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Design and synthesis of a potent macrocyclic 1,6-napthyridine anti-human cytomegalovirus (HCMV) inhibitors

Guy Falardeau,[a,*] Hugo Lachance,[a] Annie St-Pierre,[a] Constantin G. Yannopoulos,[a] Marc Drouin,[b] Jean Bédard[a] and Laval Chan[a]

[a]*ViroChem Pharma Inc., 275 Armand-Frappier Blvd., Laval, Québec, Canada H7V 4A7*
[b]*Université de Sherbrooke, Sherbrooke, Québec, Canada J1K 2R1*

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Abstract—A novel class of macrocyclic 1,6-napthyridines designed to adopt the presumed bioactive conformation of anti-HCMV acyclic 1,6-napthyridines are described. Both 14- and 15-membered macrocycles were shown to be highly potent against HCMV HSV-1 and HSV-2.

Of the DNA viruses, the herpes group is the source of the most common viral illnesses in man. The group consists of herpes simplex virus (HSV) type I and II, varicella zoster (VZV), Epstein-Barr virus (EBV), and human cytomegalovirus (HCMV).[1] As with other herpes viruses, infection with HCMV leads to a lifelong association of virus and host. Following a primary infection, virus may be shed for a number of years. Infection in healthy individuals is frequently asymptomatic and up to 80% of the adult population harbors the virus in latent form. In immunocompromised individuals, such as chemotherapy patients, organ transplant patients and particularly AIDS sufferers, latent HCMV can be re-activated resulting in microcephaly, hepatosplenomegaly, jaundice, convulsive seizures, mononucleosis, retinitis, and even death. With the introduction of HAART and improved patients' compliance, the incidence of CMV infection has greatly diminished in the past few years in the AIDS population.[1d]

A variety of drugs have been developed to treat herpes virus infection, including naturally occurring proteins and synthetic nucleoside analogs. For example, interferon, has been used in the treatment of herpes virus infections, as have the nucleoside analogs, cytosine-arabinoside, adenine-arabinoside, iodoxy-uridine, and acyclovir (ACV), which is presently the treatment of choice for herpes simplex type I infection. Unfortunately, drugs such as acyclovir that have proven effective to treat certain herpes viruses infections are not sufficiently effective to treat HCMV. And, drugs currently used to treat HCMV infection, such as ganciclovir (GCV), cidofovir (CDV), foscarnet (PFA), an valganciclovir, an orally bioavailable valine ester prodrug of ganciclovir, lack the acceptable side effect and safety profiles of the drugs approved for treatment of other herpes viruses.[2]

As part of our ongoing search for new anti-HCMV compounds, we have discovered the [1,6]-naphthyridines,[3] a novel class of inhibitors in which compound **1** (Fig. 1) is 25 times more potent than GCV. The resulting SAR study showed that the optimal positions for the nitrogens on the naphthyridine ring were 1 and 6.[3b] In addition, substitution at C8 and a bulky alkoxy group at the 2′-position were beneficial for activity.[3a] We have also demonstrated that an internal hydrogen bond (IHB) likely maintains the [1,6]-naphthyridine-2-carboxylic acid benzylamide in an active conformation.[4,5] Examination of the X-ray crystal structure of **1** reveals the presence of two conformations. Conformer **1a** with two IHB, is believed to be the active conformation[5] whereas, conformer **1b** with only one IHB is about 0.4 kcal higher in energy than **1b** thus explaining the presence of the two conformers in the crystal structure.

Keywords: Anti-proliferative agents; Anti-virals; Polycyclic heterocyclic compounds; HCMV; HSV; Naphthyridine; Macrocycle.
* Corresponding author. Tel.: +1 45 0978 8431; fax: +1 45 0978 7777; e-mail: gfalardeau@virochempharma.com

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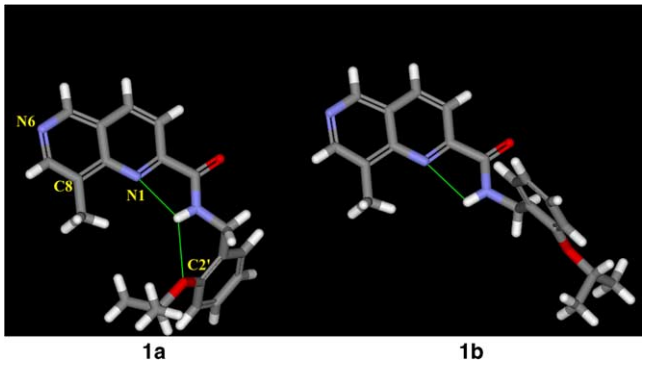
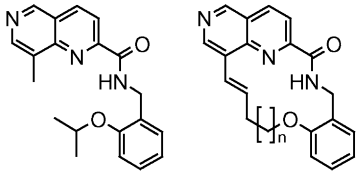


Figure 1. Crystal structure of **1**.



