SYNTHESIS OF THE AZATHIOPURINE ANALOGS

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Abstract – The effective synthesis of the azathioprine analogs - 2-substituted derivatives of 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purines 5 has been achieved by the reaction of 2-substituted 6-purinethiones 4 with 5-chloro-1-methyl-4-nitroimidazole in ethanol. In the case of 7-methyl-2,6-dithioxanthine 4j, reaction in DMF gave di(imidazolyl) product 5l. The key step in this synthesis was preparation of the appropriate 2-substituted 6-purinones 2 and 6-chloropurines 3 which were further converted to 6-purinethione 4.

INTRODUCTION

Azathioprine, 6-(1-methyl-4-nitroimidazol-5-ylthio)purine, is one of the oldest immunosuppressive agents available today. Now rarely used for chemotherapy but more for immunosuppresion in solid organ transplantation and autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, hematologic malignancies, dermatologic afflictions, ulcerative colitis or Crohn’s disease. Azathioprine is a prodrug that is converted in the liver to imidazole derivatives and 6-mercaptopurine. The metabolites of both substances have independent immunosuppressive properties. The accepted mechanism of its action is at the level of DNA. Azathioprine interferes with DNA and RNA synthesis and has numerous non-specific effects on immune system. The active metabolite of 6-mercaptopurine, thiouquarine, is incorporated into ribonucleotides, thereby exerting an antiproliferative effect on mitotically active lymphocyte populations. Azathioprine also possesses direct antiinflammatory properties by inhibiting cytotoxic T cell and natural killer cell function. The unique and unexpected role for azathioprine and its metabolites is the control of T cell apoptosis by modulation of GTP-binding protein – Rac 1 upon CD28 costimulation. Searching for prodrugs with potential biological activity, the synthesis of new analogs of azathioprine as 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purines has been described.

RESULTS AND DISCUSSION

The key step in the synthesis of the azathioprine analogs 5 was a preparation of appropriate 2-substituted 7-methyl-6-purinethiones 2 and 2-substituted 6-chloro-7-methylpurines 3 which were further transformed into appropriate 6-purinethiones 4. Problems with the synthesis of the chloro substrates 3 were overcome
exploiting regioselective reactions of identical substituted 2,6-dialkoxo- and 2,6-dialkylthio-7-methylpurines \(1\) with the hydroxide ion to give the hypoxanthine analogs \(2\). This alkaline hydrolysis of compounds \(1\) proceeded in aqueous sodium hydroxide solution in position 6 and was effective for compounds with the methoxy, ethoxy, allyloxy, methylthio and ethylthio substituents in position 2 (\(2a-c, 2e, 2f\), 85-98% yield, Scheme 1). The yields of hydrolysis of compounds \(1\) with other substituents, i.e. benzyloxy, allylthio and benzylthio were unsatisfactory (10-21% yields) in these conditions, probably for the reason of weak solubility of the 2,6-dibenzylxyo-, 2,6-diallylthio- and 2,6-dibenzylthio-7-methylpurines \(1d, 1g, 1h\) in water. When the alkaline hydrolysis was carried out in aqueous ethanol solution (water-ethanol 1:2 v/v) 6-purinones \(2d, 2g\) and \(2h\) were obtained in good yields (87-94%) but 2-allylthio-
and 2-benzylthio-6-ethoxy-7-methylpurines 1i and 1j were also obtained (in 8 and 12% yields) as a result of high reactivity of the position 6 in identical substituted purines towards the oxygen nucleophilic agents, as observed previously in other purine derivatives.\textsuperscript{10} Hydrolysis in alkaline DMSO solution led to only one product, 7-methylxanthine 2i, as the result of the double substitution (Scheme 1).

Next we wanted to convert 6-purinones 2 into corresponding 6-purinethiones 4 by the described earlier procedure of chlorination of 2-substituted 7-methyl-6-purinones 2 with phosphorus oxychloride followed by reaction with thiourea in ethanol.\textsuperscript{10a} It appeared that only 2-methoxy-, 2-methylthio- and 2-ethylthio-7-methyl-6-purinones 2a, 2e and 2f reacted smoothly with phosphorus oxychloride to give 6-chloroderivatives 3a, 3e and 3f. The other 6-purinones with the ethoxy, benzyloxy, allylthio or benzylthio groups underwent O- and S-dealkylation in position 2 during reaction with phosphorus oxychloride giving 6-chloro-7-methyl-2-purinone 3i or 2-purinethione 3j (Scheme 2). The last compound was used later for the synthesis of the 2-allylthio- and 2-benzylthio derivatives 3g and 3h by S-alkylation.

\begin{center}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Substrate & Product & \multicolumn{3}{|c|}{Yield (%)} \\
\hline
No & X & R & No & X & R \\
\hline
2a & O & Me & 3a & O & Me & 75 \\
2b & O & Et & 3i & O & 82 \\
2c & O & allyl & 3i & O & 81 \\
2d & O & Bn & 3i & O & 95 \\
2e & S & Me & 3e & S & Me & 87 \\
2f & S & Et & 3f & S & Et & 89 \\
2g & S & allyl & 3j & S & 78 \\
2h & S & Bn & 3j & S & 85 \\
3j & S & & 3g & S & allyl & 91 \\
3j & S & & 3h & S & Bn & 85 \\
\hline
\end{tabular}
\end{center}

Scheme 2

In order to obtain 6-purinethiones 4 with the alkoxy and alkylthio groups in position 2 two routes were used. In the first one 6-chloroderivatives 3 were refluxed with thiourea in ethanol and in the second one 6-purinones 2 were heated with phosphorus pentasulfide in pyridine. Treatment of 6-chloro-7-methyl-2-purinone 3i or 2-purinethione 3j with thiourea led to 7-methyl-6-thioxanthine 4i and 7-methyl-2,6-dithio-
xanthine 4j (Scheme 3).

