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Synthesis and cytotoxic activity of various 5-[alkoxy-(4-nitro-phenyl)-methyl]-uracils in their racemic form

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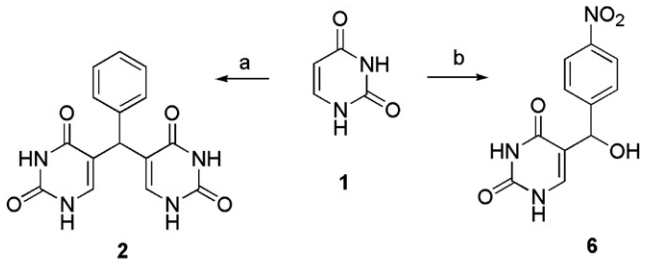
Abstract—The preparation of various 5-[alkoxy-(4-nitro-phenyl)-methyl]-uracils with alkyl chain lengths C_1–C_{12} is described. The synthesis is based on the preparation of 5-[chloro-(4-nitro-phenyl)-methyl]-uracil and subsequent substitution of chlorine with appropriate alcohols. The resulting ethers were tested for their cytotoxic activity in vitro against five cancer cell lines. The compounds were less active in lung resistance protein expressing cell lines, suggesting the involvement of this multidrug resistant protein in control of the biological activity. Cytotoxic substances induced rapid inhibition of DNA and modulation of RNA synthesis followed by induction of apoptosis. The data indicate that the biological activity of 5-[alkoxy-(4-nitro-phenyl)-methyl]-uracils depends on the alkyl chain length.

Compounds derived from 5-alkyluracil are well known for their biological activity. Some of them, for example, have been described as suitable agents for treating various diseases caused by excessive cell proliferation, such as in the treatment of various cancers[1] or treatment of proliferative diseases mediated by second messengers.[2] The most potent 5-alkyluracils inhibited the proliferation of leukemia, lymphoma, and solid tumor-derived cell lines at micromolar concentrations.[3] Our research is focused on derivatives of 5-alkoxyuracils with anticancer activity. In this paper we describe the synthesis and cytotoxic activity of 5-[alkoxy-(4-nitro-phenyl)-methyl]-uracils having various alkyl chain lengths.

The reaction of uracil with benzaldehyde is reported to afford the appropriate derivative **2**.[4] If 4-nitrobenzaldehyde is used instead, 5-[hydroxy-(4-nitro-phenyl)-methyl]-uracil **6** results as is described in the same publication (Scheme 1).

In our hands the reaction does not lead to compound **6**, but rather to a mixture of 5-[chloro-(4-nitro-phenyl)-methyl]-uracil **3** and 5,5′-(4-nitrophenyl)-methyl-bis-1*H*-pyrimidine-2,4-dione **4** instead. We successfully tried to find reaction conditions for selective preparation of each derivative (Scheme 2).

Derivative **4** has been prepared recently by nitration of 5,5′-phenylmethylene-bis-uracil and patented in the class of anti-ictogenic or anti-epileptogenic agents.[5]



a) benzaldehyde, conc. HCl, reflux, 2h;
b) 4-nitrobenzaldehyde, conc. HCl, 60°C, then reflux

Scheme 1. Reaction of uracil with benzaldehyde and p-nitrobenzaldehyde according to Ref. 4.

Keywords: Uracil; DNA; RNA; Apoptosis; Cytotoxic; Anticancer activity.
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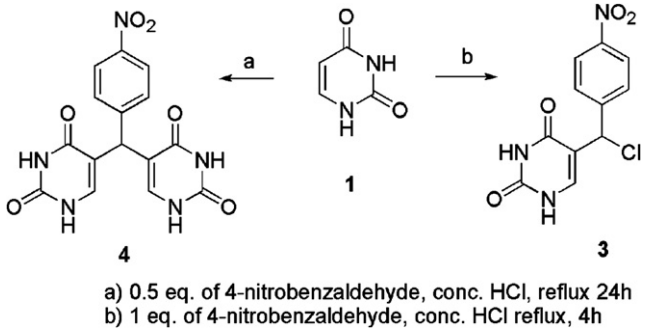


