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Modifications of C-2 on the pyrroloquinoline template aimed at the development of potent herpesvirus antivirals with improved aqueous solubility

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ABSTRACT

A series of C-2 pyrroloquinoline analogs designed to improve aqueous solubility were examined for herpesvirus polymerase and antiviral activity. Several analogs were identified that maintained the antiviral activity of the previous development candidate against HCMV, HSV-1 and VZV, but with significantly improved aqueous solubility.

The Herpesvirus family contains eight known viruses that infect humans.[1] These members of *Herpesviridae* are the herpes simplex viruses (HSV-1 and HSV-2), varicella zoster virus (VZV), Epstein-Barr Virus (EBV), human cytomegalovirus (HCMV), and human herpes viruses (HHV-6, HHV-7 and HHV-8). Infections by members of this family are responsible for a wide range of diseases. Although troublesome, infections in immunocompetent patients are typically minor and may involve periodic reactivation of a latent state. However, for patients with suppressed immune systems as a result of chemotherapy, HIV infection or old-age, serious morbidity and mortality can occur.[2]

Safe and effective treatments have been developed for infections resulting from some members of the *Herpesviridae* family, for example treatment of HSV-2 with acyclovir.[3] The treatment of other Herpesvirus infections has not proven as successful. For HCMV, agents such as ganciclovir are associated with a range of serious adverse effects and display only modest efficacy.[4] Even with these complications, ganciclovir is considered the gold standard compared to other less ideal agents such as forscarnet, fomivirsen and cidofovir.[1] The emergence of drug-resistant strains also poses an increasing problem for disease management.[4a] Therefore, the discovery of a new class of treatments for immunocompromised patients infected with HCMV represents a serious unmet medical need. Previously disclosed DNA polymerase inhibitors **1**,[5] **2**[6] and **3**[7] (Fig. 1) are broad-spectrum anti-herpetic compounds that show activity against ganciclovir resistant isolates.[5c] One consistent issue observed for these and related templates was poor aqueous solubility.[3,8] This Letter describes our C-2 analog effort

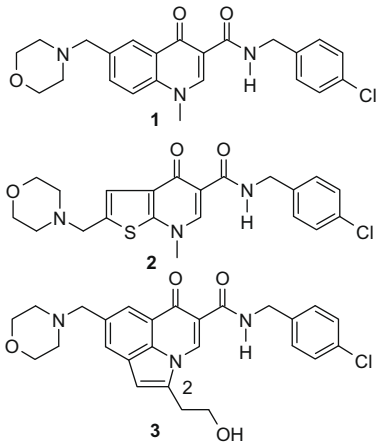

Figure 1. Previously reported polymerase inhibitors.

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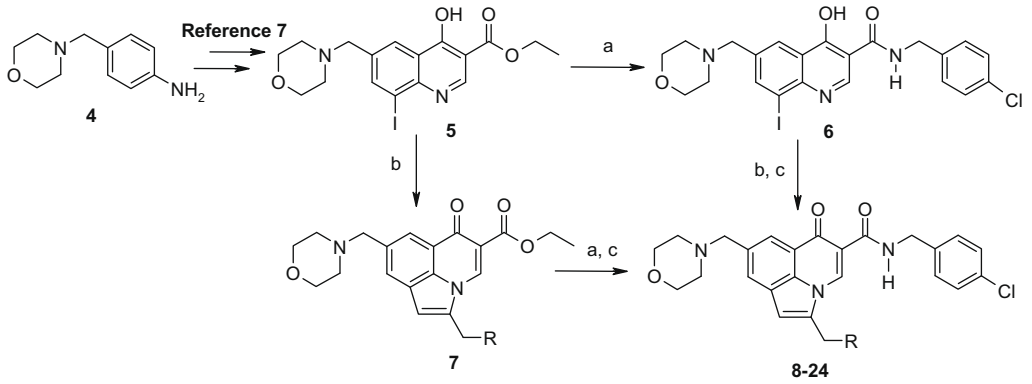


Scheme 1. Synthesis of C-2 pyrroloquinoline analogs **8**–**24**. Reagents and conditions: (a) 4-chlorobenzylamine, 135 °C, 4 h; (b) CuI, $PdCl_2(PPh_3)_2$, triethylamine, alkyne, EtOH, reflux, (c) if deprotection was required, HCl in dioxanes or methanol for Boc removal or in THF/water for ketal hydrolysis.

to alleviate the poor aqueous solubility shown by development candidate **3**, while attempting to improve, or at least retain, potent antiviral activity.

