Discovery of 1,6-Naphthyridines as a Novel Class of Potent and Selective Human Cytomegalovirus Inhibitors

Laval Chan,* Haolun Jin,* Tomislav Stefanac, Jean-François Lavallée, Guy Falardeau, Wei Wang, Jean Bédard, Suzanne May, and Leonard Yuen

BioChem Pharma Inc., 275 Armand-Frappier Boulevard, Laval, Québec, Canada H7V 4A7

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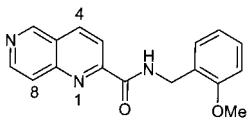
Introduction. Human cytomegalovirus (HCMV) is a species-specific DNA virus belonging to the herpesviridae family. Although HCMV infection is prevalent in the majority of the adult population, manifestation of disease is rare in immunocompetent individuals. However, in immunocompromised persons such as AIDS patients and organ transplant recipients, infection can result in retinitis, pneumonitis, or other afflictions.[1] The current anti-HCMV agents on the market (foscarnet, ganciclovir (GCV), cidofovir (CDV), and formivirsen) suffer from various toxicities and poor oral bioavailability.[2] Since management of HCMV disease often requires prophylactic treatment or maintenance therapy, adverse effects due to drug are a concern in these cases. Furthermore, the emergence of strains resistant to the currently used therapies has been observed.[3] There is therefore a need for novel antiviral agents with a more favorable safety profile. As part of our ongoing interest in the area of antiviral research, we have screened numerous compounds, both from in-house collections and from external sources, against a variety of viruses. This led to the identification of 1,6-naphthyridine **1** as



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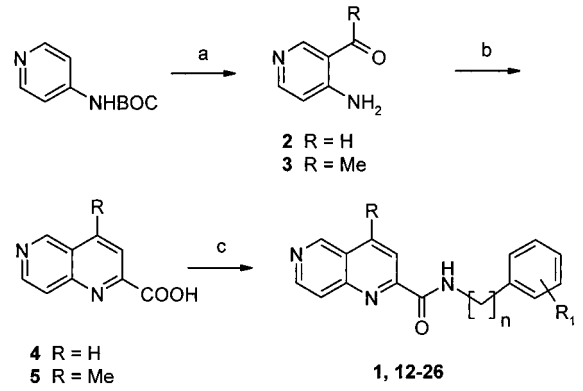
an inhibitor of HCMV with a potency and selectivity similar to that of GCV (Table 1). In this Communication, the design and synthesis of analogues of **1**, as well as the results of preliminary structure−activity relationship (SAR) studies, will be described.

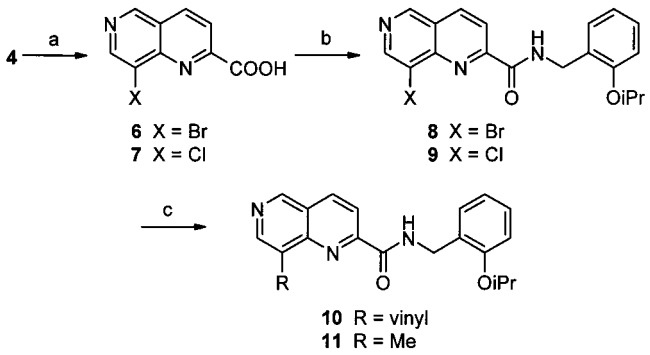
Chemistry. Our medicinal chemistry effort was directed toward the optimization of the amine portion and studying the effect of substituents on the naphthyridine moiety. Although **1** was acquired from a commercial source,[4] to the best of our knowledge, no synthesis of the corresponding carboxylic acid **4**[5] has been reported. However, we found that condensation of 4-aminonicotinaldehyde[6] (**2**) with sodium pyruvate under basic conditions provided the desired acid **4** in excellent yield (Scheme 1). This reaction was also extended to the corresponding methyl ketone **3** which was prepared from reaction of methyllithium with the Weinreb amide of 4-(*tert*-butoxycarbonyl)aminonicotinic acid; 4-methyl-substituted naphthyridine **5** was thus obtained in good yield. Formation of the amide bond was

Scheme 1. Synthesis of 1,6-Naphthyridine Carboxamides[a]



[a] Reagents and conditions: (a) **2** (R = H) (i) *t*BuLi, THF, −78 °C, DMF, 65%; (ii) TFA/CH_2Cl_2 (1:1), 90%; **3** (R = Me) (i) *t*BuLi, CO_2, THF, −78 °C, 54%; (ii) HN(OMe)Me·HCl, EDC, HOBT, DMAP, NEt_3, CH_2Cl_2 (78%); (iii) MeLi, THF, −78 °C → rt, 100%; (iv) TFA/CH_2Cl_2 (1:1), 95%; (b) sodium pyruvate, NaOH, water, 90%; (c) amine, EDC, HOBt, DMF.

