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[C001]

SUBSTITUTED 3-AMINOPYRAZINE-2,5-DICARBONITRILES AS NEW ANTIINFECTIVES

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Abstract: A series of new pyrazinamide analogues have been prepared. Report that 5-chloropyrazinamide [1] has different mode of action than pyrazinamide itself [2] promises good chance to reach new structure with high antimycobacterial activity and new action mechanism that is realy needed because of resisance increase [3] to drugs in current use. Dericatives of pyrazine-2,5-dicarbonitrile promise good starting point to further studies (best activity reached yet is 3-(3-chlorophenylamino)pyrazine-2,5-dicarbonitrile, with MIC = 8μmol.l^{-1} against classical TBC strains and with lower activity against atypical strains. Compared to pyrazinamide with MIC = 4μmol.l^{-1} against classical strains and no activity against atypical ones).

Keywords: Pyrazinamide, tuberculosis, antimycobacterial, antifungal

Introduction

Tuberculosis becomes after a period of low incidence one of major health problems worldwide [4]. This yield into a definite solution: new antitubercular drugs are urgently

needed, especially those with new mode of action.

A short time ago, there have been reported that compounds derived from pyrazinamide, first line antitubercular sterilising agent, have different mode of action than pyrazinamide itself [2,3]. Research at our dept. follows the pathway of synthesis of pyrazinamide analogues [5,6], in this case compounds derived from pyrazine-2,5-dicarbonitrile, 3-arylamino derivatives respectively. We have prepared a series of 10 derivatives with very similar structure. The synthesis is then followed by activity assays that should screen the activity of each compound against various strains of *Mycobacterium* genus and against various fungi species as well. Each compound is tested first in Czech Republic against strains found in diseased people in this country and second in the USA by TAACF project, additionally, Dept. of Biological and Medical Sciences at our Faculty carry out antifungal assays.

Results of these assays promise new possibility of activity increase. Model compound is less active than the new one we have synthesized. In case of antimycobacterial activity is the best one 3-(3-trifluoromethylphenyl)amino derivative (**7**). Best antifungal results gave 3-(2,4-dibromo-6-nitrophenyl) amino derivative (**4**). There is plan to finish the series to complete all assays and to put everything together in order to get some structure-activity solution that should yield into preparation of even more active structures.

Results and Discussion

We have prepared ten compounds. First reaction scheme shown here [**figure 1**] is pathway to reach intermediate 3-chloropyrazine-2,5-dicarbonitrile via well known method [7]. This compound is starting material for final nucleophilic substitution [8] where chlorine is replaced by arylamino group with various substitutions [**figure 2**]. Conditions of this reaction have to be optimised according to substitution on arylamino- group.

The antifungal activity of our compounds was assessed *in vitro* against *Trichophyton mentagrophytes* (TM), *Candida albicans* (CA), *C. tropicalis* (CT), *C. krusei* (CK), *C. glabrata* (CG), *Trichosporon beigelii* (TB), *Aspergillus fumigatus* (AF), and *Absidia corymbifera* (AC) by the broth micro dilution method [9,10] (for results see **Table 1**).

Antimycobacterial assays screen activity against major strains of *Mycobacterium tuberculosis* (H37Rv), *Mycobacterium avium* (80/72 and 152/73) and *Mycobacterium cansasii* (CNCTC 235/80). The conditions are: acidic pH (imitation of acidic pH found in diseased tissues) and various grounds. Amtimycobacterial activity is screened by two independent laboratories in order to exlude mistakes [11,12] (see **Table 2** for more results).

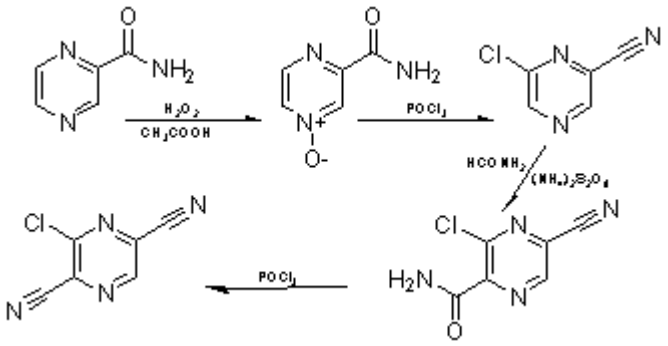

Figure 1: Preparation of 3-chloropyrazine-2,5-dicarbonitrile.