The synthesis of key intermediate **5** from **4** (Scheme 1) has previously been reported and is amenable to pilot plant synthesis.[7a] Conversion of **5** to pyrroloquinoline analogs **8**–**24** (Table 1) was accomplished by two routes. Reaction of **5** with 4-chlorobenzylamine generated compound **6**.[7,9] Sonogashira coupling of **6** with the appropriate alkyne and concomitment annulation generated the C-2 pyrroloquinoline analogs.[10] Although this approach allowed for late stage diversity, removing Pd from the target compound proved difficult in some cases.[7a] An alternate approach from **5**, that allowed an extra step for Pd removal, involved initial Sonogashira coupling/annulation generating **7**, followed by 4-chlorobenzylamide formation and, if required, deprotection.

The alkynes used in the Sonogashira couplings in Scheme 1 were commercially available or prepared in the following manners. For compound **8**, the protected alkyne (Fig. 2) was generated by periodate cleavage of the diketal of mannitol (**25**).[11] Attempted conversion of the resulting aldehyde to an alkyne by a Corey–Fuchs reaction resulted in a low yield of **26**, but the alternate diazophosphonate protocol resulted in a reasonable yield of **26**.[12] The ether-containing alkynes used in Scheme 1 were generated according to literature.[13] The alkynes with amine linkers (entries **11–24**) were prepared simply by reacting propargyl bromide or 3-butynyl *p*-toluenesulfonate and the corresponding amine in the presence of diisopropylethylamine or potassium carbonate in dichloromethane or ethanol.[14]

The HCMV DNA Polymerase and Plaque Reduction assays results are shown in Table 1 and the details of these assays and their statistical error have previously been disclosed.[3,5,6,7b] The C-2 analogs in Table 1 were designed to improve the thermodynamic aqueous solubility[15] while also attempting to pick up additional interactions with the protein. The results for compound **3** are shown for comparison purposes to its C-2 analogs.

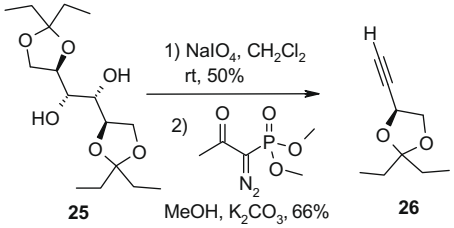


Figure 2. Preparation of alkyne **26** for generation of compound **8**.

Compounds **8** and **9** are analogs that included an additional hydroxyl group in the C-2 side-chain. Although compound **8** and **9** had improved aqueous solubility compared to **3**, they both had diminished antiviral activity, likely associated with a decrease in permeability. Entry **10**, with an increase chain length and ethereal oxygen demonstrated a significant improvement, almost 10-fold, in aqueous solubility ($\geqslant$5 μg/mL was the observation-based criteria for the team) and comparable HCMV polymerase and antiviral activity.

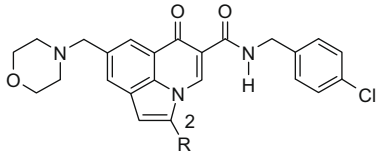
Acyclic amine derivatives **11**, **12**, **14** and **15** all displayed an improvement in aqueous solubility compared to **3**, but interestingly compound **13**, despite its basic amine, displayed no solubility improvement. Unfortunately, compound **11** showed a slight loss in HCMV polymerase inhibition, and **12** and **14** demonstrated marginally lower antiviral activities. Compound **15** was similar in HCMV polymerase and plaque reduction activity when compared to **3**, but with almost an order of magnitude improvement in aqueous solubility.

