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1-[2-(2-Benzoyl- and 2-benzylphenoxy)ethyl]uracils as potent anti-HIV-1 agents

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ABSTRACT

Non-nucleoside reverse transcriptase inhibitors (NNRTI) are key components in highly active antiretroviral therapy for treating HIV-1. Herein we present the synthesis for a series of N1-alkylated uracil derivatives bearing ω-(2-benzyl- and 2-benzoylphenoxy)alkyl substituents as novel NNRTIs. These compounds displayed anti-HIV activity similar to that of nevirapine and several of them exhibited activity against the K103N/Y181C RT mutant HIV-1 strain. Further evaluation revealed that the inhibitors were active against most nevirapine-resistant mono- and di-substituted RTs with the exception of the V106A RT. Thus, the candidate compounds can be regarded as potential lead compounds against the wild-type virus and drug-resistant forms.

1. Introduction

A third decade of pandemic AIDS is underway, despite extensive efforts from scientists across the globe. In the USA alone, almost 1.6 million patients require antiretroviral therapy, while in many developing countries this number is an order of magnitude greater.[1] To date 25 antiretroviral drugs have been approved for the treatment of HIV infection using 'highly active antiretroviral therapy' or HAART.[2] These compounds inhibit HIV replication, however their use is often hampered by toxic side-effects over long periods of therapy, as well as a loss of activity when faced with emerging drug-resistant virus strains. As a result, the search for new therapeutics with minimal side-effects and the ability to retain activity against drug-resistant virus strains is regarded as a critical goal for medicinal chemists.

These anti-HIV drugs comprise six different classes of compounds: eight nucleoside (or nucleotide) reverse transcriptase (RT) inhibitors (NRTIs), four non-nucleoside RT inhibitors (NNRTIs), ten protease inhibitors (PIs), one integrase inhibitor (INI), one fusion inhibitor (FI) and one CCR5 inhibitor.[2] The first four classes of inhibitors target three different viral enzymes: HIV–RT, HIV protease and HIV integrase, respectively. NRTI and NNRTI are primary components of HAART.

One area of ongoing research in our laboratories has focused on the design and synthesis of NNRTIs. These compounds are highly specific, non-competitive inhibitors of HIV-1 RT, the key enzyme involved in HIV replication. Since 1996, five NNRTIs (shown in Fig. 1) have been approved by the FDA for clinical use: nevirapine (Viramune®, Boehringer Ingelheim), delavirdine (Rescriptor®, Pharmacia & UpJohn), and efavirenz (Sustiva®, DuPont) are classified as first generation NNRTIs, whereas etravirine (Intelence®, Tibotec) and rilpivirine (TMC278, Tibotec) are considered as second-generation NNRTIs, since they retain activity against a variety of drug-resistant virus strains.[3] NNRTIs have been reported to bind to a non-nucleoside inhibitor binding pocket in HIV-1 RT (NNIBP), which is located in the 'palm' domain of the p66 subunit at a distance of approximately 10 Å from the enzyme catalytic site.[4,5] The pocket is primarily hydrophobic and key amino acid residues involved in inhibitor binding include aromatic (Y181, Y188, F227, W229 and Y232), hydrophobic (P59, L100, V106, V179, F227, L234 and P236) and hydrophilic (K101, K103, S105, D132 and E224) residues in the p66 RT subunit, as well as three additional amino acids (I135, E138 and T139) in the p51 subunit.[3,6] One major limitation of many NNRTIs however, is the rapid emergence of HIV-1 variants resistant to these drugs due to one or more point mutations in the RT binding site.

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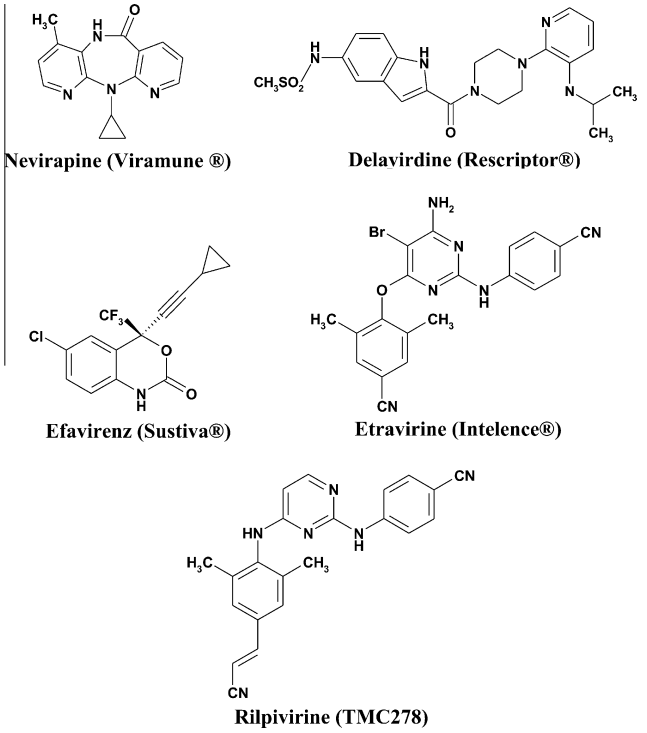


Figure 1. FDA-approved NNRTIs.

To date more than 50 different structural types of NNRTIs have been designed and described.[7–9] Several are currently regarded as potential anti-HIV drugs, either directly or following additional structural activity relationship (SAR) optimization of a lead compound. One such scaffold that has produced strong antiviral lead activity encompasses the uracil nucleobase (Fig. 2). Uracil analogues with various substituents in the C-6 position such as exemplified in scaffold **1** (Fig. 2) have proven to be fruitful leads for further development. Many of these analogues, for example, the 6-phenylthio-[10,11], 6-phenyl–selenyl-[12–14], 6-phenoxy-[15,16], 6-benzyl-[17–19], and 6-benzoyluracil-[15,16,20] derivatives such as those shown in Figure 2 have been shown to inhibit HIV replication in cell culture in nanomolar concentrations. However, the emergence of resistant virus strains[19] has hampered their clinical use.

Recently another promising NNRTI scaffold (**2**, Fig. 2) has been reported. Inhibitors of this structural type were found to be highly

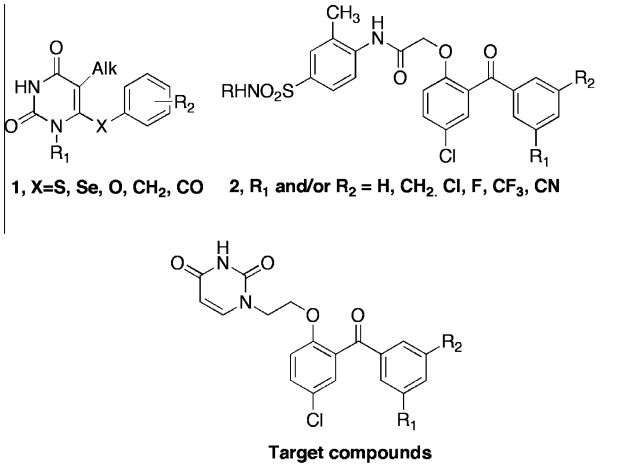


Figure 2. Uracil (**1**) and benzophenone (**2**) containing NNRTI scaffolds.

active against both wild-type HIV-1 and clinically significant drug-resistant forms.[21–23] This class of compounds is based on the benzophenone fragment bridged by a short linker to an ortho-methylaniline residue. SAR studies have further revealed that the substituents in the two meta-positions of the benzoyl moiety as well as the benzophenone fragment play crucial roles in retaining antiviral activity against mutant strains.

Thus, by combining these leads, 'chimeric' molecules containing the uracil residue such as that found in scaffold **1**, as well as the benzophenone fragment found in scaffold **2**, may not only inhibit HIV-1 RT, but also exhibit strong antiviral activity towards both wild-type HIV-1 and drug-resistant virus strains (Fig. 2). Herein we report the synthesis of N^1-alkylated uracil derivatives and the initial biological studies describing their antiviral activity.

2. Results and discussion

2.1. Chemistry

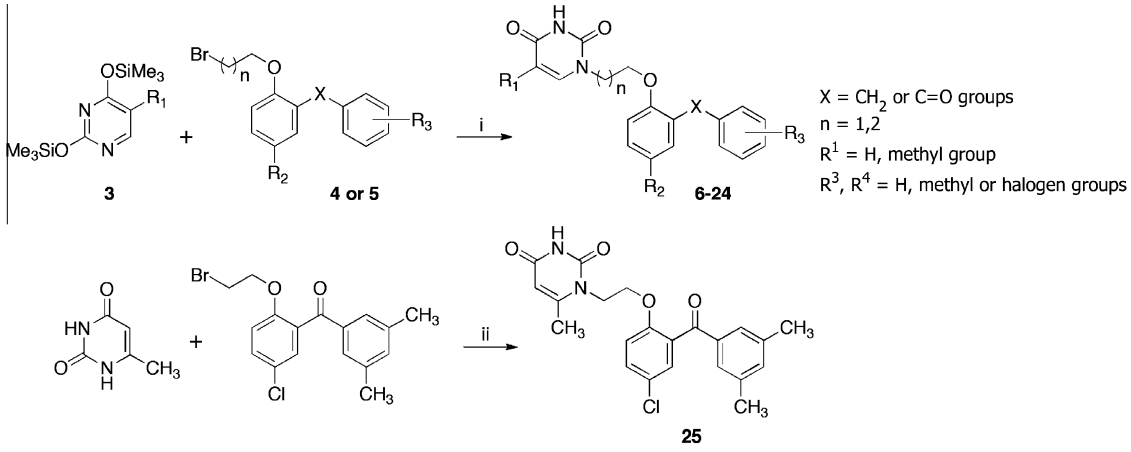
Synthesis of the target compounds was carried out by condensation of equimolar quantities 2,4-bis(trimethylsilyloxy)pyrimidine or -5-methylpyrimidine **3** with 1-bromo-ω-(2-benzylphenoxy)- **4** or 1-bromo-ω-(2-benzoylphenoxy)alkanes **5**. Starting bromides **4** and **5** were prepared according to previously described methods.[24] This synthetic approach has a key advantage: the reaction results in the N^1-substituted uracils as the only product. Formation of the N^3-substituted products was not observed, likely due to the steric hindrance of the adjacent trimethylsilyl groups blocking the approach to the N^3-nitrogen atom.

