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Synthesis and antimycobacterial evaluation of N-substituted 5-chloropyrazine-2-carboxamides

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ABSTRACT

To develop new potential antimycobacterial drugs, a series of pyrazinamide derivatives was designed, synthesized and tested for their ability to inhibit the growth of selected mycobacterial strains (*Mycobacterium tuberculosis* H37Rv, *Mycobacterium kansasii* and two strains of *Mycobacterium avium*). This Letter is focused on binuclear pyrazinamide analogues containing the –CONH–CH_2– bridge, namely on *N*-benzyl-5-chloropyrazine-2-carboxamides with various substituents on the phenyl ring and their comparison with some analogously substituted 5-chloro-*N*-phenylpyrazine-2-carboxamides. Compounds from the *N*-benzyl series exerted lower antimycobacterial activity against *M. tuberculosis* H37Rv then corresponding anilides, however comparable with pyrazinamide (12.5–25 μg/mL). Remarkably, 5-chloro-*N*-(4-methylbenzyl)pyrazine-2-carboxamide (**8**, MIC = 3.13 μg/mL) and 5-chloro-*N*-(2-chlorobenzyl)pyrazine-2-carboxamide (**1**, MIC = 6.25 μg/mL) were active against *M. kansasii*, which is naturally unsusceptible to PZA. Basic structure–activity relationships are presented.

Tuberculosis (TB) is considered to be one of the most frequent infectious diseases especially in developing countries. In 2011, there were about 8.7 million new cases of TB and 1.4 million deaths.[1] Multidrug-resistant TB (MDR-TB, characterized as resistance to, at least, isoniazid and rifampicin), extensively drug-resistant TB (XDR-TB), totally drug-resistant TB (TDR-TB) and also co-infection with HIV[2] remain a serious public health problem primarily in underdeveloped countries and underline the need to develop novel anti-tubercular agents.[1]

Pyrazinamide (PZA), a nicotinamide analogue, plays an important role in TB-therapy.[3] PZA has multiple mechanisms of action and as a prodrug, it is metabolized *via* mycobacterial enzyme pyrazinamidase (EC 3.5.1.19) to form pyrazinoic acid (POA).[4] POA accumulates intracellularly and lowers pH in mycobacterial cell, which leads to inhibition of membrane transport and depletion of energy.[5] Along with rifampicin, PZA has also a sterilizing activity, which is a crucial factor in shortening the duration of therapy.[6]

During the last years, the specific targets of PZA and/or POA were recognized. As previous studies suggested,[7–10] both PZA and POA were confirmed as inhibitors of fatty acid synthase I (FAS I), which participates in the synthesis of cell wall components. The studies of Sayahi et al. showed that PZA[11] and 5-Cl-PZA[12] are able to competitively displace the NADPH cofactor from FAS I. Finally, another specific target for POA (but not for PZA), ribosomal protein S1 (RpsA) involved in protein translation, was identified. POA prevents binding of tmRNA to RpsA and thus blocks trans-translation (the process involving the release of ribosomes stalled during translation).[13]

5-Cl-PZA proved to be active *in vitro* against both PZA susceptible *Mycobacterium tuberculosis* strains (MIC = 16 μg/mL) and PZA resistant mycobacterial strains (*Mycobacterium bovis, Mycobacterium kansasii, Mycobacterium fortuitum* and *Mycobacterium avium;* MIC = 8–64 μg/mL).[14] Therefore it became a pattern for target compounds mentioned in this paper. The series of substituted *N*-benzyl-5-chloropyrazine-2-carboxamides (**1**–**10**) and 5-chloro-*N*-phenylpyrazine-2-carboxamide (**1a**–**6a**) were synthesized and compared to study the influence of incorporated methylene moiety in the connecting bridge and also to continue to study of the substituent variability influence on the biological activity.

