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ISOQUINOLINE-6-CARBOXAMIDES AS POTENT AND SELECTIVE ANTI-HUMAN CYTOMEGALOVIRUS (HCMV) INHIBITORS

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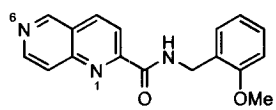
Abstract: Structure–activity relationship studies on our newly identified anti-HCMV compounds, the 1,6-naphthyridines led to the identification of isoquinoline-6-carboxamides as potent and selective anti-HCMV agents.

We have recently described the identification of a class of potent anti-HCMV compounds, the 1,6-naphthyridines **1**.[1] Structure–activity relationship studies on this class of compounds have defined the structural requirements for potency and selectivity. On the benzylamine side of the molecule, a number of modifications can be tolerated, however, the 2'-alkoxy benzylamides seem to be the most promising in terms of potency and selectivity. Substitution at C-8 of the naphthyridine ring enhances potency whereas introduction of a methyl at C-4 did not result in any improvement. In this communication, we will describe the synthesis of various naphthyridines as well as other related classes of compounds in order to assess the importance of the nitrogen atoms in the naphthyridine ring as well as the importance of their relative positions in the molecule.



1

IC_{50} 0.3 μg/mL
CC_{50} 12.5 μg/mL

Chemistry

The amides depicted in Table 1 were all prepared by reaction of the corresponding acid with 2-methoxybenzylamine in the presence of EDCI and HOBt in DMF. Quinaldic acid **2** and [1,8]naphthyridine-2-carboxylic acid **3** were both acquired from commercial sources.[2] The synthesis of the other acids is summarized in Schemes 1–4. Carboxylic acids **7**, **8**, and **9** were prepared by conversion of known 4-naphthyridones **4**,[3a] **5**,[3b] and **6**[3c] to the corresponding 4-chloronaphthyridines followed by reduction using phase transfer hydrogenation under homogeneous catalysis[4] and finally base hydrolysis and acidification yielded the desired acids (Scheme 1). Similarly, 2,7-naphthyridine-3-carboxylic acid **11** was prepared from the naphthyridone **10**[5]

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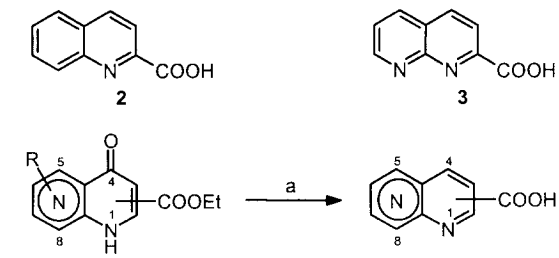
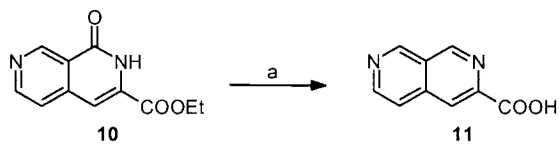
according to Scheme 2. 1,7-naphthyridine-3-carboxylic acid **13** was prepared from condensation of pyridine carboxaldehyde **12**[6] with ethyl 3-ethoxyacrylate followed by hydrolysis (Scheme 3). The isoquinoline derivative **15** was prepared by palladium catalyzed CO insertion of isoquinoline **14**[7] (Scheme 4).



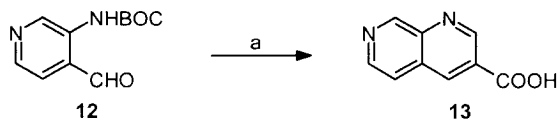
4 N-6, R = H, 3-COOEt
5 N-5, R = 6-Cl, 2-COOEt
6 N-7, R = 8-Cl, 2-COOEt

7 N-6, 3-COOH
8 N-5, 2-COOH
9 N-7, 2-COOH

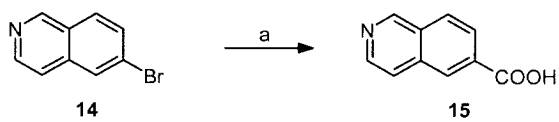
Scheme 1. *Conditions and reactions:* (a) (i) $SOCl_2$, reflux, (~100%); (ii) $Pd(PPh_3)_4$, sodium formate, DMSO, 100 °C (34-81%); (iii) LiOH, THF/H_2O then HCl (65–87%).



