Bioorganic & Medicinal Chemistry Letters 21 (2011) 1948–1952

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Isatin-β-thiosemicarbazones as potent herpes simplex virus inhibitors

Iou-Jiun Kang [a,†], Li-Wen Wang [a,†], Tsu-An Hsu [a], Andrew Yueh [a], Chung-Chi Lee [a], Yen-Chun Lee [a], Ching-Yin Lee [b], Yu-Sheng Chao [a], Shin-Ru Shih [b,*], Jyh-Haur Chern [a,*]

[a] Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC
[b] School of Medical Technology, Chang Gung University, Taoyuan 333, Taiwan, ROC

ARTICLE INFO

Article history:
Received 28 September 2010
Revised 11 January 2011
Accepted 10 February 2011
Available online 15 February 2011

Keywords:
Herpes simplex virus
HSV-1
HSV-2

ABSTRACT

A series of isatin-β-thiosemicarbazones have been designed and evaluated for antiviral activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in a plaque reduction assay. Their cytotoxicity was examined using human rhabdomyosarcoma cells (RD cells). Several derivatives of isatin-β-thiosemicarbazone exhibited significant and selective antiviral activity with low cytotoxicity. It was found that the thiourea group at thiosemicarbazone and the NH functionality at isatin were essential for their anti-herpetic activity. The synthesis and structure–activity relationship studies are presented.

Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) are double-stranded DNA viruses[1] and well known for their ability to cause one of the most common infections in man throughout the world.[2] In both cases, life-long latency is established in sensory ganglia from which the virus can reactivate to cause recurrent episodes on the mouth (labial herpes) or genitals (genital herpes) as often as several times a year.[3] HSV infections can also cause serious systemic illnesses, particularly in the growing immunocompromised population (AIDS patients, organ transplant recipients, chemotherapy patients) and neonates.[4]

Most of the existing drugs for treatment of HSV infections have been dominated by nucleoside derivatives such as acyclovir, ganciclovir, penciclovir, valaciclovir (converted to acyclovir) and famciclovir (converted to penciclovir).[5] Among these drugs, acyclovir is the most commonly used drug for HSV therapy.[6] However, drug-resistant strains of HSV are emerging[7] which is prevalent in the following therapeutic treatment.[8] In 1980, it was first reported that the mechanism of resistance to acyclovir could occur following mutation in either HSV thymidine kinase (TK) or DNA polymerase.[9] The similarity in the mechanism of current anti-herpese drugs suggests that cross-resistance is common among drug-resistant strains.[10] Thus, the discovery of new non-nucleoside anti-HSV molecules with novel mechanisms of action is an important goal for antiviral research.

As early as 1946, the discovery of thiosemicarbazones as antitubercular agents was reported by Domagk et al.[11] In 1950, thiosemicarbazones were first reported to possess antiviral activity in mice against vaccinia virus.[12] Since then, the biological effects of other thiosemicarbazones have been widely investigated. Results of these studies have demonstrated that thiosemicarbazone derivatives also possess additional antibacterial,[13] anti-fungal,[14] antimalarial,[15] antineoplastic,[16] antiparasitic,[17] anti-HIV,[18] anti-HSV[19], anti-CMV (Cytomegalovirus),[20] and anti-MLV (Moloney leukemia virus)[21] activity. Isatin-β-thiosemicarbazone (**1**, IBT) and *N*-methylisatin-β-thiosemicarbazone (**2**, methisazone, marboran) (Fig. 1) are extensively studied thiosemicarbazones that demonstrate an inhibitory effect against the replication of poxviruses.[22] Methisazone has also been used in the clinical treatment of smallpox.[23] This drug plays an important role as a smallpox chemoprophylactic agent. The spectrum of antiviral activity of this drug has been extended by additional in vitro studies to include other groups of viruses, such as adenovirus, herpesvirus, picornavirus, reovirus, arbovirus, myxo- and para-myxovirus, and retrovirus.[24]

The precise viral target and mechanism of action of IBT and its derivatives remains unknown. The biological properties of thiosemicarbazones are often related to metal ion coordination. It is possible that the function of the IBT or thiosemicarbazone is to act as a carrier for heavy metal, thereby increasing its ability to penetrate the viral envelope. The ability of thiosemicarbazones to increase the intracellular copper concentration has been reported.[25] Based on the observation that some viral DNA and structural protein is synthesized in the presence of IBT, the suggestion had been made that the compound interferes with the maturation of viral progeny.[26] Also, it was reported that the primary effect of

* Corresponding authors. Address: Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, No. 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, ROC. Tel.: +886 37 246 166; fax: +886 37 586 456.
E-mail address: jhchen@nhri.org.tw (J.-H. Chern).
† Authors with equal contribution.

