SYNTHESIS AND ANTITUBERCULOTIC ACTIVITY OF SOME SUBSTITUTED 3-ARYLAMINO-5-CYANO-2-PYRAZINECARBOXAMIDES

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Nucleophilic substitution of 3-chloro-5-cyano-2-pyrazinecarboxamide by substituted anilines afforded substituted 3-arylamino-5-cyano-2-pyrazinecarboxamides *I–X*. The structures of compounds were confirmed by elemental analysis, UV, IR and ^{1}H NMR spectra. The assessment of in vitro antimycotic and antimycobacterial activities of the compounds was carried out. The highest antituberculotic activity against *M. tuberculosis* in this series was shown by 3-anilino-5-cyano-2-pyrazinecarboxamide (*I*), whose efficacy was the same as that of pyrazinecarboxamide.

The increasing number of new cases of tuberculosis even in the industrial countries, problems with the resistance to antituberculous drugs and the human immunodeficiency virus pandemic has promoted new research interest in the synthesis of potential tuberculostatics. The first-line antituberculosis agent pyrazinecarboxamide has potent sterilising activity in the acidic pH of the intracellular environment[1].

We reported recently the synthesis[2] of a series of non-aromatic *N*-substituted 3-amino-5-cyano-2-pyrazinecarboxamides. All these compounds exhibited none or very low antimycotic and antituberculotic activity. During the other course of our search for new antimycotic and antituberculotic agents a series of 3-arylamino-5-cyano-2-pyrazinecarboxamides *I–X* i.e. more lipophilic derivatives with substituted phenyl ring has been synthesized according to the method of Foks[3]. There was no correlation between antimycotic and antituberculotic activity investigated in the series of 3-arylamino-5-cyano-2-pyrazinecarboxamides *I–X*. None of the compounds tested for their antimycotic activity was effective.

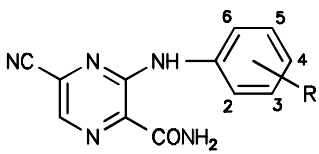
Five tested compounds (*I*, *II*, *VII*, *VIII*, and *X*) were active in vitro against *M. tuberculosis*. The compounds *VII* and especially *VIII* exhibited attractive activity against *M. kansasii*, a representative of atypical mycobacterial strains. The antituberculotic activity of these compounds is mostly influenced by the aromatic character of the substituted phenyl ring. The activity disappeared at the compounds with higher lipophilicity, i.e. at the derivatives with substituted phenyl ring by halogen atom or group with halogen atoms, by the methoxy group or by other methyl group. Positive effect on the activity showed the substitution by polar group, in our series by phenolic hydroxylic group or by the nitro group. The most significant activity in this series was shown by 3-anilino-5-cyano-2-pyrazinecarboxamide (*I*), which was found to be active in the concentration of 12.5 μg ml^{-1} against *Mycobacterium tuberculosis* $H_{31}Rv$.

EXPERIMENTAL

Melting points were determined on a Kofler apparatus and are uncorrected. All the compounds were checked for purity by TLC on Silufol UV 254 plates (Kavalier, Votice) in the following systems: toluene–acetone (1 : 1), petrolether–ethyl acetate (1 : 1). Samples for elemental analysis were dried in vacuo of about 100 Pa over phosphorus pentoxide at room temperature. Elemental analyses were obtained using a CHN Analyser (Laboratorni pristroje, Prague). Electronic spectra (λ, nm) were determined on a Specord UV-VIS apparatus (Zeiss) in ca 2 . 10^{-4} M methanolic solution. IR spectra (ν, cm^{-1}) were recorded on a Perkin–Elmer 577 spectrometer in KBr pellets. ^1H NMR spectra were measured for solutions in hexadeuteriodimethyl sulfoxide with a Bruker AMX 360 spectrometer at 360.13 MHz. The ^1H chemical shifts (δ, ppm) are related to the internal tetramethylsilane.

3-Arylamino-5-cyano-2-pyrazinecarboxamides *I–X*. General Procedure

3-Chloro-5-cyano-2-pyrazinecarboxamide[4] (1.82 g, 10 mmol) was dissolved in dry benzene (50 ml) and a corresponding aniline derivative (25 mmol) was added. The resulting mixture was refluxed for



I–X

	R		R
I	H	*VI*	3,5-$(CF_3)_2$
II	3-CH_3	*VII*	3-OH
III	2,6-$(CH_3)_2$	*VIII*	4-OH
IV	3-Br	*IX*	3-OCH_3
V	4-Cl	*X*	3-NO_2-4-CH_3

TABLE I
Physical properties, yields and elemental analyses of 3-arylamino-5-cyano-2-pyrazinecarboxamides *I–X*

