SYNTHESIS AND ANTITUBERCULOTIC PROPERTIES OF SOME SUBSTITUTED PYRAZINECARBOTHIOAMIDES

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A series of *N*-substituted 3-amino-5-thiocarbamoyl-2-pyrazinecarboxamides was prepared. The structure of compounds was confirmed by elemental analysis, IR and ^{1}H NMR spectra. The results of in vitro antifugal and antimycobacterial susceptibility testing shown no activity against pathogenic fungi and some effect on mycobacteria. The highest antituberculotic activity (MIC within 25–50 mg ml^{-1}) against *Mycobacterium tuberculosis* and other mycobacterial strains in this series was shown by 3-(3-hydroxyphenylamino)-5-thiocarbamoyl-2-pyrazinecarboxamide. The antituberculotic activity of these compounds is mostly influenced by the presence of the thioamide moiety.

Key words: Substituted aminopyrazinecarbothioamides; Structure–activity relationships; Antituberculotic activity; Antifungal activity.

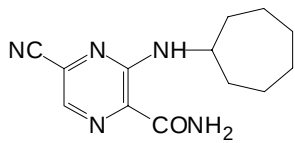
Tuberculosis (TB) is very much present disease on the rise today, which is responsible for over three millions of human victims a year in both the developed and developing countries over the world. If the present trend continues, it is likely to claim more than 30 million lives within the next decade. The increase in TB cases and other non-specific mycobacterial infections is related to HIV/AIDS, homelessness, drug abuse and immigration of persons with active infections. The firstline antituberculosis agent pyrazinamide is active in an acid environment and has the ability to kill intracellular organisms[1,2].

We reported recently the synthesis of a series of nonaromatic[3] and aromatic[4] *N*-substituted 3-amino-5-cyano-2-pyrazinecarboxamides. In pursuing our research on the synthesis of pyrazine derivatives with potential tuberculostatic and fungistatic activity we wish to describe here a preparation of 5-cyano-3-cycloheptylamino-2-pyrazinecarboxamide (**1**, prepared after the method of Foks[5]) and of a new series of *N*-substituted 3-amino-5-thiocarbamoyl-2-pyrazinecarboxamides **2a–2p** (prepared after the method of

Milczarska[6]). The aim of this work is to study the effect of thioamidic moiety on the biological activity.

There was no correlation between antimycotic and antituberculotic activity investigated in the series of *N*-substituted 3-amino-5-thiocarbamoyl-2-pyrazinecarboxamides **2a–2p**. None of the compounds had antifugal activity within the concentration ranges tested by the microdilution broth method.

Eleven tested compounds were active in vitro against *Mycobacterium tuberculosis* (MIC within 25–100 mg ml^{-1}). The compounds **2b**, **2d**, **2f**, **2g**, **2p** and especially **2o**



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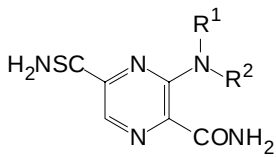

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2	R^1	R^2
a	C_2H_5	C_2H_5
b	H	$CH_3(CH_2)_2$
c	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$
d	H	CH_2=$CHCH_2$
e	CH_2=$CHCH_2$	CH_2=$CHCH_2$
f	H	$CH_3(CH_2)_3$
g	H	$(CH_3)_2CHCH_2$
h	$CH_3(CH_2)_3$	$CH_3(CH_2)_3$
i	H	cycloheptyl
j	$-(CH_2)_4-$	
k	$-(CH_2)_5-$	
l	$-(CH_2)_2O(CH_2)_2-$	
m	H	C_6H_5
n	H	3-$CH_3C_6H_4$
o	H	3-HOC_6H_4
p	H	4-HOC_6H_4