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.037

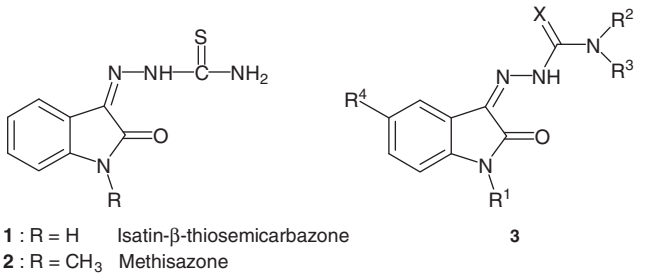


Figure 1. Isatin thiosemicarbazones with previously reported anti-poxvirus activity (**1**, **2**) and scaffold **3** for anti-HSV activity.

the IBT is to enhance viral postreplicative transcription elongation, either directly or by suppressing termination, resulting in the formation of longer-than-normal transcripts.[27] Studies by Katz et al.[28] have supported that inhibition of A18R helicase activity on the viral RNA polymerase is one possible mode of action of IBT.

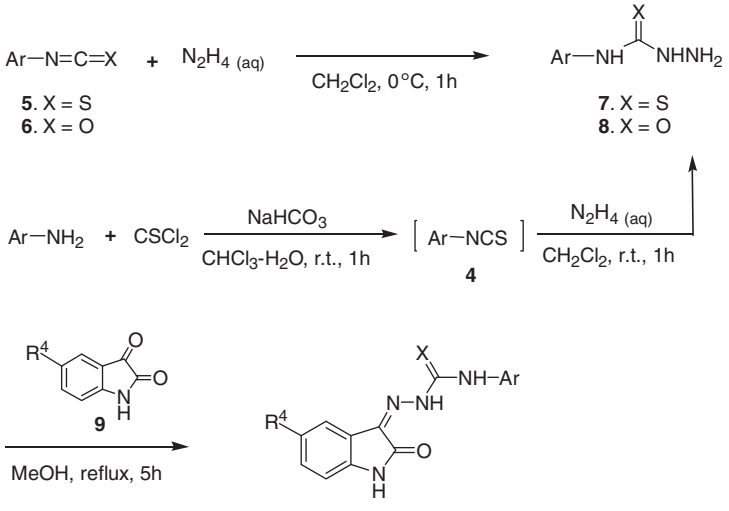
Additionally, the combinatorial optimization and a more in-depth biological evaluation of isatin-β-thiosemicarbazone derivatives against vaccinia and cowpox virus infections have been reported in 2005.[33a,b] This combinatorial library has provided compounds with significantly enhanced potency over IBT in cell-based activity against vaccinia, and with somewhat enhanced potency against cowpox. On the other hand, a series of derivatives were found to possess significant anti-HIV activity with low cytotoxicity and high selectivity index.[33c] It was recently reported that some of isatin-β-thiosemicarbazone derivatives showed selective activity toward multidrug-resistant cells.[33d] Evolution of this chemical family offers the promise of curing those cancers most resistant to chemotherapy.

Recently, an antiviral screening program was initiated to search for new isatin-β-thiosemicarbazone HSV inhibitors in our laboratory. In order to diversify the functional groups on the IBT scaffold, we synthesized compounds of general structure **3** (Fig. 1) and evaluated their anti-HSV activity via plaque reduction assay.[29] Interestingly, several derivatives of IBT were identified as potential antiherpetic agents. In this Letter, we would like to report the synthesis of these isatin-β-thiosemicarbazones and evaluation of their inhibitory activities against HSV. Details of this investigation will be described herein.