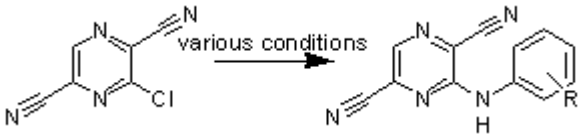

Figure 2: Synthesis of final compounds.

Experimetal

General Experimental Data

All solvents used for the synthesis were of analytical grade. TLC was performed on Merck UV 254 plates (, Germany). The spots were detected in UV (254 nm). Melting points were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany). ^{1}H and ^{13}C NMR Spectra were recorded on Varian Mercury – Vx BB 300 (299.95 MHz for ^{1}H and 75.43 MHz for ^{13}C), Varian (Palo Alto CA, USA). Chemical shifts are given relative to internal Si $(CH_3)_4$.

Synthesis of 3-[arylamino]pyrazine-2.5-dicarbonitriles

A mixture of aniline, *i.e.* 3-trifluoromethylaniline, (1.0 mmol) and triethylamine (2.0 mmol) in 20 mL of dry toluene was refluxed for 6 hours. The solvent was then removed uder reduced pressure. The crude compound was washed with water (3 x 15 mL) and dried over Na_2SO_4. Compound was then purified on column of silica using flash method.

3-[3-chlorophenylamino]pyrazine-2,5-dicarbonitrile (1) Yield: 42.6%, m.p. 154-155 °C. For $C_{12}H_7ClN_5$ (217.2) $^{-1}$: 3428 (N-H), 1680 (C=O). ^{1}H NMR (300 MHz, DMSO) δ 10.84 (1H, bs, NH), 9.29 (1H, d, *J*=1.7 Hz, H3), 8.92 (1H, d, *J*=2.5 Hz, H6), 8.80 (1H, dd, *J*=2.5 Hz, *J*=1.4 Hz, H5), 7.98-7.87 (2H, m, H2', H6'), and 7.26-7.15 (2H, m, H3', H5'). ^{13}C NMR (75 MHz, DMSO) δ 161.9, 158.8 (d, *J*=240.8 Hz), 148.0, 145.2, 144.3, 143.4, 134.8 (d, *J*=2.6 Hz), 122.7 (d, *J*=7.8 Hz), and 115.5 (d, *J*=22.0 Hz).

3-[3,5-dibromo-4-hydroxy-phenylamino]pyrazine-2,5-dicarbonitrile (2) Yield: 33.7%, m.p. 131-132 °C. For $C_{12}H_5Br_2N_5O$ (251.7) ^{1}H NMR (300 MHz, DMSO) δ 10.74 (1H, bs, NH), 9.22 (1H, d, *J*=0.6 Hz, H3), 9.05 (1H, s, H5), 7.93-7.83 (2H, m, H2', H6'),

and 7.27-7.17 (2H, m, H3', H5'). ^{13}C NMR (75 MHz, DMSO) δ 160.8, 159.0 (d, *J*=241.0 Hz), 147.7, 147.1, 145.3, 142.6, 134.5 (d, *J*=2.6 Hz), 123.0 (d, *J*=8.1 Hz), and 115.5 (d, *J*=22.3 Hz).