It is known that condensation of trimethylsilyl pyrimidines with highly reactive alkylating agents occurs in a facile manner in aprotic solvents under mild conditions and with high yields.[25,26] In contrast, treatment of 2,4-bis(trimethyl-silyloxy)pyrimidines with alkylating agents of lower reactivity requires elevated temperatures. Condensation of trimethylsilyl uracil derivatives with allylbromides[27,28] or benzylhalides[28] successfully proceeds in 1,2-dichloroethane in the presence of a catalytic amount of iodine. Heating 2,4-bis(trimethylsilyloxy)pyrimidines with an excess of alkyl halide leads to 1-substituted uracils with good yields.[29,30] Previously we have reported a method of synthesis of 1-[2-(phenoxy)ethyl]-uracils which was based on condensation of trimethylsilyl uracil derivatives with 1-bromo-2-(phenoxy)ethanes.[31]

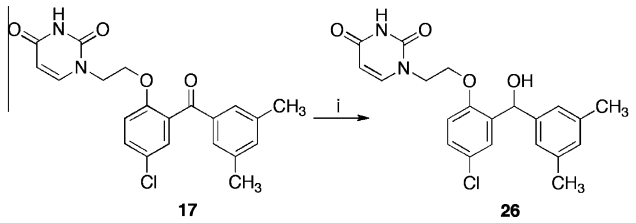
Analogous to this route, we have now synthesized a number of 1-[2-(2-benzylphenoxy)ethyl]- and 1-[ω-(2-benzoylphenoxy)ethyl]uracil derivatives **6**–**24**. The synthesis (Scheme 1) was accomplished by heating trimethylsilyl derivative **3** and bromide **4** or **5** at 160–170 °C for 2 h without a solvent. The target compounds were obtained in 62–83% yields, and formation of the N^3- and N^1,N^3-disubstituted byproducts was not observed.

The presence of a substituent at position 6 of the pyrimidine can affect regioselectivity of 6-R-uracil alkylation.[32] For example, condensation of 2,4-bis(trimethylsilyloxy)-6-methylpyrimidine with 4–5 molar excess of 1,4-dibromobutane leads to a complex mixture of N^1- and N^3-mono- as well as to N^1,N^3-disubstituted uracils, the ratio of which depends upon the reaction temperature.[33] As a result, an alternative approach for synthesis of 1-[2-[2-(3,5-dimethylbenzoyl)-4-methylphenoxy]ethyl]-6-methyluracil **25** was needed. Thus treatment of 6-methyluracil (5 molar excess) with bromide **5** in the presence of K_2CO_3 in DMF (Scheme 1) afforded **25** in a 64% yield following separation from the N^1,N^3-disubstituted product.

To study the influence of the bridging carbonyl group on the antiviral properties of the compounds, the reduced analogue of compound **17** was pursued. As shown in Scheme 2, treatment of **17** with $NaBH_4$ in the presence of NaOH resulted in the hydroxyl derivative **26** (93%).



Scheme 1. Reagents and conditions: (i) 160–170 °C, 2 h; (ii) K_2CO_3, DMF, 70 °C, 8 h.



Scheme 2. Reagents and conditions: (i) $NaBH_4$, NaOH, EtOH (aq.), 50 °C, 8 h.

2.2. Antiviral activity

The anti-HIV properties of the uracil derivatives were studied in human T-lymphocyte CEM (compounds **6–10**) or MT-4 (compounds **11–26**) cell cultures infected with HIV-1(IIIB) or HIV-2 (ROD), or the double mutant (K103N + Y181C RT) virus strain (Table 1). All compounds were found to be inactive against HIV-2, however most compounds exhibited notable antiviral activity against HIV-1. In examining the structure–activity relationship (SAR) of the compounds, it became evident that 2-(2-benzylphenoxy)ethyl uracils **6–10** displayed moderate levels of activity whereas their 2-(2-benzoylphenoxy)ethyl counterparts **11**–**22**, **24**, **25**, which contain the bridging carbonyl linker, inhibit HIV replication at much lower compound concentrations (except for **11** and **12**). Reduction of the carbonyl group of the benzophenone fragment to the respective alcohol (**26**) or addition of a methylene group to the linker (**23**) resulted in a dramatic decrease of antiviral properties. Substituent R^3 appears to play a crucial role in modulating antiviral activity of the compounds; benzophenones bearing a methyl group or fluorine atom were found to be less active than their counterparts bearing chlorine or bromine atoms (**11** and **12** vs **13** and **14**, and **16** vs **17** and **18**).

An increase in antiviral activity was obtained by inserting one or two substituents at the R^4 position(s). As such, compound **17** bearing two methyl groups exhibited more pronounced activity than the analogous **15** possessing only one methyl group or **13**, which has no methyl groups. Moreover, substitution of these methyl groups by various halogens had no significant impact on the antiviral activity as exemplified in **17** versus **19-21** and **18** versus **22**. A similar level of anti-HIV activity was exhibited by the compound **25** bearing a methyl group in the C-6-position of the pyrimidine ring whereas introduction of the same substituent at C-5 (**24**) slightly decreased the antiviral properties of the compound. The most potent anti-HIV agents were found to be uracils **17**, **20**, **22** and **25** which inhibited viral replication at nanomolar concentrations. Most compounds were found to be nontoxic in MT-4/CEM cell cultures. Benzophenone **16**, which bears a methyl group at the R^3-position, showed the highest toxicity albeit at a minor level (CC_{50}:179 μM).

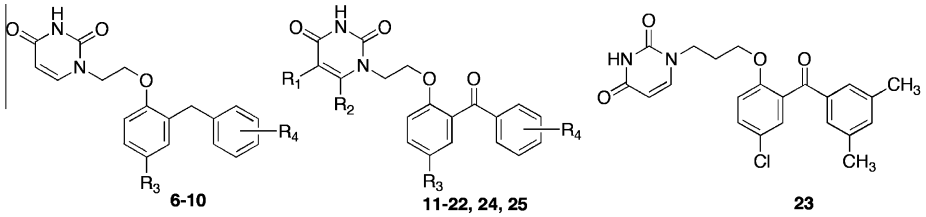
The studied compounds were also investigated as potential inhibitors of replication against a large panel of other DNA and RNA viruses. However, none of the compounds displayed notable activity against HIV-2 or any DNA or RNA viruses other than HIV-1 (HSV-1, HSV-2, Vaccinia virus, Para-influenza-3 virus, Reovirus-1, Sindbis virus, Vesicular stomatitis virus, Respiratory syncytial virus, Coxsackie virus B4, Feline Corona Virus (FIPV), Yellow Fever virus, Dengue virus, Rift Valley Fever virus, Punta Toro virus, West Nile virus).

Compounds **11–22** and **24–25** were also evaluated against a drug-resistant (double mutant) HIV-1 strain bearing the K103N and Y181C mutations in the RT. These mutations resulted in pronounced resistance to nevirapine and, to a much lesser extent, efavirenz. Most of the studied benzophenones were found to be inactive against the mutant virus (Table 1). However, **20**, **24** and **25** did inhibit HIV-1 replication, albeit at higher compound concentrations (50- to 150-fold). Compounds **12**, **15** and **22** also retained weak levels of activity against this mutant virus strain. At this time we cannot explain the differences in binding affinity for the closely related benzophenones towards the double mutant HIV strain, however investigations are currently underway that may provide additional information that may shed light on the differing levels of activity.

