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# Substituted pyrazine-2,5-dicarboxamides: Synthesis, Hydrophobicity Parameters, and Antimycobacterial Evaluation

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**Abstract:** Substituted pyrazine derivatives were synthesized and tested against *Mycobacterium tuberculosis* strain $H_{37}Rv$. The hydrophobicity of all the pyrazines was determined using the reversed phase high performance liquid chromatography (RP-HPLC) method (isocratic elution with methanol as an organic modifier in the mobile phase, end-capped non-polar $C_{18}$ stationary RP column). Experimentally derived Log *K* values (the logarithm of capacity factor *K*) were that compared with Log *P* values calculated by commercially available programmes. The synthetic approach, analytical, spectroscopic, lipophilicity and biological data of ten newly synthesized compounds are presented. Structure—activity relationships among the chemical structure, the anti-mycobacterial activity of the evaluated compounds are discussed. 3-(3-Methylphenyl)-aminopyrazine-2,5-dicarboxamide (**7**) has shown the highest activity against *M. tuberculosis* $H_{37}Rv$ (63% inhibition).

**Keywords:** Pyrazinecarboxamides; In vitro antimycobacterial activity; Lipophilicity determination.

## Introduction

Tuberculosis (TB) has added greatly to the burden of poor health in developing countries. Worldwide, TB now kills one person every 18 seconds, and because infection with HIV increases the chances that latent TB will develop into active disease, TB's effects now are being amplified. Every year, tuberculosis kills some 2 million people, many of whom also suffered from HIV. The World Health Organization (WHO) has expressed concern over the emergence of virulent drug-resistant strains of tuberculosis (TB) and is calling for measures to be strengthened and implemented to prevent the global spread of the deadly TB strains. Multidrug Resistant TB (MDR-TB) describes strains of tuberculosis that are resistant to at least the two main first-line TB drugs — isoniazid (INH) and rifampicin. Extensive Drug Resistant TB (also referred to as Extreme Drug Resistance, XDR-TB) is MDR-TB that is also resistant to three or more of the six classes of second-line drugs. The description of XDR-TB was first used earlier in 2006, following a joint survey by WHO and the US Centers for Disease Control and Prevention. This follows research showing the extent of XDR-TB, a newly identified TB threat which leaves patients (including many people living with HIV) virtually untreatable using currently available anti-TB drugs. Therefore there is an urgent need to develop new agents active against MDR bacteria possibly having different mechanism of action. [1, 2].

Pyrazinamide (PZA) was discovered through an effort to find antitubercular nicotinamide derivatives. The activity of PZA appears to be pH dependent, since it is bactericidal at pH 5.5, but inactive at neutral pH. PZA is a popular drug used in many combinations, but unfortunately resistance develops very quickly. It is especially effective against semi-dormant mycobacterium, and it is used in combinations with INH and rifampicin. Its mechanism of appears to involve its hydrolysis to pyrazinoic acid *via* the bacterial enzyme pmcA [3]. PZA can also be metabolized to pyrazinoic acid by hepatic microsomal deamidase, which becomes a substrate for xanthine oxidase, affording 5-hydroxypyrazinoic acid. The acid is believed to act as an antimetabolite of nicotinamide and interferes with NAD biosynthesis. Pyrazinoic acid disrupted membrane energetics and inhibited membrane transport function in *M. tuberculosis* [4]. Resistance on PZA arises by the absence of the enzyme, Pmc A. The major side effect of PZA is a dose-related hepatotoxicity. The different analog of PZA, 5-chloropyrazinamide, has previously been shown to inhibit mycobacterial fatty acid synthase I (FAS I) [5].