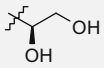
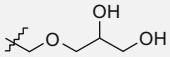
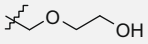
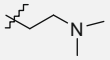
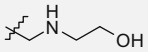
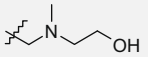
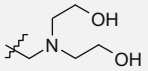
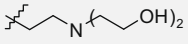
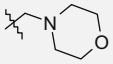
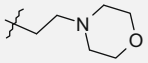
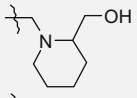
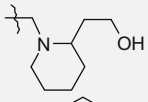
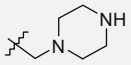
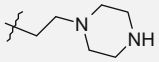
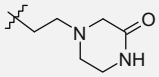
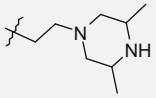
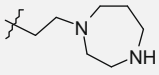
The cyclic amine containing C-2 analogs are entries **16**–**24** in Table 1. Entries **17**, **20**, **21**, **23** and **24** showed significantly better aqueous solubility than **3**, but only **17** maintained antiviral activity. In contrast, poor solubility was observed for entries **16**, **18**, **19** and **22**. It is interesting to note the dramatic difference (31-fold) in solubility between **16** and its homolog **17**. Two aqueous solubility prediction programs employed (data not shown) calculated that **16** and **17** would have similar solubility, less than threefold difference, and both programs overestimated the solubility of these compounds. This exemplifies the difficulty in accurately predicting thermodynamic aqueous solubilities.

In many examples in Table 1, as one would expect, incorporating polar functionality to improve solubility appears to have negatively impacted permeability. This is supported by the higher HCMV plaque reduction IC_{50} values despite similar DNA polymerase inhibition. It is also noteworthy that pyrroloquinoline compounds **3, 8**–**24** showed micromolar or better HCMV DNA polymerase inhibition. This implies that the C-2 substituent is not in close contact with the polymerase protein when bound. Unlike the tight structure activity relationship of the 4-chlorobenzylamide moiety, the structural tolerance displayed by the C-2 region supports the choice of this region to refine physical properties.

An alternate method to improve aqueous solubility, besides introducing polarity or removing 'grease' is to decrease the crystal packing capabilities of the analog. One means to accomplish this is to reduce the planarity of the template. Towards that goal, entry **3** and **10** were reduced generating racemic **27** and **28** (Fig. 3),[16] which showed acceptable solubilities of 7.1 and 4.9 μg/mL, respectively. However, a slight loss of HCMV DNA polymerase activity was observed (IC_{50} = 1.5 μM for both).[17]

Table 1
HCMV polymerase and plaque reduction and aqueous solubility results for compounds **3**, **8**–**24**



Entry	R	HCMV Pol. IC_{50}[a] (μM)	HCMV Plaq. IC_{50}[a,b] (μM)	Aq Sol. pH 7.0[c] (μg/mL)
3		0.35	0.04	0.6
8		0.29	0.20	1.4
9		0.44 (±0.02)	0.20	5.8
10		0.51 (±0.03)	0.03	5.8
11		0.80	nd	55
12		0.35	0.20	16.1
13		0.46	0.10	0.37
14		0.52	0.10	2.4
15		0.55 (±0.03)	0.06	5.2
16		0.65	0.03	0.2
17		0.45 (±0.06)	0.04	6.2
18		0.90	nd	0.1
19		0.80	nd	0.1
20		1.1 (±0.0)	nd	80.3
21		0.39 (±0.02)	0.35	23
22		0.72	nd	0.4
23		0.39	0.20	168
24		0.43	0.14	34.5

[a] Ref. 6a (nd = not determined).
[b] Determined by plaque reduction assay (Davis strain).
[c] Thermodynamic method used from Ref. 15.

The compounds that showed antiviral activity on par with **3** and had improved aqueous solubility (Table 1, entries **10**, **15**, and **17)** were then examined for broad-spectrum activity against HSV-1 and VZV in the DNA Polymerase Inhibition and Plaque Reduction assays as well as selectivity over human α-DNA polymerase and cytotoxicity (Table 2). As observed for other templates[3,5,6,8] all show good broad-spectrum anti-herpetic activity. In the Plaque Reduction assays the pyrroloquinoline compounds in Table 2 had better antiviral activity than ganciclovir against HCMV and acyclovir against VZV. For HSV-1, acyclovir and the pyrroloquinoline compounds had comparable antiviral activity. However, compound **17** showed human α-DNA polymerase inhibition and compound **15** displayed cytotoxicity. Compound **10** had good selectivity, no cytotoxicity and also demonstrated almost identical pharmacokinetics to **3** in rats (F = 66%, $T_{1/2}$ = 0.8 h, CL = 2.5 L/h/kg, and V_{ss} = 1.7 L/kg). Overall, compound **10** had a very similar profile to the develop candidate **3**, but with dramatically improved solubility.

