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Design and Evaluation of Dihydroisoquinolines as Potent and Orally Bioavailable Human Cytomegalovirus Inhibitors

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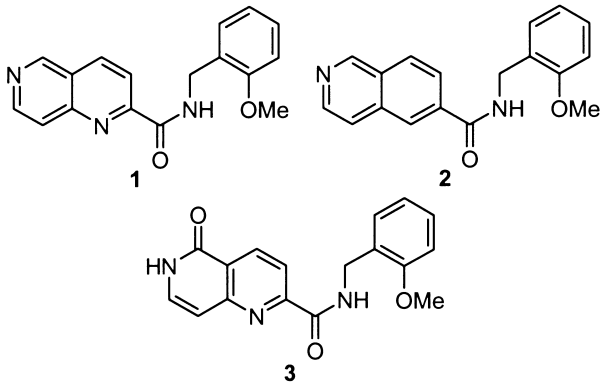
Abstract—Following the identification of first pass metabolism issues with our recently described anti-HCMV compounds, the naphthyridines and isoquinolines, we have designed a class of novel metabolically stable and orally bioavailable anti-HCMV agents, the dihydroisoquinolines.

Infections by HCMV can have debilitating effects particularly in individuals whose immune system has been weakened either by disease such as AIDS or by immunosuppressive therapy following organ transplant. These individuals are very often afflicted with a variety of problems such as encephalopathy and retinitis. Although current therapies can be effective, they are all associated with various toxicities such as nephrotoxicity (foscarnet and cidofovir, CDV) and myelotoxicity (ganciclovir, GCV). In addition, administration of these drugs require long intravenous iv infusion times; the negative impact of these therapies on quality of life of HCMV patients has been recognized as a problem.[1] There has also been growing evidence, albeit circumstantial, that HCMV plays an important role in the process of atherogenesis.[2] There is therefore a clear medical need for HCMV drugs with a more desirable profile.

Our recent effort in this area led to the identification of two novel classes of potent and selective anti-HCMV agents,[3] the 1,6-naphthyridines **1** and the 6-isoquinolines **2**. We were also encouraged by the fact that both **1** and **2** lacked features that are associated with poor oral bioavailability such as a guanine (GCV) or a phosphonic acid (CDV and foscarnet) moiety; thus both **1** and **2** were likely candidates in the identification of oral HCMV drugs. However, during preliminary pharmacological evaluation of **1** and **2**, it was found that these compounds were not stable in mouse or monkey S9 liver preparations indicating that these compounds are likely to be subject to first pass metabolism.[4] In vivo studies in mice confirmed our hypothesis; no parent compound was detected 20 min after **1** was given orally. The same primary metabolite was detected in vivo and in the S9 incubation experiment. Following a preparative scale incubation with mouse S9 homogenate, this metabolite was isolated, characterized (NMR and MS) and was assigned the 1,6-naphthyrid-5-one **3** structure. Unfortunately, **3** was devoid of any anti-HCMV activity thus indicating that neither **1**, or **2**, or their metabolites can serve as orally bioavailable anti-HCMV agents.



In this communication, we will describe our efforts to circumvent first pass metabolism issues while attempting to retain antiviral activity. Our initial approach was to block metabolism by installing a substituent at the site of oxidation; the synthesis of these compounds is depicted in Scheme 1. MCPBA oxidation of **1** gave *N*-oxide **4** in good yields and reaction with phosphorus oxychloride[5] or with triphenylphosphonium bromide gave the chloride **5**, or bromide **7**, respectively. Reaction of chloro derivative

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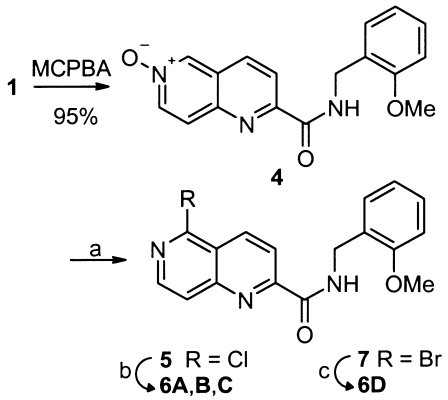


Scheme 1. Synthesis of 5-substituted naphthyridines. (a) **5** (R = Cl) $POCl_3$, rt, 66%; **7** (R = Br) PPh_3, Br_2, NEt_3, CH_2Cl_2, 31%; (b) **6A** (R = F) KF, sulfolane, 230 °C, 43%; **6B** (R = NH_2); (i) NaN_3, DMF, 80 °C, 100%; (ii) PPh_3, xylene, 150 °C then HCl, MeOH, 83%; **6C** (R = Me) Me_4Sn, $Pd(PPh_3)_4$, LiCl, DMF, 20%; (c) **6D** (R = OMe) NaOMe, THF, 75%.

