Bioorganic & Medicinal Chemistry Letters 26 (2016) 2142–2146

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# L-Aspartic and L-glutamic acid ester-based ProTides of anticancer nucleosides: Synthesis and antitumoral evaluation ☆

Ling-Jie Gao [a], Steven De Jonghe [a], Dirk Daelemans [b], Piet Herdewijn [a,*]

[a] KU Leuven, Rega Institute for Medical Research, Laboratory of Medicinal Chemistry, Minderbroedersstraat 10, 3000 Leuven, Belgium
[b] KU Leuven, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Minderbroedersstraat 10, 3000 Leuven, Belgium

ARTICLE INFO

*Article history:*
Received 1 March 2016
Revised 18 March 2016
Accepted 20 March 2016
Available online 22 March 2016

*Keywords:*
ProTides
Nucleosides
Prodrugs
Antitumoral

ABSTRACT

A series of novel aryloxyphosphoramidate nucleoside prodrugs based on L-aspartic acid and L-glutamic acid as amino acid motif has been synthesized and evaluated for antitumoral activity. Depending on the cancer cell line studied and on the nature of the parent nucleoside compound (gemcitabine, 5-iodo-2′-deoxy-uridine, floxuridine or brivudin), the corresponding ProTides are endowed with an improved or decreased cytotoxic activity.



Nucleoside analogues represent important drugs for the treatment of different cancers. Currently approved antitumoral nucleoside analogues include Cytarabine, Fludarabine, Cladribine, Gemcitabine, Clofarabine, Capecitabine, Floxuridine, Deoxycoformycin, Azacitidine and Decitabine. These agents have been licensed for a variety of oncology indications, including leukaemia, lung cancer, pancreatic cancer and colon cancer.[1] The mechanism of action for most of these compounds is very similar. They are intracellularly converted to their respective nucleotide analogues, which inhibit DNA synthesis by inhibition of DNA polymerase and/or ribonucleotide reductase. Decitabine is incorporated into DNA as its triphosphate metabolite, resulting in inhibition of DNA methylation. The cytotoxic activity of azacitidine is mainly due to its conversion to deoxynucleotides via ribonucleotide reductase and its incorporation into DNA. Floxuridine is intracellularly phosphorylated to its active form, inhibiting thymidylate synthase. Deoxycoformycin (pentostatin) is the only antimetabolite active without metabolism. It acts as a potent inhibitor of adenosine deaminase.[2]

A common problem in the treatment of cancer patients is the resistance to nucleoside analogues.[3,4] Resistance can be caused by poor conversion of the parent nucleoside to its active nucleoside monophosphate, diphosphate and triphosphate. In most cases, the first step of phosphorylation represents the rate-limiting step of the bioactivation and its inefficiency may limit the therapeutic potential of the nucleoside analogues. Another reason for the decreased activity of the nucleoside analogue is the limited uptake by the tumor cells, due to a decreased expression of nucleoside transporter proteins.

Various prodrug or 'pronucleotide' approaches have been devised and investigated in order to tackle these issues.[5] In general, the goal of these approaches has been to promote passive diffusion through the lipophilic cell membranes and to release the parent nucleotide intracellularly, where it can be further phosphorylated to the pharmacologically active species. One of the most promising approaches are the aryloxyphosphoramidates (also known as the ProTides).[6] Originally, this approach was focused on improving the biological activity of antiviral agents. The ultimate success came with the marketing of Sofosbuvir, which is currently used for the treatment of HCV infected patients.[7] Aryloxyphosphonoamidates, such as GS-9131,[8] moved into clinical trials to be evaluated as anti-HIV medication. GS-7340 (also known as tenofovir alafenamide) is an example of another phosphono-amidate prodrug that received recently marketing approval as anti-HIV drug.[9]

More recently, the aryloxyphosphoramidate prodrug strategy has also been applied to antitumoral nucleosides. Phosphoramidate ProTides of the anticancer agents gemcitabine,[3] floxuridine,[10] cladribine,[11] and brivudine[12] have been synthesized and evaluated for antitumoral activity. NUC-1031 (Fig. 1) is the phosphoramidate prodrug of gemcitabine, which is currently undergoing clinical trials in patients with solid tumors. Thymectacin, an

☆ In memory of Professor Christopher McGuigan.

* Corresponding author. Tel.: +32 16 33 73 87.
*E-mail address:* piet.herdewijn@kuleuven.be (P. Herdewijn).