2.3. Enzymatic study

Next, the inhibitory properties of the uracil derivatives against HIV-1 RT enzyme were studied in standard DNA- and RNA-dependent DNA-polymerase assays using activated DNA and wild-type p66/p66 homodimer RT or poly(rC)-oligo(dG) template–primer and p66/p51 heterodimer recombinant enzyme. Noteworthy that these enzyme preparations had similar activity: when activated DNA was used as template–primer in assay conditions as described in Section 4, p66/p66 and p66/p51 catalyzed incorporation of 0.29, and 0.30 pmol dATP per second per 1 μg of the enzyme, respectively. Nevirapine and efavirenz were used as reference standards (Table 2). A preliminary investigation of their mechanism of action was performed with compound **8** in p66/p66/activated DNA assay. It was shown that this compound acted in a non-competitive manner with regards to the dNTP substrate (Fig. S1). In addition, the uracil derivative **8** retained its efficacy when the enzymatic reactions were carried out in the presence of heparin, which was added

Table 1
Antiviral activity of the studied compounds against HIV-1



Compound	R^1	R^2	R^3	R^4	CC_{50} (μM) CEM or MT-4	HIV-1 (III_B) wild type		HIV-1(III_B) K103N + Y181C		
						EC_{50} (μM)	SI[a]	EC_{50} (μM)	SI[a]	RI[b]
6	H	H	4-Me	H	48[c]	3.3[c]	14	—	—	—
7	H	H	4-Me	3-Me	53[c]	0.22[c]	241	—	—	—
8	H	H	4-Me	3,5-Me_2	66[c]	0.13[c]	507	—	—	—
9	H	H	4-Cl	3,5-Me_2	>200[c]	0.22[c]	>909	>200	1	>909
10	H	H	4-Br	3,5-Me_2	>220[c]	0.22[c]	>1000	—	—	—
11	H	H	Me	H	>357	0.31	>1136	>357	1	>1136
12	H	H	F	H	>353	0.51	>694	171	>2	330
13	H	H	Cl	H	>337	0.089	>3787	>337	1	>3787
14	H	H	Br	H	>301	0.065	>4630	>301	1	>4630
15	H	H	Cl	3-Me	>325	0.052	>6246	52	>6	1000
16	H	H	Me	3,5-Me_2	179	0.058	3081	>179	<1	>3081
17	H	H	Cl	3,5-Me_2	>313	0.016	>19588	>313	1	>19588
18	H	H	Br	3,5-Me_2	>282	0.023	>12260	>282	1	>12260
19	H	H	Cl	3,5-F_2	>307	0.027	>11381	>307	1	>11381
20	H	H	Cl	3,5-Cl_2	>284	0.018	>15794	1.13	>251	63
21	H	H	Cl	3,5-Br_2	>236	0.032	>7390	>237	1	>7390
22	H	H	Br	3,5-Cl_2	>258	0.017	>15188	24.8	>10	1459
23	H	H	Cl	3,5-Me_2	>20[c]	5.8[c]	>3.45	—	—	—
24	Me	H	Cl	3,5-Me_2	>303	0.044	>6882	4.09	>74	93
25	H	Me	Cl	3,5-Me_2	>303	0.020	>15140	4.09	>74	205
26	H	H	Cl	3,5-Me_2	>20[c]	⩾20[c]	>1	—	—	—
Nevirapine[39]	—	—	—	—	>15	0.075	>200	>15	1	>200
Efavirenz[39]	—	—	—	—	>63	0.003	>2112	0.56	>113	186

[a] SI = selectivity index; CC_{50}/EC_{50}.
[b] RI = resistance index; EC_{50} (resistant virus)/EC_{50} (wild-type virus).
[c] Data obtained in CEM cell cultures.

Table 2
Inhibitory activity of the compounds against wild-type HIV-1 RT in DNA-dependent and RNA-dependent polymerase activity assays

Compound	K_I (μM)	
	Activated DNA (p66/p66)	Poly(rC)-oligo(dG) (p66/p51)
6	76	18
7	36	6.3
8	5.9	2.7
9	5.0	2.1
10	11	3.1
11	5.6	1.7
12	2.9	2.6
13	0.98	0.65
14	2.0	0.45
15	0.49	0.21
16	2.4	2.0
17	2.4	0.52
18	0.77	1.7
19	0.77	0.36
20	0.68	0.82
21	2.3	0.47
22	0.69	0.16
23	>50	>242
24	1.2	1.1
25	0.73	1.7
26	>50	>249

simultaneously with the inhibitor and dNTPs after a short pre-incubation period of the RT with activated DNA (Fig. S2). Since heparin prevents reinitiation of the polymerization reaction, this indicates that the inhibitor only impacts the elongation process of the RT reaction and has no effect on binding of the enzyme to nucleic acids.[34]

The inhibition constants (K_I) reflecting the affinity of each compound for RT were assessed using a Dixon plot.[35] Comparison of the results revealed that the compounds displayed similar inhibitory activity in activated DNA (p66/p66) and poly(rC)-oligo(dG) (p66/p51) assays. The correlation (r) between the K_I values of the test compounds against HIV-1 RT in these assays was 0.979 (Fig. S3). Moreover, the correlation (r) between the EC_{50}'s for HIV-1 replication in cell culture and the K_I's for HIV-1 RT inhibition using p66/p66 and p66/p51 were 0.911 and 0.952, respectively (Fig. S4).

In examining the SAR of the compounds, the results show that, in general, as also noticed in the HIV-1-infected cell culture system, 2-(2-benzoylphenoxy)ethyl uracils **11–22** exhibit greater inhibition of HIV–RT than their corresponding 2-(2-benzylphenoxy)ethyl counterparts (compounds **6–10**). The differences between the benzophenones and the diphenylmethane series were especially pronounced when comparing the efficacy of compounds **11** and **6**. In contrast, the carbonyl group had almost no influence on the activity of uracil derivatives bearing methyl groups at the R^3 and R^4 positions (**16** vs **8**).

Nonetheless, considering that the reduced benzohydroxy analogue **26** failed to inhibit HIV–RT, the benzophenone carbonyl fragment appears to be crucial for retention of activity and must be interacting with the enzyme in some fashion.

Comparison of the antiviral activity data for the compounds bearing various substituents at R^3 led to surprising results. On the one hand, substitution of the methyl group of 2-(2-(3,5-dimethyl)benzylphenoxy)ethyl uracil with a chlorine or bromine had almost no effect on the potency of the compounds (**8** vs **9** and **10**). However the same substitution on the benzophenone-containing compounds led to a marked enhancement of activity. The inhibitors bearing chlorine or bromine in R^3 position inhibited RT in lower concentrations in comparison to their counterparts with methyl or fluorine substituents (**13** and **14** vs **11** and **12**, or **17** and **18** vs **16**). It is notable that these results are in agreement with the anti-HIV activity observed for the uracil derivatives.

Interestingly, several of the compounds (**14**, **17**, and **18**) showed decreased inhibitory activity in one of the enzymatic assays. Since there were no similarities between structure and the type of assay, this discrepancy could be attributed to the poor solubility observed for some of the halogenated compounds in aqueous media, in particular, in the enzymatic reaction conditions required for the assays.

Moving forward with the SAR evaluation, the role of the R^4 substituent was less clear. On the one hand, for compounds lacking the carbonyl group (**6–10**), introduction of one or two methyl substituents significantly enhanced the inhibitory activity against RT (**8** vs **7** vs **6**). On the other hand, in the benzophenone series, the corresponding 3,5-dimethyl or 3,5-dichloro-containing inhibitors displayed activity similar to that of their unsubstituted counterparts (**16** vs **11**, **17** and **20** vs **13**). These observations were in disagreement with the activity of the compounds in infected cell culture. A correlation was found only in the pairs of the benzophenones **13** and **15** (all assays), **18** and **14** (activated DNA assay), and **22** and **14** (poly(rC)-oligo(dG) assay). In all other cases, the discrepancies again could be attributed to two effects: the greater affinity of the substituted benzophenones for the enzyme, and/or the lower solubility of the compounds bearing the halogen substituents.

Next, in order to more fully understand the activity of the benzophenones against drug-resistant HIV strains as well as to verify the reasons for the poor levels of activity of the compounds against the K103N/Y181C RT double mutant virus, a panel of RT's bearing one or two of the most clinically significant mutations (L100I, K103N, V106I, Y181C, Y188L, G190A, and K103N/Y181C) conferring resistance of the virus to NNRTIs was constructed. Based on the results of the previously highlighted antiviral studies, two structurally-related benzophenones were selected for further study. In that regard, compounds **17** and **20** both exhibited the highest activity against the wild-type virus but exhibited quite different levels of activity towards the K103N/Y181C double mutant HIV-1 strain (Table 3).

Both benzophenones **17** and **20** retained activity against most HIV-RT forms with the exception of V106A (Table 3). Although the absolute K_I values were closer to that of nevirapine, the resistance susceptibility profile of compounds **17** and **20** towards most HIV–RT mutant forms more closely resembled the profile of efavirenz. For example, nevirapine and efavirenz exhibited a 15-fold and 10-fold lower activity, respectively, towards the L100I RT mutant compared to the wild-type enzyme, whereas benzophenones **17** and **20** retained almost full activity, exhibiting only a very minor degree of resistance (1.4-fold and 2.5-fold, respectively). Similarly, against K103N, a common drug-resistant HIV–RT form, the inhibitory activity of nevirapine was 400-fold lower than that towards wtRT, whereas activity of efavirenz or the studied benzophenones **17** and **20** was only 24-, 13.8 and 8-fold lower, respectively.

