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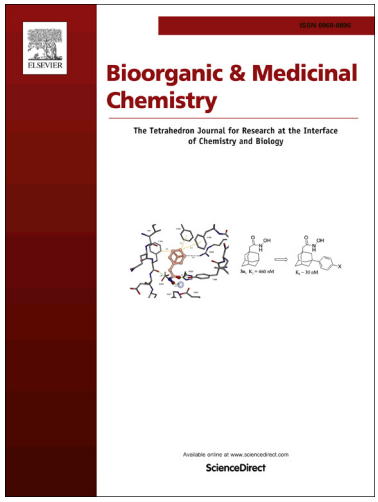


# Scaffold hopping: exploration of acetanilide-containing uracil analogues as potential NNRTIs

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## Graphical Abstract

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**Scaffold hopping: exploration of acetanilide-containing uracil analogues as potential NNRTIs**

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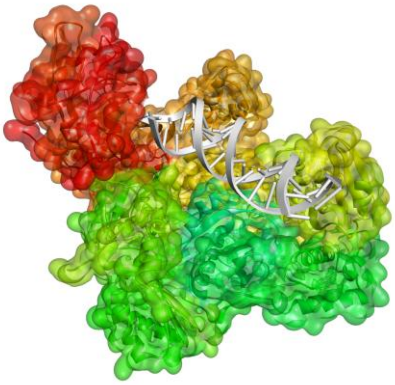


HIV-1 non-nucleoside reverse transcriptase inhibitors
$EC_{50}$ 1.7-35.8 μM

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ABSTRACT

In order to identify novel nonnucleoside inhibitors of HIV-1 reverse transcriptase two series of amide-containing uracil derivatives were designed as hybrids of two scaffolds of previously reported inhibitors. Subsequent biological evaluation confirmed acetamide uracil derivatives **15a-k** as selective micromolar NNRTIs with a first generation-like resistance profile. Molecular modeling of the most active compounds **15c** and **15i** was employed to provide insight on their inhibitory properties and direct future design efforts.

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## 1. Introduction

Human immunodeficiency virus (HIV) was identified as the causative agent of acquired immunodeficiency syndrome (AIDS) more than 3 decades ago. Since that time numerous successes in the development of various anti-HIV drugs have been achieved, in part due to the growing availability of new classes of inhibitors.[1] Despite new enzymatic targets, non-nucleoside reverse transcriptase inhibitors (NNRTI) remain a key element of highly active antiretroviral therapy (HAART) regimes. Five NNRTIs are currently used in the clinic, however, their use remains somewhat limited due to long-term toxicity[2,3] and drug resistance.[4] The latter issue has become even more problematic since drug-resistant HIV strains are arising more frequently even in treatment-naïve patients.[5,6] As a result, there remains a strong need for novel NNRTIs with acceptable levels of toxicity and advanced drug-resistance profiles.

In previous papers we have described our efforts to design and synthesize several series of uracil-based NNRTIs that exhibited potent anti-HIV activity. In particular, 1-[2-(2-benzoylphenoxy)ethyl]uracils[7] (**1**) exhibited strong inhibitory properties against HIV-1 reverse transcriptase (RT) and blocked HIV replication in cell culture. Additionally, a series of 1-(2-phenoxyethyl)-3-benzyl- (**2**) and 1-cinnamyl-3-benzyluracil derivatives[8] also showed remarkable antiretroviral activity. The main drawback of these compounds was the loss of activity against HIV RTs bearing K103N, V106A or Y181C mutations, which confer resistance for various NNRTIs.[9]

In that regard, it was hypothesized that introduction of an amide moiety might improve binding of the inhibitors to the mutant RTs due to the formation of additional polar interactions. Consequently, two novel series of acetamide-containing uracil derivatives (Fig. 1) were designed in an effort to explore their anti-HIV activity, particularly against mutant strains. Moreover, their scaffolds can be regarded as the result of molecular hybridization between two second generation NNRTIs – the benzophenone derivatives[10] (**3**) and the biaryl ethers[11] (**4**) – which have been characterized to possess a high barrier to the emergence of drug resistance.

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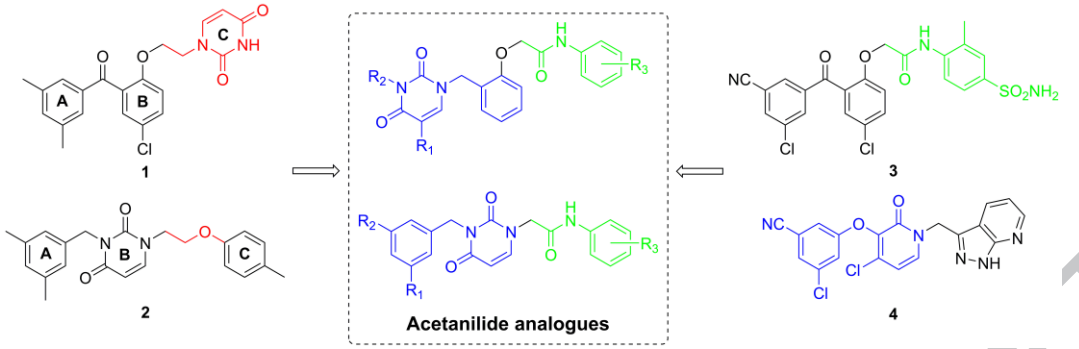



**Figure 1.** Design of novel inhibitor series by isosteric replacement of unoptimized fragments (red) with known NNRTIs pharmacophores (green and blue).

These newly designed compounds also share a feature commonly found in most of the potent NNRTIs – three aromatic ring systems connected by short linkers.[12] The acetamide group represents a logical replacement to the oxyethyl linker used in our previous inhibitor series. Specifically, introduction of the amide functionality should establish a critical H-bond with the RT backbone at the entrance of the binding pocket and thus prevent the loss of activity specifically caused by the K103N RT mutation (Fig. 2).[10,13]

Uracil is known to be associated with the bioactive conformation for several series of NNRTIs. In the case of benzophenone derivatives[7] it represents the ring **C**, while for 1,3-dibenzyl,[14] phenoxyethyl- and cinnamyl- derivatives[8] it serves as the central core (**B**) for the overall scaffold. Notably however, to the best of our knowledge, inhibitors featuring the uracil in position **A** (*i.e.* pointed to the bottom of the binding pocket) have not been previously described. As a result, this provided strong impetus for the design and development of compounds that encompass that arrangement (Fig. 1).

## 2. Results and discussion

### *2.1. Chemistry*

Target compounds **12a-k** were synthesized as outlined in Scheme 1. Bromide **7** was obtained using *ortho*-cresol and ethyl bromoacetate as building blocks. Use of the Hilbert-Johnson reaction[15,16] between 2,4-bis(trimethylsilyl)pyrimidines **8a-d** and **7** afforded compounds **9a-d**, however in unexpectedly moderate yields (51-67%). This was somewhat surprising since our previous observations for this reaction involving similarly reactive alkylating agents were quite efficient.[8,17] One explanation for the lower yields may be the steric hindrance between the bromoacetate reagent and the *o*-substituent of **7**. Next, an alkyl substituent was introduced in position 3 of the pyrimidine ring using standard procedures.[18,19] Subsequent hydrolysis of the ester functionality was accomplished in mild conditions using LiOH,[20] which was then converted to the acid chloride using standard conditions. Interestingly, we were unable to obtain the 5-bromouracil derivative, most likely due to its instability in alkaline conditions.[21] It should be noted that electrophilic bromination[22] could not be used for the **11a-c** since it lacks stereoselectivity due to the presence of the two electron-rich aromatic ring systems. Finally, compounds **12a-k** were synthesized in good yields using the appropriate substituted anilines and pyridine in dry 1,2-dichloroethane.

The synthetic route to target compounds **15a-l** is shown in Scheme 2. First, condensation of ethyl bromoacetate with 2,4-bis(trimethylsilyloxy)pyrimidine (**8a**) afforded 1-(ethoxycarbonylmethyl)uracil **13**.[23] Next, $N^3$-benzylation in the presence of solid $K_2CO_3$ in DMF, followed by hydrolysis of the $N^3$ esters, which was accomplished directly without isolation to give acids **14a-d**. Subsequent conversion to the appropriate acid chlorides using standard conditions as before, followed by coupling with the appropriate anilines was done in an analogous manner as was previously used to realize compounds **12a-k**.

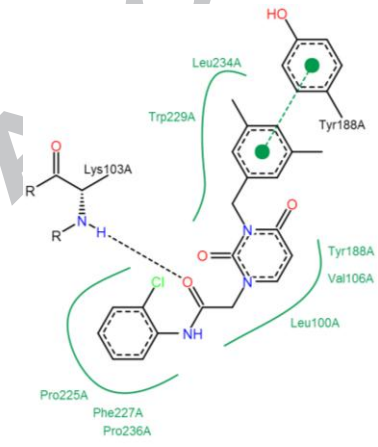
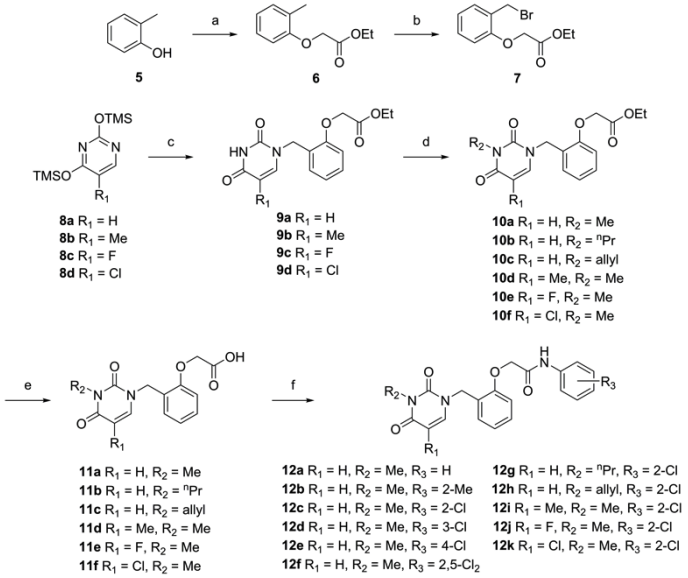



**Figure 2.** Proposed binding mode of the acetanilide analogue in the HIV-1 NNRTI binding pocket.




**Scheme 1.** Reagents and conditions: (a) $BrCH_2CO_2Et$, $K_2CO_3$, $Me_2CO$, 56 °C, 76%; (b) NBS, $CCl_4$, 77 °C, 90%; (c) **7**, DCE, 85 °C, 51-67%; (d) $(CH_3)_2SO_4$, $Me_2CO$; or alkyl halide, $K_2CO_3$, DMF, 22 °C, 91-99%; (e) LiOH, $EtOH/H_2O$, 22 °C, 89-94%; (f) i. $SOCl_2$, DCE, 85 °C; ii. $ArNH_2$, Py, -10 °C, 10-86%.

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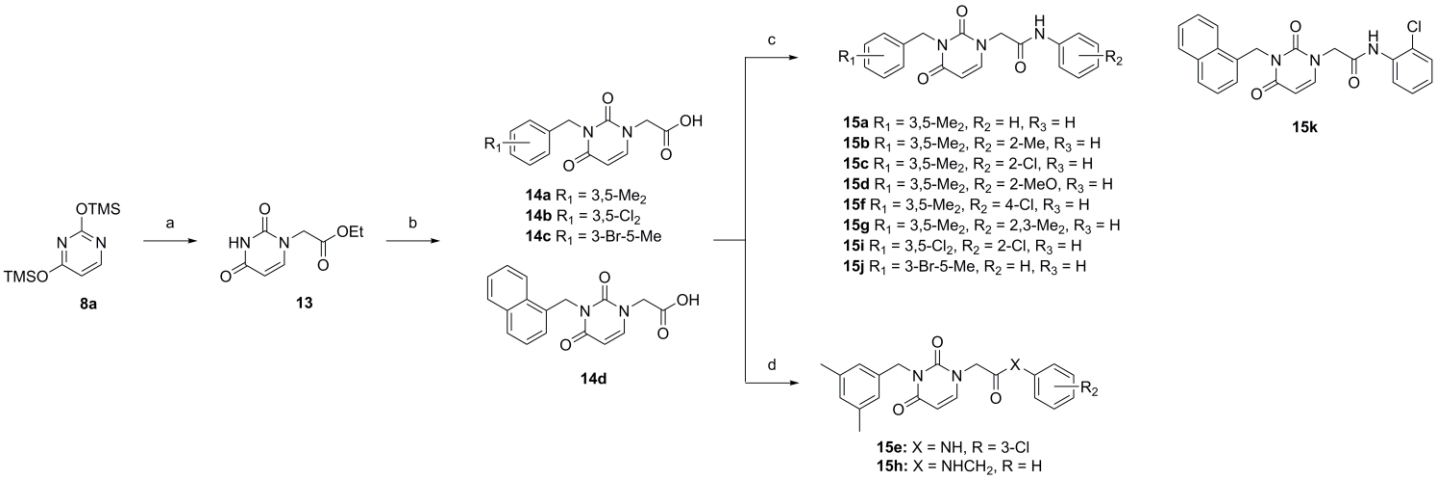


**Scheme 2.** Reagents and conditions: (a) $BrCH_2CO_2Et$, DCE, 85 °C, 92%; (b) i. $ArCH_2Br$, $K_2CO_3$, DMF, 80 °C; ii. LiOH, EtOH/$H_2O$, 22 °C, 71-87%; (c) i. $SOCl_2$, DCE, 85 °C; ii. $ArNH_2$, Py, -10 °C, 32-95%; (d) $ArNH_2$, 20 mol% $H_3BO_3$, PhMe, 110 °C, 48-50%.

