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## SYNTHESIS OF ISOMERIC ANALOGS OF AZATHIOPRINE

Alicja Kowalska,<sup>a,\*</sup> Krystian Pluta,<sup>a</sup> and Kinga Suwińska<sup>b</sup>

<sup>a</sup> Department of Organic Chemistry, The Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland, E-mail: kowalska@sum.edu.pl

<sup>b</sup> Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

**Abstract** – The effective synthesis of isomeric analogs of azathioprine - 6-substituted derivatives of 7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purines (**2**), (**4**) and (**6-8**) has been worked out using different reactivity of the imidazolylthio groups in 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**) towards selected reagents. X-Ray analysis of compound (**1**) showed an unusual conformation of the imidazolylthio groups.

## INTRODUCTION

The thiopurines (azathioprine, 6-mercaptopurine and 6-thioguanine) are one at the success stories of chemotherapy.<sup>1,2</sup> They are effective immunosuppressants and antitumor agents, widely used in the treatment of cancer, inflammatory conditions and organ transplantation.<sup>1,3-8</sup> Azathioprine has been established as drug used as an immunomodulator in different disciplines such as hematology, dermatology, rheumatology and gastroenterology.<sup>4,5,7-14</sup>

The therapeutic effect of 6-mercaptopurine and azathioprine (as a pro-drug of 6-mercaptopurine) are thought to be primarily due to the intracellular formation of the 6-thioguanine nucleotides after their incorporation into cellular DNA and RNA.<sup>1,6,15</sup> Azathioprine has also numerous non-specific effects on immune system preventing proliferation of mitotically active lymphocytes population, induced apoptosis of T cells, suppressed cell – mediated hypersensitivity reactions and is cytotoxic to NK lymphocytes.<sup>9,12,15</sup> Recently there have been reported about antiviral activity of certain riboside metabolites of azathioprine<sup>16</sup> and antibacterial activity of both 6-mercaptopurine and azathioprine against *Mycobacterium paratuberculosis*.<sup>17</sup>

Thiopurines with free SH groups (i.e. 6-mercaptopurine, 6-thioguanine, 2-thiopurine and 2,6-dithiopurine) but also S<sub>6</sub>-substituted thioethers, azathioprine and 6-methylthioguanine inhibit topoisomerase II<sub>α</sub>

ATPase activity by covalent modification of free cysteine residues.<sup>18,19</sup> DNA strand passage structure-activity data suggest that the presence of a substituent on C<sub>2</sub> in purine analogs is also important for potency and its modification has pronounced effects on their potency as cyclin-dependent (CDK) inhibitors.<sup>20</sup>

Recently we described the effective synthesis of the azathioprine analogs – 2-substituted derivatives of 7-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purines in the reaction of 2-substituted 6-purinethiones with 5-chloro-1-methyl-4-nitroimidazole in ethanol. In some cases very interesting 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**), containing three imidazole rings, was also obtained.<sup>21</sup> The aim of this paper is to determine X-ray structure of the last compound and to use it as a substrate to form new isomeric analogs of azathioprine as 6-substituted derivatives of 7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purines.

## RESULTS AND DISCUSSION

### X-Ray analysis

2,6-Dithiosubstituted purines have been seldom the subject of X-ray analysis. We found only two reports, both on the tin(IV) complexes of 2,6-dithiopurines.<sup>22,23</sup> Diimidazolylthiopurine (**1**) exhibits very unusual conformation (Figure 1). The imidazole rings are not coplanar with the purine ring. Similar to the azathioprine conformation the imidazolylthio group in position 6 is directed to the nitrogen atom N1 but the imidazole ring is upside-down (torsion angle C6–S21–C22–N23 is equal to  $-69.1$  and  $-66.2^\circ$  in azathioprine<sup>24,25</sup> against  $74.0(1)^\circ$  in (**1**), and torsion angle C6–S21–C22–C26 is  $124.2^\circ$  and  $121.1^\circ$  in azathioprine<sup>24,25</sup> against  $-117.2(1)^\circ$  in (**1**)). Despite that both substituted imidazolylthio groups are being quite large substituents, they are directed to one another (and to N1 atom), but alternately. There are donor–acceptor interactions between two aromatic imidazole rings as the rings are almost parallel (the dihedral angle between these rings is  $155.06(5)^\circ$  with the shortest distance is  $3.369(2)$  between C14 and C24, for details see Table 2). It is worth noting that two methoxy groups in 2,6-dimethoxy-7-methylpurine and 2,6-dimethoxy-7,9-dimethylpurinium iodide (being significantly smaller groups) are directed to N1 atom (6-OMe) and to N3 atom (2-OMe), respectively.<sup>26,27</sup> The purine ring system is quite planar, the dihedral angle between the pyrimidine and imidazole rings is equal to  $178.16(3)^\circ$ .

The bond lengths and bond angles are similar to those found in azathioprine.<sup>24,25</sup>

There are also donor–acceptor interactions between the sulfur atoms (S11 and S21) and the oxygen atoms of the nitro groups causing very short contacts S11–O19 and S21–O30 of  $3.080(1)$  and  $3.098(1)$  Å (less than sum of their van der Waals' radii,  $3.25$  Å).<sup>28,29</sup> The observed conformation of the molecule is additionally stabilized by two intramolecular C–H $\cdots$  $\pi$  interactions between methyl group of one of the nitroimidazole moiety and the ring of the other nitroimidazole (Table 3).

