Synthesis and properties of 2-amino-6-thioxo-, 2-amino-6-oxo-8-thioxo- and 2-amino-6,8-di-thioxo-N-alkoxyalkylpurines

Abstract of Thesis

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INTRODUCTION

In recent years, there has been a resurgence of interest in the synthesis of purine derivatives. Some of them present interesting therapeutic activities whereas others may serve as subunits of supramolecular arrays essential both in certain biological processes and binding components in artificial systems. Purine-based compounds have found new applications as inducers of interferon and lineage-committed cell dedifferentiation, agonists and antagonists of adenosine receptors, ligands of corticotropin-releasing hormone receptors, ferments inhibitors, and some of them are used in the treatment of cancer, virus infections, AIDS.

2-Amino-6-oxo-N-alkoxyalkylpurines or acyclic analogues of guanosine are a unique class of heterocyclic compounds, and they are of interest as potentially biologically active substances. Several guanosine analogues (e.g., acyclovir, ganciclovir) are widely used for the treatment of herpes virus infections. In recent years, several new guanosine analogues hold great promise not only as antiviral agents, but also as antitumor agents for the combined gene therapy/chemotherapy of cancer (antitumor suicide gene therapy). This therapy consists of the introduction into cancer cells of a gene capable of converting nontoxic prodrug into cytotoxic drug and thus to induce enzymatic cell death in those cells that express the transferred gene. The thymidine kinase gene from the herpes simplex virus, in combination with the prodrug ganciclovir, is the promising approach in suicide gene therapy.

In the search for the new bioactive agents and tools for biological studies, many 2-amino-N-alkoxyalkylpurines have been synthesized. The majority of these compounds have rather similar chemical structures. Among these compounds are only few sulfur containing guanine analogues. Introduction of sulfur into guanine molecule may improve the therapeutic effect and properties (i.e., guanine/thioguanine).

The interest of issues of the biological properties of 2-amino-N-alkoxyalkylpurine derivatives is growing. At the same time, many investigations of various guanosine analogues have been hindered by difficulties connected with their synthesis. Modern synthetic methods in the purine chemistry gain ground slowly. The scope of application of purines in biology is most certainly far from being exhausted.

Objective of the research

The objective of the present thesis is to find convenient methods of the preparation of 6-oxo-8-thioxo- and 6,8-di-thioxo-2-amino-N-alkoxyalkylpurine derivatives and investigate their cytotoxic activity.
The main tasks of the research are:

- synthesis of new 6-oxo-8-thioxo- and 6,8-di-thioxo-2-amino-N-alkoxyalkylpurine derivatives containing modified alkyl-, aralkyl- or arylsubstitents at sulfur atoms at the 8 and 6 positions of the purine heterocycle,
- preparation of target compounds by approving and optimizing traditional reactions of purine chemistry (e.g., thionation, alkylation), as well as adopting some new protocols of the organic synthesis (e.g., metal-mediated cross-coupling reactions) for the further modification of 6-oxo-8-thioxo- and 6,8-dithioxo-2-amino-N-alkoxyalkylpurines,
- confirmation of the structures of the synthesized 6-oxo-8-thioxo- and 6,8-di-thioxo-2-amino-N-alkoxyalkylpurine derivatives by spectroscopic and X-ray analysis,
- investigation of the cytotoxic activity of 2-amino-6-oxo-8-thioxo- and 2-amino-6,8-di-thioxopurine derivatives.

Novelty of the research

We have studied, adopted and optimized various methods of organic chemistry for the preparation of a series of new 6-oxo-8-thioxo- and 6,8-di-thioxo-2-amino-N-alkoxyalkylpurine derivatives. A preliminary analysis of the structure relationship for the cytotoxic action on tumor cell lines clearly indicates the strong influence of substituents in 6-oxo-8-thioxo- un 6,8-di-thioxo-2-amino-N-alkoxyalkylpurine derivatives.

Practical significance of the research

The optimization of synthetic methods and the development of new strategies to obtain sulfur containing 2-amino-N-alkoxyalkylpurines using cheaper and available substrates let to create new purine scaffolds for the investigation of biological properties. The synthesized purines, as well as their synthetic methods can be used to obtain other purine derivatives giving further contribution in the purine chemistry.

In acknowledgment of the significance of research the European Social Foundation (agreement 2004/0001/VPD/ESF/PIAA/04NP/3.2.3.1/0001/0063) have granted support of this work. The author is very grateful for this valuable assistance. Content of the thesis is disclosed in 5 published full papers and discussed in 5 international scientific conferences.
RESULTS AND DISCUSSION

1. SYNTHESIS OF 2-AMINO-6-OXO-8-TIOXOPURINE DERIVATIVES

2-Amino-N-alkoxyalkyl-6-oxo-8-tioxopurines can be obtained by 6-halopurine thionation with the following S-alkylation or by nucleophylic displacement of halogen substituent with a thiolate group.

