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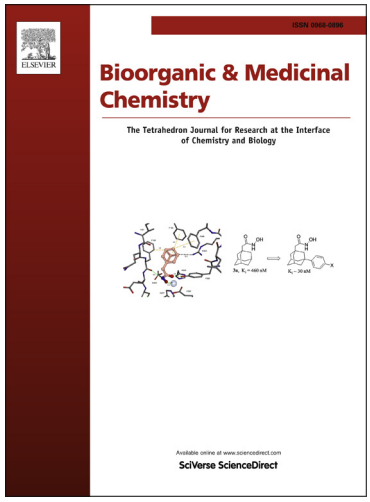

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Synthesis and evaluation of novel 3-(3,5-dimethylbenzyl)uracil analogs as potential anti-HIV-1 agents

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Abstract

A novel series of uracil derivatives with a 3,5-dimethylbenzyl group at the N^3-position were synthesized and evaluated as non-nucleoside HIV-1 reverse transcriptase inhibitors. Some of these compounds showed good-to-moderate activity with EC_{50} values in the submicromolar range. Among them, compound **10c** showed significant potency against HIV-1 activity with an EC_{50} value of 0.03 μM and a high selectivity index of 2863. Preliminary structure-activity relationships and molecular modeling analyses were used to explore the major interactions between HIV-1 reverse transcriptase and the potent inhibitor **10c**, which may serve as an important lead for further optimization.

1. Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a structurally diverse group of compounds that bind to the viral enzyme reverse transcriptase (RT), where the NNRTIs [1] interact with a specific allosteric non-substrate binding pocket site. It is interesting that some NNRTIs have an aromatic group at the 6-position of uracil skeleton. For instance, in 1989, Baba *et al.*[2a] discovered that 1-[(2-hydroxyethoxy)methyl]-6-phenylthiothymine (**1a**, HEPT) had strong anti-HIV-1 activity. Interestingly, HEPT **1a** can be regarded as an acyclonucleoside analog, yet its 5'-triphosphate derivative **1b**

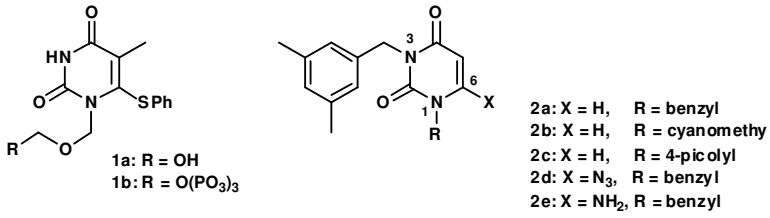


Figure 1. Structures of HEPT derivatives **(1a, b)** and 1-substituted-3-(3,5-dimethylbenzyl)uracil derivatives **(2a-e)**.

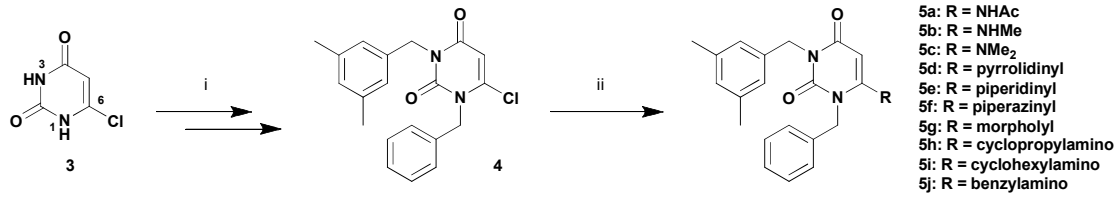
showed no inhibitory effect on HIV-1 RT,[2b] suggesting that HEPT could be undoubtedly considered an NNRTI despite having a uracil skeleton. Since the first finding of HEPT **1a**, related uracil derivatives have been synthesized.[2] We have consistently searched for an anti-HIV-1 agent using the structure-activity relationships (SAR) of the 1,3-disubstituted and 1,3,6-trisubstituted uracils.[3] As a result, we have demonstrated that the 3,5-dimethylbenzyl group at the 3-position of the uracil skeleton plays an important role in an enhancement of the anti-HIV-1 activity; notably, 1-substituted (e.g., benzyl, cyanomethyl, or 4-picolyl group) 3-(3,5-dimethylbenzyl)uracil **2a–c** showed good antiviral activity.[3a] Moreover, the introduction of an azido or amino group at the 6-position of 1-benzyl-3-(3,5-dimethylbenzyl)uracil **2d** and **2e** showed excellent potency with an EC_{50} of 0.067 ±0.011 μM and 0.069 ±0.006 μM, respectively.[3c] However, the CC_{50} and corresponding SI values of compounds **2d** and **2e** were not satisfactory (CC_{50} 45.9 ± 0.7 μM and 45.6 ±0.9 μM; SI 685 and 661, respectively).[3c] Taken together, these results prompted us to evaluate the following newly synthesized 1,3,6-trisubstituted uracils with an aim to develop higher anti-HIV-1 activity and lower cytotoxicity: i) 6-alkylamino-introduced analogs of 1-benzyl-3-(3,5-dimethylbenzyl)uracil (**5 series**) to examine the effect of *N*-alkylation of the C6-amino group on **2e**, and ii) 1-substituted derivatives of 6-azido (or amino)-3-(3,5-dimethylbenzyl)uracil (**9** and **10 series**) to investigate substituent effect when the N^1-benzyl group of **2d** or **2e** is replaced by an appropriate alkyl group such as cyanomethyl, 2-picolyl and 4-picolyl groups. We also report on the docking studies of the most promising inhibitor **10c** with an RT nevirapine binding site.

