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Il Farmaco 57 (2002) 71–78

IL FARMACO

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Synthesis and biological activity of 5-alkyl-6-(alkylsulfanyl)- or 5-alkyl-6-(arylsulfanyl)pyrazine-2-carboxamides and corresponding thioamides

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Received 8 May 2001; accepted 28 August 2001

Abstract

Nucleophilic substitution of chlorine in 5-alkyl-6-chloropyrazine-2-carboxamides with various alkyl and arylthiolates afforded 20 5-alkyl-6-(alkylsulfanyl)- and 5-alkyl-6-(arylsulfanyl)pyrazine-2-carboxamides. The reaction of the amides with Lawesson's reagent yielded the corresponding thioamides. The assessment of in vitro antimycobacterial and antifungal activity of the compounds was carried out. In both series, the antimycobacterial activity increases with increasing molecular weight of the alkylsulfanyl group in position 6 of the pyrazine ring. Thioamides exhibited higher activity than the corresponding amides. 5-Butyl-6-(phenylsulfanyl)pyrazine-2-carbothioamide (**2j**) possessed the highest activity (91% inhibition) against *Mycobacterium tuberculosis* and also the highest lipophilicity (log $P = 4.95$). Only a poor in vitro antifungal effect was noted in 5-butyl-6-(butylsulfanyl)pyrazine-2-carboxamide (**1i**) and 6-(ethylsulfanyl)-5-isobutylpyrazine-2-carbothioamide (**2q**) against *Trichophyton mentagrophytes* and *Absidia corymbifera*.

Keywords: Nucleophilic substitution; Pyrazines; Antimycobacterial activity; Antifungal evaluation; Structure–activity relationships

1. Introduction

Tuberculosis (TB) has again become epidemic in many parts of the world. The worst situation is in southeast Asia and in sub-Saharan Africa. New outbreaks also occurred in Eastern Europe, where TB-related deaths are increasing after almost 40 years of steady decline. The increase in TB and other non-specific mycobacterial infections is associated with HIV/AIDS, homelessness, drug abuse and immigration of persons with active infections. Another serious problem is the emergence of multidrug-resistant TB (TB bacilli resistant to at least isoniazid and rifampicin—the two most powerful antituberculotic drugs) [1]. Therefore new antituberculotic drugs and/or new derivatives of old drugs, such as pyrazinamide, have been prepared and studied. The antituberculotic pyrazinecarboxamide has potent sterilising activity in the acidic pH of the intracellular environment [2].

Previous studies [3–9] showed that alkylation, amidation or substitution of the pyrazine ring with chlorine increases antituberculotic activity of some functional derivatives of pyrazine-2-carboxylic acid. This paper deals with the structure, antituberculotic and antifungal activity study of amides and thioamides of 5-alkyl-6-alkylsulfanyl- and 5-alkyl-6-arylsulfanylpyrazine-2-carboxylic acid.

5-Alkyl-6-chloropyrazine-2-carboxamides [7] were used as the starting materials for the preparation of 5-alkyl-6-(alkylsulfanyl)- and 5-alkyl-6-(arylsulfanyl)-pyrazine-2-carboxamides. The general procedure is analogous to that reported for the preparation of 6-(alkylsulfanyl)- or 6-(arylsulfanyl)pyrazine-2-carbonitrile

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0014-827X/02/$ - see front matter
PII: S0014-827X(01)01156-9

[10]. The desired 5-alkyl-6-(alkylsulfanyl)- and 5-alkyl-6-(arylsulfanyl)pyrazine-2-carboxamides (**1a**–**t**, Scheme 1) were separated by column chromatography from the reaction mixtures obtained by the substitution of chlorine with alkylsulfanyl (methyl, ethyl, propyl, butyl) and arylsulfanyl group in anhydrous *N*,*N*-dimethylformamide. The isosteric 5-alkyl-6-(alkylsulfanyl)- and 5-alkyl-6-(arylsulfanyl)pyrazine-2-carbothioamides (**2a**–**t**, Scheme 1) were prepared from the corresponding amides by treatment with Lawesson's reagent [11].

2. Experimental procedures

2.1. Instrumentation

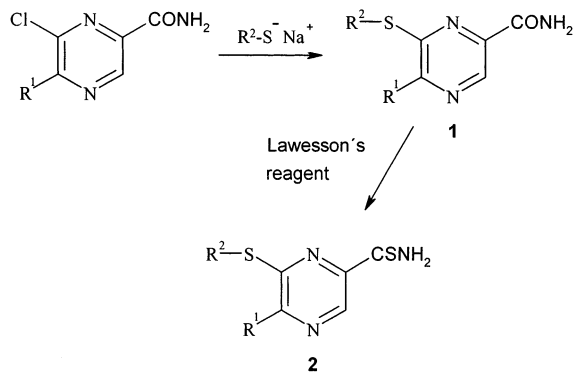
Melting points were determined on a Kofler apparatus and are uncorrected. All compounds were checked for purity by TLC on Silufol UV 254 plates (Kavalier, Votice) in acetone–toluene (1:1 or 1:2) and in ethyl acetate–light petroleum (1:3), with UV detection. Column chromatography was performed on silica gel Silpearl (Kavalier, Votice, 100 g silica gel, ethyl acetate–light petroleum, 1:2). Elemental analyses were obtained using a CHN Analyser EA 1110 CHNS–O (CE Instruments, Italy). IR spectra (ν, cm^{-1}) were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. ^1H NMR spectra were measured in dimethyl sulfoxide with a Varian Mercury-Vx BB 300 spectrometer at 300 MHz. ^1H chemical shifts (δ, ppm) are relative to tetramethylsilane (TMS) as internal standard. Calculation of lipophilicity ($\log P$) was carried out using ACD/LogP programme ver.1.0 (Advanced Chemistry Development Inc., Toronto).