Thionation of 2-acetamido-8-bromo-6-oxopurine derivatives

A green chemistry protocol was elaborated for the thionation of 2-acetamido-8-bromo-6-oxopurine derivatives 1-8. Aluminium trichloride mediated thionation proceeded in a water medium using sodium thiosulfate as a source of sulfur. The corresponding 2-acetamido-N-alkoxyalkyl-6-oxo-8-thioxopurines 9-12 were obtained in good yields (64–95%) (Table 1).

\[
\text{R} \quad \text{N}^9\text{-CH}_2\text{OCH}_2\text{CH}_2\text{OAc} \quad 75 \quad -
\]
\[
\text{R} \quad \text{N}^9\text{-CH}_2\text{OCH(CH}_3\text{)_2} \quad 87 \quad -
\]
\[
\text{R} \quad \text{N}^7\text{-CH}_2\text{OCH(CH}_3\text{)_2} \quad 95 \quad -
\]
\[
\text{R} \quad \text{N}^9\text{-CH}_2\text{OCH}_2\text{CH}_2\text{OCOCH}_2\text{CH}_2\text{COOH} \quad 64 \quad -
\]
\[
\text{R} \quad \text{N}^9\text{-CH}_2\text{OCOCH}_8\text{H}_{17} \quad \text{RN} \quad 50^1
\]
\[
\text{R} \quad \text{N}^9\text{-CH}_2\text{OBn} \quad \text{RN} \quad 70
\]
\[
\text{R} \quad \text{N}^7\text{-CH}_2\text{OBn} \quad \text{RN} \quad 57
\]
\[
\text{R} \quad \text{N}^9\text{-(2-terahydrofuryl), H} \quad \text{RN} \quad 60
\]

\( ^1\text{EtOH, reflux} \)

Our attempts to convert the purines 5-8 by Na\(_2\)S\(_2\)O\(_3\) failed due to the high lipophility of N7 or N9 substituents. Substitution of the water medium by ethanol or water-ethanol solutions didn’t give a positive result. The corresponding 9-octyloxymethyl-, 7-benzyloxymethyl- and 9-benzyloxymethyl-2-acetamido-6-oxo-8-thioxopurines (13-15) were prepared by an alternative method consisting of the treatment of the starting compounds 5-8 with thiourea in ethanol or
DMF. N2-Deacylation was not observed during the reaction of thionation. However, the substitution of halogen by sulfur for 2-(N,N-dimethylamino)methylenamino-8-bromo-N-alkoxyalkyl-6-oxopurines proceeds with the following deprotection of 2-aminogroup. 2-Acetamido-8-thioxo-6-oxo-9(7)H-purine (16) was prepared by the reaction of the starting 2-acetamido-8-bromo-6-oxo-9-(2-tetrahydrofuryl)purine (8) and thiourea in DMF in good yield (60%). The compound 16 is a useful synthon for following chemical modification.

*S-Alkylation of 2-amino-6-oxo-8-thioxopurine derivatives*

Alkylation of thiopurines is very convenient way for the preparation of various new purine derivatives. 2-Acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurine (9) was chosen as a model compound for the further derivatization. It was established that the alkylation conditions strongly depend on the nature and reactivity of the alkylation reagent.

Our investigation started with 8-thioxopurine 9 S-alkylation by alkyl and benzyl halides in sodium hydroxide water solution to provide corresponding 8-methylthio-, 8-benzylthio-, 8-(3,5-di(trifluoromethyl)benzyl)thio- and 8-(2-hydroxyethyl)thiopurine derivatives 17-20 (Table 2). It should be noted that 8-thioxopurine 9 alkylation proceeds in high yields only in case of alkyl halides which are stable in strong alkaline medium.

Mild S-alkylation of the 9 with alkyl halides in DMF in the presence of potassium carbonate opens an alternative way of the synthesis of 8-alkylthiopurines (21-26) using less stable alkylation agents.

\[
\begin{align*}
\text{RX} & \quad \text{i} \text{ vai ii} \\
\text{i) NaOH, H}_2\text{O, 6-12h r. t. or 60°C; ii) K}_2\text{CO}_3, \text{DMF, 8-24h, rt.}
\end{align*}
\]

The inspection of alkylation products shows a formation of the mixture of S- and N7-alkyl purines. A ratio of two regioisomers depends on alkylation agent nature and thermodynamic stability of the forming product. To our opinion, S-alkyl purine is the primary product formed, and then proceeds full or partial migration of the alkyl substituent to N7 atom of purine.
Alkylation of 2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurine (9)

<table>
<thead>
<tr>
<th>Compds.</th>
<th>R</th>
<th>X</th>
<th>Method</th>
<th>Yield, %</th>
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<tr>
<td>17</td>
<td>Me</td>
<td>I</td>
<td>i</td>
<td>60</td>
</tr>
<tr>
<td>18</td>
<td>Bn</td>
<td>Br</td>
<td>i/ii</td>
<td>63/32</td>
</tr>
<tr>
<td>19</td>
<td>CH₂-C₆H₃-3,5-CF₃</td>
<td>Br</td>
<td>i</td>
<td>65</td>
</tr>
<tr>
<td>20</td>
<td>CH₂CH₂OH</td>
<td>Br</td>
<td>i/ii</td>
<td>54/12</td>
</tr>
<tr>
<td>21</td>
<td>CH₂COOEt</td>
<td>Cl</td>
<td>ii</td>
<td>11</td>
</tr>
<tr>
<td>22</td>
<td>CH₂OC₆H₁₇</td>
<td>Cl</td>
<td>ii</td>
<td>30</td>
</tr>
<tr>
<td>23</td>
<td>CH₂OC₁₈H₃₇</td>
<td>Cl</td>
<td>ii</td>
<td>28</td>
</tr>
<tr>
<td>24</td>
<td>CH₂OC₁₈H₃₇</td>
<td>Cl</td>
<td>ii</td>
<td>19</td>
</tr>
<tr>
<td>25</td>
<td>CH₂OBn</td>
<td>Cl</td>
<td>ii</td>
<td>21</td>
</tr>
<tr>
<td>26</td>
<td>CH₂OBn</td>
<td>Cl</td>
<td>ii</td>
<td>12</td>
</tr>
</tbody>
</table>