exhibited promising activity against *M. kansasii* or other representatives of atypical mycobacterial strains (MIC within 25–100 mg ml^{-1}). Several conclusions can be drawn from the study of structure–activity relationships in this series. Secondary amines are more active than tertiary amines. The influence of free hydrogen atom is significant for the spread of the activity against the atypical mycobacterial strains. The activity vanished in compounds with higher lipophilicity, i.e. in derivatives with longer aliphatic chain (**2h**, **2i**) or with hydrophobic substitution (**2n**). The activity dropped or disappeared in the compounds with nonaromatic heterocyclic substitution (**2j**, **2k**, **2l**). In the series of aromatic derivatives the positive effect on the spread of the activity against atypical mycobacterial strains showed the substitution by some polar group, in our case by phenolic hydroxyl group (**2o**, **2p**). The antituberculotic activity of these compounds is mostly influenced by the presence of the thioamidic moiety. The most significant activity in this series was shown by 3-(3-hydroxyphenylamino)-5-thiocarbamoyl-2-pyrazinecarboxamide (**2o**), which is active in the concentration of 25 to 50 μg ml^{-1} against several atypical mycobacterial strains.

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), light petroleum–ethyl acetate (1 : 1). Samples for elemental analysis were dried in vacuo of about 100 Pa over phosphorus pentoxide at room temperature. IR spectra were recorded on a Perkin–Elmer model 577 spectrometer in KBr pellets; wavenumbers are given in cm^{-1}. ^1H NMR spectra were measured for solutions in $(CD_3)_2SO$ with a Bruker AMX 360 spectrometer at 360.13 MHz. The ^1H chemical shifts (δ, ppm) are related to the internal tetramethylsilane.

5-Cyano-3-cycloheptylamino-2-pyrazincarboxamide (**1**)

3-Chloro-5-cyano-2-pyrazinecarboxamide[7] (1.82 g, 10 mmol) was dissolved in dry toluene (50 ml), cycloheptylamine (2.85 g, 25 mmol) was added, and the mixture was refluxed for 1 h. After cooling, the mixture was filtered, the solvent was then removed under reduced pressure, and the crude product was recrystallized from water. The yield and analytical data are given in Table I, the IR and ^1H NMR spectra are given in Table II.

General Procedure for Preparation of Substituted 3-Amino-5-thiocarbamoyl-2-pyrazinecarboxamides (**2a–2p**)

Substituted 3-amino-5-cyano-2-pyrazinecarboxamides[3,4] (1 mmol) was dissolved in methanol (50 ml), a saturated aqueous solution of ammonium sulfide (1 ml) was added and the resulting mixture was placed in the refrigerator over night. The mixture was filtered, the solvent was then removed under reduced pressure, and the crude product was recrystallized from water with charcoal. The yields and analytical data are given in Table I, the IR and ^1H NMR spectra are given in Table II.

TABLE I
Physical properties, yields and elemental analyses of compounds **1** and **2a–2p**