5 with potassium fluoride[6] at 230 °C yielded fluoro compound **6A** in 43% yield. Similarly, displacement of chloride with azide anion followed by the Staudinger[7] reaction gave amine **6B** in good yields. The 5-methyl analogue **6C** was obtained in low yields under Stille[8] conditions. Finally, the methoxy **6D** was obtained from bromide **7** in 75% yield. The monkey S9 stability of the methoxy **6D** and amino **6B** analogues were determined and they were found to be indeed resistant to oxidation. However, all the 5 substituted 1,6-naphthyridines **5**–**7** as well as *N*-oxide **4** were devoid of HCMV activity (Table 1) indicating that substitution cannot be tolerated at this position and this approach was therefore abandoned. Interestingly, *N*-oxide **4** was also found to be stable to monkey S9 thereby indicating **4** is not an intermediate during the process of oxidation.

The second strategy adopted was more successful; we attributed the facile oxidation of the 5-position to the presence of the bicyclic aromatic system and the nitrogen atom at position 6. The reactivity of 1,6-naphthyridines and similar systems at this position is well documented.[9] We reasoned that if the right hand ring is no longer aromatic, the reactivity of the 5-position would be substantially decreased and oxidation might be prevented. We were also aware that the bicyclic portion of the lead compounds **1** and **2** is planar and disruption of this planarity may be detrimental to antiviral activity. Both fully and partially saturated systems were therefore considered; in the latter case, at least some degree of planarity can be maintained. Such strategy precludes the 1,6-naphthyridine class of compounds, but the isoquinoline analogues are amenable to this approach as the compounds resulting from this modification are the tetrahydro or dihydroisoquinolines.

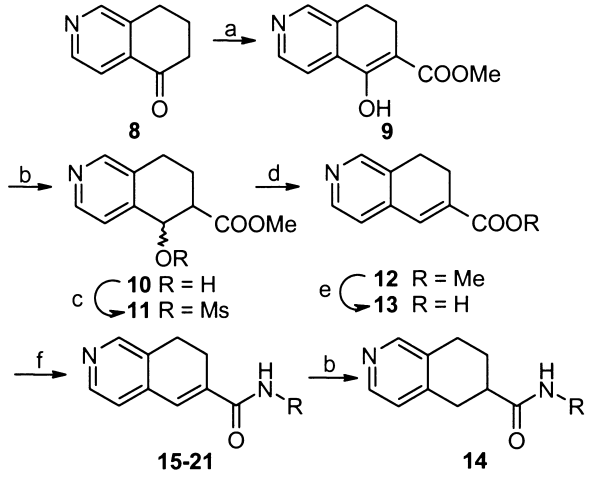
Table 1. Antiviral and cytotoxicity of 5-substituted naphthyridine

Compound	IC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
4	>25	100
5	>10	~3.2
6A	~10	~ 10
6B	~10	~12.5
6C	>10	>25
6D	>25	>50
7	>25	~ 25

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

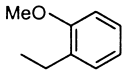
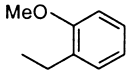
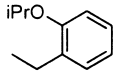
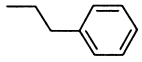
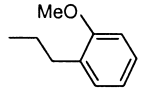
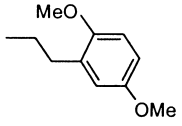
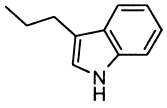
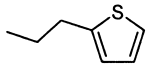
The synthesis of the desired compounds is depicted in Scheme 2. The enolate of the known isoquinolinone **8**[10] was prepared from reaction with LiHMDS and quenched with Mander's reagent[11] giving the β-keto ester as enol **9** in 52% yield. Reduction of enol **9** to β-hydroxy ester **10** was readily achieved by palladium catalyzed hydrogenation, **10** was obtained in quantitative yield as a 4:1 mixture of isomers (determined by ^1H NMR). Conversion of alcohol **10** to mesylate **11** followed by DBU catalyzed β-elimination gave the α,β-unsaturated ester **12** in 88% yield. Finally, the methyl ester was hydrolyzed with lithium hydroxide and following acidification, the desired acid **13** was obtained in 70% yield. The requisite amides were then prepared either by the mixed anhydride method (isopropyl chloroformate) or by the use of EDCI and HOBt as coupling agents. The reduction of the α,β-unsaturated amide to the corresponding 5,6,7,8-tetrahydroisoquinoline was readily achieved by palladium catalyzed hydrogenation.