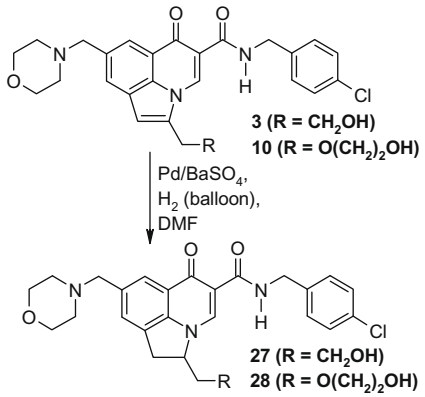


Figure 3. Reduction of **3** and **10** generating **27** and **28**, respectively.

Table 2
Comparison of select compounds, including **3**, and established therapies for broad-spectrum herpesvirus activity, selectivity and cytotoxicity

Compound	DNA polymerase IC_{50}[a] (μM)				Plaque reduction IC_{50}[a,b] (μM)			CC_{50}[c] (μM)
	HCMV	HSV-1	VZV	Human α	HCMV	HSV-1	VZV	
3	0.35	0.12	0.07	>50	0.04	0.8	0.05	>75
10	0.51	0.37	0.38	>50	0.03	1.2	0.10	>75
15	0.55	0.75	0.23	>20	0.06	1.7	nd	23
17	0.45	0.36	0.19	26	0.04	2.1	0.03	nd
Ganciclovir					1.3	nd	nd	>100
Acyclovir					>20	2.1	8.1	>100
Forscarnet	2.5							
Aphidicolin	0.487	0.438	0.473	2.6				
AZT-TP[d]	22.1	3.3	5.8					

[a] Ref. 6a (nd = not determined).
[b] Determined by plaque reduction assay with Davis strain (HMCV), KOS strain (HSV-1) and Webster strain (VZV).
[c] Ref. 8b.
[d] AZT-TP = Azidovudine triphosphate.

In conclusion, this examination of analogs at C-2 of the pyrroloquinoline template successfully demonstrated that solubilizing groups could be incorporated and the HCMV antiviral activity maintained. Analogs **10**, **15** and **17** demonstrated broad-spectrum anti-herpetic activity that was equal or better than current therapies with improved aqueous solubility over previous development candidate **3**. Compound **10** also had good selectivity over human α-DNA polymerase, no cytotoxicity and acceptable rat pharmacokinetics. The focus of our next publication will be the exploration of the C-8 position of the pyrroloquinoline system, in combination with select C-2 substituents presented here.

Acknowledgements

With much appreciation, we acknowledge Roberta Dorow and Mark Lyster for providing additional quantities of **5** and **6** and Steven Tanis for supplying **25**. We thank the Analytical Chemistry group in Kalamazoo and Eric Seest for their work. We also thank Steven Tanis and Michael Ennis for helpful discussions and advice. In addition, we would also like to recognize the yet unpublished pioneering pyrroloquinoline work by Joseph Strohbach, Audris Huang, Sandra Staley and Valerie Vaillancourt.

Supplementary data

Supplementary data (additional cytotoxicity data, synthetic procedures, and characterization data for **8**–**21**, **26** and **28**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.115.

