Design, synthesis, and anti-HIV-I activity of 1-aromatic methyl-substituted 3-(3,5-dimethylbenzyl)uracil and N-3,5-dimethylbenzyl-substituted urea derivatives

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Abstract

Background: A new series of 1-aromatic methyl-substituted 3-(3,5-dimethylbenzyl)uracil and N-3,5-dimethylbenzyl-substituted urea derivatives were synthesized and evaluated as non-nucleoside HIV-I reverse transcriptase inhibitors.

Methods: A series of new 6-azido and 6-amino derivatives of 1-substituted-3-(3,5-dimethylbenzyl)uracils were synthesized using our previously reported method, and three acyclic derivatives were synthesized from urea. The anti-HIV-I activities of these compounds were determined based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. The cytotoxicities of the compounds were evaluated using the viability of mock-infected cells.

Results: Some of these compounds showed good-to-moderate activities against HIV-I with half maximal effective concentration (EC₅₀) values in the submicromolar or subnanomolar range. Compared with emivirine, compound 6-amino-3-(3,5-dimethylbenzyl)-1-(4-aminobenzyl)uracil showed significant anti-HIV-I activity with an EC₅₀ value of 10 nM and a high selectivity index of 1923. Preliminary structure–activity relationship studies and molecular modeling analyses were carried out to explore the major interactions between HIV-I reverse transcriptase and the potent inhibitor 6-amino-3-(3,5-dimethylbenzyl)-1-(4-aminobenzyl)uracil; these results may be important for further development of this class of compounds as anti-HIV-I agents.

Conclusion: The excellent activity of 6-amino-3-(3,5-dimethylbenzyl)-1-(4-aminobenzyl)uracil (EC₅₀: 0.010 ± 0.006 μM, SI: > 1923) may serve as the basis for conducting further investigations on the behavior of this class of compounds against drug-resistant mutants.

Keywords

Anti-HIV-I agents, non-nucleoside reverse transcriptase inhibitors (NNRTIs), Uracil analogs, Molecular modeling analysis, HIV-I reverse transcriptase (RT)

Introduction

In contrast to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) are highly specific. NNRTIs bind allosterically to the enzyme, thus making the catalytic reverse transcriptase (RT) subunit inactive.^{1,2} Much effort has been made in the design and synthesis of NNRTIs, with more than 30 structurally

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Figure 1. Structures of HEPT, emivirine, and 1-substituted-3-(3,5-dimethylbenzyl)uracil derivatives (**1a–g**).

Figure 2. Structural modification on 1-substituted-3-(3,5-dimethylbenzyl)uracil derivatives **1f** and **1g**.

different classes of NNRTIs reported.^{3,4} One of the first NNRTIs, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (Figure 1, HEPT), possesses the uracil skeleton.^{5,6} Derivatives of HEPT have been investigated as NNRTIs for more than two decades. In particular, emivirine (Figure 1),^{7–10} formerly known as MKC-442 and chosen as a candidate for clinical trials with AIDS patients, is the best-known HEPT derivative and also possesses the uracil skeleton.

However, the phase III study was halted when emivirine was found to activate the liver enzyme Cytochrome P450, which metabolizes protease inhibitors.¹¹ Although several NNRTIs, such as nevirapine, delavirdine, efavirenz, etravirine, and rilpivirine, have been approved by US Food and Drug Administration (FDA) for the treatment of HIV-1 infection, these molecules rapidly induce the development of drug-resistant HIV-1 strains. Therefore, we have actively investigated the development of an anti-HIV-1 agent using the structure–activity relationship (SAR) studies of 1,3-disubstituted and 1,3,6-trisubstituted uracils.^{12–15} We have demonstrated that the 3,5-dimethylbenzyl group at the 3-position of the uracil skeleton plays an important role in the enhancement of anti-HIV-1 activity; notably, substitution at the 1-position (e.g. with a benzyl, cyanomethyl, or 4-picolyl group) of 3-(3,5-dimethylbenzyl)uracil to obtain **1a–c** resulted in

good antiviral activity against HIV-1.¹² Moreover, the azido or amino group introduced at the 6-position of 1-benzyl-3-(3,5-dimethylbenzyl)uracil **1d** and **1e**, respectively, resulted in satisfactory activity with EC₅₀ values of $0.067 \pm 0.011 \mu\text{M}$ and $0.069 \pm 0.006 \mu\text{M}$, respectively.¹⁴ Furthermore, *N*¹-picolyl-C6-azido and *N*¹-picolyl-C6-amino-uracil derivatives, **1f** and **1g**, exhibited superior activities with EC₅₀ values of $0.050 \pm 0.020 \mu\text{M}$ and $0.030 \pm 0.030 \mu\text{M}$, respectively. However, the EC₅₀ values of the abovementioned compounds **1a–g** in our previous work were not satisfactory. Therefore, we evaluated newly synthesized 1,3,6-trisubstituted uracils with the aim of achieving greater anti-HIV-1 activity and lower cytotoxicity. In particular, as shown in Figure 2, we synthesized the 1-aromatic methyl-substituted derivatives of 6-azido or amino-3-(3,5-dimethylbenzyl)uracil to investigate the substituent effect by replacing the *N*¹-picolyl group of **1f** or **1g** with an appropriate aromatic-substituted methyl group such as 4-aminobenzyl, 2-furanylmethyl, and 4-pyrimidinylmethyl groups. Moreover, *N*-3,5-dimethylbenzyl-substituted urea derivatives with acyclic rings, as shown in Figure 2, were prepared to investigate whether the uracil skeleton is essential as the ligand to bind to HIV-1 RT. We also report the molecular-docking studies of some interesting ligands (**7a** and **7b**) with the nevirapine-binding site in HIV-1 RT.

Figure 3, Scheme 1. Synthesis of 6-azido-1-substituted-3-(3,5-dimethylbenzyl)uracil (**5**) and 6-amino-1-substituted-3-(3,5-dimethylbenzyl)uracil (**6** and **7**). *Reagents and conditions:* (i) R_1X ($X = Cl, Br$), K_2CO_3 , DMF, $-70^\circ C$, 2–18 h, 25–75%; (ii) $3,5\text{-(CH}_3)_2\text{BnOH}$, PPh_3 , TMAD, THF, $50^\circ C$, 11–48 h, 60–84%; (iii) NaN_3 , DMF, rt, 15–35 min, 65–98%; iv, $LiAlH_4$, THF, $0^\circ C$, 30 min, 69–74%; v, $NaBH_4$, MeOH, THF, $65^\circ C$, 30–50 min, 93–95%; vi, $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, THF, $0^\circ C$, 30 min, 80–90%.

Methods

Chemistry

The 1-substituted 6-azidouracil derivatives (**5**) and 1-substituted 6-aminouracil derivatives (**6** and **7**) were synthesized by alkylation at the 1-position of the uracil skeleton of the readily available 6-chlorouracil **2** using our previously reported method, as shown in Figure 3, Scheme 1.^{12–15} First, **2** was reacted with appropriate alkyl halides (e.g. 4-nitrobenzyl bromide, 2-nitrobenzyl bromide, or 4-fluorobenzyl bromide) to afford the corresponding 1-alkylated products, **3**. The Mitsunobu reaction of **3** treated with 3,5-dimethylbenzyl alcohol^{14,15} in the presence of PPh_3 and TMAD (N,N,N',N' -tetramethylazodicarboxamide) yielded the 3-(3,5-dimethylbenzyl) congeners, **4**. The nucleophilic substitution of the 6-chloro derivatives, **4**, at the C6-position of the uracil moiety with sodium azide afforded the corresponding 6-azido derivatives, **5**. The reduction of the 6-azido derivatives, **5** (except for **5e**), with $LiAlH_4$ yielded the corresponding 6-amino derivatives, **6**. The conversion of **5e** was performed using $NaBH_4$ /MeOH/THF¹⁶ to afford **6e**, thus avoiding the excessive reduction of the cyanobenzyl group of **5e**. The reactions of 6-azido-1-(4-nitrobenzyl)uracil derivative **5a** and 6-azido-1-(2-nitrobenzyl)uracil derivative **5b** with $NaBH_4$ and $NiCl_2 \cdot 6H_2O$ in the presence of MeOH and THF at $0^\circ C$ afforded the corresponding diamino products (**7a** and **7b**, respectively).¹⁷

1-(4-Hydroxybenzyl) or 1-(4-pyrimidinylmethyl)-substituted uracil derivatives (**11**, **12**, **14**, and **15**) were synthesized from a previously reported intermediate,¹⁵ 6-chloro-3-(3,5-dimethylbenzyl)uracil **8**. The alkylation of **8** afforded 1-substituted uracil derivatives **9** and **13** (Figure 4, Scheme 2); subsequent C6-azidation afforded products **10** and **14**, respectively. The deprotection of the acetyl group of **10** with K_2CO_3 /MeOH afforded C6-azido compound **11**, whereas the reduction of **10** with $LiAlH_4$ /THF afforded C6-amino derivative **12**. The C6-amination of **14** with $NaBH_4$ /MeOH/THF¹⁶ afforded **15**.

Three acyclic derivatives (**20aa**, **20ab**, and **20b**) were synthesized from urea (Figure 5, Scheme 3).¹⁸ Urea **16** was refluxed with H_2O in the presence of benzylamine or 4-aminomethylpyridine to afford *N*-benzylurea **17a** and *N*-(4-picolyl)urea **17b**, respectively. Condensation of **17a** and **17b** with 3,5-dimethylbenzaldehyde in the presence of titanium(IV) isopropoxide and subsequent reduction with $NaBH_4$ ¹⁸ afforded the condensation products **18a** and **18b**, respectively. The treatment of *N'*-3,5-dimethylbenzyl derivatives **18a** or **18b** with trichloroacetyl isocyanate, followed by silica gel chromatography, afforded the intermediate **19aa** and its regioisomer **19ab**, or a single product **19b**. Subsequently, **19aa**, **19ab**, and **19b** were individually subjected to acidic methanolysis with MeOH/silica gel to afford **20aa**, **20ab**, and **20b**;¹⁹ the regioisomers were identified using ^1H -NMR and COSY spectra.