Examination of conformer **1a** reveals that the C8 methyl is in close proximity to the alkoxy moiety. We therefore decided to evaluate the effect of introducing a covalent link between C8 methyl and the alkoxy group on antiviral activity. A low energy computer generated model of a 14-membered macrocycle **2** (Fig. 2) gave two conformers with an energy difference of 0.1 kcal, the difference in the **2** conformers resided mainly in the orientation of the phenyl group. Interestingly, both conformers **2a** and **2b** could be superimposed on the open conformer **1a**. Encouraged by these results, we therefore embarked on the synthesis of 14-membered macrocycle **2** as well as 15-membered macrocycle **3**.

Both macrocycles were constructed by an intramolecular Stille coupling between an appropriate vinyl stannane and 8-bromo-napthyridine **5** (Scheme 1). Thus, bromination of the carboxylic acid **4**[3a] with bromine in acetic acid yielded the 8-bromo-[1,6]-naphthyridine-2-carboxylic acid **5** in 80% yield. Reaction of the carboxylic acid **5** with 2-hydroxy-benzylamine in the presence of EDCI/HOBt gave amide **6** in 93% yield. The stannane intermediate **7** was assembled using a Mitsunobu[6] reaction between phenol **6** and 4-tributylstannanyl-but-3-en-1-ol giving *E*-vinylstannane **2** in 53% yield. The Stille coupling[7] membered macrocycle **3** was constructed from the corresponding pent-4-en-1-ol.

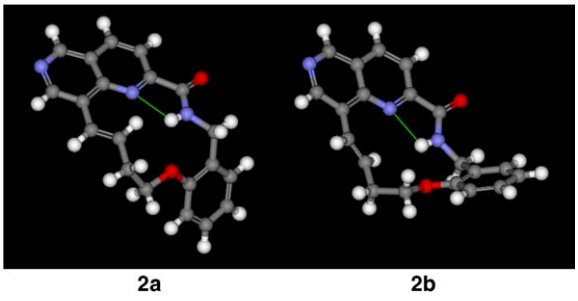
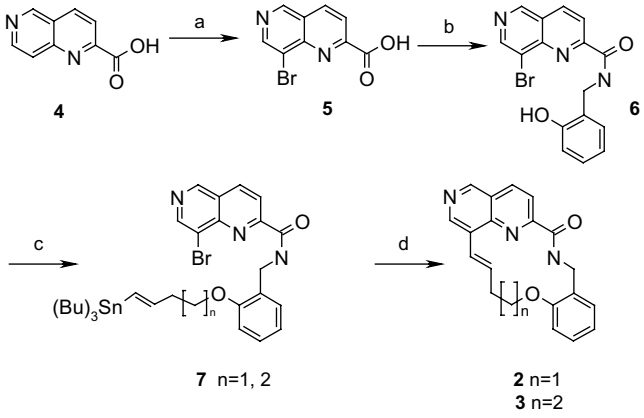


Figure 2. Molecular model of macrocycle **2**.



Scheme 1. Reagents and conditions: (a) AcOH, Br_2, 80%; (b) HOBT, EDCI, 2-hydroxy-benzylamine, Et_3N, DMF, 93%; (c) (n = 1) DEAD, 4-tributylstannanyl-but-3-en-1-ol, Et_3N, DMF (53%); (n = 2) DEAD, 4-tributylstannanyl-pent-3-en-1-ol, Et_3N, DMF (50%); (d) $Pd(dba)_3{\cdot}CHCl_3$, DMF, 110 °C, 3 h. n = 1, 35%; n = 2, 28%.

Other approaches to form the macrocycle by an intramolecular amidation, olefin metathesis or phenolic alkylation failed to give the desired product in useful yields.