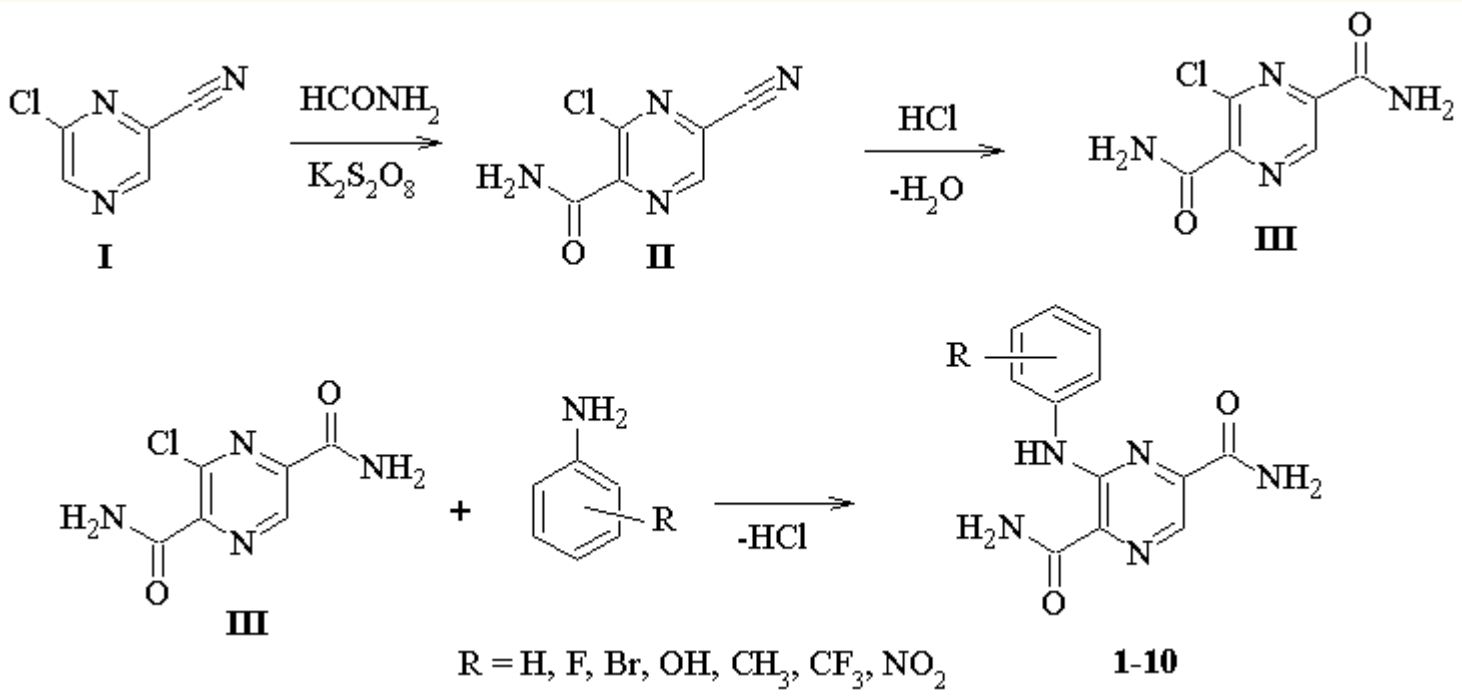
Our research was focused on PZA analogues with the doubled -$CONH_2$ moieties, which can form centro symmetric dimer pairs with the peptidic carboxamido group of some peptide, needed for binding to the receptor site, possibly by forming of hydrogen bond. All substituted amides of pyrazinecarboxylic acid studied can be interpreted as some more lipophilic aza-analogues of nicotinamide.

Most frequently the drugs cross biological barriers by means of passive transport, which strongly depends on the lipophilicity. Therefore hydrophobicity is one of the most important physical properties of biologically active compounds. This thermodynamic parameter describes the partitioning of a compound between an aqueous and an organic phase and is characterized by the partition (log *P*) coefficient [6, 7]. The report that 5-chloropyrazine-2-carboxamide has different mode of action than pyrazinamide itself makes this direction in research more than relevant [8]. This study deals with pyrazinamide structure modification and shows the compounds derived from

the newly prepared 3-chloropyrazine-2,5-dicarboxamide that are formed by nucleophillic substitution of chlorine in position 3. In this case 3-arylaminopyrazine-2,5-dicarboxamide derivatives with different substitution in aryl part of the molecule are formed.

**Results and Discussion**

Homolytic amidation of 6-chloropyrazine-2-carbonitrile (**I**) yielded in 5-cyano-3-chloropyrazine-2-carboxamide (**II**). Via the hydrolysis was obtained 3-chloropyrazine-2,5-dicarboxamide (**III**) as a starting material [9, 10]. Chlorine of **III** then undertakes nucleophilic substitution with some ring substituted anilines in dry toluene with pyridine as a base (see **Scheme 1**).