Figure 4, Scheme 2. Synthesis of 6-azido (or amino)-3-(3,5-dimethylbenzyl)-1-substituted uracils (**11**, **12**, **14** and **15**). Reagents and conditions: (a) 4-AcO-BnOH, PPh₃, TMAD, THF, 50°C, 12 h, 64%; (b) NaN₃, DMF, rt, 30 min, 89–96%; (c) K₂CO₃, MeOH, rt, 1 h, 78%; iv, LiAlH₄, THF, 0°C, 30 min, 95%; v, 4-(chloromethyl)pyrimidine, K₂CO₃, NaI, DMF, 18 h, 43%; vi, NaBH₄ MeOH, THF, 65°C, 55 min, 58%.

Anti-HIV-1 assay

MT-4 cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 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°C until use. The anti-HIV-1 activity of the test compounds was determined by the inhibition of virus-induced cytopathogenicity in MT-4 cells.²⁰ Briefly, MT-4 cells (1 × 10⁵ cells/mL) were infected with HIV-1 at a multiplicity of infection of 0.1 and were cultured in the presence of various concentrations of the test compounds. After 4-day incubation at 37°C in 5% CO₂, the number of viable cells was monitored by the water-soluble tetrazolium dye WST-8. 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 WST-8 method.

Materials

Instrumentation

¹H NMR and ¹³C NMR spectra were taken with an Ultrashield™ 400 Plus FT NMR System (BRUKER, Germany). Chemical shifts and coupling constants (*J*) were given in δ and 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).

Compounds

General procedure for the synthesis of 3a–h

A solution of compound **2** (1.03 g, 7.0 mmol), appropriate alkyl halide (8.4 mmol) and K₂CO₃ (0.51 g, 3.68 mmol), in dry DMF (25.0 mL) was heated at RT – 70°C. After 2–18 h stirring, the mixture was extracted with silica gel column chromatography (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 **3a–h**.

6-Chloro-1-(4-nitrobenzyl)uracil [**3a**]

Yield 75%; white crystal; ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.81 (1H, brs, 3-NH), 8.22 (2H, d, *J* 8.8, 4-NO₂-Bn), 7.57 (2H, d, *J* 8.8, 4-NO₂-Bn), 6.05 (1H, s, H-5), 5.29 (2H, s, 4-NO₂-Bn); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.0, 150.4, 146.8, 146.4, 144.1, 127.6, 123.8, 102.7, 47.8; HRMS (ESI) Calcd for C₁₁H₈ClN₃NaO₄⁺ [M+Na]⁺: 304.00955. Found 304.02556; mp: 111.9–114.6°C.

Figure 5, Scheme 3. Synthesis of IV-carbamoylurea derivatives (**20aa**, **20ab**, and **20b**). Reagents and conditions: (a) benzylamine, H_2O , $130^\circ C$, 2 h, **17a**: 61% yield, **17b**: 74% yield; (b) 3,5-dimethylbenzylaldehyde, titanium(iv) isopropoxide, THF, $50^\circ C$, 2 h, **18a**: 79% yield, **18b**: 20% yield; (c) trichloroacetylisocyanate, THF, rt, 1 h, **19aa**: 32% yield, **19ab**: 38% yield, **19b**: 70% yield; (4) methanol, silica gel, $50^\circ C$, 40 h, **20aa**: 74% yield, **20ab**: 83% yield, **20b**: 81% yield.

6-Chloro-1-(2-nitrobenzyl)uracil [**3b**]

Yield 44%; white crystal; ^1H NMR (400 MHz, $DMSO-d_6$): δ 11.81 (1H, brs, 3-NH), 8.08 (1H, d, J 8.0, 2- NO_2 -Bn), 7.78 (1H, dd, J 8.0 and 8.0, 2- NO_2 -Bn), 7.61 (1H, dd, J 8.0 and 8.0, 2- NO_2 -Bn), 7.39 (1H, d, J 8.0, 2- NO_2 -Bn), 6.09 (1H, s, H-5), 5.47 (2H, s, 2- NO_2 -Bn); ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 161.1, 150.4, 147.0, 146.4, 134.7, 131.5, 128.8, 127.1, 125.3, 102.9, 46.1; HRMS (ESI) Calcd for $C_{11}H_8ClN_3NaO_4^+$ [$M+Na$] $^+$: 304.00955. Found 304.00970; mp: 145.9 – $147.5^\circ C$.

6-Chloro-1-(2,6-difluorobenzyl)uracil [**3c**]

Yield 21%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 8.66 (1H, brs, 3-NH), 7.30 (1H, m, 2,6- F_2 -Bn), 6.93 (2H, m, 2,6- F_2 -Bn), 5.89 (1H, s, H-5), 5.37 (2H, s, 2,6- F_2 -Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.4 (d, J 28), 160.4 (d, J 212), 159.9, 149.6, 147.6, 130.2, 111.9, 103.1, 39.0; HRMS (ESI) Calcd for $C_{11}H_7ClF_2N_2NaO_2^+$ [$M+Na$] $^+$: 295.00563. Found 295.00600; mp: 76.2 – $77.4^\circ C$.

6-Chloro-1-(4-fluorobenzyl)uracil [**3d**]

Yield 60%; white crystal; ^1H NMR (400 MHz, $DMSO-d_6$): δ 11.75 (1H, brs, 3-NH), 7.33 (2H, m, 4- F -Bn), 7.19 (2H, m, 4- F -Bn), 6.00 (1H, s, H-5), 5.14 (2H, s, 4- F -Bn); ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 162.6, 161.0, 150.5, 146.6, 132.4 (d, J 12), 128.9 (d, J 36), 115.5 (d, J 84), 102.5, 47.5; HRMS (ESI) Calcd for $C_{11}H_9ClFN_2O_2^+$ [$M+H$] $^+$: 255.03311. Found 255.03365; mp: 93.4 – $95.0^\circ C$.

6-Chloro-1-(4-cyanobenzyl)uracil [**3e**]

Yield 59%; white crystal; ^1H NMR (400 MHz, $DMSO-d_6$): δ 11.78 (1H, brs, 3-NH), 7.84 (2H, d, J 8.4, 4-CN-Bn), 7.49 (2H, d, J 8.4, 4-CN-Bn), 6.04 (1H, s, H-5), 5.24 (2H, s, 4-CN-Bn); ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 161.0, 150.5, 146.4, 142.0, 132.6, 127.3, 118.6, 110.2, 102.7, 47.9; HRMS (ESI) Calcd for $C_{12}H_9ClN_3O_2^+$ [$M+Na$] $^+$: 262.03778. Found 262.03830; mp: 131.0 – $132.0^\circ C$.

6-Chloro-1-(4-methoxybenzyl)uracil [**3f**]

Yield 47%; white crystal; ^1H NMR (400 MHz, $DMSO-d_6$): δ 11.78 (1H, brs, 3-NH), 7.22 (2H, d, J 8.8, 4- CH_3O -Bn), 6.92 (2H, d, J 8.8, 4- CH_3O -Bn), 5.97 (1H, s, H-5), 5.08 (2H, s, 4- CH_3O -Bn), 3.74 (3H, s, 4- CH_3O -Bn); ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 160.9, 158.6, 150.5, 146.8, 128.3, 128.1, 114.0, 102.3, 55.1, 47.6; HRMS (ESI) Calcd for $C_{12}H_{12}ClN_2O_3^+$ [$M+H$] $^+$: 267.05310. Found 267.05375; mp: 123.5 – $124.8^\circ C$.

6-Chloro-1-[(2-furanyl)methyl]uracil [**3g**]

Yield 29%; brown crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 9.25 (1H, brs, 3-NH), 7.38 (1H, dd, J 2.0 and 0.4, CH_2 (furan-2-yl)), 6.41 (1H, d, J 3.2, CH_2 (furan-2-yl)), 6.35 (1H, dd, J 3.2 and 2.0, CH_2 (furan-2-yl)), 5.90 (1H, s, H-5), 5.23 (2H, s, CH_2 (furan-2-yl)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.8, 149.8, 148.1, 147.5, 143.0, 110.7, 110.1, 103.0, 42.1; HRMS (ESI) Calcd for $C_9H_7ClN_2NaO_3^+$

$[M+Na]^+$: 249.00374. Found 249.00441; mp: 130.6–132.4°C.

6-Chloro-1-[(2-thiophenyl)methyl]uracil [3h]

Yield 70%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 9.25 (1H, brs, 3-NH), 7.38 (1H, dd, J 2.0 and 0.4, CH_2 (thiophen-2-yl)), 6.41 (1H, d, J 3.2, CH_2 (thiophen-2-yl)), 6.35 (1H, dd, J 3.2 and 2.0, CH_2 (thiophen-2-yl)), 5.90 (1H, s, H-5), 5.23 (2H, s, CH_2 (thiophen-2-yl)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 149.7, 147.2, 136.4, 128.7, 126.9, 126.7, 103.0, 44.0; HRMS (ESI) Calcd for $C_9H_7ClN_2NaO_2S^+$ $[M+Na]^+$: 264.98090. Found 264.98138; mp: 138.2–139.4°C.

General procedure for the synthesis of 4a–h

A solution of compound 3a–h (1.93 mmol), triphenylphosphine (0.66 g, 2.51 mmol), 3,5-dimethylbenzylalcohol (0.28 g, 2.02 mmol), and TMAD (0.43 g, 2.51 mmol) in THF (16.3 mL) was stirred at 50°C. After 18 h stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography to form 4a–h.

6-Chloro-3-(3,5-dimethylbenzyl)-1-(4-nitrobenzyl)uracil [4a]

Yield 69%; clear oil; ^1H NMR (400 MHz, $CDCl_3$): δ 8.20 (2H, d, J 8.8, 4- NO_2 -Bn), 7.45 (2H, d, J 8.8, 4- NO_2 -Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.91 (1H, s, 3,5-Me₂-Bn), 6.01 (1H, s, H-5), 5.34 (2H, s, 4- NO_2 -Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.2, 151.1, 147.7, 145.0, 142.6, 138.1, 135.9, 129.6, 128.2, 126.6, 124.1, 103.1, 49.2, 45.0, 21.3; HRMS (ESI) Calcd for $C_{20}H_{18}ClN_3NaO_4^+$ $[M+Na]^+$: 422.08780. Found 422.08734.