Both compounds proved to be inactive against the V106A RT, while efavirenz retained activity. In contrast to nevirapine, the benzophenones retained partial activity against all other forms of RT, and in some cases the decrease was lower than that of efavirenz. For the Y181C RT mutant, compounds **17** and **20** inhibited the polymerase 3.3- and 2-fold less effectively, as compared to a decrease of 395- and 2.6-fold for nevirapine and efavirenz, respectively. In the case of the Y188L mutant RT, the activity of benzophenone **17** was 4.3-fold lower than for the wild-type enzyme, while compound **20** exhibited 12-fold lower activity. Nevirapine and efavirenz were >250 and 12-fold less active, respectively. Interestingly, benzophenone **17** displayed even higher inhibitory activity toward the mutant G190A RT form, while **20** exhibited somewhat lower activity (2.3-fold), more closely in line with efavirenz (2.7-fold decrease) but in sharp contrast to nevirapine (423-fold decrease). Lastly, benzophenones **17** and **20**, as well as efavirenz, exhibited a 23.8-, 14.9- and 19.3-fold lower efficacy towards the K103N/Y181C double mutant RT, as compared to a >250-fold lower inhibitory activity for nevirapine.

Table 3
Inhibitory activity of inhibitors **17** and **20** against mutant HIV–RT's in the activated DNA assay

HIV-1 RT mutation(s)	K_I (μM)			
	17	**20**	Nevirapine	Efavirenz
Wt	2.4	0.7	4.2	0.03
L100I	3.3	1.8	61	0.33
K103N	33	5.6	1685	0.73
V106A	>1000	>1200	482	0.08
Y181C	8.0	1.4	1655	0.08
Y188L	10	8.4	>1000	0.36
G190A	1.0	1.6	1362	0.08
K103N/Y181C	57	10	>1000	0.58

Thus, the data obtained are highly encouraging and appear to point to a resistance susceptibility profile for the synthesized benzophenones that is closer to efavirenz than to nevirapine. Moreover, the resistance index for the compounds against the Y181C, Y188L and K103N/Y181C mutant RTs was comparable to that of efavirenz, and even lower for the L100I, K103N and G190A mutants, although for the V106A mutant, efavirenz was more potent than **17** and **20**. While these data do not directly support the results of the antiviral activity studies, which revealed different resistance susceptibility profiles for the structurally-related benzophenones **17** and **20**, it is possible that this difference is due to differences in solubility for some of the compounds in aqueous media, rather than to a different mode of interaction with mutant HIV–RTs.

In that regard, it should be noted that 10% DMSO was used in the HIV-1 RT poly(rC)-oligo(dG) experiment, whereas cell cultures only contained 0.1% DMSO at most. To test this possibility, we determined solubility of several compounds in 10% DMSO solution (as was used in the RT activity assay) or in 0.1% (as was used in the cell culture experiments) by UV spectroscopy. It was found that the maximum concentration of the compounds bearing halogens (including **17** and **20**) could not exceed 2–3 μM in 10% DMSO solution (Supplementary data, Table S2). Assuming that the resistance index of the compounds **17**–**19** against K103N + Y181C HIV-1 strain is 50–200 as was shown for benzophenones **20**, **24**, and **25**, they should display antiviral activity against this strain at micromolar concentrations, that is, in the same range as they tend to precipitate. If this is indeed the case, the next generation of benzophenones should be designed with enhanced solubility characteristics, which may lead to obtaining more effective antiretroviral agents active against a variety of drug-resistant viral strains.

3. Conclusions

Herein we have described the synthesis, SAR, and biological evaluation of a series of potential NNRTI anti-HIV agents. Several of the uracil derivatives exhibited potent antiviral activity in infected cell

culture and effectively inhibited recombinant HIV–RT. Notably, the compounds did not exhibit toxicity in cell culture, and thus have high selectivity indices. Two of the uracil derivatives effectively inhibited several mutant HIV-1 RTs and also partially retained antiviral activity against several drug-resistant HIV strains. Despite these encouraging results, the poor solubility of some of the compounds hindered true assessment of their potential biological activity. Although the activity levels of the compounds towards wild-type virus and HIV-1 RT were similar to that of nevirapine, they more closely resemble the activity profile of efavirenz by retaining their inhibitory activity towards mutant RT forms resistant to nevirapine. Given the promising and potent antiviral activity exhibited by these compounds, it is clear that the benzophenone-linked uracil scaffold is an excellent lead for the development of additional compounds. As a result, current efforts are underway to improve the solubility of some of the more insoluble compounds in order to design more effective anti-HIV drugs. Those results will be reported elsewhere as they become available.

4. Materials and methods

4.1. General

Activated DNA was purchased from GE Healthcare (Little Chalfont, UK) Oligonucleotides were obtained from Lytech (Moscow, Russia). [α-^{32}P]dATP (5000 Ci/mmol) and [8-^{3}H]dGTP were from Izotop (Moscow, Russia), and Moravek Biochemicals, Brea, CA), respectively. Ni-NTA-agarose resin and Rosetta(DE3) *Escherichia coli* strain were from Novagen (Madison, WI). All other reagents of highest grade were obtained from Sigma (St. Louis, MI), and the enzymes were purchased from Sybenzyme (Novosibirsk, Russia) or Fermentas (Vilnus, Lithuania). All chemicals were obtained from commercial sources and used without further purification unless otherwise noted. Anhydrous DMF, isopropanol, and ethylene glycol were purchased from Sigma-Aldrich Co. Anhydrous acetone, CH_2Cl_2, 1,2-dichloroethane, and ethyl acetate were obtained by distillation over P_2O_5.

Melting points were measured on Mel-Temp 3.0 Pro apparatus (Laboratory Devices Inc.) and uncorrected. All ^{1}H and ^{13}C NMR spectra were registered on a Varian Mercury 300B and AMXIII-400 Bruker, operated at 300 and 400 MHz for ^{1}H; 75 and 100 MHz for ^{13}C, respectively, and tetramethylsilane (TMS) was used as an internal standard (0.0 ppm). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel precoated plates. Mass spectra were recorded at the Johns Hopkins Mass Spectrometry Facility (Baltimore, MD). X-ray structure analysis was performed on a Bruker SMART 1000 difractometer, and the data were analyzed with the SHELX 97 program. Complete structural data will be reported separately.

Yields refer to chromatographically (HPLC) and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials. The purity of the compounds was verified using HPLC and all compounds were verified to be >98% pure with the only minor impurity being uracil. Briefly, the compounds were dissolved in DMSO, and purified using a Nucleosil 100 C-8 (5 m, 4 × 150 mm) column on a Gilson chromatograph (France) supplied with a digital Gilson GSIOC 506 controller and a Gilson-315 UV detector with varied wavelengths. The compounds were purified using the following two protocols:

(1) Reversed phase regime: solution [A]: 5 mM sodium-phosphate buffer [NaPi] (pH 5.2); solution [B]: 80% CH_3CN. Gradient: 0% [B] for 5 min; 0% →20% [B] for 10 min; 20% →100% [B] for 15 min; 100% [B] for 5 min.

(2) Ion-pairing reversed phase regime: solution [A]: 0.1% heptafluorobutyric acid [$CF_3(CF_2)_2COOH$] (pH 3); solution [B]: 80% CH_3CN. Gradient: 0% [B] for 5 min; 0% →20% [B] for 10 min; 20% →100% [B] for 15 min; 100% [B] for 5 min. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer (Japan). The rate of elution was 0.5 ml/min.

4.2. Synthesis

4.2.1. General procedure for compounds 6–24

2,4-bis(trimethylsilyloxy)pyrimidine **3**, prepared from uracil or thymine (17.84 mmol) by refluxing in excess HMDS, was incubated with bromides **4** or **5** (17.85 mmol) at 160–170 °C for 2 h and then kept overnight at room temperature. The resulting product was treated with EtOAc (40 mL) and isopropanol (10 mL), the mixture kept at room temperature for 30 min then evaporated. The resulting residue was recrystallized twice from isopropanol or from a mixture isopropanol–DMF (1:1).

4.2.1.1. 1-[2-(2-Benzyl-4-methylphenoxy)ethyl]uracil (6). Yield: 66%, mp: 146–147 °C, R_f 0.55 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 2.18 (s, 3H, CH_3), 3.81 (s, 2H, $PhCH_2$), 4.07 (t, 2H, J = 6 Hz, N–CH_2), 4.14 (t, 2H, J = 6 Hz, O–CH_2), 5.48 (d, 1H, J = 8 Hz, H-5), 6.84–6.98 (m, 3H, aromatic H), 7.12–7.25 (m, 5H, aromatic H), 7.60 (s, 1H, J = 8 Hz, H-6), 11.31 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.1, 32.9, 45.3, 63.5, 98.8, 110.1, 124.2, 126.7, 127.2, 127.8, 129.1, 139.2, 144.5, 149.4, 151.9, 162.2; HRMS calcd for $C_{20}H_{20}N_2O_3$, $[M+H]^+$ 337.1547; found (FAB) m/z ($[M+H]^+$): 337.1549.

