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Thiourea inhibitors of herpesviruses. Part 3: Inhibitors of varicella zoster virus

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Abstract—The preparation of α-methylbenzyl thioureas and their biological activity against varicella zoster virus is described. Several analogs demonstrated IC_{50}s < 0.1 μM and their SAR are discussed. These compounds represent a novel class of potent and selective nonnucleoside inhibitors of varicella zoster virus.

1. Introduction

Varicella zoster virus (VZV), an alpha herpesvirus, is the causative agent of chickenpox in children and shingles in adults. Though in certain instances, this viral infection can lead to encephalitis, a potentially fatal complication for both immunocompetent and immunocompromised populations,[1] more commonly, adulthood VZV disease is accompanied by severe pain known as post-herpetic neuralgia (PHN), which can persist for months after initial skin lesions have resolved. To date, there are only four available therapies for the treatment of VZV disease: acyclovir, valacyclovir, famciclovir, and foscarnet[2] and while these agents can shorten lesion duration, they are at best only marginally effective for the treatment of PHN. Moreover, as these agents (except foscarnet) require intracellular activation by viral thymidine kinase to inhibit viral DNA polymerase, they are likely to suffer from cross-resistance. As part of a program to identify inhibitors of herpesviruses,[3a,4] we have discovered a novel class of specific and potent thiourea inhibitors of VZV replication. Additionally, their mechanism of action, inhibition of the ORF54 protein which, based on its homology to the HSV-1 U_L6 protein, is responsible for the cleavage and packaging of viral DNA into capsids, appears to be unique.[4]

We recently reported the antiviral activity of a series of bis-aryl thioureas (**1**) against human cytomegalovirus (HCMV).[3b] Refinement of this lead series by converting the aryl A-ring to an α-methylbenzyl A-ring (**2**) improved potency against this virus by 1–2 orders of magnitude.[3b] Incorporation of this structural modification into the previously reported HSV-1 inhibitor **3**[5] ultimately led to (±)-**8**, a compound that demonstrated activity against VZV (vide infra). With the goal of enhancing potency against VZV, a separate program was initiated (Fig. 1).

2. SAR

The three aromatic rings and the linker between the A-ring and the thiourea of **8** offered ample opportunity for lead optimization. The SAR of the benzylic carbon of compounds **4**–**12** is shown in Table 1.[6] The α-methyl substituent of **5** is clearly superior to the unsubstituted methylene (**4**), the *gem*-disubstituted derivative (**6**) or α-ethyl linker (compare **7** vs **8**). Moreover, unlike the case with our CMV inhibitors, there was a strict stereochemical requirement as only the *S*-enantiomers were active (**9**–**12**).[7]

With regard to the aromatic A-ring, several substituents were well tolerated, though there was a modest preference for 4-halo groups in this portion of the molecule

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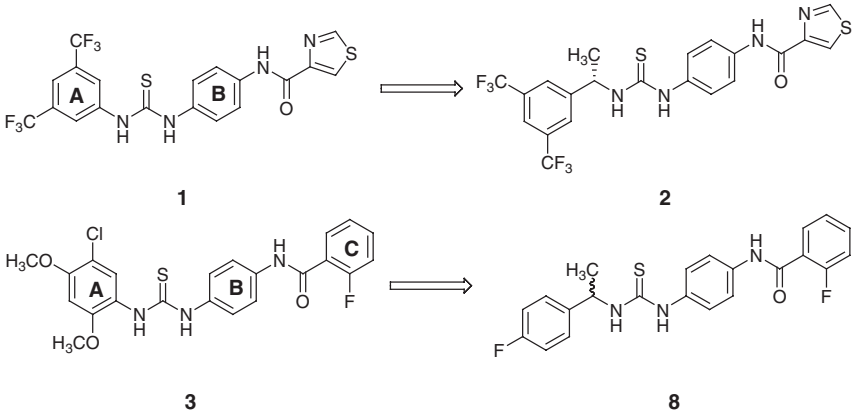
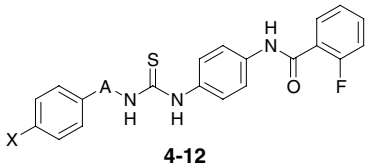


Figure 1. Structures of herpesvirus inhibitors.

Table 1. Effect of A-ring linkers on VZV activity



4-12

Compound	X	A	Chirality	IC_{50}[a]
4	H	$–CH_2–$	—	>26.3
5	H	$–C(CH_3)H–$	±	1.3
6	H	$–C(CH_3)_2–$	—	9.7
7	F	$–C(C_2H_5)H–$	±	>17.6
8	F	$–C(CH_3)H–$	±	0.60
9	H	$–C(CH_3)H–$	*S*	0.70
10	H	$–C(CH_3)H–$	*R*	>19.1
11	Br	$–C(CH_3)H–$	*S*	0.13
12	Br	$–C(CH_3)H–$	*R*	49.1

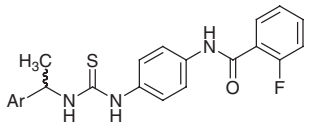
[a] IC_{50} values are μM.

