CHEMIJA. 2004. T. 15. Nr. 4. P. 53–56

Synthesis and anti-inflammatory activity of N-(4-alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl)thiocarbamides

Milda Malvina Burbulienë, Virginija Jakubkienë, Emilija Udrënaitë, Povilas Vainilavièius

Faculty of Chemistry, Vilnius University, Naugarduko 24, LT-03225, Vilnius, Lithuania

Refluxing of 2-alkylthio-4-chloro-6-methylpyrimidines with potassium thiocyanate in ethanol gave mixtures of corresponding thio- and isothiocianates, which on heating in dry xylene isomerized completely to isothiocyanates. The latter reacted with 4-alkoxyanilines to give the title compounds. N-(4-alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl) thiocarbamides possessing methylthio group at the 2nd position of pyrimidine ring stimulated the inflammation process in a rat paw, while thiocarbamides with butylthio substituent at the same position exhibited anti-inflammatory activity.

Key words: N-(4-alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl)thiocarbamides, synthesis, IR spectra, anti-inflammatory activity

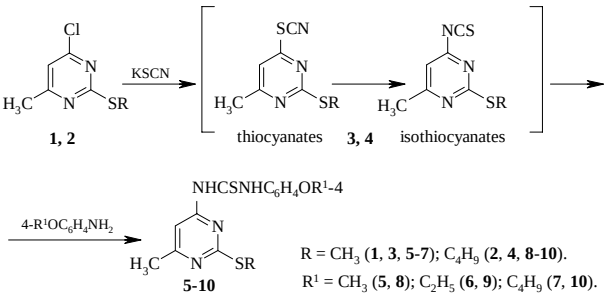
INTRODUCTION

Thiourea derivatives exhibit diverse biological and pharmacological activities. Derivatives of N-aryl- or N-heteroarylthioureas are known as potential inhibitors of HIV-1 Reverse Transcriptase and related viruses [1, 2], antihyperthyroid drugs [3], anti-inflammatory and analgetic agents [4], acaricides [5], as well as for their wide spectrum of anthelmintic activity [6].

Previously we reported on alkyl 2-{4-[4-alkoxyanilino(thioxo)methylamino]-6-methyl-2-pyrimidinylsulfanyl}acetates [7] and 2-{4-[4-alkoxyanilino(thioxo)methylamino]-6-methyl-2-pyrimidinylsulfanyl}acetic acids, their diethylammonium salts and hydrazides [8] exhibiting anti-inflammatory activity. The anti-inflammatory activity may be caused by two structural fragments, N-phenylthioureido- and thioglycolic acid, present in the molecules of these compounds. As a continuation of our search of the structure of pyrimidine derivatives and a relationship with pharmacological properties, we have synthesized thiocarbamides possessing in their structure a remainder of N-phenylthioureido- but lacking the thioglicolic acid fragment. Herein we report on the synthesis and tests of anti-inflammatory activity of N-(4-alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl)thiocarbamides.

RESULTS AND DISCUSSION

The synthesis was accomplished according to the scheme:



The starting compounds 2-alkylthio-6-chloro-4-methylpyrimidines (**1**, **2**) were prepared following the reported method [9]. Thiocyanates **3**, **4** were obtained by the treatment of 6-chloropyrimidines **1**, **2** with potassium thiocyanate in 80% ethanol (in the case of **2** in abs. ethanol) according to reference [7]. However, the formation of the mixture of two compounds was observed by the method of TLC. The resulting thiocyanates **3** undergo partial isomerization to isothiocyanates **4**. This was confirmed by literature data [7]. Complete isomerization of thiocyanates **3** to

isothiocyanates **4** was achieved by refluxing the mixture (after evaporation of ethanol) in dry xylene. Isothiocyanates **3**, **4** are viscous non-crystallizing liquids growing resinous on vacuum distillation. Therefore we used them in reactions with aromatic amines without any purification. Isothiocyanates **3**, **4** reacted completely within 2 hours. Thus, they are less active than isothiocyanates, possessing thioglycolic acid moiety at the 2nd position of pyrimidine ring. The reaction of the latter with amines proceeded 1 hour [7]. Compounds **5-10** are colourless substances insoluble in water. Solubility in organic solvents increases as the number of carbon atoms in alkoxy or alkylthio group increases. The structures of **5-10** were supported by IR, ^{1}H NMR spectroscopy and elemental analysis. IR spectra of **5-10** display absorption bands in the range 1178–1184 cm^{-1} and 1294–1303 cm^{-1} characteristic of C=S, also at 3132–3147 cm^{-1} and 3204–3219 cm^{-1} corresponding to stretching absorption of NH of tiocarbamides [10, 11].

