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The design and synthesis of N-1-alkylated-5-aminoaryalkylsubstituted-6-methyluracils as potential non-nucleoside HIV-1 RT inhibitors

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Abstract—Novel compounds **1a**–**u**, which can be considered as hybrid analogues of MKC-442 and pyridinon, have been synthesized and evaluated as inhibitors of HIV-1 reverse transcriptase (HIV-1 RT). Starting from 6-methyuracil **2**, 1-alkylated-5-bromomethyl-6-methyluracils **8** was prepared in four steps by hydroxylmethylation, etherification, N-1 alkylation, and bromination. Finally, compounds **1a**–**u** were achieved in the displacement of 5-bromomethyl group by nucleophiles with amino compounds. Some of compounds **1a**–**u** showed potent inhibitory activity against HIV-1 RT. The most active compounds showed activity in the low micromolecular range with IC_{50} values (IC_{50} 0.82–5.09 μM) comparable to that of nevirapine (IC_{50} 10.60 μM). The biological testing results are in accordance with the docking.

1. Introduction

Following the discovery of human immunodeficiency virus (HIV) as causative acquired immunodeficiency syndrome (AIDS),[1] enormous efforts have been made to search the potential targets for antiviral chemotherapy. HIV-1 reverse transcriptase (HIV-1 RT) has been successful to date. Using as inhibitors of this enzyme, the administration of Nevirapine, AZT, DDI, and DDC are useful drugs against HIV.[2] The inhibition of RT is considered as one of the most valuable and practicable approaches to suppress virus spreading.[3,4] However, their long-term use leads to significant toxicity and viral resistance. Therefore, the development of very specific and highly active inhibitors of HIV-RT was envisioned to be the potential solution for HIV-1 infection.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV are a very broad class of structurally diverse molecules[5,6] but they exhibit similarity in 3D structures and show remarkable potential and selective inhibition profiles such as 1-[(2-hydroxy-ethoxy)methyl]-6-phenylthio thymine (HEPT) (i)[7,8] and the 2-pyridinon derivatives (L-697, 661) (ii)[9] (Fig. 1). Structure–activity studies in the HEPT series have led to the synthesis of serial new promising analogues, of which 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442) (iii),[10,11] and TNK-651 (iv)[12] (Fig. 1) have been chosen as candidates for clinical trials with AIDS patients. The reason for the increased activity is that the 5-substituent in the uracil ring of MKC-442 induces a Tyr181 switch in the HIV-RT.[12] A major problem with NNRTIs therapy is the rapid development of resistance mediated by mutations of residues that line the NNRTI binding pocket of the viral RT and reduce drug binding, even though they are more active against HIV-RT with general low toxicity and favorable pharmacokinetic properties. Therefore, in NNRTIS areas, interests are focused on finding new analogues with higher binding affinity and the capability of inhibiting clinically resistant mutants.[13–16]

Keywords: HIV-1 reverse transcriptase; Non-nucleoside reverse ranscriptase inhibitors (NNRTIs); HEPT analogues.
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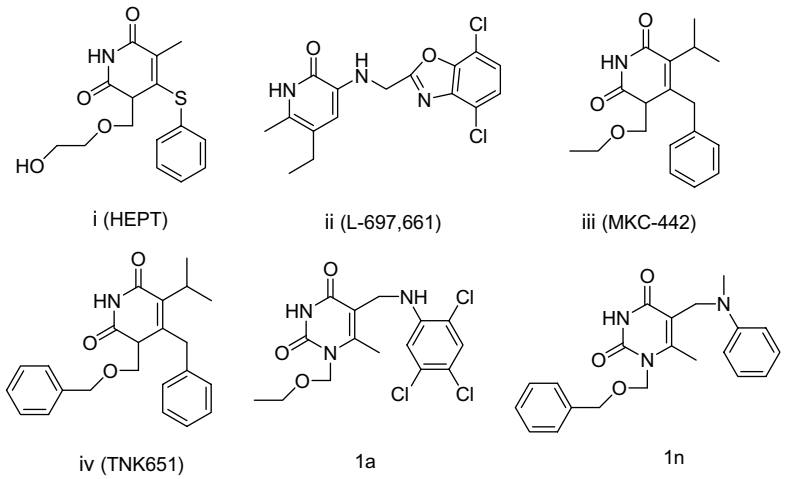


Figure 1. Structures of HEPT, L-697,661, MKC-442, TNK-651, and designed target compounds **1a**, **1n**.

2. Results and discussions

2.1. Design of target molecule

Studies of crystal structures of the RT complex with inhibitors TNK-651, MKC-442 suggest that NNRTIs share a common mode of action and interact with a hydrophobic pocket. Upon binding, MKC-442 and TNK-651 distort the RT active site and alter the conformation of Tyr 181, Tyr 183, and Tyr 188 residues with respect to that of unliganded RT. Thus the aromatic group of Tyr 181 is faced into a conformation able to perform a strong interaction with the C-6 benzyl group of the inhibitors, stabilizing the structure of the complex. This revealed that the C-5 substituent of the pyrimidine ring is very important for anti-HIV-1 RT activity.