2.2. 5-Alkyl-6-(alkylsulfanyl)- and 5-alkyl-6-(arylsulfanyl)pyrazine-2-carboxamides (*1a*–*1t*). General procedure

5-Alkyl-6-chloropyrazine-2-carboxamide (0.1 mol) and the corresponding sodium thioalcoholate or thiophenolate (0.1 mol) were dissolved in anhydrous *N*,*N*-dimethylformamide (150 ml) and stirred at 100 °C for 3 h. After cooling, water was added to the reaction mixture and the solution was evaporated at reduced pressure. The crude product was extracted with ether. The organic phase, after washing with sodium hydroxide (8%), water and drying over anhydrous sodium sulfate was evaporated at reduced pressure. The solid obtained was purified by column chromatography. The product was recrystallised from ethanol.

2.2.1. 6-(Methylsulfanyl)-5-propylpyrazine-2-carboxamide (*1a*)

Yield 68%, m.p. 115–117 °C. *Anal.* Calc. for $C_9H_{13}N_3OS$ (211.3): 51.16% C, 6.20% H, 19.89% N, 15.17% S; found: 51.56% C, 5.86% H, 19.73% N, 15.09% S, $\log P = 2.67 \pm 0.40$. IR spectrum: 1694; ^1H NMR spectrum: δ 8.70s 1H (H3), 8.07s 1H (NH_2), 7.82s 1H (NH_2), 2.73t 2H, $J = 7.4$ (CH_2), 2.59s 3H (CH_3), 1.79–1.64m 2H(CH_2), 0.91t 3H $J = 7.4$ (CH_3).



Compound	R^1	R^2	Compound	R^1	R^2
1a	C_3H_7	CH_3	**2a**	C_3H_7	CH_3
1b	C_3H_7	C_2H_5	**2b**	C_3H_7	C_2H_5
1c	C_3H_7	C_3H_7	**2c**	C_3H_7	C_3H_7
1d	C_3H_7	C_4H_9	**2d**	C_3H_7	C_4H_9
1e	C_3H_7	C_6H_5	**2e**	C_3H_7	C_6H_5
1f	C_4H_9	CH_3	**2f**	C_4H_9	CH_3
1g	C_4H_9	C_2H_5	**2g**	C_4H_9	C_2H_5
1h	C_4H_9	C_3H_7	**2h**	C_4H_9	C_3H_7
1i	C_4H_9	C_4H_9	**2i**	C_4H_9	C_4H_9
1j	C_4H_9	C_6H_5	**2j**	C_4H_9	C_6H_5
1k	iso-C_4H_9	CH_3	**2k**	iso-C_4H_9	CH_3
1l	iso-C_4H_9	C_2H_5	**2l**	iso-C_4H_9	C_2H_5
1m	iso-C_4H_9	C_3H_7	**2m**	iso-C_4H_9	C_3H_7
1n	iso-C_4H_9	C_4H_9	**2n**	iso-C_4H_9	C_4H_9
1o	iso-C_4H_9	C_6H_5	**2o**	iso-C_4H_9	C_6H_5
1p	$(CH_3)_3C$	CH_3	**2p**	$(CH_3)_3C$	CH_3
1q	$(CH_3)_3C$	C_2H_5	**2q**	$(CH_3)_3C$	C_2H_5
1r	$(CH_3)_3C$	C_3H_7	**2r**	$(CH_3)_3C$	C_3H_7
1s	$(CH_3)_3C$	C_4H_9	**2s**	$(CH_3)_3C$	C_4H_9
1t	$(CH_3)_3C$	C_6H_5	**2t**	$(CH_3)_3C$	C_6H_5

Scheme 1.

2.2.2. 6-(Ethylsulfanyl)-5-propylpyrazine-2-carboxamide ***(1b)***

Yield 75%, m.p. 97–100 °C. *Anal.* Calc. for $C_{10}H_{15}N_3OS$ (225.3): 53.31% C, 6.71% H, 18.65% N, 14.23% S; found: 53.22% C, 6.73% H, 18.67% N, 13.90% S, log $P = 3.20 \pm 0.40$. IR spectrum: 1673; ^{1}H NMR spectrum: δ 8.70s 1H (H3), 3.28q 2H, $J_1 = 14.7$, $J_2 = 7.3$ (CH_2), 2.71t 2H, $J = 7.5$ (CH_2), 1.77–1.63m 2H (CH_2), 1.27t 3H $J = 7.3$ (CH_3), 0.91t 3H $J = 7.5$ (CH_3).

2.2.3. 5-Propyl-6-(propylsulfanyl)pyrazine-2-carboxamide ***(1c)***

Yield 40%, m.p. 112–116 °C. *Anal.* Calc. for $C_{11}H_{17}N_3OS$ (239.3): 55.20% C, 7.16% H, 17.56% N, 13.40% S; found: 55.20% C, 7.11% H, 17.43% N, 13.13% S, log $P = 3.73 \pm 0.40$. IR spectrum: 1690; ^{1}H NMR spectrum: δ 8.70s 1H (H3), 7.99s 1H (NH_2), 7.83s 1H (NH_2), 3.27t 2H, $J = 7.1$ (CH_2), 2.72t 2H, $J = 7.4$ (CH_2), 1.78–1.57m 4H (CH_2), 0.91t 3H, $J = 7.1$ (CH_3), 0.91t 3H, $J = 7.4$ (CH_3).