Scheme 2. Synthesis of 8-Substituted 1,6-Naphthyridines[a]



[a] Reagents and conditions: (a) **7** (X = Br) Br_2, AcOH, 59%; **8** (X = Cl) Cl_2, AcOH, 40%; (b) 2-isopropoxybenzylamine, EDC, HOBT, DMF (Br, 69%; Cl, 40%); (c) (from **8**) **11** (R = Me) Me_4Sn, $(Ph_3P)_2PdCl_2$, DMF, 49%; **12** (R = vinyl) vinyltributyltin, $(Ph_3P)_2$-$PdCl_2$, DMF, 34%.

then readily achieved by reaction of the carboxylic acid with a variety of amines[7] using EDC/HOBt as coupling agent.

Reaction of elemental bromine or chlorine with carboxylic acid **4** resulted in the 8-halonaphthyridines **6**[8] (Br) and **7** (Cl) which were then coupled with 2-isopropoxybenzylamine giving amides **8** and **9** (Scheme 2). The formation of the bromo derivative **6** was straightforward, and a yield of 59% was obtained; however, preparation of the chloro derivative required careful monitoring of the amount of chlorine gas used since polychlorination was often observed. The 8-bromo derivative also provided us with a useful entry for further functionalization at this position; for example, Stille[9] coupling with various stannanes resulted in the introduction of methyl (**10**) and vinyl (**11**) moieties.

Results and Discussion. The compounds thus prepared were then screened for anti-HCMV activity in the Hs68 cell line, and cytotoxicity was also determined in the same cell line; the results obtained are shown in

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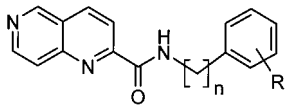
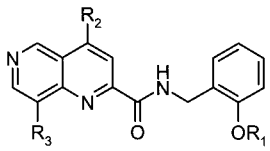
Table 1. Anti-HCMV Activity and Cytotoxicity of 1,6-Naphthyridines: Modification of Amine



compd	*n*	R	IC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
1	1	2′-OMe	0.3	12.5
12	1	3′-OMe	2.1	100
13	1	4′-OMe	>50	50
14	1	2′-Br	0.7	100
15	1	3′-Br	2.2	50
16	1	4′-Br	>10	10
17	1	2′-O*i*Pr	0.005	2.5
18	1	2′-O*t*Bu	0.04	4.4
19	1	2′-O(*R*)*s*Bu	0.003	0.1
20	1	2′-O(*S*)*s*Bu	0.03	3.1
21	1	3′-*i*OPr	3.6	8.4
22	1	H	1	71
23	0	H	50	50
24	2	H	0.78	5
25	2	2′-OMe	6	17
ganciclovir			0.3	12.5

[a] Mean of duplicate values (SD < 15%); all experiments were performed at least twice. [b] Mean of triplicate values (SD < 15%).

Table 2. Anti-HCMV Activity and Cytotoxicity of 4- and 8-Substituted 1,6-Naphthyridines



compd	R_1	R_2	R_3	IC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
26	OMe	Me	H	11	8
8	O*i*Pr	H	Br	0.15	9
9	O*i*Pr	H	Cl	0.03	6
10	O*i*Pr	H	Vinyl	0.003	17
11	O*i*Pr	H	Me	0.0004	3
ganciclovir				0.3	12.5

[a] Mean of duplicate values (SD < 15%); all experiments were performed at least twice. [b] Mean of triplicate values (SD < 15%).