Although structure–activity relationship on the anti-HIV of HEPT analogues has been studied, no or little information is available about 5-bulky substituents and 6-small alkyl substituents. In the year 2006, Chen et al. have synthesized the 1-(alkoxymethyl)-5-benzyl-6-methyluracils whose activity showed to be ca. 2-fold higher than HEPT but 10-fold lower than nevirapine. After inspection of the above-mentioned work, we think that the key point to develop such compounds is the optimal linking distance between the pyrimidine ring and the aromatic ring placed at C-5. This distance is an important prerequisite for potency. It was pointed out in an earlier study that a major determinant of increased potency in the analogues of HEPT is an improved interaction between residue Tyr181 in the protein and the 6-benzyl ring of the inhibitors which stabilizes the structure of the complex. This arises through a confirmational switching of the protein structure triggered by the steric bulk of the 5-substituent of the inhibitor pyrimidine ring.[12] Thus we think the aromatic ring at C-5 should possess the conformational similarity of C-6 benzyl group of MKC-442 or TNK-651: the chain between pyrimidine ring and aromatic ring at C-5 should possess conformational flexibility, and property linking length which should be in 2–3 carbon (nitrogen) units. From this it also can preserve 5-position group's 'trigger' role.

Therefore, we decided to select N-1-alkylated, 5-bulky aryalkyl moiety and 6-methyl group uracils **1a**, **1n** which can also be considered as HEPT-pyridinone analogue hydrid molecules with two possible orientations in RT as new potential NNRTIs.

To verify our design, these compounds (**1a**, **1n**) were flexibly docked into the binding site of HIV-1 RT (PDB:1RT2, complexed with TNK-651) using AutoDock 3.05 program.[17] Default parameters were used as described in the AutoDock manual unless otherwise specified. Each molecule was docked with 100 genetic algorithm runs of up to 250,000 energy evaluations for each run in the docking study of **1a** and **1n** with a RT non-nucleoside binding site. It was predicted that the binding modes for **1a**, **1n** have approximate conformations as in the TNK-651-RT complex. (Fig. 2) The docking results showed that the **1a**, **1n** bind to the RT with an conformation forming hydrogen bonds with the Lys101 and Lys103. Meanwhile the 5-substituted phenyl ring of **1a**, **1n** occupies a hydrophobic pocket constructed by the side chains of Try 181, Try 188, and Trp 229, making favorable π–π interactions with these residues. As seen in Figure 2, the bulky substituted aryalkyl moiety with two-atom linker on the side chain of C-5 is positioned in a favored location between isopropyl group at C-5 and benzyl group at C-6 in TNK-651, thus it can exert the function of both of them, which seems to be crucial for the inhibition of the RT. Based on results of the docking study, we hypothesized that an efficient use of C-5 substitute by strategically designed functional groups should yield more potent anti-HIV agents with higher affinity for the inhibitor binding pocket.

2.2. Chemistry

The use of the 5-bromomethyl-6-methyl **4** as intermediate gave easy access to 5-amino substitutes **5** by the use of 6-methyluracil as starting materials with a modified method of Carbon,[18] and when tested this strategy on a 6-methyluracil we obtained the corresponding 5-amino substitutes **5** in 50–60% yield (Scheme 1). However, after

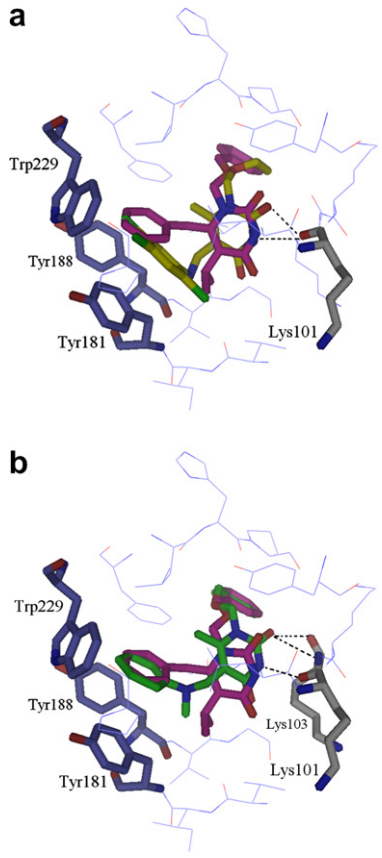
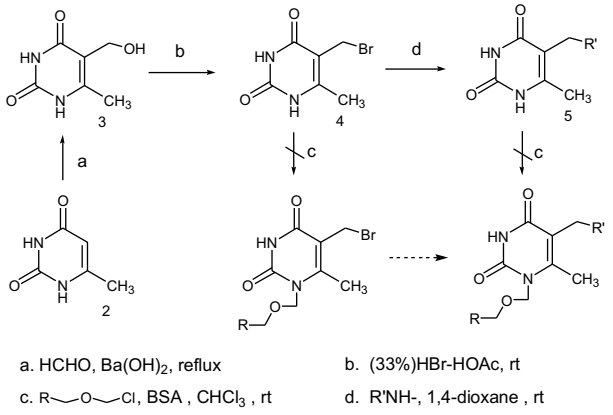
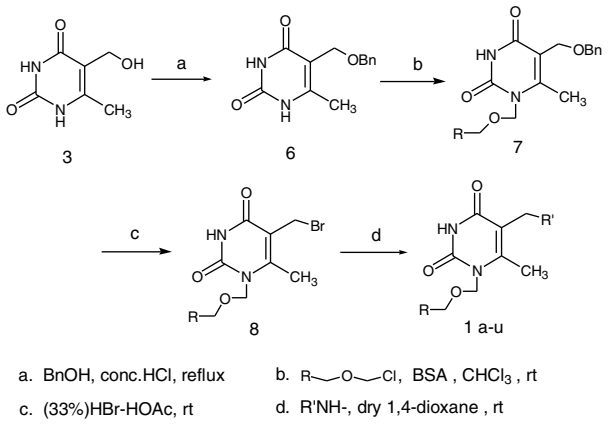