\[
\begin{align*}
3a, 3e, 3f & \xrightarrow{A} 4a-h \xrightarrow{B} 2b-d, 2g, 2h \\
3i, 3j & \xrightarrow{A} 4i, 4j
\end{align*}
\]

**Scheme 3**

Reactions of 2-substituted (with the alkoxy, alkylthio and chloro groups) 7-methyl-6-purinethiones 4a-4h and 4k with 5-chloro-1-methyl-4-nitroimidazole in 70% ethanol gave 2-substituted 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purines 5a-5h and 5k in good yields (71-98%, Scheme 4). In the case of the 2-allylthio and 2-benzylthio compounds 4g and 4h a process of S-dealkylation was also observed to give derivative 5j (in 12% and 18% yield). Whereas reaction of 7-methyl-6-thioxanthine 4i with 5-chloro-1-methyl-4-nitroimidazole in ethanol gave only expected imidazolylthio derivative 5i (in 68% yield), 7-methyldithioxanthine 4j gave 3 products: expected imidazolylthio derivative 5j (in low yield of 9%), di(imidazolylthio) product 5l (in 62% yield) and a product being the result of an action of ethanol, ethoxy derivative 5m (in 24%). Only di(imidazolylthio) product 5l in high yield of 91% was observed when the reaction was carried out in DMF (Scheme 5). Unexpected ethoxy compound 5m was considered as a result of the subsequent reaction of the formed di(imidazolylthio) product 5l rather than the substrate 4j with ethanol. The separate reaction of compound 5l with boiling ethanol for 2 h showed the
imidazolylthio group in position 6 to be a quite good leaving group to give ethoxy derivative 5m in 82% yield (Scheme 6).

\[
\text{Scheme 4}
\]

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\[
\text{Scheme 5}
\]

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Conclusion

We report here an efficient synthesis of 13 new azathioprine analogs being of 2-substituted derivatives of 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purines $5$ in the series of transformations starting from 2,6-dialkoxo- and 2-dialkylthio-7-methylpurines $1$.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Boetius melting point apparatus and are uncorrected. The $^1$H NMR spectra were recorded on a Varian Unity-Inova 300 spectrometer at 300 MHz in deuteriochloroform and dimethyl sulfoxide-$d_6$ with tetramethylsilane as the internal standard. Electron impact (EI MS) and chemical ionization mass spectra (CI MS) were run on Finnigan MAT 95 spectrometer at 70eV. Column chromatography was performed on silica gel 60 (Merck) using a mixture of chloroform-methanol (10:1, v/v) as an eluent. 2,6-Dialkoxo-7-methylpurines $1a-d$ with identical alkyl groups were prepared from 2,6-dichloro-7-methylpurine and corresponding sodium alkoxide in alcohol solution according to known procedure.$^{10c,11}$

1. 2,6-Dimethoxy-7-methylpurine ($1a$), mp 197-198 °C (EtOH), lit.,$^{10c}$ mp 198-199 °C.
2. 2,6-Diethoxy-7-methylpurine ($1b$), mp 147-148 °C (EtOH), lit.,$^{10c}$ mp 146-148 °C.
3. 2,6-Diallyloxy-7-methylpurine ($1c$), mp 109-110 °C (Et$_2$O), lit.,$^{11b}$ mp 111-112 °C.
4. 2,6-Dibenzyloxy-7-methylpurine ($1d$), (2.57g, 75%), mp 103-105 °C (Et$_2$O).

$^1$H NMR (CDCl$_3$) $\delta$: 3.94 (s, 3H, NMe), 5.52 (s, 2H, $O_2$CH$_2$), 5.58 (s, 2H, $O_6$CH$_2$), 7.36 (m, 6H, 2 m-C$_6$H$_5$, 2 p-C$_6$H$_5$), 7.45 (d, $J = 7.5$ Hz, 2H, o-C$_6$H$_5$), 7.54 (d, $J = 7.5$Hz, 2H, o-C$_6$H$_3$), 7.83 (s, 1H, H8), EI MS m/z: 346 (M$^+$, 23), 255 (M-CH$_2$C$_6$H$_5$, 33), C$_6$H$_5^+$ (100). Anal. Calcd for C$_{20}$H$_{18}$N$_4$O$_2$: C 69.35, H 5.24, N 16.17. Found C 69.07, H 5.29, N 15.96.

2,6-Dialkylthio-7-methylpurines $1e-h$ with identical alkyl groups were prepared from 2,6-dichloro-7-methylpurine and thiourea in EtOH followed S-alkylation with 2 equivalents of alkyl halides (methyl iodide, ethyl iodide, allyl bromide and benzyl chloride) in 4% KOH solution at room temperature.$^{12}$

1. 2,6-Dimethylthio-7-methylpurine ($1e$), mp 176-177 °C (EtOH), lit.,$^{12}$ mp 176-178 °C.
2. 2,6-Diethylthio-7-methylpurine (1f) (2.18g, 85%), mp 132-133 °C (EtOH).

\[ \text{H NMR (CDCl}_3\text{)} \delta: 1.41 (t, J = 7.3 \text{ Hz, } 3 \text{H, Me}), 1.45 (t, J = 7.3 \text{ Hz, } 3 \text{H, Me}), 3.24 (q, J = 7.3 \text{ Hz, } 2 \text{H, S}_2\text{CH}_2), 3.38 (q, J = 7.3 \text{ Hz, } 2 \text{H, CH}_2), 4.06 (s, 3 \text{H, NMe}), 7.86 (s, 1 \text{H, H-8}), \text{EI MS m/z: 254 } (\text{M}^+, 100), 225 (\text{M-C}_2\text{H}_5, 89). \]

Anal. Calcd for C\text{_{10}H}_{14}\text{N}_4\text{S}_2: C 47.22, H 5.55, N 22.03. Found C 47.06, H 5.59, N 21.76.