4.2.1.2. 1-[2-[2-(3-Methylbenzyl)-4-methylphenoxy]ethyl]uracil (7). Yield: 83%, mp: 127–128 °C, R_f 0.35 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 2.17 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 3.79 (s, 2H, $ArCH_2$), 4.06 (t, 2H, J = 4.9 Hz, N–CH_2), 4.13 (t, 2H, J = 5 Hz, O–CH_2), 5.46 (dd, 1H, J = 7.8 and 2.2 Hz, H-5), 6.85–6.86 (m, 2H, aromatic H), 6.90 (m, 1H, aromatic H), 6.93–6.96 (m, 3H, aromatic H), 7.09–7.12 (m, 1H, aromatic H), 7.58 (d, 1H, J = 7.8 Hz, H-6), 11.29 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 20.2, 21.1, 47.3, 65.4, 100.7, 111.7, 125.7, 126.5, 127.7, 128.1, 129.1, 129.2, 129.5, 131.0, 137.3, 140.9, 146.1, 151.1, 153.5, 163.7; HRMS calcd for $C_{21}H_{22}N_2O_3$, $[M+H]^+$ 351.1630; found (FAB) m/z ($[M+H]^+$): 351.1698.

4.2.1.3. 1-[2-[2-(3,5-Dimethylbenzyl)-4-methylphenoxy]ethyl] uracil (8). Yield: 74%, mp: 156–158 °C, R_f 0.58 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 2.12 (s, 6H, CH_3), 3.68 (s, 2H, $PhCH_2$), 4.02 (t, 2H, J = 6 Hz, N–CH_2), 4.07 (t, 2H, J = 6 Hz, O–CH_2), 5.38 (d, 1H, J = 8 Hz, H-5), 6.67 (s, 2H, H-2″, H-6″), 6.70 (s, 1H, H-4″), 6.78 (m, 3H, H-3′, H-5′, H-6′), 7.52 (d, 1H, J = 8 Hz, H-6), 11.28 (s, ^{1}H, NH); ^{13}C NMR (DMSO-d_6): δ 18.4, 19.1, 32.8, 45.5, 63.6, 98.8, 110.0, 124.4, 124.6, 125.3, 125.5, 126.0, 127.3, 127.6, 128.9, 129.2, 135.3, 138.9, 144.2, 149.2, 151.6, 161.9; HRMS calcd for $C_{22}H_{24}N_2O_3$, $[M+H]^+$ 365.1859; found (FAB) m/z ($[M+H]^+$): 365.1854.

4.2.1.4. 1-[2-[4-Chloro-2-(3,5-dimethylbenzyl)phenoxy]ethyl] uracil (9). Yield: 80%, mp: 191–192.5 °C, R_f 0.59 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 6H, CH_3), 3.71 (s, 2H, $ArCH_2$), 4.03 (t, 2H, J = 4 Hz, N–CH_2), 4.19 (t, 2H, J = 5 Hz, O–CH_2), 5.46 (dd, 1H, J = 7.9 and 2.2 Hz, H-5), 6.75 (s, 2H, H-2″, H-6″), 6.79 (s, 1H, H-4″), 6.95–6.97 (m, 1H, H-6′), 7.20 (d, 1H, J = 2.4 Hz, H-3′), 7.32 (dd, 1H, J = 8.8 and 2.5 Hz, H-5′), 7.60 (d, 1H, J = 7.8 Hz, H-6), 11.30 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 19.0, 32.5, 45.3, 63.8, 98.9, 111.6, 122.5, 124.4, 124.6, 125.0, 125.7, 127.7, 127.9, 129.9, 135.5, 138.0, 144.4, 149.2, 152.5,

161.9; HRMS calcd for $C_{21}H_{21}ClN_2O_3$, $[M+H]^+$ 385.1313; found (FAB) *m/z* ($[M+H]^+$): 385.1319 (^{35}Cl), 387.1297 (^{37}Cl).

4.2.1.5. 1-[2-[4-Bromo-2-(3,5-dimethylbenzyl)phenoxy]ethyl] uracil (10). Yield: 76%, mp: 189–190.5 °C, R_f 0.71 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 2.20 (s, 6H, CH_3), 3.78 (s, 2H, $ArCH_2$), 4.09 (t, 2H, J = 5 Hz, N–CH_2), 4.13 (t, 2H, J = 4.5 Hz, O–CH_2), 5.40 (d, 1H, J = 8 Hz, H-5), 6.69 (s, 2H, H-2″, H-6″), 6.73 (s, 1H, H-4″), 6.88–6.91 (m 1H, H-6′), 7.14 (m, 1H, H-3′), 7.25–7.28 (m, 1H, H-5′), 7.55 (d, 1H, J = 8 Hz, H-6), 11.28 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.0, 47.1, 65.7, 100.7, 112.3, 114.0, 126.4, 127.6, 130.0, 132.3, 132.5, 137.3, 139.9, 146.1, 151.0, 154.9, 163.7; HRMS calcd for $C_{21}H_{21}BrN_2O_3$, $[M+H]^+$ 429.0808; found (FAB) *m/z* ($[M+H]^+$): 429.0804 (^{79}Br), 431.0796 (^{81}Br).

4.2.1.6. 1-[2-(2-Benzoyl-4-methylphenoxy)ethyl]uracil (11). Yield: 66%, mp: 191–193 °C, R_f 0.48 (elution with ethyl acetate), ^1H NMR (DMSO-d_6): δ 2.23 (s, 3H, CH_3), 3.67 (m, 2H, N–CH_2), 4.03 (m, 2H, O–CH_2), 5.09 (d, 1H, J = 7.5 Hz, H-5), 6.66 (d, 1H, J = 7.5 Hz, H-5′), 7.02–7.08 (m, 2H, H-3′, H-6′), 7.26–7.29 (m, 1H, H-4″), 7.41–7.46 (m, 2H, H-2″, H-6″), 7.54–7.63 (m, 2H, H-5″, H-6), 11.11 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 19.8, 47.0, 65.8, 100.3, 112.8, 128.5, 129.1, 130.1, 132.3, 133.3, 137.0, 145.3, 150.6, 153.3, 163.4, 195.6; HRMS calcd for $C_{20}H_{18}N_2O_4$, $[M+H]^+$ 351.1339; found (FAB) *m/z* ($[M+H]^+$): 351.1336.

4.2.1.7. 1-[2-(2-Benzoyl-4-fluorophenoxy)ethyl]uracil (12). Yield: 71%, mp: 170.5–172 °C, R_f 0.47 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 3.69 (m, 2H, N–CH_2), 4.07 (m, 2H, O–CH_2), 5.08 (d, 1H, J = 7.5 Hz, H-5), 6.68 (d, 1H, J = 8 Hz, H-5′), 7.16–7.22 (m, 2H, aromatic H), 7.30–7.36 (m, 1H, aromatic H), 7.43–7.51 (m, 2H, aromatic H, H-6), 7.53–7.58 (m, 1H, aromatic H), 7.57–7.66 (m, 2H, aromatic H), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 46.9, 66.3, 100.3, 114.6, 114.6, 115.2, 115.4, 117.9, 118.1, 128.6, 129.1, 129.7, 129.7, 133.7, 136.3, 145.2, 150.6, 151.5, 155.1, 157.4, 163.4, 194.0; HRMS calcd for $C_{19}H_{15}FN_2O_4$, $[M+H]^+$ 355.1088; found (FAB) *m/z* ($[M+H]^+$): 355.1086.

4.2.1.8. 1-[2-(2-Benzoyl-4-chlorophenoxy)ethyl]uracil (13). Yield: 79%, mp: 219–220 °C, R_f 0.39 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 3.70 (t, 2H, J = 5 Hz, N–CH_2), 4.10 (t, 2H, J = 5 Hz, O–CH_2), 5.08 (dd, 1H, J = 8 and 2 Hz, H-5), 6.67 (d, 1H, J = 8 Hz, H-5′), 7.19 (d, 1H, J = 8 Hz, H-6′), 7.35–7.65 (m, 7H, H-3′, H-2″, H-4″, H-6″, H-6), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 46.8, 66.1, 100.4, 114.8, 125.0, 128.2, 128.6, 129.2, 130.1, 131.4, 133.7, 136.3, 145.2, 150.6, 154.0, 163.4, 193.9. HRMS calcd for $C_{19}H_{15}ClN_2O_4$, $[M+H]^+$ 371.0793; found (FAB) *m/z* ($[M+H]^+$): 371.0792 (^{35}Cl), 373.0776 (^{37}Cl).

4.2.1.9. 1-[2-(2-Benzoyl-4-bromophenoxy)ethyl]uracil (14). Yield: 76%, mp: 236–237 °C, R_f 0.42 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 3.69 (t, 2H, J = 4.5 Hz, N–CH_2), 4.09 (t, 2H, J = 4.5 Hz, O–CH_2), 5.07 (d, 1H, J = 8 Hz, H-5), 6.66 (d, 1H, J = 8 Hz, H-5′), 7.14 (d, 1H, J = 8 Hz, H-6′), 7.42–7.47 (m, 2H, aromatic H), 7.57–7.65 (m, 3H, aromatic H), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 46.8, 66.0, 100.4, 112.5, 115.2, 128.5, 129.2, 130.5, 130.9, 133.7, 134.3, 136.3, 145.2, 150.6, 154.5, 163.4, 193.8; HRMS calcd for $C_{19}H_{15}BrN_2O_4$, $[M+H]^+$ 416.0288; found (FAB) *m/z* ($[M+H]^+$): 415.0293 (^{79}Br), 417.0268 (^{81}Br).