The synthesis of N^4-monosubstituted thiosemicarbazones **10–23**, **28–33** and semicarbazones **24–27** was described in Scheme 1. Condensation of aqueous hydrazine (80% in H_2O) with commercially available isothiocyanate **5** or isocyanate **6** gave thiosemicarbazide **7** or semicarbazide **8**. Alternatively, reaction of aryl amine with thiophosgene in the presence of sodium bicarbonate gave the isothiocyanate intermediate **4**. Subsequent condensation of **4** with aqueous hydrazine (80% in H_2O) afforded thiosemicarbazide **7**. Condensation of **7** and **8** with isatin **9** in methanol afforded the desired products **10–33**.

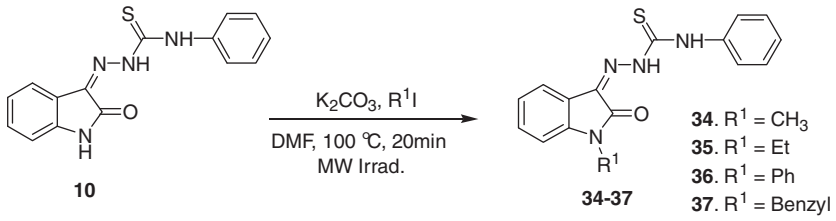
In addition, N^1-substituted thiosemicarbazones **34–37** were prepared by reacting thiosemicarbazone **10** with alkyl or aryl iodide in DMF under microwave irradiation at 100 °C[30] (Scheme 2).

The synthesis of N^4-substituted thiosemicarbazones **41–49** was described in Scheme 3. The methyl hydrazinecarbodithioate **38** was prepared by the reaction of carbon disulfide with aqueous

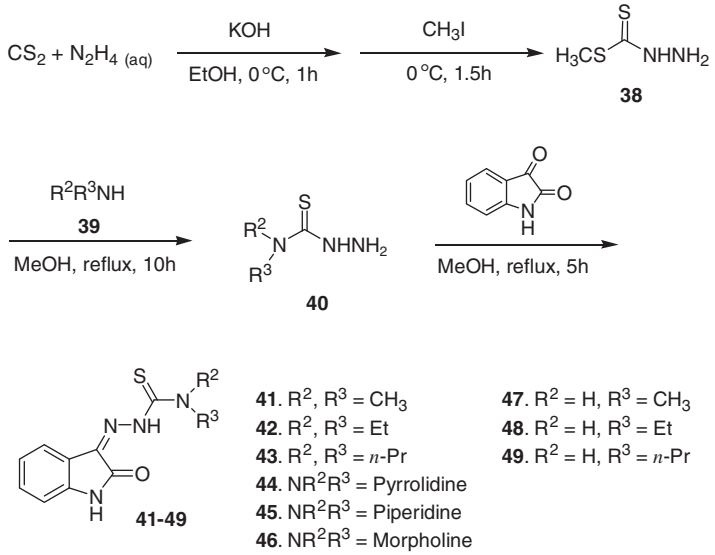


10. X = S, R^4 = H, Ar = Ph
11. X = S, R^4 = H, Ar = 2-CH_3Ph
12. X = S, R^4 = H, Ar = 3-CH_3Ph
13. X = S, R^4 = H, Ar = 4-CH_3Ph
14. X = S, R^4 = H, Ar = 2-OCH_3Ph
15. X = S, R^4 = H, Ar = 3-OCH_3Ph
16. X = S, R^4 = H, Ar = 4-OCH_3Ph
17. X = S, R^4 = H, Ar = 4-FPh
18. X = S, R^4 = H, Ar = 2-ClPh
19. X = S, R^4 = H, Ar = 3-ClPh
20. X = S, R^4 = H, Ar = 4-ClPh
21. X = S, R^4 = H, Ar = 4-BrPh
22. X = S, R^4 = H, Ar = 4-ImidazolylPh
23. X = S, R^4 = H, Ar = 4-MorpholinylPh
24. X = O, R^4 = H, Ar = Ph
25. X = O, R^4 = H, Ar = 3-CH_3Ph
26. X = O, R^4 = H, Ar = 4-CH_3Ph
27. X = O, R^4 = H, Ar = 3-OCH_3Ph
28. X = S, R^4 = F, Ar = Ph
29. X = S, R^4 = Cl, Ar = Ph
30. X = S, R^4 = Br, Ar = Ph
31. X = S, R^4 = OCH_3, Ar = Ph
32. X = S, R^4 = OCF_3, Ar = Ph
33. X = S, R^4 = CH_3, Ar = Ph

Scheme 1. General synthetic route to N^4-monosubstituted thiosemicarbazones **10–23**, **28–33**, and semicarbazones **24–27**.



