Antimicrobial Evaluation of Some Arylsulfanylpyrazinecarboxylic Acid Derivatives

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Abstract: The radical-ionic coupling of chloropyrazine-2-carboxylic acid derivatives with methoxybenzenethiols, carried out in the presence of a heterogeneous copper catalyst, provided the series of 6- or 5- or 3-(4-methoxyphenyl)sulfanylpyrazine-2-carboxylic acid derivatives as well as 6- or 5- or 3-(3-methoxyphenyl)sulfanylpyrazine-2-carboxylic acid derivatives. The prepared compounds were evaluated as potential antifungal agents and new antituberculotics. Their preliminary *in vitro* evaluation of antimycobacterial activity according to the international program with the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) is presented. Several compounds showed an interesting activity in the preliminary screening with a percentage growth inhibition of the virulent *Mycobacterium tuberculosis* $H_{37}Rv$ between 50 to 100% at the concentration 6.25 μg/mL. Structure-activity relationships among the chemical structure, the physical properties and the biological activities of the evaluated compounds are discussed in the article.

Key Words: Methoxyphenylsulfanylpyrazine-2-carboxylic acid derivatives, *in vitro* antimycobacterial activity, *in vitro* antifungal activity, lipophilicity, structure-activity relationships.

1. INTRODUCTION

Tuberculosis (TB), caused especially by *Mycobacterium tuberculosis*, is one of the most devastating diseases primarily due to several decades of neglect. It presents a global health threat of escalating proportions. TB remains a deadly disease and continues to claim approximately 2 million lives annually. TB is the second leading infectious cause of mortality today after only HIV/AIDS. This rise in TB incidence can be attributed to the development of resistance by *M. tuberculosis* to commonly used antituberculotics [1]. There is an intensified need for developing new antituberculotic drugs due to the growing number of the immuno-compromised patient population, and onset of multi-drug resistant (MDR) strains [2]. In patients with impaired cellular immune mechanism fungal infections, especially nosocomial mycoses may also develop [3]. There is a real possibility to develop the resistance to broadly used antimycotics such as azole derivatives [4].

Pyrazinamide (PZA), an analogue of nicotinamide, is a frontline tuberculosis-specific-drug that is used in combination with other drugs and contributed to shortening the TB therapy. PZA is prodrug that requires activation or conversion at acid pH condition into its active form, pyrazinoic acid (POA), by the *pyrazinamidase/nicotinamidase* enzyme encoded by *pncA* gene of susceptible *M. tuberculosis*. The target of PZA/POA appears to be the membrane, i.e. *fatty acid synthase-I* [5,6].

Recently new pyrazine-2-carboxylic acid derivatives as potential antimycobacterial agents were reported [7-11]. Also some pyrazine derivatives showed interesting *in vitro* antifungal activity [7-14].

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This presented study is a follow-up paper to the previous articles dealing with the *N*-heterocyclic derivatives as potential drugs [7-11,15-19]. This work aimed at biological evaluating of prepared pyrazine derivatives and consequently at searching for the structure-activity relationships in the mentioned series, i.e. to continuation of the study of the substituent variability influence on the biological activity.

2. EXPERIMENTAL

2.1. Instrumentation and Chemicals and Synthesis

The studied compounds are shown in Scheme **1** and listed in Table **1**. Synthesis, physico-chemical data and analytical parameters of the discussed pyrazine-2-carboxylic acid derivatives were described in reference [16].

2.2. Lipophilicity Calculations

Hydrophobicity of compounds (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). Results are shown in Table **1**.

2.3. Antimycobacterial Screening

Antimycobacterial evaluation was carried out in 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) [20]. Primary screening was conducted at 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC27294) in BACTEC 12B medium using the BACTEC 460 radiometric system [21]. The compounds showing at least 90% inhibition in this primary screening were retested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in a broth microdi-