2. Results and discussion

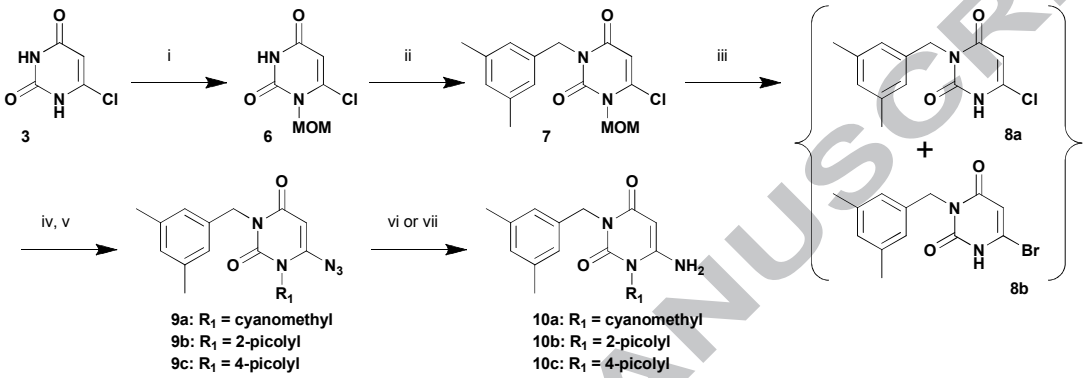
2.1. Chemistry

To obtain a series of 6-amino-substituted analogs of 1-benzyl-3-(3,5-dimethylbenzyl)uracil (**5 series**), 1-benzyl-6-chloro-3-(3,5-dimethylbenzyl)uracil **4** was prepared in two steps from the commercially available 6-chlorouracil **3** using our previously reported method shown in scheme 1.[3c] Then, compound **4** was subjected to nucleophilic substitution with a variety of acyclic or cyclic amines (e.g., dimethylamine, pyrrolidine, morpholine, or benzylamine) in the presence of Na_2CO_3 in EtOH at 50–100 ℃ for 0.5–65 h to give their 6-alkylamino-substituted analogs in 65–100% yields (Scheme 1, **5b–j**). In the case of the 6-acetamide analog **5a**, 6-amino derivative **2b** prepared from **3**[3c] was *N*-monoacetylated with Ac_2O and pyridine to obtain the corresponding derivative **5a**.

The 6-azido (and amino)-1-substituted analogs of 3-(3,5-dimethylbenzyl)uracil synthesis (**9** and **10 series**) also began with 6-chlorouracil **4** as a starting material and led to 1-methoxymethyl-6-chlorouracil **6** (Scheme 2). The resulting **6** was condensed with 3,5-dimethylbenzyl alcohol using the Mitsunobu reaction[3c] to afford 3-(3,5-dimethybenzyl) congener **7**. The product was carried out by deprotection of the N^1-MOM group using B-bromocatecholborane[4] in CH_2Cl_2 to give the corresponding N^1-deprotected inseparable mixture of **8a** and 6-bromo-substituted product **8b** in 72% combined yield (ratio **8a**/**8b**: 80/20). Next, the resulting mixture of **8a** and **8b** was treated with bromoacetonitrile, 2-picolyl chloride or 4-picolyl chloride to give the N^1-alkylated congeners, and subsequently, the mixture of 6-chloro and 6-bromo



Scheme 1. Synthesis of 6-alkylamino-substituted-1-benzyl-3-(3,5-dimethylbenzyl)uracil **(5 series)**. *Reagents and conditions*: i, see ref. 3c; ii, K_2CO_3, EtOH, 50–100 ºC, 0.5–65 h, 65–100%



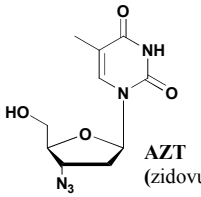
Scheme 2. Synthesis of 6-azido-1-substitueted-3-(3,5-dimethylbenzyl)uracil **(9 series)** and 6-amino-1-substitueted-3-(3,5-dimethylbenzyl)uracil **(10 series)**. *Reagents and conditions*: i, MOMCl, DBU, CH_2Cl_2, 0–C, 20 min, 90%; ii, 2,6-dimethylbenzylalcohol, TMAD, PPh_3, 50 –C, 2 h, 93%; iii, B-bromocatecholborane, CH_2Cl_2, rt, 1 h, 72%; iv, R-X (R = cyanomethyl, X = Br; R= 2-picolyl, X = Cl; R = 4-picolyl, X = Cl), K_2CO_3, DMF, 40–93%; v, NaN_3, DMF, rt, 30 min, 81–90%; vi, PPh_3, H_2O, THF, rt, 10 h, 90%; vii, $LiAlH_4$, THF, 0 –C, 5 min, 69–74%

derivatives was subjected to the nucleophlic substitution at the C6-position in the uracil moiety with sodium azide to afford the single product of 6-azido substituted analogs **9a–c**. The reduction of compounds **9b** and **9c** was carried out with lithium aluminum hydride to provide 6-amino compound **10b** and **10c**, whereas the conversion of **9a** was performed using PPh_3-H_2O condition [5] to obtain **10a**, avoiding the excessive reduction of cyanomethyl group of **9a**.

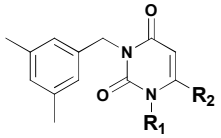
2.2. Biological activity

The antiviral activity of the 6-alkylamino-substituted 1-benzyl-3-(3,5-dimethylbenzyl)uracils **5a–j**, 6-azido congeners **9a–c**, and 6-amino analogs **10a–c** of 1-substituted-3-(3,5-dimethylbenzyl)uracils was determined by examining the inhibitory effects of these compounds on the HIV-1-induced cytopathogenicity and on cell viability in MT-4 cells. Zidovudine (AZT) was also tested as a positive control. In the series of 6-alkylamino-substituted analogs **5a–j** shown in Table 1, almost every compound had considerably reduced anti-HIV-1 activity when compared with 6-azido or amino-introduced derivatives **2d** and **2e**, which displayed EC_{50} values of 0.067 ±0.011 μM and 0.069 ±0.006 μM, respectively,[3c] suggesting that alkylation of the amino group at the 6-position on the uracil moiety diminished anti-HIV-1 activity. Even *N*-monomethylation of the amino group at the 6-position, such as for compound **5b**, resulted