Scheme 2. General synthetic route to N^1-substituted thiosemicarbazones **34**–**37**.



Scheme 3. General synthetic route to N^4-substituted thiosemicarbazones **41**–**49**.

hydrazine (80% in H_2O) and followed by treatment of methyl iodide at 0 °C in ethanol.[31] The displacement of the S-methyl group of methyl hydrazinecarbodithioate **38** by appropriate amine **39** gave the thiosemicarbazide **40**.[32] Subsequent condensation of **40** with isatin in methanol afforded the desired thiosemicarbazones **41–49** in quantitative yields.

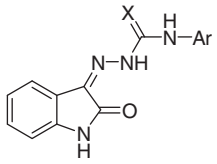
All the isatin-β-thiosemicarbazones described herein were tested in a plaque reduction assay[29] under a standard procedure. In preliminary SAR studies, a series of compounds **10–27** were synthesized according to Scheme 1 and then submitted for anti-HSV-1 and anti-HSV-2 testing as well as cytotoxicity evaluation in the RD cell lines. These biological results were summarized in Table 1. Interestingly, compound **10** exhibited significant activity against HSV-1 (IC_{50} = 2.97 ± 0.19 μM) without cytotoxicity up to the concentration of 25 μM. Surprisingly, it was completely inactive against HSV-2. Introduction of a methyl or methoxy group in the N^4 phenyl ring of compound **10** resulted in compounds (**11–16**) with different antiviral activities. The 3-methyl (**12**), 3-methoxy (**15**), and 4-methoxy (**16**) analogues show similar antiviral activity against HSV-1 (IC_{50} = 3.84 ± 0.41, 2.64 ± 0.21 and 2.20 ± 0.34 μM, respectively). However, the 2-methyl (**11**), 4-methyl (**13**), and 2-methoxy (**14**) analogues exhibit no anti-HSV activity. It is very interesting to note that compound **12** exhibited significant anti-HSV-1 activity with no cytotoxicity up to the concentration of 25 μM. Introduction of a halogen group in the N^4 phenyl ring of compound **10** (**17–21**) revealed that only 4-chloro (**20**) and 4-bromo (**21**) analogues showed activity against HSV-1 (IC_{50} = 2.69 ± 0.36 and 3.29 ± 0.14 μM, respectively). Of the heterocycles placed in the N^4 phenyl ring of compound **10** (**22**, **23**), only 4-imidazole analogue (**22**) showed anti-HSV-1 activity (IC_{50} = 6.21 ± 0.45 μM). On the basis of these results, the various substituents introduced in the N^4 phenyl ring of the thiosemicarbazone had a pronounced influence on its antiviral activity. The lack of anti-HSV activity of these thiosemicarbazones is probably associated with an increase in lipophilic, steric, and electrostatic properties caused by the substituents.

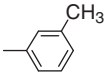
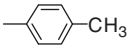
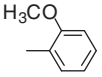
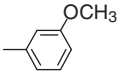
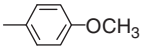
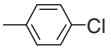
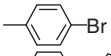
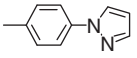
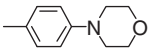
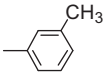
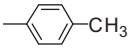
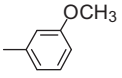
Next, replacement of the thiocarbonyl group of compounds **10**, **12**, **13** and **15** by a carbonyl group gave compounds **24–27**, respectively. All of the urea analogues (**24–27**) were found to be totally inactive, which revealed that the thiourea moiety is a critical structural feature required for antiherpetic activity.

