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Thiourea inhibitors of herpes viruses. Part 2: *N*-Benzyl-*N*′-arylthiourea inhibitors of CMV

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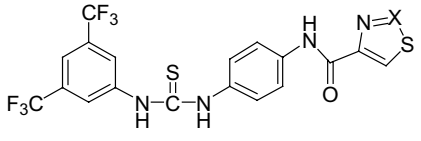
Abstract—A series of highly potent thiourea inhibitors of cytomegalovirus (CMV) with improved stability properties was prepared and evaluated. Compound **29** inhibited the virus in cultured HFF cells with IC_{50} of 0.2 nM.

We recently reported the preparation and evaluation of a series of novel bis(aryl)thiourea inhibitors of cytomegalovirus (CMV).[1] The compounds, as exemplified by structures **1a**,**b** (Fig. 1), were found to be highly potent and specific inhibitors of CMV in cultured human fibroblasts. Unfortunately, as pre-development studies progressed we noted an unexpectedly facile decomposition of the thiourea moiety, especially in acidic media under forcing conditions. Since CMV remains an important infection in certain populations,[2–4] we embarked on an effort to prepare more suitable compounds that would retain anti-CMV activity while exhibiting greater hydrolytic stability.

We reasoned that the decomposition (Fig. 2) likely resulted from protonation of the 3,5-(bis)trifluoromethylaniline[5] nitrogen and subsequent elimination of the corresponding aniline **2** and isothiocyanate **3**. We further assumed that this degradation was facilitated by the electronegative nature of the trifluoromethyl groups, augmenting the leaving group ability of the aniline. The placement of an appropriate spacer between the aniline nitrogen and the phenyl ring would be expected to modulate this effect and therefore improve the stability characteristics of the molecule. A series of *N*-benzyl and *N*-α-alkylbenzyl analogs of type **4** was prepared.



1a (X = CH)
1b (X = N)

Figure 1. Discontinued CMV inhibitors.

Thioureas **4** were easily prepared as shown in Scheme 1 by reaction of amines **5** with isothiocyanates **6**[1] in hot acetonitrile.

The preparation of amines **5** is shown in Scheme 2. In the case where $W = CH_2$ the required 3,5-bis(trifluoromethyl)benzylamine was commercially available. α-Methyl- benzylamines **5**, ($W = CH(CH_3)$) was prepared by conversion of commercially available 3,5-bis(trifluoromethyl)phenyl methyl ketone **7** to the *O*-methyl oxime **9** and subsequent reduction using the conditions reported by Itsuno et al.[7] Alternatively, using the conditions described by Leukhardt,[8] **7** could be converted to the 2-(formamido)ethylbenzene **8**, which yielded **5** upon basic hydrolysis. Enantiomerically pure *S*-(**5a**) or *R*-α-methyl-3,5-(bis)trifluorobenzylamine (**5b**) could be prepared by fractional crystallization of the L- or D-tartrate salt, respectively. The absolute configuration of **5a** and **5b** was determined according to the Mosher amide method.[9]

