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Design and Synthesis of New Potent Human Cytomegalovirus (HCMV) Inhibitors Based on Internally Hydrogen-Bonded 1,6-Naphthyridines

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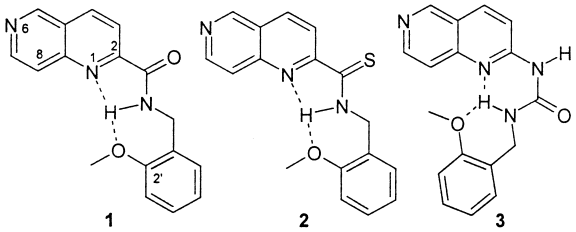
Abstract—1,6-Naphthyridine-2-carboxylic acid benzylamides are potent anti-HCMV compounds. Replacement of the amide moiety by other groups containing internal hydrogen bonds was undertaken to extend the SAR. Our results indicated that the urea derivatives showed very good activity. © 2001 Elsevier Science Ltd. All rights reserved.

HCMV is a member of the *Herpesviridae* family infecting more than 80% of the population.[1] Most infections are asymptomatic but severe manifestation of HCMV (retinitis and pneumonitis) can be seen in individuals whose immune system has been weakened either by a disease such as AIDS or by immunosuppressive therapy following organ transplant.[1] Current therapies are associated with undesirable pharmacological properties such as poor bioavailability and various toxicities, for example, nephrotoxicity (Foscarnet and HPMPC)[2] and myelotoxicity (ganciclovir).[2] There is a clear, unmet medicinal need for a convenient therapy for HCMV.

As part of our ongoing search for new anti-HCMV compounds we have discovered the 1,6-naphthyridines, a novel class of inhibitors in which the lead compound **1** has comparable activity to ganciclovir.[3] The resulting structure–activity relationship (SAR) study showed that the optimal positions for the nitrogens on the naphthyridine ring were 1 and 6, substitution at C8 and a bulky alkoxy group at the 2′ position were beneficial for activity. We recently reported[4] that the internal hydrogen bonds between the NH of the amide and the heteroatoms N1 and O2′ **1** help to maintain the molecule in an active conformation. The presence of internal hydrogen bonds was confirmed by ^{1}H NMR, molecular modeling studies and X-ray crystallography.



1 **2** **3**

IC_{50} = 0.3 μg/mL
CC_{50} = 12.5 μg/mL

In this communication, the design and synthesis of new naphthyridines where the amide moiety is replaced by other groups capable of maintaining internal hydrogen bonds will be presented. Thioamides **2** and ureas **3** were considered since they can provide some insights about the active conformation of the napthyridines.

Thioamides were selected because of their structural similarity to the amide bond and their potential gain of in vivo[5] stability. These compounds were prepared by reacting the corresponding amide (e.g., **1**) with Lawesson's[6] reagent. The anti-HCMV activity and cytotoxicity of these compounds (Table 1) were determined by plaque reduction assay and inhibition of cell proliferation,[3] respectively.

In the thioamide series, the methoxybenzylamine analogue (entry 1) is about 3 times less active than its amide homologue **1**. However, only a slight improvement of activity was observed when increasing the bulk of the alkoxy to the 2′-ethoxybenzylamine (entry 2), or to the 2′-isopropoxybenzylamine (entry 3). In the corresponding

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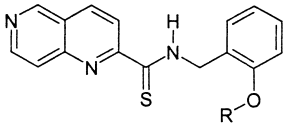
amide series, the latter modification enhances the activity by 2 log[3] indicating that replacement of the carbonyl oxygen by sulfur results in the loss of some desirable interaction at this position. This class of compounds was therefore not further pursued.

Our attention was then turned to the urea derivatives that can still accommodate the two internal hydrogen bonds as shown in **3**. Since the benzylureas are one atom longer than the corresponding benzylamides, both benzyl and phenylureas were considered even though the phenylamides were inactive in the previous series.[3] With the knowledge of our previous SAR,[3] that substitution on the naphthyridine scaffold can enhance potency, substitution at C3 and C8 were also studied. The ureas were prepared by reacting 1,6-naphthyridine-2-ylamine[7] with the appropriate isocyanate in refluxing dichloroethane. The C8 bromo analogue **5** was synthesized from the carboxylic acid[3] **4** using a Curtius rearrangement followed by a standard coupling with isocyanate (Scheme 1).

