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Original article

Hydroxyl may not be indispensable for raltegravir: Design, synthesis and SAR Studies of raltegravir derivatives as HIV-1 inhibitors

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ABSTRACT

A series of raltegravir derivatives **20**–**42** were prepared and systematically evaluated for their anti-HIV activity. The bioassay results showed that most of the compounds possess good to excellent anti-HIV activity. Especially, compounds **25** and **35** with subpicomole IC_{50} values seemed to be the most potent anti-HIV agents among all of the reported synthesized compounds. These compounds may therefore be considered as new potent anti-HIV agents. The 5-hydroxyl modification of raltegravir derivatives significantly increased the anti-HIV activity, which indicates that the hydroxyl may not be indispensable for raltegravir. The introducing of acyl at 5-position of raltegravir derivatives is favorable for antiviral activity. In addition, a high-throughput cell-based assay method with pseudotyped virus stocks was developed and used to identify HIV inhibitors.

1. Introduction

UNAIDS (Joint United Nations Programme on HIV/AIDS) estimates that there were 34.0 million (31.6 million–35.2 million) people living with HIV at the end of 2010, with 1.8 million (1.6 million–1.9 million) AIDS-related deaths and 2.7 million (2.4 million–2.9 million) new infections [1]. HIV/AIDS continues to be a great challenge, thus rendering the discovery of new drugs imperative. HIV integrase (IN) is one of the three enzymes encoded by the HIV genome and, being vital for the replication of the virus [2], so HIV IN represents an attractive target for the management of HIV infection. It catalyzes the insertion and the integration of the proviral DNA into the genome of the host cell in two steps: 3′-processing, the endonucleolytic sequence-specific hydrolysis of 3′-ends of the viral cDNA, and strand transfer, the ligation of the viral 3′–OH cDNA ends to the phosphate backbone of the host DNA acceptor. The first generation integrase inhibitors reported approximately 19 years ago [3,4] blocked the whole assembly process. More recently, 1,3-diketo acids were described which specifically inhibit the strand transfer reaction catalyzed by HIV integrase: their evolution to a molecule with more drug-like properties led to the naphthyridine series [5,6]. Recently, raltegravir (RAL, marketed as Isentress™ by Merck and Co.), a new HIV inhibitor targeting the viral integrase enzyme was approved for clinical use by the FDA. Raltegravir disrupts the critical viral process of integration in which newly made viral DNA is inserted into the host cell chromosomal DNA [7]. Raltegravir is the first approved integrase inhibitor whereas other integrase inhibitors GS-9137 and S/GSK1349572 have reached clinical development (Fig. 1) [8,9]. Like other well-known diketo acid inhibitors, these compounds share two common structural chemotypes essential for the anti-integrase activity: a diketo acid chain able to interact with Mg^{2+} metal ions (marked in bold) and a properly oriented hydrophobic benzyl moiety (marked in dashed box). They selectively inhibit strand transfer reaction, suggesting that they bind at the IN/DNA interface, acting as "interfacial inhibitors" [10,11]. Despite the lack of detailed structural information about HIV-1 IN/DNA interactions, this speculative mechanism of action tends to be validated by the recent X-ray crystal structure of integrase from the prototype foamy virus (PFV-1 IN) in complex with its cognate viral DNA and strand transfer inhibitors [12,13].

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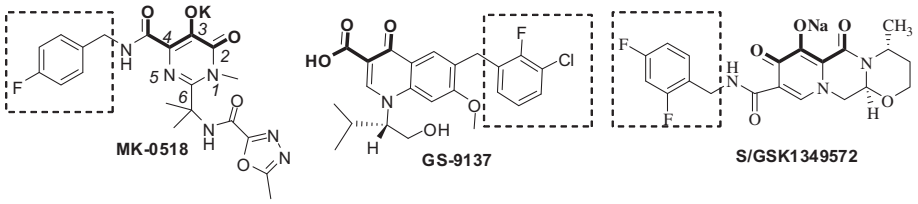


Fig. 1. Chemical structures of MK-0518, GS-9137 and S/GSK1349572.