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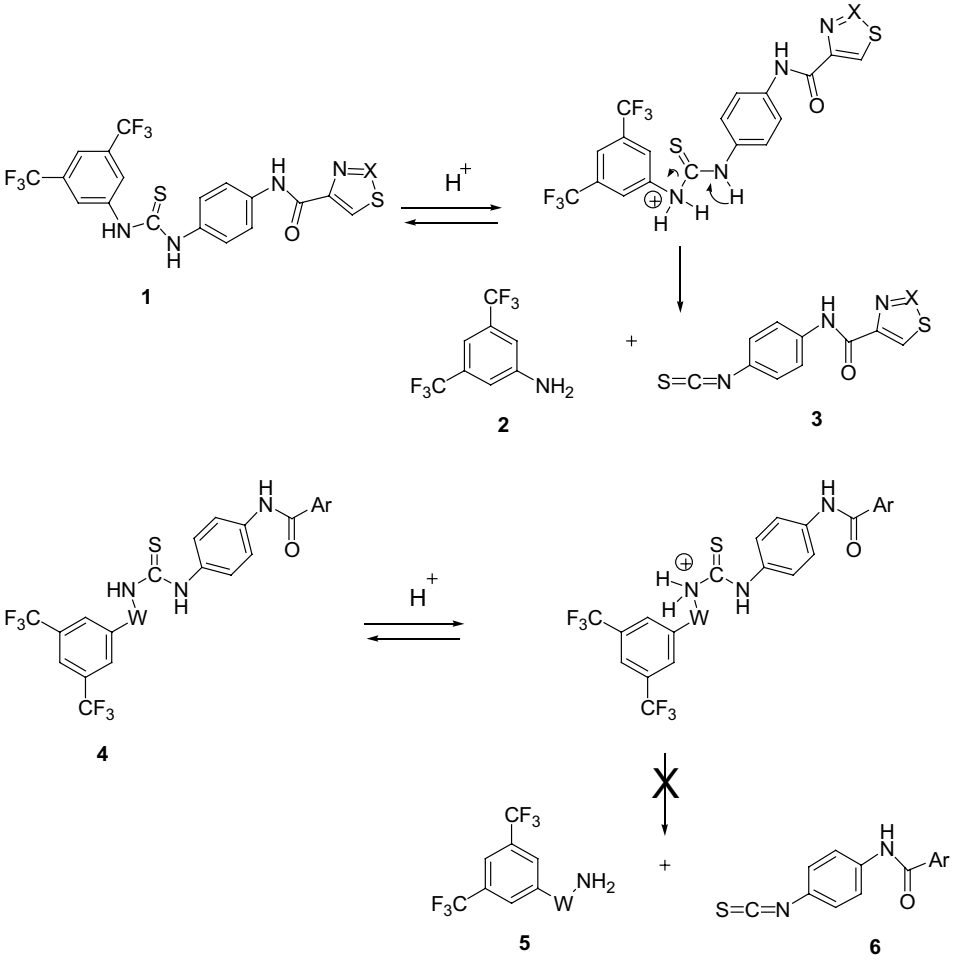
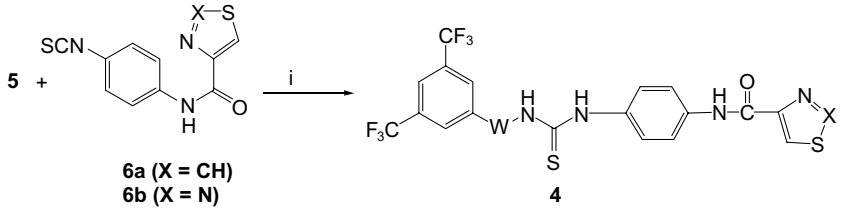
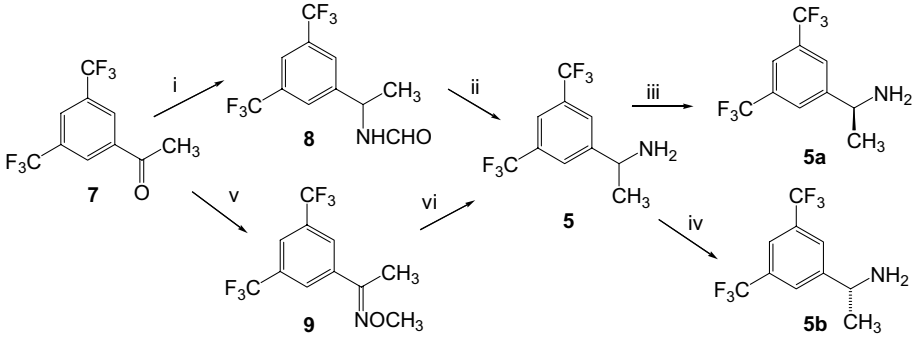