3. 2,6-Diallylthio-7-methylpurine (1g) (2.81g, 78%); mp 94-95 °C (Et\text{2}O).

\[ \text{H NMR (CDCl}_3\text{)} \delta: 3.91 (d, J = 6.9 \text{ Hz, } 2 \text{H, S}_2\text{CH}_2), 4.04 (d, J = 6.9 \text{ Hz, } 2 \text{H, S}_6\text{CH}_2), 4.08 (s, 3 \text{H, NMe}), 5.12 (d, J = 10.1 \text{ Hz, } 1 \text{H, =CH}_2), 5.20 (d, J = 10.1 \text{ Hz, } 1 \text{H, =CH}_2), 5.33 (d, J = 13.5 \text{ Hz, } 1 \text{H, =CH}_2), 5.39 (d, J = 13.5 \text{ Hz, } 1 \text{H, =CH}_2), 6.01 (m, 2 \text{H, =CH}), 8.00 (s, 1 \text{H, H8}), \text{EI MS m/z: 278 } (\text{M}^+, 27), 237 (\text{M-C}_3\text{H}_5, 100). \]


4. 2,6-Dibenzylthio-7-methylpurine (1h) (3.48g, 91%); mp 131-132 °C (EtOH).

\[ \text{H NMR (CDCl}_3\text{)} \delta: 4.01 (s, 3 \text{H, NMe}), 4.52 (s, 2 \text{H, S}_2\text{CH}_2), 4.57 (s, 2 \text{H, S}_6\text{CH}_2), 7.27 (m, 6 \text{H, 2 m-C}_6\text{H}_5, 2 \text{ p-C}_6\text{H}_5), 7.41 (d, J = 6.9 \text{Hz, } 2 \text{H, o-C}_6\text{H}_5), 7.47 (d, J = 6.9 \text{Hz, } 2 \text{H, o-C}_6\text{H}_5), 7.88 (s, 1 \text{H, H8}), \text{EI MS m/z: 378 } (\text{M}^+, 86), 287 (\text{M-C}_3\text{H}_5\text{C}_6\text{H}_5, 100). \]


**Hydrolysis of dialkoxy- and dialkylthio-7-methylpurines 1 to 2-substituted 7-methyl-6-purinones 2 - general procedure.**

To the substrates 1 (10 mmol) in 8-10% aqueous NaOH solution (100 mL, method A) or a mixture of 8% NaOH-EtOH solution (1:2 v/v, 120 mL, method B) or 5% NaOH-DMSO solution (1:2 v/v, 40 mL, method C) was added. The mixture was boiled (until all the material had gone into solution) for 1-4 h up to full clarification of a suspension. In 5% NaOH-DMSO solution, the suspension of the substrates 1d or 1h was stirred on an oil bath at 100 °C for 1.5 h. After cooling the clear solution was brought to pH = 6 by addition of glacial acetic acid. The resulting solid was filtered off, air-dried and crystallized from EtOH to give 6-purinones 2a-h. When the reaction was carried out in NaOH-EtOH solution, a mixture of two products was obtained. After cooling, EtOH was evaporated in vaccuo and the residue was neutralized with diluted hydrochloric acid. The resulting solid was filtered off to give crude products which were purified by column chromatography (silica gel, CHCl\text{3}) to give 6-purinones 2d, 2g and 2h. The filtrate was extracted with CHCl\text{3} (3 x 10 mL), the extracts were dried with anhydrous sodium sulfate and evaporated in vaccuo to dryness. The residue was purified by column chromatography (silica gel, CHCl\text{3}) to give 2-allylthio-6-ethoxy-7-methylpurine 1i or 2-benzylthio-6-ethoxy-7-methylpurine 1j.

1. 2-Methoxy-7-methyl-6-oxo-1,6-dihydropurine (2a) (1.53g, 85%); mp 219-220 °C (EtOH-water), lit.,\textsuperscript{13} mp 220 °C.
2. 2-Ethoxy-7-methyl-6-oxo-1,6-dihydropurine (2b) (1.89g, 97%); mp 214-215 °C (EtOH-water).

\[ \text{H NMR (DMSO-}d_6\text{)} \delta: 1.29 (t, J = 6.9 Hz, 3H, Me), 3.88 (s, 3H, NMe), 4.30 (q, J = 6.9 Hz, 2H, OCH}_2\text{), 7.92 (s, 1H, C8), 10.02 (s, 1H, NH), EI MS m/z: 194 (M^+, 76), 166 (M-C_2H_4, 100). Anal. Calcd for C_8H_10N_4O_2: C 49.48, H 5.19, N 28.85. Found C 49.18, H 5.25, N 28.54.\]

3. 2-Allyloxy-7-methyl-6-oxo-1,6-dihydropurine (2c) (1.95g, 95%); mp 199-200 °C (EtOH-water).

\[ \text{H NMR (DMSO-}d_6\text{)} \delta: 3.89 (s, 3H, NMe), 4.80 (d, J = 6.4 Hz, 2H, OCH}_2\text{), 5.25 (d, J = 11.1 Hz, 1H, =CH}_2\text{), 5.40 (d, J = 18.0 Hz, 1H, =CH), 6.04 (m, 1H, =CH), 7.94 (s, 1H, H8), 10.04 (s, 1H, NH), EI MS m/z: 206 (M^+, 23), 165 (M-C_3H_5, 100). Anal. Calcd for C_9H_10N_4O_2: C 52.42, H 4.89, N 27.17. Found C 52.18, H 4.96, N 26.88.\]