Figure 2. Modeled binding mode of **1a** (a, yellow) and **1n** (b, green) overlapped to crystal structure (1RT2 in PDB) of TNK651 (pink). The two inhibitors are docked into the active site of the HIV-1 RT structure 1RT2 using AutoDock 3.05[17] as rendered in Pymol (Delano Scientific, South San Francisco, CA). The docking modes are chosen on the basis of binding affinity rank. All inhibitors and important interacting residues are shown as stick figures. Hydrogen bonds are shown as black dashed lines.



Scheme 1. A strategy of synthesis 1-(alkoxymethyl)-5-(aryalkylamino)-6-methyluracil.

several attempts, it was not possible to obtain the desired target compounds by reaction **4** or **5** with *N*,*O*-bis-(trimethylsily)acetamide (BSA) in $CHCl_3$ at room temperature followed by reaction with chloromethyl ethyl ether (Scheme 1).



Scheme 2. Reagents and conditions.

Therefore it was decided to introduce the N1-substituent into the pyrimidine before making 5-bromomethyl. The inspiration came from Chen[19] who made a 5-(benzyloxymethyl)-6-methyluracil starting from 6-methyluracil in 64% yield. The advantage of this method is easy preparation of 1-alkylated-5-bromomethyl-6-methyluracil **8** starting from the reaction of 5-hydroxymethyl-6-methyluracil **3** (Scheme 2).

5-(Benzyloxymethyl)-6-methyluracil **6** prepared by benzylation of **3** according to a literature procedure[19] was used for the synthesis of the 1-alkylated uracil **7**. Therefore **6** was reacted with *N*,*O*-bis(trimethylsily)acetamide (BSA) in dry $CHCl_3$ and subsequently alkylated with chloromethyl ethyl ether or chloromethyl benzyl ether[20] to obtain the desired 1-(alkyloxymethyl)-5-(benzyloxymethyl)-6-methyluracil **7a** in 92.5% yield after column chromatography purification. The C-1 benzyloxymethyl derivative **7b** was synthesized using the same method.

For compound **7** we observed exactly the same NMR chemical shift for the N-1-CH2 as was previously observed for similar compounds.[19,20] The conversion of **7a** to 1-(alkyloxymethyl)-5-(bromomethyl)-6-methyluracil **8a** was performed by the reaction with a 33% solution of HBr in glacial acetic acid in 87.8% yield, which not afforded the cleavage of N-1 ether bond. Finally compound **8** was treated with various amines in dry 1,4-dioxane to give compounds **1a**–**u** in 9–87% yields (Scheme 2 and Table 1).

To sum up, a simple and efficient procedure for the synthesis of a family of 1-alkyl-5-aminoaryalkyl-6-methyluracil **1** has been developed. These new HEPT-pyridinone analogue hybrid molecules were obtained in an easy, rapid and profitable way using solution-phase synthesis. The whole methodology shows combinatorial potential in the synthesis of larger libraries of compounds which is ongoing in our laboratory.

2.3. Biological activity

The compounds **1a**–**u** were tested for antiviral activity against HIV-1 RT, using a poly(ra)/oligo(dT)$_{15}$

Table 1. Preparation of **1** from **8** and RT inhibitory activity values[a]

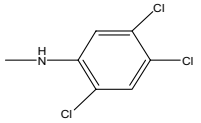
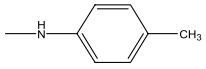
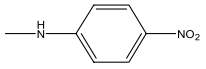
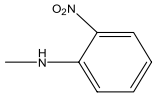
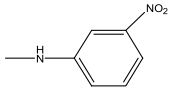
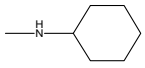
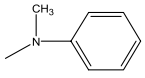
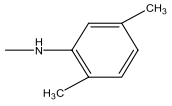
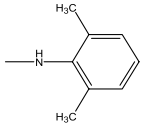
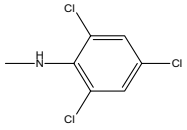
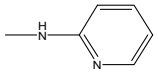
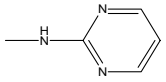
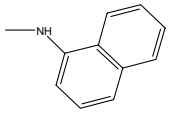
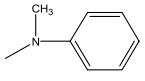
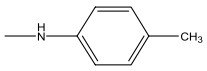
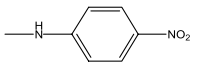
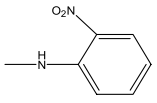
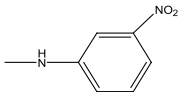
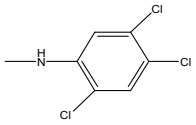
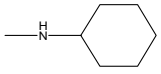
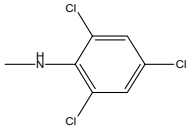
Compound	R	R′	Time (h)	Yield (%)	IC_{50}[b] (μM)
1a	CH_3		2	59	0.82
1b	CH_3		4	55	NA[c]
1c	CH_3		4	44	NA
1d	CH_3		5	86	>100
1e	CH_3		5	80	NA
1f	CH_3		Over night	9	NA
1g	CH_3		4	39	NA
1h	CH_3		1.5	26	26.2
1i	CH_3		3	32	NA
1j	CH_3		4	40	NA
1k	CH_3		4	35	54.7
1l	CH_3		1	20	NA
1m	CH_3		4	38	NA
1n	Ph		9	46	3.49