As reported in Table 2, when a substituent (F, Cl, Br, OCH_3, OCF_3, CH_3) was introduced at the C-5 position of indole moiety, to form compounds **28–33**, only 5-methoxy (**31**) and 5-trifluoromethoxy (**32**) analogues showed significant anti-HSV-1 activity (IC_{50} = 5.01 ± 0.72 and 5.90 ± 0.06 μM, respectively). It is also interesting to note that the halogen (**28–30**) and methyl (**33**) groups were considerably less active than the methoxy (**31**) and trifluoromethoxy (**32**) groups at the C-5 position. On the other hand, introduction of various substituents (CH_3, Et, Ph, Benzyl) at *N*-1 of indole moiety, to form compounds **34–37**, resulted in a complete loss of antiherpetic activity as compared with the corresponding compound **10**. These significant results indicated that the NH group in the indole moiety is critical for antiviral activity against HSV. This effect might be due to their drastically conformational change and steric requirement at the *N*-1 position of indole moiety.

In an attempt to confirm the influence on antiviral activity between mono- and di-substituents at the terminal (N^4) position of thiourea moiety, a number of thiosemicarbazones (**41–49**) were prepared (Table 3). As shown in Table 3, the introduction of a diethylamine (**42**) on the thiocarbonyl group showed significant and highly selective activity against HSV-2 (IC_{50} = 1.54 ± 0.21 μM).

Table 1
Antiherpetic activity and cytotoxicity for compounds **10–27**

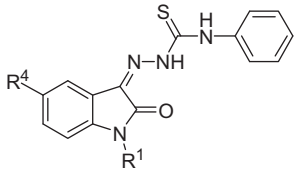


Compound	X	Ar	IC_{50} (μM)[a] HSV-1	IC_{50} (μM)[a] HSV-2	CC_{50} (μM)[b] RD[c]
10	S		2.97 ± 0.19	>25	>25
11	S		>25	>25	>25
12	S		3.84 ± 0.41	>25	>25
13	S		>25	>25	>25
14	S		>25	>25	>25
15	S		2.64 ± 0.21	>25	>25
16	S		2.20 ± 0.34	>25	>25
17	S		>25	>25	>25
18	S		>25	>25	>25
19	S		>25	>25	>25
20	S		2.69 ± 0.36	>25	>25
21	S		3.29 ± 0.14	>25	>25
22	S		6.21 ± 0.45	>25	>25
23	S		>25	>25	>25
24	O		>25	>25	>25
25	O		>25	>25	>25
26	O		>25	>25	>25
27	O		>25	>25	>25

[a] Mean of triplicate well values. All experiments were performed at least twice. Plaque reduction assay was employed.
[b] Mean of triplicate well values. All experiments were performed at least twice.
[c] RD: human rhabdomyosarcoma cells.

However, the dimethylamine (**41**) and dipropylamine (**43**) analogues showed poor activity. Additionally, replacement of the dialkylamino group with larger heterocycloalkyl groups such as pyrrolidine (**44**) and piperidine (**45**) also led to a significant and highly selective activity against HSV-2 (IC_{50} = 5.80 ± 0.52 and 3.14 ± 0.20 μM, respectively). These results demonstrated that most of compounds in this series were highly selective in inhibiting HSV-2. Interestingly, when a morpholinyl ring was introduced at the thiocarbonyl group (NR^2R^3), compound **46** showed significant activity against both HSV-1 (IC_{50} = 1.30 ± 0.16 μM) and HSV-2 (IC_{50} = 2.74 ± 0.23 μM). It is worthy to note that compound **46** was only slightly less active than acyclovir, one of the most commonly-used antiherpetic drugs. This unexpected biological result

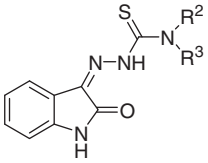
Table 2
Antiherpetic activity and cytotoxicity for compounds **28–37**

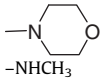


Compound	R^1	R^4	IC_{50} (μM)[a] HSV-1	IC_{50} (μM)[a] HSV-2	CC_{50} (μM)[b] RD[c]
28	H	F	>25	>25	>25
29	H	Cl	>25	>25	>25
30	H	Br	>25	>25	>25
31	H	OCH_3	5.01 ± 0.72	>25	>25
32	H	OCF_3	5.90 ± 0.06	>25	>25
33	H	CH_3	>25	>25	>25
34	CH_3	H	>25	>25	>25
35	Et	H	>25	>25	>25
36	Ph	H	>25	>25	>25
37	Benzyl	H	>25	>25	>25

[a] Mean of triplicate well values. All experiments were performed at least twice. Plaque reduction assay was employed.
[b] Mean of triplicate well values. All experiments were performed at least twice.
[c] RD: human rhabdomyosarcoma cells.

