



1-Benzyl derivatives of 5-(arylamino)uracils as anti-HIV-1 and anti-EBV agents

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ARTICLE INFO

Article history:

Received 6 August 2010

Revised 27 September 2010

Accepted 29 September 2010

Available online 7 October 2010

Keywords:

Pyrimidine

Uracil

HIV

EBV

Antiviral

ABSTRACT

Pyrimidine analogs have long found use over a broad chemotherapeutic spectrum. In an effort to further explore the antiviral potential of several uracil derivatives previously synthesized in our laboratories, a series of benzylated pyrimidines were designed and synthesized. Introduction of the benzyl residue onto the 5-phenylaminouracil scaffold was carried out using 2,4-bis(trimethylsilyloxy)pyrimidine with the corresponding benzyl bromides. Similarly, 1-benzyl-5-(benzylamino)- and 1-benzyl-5-(phenethylamino)uracils were obtained via amination of 1-benzyl-5-bromouracils with benzylamine or phenylethylamine. The results of the broad screen antiviral studies revealed that compounds **5** and **11** exhibit promising inhibitory activity against HIV-1 in CEM-SS culture. A 50% protective effect was observed at concentrations of 11.9 and 9.5 μM , respectively. Moreover, compounds **8** and **3** exhibited good inhibitory effects against EBV in AKATA cell culture with EC_{50} values of 2.3 and 12 μM , respectively. The synthesis and biological studies are detailed herein.

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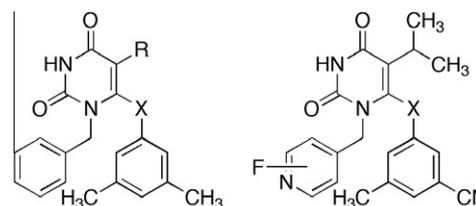
1. Introduction

Nucleosides and their corresponding nucleobases are key components in nucleic acids and many other biologically significant systems, thus modification to their structure can have a significant effect. In that regard, many nucleoside and nucleobase derivatives have exhibited a broad spectrum of activity, in particular, in the area of antiviral chemotherapy.^{1–4} Inhibition of a key step in the viral replication pathway can lead to potent activity against many viruses including HIV, hepatitis B and C (HBV and HCV), the herpes viruses (HSV-1 and -2), Epstein-Barr virus (EBV), and human cytomegalovirus (HCMV), just to name a few.^{1,5–7} In the realm of non-nucleoside pyrimidine analogs, a vast number of 6-thiophenyl-,^{7–11} 6-selenylphenyl-,^{12,13} 6-phenoxy-,¹⁴ 6-benzyl-,^{15–17} and 6-benzyluracil-¹⁸ pyrimidines bearing different substituents at position 1 are known. Potent activity against HIV in cell culture has been noted for these compounds, most notably, analogs possessing a 1-benzyl or 4-pyrimidyl-methyl substituent (Fig. 1). These compounds were found to block HIV-1 reverse transcriptase (HIV RT)^{12,16,17} at nanomolar concentrations, thus inhibiting viral replication at an early stage.¹²

Moreover, although numerous 6-aryl non-nucleoside derivatives are known, the analogous 5-aryl pyrimidines have received far

less attention. There are, however, several 5-aryl substituted pyrimidine nucleosides known in the literature, including from our laboratory (Fig. 2), some of which have exhibited potent biological activity.^{19–24} Similar to Tenofovir²⁵ and Etravirine,^{26–28} both FDA-approved HIV-1-RT inhibitors, our compounds' inherent flexibility allows them to overcome resistance mechanisms related to point mutations.^{29,30} The concepts of conformational flexibility and positional adaptability, or the ability to 'wiggle and jiggle' in a binding site when confronted with an active site mutation have now been shown^{26–28} to be critical for avoiding resistance mechanisms for non-nucleoside HIV-1-RT inhibitors, thus provide additional impetus for our approach.

Additional examples pertinent to our approach can be found in the 1,3-dibenzyl-derivatives of uracil from Maruyama et al.^{31,32}



R=Et, *i*Pr,
X=S, Se, CO, O

Figure 1.

