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Naphthalene Carboxamides as Inhibitors of Human Cytomegalovirus DNA Polymerase

Valerie A. Vaillancourt,* Michele M. Cudahy, Sandra A. Staley, Roger J. Brideau, Steven J. Conrad, Mary L. Knechtel, Nancee L. Oien, Janet L. Wieber, Yoshihiko Yagi and Michael W. Wathen

Pharmacia, 301 Henrietta Street, Kalamazoo, MI 49007, USA

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Abstract—*ortho*-Hydroxynaphthalene carboxamides have been identified as inhibitors of HCMV DNA polymerase. SAR investigations have demonstrated that both the amide and hydroxy functionalities are required for activity. Substitution on the naphthalene ring has led to inhibitors with submicromolar IC_{50}s against HCMV polymerase. These compounds have been found to be >100-fold selective for inhibition of HCMV polymerase versus human alpha polymerase and display antiviral activity in a cell-based plaque reduction assay.

Human cytomegalovirus (HCMV) is a ubiquitous member of the herpes virus family which infects over forty percent of the population. Like other herpes viruses, HCMV persists in the host in a latent state after primary infection. Although generally benign in the immunocompetent host, reactivation of the latent infection is associated with significant morbidity and mortality in immunocompromised individuals. Active HCMV infection is associated with clinical syndromes such as pneumonia, retinitis, hepatitis, gastrointestinal disease and congenital birth defects. Ganciclovir, foscarnet, cidofovir and formivirsen, the only drugs approved for treatment of HCMV infections, are less than ideal agents due to their significant toxicity, modest efficacy and required intravenous or intraocular routes of administration.[1,2] Clearly, less toxic, orally available alternatives are needed.

In order to address this unmet medical need, the herpes virus program team at Pharmacia initiated a program to develop better anti-HCMV agents. The program team has chosen HCMV DNA polymerase (HCMV pol) as a molecular target.[3] Currently marketed therapies including ganciclovir, foscarnet and cidofovir all inhibit HCMV via inhibition of this viral enzyme.[4–6] Through broad screening of the compound collection, the naphthalene carboxamide **1** was identified as an inhibitor of HCMV pol. Structure–activity relationship (SAR) studies were initiated around this template.

SAR investigations of this template were focused on three major structural features of the molecule, the amide functionality, the hydroxyl group and substitution on the naphthalene ring. The reported naphthalene carboxamides were prepared by condensation of the corresponding naphthalene carboxylic acid with the appropriate amine, utilizing reaction conditions of either PCl_3 in refluxing xylenes[7] or carbonyldiimidazole in THF.[8] Compounds were tested for their ability to inhibit HCMV pol using a scintillation proximity assay.[9] Initial SAR investigations of the naphthanilides showed that the presence of an electron withdrawing substituent on the aniline ring was essential for activity (Table 1). Unsubstituted or electron rich anilides were found to be less active, as were pyridyl amides.

To further examine the SAR around this template, optimal placement of the electron-deficient aromatic ring was explored. It was found that a one carbon linkage between the amide nitrogen and the electron-deficient aromatic group gave similar HCMV pol inhibitory activity (Table 2). Longer linkages resulted in diminished activity.

Continuing investigations focused on the hydroxyl substituent, evaluating the necessity and the placement of this functionality (Table 3). In the aniline series, derivatization of the hydroxyl group as either its methyl ether

*Corresponding author. Tel.: +1-616-833-9552; fax: +1-616-833-2232; e-mail: valerie.a.vaillancourt@am.pnu.com

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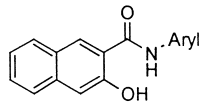
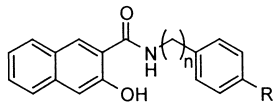
Table 1. Aryl amides



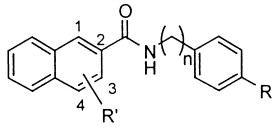
Compound	Aryl group	HCMV pol IC_{50} (μM)
1	3-NO_2-Ph	12
2	4-NO_2-Ph	22
3	4-Cl-Ph	69
4	4-Br-Ph	22
5	4-CN-Ph	19
6	Ph	>100
7	4-OCH_3-Ph	56
8	3-NH_2-Ph	>100
9	2-pyridyl	>100
10	3-pyridyl	>100

Table 2. Linker region



Compound	*n*	R	HCMV pol IC_{50} (μM)
11	1	NO_2	12
12	1	Cl	52
13	2	NO_2	>100
14	2	Cl	>100