Earlier synthesized N-phenyl-N'-pyrimidinylthiocarbamides with thioglycolic acid moiety at the 2nd position of pyrimidine ring suppressed the inflammatory process to some extent in a rat paw [7].

Table 1. **Anti-inflammatory activity of compounds 5–10**

Compound (dose 50 mg/kg)	Inhibition of rat hind paw oedema (%) induced by	
	carrageenin	bentonite
5	+6.2 ± 1.4	+4.6 ± 0.8
6	+8.1 ± 1.6	+5.2 ± 1.2
7	+24.7 ± 5.0*	+12.7 ± 3.7*
8	10.5 ± 2.4	2.0 ± 0.6
9	12.0 ± 2.2*	9.9 ± 2.3
10	18.8 ± 3.2*	21.3 ± 3.2*
Acetylsalicylic acid	20.3 ± 3.5*	21.5 ± 3.0*

* $p < 0.05$.

Compounds **5-10** influence the inflammation process depending on the 2-alkylthio substituent differently. Compounds **5-7** possessing methylthio group at the 2nd position of pyrimidine ring stimulate inflammation, and compounds **8-10** with lipofilic butylthio group at the analogous position of pyrimidine skeleton, on the contrary, inhibit this process. Increasing the carbon chain in the alkoxy fragment increased the effect in both groups. The best anti-inflammatory activity equal to that of acetylsalicylic acid was shown by N-(4-butoxyphenyl)-N'-(2-butylthio-6-methyl-4-pyrimidinyl)thiocarbamide (**10**).

EXPERIMENTAL

1. Chemistry

Melting points were determined in open capillaries and are uncorrected. The IR spectra were measured on a Spectrum BX FT-IR (Perkin-Elmer) in Nujol, ^{1}H-NMR spectra were recorded on a BS-587A (80 MHz, Tesla) in DMSO-d_6 with TMS as an internal standard. Chemical shifts (δ) are reported in ppm. The reactions were monitored by TLC on silica-gel-coated Al plates (KAVALIER). Elemental analyses were performed at the Microanalysis Laboratory of the Department of Organic Chemistry, Faculty of Chemistry of Vilnius University.

4-Chloro-6-methyl-2-methylthiopyrimidine (**1**) and 2-butylthio-4-chloro-6-methylpyrimidine (**2**) were synthesized according to the procedure reported in [9].

N-(4-Alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl)thiocarbamides (5-10). A mixture of 2-alkylthio-4-chloro-6-methylpyrimidine **1**, **2** (0.1 mol) and potassium thiocyanate (14.6 g, 0.15 mol) in 85% ethanol (100 ml) (in the case of compd. **2** – abs. ethanol) was stirred at reflux for 5 h. After a half of the solvent was removed, the residue was left to cool to room temperature. The mixture was then diluted with water (300 ml) and extracted with benzene (3 × 100 ml). The organic layer was dried over anhydrous magnesium sulfate and evaporated. The residue was dissolved in xylene (50 ml) and heated at reflux for 10 h. The solvent was removed *in vacuo*. The residue was diluted with acetonitrile (100 ml). The solution obtained was divided into 3 equal parts and each of them was treated with 4-methoxy-, 4-ethoxy- or 4-butoxyanilines (0.025 mol). The reaction mixture was kept at r. t. (18–20 °C) for 2 h. The precipitate formed was filtered off, washed with ether and crystallized.

N-(4-Methoxyphenyl)-N'-(6-methyl-2-methylthio-4-pyrimidinyl)thiocarbamide (5): yield 48%, m.p. 204–206 °C (propanol). ^{1}H NMR: 2.23 (3H, s, CH_3), 2.27 (3H, s, SCH_3), 3.54 (3H, s, OCH_3), 6.55 (1H, s, pyrimidine CH), 6.61–7.16 (4H, m, aromatic CH). IR, ν, cm^{-1}: 3138, 3219 (NH), 1183, 1300 (C=S). Found: C, 52.30; H, 5.21; N, 17.18%. Calculated for $C_{14}H_{16}N_4OS_2$: C, 52.48; H, 5.03; N, 17.48%.

N-(4-Ethoxyphenyl)-N'-(6-methyl-2-methylthio-4-pyrimidinyl)thiocarbamide (6): yield 52%, m.p. 197–198 °C (propanol). ^{1}H NMR: 1.06 (3H, m, OCH_2CH_3), 2.23 (3H, s, CH_3), 2.26 (3H, s, SCH_3), 3.86 (2H, m, OCH_2CH_2), 6.54 (1H, s, pyrimidine CH), 6.60–7.25 (4H, m, aromatic CH). IR, ν, cm^{-1}: 3132, 3204 (NH), 1180, 1294 (C=S). Found: C, 53.59; H, 5.72; N, 16.60%. Calculated for $C_{15}H_{18}N_4OS_2$: C, 53.87; H, 5.42; N, 16.75%.