Table. 1. Antiviral activity of 3-(3,5-dimethylbenzyl)uracil analogs against HIV-1



AZT
(zidovudine)



5a-j, 9a-c, 10a-c

Compound	R_1	R_2	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
AZT	—	—	0.04 ± 0.04	> 100	> 2769
5a	Bn	NHAc	> 100	> 100	—
5b	Bn	NHMe	7 ± 4	65 ± 3	9
5c	Bn	NMe_2	> 48 ± 5	48 ± 5	—
5d	Bn	pyrrolidinyl	> 31 ± 3	31 ± 3	—
5e	Bn	piperidinyl	> 47 ± 3	47 ± 3	—
5f	Bn	piperazinyl	> 16 ± 4	16 ± 4	—
5g	Bn	morpholyl	> 100	> 100	—
5h	Bn	cyclopropylamino	> 100	> 100	—
5i	Bn	cyclohexylamino	> 74 ± 19	74 ± 19	—
5j	Bn	NHBn	> 100	> 100	—
9a	cyanomethyl	N_3	3.5 ± 1	60 ± 1	17
9b	2-picolyl	N_3	0.22 ± 0.10	> 100	> 457
9c	4-picolyl	N_3	0.05 ± 0.02	59 ± 6	1162
10a	cyanomethyl	NH_2	> 100	> 100	—
10b	2-picolyl	NH_2	0.23 ± 0.06	> 100	> 443
10c	4-picolyl	NH_2	0.03 ± 0.03	> 100	> 2863

[a] EC_{50}, effective concentration; the concentration of compound required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.
[b] CC_{50}, cytotoxic concentration; the concentration of compound that reduces the normal uninfected MT-4 cell viability by 50%.
[c] SI, selectivity idex (CC_{50}/EC_{50}).

in centesimal decreased HIV-1 activity (EC_{50} = 7 ±4 μM) in comparison to the corresponding 6-free amino analog **2e**.

Next, the introduction of an alkyl group (such as cyanomethyl, 2-picolyl, and 4-picolyl) at the N^1-position of the uracil skeleton in place of N^1-benzyl group of **2d** and **2e** was investigated for the SAR. Among the compounds **9a–c** and **10a–c** shown in Table 1, N^1-2-picolyluracil congeners **9b** and **10b** showed good anti-HIV-1 activity with EC_{50} values of 0.22 ±0.10 μM and 0.23 ±0.06 μM, respectively. Because CC_{50} values for both **9b** and **10b** exceeded 100 μM, their SI values were > 457 and > 443, respectively, which were comparable to the SI values of the N^1-benzyl uracil derivatives **2d** and **2e** (658 and 661, respectively), reported in our previous study.[3c] However, the introduction of an N^1-4-picolyl group in **9c** and **10c** produced compounds with the strongest anti-HIV-1 potencies (EC_{50} 0.05 ±0.02 μM and 0.03 ±0.03 μM, respectively). In particular, the 6-amino-1-(4-picolyl) analog **10c** not only exhibited significant activity (at EC_{50} = 0.03 ± 0.03 μM), but also displayed low cytotoxicity with CC_{50} > 100 μM, resulting in an SI > 2863, which is high

and comparable to those of the positive controls AZT (SI > 2769) or nevirapine[3c] (SI > 1639). In contrast, the 6-azido-1-(4-picolyl) congener **9c** showed moderate values for CC_{50} of 59 ±6 μM and for SI of 1162. As for the similar potencies (defined by their similar EC_{50} values) among C6-azido analogs **2d**, **9b** and **9c**, and C6-amino derivatives **2e**, **10b** and **10c**, it is possible that 6-azidouracil developed antiviral activity after metabolic conversion to the 6-aminocongener. Finally, C6-azidouracil **9a** with a cyanomethyl group at the N^1 position was, unfortunately, 100 times less potent (EC_{50} = 3.5 ±1 μM) than its N^1-4-picolyl counterpart, **10c**. Moreover, the 6-amino-1-cyanomethyl analog **10a** showed no antiviral activity.

2.3. Molecular modeling analysis

The X-Ray co-crystal structure (PDB: 1VRT) of HIV-1 RT with nevirapine was taken from PDB (1VRT)[6] and used for docking studies. A docking model of ligand **10c**, which showed the most promising HIV-1 activity, bound to HIV-1 RT was constructed by conformational search using *MacroModel* (ver. 9.1). AMBER* was used as a force field, and more than 3,000 conformers for **10c** were optimized. The most stable conformation is shown in Figure 2. The hydrogen of the 6-amino group for **10c** is hydrogen bonded to the amide group of Lys101 residue (NH···O=C), and the 3,5-dimethylbenzyl moiety was oriented around the hydrophobic area (Tyr181, Tyr188, Trp229, and Leu234 residues) of HIV-1 RT. This result was significantly different from that of the C6-deaminated counterpart **2c** in our previous report.[3b] The nitrogen of the 4-picolyl formed a hydrogen bond with the amide group of the Lys101 residue (N···H–N), whereas the 3,5-dimethylbenzyl group at the N^3 position existed around another hydrophobic area (Val106, Pro225,