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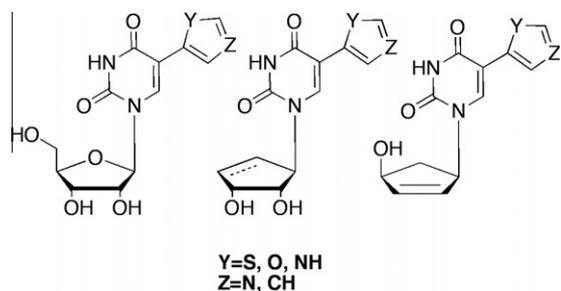


Figure 2. 5-Substituted pyrimidine nucleosides.

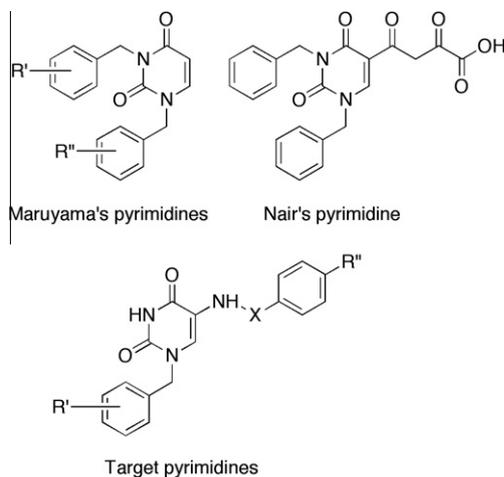


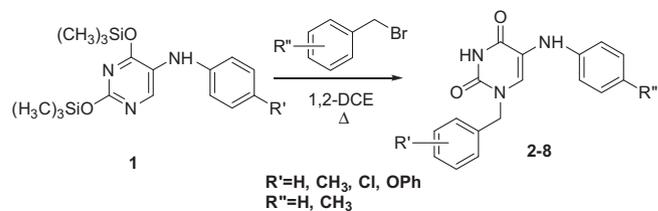
Figure 3.

(Fig. 3). These aryl substituted uracils were shown to block HIV-1 and HCMV replication in vitro in sub-micromolar concentrations.^{31,32} Potent activity was also noted for a similar 1,3-dibenzyl inhibitor developed by Nair et al., which exhibited potent activity against the HIV integrase (Fig. 3).³³ On the basis of the leads outlined above, we have designed a series of aryl substituted pyrimidines where the aromatic fragment has been relocated from C-6 or N-3 to C-5 of the uracil pharmacophore, as well as to introduce a nitrogen into the bridging group (Fig. 3) to increase potential interactions in enzymatic binding sites. We believe these strategic modifications will lead to potential inhibition in the replication of viruses of a number of biologically significant diseases.

2. Chemistry

The synthesis 5-(arylamino)-1-benzyluracils can be realized by way of a modification to the Hilbert–Johnson reaction widely used for the synthesis of pyrimidine nucleosides and their acyclic analogs. The method involves treatment of 2,4-bis(trimethylsilyloxy)pyrimidines with highly reactive alkylating agents under mild conditions.^{14,18,34–36} Use of alkylating agents with decreased reactivity however requires more harsh conditions. For example, Malik et al. have described addition of a benzyl residue to uracil by coupling 2,4-bis(trimethylsilyloxy)pyrimidine with benzyl chloride in boiling 1,2-dichloroethane for 96 h in the presence of a catalytic amount of iodine.³⁷ In an effort to optimize this reaction, we found that the use of benzyl bromides in place of benzyl chlorides considerably shortened the reaction time from 96 h to 14 h.

In that regard, 1-benzyl-5-(phenylamino)uracils **2–8** were prepared by coupling 2,4-bis(trimethylsilyloxy)-5-phenylamino-uracil **1** with the corresponding benzyl bromides in refluxing



Scheme 1.

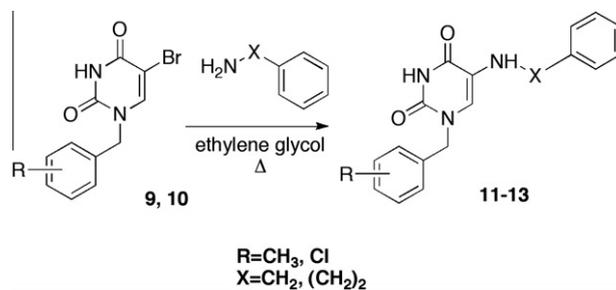
1,2-dichloroethane (Scheme 1). The silyl derivative **1** was prepared and characterized as previously reported.³⁸ The yields of compounds **2–8** ranged from 61% to 77%. It is notable that the formation of 1,3-substitution products was not observed (TLC).