N-(4-Butoxyphenyl)-N'-(6-methyl-2-methylthio-4-pyrimidinyl)thiocarbamide (7): yield 50%, m.p. 181–182 °C (propanol). ^{1}H NMR: 0.54 [3H, m, $OCH_2(CH_2)_2CH_3$], 0.81–1.64 [4H, m, $OCH_2(CH_2)_2CH_3$], 2.23 (3H, s, CH_3), 2.24 (3H, s, SCH_3), 3.74 [3H, m, $OCH_2(CH_2)_2CH_3$], 6.53 (1H, s, pyrimidine CH), 6.56–7.18 (4H, m, aromatic CH). IR, ν, cm^{-1}: 3147, 3217 (NH), 1181, 1294 (C=S). Found: C, 56.49; H, 6.45; N, 15.23%. Calculated for $C_{17}H_{22}N_4OS_2$: C, 56.33; H, 6.12; N, 15.45%.

N-(4-Methoxyphenyl)-N'-(2-butylthio-6-methyl-4-pyrimidinyl)thiocarbamide (8): yield 49%, m.p. 179–180 °C (ethanol). ^{1}H NMR: 0.42 [3H, m, $SCH_2(CH_2)_2CH_3$], 0.64–1.50 [4H, m, $SCH_2(CH_2)_2CH_3$], 2.22 (3H, s, CH_3), 2.80 [2H, m, $SCH_2(CH_2)_2CH_3$], 3.56 (3H, s, OCH_3), 6.52 (1H, s, pyrimidine CH), 6.60–7.15 (4H, m, aromatic CH). IR, ν, cm^{-1}: 3141, 3210 (NH), 1178, 1300 (C=S). Found: C, 56.55; H, 6.35; N, 15.47%. Calculated for $C_{17}H_{22}N_4OS_2$: C, 56.33; H, 6.12; N, 15.45%.

N-(4-Ethoxyphenyl)-N'-(2-butylthio-6-methyl-4-pyrimidinyl)thiocarbamide (9): yield 50%, m.p. 162-163 °C (ethanol). ^{1}H NMR: 0.40 [3H, m, $SCH_2(CH_2)_2CH_3$], 0.66–1.52 [7H, m, $SCH_2(CH_2)_2CH_3$, OCH_2CH_3], 2.23 (3H, s, CH_3), 2.82 [2H, m, $SCH_2(CH_2)_2CH_3$], 3.85 (2H, m, OCH_2CH_3), 6.53 (1H, s, pyrimidine CH), 6.59–7.14 (4H, m, aromatic CH). IR, ν, cm^{-1}: 3146, 3213 (NH), 1178, 1303 (C=S). Found: C, 57.51; H, 6.48; N, 15.12%. Calculated for $C_{18}H_{24}N_4OS_2$: C, 57.42; H, 6.42; N, 14.88%.

N-(4-Butoxyphenyl)-N'-(2-butylthio-6-methyl-4-pyrimidinyl)thiocarbamide (10): yield 52%, m.p. 171–172 °C (ethanol). ^{1}H NMR: 0.38–0.56 [6H, m, $SCH_2(CH_2)_2CH_3$, $OCH_2(CH_2)_2CH_3$], 0.64–1.66 [8H, m, $SCH_2(CH_2)_2CH_3$, $OCH_2(CH_2)_2CH_3$], 2.22 (3H, s, CH_3), 2.80 [2H, m, $SCH_2(CH_2)_2CH_3$], 3.76 [2H, m, $OCH_2(CH_2)_2CH_3$], 6.54 (1H, s, pyrimidine CH), 6.58–7.20 (4H, m, aromatic CH). IR, ν, cm^{-1}: 3146, 3209 (NH), 1184, 1295 (C=S). Found: C, 59.41; H, 7.15; N, 14.01%. Calculated for $C_{20}H_{28}N_4OS_2$: C, 59.37; H, 6.97; N, 13.85.

2. Pharmacology

For anti-inflammatory tests, adult male and female Wistar rats weighing 140–150 g were used. All test compounds and the reference drug were administered orally suspended in 1% carboxymethylcellulose with 1 drop of twin-80 solution. The effect of test compounds on rat paw oedema was compared to that in control rats.

Carrageenin-induced hind paw oedema in rats was produced by the method of Winter et al. [12]. Carrageenin solution (1.0% in sterile 0.9% NaCl solution) was injected subcutaneously into the subplanar region of the hind paw (in a volume of 0.1 ml to each paw) 1 h after administration of the test compound. Control rats received only solution of 0.5% carboxymethylcellulose with one drop of twin-80. The hind paw volume was measured with an electronic oncograph immediately before and 1, 2, 3 and 5 h after carrageenin injection. The values obtained at the each recording time were expressed as a mean ± standard deviation and are given in relative units as an arithmetical mean. Significance in the differences between the mean values of the controls and the test groups was estimated by the Student's t test. The results were statistically significant ($p < 0.05$) during the whole period of observation (5 h).