Notably, the final steps of this approach involved handling moisture-sensitive intermediates and the use of excess base. To overcome this issue, we decided to try a more "green" approach to realize compounds **15e** and **15h** – direct acid-amine coupling catalyzed by boric acid.[24,25] Disappointingly, while this method proved to be convenient and more environmentally friendly, conversion of the starting materials never exceeded 50% despite all attempts to increase the yields. Since the low yield could also have been associated with the poor solubility of acids **14** in toluene, we then employed a 5:1 toluene:DMF solvent system. Unfortunately this did not lead to a substantial increase of the yield (data not shown).

*2.2. Biological activities*

*2.2.1. HIV-1 RT inhibition assay*

The antiretroviral activity of the target compounds was evaluated *in vitro* against the recombinant HIV-1 reverse transcriptase. Unfortunately, compounds **12a-k** featuring a terminal uracil moiety were essentially inactive against wild-type RT (Table 1).

Uracil's C-4-carbonyl can be considered as the most significant structural difference between these derivatives and the benzophenone NNRTIs patented by GlaxoSmithKline (**3, Fig. 1**). Preliminary docking studies predicted an H-bond between the carbonyl and the pyrrole NH of the Trp229 side chain (data not shown). However, in light of the experimental data, it seems more likely that the oxygen lone pairs are instead, forced to face the Trp229 π-system. Such an unfavorable interaction is in contradiction to the typical NNRTIs edge-face stacking with Trp229 provided by an aromatic proton.[26]

In contrast, anilides **15a-k** showed significant inhibitory properties, except for **15g** which proved completely inactive ($IC_{50}$ > 300 μM). A close correlation between the HIV-1 RT inhibitory activity and the antiviral activity in MT-4 cells was also observed (Figure 3).

Anilide **15i** proved to be one of the most active compounds, thus was also evaluated against a panel of recombinant reverse transcriptases. These contain one or two mutations, the ones most often observed in the NNRTI-resistant virus strains. It was revealed that the inhibitory activity of **15i** was much lower against RTs bearing mutations K103N, V106A, or a combination K103N/Y181C. This was noteworthy since a similar decrease was described earlier for the previously reported series of compounds.[7,8] In addition, we observed a moderate decrease in the inhibitory activity towards RT with L100I, and G190A, which was not the case for the benzophenones[7] or the $N^1$,$N^3$-substituted uracils.[8] The largest difference between **15i** and the previously described $N^1$,$N^3$-substituted uracils was noted for Y181C RT: **15i** was only three-fold less active towards this mutant RT, whereas the previously described inhibitors suffered a complete loss of activity against this mutant. Nevertheless, the resistance profile of **15i** suggests that these compounds belong to the first generation types of NNRTIs.

Table 1. Antiviral activity of compounds **12a-k** against HIV-1.

| Comp. | $R_1$ | $R_2$ | $R_3$ | $EC_{50}$[a] (μM) | $CC_{50}$[b] (μM) | SI[c] | $K_i$[d] (μM) |
|---|---|---|---|---|---|---|---|
| **12a** | H | Me | H | >152.85 | 152.85±9.47 | <1 | >100 |
| **12b** | H | Me | 2-Me | >151.82 | 151.82±16.02 | <1 | >100 |
| **12c** | H | Me | 2-Cl | >140.94 | 140.94±0.18 | <1 | >100 |
| **12d** | H | Me | 3-Cl | >287.62 | 287.62±10.60 | <1 | >100 |
| **12e** | H | Me | 4-Cl | >100.42 | 100.42±67.38 | <1 | >100 |
| **12f** | H | Me | 2,5-$Cl_2$ | >29.47 | 29.47±1.63 | <1 | >100 |
| **12g** | H | *n*-Pr | 2-Cl | >25.36 | 25.36±1.50 | <1 | >100 |
| **12h** | H | allyl | 2-Cl | >30.64 | 30.64±0.82 | <1 | >100 |
| **12i** | Me | Me | 2-Cl | >14.76 | 14.76±8.34 | <1 | >100 |
| **12j** | F | Me | 2-Cl | >30.28 | 30.28±1.53 | <1 | >100 |
| **12k** | Cl | Me | 2-Cl | >9.37 | 9.37±0.37 | <1 | >100 |

[a]50% effective concentration or compound concentration required to inhibit HIV-induced cytopathogenic effect by 50% in MT-4 cells.

[b]50% cytotoxic concentration or compound concentration required to reduce MT-4 viability by 50%.

[c]selectivity index or ratio $CC_{50}/EC_{50}$.

[d]inhibition constant for inhibition of HIV-1 reverse transcriptase.

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Table 2. Antiviral activity of compounds **15a-k** against HIV-1.

| Comp. | $R_1$ | $R_2$ | X | $EC_{50}$[a] (μM) | $IC_{50}$[b] (μM) | $CC_{50}$[c] (μM) | SI[d] |
|---|---|---|---|---|---|---|---|
| **15a** | 3,5-$Me_2$ | H | NH | 9.68±0.58 | 79.28±6.42 | 241.72±48.43 | 25 |
| **15b** | 3,5-$Me_2$ | 2-Me | NH | 7.68±0.79 | 68.77±1.59 | 291.78±21.54 | 38 |
| **15c** | 3,5-$Me_2$ | 2-MeO | NH | 1.71±0.45 | 15.68±2.85 | 28.48±4.12 | 17 |
| **15d** | 3,5-$Me_2$ | 2-Cl | NH | 14.54±3.58 | 210.96±133.01 | 227.41±45.14 | 16 |
| **15e** | 3,5-$Me_2$ | 3-Cl | NH | 29.50±3.04 | 114.15±17.22 | 167.00±75.50 | 6 |
| **15f** | 3,5-$Me_2$ | 4-Cl | NH | 7.29±2.84 | 62.90±9.17 | 77.49±79.70 | 11 |
| **15g** | 3,5-$Me_2$ | 2,3-$Me_2$ | NH | 35.84±7.61 | >300 | 275.74±43.32 | 8 |
| **15h** | 3,5-$Me_2$ | H | $NCH_2$ | >190.84 | 193.87±33.72 | 190.84±28.38 | <1 |
| **15i** | 3,5-$Cl_2$ | 2-Cl | NH | 3.26±2.64 | 12.67±1.00 | 31.09±6.54 | 10 |
| **15j** | 3-Br-5-Me | 2-Cl | NH | 2.01±0.71 | 12.08±0.11 | 33.14±4.26 | 17 |
| **15k** | - | - | - | 7.94±1.43 | 44.54±5.04 | 207.40±56.07 | 26 |
| NVP | - | - | - | 0.31±0.06 | 1.15±0.51 | >15 | >50 |
| EFV | - | - | - | 0.006±0.001 | 0.022±0.009 | >6 | >1000 |

[a]50% effective concentration or compound concentration required to inhibit HIV-induced cytopathogenic effect by 50%.

[b]compound concentration required to inhibit in vitro HIV-1 RT wt activity by 50%.

[c]50% cytotoxic concentration or compound concentration required to reduce MT-4 viability by 50%.

[d]selectivity index or ratio $CC_{50}/EC_{50}$.

### *2.2.2. Anti-HIV activities evaluation*

To further confirm the anti-HIV potency of the newly synthesized compounds we also determined their ability to inhibit the HIV-1 $III_B$ strain-induced cytopathicity in MT-4 cell cultures. The cytotoxicity of these compounds was determined in parallel. Comparisons of the antiviral inhibitory concentration $EC_{50}$, cytotoxic concentration $CC_{50}$, and SI (selectivity, given by the $CC_{50}/EC_{50}$ ratio) values are depicted in Tables 1 and 2.

No activity was observed for series **12** in cell cultures, while compounds **15a-k** revealed rather interesting trends. Compounds **15a-k** were further evaluated for their antiretroviral activity against the WT strain $III_B$ and the NNRTI-resistant double RT mutant K103N;Y181C in MT-4 cells. The compounds behaved as typical first generation NNRTI, with a total loss of activity against the RT double mutant. Overall there was a close correlation between the anti-HIV-1 activity in MT-4 cells and the inhibitory activity of the compounds in the RT polymerase assay. This was shown by way of a linear regression plot with results for compounds **15a-k** (except for **15g** and **15h** due to lack of activity in the enzymatic assay and MT-4/MTT assay, respectively), and the reference compounds nevirapine and efavirenz, in both assays (Fig. 3). The correlation coefficient (*r*) was 0.99.

The parent structure **15a** shows moderate activity at the lower μM-range. Introducing an electron donating methoxy group at the ortho position of the aniline ring, as in compound **15c**, was the most beneficial modification that led to improved anti-HIV-1 activity over the parent structure **15a**, but resulted in a concomitant increase in cytotoxicity. Other substituents such as chlorine (compound **15d**) and methyl (compound **15b**) introduced at the same position did not markedly influence the anti-HIV activity, nor the cytotoxicity of the compound as compared to **15a**. The series of analogues **15d**, **15e** and **15f** revealed the effect resulting from shifting the chlorine on the aniline ring from ortho to meta or para position – the *para*-Cl was better than the *ortho*-Cl which was better than the *meta*-Cl. Introduction of a second methyl group on molecule **15b**, resulting in **15g,** also indicates a possible negative effect of *meta*-substitution on the antiviral activity.

When replacing all three methyl groups in **15b** by chlorine (**15i**), no pronounced differences in anti-HIV activity arose but the cytotoxicity increased. Replacing one *meta*-Me on the benzyl group in **15d** by a bromine, which has almost the same sterics and size, leads to a more active molecule (**15j**) with an $EC_{50}$ in MT-4 cells close to that of the most active molecule of the series, compound **15c**.

Table 3. Activity of **15i** against mutant forms of HIV-1 RT.

| PDB | HIV-1 RT | Ki (μM) | Fold decrease |
|---|---|---|---|
| 3DLG | WT | 4.5 | - |
| 3DOL | L100I | 15.9 | 4 |
| 3DOK | K103N | 63.3 | 14 |
| 3DMJ | V106A | 67.6 | 15 |
| 1JLA | Y181C | 15.4 | 3 |
| - | G190A | 20 | 4 |
| 2IC3 | K103N+Y181C | 69.9 | 16 |

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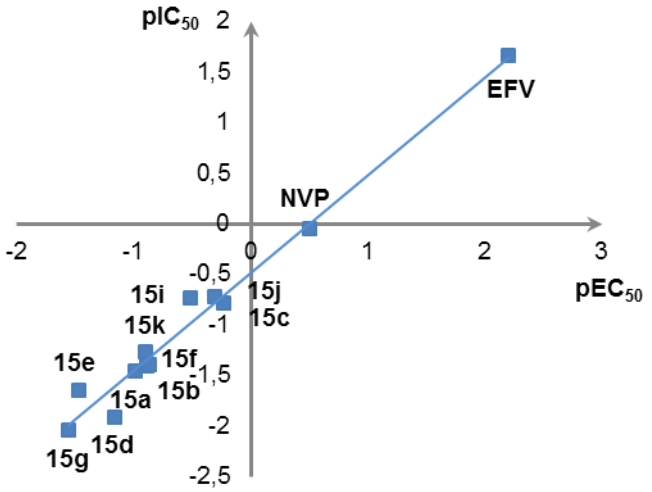


**Figure 3.** $pIC_{50}$ versus $pEC_{50}$ values for compounds **15a-f;i-k** and the reference compounds nevirapine (NVP) and efavirenz (EFV).

One last modification was done to assess the influence of lengthening the linker in the aniline moiety, creating the benzylamine analogue (**15h**) of the molecule **15a**. The elongated linker of the benzylamine derivative **15h** led to complete loss of activity, so no further modifications of this type were considered.