6-Chloro-3-(3,5-dimethylbenzyl)-1-(2-nitrobenzyl)uracil [4b]

Yield 84%; brown oil; ^1H NMR (400 MHz, $CDCl_3$): δ 8.18 (1H, d, J 8.0, 2- NO_2 -Bn), 7.62 (1H, dd, J 8.0 and 8.0, 2- NO_2 -Bn), 7.50 (1H, dd, J 8.0 and 8.0, 2- NO_2 -Bn), 7.08 (1H, d, J 8.0, 2- NO_2 -Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.91 (1H, s, 3,5-Me₂-Bn), 6.05 (1H, s, H-5), 5.60 (2H, s, 2- NO_2 -Bn), 5.05 (2H, s, 3,5-Me₂-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.3, 151.0, 147.4, 145.5, 138.1, 135.9, 134.2, 131.7, 129.6, 128.5, 126.6, 126.4, 125.8, 103.1, 47.8, 45.0, 21.3; HRMS (ESI) Calcd for $C_{20}H_{18}ClN_3NaO_4^+$ $[M+Na]^+$: 422.08780. Found 422.08740.

6-Chloro-1-(2,6-difluorobenzyl)-3-(3,5-dimethylbenzyl)uracil [4c]

Yield 60%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.27 (1H, m, 2,6-F₂-Bn), 7.00 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 6.89 (2H, m, 2,6-F₂-Bn), 5.94 (1H, s, H-5), 5.39 (1H, s, 2,6-F₂-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.4 (d, J 28), 160.5, 159.9 (d, J 28), 150.9, 145.5, 137.9, 136.1, 130.0 (t, J 40), 129.4, 126.3, 111.7 (q, J 24), 102.7, 44.9, 39.8 (t, J 16), 21.3; HRMS (ESI) Calcd for $C_{20}H_{17}ClF_2N_2NaO_2^+$ $[M+Na]^+$: 413.08388. Found 413.08344; mp: 120.5–122.2°C.

6-Chloro-3-(3,5-dimethylbenzyl)-1-(4-fluorobenzyl)uracil [4d]

Yield 84%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.27 (2H, m, 4-F-Bn), 7.00 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 6.89 (2H, m, 4-F-Bn), 5.94 (1H, s, H-5), 5.39 (2H, s, 4-F-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 161.3, 160.4, 151.2, 145.5, 138.2 (d, J 52), 136.1, 129.6 (q, J 32), 126.5, 124.9, 115.9 (d, J 84), 102.7, 49.2, 44.9, 21.3; HRMS (ESI) Calcd for $C_{20}H_{19}ClFN_2O_2^+$ $[M+Na]^+$: 373.11136. Found 373.11074; mp: 99.0–99.9°C.

6-Chloro-1-(4-cyanobenzyl)-3-(3,5-dimethylbenzyl)uracil [4e]

Yield 71%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (2H, d, J 8.4, 4-CN-Bn), 7.40 (2H, d, J 8.4, 4-CN-Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.91 (1H, s, 3,5-Me₂-Bn), 6.00 (1H, s, H-5), 5.29 (2H, s, 4-CN-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.2, 151.1, 145.1, 140.6, 138.1, 135.9, 132.7, 129.6, 128.0, 126.5, 118.3, 112.2, 103.0, 49.4, 45.0, 21.3; HRMS (ESI) Calcd for $C_{21}H_{18}ClN_3NaO_2^+$ $[M+Na]^+$: 402.09798. Found 402.09728; mp: 97.6–98.5°C.

6-Chloro-3-(3,5-dimethylbenzyl)-1-(4-methoxybenzyl)uracil [4f]

Yield 83%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 (2H, d, J 8.8, 4-CH₃O-Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 6.83 (2H, d, J 8.8, 4-CH₃O-Bn), 5.93 (1H, s, H-5), 5.19 (2H, s, 4-CH₃O-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 3.79 (3H, s, 4-CH₃O-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5, 159.5, 151.3, 145.7, 138.0, 136.2, 129.3, 127.5, 126.5, 124.8, 114.1, 102.5, 55.3, 49.4, 44.8, 21.3; HRMS (ESI) Calcd for $C_{21}H_{21}ClN_2NaO_3^+$

$[M+Na]^+$: 407.11329. Found 407.11293; mp: 96.4–97.2°C.

6-Chloro-3-(3,5-dimethylbenzyl)-1-[(2-furanyl)methyl]uracil [4g]

Yield 69%; brown crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37 (1H, m, CH_2 (furan-2-yl)), 7.04 (2H, s, 3,5-Me₂-Bn), 6.89 (1H, s, 3,5-Me₂-Bn), 6.38 (1H, d, J 3.2, CH_2 (furan-2-yl)), 6.34 (1H, dd, J 3.2 and 2.0, CH_2 (furan-2-yl)), 5.94 (1H, s, H-5), 5.23 (2H, s, CH_2 (furan-2-yl)), 5.02 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5, 150.8, 148.5, 145.3, 142.8, 138.0, 136.2, 129.5, 126.6, 110.6, 109.9, 102.7, 44.9, 42.9, 21.2; HRMS (ESI) Calcd for $C_{18}H_{17}ClN_2NaO_3^+$ $[M+Na]^+$: 367.08199. Found 367.08146; mp: 51.8–52.3°C.

6-Chloro-3-(3,5-dimethylbenzyl)-1-[(2-thiophenyl)methyl]uracil [4h]

Yield 68%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 (1H, dd, J 5.2 and 0.8, CH_2 (thiophen-2-yl)), 7.16 (1H, d, J 3.2, CH_2 (thiophen-2-yl)), 7.05 (2H, s, 3,5-Me₂-Bn), 6.97 (1H, dd, J 5.2 and 3.2, CH_2 (thiophen-2-yl)), 6.90 (1H, s, 3,5-Me₂-Bn), 5.93 (1H, s, H-5), 5.39 (2H, s, CH_2 (thiophen-2-yl)), 5.04 (2H, s, 3,5-Me₂-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 150.9, 144.9, 138.0, 136.7, 136.1, 129.5, 128.6, 126.8, 126.6, 126.5, 102.7, 44.8, 44.7, 21.3; HRMS (ESI) Calcd for $C_{18}H_{17}ClN_2NaO_2S^+$ $[M+Na]^+$: 383.05915. Found 383.05870; mp: 94.9–95.5°C.

General procedure for the synthesis of 5a–h, 10, and 14

Compound **4a–h**, **9**, or **13** (0.58 mmol) was dissolved in dry DMF (4.0 mL), and NaN_3 (0.04 g, 0.67 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 form C6-azido derivatives.

6-Azido-3-(3,5-dimethylbenzyl)-1-(4-nitrobenzyl)uracil [5a]

Yield 90%; brownish crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 8.19 (2H, d, J 8.4, 4-NO₂-Bn), 7.47 (2H, d, J 8.4, 4-NO₂-Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 5.60 (1H, s, H-5), 5.11 (2H, s, 4-NO₂-Bn), 5.05 (2H, s, 3,5-Me₂-Bn), 2.28 (6H, s, 3,5-Me₂-Bn);

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.0, 151.1, 150.3, 147.7, 142.9, 138.0, 136.2, 128.6, 126.4, 124.8, 124.0, 88.3, 46.2, 44.7, 21.3; HRMS (ESI) Calcd for $C_{20}H_{18}N_6NaO_4^+$ $[M+Na]^+$: 429.12817. Found 429.12714; mp: 143.6–146.7°C.

6-Azido-3-(3,5-dimethylbenzyl)-1-(2-nitrobenzyl)uracil [5b]

Yield 80%; colorless needle crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 8.13 (1H, dd, J 8.4 and 1.6, 2-NO₂-Bn), 7.59 (1H, m, 2-NO₂-Bn), 7.48 (1H, m, 2-NO₂-Bn), 7.09 (1H, d, J 8.0, 2-NO₂-Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 5.63 (1H, s, H-5), 5.47 (2H, s, 2-NO₂-Bn), 5.06 (2H, s, 3,5-Me₂-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.0, 151.1, 150.6, 147.8, 138.0, 136.0, 134.0, 131.9, 129.4, 128.6, 126.9, 126.5, 125.5, 88.3, 44.7, 44.5, 21.3; HRMS (ESI) Calcd for $C_{20}H_{18}N_6NaO_4^+$ $[M+Na]^+$: 429.12817. Found 429.12757; mp: 127.6–128.3°C.

6-Azido-1-(2,6-difluorobenzyl)-3-(3,5-dimethylbenzyl)uracil [5c]

Yield 98%; brown crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.25 (1H, m, 2,6-F₂-Bn), 6.99 (2H, s, 3,5-Me₂-Bn), 6.88 (2H, m, 2,6-F₂-Bn), 6.87 (1H, s, 3,5-Me₂-Bn), 5.56 (1H, s, H-5), 5.17 (1H, s, 2,6-F₂-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.26 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.5 (d, J 28), 161.3, 160.0 (d, J 32), 150.8 (d, J 28), 137.9, 136.4, 129.9 (t, J 40), 129.2, 126.2, 111.8 (d, J 24), 111.5 (t, J 12), 88.2, 44.6, 36.8 (t, J 12), 21.3; HRMS (ESI) Calcd for $C_{20}H_{17}F_2N_5NaO_2^+$ $[M+Na]^+$: 420.12425. Found 420.12268; mp: 96.8–99.8°C.

6-Azido-3-(3,5-dimethylbenzyl)-1-(4-fluorobenzyl)uracil [5d]

Yield 86%; brown crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34 (2H, m, 4-F-Bn), 7.02 (2H, s, 3,5-Me₂-Bn), 7.01 (2H, m, 4-F-Bn), 6.89 (1H, s, 3,5-Me₂-Bn), 5.56 (1H, s, H-5), 5.05 (2H, s, 3,5-Me₂-Bn), 4.99 (2H, s, 4-F-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 161.3 (d, J 40), 151.2, 150.6, 137.9, 136.4, 131.6 (d, J 12), 130.0 (d, J 24), 129.3, 126.3, 115.8 (d, J 84), 88.1, 46.3, 44.6, 21.3; HRMS (ESI) Calcd for $C_{20}H_{18}FN_5NaO_2^+$ $[M+Na]^+$: 402.13367. Found 402.13242; mp: 78.8–82.5°C.

6-Azido-1-(4-cyanobenzyl)-3-(3,5-dimethylbenzyl)uracil [5e]

Yield 96%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.62 (2H, d, J 8.0, 4-CN-Bn), 7.41 (2H, d,

J 8.0, 4-CN-Bn), 7.02 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 5.59 (1H, s, H-5), 5.07 (2H, s, 4-CN-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 161.0, 151.1, 150.3, 141.0, 138.0, 136.3, 132.6, 129.4, 128.4, 126.4, 118.4, 112.1, 88.2, 46.5, 44.7, 21.3; HRMS (ESI) Calcd for C₂₁H₁₈N₆NaO₂⁺ [M+Na]⁺: 409.13834. Found 409.13718; mp: 131.3–133.4°C.