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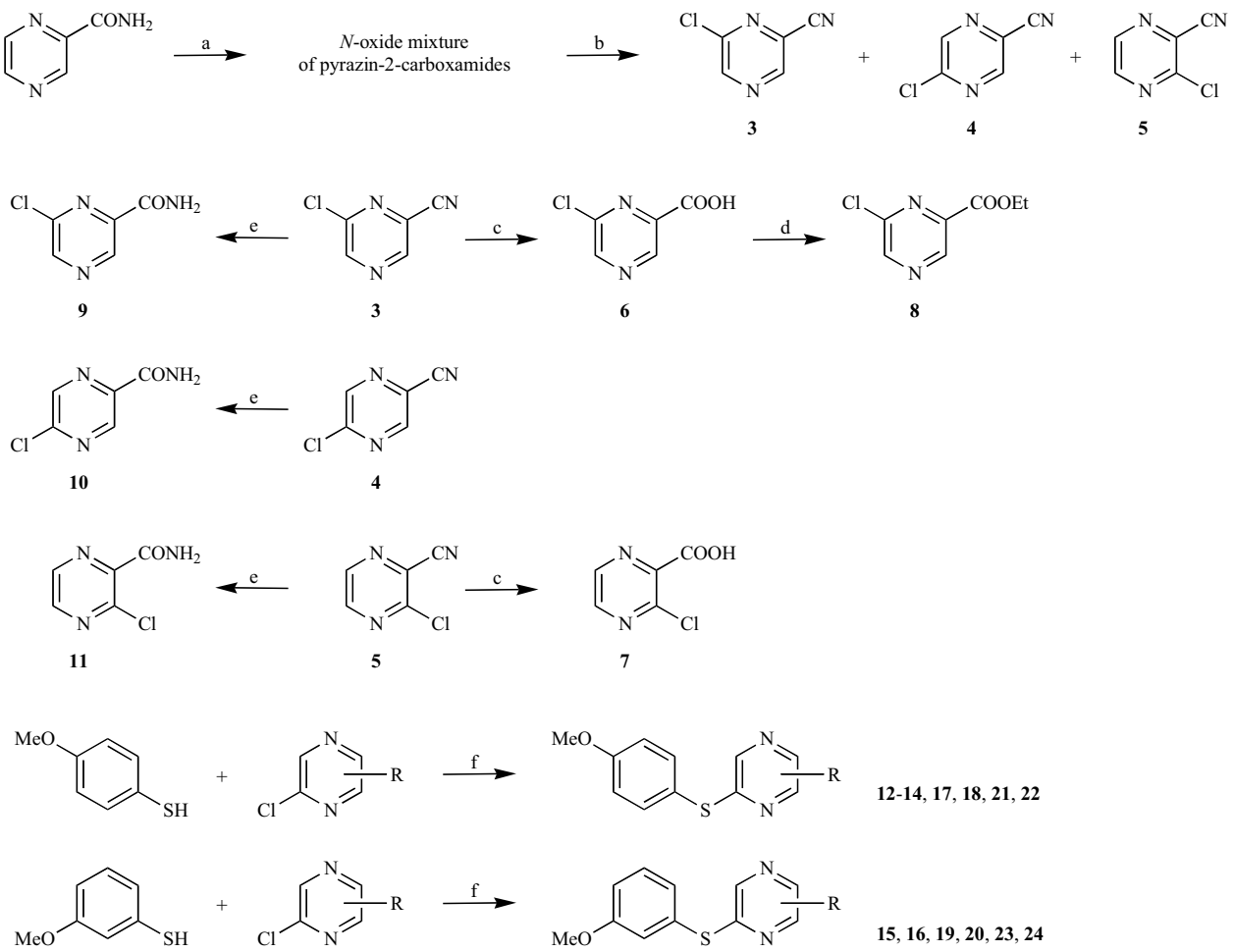


Conditions: a) H_2O_2, AcOH; b) $POCl_3$; c) *i*) NaOH, *ii*) HCl; d) toluene, $SOCl_2$, EtOH, TEA; e) H_2O_2, H_2O, NaOH; f) Cu_2O, DMF, NaH.

Scheme 1. Preparation of chloropyrazine-2-carbonitriles **3**-**5** and further intermediates **6**-**11** used for synthesis of coupled target methoxyphenylsulfanylpyrazine-2-carboxylic acid derivatives **12**-**24**.

lution Alamar Blue assay (MABA) [22]. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to controls. For the result of the compounds see Table **1**.

