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Synthesis of C3-arylated-3-deazauridine derivatives with potent anti-HSV-1 activities

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ABSTRACT

A series of 3-deazauridines (3-DU) analogues were synthesized and evaluated in vitro for their antiherpetic activity against HSV-1 on Vero cell lines by cell viability. A first campaign of tests suggested that C3-arylated-3-DU derivatives could constitute a novel family of antiherpetic agents. A second campaign of biological evaluations led to the discovery of two potent anti-HSV-1 agents with comparable activity than acyclovir.

Herpes simplex virus (HSV) infections are responsible of a wide range of diseases in humans including genital and labial herpes that could be life threatening for pregnant women, newborns and immunocompromized patients. HSV infections is a common human pathogen with between 60% and up to 95% of certain populations infected with *Herpes simplex virus* type 1 (HSV-1), and between 6 and 50% infected with *Herpes simplex virus* type 2 (HSV-2).[1] The frequency of HSV-seropositive males was significantly higher in populations infected with human immunodeficiency virus (HIV).[2] As HIV disease progresses, cutaneous and mucosal complications become more severe and occur in up to 92% of HIV-infected individuals.[3] Moreover, the recurrence of infections upon various stimuli is severely damaging for patients. The standard antiviral arsenal for the treatment of HSV infections is mainly based on nucleoside-type drugs including acyclovir **1**, famcyclovir **2** and brivudin **3** (Fig. 1).[4] These drugs have been introduced at least 15 years ago and there is still a need for the discovery of new agents with enhanced activities. While new helicase-primase inhibitors have been recently proposed as drug candidates for the treatment of HSV infections,[5] nucleoside-based agents are the only class of available drugs on the market due to their unique pharmacological profile.

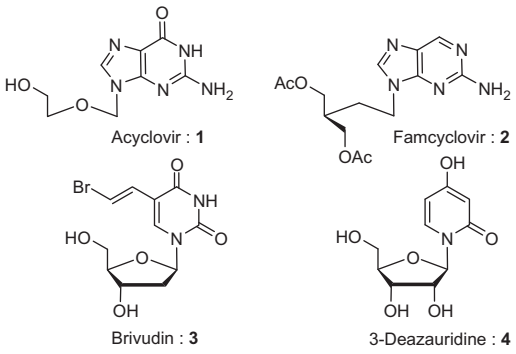

Figure 1. Structure of anti-HSV agents and 3-deazauridine **4**.

On the other hand 3-deazauridine (DU) **4** has been shown to inhibit cytidine triphosphate synthetase, thereby reducing intracellular levels of cytidine triphosphate and disrupting DNA and RNA synthesis.[6] However, DU **4** used as a single agent exerts negligible anti-tumoral clinical efficiency resulting in a lack of interest in clinical investigation.[7] Our interest in DU analogs stems from a study describing that substitution at C3 position could led to agents with anti-viral activities while cell toxicity remains weak.[8]

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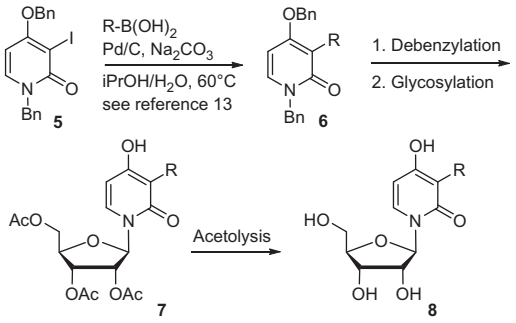


Scheme 1. General strategy for the preparation of C3-arylated-3-deazauridine compounds.

However, only 3-nitro-3-deazauridine was active and no other active compound was disclosed. We assumed that other substitution at C3 with sterically hindered and/or appropriate functional groups can modify interactions associated with base pairing and protein receptor binding. In this context, we felt that substitution at C3 by aryl groups could lead to a new class of nucleosides. Such a strategy has been recently studied on other systems[9] and lead to new promising anti-viral and cytostatic agents[10] as well as fluorescent probes.[11] Only a few papers deals with the synthesis of 3-DU analogues,[12] and to the best of our knowledge, no work has been disclosed concerning the arylation of 3-DU **4**. In this communication we report our last finding that led to the discovery of a new family of C3-arylated-3-deazauridine derivatives displaying potent anti-HSV-1 activities.