Similarly, 1-benzyl derivatives of 5-(benzylamino)- and 5-(phenethylamino)uracils **11–13** were obtained by amination of 1-(3,5-dimethylbenzyl)- and 1-(2,4-dichlorobenzyl)-5-bromouracil (**9** and **10**, respectively) with excess benzylamine or phenethylamine in refluxing ethylene glycol for 1 h (Scheme 2) in yields averaging 70–75%. Notably, formation of 1-benzyl-5-(phenylamino)uracils was not observed under these conditions. The physical properties of the synthesized compounds are shown in Table 1.

3. Biological assays

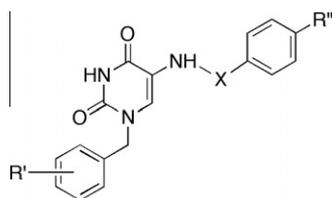
The compounds were then subjected to screening across a broad range of viruses in order to evaluate their biological potential both in Southern Research Institute's high-throughput screening program, as well as in our collaborator's laboratory at ImQuest BioSciences. Not surprisingly, two of the compounds (**5** and **11**) demonstrated promising inhibitory activity against HIV. As shown in Table 2, a 50% protective effect was observed at concentrations of 11.9 and 9.5 μM , respectively, in CEM-SS cell culture. Some of the other 1-benzyl-5-(arylamino)uracils tested were less active or, as in the case of **2**, **3**, and **7**, completely inactive. It is noteworthy that both compounds **5** and **11** possess the same benzyl fragment, that is, a 3,5-dimethylphenylmethyl residue at the N1 position of the uracil ring. From this it appears that the presence of the methyl substituents in *meta* positions of the benzyl fragment favorably affects the inhibitory properties of these compounds.

In addition to the anti-HIV activity noted, several of the compounds also exhibited activity against Epstein-Barr virus in AKATA cell culture.¹⁸ As shown in Table 3, results revealed that most of the compounds demonstrated some level of antiviral effect. The most active compound was 1-(3-phenoxybenzyl)-5-(phenylamino)uracil (**8**) with an EC_{50} value of 2.3 μM and no toxicity observed at a concentration of 100 μM . A second active compound was 1-(2-methylbenzyl)-5-(phenylamino)uracil (**3**) whose EC_{50} was 12 μM . While several uracil nucleosides modified at the sugar and/or base have demonstrated potent anti-Epstein-Barr virus (EBV) activity at the sub-micromolar level^{14,39,40} to our knowledge, similar



Scheme 2.

Table 1
Physical properties for the synthesized compounds



Compound	R'	R''	X	Yield (%)	Mp (°C)	R _f ^a
2	H	H	—	77	230–232	0.37
3	2-Me	H	—	61	191–192	0.49
4	3-Me	H	—	64	222–223	0.43
5	3,5-Me	H	—	69	197–198	0.55
6	3-Cl	H	—	72	235–236	0.51
7	2,4-Cl	4-Me	—	62	204–205	0.64
8	3-OPh	H	—	61	192–194	0.58
11	3,5-Me	H	CH ₂	71	152–153	0.71
12	2,4-Cl	H	CH ₂	75	177–178	0.78
13	2,4-Cl	H	(CH ₂) ₂	70	167–168	0.79

^a Elution with ethyl acetate.

Table 2
Anti-HIV-1 activity measured in CEM-SS cell culture

Compound	EC ₅₀ ^a (μM)	CC ₅₀ ^b (μM)	SI ^c
2	>100.0	>100.0	—
3	>100.0	>100.0	—
4	44.7	>200.0	>4.5
5	11.9	>200.0	>16.8
6	ND	ND	ND
7	ND	ND	ND
8	>100.0	>100.0	—
11	9.5	168.0	17.8
12	54.6	>100.0	>1.8
13	42.7	>100.0	>2.34
AZT	0.008	>1.0	>125.0

ND—not determined.

^a Effective concentration, giving 50% inhibition of viral replication.

^b Cytotoxic concentration, concentration producing 50% cytotoxicity.

