Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2929–2932

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Thiourea Inhibitors of Herpes Viruses. Part 1: Bis-(aryl)thiourea Inhibitors of CMV

Jonathan D. Bloom,* Martin J. DiGrandi, Russell G. Dushin, Kevin J. Curran, Adma A. Ross, Emily B. Norton, Eugene Terefenko, Thomas R. Jones, Boris Feld and Stanley A. Lang†

Wyeth Research, 401 N. Middletown Rd., Pearl River, NY 10965, USA

Received 15 January 2003; accepted 24 April 2003

Abstract—Bis-(aryl)thioureas were found to be potent and selective inhibitors of cytomegalovirus (CMV) in cultured HFF cells. Of these, the thiazole analogue **38** was investigated as a potential development candidate.

Human cytomegalovirus (CMV) is a ubiquitous pathogen belonging to the herpes family of viruses.[1] Transmission can be perinatal or through body fluids. Seroprevalence rates in adults range from 50–90%, depending on the socioeconomic population examined.[1] CMV infection in immunocompetent adults is usually asymptomatic. However, as an opportunistic pathogen, CMV causes clinically significant disease in the absence of normal immunity. In this regard, relevant clinical populations include infected infants, and immunocompromised adults. Although in recent years HAART[2] therapies have significantly restored immune function in AIDS patients, there remains a need for safer, more effective CMV therapies for infected neonates and transplant patients as well as AIDS patients in whom HAART therapy has failed.

Current therapies for CMV (Fig. 1) include ganciclovir,[3] cidofovir[4] and foscarnet.[5] These drugs typically produce significant side effects including neutropenia and nephrotoxicity as well as exhibit poor bioavailability, although valganciclovir[6] (a valine ester prodrug of gancyclovir) has shown improved bioavalability. Recently, fomivirsen, a 21-nucleotide anti-sense therapy has been approved for intravitreal injection.[7] A review of non-nucleoside inhibitors of CMV has recently appeared.[8]

High-throughput screening against a panel of viruses in cultured cells revealed thiourea **1** (Fig. 2) as a weak inhibitor of Herpes simplex virus (HSV). An early attempt to maximize this activity led to the preparation of phenyl analogue **2**, which unexpectedly displayed inhibitory activity against CMV in cultured human foreskin fibroblast (HFF) cells. Inhibitory activities of analogues against CMV and HSV quickly diverged, leading to parallel series.

Thioureas **3** could be prepared (Scheme 1) by reacting appropriately substituted anilines **7** with isothiocyanates **6** in acetonitrile or THF. Alternatively, **3** could also be prepared by sequential treatment of anilines **7** with

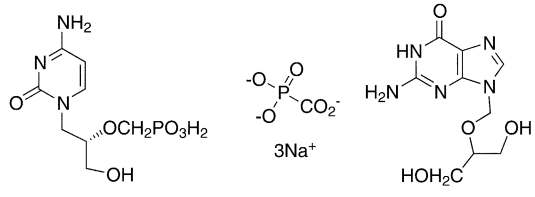


Figure 1. Current CMV therapies.

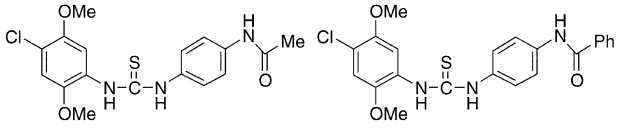
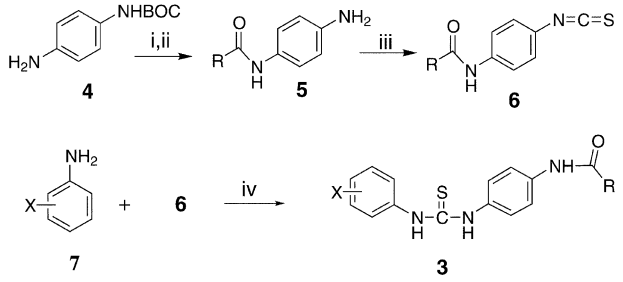


Figure 2. Initial HSV and CMV inhibitors.

*Corresponding author. Fax: +1-845-602-5561; e-mail: bloomj@wyeth.com
†Current address: Ribapharm Inc., 3300 Hyland Ave., Costa Mesa, CA 92626, USA.

0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00586-9

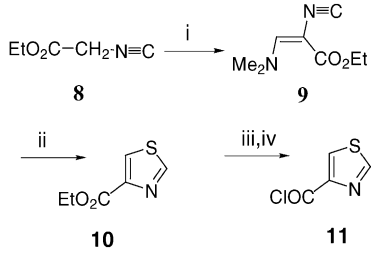


Scheme 1. (i) RC(O)Cl, TEA, CH_2Cl_2; (ii) TFA; (iii) 1,1′-thiocarbonyldiimidazole; (iv) hot CH_3CN.