6-Azido-3-(3,5-dimethylbenzyl)-*l*-(4-methoxybenzyl)uracil [5f]

Yield 87%; brown crystal; ¹H NMR (400 MHz, CDCl₃): δ 7.30 (2H, d, *J* 8.4, 4-CH₃O-Bn), 7.03 (2H, s, 3,5-Me₂-Bn), 6.88 (1H, s, 3,5-Me₂-Bn), 6.85 (2H, d, *J* 8.4, 4-CH₃O-Bn), 5.54 (1H, s, H-5), 5.05 (2H, s, 3,5-Me₂-Bn), 4.97 (2H, s, 4-CH₃O-Bn), 3.79 (3H, s, 4-CH₃O-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 161.3, 159.4, 151.2, 150.8, 137.9, 136.5, 129.6, 129.3, 127.9, 126.3, 114.1, 88.0, 55.3, 46.5, 44.6, 21.3; HRMS (ESI) Calcd for C₂₁H₂₁N₅NaO₃⁺ [M+Na]⁺: 414.15366. Found 414.15233; mp: 93.4–98.7°C.

6-Azido-3-(3,5-dimethylbenzyl)-*l*-[(2-furanyl)methyl]uracil [5g]

Yield 65%; brown oil; ¹H NMR (400 MHz, CDCl₃): δ 7.35 (1H, dd, *J* 1.6 and 0.8, CH₂(furan-2-yl)), 7.03 (2H, s, 3,5-Me₂-Bn), 6.88 (1H, s, 3,5-Me₂-Bn), 6.35 (1H, d, *J* 3.2, CH₂(furan-2-yl)), 6.32 (1H, dd, *J* 3.2 and 1.6, CH₂(furan-2-yl)), 5.56 (1H, s, H-5), 5.03 (2H, s, 3,5-Me₂-Bn), 5.01 (2H, s, CH₂(furan-2-yl)), 2.27 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 161.2, 150.8, 150.5, 148.8, 142.7, 137.9, 136.5, 129.3, 126.5, 110.6, 109.6, 88.1, 44.5, 39.9, 21.3; HRMS (ESI) Calcd for C₁₈H₁₇N₅NaO₃⁺ [M+Na]⁺: 374.12236. Found 374.12183.

6-Azido-3-(3,5-dimethylbenzyl)-*l*-[(2-thiophenyl)methyl]uracil [5h]

Yield 86%; brown oil; ¹H NMR (400 MHz, CDCl₃): δ 7.26 (1H, dd, *J* 5.2 and 0.8, CH₂(thiophen-2-yl)), 7.14 (1H, d, *J* 3.6, CH₂(thiophen-2-yl)), 7.04 (2H, s, 3,5-Me₂-Bn), 6.95 (1H, dd, *J* 5.2 and 3.6, CH₂(thiophen-2-yl)), 6.88 (1H, s, 3,5-Me₂-Bn), 5.54 (1H, s, H-5), 5.17 (2H, s, CH₂(thiophen-2-yl)), 5.04 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 161.1, 150.8, 150.2, 137.9, 137.1, 136.4, 129.3, 128.4, 126.7, 126.4, 126.4, 88.1, 44.5, 41.6, 21.3; HRMS (ESI) Calcd for C₁₈H₁₇N₅NaO₂S⁺ [M+Na]⁺: 390.09952. Found 390.09913.

***l*-[4-(Acetoxy)benzyl]-6-azido-3-(3,5-dimethylbenzyl)uracil [10]**

Yield 96%; yellow crystal; ¹H NMR (400 MHz, CDCl₃): δ 7.35 (2H, d, *J* 8.8, 4-AcO-Bn), 7.06 (2H, d, *J* 8.8, 4-AcO-Bn), 7.04 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 5.96 (1H, s, H-5), 5.24 (2H, s, 4-AcO-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.29 (3H, s, 4-AcO-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 169.4, 161.2, 151.2, 150.7, 150.4, 138.0, 136.4, 133.3, 129.3, 129.3, 126.3, 121.9, 88.0, 46.3, 44.6, 21.3, 21.1; HRMS (ESI) Calcd for C₂₂H₂₁N₅NaO₄⁺ [M+Na]⁺: 442.14858. Found 442.14776; mp: 39.6–41.5°C.

6-Azido-3-(3,5-dimethylbenzyl)-*l*-(4-pyrimidinylmethyl)uracil [14]

Yield 89%; brown oil; ¹H NMR (400 MHz, CDCl₃): δ 9.13 (1H, s, 4-pyrimidinylmethyl), 8.69 (1H, d, *J* 5.2, 4-pyrimidinylmethyl), 7.20 (1H, d, *J* 5.2, 4-pyrimidinylmethyl), 7.01 (2H, s, 3,5-Me₂-Bn), 6.89 (1H, s, 3,5-Me₂-Bn), 5.64 (1H, s, H-5), 5.13 (2H, s, 4-pyrimidinylmethyl), 5.05 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 163.5, 161.2, 158.9, 157.2, 151.1, 150.7, 137.9, 136.3, 129.3, 126.2, 118.4, 88.1, 47.1, 44.6, 21.3; HRMS (ESI) Calcd for C₁₈H₁₇N₇NaO₂⁺ [M+Na]⁺: 386.13359. Found 386.13924.

General procedure for the synthesis of 6a–d, 6f–h, and 12

Compound **5a–h** or **10** (0.64 mmol) was dissolved in dry THF (10.0 mL) under nitrogen atmosphere. To this stirred solution, we carefully added LiAlH₄ (0.02 g, 0.77 mmol) at 0°C. Stirring was continued at 0°C 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 (10% MeOH in AcOEt) to form C6-amino analogs.

6-Amino-3-(3,5-dimethylbenzyl)-*l*-(4-nitrobenzyl)uracil [6a]

Yield 71%; yellowish crystal; ¹H NMR (400 MHz, CDCl₃): δ 8.12 (2H, d, *J* 8.4, 4-NO₂-Bn), 7.30 (2H, d, *J* 8.4, 4-NO₂-Bn), 6.93 (2H, s, 3,5-Me₂-Bn), 6.84 (1H, s, 3,5-Me₂-Bn), 5.13 (2H, s, 4-NO₂-Bn), 5.07 (2H, s, 3,5-Me₂-Bn), 4.99 (1H, brs, 6-NH₂), 4.93 (1H, s, H-5), 2.23 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃):

δ 162.9, 153.7, 151.8, 147.6, 142.5, 138.0, 137.1, 129.1, 127.2, 125.8, 124.2, 78.6, 45.5, 44.4, 21.3; HRMS (ESI) Calcd for $C_{20}H_{20}N_4NaO_4^+$ $[M+Na]^+$: 403.13768. Found 403.13612; mp: 109.3–112.2°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(2-nitrobenzyl)uracil [6b]

Yield 67%; yellowish crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 8.11 (1H, dd, J 8.4 and 1.2, 2- NO_2 -Bn), 7.63 (1H, m, 2- NO_2 -Bn), 7.50 (1H, m, 2- NO_2 -Bn), 7.24 (1H, d, J 8.0, 2- NO_2 -Bn), 7.00 (2H, s, 3,5-Me₂-Bn), 6.86 (1H, s, 3,5-Me₂-Bn), 5.50 (2H, s, 2- NO_2 -Bn), 5.04 (1H, s, H-5), 5.04 (2H, s, 3,5-Me₂-Bn), 4.71 (2H, brs, 6-NH₂), 2.28 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.5, 152.9, 152.0, 147.6, 137.9, 137.2, 134.5, 130.7, 129.1, 127.4, 126.1, 126.1, 125.7, 79.1, 44.4, 43.2, 21.3; HRMS (ESI) Calcd for $C_{20}H_{20}N_4NaO_4^+$ $[M+Na]^+$: 403.13768. Found 403.13663; mp: 177.1–181.6°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(2,6-difluorobenzyl)uracil [6c]

Yield 89%; yellowish crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.23 (1H, m, 2,6-F₂-Bn), 6.97 (2H, s, 3,5-Me₂-Bn), 6.91 (2H, m, 2,6-F₂-Bn), 6.83 (1H, s, 3,5-Me₂-Bn), 5.20 (1H, s, 2,6-F₂-Bn), 5.02 (2H, s, 3,5-Me₂-Bn), 4.97 (1H, s, H-5), 4.78 (2H, brs, 6-NH₂), 2.25 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 162.4 (d, J 28), 159.9 (d, J 28), 153.3, 151.6, 137.8, 137.3, 130.3 (t, J 40), 128.8, 125.7, 112.0 (q, J 28), 111.0, 79.1, 44.3, 21.2; HRMS (ESI) Calcd for $C_{20}H_{19}F_2N_3NaO_2^+$ $[M+Na]^+$: 394.13375. Found 394.13294; mp: 53.1–57.2°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(4-fluorobenzyl)uracil [6d]

Yield 84%; white crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.21 (2H, m, 4-F-Bn), 7.04 (2H, m, 4-F-Bn), 7.01 (2H, s, 3,5-Me₂-Bn), 6.86 (1H, s, 3,5-Me₂-Bn), 5.07 (2H, s, 4-F-Bn), 5.05 (2H, s, 3,5-Me₂-Bn), 5.00 (1H, s, H-5), 4.55 (1H, brs, 6-NH₂), 2.26 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 162.7, 153.6, 152.1, 137.9, 137.3, 130.8 (d, J 12), 129.0, 128.2 (d, J 32), 126.1, 116.4 (d, J 84), 79.4, 45.6, 44.3, 21.3; HRMS (ESI) Calcd for $C_{20}H_{20}FN_3NaO_2^+$ $[M+Na]^+$: 376.14318. Found 376.14182; mp: 166.8–168.4°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(4-methoxybenzyl)uracil [6f]

Yield 88%; yellowish oil; ^1H NMR (400 MHz, $CDCl_3$): δ 7.14 (2H, d, J 8.8, 4-CH₃O-Bn), 6.99 (2H,

s, 3,5-Me₂-Bn), 6.87 (1H, s, 3,5-Me₂-Bn), 6.86 (2H, d, J 8.8, 4-CH₃O-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 5.02 (2H, s, 4-CH₃O-Bn), 4.97 (1H, s, H-5), 4.71 (1H, brs, 6-NH₂), 3.78 (3H, s, 4-CH₃O-Bn), 2.25 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 159.6, 154.1, 152.2, 137.8, 137.4, 128.9, 127.7, 127.0, 126.0, 114.7, 79.0, 55.3, 45.8, 44.3, 21.3; HRMS (ESI) Calcd for $C_{21}H_{23}N_3NaO_3^+$ $[M+Na]^+$: 388.16316. Found 388.16225.