An additional mechanism of action could be hypothesized, since similar results were reported for uracil-derived NNRTIs previously reported by Buckheit et al.[27,28] and for thiouracil derivatives by Mugnaini et al.[29] However, based on the strong correlation between the inhibition of the HIV-1 replication in vitro and the inhibitory activity in a RT polymerase assay this is unlikely. To completely exclude other mechanisms of action, we performed time of addition experiments for all active compounds using the NNRTI-resistant double mutant virus and concentrations of compound close to their $CC_{50}$ values as determined in the MT-4/MTT assay. No inhibitory effect on virus production was revealed, excluding inhibition of a process anterior to reverse transcription. To exclude possible targets after transcription, in parallel with the evaluation of the activity using the MT-4/MTT assay where cell viability is the read out, we measured the p24 production to assess the inhibitory activity of the compounds. As no inhibition of virus production, based on p24 ELISA, was observed, targets after the RT step can also be excluded. Finally all active compounds were subjected to an RT RNaseH assay. None of the compounds **15a-k** inhibited the RNaseH activity, except for compound **15i** which exhibited marginal activity ($IC_{50}$: 149.2 ± 43.9 µM).

As a result, it is clear that the NNRTI mode of action is the sole mechanism responsible for the observed HIV-1 inhibitory activity of the compounds **15a-k** in cell culture.

### 3. Molecular modeling

In order to rationalize the HIV-RT inhibitory properties observed we employed extensive docking experiments. Crystal structures of WT and mutant HIV-1 RTs with structurally relevant bound inhibitors were selected from the RCSB Protein Data Bank for these studies (Table 3).

The binding conformation predicted for the most active compound **15c** in WT RT represents the typical "seahorse"[30] conformation (Fig. 4a). The benzyl moiety is anchored at the aromatic bottom of the binding pocket by means of π-stacking interactions with Trp229 and Tyr188, while contacts with Tyr181 are diminished. A key hydrogen bond with Lys103 in the backbone is formed by the anilide carbonyl, and the aniline and uracil moieties are sandwiched between Leu100, Val106 and Pro236 residues of RT. Additional hydrophobic interactions with the Leu234 side chain were observed as well as weak hydrogen bonding between H-6 of uracil and a carbonyl of Lys101 backbone.

The binding modes of **15i** were investigated for mutant RT crystallographic structures and the results demonstrate negligible differences with RMSD values ranging from 0.25 to 2.37 Å upon molecular overlay. As a result, possible reorganization of the binding pockets of the mutated enzymes could serve as explanation for the observed differences in $K_i$ values. The chlorine in position 2 of the aniline piece of the scaffold occupies space nearby several hydrophobic residues. Notably, this chorine is computed to be at a distance of 2.98 Å from the carbonyl oxygen of the Lys/Asn103 backbone. Hence, it is feasible that a halogen bond is likely occuring.[26] This finding not only supports

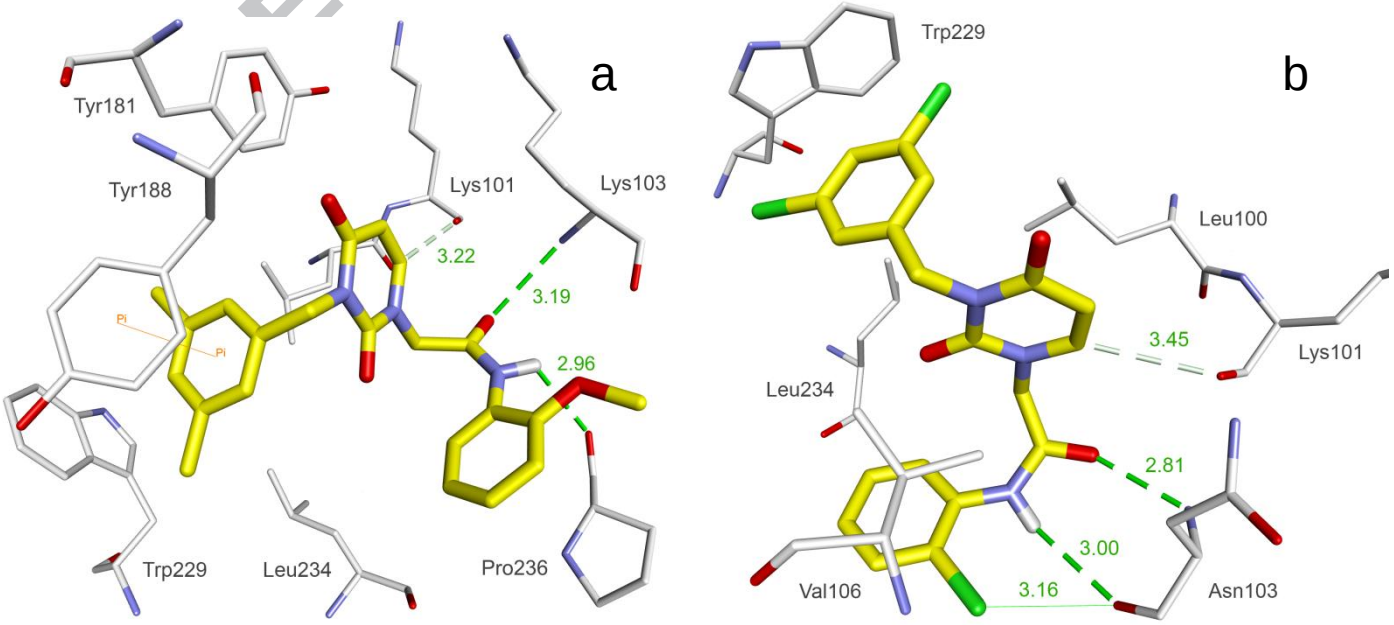


**Figure 4.** Predicted binding conformations: a) **15c** WT HIV-1 RT (Val106 is omitted for clarity); b) **15i** in K103N mutant of HIV-1 RT. Notice close contacts with Val106 and spacial gap extending to the Asn103 side-chain. Distance for hydrogen and halogen bonds are indicated in angstroms.

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the influence of the *ortho*-chloro substituent seems to be having on the anti-HIV RT activity, but could also be responsible for the *cis*-conformation of the amide bond. This last point is of particular interest since our previously reported cinnamyl derivatives comprise a trans-configured double C-C bond.

The L100I mutation generally affects binding of NNRTIs either by destabilizing the hydrogen bond to the backbone of Lys101 or by introducing unfavorable protein-ligand interactions.[31] Notably **15i** remained effective against this mutant enzyme with the hydrogen bond to Lys103 computed to have similar lengths in both WT and L100I HIV-RT (3.00 and 2.95 Å, respectively).

The K103N mutation confers resistance to a wide variety of NNRTIs. Despite the prediction that the key stabilizing interactions for this mutant RT would be retained, the significant (14-fold) loss of inhibitory activity could be attributed to unfavorable interactions between the amide functionality of Asn103 and the uracil ring. It appears to be unable to effectively accommodate the space generated by the replacement of the Lys103 side chain (Fig. 4b). Moreover, the central region of **15i** lacks the ability to establish a halogen bond with the Tyr181 RT backbone, which is known to contribute to improved resistance profiles for several of the NNRTI families.[32,33]

The effects of the V106A mutation in HIV-1 RT on the binding of NNRTIs has been much less studied, thus there are only a limited number of crystal structures of this mutant available, and those that are, feature structurally irrelevant inhibitors bound in the active site. Nonetheless, inspection of the predicted conformation of **15i** for WT HIV-1 RT demonstrates an extensive hydrophobic interaction between the side chain of Val106 and the uracil moiety that is lost upon mutation of V106A. This is supported by the analogous loss of activity reported previously for the 1-cinnamyl-3-benzyluracil derivatives.[8] Consequently, the 3-benzyluracil scaffold appears to be responsible for the failure to adapt to the mutated binding pocket; however a more satisfactory explanation for the influence of the V106A mutation on **15i**'s activity has not been uncovered.

Finally, reduced activity against the Y181C RT mutant has mostly been attributed to the loss of important π-π interactions between the tyrosine residue and aryl or allyl groups of many NNRTIs (*e.g.*, nevirapine, MKC-442, and 9-Cl-TIBO).[34] Since **15i** makes a much less significant contact with Tyr181, while π-stacking still occurs with Tyr188, the diminished interaction with Tyr181 appears to have little impact on the activity of **15i**.

## 4. Conclusions

In summary, we have reported the design and synthesis of a series of acetanilide-containing uracil analogues based on molecular hybridization of two other NNRTI scaffolds. All newly synthesized compounds were evaluated for their anti-HIV activity in MT-4 cells and their inhibitory activity against the HIV-1 RT polymerase. The compounds bearing the uracil in position **A** (**12a-k**) were revealed to be inactive whereas their analogues with the uracil in position **B** (**15a-k**) displayed in vitro anti-HIV-1 activities ranging from 1.7 to 35.8 μM in MT-4 cells, with the exception of the benzylamine analogue that was devoid of any anti-HIV activity. All of the active compounds owe their inhibitory activity on HIV-1 replication exclusively to an NNRTI type mode of action. Unfortunately, the active compounds behaved like first generation NNRTIs.

In conclusion, we have presented a versatile approach to construct novel uracil-based small-molecule inhibitors and thus have expanded the NNRTI chemical space. Simple flexible docking protocols proved effective for elucidating the structural basis responsible for the observed activity trends. Overall the results of the present study have added to our understanding of the impact certain structural modifications have on anti-HIV activity. This information will allow for further rational design of more potent and efficacious NNRTIs.

## 5. Experimental

### *5.1. General*

All reagents were obtained at the highest grade available from Sigma and Acros Organics and used without further purification unless otherwise is noted. Anhydrous DMF and isopropanol were purchased from Sigma-Aldrich Co. Anhydrous acetone, 1,2-dichloroethane, and ethyl acetate were obtained by distillation over $P_2O_5$. NMR spectra were performed on a Bruker Avance 400 spectrometer (400 MHz for $^1$H and 100 MHz for $^{13}$C) in DMSO-$d_6$ with tetramethylsilane as an internal standard. Data are reported in the following order: multiplicity (br, broad; s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; q, quartet; qu, quintet). TLC was performed on Merck TLC Silica gel 60 $F_{254}$ plates eluted with ethyl acetate or chloroform/MeOH (10:1) and developed with UV-lamp VL-6.LC (France). Acros Organics (Belgium) silica gel (Kieselguhr 60-200 μm, 60A) was used for column chromatography. Melting points were determined in glass capillaries on a Mel-Temp 3.0 (Laboratory Devices Inc., US). Yields refer to spectroscopically ($^1$H and $^{13}$C NMR) homogeneous materials. High resolution mass spectra were measured on Bruker micrOTOF II instruments using electrospray ionization (HRESIMS). The measurements were done in a positive ion mode (interface capillary voltage –4500 V) in a mass range from m/z 50 to m/z 3000 Da; external or internal calibration was done with ESI Tuning Mix™ (Agilent Technologies). A syringe injection was used for solutions in acetonitrile (flow rate 3 μl/min). Nitrogen was applied as a dry gas; the interface temperature was set at 180 °C.

### *5.2. Synthesis*

#### *5.2.1. General procedure for the synthesis of **9a-d***

Anhydrous $K_2CO_3$ (20.09 g, 145.36 mmol) was added to a solution of *o*-cresol (10.0 ml, 96.91 mmol) in 70 ml of acetone followed with ethyl bromoacetate (11.80 ml, 106.60 mmol). Resulting suspension was refluxed for 24 hr, filtered off and evaporated. The residue was dissolved in 40 ml of $CHCl_3$, washed with 50 × 2 ml of 5% NaOH solution, water and evaporated. Vacuum distillation afforded 14.33 g of **6**[35,36] as a clear oil; yield 76%; bp 125-128 °C/5 mm. A mixture of **6** (14.33 g, 73.78 mmol) and NBS (13.80 g, 77.53 mmol) in 100 ml of $CCl_4$ was refluxed under irradiation of 100 W tungsten lamp for 4 hr, cooled to room temperature, filtered and evaporated. The residual oil was distilled to afford 18.10 g of bromide **7**[37] as pale yellow oil; yield 90%; bp 145-148 °C/5 mm.

A mixture of uracil, thymine, 5-fluorouracil or 5-chlorouracil (53.53 mmol) and ammonium chloride (0.3 g, 5.60 mmol) in HMDS (15 mL) was refluxed for 10 hr with exclusion of moisture until a clear solution was obtained. The excess of silylating agent was removed under vacuum. The residual clear oil of 2,4-bis(trimethylsilyloxy)pyrimidines[38] **8a-d** were dissolved in 100 ml of anhydrous 1,2-dichloroethane and bromide **7** (14.62 g, 53.53 mmol) was added. Reaction mixture was heated at reflux for 30 hr, cooled to room temperature and treated with 15 ml of $^i$PrOH. Resulting precipitate was collected and purified by flash chromatography eluting with 1:10 EtOH/1,2-dichloroethane.