Substituent migration S→N7 was confirmed experimentally: 8-(benzyloxymethyl)thio derivative 25 was quantitatively converted to 7-benzyl-oxymethyl-8-thioxopurine 26 by heating in DMF for 3h. Benzyloxymethyl group migration became possible due to the formation of a “pseudooxirane” carbocation cycle.

To study the influence of protecting group onto the reaction regioselectivity N2-(N,N-dimethylamino)methylene protected 2-amino-6-oxo-8-tioxopurines also have been investigated in the reaction of alkylation. Initial 2-(N,N-dimethylamino)methyleneamino-6-oxo-8-thioxo-9-alkoxyalkyl-purines 27a, b were obtained from the corresponding 2-amino-8-thioxopurines in the reaction with N,N-di-butylacetal of DMF or its homologues.

The results of alkylation with alkyl, alkoxyalkyl, benzyl halogenides, 1-chlorocarbonic acid esters of compounds 27a, b are presented in Table 3 and compared with the experimental data using N2-acetyl protected analogue 9 under the same reaction conditions.
Table 3

<table>
<thead>
<tr>
<th>Compds.</th>
<th>R²</th>
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<th>R¹</th>
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<th>Yield., %¹</th>
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<tr>
<td>28</td>
<td>iPr</td>
<td>I</td>
<td>Ac</td>
<td>35</td>
<td>-</td>
</tr>
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<td>29</td>
<td>CH₂CH₂OH</td>
<td>Br</td>
<td>H</td>
<td>60</td>
<td>&lt;5</td>
</tr>
<tr>
<td>30</td>
<td>Bn</td>
<td>Br</td>
<td>H</td>
<td>68</td>
<td>25</td>
</tr>
<tr>
<td>31</td>
<td>CH₂COOMe</td>
<td>Br</td>
<td>H</td>
<td>94</td>
<td>11</td>
</tr>
<tr>
<td>32</td>
<td>CH(CH₃)COOEt</td>
<td>Br</td>
<td>Ac</td>
<td>76</td>
<td>-</td>
</tr>
<tr>
<td>33</td>
<td>CH₂OC₈H₁₇</td>
<td>Cl</td>
<td>Ac</td>
<td>53</td>
<td>30</td>
</tr>
</tbody>
</table>

¹yields for 2-acetamido-6-oxo-8-thioxopurine 9 alkylation

It should be noted that the utilization of N2-(N,N-dimethylamino)methylene protected 2-amino-6-oxo-8-thioxopurines in the reaction of alkylation is more preferable than it of the N2-acetyl protected analogues. It opens a convenient way to prepare different S-alkylpurines using less electrophilic or unstable alkylating agents under mild reaction conditions. Amidine-type protecting group didn’t influence the alkylation regioselectivity (S vs. N).

Reaction of 2-amino-N-substituted-8-bromo-6-oxopurines with thioles

Interaction of 2-amino-8-bromo-6-oxopurine with arylthioles in the presence of sodium acetate leads to the formation of corresponding 2-amino-8-aryl-6-oxopurines. This procedure was elaborated for the synthesis of various new 8-arylpurines 38-48. We observed that treating of 7-(2-chloroethoxymethyl)purine 48 with 3-methoxyphenylthiol proceeded with the simultaneous substitution of 8-bromo group and chloro atom in the acyclic chain to yield derivative 49.

Under the current experimental conditions the hydrolysis of the N-acetyl group was observed. The yields of 2-amino-8-aryltio-6-oxopurines depend on the activity of aryl thiole, but alkoxyalkyl- and alkyl substituents at the N7 or N9 positions of the purine ring have no
significant influence on the yields of products. It should be noted, that this method is not suitable for the synthesis of 2-amino-6-oxo-8-alkylthiopurine derivatives.

\[
\begin{align*}
\text{HN} & \text{O} \\
\text{N} & \text{N} \\
\text{HN} & \text{Ac} \\
1-3, 34-37 & \text{NaOAc, MeOH, H}_2\text{O, 3-4h, v.t.} \\
\text{HN} & \text{N} \\
\text{O} & \text{N} \\
\text{HN} & \text{OAc} \\
30-62\% & \text{SR}^2 \\
38-49 & \end{align*}
\]

38 $R^1=N9-\text{CH}_2\text{COCH}_2\text{CH}_2\text{OAc}$, $R^2=\text{Ph}$; 39 $R^1=N9-\text{CH}_2\text{OCH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OMe;
40 $R^1=N7-\text{CH}_2\text{OCH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
41 $R^1=-\text{N9-CH}_2\text{OCH}_2\text{CH}_2\text{CCCH}$, $R^2=\text{C}_6\text{H}_4$-3-OMe;
42 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH;
43 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
44 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
45 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
46 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
47 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
48 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
49 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
50 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
51 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;
52 $R^1=N7-\text{CH}_2\text{OCH}_2\text{CH}(\text{CH}_3)_2$, $R^2=\text{C}_6\text{H}_4$-3-OH, $R^2=\text{Ph}$;

2. SYNTHESIS OF 2-AMINO-N-ALKOXYALKYL-6,8-DI-THIOXOPURINE DERIVATIVES

According to the literature data, only few papers describe chemical transformations of 2-amino-N-alkoxyalkyl-6,8-di-thioxopurines. These compounds can be obtained by alkylation or arylation of 6,8-di-thioxopurines or by the nucleophilic substitution of the halogen substituent with the thiolate group at the 6 and 8 positions.