6-Amino-3-(3,5-dimethylbenzyl)-1-[(2-furanyl)methyl]uracil [6g]

Yield 53%; red-brown crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37 (1H, m, CH₂(furan-2-yl)), 7.04 (2H, s, 3,5-Me₂-Bn), 6.86 (1H, s, 3,5-Me₂-Bn), 6.45 (1H, d, J 3.2, CH₂(furan-2-yl)), 6.37 (1H, dd, J 3.2 and 2.0, CH₂(furan-2-yl)), 5.05 (2H, s, 3,5-Me₂-Bn), 5.04 (1H, s, H-5), 5.01 (2H, s, CH₂(furan-2-yl)), 4.89 (1H, brs, 6-NH₂), 2.26 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 153.6, 151.6, 148.9, 142.7, 137.8, 137.3, 129.0, 126.3, 111.2, 110.1, 78.0, 44.3, 39.2, 21.3; HRMS (ESI) Calcd for $C_{18}H_{19}N_3NaO_3^+$ $[M+Na]^+$: 348.13186. Found 348.13110; mp: 69.8–74.8°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-[(2-thiophenyl)methyl]uracil [6h]

Yield 79%; yellow crystal; ^1H NMR (400 MHz, $CDCl_3$): δ 7.26 (1H, m, CH₂(thiophen-2-yl)), 7.01 (1H, d, J 3.6, CH₂(thiophen-2-yl)), 7.00 (2H, s, 3,5-Me₂-Bn), 6.94 (1H, dd, J 5.2 and 3.6, CH₂(thiophen-2-yl)), 6.85 (1H, s, 3,5-Me₂-Bn), 5.21 (2H, s, CH₂(thiophen-2-yl)), 5.04 (2H, s, 3,5-Me₂-Bn), 4.97 (1H, s, H-5), 4.82 (1H, brs, 6-NH₂), 2.25 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 153.6, 151.7, 137.9, 137.7, 137.2, 129.0, 127.0, 126.4, 126.4, 125.9, 79.4, 44.3, 41.6, 21.3; HRMS (ESI) Calcd for $C_{18}H_{19}N_3NaO_2S^+$ $[M+Na]^+$: 364.10902. Found 364.10866; mp: 72.3–78.5°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(4-hydroxybenzyl)uracil [12]

Yield 95%; colorless oil; ^1H NMR (400 MHz, CD_3OD): δ 6.95 (2H, d, J 8.4, 4-HO-Bn), 6.76 (2H, s, 3,5-Me₂-Bn), 6.72 (1H, s, 3,5-Me₂-Bn), 6.62 (2H, d, J 8.4, 4-HO-Bn), 4.89 (2H, s, 3,5-Me₂-Bn), 4.89 (2H, s, 4-HO-Bn), 4.86 (1H, s, H-5), 2.11 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, CD_3OD): δ 165.2, 158.2, 157.1, 153.5, 138.9, 138.8, 129.6, 129.0, 127.6, 126.3, 116.5, 77.1, 46.2, 45.0, 21.4; HRMS (ESI) Calcd for $C_{20}H_{21}N_3NaO_3^+$ $[M+Na]^+$: 374.14751. Found 374.14671.

6-Amino-1-(4-cyanobenzyl)-3-(3,5-dimethylbenzyl)uracil [6e]

To a refluxing mixture of compound **5e** (0.08 g, 0.20 mmol) and NaBH₄ (0.008 g, 0.20 mmol) in THF (3.0 mL), MeOH (0.3 mL) was added over a period of 15 min. The mixture was allowed to cool to room temperature, sat. NaHCO₃ aq (5.0 mL) was added, and the solution was extracted with AcOEt, washed with saturated aqueous sodium chloride solution, dried with sodium sulfate, and then evaporated. The residual solution was purified by silica gel column chromatography (20% MeOH in AcOEt) to form a white crystal **6e** (0.07 g, 0.19 mmol, 95%). ¹H NMR (400 MHz, CDCl₃): δ 7.67 (2H, d, *J* 8.0, 4-CN-Bn), 7.35 (2H, d, *J* 8.0, 4-CN-Bn), 7.04 (2H, s, 3,5-Me₂-Bn), 6.89 (1H, s, 3,5-Me₂-Bn), 5.18 (2H, s, 4-CN-Bn), 5.06 (1H, s, H-5), 5.05 (2H, s, 3,5-Me₂-Bn), 4.27 (1H, brs, 6-NH₂), 2.27 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 162.4, 152.7, 151.9, 140.4, 137.9, 137.0, 133.1, 129.2, 127.0, 126.3, 118.1, 112.5, 80.1, 45.9, 44.4, 21.3; HRMS (ESI) Calcd for C₂₁H₂₀N₄NaO₂⁺ [M+Na]⁺: 383.14785. Found 383.14664; mp: 128.5–131.6°C.

General procedure for the synthesis of 7a,b

Compound **5a,b** (0.20 mmol) was dissolved in the 3:1 mixture of dry MeOH (3.0 mL) and dry THF (1.0 mL), and NaBH₄ (0.03 g, 0.92 mmol) and NiCl₂·6H₂O (0.08 g, 0.32 mmol) were added to the solution, which was stirred for 30 min at 0°C. 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 (20% MeOH in AcOEt) to form diamino derivative **7a,b**.

6-Amino-3-(3,5-dimethylbenzyl)-1-(4-aminobenzyl)uracil [7a]

Yield 80%; white crystal; ¹H NMR (400 MHz, CDCl₃): δ 7.01 (2H, d, *J* 8.4, 4-NH₂-Bn), 7.00 (2H, d, *J* 8.4, 4-NH₂-Bn), 6.85 (2H, s, 3,5-Me₂-Bn), 6.61 (1H, s, 3,5-Me₂-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 4.97 (2H, s, 4-NH₂-Bn), 4.91 (1H, s, H-5), 4.65 (2H, brs, 6-NH₂), 3.76 (2H, brs, 4-NH₂-Bn), 2.26 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 162.8, 154.2, 152.2, 146.5, 137.8, 137.5, 128.9, 127.7, 126.0, 124.5, 115.7, 79.0, 46.0, 44.3, 21.3; HRMS (ESI) Calcd for C₂₀H₂₂N₄NaO₂⁺ [M+Na]⁺: 373.16350. Found 373.16279; mp: 167.5–168.9°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(2-aminobenzyl)uracil [7b]

Yield 80%; yellowish needle crystal; ¹H NMR (400 MHz, CD₃OD): δ 6.96 (1H, m, 2-NH₂-Bn), 6.88 (1H, d, *J* 7.2, 2-NH₂-Bn), 6.80 (2H, s, 3,5-Me₂-Bn), 6.74 (1H, s, 3,5-Me₂-Bn), 6.70 (1H, d, *J* 7.6, 2-NH₂-Bn), 6.61 (1H, m, 2-NH₂-Bn), 4.91 (2H, s, 2-NH₂-Bn), 4.91 (2H, s, 3,5-Me₂-Bn), 4.86 (1H, s, H-5), 2.14 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CD₃OD): δ 165.1, 157.1, 135.7, 154.8, 138.9, 138.8, 129.8, 129.6, 128.0, 126.3, 122.7, 120.6, 118.7, 76.9, 45.1, 42.6, 21.4; HRMS (ESI) Calcd for C₂₀H₂₂N₄NaO₂⁺ [M+Na]⁺: 373.16350. Found 373.16246; mp: 157.3–162.2°C.

1-[4-(Acetoxy)benzyl]-6-chloro-3-(3,5-dimethylbenzyl)uracil [9]

A solution of compound **8** (0.26 g, 1.00 mmol), triphenylphosphine (0.34 g, 1.30 mmol), 4-(acetoxy)benzyl alcohol (0.28 g, 2.02 mmol), and TMAD (0.22 g, 1.30 mmol) in THF (8.5 mL) was stirred at 50°C. After 24 h stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography (50% AcOEt in hexane) to form syrup **9** (0.26 g, 0.64 mmol, 64%). ¹H NMR (400 MHz, CDCl₃): δ 7.35 (2H, d, *J* 8.8, 4-AcO-Bn), 7.06 (2H, d, *J* 8.8, 4-AcO-Bn), 7.04 (2H, s, 3,5-Me₂-Bn), 6.90 (1H, s, 3,5-Me₂-Bn), 5.96 (1H, s, H-5), 5.24 (2H, s, 4-AcO-Bn), 5.04 (2H, s, 3,5-Me₂-Bn), 2.29 (3H, s, 4-AcO-Bn), 2.28 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 169.3, 160.5, 151.2, 150.5, 145.6, 138.0, 136.1, 132.9, 129.5, 128.9, 126.5, 122.0, 102.7, 49.3, 44.9, 21.3, 21.1; HRMS (ESI) Calcd for C₂₂H₂₁ClN₂NaO₄⁺ [M+Na]⁺: 435.10821. Found 435.10700.

6-Azido-3-(3,5-dimethylbenzyl)-1-(4-hydroxybenzyl)uracil [11]

Compound **10** (0.06 g, 0.15 mmol) was dissolved in dry MeOH (5.0 mL), and K₂CO₃ (0.42 g, 3.00 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 AcOEt to form a red-brown crystal **11** (0.04 g, 0.12 mmol, 78%). ¹H NMR (400 MHz, CDCl₃): δ 7.17 (2H, d, *J* 8.8, 4-HO-Bn), 7.02 (2H, s, 3,5-Me₂-Bn), 6.86 (1H, s, 3,5-Me₂-Bn), 6.74 (2H, d, *J* 8.8, 4-HO-Bn), 6.16 (1H, brs, 4-HO-Bn), 5.14 (1H, s, H-5), 5.05 (2H, s, 3,5-Me₂-Bn), 4.97 (2H, s, 4-HO-Bn), 2.25 (6H, s, 3,5-Me₂-Bn); ¹³C NMR (100 MHz, CDCl₃): δ 163.3, 161.3, 155.8, 151.2, 137.9, 136.8, 129.7, 129.2, 126.2, 115.5, 78.2, 57.0, 45.0, 44.4,

21.3; HRMS (ESI) Calcd for $C_{20}H_{19}N_5NaO_3^+$ $[M+Na]^+$: 400.13801. Found 400.11876; mp: 79.6–83.8°C.