(Table 2; **13**, **14**). Interestingly, the 4-fluorophenyl A-ring derivative **8** yielded the weakest inhibitor of the halogen series but when combined with different B-rings (vide infra), this moiety was most useful. Other aryl groups were examined and initially the potency of benzofuran **16** was encouraging. However, numerous additional fused bicyclic A-rings, including 3-benzothiophenyl (**17**), 2-naphthyl (**18**), 3-indolyl (**19**) as well as a variety of quinoline and isoquinoline derivatives, had no appreciable activity against VZV (3.7 to >17 μM).

In the B-ring, 3-substituted phenyl rings were generally more potent than the corresponding unsubstituted analogs. For instance, the 3-CF_3 and 3-CN derivatives of compound **8**, (compounds **20** and **23**, Table 3) improved potency by 5-10-fold. That electronically dissimilar groups (cf., **20**–**24**) at this position afforded improved activity in most instances argues for a conformational effect of this substituent. However, there is a size limit as increasing from methoxy to ethoxy (**25**) or *N*-phenyl benzamide (**26**) diminishes potency. The 2-substituted analogs (**27**–**29**) did not offer any improvement over compound **8**.

About the C-ring, there was little correlation between this ring or its linker, and potency (Table 4). For instance, aryl (**30**) or heteroaryl (**31**, **32**) groups linked through an amide bond were well tolerated as were

Table 2. A-ring modified VZV inhibitors

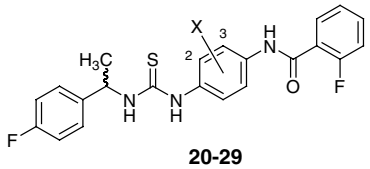


13-19

Compound	Ar	IC_{50}[a]
8	4-F–Ph	0.60
13	4-Cl–Ph	0.21
14	4-Br–Ph	0.42
15	4-CN–Ph	0.96
16	2-Benzofuran	0.18
17	3-Benzothiophene	3.56
18	2-Naphthalene	4.51
19	3-Indole	9.48

[a] IC_{50} values are μM.

Table 3. Racemic B-ring modified VZV inhibitors

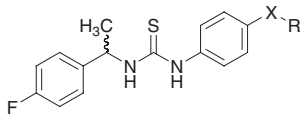


20-29

Compound	X	IC_{50}[a]
8	H	0.60
20	3-CF_3	0.06
21	3,5-Br_2	0.07
22	3-OCH_3	0.09
23	3-CN	0.11
24	3-CH_3	0.12
25	3-OC_2H_5	0.57
26	3-CONHPh	0.26
27	2-CH_3	0.99
28	2-Cl	1.37
29	2-OCH_3	1.54

[a] IC_{50} values are μM.

Table 4. Racemic C-ring substituted VZV inhibitors

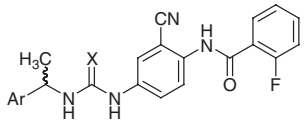


30-34

Compound	X	R	IC_{50}[a]
30	NHCO	2-(OCH_3)-Ph	0.19
31	NHCO	3-Isoquinoline	0.18
32	NHCO	2-Quinoline	0.09
33	3-Isoxazole	4-Quinoline	0.19
34	4-Isoxazole	$PhCH_2$	0.19

[a] IC_{50} values are μM.

Table 5. Urea versus thiourea comparison



23, 35-37

Compound[a]	Ar	X	IC_{50}[b]
35 (***S***)	2-Benzofuran	O	0.11
36 (±)	2-Benzofuran	S	0.11
37 (***S***)	4-F–Ph	O	0.03
23 (±)	4-F–Ph	S	0.11

[a] Compounds are either pure (*S*)-enantiomer or racemic (±) as indicated.