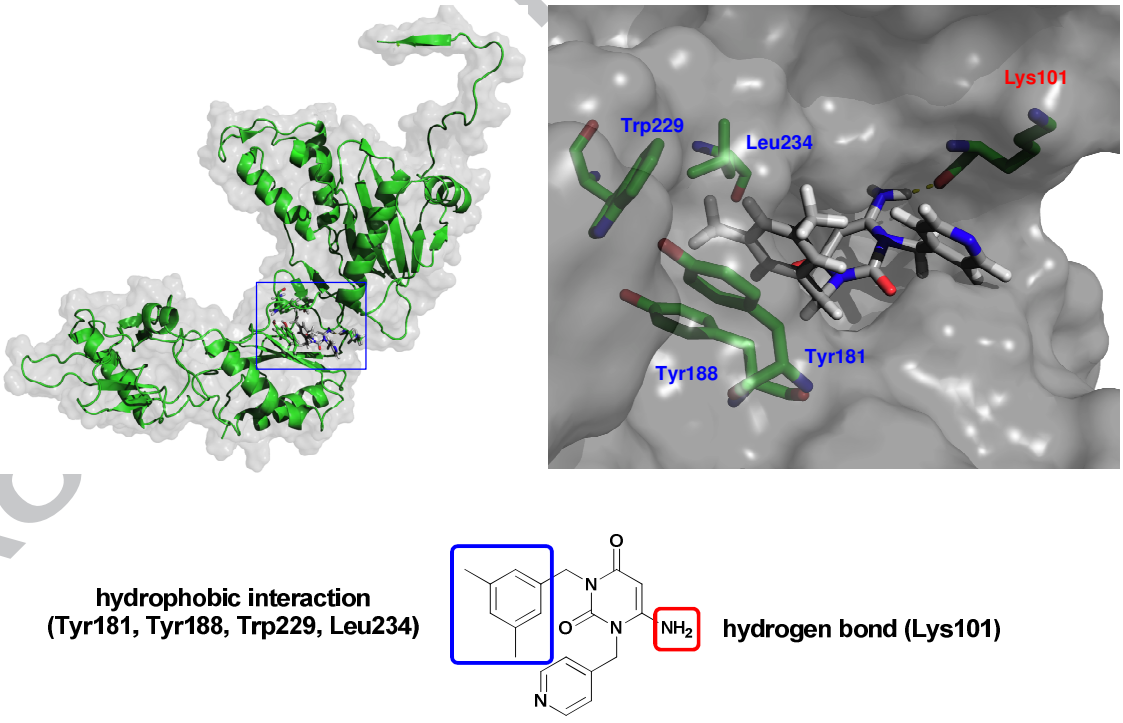


Figure 2. Docking structure between HIV-RT and 6-amino-3-(3,5-dimethylbenzyl)-1-(4-picolyl)uracil **(10c).**

Phe227, Leu234, and Pro236 residues) of HIV-1 RT. Most NNRTIs are known to be engaged in the hydrogen bond with the backbone of the amino acids Lys101.[7] Thus, the results from our docking study with compound **10c** suggest a role for the hydrogen bond in the affinity with RT.

2.4. Conclusion

In the present study, a series of newly synthesized 1-substituted analogs of 6-azido or 6-amino-3-(3,5-dimethylbenzyl)uracil exhibited good potency against HIV-1 activities. In particular, compound **10c** displayed excellent activity with an EC_{50} value of 0.03 ±0.03 μM and an SI value of 2863. Furthermore, the simulated binding model of **10c** with HIV-1 RT indicated that the hydrogen of the 6-amino group for **10c** is hydrogen bonded to the amide group of the Lys101 residue, and that the 3,5-dimethylbenzyl moiety was oriented around the hydrophobic area (Tyr181, Tyr188, Trp229, and Leu234 residues) of HIV-1 RT. Overall, this compound may serve as the basis for further modification in the search for more potent candidates for anti-HIV-1 chemotherapy.

3. Experimental section

3.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were taken with an Ultrashield™ 400 Plus FT NMR System (BRUKER, Germany). Chemical shifts and coupling constants (*J*) were given in δand Hz, respectively. Melting points were determined on a Yanaco MP-500D. High-resolution mass spectrometry was performed on an APEX IV mass spectrometer (BRUKER) with electrospray ionization mass spectroscopy (ESI-MS).

3.2. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-acetamidouracil (5a)

A solution of compound **2e** (33.5 mg, 0.1 mmol) and Ac_2O (189.1 μl, 2.0 mmol) in dry pyridine (0.3 mL) was stirred for 7 days at room temperature. The mixture was then evaporated in vacuo and the residue was extracted with AcOEt. The organic extracts were washed with water and saturated sodium chloride solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to a white crystal **5a** (13.2 mg, 0.035 mmol, 35%). ^{1}H NMR (400MHz, $CDCl_3$): δ 11.60 (1H, brs, AcNH), 7.21-7.40 (5H, m, Bn), 7.06 (2H, s, 3,5-Me_2-Bn), 6.90 (1H, s, 3,5-Me_2-Bn), 5.40 (1H, s, H-5), 5.21 (2H, s, Bn), 5.13 (2H, s, 3,5-Me_2-Bn), 2.64 (6H, s, Ac), 2.30 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 199.4, 161.3, 158.2, 150.4, 138.0, 136.9, 133.7, 129.6, 129.2, 128.7, 126.1, 126.1, 91.9, 46.1, 44.6, 32.5, 21.3; HRMS (ESI) Calcd for $C_{22}H_{23}N_3NaO_3^+$ $[M+Na]^+$:400.16316. Found 400.16349; mp: 212.3 ℃.

3.3. General procedure for the synthesis of 5b-j

A solution of compound **4** (177.4 mg, 0.5 mmol), appropriate amine (1.0 mmol) and Na_2CO_3 (106.0 mg,

1.0 mmol), in dry EtOH (3.0 mL) was heated at 50–100 ℃. After 0.5–65 h stirring, the mixture was evaporated *in vacuo* and the residue was extracted with AcOEt. The organic extracts were washed with water and saturated sodium chloride solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography to afford **5b–j** in 65–100% yield.