**Scheme 1.** Synthesis of substituted pyrazine-2,5-dicarboxamides **1—10**.

All compounds prepared were screened at the TAACF screening (Tuberculosis Antimicrobial Acquisition and Coordinating Facility by The National Institute of Health of the US government) [11]. The primary screen was conducted at 6.25 μg/ml (or molar equivalent of highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [12]. Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system. Compounds effecting <90% inhibition in the primary screen (MIC >6.25 μg/ml) were not generally evaluated further.

Some interesting results were obtained, as shown in **Table 1**. All compounds prepared possess anti-mycobacterial effect (34—63%). The highest activity against *Mycobacterium tuberculosis* $H_{37}Rv$ was found for 3-(3-methylphenyl)-aminopyrazine-2,5-dicarboxamide (**7**, 63% inhibition). 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.

Hydrophobicity parameters of the discussed compounds 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 2.12 to 4.83. The increasing lipophilicity of the substituents 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, see Table 1. The linear dependence of log *K* on log *P* is illustrated in Figure 1. The parameters of this correlation (r = 0.9694, n = 10) confirmed in accordance between experimental log *K* values with the corresponding calculated log *P* values. The lowest lipophilicity showed 2-(4-hydroxyphenyl)-pyrazine-3,5-dicarboxamide (**1**), whereas 2-(2,4-dibromo-6-nitrophenyl)pyrazine-3,5-dicarboxamide (**10**) possesses the highest lipophilicity, as expected.

Distributive parameters π have been firmly established as the parameter of choice for correlating both binding to biological macromolecules and transport through a biological system. Constant π describes lipophilicity contribution of individual moieties substituted in some skeleton. These π parameters characterizing hydrophobicity of the individual substituents were calculated according to the formula $\pi = \log K_S - \log K_U$, where log $K_S$ is the determined capacity factor logarithm of the individual substituted compounds, whereas log $K_U$ denotes the determined capacity factor logarithm of the unsubstituted compound, it means $\pi_{\mathbf{4}} = 0$. The π values of the individual substituents in the discussed compounds are shown in Table 2.

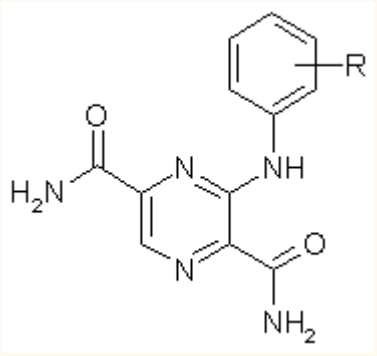
Distributive constants π of individual substituents are dependent on the basic skeleton (aliphatic, aromatic, heteroaromatic), as well as on the character of heteroaromatic system. A number of distributive parameters π for various substituents for the all three substituent positions in the benzene ring have been described [13]. Pyrazine derivatives possess very special properties due to the pyrazine ring that shows as aliphatic / aromatic as electron-releasing / electron-withdrawing characteristic [14, 15]. Therefore the distributive parameters π of the discussed pyrazine derivatives were tried to describe and to compare with π and $\pi^{(-)}$ substituent constants of benzene systems obtained from the literature [16]. All the distributive parameters π and $\pi^{(-)}$ are shown in **Table 2**. The determined π parameters of substituents can be used for describing relationships between physicochemical properties and activity of prepared ring substituted pyrazine based compounds.

Based on the log *K* data, the lipophilicity of the substituents in the aromatic part of the molecule increased in the following order: 4-OH < 3-OH < 2-OH < H < 3-F < 3-$CH_3$ < 3-Cl < 3-$CF_3$ < 3-Br < 2,4-Br-6-$NO_2$. This is in accordance with the values of corresponding π-parameters characterizing hydrophobicity of the substituents with exceptions 3-$CF_3$ and 3-Br moieties, see Tables 1 and 2. For *ortho* $NO_2$ moiety the value of the π-parameter is not published.