In a recent paper we reported the rapid preparation of 3-aryl-4-oxypyridin-2-(*1H*)-ones **6** through a practical Pd/C-catalyzed Suzuki–Miyaura reaction.[13] These useful intermediates can be viewed as benzylated nucleobases for the preparation of novel nucleosides as depicted in the Scheme 1.

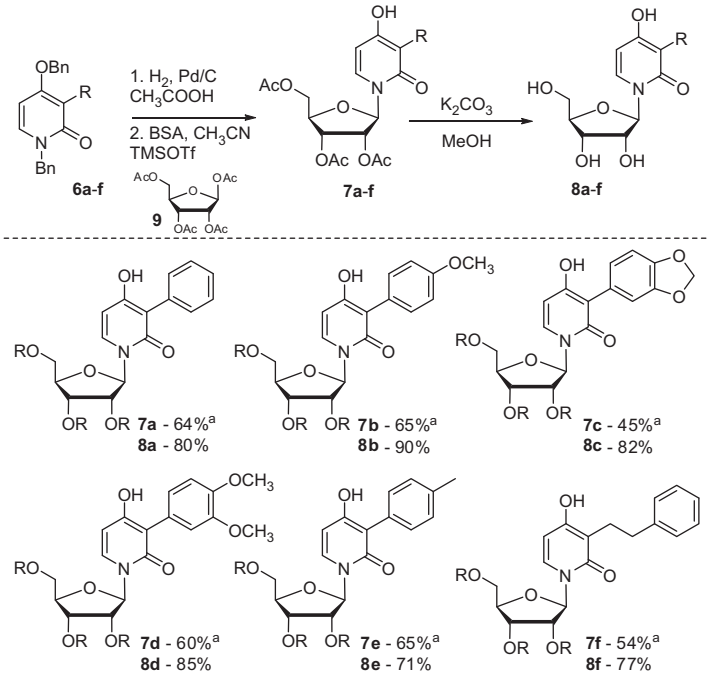
Table 2
Evaluation of anti-HSV-1 activity on Vero cell line by neutral red dye method

Compounds	Vero EC_{50} μM[a]	Vero CC_{50} μM[b]	Efficiency[c] (%)
Acyclovir	0.76	>400	100
7a	>100	>400	ND
7b	10.5	>400	7.2
7c	>100	>400	ND
7d	36.6	>400	2.1
7e	>100	>400	ND
7f	>100	>400	ND
8a	>100	>400	ND
8b	20.0	>400	3.8
8c	>100	>400	ND
8d	>100	>400	ND
8e	>100	>400	ND
8f	>100	>400	ND

[a] Represents the concentration that achieves 50% protection of virus-infected cells from the HSV-induced destruction.
[b] Represents the concentration that reduces the absorbance of mock-infected cells to 50% of that of control.
[c] The efficiency is expressed in % as the ratio of EC_{50} for acyclovir relative to the 3-DU analogues.

In this context, we initially prepared six benzylated nucleobases **6a–f**[13]for the design of new potential antiherpetic 3-DU analogues (Table 1). The benzyl group of compounds **6a–f** was cleaved under standard conditions with the Pearlman's catalysts and the corresponding deprotected products were immediately reacted with the tetra-*O*-acetyl-D-ribose **9** under Vorbrüggen conditions[14] to give the acetylated nucleosides **7a–f**. The modest yield obtained for nucleoside **7c** could be explained by partial instability of the methylenedioxy group in the presence of the strong Lewis acid,