3.3.1. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-methylaminouracil (5b)

Yield 92%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.20-7.39 (5H, m, Bn), 7.08 (2H, s, 3,5-Me_2-Bn), 6.88 (1H, s, 3,5-Me_2-Bn), 5.14 (2H, s, 3,5-Me_2-Bn), 5.10 (2H, s, Bn), 4.88 (1H, s, H-5), 4.17 (1H, m, NH), 2.69 (3H, d, *J* 5.2, methylamino), 2.30 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 162.7, 154.0, 152.1, 137.8, 137.5, 135.1, 129.3, 128.9, 128.2, 126.1, 126.0, 76.0, 45.7, 44.2, 29.9, 21.3; HRMS (ESI) Calcd for $C_{21}H_{23}N_3NaO_2^+$ $[M+Na]^+$: 372.16825. Found 372.16855; mp: 186.7 ℃.

3.3.2. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-dimethylaminouracil (5c)

Yield 100%; pale yellow oil; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.15-7.31 (5H, m, Bn), 6.99 (2H, s, 3,5-Me_2-Bn), 6.85 (1H, s, 3,5-Me_2-Bn), 5.29 (1H, s, H-5), 5.07 (2H, s, Bn), 5.00 (2H, s, 3,5-Me_2-Bn), 2.68 (6H, s, 6-NMe_2), 2.25 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 162.9, 160.6, 152.9, 137.7, 137.1, 136.9, 129.1, 128.6, 127.5, 126.8, 126.3, 88.4, 48.8, 44.1, 42.5, 21.3; HRMS (ESI) Calcd for $C_{22}H_{26}N_3O_3^+$ $[M+H]^+$:364.20195. Found 364.20256.

3.3.3. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-pyrrolidinyluracil (5d)

Yield 84%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$) : δ 7.12-7.30 (5H, m, Bn), 7.04 (2H, s, 3,5-Me_2-Bn), 6.86 (1H, s, 3,5-Me_2-Bn), 5.21 (1H, s, H-5), 5.10 (2H, s, 3,5-Me_2-Bn), 5.04 (2H, s, Bn), 3.13 (4H, m, pirrolidinyl), 2.26 (6H, s, 3,5-Me_2-Bn), 1.86 (4H, m, pirrolidinyl); ^{13}C NMR (100MHz, $CDCl_3$): 162.8, 157.2, 153.3, 137.7, 137.3, 136.9, 129.0, 128.6, 127.3, 126.4, 126.0, 84.0, 51.5, 49.9, 44.1, 25.3, 21.3; HRMS (ESI) Calcd for $C_{24}H_{27}N_3NaO_2^+$ $[M+Na]^+$: 412.19955. Found 412.19893; mp: 132.2 ℃.

3.3.4. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-piperidinyluracil (5e)

Yield 96%; pale yellow oil; ^{1}H NMR (400MHz, $CDCl_3$) : δ 7.19-7.29 (5H, m, Bn), 7.00 (2H, s, 3,5-Me_2-Bn), 6.86 (1H, s, 3,5-Me_2-Bn), 5.30 (1H, s, H-5), 5.04 (2H, s, 3,5-Me_2-Bn), 5.00 (2H, s, Bn), 2.85 (4H, m, piperidinyl), 2.24 (6H, s, 3,5-Me_2-Bn), 1.56 (6H, m, piperidinyl); ^{13}C NMR (100MHz, $CDCl_3$): 163.1, 160.6, 152.9, 137.8, 137.8, 137.1, 129.1, 128.6, 127.5, 127.0, 126.4, 89.7, 52.3, 48.1, 44.1, 25.3, 23.9, 21.3; HRMS (ESI) Calcd for $C_{25}H_{29}N_3NaO_2^+$ $[M+Na]^+$: 426.21520. Found 426.21293.

3.3.5. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-piperazinyluracil (5f)

Yield 92%; pale yellow oil; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.19-7.32 (5H, m, Bn), 7.01 (2H, s, 3,5-Me_2-Bn), 6.86 (1H, s, 3,5-Me_2-Bn), 5.33 (1H, s, H-5), 5.07 (2H, s, Bn), 5.01 (2H, s, 3,5-Me_2-Bn), 2.93 (4H, s, piperazinyl), 2.87 (4H, s, piperazinyl), 2.25 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$):

162.9, 160.0, 152.8, 137.8, 136.9, 129.1, 128.7, 127.6, 126.8, 126.3, 90.1, 52.2, 48.1, 45.3, 44.2, 21.3; HRMS (ESI) Calcd for $C_{24}H_{29}N_4NaO_2^+$ $[M+Na]^+$:405.22850. Found 405.22688.

3.3.6. 1-Benzyl-3-(3,5-dimethylbenzyl)-6-morpholyluracil (5g)

Yield 100%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.24-7.33 (5H, m, Bn), 7.02 (2H, s, 3,5-Me_2-Bn), 6.87 (1H, s, 3,5-Me_2-Bn), 5.35 (1H, s, H-5), 5.09 (2H, s, Bn), 5.02 (2H, s, 3,5-Me_2-Bn), 3.74 (4H, s, morpholyl), 2.88 (4H, s, morpholyl), 2.25 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 162.8, 159.5, 152.7, 137.8, 136.9, 136.8, 129.2, 128.8, 127.7, 126.7, 126.4, 90.3, 66.1, 51.4, 48.1, 44.2, 21.3; HRMS (ESI) Calcd for $C_{24}H_{27}N_3NaO_3^+$ $[M+Na]^+$:428.19446. Found 428.19306; mp: 133.2 ℃.

3.3.7. 1-Benzyl-6-cyclopropylamino-3-(3,5-dimethylbenzyl)uracil (5h)

Yield 94%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$) : δ 7.26-7.38 (5H, m, Bn), 7.10 (2H, s, 3,5-Me_2-Bn), 6.89 (1H, s, 3,5-Me_2-Bn), 5.31 (1H, s, H-5), 5.10 (2H, s, Bn), 5.08 (2H, s, 3,5-Me_2-Bn), 4.46 (1H, brs, NH), 2.29 (7H, m, 3,5-Me_2-Bn and cyclopropylamino), 0.42-0.75 (4H, m, cyclopropylamino); ^{13}C NMR (100MHz, $CDCl_3$): 161.2, 154.1, 151.5, 137.9, 137.1, 136.5, 128.4, 128.2, 127.1, 126.1, 124.9, 75.5, 44.3, 42.9, 24.2, 20.9, 6.6; HRMS (ESI) Calcd for $C_{23}H_{25}N_3NaO_2^+$ $[M+Na]^+$: 398.18390. Found 398.18193; mp: 214.4 ℃.