2.2.4. 6-(Butylsulfanyl)-5-propylpyrazine-2-carboxamide ***(1d)***

Yield 37%, m.p. 98–102 °C. *Anal.* Calc. for $C_{12}H_{19}N_3OS$ (253.4): 56.89% C, 7.56% H, 16.58% N, 12.65% S; found: 57.33% C, 7.94% H, 16.46% N, 12.30% S, log $P = 4.27 \pm 0.40$. IR spectrum: 1675; ^{1}H NMR spectrum: δ 8.70s 1H (H3), 7.97s 1H (NH_2), 7.83s 1H (NH_2), 3.28t 2H, $J = 7.2$ (CH_2), 2.72t 2H, $J = 7.4$ (CH_2), 1.77–1.53m 4H (CH_2), 1.47–1.33m 2H (CH_2), 0.91t 3H, $J = 7.4$ (CH_3), 0.87t 3H, $J = 7.2$ (CH_3).

2.2.5. 6-(Phenylsulfanyl)-5-propylpyrazine-2-carboxamide ***(1e)***

Yield 75%, m.p. 138–142 °C. *Anal.* Calc. for $C_{14}H_{15}N_3OS$ (273.2): 61.52% C, 5.53% H, 15.37% N, 11.73% S; found: 62.00% C, 5.75% H, 15.36% N, 11.82% S, log $P = 4.42 \pm 0.44$. IR spectrum: 1698; ^{1}H NMR spectrum: δ 8.77s 1H (H3), 7.75s 1H (NH_2), 7.61–7.54m 2H (H2′, H6′), 7.52–7.46m 3H (H3′, H4′, H5′), 6.70s 1H (NH_2), 2.85t 2H, $J = 7.4$ (CH_2), 1.85–1.70m 2H (CH_2), 0.96t 3H, $J = 7.4$ (CH_3).

2.2.6. 5-Butyl-6-(methylsulfanyl)pyrazine-2-carboxamide ***(1f)***

Yield 45%, m.p. 114–118 °C. *Anal.* Calc. for $C_{10}H_{15}N_3OS$ (225.3): 53.31% C, 6.71% H, 18.65% N, 14.23% S; found: 53.00% C, 6.58% H, 18.36% N, 14.00% S, log $P = 3.02 \pm 0.41$. IR spectrum: 1677; ^{1}H NMR spectrum: δ 8.77s 1H (H3), 8.11s 1H (NH_2), 7.87s 1H (NH_2), 2.67s 3H (CH_3), 2.82t 2H (CH_2), 1.74m 2H (CH_2), 1.39m 2H (CH_2), 0.95t 3H (CH_3).

2.2.7. 5-Butyl-6-(ethylsulfanyl)pyrazine-2-carboxamide ***(1g)***

Yield 55%, m.p. 70–74 °C. *Anal.* Calc. for $C_{11}H_{17}N_3OS$ (239.3): 55.20% C, 7.16% H, 17.56% N, 13.40% S; found: 55.34% C, 7.19% H, 17.24% N, 13.40% S, log $P = 3.55 \pm 0.41$. IR spectrum: 1671; ^{1}H NMR spectrum: δ 8.77s 1H (H3), 8.05s 1H (NH_2), 7.87s 1H (NH_2), 3.35q 2H (CH_2), 1.34t 3H (CH_3), 2.81t 2H (CH_2), 1.73m 2H (CH_2), 1.39m 2H (CH_2), 0.95t 3H (CH_3).

2.2.8. 5-Butyl-6-(propylsulfanyl)pyrazine-2-carboxamide ***(1h)***

Yield 54%, m.p. 90–92 °C. *Anal.* Calc. for $C_{12}H_{19}N_3OS$ (253.4): 56.89% C, 7.56% H, 16.58% N, 12.65% S; found: 56.91% C, 7.89% H, 16.84% N, 12.89% S, log $P = 4.08 \pm 0.41$. IR spectrum: 1671; ^{1}H NMR spectrum: δ 8.76s 1H (H3), 8.03s 1H (NH_2), 7.88s 1H (NH_2), 3.34t 2H (CH_2), 1.72m 2H (CH_2), 1.03t 3H (CH_3), 2.81t 2H(CH_2), 1.74m 2H (CH_2), 1.38m 2H (CH_2), 0.94t 3H (CH_3).

2.2.9. 5-Butyl-6-(butylsulfanyl)pyrazine-2-carboxamide ***(1i)***

Yield 46%, m.p. 75–79 °C. *Anal.* Calc. for $C_{13}H_{21}N_3OS$ (267.4): 58.40% C, 7.92% H, 15.71% N, 11.99% S; found: 58.26% C, 7.90% H, 15.98% N, 11.71% S, log $P = 4.61 \pm 0.41$. IR spectrum: 1675; ^{1}H NMR spectrum: δ 8.76s 1H (H3), 8.02s 1H (NH_2), 7.88s 1H (NH_2), 3.36t 2H (CH_2), 1.65m 2H (CH_2), 1.48m 2H (CH_2), 0.94t 3H (CH_3), 2.81t 2H (CH_2), 1.73m 2H (CH_2), 1.39m 2H (CH_2), 0.94t 3H (CH_3).

2.2.10. 5-Butyl-6-(phenylsulfanyl)pyrazine-2-carboxamide ***(1j)***

Yield 58%, m.p. 149–152 °C. *Anal.* Calc. for $C_{15}H_{17}N_3OS$ (287.4): 62.69% C, 5.96% H, 14.62% N, 11.16% S; found: 62.56% C, 6.15% H, 14.44% N, 11.31% S, log $P = 4.76 \pm 0.44$. IR spectrum: 1695; ^{1}H NMR spectrum: δ 8.84s 1H (H3), 7.80s 1H (NH_2), 6.76s 1H (NH_2), 7.65m 2H, 7.56m 3H, 2.94t 2H (CH_2), 1.79m 2H (CH_2), 1.44m 2H (CH_2), 0.98t 3H (CH_3).