Table 1
Preparation of the first generation anti-HSV-1 nucleosides



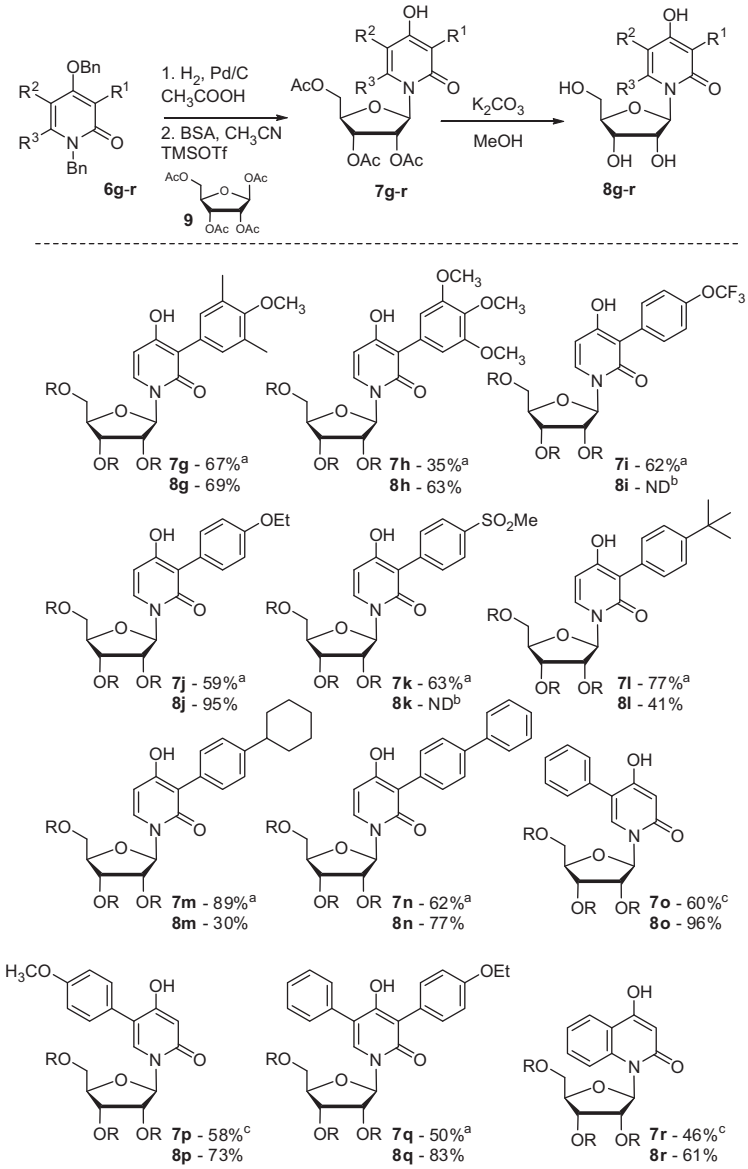
[a]Yield over two steps.

TMSOTf. The acetyl functions of compounds **7a–f** were then removed by methanolysis to afford the free nucleoside **8a–f**.

Having led the foundation of a solid synthetic pathway for the preparation of C3-functionnalized-3-DU compounds we carried out preliminary biological evaluations. The antiviral properties against HSV-1 on Vero cell line of our set of 12 nucleosides (**7a–f** and **8a–f**) were evaluated and the results are reported in the Table 2. In comparison with Vero cell lines, after 3 days of treatment, microscopically visible alteration of normal cell morphology was observed and viability assay showed destruction of cell layer. The range of concentrations assayed for all compounds tested on Vero cell lines corresponds to 200, 50, 10, 5, 1 μg/ml. No cytotoxic effect of the compounds on the Vero cells was observed in the range of the concentrations assayed for all compounds. But more importantly, among the set of compounds prepared (**7a–f** and **8a–f**) in this first campaign of synthesis, three of them displayed interesting anti-HSV-1 activity with an EC_{50}<50 μM. For instance, at a multiplicity of infection (MOI) of 0.001 ID_{50}/cells, 85% cellular protection was obtained for 200 μg/ml of **7b** at 72 h after infection and 82% cellular protection was obtained for 200 μg/ml of **8b** at 72 h after infection. For **7d**, 75% cellular protection was obtained at 200 μg/ml. It is worth mentioning that acylated nucleosides display a higher activity relative to their deprotected congeners. Although these results were very encouraging for the discovery of a lead, the efficiency, relative to the selected drug of reference (acyclovir), still remained to be improved.