Bentonite-induced hind paw oedema was analogously studied [13]. Bentonite suspension (5% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was used. The data were evaluated statistically using Student's t test.

CONCLUSIONS

1. Under treatment of 2-alkylthio-6-methyl-4-pyrimidinylisothiocyanates with 4-alkoxyanilines N-(4-alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl)thiocarbamides were obtained.

2. IR spectra of N-(4-alkoxyphenyl)-N'-(2-alkylthio-6-methyl-4-pyrimidinyl)thiocarbamides display characteristic absorption bands in the range 1178–1184, 1294–1303 cm^{-1} (C = S) and 3132–3147, 3204–3219 cm^{-1} (NH).

3. N-(4-Alkoxyphenyl)-N'-(2-methylthio-6-methyl-4-pyrimidinyl)thiocarbamides stimulate the inflammatory process, while N-(4-alkoxyphenyl)-N'-(2-butylthio-6-methyl-4-pyrimidinyl)thiocarbamides suppress it.

4. The best anti-inflammatory activity equal to that of acetysalicylic acid was exhibited by N-(4-butoxyphenyl)-N'-(2-butylthio-6-methyl-4-pyrimidinyl)thiocarbamide.

Received 11 October 2004
Accepted 2 November 2004

References

1. P. T. Lind, J. M. Morin, R. Noreen and R. J. Ternansky, PTC Int. Appl. WO 9303022 (1993); *C. A.* **119**, 160110 (1993).
2. F. W. Bell, A. S. Cantrell, M. Holgberg, S. R. Jaskunas, N. G. Johasson, Ch. L. Jordan, M. D. Kinnick, P. Lind, J. M. Morin et al. *J. Med. Chem*., **38** (25), 4929 (1995).
3. D. R. Doerge, C. J. Decker and R. S. Takazawa, *Biochemistry*, **32** (1), 58 (1993).
4. Ch. S. J. Walpole and R. Wrigglesworth, Eur. Pat. Appl. EP 462933 (1991); *C. A.*, **116**, 151346 (1992).
5. A. Pascual and A. Rindlisbacter, *Pestic. Sci*. **42** (4), 253 (1994); *C. A.*, **122**, 49049 (1995).
6. M. D. Brewer, R. J. J. Dorgan, B. R. Manger, P. Mamalis and R. A. B. Webster, *J. Med. Chem*., **30** (10), 1848 (1987).

7. M. M. Burbulienë, V. Jakubkienë, V. S. Roèka, E. Udrënaitë, P. Gaidelis and P. Vainilavièius, *Chemija*, **10** (2), 136 (1999).
8. M. M. Burbulienë, V. Jakubkienë, V. S. Roèka, E. Udrënaitë, P. Gaidelis and P. Vainilavièius, *Chemija*, **11** (2), 82 (2000).
9. K. Takagi and T. Ueda, *Chem. Pharm. Bull*, **11**, 1382 (1963).
10. L. J. Bellamy, *Advances in infrared group frequences* (in Russian), 230, Mir, Moscow (1971).
11. Ê. Nakanisi, *Infrared spectra and structure of organic compounds* (in Russian), 66, Mir, Moscow (1965).
12. C. A. Winter, E. A. Rielsy and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
13. J. Marek, *Pharmazie*, **36**, 46 (1981).

Milda Malvina Burbulienë, Virginija Jakubkienë, Emilija Udrënaitë, Povilas Vainilavièius

N-(4-ALKOKSIFENIL)-N'-(2-ALKILTIO-6-METIL-4-PIRIMIDINIL)TIOKARBAMIDØ SINTEZË IR JØ PRIEÐUÞDEGIMINIS AKTYVUMAS

Santrauka

Virinant 2-alkiltio-4-chlor-6-metilpirimidinus etanolio tirpale su kalio tiocianatu susidaro atitinkamø tio- ir izotiocianatø miðiniai. Pastarieji, kaitinami abs. ksilene, visiðkai izomerizuojasi á izotiocianatus. Izotiocianatai, reaguodami su 4-alkoksianilinais, virsta N-(4-alkoksifenil)-N'-(2-alkiltio-6-metil-4-pirimidinil)tiokarbamidais. Tiokarbamidai, turintys 2-oje pirimidino þiedo padëtyje metiltiogrupæ, skatina uþdegiminá procesà, o tiokarbamidai, turintys 2-oje pirimidino þiedo padëtyje butiltiogrupæ, pasiþymi prieðuþdegiminiu aktyvumu.