3-[2-cyano-4-nitro-phenylamino]pyrazine-2,5-dicarbonitrile (3) Yield: 66.5%, m.p. 178 °C. For $C_{13}H_5N_7O_2$ (273.3) ^{1}H NMR (300 MHz, DMSO) δ 10.45 (1H, bs, NH), 9.19 (1H, d, *J*=1.5 Hz, H3), 8.84 (1H, d, *J*=1.5 Hz, H6), 7.96-7.86 (2H, m, H2', H6'), 7.25-7.15 (2H, m, H3', H5'), and 1.39 (9H, s, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 166.9, 162.0, 158.7 (d, *J*=240.8 Hz), 142.6, 142.6, 139.9, 134.9 (d, *J*=2.6 Hz), 122.6 (d, *J*=8.0 Hz), 115.5 (d, *J*=22.3 Hz), 37.0, and 29.6.

3-[2,4-dibromo-6-nitro-phenylamino]pyrazine-2,5-dicarbonitrile (4) Yield: 44.3%, m.p. 171-172 °C. For $C_{12}H_4BrN_6O_2$ ^{1}H NMR (300 MHz, DMSO) δ 10.62 (1H, bs, NH), 9.12 (1H, s, H3), 7.91-7.81 (2H, m, H2', H6'), 7.26-7.16 (2H, m, H3', H5'), and 1.50 (9H, s, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 163.0, 160.9, 158.9 (d, *J*=241.4 Hz), 145.4, 142.8, 140.6, 134.6 (d, *J*=2.6 Hz), 122.9 (d, *J*=8.0 Hz), 115.5 (d, *J*=22.3 Hz), 38.7, and 28.2.

3-[2-chloro-5-hydroxy-phenylamino]pyrazine-2,5-dicarbonitrile (5) Yield: 50.2%, m.p. 139-140 °C. For $C_{12}H_6ClN_5O$ (233.7) ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.68 (bs, 1H, NH), 9.50 (s, 1H, H3), 8.83 (d, 1H, J=2.19 Hz, H6), 8.62-8.57 (m, 1H, H5), 7.92-7.86 (m, 1H, H2'), 7.65-7.56 (m, 1H, H6'), 7.31 (t, 1H, J=1.97 Hz, H5'), and 7.18-7.11 (m, 1H, H4'). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 147.7, 144.7, 144.0, 142.4, 138.3, 134.8, 130.2, 124.9, 119.9, and 117.7.

3-[4-fluorophenylamino]pyrazine-2,5-dicarbonitrile (6) Yield: 54.8%, m.p. 107-108 °C. For $C_{12}H_6FN_5$ (268.1) ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.44-9.35 (m, 2H, NH, H3), 8.82 (s, 1H, H5), 7.88 (t, 1H, J=1.93 Hz, H2'), 7.60 (ddd, 1H, J=7.97 Hz, J=1.93 Hz, J=0.83 Hz, H6'), 7.32 (t, 1H, J=7.96 Hz, H5'), and 7.17 (ddd, 1H, J=7.97 Hz, J=1.92 Hz, J=0.82 Hz, H4'). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.4, 147.8, 147.5, 143.6, 142.2, 137.9, 134.9, 130.2, 125.2, 120.1, and 118.0.

3-[3-trifluoromethylphenylamino]pyrazine-2,5-dicarbonitrile (7) Yield: 64.7%, m.p. 117-118 °C. For $C_{13}H_6F_3N_5$ (289.8) ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.67 (bs, 1H, NH), 9.38 (d, 1H, J=1.37 Hz, H3), 8.62 (d, 1H, J=1.37 Hz, H6), 7.89 (t, 1H, J=2.07 Hz, H2'), 7.59 (ddd, 1H, J=7.96 Hz, J=2.07 Hz, J=1.10 Hz, H4'), 7.30 (t, 1H, J=7.96 Hz, H5'), 7.13 (ddd, 1H, J=7.96 Hz, J=2.07 Hz, J=1.10 Hz, H6'), and 1.45 (s, 9H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 161.1, 143.0, 141.0, 139.0, 138.5, 134.8, 130.1, 124.6, 119.8, 117.6, 37.1, and 29.7.