Table 1 (*continued*)

Compound	R	R′	Time (h)	Yield (%)	IC_{50}[b] (μM)
1o	Ph		9	37	13.8
1p	Ph		9	29	NA
1q	Ph		10	29	NA
1r	Ph		10	16	NA
1s	Ph		3	45	>100
1t	Ph		9	54	5.09
1u	Ph		3	28	NA

[a] Nevirapine was used as a reference compound here; IC_{50} for nevirapine was 10.60 μM.
[b] Compound dose (μM) required to inhibit the HIV-1 rRT activity by 50%; Data represent mean values for three separate experiments, variation among triplicate samples was less than 15%.
[c] No inhibition of reverse transcriptase activity was observed up to a concentration of 100 μM.

homopolymer template with HIV antigen detection ELISA[21] for quantifying expression of HIV-1 RT in culture medium, and nevirapine as a reference compound. Oligo(dT) was immobilized via its 5′-terminal phosphate to Covalink-NH microtiter plates. The biotin-dUTP was incorporated by reverse transcriptase. The reaction mixture contained 50 mmol/L Tris–HCl (pH 8.3), 3 mmol/L $MgCl_2$, 75 mmol/L KCl_2, 5 mmol/L DTT (DL-dithiothreitol), 0.13 mg/ml BSA (Albumin Bovine V), 10 μg/ml poly (A), 0.75 μM biotin-11-dUTP, and 1.5 μM dTTP. After incubation at 37 °C for 1 h, the plate was washed three times with a wash buffer containing 50 mmol/L Tris–HCl (pH7.5), 0.15 mol/L NaCl, 0.05 mmol/L $MgCl_2$, and 0.02% Tween-20. After 100 μl of 1% BSA was added to each well and incubated for 30 min at room temperature, the plate was washed with the same buffer. Subsequently 50 μl of SA-ALP (Alkaline Phosphatase Streptavidin) solution (100 ng/ml) was added per well and then incubated for 1 h at 37 °C. The plate was washed as above and to which then was added 50 μl of PNPP (*p*-nitrophenyl phosphate, disodium) (1 mg/ml, pH 9.5), after 30 min at 37 °C, the reaction was stopped by addition of 0.5 M NaOH. The products were detected and quantified using a colorimetric streptavidin alkaline phosphatase reporter system. It is interesting to note that some of the reported compounds showed high inhibitory activity against HIV-1 RT. It is noteworthy that compounds **1a** (IC_{50} 0.82 μM), **1n** (IC_{50} 3.49 μM), and **1t** (IC_{50} 5.09 μM) were more active than nevirapine (IC_{50} 10.60 μM) at concentrations 2- to 13-fold compared to nevirapine by introducing the terminal 2,4,5-trichloro-anilino, benzenmethanamine, and cyclohexylamino groups at pyrimidine C-5. However, an increase of the other steric bulkynesses at C-5 showed lower activity compared to nevirapine.

2.4. Conclusions

We have developed novel HIV-1 RT non-nucleoside inhibitors characterized by micromolar potency. Among our study compounds (**1a**–**u**), the higher potency of **1a**, **1n**, and **1t**, compared with nevirapine and other synthesizing target molecules (TMs), might depend on switching the conformation of Tyr 181, Tyr 183, and Tyr 188 residues with respect to that of unliganded RT after binding. Additional reasons for the relatively high activity of these novel inhibitors might be the existing 5-substituted phenyl ring which is located at suitable distance from the pyrimidine ring. In designing the

new TMs, it was also hoped that the favorable effects of the hydrogen bonds attributed to the virtual RT/inhibitor complexes. Encouraged by these results, more detailed SAR studies on these compounds (5-bulky substituents and 6-small alkyl substituents) are underway, with a focus on exploring the important role of these unique structure features.

3. Experimental

In general, reagents and solvents were used as purchased without further purification. Melting points were determined with an X4-type melting-point apparatus and are left uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-AL-300 or a VarianINOVA-500 spectrometer. Chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak). Mass spectra were recorded on a VG-ZAB-HS spectrometer. IR Spectra were recorded with Avatar 360 FT-IR and reported in cm^{-1}. Silica gel (0.040–0.064 mm) was used for column chromatography.