References and notes

1. Wathen, M. W. *Rev. Med. Virol.* **2002**, *12*, 167.
2. Villarreal, E. C. *Prog. Drug Res.* **2003**, *60*, 263.
3. Oien, N. L.; Brideau, R. J.; Hopkins, T. A.; Wieber, J. L.; Knetchel, M. L.; Shelly, J. A.; Anstadt, R. A.; Wells, P. A.; Poorman, R. A.; Huang, A.; Vaillancourt, V. A.; Clayton, T. L.; Tucker, J. A.; Wathen, M. W. *Antimicrob. Agents Chemother.* **2002**, *46*, 724.
4. (a) Mercorelli, B.; Sinigalia, E.; Loregian, A.; Palu, G. *Rev. Med. Virol.* **2008**, *18*, 177; (b) Buhles, W. C. In *Ganciclovir Therapy for Cytomegalovirus Infections*; Spector, S. A., Ed.; Marcel Dekker: New York, 1991; pp 31–40.
5. (a) Brideau, R. J.; Knetchel, M. L.; Huang, A.; Vaillancourt, V. A.; Vera, E. E.; Oien, N. L.; Hopkins, T. A.; Wieber, J. L.; Wilkinson, K. F.; Rush, B. D.; Schwende, F. J.; Wathen, W. M. *Antiviral Res.* **2002**, *54*, 19; (b) Knechtel, M. L.; Huang, A.; Vaillancourt, V. A.; Brideau, R. J. *J. Med. Virol.* **2002**, *28*, 234; (c) Hartline, C. B.; Harden, E. A.; Williams-Aziz, S. L.; Kushner, N. L.; Brideau, R. J.; Kern, E. R. *Antiviral Res.* **2005**, *65*, 97.
6. (a) Schnute, M. E.; Cudahy, M. M.; Brideau, R. J.; Homa, F. L.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Pitts, T. W.; Poorman, R. A.; Wathen, M. W.; Wieber, J. L. *J. Med. Chem.* **2005**, *48*, 5794; (b) Schnute, M. E.; Anderson, D. J.; Brideau, R. J.; Ciske, F. L.; Collier, S. A.; Cudahy, M. M.; Eggen, M.; Genin, M. J.; Hopkins, T. A.; Judge, T. M.; Kim, E. J.; Knechtel, M. L.; Nair, S. K.; Nieman, J. A.; Oien, N. L.; Scott, A.; Tanis, S. P.; Vaillancourt, V. A.; Wathen, M. W.; Wieber, J. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3349. and references within.
7. (a) Dorow, R. L.; Herrinton, P. M.; Hohler, R. A.; Maloney, M. T.; Mauragis, M. A.; McGhee, W. E.; Moeslein, J. A.; Strohbach, J. W.; Veley, M. F. *Org. Process Res. Dev.* **2006**, *10*, 493; (b) Vaillancourt, V. A.; Staley, S.; Huan, A.; Nugent, R. A.; Chen, K.; Nair, S. K.; Nieman, J. A.; Strohbach, W. A. U.S. Patent 6,525,049, 2003
8. (a) Tanis, S. P.; Strohbach, J. W.; Parker, T. T.; Moon, M. W.; Thaisrivongs, S.; Perrault, W. R.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Wieber, J. L.; Stephanski, K. J.; Wathen, M. W. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1994; (b) Schnute, M. E.; Brideau, R. J.; Collier, S. A.; Cudahy, M. M.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Sackett, R. S.; Scott, A.; Sephan, M. L.; Wathen, M. W.; Wieber, J. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3856; (c) Larsen, S. D.; Zhang, Z.; DiPaolo, B. A.; Manninen, P. R.; Rohrer, D. C.; Hageman, M. J.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Rush, B. D.; Schende, F. J.; Stefanski, K. J.; Wieber, J. L.; Wilkinson, K. F.; Zamora, K. M.; Wathen, M. W.; Brideau, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3840.
9. General procedure for amide bond formation: To the desired ester was added 4-chlorobenzylamine (3–10 equiv) and the mixture was heated to 135 °C for 4 h. Excess 4-chlorobenzylamine was removed by Kugelrohr distillation at 120 °C (0.1 Torr). The product was purified by silica gel chromatography.
10. General procedure for coupling/cyclization reaction: To a solution of the desired aryl iodide, copper(I) iodide (0.1 equiv), and $PdCl_2(PPh_3)_2$ (0.05 equiv) in EtOH (5 mL) was added sequentially triethylamine (2 equiv) and the appropriate alkyne (1.5 equiv) at room temperature. The reaction mixture is heated to reflux (0.5–2 h) and cooled to room temperature. The solvent is removed in vacuo. The product was purified by silica gel chromatography.
11. Schmid, C. R.; Bradley, D. A. *Synthesis* **1992**, 2540–2541.
12. Pietruszka, J.; Witt, A. *J. Chem. Soc., Perkin Trans. 1* **2000**, 4293.
13. Hernandez, E.; Galan, A.; Rovira, C.; Veciana, J. *Synthesis* **1992**, 1164.
14. Entries **20**, **21** and **24** had a Boc protected nitrogen and thus required deprotection to form the final compound.
15. For the protocol used to measure thermodynamic solubility see: Guo, J.; Elzinga, P. A.; Hageman, M. J.; Herron, J. N. *J. Pharm Sci.* **2008**, *97*, 1427.
16. Alternatively, intermediate **7** could be reduced followed by 4-chlorobenzylamide formation.
17. The enantiomers of **28** were separated and displayed HCMV DNA polymerase IC_{50} values of 3.0 and 0.7 μM.