4. 2-Benzylthio-7-methyl-6-oxo-1,6-dihydropurine (2d) (method A: 0.54g, 21%, method B: 2.41g, 94%); mp 191-192 °C (EtOH).

\[ \text{H NMR (DMSO-}d_6\text{)} \delta: 3.87 (s, 3H, NMe), 5.32 (s, 2H, CH}_2\text{), 7.32 (t, J = 7.4 Hz, 1H, p-C}_6\text{H}_5\text{), 7.38 (t, J = 7.4 Hz, 2H, m-C}_6\text{H}_5\text{), 7.44 (d, J = 7.4 Hz, 2H, o-C}_6\text{H}_5\text{), 7.89 (s, 1H, H8), 10.12 (s, 1H, NH), EI MS m/z: 256 (M^+, 30), 165 (M-C}_2\text{H}_4\text{C}_6\text{H}_5\text{, 13), C}_6\text{H}_5\text{CH}_2\text{OS}^+\text{ (100). Anal. Calcd for C}_{13}\text{H}_{12}\text{N}_4\text{O}_2: C 60.93, H 4.72, N 21.86. Found C 60.81, H 4.77, N 21.49.\]

5. 2-Methylthio-7-methyl-6-oxo-1,6-dihydropurine (2e) (1.92g, 98%); mp 303-305 °C (water), lit.\textsuperscript{10b} mp > 300 °C.

6. 2-Ethylthio-7-methyl-6-oxo-1,6-dihydropurine (2f) (1.89g, 90%); mp 275-276 °C (EtOH-water).

\[ \text{H NMR (DMSO-}d_6\text{)} \delta: 1.27 (t, J = 7.2 Hz, 3H, Me), 3.03 (q, J = 7.2 Hz, 2H, OCH}_2\text{), 3.88 (s, 3H, NMe), 7.82 (s, 1H, H8), 10.19 (s, 1H, NH), EI MS m/z: 210 (M^+, 100), 182 (M-C}_2\text{H}_4\text{, 43). Anal. Calcd for C}_8\text{H}_{10}\text{N}_4\text{OS: C 45.70, H 4.79, N 26.65. Found C 45.49, H 4.69, N 26.39.\]

7. 2-Allylthio-7-methyl-6-oxo-1,6-dihydropurine (2g) (method A: 0.42 g, 19%, method B: 1.05g, 88%); mp 216-218 °C (EtOH-water).

\[ \text{H NMR (DMSO-}d_6\text{)} \delta: 3.83 (d, J = 6.9 Hz, 2H, SCH}_2\text{), 3.91 (s, 3H, NMe), 4.60 (q, J = 6.9 Hz, 2H, OCH}_2\text{), 5.31 (d, J = 17.4 Hz, 1H, =CH), 5.95 (m, 1H, =CH), 8.07 (s, 1H, H8), 10.38 (s, 1H, NH), EI MS m/z: 222 (M^+, 100), 181 (M-C}_3\text{H}_5\text{, 55). Anal. Calcd for C}_{11}\text{H}_{14}\text{N}_4\text{OS: C 48.64, H 5.64, N 22.38. Found C 48.41, H 4.55, N 24.92.\]

In the case of method B an additional product was isolated: 2-allylthio-6-ethoxy-7-methylpurine (1i) (0.20g, 8%); mp 165-166 °C (EtOH).

\[ \text{H NMR (CDCl}_3\text{)} \delta: 1.47 (t, J = 7.2 Hz, 3H, Me), 3.90 (d, J = 6.9 Hz, 2H, SCH}_2\text{), 4.02 (s, 3H, NMe), 4.60 (q, J = 7.2 Hz, 2H, OCH}_2\text{), 5.11 (d, J = 10.2 Hz, 1H, =CH), 5.34 (d, J = 17.3 Hz, 1H, =CH), 6.04 (m, 1H, =CH), 8.03 (s, 1H, H8), EI MS m/z: 250 (M^+, 39), 209 (M-C}_3\text{H}_5\text{, 65), 222 (M-C}_2\text{H}_4\text{, 100). Anal. Calcd for C}_{11}\text{H}_{14}\text{N}_4\text{OS: C 52.78, H 5.64, N 22.38. Found C 52.52, H 5.74, N 22.09.\]
8. 2-Benzylthio-7-methyl-6-oxo-1,6-dihydropurine (2h) (method A: 0.27 g, 10%, 2.37 g, 87%); mp 246-248 °C (EtOH).

\[ \text{H NMR (DMSO-} d_6 \text{)} \delta: \]
- 3.91 (s, 3H, NMe)
- 4.44 (s, 2H, CH2)
- 7.32 (m, 3H, m-C₆H₅, p-C₆H₅)
- 7.43 (d, J = 6.9 Hz, 2H, o-C₆H₅)
- 8.10 (s, 1H, H8)
- 12.28 (s, 1H, NH)

EI MS m/z: 272 (M⁺, 100), 181 (M-CH₂C₆H₅, 22), C₆H₅CH₂⁺ (78).


In the case of method B an additional product was isolated: 2-benzylthio-6-ethoxy-7-methylpurine (1j) (0.36 g, 12%); mp 142-143 °C (EtOH).

\[ \text{H NMR (CDCl₃)} \delta: \]
- 1.39 (t, J = 6.9 Hz, 3H, Me)
- 3.96 (s, 3H, NMe)
- 4.51 (s, 2H, SCH₂)
- 4.53 (q, J = 6.9 Hz, 2H, OCH₂)
- 7.25 (m, 3H, m-C₆H₅, p-C₆H₅)
- 7.42 (d, J = 6.9 Hz, 2H, o-C₆H₅)
- 8.04 (s, 1H, H8)

EI MS m/z: 300 (M⁺, 32), 209 (M-CH₂C₆H₅, 19), 272 (M-C₂H₄, 100). Anal. Calcd for C₁₅H₁₆N₄OS: C 59.98, H 5.37, N 18.65. Found C 59.66, H 5.41, N 18.39.