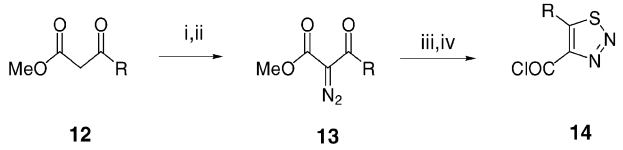
1,1′-thiocarbonyldi-(1,2,4)-triazole,[9] followed by addition of anilines **5**. This latter approach was advantageous when employing less nucleophilic anilines. Requisite isothiocyanates **6** were prepared in an efficient three-step process beginning with the acylation of mono-BOC phenylenediamine **4**, deprotection with TFA and treatment of the resulting free anilines **5** with 1,1′-thiocarbonyldiimidazole. Anilines **7** reported herein are commercially available, with the exception of 3-chloro-4-trifluoromethylaniline, which was prepared in two steps by reaction of trifluoromethylcopper[10] with 3-chloro-4-iodonitrobenzene, followed by reduction of the nitro group.

The preparation of non-commercially available carboxylic acid chlorides is shown in Scheme 2[11] and Scheme 3.[12] Condensation of ethyl isocyanoacetate, **8** with *N*,*N*-dimethylformamide dimethyl acetal gave ethyl 3-dimethylamino-2-isocyano acrylate, **9**. Treatment of **9** with hydrogen sulfide and TEA yielded 4-carboethoxy thiazole, **10** which was converted to the acid chloride, **11** by basic hydrolysis followed by exposure to oxalyl chloride.

Substituted 1,2,3-thiadiazole 4-carboxylates (Scheme 3)[12] were prepared by reaction of the appropriately substituted β-keto ester **12** with *p*-toluenesulfonyl azide to form the intermediate diazo ketone **13**. Subsequent treatment with Lawesson's reagent in refluxing toluene



Scheme 2. (i) $Me_2NCH(OMe)_2$; (ii) H_2S, TEA; (iii) NaOH; (iv) $(COCl)_2$.



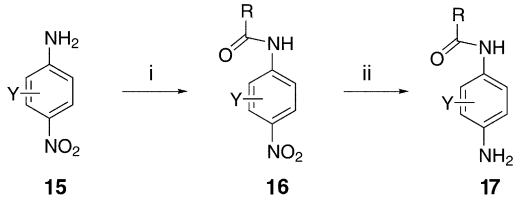
Scheme 3. (i) TsN_3, TEA (ii) Lawesson's reagent; (iii) NaOH; (iv) $(COCl)_2$.

gave the 4-carboxy-1,2,3-thiadiazole methyl ester, which could be converted to the acid chloride **14** as described in Scheme 2. 4-Carboxy-1,2,3-thiadiazole was purchased.

The preparation of analogues in which the central phenylenediamine ring was substituted (Scheme 4) was accomplished by acylation of the commercially available substituted nitroanilines **15** to yield amides **16**. Reduction of the nitro group with palladium and cyclohexene[13] in refluxing ethanol or with iron and ammonium chloride[14] in ethanol gave the substituted anilines 17. Conversion to the desired thiourea was identical to the procedure described in Scheme 1.

All compounds in the program were assayed[15] against the herpes viruses CMV, HSV and varicella zoster (VZV). Additionally, screening against non-herpes viruses such as respiratory syncytial virus (RSV) and the MTS cellular toxicity assay[16] were performed to distinguish between specific anti-viral activity and non-specific host cell toxicity. All of the compounds reported herein (Fig. 3) have RSV and MTS IC_{50} values of >10 μg/mL. These compounds are considered to be non-cytotoxic and RSV and MTS data are not included in the tables.

The SAR resulting from variation of the acyl group is shown in Table 1. Replacement of the methyl group in **1** with larger alkyls such as ethyl (**18**) did not improve activity versus HSV. However, introduction of a phenyl group (**2**) led to a 10-fold reduction in the IC_{50} against both HSV and CMV. Replacement of phenyl by a heteroaromatic group such as 2-furoyl (**19**) further increased the potency against CMV 20-fold, to 0.2 μg/mL, but HSV activity was lost. At this point, the HSV



Scheme 4. (i) Excess RC(O)Cl, THF, reflux; (ii) Pd/C/cyclohexene, EtOH; or Fe/NH_4Cl, EtOH.

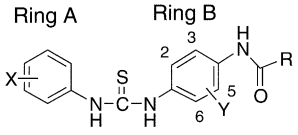


Figure 3. Generic structure of CMV inhibitors **18**–**38**.