3.3.8. 1-Benzyl-6-cyclohexylamino-3-(3,5-dimethylbenzyl)uracil (5i)

Yield 89%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$) : δ 7.22-7.34 (5H, m, Bn), 7.10 (2H, s, 3,5-Me_2-Bn), 6.88 (1H, s, 3,5-Me_2-Bn), 5.16 (2H, s, 3,5-Me_2-Bn), 5.10 (2H, s, Bn), 4.88 (1H, s, H-5), 4.13 (1H, m, cyclohexylamino), 2.30 (6H, s, 3,5-Me_2-Bn), 2.05 (1H, brs, NH), 1.53-1.76 (4H, m, cyclohexylamino), 1.11-1.47 (6H, m, cyclohexylamino); ^{13}C NMR (100MHz, $CDCl_3$): 162.8, 152.4, 151.9, 137.8, 137.6, 135.3, 129.4, 129.0, 128.4, 126.3, 126.2, 76.2, 51.3, 46.0, 44.2, 31.9, 25.3, 24.0, 21.3; HRMS (ESI) Calcd for $C_{26}H_{31}N_3NaO_2^+$ $[M+Na]^+$: 440.23085. Found 440.22996; mp: 71.8 ℃.

3.3.9. 1-Benzyl-6-benzylamino-3-(3,5-dimethylbenzyl)uracil (5j)

Yield 64%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$) : δ 7.26-7.36 (5H, m, Bn), 7.18-7.19 (2H, s, 3,5-Me_2-Bn), 6.89-6.95 (5H, m, benzylamino), 5.18 (2H, s, Bn), 5.10 (2H, s, 3,5-Me_2-Bn), 4.92 (1H, s, H-5), 4.46 (1H, m, NH), 4.11 (2H, d, *J* 5.2, benzylamino), 2.29 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 162.7, 152.7, 152.2, 137.8, 137.4, 135.9, 135.2, 129.3, 129.0, 128.9, 128.3, 128.0, 127.1, 126.3, 126.2, 76.8, 47.1, 45.8, 44.2, 21.3; HRMS (ESI) Calcd for $C_{27}H_{27}N_3NaO_2^+$ $[M+Na]^+$: 448.19955. Found 448.19661; mp: 189.1 ℃.

3.4. 6-Chloro-1-methoxymethyluracil (6)

6-Chlorouracil (**3**) (1.46 g, 10.0 mmol) was dissolved in dry CH_2Cl_2 (30 ml), and DBU (1646.3 μl, 11.0 mmol) was added to the solution at room temperature, and after stirring an additional 10 min, the mixture

became clear. After the mixture was cooled to 0 ℃, methyl chloromethyl ether (911.1 μl, 12.0 mmol, MOMCl) was added dropwise, and stirring continued for an additional 20 min at 0 ℃. The mixture was evaporated, and the residue was purified by silica gel column chromatography (AcOEt) to white powder **6** (1.71 g, 8.99 mmol, 90%). ^{1}H NMR (400MHz, $CDCl_3$): δ8.58 (1H, s, NH), 5.94 (1H, s, 5-H), 5.43 (2H, s, MOM), 3.47 (3H, s, MOM).

3.5. 6-Chloro-1-methoxymethyl-3-(3,5-dimethylbenzyl)uracil (7)

A solution of compound **6** (1.44 g, 7.56 mmol), triphenylphosphine (2.58 g, 9.83 mmol), 3,5-dimethylbenzylalcohol (1.17 ml, 7.94 mmol) and TMAD (*N*,*N*,*N*',*N*'-tetramethylazodicarboxamide, 1.69 g, 9.83 mmol) in THF (28.0 ml) was stirred at 50 ℃. After 2 h stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography (30% AcOEt in hexane) to give as a syrup **7** (2.17 g, 7.03 mmol, 93%). ^{1}H NMR (400MHz, $CDCl_3$): δ7.04 (2H, s, 3,5-Me_2-Bn), 6.90 (1H, s, 3,5-Me_2-Bn), 5.98 (1H, s, 5-H), 5.44 (2H, s, MOM), 5.03 (2H, s, 3,5-Me_2-Bn), 3.44 (3H, s, MOM), 2.28 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 160.5, 151.4, 145.2, 138.0, 136.0, 129.5, 126.6, 103.3, 57.5, 44.8, 21.3; HRMS (ESI) Calcd for $C_{15}H_{17}ClN_2NaO_3$ $[M+Na]^+$: 331.08199. Found 331.08154.

3.6. 6-Chloro-3-(3,5-dimethylbenzyl)uracil (8a) and **6-bromo-3-(3,5-dimethylbenzyl)uracil (8b)** [4]

A solution of compound **7** (1.90 g, 6.15 mmol) and B-bromocatecholborane (1.47 g, 7.38 mmol, 0.2 M in CH_2Cl_2) in CH_2Cl_2 (20 mL) was stirred at room temperature. After 2 h stirring, the mixture was evaporated *in vacuo*, and the residue was extracted with AcOEt. The organic extracts were washed with saturated aqueous $NaHCO_3$ and saturated NaCl solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (40% AcOEt in hexane) to give the inseparable mixture of **8a** and **8b** as a needle crystal (1.21 g, 4.42 mmol, 72% combined yield), which was employed in the next reaction without further purification, in a ratio of 80:20 according to ^{1}H-NMR spectrum. ^{1}H NMR (400MHz, $CDCl_3$): δ7.06 (2H, s, 3,5-Me_2-Bn), 6.90 (1H, s, 3,5-Me_2-Bn), 6.05 (0.2H, s, 5-H, **8b**), 5.89 (0.8H, s, 5-H, **8a**), 4.99 (2H, s, 3,5-Me_2-Bn), 2.27 (6H, s, 3,5-Me_2-Bn); HRMS (ESI) Calcd for $C_{13}H_{13}ClN_2NaO_2$ $[M+Na]^+$: 287.05578. Found 278.05656 (**8a**), Calcd for $C_{13}H_{13}BrN_2NaO_2$ $[M+Na]^+$: 331.00526. Found 331.00300 (**8b**).