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*5.2.1.1. Ethyl 2-(2-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**9a**)*

Yield 58%; white crystals; mp 157-159 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 1.20 (3 H, t, *J*=7.1 Hz, $CH_3$), 4.17 (2 H, q, *J*=7.1 Hz, $COOCH_2$), 4.84 (4 H, s, $CH_2$), 5.53 (1 H, dd, *J*=7.9, 1.8 Hz, H-5), 6.91 - 7.02 (2 H, m, H-5', H-6'), 7.19 (1 H, d, *J*=7.4 Hz, H-3'), 7.27 (1 H, t, *J*=7.7 Hz, H-4'), 7.74 (1 H, d, *J*=7.9 Hz, H-6), 11.25 (1 H, br. s., H-3); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ ppm 18.2, 43.3, 43.4, 43.5, 43.7, 43.8, 43.9, 44.1, 50.9, 65.0, 69.2, 104.8, 116.3, 125.4, 128.6, 133.5, 134.0, 150.5, 155.2, 159.7, 168.0, 172.8.

*5.2.1.2. Ethyl 2-(2-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**9b**)*

Yield 51%; white crystals; mp 189.5-191.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.21 (3 H, t, *J*=7.2 Hz, $CH_3$), 1.74 (3 H, d, *J*=1.0 Hz, $CH_3$), 4.18 (2 H, q, *J*=7.1 Hz, $COOCH_2$), 4.81 (2 H, s, $OCH_2$), 4.85 (2 H, s, $NCH_2$), 6.89 - 7.02 (2 H, m, H-5', H-6'), 7.16 (1 H, dd, *J*=7.5, 1.4 Hz, H-3'), 7.21 - 7.30 (1 H, m, H-4'), 7.65 (1 H, d, *J*=1.3 Hz, H-6), 11.22 (1 H, s, H-3); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 12.2, 14.3, 39.4, 39.6, 39.8, 40.1, 40.3, 46.6, 61.1, 65.3, 108.6, 112.3, 121.5, 125.0, 129.5, 129.9, 142.4, 151.3, 155.7, 164.6, 169.0.

*5.2.1.3. Ethyl 2-(2-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**9c**)*

Yield 67%; white crystals; mp 123.5-125°C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.20 (3 H, t, *J*=7.1 Hz, $CH_3$), 4.17 (2 H, q, *J*=7.2 Hz, $COOCH_2$), 4.71 - 4.95 (4 H, m, $CH_2$), 6.89 - 7.05 (2 H, m, H-5', H-6'), 7.16 - 7.37 (2 H, m, H-3', H-4'), 8.13 (1 H, d, *J*=6.8 Hz, H-6), 11.77 (1 H, d, *J*=4.5 Hz, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 14.3, 39.4, 39.6, 39.8, 40.0, 40.2, 47.5, 61.1, 65.3, 112.3, 121.4, 124.4, 129.7, 130.3, 131.0, 131.3, 150.0, 155.7, 169.0.

*5.2.1.4. Ethyl 2-(2-((5-chloro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**9d**)*

Yield 55%; white crystals; mp 200-201 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.20 (3 H, t, *J*=7.1 Hz, $CH_3$), 4.17 (2 H, q, *J*=7.1 Hz, $COOCH_2$), 4.85 (4 H, s, $CH_2$), 6.88 - 7.04 (2 H, m, H-5', H-6'), 7.14 - 7.37 (2 H, m, H-3', H-4'), 8.19 (1 H, s, H-6), 11.79 (1 H, br. s., H-3); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 14.3, 39.4, 39.6, 39.8, 40.0, 40.2, 47.7, 61.1, 65.3, 112.3, 121.5, 124.3, 129.7, 130.3, 143.9, 155.8, 168.9.

*5.2.2. General procedure for the synthesis of **10a**, **d-f***

To a magnetically stirred mixture of **9** (13.97 mmol) and anhydrous $K_2CO_3$ (7.72 g, 55.86 mmol) in 50 ml of acetone, dimethyl sulfate (2 ml, 21.14 mmol) was added in one portion at room temperature. After 20 hr, a solution of NaOH (3.5 g) in 100 ml of water was added, and stirring was continued for 0.5 hr. The mixture was concentrated and the resulting precipitate filtered off after cooling in a refrigerator. The products **10a-d** were sufficiently pure to be used in the next step without any additional purification.

*5.2.2.1. Ethyl 2-(2-((3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**10a**)*

Yield 92%; white crystals; mp 123.5-126 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.20 (3 H, br. s., $CH_3$), 3.13 (3 H, br. s. , $NCH_3$), 4.16 (2 H, d, *J*=6.3 Hz, $COOCH_2$), 4.63 - 5.08 (4 H, m, $CH_2$), 5.65 (1 H, d, *J*=7.1 Hz, H-5), 6.96 (2 H, d, *J*=7.3 Hz, H-5', H-6'), 7.12 - 7.40 (2 H, m, H-3', H-4'), 7.79 (1 H, d, *J*=6.8 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 14.3, 27.5, 39.4, 39.7, 39.9, 40.1, 40.3, 48.3, 61.1, 65.3, 99.9, 112.4, 121.4, 124.5, 129.6, 130.4, 144.9, 151.6, 155.9, 162.9, 168.9.

*5.2.2.2. Ethyl 2-(2-((3,5-dimethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**10d**)*

Yield 99%; white crystals; mp 125-126.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.22 (2 H, t, *J*=7.1 Hz, $CH_3$), 1.81 (3 H, d, *J*=1.1 Hz, $CH_3$), 3.16 (3 H, s, $NCH_3$), 4.19 (2 H, q, *J*=7.2 Hz, $COOCH_2$), 4.85 (2 H, s, $OCH_2$), 4.88 (2 H, s, $NCH_2$), 6.92 - 7.00 (2 H, m, H-5', H-6'), 7.21 (1 H, dd, *J*=7.5, 1.7 Hz, H-3'), 7.27 (1 H, td, *J*=7.9, 1.7 Hz, H-4'), 7.70 - 7.75 (1 H, m, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 12.6, 14.1, 27.5, 39.2, 39.4, 39.6, 39.8, 40.0, 47.6, 60.8, 65.0, 107.3, 112.1, 121.2, 124.5, 129.3, 130.0, 140.6, 151.2, 155.5, 163.4, 168.7.

*5.2.2.3. Ethyl 2-(2-((5-fluoro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**10e**)*

Yield 81%; white crystals; mp 123.5-125°C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 1.23 (3 H, t, *J*=7.2 Hz, $CH_3$), 3.18 (3 H, s, $NCH_3$), 4.20 (2 H, q, *J*=7.1 Hz, $COOCH_2$), 4.88 (4 H, s, $CH_2$), 6.91 - 7.05 (2 H, m, H-5', H-6'), 7.23 - 7.35 (2 H, m, H-3', H-4'), 8.23 (1 H, d, *J*=6.4 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 18.2, 32.1, 43.4, 43.5, 43.7, 43.8, 43.9, 44.1, 44.2, 52.6, 65.0, 69.2, 116.2, 125.3, 128.1, 133.6, 134.5, 154.0, 159.8, 172.8.

*5.2.2.4. Ethyl 2-(2-((5-chloro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**10f**)*

Yield 88%; white crystals; mp °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 1.23 (3 H, t, *J*=7.1 Hz, $CH_3$), 3.32 (3 H, s, $NCH_3$), 4.20 (2 H, q, *J*=7.2 Hz, $COOCH_2$), 4.86 (2 H, s, $OCH_2$), 4.93 (2 H, s, $NCH_2$), 6.93 - 7.02 (2 H, m, H-5', H-6'), 7.25 - 7.34 (2 H, m, H-3', H-4'), 8.28 (1 H, s, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 18.2, 32.7, 43.4, 43.5, 43.7, 43.8, 44.0, 44.1, 44.2, 52.8, 65.0, 69.2, 109.6, 116.3, 125.4, 128.0, 133.7, 134.5, 146.2, 159.8, 172.7.

*5.2.3. General procedure for the synthesis of **10b** and **10c***

A suspension of **9a** (1.11 g, 3.66 mmol) and $K_2CO_3$ (0.6 g, 4.34 mmol) in anhydrous DMF (8 mL) was stirred at 80 °C for 1 hr. After addition of *n*-propyl iodide (0.37 ml, 3.82 mmol) for **10b** or allyl bromide (0.33 ml, 3.82 mmol) for **10c**, stirring was continued at room temperature for 24 hr. The reaction mixture was filtered, evaporated and the residue treated with water, filtered off and purified by flash chromatography eluting with 1:10 hexane/ethyl acetate.

*5.2.3.1. Ethyl 2-(2-((2,4-dioxo-3-propyl-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**10b**)*

Yield 94%; white crystals; mp 187-188 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.20 (3 H, t, *J*=7.1 Hz, $CH_3$), 4.17 (2 H, q, *J*=7.1 Hz, $CH_2$), 4.84 (4 H, s, ), 5.52 (1 H, dd, *J*=7.8, 2.3 Hz, H-5), 6.90 - 7.03 (2 H, m, H-5', H-6'), 7.18 (1 H, dd, *J*=7.8, 1.8 Hz, H-3'), 7.27 (1 H, td, *J*=7.8, 1.5 Hz, H-4'), 7.73 (1 H, d, *J*=7.8 Hz, H-6), 11.23 (1 H, br. s., NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 14.3, 39.4, 39.6, 39.9, 40.1, 40.3, 47.0, 61.1, 65.3,

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100.9, 112.4, 121.5, 124.8, 129.5, 130.0, 146.5, 151.3, 155.8, 164.1, 168.9.

*5.2.3.2. Ethyl 2-(2-((3-allyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetate (**10c**)*

Yield 91%; white crystals; mp 86.5-87.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.21 (3 H, t, *J*=7.2 Hz, $CH_3$), 4.18 (2 H, q, *J*=7.2 Hz, $CH_2$), 4.38 (2 H, d, *J*=5.2 Hz, $CH_2$), 4.84 (2 H, s, $CH_2$), 4.91 (2 H, s, $CH_2$), 5.00 - 5.11 (2 H, m, =$CH_2$), 5.68 (1 H, d, *J*=7.9 Hz, H-5), 5.80 (1 H, ddt, *J*=17.2, 10.5, 5.3, 5.3 Hz, -CH=), 6.93 - 7.01 (2 H, m, H-5', H-6'), 7.23 (1 H, dd, *J*=7.4, 1.2 Hz, H-3'), 7.25 - 7.31 (1 H, m, H-4'), 7.80 (1 H, d, *J*=7.9 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 13.9, 39.3, 39.5, 39.7, 42.2, 47.9, 60.7, 65.0, 99.7, 112.0, 116.4, 121.1, 124.2, 129.3, 129.9, 132.4, 144.8, 150.8, 155.5, 162.0, 168.5.

*5.2.4. General procedure for the synthesis of **11a-f***

To a magnetically stirred mixture of ester **10** (21.52 mmol) in 50 ml of THF and 30 ml of water LiOH·$H_2O$ (0.95 g, 22.64 mmol) was added. After 5 hr at room temperature, the resulting clear solution was acidified with dilute HCl to precipitate the product. After filtration the product was obtained as a white crystalline solid, which was used in subsequent steps without further purification. An analytically pure sample was recrystallized from *i*-PrOH/hexane mixture.

*5.2.4.1. 2-(2-((3-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetic acid (**11a**)*

Yield 89%; white crystals; mp 220.5-221.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.13 (3 H, s, $NCH_3$), 4.75 (2 H, s, $OCH_2$), 4.89 (2 H, s, $NCH_2$), 5.64 (1 H, d, *J*=7.8 Hz, H-5), 6.89 - 7.02 (2 H, m, H-5', H-6'), 7.17 - 7.33 (2 H, m, H-3', H-4'), 7.84 (1 H, d, *J*=8.1 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 27.3, 39.1, 39.3, 39.5, 39.7, 39.9, 48.1, 64.8, 99.6, 112.0, 121.0, 124.2, 129.3, 130.1, 144.7, 151.3, 155.7, 162.7, 170.1.

*5.2.4.2. 2-(2-((2,4-Dioxo-3-propyl-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetic acid (**11b**)*

Yield 94%; white crystals; mp 187-188 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 0.83 (3 H, t, *J*=7.5 Hz, $CH_3$), 1.51 (2 H, sxt, *J*=7.5 Hz, $CH_2$), 3.74 (2 H, t, *J*=7.5 Hz, $NCH_2$), 4.76 (2 H, s, $OCH_2$), 4.91 (2 H, s, $NCH_2$), 5.64 (1 H, d, *J*=7.8 Hz, H-5), 6.92 - 7.00 (2 H, m, H-5', H-6'), 7.21 (1 H, d, *J*=7.4 Hz, H-3'), 7.25 - 7.31 (1 H, m, H-4'), 7.82 (1 H, d, *J*=7.9 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 15.3, 24.6, 43.4, 43.5, 43.6, 43.8, 43.9, 44.0, 44.2, 46.0, 52.0, 69.1, 104.0, 116.2, 125.2, 128.5, 133.5, 134.1, 148.9, 159.9, 166.6, 174.2.