The differences between the experimental data (log *K* or π) and the calculated lipophilicity parameters (log *P*) or π distributive parameters obtained from ref [1] were observed at 3-$CF_3$ and 3-Br substituents. *N*-Phenyl derivative substituted by the 3-$CF_3$ moiety showed lower hydrophobicity than 3-Br moiety substituted compound according to experimental data. All these differences between π parameters of substituents are probably caused by physicochemical properties of the pyrazine core and intramolecular interactions of heteroatoms with the individual substituents.

It can be assumed, that capacity factor *K* / calculated log *K* values or experimentally determined π parameters specify lipophilicity within the individual series of compounds.

**Table 1.** Comparison of the inhibition (%, antimycobacterial activity) with the calculated lipophilicities (log *P* data) determined log *K* values of the discussed substituted pyrazine derivatives **1**—**10**, as well as the determined distributive parameters π calculated from log *K*. The distributive parameters π of the discussed substituted pyrazine derivatives obtained from literature [16].




| Comp. | R | Inhibition (%) | UV $\lambda_{max}$ / log ε | Purity (%) | log *K* | log *P* ACD/Log P | $\pi_{determined}$ |
|---|---|---|---|---|---|---|---|
| **1** | 4-OH | 38 | 295.0 / 3.41 | 92.58 | 0.5633 | 2.12 ± 0.53 | -0.15 |
| **2** | 3-OH | 34 | 302.2 / 3.41 | 98.89 | 0.5801 | 2.51 ± 0.54 | -0.13 |
| **3** | 2-OH | 42 | 308.1 / 3.62 | 97.96 | 0.6303 | 2.50 ± 0.54 | -0.08 |
| **4** | H | 52 | 296.2 / 3.53 | 99.83 | 0.7130 | 2.86 ± 0.53 | 0.00 |
| **5** | 3-F | 54 | 297.4 / 3.47 | 99.70 | 0.7345 | 3.35 ± 0.60 | 0.02 |
| **6** | 3-$CH_3$ | 63 | 297.4 / 3.33 | 99.74 | 0.8048 | 3.32 ± 0.53 | 0.09 |
| **7** | 3-Cl | 45 | 297.4 / 3.34 | 99.76 | 0.8307 | 3.89 ± 0.55 | 0.12 |
| **8** | 3-$CF_3$ | 57 | 293.8 / 3.50 | 99.64 | 0.8337 | 4.07± 0.58 | 0.12 |
| **9** | 3-Br | 50 | 297.4 / 3.37 | 99.40 | 0.8603 | 4.07 ± 0.60 | 0.15 |
| **10** | 2,4-Br-6-$NO_2$ | 35 | 237.0 / 3.38 | 99.43 | 0.9062 | 4.53 ± 0.68 | 0.19 |

**Figure 1.** Dependence of experimentally determined log *K* values on calculated log *P* values of the discussed compounds.