Table 3. Significance of hydroxy substituent

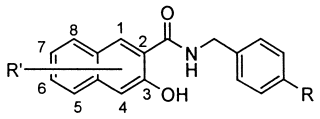


Compound	R	R′	*n*	HCMV pol IC_{50} (μM)
15	NO_2	3-OCH_3	0	>100
16	NO_2	3-OMOM	0	>100
17	Cl	3-OCH_3	1	8.0
18	NO_2	H	0	>100
19	Cl	H	1	>100
20	Cl	1-OH	0	37
21	Cl	1-OH	1	13
22	NO_2	1-OH	1	14.5

(**15**) or its methoxymethyl ether (**16**) resulted in reduction of activity. This was not true for the benzylamide series, in which the corresponding methyl ether (**17**) was actually more active than the free hydroxyl group. Removal of the hydroxyl functionality resulted in reduction of activity in both series (**18** and **19**). Of note in this study was that the hydroxy group could be placed at either the 1- or 3-position of the naphthalene ring *ortho* to the carboxamide with little difference in activity. Again, both anilides and benzylamides were active within this new series (**20**, **21**, and **22**).

The next phase of SAR investigations involved the study of the effects of substitution on the naphthalene ring system. Toward this end, commercially available naphthalene carboxylic acids were condensed with 4-chloro- or 4-nitrobenzylamine to form the desired carboxamides. The results of this study are shown in Table 4. In general, substitution at the 5- and 7-positions of the naphthalene ring had beneficial effects on HCMV pol inhibitory activity, with the 7-methoxy substituted analogues being the first compounds in this series to display submicromolar activity.

Table 4. Naphthalene substitution effects



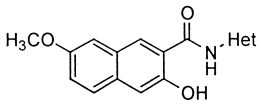
Compound	R	R′	HCMV pol IC_{50} (μM)
23	NO_2	4,7-di-Br	2.9
24	NO_2	5-OH	3.8
25	Cl	5-OH	3.3
26	NO_2	7-OH	10.6
27	Cl	7-OH	2.4
28	NO_2	7-OCH_3	0.82
29	Cl	7-OCH_3	0.76

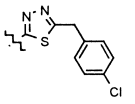
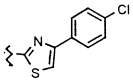
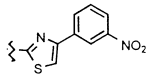
Having identified methoxy as a favored substituent at the 7-position, the identity and necessity of the amide group was revisited within this series. Heterocyclic amides were investigated. Again, the desired analogues could be prepared by PCl_3 or carbonyldiimidazole promoted condensations of the naphthalene carboxylic acid with the appropriate aminoheterocycle. The compounds prepared and their HCMV pol inhibitory activities are shown in Table 5. As can be seen in this table, although electron deficient heterocyclic amides displayed activity in the polymerase assay, they appeared to be less potent than the benzyl amides. To further evaluate the necessity of the amide functionality, the amide of **29** was reduced with $LiAlH_4$ to provide **35**. This led to a marked decrease in activity.

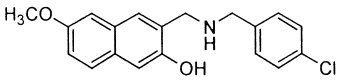
The next step in the biological investigations of these compounds was to determine the selectivity of the polymerase activity. The initial lead **1** was found to only be 2- to 3-fold selective for inhibition of HCMV pol versus human alpha polymerase. In contrast, many of the new analogues showed remarkable selectivity (10- to 100-fold) for inhibition of HCMV pol versus human alpha polymerase. Additionally, antiviral activity in a plaque reduction assay was observed for many of the more potent analogues, while less potent HCMV pol inhibitors (e.g. **14** and **21**) did not exhibit measurable antiviral activity.[10] Data for select analogues is shown in Table 6.

In summary, *ortho*-hydroxynaphthalenecarboxamides have been identified as HCMV DNA polymerase inhibitors. SAR investigations of these compounds show that both the amide and hydroxy functionalities are required for activity. Electron-deficient phenyl, and benzylic amides provide the highest levels of inhibition. Substitution at the 5- or 7-postion of the naphthalene ring with either hydroxy or methoxy groups enhances