9. 7-Methylxanthine (2i) (1.53 g, 92% from 1d, 1.49 g, 90% from 1h); mp > 300 °C (EtOH), lit., mp > 300 °C.

**Synthesis of 2-substituted 6-chloro-7-methylpurines 3**

2-Substituted 6-chloro-7-methylpurines 3a, 3b and 3f were obtained by boiling appropriate 6-purinones 2 with phosphorus oxychloride, as described previously. When in position 2 in derivatives 2 the ethoxy, allyloxy, benzyloxy 2b-d or allythio 2g and benzylthio 2h groups were present, O- or S-dealkylation took place and the products were identified as 6-chloro-7-methyl-2-purinone 3i or 2-purinethione 3j.

6-Chloroderivatives 3g and 3h were prepared by S-alkylation with allyl bromide or benzyl chloride of 2-purinethione 3j according to earlier described procedure.

1. 2-Methoxy-6-chloro-7-methylpurine (3a) (1.49 g, 75%); mp 156-157 °C (EtOH), lit., mp 156-158 °C.
2. 2-Methylthio-6-chloro-7-methylpurine (3e) (1.86 g, 87%); mp 178-179 °C (EtOH), lit., mp 176-177 °C.
3. 2-Ethylthio-6-chloro-7-methylpurine (3f) (2.03 g, 89%); mp 174-175 °C (EtOH).

\[ \text{H NMR (CDCl₃)} \delta: \]
- 1.43 (t, J = 7.2 Hz, 3H, Me)
- 3.32 (q, J = 7.2 Hz, 2H, CH₂)
- 4.06 (s, 3H, NMe)
- 7.75 (s, 1H, C₈)

EI MS m/z: 228 (M⁺, 100), 200 (M-C₂H₄, 23). Anal. Calcd for C₈H₉ClN₄S: C 42.02, H 3.97, N 24.50. Found C 41.84, H 3.91, N 24.50.

4. 2-Allylthio-6-chloro-7-methylpurine (3g) (2.18 g, 91%); mp 206-207 °C (EtOH).

\[ \text{H NMR (CDCl₃)} \delta: \]
- 3.97 (d, J = 6.9 Hz, 2H, SCH₂)
- 4.06 (s, 3H, NMe)
- 5.17 (d, J = 10.3 Hz, 1H, =CH₂)
- 5.36 (d, J = 17.2 Hz, 1H, =CH₂)
- 5.97 (m, 2H, =CH)

EI MS m/z: 240 (M⁺, 100), 199 (M-C₃H₅, 83). Anal. Calcd for C₉H₁₀ClN₄S: C 44.91, H 3.77, N 23.28. Found C 44.63, H 3.79, N 23.01.
5. 2-Benzylthio-6-chloro-7-methylpurine (3h) (2.47g, 85%); mp 163-164 °C (EtOH).

\[ \text{\^H NMR (CDCl}_3\text{)} \delta: 4.02 \text{ (s, 3H, NMe), 4.45 \text{ (s, 2H, CH}_2\text{)}, 7.31 \text{ (m, 3H, m-C}_6\text{H}_5\text{, p-C}_6\text{H}_5\text{), 7.45 \text{ (d, } J = 6.9 \text{ Hz, 2H, o-C}_6\text{H}_5\text{), 8.66 \text{ (s, 1H, H8), EI MS m/z: 290 (M}^+, 100), 199 (M-CH}_2\text{C}_6\text{H}_5\text{, 28), C}_6\text{H}_5\text{CH}_2\text{} (96).} \]


6. 6-Chloro-7-methyl-2-oxo-2,3-dihydropurine (3i); mp 290-292 °C (water).

\[ \text{\^H NMR (DMSO-d}_6\text{)} \delta: 3.82 \text{ (s, 3H, NMe), 7.90 \text{ (s, 1H, H8), 11.52 \text{ (s, 1H, NH), EI MS m/z: 184 (M}^+, 100).} \]

Anal. Calcd for C\text{_6H}_5\text{ClN}_4\text{O}: C 39.04, H 2.73, N 30.35. Found C 38.82, H 2.79, N 30.03.

7. 6-Chloro-7-methyl-2-thioxo-2,3-dihydropurine (3j); mp > 300 °C (water), lit., 10a mp > 300 °C.

Synthesis of 6-purinethiones 4a-h

A. Reaction with thiourea.

A solution of 6-chloroderivative 3a and 3e-h (5 mmol) and thiourea (0.76 g, 10 mmol) in absolute EtOH (75 mL) was refluxed for 1-2 h. The solvent was removed \textit{in vacuo} and the residue was dissolved in 5% NaOH solution. The reaction product was precipitated with 15% hydrochloric acid and the process was repeated twice to give 6-purinethiones 4a and 4e-h in 73-86% yields, and 4i, 4j in 95% and 98% yields, respectively.

1. 2-Methoxy-7-methyl-6-thioxo-1,6-dihydropurine (4a) (0.72g, 73%); mp 238-240 °C (EtOH), lit., 10a mp 239-240 °C.

2. 2-Methylthio-7-methyl-6-thioxo-1,6-dihydropurine (4e) (0.91g, 86%); mp 176-177 °C (EtOH), lit., 10a mp 176-177 °C.