The initial compound in both methods is 6,8-di-chloropurine, which can be directly treated with thiols, or chloro atoms previously substituted with thiogroup, and then alkylated or arylated to yield new 2-amino-6,8-di-thiopurine derivatives. A model compound was prepared from available 2-acetamido-9-(2-acetoxyethoxymethyl)-8-bromo-6-oxopurine (1) by chlorination with phosphorus oxychloride in acetonitrile to yield the corresponding 6,8-di-chloropurine 51. Our attempts to convert N7-substituted regioisomer 50, or the mixture of regioisomers lead to the formation of the 51 as a single product of chlorination. Notably, during the reaction a migration of the 2-acetoxyethoxymethyl group N7→N9 was observed.

According to the literature data, 6-(4-toluenesulfonyl)oxyderivatives can be utilized instead of 6-chloro analogues. Simplicity of the tosylation protocol and low toxicity of reagents were accepted as advantages of the current method. 2-Acetamido-9-(2-acetoxyethoxymethyl)-8-bromo-6-(4-toluenesulfonyloxy)purine (52) was successfully prepared by treating of purine 1 with 4-toluenesulfonylchloride and DMAP in dichloromethane.
Interaction of 6,8-di-chloropurine 51 with sodium thiosulfate leads to the formation of 2-acetamido-9-(2-acetoxyethoxymethyl)-6,8-di-thioxopurine (53) in 76% yield. We have found that the compound 61 can be alternatively obtained by the same method from 6-(4-toluenesulfonyl)oxyderivative 52. However, during the reaction detosylation process was observed to yield 8-thioguanine 9.

\[
\begin{align*}
\text{i) } & \text{POCl}_3, \text{ DEA, CH}_3\text{CN, 10 min}-1\text{h, reflux.; ii) } \text{TsCl, Et}_3\text{N, DMAP, } \text{CH}_2\text{Cl}_2, \text{ 3h, rt.;} \text{ iii) } \text{Na}_2\text{S}_2\text{O}_3, \text{ AlCl}_3, \text{ H}_2\text{O, 4h, reflux.} \text{ iv) } \text{RBr, K}_2\text{CO}_3, \text{ DMF, 3-6h, rt.;} \text{ v) } \text{RSH, Et}_3\text{N, MeOH, 8h, reflux;} \\
& \text{ vi) } \text{HOCH}_2\text{CH}_2\text{SH, NaH, 3h, DMF, rt.; vii) } \text{PhSH, NaOAc, MeOH, 14h, reflux;} \text{ viii) thiourea, EtOH, 8h, reflux.}
\end{align*}
\]

6,8-Di-thioxopurine 53 was used as a model for the further transformations. The treatment of the 53 by two equivalents of benzyl bromide in sodium hydroxide aqueous solution leads to the formation of 6,8-dibenzylthiopurine 54 in good yield. Our attempts to use less reactive alkylating agents (for example 1-bromoctane) failed. Besides that, the alternative alkylation method in the DMF medium allowed further derivatization of the 53. As a result 6,8-di-octylthio-, 6,8-di-iso-propylthio-, 6,8-di-allylthio-, 6,8-di-(2-hydroxyethyl)thio-, 6,8-di-(2-ethoxy-2-oxyethyl)thio-, 6,8-di-(2-pyridinylmethyl)thiopurine derivatives (54-60) were obtained in good to excellent yields (55-93%). We have tried to prepare monoalkylthio purine using one equivalent of alkyl halide, however, only dialkylated and unreacted 6,8-di-thioxoguanine was...
detected in the reaction mixture. Amino- and hydroxyl groups of the purines 54-60 were successfully deacylated by methylamine or sodium hydroxide solution in water. Simultaneous hydrolysis of the ester fragment of purine proceeds to yield the corresponding acid 59.

As the next step, we have investigated nucleophilic substitution of chloro atom in guanines by various thiols. Traditionally, the interaction of thiols with chloropurines proceeds in basic medium. With the purpose to find the best reaction conditions, an optimization was made for each single thiol. 2-Acetamido-9-(2-acetoxyethoxymethyl)-6,8-di-(benzylthio)purine (54) was prepared by the reaction of 6,8-di-chloropurine 51 with benzyl thiol in refluxing methanol in the presence of triethylamine in 51% yield. We have observed that the treatment of 51 with 1-pentylthiol or 2-hydroxyethylthiol in Et3N/MeOH leads to the substitution of one chloro atom only to form 8-(1-pentylthio) and 8-(2-hydroxyethoxymethyl)thio-2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxopurines (61, 62). Using excess of thiol didn’t give any positive result.