*5.2.4.3. 2-(2-((3-Allyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetic acid (**11c**)*

Yield 90%; white crystals; mp 183.5-185°C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 4.37 (2 H, dt, *J*=5.3, 1.5 Hz, $NCH_2$), 4.74 (2 H, s, $OCH_2$), 4.90 (2 H, s, $NCH_2$), 4.99 - 5.11 (2 H, m, =$CH_2$), 5.65 (1 H, d, *J*=7.9 Hz, H-5), 5.79 (1 H, ddt, *J*=17.1, 10.4, 5.3, 5.3 Hz, -CH=), 6.90 - 7.00 (2 H, m, H-5', H-6'), 7.20 (1 H, dd, *J*=7.8, 1.6 Hz, H-3'), 7.28 (1 H, td, *J*=7.8, 1.7 Hz, H-4'), 7.83 (1 H, d, *J*=7.9 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 39.2, 39.4, 39.6, 39.8, 40.0, 42.3, 48.0, 64.9, 99.8, 112.1, 116.5, 121.0, 124.2, 129.4, 129.9, 132.5, 145.0, 155.8, 170.1.

*5.2.4.4. 2-(2-((3,5-Dimethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetic acid (**11d**)*

Yield 94%; white crystals; mp 233.5-234.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.80 (3 H, d, *J*=1.1 Hz, $CH_3$), 3.16 (3 H, s, $NCH_3$), 4.75 (2 H, s, $OCH_2$), 4.86 (2 H, s, $NCH_2$), 6.91 - 6.98 (2 H, m, H-5', H-6'), 7.21 - 7.29 (2 H, m, H-3', H-4'), 7.78 (1 H, d, *J*=1.3 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 12.5, 27.4, 39.1, 39.3, 39.5, 39.7, 39.9, 47.5, 64.8, 107.2, 111.9, 120.9, 124.3, 129.2, 130.1, 140.6, 151.1, 155.6, 163.3, 170.0.

*5.2.4.5. 2-(2-((5-Fluoro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetic acid (**11e**)*

Yield 71%; white crystals; mp 220.5-222.5 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 3.15 (3 H, s, $NCH_3$), 4.74 (2 H, s, $NCH_2$), 4.85 (2 H, s, $OCH_2$), 6.89 - 7.01 (2 H, m, H-5', H-6'), 7.21 - 7.35 (2 H, m, H-3', H-4'), 8.25 (1 H, d, *J*=6.3 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 32.1, 43.1, 43.2, 43.4, 43.5, 43.6, 43.8, 43.9, 44.0, 52.7, 68.9, 116.0, 125.1, 127.9, 133.6, 133.7, 133.8, 134.8, 154.0, 159.9, 174.4.

*5.2.4.6. 2-(2-((5-Chloro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetic acid (**11f**)*

Yield 64%; white crystals; mp 209-210.5 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 3.20 (3 H, s, $NCH_3$), 4.76 (2 H, s, $NCH_2$), 4.92 (2 H, s, $OCH_2$), 6.92 - 7.02 (2 H, m, H-5', H-6'), 7.25 - 7.34 (2 H, m, H-3', H-4'), 8.33 (1 H, s, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 32.7, 43.4, 43.5, 43.6, 43.8, 43.9, 44.1, 44.2, 44.3, 52.8, 69.0, 109.6, 116.2, 125.2, 128.0, 133.7, 134.7, 146.3, 154.5, 159.9, 163.2.

*5.2.5. General procedure for the synthesis of **12a-k***

A mixture of **11** (5.68 mmol) and thionyl chloride (0.42 ml, 5.76 mmol) in 10 ml of anhydrous 1,2-dichloroethane was refluxed for 2 hr. with the exclusion of moisture. The volatile materials were evaporated under reduced pressure, and the residue dissolved in 10 ml of anhydrous 1,2-dichloroethane and cooled to -15 °C. To the magnetically stirred solution, the appropriate aniline (5.71 mmol) was added in one portion, followed by 3 ml of pyridine after 5 min. The reaction mixture was stirred for 2 hr. and left to warm to room temperature overnight. The reaction mixture was washed portions of dilute HCl, 2% aqueous NaOH solution, then water, and evaporated. The residual solid was purified by flash chromatography eluting with 1:10 EtOH/1,2-dichloroethane.

*5.2.5.1. 2-(2-((3-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)-N-phenylacetamide (**12a**)*

Yield 73%; white crystals; mp 145-146.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.14 (3 H, s, $NCH_3$), 4.78 (2 H, s, $OCH_2$), 5.00 (2 H, s, $NCH_2$), 5.68 (1 H, d, *J*=7.8 Hz, H-5), 6.94 - 7.04 (2 H, m, H-5', H-6'), 7.06 - 7.12 (1 H, m, H-4''), 7.24 - 7.30 (1 H, m, H-3'), 7.30 - 7.37 (3 H, m, H-4', H-3'', H-5''), 7.66 (2 H, d, *J*=7.6 Hz, H-2'', H-6''), 7.83 (1 H, d, *J*=7.8 Hz, H-6), 10.08 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 27.3, 39.1, 39.3, 39.5, 39.7, 39.9, 47.6, 67.3, 100.0, 112.1, 119.7, 121.2, 123.8, 124.3, 128.8, 129.5, 130.1, 138.3, 144.4, 151.5, 155.7, 162.6, 166.3. HRESIMS *m/z*: calculated for $C_{20}H_{19}N_3O_4$ $[M+H]^+$ 366.1448, found 366.1448; calculated for $C_{16}H_{12}Cl_2N_2O_4$ $[M+Na]^+$ 388.1268, found 388.1263.

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*5.2.5.2. 2-(2-((3-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)-N-(o-tolyl)acetamide (**12b**)*

Yield 10%; white crystals; mp 160-161.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.17 (3 H, s, $CH_3$), 3.11 (3 H, s, $NCH_3$), 4.80 (2 H, s, $OCH_2$), 5.02 (2 H, s, $NCH_2$), 5.68 (1 H, d, *J*=7.8 Hz, H-5), 6.98 (1 H, t, *J*=7.3 Hz, H-5'), 7.03 (1 H, d, *J*=8.3 Hz, H-6'), 7.08 - 7.15 (1 H, m, H-4''), 7.18 (1 H, d, *J*=7.8 Hz, H-3''), 7.23 (2 H, d, *J*=7.6 Hz, H-3', H-5''), 7.32 (1 H, t, *J*=7.7 Hz, H-4'), 7.41 (1 H, d, *J*=7.3 Hz, H-6''), 7.79 (1 H, d, *J*=7.8 Hz, H-6), 9.43 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 18.0, 27.6, 39.2, 39.5, 39.7, 39.9, 40.1, 40.3, 40.5, 47.8, 67.6, 100.3, 112.3, 121.5, 124.7, 125.7, 126.1, 126.4, 129.7, 130.0, 130.7, 135.8, 144.6, 151.8, 155.8, 162.9, 166.7. HRESIMS *m/z*: calculated for $C_{21}H_{21}N_3O_4$ $[M+H]^+$ 380.1605, found 380.1601; calculated for $C_{21}H_{21}N_3O_4$ $[M+Na]^+$ 402.1424, found 402.1420.

*5.2.5.3. N-(2-Chlorophenyl)-2-(2-((3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12c**)*

Yield 70%; white crystals; mp 139-140.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.13 (3 H, s, $NCH_3$) 4.78 (2 H, s, $CH_2$) 5.00 (2 H, s, $CH_2$) 5.67 (1 H, d, *J*=7.8 Hz, H-5) 6.95 - 7.03 (2 H, m) 7.24 - 7.34 (2 H, m) 7.36 - 7.43 (2 H, m) 7.66 - 7.73 (2 H, m) 7.82 (1 H, d, *J*=7.8 Hz, H-6) 10.20 (1 H, s, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 27.35, 39.10, 39.31, 39.52, 39.73, 39.94, 47.58, 67.32, 100.01, 112.12, 121.21, 121.28, 124.35, 127.41, 128.73, 129.47, 130.05, 137.29, 144.34, 151.46, 155.67, 162.58, 166.50.

*5.2.5.4. N-(3-Chlorophenyl)-2-(2-((3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12d**)*

Yield 80%; white crystals; mp 172.5-174 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.14 (3 H, s, $NCH_3$), 4.79 (2 H, s, $OCH_2$), 5.00 (2 H, s, $NCH_2$), 5.68 (1 H, d, *J*=7.8 Hz, H-5), 6.95 - 7.04 (2 H, m, H-5', H-6'), 7.12 - 7.18 (1 H, m, H-4''), 7.25 - 7.33 (2 H, m, H-3', H-4'), 7.36 (1 H, t, *J*=8.1 Hz, H-5''), 7.54 - 7.60 (1 H, m, H-6''), 7.81 (1 H, d, *J*=8.1 Hz, H-6), 7.86 (1 H, t, *J*=1.9 Hz, H-2''), 10.25 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 27.3, 39.1, 39.3, 39.5, 39.7, 39.9, 47.6, 67.3, 100.0, 112.1, 118.1, 119.2, 121.2, 123.5, 124.4, 129.5, 130.1, 130.5, 133.1, 139.8, 144.3, 151.5, 155.6, 162.6, 166.8. HRESIMS *m/z*: calculated for $C_{20}H_{18}ClN_3O_4$ $[M+H]^+$ 400.1059, found 400.1056; calculated for $C_{20}H_{18}ClN_3O_4$ $[M+Na]^+$ 422.0878, found 422.0877.

*5.2.5.5. N-(4-Chlorophenyl)-2-(2-((3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12e**)*

Yield 70%; white crystals; mp 209-210.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.13 (3 H, s, $NCH_3$), 4.77 (2 H, s, $OCH_2$), 4.99 (2 H, s, $NCH_2$), 5.66 (1 H, d, *J*=7.8 Hz, H-5), 6.90 - 7.05 (2 H, m, H-5', H-6'), 7.21 - 7.34 (2 H, m, H-3', H-4'), 7.38 (2 H, d, *J*=8.8 Hz, H-3'', H-5''), 7.68 (2 H, d, *J*=8.8 Hz, H-2'', H-6''), 7.80 (1 H, d, *J*=7.8 Hz, H-6), 10.17 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 27.6, 39.2, 39.5, 39.7, 39.9, 40.1, 40.3, 40.5, 47.9, 67.7, 100.3, 112.4, 121.5, 121.6, 124.7, 127.7, 129.0, 129.8, 130.3, 137.6, 144.6, 151.8, 156.0, 162.9, 166.8. HRESIMS *m/z*: calculated for $C_{20}H_{18}ClN_3O_4$ $[M+H]^+$ 400.1059, found 400.1049; calculated for $C_{20}H_{18}ClN_3O_4$ $[M+Na]^+$ 422.0878, found 422.0871.

*5.2.5.6. N-(2,5-Dichlorophenyl)-2-(2-((3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12f**)*

Yield 50%; white crystals; mp 162.5-164.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.12 (3 H, s, $NCH_3$), 4.88 (2 H, s, $OCH_2$), 5.01 (2 H, s, $NCH_2$), 5.68 (1 H, d, *J*=8.1 Hz, H-5), 6.98 (1 H, t, *J*=7.5 Hz, H-5'), 7.04 (1 H, d, *J*=8.3 Hz, H-6'), 7.18 - 7.25 (1 H, m, H-3'), 7.25 - 7.34 (2 H, m, H-4', H-4''), 7.55 (1 H, d, *J*=8.8 Hz, H-3''), 7.79 (1 H, d, *J*=7.8 Hz, H-6), 7.98 (1 H, d, *J*=2.3 Hz, H-6''), 9.69 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 27.6, 39.5, 39.7, 39.9, 40.1, 40.3, 47.9, 67.5, 100.3, 112.5, 121.7, 124.7, 124.9, 126.5, 129.7, 129.9, 131.2, 132.0, 135.7, 144.6, 151.7, 155.6, 162.9, 167.3. HRESIMS *m/z*: calculated for $C_{20}H_{17}Cl_2N_3O_4$ $[M+H]^+$ 434.0669, found 434.0658; calculated for $C_{20}H_{17}Cl_2N_3O_4$ $[M+Na]^+$ 456.0488, found 456.0484.