^c Selectivity index, CC₅₀/EC₅₀.

Table 3
Anti-EBV activity in AKATA cell culture

Compound	EC ₅₀ ^a (μM)	CC ₅₀ ^b (μM)	SI ^c
2	ND	ND	ND
3	12	>100	>8.3
4	99.1	>100	>1
5	>20	96.2	<4.8
6	20	>100	>5
7	43.3	92.3	2.1
8	2.3	>100	>43
11	ND	ND	ND
12	39.6	96.3	2.4
13	67.2	>100	>1.5
L-I-OddU	0.033	>100	>3030

ND—not determined.

^a Effective concentration, concentration which reduces viral replication by 50%

^b Cytotoxic concentration, concentration which 50% of the cells are destroyed.

^c Selectivity index, CC₅₀/EC₅₀.

activity has not been noted for non-nucleoside pyrimidine-like inhibitors such as ours.

Although the activity noted for some of these compounds is encouraging, one problem noted during their synthesis and screening was their poor solubility in aqueous solution. Even in the presence of DMSO a precipitate was noted for some analogs, which could be one explanation for why some of the compounds with

very similar substituents showed a significant difference in activity. Efforts are currently underway to address this issue by investigating additional substituents that would impart greater solubility under physiological conditions.

4. Summary

As an extension of the ongoing research in our laboratories, the search for new and more effective antiviral agents based on the pyrimidine scaffold was undertaken. Results showed that the electronic nature of the aromatic amine moiety was critical to the yield. As a result, a new and more facile route was developed to realize the synthesis of several new types of 1-benzyl-substituted 5-arylamino uracils. The antiviral properties of the target compounds were studied and several compounds were observed to have promising activity against HIV and EBV. Issues with solubility may be a factor in truly realizing their full potential however, thus current SAR efforts are now underway to explore alternative substituents as a solution to this problem. Additional biological studies are also currently underway and the results of those investigations will be published elsewhere as they become available.

5. Experimental

All chemicals were obtained from commercial sources and used without further purification unless otherwise noted. Anhydrous DMF, isopropanol, and ethylene glycol were purchased from Sigma–Aldrich Co. Anhydrous acetone, CH₂Cl₂, 1,2-dichloroethane, and ethyl acetate were obtained by distillation over P₂O₅. Melting points were measured on Mel-Temp 3.0 Pro apparatus (Laboratory Devices Inc.) and uncorrected. All ¹H and ¹³C NMR spectra were obtained on a Varian Mercury 300B (300 MHz) NMR, operated at 300 and 75 MHz, respectively, and referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel precoated plates. Column chromatography was performed using silica gel (63–200 m) from Merck Chemicals, and eluted with ethyl acetate. Yields refer to chromatographically and spectroscopically (¹H and ¹³C NMR) homogeneous materials. Mass spectra were recorded at the Johns Hopkins Mass Spectrometry Facility.

5.1. General procedure for preparation of 1-benzyl-5-(phenylamino)uracils 2–8

A solution of the specific benzyl bromide (6.81 mmol) in 1,2-dichloroethane (20 mL) was added to a solution of 2,4-bis(trimethylsilyloxy)-5-(arylamino)pyrimidine **1** in 1,2-dichloroethane (50 mL). The resulting mixture is refluxed for 14 h, cooled to room temperature and EtOH (10 mL) added. The precipitate was filtered and air dried. The filtrate was evaporated by two thirds and cooled to 0 °C. The resulting precipitate was filtered and combined with the previously obtained solid and purified by column chromatography eluting with EtOAc/CH₂Cl₂ (1:1) or by recrystallization from isopropanol/DMF (2:1).

5.1.1. 1-Benzyl-(phenylamino)uracil (2)

¹H (DMSO-*d*₆): δ 4.85 (s, 2H, CH₂), 6.61 (t, 1H, *J* = 7, H-6'), 6.69–6.71 (m, 2H, aromatic H), 7.00–7.07 (m, 3H, aromatic H, NH), 7.22–7.34 (m, 5H, C₆H₅), 7.65 (s, 1H, H-6), 11.54 (s, 1H, NH). ¹³C (DMSO-*d*₆): δ 48.2, 112.4, 114.7, 116.1, 125.5, 126.6, 126.8, 132.9, 135.1, 143.6, 148.0, 159.7. HRMS calculated for C₁₇H₁₅N₃O₂, [M+H]⁺ 293.11643; found 293.11677.

