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Synthesis and Hydrophobic Properties of Some Substituted 3-Arylaminopyrazine-2,5-dicarbonitriles

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Abstract: The series of thirteen mono-substituted 3-arylaminopyrazine-2,5-dicarbonitriles was prepared by the reaction of 3-chloropyrazine-2,5-dicarbonitrile with the appropriated anilines. The general synthetic approach of all newly synthesized compounds is presented. All the substituted 3-arylaminopyrazine-2,5-dicarbonitriles, which showed significant antifungal and antimycobacterial activity, were analyzed using the reversed phase high performance liquid chromatography (RP-HPLC) method for the lipophilicity measurement. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP column. In the present study the correlation between RP-HPLC retention parameter log *K* (the logarithm of capacity factor *K*) and log *P* data calculated in various ways is shown. The relationships between the lipophilicity and the chemical structure of the studied compounds are discussed as well.

Keywords: 3-Arylaminopyrazine-2,5-dicarbonitriles; Lipophilicity measurement; Structure-lipophilicity relationships.

Introduction

Pyrazinamide (PZA) is a frontline tuberculosis-specific-drug that is used in combination with other drugs. Modification of its molecule is still up to date. Based on the results described in refs. [1-6] a series of new pyrazinamide analogues were prepared [7, 8]. Most compounds showed antituberculotic activity against *M. tuberculosis* comparable with or higher than the standard PZA. Several compounds possessed excellent antituberculotic activities against opportunistic pathogen *M. kansasii* and *M. avium*, as well [7, 8].

One of the major prerequisites for drug development is the prediction of the transport of a molecule through cellular membranes. Most frequently the drugs cross the 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.

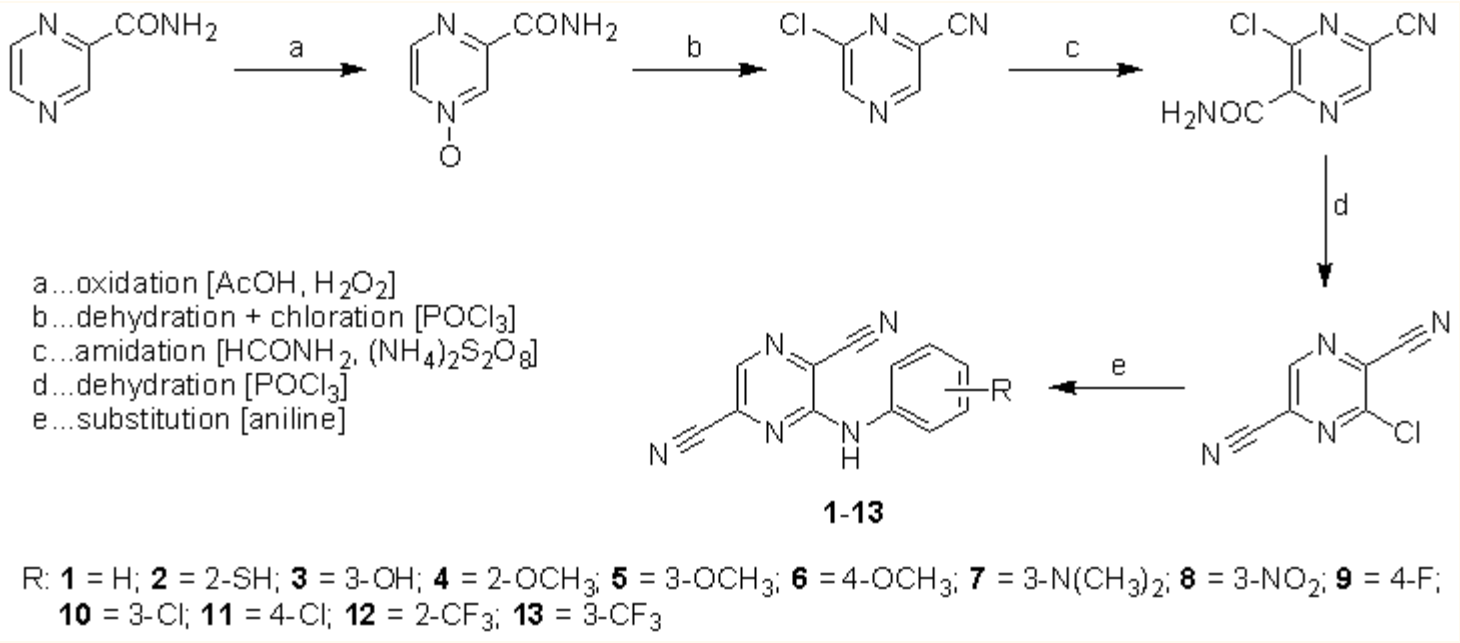
Substituted 3-arylaminopyrazine-2,5-dicarbonitriles, which showed significant antimicrobial activity, were analyzed using the reversed phase high performance liquid chromatography (RP-HPLC) method for the lipophilicity measurement. A general procedure is the measurement of the directly accessible retention time under isocratic conditions with varying amounts of an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP columns and calculating the capacity factor *K*. Log *K*, calculated from the capacity factor *K*, is used as the lipophilicity index converted to log *P* scale [9].