2.2.11. 5-Isobutyl-6-(methylsulfanyl)pyrazine-2-carboxamide ***(1k)***

Yield 29%, m.p. 96–99 °C. *Anal.* Calc. for $C_{10}H_{15}N_3OS$ (225.3): 53.31% C, 6.71% H, 18.65% N, 14.23% S; found: 53.62% C, 7.20% H, 18.33% N, 14.03% S, log $P = 3.02 \pm 0.41$. IR spectrum: 1701; ^{1}H NMR spectrum: δ 8.79s 1H (H3), 8.09s 1H (NH_2), 7.88s 1H (NH_2), 2.70t 3H (CH_3), 2.69d 2H (CH_2), 2.23m 1H (CH), 0.95d 6H (CH_3).

2.2.12. 6-(Ethylsulfanyl)-5-isobutylpyrazine-2-carboxamide ***(1l)***

Yield 49%, m.p. 104–109 °C. *Anal.* Calc. for

$C_{11}H_{17}N_3OS$ (239.3): 55.20% C, 7.16% H, 17.56% N, 13.40% S; found: 55.05% C, 7.32% H, 17.27% N, 13.09% S, log $P = 3.55 \pm 0.41$. IR spectrum: 1691; ^{1}H NMR spectrum: δ 8.79s 1H (H3), 8.06s 1H (NH_2), 7.88s 1H (NH_2), 3.35q 2H(CH_2), 1.34t 3H (CH_3), 2.70d 2H (CH_2), 2.23m 1H (CH), 0.95d 6H (CH_3).

*2.2.13. 5-Isobutyl-6-(propylsulfanyl)pyrazine-2-carboxamide (**1m**)*

Yield 76%, m.p. 90–93 °C. *Anal.* Calc. for $C_{12}H_{19}N_3OS$ (253.4): 56.89% C, 7.56% H, 16.58% N, 12.65% S; found: 56.74% C, 7.69% H, 16.21% N, 12.38% S, log $P = 4.08 \pm 0.41$. IR spectrum: 1671; ^{1}H NMR spectrum: δ 8.78s 1H (H3), 8.04s 1H (NH_2), 7.89s 1H (NH_2), 3.34t 2H (CH_2), 1.72m 2H (CH_2), 1.03t 3H (CH_3), 2.71d 2H (CH_2), 2.23m 1H (CH), 0.95d 6H (CH_3).

*2.2.14. 6-(Butylsulfanyl)-5-isobutylpyrazine-2-carboxamide (**1n**)*

Yield 80%, m.p. 86–89 °C. *Anal.* Calc. for $C_{13}H_{21}N_3OS$ (267.4): 58.40% C, 7.92% H, 15.71% N, 11.99% S; found: 58.67% C, 7.83% H, 15.50% N, 11.81% S, log $P = 4.61 \pm 0.41$. IR spectrum: 1671; ^{1}H NMR spectrum: δ 8.78s 1H (H3), 8.02s 1H (NH_2), 7.88s 1H (NH_2), 3.36t 2H (CH_2), 1.66m 2H (CH_2), 1.47m 2H (CH_2), 0.94t 3H (CH_3), 2.71d 2H (CH_2), 2.23m 1H (CH), 0.95d 6H (CH_3).

*2.2.15. 5-Isobutyl-6-(phenylsulfanyl)pyrazine-2-carboxamide (**1o**)*

Yield 50%, m.p. 125–128 °C. *Anal.* Calc. for $C_{15}H_{17}N_3OS$ (287.4): 62.69% C, 5.96% H, 14.62% N, 11.16% S; found: 62.66% C, 5.98% H, 14.31% N, 11.45% S, log $P = 4.76 \pm 0.44$. IR spectrum: 1687; ^{1}H NMR spectrum: δ 8.78s 1H (H3), 7.60–7.54m 2H (H2′, H6′), 7.52–7.46m 3H (H3′, H4′, H5′), 2.76d 2H $J = 6.9$ (CH_2), 2.33–2.17m 1H (CH), 0.94d 6H $J = 6.9$ (CH_3).

*2.2.16. 5-tert-Butyl-6-(methylsulfanyl)pyrazine-2-carboxamide (**1p**)*

Yield 32%, m.p. 149–151 °C. *Anal.* Calc. for $C_{10}H_{15}N_3OS$ (225.3): 53.31% C, 6.71% H, 18.65% N, 14.23% S; found: 53.36% C, 7.10% H, 18.79% N, 14.15% S, log $P = 2.84 \pm 0.41$. IR spectrum: 1682; ^{1}H NMR spectrum: δ 8.79s 1H (H3), 8.08s 1H (NH_2), 7.82s 1H (NH_2), 2.56t 3H (CH_3), 1.31m 9H (CH_3).

*2.2.17. 5-tert-Butyl-6-(ethylsulfanyl)pyrazine-2-carboxamide (**1q**)*

Yield 30%, m.p. 109–111 °C. *Anal.* Calc. for $C_{11}H_{17}N_3OS$ (239.3): 55.20% C, 7.16% H, 17.56% N, 13.40% S; found: 55.09% C, 6.96% H, 17.37% N, 13.35% S, log $P = 3.37 \pm 0.41$. IR spectrum: 1682; ^{1}H NMR spectrum: δ 8.79s 1H (H3), 8.16s 1H (NH_2), 7.70s 1H (NH_2), 3.00q 2H (CH_2), 1.50t 3H (CH_3), 1.36m 9H (CH_3).