5.1.2. 1-(2-Methylbenzyl)-5-(phenylamino)uracil (3)

^1H (DMSO- d_6): δ 2.23 (s, 3H, CH₃), 4.85 (s, 2H, CH₂), 6.61 (t, 1H, $J = 7$, aromatic H), 6.68–6.71 (m, 1H, H-5'), 6.99–7.05 (m, 6H, C₆H₅, NH), 7.13–7.16 (m, 2H, H-3', H-6'), 7.43 (s, 1H, H-6), 11.58 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 16.6, 46.2, 112.5, 114.9, 116.2, 124.1, 124.8, 125.4, 126.8, 128.4, 131.9, 132.8, 133.6, 143.4, 147.9, 159.7. HRMS calculated for C₁₈H₁₇N₃O₂, [M+H]⁺ 307.13208; found 307.13138.

5.1.3. 1-(3-Methylbenzyl)-5-(phenylamino)uracil (4)

^1H (DMSO- d_6): δ 2.24 (s, 3H, CH₃), 4.81 (s, 2H, CH₂), 6.61 (t, 1H, $J = 7$, aromatic H), 6.69–6.72 (m, 1H, aromatic H), 6.99–7.09 (m, 6H, C₆H₅, NH), 7.17–7.22 (m, 2H, H-2', H-6'), 7.63 (s, 1H, H-6), 11.53 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 18.9, 48.1, 112.3, 114.6, 116.1, 122.6, 126.0, 126.3, 126.6, 126.8, 133.1, 135.0, 135.8, 143.8, 147.9, 159.8. HRMS calculated for C₁₈H₁₇N₃O₂, [M+H]⁺ 307.13208; found 307.13279.

5.1.4. 1-(3,5-Dimethylbenzyl)-5-(phenylamino)uracil (5)

^1H (DMSO- d_6): δ 2.20 (s, 6H, CH₃), 4.77 (s, 2H, CH₂), 6.61 (t, 1H, $J = 7$, aromatic H), 6.68–6.71 (m, 2H, aromatic H), 6.88 (s, 3H, aromatic H, NH), 7.00–7.07 (m, 2H, aromatic H), 7.61 (s, 1H, H-6), 11.51 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 18.8, 48.0, 112.3, 114.6, 116.1, 123.3, 126.8, 127.1, 133.2, 134.9, 135.7, 143.8, 148.0, 159.8. HRMS calculated for C₁₉H₁₉N₃O₂, [M+H]⁺ 321.14773; found 321.14809.

5.1.5. 1-(3-Chlorobenzyl)-5-(phenylamino)uracil (6)

^1H (DMSO- d_6): δ 4.85 (s, 2H, CH₂), 6.61 (t, 1H, $J = 7$, aromatic H), 6.69–6.72 (m, 2H, aromatic H), 7.00–7.07 (m, 3H, aromatic H, NH), 7.22–7.29 (m, 4H, aromatic H), 7.65 (s, 1H, H-6), 11.55 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 47.7, 112.4, 114.9, 116.2, 124.2, 125.4, 125.6, 126.8, 128.6, 131.2, 132.6, 137.6, 143.6, 147.9, 159.8. HRMS calculated for C₁₇H₁₄ClN₃O₂, [M+H]⁺ 327.07745; found 327.07758.

5.1.6. 1-(2,4-Dichlorobenzyl)-5-[(4-methylphenyl)amino]uracil (7)

^1H (DMSO- d_6): δ 2.11 (s, 3H, CH₃), 4.89 (s, 2H, CH₂), 6.71–6.73 (m, 2H, aromatic H), 6.87–6.89 (m, aromatic H, NH), 7.17–7.20 (m, 1H, H-6'), 7.37–7.40 (m, 1H, H-5'), 7.46 (s, 1H, H-3'), 7.62 (s, 1H, H-6), 11.60 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 18.2, 46.3, 113.5, 116.0, 125.6, 126.9, 127.3, 127.9, 128.6, 130.8, 131.6, 140.2, 147.6, 159.6. HRMS calculated for C₁₈H₁₅Cl₂N₃O₂, [M+H]⁺ 375.05413; found 375.05409.