4.2.1.10. 1-[2-[4-Chloro-2-(3-methylbenzoyl)phenoxy]ethyl] uracil (15). Yield: 79%, mp: 191–192 °C, R_f 0.37 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 3.71 (t, 2H, J = 4.5 Hz, N-CH_2), 4.10 (t, 2H, J = 4.5 Hz, O–CH_2), 5.04 (dd, 1H, J = 8 and 2 Hz, H-5), 6.69 (d, 1H, J = 7 Hz, H-5′), 7.19 (d, 1H, J = 9 Hz, H-6′), 7.29–7.44 (m, 5H, H-3′, H-2″, H-4″, H-6″), 7.51 (dd, 1H, J = 8 and 2 Hz, H-6), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 20.7, 46.9, 66.1, 100.3, 114.8, 125.0, 126.5, 128.1, 128.5, 129.4, 130.3, 131.3, 134.5, 136.3, 138.1, 145.2, 150.6, 154.0, 163.4, 193.9; HRMS calcd for $C_{20}H_{17}ClN_2O_4$, $[M+H]^+$ 385.0949; found (FAB) *m/z* ($[M+H]^+$): 385.0959 (^{35}Cl), 387.0932 (^{37}Cl).

4.2.1.11. 1-[2-[2-(3,5-Dimethylbenzoyl)-4-methylphenoxy)ethyl]uracil (16). Yield: 66%, mp: 215.5–217 °C, R_f 0.53 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 2.22 (s, 9H, CH_3), 3.68 (t, 2H, J = 4.5 Hz, N–CH_2), 4.04 (t, 2H, J = 4.5 Hz, O–CH_2), 5.02 (d, 1H, J = 7.5 Hz, H-5), 6.70 (d, 1H, J = 7.5 Hz, H-5′), 7.02–7.05 (m, 2H, aromatic H), 7.16–7.27 (m, 4H, aromatic H, H-6), 11.11 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 19.8, 20.6, 47.2, 65.7, 100.1, 112.9, 126.7, 128.7, 129.0, 130.2, 132.2, 134.9, 137.1, 137.7, 145.3, 150.6, 153.2, 163.4, 195.7; HRMS calcd for $C_{22}H_{22}N_2O_4$, $[M+H]^+$ 379.1652; found (FAB) *m/z* ($[M+H]^+$): 379.1661.

4.2.1.12. 1-[2-[4-Chloro-2-(3,5-dimethylbenzoyl)phenoxy]ethyl] uracil (17). Yield: 64%, mp: 236–238 °C, R_f 0.45 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 2.29 (s, 6H, CH_3), 3.77 (t, 2H, J = 5 Hz, N–CH_2), 4.16 (t, 2H, J = 5 Hz, O–CH_2), 5.07 (d, 1H, J = 7.8 Hz, H-5), 6.77 (d, 1H, J = 7.9 Hz, H-5′), 7.23–7.28 (m, 4H, H-3′, H-2″, H-4″, H-6″), 7.35 (m, 1H, H-6′), 7.55 (dd, 1H, J = 8.8 and 2.8 Hz, H-6), 11.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 20.6, 47.0, 66.1, 100.2, 114.8, 125.0, 126.8, 128.1, 130.5, 131.2, 135.3, 136.4, 137.9, 145.1, 150.6, 154.0, 163.3, 193.9; HRMS calcd for $C_{21}H_{19}ClN_2O_4$, $[M+H]^+$ 399.1106; found (FAB) *m/z* ($[M+H]^+$): 399.1108 (^{35}Cl), 401.1091 (^{37}Cl).

4.2.1.13. 1-[2-[4-Bromo-(3,5-dimethylbenzoyl)phenoxy]ethyl] uracil (18). Yield: 69%, mp: 236–237 °C, R_f 0.50 (elution with ethyl acetate); ^1H NMR(DMSO-d_6): δ 2.23 (s, 6H, CH_3), 3.71 (t, 2H, J = 4.5 Hz, N–CH_2), 4.10 (t, 2H, J = 4.5 Hz, O–CH_2), 4.99 (d, 1H, J = 8 Hz, H-5), 6.96–6.99 (m, 1H, H-5′), 7.21 (d, 1H, J = 9 Hz, H-6′), 7.19–7.21 (m, 3H, H-2″, H-4″, H-6″), 7.41 (d, 1H, J = 2 Hz, H-3′),7.63 (dd, 1H, J = 8 and 2 Hz, H-6), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 20.6, 47.0, 66.0, 100.2, 112.6, 115.3, 126.8, 130.8, 134.1, 135.3, 136.4, 137.9, 145.1, 150.6, 154.4, 163.3, 193.8; HRMS calcd for $C_{19}H_{20}BrN_2O_4$, $[M+H]^+$ 444.0601; found (FAB) *m/z* ($[M+H]^+$): 443.0606 (^{79}Br), 445.0589 (^{81}Br).

4.2.1.14. 1-[2-[4-Chloro-2-(3,5-difluorobenzoyl)phenoxy]ethyl] uracil (19). Yield: 77%, mp: 233.5–235 °C, R_f 0.43 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 3.77 (t, 2H, J = 4.5 Hz, N–CH_2), 4.14 (t, 2H, J = 4.5 Hz, O–CH_2), 5.13 (d, 1H, J = 7.5 Hz, H-5), 6.96 (d, 1H, J = 7.5 Hz, H-5′), 7.20–7.26 (m, 3H, aromatic H), 7.39–7.50 (m, 2H, aromatic H), 7.55 (dd, 1H, J = 8 and 2.5 Hz, H-6), 11.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 47.1, 66.2, 100.2, 114.9, 122.9, 125.1, 128.4, 128.7, 130.6, 132.3, 138.2, 139.7, 145.2, 150.6, 154.2, 163.3, 191.3; HRMS calcd for $C_{19}H_{13}ClF_2N_2O_4$, $[M+H]^+$ 407.0605; found (FAB) *m/z* ($[M+H]^+$): 407.0608 (^{35}Cl), 409.0589 (^{37}Cl).

4.2.1.15. 1-[2-[4-Chloro-2-(3,5-dichlorobenzoyl)phenoxy]ethyl] uracil (20). Yield: 70%, mp: 240–242 °C, R_f 0.35 (elution with ethyl acetate); ^1H NMR (DMSO-d_6): δ 3.81 (t, 2H, J = 5 Hz, N–CH_2), 4.20 (t, 2H, J = 5 Hz, O–CH_2), 5.16 (d, 1H, J = 7.8 Hz, H-5), 6.98 (d, 1H, J = 7.8 Hz, H-5′), 7.27 (d, 1H, J = 8.9 Hz, H-6′), 7.46 (d, 1H, J = 2.7 Hz, H-3′), 7.59–7.59 (m, 2H, H-2″, H-4″), 7.61 (dd, 1H, J = 8.9 and 2.7 Hz, H-6), 7.84 (t, 1H, J = 1.9 Hz, H-6″), 11.14 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 47.0, 66.2, 100.1, 114.9, 125.1, 127.4, 128.5, 128.6, 132.2, 132.9, 134.6, 139.3, 145.2, 150.6, 154.2, 163.2, 191.4; HRMS calcd for $C_{19}H_{13}Cl_3N_2O_4$, $[M+H]^+$ 439.00137; found (FAB) *m/z* ($[M+H]^+$): 439.0023 (^{35}Cl), 440.9998 ($2 \times {}^{35}Cl$, $1 \times {}^{37}Cl$), 442.9972 ($1 \times {}^{35}Cl$, $2 \times {}^{37}Cl$).

4.2.1.16. 1-[2-[4-Chloro-2-(3,5-dibromobenzoyl)phenoxy]ethyl] uracil (21). Yield: 75%, mp: 261–262 °C, R_f 0.47 (elution with ethyl acetate); ^{1}H NMP-spectrum (DMSO-d_6): δ 3.75 (t, 2H, J = 5 Hz, N–CH_2), 4.13 (t, 2H, J = 5 Hz, O–CH_2), 5.08 (d, 1H, J = 8 Hz, H-5), 6.87 (d, 1H, J = 8 Hz, H-5′), 7.21 (d, 1H, J = 8 Hz, H-6′), 7.41 (d, 1H, J = 1.2 Hz, H-4″), 7.57 (d, 1H, J = 8 Hz, H-6), 6.69 (s, 2H, H-2″, H-6″), 8.03 (d, 1H, J = 1.5 Hz, H-4″), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 47.0, 66.2, 100.2, 114.9, 122.9, 125.1, 128.4, 128.7, 128.6, 130.6, 132.3, 138.2, 139.7, 145.2, 150.6, 154.2, 163.2, 191.2; HRMS calcd for $C_{19}H_{13}Br_2ClN_2O_4$, $[M+H]^+$ 526.9003; found (FAB) m/z ($[M+H]^+$): 526.9011 ($2\times{}^{79}$Br, ^{35}Cl), 528.8991 (^{79}Br, ^{81}Br, ^{35}Cl), 530.8974 (^{79}Br, ^{81}Br, ^{37}Cl).

