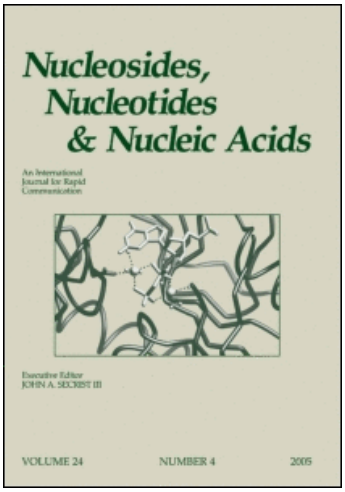

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ANTIVIRAL ACTIVITY OF 3-(3,5-DIMETHYLBENZYL)URACIL DERIVATIVES AGAINST HIV-1 AND HCMV

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□ *Antiviral activity of 1,3-disubstituted uracil derivatives was evaluated against HIV-1 and HCMV. It appears that the nitrogen of the 1-cyanomethyl group is important for anti-HIV-1 activity, suggesting interaction with the amino acid residues of HIV-1 reverse transcriptase. 1-Arylmethyl derivatives also exhibited good anti-HIV-1 activity; and that of the 2- and 4-picolyl derivatives was particularly excellent.*

Keywords Uracil derivatives; human immunodeficiency virus; human cytomegalovirus; antiviral activity

INTRODUCTION

New non-nucleoside reverse transcriptase inhibitors (NNRTIs) are one of the efficient therapeutic agents against human immunodeficiency virus (HIV) and 6-substituted uracil derivatives form one group of these NNRTIs. In 1989, Baba and coworkers reported potent anti-HIV activity for 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)[1] and emivirine has entered in phase III clinical trials.[2] Recently, 1-(3-cyclopenten-1-yl) methyl-6-(3,5-dimethylbenzoyl)-5-ethyl-2,4-pyrimidinedione (SJ-3366) also was identified as an anti-HIV agent.[3] It is not easy, however, to introduce a substituent at 6-position of uracil. These background facts prompted us to develop 1,3-disubstituted uracils as an anti-HIV agent candidates.[4,5] Initially, 3-substituted derivatives of 1-cyanomethyluracil were challenged

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since these compounds had been explored as candidates of antitumor agents.[6]

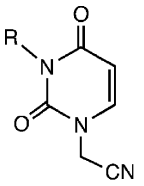
ANTI-HIV-1 ACTIVITY

Effect of the Methyl Function of the 3-Benzyl Group in the 1-Cyanomethyluracils

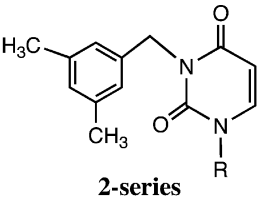
Starting from the 1-cyanomethyluracil **1a**, modifications in the N3 benzyl group were attempted to enhance the antiviral activity. The introduction of methyl functions in the *ortho* and *para* position of the N3 benzyl group was proved to be undesirable. However, moderate anti-HIV activity was observed with a methyl function substituted in *meta* **1c**, indicating the importance of hydrophobicity at the *meta* position. This was further supported by the significant anti-HIV activity of **1e**, which holds dimethyl groups in the *meta* position in the N^3-benzyl group. Some anti-HIV activity was still preserved in the case of dimethylation in the *meta* and *para* positions **1f**. Methyl function in the *meta* positions was changeable to methoxy, halogen or cyano function, since compounds **1g-h** exhibited good anti-HIV activity (Table 1).

Change of 1-Substituent in the 3-(3,5-Dimethylbenzyl)uracils

After basic research of N^3-benzylated uracil, a trial to change the 1-cyanomethyl group of **1e** was carried out. Initially, the 1-ethoxymethyl derivative **2a** was prepared to resemble emivirine.[2] Allyl **2b** and the 2-(methylthio)ethyl **2c** function were also introduced into N1 as non-cyclic substituents. However, the anti-HIV activity of these compounds was weak compared to that of **1e**, indicating the importance of the cyano group to adopt the binding site of HIV-1 reverse transcriptase (RT). Then, under the hypothesis that an electron-withdrawing group at N1 is essential for the anti-HIV activity of **1e**, ethoxycarbonylmethyl **2d**, 2-propanonyl **2e** and the 2,2,2-trifluoroethyl derivatives **2f** were prepared. However, these compounds also exhibited only weak activity. It is concluded that the nitrogen of the cyano group is important as a hydrogen receptor for hydrogen bonding. Another trial was undertaken to change the cyano of **1e** to the phenyls **2g,h** in expectation of a hydrophobic interaction with HIV-1 RT. This modification brought about a change to good anti-HIV activity these compounds. 1-Furanylmethyl was also compatible with anti-HIV-1 activity. Since the picolyl function may be able to act as a hydrogen receiving and hydrophobic functional group, an exchange of 1-benzyl in **2g** to 1-picolyl was explored. It is interesting that the anti-HIV-1 activity of 1-(2-picolyl)- **2l** and 1-(4-picolyl) derivative **2n** increased ten times compared with that of **2g**, and the anti-HIV activity of the 1-(3-picolyl) derivative **2m** resembled to that of **2g** (Table 1).