5.1.7. 1-(3-Phenoxybenzyl)-5-(phenylamino)uracil (8)

^1H (DMSO- d_6): δ 4.83 (s, 2H, CH₂), 6.62 (t, 1H, $J = 7$, aromatic H), 6.68–6.71 (m, 2H, aromatic H), 6.86–7.11 (m, 9H, aromatic H, NH), 7.29–7.35 (m, 3H, aromatic H), 7.66 (s, 1H, H-6), 11.53 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 47.9, 112.4, 114.8, 115.6, 116.1, 116.6, 120.5, 121.7, 126.8, 128.1, 128.4, 132.7, 137.3, 143.6, 147.9, 154.3, 159.7, 181.3. HRMS calculated for C₂₃H₁₉N₃O₃, [M+H]⁺ 385.14264; found 385.14412.

5.2. General procedure for preparation of 5-benzylamino- and 5-phenethylamino-derivatives of 1-benzyluracil 11–13

A mixture of 1-(3,5-dimethylbenzyl)- (9) or 1-(2,4-dichlorobenzyl)-5-bromouracil (10) (5.08 mmol), benzylamine or phenethylamine (18.31 mmol) and ethylene glycol (10 mL) was refluxed for 1 h, cooled to room temperature and the crystallized mass was treated with cold water (100 mL). The precipitate was filtered off, dried in air, and recrystallized from acetone/DMF 1:1 mixture.

5.2.1. 5-(Benzylamino)-1-(3,5-dimethylbenzyl)uracil (11)

^1H (DMSO- d_6): δ 2.14 (s, 6H, CH₃), 4.02 (d, 2H, $J = 6$, CH₂Ph), 4.59 (s, 2H, CH₂Ar), 5.10 (t, 1H, $J = 6$, NHCH₂Ph), 6.47 (s, 1H, H-4'), 6.68 (s, 2H, H-2', H-6'), 6.81 (s, 1H, aromatic H), 7.14–7.22 (m, 4H, aromatic H), 7.23 (s, 1H, H-5), 11.37 (s, 1H, NH). ^{13}C (DMSO- d_6): δ 18.9, 44.6, 48.0, 114.9, 123.1, 124.8, 125.2, 126.1, 126.8, 135.4, 146.6, 158.7.

5.2.2. 5-(Benzylamino)-1-(2,4-dichlorobenzyl)uracil (12)

^1H (DMSO- d_6): δ 3.96 (d, 2H, $J = 6.3$, CH₂Ph), 4.73 (s, 2H, CH₂Ar), 5.13 (t, 1H, $J = 6.3$, NHCH₂Ph), 6.37 (s, 1H, H-3'), 6.93–6.95 (m, 1H, H-6'), 7.12–7.19 (m, 5H, C₆H₅), 7.26–7.30 (m, 1H, H-5'), 7.54 (s, 1H, H-6), 11.48 (s, 1H, N³-H). ^{13}C (DMSO- d_6): δ 44.6, 45.7, 114.5, 122.1, 124.7, 125.1, 125.5, 126.2, 126.8, 127.9, 130.8, 131.5, 136.7, 146.7, 158.8. HRMS calculated for C₁₈H₁₅Cl₂N₃O₂, [M+H]⁺ 375.05413; found 375.05395.

5.2.3. 1-(2,4-Dichlorobenzyl)-5-(phenethylamino)uracil (13)

^1H (DMSO- d_6): δ 2.73 (m, 2H, $J = 7$, CH₂CH₂Ph), 2.94 (m, 2H, $J = 7$, CH₂CH₂Ph), 4.45 (t, 1H, $J = 6$, NH), 4.82 (s, 2H, CH₂Ar), 6.65 (s, 1H, H-3'), 7.02–7.05 (m, 1H, H-6'), 7.10–7.24 (m, 5H, C₆H₅), 7.34–7.38 (m, 1H, H-5'), 7.61 (s, 1H, H-6), 11.49 (s, 1H, N³-H). ^{13}C (DMSO- d_6): δ 32.1, 43.0, 46.0, 114.3, 122.7, 124.1, 125.6, 126.2, 126.6, 126.8, 127.2, 130.6, 131.8, 146.8, 158.8. HRMS calculated for C₁₉H₁₇Cl₂N₃O₂, [M+H]⁺ 389.06978; found 389.07049.