3. 2-Ethylthio-7-methyl-6-thioxo-1,6-dihydropurine (4f) (0.95g, 84%); mp 194-196 °C (EtOH).

\[ \text{\^H NMR (DMSO-d}_6\text{)} \delta: 1.29 \text{ (t, } J = 7.1 \text{ Hz, 3H, Me), 3.06 \text{ (q, } J = 7.1\text{Hz, 2H, CH}_2\text{), 3.91 \text{ (s, 3H, NMe), 7.97 \text{ (s, 1H, H8), 13.82 \text{ (s, 1H, N1), EI MS m/z: 226 (M}^+, 100), 198 (M-C}_2\text{H}_4\text{, 12).} \]

Anal. Calcd for C\text{_8H}_{10}\text{N}_4\text{S}_2: C 42.46, H 4.45, N 24.76. Found C 42.16, H 4.49, N 24.49.

4. 2-allylthio-7-methyl-6-thioxo-1,6-dihydropurine (4g) (0.87 g, 73%); mp 186-188 °C (EtOH).

\[ \text{\^H NMR (DMSO-d}_6\text{)} \delta: 3.85 \text{ (d, } J = 6.9 \text{ Hz, 2H, SCH}_2\text{), 3.93 \text{ (s, 3H, NMe), 5.13 \text{ (d, } J \text{ =10.2 Hz, 1H, =CH}_2\text{), 5.32 \text{ (d, } J = 17.1 \text{ Hz, 1H, =CH}_2\text{), 5.96 \text{ (m, 1H, =CH), 8.10 \text{ (s, 1H, H8), 13.92 \text{ (s, 1H, N1), EI MS m/z: 238 (M}^+, 100), 197 (M-C}_3\text{H}_5\text{, 42).} \]


5. 2-Benzylthio-7-methyl-6-thioxo-1,6-dihydropurine (4h) (1.12g, 78%); mp 201-203 °C (EtOH).

\[ \text{\^H NMR (DMSO-d}_6\text{)} \delta: 4.15 \text{ (s, 3H, NMe), 4.41 \text{ (s, 2H, CH}_2\text{), 7.29 \text{ (m, 3H, m-C}_6\text{H}_5\text{, p-C}_6\text{H}_5\text{), 7.44 \text{ (d, } J = 6.9 \text{ Hz, 2H, o-C}_6\text{H}_5\text{), 8.18 \text{ (s, 1H, H8), 13.59 \text{ (s, 1H, N1), EI MS m/z: 288 (M}^+, 100), 197 (M-CH}_2\text{C}_6\text{H}_5\text{, 87).} \]

5. 7-Methyl-2-oxo-6-thioxo-1,2,3,6-tetrahydropurine (4i) (0.87g, 95%); mp 342-344 °C (water), lit.,
mp 343-344 °C.
6. 7-Methyl-2,6-dithioxo-1,2,3,6-tetrahydropurine (4j) (0.97g, 98%); mp >300 °C (EtOH), lit.,
mp >300 °C.
B. Reaction with phosphorus pentasulfide
A mixture of 2-alkoxy- or 2-alkylthio-7-methyl-6-purinone 2b-d, 2g and 2h (1 mmol) and phosphorus pentasulfide (0.67g, 3 mmol) in dry pyridine (30 mL) was stirred on oil bath at boiling temperature for 3.5 h. After cooling, the pyridine was removed in vacuo and the residue was treated with boiling water (15 mL) for 15 min. The insoluble portion was dissolved in 25% NH₄OH solution and the products were precipitated by acidification with glacial acetic acid to pH = 5. The resulting solid was filtered off and air-dried to give 6-purinethiones 4b-d, 4g and 4h in 56-76% yields.
1. 2-Ethoxy-7-methyl-6-thioxo-1,6-dihydropurine (4b) (0.16g, 76%); mp 206-208 °C (EtOH).
1H NMR (DMSO-d₆) δ: 1.29 (t, J = 7.1 Hz, 3H, Me), 3.68 (s, 3H, NMe), 4.33 (q, J = 7.1 Hz, 2H, OCH₂), 7.98 (s, 1H, C8), 12.05 (s, 1H, NH), EI MS m/z: 210 (M⁺, 83), 182 (M-C₂H₄, 100). Anal. Calcd for C₈H₁₀N₄OS: C 45.70, H 4.79, N 26.65. Found C 45.41, H 4.69, N 26.37.
2. 2-Allyloxy-7-methyl-6-thioxo-1,6-dihydropurine (4c) (0.12g, 56%); mp 187-189 °C (EtOH).
1H NMR (DMSO-d₆) δ: 3.77 (s, 3H, NMe), 4.81 (d, J = 6.9 Hz, 2H, OCH₂), 5.25 (d, J = 10.1 Hz, 1H, =CH₂), 5.42 (d, J = 17.4 Hz, 1H, =CH₂), 6.02 (m, 1H, =CH), 8.05 (s, 1H, H8), 12.15 (s, 1H, NH), EI MS m/z: 222 (M⁺, 19), 181 (M-C₃H₅, 100). Anal. Calcd for C₉H₁₀N₄OS: C 48.64, H 4.53, N 25.21. Found C 48.39, H 4.56, N 24.92.
3. 2-Benzyloxy-7-methyl-6-thioxo-1,6-dihydropurine (4d) (0.17g, 62%); mp 228-230 °C (EtOH).
1H NMR (DMSO-d₆) δ: 4.01 (s, 3H, NMe), 5.80 (s, 2H, CH₂), 7.24 (t, J = 7.2 Hz, 1H, p-C₆H₅), 7.44 (t, J = 7.2 Hz, 2H, m-C₆H₅), 7.56 (d, J = 7.2 Hz, 2H, o-C₆H₅), 8.10 (s, 1H, H8), 12.27 (s, 1H, NH), EI MS m/z: 272 (M⁺, 26), 181 (M-C₂H₅C₆H₅, 18), C₆H₅CH₂⁺ (100). Anal. Calcd for C₁₇H₁₄N₄OS: C 57.34, H 4.44, N 20.57. Found C 57.38, H 4.48, N 20.32.
4. 2-Allylthio-7-methyl-6-thioxo-1,6-dihydropurine (4g) (0.15g, 63%); mp 186-188 °C (EtOH).
5. 2-Benzylthio-7-methyl-6-thioxo-1,6-dihydropurine (4h) (0.19g, 66%); mp 201-203 °C (EtOH).