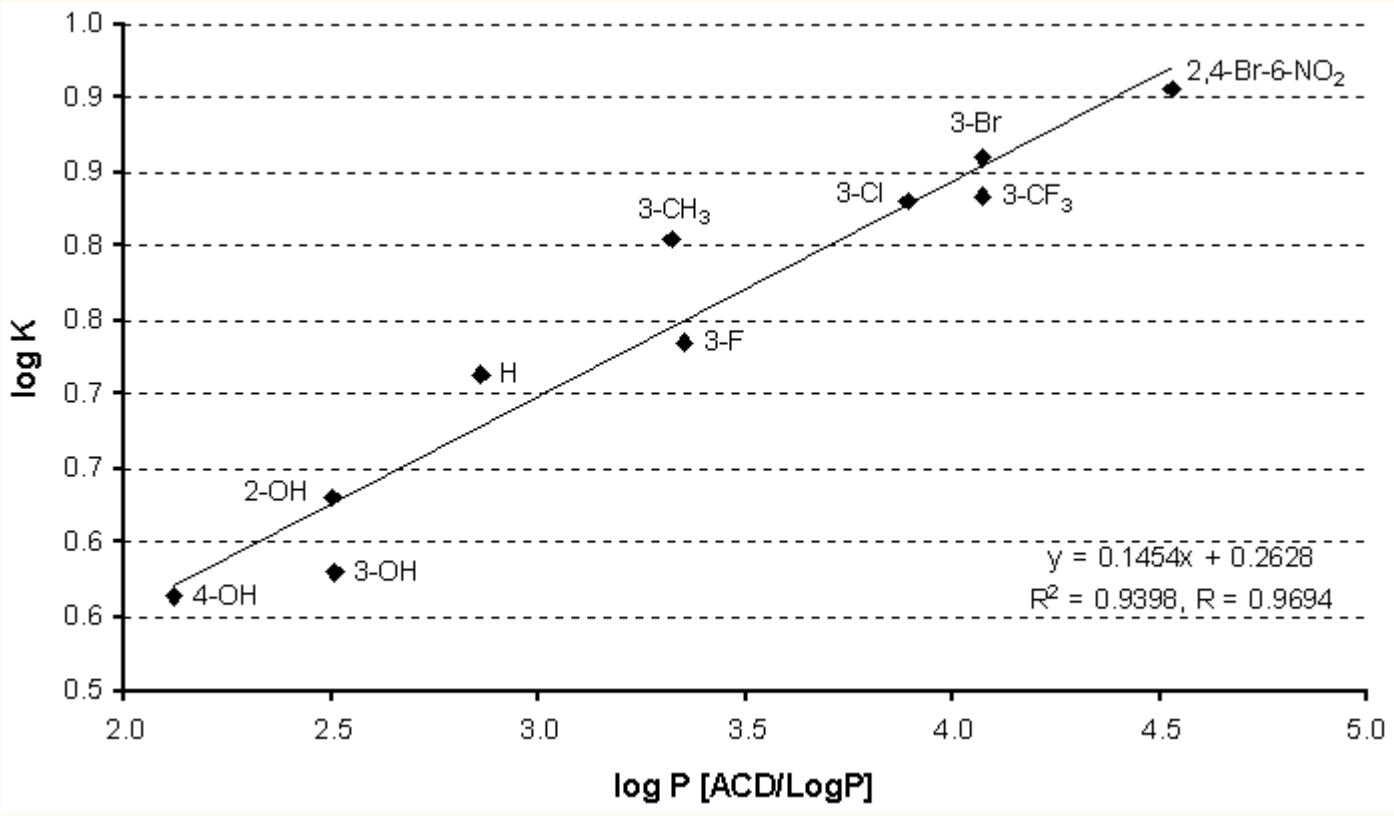
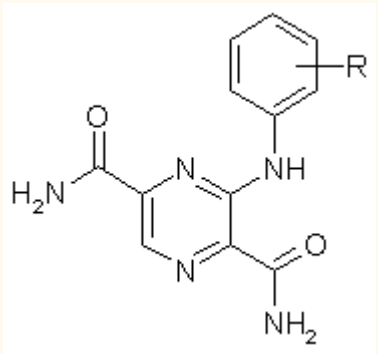



**Table 2.** The distributive parameters π and $\pi^{(-)}$, the electronic parameters F and R and the bulk parameters MR of the discussed substituted pyrazine derivatives obtained from the literature [17]. ($\pi^{(-)}$: the distributive parameters for substituents on electron-donating benzene systems, *i.e.* aniline, phenol; F: the Swain-Lupton constant for inductive effect of substituents; R: the Swain-Lupton constant for mesomeric effect of substituents).




| Comp. | R | π | $\pi^{(-)}$ | F | R | MR |
|---|---|---|---|---|---|---|
| **MD 391/II** | 4-OH | -0.61 | -0.87 | 0.49 | -0.64 | 1.5 |
| **MD 390/II** | 3-OH | -0.50 | -0.66 | 0.48 | -0.22 | 1.5 |
| **MD 386/II** | 2-OH | -0.41 | -0.58 | 0.61 | -0.56 | 1.5 |
| **MD 395/II** | H | 0 | 0 | 0 | 0 | 0 |
| **MD 389/II** | 3-F | 0.22 | 0.47 | 0.69 | -0.12 | -0.4 |
| **MD 392/II** | 3-$CH_3$ | 0.52 | 0.50 | -0.05 | -0.05 | 4.7 |
| **MD 387/II** | 3-Cl | 0.77 | 1.04 | 0.68 | -0.06 | 4.8 |
| **MD 393/II** | 3-$CF_3$ | 1.10 | 1.49 | 0.62 | 0.07 | 4.0 |
| **MD 388/II** | 3-Br | 0.96 | 1.17 | 0.71 | -0.06 | 7.6 |
| **MD 394/II** | 2,4-Br-6-$NO_2$ | *a* | *a* | *a* | *a* | 21.2 |

[a] data for -$NO_2$ moiety in *ortho* position of benzene ring have not been found.