6. Biological evaluation

Antiviral activity of the compounds was measured by inhibition of virus-induced cytopathic effects (CPE) using CEM-SS cells infected with HIV-1.⁴¹ Briefly, compound-induced protection from virus-induced cytopathic effects (cytoprotection) was measured using a mitochondria-activated dye with quantification by a colorimetric endpoint. AZT was utilized as a positive control compound in all studies performed and exhibited the expected level of activity ranging between 1 and 10 nM. For the CEM-SS assay 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) dye reduction following activation with phenazine methosulfate (PMS) at 450/650 nM was used to measure cell survival at 6 days post infection. For both assays the EC₅₀ (concentration resulting in 50% reduction in virus replication) and CC₅₀ (concentration resulting 50% loss of cell viability in cells without virus) values are calculated by linear regression analysis. A therapeutic index (TI) is calculated by dividing the TC₅₀ by the EC₅₀.

Acknowledgments

This work was supported by grants from the Russian Foundation For Basic Research (10-04-0056, 09-04-12204, and 08-04-00549), and by the Presidium of the Russian Academy of Sciences (Programme 'Molecular and Cellular Biology'). The authors would also like to thank Southern Research Institute for additional antiviral screening, including the EBV assays described herein as part of their high-throughput screening program.

References and notes

- De Clercq, E. *J. Med. Chem.* **2010**, *53*, 1438.
- Este, J. A.; Cihlar, T. *Antiviral Res.* **2010**, *85*, 25.
- Cihlar, T.; Ray, A. S. *Antiviral Res.* **2010**, *85*, 39.
- De Clercq, E. *Expert Opin. Emerg. Drugs* **2008**, *13*, 393.
- Schinazi, R. F.; Bassit, L.; Gavegnano, C. *J. Viral Hepat.* **2010**, *17*, 77.
- Wen, Y. M.; Lin, X.; Ma, Z. M. *Curr. Drug Targets Infect. Disord.* **2003**, *3*, 241.
- De Clercq, E.; Andrei, G.; Snoeck, R.; De Bolle, L.; Naesens, L.; Degreve, B.; Balzarini, J.; Zhang, Y.; Schols, D.; Leysen, P.; Ying, C.; Neyts, J. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 271.

8. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Inouye, N.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1995**, *38*, 2860.
9. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1992**, *35*, 337.
10. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1992**, *35*, 4713.
11. Tanaka, H.; Matsuda, A.; Iijima, S.; Hayakawa, H.; Miyasaka, T. *Chem. Pharm. Bull. (Tokyo)* **1983**, *31*, 2164.
12. Buckheit, R. W., Jr.; Hartman, T. L.; Watson, K. M.; Chung, S. G.; Cho, E. H. *Antimicrob. Agents Chemother.* **2008**, *52*, 225.
13. Buckheit, R. W., Jr.; Hartman, T. L.; Watson, K. M.; Kwon, H. S.; Lee, S. H.; Lee, J. W.; Kang, D. W.; Chung, S. G.; Cho, E. H. *Antiviral Chem. Chemother.* **2007**, *18*, 259.
14. Beauchamp, L. M.; Serling, B. L.; Kelsey, J. E.; Biron, K. K.; Collins, P.; Selway, J.; Lin, J. C.; Schaeffer, H. J. *J. Med. Chem.* **1988**, *31*, 144.
15. El-Brollosy, N. R.; Jorgensen, P. T.; Dahan, B.; Boel, A. M.; Pedersen, E. B.; Nielsen, C. *J. Med. Chem.* **2002**, *45*, 5721.
16. Mitchell, M. L.; Son, J. C.; Guo, H.; Im, Y. A.; Cho, E. J.; Wang, J.; Hayes, J.; Wang, M.; Paul, A.; Lansdon, E. B.; Chen, J. M.; Graupe, D.; Rhodes, G.; He, G. X.; Geleziunas, R.; Xu, L.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1589.
17. Mitchell, M. L.; Son, J. C.; Lee, I. Y.; Lee, C. K.; Kim, H. S.; Guo, H.; Wang, J.; Hayes, J.; Wang, M.; Paul, A.; Lansdon, E. B.; Chen, J. M.; Eisenberg, G.; Geleziunas, R.; Xu, L.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1585.
18. Ogilvie, K. K.; Hamilton, R. J.; Gillen, M. F.; Radatus, B. K. *Can. J. Chem.* **1984**, *62*, 16.
19. Sadler, J. M.; Zimmermann, S. C.; Balzarini, J.; Seley-Radtke, K. L. *Med. Chem. Lett.*, submitted for publication.
20. Sadler, J. M.; Zimmermann, S. C.; O'Daniel, P. I.; Kim, N.; Seley-Radtke, K. L. *J. Med. Chem.*, submitted for publication.
21. Wigerinck, P.; Pannecouque, C.; Snoeck, R.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1991**, *34*, 2383.
22. Wigerinck, P.; Snoeck, R.; Claes, P.; de Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1991**, *34*, 1767.
23. Herdewijn, P. A. M. *Antiviral Chem. Chemother.* **1994**, *5*, 131.
24. Sadler, J. M.; Ojowoye, O.; Seley-Radtke, K. L. *Nucleic Acids Symp. Ser. (Oxf.)* **2008**, 571.
25. Tuske, S.; Sarafianos, S. G. *Nat. Struct. Mol. Biol.* **2004**, *11*, 469.
26. Das, K.; Bauman, J. D.; Clark, A. D., Jr.; Frenkel, Y. V.; Lewi, P. J.; Shatkin, A. J.; Hughes, S. H.; Arnold, E. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1466.
27. Das, K.; Clark, A. D., Jr.; Lewi, P. J.; Heeres, J.; De Jonge, M. R.; Koymans, L. M.; Vinkers, H. M.; Daeyaert, F.; Ludovici, D. W.; Kukla, M. J.; De Corte, B.; Kavash, R. W.; Ho, C. Y.; Ye, H.; Lichtenstein, M. A.; Andries, K.; Pauwels, R.; De Bethune, M. P.; Boyer, P. L.; Clark, P.; Hughes, S. H.; Janssen, P. A.; Arnold, E. *J. Med. Chem.* **2004**, *47*, 2550.
28. Das, K.; Lewi, P. J.; Hughes, S. H.; Arnold, E. *Prog. Biophys. Mol. Biol.* **2005**, *88*, 209.
29. Quirk, S.; Seley, K. L. *Biochemistry* **2005**, *44*, 13172.
30. Quirk, S.; Seley, K. L. *Biochemistry* **2005**, *44*, 10854.
31. Maruyama, T.; Kozai, S.; Demizu, Y.; Witvrouw, M.; Pannecouque, C.; Balzarini, J.; Snoecks, R.; Andrei, G.; De Clercq, E. *Chem. Pharm. Bull. (Tokyo)* **2006**, *54*, 325.
32. Maruyama, T.; Demizu, Y.; Kozai, S.; Witvrouw, M.; Pannecouque, C.; Balzarini, J.; Snoecks, R.; Andrei, G.; De Clercq, E. *Nucleosides Nucleotides Nucleic Acids* **2007**, *26*, 1553.
33. Nair, V.; Chi, G.; Ptak, R.; Neamati, N. *J. Med. Chem.* **2006**, *49*, 445.
34. Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.
35. Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21.
36. Sharma, R. A.; Bobek, M. *J. Org. Chem.* **1975**, *40*, 2377.
37. Malik, V.; Singh, P.; Kumar, S. *Tetrahedron* **2005**, *61*, 4009.
38. Ozerov, A. A.; Novikov, M. S.; Brel, A. K.; Solodunova, G. N. *Chem. Heterocycl. Compd.* **1998**, *34*, 611.
39. Lin, J. S.; Kira, T.; Gullen, E.; Choi, Y.; Qu, F.; Chu, C. K.; Cheng, Y. C. *J. Med. Chem.* **1999**, *42*, 2212.
40. Hegde, V. R.; Seley, K. L.; Schneller, S. W. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 269.
41. Buckheit, R. W., Jr.; Fliakas-Boltz, V.; Decker, W. D.; Roberson, J. L.; Stup, T. L.; Pyle, C. A.; White, E. L.; McMahon, J. B.; Currens, M. J.; Boyd, M. R., et al. *Antiviral Res.* **1995**, *26*, 117.