Synthesis of 2-substituted 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purines 5 - general procedure
A. A mixture of 6-purinethione 4a-k (1 mmol), 5-chloro-1-methyl-4-nitroimidazole (0.16g, 1 mmol), 10% NaOH (0.4 mL) and 70% EtOH (10 mL) was refluxed for 2 h. After cooling the resulting solid was filtered off, washed with water, air dried and recrystallized or purified by column chromatography (silica
gel, CHCl3-EtOH, 10:1) to give 5a-k. The ethanolic filtrate was concentrated in vacuo to 5 mL to give an additional amount of products 5a-h and 5k or 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)-2-purinone 5i and 2-purinethione 5j. Compounds 5l and 5m were separated by column chromatography (silica gel, CHCl3).

1. 2-Methoxy-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5a) (0.3g, 94%); mp 218-219 °C (EtOH).

\[ \text{NMR (DMSO-}d_6\text{)} \delta: 3.68 (s, 3H, OMe), 3.74 (s, 3H, N7Me), 4.14 (s, 3H, NMe), 8.27 (s, 1H, C8), 8.53 (s, 1H, CH), Cl MS m/z: 322 (M+1, 100). \]

Anal. Calcd for C_{11}H_{11}N_{7}O_{3}S: C 41.12, H 3.45, N 30.51. Found C 41.01, H 3.40, N 30.22.

2. 2-Ethoxy-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5b) (0.26g, 78%); mp 182-183 °C (EtOH).

\[ \text{NMR (DMSO-}d_6\text{)} \delta: 1.15 (t, J = 7.1 Hz, 3H, Me), 3.72 (s, 3H, N7Me), 4.06 (q, J = 7.1 Hz, 2H, OCH₂), 4.12 (s, 3H, NMe), 8.26 (s, 1H, C8), 8.50 (s, 1H, CH), Cl MS m/z: 336 (M+1, 60), 307 (M-C_2H_5 +1, 100). \]

Anal. Calcd for C_{12}H_{13}N_{7}O_{3}S: C 42.98, H 3.91, N 29.24. Found C 42.69, H 3.95, N 29.01.

3. 2-Allyloxy-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5c) (0.25g, 72%); mp 177-178 °C (EtOH).

\[ \text{NMR (DMSO-}d_6\text{)} \delta: 3.70 (s, 3H, N7Me), 4.11 (s, 3H, NMe), 4.85 (d, J = 6.9 Hz, 2H, OCH₂), 5.18 (d, J = 11.2 Hz, 1H, =CH₂), 5.32 (d, J = 18.2 Hz, 1H, =CH₂), 5.93 (m, 1H, =CH), 8.23 (s, 1H, H8), 8.45 (s, 1H, CH), Cl MS m/z: 348 (M+1, 16), 307 (M-C_3H_5 +1, 100). \]

Anal. Calcd for C_{13}H_{13}N_{7}O_{3}S: C 44.95, H 3.77, N 28.23. Found C 44.66, H 3.79, N 27.94.

4. 2-Benzylloxy-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5d) (0.28g, 71%); mp 192-193 °C (EtOH).

\[ \text{NMR (DMSO-}d_6\text{)} \delta: 3.74 (s, 3H, N7Me), 3.85 (s, 3H, NMe), 5.15 (s, 2H, CH₂), 7.26 (m, 3H, m-C_6H_5, p-C_6H_5), 7.41 (d, J = 7.2Hz, 2H, o-C_6H_5), 7.46 (s, 1H, H8), 7.88 (s, 1H, CH), Cl MS m/z: 398 (M+1, 14), 307 (M-C_2H_5C_6H_5+1, 100). \]


5. 2-Methylthio-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5e) (0.28g, 83%); mp 224-225 °C (DMF/water).

\[ \text{NMR (DMSO-}d_6\text{)} \delta: 2.17 (s, 3H, SMe), 3.73 (s, 3H, N7Me), 4.15 (s, 3H, NMe), 8.28 (s, 1H, H8), 8.58 (s, 1H, NH), Cl MS m/z: 338 (M+1, 100). \]

Anal. Calcd for C_{11}H_{11}N_{7}O_{2}S_{2}: C 39.16, H 3.29, N 29.06. Found C 38.87, H 3.35, N 28.82.

6. 2-Ethylthio-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5f) (0.27g, 77%); mp 238-239 °C (EtOH).
1H NMR (DMSO-d$_6$) $\delta$: 1.24 (t, $J = 7.5$ Hz, 3H, Me), 2.95 (q, $J = 7.5$ Hz, 2H, SCH$_2$), 3.06 (s, 3H, N7Me), 3.85 (s, 3H, NMe), 6.99 (s, 1H, H8), 7.64 (s, 1H, CH), CI MS m/z: 352 (M+1, 100), 323 (M-C$_2$H$_5$+1, 66). Anal. Calcd for C$_{12}$H$_{13}$N$_7$O$_2$S$_2$: C 41.02, H 3.73, N 27.90. Found C 40.82, H 3.77, N 27.57.

7. 2- Allylthio-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5g) (0.26g, 72%); mp 174-175 °C (EtOH).