*5.2.5.7. N-(2-Chlorophenyl)-2-(2-((2,4-dioxo-3-propyl-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12g**)*

Yield 72%; white crystals; mp 135.5-137.5 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 0.79 (3 H, t, *J*=7.5 Hz, $CH_3$), 1.49 (2 H, sxt, *J*=7.4 Hz, $CH_2$), 3.72 (2 H, dd, *J*=7.9, 6.8 Hz, $CH_2$), 4.87 (2 H, s, $OCH_2$), 5.04 (2 H, s, $NCH_2$), 5.69 (1 H, dd, *J*=7.9, 0.9 Hz, H-5), 7.01 (1 H, t, *J*=7.5 Hz, H-5'), 7.08 (1 H, d, *J*=8.3 Hz, H-6'), 7.21 - 7.26 (2 H, m, H-3', H-5"), 7.34 (1 H, t, *J*=7.8 Hz, H-4'), 7.37 (1 H, t, *J*=7.7 Hz, H-4"), 7.53 (1 H, d, *J*=8.0 Hz, H-3"), 7.80 (1 H, d, *J*=7.9 Hz, H-6), 7.83 (1 H, d, *J*=8.1 Hz, H-6"), 9.62 (1 H, s, NH); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ ppm 15.3, 24.6, 43.4, 43.5, 43.7, 43.8, 43.9, 44.1, 44.2, 46.0, 51.6, 71.5, 104.5, 116.5, 125.6, 128.6, 130.9, 131.8, 133.6, 133.7, 133.8, 148.5, 166.5. HRESIMS *m/z*: calculated for $C_{22}H_{22}ClN_3O_4$ $[M+H]^+$ 428.1372, found 428.1364; calculated for $C_{22}H_{22}ClN_3O_4$ $[M+Na]^+$ 450.1191, found 450.1183.

*5.2.5.8. 2-(2-((3-Allyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)-N-(2-chlorophenyl)acetamide (**12h**)*

Yield 86%; white crystals; mp 102-103 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 4.38 (2 H, d, *J*=5.2 Hz, $NCH_2$), 4.78 (2 H, s, $OCH_2$), 4.97 - 5.09 (2 H, m, $=CH_2$), 5.02 (2 H, s, $NCH_2$), 5.70 (1 H, d, *J*=7.9 Hz, H-5), 5.79 (1 H, ddt, *J*=17.1, 10.4, 5.3, 5.3 Hz, -CH=), 6.99 (2 H, m, *J*=7.8, 7.8 Hz,H-4", H-5"), 7.15 (1 H, ddd, *J*=8.0, 2.1, 0.9 Hz, H-6'), 7.26 - 7.30 (1 H, m, H-5'), 7.31 (1 H, t, *J*=7.0 Hz, H-4'), 7.36 (1 H, t, *J*=8.1 Hz, H-3"), 7.57 (1 H, dt, *J*=7.3, 1.0 Hz, H-3'), 7.81 (1 H, d, *J*=7.9 Hz, H-6), 7.86 (1 H, t, *J*=2.1 Hz, H-6"), 10.19 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 39.4, 39.6, 39.8, 42.4, 47.5, 67.4, 100.3, 112.3, 116.5, 118.2, 119.3, 121.3, 123.6, 124.4, 129.6, 130.1, 130.5, 132.4, 139.8, 144.6, 155.7, 166.7. HRESIMS *m/z*: calculated for $C_{22}H_{20}ClN_3O_4$ $[M+H]^+$ 426.1215, found 426.1204; calculated for $C_{22}H_{20}ClN_3O_4$ $[M+Na]^+$ 448.1035, found 448.1024.

*5.2.5.9. N-(2-Chlorophenyl)-2-(2-((3,5-dimethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12i**)*

Yield 82%; white crystals; mp 139-140.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.79 (3 H, d, *J*=0.8 Hz, $CH_3$), 3.15 (3 H, s, $NCH_3$), 4.87 (2 H, s, $OCH_2$), 5.01 (2 H, s, $NCH_2$), 6.99 (1 H, t, *J*=7.4 Hz, H-5'), 7.06 (1 H, d, *J*=8.3 Hz, H-6'), 7.19 - 7.26 (2 H, m, H-3', H-4'), 7.31 (1 H, td, *J*=7.8, 1.4 Hz, H-4''), 7.37 (1 H, td, *J*=7.8, 1.4 Hz, H-5''), 7.52 (1 H, dd, *J*=7.9, 1.4 Hz, H-3''), 7.71 (1 H, d, *J*=1.0 Hz, H-6), 7.85 (1 H, dd, *J*=8.0, 1.5 Hz, H-6''), 9.61 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 12.5, 27.5, 39.1, 39.3, 39.5, 39.7, 39.9, 47.0, 67.2, 107.7, 112.1, 121.3, 124.5, 125.6, 126.6, 127.5, 129.2, 129.5, 134.0, 140.1, 151.2, 155.2, 163.2, 166.6. HRESIMS *m/z*: calculated for $C_{21}H_{20}ClN_3O_4$ $[M+H]^+$ 414.1215, found 414.1209; calculated for $C_{21}H_{20}ClN_3O_4$ $[M+Na]^+$ 436.1035, found 436.1026.

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*5.2.5.10. N-(2-Chlorophenyl)-2-(2-((5-fluoro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)acetamide (**12j**)*

Yield 56%; white crystals; mp 156-158 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 3.15 (3 H, s, $NCH_3$), 4.86 (2 H, s, $OCH_2$), 4.98 (2 H, s, $NCH_2$), 6.98 (1 H, t, *J*=7.5 Hz, H-5'), 7.04 (1 H, d, *J*=8.2 Hz, H-6'), 7.22 (1 H, t, *J*=7.7 Hz, H-4"), 7.28 (1 H, d, *J*=7.5 Hz, H-3'), 7.32 (1 H, t, *J*=7.8 Hz, H-4'), 7.35 (1 H, t, *J*=7.8 Hz, H-5"), 7.50 (1 H, dd, *J*=8.0, 1.3 Hz, H-3"), 7.80 (1 H, d, *J*=8.0 Hz, H-6), 8.23 (1 H, d, *J*=6.4 Hz, H-6"), 9.66 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 32.2, 43.1, 43.3, 43.4, 43.5, 43.7, 43.8, 43.9, 52.2, 71.3, 116.2, 125.5, 128.1, 130.0, 131.0, 131.8, 133.7, 134.2, 138.2, 154.1, 159.6, 171.0. HRESIMS *m/z*: calculated for $C_{20}H_{17}ClFN_3O_4$ $[M+H]^+$ 418.0964, found 418.0956; calculated for $C_{20}H_{17}ClFN_3O_4$ $[M+Na]^+$ 440.0784, found 440.0776.

*5.2.5.11. 2-(2-((5-Chloro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)phenoxy)-N-(2-chlorophenyl)acetamide (**12k**)*

Yield 58%; white crystals; mp 169-171 °C; $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 3.20 (3 H, s, $NCH_3$), 4.88 (2 H, s, $OCH_2$), 5.06 (2 H, s, $NCH_2$), 7.00 (1 H, t, *J*=7.5 Hz, H-5'), 7.07 (1 H, d, *J*=8.3 Hz, H-6'), 7.24 (1 H, t, *J*=7.8 Hz, H-4"), 7.29 (1 H, d, *J*=7.3 Hz, H-3'), 7.34 (1 H, t, *J*=7.8 Hz, H-4'), 7.38 (1 H, t, *J*=7.7 Hz, H-5"), 7.53 (1 H, dd, *J*=8.0, 1.1 Hz, H-3"), 7.86 (1 H, d, *J*=8.2 Hz, H-6"), 8.30 (1 H, s, H-6), 9.62 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 32.8, 43.4, 43.5, 43.7, 43.8, 43.9, 44.1, 44.2, 52.3, 71.5, 110.0, 116.4, 125.6, 128.3, 129.9, 130.9, 131.8, 133.7, 133.7, 133.9, 138.3, 145.9, 154.6, 163.2, 170.9. HRESIMS *m/z*: calculated for $C_{20}H_{17}Cl_2N_3O_4$ $[M+H]^+$ 434.0669, found 434.0658; calculated for $C_{20}H_{17}Cl_2N_3O_4$ $[M+Na]^+$ 456.0488, found 456.0483.

*5.2.6. Ethyl 2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**13**)*

A mixture of uracil (6.00 g, 53.53 mmol) and ammonium chloride (0.3 g, 5.60 mmol) in HMDS (15 mL) was refluxed for 10 hr with the exclusion of moisture until a clear solution was obtained. The excess of silylating agent was removed under vacuum. The residual clear oil of 2,4-bis(trimethylsilyloxy)pyrimidine was dissolved in 25 ml of anhydrous 1,2-dichloroethane, and ethyl bromoacetate (5.92 ml, 53.53 mmol) was added. The reaction mixture was heated at reflux for 20 hr, cooled to room temperature and treated with 15 ml of $^i$PrOH. The resulting precipitate was collected and purified by flash chromatography eluting with 1:10 EtOH/1,2-dichloroethane to give **13** (9.45 g, 92%) as white crystals, mp °C, $R_f$ (); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 1.21 (3 H, t, *J*=7.1 Hz, $CH_3$), 4.15 (2 H, q, *J*=7.1 Hz, $CH_2$), 4.51 (2 H, s, $NCH_2$), 5.62 (1 H, d, *J*=7.8 Hz, H-5), 7.62 (1 H, d, *J*=7.8 Hz, H-6), 11.40 (1 H, br. s., H-3); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 14.0, 39.1, 39.3, 39.5, 39.7, 39.9, 48.6, 61.2, 101.1, 145.9, 151.0, 163.8, 168.2.

*5.2.7. General procedure for the synthesis of **14a-d***

A suspension of **13** (0.85 g, 3.66 mmol) and $K_2CO_3$ (0.6 g, 4.34 mmol) in anhydrous DMF (8 mL) was stirred at 80 °C for 1 hr. A solution of the appropriate benzyl bromide (3.82 mmol) in 5 ml of DMF was added and the mixture stirred at room temperature for 24 hr. The reaction mixture was filtered and the filtrate evaporated. The residual oil was dissolved in 50 ml of EtOH, then 30 ml of water and $LiOH{\cdot}H_2O$ (0.95 g, 22.64 mmol) was added. The mixture was magnetically stirred for 5 hr at room temperature and the resulting clear solution was acidified with dilute HCl to precipitate the product. After filtration the resulting acids were obtained as white crystalline solids, which were used in subsequent steps without further purification. Analytical samples were recrystallized from *i*-PrOH/hexane mixture.

*5.2.7.1. 2-(3-(3,5-Dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**14a**)*

Yield 78%, white crystals; mp: 211-212 °C, $R_f$ 0.51 ($^i$PrOH/EtOAc/$NH_4OH_{aq.}$ 9:6:5); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.21 (6 H, s, $CH_3$), 4.50 (2 H, s, $NCH_2$), 4.91 (2 H, s, $NCH_2$), 5.79 (1 H, d, *J*=7.8 Hz, H-5), 6.85 (3 H, s, H-2',4',6'), 7.71 (1 H, d, *J*=7.9 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.9, 39.0, 39.2, 39.5, 39.7, 39.9, 43.3, 49.8, 100.2, 125.2, 128.6, 136.9, 137.3, 144.8, 151.3, 162.4, 169.5.

*5.2.7.2. 2-(3-(3,5-Dichlorobenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**14b**)*

Yield 71%; white crystals; mp 226-228 °C; $R_f$ 0.55 ($^i$PrOH/EtOAc/$NH_4OH_{aq.}$ 9:6:5); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 4.49 (2 H, s, $NCH_2$), 4.96 (2 H, s, $NCH_2$), 5.80 (1 H, d, *J*=7.8 Hz, H-5), 7.25 (2 H, d, *J*=1.7 Hz, H-2', H-6'), 7.46 (1 H, d, *J*=1.7 Hz, H-4'), 7.71 (1 H, d, *J*=7.8 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 39.2, 39.4, 39.6, 39.8, 40.0, 42.9, 50.2, 100.5, 126.5, 127.3, 134.3, 141.4, 145.5, 162.7, 169.7.

*5.2.7.3. 2-(3-(3-Bromo-5-methylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**14c**)*

Yield 87%; white crystals; mp 227-229°C; Rf 0.52 ($^i$PrOH/EtOAc/$NH_4OH_{aq.}$ 9:6:5); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.24 (3 H, s, $CH_3$), 4.48 (2 H, s, $NCH_2$), 4.92 (2 H, s, $NCH_2$), 5.79 (1 H, d, *J*=7.8 Hz, H-5), 7.04 (1 H, s, H-6'), 7.19 (1 H, s, H-2'), 7.27 (1 H, s, H-4'), 7.70 (1 H, d, *J*=8.1 Hz, H-6); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.9, 39.2, 39.4, 39.6, 39.8, 40.1, 43.1, 50.2, 100.5, 121.7, 127.5, 130.8, 139.7, 140.7, 145.4, 169.7.