Both macrocycles were almost equipotent (Table 1) and macrocycle **2** showed an impressive gain in activity compared to the acyclic analog **1**. The 14-membered macrocycle **2** with an IC_{50} of 0.6 ng/mL and a selectivity index of 3000 is probably one of the most potent anti-HCMV compound reported to date. In addition, as with the previously reported napthyridines, activity against a clinical isolate (P8 low passage) and GCV resistant strains (UL 97 and UL 54 mutants) was retained thus, indicating that the mode of action of **2** does not involve phosphotransferase or viral DNA polymerase enzymes as targets. Macrocycles **2** and **3** were also tested against herpes simplex virus (HSV) in order to determine the specificity toward the herpesviredae family (Table 2). The results are reported in Table 2. The 15-membered macrocycle **3** is more potent than the 14-membered macrocycle **2** against HSV-1 but interestingly, both are far more potent against HSV-2 than both GCV and cidofovir (CDV). Both macrocycles show IC_{50}'s of 4–9 ng/mL against this virus and good selectivity indices.

Furthermore, macrocycle **2** was successfully crystallized and the X-ray structure (Fig. 3) indicated the presence of two conformations similar to what was predicted by modeling.

In summary, we have designed novel macrocyclic naphthyridines which are believed to adopt the bioactive conformation of the previously described acyclic napthyridines. Subsequent X-ray crystallographic studies and the 2–3 log enhancement in potency provide evidence that our hypothesis is correct. Since both the

Table 1. Anti-cytomegaloviral activity of acyclic and macrocyclic naphthyridine analogs against HCMV laboratory strains (Davis, Ad169), clinical isolate (P8), and GCV resistant clinical isolates (C8704, D16)

Compound	Anti-viral activity: IC_{50}[a] (μg/ml)					Cytotoxicity (μg/ml)
	Davis[b]	Ad169[c]	P8[c]	C8704 (UL97 mutation)[c]	D16 (UL54 mutation)[c]	
1		0.09	0.13	0.11	0.1	2.4[d]
2	<0.0015	0.0006	0.0052	0.00024	0.0056	1.8[d]
3	0.003	ND	ND	ND	ND	0.2[e]
GCV	2.7	2.2	1.2	8.7	7.8	>1000[d]

[a] $IC_{50,}$ represents the compound concentration required to reduce virus plaque formation by 50% when compared to control samples without compound.
[b] Numbers represent mean of duplicate values (SD < 15%), all experiments were performed at least twice; HEL cell line was used for the anti-viral assay.
[c] Numbers represent mean of duplicate values (SD < 15%); MRC-5 cell line was used for the anti-viral assay.
[d] Number represents CC_{50}, which represents the compound concentration required to reduce neutral red uptake by 50% when compared to control samples without compound, mean of duplicate values (SD < 15%), MRC-5 cell line was used for the cytotoxicity assay.
[e] Number represents MTC value, which is defined as the minimum concentration of compound required to induce microscopically detectable alteration of cell morphology, mean of duplicate values (SD < 15%), HEL cell line was used for the cytotoxicity assay.

Table 2. Anti-herpes simplex activity (IC_{50}) and cytotoxicity (MTC) of macrocycles **2** and **3**

Compound	Anti-viral activity: IC_{50}[a] (μg/ml)	Anti-viral activity: IC_{50}[a] (μg/ml)	Cytotoxicity[b] (μg/ml)
	HSV-1 (KOS)	HSV-2 (G)	MTC
2	0.391	0.009	6
3	0.098	0.004	25
CDV	3.937	1.276	>100
GCV	1.274	1.220	>100

[a] IC_{50}, represents the compound concentration required to reduce virus cytopathic effect by 50% when compared to control samples without compound, number represents mean of duplicate values (SD < 15%), all experiments were performed at least twice. Vero cell line was used for the anti-viral assay.
[b] MTC is defined as the minimum concentration of compound required to induce microscopically detectable alteration of cell morphology, mean of triplicate values (SD < 15%). Vero cell line was used for the cytotoxicity assay.

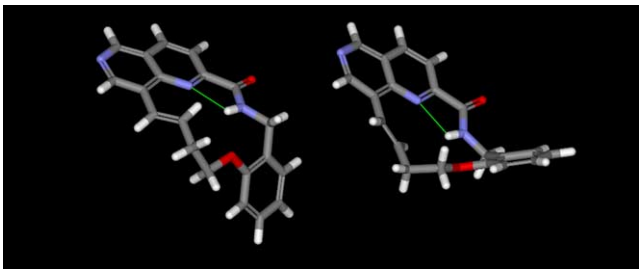


Figure 3. Crystal structure of macrocycle **2**.

14- and 15-membered macrocycles were active, it remains to be seen whether varying the nature (length, rigidity) of the linker between the alkoxy and the 8-position of the naphthyridine will provide any further enhancement in potency. The mechanism of action of these compounds is believed to involve in part the inhibition of HCMV immediate-early antigen expression; these studies as well as further SAR evaluation will be reported in due course.

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