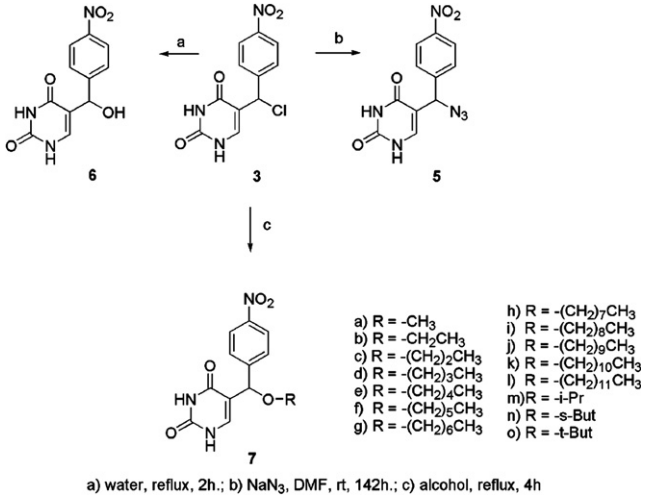
Scheme 2. Reaction of uracil with *p*-nitrobenzaldehyde.

The chlorine atom of derivative **3** is very reactive. When treated with sodium azide the appropriate azide **5** results, with DMF/water the alcohol **6** is obtained and with alcohols the desired ethers **7** are formed (Scheme 3).

The prepared compounds **4**–**7** were tested for their cytotoxic activity against five tumor cell lines under in vitro conditions. Compounds **4**, **5** were found to be quite inactive with IC_{50} above 100 μM. However, the activity of first prepared ethers differed. For this reason, we decided to prepare a series of alkoxyderivatives **7** with various alkyl chain lengths. Compounds with longer alkyl chains (heptyl **7g** to dodecyl **7l**) exhibited relatively higher cytotoxic activity in CEM and K562-Tax leukemia cells. In these lines, the only exception in the activity trend is derivative **7f** (Fig. 1).

In cell lines A549, CEM-DNR-B, and K-562, the cytotoxic activity also increases with increasing chain length (7-9 carbons) and then decreases. Again, derivative **7f** exhibits exceptions in the activity trend (Fig. 2).

Branching of alkyls reduces the activity, because compounds **7m**, **n**, and **o** are quite inactive.



Scheme 3. Preparation of 5-[(alkoxy-(4-nitrophenyl)-methyl]-uracil 7 and other derivatives.

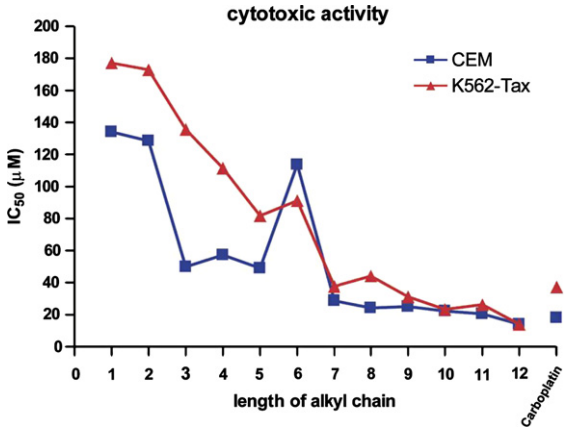


Figure 1. Cytotoxic activity of compounds **7a**–**7j** and control agent (carboplatin) as a function of chain length in LRP negative CEM and K562-Tax leukemia cell lines.

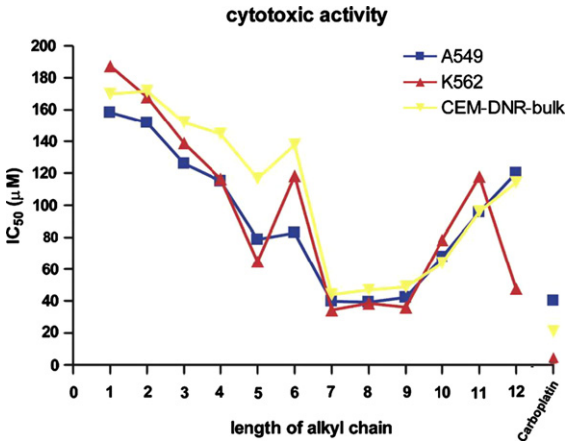


Figure 2. Cytotoxic activity of compounds **7a**–**7j** and control agent (carboplatin) related to chain length in LRP positive A549, CEM-DNRB, and K562 cell lines.

For better understanding of the biological uniqueness of compound **7f** we have studied its activity in more detailed analysis of the cell cycle, apoptosis, DNA and RNA synthesis using CEM T-lymphoblastic leukemia cells.[6,7] The potency of compound **7f** was compared to those of the two most potent substances **7g** and **7h**, whose alkyl chain lengths differ only by one or two carbon lengths, respectively. Analyses were performed at equiactive concentrations corresponding to 1× IC_{50} and 5× IC_{50}.