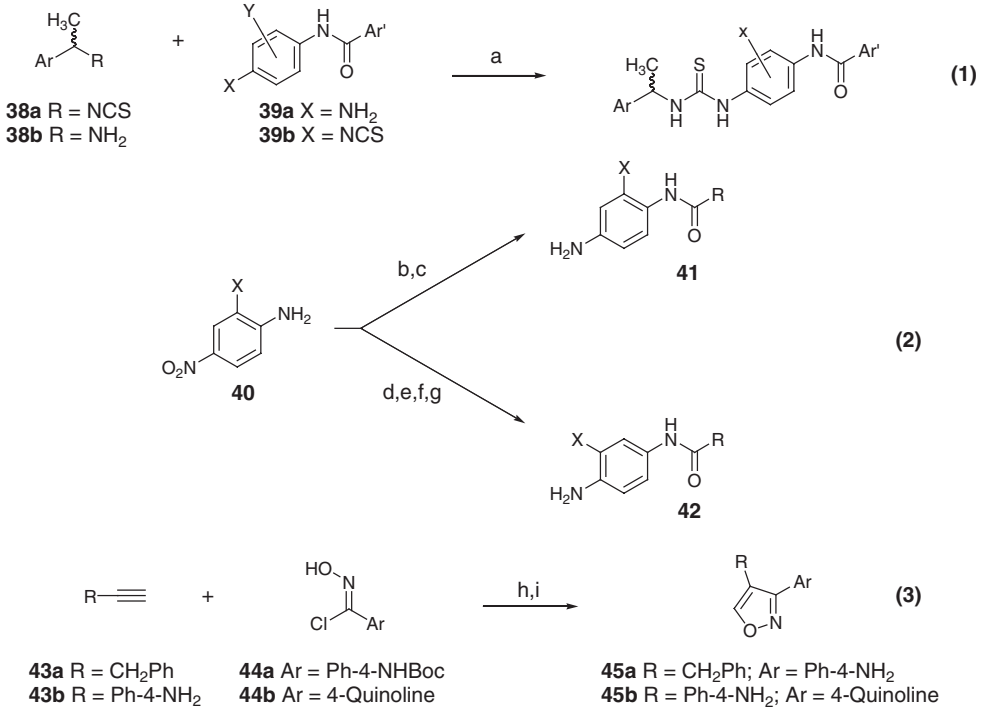
[b] IC_{50} values are μM.

heteroaromatics attached directly to the B-ring (**33**, **34**). We therefore sought to install functional groups at this position that would enhance the pharmacokinetic (PK) profile of these compounds but this effort was unsuccessful.

We also investigated the necessity of the thiourea functionality (Table 5) and found the corresponding ureas to be essentially equipotent with their thiourea counterparts, in contrast to the previously reported CMV inhibitors.[8] However, the ureas suffered from very poor PK properties and were dropped from further consideration.

3. Chemistry

The final products were synthesized in a straightforward manner using the two routes shown (Scheme 1). Treating a hot acetonitrile solution of isothiocyanate **38a** or **39b** with either aniline **39a** or benzyl amine **38b**, respectively, afforded the desired thioureas. The preparation of both the 2- and 3-substituted B-rings started with commercially available 2-substituted-4-nitroanilines (**40**). Acylation of this unreactive aniline under forcing conditions (5 equiv acid chloride, refluxing THF overnight), followed by reduction of the nitro group[9] gave the desired 3-substituted B-rings (**41**). Alternatively, if the amino moiety of **40** was first protected as its trifluoroacetamide, followed by reduction, acylation and deprotection, the result was the requisite 2-substituted B-rings (**42**). The 3- and 4-substituted isoxazole containing B-rings (i.e., **33**, **34**) were prepared via aryl nitrile



Scheme 1. Reagents: (a) hot acetonitrile; (b) acyl chloride, THF, reflux; (c) reduction; (d) TFAA; (e) reduction; (f) acyl chloride, TEA; (g) K_2CO_3, MeOH; (h) TEA; (i) TFA.

oxide addition across an appropriately substituted acetylene (**43**, Scheme 1, reaction 3)[10] followed by deprotection with TFA as necessary. These anilines were then acylated with the appropriate isothiocyanate as described above.

4. Conclusions

In this paper, we have described the synthesis of a series of thioureas with potent and selective cellular activity against VZV. The SAR pattern indicated a strong preference for the *S*-enantiomers about the A-ring linker, a bias toward 3-substituted phenyl B-rings, the presence of some structural tolerance about the C-ring position and finally that ureas are equipotent with their thiourea counterparts. Furthermore, these compounds generated resistance consistent with the inhibition of the cleavage and packaging of viral DNA. Despite the unique mechanism of action of these selective VZV inhibitors, the PK properties of these compounds were limiting.

Acknowledgements

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6. All compounds were assayed as previously described against VZV, CMV, and RSV (respiratory syncytial virus)—see Refs. 3 and 4; 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; general cellular toxicity was measured in the MTS assay (Barltrop, J. A.; Owen, T. C.; Cory, A. H.; Cory, J. G. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 611–614). All compounds reported had RSV and MTS IC_{50} values of >20 μM (data not shown) and were substantially less active against CMV.
7. The trend shown in Table 1 held true for most analogs; in the few instances where little to no preference for either enantiomer was observed, both isomers were essentially inactive.
8. In the case of our CMV inhibitors, the corresponding ureas were about 10–15 times less active; see Ref. 3a.
9. Reduction was accomplished by hydrogenation (H_2, 10% Pd–C), transfer hydrogenation (cyclohexene, 10% Pd–C, refluxing EtOH) or by dissolving metal reduction ($SnCl_2$, MeOH or Fe/NH_4Cl, EtOH).
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