## Experimental procedures

### Chemistry

#### *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 $9\times10^{-4}$ mol methanolic solution and log ε (the logarithm of molar absorption coefficient ε) was calculated for the absolute maximum $\lambda_{max}$ 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 $^{13}C$), Varian (Palo Alto CA, USA) 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 compounds was checked by HPLC. 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. A purity of the individual compounds was determined from area peaks in the chromatogram of the sample solution.

UV spectra (λ, nm) were determined on a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) in ca $8.10^{-4}$ M methanolic solution. Log ε (the logarithm of molar absorption coefficient ε) was calculated for the absolute maximum $\lambda_{max}$ of the individual compounds.

#### *General coupling method*

Substituted 3-chloropyrazine-2,5-dicarboxamide (1 mmol) was dissolved in dry toluene (50 mL), arylamine (1 mmol) and dry pyridine (1 mmol) was added, and the mixture was refluxed for 1 hour at 110 °C. After cooling, the mixture was filtered, the solvent was then removed under reduced pressure and the crude product was recrystallised from water / ethanol.

**3-(4-Hydroxyphenyl)-aminopyrazine-2,5-dicarboxamide** (**1**). Yield: 49%. Anal. Calc. for $C_{12}H_{11}N_5O_3$

(273.25): 52.75%, H 4.06%, N 25.63%; Found: C 52.76%, H 4.12%, N 25.49%. Mp. 223—224°C. Appearance: red crystals. HPLC purity 92.58%. IR (KBr), $cm^{-1}$: 1680 (C=O), 1611 (C=O). $^{1}H$ NMR (300 MHz, DMSO) δ: 11.15 (1H, bs, NH), 9.36 (1H, s, OH), 8.56 (1H, bs, $NH_2$), 8.39 (1H, s, H6), 8.16 (1H, bs, $NH_2$), 7.43-7.32 (2H, m, AA', BB', H2', H6'), 6.84-6.71 (2H, m, AA', BB', H3', H5'). $^{13}C$ NMR (75 MHz, DMSO) δ: 167.7, 154.3, 151.3, 134.5, 130.9, 129.5, 129.0, 122.8, 116.3, 115.7.

**3-(3-Hydroxyphenyl)-aminopyrazine-2,5-dicarboxamide** (**2**). Yield: 39%. Anal. Calc. for $C_{12}H_{11}N_5O_3$ (273.25): 52.75%, H 4.06%, N 25.63%; Found: 52.89%, H 3.87%, N 25.77%. Mp. 203—206°C. Appearance: orange crystals. HPLC purity 98.89%. IR (KBr), $cm^{-1}$: 1677 (C=O), 1609 (C=O). $^{1}H$ NMR (300 MHz, DMSO) δ: 11.48 (1H, s, OH), 9.56 (1H, bs, NH), 8.65 (1H, bs, $NH_2$), 8.49 (1H, s, H6), 8.24 (1H, bs, $NH_2$), 7.19 (1H, H2'), 7.17-7.12 (1H, H5'), 6.97-6.92 (1H, H6'), 6.59-6.49 (1H, H4'). $^{13}C$ NMR (75 MHz, DMSO) δ: 167.7, 158.1, 151.0, 139.3, 135.3, 131.3, 130.0, 128.8, 115.3, 111.1, 111.0, 107.1.

**3-(2-Hydroxyphenyl)-aminopyrazine-2,5-dicarboxamide** (**3**). Yield: 27%. Anal. Calc. for $C_{12}H_{11}N_5O_3$ (273.25): 52.75% C, 4.06% H, 25.63% N; Found: 52.69% C, 3.95% H, 25.53% N. Mp. 276—278°C. Appearance: orange crystals. HPLC purity 97.96%. IR (KBr), $cm^{-1}$: 3444 (NH), 3331 (OH), 1679 (CO), (1612), 1578 (phenyl), 1520 (NH), 1459, 1340, 1283, 1190, 1105 (pyrazine). $^{1}H$ NMR (300 MHz, DMSO) δ: 11.56 (1H, s, OH), 10.05 (1H, bs, NH), 8.54 (1H, s, H6), 8.44 (1H, bs, $NH_2$), 8.26-8.22 (1H, H4'), 8.10 (1H, H5'), 6.90 (1H, H3'), 6.88-6.80 (1H, H6'). $^{13}C$ NMR (75 MHz, DMSO) δ: 167.7, 150.8, 147.4, 134.8, 131.7, 128.7, 126.9, 123.7, 119.8, 119.2, 116.3, 114.8.

