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Research paper

New antiglioma zwitterionic pronucleotides with an FdUMP framework

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ABSTRACT

We have designed and synthesized new 5-fluoro-2′-deoxyuridine 5′-phosphate pronucleotides which can function as potential agents against the *glioblastoma multiforme* tumor. Their anti-malignant potency has been tested against T98G, U-118 MG, U-87 MG gliomas, HeLa, and Caco-2 cancer cell lines, using MRC-5 healthy cells as a reference. Five of the sixteen compounds (**4c**, **4f-i**) exhibited significant anti-cancer potency and high selectivity indices (SI 12–66). It is likely that these zwitterionic pronucleotides may function in a similar manner to zwitterionic phospholipids, by inducing cell membrane charge disorder, making the cell permeable to bioactive agents. The most promising therapeutic pronucleotides **4c**, **4f-h**, have high intestinal-blood uptake potency (Caco-2 cell line), and may be considered as potential, orally administrated, anticancer drugs.

1. Introduction

Glioblastoma multiforme (GBM) still remains one of the most lethal types of brain tumor, and medicine is far from finding a solution to overcome this [1]. Contemporary therapies against GBM (surgical resection, radiotherapy and chemotherapy) are hardly successful [1], with five-year survival rates lower than 5% (according to the Central Brain Tumor Registry of the United States, 2012). Therefore, novel efficient therapeutic agents for treatment of GBM are urgently needed. The examples of new approaches to combating glioblastoma encompass application of a sequence-specific RNAi in brain tumor therapy [2,3] and the use of new analogues of 5-fluoro-2′-deoxyuridine 5′-phosphate (FdUMP) that were designed as pronucleotides (vide infra). The choice of FdUMP as a leading compound was based on findings indicating that (i) thymidylate synthetase (TS) is overexpressed in glioblastoma cells [4], and that (ii) FdUMP proves to function as a true and specific inhibitor of this enzyme, with the K_m value of three orders of magnitude lower than that of the natural substrate, dUMP (2.2×10^{-8} vs 3.7×10^{-5}, respectively) [5]. Since FdUMP, as a di-anionic molecule, is not able to penetrate the cellular membrane [6,7], multiple efforts were made to deliver it into the cell in a less charged, masked form, e.g., as uncharged phosphotriesters [8–10], lipophilic phosphodiesters [8], dinucleoside phosphates [8], carboxylic-phosphate mixed anhydrides [8], or amino acid phosphoramidates [8,11–20]. Irrespectively of the masking system, all of the above-mentioned compounds were designed as prodrugs (pronucleotides) which should release FdUMP, the true drug, after chemical or enzyme-assisted conversion within the cell. However, other studies on FdUMP pronucleotides [21–27] and other type of biologically active compounds (for instance, phospholipids [28–34]), proved that the presence of a charge in combination with specific structural fragments, e.g., lipophilic or zwitterionic elements [27,35–37], did not prevent cellular internalization of the compounds and in consequence expression of their biological activity.

In this paper, we described the synthesis of new FdU-based pronucleotides carrying zwitterionic phosphate masking systems (Fig. 1, abbreviations as in Scheme 1), and evaluated their anti-proliferative potency on several glioma cell lines. By choosing charged zwitterionic pronucleotides, we expected, besides of potential therapeutic advantages, an increase of solubility in

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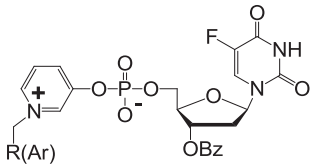


Fig. 1. Zwitterionic FdUMP pronucleotides.

physiological media. Additionally, we hoped that cellular membrane charge disorder, caused by ionic compounds, including zwitterions [38,39], can be different in healthy and malignant cells, and be beneficial with regard to combating the neoplasm.