As the diketo acid chain was commonly known as the active core against HIV, rare few efforts have been directed at evaluating the tolerance of structural changes to the core. To understand the SAR of raltegravir derivatives and develop novel integrase inhibitors with improved microsomal stability, while maintaining equal or better anti-HIV potency, the design, synthesis and bioassay of raltegravir derivatives were accomplished. In addition, a high-throughput cell-based assay method with pseudotyped virus stocks has been developed and used to identify HIV inhibitors. Herein, we wish to report our recent results.

2. Chemistry

As shown in Scheme 1, MK-0518 (**12**) was synthesized starting from commercial 2-aminoisobutyric acid (**1**). After Cbz-protection and amidation, acid **1** was converted to amide **2**, which was then subjected to dehydration to give nitrile **3**. Conversion of nitrile **3** to amidoxime **4** and reaction of **4** with dimethyl acetylenedicarboxylate provided the dihydroxypyrimidine-2-carboxylate **5**. Benzoylation of the 5-hydroxyl and subsequent methylation afforded **7** together with small amounts of the *O*-methylated derivative **8**, which could be easily separated via chromatography. Hydrogenation of **7** and subsequent amidation with **17** and aminolysis afforded raltegravir (**11**). After alkalization, compound **11** was easily converted to MK-0518 (**12**) [7]. Carbonyl chloride **17** was prepared using reported procedures [14], which is shown in Scheme 2.

To explore the tolerance of the diketo acid chain, compound **21** was prepared by using the same procedures as **12** starting from intermediate **8** (Scheme 3). Interestingly, treatment of ester **19** with excess amount of 4-fluorobenzylamine can lead to a novel raltegravir analogs **22** (Scheme 4). As shown in Scheme 5, treatment of compounds **11** and **20** with varieties of carbonyl or sulfonyl chlorides can easily afford 5-hydroxyl substituted compounds **23**–**30**.

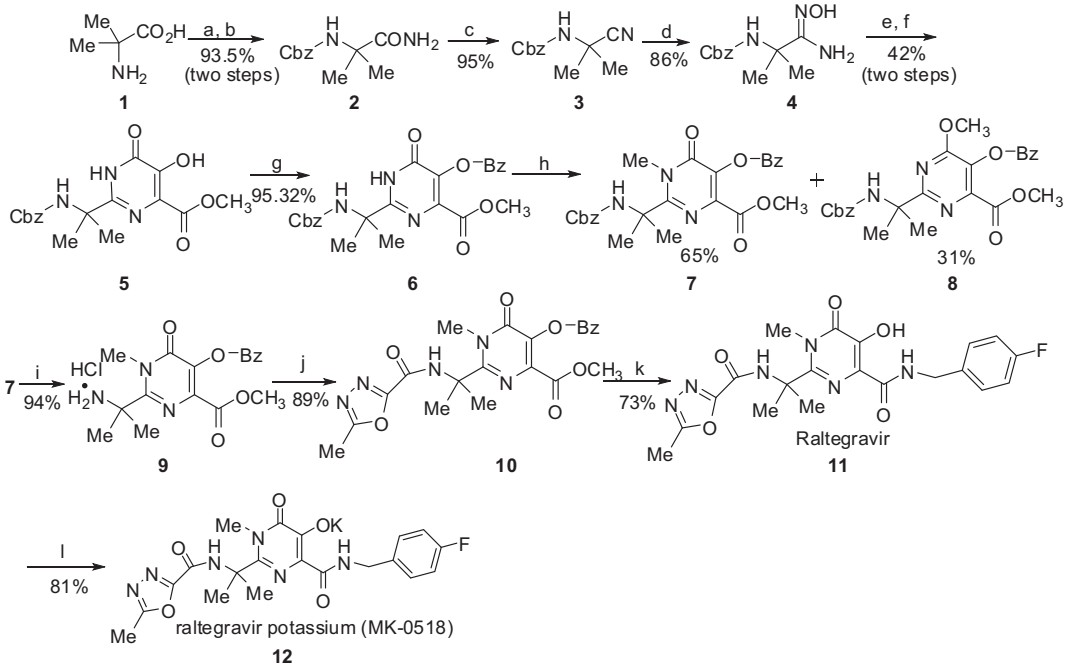
To explore the synergy effects of these compounds, a series of 5-hydroxyl substituted *N*,*N*′-diethyloxamino group containing compounds **31**–**42** were prepared by using the similar procedures for preparation compounds **20**–**30** (Scheme 6).