*5.2.7.4. 2-(3-(Naphthalen-1-ylmethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**14d**)*

Yield 72%; white crystals; mp 200-203 °C; $R_f$ 0.56 ($^i$PrOH/EtOAc/$NH_4OH_{aq.}$ 9:6:5); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 4.52 (2 H, s, $NCH_2$), 5.47 (2 H, s, $NCH_2$), 5.87 (1 H, d, *J*=7.8 Hz, H-5), 7.00 (1 H, d, *J*=7.1 Hz, H-2'), 7.39 (1 H, t, *J*=7.7 Hz, H-3'), 7.51 - 7.64 (2 H, m, H-6',7'), 7.79 (1 H, d, *J*=7.8 Hz, H-6), 7.82 (1 H, d, *J*=8.3 Hz, H-4'), 7.95 (1 H, d, *J*=8.3 Hz, H-5'), 8.19 (1 H, d, *J*=8.3 Hz, H-8'); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 39.2, 39.4, 39.6, 39.9, 40.1, 40.3, 41.6, 50.2, 100.5, 122.3, 123.4, 125.6, 126.3, 126.7, 127.6, 128.9, 132.1, 145.4, 169.7.

*5.2.8. General procedure for the synthesis of **15a-d**, **f**, **g**, **i-k***

A mixture of **14** (5.68 mmol) and thionyl chloride (0.42 ml, 5.76 mmol) in 10 ml of anhydrous 1,2-dichloroethane was refluxed for 2 hr with the exclusion of moisture. The volatile materials were evaporated under reduced pressure and the residue dissolved in 10 ml of anhydrous 1,2-dichloroethane and cooled to -15 °C. To the magnetically stirred solution the appropriate aniline (5.71 mmol) was added in one portion, followed by 3 ml of pyridine after 5 min. The reaction mixture was stirred for 2 hr and left to warm to room temperature overnight. The reaction mixture was then washed with dilute HCl, 2% aqueous NaOH solution, water and then evaporated. The product was purified by flash chromatography eluting with 1:10 EtOH/1,2-dichloroethane.

*5.2.8.1. 2-(3-(3,5-Dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-phenylacetamide (**15a**)*

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Yield 78%; white crystals; mp 201-202 °C; $R_f$ 0.59 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.22 (6 H, s, $CH_3$), 4.63 (2 H, s, $NCH_2$), 4.92 (2 H, s, $NCH_2$), 5.81 (1 H, d, *J*=7.8 Hz, H-5), 6.87 (3 H, br. s. , H-2',4',6'), 7.07 (1 H, t, *J*=7.3 Hz, H-4"), 7.32 (2 H, t, *J*=7.8 Hz, H-3",5"), 7.59 (2 H, d, *J*=7.9 Hz, H-2",6"), 7.73 (1 H, d, *J*=7.8 Hz, H-6), 10.33 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.9, 38.9, 39.1, 39.3, 39.5, 39.7, 39.9, 40.1, 43.3, 51.4, 99.9, 119.1, 123.6, 125.3, 128.6, 128.9, 136.9, 137.3, 138.6, 145.5, 162.6, 165.5. HRESIMS *m/z*: calculated for $C_{21}H_{21}N_3O_3$ $[M+Na]^+$ 386.1475, found 386.1468.

*5.2.8.2. 2-(3-(3,5-Dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(o-tolyl)acetamide* ***(15b)***

Yield 69%; white crystals; mp 231.5-233 °C; $R_f$ 0.59 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.20 (9 H, br. s., $CH_3$), 4.64 (2 H, br. s., $NCH_2$), 4.91 (2 H, br. s., $NCH_2$), 5.77 (1 H, dt, *J*=7.8, 2.0 Hz, H-5), 6.86 (3 H, br. s. , H-2',4',6'), 7.03 - 7.26 (3 H, m, H-3",4",5"), 7.37 (1 H, d, *J*=7.1 Hz, H-6"), 7.70 (1 H, dd, *J*=5.3, 2.3 Hz, H-6), 9.64 (1 H, br. s., NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) 18.1, 21.2, 39.2, 39.5, 39.7, 39.9, 40.1, 40.3, 43.6, 51.6, 100.2, 125.2, 125.6, 125.8, 126.3, 128.9, 130.7, 132.1, 135.9, 137.2, 137.6, 145.8, 151.7, 162.9, 166.0. HRESIMS *m/z*: calculated for $C_{22}H_{23}N_3O_3$ $[M+Na]^+$ 400.1632, found 400.1623; calculated for $C_{22}H_{23}N_3O_3$ $[M+K]^+$ 416.1371, found 416.1366.

*5.2.8.3. 2-(3-(3,5-Dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(2-methoxyphenyl)acetamide* ***(15c)***

Yield 62%; white crystals; mp 197-199 °C; $R_f$ 0.63 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.22 (6 H, s, $CH_3$), 3.39 (3 H, s, $OCH_3$), 4.73 (2 H, s, $NCH_2$), 4.92 (2 H, s, $NCH_2$), 5.80 (1 H, d, *J*=7.8 Hz, H-5), 6.84 - 6.95 (4 H, m, H-2',4',6', 3"), 7.02 - 7.15 (2 H, m, H-4", 6"), 7.73 (1 H, d, *J*=7.9 Hz, H-6), 7.98 (1 H, d, *J*=7.7 Hz, H-5"), 9.64 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.9, 39.1, 39.3, 39.5, 39.7, 39.9, 43.3, 51.5, 55.7, 99.9, 111.2, 120.3, 121.5, 124.6, 125.3, 126.9, 128.6, 136.9, 137.3, 145.5, 151.4, 162.6, 165.8. HRESIMS *m/z*: calculated for $C_{22}H_{23}N_3O_4$ $[M+H]^+$ 394.1761, found 394.1765; calculated for $C_{22}H_{23}N_3O_4$ $[M+Na]^+$ 416.1581, found 416.1575.

*5.2.8.4. N-(2-Chlorophenyl)-2-(3-(3,5-dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide* ***(15d)***

Yield 44%; white crystals; mp 222.5-224 °C; $R_f$ 0.50 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.22 (6 H, s, $CH_3$), 4.74 (2 H, s, $NCH_2$), 4.92 (2 H, s, $NCH_2$), 5.81 (1 H, d, *J*=7.8 Hz, H-5), 6.82 - 6.92 (3 H, m, H-2',4',6'), 7.20 (1 H, td, *J*=7.8, 1.5 Hz, H-3"), 7.33 (1 H, td, *J*=7.8, 1.5 Hz, H-4"), 7.51 (1 H, dd, *J*=8.0, 1.4 Hz, H-"6), 7.75 (1 H, d, *J*=7.8 Hz, H-6), 7.76 (1 H, br. dd, *J*=7.8, 1.5 Hz, H-5"), 9.95 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.9, 39.1, 39.3, 39.5, 39.7, 40.0, 43.3, 51.3, 100.0, 125.3, 125.7, 126.4, 127.5, 128.6, 129.6, 134.4, 136.9, 137.3, 145.4, 151.4, 162.5, 166.3. HRESIMS *m/z*: calculated for $C_{21}H_{20}ClN_3O_3$ $[M+H]^+$ 398.1266, found 398.1256; calculated for $C_{21}H_{20}ClN_3O_3$ $[M+Na]^+$ 420.1085, found 420.1080.

*5.2.8.5. N-(4-Chlorophenyl)-2-(3-(3,5-dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide* ***(15f)***

Yield 88%; white crystals; mp 247-248.5 °C; $R_f$ 0.51 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.21 (6 H, br. s, $CH_3$), 4.62 (2 H, s, $NCH_2$), 4.91 (2 H, s, $NCH_2$), 5.80 (1 H, d, *J*=7.9 Hz, H-5), 6.82 - 6.89 (3 H, m, H-2',4',6'), 7.37 (2 H, d, *J*=8.9 Hz, H-3", 5"), 7.61 (2 H, d, *J*=8.9 Hz, H-2", 6"), 7.71 (1 H, d, *J*=7.8 Hz, H-6), 10.44 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.8, 39.1, 39.3, 39.5, 39.7, 39.9, 43.2, 51.4, 99.9, 120.6, 125.2, 127.1, 128.5, 128.7, 136.8, 137.2, 137.5, 145.3, 151.3, 162.4, 165.6. HRESIMS *m/z*: calculated for $C_{21}H_{20}ClN_3O_3$ $[M+Na]^+$ 420.1085, found 420.1078.

*5.2.8.6. 2-(3-(3,5-Dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(2,3-dimethylphenyl)acetamide* ***(15g)***

Yield 34%; white crystals; mp 222-224 °C; $R_f$ 0.51 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.07 (3 H, s, $CH_3$), 2.20 (6 H, s, $CH_3$), 2.23 (3 H, s, $CH_3$), 4.63 (2 H, s, $NCH_2$), 4.91 (2 H, s, $NCH_2$), 5.77 (1 H, d, *J*=7.8 Hz, H-5), 6.86 (3 H, br. s. , H-2',4',6'), 7.03 (2 H, q, *J*=7.7 Hz, H-4",H-5"), 7.11 (1 H, d, *J*=7.3 Hz, H-6"), 7.71 (1 H, d, *J*=7.8 Hz, H-6), 9.74 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 14.3, 20.4, 21.2, 39.2, 39.4, 39.7, 39.9, 40.1, 43.6, 51.6, 100.2, 123.6, 125.6, 127.5, 128.9, 137.2, 137.4, 137.6, 145.8, 162.9, 166.1. HRESIMS *m/z*: calculated for $C_{23}H_{25}N_3O_3$ $[M+H]^+$ 392.1969, found 392.1964; calculated for $C_{23}H_{25}N_3O_3$ $[M+Na]^+$ 414.1788, found 414.1780.

*5.2.8.7. N-(2-Chlorophenyl)-2-(3-(3,5-dichlorobenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide* ***(15i)***

Yield 68%; white crystals; mp 214-216 °C; $R_f$ 0.68 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 4.72 (2 H, br. s., $NCH_2$), 4.98 (2 H, s, $NCH_2$), 5.81 (1 H, d, *J*=8.1 Hz, H-5), 7.18 (1 H, t, *J*=7.8 Hz, H-4"), 7.28 (2 H, s, H-2',6'), 7.31 (1 H, t, *J*=7.7 Hz, H-5"), 7.45 (1 H, s, H-4'), 7.48 (1 H, d, *J*=8.1 Hz, H-2"), 7.73 (1 H, d, *J*=8.1 Hz, H-6), 7.75 (1 H, br. d, *J*=7.8 Hz, H-6"), 9.94 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) 39.3, 39.5, 39.7, 39.9, 40.1, 43.0, 51.7, 100.3, 126.0, 126.6, 126.8, 127.3, 127.8, 129.9, 134.3, 134.6, 141.5, 146.1, 151.7, 162.8. HRESIMS *m/z*: calculated for $C_{19}H_{14}Cl_3N_3O_3$ $[M+ Na]^+$ 459.9993, found 459.9979.

*5.2.8.8. 2-(3-(3-Bromo-5-methylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(2-chlorophenyl)acetamide* ***(15j)***

Yield 32%; white crystals; mp 216-217.5 °C; $R_f$ 0.71 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.24 (3 H, s, $CH_3$), 4.71 (2 H, br. s., $NCH_2$), 4.93 (2 H, s, $NCH_2$), 5.80 (1 H, d, *J*=7.8 Hz, H-5), 7.07 (1 H, s, H-6'), 7.19 (1 H, t, *J*=7.7 Hz, H-4"), 7.22 (1 H, br. s. , H-2'), 7.27 (1 H, s, H-4'), 7.31 (1 H, t, *J*=7.7 Hz, H-5"), 7.49 (1 H, d, *J*=7.8 Hz, H-3"), 7.73 (1 H, d, *J*=7.8 Hz, H-6), 7.73 (1 H, d, *J*=7.8 Hz, H-6"), 9.94 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 20.9, 39.0, 39.3, 39.5, 39.7, 39.9, 40.1, 40.3, 43.2, 51.6, 100.3, 121.7, 126.0, 126.8, 127.6, 127.8, 129.9, 130.8, 134.6, 139.7, 140.7, 145.9, 151.7, 162.9, 166.5. HRESIMS *m/z*: calculated for $C_{20}H_{17}BrClN_3O_3$ $[M+ H]^+$ 462.0215, found 462.0221.