Table 5. Heterocyclic amides



Compound	Het	HCMV pol IC_{50} (μM)
30		4
31		6.4
32		6
33		59
34		2.4



35

HCMV pol IC_{50} > 100 μM

Table 6. Polymerase selectivity and antiviral activity for select compounds

Compound	HCMV pol IC_{50} (μM)	Human α-pol IC_{50} (μM)	Plaque reduction IC_{50} (μM)
1	12.3	32	nd
14	>100	nd[a]	>20
21	13	nd	>20
24	3.8	>100	1.5
25	3.3	63	0.6
27	2.4	18	1.0
28	0.82	>100	3.7
31	6.4	93	>5
Aphidicolin	0.4	3.0	—
Ganciclovir	—	—	0.6

[a]nd not determined.

polymerase inhibition activity, resulting in compounds (e.g. **28**) which display submicromolar IC_{50}s against HCMV pol. This compound was also found to be greater than 100-fold selective for inhibition of HCMV pol versus human alpha polymerase and shows antiviral activity in a cell-based antiviral assay.

References and Notes

1. deJong, M. D.; Galasso, G. J.; Gazzard, B.; Griffiths, P. D.; Jabs, D. A.; Kern, E. R.; Spector, S. A. *Antiviral Res.* **1998**, *39*, 141.
2. Hoffman, V. F.; Skiest, D. J. *Exp. Opin. Invest. Drugs* **2000**, *9*, 207.
3. Yukihiro, N.; Koichiro, M.; Shonen, Y. *Virology* **1983**, *124*, 221.
4. Biron, K. K.; Stenbuck, P. J.; Sorrell, J. B. *UCLA Syp. Mol. Cell. Biol., New Ser.* **1984**, *21*, 677.
5. Eriksson, B.; Tao, P. Z.; Wahren, B.; Oeberg, B. In *Proc. 13th Int. Congr. Chemother.*; Spitzy, K. H.; Karrer, K., Eds.; Verlag H. Egermann: Vienna, 1983; p 6.
6. Xiong, X.; Smith, J. L.; Chen, M. S. *Antimicrob. Agents Chemother.* **1997**, *41*, 594.
7. Singh, H.; Singh, A. K.; Sharma, S.; Iyer, R. N. *J. Med. Chem.* **1977**, *20*, 826 and references cited therein. A typical experimental procedure for a PCl_3 promoted coupling is as follows: A solution of 3-hydroxy-2-naphthoic acid (2.82 g, 15 mmol) and 4-nitroaniline (2.07 g, 15 mmol) in 80 mL xylenes was heated to reflux. To this was added dropwise PCl_3 (0.52 mL, 6 mmol). Refluxing was continued until HCl evolution had ceased (ca. 1 h). The reaction was then cooled and water was added to destroy excess PCl_3. The solvents were evaporated and the residual solid was crystallized from 3:1 THF:water by the addition of MeOH. The solid was collected and dried to yield 1.83 g (40%) of **2** as an olive-green solid.
8. Staab, H. A. *Angew. Chem., Int. Ed. Engl.* **1962**, *1*, 351. A typical carbonyldiimidazole promoted coupling is as follows: To a solution of 1,1′-carbonyldiimidazole (1.70 g, 10.5 mmol) in THF (20 mL) was added 3-hydroxy-2-naphthoic acid (0.94 g, 5.00 mmol). The resulting solution was stirred at room temperature for 1 h. The reaction was cooled to 0 °C and a solution of 4-chlorobenzylamine (1.2 mL, 10.0 mmol) in THF (10 mL) was added dropwise. The reaction was allowed to warm slowly to room temperature and was stirred for 18 h. The reaction mixture was concentrated in vacuo. The resulting yellow oil was chromatographed eluting with 5% CH_3OH/CH_2Cl_2. Fractions homogeneous by TLC were combined and concentrated in vacuo. The resulting yellow solid was recrystallized twice from EtOAc to yield 0.557 g (36%) of **12** as a yellow crystalline solid.
9. The assay was run as previously described: Tucker, J. A.; Clayton, T. L.; Chidester, C. G.; Schulz, M. W.; Harrington, L. E.; Conrad, S. J.; Yagi, Y.; Oien, N. L.; Yurek, D.; Kuo, M.-S. *Bioorg. Med. Chem.* **2000**, *8*, 601. Foscarnet was used as a positive control in this assay. A typical HCMV polymerase IC_{50} value for foscarnet is 3.5 μM.
10. The antiviral assay was run in a standard manner (see Bedard, J.; May, S.; L'Heureux, L.; Stamminger, T.; Copsey, A.; Drach, J.; Huffman, J.; Chan, L.; Jin, H.; Rando, R. F. *Antimicrob. Agents Chemother.* **2000**, *44*, 929) using an HFF cell line and the Davis strain of HCMV.