Table 1. Variation of the acyl group

Compd	X	Y	R	CMV[a]	HSV[a]	VZV[a]
1	2,4-$(OMe)_2$-5-Cl	H	Me	45	3.0	>10
18	2,4-$(OMe)_2$-5-Cl	H	Et	NT	3.0	>10
2	2,4-$(OMe)_2$-5-Cl	H	Ph	4	0.3	>10
19	2,4-$(OMe)_2$-5-Cl	H	2-Fur[b]	0.2	>10	NT

NT = not tested; MTS > 10 μg/mL for all compounds.
[a]IC_{50}, μg/mL.
[b]2-Fur = 2-furoyl.

and CMV programs diverged, and all SARs reported herein involved our efforts directed toward finding potent CMV inhibitors. A paper describing the SARs of the HSV series will be forthcoming.

With R held constant as 2-furoyl, we explored the SAR of Ring A (Table 2). Alkyl and electron withdrawing groups, especially *meta* and *para* to the thiourea nitrogen, showed increased potency over non-substituted analogues (**20**). Disubstituted analogues (**21**–**23** and **25**) were approximately as potent as monosubstituted (**24** and **26**). The most potent furan-containing inhibitor was the 3-trifluoromethyl-4-chloro analogue **22**, which had an IC_{50} of 0.03 μg/mL.

Substitution on Ring B (Table 3) diminished or eliminated activity altogether in every case. This was true for analogues bearing electron withdrawing groups (**30** and **31**) and electron-donating groups (**27** and **32**). The only analogue to retain good activity was **28** (Y = 2-Me), although it was 3-fold less potent than the corresponding unsubstituted analogue **19**.

Heterocycles other than furan were synthesized and this resulted in the most potent analogues in the series (Table 4). We had previously demonstrated a large advantage in activity of a five-membered ring (furan) over a six-membered ring (benzene) for R (**2** vs **19**) and this trend continued for other aromatic groups. The 2-pyridyl analogue **33** had an IC_{50} of >10 μg/mL. However, a number of five-membered heterocycles showed activity that was equipotent (1,3-oxazoles **34** and **35**) or superior (thiadiazole **36** and thiazole **37**) to the corresponding 2-furoyl analogue. Thiazoles **37** and **38** were the most active compounds in the series with IC_{50}s of 0.006 and 0.008 μg/mL, respectively.

Thiazole **38**[17] (Fig. 4) was found to be unstable under forcing conditions, especially in acidic media at elevated temperatures, suggesting that the in vivo stability of the compound might be problematic. Although the compounds in series were stable under conditions of the in vitro assay, this unexpected instability (attributed to hydrolysis of the thiourea group) prompted us to abandon this series and focus on modified structures with improved stability characteristics. This will be the subject of a future paper.

HTS identified a weak inhibitor of HSV. Chemical synthesis improved this activity, but also revealed activity against CMV, another herpes virus. During the course of this program the activity versus CMV was improved 7500-fold, yielding an extremely potent (0.006 μg/mL) inhibitor of CMV in cultured HFF cells without loss of selectivity. Unsuitable physical properties precluded further development of any compounds in this series.

Table 2. Ring A SAR

Compd	X	Y	R	CMV[a]	HSV[a]	VZV[a]
20	H	H	2-Furoyl	0.30	>10	>10
21	3-Cl–4-Me	H	2-Furoyl	0.09	>10	>10
22	3-CF_3–4-Cl	H	2-Furoyl	0.03	>10	>10
23	3-Cl–4-F	H	2-Furoyl	0.06	>10	>10
24	3-CF_3	H	2-Furoyl	0.06	>10	>10
25	3,5-$(CF_3)_2$	H	2-Furoyl	0.04	>10	7
26	3-CH_3	H	2-Furoyl	0.06	>10	>10

[a]IC_{50}, μg/mL.

Table 3. Ring B SAR

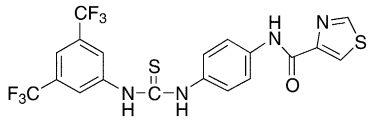
Compd	X	Y	R	CMV[a]	HSV[a]	VZV[a]
27	2,4-$(OMe)_2$–5-Cl	3-OMe	2-Fur[b]	>10	8	>10
28	2,4-$(OMe)_2$–5-Cl	2-Me	2-Fur	0.6	>10	>10
29	2,4-$(OMe)_2$–5-Cl	3-Me	2-Fur	>10	>10	>10
30	2,4-$(OMe)_2$–5-Cl	2-CN	2-Fur	>10	>10	>10
31	2,4-$(OMe)_2$–5-Cl	2-CF_3	2-Fur	2.0	2.5	>10
32	2,4-$(OMe)_2$–5-Cl	3,6-$(OMe)_2$	2-Fur	>10	>10	4