6-Chloro-3-(3,5-dimethylbenzyl)-1-(4-pyrimidinylmethyl)uracil [13]

A solution of compound **8** (0.27 g, 1.00 mmol), 4-(chloromethyl)pyrimidine (0.22 g, 1.72 mmol), K_2CO_3 (0.24 g, 1.72 mmol), and NaI (0.03 g, 0.20 mmol) in DMF (4.0 mL) was stirred at room temperature. After 48 h stirring, the solution was extracted with AcOEt, washed with saturated aqueous sodium chloride solution, dried with sodium sulfate, and then evaporated. The residual solution was purified by silica gel column chromatography (50% AcOEt in hexane) to form a brown crystal **13** (0.16 g, 0.44 mmol, 44%). ^1H NMR (400 MHz, $CDCl_3$): δ 9.15 (1H, s, 4-pyrimidinylmethyl), 8.70 (1H, d, J 5.2, 4-pyrimidinylmethyl), 7.22 (1H, d, J 5.2, 4-pyrimidinylmethyl), 7.01 (2H, s, 3,5-Me₂-Bn), 6.89 (1H, s, 3,5-Me₂-Bn), 6.03 (1H, s, H-5), 5.34 (2H, s, 4-pyrimidinylmethyl), 5.04 (2H, s, 3,5-Me₂-Bn), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 160.4, 159.0, 157.3, 151.1, 154.6, 138.0, 135.9, 129.4, 126.3, 118.3, 102.7, 49.9, 44.9, 21.3; HRMS (ESI) Calcd for $C_{18}H_{17}ClN_4NaO_2^+$ $[M+Na]^+$: 379.09322. Found 379.09275; mp: 97.1–98.2°C.

6-Amino-3-(3,5-dimethylbenzyl)-1-(4-pyrimidinylmethyl)uracil [15]

To a refluxing mixture of compound **14** (0.07 g, 0.21 mmol) and $NaBH_4$ (0.008 g, 0.21 mmol) in THF (3.0 mL), MeOH (0.3 mL) was added over a period of 15 min. The mixture was then allowed to cool to room temperature, sat. $NaHCO_3$ aq (5.0 mL) was added, and the solution was extracted with AcOEt, washed with saturated aqueous sodium chloride solution, dried with sodium sulfate, and then evaporated. The residual solution was purified by silica gel column chromatography (20% MeOH in AcOEt) to form a yellowish crystal **15** (0.04 g, 0.12 mmol, 58%). ^1H NMR (400 MHz, $CDCl_3$): δ 9.14 (1H, d, J 0.8, 4-pyrimidinylmethyl), 8.78 (1H, d, J 5.2, 4-pyrimidinylmethyl), 7.59 (1H, dd, J 5.2 and 0.8, 4-pyrimidinylmethyl), 7.00 (2H, s, 3,5-Me₂-Bn), 6.85 (1H, s, 3,5-Me₂-Bn), 5.86 (1H, brs, 6-NH₂), 5.08 (1H, s, H-5), 5.07 (2H, s, 3,5-Me₂-Bn), 4.99 (2H, s, 4-pyrimidinylmethyl), 2.25 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 162.7, 158.7, 158.4, 154.6, 151.9, 137.9, 137.2, 129.1, 126.1, 121.6, 79.9, 47.8, 44.3, 21.3; HRMS (ESI) Calcd for $C_{18}H_{19}N_5NaO_2^+$ $[M+Na]^+$: 360.14310. Found 360.14230; mp: 85.8–89.0°C.

General procedure for the synthesis of **17a** and **17b**

A mixture of urea **16** (4.32 g, 72.0 mmol) and benzylamine or 4-aminomethylpyridine (60.0 mmol) in H_2O (30.0 mL) was stirred at 130 °C. After 12 h stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography to form a benzylated or picolylated product.

N-Benzylurea [17a]

Yield 61%; white crystal; ^1H NMR (400 MHz, DMSO- d_6): δ 7.19–7.32 (5H, m, Ph), 6.39 (1H, t, J 6.0, $NH-CH_2-Ph$), 5.50 (2H, s, NH₂), 4.16 (2H, d, J 6.0, $NH-CH_2-Ph$); ^{13}C NMR (100 MHz, DMSO- d_6): δ 158.7, 140.9, 128.2, 127.0, 126.5, 42.8; HRMS (ESI) Calcd for $C_8H_{10}N_2NaO$ $[M+Na]^+$: 173.06853. Found 173.06964; mp 146.2–149.2°C.

N-(4-picolyl)urea [17b]

Yield 74%; white crystal; ^1H NMR (400 MHz, DMSO- d_6): δ 8.47–8.48 (2H, m, picolyl), 7.22–7.23 (2H, m, picolyl), 6.53 (1H, t, J 6.0, $NH-CH_2-$), 5.63 (2H, s, NH₂), 4.19 (2H, d, J 6.0, $NH-CH_2-$); ^{13}C NMR (100 MHz, DMSO- d_6): δ 158.7, 150.1, 149.4, 121.9, 41.8; HRMS (ESI) Calcd for $C_7H_9N_3NaO$ $[M+Na]^+$: 174.06378. Found 174.06470; mp 187.0–187.2°C.

General procedure for the synthesis of **18a** and **18b**

A mixture of 3,5-dimethylbenzaldehyde (0.40 mL, 3.0 mmol), benzylurea **17a** or **17b** (1.5 mmol), and titanium (IV) isopropoxide (0.50 mL, 1.7 mmol) were slurred in THF (2.0 mL). This slurry was stirred at 50°C under nitrogen. After 2 h stirring, the solution was then cooled to 0°C, and $NaBH_4$ (0.06 g, 1.5 mmol) was added. The ice bath was removed, and the resulting slurry was allowed to stir for 30 min. The slurry was again cooled to 0°C and quenched by dropwise addition of 1 N HCl aq (6.0 mL), the solution was extracted with AcOEt, washed with saturated aqueous sodium chloride solution, dried with sodium sulfate, and then evaporated. The residual solution was purified by silica gel column chromatography to form a crystal **18a** or **18b** (0.32 g, 1.18 mmol, 79%).

N-benzyl-N'-(3,5-dimethylbenzyl)urea [18a]

Yield 79%; white crystal; ^1H NMR (400 MHz, DMSO- d_6): δ 7.20–7.32 (5H, m, Ph), 6.84 (3H, s, 3,5-Me₂-Bn), 6.40 (1H, t, J 6.0, $NH-CH_2-Ph$), 6.37 (1H, t, J 6.0, $NH-CH_2-Ph$), 4.22 (2H, d, J 6.0, $NH-CH_2-Ph$), 4.16 (2H, d, J 6.0, $NH-CH_2-Ph$), 2.23 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, DMSO- d_6): δ 158.1, 141.0, 140.7,

137.1, 128.2, 127.9, 126.9, 126.5, 124.7, 42.9, 42.9, 20.9; HRMS (ESI) Calcd for $C_{17}H_{20}N_2NaO$ $[M+Na]^+$: 291.14678. Found 291.14575; mp 146.0–147.6°C.

N'-3,5-dimethyl-*N*-(4-picolyl)urea [**18b**]

Yield 20%; white crystal; ^1H NMR (400 MHz, DMSO- d_6): δ 8.47–8.48 (2 H, m, picolyl), 7.22–7.24 (2 H, m, picolyl), 6.84 (3 H, s, 3,5-Me₂-Bn), 6.50–6.56 (2 H, m, *NH*), 4.25 (2 H, d, J 6.4, *NH-CH*₂), 4.15 (2 H, d, J 6.0, *NH-CH*₂), 2.24 (6 H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, DMSO- d_6): δ 158.04, 150.16, 149.35, 140.57, 137.08, 127.92, 124.73, 121.86, 42.91, 41.99, 20.90; HRMS (ESI) Calcd for $C_{16}H_{19}N_3NaO_2$ $[M+Na]^+$: 292.14203. Found 292.14097; mp 138.1–138.8°C.

N-benzyl-*N'*-3,5-dimethylbenzyl-*N*-trichloroacetylcarbamoylurea [**19aa**] and *N*-benzyl-*N'*-3,5-dimethylbenzyl-*N'*-trichloroacetylcarbamoylurea [**19ab**]

To a solution of compound **18a** (0.13 g, 0.5 mmol) in THF (5.0 mL), trichloroacetyl isocyanate (0.24 mL, 2.0 mmol) was added dropwise. After 1 h stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography (50% *t*-butyl methyl ether in CH₂Cl) to form milky white oils **19aa** (0.07 g, 0.16 mmol, 32%) and **19ab** (0.09 g, 0.19 mmol, 38%), respectively.

Compound 19aa. ^1H NMR (400 MHz, CDCl₃): δ 7.18–7.39 (5H, m, Ph), 6.85 (1H, s, 3,5-Me₂-Bn), 6.82 (2H, s, 3,5-Me₂-Bn), 5.11 (2H, s, *N-CH*₂-Ph), 4.28 (2H, d, J 4.8, *NH-CH*₂-Ph), 2.16 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, CDCl₃): δ 161.0, 157.1, 152.1, 138.3, 138.1, 137.1, 128.5, 128.2, 127.3, 126.2, 124.6, 92.2, 44.9, 43.5, 20.9.

Compound 19ab. ^1H NMR (400 MHz, CDCl₃): δ 6.95–7.28 (5H, m, Ph), 6.94 (1H, s, 3,5-Me₂-Bn), 6.79 (2H, s, 3,5-Me₂-Bn), 4.91 (2H, s, *N-CH*₂-Ph), 4.34 (2H, d, J 5.2, *NH-CH*₂-Ph), 2.23 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, CDCl₃): δ 156.5, 151.2, 139.5, 136.2, 134.9, 130.3, 128.9, 128.0, 124.4, 92.2, 47.0, 45.1, 21.2.