4.2.1.17. 1-[2-[4-Bromo-(3,5-dichlorobenzoyl)phenoxy]ethyl] uracil (22). Yield: 70%, mp: 258–259.5 °C, R_f 0.47 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 3.81 (t, 2H, J = 5.1 Hz, N–CH_2), 4.19 (t, 2H, J = 5.1 Hz, O–CH_2), 5.16 (dd, 1H, J = 8 and 2.1 Hz, H-5), 6.91 (d, 1H, J = 8 Hz, H-5′), 7.16 d (1H, J = 8 Hz, H-6′), 7.56–7.59 (m, 3H, H-3′, H-2″, H-4″), 7.74 (dd, 1H, J = 8.9 and 2.7 Hz, H-6), 7.84–7.85 (m, 1H, H-4″), 11.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 46.9, 66.1, 100.2, 112.6, 115.3, 127.4, 128.9, 131.4, 132.9, 134.6, 135.1, 139.3, 145.2, 150.5, 154.6, 163.1, 191.3; HRMS calcd for $C_{19}H_{13}BrCl_2N_2O_4$, $[M+H]^+$ 482.9509; found (FAB) m/z ($[M+H]^+$): 482.9511 ($2\times{}^{35}$Cl, ^{79}Br), 484.9486 (alternative isotope), 486.9469 (alternative isotope).

4.2.1.18. 1-[3-[4-Chloro-2-(3,5-dimethylbenzoyl)phenoxy]propyl]uracil (23). Yield: 62%, mp: 200–201 °C, R_f 0.70 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 1.70 (m, 2H, J = 6.7 Hz, CH_2), 3.31 (t, 2H, J = 6.7 Hz, CH_2), 3.95 (t, 2H, J = 6.1 Hz, CH_2), 5.46 (dd, 1H, J = 7.8 and 1.9 Hz, H-5), 7.14 (dd, 1H, J = 7.8 and 1.2 Hz, H-5″), 7.19 (d, 1H, J = 8.9 Hz, H-6″), 7.28 (s, 1H, H-4′), 7.32 (s, 1H, H-2′, H-6′), 7.19 (d, 1H, J = 8.7 Hz, H-5″), 7.38 (d, 1H, J = 2.6 Hz, H-3″), 7.56 (dd, 1H, J = 8.9 and 2.8 Hz, H-6), 11.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 20.6, 27.7, 44.5, 65.5, 100.9, 114.9, 124.6, 126.7, 128.2, 130.4, 131.5, 134.8, 137.3, 137.9, 145.0, 150.6154.6, 163.6, 194.5; HRMS calcd for $C_{22}H_{21}ClN_2O_4$, $[M+H]^+$ 413.1263; found (FAB) m/z ($[M+H]^+$): 413.1269 (^{35}Cl), 415.1248 (^{37}Cl).

4.2.1.19. 1-[2-[4-Chloro-2-(3,5-dimethylbenzoyl)phenoxy]ethyl] thymine (24). Yield: 69%, mp: 244–245 °C, R_f 0.54 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 1.53 (d, 3H, J = 1.1 Hz, CH_3), 2.29 (s, 6H, CH3), 3.75 (t, 2H, J = 5.1 Hz, N–CH_2), 4.18 (t, 2H, J = 5 Hz, O–CH_2), 6.80–6.81 (m, 1H, H-5′), 7.25–7.28 (m, 4H, H-3′, H-2″, H-4″, H-6″), 7.32 (d, 1H, J = 2.7 Hz, H-6′), 7.55 (dd, 1H, J = 8.9 and 2.7 Hz, H-6), 11.08 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 11.6, 20.6, 46.8, 66.2, 107.8, 115.0, 124.9, 126.8, 128.0, 130.5, 131.1, 135.2, 136.2, 137.8, 141.3, 150.6, 154.0, 163.9, 193.7; HRMS calcd for $C_{22}H_{21}ClN_2O_4$, $[M+H]^+$ 413.1263; found (FAB) m/z ($[M+H]^+$): 413.1267 (^{35}Cl), 415.1244 (^{37}Cl).

4.2.2. Synthesis of 1-[2-[4-chloro-2-(3,5-dimethylbenzoyl)phenoxy]ethyl]-6-methyluracil (25)

A suspension of 6-methyluracil (5.0 g, 39.65 mmol) and K_2CO_3 (1.7 g, 12.30 mmol) in 50 mL DMF was stirred at ~70 °C for 1 h and then treated with a solution of 1-bromo-2-[4-chloro-2-(3,5-dimethylbenzoyl)phenoxy]ethane **5** (2.9 g, 7.89 mmol) in DMF (10 mL). The mixture was stirred at ~70 °C for 16 h, then was kept at room temperature overnight and filtered. The inorganic precipitate was washed with DMF, and the filtrate was evaporated in vacuum. The residue was dissolved in water (80 mL), extracted with chloroform (50 mL × 3), and the combined organic phases were dried on $CaCl_2$ and evaporated. The target compound **25** was obtained by double recrystallization from a isopropanol:DMF (1:1) mixture. Yield: 64%, mp: 192–194 °C, R_f 0.59 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 1.78 (d, 3H, J = 0.6 Hz, CH_3), 2.27 (s, 6H, CH_3), 3.85 (t, 2H, J = 5 Hz, N–CH_2), 4.19 (t, 2H, J = 5 Hz, O–CH_2), 5.01 (s, 1H, H-5), 7.21 (m, 1H, H-5′), 7.24–7.26 (m, 3H, H-3′, H-2″, H-6″), 7.55 (d, 1H, J = 2.6 Hz, H-4″), 7.55 (dd, 1H, J = 8.9 and 2.8 Hz, H-6′), 11.04 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 19.4, 20.6, 43.0, 66.2, 100.9, 114.7, 125.0, 126.9, 127.7, 130.6, 131.2, 135.5, 135.9, 137.9, 151.3, 153.7, 153.9, 162.1, 194.0; HRMS calcd for $C_{22}H_{21}ClN_2O_4$, $[M+H]^+$ 413.1263; found (FAB) m/z ($[M+H]^+$): 413.1265 (^{35}Cl), 415.1250 (^{37}Cl).

4.3. Synthesis of 1-[2-[4-chloro-2-[(3,5-dimethylphenyl)hydroxymethyl]phenoxy]ethyl]uracil (26)

To a solution of 1-[2-[4-chloro-2-(3,5-dimethylbenzoyl)-phenoxy]ethyl]uracil (**17**) (0.98 g, 2.46 mmol) in a mixture of ethanol (20 mL) and water (10 mL) containing NaOH (0.2 g, 5.0 mmol) $NaBH_4$ (0.1 g, 2.64 mmol) was added. The mixture was stirred during 8 h at 50 °C and then kept at room temperature overnight. After treatment with 2% aqueous HCl the flask was kept at 4 °C overnight. The crystalline precipitate was filtered and recrystallized twice from isopropanol:DMF (1:1) mixture. Yield: 93%, mp: 206–207.5 °C, R_f 0.55 (elution with ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 2.17 (s, 6H, CH_3), 4.05 (t, 2H, J = 5.4 Hz, N–CH_2), 4.14 (t, 2H, J = 4.2 Hz, O–CH_2), 5.44 (d, 1H, J = 7.8 Hz, H-5), 5.72 (d, 1H, J = 3.9 Hz, CH), 5.83 (d, 1H, J = 3.9 Hz, OH), 6.77 (s, 1H, H-4′), 6.85 (s, 2H, H-2′, H-6′), 6.96 (d, 1H, J = 8.7 Hz, H-6″), 7.19 (d, 1H, J = 8.7 Hz, H-5″), 7.38 (s, 1H, H-3″), 7.56 (d, 1H, J = 7.8 Hz, H-6′), 11.31 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 20.9, 47.0, 65.8, 67.4, 100.7, 113.5, 123.9, 124.7, 126.5, 127.3, 128.2, 136.1, 136.9, 144.3, 145.8, 151.0, 153.1, 163.6; HRMS calcd for $C_{21}H_{21}ClN_2O_4$, $[M]^+$ 400.1190; found (FAB) m/z ($[M+H]^+$): 400.1190 (^{35}Cl), 402.1168 (^{37}Cl).

5. Biological evaluation

5.1. Antiviral assays

The methodology of the anti-HIV assays was as follows: human CEM (~3×10^5 cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV(III_B) or HIV-2(ROD)/ml and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

The methodology of the anti-HIV assays in MT-4 cell cultures was as follows: virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1×10^5 cells/ml and infected with HIV at a multiplicity of infection of 0.02. Immediately after viral infection, 100 μl of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in 100% DMSO at 50 mM or higher. After 4 days of incubation at 37 °C, the number of viable cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The selection and characterization of mutant virus strains have been performed previously.