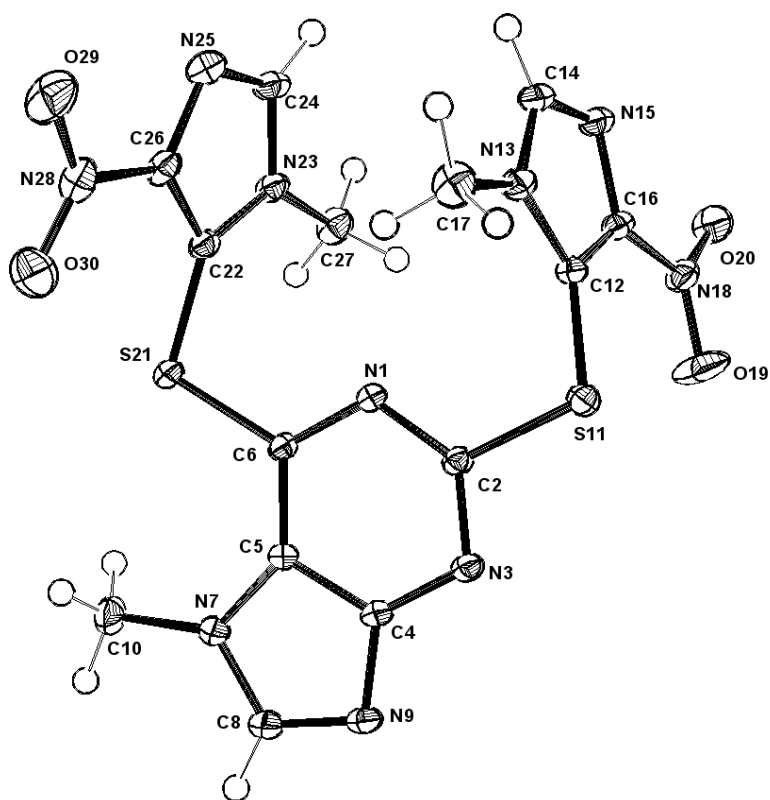


Figure 1. ORTEP drawing of compound (1).

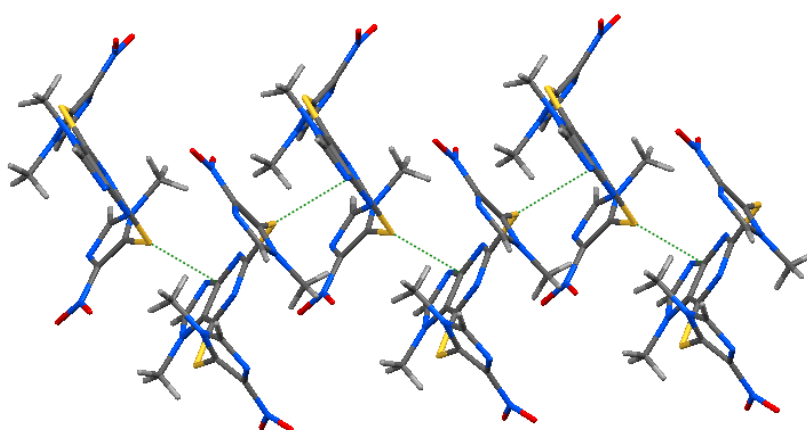


Figure 2. Stacking of the molecules along crystallographic *b* axis in 1D infinite chain based on S... $\pi$  interactions.

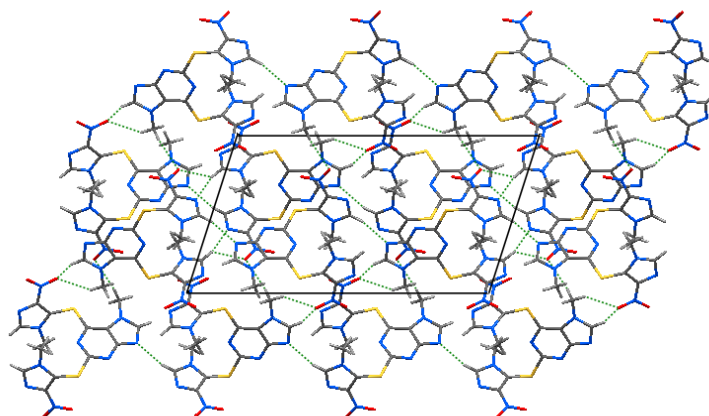


Figure 3. Packing diagram of compound (1) along *b* axis (*a* axis down and *c* axis across). Non-classical hydrogen bonds C–H...O and C–H...N are shown with dotted lines.

In the solid state one S... $\pi$  type interaction between S11 and the pyrimidine ring of the adjacent molecule at  $1-x, 1/2+y, 1/2-z$  is observed ( $d(\text{S}\cdots\pi) = 3.080(1) \text{ \AA}$ ). Due to this interactions the molecules are stacked along crystallographic *b* axis into 1D polymeric chain (Figure 2). A number of C–H...O, C–H...N,  $\pi\cdots\pi$  and Y–X... $\pi$  type interactions between neighboring molecules is also present in the crystal structure (Tables 1, 2 and 4).