This study is a follow-up paper to the previous articles [1-8, 10-13] and deals with the synthesis and physico-chemical properties of the newly prepared pyrazine derivatives as potential drugs.

Results and Discussion

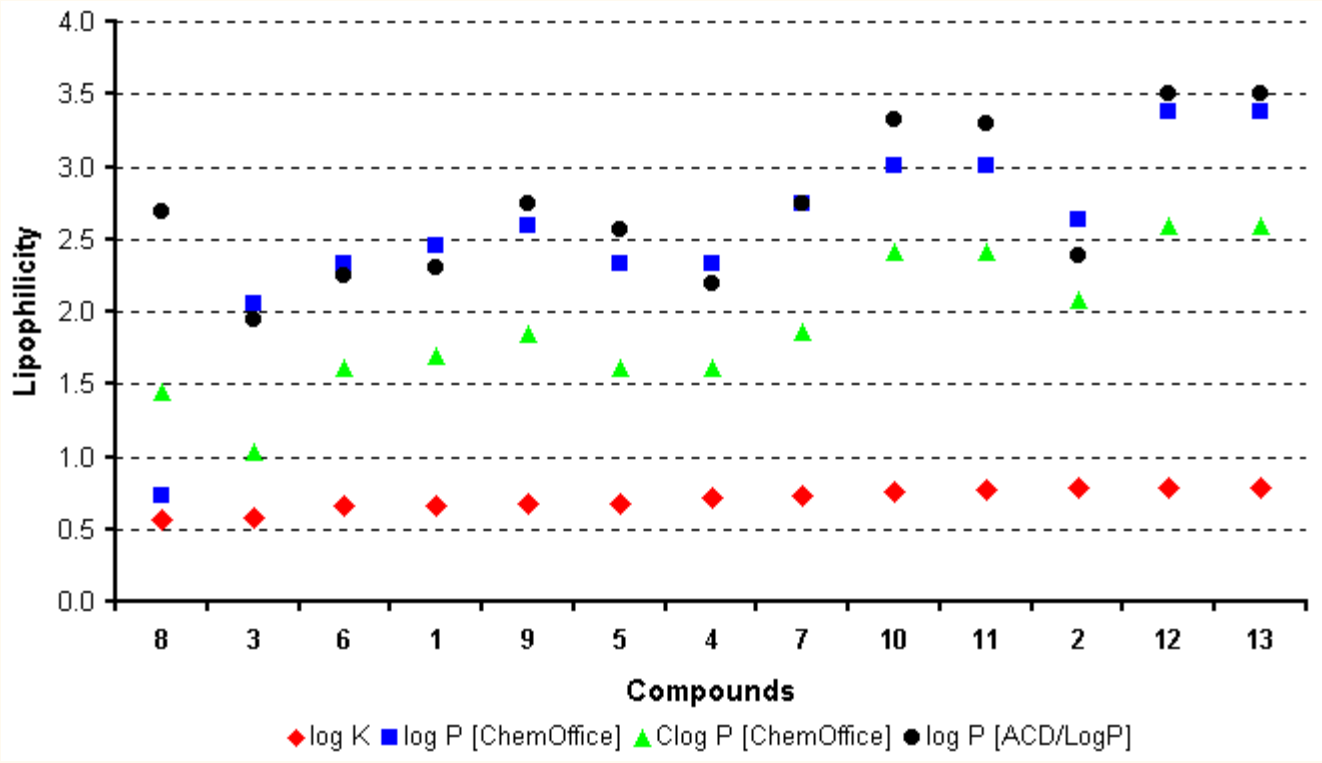
The formation of the target compounds **1**-**13** is a multistep process. The studied compounds were synthesized according to Dolezal [1, 5] see Scheme 1. Synthesis and characterization of the discussed pyrazine-2-carboxylic acid derivatives are described in reference [7, 8]. The substitution on the benzene ring was chosen in accordance with Topliss [14].

Scheme 1. Synthesis and structures of the prepared substituted 3-arylaminopyrazine-2,5-dicarbonitriles **1**-**13**.



Hydrophobicities (log *P* / Clog *P* data) of the studied compounds were calculated using two commercially available programs and measured by means of RP-HPLC determination of capacity factors *K* with a subsequent calculation of log *K*. The results are shown in Table 1. All the discussed hydrophobicity data of individual compounds are illustrated in Figure 1 and they are ordered according to the experimental log *K* values increase.

Figure 1. Comparison of log *P* / Clog *P* data calculated using the two programs with the experimentally found log *K* values. The discussed compounds are ordered according to the log *K* values increase.



The program ChemOffice has not resolved various lipophilicity values of individual positional isomers, *e.g.* the compounds **4**-**6**, **10** and **11**, or **12** and **13**, respectively.