Final structures were prepared by convenient two-step synthesis[15] using 5-hydroxypyrazine-2-carboxylic acid (5-hydroxy-POA) as a starting material (see Scheme 1). During the first step 5-hydroxy-POA was treated with thionyl chloride to form 5-chloropyrazine-2-carbonyl chloride.[16] Dimethylformamide (DMF) was added to the reaction mixture as a catalyst.[17] Final structures were prepared by aminolysis of the corresponding acyl chloride by various anilines and benzylamines. Reaction proceeded under mild conditions (at RT in acetone), triethylamine (TEA) was used to neutralize the originating HCl. All prepared compounds (white solid or crystalline) were characterized by analytical data (^{1}H NMR, ^{13}C NMR, IR spectroscopy, melting point and elementary analysis). The analytical data were fully

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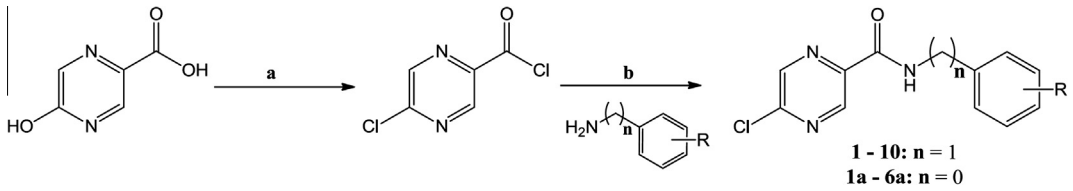

Scheme 1. Synthesis of final compounds **1–10** and **1a–6a**. Reagents: (a) $SOCl_2$, DMF, toluene; (b) TEA, acetone.

consistent with proposed structures and are enclosed in the Supplementary data.

Compound's lipophilicity plays important role in drug's passage through the mycobacterial cell wall, which contains a large amount of lipid components[18] and thus our attention was paid to lipophilicity determination. Parameters log*P*/*C*log*P* were calculated by commercially available program (ChemDraw Ultra, ver. 12.0),[19] measured using RP-HPLC determination[15] of capacity factor *k* expressed as log*k* and are shown in Table 1. The dependences of the calculated log*P*/*C*log*P* values on the measured log*k* parameters for *N*-benzyl series showed an approximate linearity and are illustrated in Figure 1. The corresponding correlations can be expressed by the following regression equations:

$$ClogP = 2.936(\pm 0.2359)\log k + 1.740(\pm 0.1022) \quad (1)$$
$$R = 0.9509\ s = 0.1150\ F = 154.8\ n = 10$$

$$LogP = 2.981(\pm 0.3301)\log k + 0.791(\pm 0.1430) \quad (2)$$
$$R = 0.9107\ s = 0.1608\ F = 81.56\ n = 10$$

Similar dependence of lipophilicity parameters was not found for *N*-phenyl series, possibly due to the formation of intramolecular hydrogen bond by *m*-OCH_3 and NH group by compound **3a**.

Prepared compounds were screened for their antimycobacterial activity against four mycobacterial strains by microdilution panel method, described in our previous published papers[15] and referred in Supplementary data. Results were expressed as minimal inhibition concentration (MIC) in μg/mL, or with respect to the molecular weight of final products in μmol/L (values in parentheses), for the results see Table 1.

The vast majority of compounds from *N*-benzyl series exhibited antimycobacterial activity against *M. tuberculosis* H37Rv comparable with pyrazinamide (MIC = 12.5–25 μg/mL). Taking into account the higher molecular weight of the prepared derivatives, compounds **6** (MIC = 38 μmol/L) and **9** (MIC = 39 μmol/L) showed almost three times better activity than PZA (MIC = 102–203 μmol/L). On the other hand, in comparison with analogously substituted anilides (**1a–6a**), whose antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv is mostly in range MIC = 0.78–6.25 μg/mL, the activity is lower. We inferred a conclusion that incorporation of methylene moiety leads to the drop in the activity but not to the complete loss.

On the other hand, 5-chloro-*N*-(4-methylbenzyl)pyrazine-2-carboxamide (**8,** MIC = 3.13 μg/mL) and 5-chloro-*N*-(2-chlorobenzyl)pyrazine-2-carboxamide (**1**, MIC = 6.25 μg/mL) showed significant activity against *M. kansasii*, which is naturally unsusceptible to PZA (MIC >100 μg/mL). Except for the compound **2a**, none of the prepared compounds exhibited any activity against the tested strains of *M. avium*. No significant differences in antimycobacterial activity between compounds with electron-withdrawing and electron-donating substitution were observed. Also no direct correlation between lipophilicity (log*k*) and antimycobacterial activity (log (1/MIC)) was found.