*2.2.18. 5-tert-Butyl-6-(propylsulfanyl)pyrazine-2-carboxamide (**1r**)*

Yield 26%, m.p. 105–106 °C. *Anal.* Calc. for $C_{12}H_{19}N_3OS$ (253.4): 56.89% C, 7.56% H, 16.58% N, 12.65% S; found: 56.74% C, 7.69% H, 16.21% N, 12.38% S, log $P = 3.90 \pm 0.41$. IR spectrum: 1684; ^{1}H NMR spectrum: δ 8.88s 1H (H3), 8.06s 1H (NH_2), 7.82s 1H (NH_2), 3.44t 2H (CH_2), 2.10m 2H (CH_2), 1.13t 3H (CH_3), 1.51m 9H (CH_3).

*2.2.19. 5-tert-Butyl-6-(butylsulfanyl)pyrazine-2-carboxamide (**1s**)*

Yield 26%, m.p. 102–103 °C. *Anal.* Calc. for $C_{13}H_{21}N_3OS$ (267.4): 58.40% C, 7.92% H, 15.71% N, 11.99% S; found: 58.67% C, 7.83% H, 15.50% N, 11.81% S, log $P = 4.43 \pm 0.41$. IR spectrum: 1682; ^{1}H NMR spectrum: δ 8.98s 1H (H3), 8.02s 1H (NH_2), 7.88s 1H (NH_2), 3.46t 2H (CH_2), 2.16m 2H (CH_2), 1.87m 2H (CH_2), 0.94t 3H (CH_3), 1.51m 9H (CH_3).

*2.2.20. 5-tert-Butyl-6-(phenylsulfanyl)pyrazine-2-carboxamide (**1t**)*

Yield 28%, m.p. 185–187 °C. *Anal.* Calc. for $C_{15}H_{17}N_3OS$ (287.4): 62.69% C, 5.96% H, 14.62% N, 11.16% S; found: 62.93% C, 5.88% H, 14.35% N, 11.11% S, log $P = 4.58 \pm 0.44$. IR spectrum: 1694; ^{1}H NMR spectrum: δ 9.18s 1H (H3), 8.10s 1H (NH_2), 7.60s 1H (NH_2), 7.59m 2H (CH_2), 7.48m 3H (CH_3), 1.59m 9H (CH_3).

*2.3. 5-Alkyl-6-(alkylsulfanyl)- and 5-alkyl-6-(arylsulfanyl)pyrazine-2-carbothioamides (**2a**–**2t**). General procedure*

The corresponding amide (10 mmol) and Lawesson's reagent (2.2 g, 5.5 mmol) in anhydrous toluene (10 ml) were heated at 110 °C for 4 h. The reaction mixture was evaporated at reduced pressure and the crude product was further purified by column chromatography and recrystallised from ethanol.

*2.3.1. 6-(Methylsulfanyl)-5-propylpyrazine-2-carbothioamide (**2a**)*

Yield 47%, m.p. 150–154 °C. *Anal.* Calc. for $C_9H_{13}N_3S_2$ (227.4): 47.55% C, 5.76% H, 18.48% N, 28.20% S; found: 47.75% C, 5.80% H, 18.13% N, 27.92% S, log $P = 2.86 \pm 0.45$. ^{1}H NMR spectrum: δ 9.07s 1H (H3), 2.73t 2H, $J = 7.5$ (CH_2), 2.60s 3H (CH_3), 1.79–1.63m 2H (CH_2), 0.91t 3H, $J = 7.5$ (CH_3).

*2.3.2. 6-(Ethylsulfanyl)-5-propylpyrazine-2-carbothioamide (**2b**)*

Yield 63%, m.p. 164–166 °C. *Anal.* Calc. for

$C_{10}H_{15}N_3S_2$ (241.4): 49.76% C, 6.26% H, 17.41% N, 26.57% S; found: 49.98% C, 6.46% H, 16.96% N, 26.61% S, log $P = 3.39 \pm 0.45$. ^{1}H NMR spectrum: δ 9.08s 1H (H3), 3.29q 2H, $J_1 = 14.7$, $J_2 = 7.3$ (CH_2), 2.71t 2H, $J = 7.4$ (CH_2), 1.78–1.62m 2H (CH_2), 1.26t 3H, $J = 7.3$ (CH_3), 0.91t 3H, $J = 7.4$ (CH_3).

2.3.3. 5-Propyl-6-(propylsulfanyl)pyrazine-2-carboxamide ***(2c)***

Yield 63%, m.p. 144–148 °C. *Anal.* Calc. for $C_{11}H_{17}N_3S_2$ (255.4): 51.73% C, 6.71% H, 16.45% N, 25.11% S; found: 51.41% C, 7.03% H, 16.08% N, 25.11% S, log $P = 3.92 \pm 0.45$. ^{1}H NMR spectrum: δ 9.07s 1H (H3), 3.28t 2H, $J = 7.1$ (CH_2), 2.72t 2H, $J = 7.4$ (CH_2), 1.78–1.56m 4H (CH_2), 0.96t 3H, $J = 7.4$ (CH_3), 0.91t 3H, $J = 7.1$ (CH_3).