Compound	M.p., °C Yield, %	Formula M.w.	Calculated/Found		
			% C	% H	% N
1	180–182	$C_{13}H_{17}N_5O$	60.21	6.61	27.01
	84	259.3	60.16	6.69	27.14
2a	167–170	$C_{10}H_{15}N_5OS$	47.41	5.97	27.65
	75	253.3	47.48	6.02	27.55
2b	218–220	$C_9H_{13}N_5OS$	45.17	5.48	29.27
	72	239.3	45.38	5.53	29.01
2c	198–199	$C_{12}H_{19}N_5OS$	51.22	6.81	24.89
	71	281.4	51.05	6.80	24.78
2d	204–207	$C_9H_{11}N_5OS$	45.56	4.67	29.52
	62	237.4	45.50	4.60	29.20
2e	162–163	$C_{12}H_{15}N_5OS$	51.97	5.45	25.25
	72	277.3	52.14	5.64	25.21
2f	195–197	$C_{10}H_{15}N_5OS$	47.41	5.97	27.65
	70	253.3	47.71	6.08	27.49
2g	196–198	$C_{10}H_{15}N_5OS$	47.41	5.97	27.65
	74	253.3	47.40	5.68	27.46
2h	202–205	$C_{14}H_{23}N_5OS$	54.34	7.49	22.63
	67	309.4	54.30	7.58	22.85
2i	231–233	$C_{13}H_{19}N_5OS$	53.22	6.53	23.87
	84	293.4	53.38	6.57	23.69
2j	225–227	$C_{10}H_{13}N_5OS$	47.79	5.21	27.87
	53	251.3	47.72	5.31	27.58
2k	237–239	$C_{11}H_{15}N_5OS$	49.79	5.70	26.39
	67	265.3	49.73	5.69	26.49
2l	200–201	$C_{10}H_{13}N_5O_2S$	44.93	4.90	26.20
	71	267.3	45.15	4.85	26.28
2m	254–255	$C_{12}H_{11}N_5OS$	52.74	4.06	25.62
	68	273.3	52.84	4.09	25.80
2n	253–255	$C_{13}H_{13}N_5OS$	54.34	4.56	24.37
	70	287.3	54.36	4.55	24.26
2o	268–270	$C_{12}H_{11}N_5O_2S$	49.82	3.83	24.21
	48	289.3	50.02	3.74	24.25
2p	247–250	$C_{12}H_{11}N_5O_2S$	49.82	3.83	24.21
	50	289.3	49.55	3.93	24.49

TABLE II
IR and ^{1}H NMR spectra of compounds **1** and **2a–2p**

Compound	IR (ν, cm^{-1})	^{1}H NMR (δ, ppm; *J* in Hz)				
	C=O	H arom.[a]	$CONH_2$[b]	$CSNH_2$[b]	NH	R
1[c]	1 680	8.29	8.45, 8.04	–	9.24 d, *J* = 5.8	4.07 m (CH); 1.54–1.96 m (6 × CH_2)
2a	1 660	8.67	8.18, 7.74	10.25, 9.42	–	3.58 q, *J* = 6.9 (CH_2); 1.16 t, *J* = 6.9 (2 × CH_3)
2b	1 660	8.68	8.32, 7.85	10.33, 9.80	8.92 t, *J* = 5.8	3.55 dt, *J* = 7.4 and 6.0 (CH_2); 1.61 m (CH_2); 0.98 t, *J* = 7.4 (CH_3)
2c	1 660	8.66	8.19, 7.76	10.25, 9.58	–	3.50 t, *J* = 7.5 (CH_2); 1.61 m (CH_2); 0.87 t, *J* = 7.3 (CH_3)
2d	1 670	8.73	8.37, 7.89	10.34, 9.83	8.89 t, *J* = 5.6	4.27 m (CH_2); 6.01 m (CH); 5.13–5.31 m (=CH_2)
2e	1 640	8.75	8.19, 7.78	10.26, 9.65	–	4.15 m (CH_2); 5.88 m (CH); 5.23 dd, *J* = 1.8 and 17.3; 5.19 dd, *J* = 1.8 and 10.2 (=CH_2)
2f	1 670	8.67	8.30, 7.83	10.33, 9.78	8.89 d, *J* = 5.6	3.58 dt, *J* = 7.4 and 5.6 (CH_2); 1.59 m (CH_2); 1.40 m (CH_2); 0.96 t, *J* = 7.3 (CH_3)
2g	1 665	8.68	8.33, 7.84	10.33, 9.80	9.02 t, *J* = 5.8	3.46 dd, *J* = 7.3 and 5.8 (CH_2); 1.90 m (CH); 0.98 d, *J* = 6.7 (2 × CH_3)
2h	1 660	8.65	8.19, 7.75	10.25, 9.56	–	3.54 t, *J* = 7.5 (CH_2); 1.56 m (CH_2); 0.92 t, *J* = 7.3 (CH_3)
2i	1 700	8.66	8.31, 7.83	10.33, 9.73	8.99 d, *J* = 8.1	4.43 m (CH); 1.66–1.96 m (6 × CH_2)
2j	1 670	8.67	8.12, 7.70	10.23, 9.68	–	3.52 m (2 × CH_2); 1.92 m (2 × CH_2)
2k	1 675	8.73	8.13, 7.71	10.25, 9.76	–	3.57 m (2 × NCH_2); 1.61 m (3 × CH_2)
2l	1 675	8.81	8.19, 7.76	10.29, 9.83	–	3.71 m (2 × CH_2); 3.61 m (2 × CH_2)
2m	1 670	8.78	8.61, 8.15	10.47, 9.57	11.49 bs	7.75 m (H2,6); 7.42 m (H3,5); 7.11 t, *J* = 7.4 (H4)
2n	1 670	8.76	8.60, 8.15	10.47, 9.55	11.47 bs	7.66 dd, *J* = 1.6 and 7.6 (H6); 7.50 d, *J* = 1.6 (H2); 7.30 dd, *J* = 7.3 and 7.3 (H5); 6.94 d, *J* = 7.3 (H4); 2.37 s (CH_3)
2o	1 660	8.81	8.60, 8.15	10.54, 9.45	11.47 bs	7.05 dd, *J* = 2.0 and 7.9 (H6); 7.28 dd, *J* = 2.0 and 2.0 (H2); 7.19 dd, *J* = 7.9 and 7.8 (H5); 6.52 dd, *J* = 2.0 and 7.8 (H4); 9.56 bs (OH)
2p	1 660	8.71	8.56, 8.07	10.42, 9.41	11.13 bs	7.50 m (H2,6); 6.80 m (H3,5); 9.32 bs (OH)