3-[2-hydroxy-4-nitrophenylamino]pyrazine-2,5-dicarbonitrile (8) Yield: 71.1%, m.p. 86-87 °C. For $C_{12}H_6N_6O_3$ (324.2) ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.39 (bs, 1H, NH), 9.26 (s, 1H, H3), 7.88 (t, 1H, J=2.07 Hz, H2'), 7.60 (ddd, 1H, J=7.97 Hz, J=2.07 Hz, J=1.10 Hz, H6'), 7.31 (t, 1H, J=7.97 Hz, H5'), 7.15 (ddd, 1H, J=7.97 Hz, J=2.07 Hz, J=1.10 Hz, H4'), and 1.55 (s, 9H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.9, 159.9, 145.8, 140.7, 140.3, 138.2, 134.8, 130.1, 125.0, 120.0, 117.9, 39.0, and 28.20.

3-[2-trifluoromethylphenylamino]pyrazine-2,5-dicarbonitrile (9) Yield: 46.2%,

m.p. 223-224 °C. For $C_{13}H_6F_3N_5$ (249.5) ^{1}H NMR (300 MHz, DMSO) δ 10.27 (1H, bs, NH), 9.93 (1H, bs, OH), 9.32 (1H, d, *J*=1.4 Hz, H3), 8.97 (1H, d, *J*=2.5 Hz, H6), 8.83-8.81 (1H, m, H5), 7.87 (1H, d, *J*=2.7 Hz, H6'), 7.32 (1H, d, *J*=8.8 Hz, H3'), and 6.61 (1H, dd, *J*=8.8 Hz, *J*=2.7 Hz, H4'). ^{13}C NMR (75 MHz, DMSO) δ 160.9, 157.1, 148.7, 144.0, 143.9, 143.6, 134.5, 130.0, 113.5, 113.2, and 109.3.

3-[3-bromophenylamino]pyrazine-2,5-dicarbonitrile (10) Yield: 65.4%, m.p. 255 ° C. For $C_{12}H_7BrN_5$ (284.1) ^{1}H NMR (300 MHz, DMSO) δ 10.10 (1H, bs, NH), 9.93 (1H, bs, OH), 9.26 (1H, s, H3), 9.11 (1H, s, H5), 7.71 (1H, d, *J*=2.8 Hz, H6'), 7.33 (1H, d, *J*=8.7 Hz, H3'), and 6.64 (1H, dd, *J*=8.7 Hz, *J*=2.8 Hz, H4'). ^{13}C NMR (75 MHz, DMSO) δ 159.9, 157.1, 148.3, 147.0, 143.9, 142.2, 134.4, 130.0, 114.4, 113.7, and 110.2.

In vitro antifungal susceptibility testing

The broth microdilution test was used for the assessment of *in vitro* antifungal activity of the synthesized compounds and ketoconazole (standard) against *Candida albicans* ATCC 44859 **(CA)**, *Candida tropicalis* 156 **(CT)**, *Candida krusei* E28 **(CK)**, *Candida glabrata* 20/I **(CG)**, *Trichosporon beigelii* 1188 **(TB)**, *Trichophyton mentagrophytes* 445 **(TM)**, *Aspergillus fumigatus* 231 **(AF)**, and *Absidia corymbifera* 272 **(AC)**. The procedure was performed with twofold dilutions of the compounds in RPMI 1640 buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulfonic acid. The final concentrations of the compounds ranged from 1000 to 0.975 μmol l^{-1}. Drug–free controls were included. The MICs were determined after 24 h and 48 h of static incubation at 35 °C. With *Trichophyton mentagrophytes*, the final MICs were read after 72 h and 120 h of incubation. See **Table 1** for more details.

Table 1. In vitro antifungal susceptibility testing of nitriles **1-10** in comparison with ketoconazole (KET).