2.3.4. 6-(Butylsulfanyl)-5-propylpyrazine-2-carbothioamide ***(2d)***

Yield 71%, m.p. 141–144 °C. *Anal.* Calc. for $C_{12}H_{19}N_3S_2$ (269.4): 53.50% C, 7.11% H, 15.60% N, 23.80% S; found: 53.63% C, 7.12% H, 15.49% N, 23.59% S, log $P = 4.46 \pm 0.45$. ^{1}H NMR spectrum: δ 9.07s 1H (H3), 3.30t 2H, $J = 7.2$ (CH_2), 2.72t 2H, $J = 7.4$ (CH_2), 1.78–1.53m 4H (CH_2), 1.47–1.32m 2H (CH_2), 0.91t 3H, $J = 7.2$ (CH_3), 0.87t 3H, $J = 7.4$ (CH_3).

2.3.5. 6-(Phenylsulfanyl)-5-propylpyrazine-2-carbothioamide ***(2e)***

Yield 32%, m.p. 134–137 °C. *Anal.* Calc. for $C_{14}H_{15}N_3S_2$ (289.2): 58.10% C, 5.22% H, 14.52% N, 22.16% S; found: 57.83% C, 5.06% H, 14.12% N, 22.37% S, log $P = 4{,}61 \pm 0{,}49$. ^{1}H NMR spectrum: δ 9.12s 1H (H3), 7.62–7.55m 2H (H2′, H6′), 7.52–7.46m 3H (H3′, H4′, H5′), 2.84t 2H, $J = 7.4$ (CH_2), 1.84–1.70m 2H (CH_2), 0.96t 3H, $J = 7.4$ (CH_3).

2.3.6. 5-Butyl-6-(methylsulfanyl)pyrazine-2-carbothioamide ***(2f)***

Yield 84%, m.p. 135–139 °C. *Anal.* Calc. for $C_{10}H_{15}N_3S_2$ (241.4): 49.76% C, 6.26% H, 17.41% N, 26.57% S; found: 49.79% C, 6.38% H, 17.70% N, 26.52% S, log $P = 3{,}21 \pm 0{,}41$. ^{1}H NMR spectrum: δ 9.14s 1H (H3), 10.32s 1H (NH_2), 9.77s 1H (NH_2), 2.67s 3H (CH_3), 2.82t 2H (CH_2), 1.74m 2H (CH_2), 1.38m 2H (CH_2), 0.95t 3H (CH_3).

2.3.7. 5-Butyl-6-(ethylsulfanyl)pyrazine-2-carbothioamide ***(2g)***

Yield 70%, m.p. 139–143 °C. *Anal.* Calc. for $C_{11}H_{17}N_3S_2$ (255.4): 51.73% C, 6.71% H, 16.45% N, 25.11% S; found: 51.66% C, 6.98% H, 16.43% N, 25.04% S, log $P = 3.74 \pm 0.45$. ^{1}H NMR spectrum: δ 9.15s 1H (H3), 10.32s 1H (NH_2), 9.74s 1H (NH_2), 3.36q 2H (CH_2), 1.34t 3H (CH_3), 2.81t 2H (CH_2), 1.73m 2H (CH_2), 1.39m 2H (CH_2), 0.95t 3H (CH_3).

2.3.8. 5-Butyl-6-(propylsulfanyl)pyrazine-2-carbothioamide ***(2h)***

Yield 82%, m.p. 118–122 °C. *Anal.* Calc. for $C_{12}H_{19}N_3S_2$ (269.4): 53.50% C, 7.11% H, 15.60% N, 23.80% S; found: 53.55% C, 7.36% H, 15.43% N, 23.94% S, log $P = 4.27 \pm 0.45$. ^{1}H NMR spectrum: δ 9.13s 1H (H3), 10.32s 1H (NH_2), 9.74s 1H (NH_2), 3.36t 2H (CH_2), 1.72m 2H (CH_2), 1.03t 3H (CH_3), 2.82t 2H (CH_2), 1.73m 2H (CH_2), 1.39m 2H (CH_2), 0.95t 3H (CH_3).

2.3.9. 5-Butyl-6-(butylsulfanyl)pyrazine-2-carbothioamide ***(2i)***

Yield 94%, m.p. 120–123 °C. *Anal.* Calc. for $C_{13}H_{21}N_3S_2$ (283.5): 55.09% C, 7.47% H, 14.82% N, 22.62% S; found: 54.99% C, 7.42% H, 14.51% N, 22.31% S, log $P = 4.80 \pm 0.45$. ^{1}H NMR spectrum: δ 9.14s 1H (H3), 10.32s 1H (NH_2), 9.73s 1H (NH_2), 3.37t 2H (CH_2), 1.65m 2H (CH_2), 1.47m 2H (CH_2), 0.95t 3H (CH_3), 2.82t 2H (CH_2), 1.74m 2H (CH_2), 1.38m 2H (CH_2), 0.95t 3H (CH_3).

2.3.10. 5-Butyl-6-(phenylsulfanyl)pyrazine-2-carbothioamide ***(2j)***

Yield 69%, m.p. 99–102 °C. *Anal.* Calc. for $C_{15}H_{17}N_3S_2$ (303.4): 59.37% C, 5.65% H, 13.85% N, 21.13% S; found: 59.58% C, 5.61% H, 13.57% N, 20.96% S, log $P = 4.95 \pm 0.50$. ^{1}H NMR spectrum: δ 9.19s 1H (H3), 10.21s 1H (NH_2), 8.56s 1H (NH_2), 7.65m 2H (CH_2), 7.55m 3H (CH_3), 2.93t 2H (CH_2), 1.79m 2H (CH_2), 1.44m 2H (CH_2), 0.98t 3H (CH_3).