Interestingly, the cytotoxic activity of the alkoxyderivatives **7f–h** was accompanied by rapid inhibition of DNA synthesis at concentration 5× IC_{50} (Fig. 3).

However, at concentration 1× IC_{50} inhibition of DNA synthesis is significant only for derivative **7f**. Moreover, there was an apparent increase of RNA synthesis in compounds **7g** and **7h** but inhibition in **7f** at 1× IC_{50}, while in 5× IC_{50} the total RNA synthesis was inhibited in cells treated with any of the compounds. Based on these data we hypothesize that the transient increase of

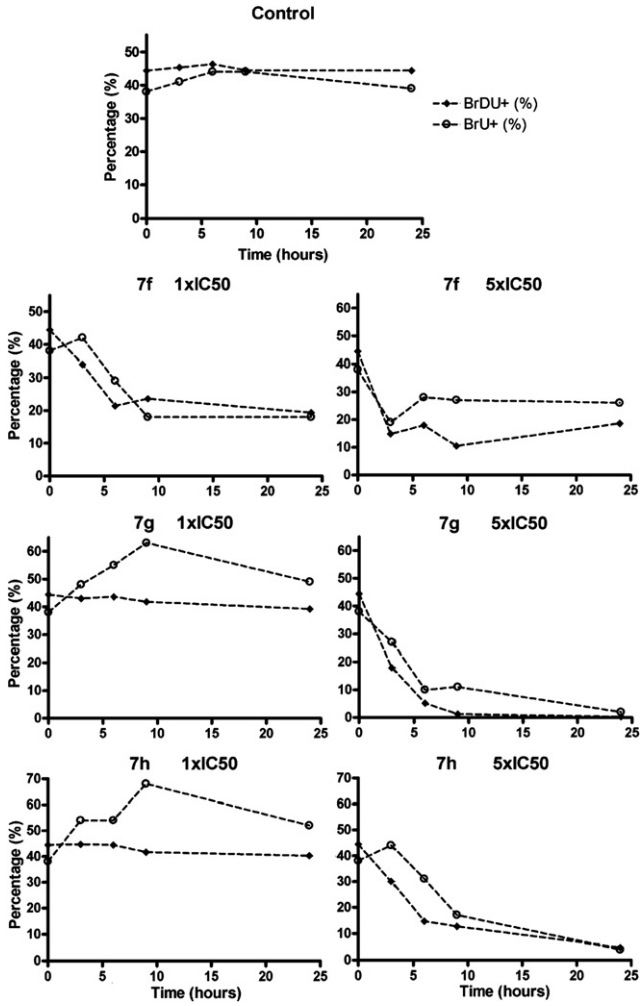


Figure 3. Summary of RNA/DNA analysis for CEM cancer cell line treated with compounds **7f–h**. Data are expressed as a percentage of positive cells in the total cellular population. [6,7]

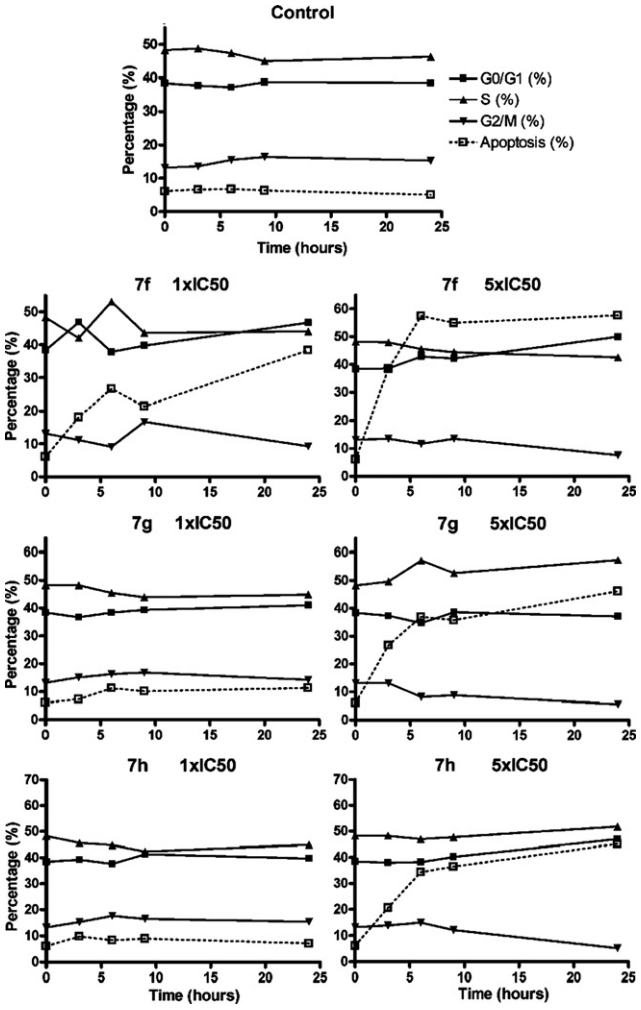


Figure 4. Summary of conventional cell cycle and apoptosis analysis for CEM cancer cell line treated with compounds **7f–h**. Data are expressed as a percentage of cells with corresponding DNA content in the total cellular population. [6,7]