1-series



2-series

(3-series; 4-thio analogues)

1a;	R = H	2a;	R = $CH_2OCH_2CH_3$
1b;	R = CH_2-C_6H_4-2-CH_3	2b;	R = $CH_2OCH{=}CH_2$
1c;	R = CH_2-C_6H_4-3-CH_3	2c;	R = $CH_2CH_2SCH_3$
1d;	R = CH_2-C_6H_4-4-CH_3	2d;	R = $CH_2COOC_2H_5$
1e;	R = CH_2-C_6H_3-3,5-CH_3	2e;	R = CH_2COCH_3
1f;	R = CH_2-C_6H_3-3,4-CH_3	2f;	R = CH_2CF_3
1g;	R = CH_2-C_6H_3-3,5-OCH_3	2g;	R = CH_2-C_6H_5
1h;	R = CH_2-C_6H-3-CN	2h;	R = CH_2-C_6H_3-2,6-F
1i;	R = CH_2-C_6H_3-3,5-F	2i;	R = CH_2-(furan-2-yl)
1j;	R = CH_2-C_6H_3-3,5-Cl	2j;	R = CH2-(furan-3-yl)
		2k;	R = CH_2-C_6H_4-4-Cl
4;	1-Benzyl-3-[(4,6-dimethylpyridin-2-yl)methyl]uracil	2l;	R = CH_2-2-C_5H_4N
		2m;	R = CH_2-3-C_5H_4N
5;	1-(2,6-Difluorobenzyl-3-[(2,6-dimethylpyridin-4-yl)methyl]uracil	2n;	R = CH_2-4-C_5H_4N
		2o;	R = CH_2-C_6H_5 (base = thymine)
		2p;	R = CH_2-C_6H_5 (base = 5-iodouraicl)

4-Thio Modification

As compared to these 4-oxo analogs **2a-n**, all the 4-thio analogs **3a-n** exhibited improved anti-HIV activity, which makes **3g** one of the most potent anti-HIV compound of the present series. Although the N1-picolyl derivatives of uracil **2l-n** and 4-thiouracil **3l-n** display similar anti-HIV-1 activity, the cytotoxicity profile is better for the 4-oxo analogs (Table 1).

INTRODUCTION OF A SUBSTITUENT IN THE 5-POSITION

Methylation or iodination in the C-5 position of the uracil moiety of **2g**, as in **2o** and **2p**, did not increase the antiviral activity of **2g**.

Anti –HCMV Activity

With regard to HCMV (human cytomegalovirus), it appeared that compound **2g** was the most potent inhibitor. Introduction of 2 fluorines in the

TABLE 1 Antiviral activity of 1,3-disubstituted uracils against HIV-1 and HCMV[4,5]

Compound	HCMV EC_{50} (μM) AD-169 strain	EC_{50} (μM)	HIV-1 (III_B) CC_{50} (μM)	SI
AZT		0.0019±0.0001	4.24±1.61	
(*s*)-HPMPC	0.4	>150		
1b	78	192	>490	>3
1c	>196	6.70	302	45
1d	78	94.8	>490	>5
1e	>74	0.59	>464	>786
1f	117	3.75	434	116
1g	>188	7.44	>469	>63
1h	>166	5.64	>415	>74
1i	>180	13.5	>451	>33
1j	64	0.77	214	278
4	20	7.31	>389	>53
5	5.9	130	322	2
2a	>170	5.2	209	40
2b	190	1.48	155	105
2c	>160	7.39	131	18
2d	>160	22.4	>395	>18
2e	>570	13.7	>359	>26
2f	>640	7.75	>400	>52
2g	1.00	0.234	189	808
2h	>42	0.115	326	2835
2i	64	0.164	115	701
2j	34	0.23	115	500
2k	>56	0.211	44.0	209
2l	>160	0.040	145	3625
2m	31	0.40	279	698
2n	>160	0.053	135	2547
2o	12	1.44	58	40
2p	>4.7	41.7	201	5
3a	55	1.31	97.9	75
3b	28	0.34	77.5	228
3c	>160	1.25	34.3	27
3d	>160	22.4	>395	>18
3e	>570	13.7	>359	>26
3f	>640	7.75	>400	>52
3g	9.5	0.051	>371	>7275
3h	>13	0.059	>336	>5695
3i	13	0.077	23.7	308
3j	12	0.064	27.0	422
3k	>54	1.19	27.5	23
3l	30	0.041	37.9	924
3m	>59	0.122	13.6	111
3n	>15	0.041	31.4	766

ortho position of the N1-benzyl group **2h** considerably reduced the anti-HCMV activity. Replacement of the phenyl moiety of the N3- *meta*-dimethyl substituted benzyl group by a pyridinyl moiety, as in **4** and **5**, significantly reduced the activity against HCMV. Methylation or iodination in the C-5 position of the uracil moiety of compound **2g**, as in **2o** and **2p**, did not increase the antiviral activity of **2g** against HCMV (Table 1).

Docking Studies of 1,3-Disubstituted Uracils

These results were confirmed by docking studies using the program, Glide Ligand Docking Jobs. The methyl function in the *meta* position of the N3-benzyl group **1c** was a better fit than the *ortho* **1b** or *para* **1d** position. The *meta*-dimethyl (3,5-Me) analog **1e** also fit better than the other dimethyl analogs. In the case of compounds **1c**,**e**, hydrogen bonding between the nitrogen of the 1-cyanomethyl group and the amide group of the Lys101 residue (CN· · ·H-N) is suggested. The affinity of **2g** to HIV-RT by hydrophobic interaction was also proven by the presence of the **2g** phenyl moiety in the proximity of the hydrophobic area (Tyr181, Tyr188, Trp229, and Leu234 residues). Compound **2n** forms a hydrogen bond between the nitrogen of the 4-picolyl with the amide group of Lys101 residue (N· · ·H-N), as with the 1-cyanomethyl group of **1e**. However, the hydrophobic interaction is different from that of **2g**, as the phenyl moiety of **2n** was present in the proximity of another hydrophobic area of HIV-RT (Val106, Pro225, Phe227, Leu234, and Pro236 residues). It has been reported that most NNRTIs are known to engage in the hydrogen bonding with the backbone of the amino acids Lys101 and/or Lys103.[7] In conclusion, both hydrogen bonding and hydrophobic interaction are important for affinity with RT.

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