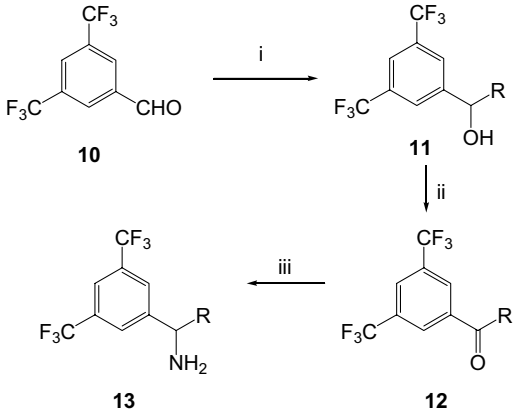
Figure 2. Proposed mechanism of thiourea decomposition.



Scheme 1. Reagents and conditions: (i) hot acetonitrile.



Scheme 2. Reagents and conditions: (i) ammonium formate, formic acid, formamide; (ii) NaOH, H_2O; (iii) L-tartaric acid, EtOH crystallization; (iv) D-tartaric acid, EtOH crystallization; (v) $MeONH_2 \cdot HCl$, pyr. EtOH; (vi) $NaBH_4$, $ZrCl_4$.



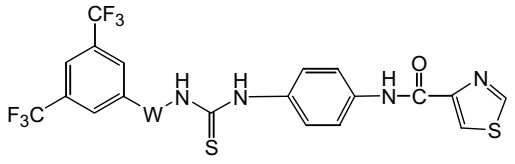
Scheme 3. Reagents and conditions: (i) RMgCl/Et_2O; (ii) Jones oxidation; (iii) see Scheme 2.

More highly substituted ketones **12** were prepared as shown in Scheme 3. This was accomplished by addition of the appropriate Grignard reagent to 3,5-bis(trifluoromethyl)benzaldehyde **10** to give alcohols **11**. Jones oxidation gave the elaborated ketones **12**, which were converted into the α-substituted benzylamines **13** as described in Scheme 2. The *O*-methyl oxime/reduction sequence[7] also worked well for preparation of intermediates **12**.

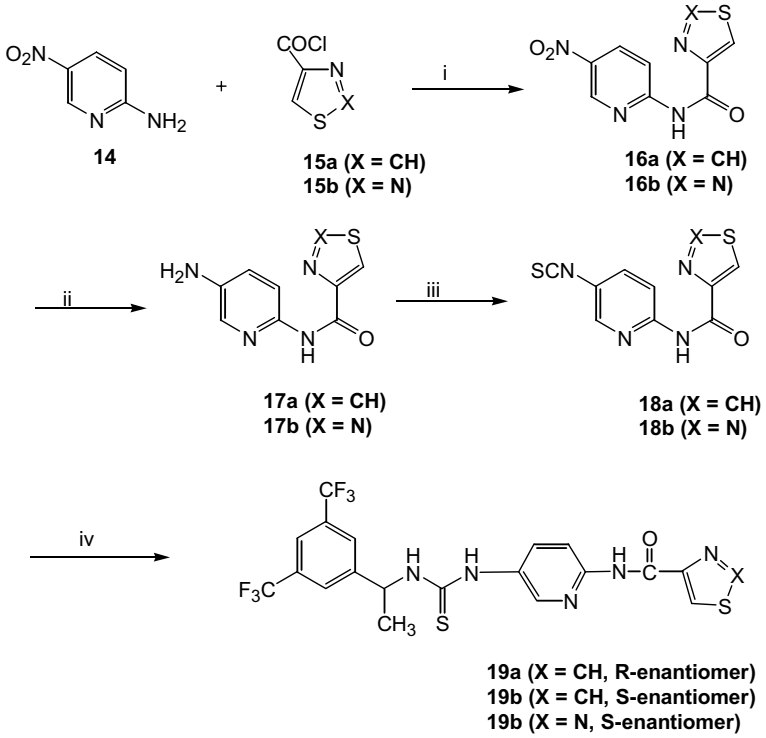
Compounds in which the central phenylenediamine functionality was replaced by the corresponding diaminopyridine group were prepared according to Scheme 4. 2-Amino-4-nitropyridine **14** was heated in THF with excess acid chlorides **15a**,**b** to yield the amides **16a**,**b**, respectively. Reduction of the nitro group using iron and ammonium chloride afforded the aminopyridines **17a**,**b**, which were converted to the corresponding isothiocyanates **18a**,**b** upon treatment with 1,1′-thiocarbonyldiimidazole. Reaction with amines **5a**,**b** provided the thioureas **19a**–**c** as described above.