RNA synthesis at 1× IC_{50} in cells treated with **7g** and **7h** is due to compensatory mechanisms, for example, treated cells are compensating insufficiency of macromolecules via increased RNA biosynthesis.

All three compounds **7f–h** induced apoptosis at 5× IC_{50} concentrations, but did not cause any significant cell cycle alterations in treated CEM cells (Fig. 4). Interestingly, at concentration 1× IC_{50} only **7f** caused significant apoptosis within 24 h. This finding rather contrasts with the lower cytotoxic potency of compound **7f** in 3-day cytotoxic MTT assay compared to structures **7g**, **7h** and suggests that chemical or metabolic stability of **7f** could explain the incoherent biological behavior of the compound.

Cell lines A549, CEM-DNR-B, and K-562 were generally more resistant to this class of compounds. Indeed, cell lines A549, CEM-DNR-B, and K-562, express consistently significant concentrations of lung resistance protein (LRP) but not other multidrug resistance associated proteins compared to CEM and K562-Tax cells.[8] Thus, we hypothesize that the cytotoxic activity of 5-[alkoxy-(4-nitro-phenyl)-methyl]-uracils is controlled by the multidrug resistance associated protein LRP. Interestingly, the cytotoxic activity of substances **7g** and **7h** in contrast to derivative **7f** is active in LRP negative, but p-glycoprotein (PgP) positive K562-Tax cells. This means that they were not influenced by expression of multidrug resistance associated protein PgP, suggesting activity of compounds in PgP overexpressing tumors.[8] However, the cytotoxic potency of all compounds was reduced in CEM-DNR-bulk cells, which are, in addition to LRP positivity, characterized by overexpression of the multidrug resistance protein 1 (MRP) and decreased expression of topoisomerase IIα gene.[8] These data suggest that compounds **7f–h** are transported via LRP and may, directly or indirectly, target topoisomerase IIα. The involvement of MRP1 dependent transportation is not clear, since derivatives were relatively potent in another MRP1 positive cell line A549.[8]

In conclusion, the synthesized derivatives of 5-[(azido-(4-nitrophenyl)-methyl]-uracil (**5**), 5-[hydroxy-(4-nitro-

phenyl)-methyl]-1H-pyrimidine-2,4-dione (**6**), 5,5′-(4-nitrophenyl)-methyl-bis-1H-pyrimidine-2,4-dione (**4**) as well as the 5-[(alkoxy-(4-nitrophenyl)-methyl]-uracil (**7**) substituted with low alkyl chain did not exhibit any significant cytotoxic activity against cancer lines. Activity increases with chain lengths continuously in lines CEM and K562-Tax. In ethers **7** with longer chains (from nonyl to undecyl), the activity decreases in LRP positive cell lines A549, K562, and CEM-DNR-B. Compounds **7f–h**, which were studied in the DNA/RNA synthesis inhibition test, exhibited similar activity in concentration 5× IC_{50}, where the synthesis of both nucleic acids was inhibited. Inhibition of de novo DNA and RNA synthesis is the most probable reason for rapid induction of apoptosis in treated cells.

At the concentration 1× IC_{50}, only compound **7f** effectively inhibited both synthesis of DNA and RNA and induced apoptosis. However, the structure was relatively inactive in a 3-day cytotoxic assay. The exceptional behavior of the hexylderivative **7f** may be caused by chemical or metabolic stability or differential affinity to the multidrug resistance associated proteins. Compounds **7g**, **7h** left the DNA synthesis uninfluenced, but caused an increase in RNA synthesis. This may be caused by promotion of compensatory mechanisms. Therefore, the apoptosis at this concentration was not apparent within the first 24 h of treatment.

The bulky alcohols used to form ethers **7m–o** did not enhance the activity to any noticeable extent.

The activity of individual enantiomers and nucleosides derived from derivatives **7** is now under intensive examination and will be published separately.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.022.

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