**Table 1.** Non-classical hydrogen bonds ( $\text{\AA}$  and  $^\circ$ ).

D–H...A	d(D–H)	d(H...A)	d(D...A)	$\angle(\text{D–H}\cdots\text{A})$
C8–H8...O30 <sup>1</sup>	0.95	2.37	3.177(2)	142.5
C10–H10A...O30 <sup>1</sup>	0.98	2.58	3.440(2)	147.1
C10–H10C...O20 <sup>2</sup>	0.98	2.49	3.372(2)	149.3
C14–H14...N9 <sup>3</sup>	0.95	2.39	3.312(2)	163.2
C24–H24...O20 <sup>4</sup>	0.95	2.45	3.152(2)	130.7

**Table 2.** Analysis of  $\pi\cdots\pi$  interactions (Cg...Cg distances  $<6.0 \text{ \AA}$  and  $\beta < 60.0^\circ$ ).

CgI...CgJ	CgI...CgJ	$\alpha$	$\beta$	$\gamma$	$\perp\text{CgI}$	$\perp\text{CgJ}$	Slippage
Cg1...Cg2 <sup>5</sup>	3.708(1)	6.19(7)	17.98	21.36	3.453(1)	3.527(1)	
Cg3...Cg3 <sup>6</sup>	3.763(1)	0.0	27.54	27.54	3.337(1)	3.337(1)	1.740

**Table 3.** Analysis of intramolecular C–H... $\pi$  interactions ( $\text{H}\cdots\text{Cg} < 3.0 \text{ \AA}$  and  $\gamma < 30.0^\circ$ ).

C–H...CgJ	H...Cg	$\perp\text{H}\cdots\text{Cg}$	$\gamma$	$\angle\text{C–H}\cdots\text{Cg}$	C...Cg	C–H, $\pi$
C17–H17C...Cg3	2.95	2.91	9	123	3.582(1)	42
C27–H27C...Cg2	2.91	2.89	8	131	3.634(1)	49

**Table 4.** Analysis of Y–X··· $\pi$  interactions ( $X\cdots Cg < 4.0 \text{ \AA}$  and  $\gamma < 30.0^\circ$ ).

Y–X···CgJ	X···Cg	$\perp X\cdots Cg$	$\gamma$	$\langle X-X\cdots Cg$	Y···Cg	Y–X, Pi
N18–O19···Cg4 <sup>5</sup>	3.612(1)	3.327	22.90	83.27(8)	3.676(1)	5.99
N18–O20···Cg1 <sup>5</sup>	3.433(1)	3.032	27.96	118.42(8)	4.158(1)	5.68
N28–O30···Cg3 <sup>6</sup>	3.565(1)	3.164	27.46	78.70(8)	3.536(1)	5.16

Symmetry transformations used to generate equivalent atoms:

(1)  $-x, y-1/2, -z+1/2$ ; (2)  $x-1, y, z$ ; (3)  $x, -y+3/2, z-1/2$ ; (4)  $1-x, 1-y, -z$ ; (5)  $1-x, -1/2+y, 1/2-z$ ; (6)  $-x, 1-y, -z$

Cg1: N7, C5, C4, N9, C8; Cg2: N13, C12, C16, N15, C14; Cg3: N23, C22, C26, N25, C24; Cg4: N1, C2, N3, C4, C5, C6

CgI – center of gravity of ring number I

$\alpha$  – dihedral angle between planes I and J ( $^\circ$ )

$\beta$  – angle between CgI···CgJ vector and normal to plane I ( $^\circ$ )

$\gamma$  – angle between CgI···CgJ vector and normal to plane J ( $^\circ$ )

Cg···Cg – distance between ring centroids ( $\text{\AA}$ )

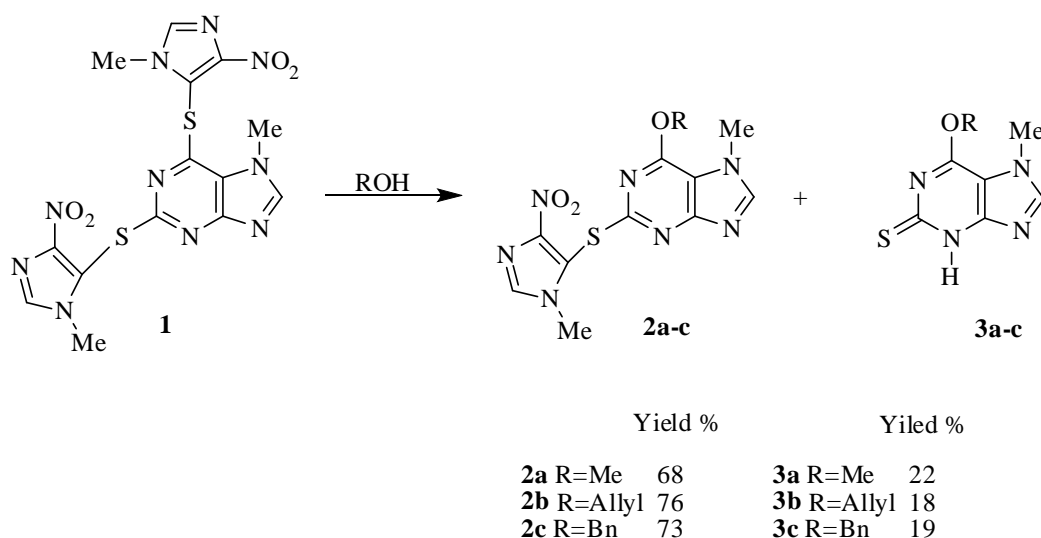
$\perp CgI$  – perpendicular distance of CgI on ring J ( $\text{\AA}$ )

