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Synthesis, cytostatic activity and ADME properties of C-5 substituted and N-acyclic pyrimidine derivatives

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ABSTRACT

The synthesis of the novel 5-alkyl pyrimidine derivatives, 5,6-dihydrofuro[2,3-*d*]pyrimidines and 5-alkyl *N*-methoxymethyl pyrimidine derivatives and evaluation of their cytostatic activities are described. The mechanism of antiproliferative effect of 5-(2-chloroethyl)-substituted pyrimidine **3** that exerted the pronounced cytostatic activity was studied in further details on colon carcinoma (HCT116) cells. The cell cycle perturbation analysis demonstrated severe DNA damage (G2/M arrest) pointing to a potential DNA alkylating ability of **3**. Preliminary ADME data of **3** and its 6-methylated structural congener (**6-Me-3**) showed their high permeability and good metabolic stability.

Synthetic nucleoside mimics with modified nucleobase and/or sugar moieties are of considerable importance in the search for new structural leads exhibiting biologically interesting activity.[1] Some nucleoside analogues substituted at various positions on the heterocycle, are known to have potent biological properties and have been investigated, for instance, as antiviral agents (against HSV, VZV, CMV, HIV, HBV and HCV), non-radioactive fluorescent labels for DNA and as anticancer drugs.[2] Extensive studies have been carried out on 5-substituted uracil analogues for use in cancer and viral chemotherapy, as enzyme inhibitors.[3] Notable among them are 5-fluorouracil (5-FU) and the corresponding 2′-deoxyribonucleoside (FdU) which have been used in cancer chemotherapy for decades.[4] Furthermore, bicyclic furo[2,3-*d*]pyrimidine nucleosides have demonstrated antiviral and antileukemic activity which has led to increased interest in preparation of corresponding nucleoside analogues.[5] Among fluorinated pyrimidine nucleoside analogues, 2′-deoxy-2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) and other small side-chain 5-substituted derivatives are phosphorylated with different efficacy by human and other mammalian nucleoside kinases including thymidine kinases TK1 and/or TK2; viral kinases such as herpes simplex virus type 1 and 2 (HSV1-TK and HSV2-TK).[6] Thymine derivatives with 6-(1,3-dihydroxyisobutenyl) and 6-(1,3-dihydroxyisobutyl) side-chain, as ligands for HSV1-TK, were developed as leads for gene expression imaging by positron emission tomography (PET).[7] In view of the importance of 5-substituted uracil derivatives in cancer chemotherapy[8] and as antiviral agents[9] we became interested in the synthesis of the novel C-5 and/or C-6 substituted pyrimidine derivatives.[10]

As part of our ongoing research in drug discovery we initiated the preparation of 5-alkyl pyrimidine derivatives (**3**–**6**, **8**, **9**, **11** and **17**), 5,6-dihydrofuro[2,3-*d*]pyrimidines (**7** and **10**) and 5-alkyl *N*-methoxymethyl pyrimidine derivatives (**12**–**16**). Construction of the pyrimidine ring was performed using the classical intramolecular cyclization of α-(1-carbamyliminomethylene)-γ-butyrolactone (**1**) with sodium ethoxide to afford 5-(2-hydroxyethyl)uracil (**2**) following a literature method.[11] Initially, the sodium α-hydroxymethylene-γ-butyrolactone was prepared by reaction of γ-butyrolactone and methylformate in dry ether with the presence of sodium methoxide, which was subsequently reacted with urea to give **2**. Transformation of hydroxyl and carbonyl functionalities to chlorine was performed using phosphoryl chloride affording chlorinated pyrimidine derivative **3** (Scheme 1).[11] The primary hydroxyl group of compound **2** was protected to give 5-(2-acetoxyethyl) substituted uracil **4**. Chlorination of **4** with phosphoryl chloride and *N*,*N*-diethylaniline yielded 2,4-dichloropyrimidines with 2-acetoxyethyl (**5**) and 2-hydroxyethyl (**6**) side-chain as well as bicyclic 5,6-dihydrofuro[2,3-*d*]pyrimidine derivative (**7**). Methoxylation of **5** gave desired 2,4-dimethoxypyrimidine (**8**) with 2-hydroxyethyl side-chain in moderate yield. 2-Chloro-4-methoxypyrimidine (**9**) and 2-methoxy-5,6-dihydrofuro[2,3-*d*]pyrimidine (**10**) were also isolated as minor products. Fluorination of **9**