5.2. Reverse transcriptase plasmids, RT expression and purification

Plasmid encoding the HIV-1 reverse transcriptase (RT) was obtained out as previously reported.[36] Plasmids encoding RT in which one or two amino acid residues were substituted (L100I, K103N, V106A, Y181C, Y188L, G190A and K103N/Y181C) were constructed by amplifying the gene in two fragments using the oligonucleotides listed in Table S1. 'Head' and 'Tail' PCR-products were mixed

together in the absence of oligos and 30 additional amplification cycles were performed in order to obtain the full-length genes. The resulting products were digested with *Xho*I and *Xba*I restriction endonucleases and ligated into pBRP-HR or pET-21d-2c vectors.[37] RT genes containing mutations K103N and G190A were inserted into a pBRP-HR vector, other mutant genes were subcloned into pET-21d-2c. The wild-type and mutant RTs were expressed in *E. Coli* Rosetta (DE3) strain and purified on Ni-NTA–agarose resin as described earlier for HCV RNA polymerase.[37]

5.3. RT enzyme assay

The RT assays using activated DNA were performed as follows: the standard reaction mixture (20 μl) contained 0.75 μg of activated DNA, 0.05 μg p66/p66 RT, 3 μM dATP, 30 μM of dCTP, dGTP and dTTP, 1 μCi [α-^{32}P]dATP in a Tris-HCl buffer (50 mM, pH 8.1) containing also 10 mM $MgCl_2$, and 0.2 M KCl. The RT assays using poly(rC)-oligo(dG) as the template–primer complex were performed as follows: the reaction mixture (40 μl) contained 0.1 mM poly(rC)-oligo(dG)[12–18], 0.02–0.1 μg p66/p51 RT, 1.6 μM radiolabeled [8-^{3}H]dGTP (1 μCi), and 0.5 μg BSA in a 25 mM Tris-HCl (pH 7.5) supplemented with 0.3 mM DTT, 3 mM $MgCl_2$, 25 mM NaCl, and 5% glycerol. The test compounds were dissolved in DMSO and added to both assays to the 10% final DMSO concentration. The reaction mixtures were incubated for 30 minutes at 37 °C, and applied onto Whatman 3MM filters. After drying on air the filters were washed twice with 10% trichloracetic acid, then twice with 5% trichloracetic acid, once with ethanol and dried on air. The radioactivity was measured by the Cherenkov method.[38] Alternatively, 1 ml ice-cold 5% TCA in 0.02 M $Na_4P_2O_7$ was added to the reaction mixtures after incubation to terminate the polymerization reaction, after which the acid-insoluble precipitate (radiolabeled DNA) was captured onto Whatman glass fiber filters type GF/C (GE Healthcare UK Limited, Buckinghamshire, UK) and further washed with 5% TCA and ethanol to remove free radiolabeled dNTP. Radioactivity was determined in a Perkin Elmer Tri-Carb 2810 TR liquid scintillation counter.

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Supplementary data

Supplementary data (^{1}H, ^{13}C NMR and solubility data, as well as Dixon plots for the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.025.

References and notes

1. Hall, H. I.; Song, R.; Rhodes, P.; Prejean, J.; An, Q.; Lee, L. M.; Karon, J.; Brookmeyer, R.; Kaplan, E. H.; McKenna, M. T.; Janssen, R. S. *JAMA* **2008**, *300*, 520.
2. De Clercq, E. *Int. J. Antimicrob. Agents* **2009**, *33*, 307.
3. de Bethune, M. P. *Antiviral Res.* **2010**, *85*, 75.
4. Esnouf, R.; Ren, J.; Ross, C.; Jones, Y.; Stammers, D.; Stuart, D. *Nat. Struct. Biol.* **1995**, *2*, 303.
5. Ivetac, A.; McCammon, J. A. *J. Mol. Biol.* **2009**, *388*, 644.
6. Zhou, Z.; Lin, X.; Madura, J. D. *Infect. Disord.: Drug Targets* **2006**, *6*, 391.
7. Balzarini, J. *Curr. Top. Med. Chem.* **2004**, *4*, 921.
8. De Clercq, E. *Antiviral Res.* **1998**, *38*, 153.
9. Jochmans, D. *Virus Res.* **2008**, *134*, 171.
10. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1992**, *35*, 337.
11. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1992**, *35*, 4713.
12. Goudgaon, N. M.; Schinazi, R. F. *J. Med. Chem.* **1991**, *34*, 3305.
13. Pan, B.-C.; Chen, Z.-H.; Piras, G.; Dutschman, G. E.; Rowe, E. C.; Chen, Y.-C.; Chu, S.-H. *J. Heterocycl. Chem.* **1994**, *31*, 177.
14. Kim, D.-K.; Kim, Y.-W.; Gam, J.; Kim, G.; Lim, J.; Lee, N.; Kim, H.-T.; Kim, K. H. *J. Heterocycl. Chem.* **1996**, *33*, 1275.
15. Buckheit, R. W., Jr.; Hartman, T. L.; Watson, K. M.; Chung, S. G.; Cho, E. H. *Antimicrob. Agents Chemother.* **2008**, *52*, 225.
16. Buckheit, R. W., Jr.; Hartman, T. L.; Watson, K. M.; Kwon, H. S.; Lee, S. H.; Lee, J. W.; Kang, D. W.; Chung, S. G.; Cho, E. H. *Antiviral Chem. Chemother.* **2007**, *18*, 259.
17. El-Brollosy, N. R.; Jorgensen, P. T.; Dahan, B.; Boel, A. M.; Pedersen, E. B.; Nielsen, C. *J. Med. Chem.* **2002**, *45*, 5721.
18. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Inouye, N.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1995**, *38*, 2860.
19. Baba, M.; Shigeta, S.; Yuasa, S.; Takashima, H.; Sekiya, K.; Ubasawa, M.; Tanaka, H.; Miyasaka, T.; Walker, R. T.; De Clercq, E. *Antimicrob. Agents Chemother.* **1994**, *38*, 688.
20. Mitchell, M. L.; Son, J. C.; Lee, I. Y.; Lee, C. K.; Kim, H. S.; Guo, H.; Wang, J.; Hayes, J.; Wang, M.; Paul, A.; Lansdon, E. B.; Chen, J. M.; Eisenberg, G.; Geleziunas, R.; Xu, L.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1585.
21. Ren, J.; Chamberlain, P. P.; Stamp, A.; Short, S. A.; Weaver, K. L.; Romines, K. R.; Hazen, R.; Freeman, A.; Ferris, R. G.; Andrews, C. W.; Boone, L.; Chan, J. H.; Stammers, D. K. *J. Med. Chem.* **2008**, *51*, 5000.
22. Chan, J. H.; Freeman, G. A.; Tidwell, J. H.; Romines, K. R.; Schaller, L. T.; Cowan, J. R.; Gonzales, S. S.; Lowell, G. S.; Andrews, C. W., 3rd; Reynolds, D. J.; St Clair, M.; Hazen, R. J.; Ferris, R. G.; Creech, K. L.; Roberts, G. B.; Short, S. A.; Weaver, K.; Koszalka, G. W.; Boone, L. R. *J. Med. Chem.* **2004**, *47*, 1175.
23. Romines, K. R.; Freeman, G. A.; Schaller, L. T.; Cowan, J. R.; Gonzales, S. S.; Tidwell, J. H.; Andrews, C. W., 3rd; Stammers, D. K.; Hazen, R. J.; Ferris, R. G.; Short, S. A.; Chan, J. H.; Boone, L. R. *J. Med. Chem.* **2006**, *49*, 727.
24. Marvel, C. S. *Org. Synth.* **1941**, *1*, 435.
25. Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.
26. Ogilvie, K. K.; Hamilton, R. J.; Gillen, M. F.; Radatus, B. K. *Can. J. Chem.* **1984**, *62*, 16.
27. Malik, V.; Singh, P.; Kumar, S. *Tetrahedron* **2006**, *62*, 5944.
28. Malik, V.; Singh, P.; Kumar, S. *Tetrahedron* **2005**, *61*, 4009.
29. Tjoeng, F.-S.; Kraas, E.; Breitmaier, E.; Jung, G. *Chem. Ber.* **1976**, *109*, 2615.
30. Nowick, J. S.; Chen, J. S.; Noronha, G. *J. Am. Chem. Soc.* **1993**, *115*, 7636.
31. Novikov, M. S.; Ozerov, A. A. *Chem. Heterocycl. Comp.* **2005**, *41*, 905.
32. Wu, F.; Buhendwa, M. G.; Weaver, D. F. *J. Org. Chem.* **2004**, *69*, 9307.
33. Salikhov, I. S.; Reznik, V. S.; Shvetsov, Y. S.; Petrova, V. V. *Russ. Chem. Bull.* **1986**, *35*, 1064.
34. Gu, B.; Johnston, V. K.; Gutshall, L. L.; Nguyen, T. T.; Gontarek, R. R.; Darcy, M. G.; Tedesco, R.; Dhanak, D.; Duffy, K. J.; Kao, C. C.; Sarisky, R. T. *J. Biol. Chem.* **2003**, *278*, 16602.
35. Dixon, M. *Biochem. J.* **1953**, *55*, 170.
36. Pokholok, D. K.; Gudima, S. O.; Yesipov, D. S.; Dobrynin, V. N.; Rechinsky, V. O.; Kochetkov, S. N. *FEBS Lett.* **1993**, *325*, 237.
37. Ivanov, A. V.; Korovina, A. N.; Tunitskaya, V. L.; Kostyuk, D. A.; Rechinsky, V. O.; Kukhanova, M. K.; Kochetkov, S. N. *Protein Expr. Purif.* **2006**, *48*, 14.
38. Rengan, K. *J. Chem. Ed.* **1983**, *60*, 682.
39. Liang, Y.-H.; He, Q.-Q.; Zeng, Z.-S.; Liu, Z.-Q.; Feng, X.-Q.; Chen, F.-R.; Balzarini, J.; Pannecouque, C.; De Clercq, E. *Bioorg. Med. Chem.* **2010**, *18*, 4601.