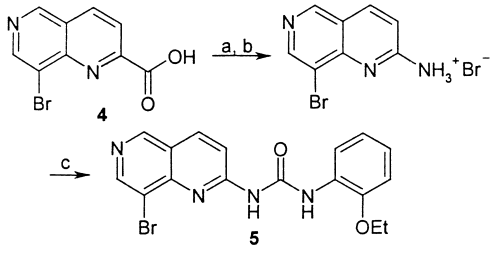
The C3 analogues were easily accessible from established methods.[7] Since the cyano group at position 3 deactivates the amino group in position 2, deprotonation of the amino group using KHMDS prior to condensation with the isocyanate was necessary to give the compound in entry 9, Table 2 (Scheme 2).

Table 1. Antiviral activity (IC_{50}) and cytotoxicity (CC_{50}) of thioamides in Hs68 cell line

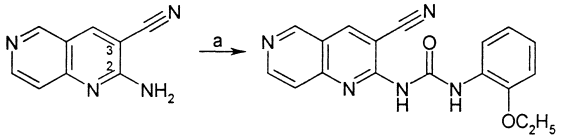


Entry	R	IC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[b]
1	OCH_3	0.93	8.1
2	OC_2H_5	0.1	4.6
3	O*i*Pr	0.16	11.1
Compound 1		0.3	12.5

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



Scheme 1. (a) *t*-BuOH, Et_3N, diphenylphosphoryl azide, reflux, 3 h, 35%; (b) HBr/CH_3CO_2H, $CHCl_3$, 2 h, rt, 97%; (c) isocyanate, dichloroethane, reflux, 1 h 40–60%.



Scheme 2. (a) KHMDS, THF, 0 °C, isocyanate, 3 h, 59%.

Results and Discussion

Our results are summarized in Table 2. In the benzylurea series, the unsubstituted analogue (entry 1) is not selective. The 2′-methoxy (entry 2) showed activity with an IC_{50} of 1 μg/mL and a selectivity index (SI) of 10. By increasing the bulk of the substituent to an isopropoxy (entry 3), the activity was enhanced to 10 ng/mL and the SI reached 1000. Similar results were also obtained in the case of the phenylurea. The unsubstituted phenylurea analogue (entry 4) was also inactive. However, introduction of an alkoxy group resulted in active compounds (entries 5–7) and, as with the corresponding amides, activity was enhanced with the increase in bulk of the alkoxy group. The 2′-isopropoxy derivative was the most active and selective of the series (entry 7) with an IC_{50} of 30 ng/mL and an SI of 1000. Substitution on the 1,6-naphthyridine scaffold indicated that incorporation of a cyano or a methyl substituent at the C3 position (entries 8 and 9) abolished the activity. Surprisingly, the presence of a bromine on C8 (entry 10) did not improve the potency in the urea series.

The observed SAR of the 2′-alkoxy substituent, isopropoxy > ethoxy > methoxy, is similar to that described previously in the benzylamide series,[3] suggesting that the two series, ureas and amides, are maintained in a similar active conformation by internal hydrogen bonds. The two examples lacking the 2′-alkoxy (entries 1 and 4), have only one internal hydrogen bond and therefore more degrees of freedoms are present around the amide bond. They showed only toxicity or no activity; these results are in line with our findings.

In conclusion, based on our previous findings that internal hydrogen bonds are an important feature in the 1,6-naphthyridine series for activity against HCMV, we have designed new potent HCMV inhibitors, the 1,6-

Table 2. Antiviral activity (IC_{50}) and cytotoxicity (CC_{50}) of various 1,6-naphthyridine-2-yl-ureas on Hs68 cell line

Entry	*n*	R^1	R^2	R^3	IC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[b]
1	1	H	H	H	0.29	0.37
2	1	OCH_3	H	H	1	13
3	**1**	**O*i*Pr**	**H**	**H**	**0.01**	**18.9**
4	0	H	H	H	>25	>100
5	0	OCH_3	H	H	1.2	33.9
6	0	OC_2H_5	H	H	0.14	1.7
7	**0**	**O*i*Pr**	**H**	**H**	**0.03**	**30.2**
8	0	OC_2H_5	CH_3	H	>25	>100
9	0	OC_2H_5	CN	H	>25	<25
10	0	OC_2H_5	H	Br	0.46	9.3

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

naphthyridinyl-ureas, showing good activity throughout a range of substitutions with the 2′-isopropoxy analogues showing antiviral activity in the low ng/mL range. Further work to determine the mode of action of this novel class of HCMV agents and to improve the potency is ongoing.

Acknowledgements

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