The anti-HCMV activity and cytotoxicity of the compounds were determined by plaque reduction assay and inhibition of cell proliferation,[12] respectively, and the results are summarized in Table 2. The tetrahydroisoquinoline analogue **14** is devoid of anti-HCMV activity suggesting that planarity of the left-hand part is indeed a significant factor. The corresponding 7,8-dihydroisoquinoline derivative **15** shows activity comparable to parent compound. However, as with the isoquinoline class, potency cannot be enhanced by the introduction of bulkier alkoxy groups on the 2′ position of the phenyl ring. The absence of a hydrogen bond acceptor at position



Scheme 2. Synthesis of isoquinoline analogues. (a) LiHMDS, THF, −78 °C, methyl cyanoformate, 52%; (b) Pd/C, MeOH, H_2, 100%; (c) MsCl, NEt_3, CH_2Cl_2, 0 °C, 100%; (d) DBU, CH_2Cl_2, 88%; (e) LiOH, THF/water then aq HCl, 70%; (f) isopropyl chloroformate, NEt_3, RNH_2, THF, 0 °C or RNH_2, EDCI, HOBt, DMF, 70–80%.

Table 2. Antiviral activity and cytotoxicity of in Hs68 cell line

Compound	R	IC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
2	—	0.9	43
14		25	100
15		0.64	>50
16		0.68	12.5
17		0.71	25
18		10	>25
19		0.23	>50
20		0.1	5
21		0.17	15
GCV		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%).

5 is believed to account for this lack of enhancement of potency.[13] We therefore turned our attention to non-benzylic amides in an effort to obtain the right combination for higher potency. Phenethylamines and other systems with a 2 carbon linker between the amide moiety and an aryl ring were found to provide good potency and selectivity. For example, the phenethylamine **17** and the 2,5-dimethoxy phenethylamine **19** showed activity similar to that of **15** and a selectivity index of greater than 200 but surprisingly the 2-methoxy derivative **18** was not active. Other aryl systems such the tryptamine **20** and the thiophene **21** derivatives were also found to be active.

The stability of **15** and **20** towards monkey and mouse S9 homogenate was then determined. Both compounds were found to be stable to these liver preparations, almost no metabolites were detected under these conditions. Preliminary in vivo experiments also indicated that these compounds were stable and well absorbed when administered po in mice (Table 3). In contrast, under similar conditions, 1,6-naphthyridine **1** was virtually undetectable after oral dosing. The oral bioavailability in mice (at a dose of 50 mg/kg) of compound **20** was determined to be 60.4%.[14]

Table 3. Pharmacokinetic studies in mice[14,a]

Dose (mg/Kg)	Route	C_{max} (μg/mL)	$AUC_{(0-\infty)}$ (μg/mL min)	$t_{1/2}$ (min)	BA (%)
Compd **15**					
50	po	12.47	734	42.3	
5	iv	6.94	57	14.2	
Compd **20**					
5	po	0.60	21	15.2	22.5
50	po	15.1	1762	72.5	60.4
5	iv	5.56	94	14.2	
50	iv	57.8	2914	43.9	

[a]Mean of triplicate data (SD < 8%).

In conclusion, it was found that the 7,8-dihydroisoquinoline class of compounds offer a metabolically stable and orally bioavailable alternative to the 1,6-napthyridines and isoquinolines. The favorable pharmacokinetic profile of this class of compounds will allow us to evaluate these compounds as potential oral anti-HCMV agents. Further work to determine the mode of action of this novel class of HCMV agents and to improve potency is ongoing.

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References and Notes

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14. Pharmacokinetic parameters were generated by WinNonLin (Scientific Consulting Inc. Apex, N.C.) using mean plasma concentrations of triplicate data at each time point. The area under the concentration-time curve (AUC) was estimated by the linear trapezoidal method from time zero to the last measured sample time with extrapolation to infinity by using the terminal slope (λz) generated by WinNonlin non-compartmental model. The oral bioavailability (BA%) was calculated from $AUC_{po,0\text{-}\infty}/AUC_{iv,0\text{-}\infty}$at the corresponding dose. Half-life ($t_{1/2}$) was calculated from $0.693/\lambda z$. Chen, Y.; Mongrain, I.; Richard, A.; Chan, L.; Winocour, P.; Hu, Z. *Antiviral Chem. Chemother*. submitted.