2. Results and discussion

2.1. The rationale behind the choice of FdUMP derivatives

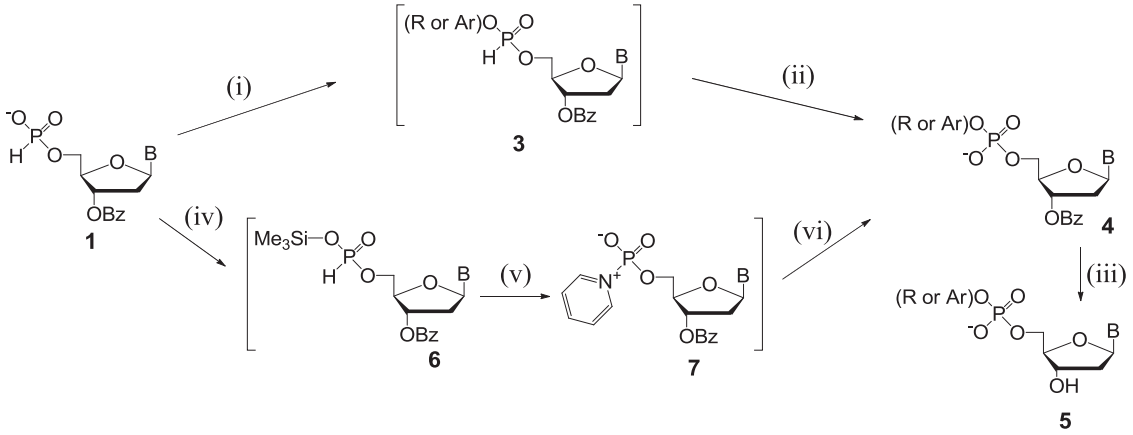
We focused our attention on the FdU 5′-phosphodiester derivatives, that should meet the criteria typical of pronucleotides. In most cases, the investigated pronucleotides of type **4** (Scheme 1) have 3′-*O*-benzoyl-5-fluoro-2′-deoxyuridin-5′-yl residue in the nucleoside part, that *per se* demonstrates advantageous antiglioma potency [40]. For comparison, nucleotide analogues **5a,c-d** with free 3′-OH function were also evaluated. With exception of compounds **4a** and **4b**, a common structural motif of the examined nucleoside phosphodiesters **4c-m** and **5c-d** is an aliphatic or aromatic amine that together with a phosphate residue can form a zwitterionic structure (e.g. **4c** and **4d** [37]) or are permanent zwitterions (**4e-m**). Phosphodiesters **4e-m**, in addition to a zwitterionic part, have also different lipophilic handles that may further modulate their ability of cellular membrane penetration. Compounds **4h** and **4i** were designed as nucleotide analogues of the known biocidal, membrane interactive drug, 12-methacryloyloxydodecylpyridinium bromide [38]. For similar reasons, phosphodiesters **4j** and **4k** were appended with 2-*N*,*N*,*N*-trimethylethanaminum or *N*,*N*-dimethylpiperidinium zwitterionic residues that are part of the known cytostatic compounds, e.g. miltefosine [36] and perifosine [35], respectively. In the studied compounds, the aryl and alkyl groups were selected as lipophilic parts of pronucleotides, in order to find out which type of the group will turn out to be more beneficial for antiglioma activity, and thus worthy of further development.

2.2. Chemistry

2.2.1. Synthesis of aryl(alkyl) nucleoside 5′-phosphate diesters of type *4* and *5*

*A route via H-phosphonate diesters of type **3** as intermediates.*

For the purpose of the synthesis of aryl nucleoside phosphate diesters, we used an approach that was previously developed in our laboratory [41]. Consequently, nucleoside 5′-*H*-phosphonates of type **1** were coupled with phenols **2a-d** or alkanols **2j-m,** with the aid of diphenyl chlorophosphate (Scheme 1). The reactions were carried out in CH_2Cl_2/pyridine 9: 1 (v/v) to avoid double activation of the *H*-phosphonate component [42]. Formation of the respective *H*-phosphonate diesters of type **3** as well as their oxidation with iodine towards phosphodiesters **4**, proceeded smoothly, practically quantitatively (as judged from ^{31}P NMR spectra), and could be



B = 5-fluorouracyl-1-yl
Bz = benzoyl;
2a, 3a, 4a, 5a; Ar = phenyl;
2b, **3b**, **4b**; Ar = 4-nonylphenyl
2c, 3c, 4c, 5c; Ar = pyridin-3-yl;
2d, 3d, 4d, 5d; Ar = quinolin-6-yl
2e, 4e,; Ar = *N*-methylpyridinium-3-yl;
2f, 4f; Ar = *N*-decylpyridinium-3-yl;
2g, 4g; Ar = *N*-benzylpyridinium-3-yl;
2h, 4h; Ar = *N*-(2-ethoxy-2-oxoethyl)pyridinium-3-yl;
2i, 4i; Ar = *N*-(2-ethoxy-2-oxoethyl)quinolinium-6-yl;
2j, 3j, 4j; R = 2-(*N*,*N*,*N*-trimethylaminium)ethyl;
2k, 3k, 4k; R = *N*,*N*-dimethylpiperidinium-4-yl;
2l, 3l, 4l; R = 2-(*N*-benzyl-*N*,*N*-dimethylaminium)ethyl;
2m, 3m, 4m; R = *N*-benzyltropanium-3-yl;

Scheme 1. Synthesis of FdUMP pronucleotides of type **4** and **5**. (i) 1 mmol of **1** in 10 mL of CH_2Cl_2/pyridine 9: 1 (v/v), ArOH or ROH, **2**, 1.5 mmol, diphenylchlorophosphate 1.5 mmol, 5 min, r. t.; (ii) 2 mmol of I_2 in 5 mL pyridine, after 10 s H_2O > 10 mmol, 5 min; (iii) 40% of $MeNH_2$ aq., rt, overnight; (iv) 1 mmol of **1** in 10 mL of acetonitrile, trimethylsilyl chloride, 2 mmol and Et_3N, 2 mmol, < 3 min; (v) 2 mmol of I_2 in 5 mL pyridine, < 3 min; (vi) 2.5 mmol of ArOH **2**.