http://dx.doi.org/10.1016/j.bmcl.2016.03.076
0960-894X/

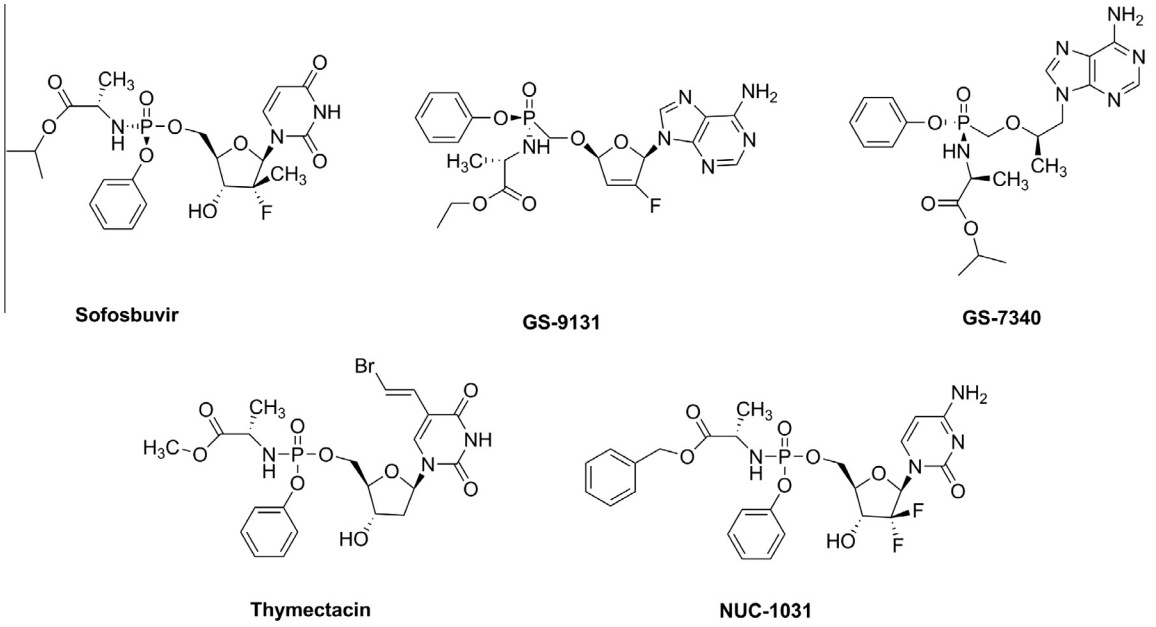



**Figure 1.** Known ProTides.

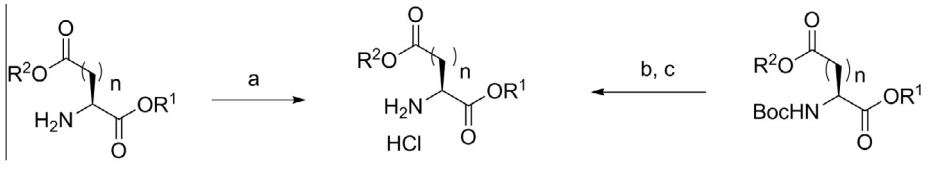
aryloxyphosphoramidate prodrug of brivudine, is currently undergoing clinical trials in colon cancer.

In all phosphoramidate ProTides studied so far (antiviral, as well as, antitumoral congeners), L-alanine is the preferred amino acid motif. Other amino acids, such as the L-aspartic acid dimethylester have been evaluated and were found to be less active and have therefore not been further pursued.[13] However, we recently reported the synthesis and antiviral evaluation of phosphoramidate prodrugs of 2′-*C*-Me-cytidine, 2′-*C*-Me-uridine and 2′-*C*-Me-2′-F-uridine using L-aspartic acid as amino acid.[14] These compounds were endowed with very potent antiviral activity when tested against the hepatitis C virus. As their activity is higher than the classical L-alanine containing ProTides, it suggests that L-aspartic acid should be considered as amino acid alternative in prodrug design. These findings prompted us to synthesize a series of L-aspartic acid phosphoramidate analogues as potential prodrugs of antitumoral nucleosides.

Although different methods are available for the synthesis of the aryloxyphosphoramidate prodrugs, we selected the classical procedure in which the (protected) nucleoside is coupled with a suitable chlorophosphoramidate reagent, which is activated as its imidazolium intermediate using *N*-methylimidazole (NMI).[15]

The synthesis of the amino acid ester building blocks is shown in Scheme 1. Diisoamyl L-aspartic acid **3a** was prepared from L-aspartic acid **1a** using thionyl chloride and isoamyl alcohol, according to a known method.[14] The corresponding diisoamyl L-glutamic acid **3b**, was prepared similarly in a good yield, starting from L-glutamic acid **1b**. The asymmetric esters of L-aspartic acid (compounds **3c** and **3d**) were prepared from the commercially available Boc-protected L-aspartic acid analogues **2a** and **2b** in a two step procedure. Reaction of the carboxylic acid moiety with isoamyl alcohol using *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(6-chloro-1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HCTU) as coupling agent yielded the corresponding ester derivative. Acidic cleavage of the Boc group afforded the desired di-esterified L-aspartic acid derivatives (**3c**–**d**), as their hydrochloride salts.

The ProTides of 5-Iodo-2′-deoxy-uridine (IDU), Floxuridine (2′-deoxy-5-fluoro-uridine, FDU) and Brivudin ((*E*)-5-(2-bromovinyl)-2′-deoxyuridine, BVDU) were prepared from the unprotected, parent nucleosides **4**–**6** (Scheme 2). The key reagent **7** was prepared in situ by reacting phenyl phosphorodichloridate with the esterified L-aspartic acid **3a**, after which the nucleosides **4**–**6** were coupled in the presence of *N*-methylimidazole (NMI), in a one pot procedure, according to a known method.[16] Literature precedents suggest that these reactions preferentially take place in dichloromethane. However, in our hands, the reaction did not work well, due to the poor solubility of the reactants in dichloromethane. The problem could be easily resolved by running the reaction in




**1a:** n = 1, $R^1 = R^2 = H$
**1b:** n = 2, $R^1 = R^2 = H$

**3a:** n = 1, $R^1 = R^2$ = isoamyl
**3b:** n = 2, $R^1 = R^2$ = isoamyl
**3c:** n = 1, $R^1$ = isoamyl, $R^2$ = Bn
**3d:** n = 1, $R^1$ = Bn; $R^2$ = isoamyl

**2a:** n = 1, $R^1$ = H, $R^2$ = Bn
**2b:** n = 1, $R^1$ = Bn, $R^2$ = H

**Scheme 1.** Reagents and conditions: (a) $SOCl_2$, isoamyl alcohol, 0 °C to rt, 12 h, then reflux, 3 h, 80%; (b) HCTU, isoamyl alcohol, $Et_3N$, $CH_2Cl_2$, rt, 4 h, 95%; (c) 5 N HCl in *i*-PrOH, $CH_2Cl_2$, rt, 2 h, 95%.