[a]IC_{50}, μg/mL.
[b]2-Fur, 2-Furoyl

Table 4. Heteroaryl R groups

Compd	X	Y	R	CMV[a,b]
33	3-Cl–4-CF_3	H	2-Pyridyl	>10
34	3-Cl–4-CF_3	H	4-(1,3-Oxazoyl)	0.02
35	3,5-$(CF_3)_2$	H	4-(1,3-Oxazoyl)	0.03
36	3,5-$(CF_3)_2$	H	4-(1,2,3-Thiadiazolyl)	0.02
37	3-Cl–4-CF_3	H	4-(1,3-Thiazolyl)	0.006
38	3,5-$(CF_3)_2$	H	4-(1,3-Thiazolyl)	0.008

[a]IC_{50}, μg/mL.
[b]VZV and HSV > 10 mg/mL for all analogues in table.



38

Figure 4. Advanced lead candidate.

References and Notes

1. Britt, W. J.; Alford, C. A. In *Fields Virology*, 3rd ed; Fields B. N., Knipe, D. M., Howley, P. M. Eds.; Lippincott-Raven: Philadelphia, 1996; Vol. 2, p 2493.
2. Palella, F. J.; Delaney, K. M.; Moorman, A. C.; Loveless, M. O.; Fuhrer, J.; Satten, G. A.; Aschman, D. J.; Holmberg, S. D. *N. Engl. J. Med.* **1998**, *338*, 853.
3. (a) Holland, G. N.; Buhles, W. C., Jr.; Mastre, B. *Arch. Ophthalmol.* **1989**, *107*, 1759. (b) Spector, S. A.; Weingeist, T.; Pollard, R. B. *J. Infect. Dis.* **1993**, *168*, 557.
4. (a) Drew, W. L. *J. Infect. Dis.* **1988**, *158*, 449. (b) Hitchcock, M. J. M.; Jaffe, H. S.; Martin, J. C.; Stagg, R. J. *Antiviral Chem. Chemother.* **1996**, *7*, 115.
5. Jacobson, M. A.; Wulfsohn, M.; Feinberg, J. E. *AIDS* **1994**, *8*, 451.
6. Martin, D.; Sierra-Madero, J.; Walmsley, S.; Wolitz, R.; Brown, F.; Robinson, C. *Program and Abstracts*, 7th Conference on Retroviruses and Opportunistic Infections: San Francisco, CA, 2000; Abstr. 231.
7. Grillone, L. R.; Lanz, R. *Drugs Today* **2001**, *37*, 245.
8. Ogilvie, W. W. *Curr. Med. Chem. Anti-Infect. Agents* **2002**, *1*, 177.
9. Larsen, C.; Steliou, K.; Harpp, D. N. *J. Org. Chem.* **1978**, *43*, 337.
10. Urata, H.; Fuchikami, T. *Tetrahedron Lett.* **1991**, *32*, 91.

11. Schollkopf, U.; Porsch, P.; Lau, H. *Liebigs Ann. Chem.* **1979**, 1444.
12. (a) Looker, J. H.; Thatcher, D. N. *J. Org. Chem.* **1957**, *22*, 1233. (b) Hurd, C. D.; Mori, R. I. *J. Am. Chem. Soc.* **1955**, *77*, 5359.
13. Entwistle, I. D.; Johnstone, R. A. W.; Povall, T. J. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1300.
14. Ramadas, K.; Srinivasan, N. *Synth. Commun.* **1992**, *22*, 3189.
15. To calculate IC_{50}s, the compounds were serially diluted and tested in duplicate. The Sigmoidal Hill Slope (0–100) model was used for curve fitting and IC_{50} determination. Each compound was tested at least in two independent runs on different days.
16. Barltrop, J. A.; Owen, T. C.; Cory, A. H.; Cory, J. G. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 611.
17. Physical data for **38**: calcd For $C_{19}H_{12}F_6N_4OS_2$: C 46.53; H 2.47; N 11.42. Found: C 46.31; H 2.42; N 11.34. EI-MS $[M+H]^+ = 491$. ^1H NMR (300 MHz, DMSO-d_6) δ 7.41 (d, 2H), 7.77 (b s, 1H), 7.86 (d, 2H), 8.27 (s, 2H), 8.52 (d, 1H), 9.29 (d, 1H), 10.15 (s, 1H), 10.28 (s, 1H), 10.44 (s, 1H).