Compound	M.p., °C Yield, %	Formula M.w.	Calculated/Found		
			% C	% H	% N
I	193–195	$C_{12}H_9N_5O$	60.25	3.79	29.27
	84	239.2	60.22	3.80	29.36
II	198–201	$C_{13}H_{11}N_5O$	61.65	4.38	27.65
	78	253.3	61.89	4.27	27.47
III	233–235	$C_{14}H_{13}N_5O$	62.91	4.90	26.20
	86	267.3	62.80	4.77	26.41
IV[a]	245–246	$C_{12}H_8BrN_5O$	45.31	2.53	22.01
	86	318.1	45.70	2.65	22.18
V[b]	326–328	$C_{12}H_8ClN_5O$	52.66	2.95	25.59
	79	273.7	52.55	3.04	25.97
VI	195–198	$C_{14}H_7F_6N_5O$	44.81	1.88	18.66
	86	375.2	45.03	1.85	18.85
VII	219–220	$C_{12}H_9N_5O_2$	56.47	3.55	27.44
	55	255.2	56.31	3.75	27.50
VIII	237–238	$C_{12}H_9N_5O_2$	56.47	3.55	27.44
	43	255.2	56.67	3.64	27.77
IX	247–248	$C_{13}H_{11}N_5O_2$	57.99	4.12	26.01
	87	269.3	57.89	4.20	26.28
X	259–261	$C_{13}H_{10}N_6O_3$	52.35	3.38	28.18
	82	298.3	52.32	3.58	27.87

[a] Calculated: 25.12% Br, found: 25.08% Br. [b] Calculated: 12.95% Cl, found: 13.16% Cl.

1 h. After cooling, the mixture was filtered, the solvent was then removed under reduced pressure, and the crude product was recrystallized from water (charcoal). The yields and analytical data are given in Table I, the IR, ^{1}H NMR and UV spectra are given in Table II.

TABLE II
IR, ^{1}H NMR, and UV spectra of 3-arylamino-5-cyano-2-pyrazinecarboxamides

Compound	IR ν(CN) ν(CO)	^{1}H NMR =CH– $CONH_2$	NH H-2	H-3 H-4	H-5 H-6	UV λ_{max}/log ε
I	2 280	8.55	11.57	7.44	7.44	410
	1 680	8.70 and 8.31	7.67	7.16	7.67	3.53
II	2 240	8.52	11.53	[a]	7.30	410
	1 690	8.68 and 8.30	7.38	6.96	7.55	3.33
III	2 240	8.45	10.60	7.17	7.17	390
	1 680	8.64 and 8.24	[b]	7.17	[b]	3.84
IV	2 240	8.63	11.67	–	7.40	410
	1 680	8.75 and 8.35	8.05	7.34	7.60	3.60
V	2 290	8.59	11.63	7.49	7.49	410
	1 680	8.72 and 8.33	7.71	–	7.71	3.45
VI	2 240	8.72	11.89	–	–	410
	1 680	8.80 and 8.37	8.42	7.81	8.42	3.61
VII	2 230	8.52	11.55	[c]	7.20	410
	1 680	8.68 and 8.27	7.25	6.56	7.00	3.48
VIII	2 240	8.43	11.21	6.82	6.82	415
	1 680	8.62 and 8.23	7.43	[d]	7.43	3.37
IX	2 240	8.55	11.58	[e]	7.32	410
	1 680	8.70 and 8.30	7.37	6.72	7.17	3.50
X	2 230	8.65	11.72	–	7.81	395
	1 700	8.75 and 8.36	8.50	[f]	7.54	3.56

Methyl group: [a] 2.35, [b] 2.17, [f] 3.40. Hydroxyl group: [c] 9.62, [d] 9.43. Methoxy group: [e] 3.81.

Microbiological Assays

The prepared compounds were tested for their antimycotic activity (expressed as a minimal inhibitory concentration – MIC) by the microdilution broth method. The procedure was performed with twofold compound dilutions in RPMI 1640 buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid. The final concentrations of the compounds ranged from 1 000 to 0.975 μM. Drug free controls were included. The MICs were determined after 24 and 48 h of static incubation at 35 °C. In case of *Trichophyton mentagrophytes* the MICs were recorded after 48 and 72 h incubation. The MIC of the compounds *I–X* was measured in *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, *Candida glabrata* 20/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231, and *Absidia corymbifera* 272. None of the compounds studied was effective (MIC > 0.5–2.0 . 10^{-6} mol l^{-1}).

Antimycobacterial evaluation was carried out on a semisynthetic liquid protein containing Sula medium (Institute of Sera and Inoculation Substances, Prague) buffered to pH 5.4. The following mycobacterial strains were used: *Mycobacterium tuberculosis* $H_{37}Rv$, *M. kansasii* PKG 8, *M. avium* 80/72 and *M. fortuitum* 1021. The final concentration of the compounds in the medium was 6.2, 12.5, 25, 50, and 100 μg ml^{-1}. The MICs were determined after 14 days of incubation at 37 °C. For the results see Table III.

TABLE III

Minimum inhibitory concentration against *Mycobacterium tuberculosis* $H_{31}Rv$, *M. kansasii* PKG 8, *M. avium* 80/72, and *M. fortuitum* 1021 in the series of 3-arylamino-5-cyano-2-pyrazinecarboxamides

Compound	MIC μg ml^{-1} (μmol l^{-1})			
	M. tbc.	*M. kans.*	*M. avium*	*M. fort.*
I	12.5 (52.3)	>100	>100	>100
II	25 (98.7)	>100	>100	>100
III	>100	>100	>100	>100
IV	>100	>100	>100	>100
V	>100	>100	>100	>100
VI	>100	>100	>100	>100
VII	25 (97.9)	100 (391.8)	>100	>100
VIII	25 (97.9)	50 (195.9)	>100	>100
IX	>100	>100	>100	>100
X	25 (83.82)	>100	>100	>100
Pyrazinecarboxamide	12.5 (101.54)	>100 (812.3)	>100	>100

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