3.7. General procedure for the synthesis of 9a-c

The mixture of **8a** and **8b** (264.7 mg, 0.97 mmol) was dissolved in dry DMF (5.0 mL) under nitrogen atmosphere. To this stirred solution, we carefully added the appropriate alkyl halide (bromoacetonitrile, 2-(chloromethyl)pyridine hydrochloride or 4-(chloromethyl)pyridine hydrochloride; 2.0 mmol) and K_2CO_3 (276.4 mg, 2.0 mmol). Stirring was continued at room temperature for 2–12 h, and the mixture was extracted with AcOEt. The organic extracts were washed with water and saturated aqueous sodium chloride solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column

chromatography to give the inseparable mixture of C6-chloro and C6-bromo congeners, ratio 80:20 according to ^{1}H-NMR spectrum.

The resulting mixture (258.1 mg, 0.83 mmol) was dissolved in dry DMF (5.0 mL), and NaN_3 (66.3 mg, 1.02 mmol) was added to the solution, which was stirred for 30 min at room temperature. The mixture was extracted with AcOEt, washed with saturated aqueous sodium chloride solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (70% AcOEt in hexane) to give C6-azido derivative **9a–c**.

3.7.1. 6-Azido-1-cyanomethyl-3-(3,5-dimethylbenzyl)uracil (9a)

Combined yield 82%; brown oil; ^{1}H NMR (400MHz, $CDCl_3$): δ7.04 (2H, s, 3,5-Me_2-Bn), 6.91 (1H, s, 3,5-Me_2-Bn), 5.64 (1H, s, 5-H), 5.02 (2H, s, 3,5-Me_2-Bn), 4.75 (2H, s, cyanomethyl), 2.28 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 160.5, 150.0, 148.9, 138.1, 135.8, 129.6, 126.6, 113.8, 88.9, 44.9, 30.8, 21.3; HRMS (ESI) Calcd for $C_{15}H_{15}N_6NaO_2$ $[M+Na]^+$: 333.10704. Found 333.10687.

3.7.2. 6-Azido-3-(3,5-dimethylbenzyl)-1-(2-picolyl)uracil (9b)

Combined yield 32%; brown oil; ^{1}H NMR (400MHz, $CDCl_3$): δ8.54 (1H, d, *J* 4.0, 2-picolyl), 7.66 (1H, m, 2-picolyl), 7.20 (2H, m, 2-picolyl), 7.02 (2H, s, 3,5-Me_2-Bn), 6.88 (1H, s, 3,5-Me_2-Bn), 5.61 (1H, s, 5-H), 5.17 (2H, s, 2-picolyl), 5.05 (2H, s, 3,5-Me_2-Bn), 2.27 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 161.4, 154.9, 151.2, 151.2, 149.6, 137.9, 136.7, 136.5, 129.2, 126.2, 122.6, 121.1, 88.0, 48.0, 44.5, 21.2; HRMS (ESI) Calcd for $C_{19}H_{18}N_6NaO_2$ $[M+Na]^+$: 385.13834. Found 385.13740.

3.7.3. 6-Azido-3-(3,5-dimethylbenzyl)-1-(4-picolyl)uracil (9c)

Combined yield 59%; brown oil; ^{1}H NMR (400MHz, $CDCl_3$): δ8.58 (2H, d, *J* 6.0, 4-picolyl), 7.16 (2H, d, *J* 5.6, 4-picolyl), 7.03 (2H, s, 3,5-Me_2-Bn), 6.90 (1H, s, 3,5-Me_2-Bn), 5.60 (1H, s, 5-H), 5.06 (2H, s, 4-picolyl), 5.03 (2H, s, 3,5-Me_2-Bn), 2.28 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 161.0, 151.1, 150.4, 150.3, 144.6, 138.0, 136.3, 129.4, 126.4, 122.1, 88.2, 45.8, 44.7, 21.3; HRMS (ESI) Calcd for $C_{19}H_{18}N_6NaO_2$ $[M+Na]^+$: 385.13834. Found 385.13777.

3.8. 6-Amino-1-cyanomethyl-3-(3,5-dimethylbenzyl)uracil (10a)

A solution of compound **9a** (73.0 mg, 0.23 mmol), triphenylphosphine (74.0 mg, 0.28 mmol) and H_2O (6.2 µl, 0.35 mmol) in THF (1.0 ml) was stirred at room temperature. After stirring for 10 h, the solution was concentrated to a small volume. The residual solution was purified by silica gel column chromatography (20% MeOH in $CHCl_3$) to give as a yellowish crystal **10a** (60.0 mg, 0.21 mmol, 90%). ^{1}H NMR (400MHz, $CDCl_3$): δ6.98 (2H, s, 3,5-Me_2-Bn), 6.88 (1H, s, 3,5-Me_2-Bn), 5.07 (2H, brs, NH_2), 5.05 (1H, s, 5-H), 4.99 (2H, s, cyanomethyl), 4.80 (2H, s, 3,5-Me_2-Bn), 2.27 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 163.0, 153.2, 150.8, 138.2, 136.6, 129.3, 125.6, 114.3, 78.4, 44.6, 30.4, 21.2; HRMS (ESI) Calcd for $C_{15}H_{16}N_4NaO_2$ $[M+Na]^+$: 307.11655. Found 307.11537; mp: 350.0 ℃.