The stabilizing influence of an alkyl spacer group is illustrated (Table 1) by comparing the rate of degradation of compounds **1** and **28** under forcing conditions.

Table 1. Comparative stability of thioureas **1a** and **28**



Compound, conditions		% Remaining	
		1a	**28**
Water @ 37 °C	1 Day	89	100
	7 Days	44	99
Water @ 60 °C	1 Day	11	ND
	7 Days	0	75
0.1 N HCl @ 37 °C	1 Day	71	100
	7 Days	22	96
1.0 N HCl @ 37 °C	1 Day	52	88
	7 Days	14	34
0.1 N NaOH @ 37 °C	1 Day	98	100
	7 Days	97	99



Scheme 4. Reagents and conditions: (i) refluxing THF; (ii) Fe/NH_4Cl; (iii) 1,1′-thiocarbonyldiimidazole; (iv) **5a**, hot acetonitrile.

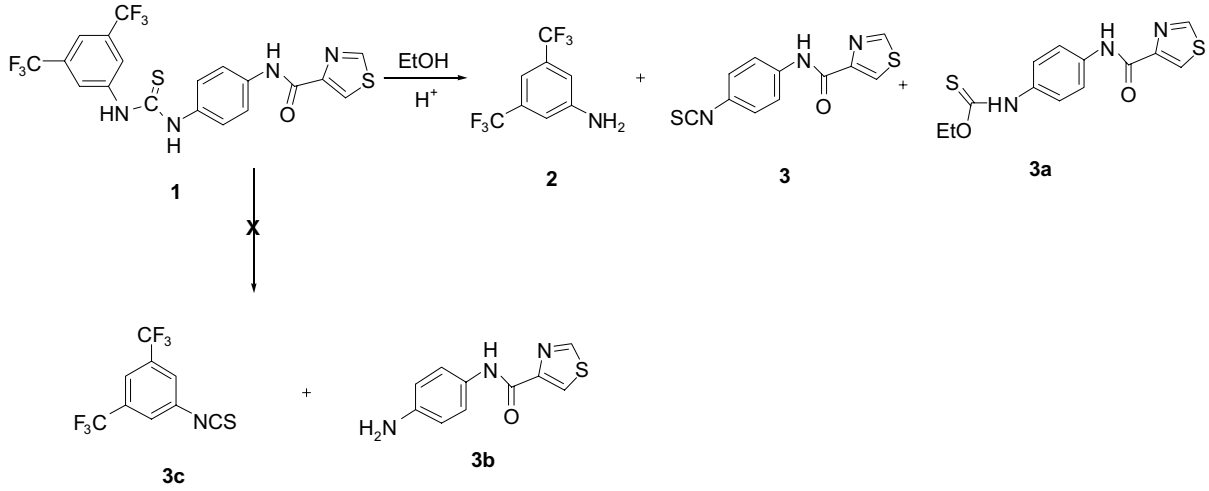


Figure 3. Ethanol trapping experiment.