The desired 6,8-di-(2-hydroxyethyl)tiopurine 58 was prepared by reaction of 51 with 2-hydroxyethylthiol and sodium hydride in DMF. Spectroscopic data are identical with the derivative 58 prepared by alkylation of 6,8-di-thioxopurine 53 with 2-bromoethanol. It was found that the introduction of phenylthiofragment is convenient in the presence of sodium acetate to obtain 2-acetamido-9-(2-acetoxyethoxymethyl)-6,8-di-(phenylthio)purine (63). Unlike to 2-acetamido-8-bromo-6-oxopurine interaction with thiophenol, the current reaction does not touch N-acetyl protecting group in purine. 2-Acetamido-9-(2-acetoxyethoxymethyl)-8-(1-pentylthio)-6-thioxopurine 64 was obtained from the reaction of thiourea with 61 in ethanol.

It may be concluded that 6,8-di-chloropurine thionation with the following S-alkylation of 6,8-di-thioxopurine derivatives opens a convenient way for the preparation of various purines containing alkylthio or arylthio substituents at the 6 and 8 positions of the purine cycle. Contrary the literature data, we have found that the 8 position is more active under current experimental conditions than the position 6 to give 8-monosubstituted purines.

3. SYNTHESIS OF 6-ARYL-, 6-ARYL-8-ALKYLTHIO-, 6-ARYLTHIO- AND 6,8-DI-ARYLTHIO-2-AMINO-9-ALKOXYALKYLPURINES

In the last decade, the interest of utilization of arylboronic acids for the formation of C–C bond is extremely increased. We have adopted the transition metal mediated cross-coupling reaction in the series of 2-amino-N-alkoxyalkylpurine derivatives
C-Arylation of 6-substituted-2-acetamido-8-alkylthio-9-(2-acetoxyethoxymethyl)purines

At the beginning, Suzuki-Miyaura method was used for the introduction of aryl substituent at the 6 position of purine heterocycle. Initial 6-chloro- and 6-(4-toluene-sulfonyl)oxopurines 62, 65-68 were converted into the corresponding 6-aryl derivatives 69-73 by palladium acetate/tri(o-tolyl)phosphine/K$_3$PO$_4$ mediated cross-coupling reaction in toluene (Table 4). It should be noted, that substituent at the 8 position can affect which substrate is preferable (6-chloro- or 6-(4-toluene-sulfonyl)oxopurine).

\[
\text{i) ArB(OH)$_2$, Pd(OAc)$_2$, tri(o-tolyl)phosphine, K$_3$PO$_4$, toluene, 3-8h, reflux}
\]

Table 4

Reaction of 6-chloro- or 6-(4-toluene-sulfonyl)oxo-2-acetamido-9-(2-acetoxyethoxymethyl)-8-alkylthiopurines 62, 65-68 with arylboronic acids

<table>
<thead>
<tr>
<th>Compds.</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>$R^3$</th>
<th>Yield, %</th>
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<td>21</td>
</tr>
<tr>
<td>66, 69</td>
<td>Me</td>
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<td>H</td>
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</tr>
<tr>
<td>67, 70</td>
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<td>Cl</td>
<td>H</td>
<td>48</td>
</tr>
<tr>
<td>68, 70</td>
<td>Bn</td>
<td>OTs</td>
<td>H</td>
<td>55</td>
</tr>
<tr>
<td>68, 71</td>
<td>Bn</td>
<td>OTs</td>
<td>OCH(CH$_3$)$_2$</td>
<td>53</td>
</tr>
<tr>
<td>68, 72</td>
<td>Bn</td>
<td>OTs</td>
<td>C(CH$_3$)$_3$</td>
<td>62</td>
</tr>
<tr>
<td>62, 73</td>
<td>C$<em>5$H$</em>{11}$</td>
<td>Cl</td>
<td>H</td>
<td>56</td>
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S-Arylation of 6-thioxo-, 6-thioxo-8-alkylthio- and 6,8-di-thioxo-2-acetamido-9-(2-acetoxyethoxymethyl)purines

Investigations in the field of S-arylation of 6-thioxo-, 6-oxo-8-thioxo-, 6,8-di-thioxo- and 6-thioxo-8-alkylthio-2-amino-9-alkoxyalkylpurines were performed by copper mediated Chan-Evans-Lam method. Interaction of 2-acetamido-9-(2-acetoxyethoxymethyl)-6-thioxopurine (74) with 4-trifluoromethylphenyl boronic acid was chosen as a model reaction with the purpose to find the best conditions for the cross-coupling. The desired 2-acetamido-9-(2-
acetoxyethoxymethyl)-6-(4-trifluoromethylphenyl)thiopurine (75) was obtained using copper (II) acetate – phenanthroline complex in dichloromethane in moderate yield (50%). After the optimization of copper salt, ligand, and solvent, we have found that the best conditions are Cu(OAc)$_2$/Et$_3$N/DMF at 70°C (Table 5). Various 6-aryl(hetaryl)thiopurines (75–85) were prepared by this method.