Scheme 2. *Conditions and reactions:* (a) (i) $POBr_3$, (65%); (ii) H_2, 50 psi, $PdCl_2$, NaOAc, MeOH, (42%); (iii) LiOH, THF/H_2O then HCl (78%).

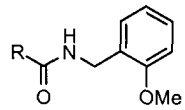


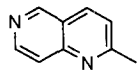
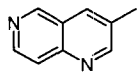
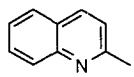
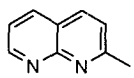
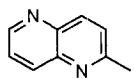
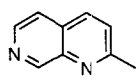
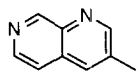
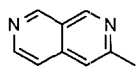
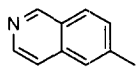
Scheme 3. *Conditions and reactions:* (a) (i) Ethyl 3-ethoxyacrylate, TFA, CH_2Cl_2; (ii) LiOH, THF/H_2O then HCl (86%, 2 steps).



Scheme 4. *Conditions and reactions:* (a) (i) $Pd(OAc)_2$, CO, DMF/MeOH, 100 °C, (61%); (ii) LiOH, THF/H_2O then HCl (81%).

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



Entry	R	IC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
1		0.3	12.5
2		>25	>25
3		>25	10
4		>25	>25
5		>25	25
6		>25	25
7		>25	>100
8		>25	<25
9		0.9	43
10		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%)

Results and Discussion

The anti-HCMV activity and cytotoxicity of the compounds were determined by plaque reduction assay and inhibition of cell proliferation,[8] respectively, and the results are summarized in Table 1. Only the isoquinoline derivative (entry 9) showed anti-HCMV activity; the potency and selectivity was comparable to that of our lead compound **1.** Moving the carboxamide moiety from the 2 to 3 position (entry 2) of the 1,6-naphthyridine abolishes activity indicating that position 2 is optimal. Compounds lacking a nitrogen atom at the 6 position do not show any activity (entries 3–6) whereas in the right hand ring, a nitrogen atom can only be tolerated at position 1 (entries 7 and 8). However, a nitrogen atom at this position is not a requisite for anti-HCMV activity as evidenced by the isoquinoline derivative (entry 9). Introduction of a larger alkoxy group on the ortho position of the benzylamide did not result in a dramatic increase in anti-HCMV activity as was the case in the 1,6-naphthyridine derivatives;[1] the corresponding isopropoxy derivative had an IC_{50} of 0.5 μg/mL.

From the present SAR studies, a novel class of potent HCMV inhibitors with a good selectivity index have been identified. Further studies aimed at determining other factors that are important for antiviral activity as well as other issues such as oral bioavailability will be described in the near future.

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References and Notes

1. Jin, H.; Chan, L.; Stefanac, T.; Lavallée, J. F.; Falardeau, G.; Wang, W.; Bédard, J.; May, S.; Yuen, L. *J. Med. Chem.* **1999**, *42*, (16), in press.
2. Quinaldic acid **2** and 1,8-naphthyridine **3** were purchased from Aldrich Chemical Company and Peakdale Fine Chemicals (UK) respectively.
3. (a) Hauser, C. R.; Reynolds, G. A. *J. Org. Chem.* **1950**, *15*, 1224. (b) Pomorski, J. *Roz. Chem.* **1974**, *48*, 321. (c) Compound **6** was prepared according the procedure for **5** but starting from 3-amino 2-chloropyridine.
4. Pri-Bar, I.; Buchman, O. *J. Org. Chem.* **1986**, *51*, 734.
5. Sheffield, D. J.; Woolridge, R. H. *J. Chem. Soc. Perkin 1* **1972**, 2506.
6. Venuti, M. C.; Stephenson, R. A.; Alvarez, R.; Bruno, J. J.; Strosberg, A. M. *J. Med. Chem.* **1988**, *31*, 2136.
7. Tyson, F. T. *J. Am. Chem. Soc.* **1939**, *61*, 183.
8. **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% of 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 per well and incubated overnight. The supernatant medium was removed and replaced with compounds. Six serial twofold dilution (3.2 to 100 μg/mL) were tested in triplicate. After incubation for 72 hours, ^{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% of inhibition was established as CC_{50}.