**3-Phenylaminopyrazine-2,5-dicarboxamide** (**4**). Yield: 43%. Anal. Calc. for $C_{12}H_{11}N_5O_2$ (257.25): C 56.03 %, H 4.31 %, N 27.22%; Found: C 55.83%, H 4.47%, N 27.61%. Mp. 194—195°C. Appearance: orange crystals. HPLC purity 99.83%. IR (KBr), $cm^{-1}$: 1688 (C=O), 1619 (C=O). $^{1}H$ NMR (300 MHz, DMSO) δ: 11.50 (1H, bs, NH), 8.64 (1H, bs, NH2), 8.49 (1H, s, H6), 8.24 (1H, bs, NH2), 7.64-7.60 (2H, AA', BB', H2', H6'), 7.40-7.34 (2H, AA', BB', H3', H5'), 7.14-7.07 (1H, H4'). $^{13}C$ NMR (75 MHz, DMSO) δ: 138.3, 135.4, 131.3, 129.3, 128.7, 123.8, 120.4, 116.2.

**3-(3-Fluorfenyl)-aminopyrazin-2,5-dicarboxamide** (**5**). Yield: 68%. Anal. Calc. for $C_{12}H_{10}FN_5O_2$ (275.24): C 52.37%, H 3.66%, N 25.44%; Found: C 52.10%, H 3.51%, N 25.28%. Mp. 189—190°C. HPLC purity 99.70%. IR (KBr), $cm^{-1}$: 1680 (C=O), 1611 (C=O). $^{1}H$ NMR (300 MHz, DMSO) δ: 11.66 (1H, bs, NH), 8.68 (1H, bs, $NH_2$), 8.56 (1H, s, H6), 8.38 (1H, bs, $NH_2$), 7.70-7.62 (1H, H2'), 7.42-7.35 (1H, H6'), 7.35-7.29 (1H,H5'), 6.93-6.88 (1H, H4'). $^{13}C$ NMR (75 MHz, DMSO) δ: 167.5, 164.1, 160.9, 150.7, 140.1, 140.0, 136.0, 131.6, 130.9, 130.7, 128.5, 116.3, 116.2, 110.2, 110.0, 107.1, 106.8.

**3-(3-Methylphenyl)-aminopyrazine-2,5-dicarboxamide** (**6**). Yield: 46%. Anal. Calc. for $C_{13}H_{13}N_5O_2$ (271.28): C 57.56%, H 4.83%, N 25.82% N; Found: C 57.59%, H 4.90%, N 25.98%. Mp. 194—195°C. Appearance: red crystals. HPLC purity 99.74%. IR (KBr), $cm^{-1}$: 1689 (C=O), 1616 (C=O). $^{1}H$ NMR (300 MHz, DMSO) δ: 11.47 (1H, bs, NH), 8.63

(1H, bs, $NH_2$), 8.48 (1H, s, H6), 8.23 (1H, bs, $NH_2$), 7.51 (1H, dd, *J*=7.7 Hz, *J*=1.6 Hz, H6'), 7.33 (1H, bs, H2'), 7.25 (1H, t, *J*=7.7 Hz, H5'), 6.91 (1H, d, *J*=7.7 Hz, H4'), 2.30 (3H, s, $CH_3$). $^{13}$C NMR (75 MHz, DMSO) δ: 167.7, 151.0, 138.6, 138.2, 135.3, 131.2, 129.1, 128.8, 124.6, 120.9, 117.4, 116.2, 21.3.