Motivated by the promising results reported above, we engaged a second campaign of synthesis of anti-HSV-1 nucleosides with a higher molecular diversity. Toward this end, we prepared a variety of C3-, C5- and C6-subtituted 3-DU taking advantage of the meth-

Table 3
Preparation of the second generation of anti-HSV-1 nucleosides



[a] Yield over two steps
[b] Not determined due to extensive decomposition
[c] Yield of the glycosidation step, see text.

Table 4
Evaluation of anti-HSV-1 activity on vero cell line by neutral red dye method

Compounds	Vero EC_{50} μM[a]	Vero CC_{50} μM[b]	Efficiency (%)[c]
Acyclovir	22	>200	100
4	>100	>200	ND
7b	>100	>200	ND
7d	>100	>200	ND
7g	>100	>200	ND
7h	>100	>200	ND
7i	>100	>200	ND
7j	>100	>200	ND
7k	>100	>200	ND
71	>100	>200	ND
7m	49	>200	45
7n	33	>200	67
7o	>100	>200	ND
7p	>100	>200	ND
7q	>100	>200	ND
7r	>100	>200	ND
8b	>100	>200	ND
8g	>100	>200	ND
8h	>100	>200	ND
8j	>100	>200	ND
81	>100	>200	ND
8m	>100	>200	ND
8n	>100	>200	ND
8o	>100	>200	ND
8p	>100	>200	ND
8q	>100	>200	ND
8r	>100	>200	ND

[a] Represents the concentration that achieves 50% protection of virus-infected cells from the HSV-induced destruction.
[b] Represents the concentration that reduces the absorbance of mock-infected cells to 50% of that of control.
[c] The efficiency is expressed in % as the ratio of EC_{50} for acyclovir relative to the 3-DU analogues.

odologies developed in our laboratory (Table 3).[13] The collection of the second generation of nucleosides prepared are reported in the Scheme 3. It should be noted that the nucleobases of compounds **7o**, **7p** and **7r** were not prepared according to the general procedure. Indeed, the corresponding 5-aryl-3-hydroxypyridones for **7o** and **7p** were prepared following literature precedents[15] and the 4-hydroxy-2-quinolone for **7r** was commercially available.

For this second campaign of tests, we worked with virus-infected cell suspensions at a higher MOI (0.01 ID_{50}/cells) in order to exclude any structures having only a marginal anti-HSV-1 activity. As a consequence, the EC_{50} of the reference (acyclovir) increased to 22 μM (vs 0.76 μM at a MOI of 0.001 ID_{50}/cells). However, the determination of the drug efficiency expressed as the ratio of EC_{50} for acyclovir relative to the 3-DU-analogues allows a direct comparison of the results with those reported in the Table 2. All drugs reported in this panel of tests didn't display any cytotoxic effect on Vero cell lines at concentration as high as 200 μM. At a MOI of 0.01 ID_{50}/cells we found that compound **7m** and **7n** presented a remarkable antiherpetic activity with EC_{50} of respectively 49 μM and 33 μM (Table 4). When compared to the value of reference measured for acyclovir, a very promising efficiency of 45% for **7m** and even 67% for **7n** was calculated. In these conditions, the 3-deazauridine **4** and compound **7b** were inactive at 100 μM. Nucleosides having a structurally modified base-arrangement (**7o–r** and **8o–r**) did not display any biological activity. As already observed with the first set of compounds (see Table 1), none of the deacetylated nucleosides displayed a significant activity. Hydrophobic factors are likely responsible of this general trend. Although the agents **7m** and **7n** reveals structural similarities, it seems still premature to build a SAR. However, it appeared that the introduction of an apolar substituent at C4′ of the aryl group could enhance the anti-HSV-1 activity. Conformational factors could also play a crucial role since the nucleoside **7l**, bearing a *tert*-butyl group, was inactive. The elucidation of the mechanism of action could help us in determining the interactions with the biological target.

The discovery of new antiherpetic agents, able to substitute ageing marketed drugs, is still of need for public health consideration. In this work we have disclosed a new family of potent anti-HSV-1 nucleosides, analogues to the well known 3-deazauridine **4**. From the preparation and the biological evaluations of more than thirty C3-arylated-3-DU derivatives, two compounds emerged as potential antiherpetic agents. Further studies toward the determination of the mechanism of action are currently underway and should provide us essential information for the design of agents with an improved biological profile.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.047.

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