N'-3,5-dimethylbenzyl-*N'*-trichloroacetylcarbamoyl-*N*-(4-picolyl)urea [**19b**]

To a solution of compound **18b** (0.29 g, 1.06 mmol) in THF (15.0 mL), trichloroacetyl isocyanate (0.15 mL, 1.27 mmol) was added dropwise. After 10 min stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography (20% MeOH in AcOEt)

to form yellowish crystal **19b** (0.34 g, 0.74 mmol, 70%). ^1H NMR (400 MHz, DMSO- d_6): δ 9.03 (1H, t, J 6.0, *NH-CH*₂), 8.47–8.48 (2H, m, picolyl), 7.25–7.26 (2H, m, picolyl), 6.79–6.88 (3H, m, 3,5-Me₂-Bn), 4.89 (2H, s, *N-CH*₂), 4.39 (2H, d, J 6.0, *NH-CH*₂), 2.20 (6H, s, 3,5-Me₂-Bn); mp 186.1–186.9°C.

General procedure for the synthesis of **20aa**, **20ab**, and **20b**

A mixture of urea **19aa**, **19ab**, or **19b** (0.046 mmol) and silica gel (0.5 g) in MeOH (5.0 mL) was stirred at 50°C. After 18 h stirring, the solution was filtered and concentrated to a small volume. The residual solution was purified by silica gel column chromatography to form **20aa**, **20ab**, or **20b** (6.54 g, 43.6 mmol, 61%).

N-benzyl-*N*-carbamoyl-*N'*-(3,5-dimethylbenzyl)urea [**20aa**]

Yield 74%; white crystal; ^1H NMR (400 MHz, CDCl₃): δ 7.26–7.39 (5H, m, Ph), 6.88 (1H, s, 3,5-Me₂-Bn), 6.75 (2H, s, 3,5-Me₂-Bn), 5.02 (2H, s, *N-CH*₂-Ph), 4.36 (2H, d, J 5.2, *NH-CH*₂-Ph), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, CDCl₃): δ 157.5, 156.1, 138.2, 137.9, 136.7, 129.3, 129.0, 128.0, 126.3, 125.0, 47.0, 44.7, 21.2; HRMS (ESI) Calcd for $C_{18}H_{21}N_3NaO_2$ $[M+Na]^+$: 334.15260. Found 334.15086; mp 123.5–124.6°C.

N-benzyl-*N'*-carbamoyl-*N'*-3,5-dimethylbenzylurea [**20ab**]

Yield 83%; white crystal; ^1H NMR (400 MHz, CDCl₃): δ 7.17–7.32 (5H, m, Ph), 6.91 (1H, s, 3,5-Me₂-Bn), 6.84 (2H, s, 3,5-Me₂-Bn), 4.93 (2H, s, *N-CH*₂-Ph), 4.44 (2H, d, J 5.2, *NH-CH*₂-Ph), 2.27 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, CDCl₃): δ 157.8, 156.6, 139.0, 138.2, 136.6, 129.7, 128.6, 127.3, 127.3, 124.0, 46.9, 44.7, 21.3; HRMS (ESI) Calcd for $C_{18}H_{21}N_3NaO_2$ $[M+Na]^+$: 334.15260. Found 334.15074; mp 118.1–119.5°C.

N'-carbamoyl-*N'*-3,5-dimethylbenzyl-*N*-(4-picolyl)urea [**20b**]

Yield 81%; white crystal; ^1H NMR (400 MHz, DMSO- d_6): δ 8.96 (1H, t, J 5.6, *NH-CH*₂), 8.37–8.38 (2H, m, picolyl), 7.22 (2H, s, *NH*₂), 7.06–7.07 (2H, m, picolyl), 6.81 (1H, s, 3,5-Me₂-Bn), 6.71 (2H, s, 3,5-Me₂-Bn), 4.82 (2H, s, *N-CH*₂), 4.29 (2H, d, J 5.6, *NH-CH*₂), 2.17 (6H, s, 3,5-Me₂-Bn); ^{13}C NMR (100 MHz, DMSO- d_6): δ 157.0, 156.1, 149.4, 148.8, 138.1, 137.2, 128.1, 123.9, 121.8, 44.9, 42.5, 20.9; HRMS (ESI) Calcd for $C_{17}H_{20}N_4NaO_2$ $[M+Na]^+$: 335.14785. Found 335.14757; mp 151.2–153.3°C.

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

Emivirine 5a-h, 6a-h, 7a-b, 11, 12, 14, 15 20aa 20ab: X = Bn 20b: X = picolyl					
Compound	R ₁	R ₂	EC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b	SI ^c
Emivirine	–	–	0.013 ± 0.001	>20	>1539
5a	4-NO ₂ -Bn	N ₃	0.37 ± 0.24	13.6 ± 3.8	>27
5b	2-NO ₂ -Bn	N ₃	0.94 ± 0.16	>20	>21
5c	2,6-F ₂ -Bn	N ₃	0.10 ± 0.01	>20	>192
5d	4-F-Bn	N ₃	0.23 ± 0.01	>20	>87
5e	4-CN-Bn	N ₃	0.13 ± 0.05	>20	>156
5f	4-MeO-Bn	N ₃	0.18 ± 0.07	>20	>111
5g	CH ₂ (furan-2-yl)	N ₃	0.12 ± 0.04	>20	>176
5h	CH ₂ (thiophen-2-yl)	N ₃	0.12 ± 0.01	>20	>174
6a	4-NO ₂ -Bn	NH ₂	0.068 ± 0.005	>20	>294
6b	2-NO ₂ -Bn	NH ₂	0.35 ± 0.13	>20	>58
6c	2,6-F ₂ -Bn	NH ₂	0.042 ± 0.012	>20	>476
6d	4-F-Bn	NH ₂	0.037 ± 0.008	>20	>548
6e	4-CN-Bn	NH ₂	0.099 ± 0.012	>20	>203
6f	4-MeO-Bn	NH ₂	0.096 ± 0.001	>20	>209
6g	CH ₂ (furan-2-yl)	NH ₂	0.038 ± 0.009	>20	>533
6h	CH ₂ (thiophen-2-yl)	NH ₂	0.093 ± 0.004	>20	>215
7a	4-NH ₂ -Bn	NH ₂	0.010 ± 0.006	>20	>1923
7b	2-NH ₂ -Bn	NH ₂	2.0 ± 1.1	>20	>10
11	4-HO-Bn	N ₃	1.8 ± 0.2	>20	>11
12	4-HO-Bn	NH ₂	0.043 ± 0.014	>20	>465
14	CH ₂ (pyrimidin-4-yl)	N ₃	1.8 ± 0.6	>20	>11
15	CH ₂ (pyrimidin-4-yl)	NH ₂	1.8 ± 0.7	>20	>11
20aa	–	–	>20	>20	–
20ab	–	–	>20	>20	–
20b	–	–	>20	>20	–

^aEC₅₀: effective concentration; the concentration of compound required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.

^bCC₅₀: cytotoxic concentration; the concentration of compound that reduces the normal uninfected MT-4 cell viability by 50%.

^cSI: selectivity index (CC₅₀/EC₅₀).

Results

Biological activity

The antiviral activities of the 6-azido (**5a–h**, **11**, and **14**) and 6-amino (**6a–h**, **7a–b**, **12**, and **15**) derivatives of 1-substituted-3-(3,5-dimethylbenzyl)uracils and *N*-3,5-dimethylbenzyl-substituted urea derivatives **20aa–20b** were determined by examining the inhibitory effects of these compounds on the HIV-1-induced cytopathogenicity and cell viability in MT-4 cells. The

cytotoxicities of these compounds, which were dissolved in dimethyl sulfoxide at concentrations up to 20 μM, were evaluated based on the viability of mock-infected cells, as determined by the MTT method. Emivirine (MKC-442) was also tested as a positive control. As shown in Table 1, almost every compound in the series of 6-azido and 6-amino derivatives of 1-substituted-3-(3,5-dimethylbenzyl)uracils exhibited good-to-moderate anti-HIV-1 activity with EC₅₀ values ranging from 10 nM to 2.0 μM. Almost every

Figure 6. (a) Molecular docking of *N*¹-(4-aminobenzyl) derivative **7a** into the allosteric site of HIV-1 RT (PDB code: 1VRT); (b) molecular docking of *N*¹-(2-aminobenzyl) derivative **7b** into the allosteric site of HIV-1 RT (PDB code: 1VRT); (c) superimposition of the docked conformation of **7a** (green) and **7b** (magenta) in the HIV-1 RT (PDB code: 1VRT).

6-azido derivative exhibited considerably less anti-HIV-1 activity than the 6-amino derivatives; for example, **5a** (6-azidouracil with a 4-nitrobenzyl group at the *N*¹ position) was approximately 10 times less potent ($EC_{50} = 0.37 \pm 0.24 \mu M$, SI = 27; SI: selectivity index) than its 6-amino counterpart, **6a** ($EC_{50} = 0.068 \pm 0.005 \mu M$, SI > 294).

Subsequently, the 6-amino derivatives were further investigated for SAR studies. Among the compounds shown in Table 1, the 6-amino derivatives of *N*¹-(2,6-difluorobenzyl)uracil (**6c**), *N*¹-(4-fluorobenzyl)uracil (**6d**), *N*¹-(2-furanyl)methyluracil (**6g**), *N*¹-(4-aminobenzyl)uracil (**7a**), and *N*¹-(4-hydroxybenzyl)uracil (**12**) showed satisfactory anti-HIV-1 activities with EC_{50} values of $0.042 \pm 0.012 \mu M$, $0.037 \pm 0.008 \mu M$, $0.038 \pm 0.009 \mu M$, $0.010 \pm 0.006 \mu M$, and $0.043 \pm 0.014 \mu M$, respectively. The introduction of fluorine atom(s) on the benzene ring of the benzyl group at the *N*¹ position in uracil (**6c** and **6c**) made the compounds more active than the unsubstituted compound **1e** ($EC_{50} = 0.069 \pm 0.006 \mu M$), as reported in our previous study.¹⁵ Moreover, the introduction of polar groups at the *para*-position of the benzyl group (e.g. the amino group in **7a** or hydroxyl group in **12**) increased the anti-HIV-1 activity. In particular, **7a** exhibited excellent activity with an EC_{50} value of $0.010 \pm 0.006 \mu M$ and an SI value of >1923, which are

high and comparable to those of the positive control emivirine ($EC_{50} = 0.013 \pm 0.001 \mu M$, SI > 1539). However, the corresponding regioisomer of **7a**, *N*¹-(2-aminobenzyl)uracil derivative **7b**, exhibited decreased antiviral activity with an EC_{50} value of $2.0 \pm 1.1 \mu M$, which was almost two orders of magnitude lower than the EC_{50} value of **7a** ($0.010 \pm 0.006 \mu M$). The probable cause of this decreased anti-HIV-1 activity is described in detail in the next paragraph, "Molecular modeling analysis." Unfortunately, in the case of *N*-3,5-dimethylbenzyl-substituted urea derivatives **20aa–20b**, no significant anti-HIV-1 activity was observed ($EC_{50} > 20 \mu M$ and $CC_{50} > 20 \mu M$), indicating that the conversion of the uracil skeleton into the acyclic derivative may decrease the binding affinity of these compounds to the HIV-1 RT; that is, the uracil skeleton plays an important role in exerting HIV-1 activity in the 1-aromatic methyl-substituted 3-(3,5-dimethylbenzyl)uracil series.