$\perp CgJ$  – perpendicular distance of CgJ on ring I ( $\text{\AA}$ )

Slippage – distance between CgI and perpendicular projection of CgJ on ring I ( $\text{\AA}$ )

## Synthesis

In our previous paper,<sup>21</sup> we reported that compound (**1**) was the only product or main product in reaction of 7-methyl-2,6-dithioxanthine with 5-chloro-1-methyl-4-nitroimidazole in DMF or 70% ethanol, respectively. The existence of 6-ethoxy-7-methyl-2-(1-methyl-4-nitroimidazolyl-5-ylthio)purine in the reaction products in ethanol (in 24% yield) and further reaction of compound (**1**) with boiling ethanol showed the imidazolylthio group in position 6 to be a quite good leaving group (giving the 6-ethoxy derivative in 82% yield). A different reactivity of the imidazolylthio groups towards nucleophilic reagents prompted us to work out synthesis of isomeric analogs of azathioprine.

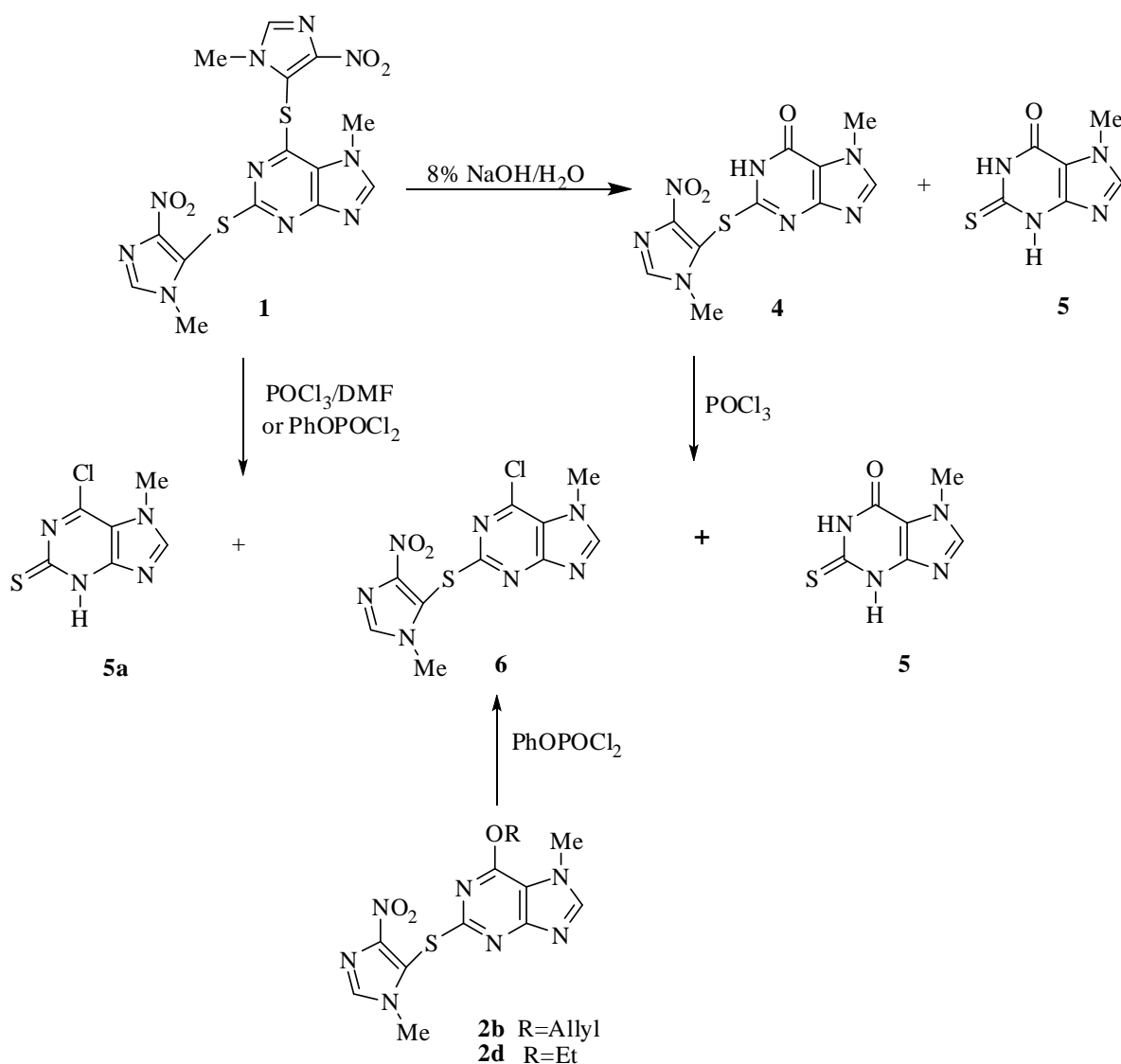


Scheme 1

Reaction of diimidazolylthiopurine (**1**) with alcohols (methyl, allyl and benzyl) at higher temperature (boiling methanol or 80-90  $^\circ\text{C}$ ) led to two products: the expected 6-alkoxy-2-imidazolylthio compounds