3.9. General procedure for the synthesis of 10b and 10c

Compound **9b** or **9c** (109.4 mg, 0.30 mmol) was dissolved in dry THF (5.4 ml) under nitrogen atmosphere. To this stirred solution we carefully added $LiAlH_4$ (13.7 mg, 0.36 mmol) at 0 ℃. Stirring was continued at 0 ℃ for 5 min, and the reaction quenched by the addition of AcOEt (5.0 ml) until no effervescence was observed. Aqueous 1N HCl (2.2 ml) was then added, and the product was extracted with AcOEt. The combined organic extracts were washed with water and saturated sodium chloride solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (20% MeOH in $CHCl_3$) to give a white crystal **10b** or **10c**.

3.9.1. 6-Amino-3-(3,5-dimethylbenzyl)-1-(2-picolyl)uracil (10b)

Yield 69%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$): δ 8.48 (1H, d, *J* 4.0, 2-picolyl), 7.73 (1H, m, 2-picolyl), 7.61 (1H, d, *J* 8.0, 2-picolyl), 7.27 (1H, m, 2-picolyl), 7.03 (2H, s, 3,5-Me_2-Bn), 6.85 (1H, s, 3,5-Me_2-Bn), 6.21 (2H, brs, NH_2), 5.13 (2H, s, 2-picolyl), 5.03 (1H, s, 5-H), 5.01 (2H, s, 3,5-Me_2-Bn), 2.26 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 162.8, 155.5, 155.3, 152.0, 148.7, 138.0, 137.8, 137.4, 128.9, 126.2, 125.0, 123.6, 79.4, 48.7, 44.2, 21.3; HRMS (ESI) Calcd for $C_{19}H_{20}N_4NaO_2$ $[M+Na]^+$: 359.14785. Found 359.14714; mp: 189.3 ℃.

3.9.2. 6-Amino-3-(3,5-dimethylbenzyl)-1-(4-picolyl)uracil (10c)

Yield 74%; white crystal; ^{1}H NMR (400MHz, $CDCl_3$): δ8.61 (2H, d, *J* 4.0, 4-picolyl), 7.14 (2H, d, *J* 8.0, 4-picolyl), 7.04 (2H, s, 3,5-Me_2-Bn), 6.88 (1H, s, 3,5-Me_2-Bn), 5.14 (2H, s, 4-picolyl), 5.06 (2H, s, 3,5-Me_2-Bn), 5.02 (1H, s, 5-H), 4.32 (2H, brs, NH_2) 2.28 (6H, s, 3,5-Me_2-Bn); ^{13}C NMR (100MHz, $CDCl_3$): 165.1, 156.9, 153.2, 150.3, 147.9, 138.9, 138.7, 129.7, 126.5, 122.9, 77.1, 45.8, 45.1, 21.4; HRMS (ESI) Calcd for $C_{19}H_{20}N_4NaO_2$ $[M+Na]^+$: 359.14785. Found 359.14734; mp: 142.0 ℃.

3.10. Anti-HIV-1 Assay

MT-4 cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL of penicillin G, and 100 mg/mL of streptomycin. The III_B strain of HIV-1 was used throughout the experiment. The virus was propagated and titrated in MT-4 cells. Virus stocks were stored at -80 ℃ until use. The anti-HIV-1 activity of the test compounds was determined by the inhibition of virus-induced cytopathogenicity in MT-4 cells.[8] Briefly, MT-4 cells (1×10^5 cells/mL) were infected with HIV-1 at a multiplicity of infection (MOI) of 0.1 and were cultured in the presence of various concentrations of the test compounds. After 4-day incubation at 37 ℃ in 5% CO_2, the number of viable cells was monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method.[9] The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity, based on the viability of mock-infected cells, as determined by the MTT method.

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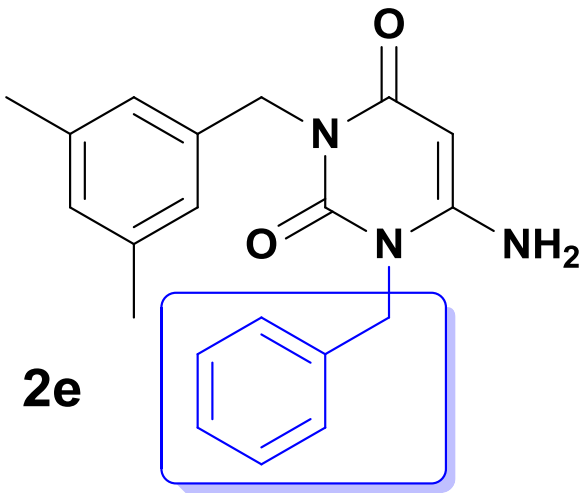
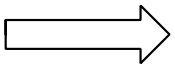


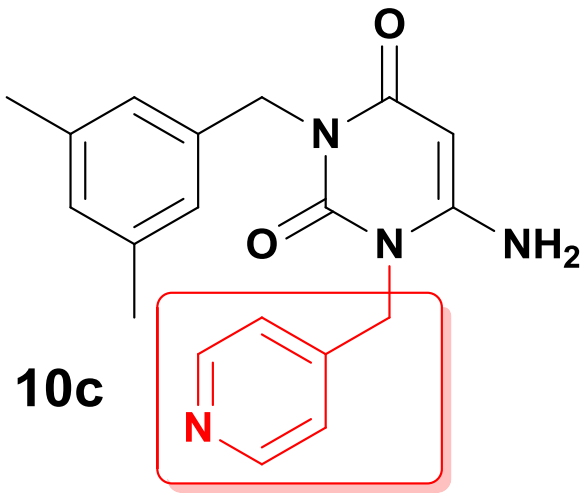
2e

EC_{50} = 0.07 μM

SI = 661

HIV-1 activity





10c

EC_{50} = 0.03 μM

SI = > 2863