2.3.11. 5-Isobutyl-6-(methylsulfanyl)pyrazine-2-carbothioamide ***(2k)***

Yield 61%, m.p. 153–157 °C. *Anal.* Calc. for $C_{10}H_{15}N_3S_2$ (241.4): 49.76% C, 6.26% H, 17.41% N, 26.57% S; found: 49.65% C, 6.09% H, 17.32% N, 26.35% S, log $P = 3.21 \pm 0.41$. ^{1}H NMR spectrum: δ 9.16s 1H (H3), 10.33s 1H (NH_2), 9.75s 1H (NH_2), 2.62s 3H (CH_3), 2.71d 2H (CH_2), 2.26m 1H (CH), 0.96d 6H (CH_3).

2.3.12. 6-(Ethylsulfanyl)-5-isobutylpyrazine-2-carbothioamide ***(2l)***

Yield 75%, m.p. 130–134 °C. *Anal.* Calc. for $C_{11}H_{17}N_3S_2$ (255.4): 51.73% C, 6.71% H, 16.45% N, 25.11% S; found: 52.06% C, 6.51% H, 16.61% N, 25.90% S, log $P = 3.74 \pm 0.45$. ^{1}H NMR spectrum: δ 9.16s 1H (H3), 10.33s 1H (NH_2), 9.75s 1H (NH_2), 3.36q 2H (CH_2), 1.33t 3H (CH_3), 2.71d 2H (CH_2), 2.26m 1H (CH), 0.96d 6H (CH_3).

Table 1
Antituberculotic activity (% inhibition) of selected 5-alkyl-6-alkylsulfanyl- resp. 5-alkyl-6-arylsulfanylpyrazine-2-carboxamides (**1**) and corresponding thioamides (**2**) and calculated lipophilicity (log *P*) in comparison with rifampicin (RMP)

Comp.	MIC (μg ml^{-1})	% Inhibition	log *P*
1h	>12.5	62	4.08 ± 0.41
1i	>12.5	79	4.61 ± 0.41
1j	>6.25	61	4.76 ± 0.44
2e	>6.25	63	4.61 ± 0.49
2f	>12.5	62	3.21 ± 0.41
2g	>12.5	77	3.74 ± 0.45
2h	>12.5	83	4.27 ± 0.45
2i	>12.5	87	4.80 ± 0.45
2j	<6.25	91	4.95 ± 0.50
2m	>6.25	64	4.27 ± 0.45
2o	>6.25	78	4.95 ± 0.50
2p	>6.25	72	3.03 ± 0.46
RMP	0.125	100	0.49 ± 0.74

*2.3.13. 5-Isobutyl-6-(propylsulfanyl)pyrazine-2-carbothioamide (**2m**)*

Yield 81%, m.p. 120–123 °C. *Anal.* Calc. for $C_{12}H_{19}N_3S_2$ (269.4): 53.50% C, 7.11% H, 15.60% N, 23.80% S; found: 53.83% C, 7.21% H, 15.60% N, 23.50% S, log $P = 4.27 \pm 0.45$. ^{1}H NMR spectrum: δ 9.16s 1H (H3), 10.33s 1H (NH_2), 9.74s 1H (NH_2), 3.35t 2H (CH_2), 1.70m 2H (CH_2), 1.03t 3H (CH_3), 2.71d 2H (CH_2), 2.23m 1H (CH), 0.96d 6H (CH_3).

*2.3.14. 6-(Butylsulfanyl)-5-isobutylpyrazine-2-carbothioamide (**2n**)*

Yield 61%, m.p. 119–122 °C. *Anal.* Calc. for $C_{13}H_{21}N_3S_2$ (283.5): 55.09% C, 7.47% H, 14.82% N, 22.62% S; found: 55.39% C, 7.45% H, 14.53% N, 22.35% S, log $P = 4.80 \pm 0.45$. ^{1}H NMR spectrum: δ 9.15s 1H (H3), 10.34s 1H (NH_2), 9.74s 1H (NH_2), 3.37t 2H (CH_2), 1.66m 2H (CH_2), 1.46m 2H (CH_2), 0.95t 3H (CH_3), 2.71d 2H (CH_2), 2.23m 1H (CH), 0.96d 6H (CH_3).

*2.3.15. 5-Isobutyl-6-(phenylsulfanyl)pyrazine-2-carbothioamide (**2o**)*

Yield 47%, m.p. 127–131 °C. *Anal.* Calc. for $C_{15}H_{17}N_3S_2$ (303.4): 59.37% C, 5.65% H, 13.85% N, 21.13% S; found: 59.46% C, 5.69% H, 13.83% N, 21.44% S, log $P = 4.95 \pm 0.50$. ^{1}H NMR spectrum: δ 9.13s 1H (H3), 7.62–7.55m 2H (H2′, H6′), 7.52–7.45m 3H (H3′, H4′, H5′), 2.76d 2H, $J = 6.9$ (CH_2), 2.32–2.16m 1H (CH), 0.94d 6H, $J = 6.9$ (CH_3).

*2.3.16. 5-tert-Butyl-6-(methylsulfanyl)-pyrazine-2-carbothioamide (**2p**)*

Yield 32%, m.p. 116–118 °C. *Anal.* Calc. for $C_{10}H_{15}N_3S_2$ (241.4): 49.76% C, 6.26% H, 17.41% N, 26.57% S; found: 50.06% C, 6.09% H, 17.36% N, 26.30% S, log $P = 3.03 \pm 0.46$. ^{1}H NMR spectrum: δ 9.40s 1H (H3), 10.10s 1H (NH_2), 8.28s 1H (NH_2), 2.56m 3H (CH_3), 1.29m 9H (CH_3).