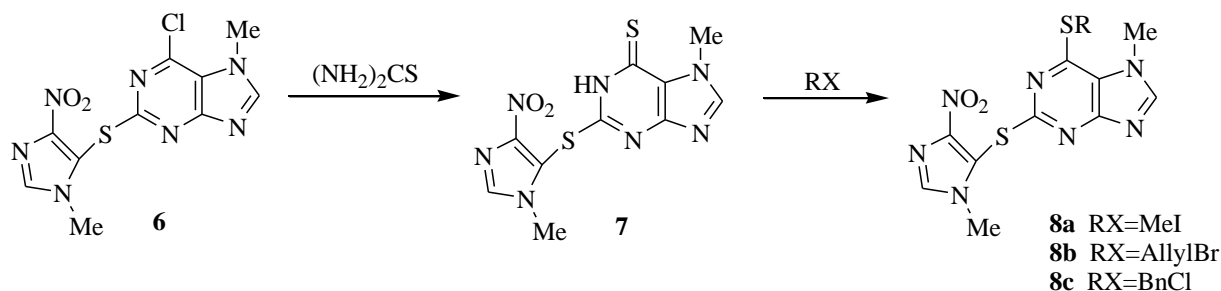
(**2a-2c**) in 68-73% yield and the products of a cleavage of the imidazolyl-sulfur bond, 6-alkoxy-2-thio compounds (**3a-3c**) in 18-22% yield (Scheme 1).

Hydrolysis of compound (**1**) with aqueous sodium hydroxide led to 2-oxo compound (**4**) (in 26% yield) and 7-methyl-2-thioxanthine (**5**) (in 66% yield) as the result of the imidazolyl-sulfur bond cleavage. Reaction of 6-oxo compound (**4**) with boiling phosphorus oxychloride led to 6-chloro compound (**6**) in very low yield (15%) giving mainly compound (**5**) (in 66% yield, Scheme 2). Next we tried to use 6-allyloxy (**2b**) and 6-ethoxy (**2d**) compounds. Whereas reaction with boiling phosphorus oxychloride failed, the reaction with phenyldichlorophosphate at 105-110 °C provided the expected 6-chloro compound (**6**) in 82% and 76% yields, respectively. We also checked a direct transformation of compound (**1**) in various conditions. The reaction failed in boiling phosphorus oxychloride but in a POCl<sub>3</sub>-DMF mixture at 105-110 °C and in phenyldichlorophosphate at 145-150 °C led to chloro compound (**6**) in low yield (12% and 37%) together with 6-chloropurinethione (**5a**, in 70% and 55% yields, respectively).



Scheme 2

The chloro compound (**6**) was transformed into the thioxo compound (**7**) in the reaction with thiourea (in 96% yield) and further into the 6-alkylthio derivatives (**8a-8c**) in 82-86% yield (Scheme 3).



Scheme 3

7-Methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**) show promising potential cytostatic, immunosuppressive, antimetabolite, antineoplastic and cytokine modulator activity and could be useful as antiinflammatory, anticancer, antiarthritic and dermatologic agent.<sup>30</sup>

## CONCLUSION

We report here an efficient synthesis of 9 new isomeric analogs of azathioprine being 6-substituted derivatives of 7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purines (**2**), (**4**) and (**6-8**) from 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**) exploiting different reactivity of the imidazolylthio groups towards nucleophilic reagents. X-ray analysis of compound (**1**) showed an unusual conformation of imidazolylthio groups.

## EXPERIMENTAL

Melting points were determined in open capillary tubes on a Boetius melting point apparatus and were uncorrected. The <sup>1</sup>H NMR spectra were recorded on a Varian Unity-Inova 300 spectrometer at 300 MHz in deuteriochloroform and dimethyl sulphoxide-*d*<sub>6</sub> with tetramethylsilane as the internal standard. Electron impact (EI MS) and chemical ionization (CI MS) mass spectra were run on a Finnigan MAT 95 spectrometer at 70eV.

### Reaction of 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**) with alcohols

A solution of di(imidazolylthio) compound (**1**) (0.224 g, 0.5 mmol) in dry alcohol (5 mL) was refluxed (methanol) or heated and stirred on an oil bath at 80-90 °C for 2 h. After cooling a resulted solid (**3a-c**) was filtered off and the alcoholic filtrate was evaporated to dryness *in vacuo*. The residue was crystallized from 70% EtOH and purified by column chromatography (silica gel, CHCl<sub>3</sub>-EtOH, 10:1 v/v) to give (**2a-c**) in 68-76% yields. Compounds (**3a-c**) were dissolved in 5% NaOH solution and precipitated with 15% hydrochloric acid to give 2-purinethiones (**3a-c**) in 18-22% yields.