3.1. 5-Hydroxymethyl-6-methyluracil (3)

Introduced formaldehyde (37% aq solution) (4.14 ml, 51.0 mmol) and 6-methyluracil (2.0 g, 15.8 mmol) to a filtered solution of $Ba(OH)_2{\cdot}8H_2O$ (1.5 g, 36.0 mmol) in water (20 ml), heated the mixture to gentle refluxing until the solid completely dissolved. Left to stand for 1 day, CO_2 (gas) was bubbled into the reaction mixture to precipitate $BaCO_3$. Removed the water on a rotary evaporator, and the viscous residue was dissolved at reflux in EtOH (20 ml). Kept the mixture overnight at room temperature, collected **3** as a pure white solid by filtration. The yield was 80% (1.97 g), mp >300 °C. ^{1}H NMR (300 MHz, DMSO): δ 10.94 (s, 1H, N*H*), 10.74 (s, 1H, N*H*), 4.52 (s, 1H, O*H*), 4.14 (s, 2H, CH_2), 2.12 (s, 3H, CH_3). ^{13}C NMR (300 MHz, DMSO): δ 164.2 (*C*4), 151.4 (*C*2), 150.9 (*C*6), 109.2 (*C*5), 62.8 (*C*H$_2$), 15.8 (*C*H$_3$). MS (EI) *m*/*z*: 156 (M, 70), 155 (38), 138 (100), 127 (39). IR (KBr) cm^{-1} 3414, 2933, 1704, 1665, 1447.

3.2. 5-Benzyloxymethyl-6-methyluracil (6)

A mixture of 5-hydroxymethyl-6-methyluracil **3** (2.0 g, 12.8 mmol) and aqueous HCl (1 ml) in benzyl alcohol (50 ml) was refluxed for 1 h. After being cooled to room temperature, the reaction mixture was poured into ether (250 ml). The resulting fine precipitate was filtered and washed several times with ether to give the pure product **6**. The yield was 80% (2.51 g). Mp 300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.04 (s, 1H, N*H*), 10.90 (s, 1H, N*H*), 7.31 (s, 5H, Ar*H*), 4.49 (s, 2H, CH_2), 4.22 (s, 2H, CH_2), 2.10 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.1 (*C*4), 153.0 (*C*2), 150.8 (*C*6), 138.6, 128.2, 127.6, 127.4 (*C*1′–*C*6′), 105.9 (*C*5), 71.2 (*C*H$_2$), 61.8 (*C*H$_2$), 15.9 (*C*H$_3$). MS (EI) *m*/*z*: 247 (M+H, 5), 155 (100), 140 (100). IR (KBr) cm^{-1} 3433, 1721, 1643, 1447, 1071.

3.3. 1-(Ethoxymethyl)-5-(benzyloxymethyl)-6-methyluracil (7a)

Compound **6** (0.35 g, 1.4 mmol) was suspended in dry $CHCl_3$ (15 ml), and *N*,*O*-bis(trimethylsily)acetamide (BSA) (0.8 ml, 3.2 mmol) was added to the suspension. Introduced (0.198 ml, 1.4 mmol) (chloromethyl)-ethylether after the entire solid completely dissolved. The reaction mixture was allowed to stir for 4 h until no change in the amount of the starting material could be noticed on TLC. After evaporation of the solvent in vacuum, the resulting residue was purified by silica gel column chromatography using $CHCl_3$-MeOH (9:1) to give **7a** (0.40 g, 31% yield) as white solid. Mp 104–105 °C. ^{1}H NMR(300 MHz, $CDCl_3$): δ 8.80 (s, 1H, N*H*), 7.36–7.26 (m, 5H, Ar*H*), 5.34 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.42 (s, 2H, CH_2), 3.62 (q, J = 7.2 Hz, 2H, CH_2), 2.42 (s, 3H, CH_3), 1.20 (t, 3H, J = 6.9 Hz, CH_3). *m*/*z* (MS) found 305.1067 ([MH]$^+$ $C_{16}H_{21}N_2O_4$ requires 305.1501), found 327. 0861 ([MNa]$^+$$C_{16}H_{20}N_2Na\ O_4$ requires 327.1321).

3.4. 1-(Benzyloxymethyl)-5-(benzyloxymethyl)-6-methyluracil (7b)

Compound **6** (2.17 g, 8.8 mmol) was converted to **7b** (0.613 g, 20.6% yield) according to the similar procedure used in the preparation of compound **7a**. Mp 143–145 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (s, 1H, N*H*), 7.35–7.28 (m, 10H, Ar*H*), 5.42 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.54 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 2.43 (s, 3H, CH_3). *m*/*z* (MS) found 367.1658 ([MH]$^+$ $C_{16}H_{21}N_2O_4$ requires 367.1607), found 389. 1477 ([MNa]$^+$$C_{16}H_{20}N_2NaO_4$ requires 389.1378).

3.5. 1-(Ethoxymethyl)-5-(bromomethyl)-6-methyluracil (8a)

Dry 1,4-dioxane (0.35 ml) and 33% HBr–AcOH (130 μl, 0.34 mmol) were added to 1,3-dibenzyl-5-benzyloxy-6-methyluracil **7a** (0.1 g, 0.33 mmol), resulting in the formation of a clear solution. After the reaction mixture was stirred for 4 h at room temperature, the solvent was removed by vacuum to afford an oily residue that crystallizes after the addition of a few drops of ether. It was filtered and washed with ether (10 ml). The resulting product (80 mg, 80% yield) was analytically pure: mp 182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (s, 1H, N*H*), 5.36 (s, 2H, CH_2), 4.42 (s, 2H, CH_2), 3.64 (q, J = 7.2 Hz, 2H, CH_2), 2.51 (s, 3H, CH_3), 1.22 (t, J = 10.2 Hz, 3H, CH_3). MS (EI) *m*/*z*: 277 (M, 1), 197 (95), 59 (100).