3. Biological results and discussion

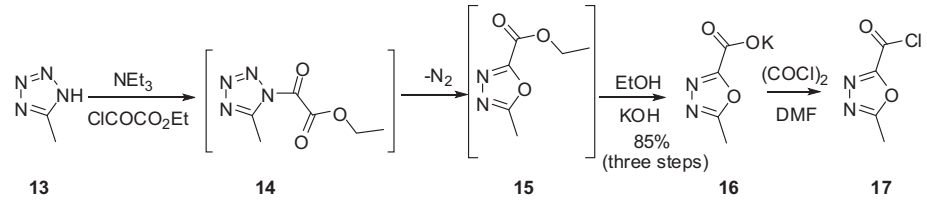
3.1. Method for high-throughput cell-based assay

3.1.1. Pseudotyped virus

The pseudotyped virus was generated by transfection of two plasmids, one coding for envelope, the other for the backbone. The glycoprotein G from Vesicular Stomatitis Virus (VSV-G) was selected as the envelope protein, because it allows the infection of a very wide range of different cell types [15]. Furthermore, VSV-G pseudotyped viruses have been shown to provide high titers [15]. Because the target of our screening was HIV-1 replication, the pSG3$^{\Delta env}$ was used as the backbone, which was derived from pSG3.1 by SpeI partial digestion, Klenow filling of the 3′ recessed



Scheme 1. Synthesis of raltegravir potassium (MK-0518). a: Cbz-Cl, K_2CO_3; b: $ClCO_2C_2H_5$, $NH_3{\cdot}H_2O$; c: $(CF_3CO)_2O$, NEt_3; d: NH_2OH HCl, KOH; e: DMADC, $CHCl_3$; f: xylene, reflux; g: pyridine, $(PhCO)_2O$; h: Me_2SO_4, LiH, 1,4-Dioxane; i: 10% Pd/C, H_2, HCl; j: **17**, NEt_3; k: 4-Fluorobenzylamine, MeOH; l: 0.5 N KOH, CH_3CN.



Scheme 2. Synthesis of 5-Methyl-1,3,4-oxadiazole-2-carbonyl chloride (**17**).

ends and religation. This introduced a four nucleotide insertion mutation (CTAG) in *env* and a stop codon. In the producer cells only the transcript from the proviral plasmid was packaged into the capsid due to the presence of the packaging signal. During the budding process, capsids were enveloped by the VSV-G proteins expressed on the cell surface prior to their release into the medium. Production of pseudotyped viruses is a single infectious cycle.

3.1.2. TZM-bl cell line

The TZM-bl cell line used as indicator cell in our experiment, obtained through the AIDS Research and Reference Reagent Program, is a HeLa cell line derivative that expresses high levels of CD4 and CCR5 along with endogenously expressed CXCR4. TZM-bl cells contain HIV LTR-driven β-galactosidase and luciferase reporter cassettes that are activated by HIV tat expression. These virions are valuable reagents for antiviral screening since they allow a reduction in viral RNA replication to be measured by direct enzymatic quantification of luciferase in the cell lysate using a simple add-and-read type of cell-based assay.

3.1.3. Cellular cytotoxicity screening

The cytotoxicity of compounds was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method [16]. Briefly, TZM-bl cells were plated in 96-well plates and incubated for 18 h at 37 °C, followed by a second incubation for 48 h in the presence of the test compounds. MTT (20 uL at 5 mg/mL) was added to each well and incubated for 3 h at 37 °C. The plates were placed on a shaker for 10 min at room temperature and the OD_{550} measured using a Spectra MAX340 microplate reader (Molecular Devices) with a reference wave length at 690 nm.