1. 6-Methoxy-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**2a**) (0.11 g, 68%); mp 189-190 °C (EtOH).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$ : 3.77 (s, 3H, N(7)CH<sub>3</sub>), 3.97 (s, 3H, NCH<sub>3</sub>), 4.22 (s, 3H, OCH<sub>3</sub>), 7.67 (s, 1H, H8), 7.86 (s, 1H, CH), CIMS m/z: 322 (M+1, 100). Anal. Calcd for C<sub>11</sub>H<sub>11</sub>N<sub>7</sub>O<sub>3</sub>S: C 41.12, H 3.45, N 30.51. Found C 40.91, H 3.41, N 30.26.
2. 6-Allyloxy-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**2b**) (0.13 g, 76%); mp 146-147 °C (EtOH).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$ : 3.75 (s, 3H, N(7)CH<sub>3</sub>), 3.99 (s, 3H, NCH<sub>3</sub>), 4.87 (d,  $J = 5.5$  Hz, 2H, OCH<sub>2</sub>), 5.31 (d,  $J = 10.6$  Hz, 2H, =CH<sub>2</sub>), 5.36 (d,  $J = 17.2$  Hz, 2H, =CH<sub>2</sub>), 6.01 (m,  $J = 6.3$  Hz, 1H, =CH), 7.67 (s, 1H, H8), 7.87 (s, 1H, CH), CI MS m/z: 348 (M+1, 100), 307 (M+1-C<sub>3</sub>H<sub>5</sub>, 76). Anal. Calcd for C<sub>13</sub>H<sub>13</sub>N<sub>7</sub>O<sub>3</sub>S: C 44.95, H 3.77, N 28.23. Found C 44.62, H 3.79, N 27.95.
3. 6-Benzyloxy-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**2c**) (0.14 g, 73%); mp 151-152 °C (EtOH).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$ : 3.86 (s, 3H, N(7)CH<sub>3</sub>), 3.95 (s, 3H, NCH<sub>3</sub>), 4.50 (s, 2H, CH<sub>2</sub>), 7.26 (t,  $J = 7.2$  Hz, 1H, p-C<sub>6</sub>H<sub>5</sub>), 7.31 (t,  $J = 7.2$  Hz, 2H, m-C<sub>6</sub>H<sub>5</sub>), 7.39 (d,  $J = 7.2$ , 2H, o-C<sub>6</sub>H<sub>5</sub>), 7.97 (s, 1H, H8), 8.49 (s, 1H, CH), CI MS m/z: 398 (M+1, 21), 307 (M+1-CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, 100). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>N<sub>7</sub>O<sub>3</sub>S: C 51.38, H 3.80, N 24.67. Found C 51.22, H 3.88, N 24.35.
4. 6-Methoxy-7-methyl-2-thioxo-2,3-dihydropurine (**3a**) (0.02 g, 22%); mp 152-153 °C (EtOH).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$ : 3.91 (s, 3H, NCH<sub>3</sub>), 4.04 (s, 3H, OCH<sub>3</sub>), 8.30 (s, 1H, H8), 12.41 (s, 1H, N(3)H), EI MS m/z: 196 (M<sup>+</sup>, 100). Anal. Calcd for C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>OS: C 42.85, H 4.11, N 28.55. Found C 42.61, H 4.03, N 28.34.
5. 6-Allyloxy-7-methyl-2-thioxo-2,3-dihydropurine (**3b**) (0.02 g, 18%); mp 168-169 °C (EtOH).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$ : 3.93 (s, 3H, NCH<sub>3</sub>), 5.02 (d,  $J = 6.2$  Hz, 2H, OCH<sub>2</sub>), 5.09 (d,  $J = 10.2$  Hz, 2H, =CH<sub>2</sub>), 5.45 (d,  $J = 17.2$  Hz, 2H, =CH<sub>2</sub>), 5.95 (m,  $J = 6.8$  Hz, 1H, =CH) 8.33 (s, 1H, H8), 12.23 (s, 1H, N(3)H), EI MS m/z: 222 (M<sup>+</sup>, 24), 181 (M-C<sub>3</sub>H<sub>5</sub>, 100). Anal. Calcd for C<sub>9</sub>H<sub>10</sub>N<sub>4</sub>OS: C 48.64, H 4.53, N 25.21. Found C 48.37, H 4.50, N 24.84.
6. 6-Benzyloxy-7-methyl-2-thioxo-2,3-dihydropurine (**3c**) (0.025 g, 19%); mp 205-206 °C (EtOH).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$ : 3.89 (s, 3H, NCH<sub>3</sub>), 4.42 (s, 2H, CH<sub>2</sub>), 7.23 (t,  $J = 7.2$  Hz, 1H, p-C<sub>6</sub>H<sub>5</sub>), 7.29 (t,  $J = 7.2$  Hz, 2H, m-C<sub>6</sub>H<sub>5</sub>), 7.42 (d,  $J = 7.2$  Hz, 2H, o-C<sub>6</sub>H<sub>5</sub>), 8.06 (s, 1H, H8), 12.51 (s, 1H, N(3)H), EI MS m/z: 272 (M<sup>+</sup>, 12), 181 (M-CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, 23), 91 (C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub><sup>+</sup>, 100). Anal. Calcd for C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>OS: C 57.34, H 4.44, N 20.57. Found C 57.33, H 4.49, N 20.36.

### Hydrolysis of 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (1)

A solution of di(imidazolylthio) compound (**1**) (0.448 g, 1 mmol) in 8% aqueous NaOH solution (15 mL) was refluxed for 1 h. After cooling the clear solution was brought to pH = 6 by addition of glacial acetic acid. The resulting solid was filtered off to give the mixture of products (**4**) and (**5**), which was separated by extraction with absolute EtOH (3 x 15 mL). The solvent was removed *in vacuo* and the residue was

dissolved in 5% aqueous NaOH solution. The product (**4**) was precipitated with glacial acetic acid in 26% yield. The residue after extraction with EtOH was also dissolved in 5% aqueous NaOH solution and the reaction product (**5**) (0.03g, 16%) was precipitated by acidification with 15% aqueous hydrochloric acid to pH = 3. The first aqueous filtrate separated after precipitation of products (**4**) and (**5**) with glacial acetic acid was acidified also with 15% aqueous hydrochloric acid to pH = 3. The resulted solid was filtered off to give only compound (**5**) (0.09 g, 49%).