3.6. 1-(Benzyloxymethyl)-5-(bromomethyl)-6-methyluracil (8b)

Compound **7b** (350 mg, 0.96 mmol) was converted to **8b** (150 mg, 46% yield) according to the similar procedure used in the preparation of compound **8a**. Mp 154–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.21 (s, 1H, N*H*), 7.26–7.39 (m, 5H, Ar*H*), 5.47 (s, 2H, CH_2), 4.67 (d, J = 6.6 Hz, 2H, CH_2), 4.39 (s, 2H, CH_2), 2.48 (s, 3H, CH_3). MS (ES)*m*/*z*: 259 (M−Br, 85).

3.7. General procedure for the synthesis of 1a–1m

Mixed 1-(ethoxymethyl)-5-(bromomethyl)-6-methyluracil **8a** (80 mg, 0.29 mmol) and dry 1,4-dioxane (2 ml), followed by different amino compounds (1.93 mmol). The reaction mixture was allowed to stir for several hours and then was evaporated in vacuum. The resulting residue was purified by silica gel column chromatography using chloroform and methanol (10:1), as the eluent to give the target compound (**1a**–**1m**'s reaction time is illustrated in Table 1).

3.7.1. 1-(Ethoxymethyl)-5-[(2,4,5-trichloro-phenylamino)-methyl]-6-methyluracil (1a). Yield: 59%. White solid. Mp >300 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (s, 1H, Ar*H*), 6.83 (s, 1H, Ar*H*), 5.37 (s, 2H, CH_2), 4.49 (s, 1*H*), 4.12 (d, J = 4.5 Hz, 2H, CH_2), 3.65 (q, J = 7.2 Hz, 2H, CH_2), 2.25 (s, 3H, CH_3), 1.21 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 392 (M, 35), 197 (100).

3.7.2. 1-(Ethoxymethyl)-5-(*p*-tolylamino-methyl)-6-methyluracil (1b). Yield: 55%. Purple solid. Mp 44–45 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (s, 1H, N*H*), 6.99 (d, J = 8.1 Hz, 1H, Ar*H*), 6.68 (d, J = 8.4 Hz, 2H, Ar*H*), 5.33 (s, 2H, CH_2), 4.10 (s, 2H,CH_2), 3.61 (q, J = 7.2 Hz, 2H, CH_2), 2.48 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 1.20 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 304 (M+H, 100), 197 (20).

3.7.3. 1-(Ethoxymethyl)-5-[(4-nitro-phenylamino)-methyl]-6-methyluracil (1c). Yield: 44%. Yellow solid. Mp 147–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.53 (s, 1H, N*H*), 8.00 (d, J = 9.0 Hz, 2H, Ar*H*), 6.73 (d, J = 9.0 Hz, 2H, Ar*H*), 5.28 (s, 2H, CH_2), 4.06 (s, 2H, CH_2), 3.52 (q, J = 7.2 Hz, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.12 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.7 (*C*4), 154.3 (*C*2), 153.3 (*C*6), 151.2, 135.8, 126.2, 110.9 (*C*1′–*C*6′), 108.0 (*C*5), 72.3 (*C*H$_2$), 63.8 (*C*H$_2$), 37.39 (*C*H$_2$), 15.3 (*C*H$_3$), 14.9 (*C*H$_3$). MS (ES) m/z: 335 (M+H, 25), 197 (50).

3.7.4. 1-(Ethoxymethyl)-5-[(2-nitro-phenylamino)-methyl]-6-methyluracil (1d). Yield: 86%. Orange solid. Mp 138–140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (m, 1H, Ar*H*), 7.48 (m, 1H, Ar*H*), 7.03 (d, J = 8.7 Hz, 1H, Ar*H*), 6.70 (t, J = 7.8 Hz, 1H, Ar*H*), 5.37 (s, 2H, CH_2), 4.33 (d, J = 5.1 Hz, 2H, CH_2), 3.65 (q, J = 7.2 Hz, 2H, CH_2), 2.48 (s, 3H, CH_3), 1.21 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 335 (M+H, 30), 197 (60).

3.7.5. 1-(Ethoxymethyl)-5-[(3-nitro-phenylamino)-methyl]-6-methyluracil (1e). Yield: 80%. Yellow solid. Mp 169–171 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55 (m, 1H, Ar*H*), 7.44 (m, 1H, Ar*H*), 7.27 (m, 1H, Ar*H*), 6.92 (m, 1H, Ar*H*), 5.36 (s, 2H, CH_2), 4.45 (s, 1*H*), 4.17 (d, J = 5.1 Hz, 2H, CH_2), 3.58 (q, J = 7.2 Hz, 2H, CH_2), 2.54 (s, 3H, CH_3), 1.21 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 335 (M+H, 70), 197 (90).

3.7.6. 1-(Ethoxymethyl)-5-(cyclohexylaminomethyl)-6-methyluracil (1f). Yield: 9%. White solid. Mp 201–203 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.33 (s, 2H, CH_2), 3.86 (s, 2H, CH_2), 3.61 (q, J = 7.2 Hz, 2H, CH_2), 3.65 (s, 3H, CH_3), 3.04 (s, 1H, C*H*), 2.44–2.05 (m, 10H, CH_2), 1.18 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 296 (M+H, 100), 197 (16).

