.6, 120.9, 116.1, and 110.1.

5-tert-Butylpyrazine-2-carboxylic acid (5-bromo-2-hydroxyphenyl)amide (**26**). Yield: 86%; m.p. 184-185 °C; For $C_{15}H_{16}BrN_3O_2$ (350.2) calculated: 51.44% C, 4.60% H, 12.00% N; found: 51.39% C, 5.61% H, 11.94% N; R_F = 0.82; IR cm^{-1}: 3368 (NH), 1685 (CO); ^{1}H NMR ($CDCl_3$) δ 9.55 (bs, 1H, NH), 9.37 (d, 1H, *J*=1.1 Hz, H3), 8.60 (d, 1H, *J*=1.1 Hz, H6), 8.08 (d, 1H, *J*=2.47 Hz, H3´), 7.47 (dd, 1H, *J*=8.79 Hz, *J*=2.47 Hz, H5´), 7.02 (d, 1H, *J*=8.79 Hz, H6´), 5.66 (bs, 1H, OH), and 1.44 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 167.9, 161.0, 149.4, 142.9, 141.1, 139.0, 131.2, 123.6, 120.9, 116.1, 110.1, 37.1, and 29.7.

5-tert-Butyl-6-chloropyrazine-2-carboxylic acid (5-bromo-2-hydroxyphenyl)amide (**27**). Yield: 77%; m.p. 160-161 °C; For $C_{15}H_{15}BrClN_3O_2$ (384.7) calculated: 46.84% C, 3.93% H, 10.92% N; found: 47.09% C, 4.12% H, 11.13% N; R_F = 0.86; IR cm^{-1}: 3373 (NH), 1691 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.28 (bs, 1H, NH), 9.25 (s, 1H, H3), 8.06 (d, 1H, *J*=2.47 Hz, H3´), 7.49 (dd, 1H, *J*=8.79 Hz, *J*=2.47 Hz, H5´), 7.03 (d, 1H, *J*=8.79 Hz, H6´), 5.65 (bs, 1H, OH), and 1.55 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 164.7, 159.7, 149.7, 145.8, 140.8, 140.2, 130.8, 123.8, 121.2, 116.1, 110.1, 39.0, and 28.3.

6-Chloropyrazine-2-carboxylic acid (3,4-dichlorophenyl)amide (**28**). Yield: 83%; m.p. 132-133 °C; For $C_{11}H_6Cl_2N_3O$ (302.5) calculated: 43.67% C, 2.00% H, 13.89% N; found: 43.51% C, 1.78% H, 14.11% N; R_F = 0.88; IR cm^{-1}: 3370 (NH), 1690 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.41 (bs, 1H, NH), 9.38 (s, 1H, H3), 8.83 (s, 1H, H5), 8.00 (d, 1H, *J*=2.47 Hz, H2´), 7.59 (dd, 1H, *J*=8.79 Hz, *J*=2.47 Hz, H6´), and 7.45 (d, 1H, *J*=8.79 Hz, H5´); ^{13}C-NMR ($CDCl_3$) δ 159.3, 147.8, 147.4, 143.2, 142.1, 136.1, 132.9, 130.7, 130.6, 128.3, 121.5, and 119.0.

5-tert-Butylpyrazine-2-carboxylic acid (3,4-dichlorophenyl)amide (**29**). Yield: 76%; m.p. 143-144 °C; For $C_{15}H_{15}Cl_2N_3O$ (324.2) calculated: 55.57% C, 4.66% H, 12.96% N; found: 55.63% C, 4.71% H,

13.08% N; R_F = 0.92; IR cm^{-1}: 3365 (NH), 1685 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.67 (bs, 1H, NH), 9.37 (d, 1H, *J*=1.37 Hz, H3), 8.61 (d, 1H, *J*=1.37 Hz, H6), 8.01 (d, 1H, *J*=2.48 Hz, H2´), 7.58 (dd, 1H, *J*=8.79 Hz, *J*=2.47 Hz, H6´), 7.43 (d, 1H, *J*=8.79 Hz, H5´), and 1.45 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 168.2, 161.2, 143.0, 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-Chloropyrazine-2-carboxylic acid (3,4-dichlorophenyl)amide (**30**). Yield: 83%, m.p. 113-114 °C For $C_{15}H_{14}Cl_3N_3O$ (358.7) calculated: 50.23% C, 3.93% H, 11.72% N; found: 55.63% C, 4.71% H, 13.08% N; R_F = 0.95; IR cm^{-1}: 3390 (NH), 1685 (CO); ^{1}H-NMR ($CDCl_3$) δ 9.38 (bs, 1H, NH), 9.25 (s, 1H, H3), 8.01 (d, 1H, *J*=2.47 Hz, H2´), 7.59 (dd, 1H, *J*=8.79 Hz, *J*=2.48 Hz, H6´), and 7.44 (d, 1H, *J*=8.79 Hz, H5´), 1.55 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 165.1, 159.9, 145.8, 140.5, 140.3, 136.5, 133.0, 130.7, 128.2, 121.6, 119.1, 39.1, and 28.2.

Antimycobacterial assay

Antimycobacterial evaluation was carried out at 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 $\mu g \cdot mL^{-1}$ against *Mycobacterium tuberculosis* strain $H_{37}Rv$ in BACTEC 12B medium using the BACTEC 460 radiometric system [22,23]. The results are presented in Table 1.

In vitro antifungal susceptibility testing

The broth microdilution test [24,14] 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 $\mu mol \cdot L^{-1}$. 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

The inhibition of oxygen evolution rate (OER) in spinach chloroplasts by the studied compounds was investigated spectrophotometrically (Specord UV VIS, Zeiss, Jena) in the presence of an electron acceptor 2,6-dichlorophenol-indophenol, using method described in Ref. [25]. The compounds were dissolved in DMSO because of their low water solubility. The used DMSO volume fractions (up to 5 vol. %) did not affect the oxygen evolution. The inhibitory efficiency 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 atrazine [26] is about 1.0 μmol·L^{-1}, the result are summarized in Table 1.

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