1. 7-Methyl-6-oxo-1,6-dihydro-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**4**) (0.08 g, 26%); mp 242-244 °C (EtOH-water). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 3.79 (s, 3H, N(7)CH<sub>3</sub>), 3.99 (s, 3H, NCH<sub>3</sub>), 7.78 (s, 1H, H8), 7.96 (s, 1H, CH), 10.65 (s, 1H, N(1)H), CI MS m/z: 308 (M+1, 100). Anal. Calcd for C<sub>10</sub>H<sub>9</sub>N<sub>7</sub>O<sub>3</sub>S: C 39.09, H 2.95, N 31.91. Found C 38.95, H 2.91, N 31.63.
2. 7-Methyl-2-thioxanthine (**5**) (0.12g, 66%); mp > 300 °C (EtOH-water), lit.,<sup>31</sup> mp > 300 °C. EI MS m/z: 182 (M<sup>+</sup>, 100)

### Synthesis of 6-chloro-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**6**)

a. From 7-methyl-6-oxo-1,6-dihydro-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**4**).

A solution of anhydrous 7-methyl-2-substituted hypoxanthine (**4**) (0.31 g, 1 mmol) in phosphorus oxychloride (3 mL) was refluxed for 4 h. The excess of phosphorus oxychloride was removed under reduced pressure and the residue was added to 3 g of crushed ice. The reaction was then neutralized with concentrated NH<sub>4</sub>OH solution at 0-5 °C up to pH = 7 and the resulted solid was filtered off to give 7-methyl-2-thioxanthine (**5**) (0.12 g, 66%). The filtrate was extracted with CHCl<sub>3</sub> (3 x 15 mL), the extracts were combined and dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was purified by column and thin layer chromatography (silica gel, CHCl<sub>3</sub> and CHCl<sub>3</sub>-EtOH, 10:1 v/v) to give compound (**6**) (0.05 g, 15%).

6-Chloro-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**6**) (0.05 g, 15%); mp 175-176 °C (EtOH). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 3.69 (s, 3H, N(7)CH<sub>3</sub>), 4.11 (s, 3H, NCH<sub>3</sub>), 8.10 (s, 1H, H8), 8.64 (s, 1H, CH), CI MS m/z: 326 (M+1, 100). Anal. Calcd for C<sub>10</sub>H<sub>8</sub>ClN<sub>7</sub>O<sub>2</sub>S: C 36.87, H 2.48, N 30.10. Found C 36.76, H 2.41, N 29.88

b. From 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**).

A solution of anhydrous substrate (**1**) (0.448 g, 1 mmol) in a mixture of POCl<sub>3</sub>-DMF (1:2 v/v, 9 mL) or in phenyldichlorophosphate (5 mL) was stirred on oil bath at 105-110 °C for 4 h or at 145-150 °C for 3 h, respectively. After cooling the excess of the CHCl<sub>3</sub>-EtOH mixture was removed under reduced pressure and the residue was added to 3 g of crushed ice. The reaction was then neutralized with concentrated NH<sub>4</sub>OH solution at 0-5 °C up to pH = 7 and the resulted solid was filtered off to give 6-chloropurinethione (**5a**) (0.14 g, 70%); mp > 300 °C, lit.,<sup>31</sup> mp > 300 °C. EI MS m/z: 200 (M<sup>+</sup>, 100).

The filtrate was extracted with  $\text{CHCl}_3$  (3 x 15 mL), the extracts were combined and dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was purified by column and thin layer chromatography (silica gel,  $\text{CHCl}_3$  and  $\text{CHCl}_3$ -EtOH, 10:1 v/v) to give compound (**6**) (0.04 g, 12%). In case of the reaction in phenyldichlorophosphate, the cooled reaction mixture was poured into crushed ice (20 g), neutralized with concentrated  $\text{NH}_4\text{OH}$  solution at 0-5 °C up to pH = 7 and the resulted solid was filtered off to give 6-chloropurinethione (**5a**) (0.11 g, 55%). The filtrate was extracted with  $\text{CHCl}_3$  (3 x 15 mL), the extracts were combined and dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was purified by column and thin layer chromatography (silica gel,  $\text{CHCl}_3$  and  $\text{CHCl}_3$ -EtOH, 10:1 v/v) to give compound (**6**) (0.12 g, 37%).

Similar reaction in boiling phosphorus oxychloride (5 mL) failed.

c. From 6-alkoxy-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**2b**) (R = Allyl) (**2d**) (R = Et) A solution 6-allyloxy (**2b**) and 6-ethoxy (**2d**) derivatives (1 mmol) in phenyldichlorophosphate (5 mL) was stirred on oil bath at 105-110 °C for 4 h. After cooling the reaction mixture was poured into crushed ice (20 g), neutralized with concentrated  $\text{NH}_4\text{OH}$  solution at 0-5 °C up to pH = 7 and extracted with  $\text{CHCl}_3$  (3 x 15 mL). The combined extracts were dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was purified by column and thin layer chromatography (silica gel,  $\text{CHCl}_3$  and  $\text{CHCl}_3$ -EtOH, 10:1 v/v) to give compound (**6**) (0.27 g, 82%) and (0.25 g, 76%), respectively. Similar reaction in boiling phosphorus oxychloride (5 mL) failed.