Tables 1 and 2.[10] Table 1 summarizes the optimization study of the amine portion of the molecule; it is apparent that a 2-substituted benzylamine confers the best potency to this class of compound. There is a distinct preference in the methoxy, bromo, and isopropoxy cases (compounds **1**, **14**, **17**) for ortho substitution. While the meta analogues (**12**, **15**, **21**) retain some activity, the para isomers (**13**, **16**) in the methoxy and bromo cases are devoid of anti-HCMV activity. In addition, there is a preference for a bulkier alkoxy group at the ortho position, the isopropoxy analogue **17** is about 100-fold more potent than methoxy analogue **1** with an IC_{50} of 5 ng/mL (IC_{90} of 0.06 μg/mL) and a selectivity index of 500. Further increasing the size to a *sec*-butoxy group did not result in any appreciable improvement. The *R* enantiomer **19** which is about 10-fold more active than the *S* enantiomer **20** is almost equipotent to the isopropoxy **17** but has a lower selectivity index. The *tert*-butoxy derivative **18** is 10-fold less active than the isopropoxy analogue **17**, indicating a preference for a secondary alkoxy group. The 3-methoxy analogue **12** had a better selectivity index than the 2-methoxy **1**, but potency could not be increased by changing to the bulkier 3-isopropoxy **21**. Finally, the distance between the phenyl ring and the amide nitrogen is also important for activity; while the benzyl analogue **22** is moderately active, anilide **23** is devoid of any activity, whereas a two-carbon linker as in phenethyl **25** provides moderate activity which cannot be improved by introduction of a 2-methoxy moiety (**25**).

Introduction of a methyl group at the 4-position (compound **26**) resulted in weak activity and a poor selectivity index, whereas substitution at the 8-position was in general beneficial (Table 2). Both halo derivatives **8** (Br) and **9** (Cl) show activity with the chloro analogue being more potent and more selective. Introduction of a carbon substituent had a dramatic effect on potency; both the methyl and vinyl analogues were extremely potent and selective. The vinyl derivative **10** had an IC_{50} of 3 ng/mL (IC_{90} of 0.25 μg/mL) and a selectivity index of more than 5000, whereas the methyl analogue **11** was even more potent with an IC_{50} of 0.4 ng/mL (IC_{90} of 0.09 μg/mL) and a selectivity index of greater than 7000. Preliminary experiments have shown that this novel class of HCMV inhibitors is not cross-resistant to GCV and CDV; HCMV strains resistant to GCV (C8704 and C8805-37, UL97 phosphotransferase mutants and D16, UL54 DNA polymerase mutant)[11] as well as to CDV (1117r3-1-2, genotype unknown)[11] are still sensitive to **17**. In addition, since the naphthyridines and these nucleoside analogues do not share any common structural features, the mode of action of this class of inhibitors is presumed to be different.

In summary, based on a lead from random screening, we have identified and synthesized a novel class of HCMV inhibitors.[12] We have defined the structural requirements for potency and selectivity; our SAR investigation suggests that an isopropoxy group at the ortho position and substitution at C-8 are highly desirable. Some of the compounds described, such as **10**, **11**, and **17**, exhibit high potency and have an excellent selectivity index. Further SAR studies as well as mechanistic and pharmacokinetic studies on this class of compounds are ongoing and will be reported in due course.

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(5) Carboxylic acid **4** is also available from Peakdale Fine Chemicals; however, in-house preparation was more economical.

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(7) The alkoxybenzylamines that are not commercially available were prepared by alkylation (NaH, RX or ROH, DEAD, PPh_3) of hydroxybenzonitrile followed by reduction (H_2, Pd/C) to the benzylamine.

(8) The regioselectivity of the 8-bromo derivative **8** was deduced by comparison of coupling constants with those of related compounds. Further confirmation was obtained from an X-ray crystal structure of **11**.

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(10) **HCMV Plaque Reduction Assay: Determination of IC_{50}.** Human fibroblast Hs68 cells were infected by HCMV strain AD169 at a MOI of 0.001 in a 12-well tissue culture dish with and without compounds and incubated for 7 days. After necessary process, the monolayers were examined for the presence of plaques under microscope. The percentage of plaque reduction caused by compounds in wells was calculated by comparison with controls containing no compound. The concentration required to cause 50% plaque reduction was expressed as IC_{50}. **Cellular Proliferation Inhibitory Assay: Determination of CC_{50}.** Hs68 fibroblasts were plated in a 96-well dish at a density of 1000 cells/well and incubated overnight. The supernatant medium was removed and replaced with compounds. Six serial 2-fold dilutions (3.2−100 μg/mL) were tested in triplicate. After incubation for 72 h, [^{3}H]thymidine uptake experiment was conducted, and counts were measured using the liquid scintillation counter Microbeta 1450 Wallac. The percentage of cell proliferation as compared to the control without test compound was obtained, and the concentration of test compound required for 50% inhibition was established as CC_{50}.

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