	MIC (μmol l^{-1})							
	CA	**CT**	**CK**	**CG**	**TB**	**AF**	**AC**	**TM**
Comp.	*24h* *48h*	*24h* *48h*	*24h* *48h*	*24h* *48h*	*24h* *48h*	*24h* *48h*	*24h* *48h*	*72h* *120h*
1	**15,63** **62,5**	**15,63** **62,5**	**15,63** **31,25**	**31,25** **62,5**	**31,25** **125**	**15,63** **15,63**	**31,26** **62,5**	**15,63** **31,25**
2	**62,5** >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	125 125
3	**3,91** **31,25**	**15,63** **31,25**	**7,82** **31,25**	**15,63** **31,25**	**15,63** **62,5**	**7,82** **31,25**	**31,25** **62,5**	**31,25** **31,25**
4	**1,95** **15,63**	**7,82** **7,82**	**3,91** **15,63**	**7,82** **15,63**	**3,91** **15,63**	**3,91** **15,63**	**7,81** **31,25**	**15,63** **15,63**
	125	250	>250	>250	>250	>250	>250	**125**

5	>250	>250	>250	>250	>250	>250	>250	**125**
6	**125** **500**	**250** **>500**	**500** >500	**500** >500	>500 >500	125 500	500 500>	62,5 62,5
7	62,5 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	125 125
8	>250 >250	**250** >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250
9	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250
10	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250
KET	**0.98** **1.95**	**<0.24** **<0.24**	**1.95** **3.91**	**0.98** **1.95**	**0.49** **1.95**	**<0.24** **<0.24**	**7.81** **7.81**	**31.25** **31.25**

Compounds assayed:

1. 3-[3-chlorophenylamino]pyrazine-2,5-dicarbonitrile

2. 3-[3,5-dibromo-4-hydroxy-phenylamino]pyrazine-2,5-dicarbonitrile

3. 3-[2-cyano-4-nitro-phenylamino]pyrazine-2,5-dicarbonitrile

4. 3-[2,4-dibromo-6-nitro-phenylamino]pyrazine-2,5-dicarbonitrile

5. 3-[2-chloro-5-hydroxy-phenylamino]pyrazine-2,5-dicarbonitrile

6. 3-[4-fluorophenylamino]pyrazine-2,5-dicarbonitrile

7. 3-[3-trifluoromethyl-phenylamino]pyrazine-2,5-dicarbonitrile

8. 3-[2-hydroxy-4-nitro-phenylamino]pyrazine-2,5-dicarbonitrile

9. 3-[2-trifluoromethyl-phenylamino]pyrazine-2,5-dicarbonitrile

10. 3-[3-bromo-phenylamino]pyrazine-2,5-dicarbonitrile

Antimycobacterial screening

Dept. of Microbiology at General Faculty Hospital is the first laboratory performin antitubercular assays for us. Used strains are *Mycobacterium tuberculosis* $H_{37}Rv$, *Mycobacterium cansasii* CNCTC My 238/80, *Mycobacterium avium* My 80/72 and *Mycobacterium avium* My 152/74. Culture was 10 days old and used culture medium was Šula's medium at pH 6,0 and 37°C. Used method was microdilution panel method. Second laboratory - **TAACF - world screening programme for new antimycobacterial drugs** performs assays first on *Mycobacterium tuberculosis* $H_{37}Rv$ via per cent of inhibition at 6,25 $\mu mol.l^{-1}$

Table 2.Antimycobacterial activity

Strain	*M. tuberculosis* $H_{37}Rv$			*M. kansasii* My 235/80			*M. avium* My 80/72			*M. avium* My 152/74		
Incubation time (days) / Comp.	10	13	15	10	13	15	18	24	30	18	30	
1	4	4	4	16	16	32	>128	>128	>128	>128	>128	
2	2	8	16	16	32	32	64	64	64	128	>128	
3	8	16	16	16	16	16	128	-	128	128	>128	
6	-	8	8	>128	>128	>128	>128	>128	>128	>128	>128	
7	-	8	16	64	128	128	>128	>128	>128	>128	>128	
8	-	8	8	16	32	32	64	64	64	64	128	
9	-	8	8	64	128	128	>128	>128	>128	>128	>128	
10	4	8	8	128	>128	>128	>128	>128	>128	>128	>128	
PZA	8	8	8	>128	>128	>128	>128	>128	>128	>128	>128	

[a]MIC = 12.5 μg mL^{-1}, data from [8]

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