As expected, significant increases in stability were noted in the α-methylbenzyl analog **28** under neutral, mildly acidic, and strongly acidic conditions when compared to **1**.[6] These data support the assumption that the mechanism of the rapid decomposition of **1** is acid catalyzed and facilitated by the trifluoromethyl groups. The observation that 0.1 N NaOH retarded the decomposition of **1** when compared to water alone is further support for the proposed acid-catalyzed mechanism.

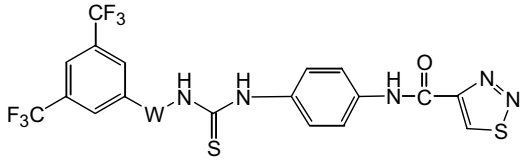
Further supporting evidence for the above mechanism was derived from an ethanol trapping experiment (Fig. 3) in which **1** was exposed to anhydrous acidic ethanol. After 1 day, the reaction products were identified by LC/MS. Aniline **2** and isothiocyanate **3** were identified, in addition to **3a**, the ethanol adduct of **3**. Neither aniline **3b** nor isothiocyanate **3c** were detected. These data support the above mechanism and suggest that while both thiourea nitrogens are probably reversibly protonated, the rate determining step is the elimination of the amino group, which is faster in the case of **2** group than for **5**, presumably due to the difference in leaving group ability.

One possible weakness of the proposed mechanism is that it requires that the weakly basic thiourea nitrogens be protonated. Although protonation would certainly promote the forward reaction, it is also possible that the rate-determining step is, in fact, the breakdown of the tetrahedral intermediate resulting from the direct addition of water to the thiocarbonyl group. This breakdown could then be inhibited by ionization of the (bis)-trifluoromethyl aniline NH bond under basic conditions and subsequent decreased leaving group capability. This alternative mechanism would also explain the base stability of the compounds.

All compounds cited herein (Tables 2–4) were tested[10] against the herpes viruses CMV, HSV (herpes simplex), and VZV (varicella zoster). Additionally, screening against non-herpes viruses such as RSV (respiratory syncytial virus) and analysis of MTS host cell toxicity[11] were performed to distinguish between specific anti-viral activity and nonspecific host cell toxicity.

In addition to demonstrating significant improvement in hydrolytic stability compared to the earlier analogs, the

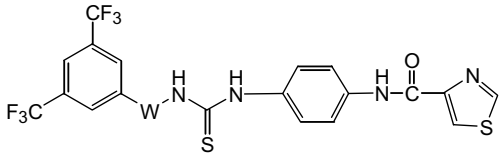
Table 2. Thiadiazole analog SAR



Compound	W	CMV[a]	HSV[a]	VZV[a]	RSV[a]	MTS[a]
1b	None	0.037	>5	>5	5	ND
20	CH_2	0.002	>5	>5	>5	ND
21	*rac*-$CH(CH_3)$	0.003	>5	>3.7	>5	>7.5
22	*S*-$CH(CH_3)$	0.003	>5	>3.7	>5	>7.5
23	CH(Et)	0.001	5	>3.7	>5	>7.5
24	CH(*n*-Pr)	0.001	>5	>3.7	>5	>7.5
25	CH(Ph)	0.002	>5	>3.7	>5	>7.5
26	CMe_2	0.004	>5	>3.7	>5	>7.5

ND = Not determined.
[a] IC_{50}, μM.

Table 3. Thiazole analog SAR



Compound	W	CMV[a]	HSV[a]	VZV[a]	RSV[a]	MTS[a]
1a	None	0.016	>5	>5	>5	ND
27	*R*-CH(CH_3)	0.0015	>5	>3.7	2.0	>7.5
28	*S*-CH(CH_3)	0.0006	>5	>3.7	>5	>7.5
29	CH(Et)	0.0002	4.1	>3.7	>5	>7.5

ND = Not determined.
[a] IC_{50}, μM.