1H NMR (DMSO-d$_6$) $\delta$: 3.54 (s, 3H, N7Me), 3.88 (d, $J = 6.9$ Hz, 2H, SCH$_2$), 3.96 (s, 3H, NMe), 5.05 (d, $J = 10.1$ Hz, 1H, =CH$_2$), 5.22 (d, $J = 16.9$ Hz, 1H, =CH$_2$), 5.82 (m, 1H, =CH), 7.11 (s, 1H, C8), 7.83 (s, 1H, CH), CI MS m/z: 364 (M+1, 100), 323 (M-C$_3$H$_5$+1, 83). Anal. Calcd for C$_{13}$H$_{13}$N$_7$O$_2$S$_2$: C 42.97, H 3.61, N 26.98. Found C 42.71, H 3.51, N 26.66.

8. 2-Benzylthio-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5h) (0.31g, 75%); mp 234-235 °C (EtOH).

1H NMR (DMSO-d$_6$) $\delta$: 3.77 (s, 3H, N7Me), 3.87 (s, 3H, NMe), 4.27 (s, 2H, CH$_2$), 7.19 (t, $J = 7.2$ Hz, 1H, p-C$_6$H$_5$), 7.27 (t, $J = 7.2$Hz, 2H, m-C$_6$H$_5$), 7.39 (d, $J = 7.2$Hz, 2H, o-C$_6$H$_5$), 7.65 (s, 1H, H8), 8.09 (s, 1H, CH), CI MS m/z: 414 (M+1, 100), 323 (M-CH$_2$C$_6$H$_5$+1, 88). Anal. Calcd for C$_{17}$H$_{15}$N$_7$O$_2$S$_2$: C 49.38, H 3.66, N 23.71. Found C 49.21, H 3.56, N 23.48.

9. 7-Methyl-2-oxo-2,3-dihydro-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5i) (0.21g, 68%); mp 216-218 °C (EtOH-water).

1H NMR (DMSO-d$_6$) $\delta$: 3.77 (s, 3H, N7Me), 3.99 (s, 3H, NMe), 7.99 (s, 1H, H8), 8.09 (s, 1H, CH), 12.24 (s, 1H, N3H), CI MS m/z: 308 (M+1, 100). Anal. Calcd for C$_{10}$H$_9$N$_7$O$_3$S: C 39.09, H 2.95, N 31.91. Found C 39.01, H 2.90, N 31.66.

10. 7-Methyl-2-thioxo-2,3-dihydro-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5j) (0.04g, 12% from 4g, 0.06g, 18% from 4h, 0.03g, 9% from 4j); mp 223-225 °C (EtOH-water).

1H NMR (DMSO-d$_6$) $\delta$: 3.78 (s, 3H, N7Me), 4.12 (s, 3H, NMe), 8.12 (s, 1H, H8), 8.66 (s, 1H, CH), 12.35 (s, 1H, N1H), CI MS m/z: 324 (M+1, 100). Anal. Calcd for C$_{10}$H$_9$N$_7$O$_3$S: C 37.15, H 2.81, N 30.32. Found C 36.91, H 2.88, N 30.02.

11. 2-Chloro-7-methyl-6-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5k) (0.32g, 98%); mp 257-258 °C (EtOH), lit.,16 mp 254-255 °C.

1H NMR (DMSO-d$_6$) $\delta$: 3.76 (s, 3H, N7Me), 4.19 (s, 3H, NMe), 8.19 (s, 1H, H8), 8.29 (s, 1H, CH), CI MS m/z: 326 (M+1, 100). Anal. Calcd for C$_{14}$H$_{12}$N$_7$O$_3$S: C 37.15, H 2.81, N 30.02.

12. 7-Methyl-2,6-bis(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5l) (0.28g, 62%); mp 165-166 °C (EtOH).

1H NMR (DMSO-d$_6$) $\delta$: 3.68 (s, 3H, N7Me), 3.76 (s, 3H, NMe), 3.89 (s, 3H, NMe), 8.07 (s, 1H, H8), 8.21 (s, 1H, CH), 8.36 (s, 1H, CH), CI MS m/z: 449 (M+1, 100). Anal. Calcd for C$_{14}$H$_{12}$N$_7$O$_3$S: C 37.50,

13. 6-Ethoxy-7-methyl-2-(1-methyl-4-nitroimidazolyl-5-ylthio)purine (5m) (0.08g, 24%); mp 154-156 °C (EtOH).

1H NMR (DMSO-\textit{d}_6) \delta: 1.28 (t, J = 6.9 Hz, 3H, Me), 3.69 (s, 3H, N7Me), 3.90 (s, 3H, NMe), 4.31 (q, J = 6.9 Hz, 2H, CH2), 8.03 (s, 1H, H8), 8.35 (s, 1H, CH), CI MS m/z: 336 (M+1, 12), 307 (M-C2H5+1, 100).


B. A mixture of 7-methyl-2,6-thioxanthine 4j (0.20 g, 1 mmol) and NaH (0.072 g, 3 mmol, washed out with hexane from mineral oil) in dry DMF (6 mL) was stirred at rt for 1 h. 5-Chloro-1-methyl-4-nitroimidazole (0.48 g, 3 mmol) was added and the stirring was continued for 24 h. The reaction mixture was poured into water (15 mL) and extracted with CHCl3 (3 x 5 mL). The extracts were dried with anhydrous Na2SO4, and evaporated \textit{in vacuo}. The residue was recrystallized from EtOH to give compound 5l (0.41g, 91%);

**Reaction of di(imidazolylthio) compound 5l with boiling ethanol**

A solution of di(imidazolylthio) compound 5l (0.224 g, 0.5 mmol) in dry EtOH (5 mL) was refluxed for 2 h. After cooling a resulted small amount of solid was filtered off and the ethanolic filtrate was was evaporated to dryness \textit{in vacuo}. The residue was crystallized from 70% EtOH to give compound 5m (0.14 g, 82%), mp 154-156 °C.

**REFERENCES**