Table 3
Antiherpetic activity and cytotoxicity for compounds **41–49**



Compound	$-NR^2R^3$	IC_{50} (μM)[a] HSV-1	IC_{50} (μM)[a] HSV-2	CC_{50} (μM)[b] RD[c]
41		>25	>25	>25
42		>25	1.54 ± 0.21	>25
43		>25	>25	>25
44		>25	5.80 ± 0.52	>25
45		>25	3.14 ± 0.20	>25
46		1.30 ± 0.16	2.74 ± 0.23	>25
47	$-NHCH_3$	>25	>25	>25
48	–NHEt	>25	>25	>25
49	–NH*n*-Pr	>25	>25	>25
Acyclovir[d]		0.30 ± 0.04	1.27 ± 0.14	>25

[a] Mean of triplicate well values. All experiments were performed at least twice. Plaque reduction assay was employed.
[b] Mean of triplicate well values. All experiments were performed at least twice.
[c] RD: human rhabdomyosarcoma cells.
[d] Standard as antiviral agent.

is not fully understood and is worthy of further study. However, the monoalkylamine analogues **47–49** were completely inactive against both HSV-1 and HSV-2.

In summary, we have developed an efficient synthesis to provide a new class of isatin-β-thiosemicarbazone HSV inhibitors. Several derivatives were found to possess significant and selective antiherpetic activity in a plaque reduction assay. According to our SAR investigation, the thiourea moiety of thiosemicarbazone and the NH functionality at isatin play a very important role in antiherpetic activity of this class of antiviral agents. The mechanism of action of this class of compounds is not yet fully understood. Further SAR studies and mechanistic studies on these new antiherpetic compounds are currently under active investigation and will be reported in due course.

Acknowledgment

We gratefully acknowledge the financial support of the National Health Research Institutes in Taiwan (ROC).