The results show that the experimentally determined lipophilicities (log *K* values) are lower than those indicated by the calculated log *P* / Clog *P* and correlate relatively poorly with the calculated parameters. All the showed differences between experimental and calculated lipophilicity values are probably caused by the presence of nitrile moieties and interactions of substituents with these moieties and pyrazine nitrogen in individual compounds.

As expected, 3-(3-nitrophenylamino)pyrazine-2,5-dicarbonitrile (**8**) and 3-(3-hydroxyphenylamino)pyrazine-2,5-dicarbonitrile (**3**) show the lowest lipophilicity, whereas 3-(trifluoromethylphenylamino)pyrazine-2,5-dicarbonitriles (**12** and **13**) possess the highest hydrophobicities within the discussed series of pyrazine derivatives. The 3-CF_3 substituted compound **13** is more lipophilic than the positional isomer 2-CF_3 substituted pyrazine-2,5-dicarbonitrile (**12**). This fact corresponds with the calculated log *P* using the program ACD/LogP.

According to our expectation hydrophilic nitro and phenolic moieties (compounds **8** and **3**) show lower lipophilicity than unsubstituted 3-phenylaminopyrazine-2,5-dicarbonitrile (**1**). The compound **4** substituted by 3-methoxy moiety is less lipophilic than the 3-*N*,*N*-dimethylamino substituted compound **10**, as expected [15]. The compound **4** possesses higher hydrophobicity than the unsubstituted compound **1**, as well.

The substitutions by chlorine atoms (compounds **10** and **11**) show lower lipophilicity than the trifluoro substitutions (compounds **12** and **13**) but higher hydrophobicity than the 4-fluoro substituted compound **9**, which is more lipophilic than the compound **1**. According to the log *K* data the 4-Cl derivative **11** is more lipophilic than the 3-Cl pyrazine derivative **10**, contrary to the results obtained using the program ACD/LogP.

According to the program ACD/LogP lipophilicity of the compounds substituted by methoxy moiety in phenyl ring increases $C_{(2)} < C_{(4)} < C_{(3)}$, but experimental log *K* data for these compounds increase $C_{(4)} < C_{(3)} < C_{(2)}$. The compound **6** substituted by 4-methoxy group possesses lower lipophilicity than the unsubstituted compound **1**, which is in good agreement with all the calculated data.

Great differences could be observed at the compound **2**. According to all the calculated data 3-(2-mercaptophenylamino)pyrazine-2,5-dicarbonitrile (**2**) shows lower lipophilicity contrary to experimentally found log *K*. The 2-sulfanyl pyrazine derivative **2** possesses high hydrophobicity due to 2-SH moiety probably because of intramolecular interactions of $C_{(2)}$ mercapto moiety with heteroatoms of pyrazine nucleus.

Experimetal

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

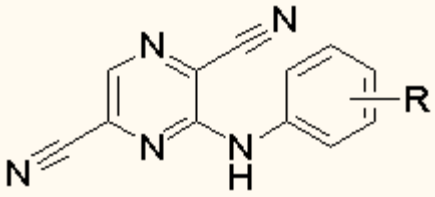
MeOH p.a. (70.0%) and H_2O-HPLC – Mili-Q Grade (30.0%) was used as a mobile phase. The total flow of the column was 1.0 mL/min, injection 30 µL, column temperature 30 °C and sample temperature 10 °C. The detection wavelength 210 nm was chosen. The KI solution was used for the dead time (T_D) determination. 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. The log *K* values of the individual compounds, calculated from the capacity factor *K*, 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 programs CS ChemOffice Ultra ver. 9.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/LogP ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). Clog *P* values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra ver. 9.0 (CambridgeSoft, Cambridge, MA, U.S.A.) software. The results are shown in Table 1.

Table 1. Calculated lipophilicities (log *P* / Clog *P*) and determined log *K* of the studied substituted 3-phenylaminopyrazine-2,5-dicarbonitriles substituted **1**-**13**.



Compound	R	log *K*	log *P* / Clog*P* ChemOffice	log *P* ACD/LogP
1	H	0.6632	2.45 / 1.69623	2.30 ± 0.50
2	2-SH	0.7829	2.64 / 2.08923	2.39 ± 0.57
3	3-OH	0.5826	2.06 / 1.02923	1.95 ± 0.51
4	2-OCH_3	0.7202	2.33 / 1.61798	2.20 ± 0.52
5	3-OCH_3	0.6799	2.33 / 1.61798	2.56 ± 0.52
6	4-OCH_3	0.6607	2.33 / 1.61798	2.25 ± 0.51
7	3-$N(CH_3)_2$	0.7334	2.74 / 1.86123	2.75 ± 0.52
8	3-NO_2	0.5634	0.73 / 1.44895	2.69 ± 0.53
9	4-F	0.6789	2.60 / 1.84376	2.75 ± 0.57
10	3-Cl	0.7578	3.01 / 2.41376	3.33 ± 0.52
11	4-Cl	0.7730	3.01 / 2.41376	3.29 ± 0.52
12	2-CF_3	0.7831	3.38 / 2.58717	3.50 ± 0.56
13	3-CF_3	0.7836	3.38 / 2.58717	3.51 ± 0.55

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