![Chemical Structure](image)

i) ArB(OH)$_2$, Cu(OAc)$_2$, conditions A or B

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Ar</th>
<th>A Yield, %</th>
<th>B Yield, %</th>
<th>Compds.</th>
<th>Ar</th>
<th>A Yield, %</th>
<th>B Yield, %</th>
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<tbody>
<tr>
<td>75</td>
<td>CF$_3$</td>
<td>81</td>
<td>75</td>
<td>81</td>
<td>N</td>
<td>72</td>
<td>5</td>
</tr>
<tr>
<td>76</td>
<td></td>
<td>83</td>
<td>90</td>
<td>82</td>
<td>F</td>
<td>83</td>
<td>47</td>
</tr>
<tr>
<td>77</td>
<td>O</td>
<td>65</td>
<td>73</td>
<td>83</td>
<td>S</td>
<td>41</td>
<td>57</td>
</tr>
<tr>
<td>78</td>
<td>Cl</td>
<td>76</td>
<td>45</td>
<td>84</td>
<td>SMe</td>
<td>32</td>
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<td>Cl</td>
<td>50</td>
<td>37</td>
<td>85</td>
<td>S</td>
<td>63</td>
<td>75</td>
</tr>
<tr>
<td>80</td>
<td>NO$_2$</td>
<td>56</td>
<td>39</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Table 5**

Arylation of 2-acetamido-9-(2-acetoxyethoxymethyl)-6-thioxopurine (74)

A green chemistry protocol was elaborated for the alternative method for the S-arylation. The reaction was performed in methanol/water medium at 0°C using TMEDA-copper acetate complex as a catalyst. The inspection of both methods for the preparation of 76-85 shows that a choice of the method strongly depends on aryl(hetaryl) boronic acid nature. Our attempts to use Chan-Evans-Lam method for the modification of 2-acetamido-6-oxo-8-thioxopurine 9 failed.
Besides that, the S-arylation of 6,8-di-thioxopurine 53 proceeds smoothly in the \( \text{Et}_3\text{N}/\text{Cu(OAc)}_2/\text{DMF} \) system to yield 6,8-di-(phenylthio)- and 6,8-di-(4-trifluoromethylphenyl) thio-2-acetamido-9-(2-acetoxyethoxymethyl)purines (63, 86) in good yields.

\[
\begin{align*}
\text{53} & \xrightarrow{i} \text{63, 86} & \text{iii) } & \text{89} \\
& \text{HN} & & \text{HN} \\
& \text{OAc} & & \text{OAc} \\
& \text{NH}_2 & & \text{H}_2\text{N} \\
& \text{OH} & & \text{OH} \\
& \text{Ph} & & \text{Ph} \\
& \text{HN} & & \text{HN} \\
& \text{OAc} & & \text{OAc} \\
& \text{NH}_2 & & \text{H}_2\text{N} \\
& \text{OH} & & \text{OH} \\
& \text{Ph} & & \text{Ph} \\
\end{align*}
\]

i) \( \text{ArB(OH)}_2, \text{Cu(OAc)}_2, \text{Et}_3\text{N}, \text{DMF}, 4\,\text{Å MS}, 4h, 70^\circ\text{C} \); ii) \( \text{NH}_2\text{CH}_3, \text{H}_2\text{O}, 3h, \text{rt.} \);

iii) \( \text{NaOH, H}_2\text{O, 8h, rt.} \)

For the deacylation of compound 63, methylamine in water was used. Unexpectedly, according to spectroscopic data two products in almost equal ratio were formed. After the isolation of both products, we have found that the protecting groups were successfully removed, in addition one of the phenylthio fragments of the 63 was simultaneously substituted by the methylamino group to yield the corresponding 6-methyamino-8-phenylthio- and 6-phenylthio-8-methylamino-2-amino-9-(2-hydroxyethoxymethyl)purines (87, 88). By treating of the 63 with sodium hydroxide in water the desired deprotected 2-amino-6,8-di-(phenylthio)-9-(2-hydroxyethoxymethyl)purine (89) was successfully obtained.

4. CYTOTOXIC ACTIVITY OF 6-OXO-8-THIOXO- AND 6,8-DI-THIOXO-2-AMINO-9-ALKOXYALKYLPURINE DERIVATIVES

The cytotoxic activity of 2-amino-9-alkoxyalkyl-6-oxo-8-thioxopurine derivatives was investigated by a group of Dr. biol. Irina Shestakova (Experimental Chemotherapy group, Medicinal Chemistry department, Latvian Institute of Organic synthesis). Table 6 presents in vitro studies of the compounds on human fibrosarcoma HT-1080, mouse hepatoma MG-22A,
mouse melanoma B16, human T cell leukemia Jurkat, and mouse embryonic fibroblasts NIH 3T3 cell lines. Cell morphology also was investigated.

It has been found that 2-amino-9-alkoxyalkyl-6-oxo-8-thioxopurine derivatives exhibit moderate cytotoxicity on HT-1080 and MG-22A cell lines (2-38 \( \mu \)g/ml) in combination with very low acute toxicity on normal cell line (LD\(_{50}\)=872 – 2517 mg/kg). The inspection of the cytotoxic effect of 2-amino-9-alkoxyalkyl-6,8-di-thioxopurines on leukemia Jurkat cell line shows high level of activity. Moreover, cytotoxic activities of several derivatives (70, 76, 101) on Jurkat cells are by an order of magnitude greater than widely known antitumor agent 6-thioxoguanine. It can be concluded that these purine derivatives are perspective for the further investigations.