3.7.7. 1-(Ethoxymethyl)-5-[(methyl-phenylamino)-methyl]-6-methyluracil (1g). Yield: 39%. Yellowish brown solid. Mp 161–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29–7.24 (m, 2H, Ar*H*), 6.97 (m, 2H, Ar*H*), 6.82 (m, 1H, Ar*H*), 5.35 (s, 2H, CH_2), 4.22 (s, 1H, CH_2), 3.62 (q, J = 7.2 Hz, 2H, CH_2), 2.80 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 1.20 (t, J = 6.9 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.3 (*C*4), 153.5 (*C*2), 151.6 (*C*1′), 150.5 (*C*1), 129.1, 118.3, 114.8 (*C*2′–*C*6′), 109.7 (*C*5), 73.0 (*C*H$_2$), 65.0 (*C*H$_2$), 46.7 (*C*H$_2$), 37.4 (*C*H$_3$), 15.5 (*C*H$_3$), 15.0 (*C*H$_3$). MS (ES) m/z: 304 (M+H, 100), 197 (30).

3.7.8. 1-(Ethoxymethyl)-5-[(2,5-dimethyl-phenylamino)-methyl]-6-methyluracil (1h). Yield: 26%. Deep orange solid. Mp 67–69 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18–6.54 (m, 3H, Ar*H*), 5.35 (s, 2H, CH_2), 4.14 (s, 2H, CH_2), 3.63 (q, J = 7.2 Hz, 2H, CH_2), 2.33 (s, 6H, CH_3), 2.25 (s, 3H, CH_3), 1.19 (t, J = 3.0 Hz, 3H, CH_3). MS (ES) m/z: 318 (M+H, 100), 197 (22).

3.7.9. 1-(Ethoxymethyl)-5-[(2,6-dimethyl-phenylamino)-methyl]-6-methyluracil (1i). Yield: 32%. Light brown solid. Mp 168–170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.99 (d, J = 7.5 Hz, 2H, Ar*H*), 6.88 (m, 1H, Ar*H*), 5.32 (s, 2H, CH_2), 3.89 (s, 2H, CH_2), 3.58 (q, J = 7.2 Hz, 2H, CH_2), 2.33 (s, 6H, CH_3), 2.25 (s, 3H, CH_3), 1.18 (t, J = 7.2 Hz, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$): δ 163.6 (*C*4), 151.6 (*C*2), 144.9 (*C*6), 131.1, 128.7, 123.0, 111.4 (*C*1′–*C*6′), 73.1 (*C*H$_2$), 64.8 (*C*H$_2$), 43.4 (*C*H$_2$), 18.3 (*C*H$_3$), 15.0 (*C*H$_3$), 14.9 (*C*H$_3$). MS (ES) m/z: 318 (M+H, 100), 197 (45).

3.7.10. 1-(Ethoxymethyl)-5-[(2,4,6-trichloro-phenylamino)-methyl]-6-methyluracil (1j). Yield: 40%. Brown solid. Mp 145–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (s, 2H, Ar*H*), 5.30 (s, 2H, CH_2), 4.45 (s, 1*H*), 4.25 (s, 2H, CH_2), 3.58 (q, J = 7.2 Hz, 2H, CH_2), 2.28 (s, 3H, CH_3), 1.17 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.4 (*C*4), 151.5 (*C*2), 151.4 (*C*6), 140.9, 128.8, 128.5, 127.3 (*C*1′–*C*6′), 110.8 (*C*5), 73.0 (*C*H$_2$), 64.8 (*C*H$_2$), 42.4 (*C*H$_2$), 15.1 (*C*H$_3$), 15.0 (*C*H$_3$). MS (ES) m/z: 394 (M+H, 20), 197 (100).

3.7.11. 1-(Ethoxymethyl)-5-(pyridin-2-ylaminomethyl)-6-methyluracil (1k). Yield: 35%. White solid. Mp 161–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (m, 1H, 6′*H*), 7.42 (m, 1H, 4′*H*), 6.65 (m, 1H, 3′*H*), 6.53 (m, 1H, 5′*H*), 5.34 (s, 2H, CH_2), 4.39 (d, J = 6.0 Hz, 2H, CH_2), 3.62 (q, J = 7.2 Hz, 2H, CH_2), 2.62 (s, 3H, CH_3), 1.19 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 314 (M+Na, 50), 292 (M+H, 100), 197 (35).

3.7.12. 1-(Ethoxymethyl)-5-(pyrimidin-2-ylaminomethyl)-6-methyluracil (1l). Yield: 20%. Light yellow solid. Mp >300 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.42 (s, 1H, N*H*), 8.31 (m, 2H, 4′ and 6′*H*), 6.51 (m, 1H, 5′*H*),

5.34 (s, 2H, CH_2), 4.39 (d, J = 6.3 Hz, 2H, CH_2), 3.36 (q, J = 7.2 Hz, 2H, CH_2), 2.62 (s, 3H, CH_3), 1.19 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 291 (M+H, 100), 197 (30).