### Synthesis of 6-alkylthio-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**8**)

A solution of 6-chloropurin (**6**) (0,325 g, 1 mmol) and thiourea (0,15g, 2 mmol) in dry EtOH (40 mL) was refluxed for 1,5 h. The solvent was removed *in vacuo* and the residue was dissolved in 5% aqueous NaOH solution. The reaction product was precipitated with 15% hydrochloric acid and the process was repeated twice to give 7-methyl-6-thioxo-1,6-dihydro-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**7**) (0.31 g, 96%); mp 267- 269 °C (EtOH).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$ : 3.92 (s, 3H, N(7) $\text{CH}_3$ ), 4.13 (s, 3H, N $\text{CH}_3$ ), 8.07 (s, 1H, H8), 8.33 (s, 1H, CH), 10.84 (s, 1H, N(1)H), CI MS m/z: 324 (M+1, 100). Anal. Calcd for  $\text{C}_{10}\text{H}_9\text{N}_7\text{O}_2\text{S}_2$ : C 37.15, H 2.81, N 30.32. Found C 36.88, H 2.87, N 30.06.

To a stirred solution of compound (**7**) (0.324 g, 1 mmol) in 4% aqueous KOH solution at room temperature methyl iodide (0.28 g, 2 mmol), allyl bromide (0.24 g, 2 mmol) or benzyl chloride (0.34 g, 2 mmol) was added. After 0.5 h the resulting solid was filtered off and washed with water. The crude product was purified by column chromatography (silica gel,  $\text{CHCl}_3$  and  $\text{CHCl}_3$ -EtOH, 10:1 v/v) to give compounds:

1. 6-Methylthio-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**8a**) (0.29 g, 86%); mp 187-188 °C (EtOH).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$ : 2.74 (s, 3H,  $\text{SCH}_3$ ), 3.97 (s, 3H, N(7) $\text{CH}_3$ ), 4.30 (s, 3H, N $\text{CH}_3$ ), 8.14

(s, 1H, H8), 8.56 (s, 1H, CH), CI MS  $m/z$ : 338 (M+1, 100). Anal. Calcd for  $C_{11}H_{11}N_7O_2S_2$ : C 39.16, H 3.29, N 29.06. Found C 38.89, H 3.31, N 28.80.

2. 6-Allylthio-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**8b**) (0.30 g, 82%); mp 137-138 °C (EtOH).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$ : 3.56 (s, 3H, N(7)CH<sub>3</sub>), 4.00 (s, 3H, NCH<sub>3</sub>), 4.25 (d,  $J = 6.9$  Hz, 2H, SCH<sub>2</sub>), 5.11 (d,  $J = 10.2$  Hz, 2H, =CH<sub>2</sub>), 5.32 (d,  $J = 16.8$  Hz, 2H, =CH<sub>2</sub>), 6.03 (m,  $J = 6.9$  Hz, 1H, =CH), 7.49 (s, 1H, H8), 7.95 (s, 1H, CH), CI MS  $m/z$ : 364 (M+1, 100), 323 (M+1-C<sub>3</sub>H<sub>5</sub>, 52). Anal. Calcd for  $C_{13}H_{13}N_7O_2S_2$ : C 42.97, H 3.61, N 26.98. Found C 42.69, H 3.50, N 26.62.

3. 6-Benzylthio-7-methyl-2-(1-methyl-4-nitroimidazol-5-ylthio)purine (**8c**) (0.35 g, 85%); mp 228-229 °C (EtOH).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$ : 3.69 (s, 3H, N(7)CH<sub>3</sub>), 3.93 (s, 3H, NCH<sub>3</sub>), 4.15 (s, 2H, CH<sub>2</sub>), 7.18 (t,  $J = 7.2$  Hz, 1H, p-C<sub>6</sub>H<sub>5</sub>), 7.24 (t,  $J = 7.2$  Hz, 2H, m-C<sub>6</sub>H<sub>5</sub>), 7.38 (d,  $J = 7.2$  Hz, 2H, o-C<sub>6</sub>H<sub>5</sub>), 8.00 (s, 1H, H8), 8.59 (s, 1H, CH), CI MS  $m/z$ : 414 (M+1, 100), 323 (M+1-CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, 72). Anal. Calcd for  $C_{17}H_{15}N_7O_2S_2$ : C 49.38, H 3.66, N 23.71. Found C 49.24, H 3.52, N 23.45.

### X-Ray analysis

Data for 7-methyl-2,6-di(1-methyl-4-nitroimidazol-5-ylthio)purine (**1**) were collected on a KappaApexII diffractometer with graphite monochromated MoK $\alpha$  radiation ( $\lambda = 0.71073$  Å) at 100 K. The structure was solved by direct methods (SHELXS-97)<sup>32</sup> and refined by full-matrix least-squares minimization based on all unique  $F^2$  (SHELXL-97-2).<sup>33</sup> The crystals were grown from methanol solution.

Crystal data:  $C_{14}H_{12}N_{10}O_4S_2$ ,  $M_r = 448.46$ , monoclinic,  $a = 11.3234$  (1),  $b = 8.1290$  (1),  $c = 20.8121$  (3) Å,  $\beta = 107.839$  (1)°, space group  $P2_1/c$ ,  $V = 1823.60$  (3) Å<sup>3</sup>,  $Z = 4$ ,  $\mu = 0.34$  mm<sup>-1</sup>. 47752 reflections were collected of which 7987 were independent and 7047 with  $I > 2\sigma(I)$  ( $R_{int} = 0.056$ ). The structure was refined to  $R[F^2 > 2\sigma(F^2)] = 0.045$  and  $wR(F^2) = 0.112$ .

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