References and notes

1. Roizman, B.; Pellett, P. E. In *Fields Virology*; Knipe, D. M., Howley, P. M., Eds.; Lippincott Williams and Wilkins: Philadelphia, PA, 2001; Vol. 2, p 2381.
2. (a) Spruance, S. L. In *Clinical Management of Herpes Viruses*; Sachs, S. L., Strauss, S. E., Whitley, R. J., Griffiths, P. D., Eds.; IOS: Washington, DC, 1995; pp 3–42; (b) Stanberry, L. R.; Jorgensen, D. M.; Nahmias, A. J. In *Viral Infections of Humans: Epidemiology and Control*; Evans, A. S., Kaslow, R. A., Eds., 4th ed.; Plenum Medical Book Company: New York, 1997; pp 419–454.
3. (a) Roizman, B.; Sears, A. E. In *Fundamental Virology*; Fields, B. N., Knipe, D. M., Howley, P. M., Eds., 2nd ed.; Lippincott-Raven: Philadelphia, 1996; p 1043; (b) Whitley, R. J.; Roizman, B. *Lancet* **2001**, *357*, 1513; (c) Corey, L.; Wald, A. In *Clinical Management of Herpes Viruses*; Sachs, S. L., Strauss, S. E., Whitley, R. J., Griffiths, P. D., Eds.; IOS: Washington, DC, 1995; pp 43–53; (d) Reeves, W. C.; Corey, L.; Adams, H. G.; Vontver, L. A.; Holmes, K. K. *N. Eng. J. Med.* **1981**, *305*, 315; (e) Whitley, R. J.; Roizman, B. In *Clinical Virology*; Richman, D. D., Whitley, R. J., Hayden, F. G., Eds.; Churchill Livingstone: New York, 1997; pp 380–382.
4. (a) Wood, M. J. *J. Antimicrob. Chemother.* **1996**, *37*, 97; (b) Brady, R. C.; Bernstein, David I. *Antiviral Res.* **2004**, *61*, 73; (c) Gilbert, C.; Bestman-Smith, J.; Boivin, G. *Drug Resist. Updates* **2002**, *5*, 88–114.
5. Balfour, H. H., Jr. *N. Eng. J. Med.* **1999**, *340*, 1255.
6. Whitley, R. J.; Gnann, J. W. In *The Human Herpes Viruses*; Roizman, B., Whitley, R. J., Lopez, C., Eds.; Raven Press: New York, 1993; pp 69–105.
7. (a) Field, A. K.; Biron, K. K. *Clin. Microbiol. Rev.* **1994**, *7*, 1; (b) Coen, D. M. In *Antiviral Chemotherapy 4: New Directions for Clinical Application and Research*; Mills, J., Volberding, P., Corey, L., Eds.; Plenum: New York, 1996; p 49.
8. Whitley, R. J.; Kimberlin, D. W.; Roizman, B. *Clin. Infect. Dis.* **1998**, *26*, 541.
9. (a) Coen, D. M.; Schaffer, P. A. *Proc. Natl. Acad. Sci.* **1980**, *77*, 2265; (b) Schnipper, L. E.; Crumpacker, C. S. *Proc. Natl. Acad. Sci.* **1980**, *77*, 2270.
10. (a) Baba, M. *Antiviral Res.* **1997**, *33*, 141; (b) Erice, A. *Clin. Microbiol. Rev.* **1999**, *12*, 286.
11. Domagk, G.; Behnish, R.; Mietzch, F.; Schmidt, H. *Naturwissenshaften* **1946**, *33*, 315.
12. Hamre, D.; Bernstein, J.; Donovick, R. *Proc. Soc. Exp. Biol. Med.* **1950**, *73*, 275.
13. (a) Wagner, W. H.; Winkelmann, E. *Arzneimittelforschung* **1972**, *22*, 1713; (b) Dobek, A. S.; Klayman, D. L.; Dickson, E. T.; Scovill, J. P.; Tramont, E. C. *Antimicrob. Agents Chemother.* **1980**, *18*, 27; (c) Dobek, A. S.; Klayman, D. L.; Dickson, E. T.; Scovill, J. P.; Oster, C. N. *Drug Res.* **1983**, *33*, 1583.
14. Jensen, K. A.; Jensen, C. L. *Acta Chem. Scand.* **1952**, *6*, 957.
15. Klayman, D. L.; Scovill, J. P.; Bartosevich, J. F.; Mason, C. J. *J. Med. Chem.* **1979**, *22*, 1367.
16. (a) French, F. A.; Blanz, E. J., Jr. *J. Med. Chem.* **1966**, *9*, 585; (b) Klayman, D. L.; Scovill, J. P.; Mason, C. J.; Bartosevich, J. F.; Bruce, J.; Lin, A. J. *Drug Res.* **1983**, *33*, 909; (c) Karali, N. *Eur. J. Med. Chem.* **2002**, *37*, 909.
17. (a) Wilson, H. R.; Revankar, G. R.; Tolman, R. L. *J. Med. Chem.* **1974**, *17*, 760; (b) Casero, R. A., Jr.; Klayman, D. L.; Childs, G. E.; Scovill, J. P.; Desjardins, R. E. *Antimicrob. Agents Chemother.* **1980**, *18*, 317.