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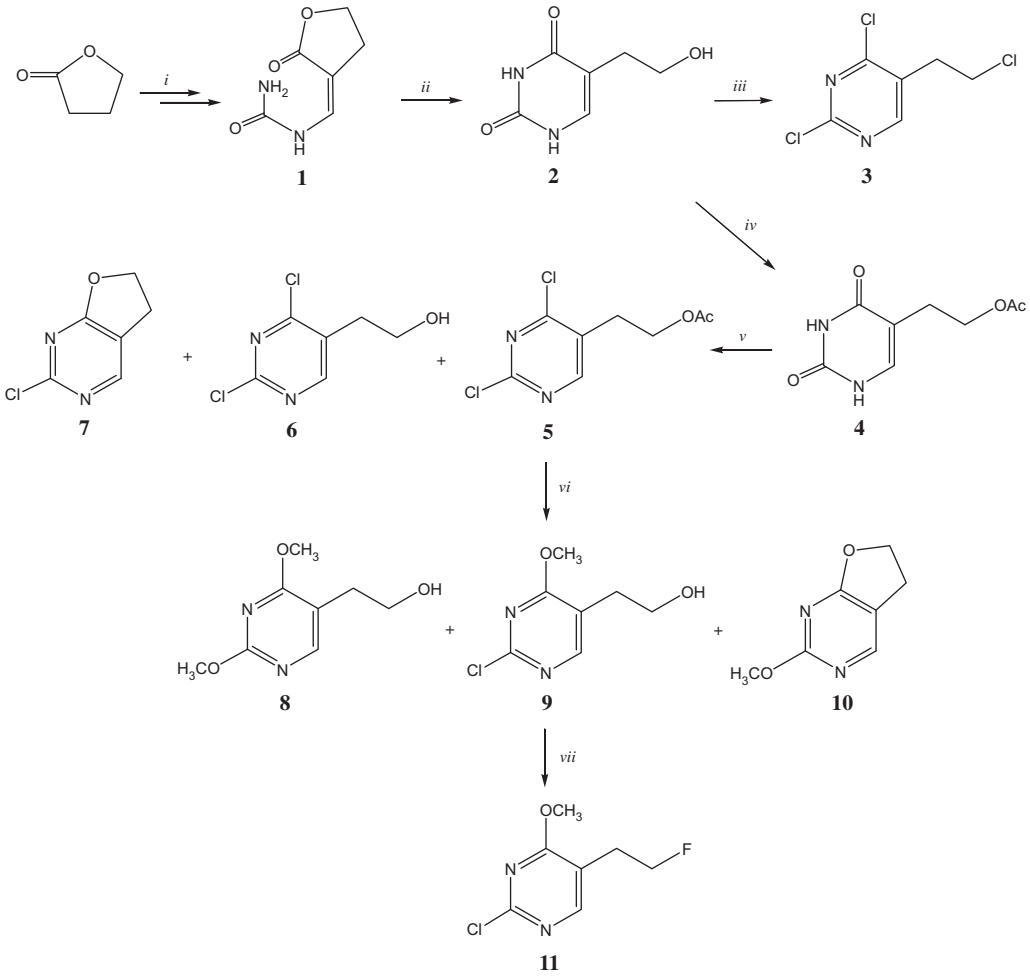

Scheme 1. Reagents and conditions: (i) NaOMe, ether, $HCOOCH_3$, rt, 24 h; urea, 3 M HCl, 4 °C, 24 h; (ii) NaOEt, EtOH, reflux, 6 h; (iii) $POCl_3$, *N*,*N*-diethylaniline, reflux, 1 h; (iv) Ac_2O, pyridine, reflux, 1 h; (v) $POCl_3$, reflux, 1 h; (vi) NaOMe, reflux, 1 h; (vii) DAST, CH_2Cl_2, −75 °C, 0.5 h.

with diethylaminosulfur trifluoride (DAST) as the fluorinating reagent afforded 2-chloro-4-methoxypyrimidine **11** with 2-fluoroethyl substituent at C-5.