3.7.13. 1-(Ethoxymethyl)-5-(naphthalen-1-ylaminomethyl)-6-methyluracil (1m). Yield: 38%. Purple solid. Mp 69–71 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.24 (s, 1H, NH), 7.83–7.29 (m, 6H, 3′, 4′, 5′, 6′, 7′, and 8′H), 6.55 (m, 1H, 2′H), 5.33 (s, 2H, CH_2), 4.24 (s, 2H, CH_2), 3.62 (q, J = 7.2 Hz, 2H, CH_2), 2.47 (s, 3H, CH_3), 1.20 (t, J = 7.2 Hz, 3H, CH_3). MS (ES) m/z: 362 (M+Na, 40), 340 (M+H, 90), 197 (30).

3.8. General procedure for the synthesis of 1n–1u

Mixed 1-(benzyloxymethyl)-5-(bromomethyl)-6-methyluracil **8b** (45 mg, 0.13 mmol) and dry 1,4-dioxane (5 ml), followed by different amino compounds (0.89 mmol). The reaction mixture was allowed to stir for several hours and then was evaporated in vacuo. The resulting residue was purified by silica gel column chromatography using chloroform and methanol (10:1), as the eluent to give the target compound (**1n–1u**'s reaction time is illustrated in Table 1).

3.8.1. 1-(Benzyloxymethyl)-5-[(methyl-phenylamino)-methyl]-6-methyluracil (1n). Yield: 46%. Brown solid. Mp 45–47 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.11 (s, 1H, NH), 7.29–7.14 (m, 7H, ArH), 6.85 (d, J = 7.8 Hz, 2H, ArH), 6.73 (t, J = 7.2 Hz, 1H, ArH), 5.36 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 4.11 (s, 2H, CH_2), 2.69 (s, 3H, CH_3), 2.27 (s, 3H, CH_3). MS (ES) m/z: 366 (M+H, 100), 274 (18), 259 (22).

3.8.2. 1-(Benzyloxymethyl)-5-(*p*-tolylamino-methyl)-6-methyluracil (1o). Yield: 37%. Brown solid. Mp 42–44 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.41–7.29 (m, 5H, ArH), 6.98 (m, 2H, ArH), 6.56 (d, J = 8.7 Hz, 2H, ArH), 5.41 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.03 (s, 2H, CH_2), 2.44 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). MS (ES) m/z: 366 (M+H, 75), 274 (20), 259 (20).

3.8.3. 1-(Benzyloxymethyl)-5-[(4-nitro-phenylamino)-methyl]-6-methyluracil (1p). Yield: 29%. Brown solid. Mp 147–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.54 (s, 1H, NH), 7.97 (d, J = 9.3 Hz, 2H, ArH), 7.32–7.26 (m, 5H, ArH), 6.68 (d, J = 9.3 Hz, 2H, ArH), 5.38 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 4.39 (d, J = 4.5 Hz, 2H, CH_2), 2.37 (s, 3H, CH_3). MS (ES) m/z: 274 (30).

3.8.4. 1-(Benzyloxymethyl)-5-[(2-nitro-phenylamino)-methyl]-6-methyluracil (1q). Yield: 29%. Yellow solid. Mp 216–218 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (m, 1H, ArH), 7.46 (t, J = 6.9 Hz, 1H, ArH), 7.32–7.24 (m, 5H, ArH), 7.00 (t, J = 8.1 Hz, 1H, ArH), 6.66 (t, J = 7.2 Hz, 1H, ArH), 5.44 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.27 (d, J = 5.1 Hz, 2H, CH_2), 2.47 (s, 3H, CH_3). MS (ES) m/z: 397 (M+H, 60), 259 (83).

3.8.5. 1-(Benzyloxymethyl)-5-[(3-nitro-phenylamino)-methyl]-6-methyluracil (1r). Yield: 16%. Orange solid. Mp 196–198 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63–7.48 (m, 3H, ArH), 7.32–7.27 (m, 5H, ArH), 7.03 (m, 1H, ArH), 5.46 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.26 (d, J = 5.1 Hz, 2H, CH_2), 2.52 (s, 3H, CH_3). MS (ES) m/z: 419 (M+Na, 10), 274 (28).

3.8.6. 1-(Benzyloxymethyl)-5-[(2,4,5-trichloro-phenylamino)-methyl]-6-methyluracil (1s). Yield: 45%. White solid. Mp 180–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (s, 1H, NH), 7.30–7.18 (m, 6H, ArH), 6.74 (s, 1H, ArH), 5.38 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 4.00 (s, 2H, CH_2), 2.40 (s, 3H, CH_3). MS (ES) m/z: 478 (M+Na, 70), 454 (M, 30), 259 (95), 274 (82).

3.8.7. 1-(Benzyloxymethyl)-5-(cyclohexylaminomethyl)-6-methyluracil (1t). Yield: 54%. Yellow solid. Mp 200–202 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.19 (m, 5H, ArH), 5.34 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 3.65 (m, 2H, CH_2), 2.76 (m, 1H, CH), 2.48 (s, 3H, CH_3), 1.94–1.24 (m, 10H, CH_2). MS (ES) m/z: 358 (M+H, 55).

3.8.8. 1-(Benzyloxymethyl)-5-[(2,4,6-trichloro-phenylamino)-methyl]-6-methyluracil (1u). Yield: 28%. White solid. Mp 152–154 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.27 (s, 1H, NH), 7.39–7.26 (m, 6H, ArH), 5.42 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.24 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). MS (ES) m/z: 478 (M+Na, 25), 454 (M, 10).

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References and notes

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