*2.3.17. 5-tert-Butyl-6-(ethylsulfanyl)pyrazine-2-carbothioamide (**2q**)*

Yield 35%, m.p. 96–97 °C. *Anal.* Calc. for $C_{11}H_{17}N_3S_2$ (255.4): 51.73% C, 6.71% H, 16.45% N, 25.11% S; found: 51.62% C, 6.73% H, 16.70% N, 25.33% S, log $P = 3.56 \pm 0.45$. ^{1}H NMR spectrum: δ 9.40s 1H (H3), 10.23s 1H (NH_2), 8.13s 1H (NH_2), 3.06q 2H (CH_2), 1.52t 3H (CH_3), 1.20m 9H (CH_3).

*2.3.18. 5-tert-Butyl-6-(propylsulfanyl)pyrazine-2-carbothioamide (**2r**)*

Yield 21%, m.p. 92–93 °C. *Anal.* Calc. for $C_{12}H_{19}N_3S_2$ (269.4): 53.50% C, 7.11% H, 15.60% N, 23.80% S; found: 53.83% C, 7.21% H, 15.60% N, 23.50% S, log $P = 4.09 \pm 0.45$. ^{1}H NMR spectrum: δ 9.38s 1H (H3), 10.34s 1H (NH_2), 8.18s 1H (NH_2), 3.45t 2H (CH_2), 2.10m 2H (CH_2), 0.98t 3H (CH_3), 1.20m 9H (CH_3).

*2.3.19. 5-tert-Butyl-6-(butylsulfanyl)pyrazine-2-carbothioamide (**2s**)*

Yield 61%, m.p. 90–91 °C. *Anal.* Calc. for $C_{13}H_{21}N_3S_2$ (283.5): 55.09% C, 7.47% H, 14.82% N, 22.62% S; found: 55.39% C, 7.45% H, 14.53% N, 22.35% S, log $P = 4.62 \pm 0.46$. ^{1}H NMR spectrum: δ 9.45s 1H (H3), 10.24s 1H (NH_2), 8.74s 1H (NH_2), 3.35t 2H (CH_2), 1.66m 2H (CH_2), 1.46m 2H (CH_2), 0.95t 3H (CH_3), 1.22m 9H (CH_3).

*2.3.20. 5-tert-Butyl-6-(phenylsulfanyl)pyrazine-2-carbothioamide (**2t**)*

Yield 20%, m.p. 153–155 °C. *Anal.* Calc. for $C_{15}H_{17}N_3S_2$ (303.4): 59.37% C, 5.65% H, 13.85 (H3), 10.34s 1H (NH_2), 8.13s 1H (NH_2), 7.58m 2H (CH_2), 7.48m 3H (CH_3), 1.20m 9H (CH_3).

2.4. Antimycobacterial assay

Antimycobacterial evaluation was carried out in Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL, USA, which is a part of the National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.5 or 12.5 μg ml^{-1} against *Mycobacterium tuberculosis* H_{37}Rv in BACTEC 12B medium using the BACTEC 460 radiometric system [12]. Compounds showing at least 90% inhibition in this primary screening were retested at lower concentrations against *M. tuberculosis* H_{37}Rv to determine the actual minimum inhibitory concentration (MIC) in a broth microdilution Alamar Blue assay (MABA). The MIC was defined as the lowest concentration effecting a decrease in fluorescence of 99% relative to controls. For

the results of the compounds with a minimal 50% inhibition, see Table 1.

2.5. In vitro antifungal susceptibility testing

The broth microdilution test [13,14] was used for the assessment of in vitro antifungal activity of the synthetised compounds against *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 in comparison with ketoconazole. The procedure was performed with twofold compound dilutions 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 and 48 h of static incubation at 35 °C. With *Trichophyton mentagrophytes*, the MICs were determined after 72 and 120 h of incubation.

3. Results and discussion

The study describes 40 newly prepared compounds (20 amides and 20 corresponding thioamides). Primary antimycobacterial and antifungal screening was provided for all prepared compounds.

In both series, the antimycobacterial activity increases with increasing molecular weight of the alkylsulfanyl group in position 6 of the pyrazine ring. Thioamides exhibited higher activity than the corresponding amides. 5-Butyl-6-phenylsulfanylpyrazine-2-carbothioamide (**2j**) showed the highest antituberculotic activity that achieved 91% inhibition of mycobacterial growth at concentration 12.5 μg ml^{-1} and was retested (MABA) to determine the actual minimal inhibitory concentration (MIC > 12.5 μg ml^{-1}). This compound also exhibited the highest lipophilicity ($\log P = 4.95$). Twelve compounds (**1h**, **1i**, **1j**, **2e**, **2f**, **2g**, **2h**, **2i**, **2j**, **2m**, **2o**, **2p**) showed higher than 60% inhibition (see Table 1). Compounds that displayed marginal inhibition > 30–40% are of interest, since subtle alterations in physical properties may result in drastic changes in biological effects. The activity is further positively influenced by the introduction of a four-carbon chain (in particular butyl) into position 5 of the pyrazine ring. The only three amides with higher than 60% inhibition contain butyl in this position as well.

The evaluation of in vitro antifungal activity of the synthetised compounds showed only compound **2q** and, partly, compounds **1m** and **2s** having a considerable antifungal effect on all the fungal strains tested. The most susceptible was *Trichophyton mentagrophytes* strain (MIC = 31.25–500 μmol l^{-1}), especially to compound **1i** and *Absidia corymbifera* (MIC = 62.50–1000 μmol l^{-1}) to compounds **1i**, **2o** and **2q**, which MICs were approaching those of ketoconazole (MIC = 31.25 μmol l^{-1} against *Absidia corymbifera*). The negative results of antifungal screening do not allow us to draw conclusions on some structure–activity relationships.

Acknowledgements

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (research plans No. 11160001 and No. 11160002). Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the US National Institute of Allergy and Infectious Diseases.

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