Table 4. SAR of pyridyl analogs

Compound	W	X	CMV[a]	HSV[a]	VZV[a]	RSV[a]	MTS[a]
30	CH_2	N	0.0044	>5	>3.7	>5	>7.5
19a	*R*-CH(CH_3)	CH	0.0028	>5	>3.7	1.0	>7.5
19b	*S*-CH(CH_3)	CH	0.0020	>5	>3.7	>5	>7.5
19c	*S*-CH(CH_3)	N	0.0030	4.1	>3.7	>5	8

[a] IC_{50}, μM.

spacer-containing thioureas were also significantly more potent than the earlier compounds, regardless of the linker chosen (Table 2).

Compound **1b** was previously found[1] to be a highly potent inhibitor against CMV in cells with IC_{50} of 37 nM. Inclusion of a methylene spacer (**20**) improved the activity 15-fold to 0.002 μM (2 nM). Addition of single branched alkyl substituents had little effect on potency. The racemic methyl analog **21** had the same activity as **20** and the *S*-enantiomer **22**. Elaboration of the methyl group resulted in a small increase in potency, with the ethyl (**23**) and *n*-propyl (**24**) analogs being about twofold more active than **15**. The *gem*-dimethyl (**26**) and phenyl (**25**) analogs had similar activity to **20**. Noting that the minimum MTS and other virus IC_{50} values for this series is at least 3.7 μM, one can estimate the selectivity index (SI) to be at least 7500 for the compounds in Table 2.

A similar SAR pattern was found in analogs of the thiazole series (Table 3). As previously reported,[1] the thiazole group affords about fivefold more potency when compared to the corresponding 1,2,3-thiadiazole analogs (**1a** vs **1b**, **22** vs **28**).

There was little difference between the *R*-(**27**) and *S*-(**28**) enantiomers, as shown in Table 3. The branched ethyl analog **29** was the most potent compound in the series (0.2 nM).

Several pyridine analogs were prepared (Fig. 4) to improve solubility characteristics of the molecules. These compounds were about twofold less active than the comparable phenyl analogs, but still retained significant activity and selectivity indices. (Table 4). The

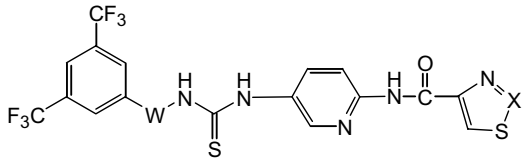


Figure 4. Structures of pyridine analogs.

absence of a stereochemical preference at the benzyl center was once again apparent (**19a** vs **19b**).

This study demonstrated that it was possible to address the stability issues of a previous series that prevented development of the compounds as a potential CMV therapy. The cause of the instability of the original compounds was uncovered and addressed mechanistically by addition of spacer groups to stabilize the thiourea group. Furthermore, these spacer groups afforded greater potency to the molecules. Single digit and subnanomolar inhibitors resulted without any appreciable MTS toxicity or loss of specificity. The most potent compound, **29**, was a 0.2 nM inhibitor of CMV with a selectivity index of at least 150,000. Unfortunately, all of the compounds in the series exhibited very poor bioavailability, which precluded the selection of any development candidates (Fig. 5).

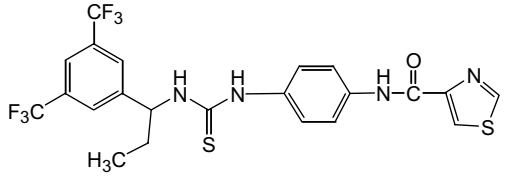


Figure 5. Compound **29**, a 0.2 nM inhibitor of CMV.

References and notes

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5. Although several other substituents (electron withdrawing) on the left-hand phenyl ring are consistent with excellent CMV activity, the 3,5-bis(trifluoro) group was found to be superior. Other substituents are not included in this manuscript.
6. It should be noted that the α-methyl group plays no obvious part in this stabilization, as corresponding analogs with a simple methylene spacer show similar stability characteristics. The selection of α-methyl compounds was made based on PK considerations.
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10. 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.
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