2.4. *In Vitro* Antifungal Susceptibility Testing

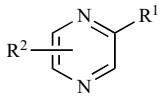
The broth microdilution test [23] was used for the assessment of *in vitro* antifungal activity of the synthesized compounds against *Candida albicans* ATCC 44859 (CA), and *Trichophyton mentagrophytes* 445 (TM). Fluconazole (FLU) was used as a reference drug. The procedure was performed with twofold dilution of the compounds in RPMI 1640 medium (Sevapharma a.s., Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulfonic acid (Sigma, Germany). The final concentrations of the compounds ranged from 500 to 0.975 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. Owing to the trailing effect, the MIC was defined as an 80% and more inhibition of growth (IC_{80}) of a fungal strain in comparison with control (a drug free medium). The results are summarized in Table **1**.

3. RESULTS AND DISCUSSION

3.1. Synthesis

The starting material pyrazine-2-carboxamide was transformed to chloropyrazine-2-carbonitriles **3**-**5** according to the conditions described in reference [16], see Scheme **1**. The intermediates **6**-**11** were prepared by well-known reactions. Three regioisomers of 6-chloropyrazine- or 5-chloropyrazine- or 3-chloropyrazine-2-carboxylic acid derivatives **6**-**11** were coupled with 4- or 3-methoxybenzene-1-thiols under catalysis by powdered copper oxide. This nucleophilic substitution provided a series of 6- or 5- or 3-(4-methoxyphenyl)sulfanylpyrazine-2-carboxylic acid derivatives **12**-**14**, **17**, **18**, **21**, **22** and 6- or 5- or 3-(3-methoxyphenyl)sulfanylpyrazine-2-carboxylic acid derivatives **15**, **16**, **19**, **20**, **23**, **24** [16], which were tested for their antimicrobial activity.

Table 1. Structure and Calculated Lipophilicity (log *P*) of the Studied Compounds 12-24. *In Vitro* Biological Properties of the Selected Methoxyphenylsulfanylpyrazine-2-Carboxylic Acid Derivatives: Antimycobacterial Activities Against *M. tuberculosis* Expressed as Inhibition (%) and Antifungal Activity Against *C. albicans* and *T. mentagrophytes* in Comparison with the Standard Fluconazole (FLU) Expressed as MIC (μmol/l)



Comp.	R^1	R^2	log *P*	*M. tuberculosis* $H_{37}Rv$		MIC/IC_{80} (μmol/l)	
						CA	TM
				MIC (μg/ml)	Inhibition (%)	*24 h* *48 h*	*72 h* *120 h*
12	$COOC_2H_5$	6-(4-MeOPhS)	3.41 ± 0.42	<6.25	94	62.5 250	125 250
13	CN	6-(4-MeOPhS)	3.17 ± 0.45	>6.25	*a*	31.25 125	31.25 62.5
14	$CONH_2$	6-(4-MeOPhS)	2.84 ± 0.45	>6.25	*a*	125 250	125 250
15	CN	6-(3-MeOPhS)	3.24 ± 0.45	>6.25	*a*	>125 >125	125 125
16	$CONH_2$	6-(3-MeOPhS)	2.91 ± 0.45	>6.25	*a*	>125 >125	250 500
17	CN	5-(4-MeOPhS)	3.33 ± 0.46	>6.25	*a*	62.5 250	62.5 125
18	$CONH_2$	5-(4-MeOPhS)	3.12 ± 0.46	>6.25	*a*	250 500	125 250
19	CN	5-(3-MeOPhS)	3.40 ± 0.46	>6.25	55	>500 >500	>500 >500
20	$CONH_2$	5-(3-MeOPhS)	3.19 ± 0.46	>6.25	88	125 500	250 500
21	CN	3-(4-MeOPhS)	3.17 ± 0.45	>6.25	*a*	31.25 125	62.5 62.5
22	$CONH_2$	3-(4-MeOPhS)	2.96 ± 0.46	>6.25	*a*	125 250	125 250
23	CN	3-(3-MeOPhS)	3.24 ± 0.46	>6.25	*a*	>125 >125	>125 >125
24	$CONH_2$	3-(3-MeOPhS)	3.03 ± 0.46	>6.25	*a*	>125 >125	>125 >125
FLU	–	–	0.31 ± 0.74	–	–	0.06 0.12	1.95 3.91

[a] moderate or no antituberculotic activity was found.

3.2. Lipophilicity

Hydrophobicities (log *P* values) of the studied compounds **12**-**24** were calculated using commercially available program. The results are shown in Tables **1**.