*5.2.8.9. N-(2-Chlorophenyl)-2-(3-(naphthalen-1-ylmethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide* ***(15k)***

Yield 74%; white crystals; mp 225-227 °C; $R_f$ 0.68 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 4.75 (2 H, s, $NCH_2$), 5.48 (2 H, s, $NCH_2$), 5.88 (1 H, d, *J*=7.8 Hz, H-5), 7.02 (1 H, d, *J*=7.1 Hz, H-2'), 7.14 - 7.25 (1 H, m, H-3"), 7.32 (1 H, t, *J*=7.7 Hz, H-4"), 7.40 (1 H, t, *J*=7.7 Hz, H-3'), 7.49 (1 H, d, *J*=8.1 Hz, H-6"), 7.52 - 7.64 (2 H, m, H-6',H-7'), 7.72 (1 H, d, *J*=7.8 Hz, H-5"), 7.81 (1 H, d, *J*=8.1 Hz, H-4'), 7.82 (1 H, d, *J*=7.8 Hz, H-6), 7.95 (1 H, d, *J*=7.8 Hz, H-5'), 8.19 (1 H, d, *J*=8.1 Hz, H-8'), 9.96 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 39.0, 39.2,

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39.5, 39.7, 39.9, 40.1, 40.3, 41.7, 51.6, 100.3, 122.3, 123.4, 125.7, 126.1, 126.3, 126.7, 127.6, 127.8, 128.9, 129.9, 130.7, 132.1, 133.5, 134.6, 146.0, 163.0, 166.5. HRESIMS *m/z*: calculated for $C_{23}H_{18}ClN_3O_3$ $[M+H]^+$ 420.1109, found 420.1101; calculated for $C_{23}H_{18}ClN_3O_3$ $[M+Na]^+$ 442.0929 found 442.0920.

*5.2.9. General procedure for the synthesis of **15e**, **h***

To a mixture of **14a** (1.64 g, 5.68 mmol) and boric acid (0.07 g, 1.13 mmol) in 50 ml of toluene, the appropriate amine (5.71 mmol) was added in one portion. Heating at reflux was continued for 20 hr with water being azeotropically removed using a Dean-Stark trap. After cooling, the reaction mixture was concentrated, taken up in 1,2-dichloroethane, washed with dilute HCl, 2% aqueous NaOH solution, water and evaporated. The product was purified by flash chromatography eluting with 1:10 EtOH/1,2-dichloroethane.

*5.2.9.1. N-(3-Chlorophenyl)-2-(3-(3,5-dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**15e**)*

Yield 48%; white crystals; mp 214-215.5 °C; $R_f$ 0.69 (EtOAc); $^1$H NMR (600 MHz, DMSO-$d_6$) δ ppm 2.22 (6 H, s, $CH_3$), 4.64 (2 H, s, $NCH_2$), 4.92 (2 H, s, $NCH_2$), 5.81 (1 H, d, *J*=7.8 Hz, H-5), 6.87 (3 H, s, H-2',4',6'), 7.14 (1 H, d, *J*=7.9 Hz, H-4"), 7.36 (1 H, t, *J*=8.0 Hz, H-5"), 7.44 (1 H, d, *J*=8.0 Hz, H-6"), 7.73 (1 H, d, *J*=7.9 Hz, H-6), 7.81 (1 H, s, H-2"), 10.52 (1 H, s, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 25.1, 43.4, 43.5, 43.7, 43.8, 43.9, 44.1, 44.2, 47.5, 55.7, 104.2, 121.7, 122.9, 127.5, 129.5, 132.8, 134.8, 137.4, 141.1, 141.4, 144.2, 149.6, 155.6, 166.7, 170.2. HRESIMS *m/z*: calculated for $C_{22}H_{23}N_3O_3$ $[M+H]^+$ 378.1812, found 378.1812; calculated for $C_{22}H_{23}N_3O_3$ $[M+Na]^+$ 400.1632, found 400.1624.

*5.2.9.2. N-Benzyl-2-(3-(3,5-dimethylbenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**15h**)*

Yield 50%; white crystals; mp 184-185 °C; $R_f$ 0.69 (EtOAc); $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.20 (6 H, s, $CH_3$), 4.32 (2 H, d, *J*=5.6 Hz, $CH_2$), 4.47 (2 H, s, $NCH_2$), 4.90 (2 H, s, $NCH_2$), 5.76 (1 H, dd, *J*=7.9, 1.8 Hz, H-5), 6.79 - 6.94 (3 H, m, H-2',4',6'), 7.17 - 7.39 (5 H, m, H-2", 3", 4", 5", 6"), 7.68 (1 H, d, *J*=7.8 Hz, H-6), 8.70 (1 H, t, *J*=5.4 Hz, NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 21.2, 39.4, 39.6, 39.8, 40.0, 40.3, 42.6, 43.6, 51.2, 100.2, 125.7, 127.2, 127.6, 128.6, 128.9, 137.3, 137.5, 139.3, 145.8, 151.7, 162.9, 167.1. HRESIMS *m/z*: calculated for $C_{22}H_{23}N_3O_3$ $[M+H]^+$ 378.1812, found 378.1812; calculated for $C_{22}H_{23}N_3O_3$ $[M+Na]^+$ 400.1632, found 400.1624.

*5.3. Reverse transcriptase assay*

Recombinant wild type p66/p51 HIV-1 RT was expressed and purified as described by Auwerx et al.[39] The RT assay is performed with the EnzCheck Reverse Transcriptase Assay kit (Molecular Probes, Invitrogen), as described by the manufacturer. The assay is based on the dsDNA quantitation reagent PicoGreen. This reagent shows a pronounced increase in fluorescence signal upon binding to dsDNA or RNA-DNA heteroduplexes. Single-stranded nucleic acids generate only minor fluorescence signal enhancement when a sufficiently high dye:base pair ratio is applied.[40] This condition is met in this assay.

A poly(rA) template of approximately 350 bases long, and an oligo(dT)16 primer, are annealed in a molar ratio of 1:1.2 (60 min. at room temperature). Fifty-two ng of the RNA/DNA is brought into each well of a 96-well plate in a volume of 20 µl polymerization buffer (60 mM Tris-HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 100 µM dTTP, pH 8.1). Five µl of RT enzyme solution, diluted to a suitable concentration in enzyme dilution buffer (50 mM Tris-HCl, 20% glycerol, 2 mM DTT, pH 7.6), is added. The reactions are incubated at 25°C for 40 minutes and then stopped by the addition of EDTA (15 mM fc). Heteroduplexes are then detected by addition of PicoGreen. Signals are read using an excitation wavelength of 490 nm and emission detection at 523 nm using a spectrofluorometer (Safire2, Tecan). To test the activity of compounds against RT, 1 µl of compound in DMSO is added to each well before the addition of RT enzyme solution. Control wells without compound contain the same amount of DMSO. Results are expressed as relative fluorescence i.e. the fluorescence signal of the reaction mix with compound divided by the signal of the same reaction mix without compound.

Inhibitory activity of the compound **15i** towards a panel of RTs bearing the mutations L100I, K103N, V106A, Y181C, Y188Lor G190A or the double mutant K103N/Y181C was studied using radioactively-labeled [α-$^{32}$P]ATP substrate and an activated DNA as a template-primer complex according to the previously published procedures.[7]

*5.4. Time-of-addition Experiments*

Time-of-addition experiments were adapted from Pauwels et al.[41] and Daelemans et al.[42] Briefly, MT-4 cells were infected with HIV-1($III_B$) or the RT double mutant virus (K103N;Y181C) at an m.o.i. of 0.5. Following a 1 hour adsorption period cells were distributed in a 96-well tray at 45,000 cells/well and incubated at 37 °C. Test compounds were added at different times (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 24, and 25h) after infection. HIV-1 production was determined at 31 hr postinfection via a p24 enzyme-linked immunosorbent assay (Perkin Elmer, Brussels, Belgium). Dextran sulfate was used at 12.5 µM, AZT at 1.9 µM, nevirapine at 7.5 µM, Compounds **15a-k**, except for the inactive compound **15h** were added at their $CC_{50}$ concentration obtained in the MT-4/MTT assay.

*5.5. RnaseH assay*

The RNaseH assay was developed also employing the dsDNA quantitation reagent PicoGreen. A RNA/DNA heteroduplex is formed by annealing a 40 bases long RNA oligonucleotide (5'-CCAGCAGGAAACAGCUAUGACGAUCUGAGCCUGGGAG CU-3') and 120 bases long DNA oligonucleotide (5'-AGCTCCCAGGCTCAGATCGTCATAGCTGTTTCCTGCTGG CAGCTCCCAGGCTCAGATCGTCATAGCTGTTTCCTGCTG GCAGCTCCCAGGCTCAGATCGTCATAGCTGTTTCCTGCT GGC-3') in a 4:1 molar ratio.

An amount of 76 ng of the annealed complex is brought into each well of a 96-well plate in a volume of 20 µl RNaseH buffer (60 mM Tris-HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, *p*H 8.1) and 5 µl of suitably diluted RT solution is added. Reactions are stopped after 60 min. at 25 °C by the addition of EDTA (15 mM fc). PicoGreen is then added to measure the amount of heteroduplexes and thus the decrease in signal upon RNA hydrolysis in the presence of enzyme activity and signals are read as described for the reverse transcriptase assay. The results are expressed relative to the amount of heteroduplexes measured in a negative control sample without RT enzyme, after subtraction of the background signal obtained with only ssDNA.

To test compounds for activity against RNaseH, 1 µl of compound in DMSO is added to each well before the addition of RT enzyme solution. Control wells without compound contain the same amount of DMSO. Positive compounds were tested for autofluorescence in a separate test.

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*5.6. Molecular Modeling*

All protein structures followed similar preparation procedure with minimal user intervention. Cognate ligands, co-crystalized ions and solvent molecules were deleted. All hydrogen atoms were added. After computing Gasteiger charges non-polar hydrogen atoms were merged and all atoms were AutoDock-typed to prepare Vina PDBQT input file. Ligand structures were processed automatically. Docking simulations were performed with AutoDock Vina 1.1.2.[43] Cubic grid box centered on cognate ligand was adjusted for each complex to include the entire concave region around the ligand and the solvent accessible entrance of the pocket. Only top-score binding poses were used in subsequent analysis.

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**Supplementary Material**

Supplementary data (NMR spectra for all of the synthesized compounds) associated with this article can be found online.

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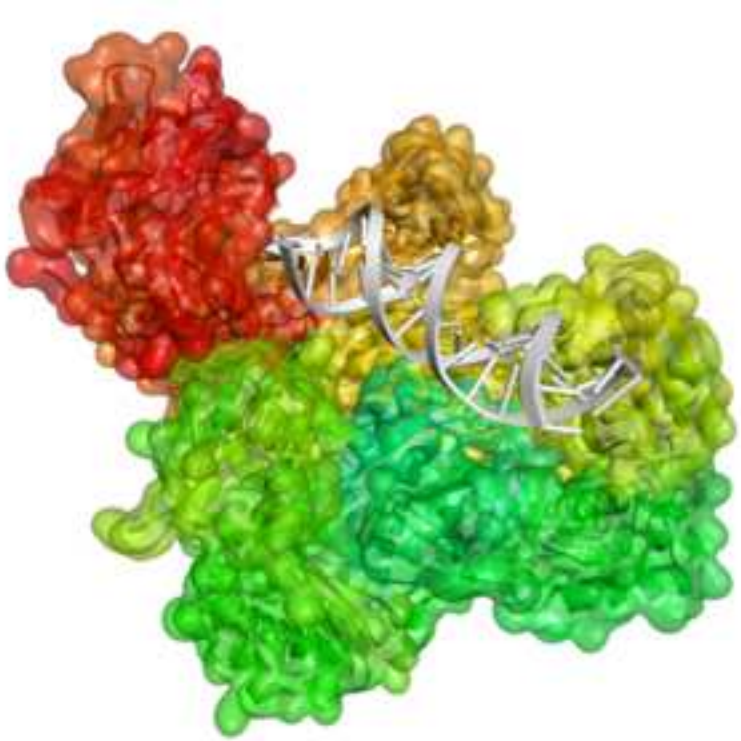

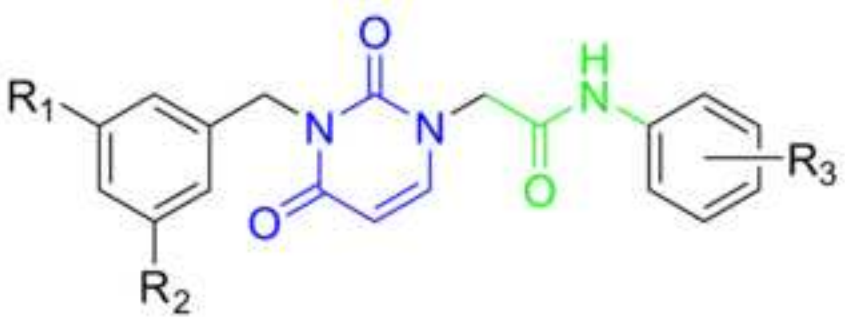


HIV-1 non-nucleoside reverse transcriptase inhibitors

$EC_{50}$ 1.7-35.8 μM

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