Discussion

Molecular modeling analysis

The X-ray co-crystal structure (PDB: 1VRT) of HIV-1 RT with nevirapine was taken from PDB (1VRT)² and used for docking studies. A docking model consisting

of ligand **7a**, which showed the most promising anti-HIV-1 activity, **7b**, which is the regioisomer of **7a**, or 1-benzyluracil derivative **1e** bound to the HIV-1 RT was constructed by a conformational search using *MacroModel* (ver. 9.1). *AMBER** was used as the force field, and more than 3,000 conformers of the corresponding ligands were optimized. Figure 6a shows the molecular docking of **7a** in the allosteric site of HIV-1 RT; this docking structure was found to be significantly different from that of the nevirapine-binding site in the HIV-1-emivirine complex.²¹ The 6-amino group of **7a** is hydrogen bonded to the amide group of the Lys101 residue ($\text{NH}\cdots\text{O}=\text{C}$). Moreover, the 3,5-dimethylbenzyl moiety, which was oriented around the hydrophobic area, enhanced the π - π stacking of the benzene rings of the Tyr181 and Tyr188 residues. Moreover, a CH- π interaction was observed between the methyl group of the 3,5-dimethylbenzyl moiety and the indole skeleton of the Trp229 residue, or between the benzene rings of the 3,5-dimethylbenzyl moiety and the isobutyl groups of the Leu234 residue. The results of this calculation were the same as that for the unsubstituted 1-benzyluracil **1e** (data not shown) or 1-(4-picolyl)uracil **1g**;¹⁵ however, **7a** with a 4-aminobenzyl group at the *N*¹ position was more potent ($\text{EC}_{50} = 0.010 \pm 0.006 \mu\text{M}$, Table 1) than **1e** ($0.069 \pm 0.006 \mu\text{M}$) or **1g** ($0.030 \pm 0.030 \mu\text{M}$).^{14,15} Based on these results, we believe that the water solubility of the 4-aminobenzyl group of **7a** may result in the formation of hydrogen bonds between the nitrogen atom of this amino group and water molecules outside the HIV-1 RT, which would stabilize ligand **7a**, and thus improve the anti-HIV-1 activity.^{21,22}

On the other hand, the regioisomer **7b**, with severely decreased anti-HIV-1 activity ($2.0 \pm 1.1 \mu\text{M}$, Table 1) was also docked with the HIV-1 RT, and the resulting most stable conformation is shown in Figure 6b. The conformation of ligand **7b** was substantially different from that of **7a** because of steric repulsion between the amino groups at the *ortho*-position on the benzene ring and the 3,5-dimethylbenzyl group at the *N*³ position of uracil, or steric repulsion between the amino group at the *ortho*-position on the benzene ring and HIV-1 RT itself. Therefore, hydrophobic interactions between the HIV-1 RT and the 3,5-dimethylbenzyl group at the *N*³ position decreased. Moreover, it may be difficult to stabilize the hydrogen bonding between the amino group at the uracil C6-position and the amide group of the Lys101 residue ($\text{NH}\cdots\text{O}=\text{C}$), which plays an important role for the exertion of anti-HIV-1 activity.^{23–28} Figure 6c shows the overlay of the docked conformations of **7a** (green) and its regioisomer **7b** (magenta) bound to the HIV-1 RT at the nevirapine-binding site, showing that the most stable conformations of **7a** and **7b** differ substantially. Thus, the activity of **7b** against HIV-1 is decreased compared with that of **7a**.

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Conflict of interest

The authors indicated no potential conflict of interest.

References

- Kohlstaedt LA, Wang J, Friedman JM, et al. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* 1992; 256: 1783–1790.
- Ren JS, Esnouf R, Garman E, et al. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. *Nat Struct Mol Biol* 1995; 2: 293–302.
- De Clercq E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antiviral Res* 1998; 38: 153–179.
- Pedersen OS and Pedersen EB. Non-nucleoside reverse transcriptase inhibitors: the NNRTI boom. *Antiviral Chem Chemother* 1999; 10: 285–314.
- Baba M, Tanaka H, De Clercq E, et al. Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. *Biochem Biophys Res Commun* 1989; 165: 1375–1381.
- Miyasaka T, Tanaka H, Baba M, et al. A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J Med Chem* 1989; 32: 2507–2509.
- Tanaka H, Takashima H, Ubasawa M, et al. Synthesis and antiviral activity of 6-benzyl analogs of 1-[(2-hydroxyethoxy)methyl]-5-(phenylthio)thymine (HEPT) as potent and selective anti-HIV-1 agents. *J Med Chem* 1995; 38: 2860–2865.
- Tanaka H, Baba M, Saito S, et al. Specific anti-HIV-1 acyclonucleosides which cannot be phosphorylated: synthesis of some deoxy analogs of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J Med Chem* 1991; 34: 1508–1511.
- Tanaka H, Takashima H, Ubasawa M, et al. Synthesis and antiviral activity of deoxy analogs of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) as potent and selective anti-HIV-1 agents. *J Med Chem* 1992; 35: 4713–4719.
- Tanaka H, Takashima H, Ubasawa M, et al. Structure-activity relationships of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine analogs: effect of substitutions at the C-6 phenyl ring and at the C-5 position on anti-HIV-1 activity. *J Med Chem* 1992; 35: 337–345.
- Szczeczek GM, Furman P, Painter GR, et al. Safety assessment, in vitro and in vivo, and pharmacokinetics of emivirine, a potent and selective nonnucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 2000; 44: 123–130.
- Maruyama T, Kozai S, Yamasaki T, et al. Synthesis and antiviral activity of 1,3-disubstituted uracils against

- HIV-1 and HCMV. *Antivir Chem Chemother* 2003; 14: 271–279.
13. Maruyama T, Kozai S, Demizu Y, et al. Synthesis and anti-HIV-1 and anti-HCMV activity of 1-substituted 3-(3,5-dimethylbenzyl)uracil derivatives. *Chem Pharm Bull* 2006; 54: 325–333.
14. Isono Y, Sakakibara N, Ordonez P, et al. Synthesis of 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives with potential anti-HIV activity. *Antivir Chem Chemother* 2011; 22: 57–65.
15. Sakakibara N, Hamasaki T, Baba M, et al. Synthesis and evaluation of novel 3-(3,5-dimethylbenzyl) uracil analogs as potential anti-HIV-1 agents. *Bioorg Med Chem* 2013; 21: 5900–5906.
16. Soai K, Yokoyama S and Ookawa A. Reduction of azides to amines with sodium borohydride in tetrahydrofuran with dropwise addition of methanol. *Synthesis* 1987; 1: 48–49.
17. White RD and Wood JL. Progress toward the total synthesis of kalihinane diterpenoids. *Org Lett* 2001; 3: 1825–1827.
18. Armstrong JD III, Wolfe CN, Keller JL, et al. A novel synthesis of disubstituted ureas using titanium(IV) isopropoxide and sodium borohydride. *Tetrahedron Lett* 1997; 38: 1531–1532.
19. Schnell B, Krenn W, Faber K, et al. Synthesis and reactions of biginelli-compounds, Part 23: chemoenzymatic syntheses of enantiomerically pure 4-aryl-3,4-dihydropyrimidin-2(1H)-ones. *J Chem Soc Perkin Trans 1* 2000; 4382–4389.
20. Baba M, De Clercq E, Tanaka H, et al. Potent and selective inhibition of human immunodeficiency virus type 1 (HIV-1) by 5-ethyl-6-phenylthiouracil derivatives through their interaction with the HIV-1 reverse transcriptase. *Proc Natl Acad Sci* 1991; 88: 2356–2360.
21. Andrew LH, Jingshan R, Robert ME, et al. Complexes of HIV-1 reverse transcriptase with inhibitors of the HEPT series reveal conformational changes relevant to the design of potent non-nucleoside inhibitors. *J Med Chem* 1996; 39: 1589–1600.
22. Demizu Y, Nagoya S, Shirakawa M, et al. Development of stapled short helical peptides capable of inhibiting vitamin D receptor (VDR)–coactivator interactions. *Bioorg Med Chem Lett* 2013; 23: 4292–4296.
23. Ribone SR, Quevedo MA, Madrid M, et al. Rational approaches for the design of effective human immunodeficiency virus type 1 nonnucleoside reverse transcriptase inhibitors. *J Chem Inform Model* 2011; 51: 130–138.
24. Figueiredo A, Zelina S, Sluis-Cremer N, et al. Impact of residues in the nonnucleoside reverse transcriptase inhibitor binding pocket on HIV-1 reverse transcriptase heterodimer stability. *Curr HIV Res* 2008; 6: 130–137.
25. Ren J, Nichols CE, Stamp A, et al. Structural insights into mechanisms of non-nucleoside drug resistance for HIV-1 reverse transcriptases mutated at codons 101 or 138. *FEBS J* 2006; 273: 3850–3860.
26. David RM, Jeffrey AR, Richard BS, et al. Synthesis of novel potent dipeptidyl peptidase IV inhibitors with enhanced chemical stability: interplay between the N-terminal amino acid alkyl side chain and the cyclopropyl group of α -aminoacyl-L-cis-4,5-methanoprolinonitrile-based inhibitors. *J Med Chem* 2004; 47: 2587–2598.
27. Joseph HC, Jean SH, Robert NH III, et al. 2-Amino-6-arylsulfonylbenzonitriles as non-nucleoside reverse transcriptase inhibitors of HIV-1. *J Med Chem* 2001; 44: 1866–1882.
28. Ren J, Milton J, Weaver KL, et al. Structural basis for the resilience of efavirenz (DMP-266) to drug resistance mutations in HIV-1 reverse transcriptase. *Structure* 2000; 8: 1089–1094.