N-methoxymethylation of **2** was accomplished with methoxymethyl chloride (MOMCl) as alkylating reagent and potassium carbonate to yield *N*-1-MOM (**12a**), *N*-3-MOM (**12b**) and *N*,*N*-1,3-diMOM (**12c**) 5-(2-hydroxyethyl)pyrimidine derivatives in 24%, 15% and 3% yields, respectively (Scheme 2). To the best of our knowledge there are only a few reports describing the N-methoxymethylated pyrimidine derivatives.[12]

The fluorine in side-chain of **12a** was introduced using DAST affording *N*-MOM pyrimidine derivative **13** containing 2-fluoroethyl at C-5 position. N-methoxymethylation of acetylated analog **4** also gave *N*-1-MOM (**14a**) and *N*-3-MOM (**14b**) regioisomers as well as *N*,*N*-1,3-diMOM (**14c**) in 22%, 2% and 17% yields, respectively. We can deduce that N-alkylation of uracil with both hydroxyethyl and acetoxyethyl substituents gave target *N*-1-MOM uracil derivatives in rather low yield (∼20%). Thus, we applied a more efficient N-methoxymethylation using same reaction conditions but starting from 2,4-dimethoxy-5-(2-hydroxyethyl)uracil (**8**) to give *N*-1-MOM (**15**) in improved yield of 46%. Compounds **16** and **17** with 2-(methoxymethoxy)ethyl substituent at C-5 were also isolated as byproducts.

Compounds **3**–**17** were evaluated for their cytostatic activities against three human malignant tumor cell lines: breast carcinoma (MCF-7), colon carcinoma (HCT116), lung carcinoma (H 460) and compared to the reference compound 5-fluorouracil. In general, compounds **4**, **6** and **8**–**16** showed no inhibitory activity on tumor-cell growth up to 100 μM (data not shown). However, moderate inhibition of cell growth was obtained with compounds **5**, **7** and to a certain extent **17** (Table 1).

While 2,4-dichloropyrimidine (**6**) with 5-(2-hydroxyethyl) side chain did not show any inhibitory effect, its acetylated structural analog **5** exhibited cytostatic activity (IC_{50} ∼30 μM). Similar activity (IC_{50} ∼27 μM) was found for bicyclic 2-chloro-5,6-dihydro furo[2,3-*d*]pyrimidine (**7**). The 2,4-dimethoxypyrimidine (**17**) with 5-(2-(methoxymethoxy)ethyl) side chain showed only slight inhibitory effect against colon carcinoma (HCT116). Of all compounds tested, 5-(2-chloroethyl)-2,4-dichloropyrimidine (**3**) exerted the most potent antiproliferative activity (in the low micromolar range), especially and rather selectively towards HCT116 colon cancer cell line (IC_{50} = 0.8 μM) (Table 1 and Fig. 1). Similar results showing significant impact of chlorine atoms on biological activity were described previously.[13] Interestingly, the increase in the chlorine content also enhanced the antiproliferative effects of 2,4-dichloro-6-methylpyrimidine structural congener bearing the same 5-(2-chloroethyl)-substituent.[13]

In order to shed more light on the mechanism of action of the most active compound **3**, we attempted the flow cytometry analysis of potential cell cycle perturbations. The compound was tested at two concentrations being close or slightly higher than its IC_{50} (1 and 5 μM) on HCT116 cells (Table 2). Interestingly, the results