3.1.4. Drug screening

Compounds were prepared from initial dimethyl sulfoxide (DMSO) stocks and plated as described. TZM-bl cells were seeded at concentration of 10^4 cells/well. The following day, samples and controls (AZT and MK-0518) were then added. Finally, the pseudotyped virus was added with final concentration of DMSO in all wells was maintained at 2%. The plates were incubated at 37 °C in a humidified CO_2 incubator for 48 h. Steady-glow substrate (Promega) was added directly to each well and cell lysis was allowed to proceed in the dark for 20 min. Luciferase activity was measured using the Envision microplate luminometer (PerkinElmer).

The above assay is robust, flexible and compatible with medium to high throughput. We have validated it for inhibitors of HIV replication using commercial AZT and MK-0518 as controls. And the similar method also reported by Garcia and co-workers [17].

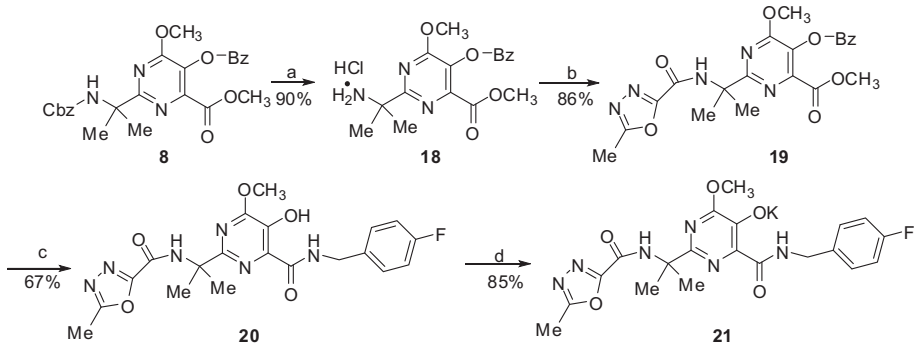
3.2. Biological evaluation of synthesized compounds against HIV

The first toxic testing toward TZM-bl cells indicates that all the synthesized compounds **20**–**42** showed no toxicity towards TZM-bl cells.

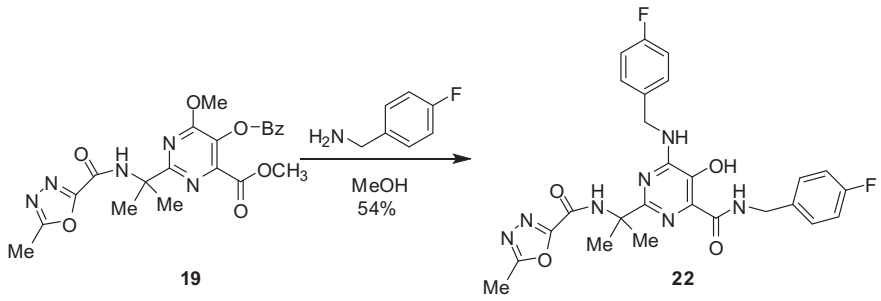
A primary inhibition assay of compounds **20**–**42** at 10 μM were evaluated by using cell-based method above. As shown in Table 1, most of synthesized compounds exhibited high anti-HIV activity. Replacement of 4-fluorobenzylamino group with methoxyl abolished activity underlining the importance of the benzylamino moiety in this part of the molecule. Replacement of methoxyl of **20** with 4-fluorobenzylamino group decreased the anti-HIV activity. Maintaining the 6-methoxy and 4-fluorobenzylamino group, replacing of 5-methyl-1,3,4-oxadiazole-2-carboxamino group with *N*,*N*′-diethyloxamino group also decreased anti-HIV activity. In addition, the 6-*O*-methylated compounds (**20**, **21**, **27**–**30** and **38**–**42**) exhibited lower inhibition rate than their corresponding 1-*N*-methylated compounds (MK-0518, **23**–**26** and **32**–**36**), which indicates that the 1-N-methylation is favorable for antiviral activity.

To further evaluate the anti-HIV activity of the raltegravir derivatives and systematically study the SAR, the IC_{50} values of **21**, **23**–**30** and **32**–**36** were calculated and results were shown in Table 1. Commercially available inhibitors MK-0518 and AZT were used as the controls, which exhibited 8 nM and 30 nM of IC_{50} values respectively.