The *N*-substituted 5-Cl-PZA derivatives presented in this paper are stable under neutral pH conditions so we do not expect them to be hydrolysed in the testing media. It is also probable that the large substituent on the carboxamide nitrogen will prevent the pyrazinamidase from converting the compounds to 5-chloropyrazine-2-carboxylic acid (5-Cl-POA). This leads us to

Table 1
Physicochemical data of prepared compounds, their antimycobacterial activity expressed as minimal inhibition concentration (MIC) in μg/mL or μmol/L (data in parentheses), comparison of calculated and determined lipophilicity parameters

Structure			Antimycobacterial activity (μg/mL) (μmol/L)				Lipophilicity		
No.	R	MW	*M. tbc* H37RV	*M. kansasii*[a]	*M. avium*[b]	*M. avium*[c]	log*k*	log*P*	*C*log*P*
1	2-Cl	282.13	25 (89)	6.25	>100	>100	0.41683	2.12	3.09819
1a	2-Cl	268.10	0.78 (3)	n.a.[d]	>100	>100	0.81931	2.05	2.08469
2	3-Cl	282.13	25 (89)	50	>100	>100	0.42294	2.12	3.09819
2a	3-Cl	268.10	3.13 (12)	25	25	25	0.58731	2.05	2.93464
3	2,4-OCH_3	307.73	>100	>100	>100	>100	0.22665	1.31	2.39319
3a	2,4-OCH_3	293.71	>100	>100	>100	>100	0.57911	1.24	1.51424
4	2-F	265.67	25 (94)	50	>100	>100	0.33949	1.72	2.52819
4a	2-F	251.64	6.25 (25)	12.5	>100	>100	0.49028	1.65	1.76469
5	3-NO_2	292.68	>100	12.5	>100	>100	0.11886	1.06	2.12819
5a	3-NO_2	278.65	3.13 (11)	n.a.[d]	>100	>100	0.34426	1.39	2.00071
6	4-Br	326.58	12.5 (38)	50	>100	>100	0.49806	2.39	3.24819
6a	4-Br	312.55	3.13 (10)	6.25	>100	>100	0.66007	2.32	3.08469
7	2-CH_3	261.71	50 (191)	25	>100	>100	0.39471	2.05	2.83419
8	4-CH_3	261.71	25 (96)	3.13	>100	>100	0.41989	2.05	2.88419
9	2,4-Cl	316.57	12.5 (39)	>100	>100	>100	0.72699	2.68	3.81119
10	2-CF_3	315.68	25 (79)	100	>100	>100	0.48509	2.48	3.26819
PZA	––	123.11	12.5–25 (102–203)	>100	>100	>100	n.d.	−1.31	−0.676
INH	––	137.14	1.56 (11)	12.5	25	6.25	n.d.	−0.60	−0.668
INH	––	137.14	1.56 (11)	12.5	25	6.25	n.d.	−0.60	−0.668

[a] CNCTC My 235/80.
[b] CNCTC My 80/72.
[c] CNCTC My 152/73.
[d] Data not available due to poor sensitivity of the tested strain.

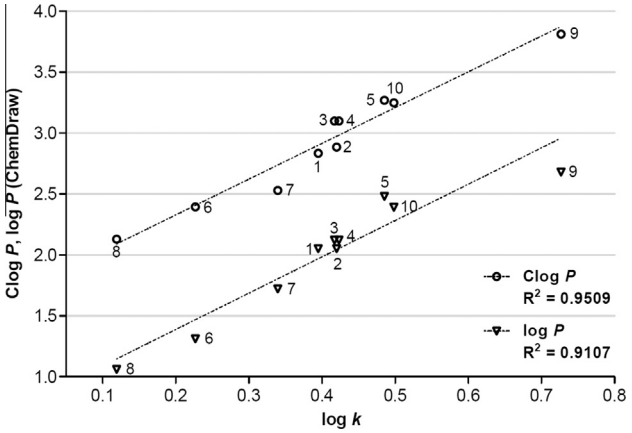

Figure 1. Relationships between calculated log*P*/C log*P* parameters and experimentally found log*k* values of *N*-benzyl-5-chloropyrazine-2-carboxamides.

the idea that the title compounds act rather as they are and not as prodrugs of 5-Cl-PZA or 5-Cl-POA.

All prepared compounds were also screened for their antifungal[15,20] and antibacterial[21,22] activities, which were negligible in comparison with used standards. Methods of biological screening are included in the Supplementary data.

Sixteen new compounds were prepared, characterized by analytical data, screened for biological activity and mutually compared in terms of antimycobacterial activity. Based on the results of biological evaluation, the incorporation of methylene moiety seems to be disadvantageous, since anilides of 5-chloropyrazine-2-carboxamide showed better activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.021.

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