**3-(3-Chlorophenyl)-aminopyrazine-2,5-dicarboxamide** (**7**). Yield: 20%. Anal. Calc. for $C_{12}H_{10}ClN_5O_2$ (291,70): C 49.41%, H 3.46%, N 24.01%; Found: C 49.49%, H 3.59%, N 24.18%. Mp. 239—240°C. Appearance: orange crystals. HPLC purity 99.76%. IR (KBr), $cm^{-1}$: 1694 (C=O), 1597 (C=O). $^1$H NMR (300 MHz, DMSO) δ: 11.62 (1H, bs, NH), 8.66 (1H, bs, $NH_2$), 8.56 (1H, s, H6), 8.27 (1H, bs, $NH_2$), 7.84 (1H, t, *J*=2.1 Hz, H2'), 7.52-7.45 (1H, m, H6'), 7.38 (1H, t, *J*=8.0 Hz, H5'), 7.17-7.11 (1H, m, H4'). $^{13}$C NMR (75 MHz, DMSO) δ: 167.5, 150.7, 139.8, 136.1, 133.5, 131.6, 130.8, 128.5, 123.4, 119.6, 119.0, 116.1.

**3-(3-Trifluoromethylfenyl)-aminopyrazin-2,5-dicarboxamide** (**8**). Yield: 22%. Anal. Calc. for $C_{13}H_{10}F_3N_5O_2$ (325.25): C 48.01%, H 3.10%, N 21.53%; Found: C 47.78%, H 3.18%, N 21.43%. Mp. 197—199°C. Appearance: yellow crystals. HPLC purity 99.64%. IR (KBr), $cm^{-1}$: 1684 (CO), 1611 (CO). $^1$H NMR (300 MHz, DMSO) δ: 9.26 (1H, s, H6), 8.30 (1H, bs, $NH_2$), 8.16 (1H, bs, $NH_2$). $^{13}$C NMR (75 MHz, DMSO) δ: 164.6, 151.4, 146.4, 145.1, 128.7, 114.9.

**3-(3-Bromophenyl)-aminopyrazine-2,5-dicarboxamide** (**9**). Yield: 59%. Anal. Calc. for $C_{12}H_{10}BrN_5O_2$ (336.15): C 42.88%, H 3.00%, N 20.83%; Found: 42.78%, H 2.87%, N 20.89%. Mp. 243—244°C. Appearance: orange crystals. HPLC purity 99.40%. IR (KBr), $cm^{-1}$: 1691 (C=O), 1615 (C=O). $^1$H NMR (300 MHz, DMSO) δ: 11.60 (1H, bs, NH), 8.68 (1H, bs, $NH_2$), 8.57 (1H, s, H6), 8.26 (1H, bs, $NH_2$), 7.97 (1H, H2'), 7.57-7.51 (1H, H6'), 7.36-7.33 (1H, H4'), 7.33-7.25 (1H, H5'). $^{13}$C NMR (75 MHz, DMSO) δ: 167.5, 150.7, 139.9, 136.1, 131.7, 131.1, 128.5, 126.3, 122.5, 121.9, 119.4, 116.1.

**3-(2,4-Dibromo-6-nitrophenyl)-aminopyrazine-2,5-dicarboxamide** (**10**). Yield: 49%. Anal. Calc. for $C_{12}H_8Br_2N_6O_4$ (460.04): C 31.33%, H 1.75%, N 18.27 %; Found: C 31.15%, H 1.69 %,N 18.59%. Mp. 131—132°C. Appearance: orange-yellow crystals. HPLC purity 99.43%. IR (KBr), $cm^{-1}$: 1690 (C=O), 1614 (C=O). $^1$H NMR (300 MHz, DMSO) δ: 8.30 (1H, bs, NH), 8.15 (1H, bs, $NH_2$), 8.06 (1H, bs, $NH_2$), 7.28 (1H, s, H6). $^{13}$C NMR (75 MHz, DMSO) δ: 142.2, 140.8, 132.3, 127.7, 112.7, 105.3, 79.4.

***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 [18]. 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 program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). The results are shown in Table 1.

## Biological evaluation

### *Antimycobacterial assay*

Antimycobacterial evaluation was carried out in the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL, USA, which is a part of the National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.25 µg/mL against *M. tuberculosis* $H_{37}$Rv (ATCC27294) in BACTEC 12B medium using both BACTEC 460 radiometric system and the Microplate Alamar Blue Assay (MABA).

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