Most of the tested compounds exhibited higher antiviral activity than that of MK-0518 and AZT. The 5-hydroxyl modification of raltegravir significantly increased the anti-HIV activity, which indicates that the 5-hydroxyl may not be indispensable for raltegravir and the introducing of acyl at 5-hydroxyl is favorable for



Scheme 3. Synthesis of compound **21**. a: 10% Pd/C, H_2, HCl; b: **17**, NEt_3; c: 4-Fluorobenzylamine, MeOH; d: 0.5 N KOH, MeOH.



Scheme 4. Synthesis of compound **22**.

antiviral activity. The similar tendency also reflected by other compounds. Among the 5-substituents, the benzoyl group showed the best activity, since both **25** and **35** displayed subpicomole IC_{50} values.

The structural difference between **21** and MK-0518 lies in the position of methyl on the pyrimidine ring, which led to a 25-fold decrease in antiviral activity. The similar tendency also reflected by other compounds. Above results further indicate that the 2-N-methylation is favorable for antiviral activity.

Interestingly, among the compounds **27**–**30**, acyl substituted compound **27** exhibited higher antiviral activity, the tendency is different from that of **23**–**26** (IC_{50}: **25** < **26** < **23** < **24**) and **33**–**36** (IC_{50}: **35** < **33** < **36** < **34**). In addition, maintaining the 1-*N*-methyl group and replacing of 5-methyl-1,3,4-oxadiazole-2-carboxamino group with *N*,*N*′-diethyloxamino group increased the anti-HIV activity except for **35** and **36**. However, maintaining the 6-*O*-methyl group and replacing of 5-methyl-1,3,4-oxadiazole-2-carboxamino group with *N*,*N*′-diethyloxamino group dramatically decreased the anti-HIV activity. The above results suggest that the synergy effects are conspicuous for raltegravir derivatives. It should be mentioned that, new compounds **25** and **35** with subpicomole IC_{50} values seemed to be the most potent anti-HIV agents among all of the reported synthesized compounds.

4. Conclusion

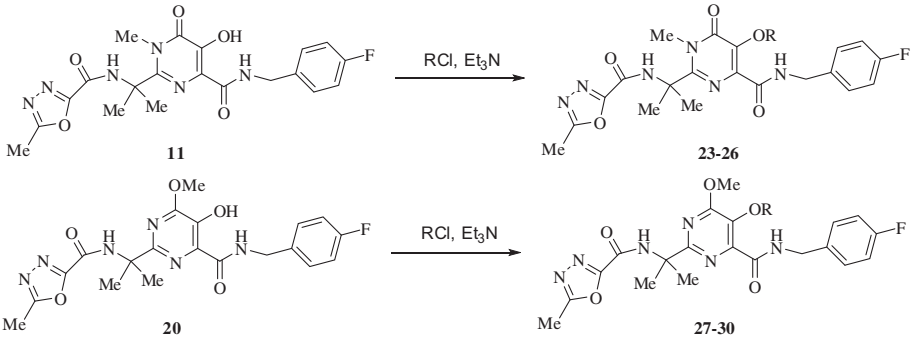
To systematically study the SAR of raltegravir derivatives, compounds **20**–**42** were designed, synthesized and evaluated for their antiviral activity against HIV. The bioassay results indicate that most of these compounds possess good anti-HIV activity. Especially, compounds **25** and **35** with subpicomole IC_{50} values seemed to be the most potent anti-HIV agents among all of the reported synthesized compounds, so and thus emerged as new potent anti-HIV agents. Replacement of 4-fluorobenzylamino group with methoxy group abolished activity underlining the importance of the benzylamino moiety in this part of the molecule. The 5-hydroxyl modification of raltegravir significantly increased the anti-HIV activity, which indicates that the hydroxyl may not be indispensable for raltegravir and the introducing of acyl at 5-position is favorable for antiviral activity. A high-throughput cell-based assay with pseudotyped virus stocks was developed and used to identify HIV inhibitors.