<table>
<thead>
<tr>
<th>Compds.</th>
<th>HT-1080</th>
<th>MG-22A</th>
<th>B16</th>
<th>Jurkat</th>
<th>NIH-3T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TD(_{50})</td>
<td>NO, 100%</td>
<td>TD(_{50})</td>
<td>NO, 100%</td>
<td>TD(_{50})</td>
</tr>
<tr>
<td>22(^{2})</td>
<td>90</td>
<td>28</td>
<td>*</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>23(^{2})</td>
<td>14</td>
<td>367</td>
<td>2,0</td>
<td>275</td>
<td>-</td>
</tr>
<tr>
<td>42(^{2})</td>
<td>38</td>
<td>75</td>
<td>*</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>43(^{3})</td>
<td>7</td>
<td>650</td>
<td>100</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>69(^{2})</td>
<td>13</td>
<td>72</td>
<td>26</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>70(^{2})</td>
<td>38</td>
<td>150</td>
<td>31</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>71(^{2})</td>
<td>80</td>
<td>29</td>
<td>34</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>76(^{4})</td>
<td>*</td>
<td>12</td>
<td>*</td>
<td>9,0</td>
<td>*</td>
</tr>
<tr>
<td>101(^{2})</td>
<td>55</td>
<td>14</td>
<td>55</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

\(^{1}\)TD\(_{50}\) – concentration (\(\mu\)g/mL) providing 50% cell killing effect; \(^{2}\)N2- and O-deacylated product; \(^{3}\)N2-deacylated product, \(^{4}\)N2- and O-deacylated un deesterified product

5. STRUCTURE DETERMINATION OF 6-THIOXO, 6-OXO-8-THIOXO-, 6,8-DI-THIOXO-2-AMINO-N-ALKOXYALKYLPURINE DERIVATIVES

The proving of the structure of 6-thioxo-, 6-oxo-8-thioxo- and 6,8-di-thioxo-N-alkoxyalkylpurine derivatives by spectroscopic and elemental analysis data is very necessary. The introduction of thioxo groups at the 6 and 8 positions of the purine heterocycle shows new proton signal formation (N7H- and N1H) in \(^{1}\)H NMR spectra at \(\delta\sim13.1-13.5\) ppm, also the singlets of N7CH2- and N9CH2-groups are shifted in lower fields by \(-0.06\) ppm in comparison with to the starting compound.
Due to the fact that there are different alkylation centres in 6-oxo-8-thioxo- and 6-oxo-8-thioxo-2-amino-9-(2-acetoxyethoxymethyl)purine derivatives, the determination of the alkylation place cause difficulties. As it is known, SCH₂ group shift in ¹H NMR spectra usually appears in a stronger field than the signal of NCH₂ protons. Thus, it can be concluded that the compounds 17-21, 23, 25 are S-alkylated, but the derivatives 22, 24, 26– N-alkylated (Table 7). The variation of the substituent structure changes absolute values of the shifts, but does not affect general rules.

Table 7

<table>
<thead>
<tr>
<th>Compds.</th>
<th>N9CH₂ (s, 2H)</th>
<th>N7CH₂ (s, 2H)</th>
<th>SCH₂ (s, 2H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>5,35</td>
<td>-</td>
<td>2,64 (s, 3H, CH₃)</td>
</tr>
<tr>
<td>18</td>
<td>5,31</td>
<td>-</td>
<td>4,49</td>
</tr>
<tr>
<td>19</td>
<td>5,33</td>
<td>-</td>
<td>4,55</td>
</tr>
<tr>
<td>20</td>
<td>5,41</td>
<td>-</td>
<td>3,46 (m, 2H)</td>
</tr>
<tr>
<td>21</td>
<td>5,43</td>
<td>-</td>
<td>4,16</td>
</tr>
<tr>
<td>22</td>
<td>5,58</td>
<td>5,72</td>
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</tr>
<tr>
<td>23</td>
<td>5,51</td>
<td>-</td>
<td>5,44</td>
</tr>
<tr>
<td>24</td>
<td>5,67</td>
<td>5,85</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>5,52</td>
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<tr>
<td>26</td>
<td>5,56</td>
<td>5,83</td>
<td>-</td>
</tr>
</tbody>
</table>

The molecular structures of the products 17 and 20 were unambiguously confirmed by X-ray diffraction analysis. The structures of the alkylated 6,8-di-thioxopurine derivatives 54-60 structure also are confirmed by ¹H NMR spectroscopic data. The observed proton signals better correspond to S-substituted purines (δ~3.3-4.7 ppm). Additionally, the molecular structure of the 57 was also confirmed by the X-ray diffraction analysis (Figure 1).

Figure 1. Structures of 2-acetamido-9-(2-acetoxyethoxymethyl)-6,8-di-(allyl)thiopurine (57) and 2-acetamido-9-(2-acetoxyethoxymethyl)-6-(4-trifluoromethylphenyl)thiopurine (75)
Since $^1$H NMR spectra of the compounds 61, 62 didn’t give a clear answer about the alkylation regioselectivity, extended two-dimensional HMBC $^{13}$C NMR spectroscopic investigations for the 61 were made (Figure 2). Moreover, the molecular structure of the 8-pentylthio-6-thioxopurine 64, derived from the 61 was confirmed by X-ray diffraction analysis.