[a] Singlet; [b] broad singlet; [c] other band 2 240 (C≡N).

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 morpholinopropanesulfonic acid (Sigma). 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 **1** and **2a–2p** was measured in *Candida albicans* ATCC 44859, *C. tropicalis* 156, *C. krusei* E28, *C. 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}).

TABLE III
Minimum inhibitory concentration against *Mycobacterium tuberculosis* $H_{37}Rv$, *M. kansasii* PKG 8, *M. avium* No. 80/72, and *M. fortuitum* 1021 of compounds **1** and **2a–2p**

Compound	MIC μg ml^{-1} (μmol l^{-1})			
	M. tuberculosis	*M. kansasii*	*M. avium*	*M. fortuitum*
1	>100	>100	>100	>100
2a	50 (197)	50 (197)	>100	>100
2b	100 (418)	>100	>100	>100
2c	25 (89)	>100	>100	>100
2d	25 (105)	25 (105)	50 (210)	>100
2e	25 (90)	>100	>100	>100
2f	25 (99)	25 (99)	100 (395)	>100
2g	50 (197)	100 (395)	>100	>100
2h	100 (323)	100 (323)	>100	>100
2i	>100	>100	>100	>100
2j	>100	>100	>100	>100
2k	100 (377)	100 (377)	>100	>100
2l	50 (187)	>100	>100	>100
2m	50 (183)	50 (183)	100 (366)	100 (366)
2n	>100	>100	>100	>100
2o	25 (86)	25 (86)	50 (173)	50 (173)
2p	25 (86)	25 (86)	100 (346)	100 (346)
Pyrazinamide[a]	12.5 (102)	>100 (>812)	>100	>100

[a] Pyrazinecarboxamide.

Antimycobacterial evaluation was carried out on a semisynthetic liquid protein-containing Sula medium (IMUNA, Sarisske Michalany) buffered to pH 5.7. The following mycobacterial strains were used: *Mycobacterium tuberculosis* $H_{37}Rv$, *M. kansasii* PKG 8, *M. avium* No. 80/72 and *M. fortuitum* 1021. The final concentration of the compounds in the medium was 3.1, 6.2, 12.5, 25, 50, and 100 μg ml^{-1}. The MICs were determined after 3 to 4 weeks of incubation at 37 °C. For the results see Table III.

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