As expected, lipophilicity increased from amide, nitrile to ester groups. The compounds substituted by methoxyphenylsulfanyl moiety in the $C_{(5)}$ position of pyrazine nucleus showed higher lipophilicity according to the program ACD/Log P than the compounds with $C_{(3)}$ or $C_{(6)}$ methoxyphenylsulfanyl substitution. Both $C_{(3)}$ or $C_{(6)}$ methoxyphenylsulfanyl substitution possessed the same lipophilicity. The compounds substituted by methoxy moiety in the $C_{(3)}$ position of benzene ring showed higher lipophilicity according to ACD/Log P than the compounds substituted in $C_{(4)}$.

3.3. Antimycobacterial Screening

Thirteen studied pyrazine-2-carboxylic acid derivatives were tested for their antimycobacterial activity. Three compounds showed an interesting activity, see Table **1**.

For antituberculotic activity seems to be important sufficient lipophilicity parameter for the molecule crossed by passive transport through cellular membranes. Further important point for a high activity seems to be easily-hydrolysed moiety on pyrazine nucleus (ester **12** showed higher activity than amide **20**), because carboxylic acid POA is probably an active compound [5,6]. Substitution by methoxyphenylsulfanyl moiety of position $C_{(3)}$ of pyrazine nucleus caused a loss of activity. The methoxy group position is not important for the antimycobacterial activity.

3.4. *In Vitro* Antifungal Susceptibility Testing

Thirteen studied compounds were tested for their *in vitro* antifungal activity against *C. albicans* and *T. mentagrophytes*. All experiments were performed in comparison with fluconazole [24,25]. Several compounds showed medium antifungal activities with MIC range of 31.25 to 500 μmol/l, see Table **1**.

The methoxy group position was very important for the antifungal activity. The methoxy moiety in $C_{(3)}$ of the benzene ring caused compounds solubility loss in the testing medium, therefore most derivatives with the methoxy group in $C_{(3)}$ showed lower activities in comparison with the compounds substituted by the methoxy moiety in $C_{(4)}$.

Substitution by methoxyphenylsulfanyl moiety of position $C_{(6)}$ or $C_{(5)}$ or $C_{(3)}$ of pyrazine nucleus showed further influence on the biological activity. The compounds substituted in $C_{(6)}$ seem to be most advantageous.

The variety of the $C_{(2)}$ substitution of pyrazine nucleus proved to be the most important. The carbonitrile moiety showed higher activity than the amide or ester group, see Table **1** for the results.

The comparison of antifungal activity with the hydrophobicity indicated an optimal value of log *P* around 3.17. Compounds **13** and **21** (log *P* = 3.17) showed the highest antifungal activity against all strains tested. However, it could be generally assumed, that the described antifungal activity of the evaluated compounds, in fact, practically did not depend on lipophilicity but only on the moieties variety and position of the substitutions of pyrazine nucleus.

In summary, the most efficient compounds in the series of the substituted pyrazine-2-carboxylic acid derivatives were 6-[(4-methoxyphenyl)sulfanyl]pyrazine-2-carbonitrile (**13**) and 3-[(4-methoxyphenyl)sulfanyl]pyrazine-2-carbonitrile (**21**), both with log *P* = 3.17.

CONCLUSIONS

The thirteen new methoxyphenylsulfanylpyrazine-2-carboxylic acid derivatives were tested for their *in vitro* antituberculotic and antifungal activities. Ethyl 6-[(4-methoxyphenyl)sulfanyl]pyrazine-2-carboxylate (**12**), log *P* = 3.41 and 5-[(3-methoxyphenyl)sulfanyl]pyrazine-2-carboxamide (**20**), log *P* = 3.19 showed the highest *in vitro* activity against *M. tuberculosis* $H_{37}Rv$. Sufficient lipophilicity for the molecule cross mycobacterial wall and easily-hydrolysed groups (ester, amide) in pyrazine nucleus are necessary for the activity. 6-[(4-Methoxyphenyl)sulfanyl]pyrazine-2-carbonitrile (**13**) and 3-[(4-methoxyphenyl)sulfanyl]pyrazine-2-carbonitrile (**21**), log *P* = 3.17 showed an interesting *in vitro* antifungal activities against *C. albicans* and *T. mentagrophytes* in the series of the studied compounds. The positions of methoxyphenylsulfanyl moiety on the pyrazine nucleus are important for these activities.

Received: 31 July, 2006 Revised: 08 September, 2006 Accepted: 08 September, 2006

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education of the Czech Republic (MSM 0021620822). Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases.

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