18. (a) Teitz, Y.; Ronen, D.; Vansover, A.; Stematsky, T.; Riggs, J. L. *Antiviral Res.* **1994**, *24*, 305; (b) Mishra, V.; Pandeya, S. N.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *5*, 183; (c) Bal, T. R.; Anand, B.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4451.
19. (a) Brockman, R. W.; Sidwell, R. W.; Arnett, G.; Shaddix, S. *Proc. Soc. Exp. Biol. Med.* **1970**, *133*, 609; (b) Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. *Antimicrob. Agents Chemother.* **1981**, *19*, 682; (c) Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. *Antiviral Res.* **1986**, *6*, 197; (d) Altun, A.; Kumru, M.; Dimoglo, A. *J. Molec. Struct. (Teochem.)* **2001**, *535*, 235.
20. (a) Sidwell, R. W.; Arnett, G.; Dixon, G. J.; Schabel, F. M., Jr. *Proc. Soc. Exp. Biol. Med.* **1969**, *131*, 1223; (b) Sidwell, R. W.; Arnett, G.; Schabel, F. M., Jr. *Chemotherapy* **1972**, *17*, 259.
21. (a) Sherman, L.; Edelstein, F.; Schtacher, G.; Avramoff, M.; Teitz, Y. *J. Gen. Virol.* **1980**, *46*, 195; (b) Ronen, D.; Nir, E.; Teitz, Y. *Antiviral Res.* **1985**, *5*, 249.
22. Bauer, D.; Sadler, P. *Br. J. Pharmacol.* **1960**, *15*, 101.
23. (a) Bauer, D. J.; St. Vincent, L.; Kempe, C. H.; Downie, A. W. *Lancet* **1963**, *2*, 494; (b) Bauer, D. J. *Ann. N. Y. Acad. Sci.* **1965**, *130*, 110; (c) Heiner, G. G.; Fatima, N.; Russell, P. K.; Haase, A. T.; Ahmad, N.; Mohammed, N.; Thomas, D. B.; Mack, T. M.; Khan, M. M.; Knatterud, G. L.; Anthony, R. L.; McCrumb, F. R. *Am. J. Epidemiol.* **1971**, *94*, 435.
24. (a) Bauer, D.; Apostolov, K. *Science* **1966**, *154*, 796; (b) Bauer, D.; Apostolov, K.; Selway, J. *Ann. N. Y. Acad. Sci.* **1970**, *173*, 314; (c) Levinson, W.; Faras, A.; Woodson, B.; Jackson, J.; Bishop, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 164.
25. Booth, B.; Sartorelli, A. *Mol. Pharmacol.* **1967**, *3*, 290.
26. (a) Easterbrook, K. B. *Virology* **1962**, *17*, 244; (b) Bach, M. K.; Magee, W. E. *Proc. Soc. Exp. Biol. Med.* **1962**, *110*, 565.
27. Xiang, Y.; Simpson, D. A.; Spiegel, J.; Zhou, A.; Silverman, R. H.; Condit, R. C. *J. Virol.* **1998**, *72*, 7012.
28. (a) Katz, E.; Margalith, E.; Winer, B.; Goldblum, N. *Antimicrob. Agents Chemother.* **1973**, *4*, 44; (b) Katz, E.; Margalith, E.; Winer, B. *J. Gen. Virol.* **1974**, *25*, 239; (c) Katz, E.; Margalith, E.; Winer, B. *J. Gen. Virol.* **1973**, *21*, 477; (d) Katz, E.; Winer, B.; Margalith, E.; Goldblum, N. *J. Gen. Virol.* **1973**, *19*, 161; (e) Katz, E.; Margalith, E.; Winer, B. *J. Gen. Virol.* **1978**, *40*, 695.
29. Otto, M. J.; Fox, M. P.; Fancher, M. J.; Kuhrt, M. F.; Diana, G. D.; McKinlay, M. A. *Antimicrob. Agents Chemother.* **1985**, *27*, 883.
30. Azizian, J.; Fallah-Bagher-Shaidaei, H.; Kefayati, H. *Synth. Commun.* **2003**, *33*, 789.
31. (a) Audrieth, L. F.; Scott, E. S.; Kippur, P. S. *J. Org. Chem.* **1954**, 733; (b) Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 855.
32. Agrawal, K. C.; Booth, B. A.; Sartorelli, A. C. *J. Med. Chem.* **1973**, *16*, 715.
33. (a) Pirrung, M. C.; Pansare, S. V.; Sarma, K. D.; Keith, K. A.; Kern, E. R. *J. Med. Chem.* **2005**, *48*, 3045; (b) Quenelle, D. C.; Keith, K. A.; Kern, E. R. *Antiviral Res.* **2006**, *71*, 24; (c) Bal, T. R.; Anand, B.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4451; (d) Hall, M. D.; Salam, N. K.; Hellawell, J. L.; Fales, H. M.; Kensler, C. B.; Ludwig, J. A.; Szakács, G.; Hibbs, D. E.; Gottesman, M. M. *J. Med. Chem.* **2009**, *52*, 3191.