![Figure 2. $^{13}$C-chemical shifts and main $^1$H-$^{13}$C correlation determined of the compound 61 by HMBC experiment](image)

The arylation of the purines 63 and 74 theoretically is possible at the N1 and S atoms. The arylation place was unequivacality determined by X-ray diffraction analysis of 6-(4-trifluoromethylphenyl)thiopurine crystals (Figure 1). The extrapolation of $^1$H NMR spectroscopic data for the derivatives 76-85 has allowed us to conclude that only S-arylation proceeded during the reaction.

![Figure 3. Structure of 2-amino-6-phenylthio-8-methylamino-9-(2-hydroxyethoxymethyl)purine (88)](image)

The substitution of one of the phenylthio group by the methylamino group in the compounds 87 and 88 was confirmed by $^1$H NMR spectra and elemental analysis. The structure of 8-methylaminopurine 88 was confirmed by X-ray diffraction analysis (Figure 3). Additionally, the structure of the 87 was confirmed by the NOE experiment.
CONCLUSIONS

1. For the thionation of 2-acetamido-8-bromo-6-oxopurine derivatives by sodium thiosulfate the green chemistry protocol was elaborated. It should be noted, that the nature of the alkoxyalkyl fragment affects the product yield. Alternatively, 2-amino-N-alkoxyalkyl-8-thioxopurines are prepared by treating initial 8-bromoguanines with thiourea in ethanol or DMF. N2- and O-acetyl protecting groups are stable upon above mentioned reaction conditions, however, N2-(N,N-dimethylamino)methylene and N9-tetrahydrofuranyl groups undergo simultaneous hydrolysis.

2. The S-alkylation of 2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurine was developed in NaOH/H₂O and DMF/K₂CO₃ systems. The choice of the alkylation method and the regioselectivity (S vs. N) is dependent on the nature of alkyl halide.

3. It was found that the reaction of 2-acetamido-N-alkoxyalkyl-8-bromo-6-oxopurine derivatives with arylthiols in the presence of sodium acetate is a convenient method for the introduction of the arylthio substituent into the 8 position of the purine cycle.

4. During chlorination reaction of the mixture of 9-(2-acetoxyethoxymethyl)- and 7-(2-acetoxethoxymethyl)-2-acetamido-8-bromo-6-oxopurine with phosphorus oxychloride, the alkoxyalkyl fragment migration from N7 to N9 in the latter compound was observed with simultaneous substitution of the bromo to chloro atom at the 8 position yielding a single product - 2-acetamido-9-(2-acetoxyethoxymethyl)-6,8-dichloropurine.

5. The thionation of 2-acetamido-9-(2-acetoxyethoxymethyl)-6,8-dichloropurine by sodium thiosulfate is the most convenient procedure of the synthesis of 2-acetamido-9-(2-acetoxyethoxymethyl)-6,8-dithioxopurine. This compound can be prepared by an alternative protocol from 2-acetamido-9-(2-acetoxyethoxy-methyl)-8-bromo-6-(4-toluenesulfonyl)-oxypurine, however, in lower yield.

6. The best way of the synthesis of 2-amino-9-alkoxyalkyl-6,8-dialkylthiopurines is the thionation of the corresponding 6,8-dichloropurine with the following alkylation by alkyl halides. The direct treating of 6,8-dichloropurine derivatives with thiols is less preferable because of the decreased selectivity of the reaction and more pronounced dependence on the thiol activity.

7. In order to obtain the corresponding 2-acetamido-9-(2-acetoxyethoxymethyl)-8-alkylthio-6-arylpurines in the series of 6-hloro- and 6-(4-toluenesulfonyl)oxypurines the Suzuki-Miyaura coupling conditions were adopted.
8. The arylation of 6-thioxo-, 6,8-dithioxo-, and 6-thioxo-8-alkylthio-2-acetamido-9-(2-acetoxyethoxymethyl)purines with aryl(hetaryl)boronic acids by the Chan-Evans-Lam method was performed. In the case of 2-acetamido-9-(2-acetoxy-ethoxymethyl)-6-oxo-8-thioxopurine this method gave diminished yields of the products. It was established that the optimal reaction conditions are dependent on the structure of boronic acid used.

9. The results of the in vitro studies on tumor cell lines show that 2-amino-9-(2-acetoxyethoxymethyl)-6-oxo-8-octadecyloxymethylpurine exhibits the highest activity on the MG-22A cell line (2.0 μg/ml), but 2-amino-9-(2-acetoxyethoxymethyl)-8-(3-methoxyphenyl)thio-6-oxopurine on the human fibrosarcoma HT-1080 cell line (7.0 μg/ml). The testing of thiopurine derivatives on human leukemia Jurkat cell line shows that 6,8-di-iso-propylthio-, 6,8-di-(2-hydroxy-2-oxyethyl)thio-, and 6-phenylthio-8-pentylthio-2-amino-9-(2-hydroxyethoxymethyl)purines possess the highest cytotoxicity in vitro (1.0-1.6 μg/ml). Moreover, all the compounds studied exhibit low acute toxicity. These preliminary results open a perspective field for further investigation of 6-okso-8-thioxo- and 6,8-di-thioxopurine derivatives.
LIST OF PUBLICATION

Papers:

Conference proceedings: