# SYNTHESIS AND ANTITUBERCULOTIC ACTIVITY OF 5-ALKYL-6-CHLORO-2-PYRAZINECARBOXAMIDES AND CORRESPONDING THIOAMIDES

Jiri HARTL[a1], Martin DOLEZAL[a2], Jana KRINKOVA[a3], Antonin LYCKA[b] and Zelmira ODLEROVA[c]

[a] *Department of Medicinal Chemistry and Drug Control, Faculty of Pharmacy, Charles University, 500 05 Hradec Kralove, Czech Republic; e-mail: [1] hartl@faf.cuni.cz, [2] dolezal@faf.cuni.cz, [3] krinkova@faf.cuni.cz*

[b] *Research Institute of Organic Syntheses, 532 18 Pardubice-Rybitvi, Czech Republic; e-mail: slnmr@pol.upce.cz*

[c] *Institute of Preventive and Clinic Medicine, 833 01 Bratislava, Slovak Republic*

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Homolytic alkylation of 6-chloro-2-pyrazinecarbonitrile by alkanoic acid and subsequent partial hydrolysis afforded 5-alkyl-6-chloro-2-pyrazinecarboxamides **1a–1e**. Reaction of amides **1a–1e** by Lawesson's reagent afforded corresponding thioamides **2a–2e**. The structure of compounds was confirmed by elemental analysis, IR and $^1$H NMR spectra. The assessment of in vitro antimycobacterial activity of the compounds was carried out. The highest antituberculotic activity against *Mycobacterium tuberculosis* and other mycobacterial strains in this series was shown by 5-(1,1-dimethylethyl)-6-chloro-2-pyrazinecarbothioamide (**2e**).

**Key words:** Homolytic alkylation; Pyrazinecarboxamides; Pyrazinecarbothioamides; Tuberculostatic activity.

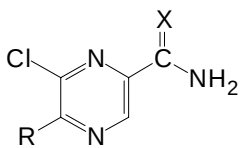
Tuberculosis has again become epidemic in many parts of the world. The increase in tuberculosis cases and other non-specific mycobacterial infections is related to HIV/AIDS, homelessness, drug abuse and imigration of persons with active infections[1]. Therefore new antituberculosis drugs and/or new derivatives of old drugs, e.g. pyrazinamide, have been discovered and studied. The antituberculotic agent pyrazinecarboxamide has potent sterilising activity in the acidic pH of the intracellular environment[2].

Earlier studies[3–6] showed that alkylation of the pyrazine nucleus increase antituberculotic activity of some functional derivatives of 2-pyrazinecarboxylic acid. The most significant activity was shown by 5-propyl- and 5-(2-propyl)-2-pyrazinecarbothioamides[5] the activity of which was higher than that of pyrazinecarboxamide used as a standard. This paper presents structure–antituberculotic activity study of amides and thioamides of 5-alkyl-6-chloro-2-pyrazinecarboxylic acid **1a–1e**, **2a–2e**.

6-Chloro-2-pyrazinecarbonitrile, prepared by a reaction of 2-pyrazinecarboxamide-4-oxide with phosphorylchloride[7], was used as a starting material for the preparation of

5-alkyl-6-chloro-2-pyrazinecarboxamides **1a–1e** (Table I). It was alkylated using a procedure analogous to that reported for the preparation of 5-alkyl-2-pyrazinecarboxamides[8]. 5-Alkyl-6-chloro-2-pyrazinecarbonitriles were the main products which is in agreement with the literature[9]. Alkyl radicals were generated by oxidative decarboxylation of alkanoic acid (butyric acid, isobutyric acid, valeric acid, isovaleric acid, pivalic acid). Desired 5-alkyl-6-chloro-2-pyrazinecarboxamides **1a–1e** were isolated by crystallization from reaction mixtures obtained by hydrolysis of 5-alkyl-6-chloro-2-pyrazinecarbonitriles with hydrogen peroxide in an alkaline medium[10]. The attempted direct alkylation of 6-chloro-2-pyrazinecarboxamide gave only low yields of 5-alkyl-6-chloro-2-pyrazinecarboxamides. Isosteric 5-alkyl-6-chloro-2-pyrazinecarbothioamides **2a–2e** were prepared from the corresponding amides **1a–1e** reaction with Lawesson's reagent[11]. Spectral characteristics of the compounds **1**, **2** are given in Table II.

The results of antimycobacterial evaluation (Table III) allow us to conclude some structure–activity relationship in the series of substituted pyrazinecarboxamides **1a–1e** and pyrazinecarbothioamides **2a–2e**. The length of the alkyl chain seems to be less important than the presence of thioamide moiety. The series of 5-alkyl-6-chloro-2-pyrazinecarboxamides **1** exhibited none or only low antituberculotic activity. The isosteric change from amide group to thioamide one with the same length of the alkyl chain on pyrazine nucleus leads to a significant increase in antituberculotic activity. Five tested thioamides **2a–2e** were active in vitro against *M. tuberculosis* (MIC range 12.5–25 μg $ml^{-1}$) and against other atypical mycobacterial strains. Especially compounds **2c** and **2e** exhibited promising activity against *M. kansasii* and *M. fortuitum*. The most significant activity in this series was shown by 5-(1,1-dimethylethyl)-6-chloro-2-pyrazinecarbo-




**1,** X = O
**2,** X = S

| **1, 2** | R |
|---|---|
| **a** | $CH_3(CH_2)_2$ |
| **b** | $(CH_3)_2CH$ |
| **c** | $CH_3(CH_2)_3$ |
| **d** | $(CH_3)_2CHCH_2$ |
| **e** | $(CH_3)_3C$ |

thioamide (**2e**) which is twice as active against *M. tuberculosis* and more active against atypical strains than pyrazinecarboxamide.

## 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 aceton–toluene (1 : 1 or 1 : 2) ethyl acetate–petroleum ether (1 : 4), ether–petroleum ether (1 : 2). Detection was carried out by

TABLE I
Characteristic data of 5-alkyl-6-chloro-2-pyrazinecarboxamides **1** and thioamides **2**

| Compound | M.p., °C<br>Yield, % | Formula<br>M.w. | Calculated/Found | | | | |
|---|---|---|---|---|---|---|---|
| | | | % C | % H | % Cl | % N | % S |
| **1a** | 145–147 | $C_8H_{10}ClN_3O$ | 48.13 | 5.05 | 17.76 | 21.05 | – |
| | 48 | 199.6 | 48.26 | 4.91 | 17.77 | 21.22 | – |
| **1b** | 134–135 | $C_8H_{10}ClN_3O$ | 48.13 | 5.05 | 17.76 | 21.05 | – |
| | 54 | 199.6 | 48.39 | 5.01 | 17.68 | 20.83 | – |
| **1c** | 123–125 | $C_9H_{12}ClN_3O$ | 50.59 | 5.66 | 16.59 | 19.67 | – |
| | 50 | 213.7 | 50.66 | 5.72 | 16.41 | 19.58 | – |
| **1d** | 155–156 | $C_9H_{12}ClN_3O$ | 50.59 | 5.66 | 16.59 | 19.67 | – |
| | 47 | 213.7 | 50.69 | 5.88 | 16.72 | 19.53 | – |
| **1e** | 148–150 | $C_9H_{12}ClN_3O$ | 50.59 | 5.66 | 16.59 | 19.67 | – |
| | 58 | 213.7 | 50.54 | 5.56 | 16.25 | 19.87 | – |
| **2a** | 89–92 | $C_8H_{10}ClN_3S$ | 44.55 | 4.67 | 16.44 | 19.48 | 14.86 |
| | 79 | 215.7 | 44.77 | 4.89 | 16.58 | 19.46 | 14.58 |
| **2b** | 79–80 | $C_8H_{10}ClN_3S$ | 44.55 | 4.67 | 16.44 | 19.48 | 14.86 |
| | 78 | 215.7 | 44.54 | 4.70 | 16.65 | 19.56 | 14.69 |
| **2c** | 80–82.5 | $C_9H_{12}ClN_3S$ | 47.06 | 5.27 | 15.43 | 18.29 | 13.96 |
| | 69 | 229.7 | 47.30 | 5.43 | 15.27 | 17.98 | 13.78 |
| **2d** | 91–92 | $C_9H_{12}ClN_3S$ | 47.06 | 5.27 | 15.43 | 18.29 | 13.96 |
| | 73 | 229.7 | 47.25 | 5.49 | 15.59 | 18.30 | 14.13 |
| **2e** | 149–151 | $C_9H_{12}ClN_3S$ | 47.06 | 5.27 | 15.43 | 18.29 | 13.96 |
| | 86 | 229.7 | 46.95 | 5.22 | 15.39 | 18.40 | 13.91 |

TABLE II
IR and $^1$H NMR spectra of 5-alkyl-6-chloro-2-pyrazinecarboxamides **1** and thioamides **2**

| Compound | IR | | $^1$H NMR | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ν(C=O) | ν($NH_2$) | H-3 | $NH_2$ | R | | | |
| **1a** | 1 693 | 3 431 | 9.20 | 7.45, 6.10 | 3.00 ($CH_2$) | 1.82 ($CH_2$) | 1.02 ($CH_3$) | |
| **1b** | 1 691 | 3 450 | 9.22 | 7.56, 6.14 | 3.57 (CH) | 1.32 $(CH_3)_2$ | | |
| **1c** | 1 690 | 3 442 | 9.19 | 7.45, 6.07 | 3.02 ($CH_2$) | 1.75 ($CH_2$) | 1.43 ($CH_2$) | 0.96 ($CH_3$) |
| **1d** | 1 693 | 3 435 | 9.21 | 7.47, 8.24 | 2.91 ($CH_2$) | 2.26 (CH) | 0.98 $(CH_3)_2$ | |
| **1e** | 1 695 | 3 452 | 9.17 | 7.46, 6.20 | 1.52 $(CH_3)_3$ | | | |
| **2a** | | 3 395 | 9.61 | 8.93, 7.74 | 2.99 ($CH_2$) | 1.80 ($CH_2$) | 1.02 ($CH_3$) | |
| **2b** | | 3 396 | 9.63 | 8.93, 7.78 | 3.57 (CH) | 1.32 $(CH_3)_2$ | | |
| **2c** | | 3 397 | 9.61 | 8.93, 7.68 | 3.01 ($CH_2$) | 1.75 ($CH_2$) | 1.43 ($CH_2$) | 0.96 ($CH_3$) |
| **2d** | | 3 386 | 9.62 | 8.93, 7.73 | 2.90 ($CH_2$) | 2.26 (CH) | 0.97 $(CH_3)_2$ | |
| **2e** | | 3 387 | 9.57 | 8.92, 7.71 | 1.52 $(CH_3)_3$ | | | |

TABLE III
Minimum inhibitory concentrations in the series of 5-alkyl-6-chloro-2-pyrazinecarboxamides **1** and thioamides **2**

| Compound | MIC, μg $ml^{-1}$ (μmol $l^{-1}$) | | | |
|---|---|---|---|---|
| | *M. tuberculosis* | *M. kansasii* | *M. avium* | *M. fortuitum* |
| **1a** | >100 | >100 | >100 | >100 |
| **1b** | >100 | >100 | >100 | >100 |
| **1c** | 50(234) | >100 | >100 | >100 |
| **1d** | 50(234) | >100 | >100 | >100 |
| **1e** | 50(234) | 50(234) | >100 | >100 |
| **2a** | 25(116) | 50(231) | 100(464) | 100(464) |
| **2b** | 25(116) | 50(231) | 100(464) | 100(464) |
| **2c** | 25(108) | 50(217) | 100(435) | 50(217) |
| **2d** | 25(108) | 50(217) | 100(435) | 100(435) |
| **2e** | 12.5(54) | 25(108) | 50(217) | 50(217) |
| Pyrazinecarboxamide | 12.5(102) | >100(>812) | >100 | >100 |

UV-irradiation. Column chromatography was performed on Silica gel Silpearl (Kavalier, Votice) (100 g silica gel, ether–petroleum ether 1 : 2). Elemental analyses were obtained using a CHN Analyser (Laboratorni pristroje, Prague). IR spectra ($\tilde{\nu}$, $cm^{-1}$) were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. $^1H$ NMR spectra were measured in deuteriochloroform with a Bruker AMX 360 Spectrometer at 360.13 MHz. The $^1H$ chemical shifts ($\delta$, ppm) are related to the internal tetramethylsilane.

5-Alkyl-6-chloro-2-pyrazinecarboxamide **1a–1e**. General Procedure

Silver nitrate (1.7 g, 0.01 mol) and alkanoic acid (0.11 mol) were added to a solution of 6-chloro-2-pyrazinecarbonitrile (13.9 g, 0.1 mol) in water (300 ml) stirred at 80 °C and then a solution of ammonium peroxydisulfate (25 g, 0.11 mol) was added dropwise. The stirring at 80 °C continued for additional 1 h. After cooling the mixture was made slightly basic by sodium hydroxide (5%) and continuously extracted with chloroform. The organic phase, after washing with water and drying over anhydrous sodium sulfate was evaporated at reduced pressure.

A solution of hydrogen peroxide (35%, 25 ml) in water (160 ml) was adjusted to pH 9 with solution of sodium hydroxide (2 mol $l^{-1}$) and treated dropwise under stirring with the crude reaction mixture. The emulsion was stirred at 55 °C for 3 h, cooled down and the precipitate washed with water. The white solid obtained was dried and recrystallized from ethanol (charcoal). The yields and analytical data of 5-alkyl-6-chloro-2-pyrazinecarboxamides **1a–1e** are given in Table I. The IR and $^1H$ NMR spectra are given in Table II.

5-Alkyl-6-chloro-2-pyrazinecarbothioamides **2a–2e**. General Procedure

The corresponding amide **1a–1e** (10 mmol) and Lawesson's reagent (2.2 g, 5.5 mmol) in anhydrous toluene (10 ml) were kept at 110 °C for 4 h. The reaction mixture was evaporated at reduced pressure and the crude product was further purified by column chromatography. The solid obtained was recrystallized from ethanol (charcoal). The yields and analytical data of 5-alkyl-6-chloro-2-pyrazinecarbothioamides **2a–2e** are given in Table I. The IR and $^1H$ NMR spectra are given in Table II.

Microbiological Assays

Antimycobacterial evaluation was carried out on a semisynthetic liquid protein containing Sula medium (IMUNA, Sarisske Michalany) buffered to pH 5.3. 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 tested compounds in the medium was 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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