5. Experimental

The melting points were determined with an X-4 binocular microscope melting-point apparatus (Beijing Tech Instruments Co., Beijing, China) and were uncorrected. ^{1}H NMR spectra were obtained by using Bruker AV 400, Bruker AV300 and a Varian Mercury Plus 400 MHz spectrometer. Chemical shifts (δ) were given in parts per million (ppm) and were measured downfield from internal tetramethylsilane. ^{13}C NMR spectra were recorded by using Bruker AV 400 (100 MHz) and Bruker AV300 (75 MHz) with $CDCl_3$ or DMSO-d_6 as a solvent. Chemical shifts (δ) are reported in parts per million using the solvent peak. Elemental analyses were determined on a Yanaco C, H, N Corder MT-3 elemental analyzer. High-resolution mass spectra were obtained with an FT-ICR MS spectrometer (Ionspec, 7.0 T). All anhydrous solvents were dried and purified by standard techniques just before use.

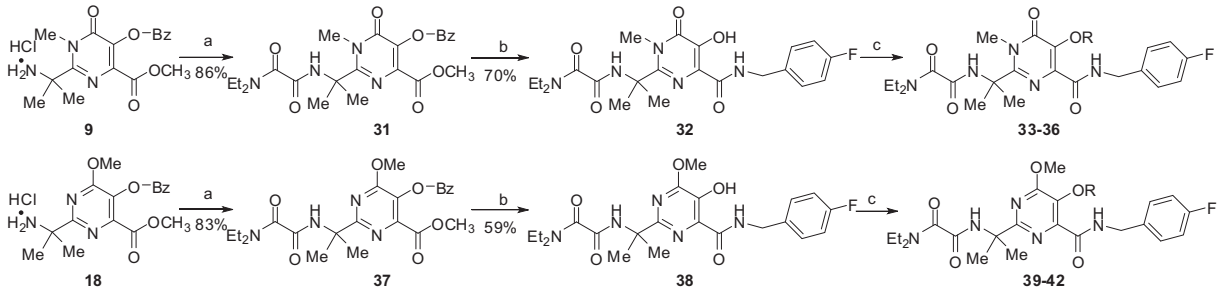
5.1. Benzyl 1-amino-2-methyl-1-oxopropan-2-ylcarbamate (*2*)

To a solution of acid **1** (0.49 mol) and K_2CO_3 (1.46 mol) in H_2O (1 L) and 1,4-dioxane (0.5 L) was added dropwise the solution of Cbz-Cl (0.73 mol) in 1,4-dioxane (50 mL) at 0 °C. The mixture was stirred at room temperature for 10 h, then washed with diethyl ether (0.5 L) and acidified to a pH of 2 with 10% HCl at 0 °C, then extracted with ethyl acetate (3 × 0.5 L). The combined organic



23, **27**: R = Ac; **24**, **28**: R = Ms; **25**, **29**: R = Bz; **26**, **30**: R = mesitylene sulfonyl.

Scheme 5. Synthesis of compounds **23**–**30**.



33, **39**: R = Ac; **34**, **40**: R = Ms; **35**, **41**: R = Bz; **36**, **42**: R = mesitylene sulfonyl.

Scheme 6. Synthesis of compounds **31**–**42**. a: $ClCOCONEt_2$, NEt_3; b: 4-Fluorobenzylamine, MeOH; c: RCl, NEt_3.

extracts were dried with anhydrous $MgSO_4$ and concentrated in vacuo to give crude 2-(benzyloxycarbonylamino)-2-methylpropanoic acid as a colorless oil, which was directly dissolved with THF (1.6 L) and Et_3N (0.63 mol). To the stirred mixture was added dropwise ethyl chloroformate (0.58 mol) at −15 °C and reacted for 1 h at this temperature, then 25% $NH_3 \cdot H_2O$ (99 g) was added. The mixture was allowed to warm to room temperature for 10 h and 60 °C for 1 h, then concentrated in vacuo, brine (200 mL) was added, and stayed at 0 °C for 1 h, filtered to give compound **2** (107 g, 93.5% yield for two steps). 2-(Benzyloxycarbonylamino)-2-methylpropanoic acid: mp 65–66 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.24 (brs, 1H, COOH), 7.35 (s, 5H, ArH), 5.39 (brs, 1H, NH), 5.10 (s, 2H, $ArCH_2$), 1.58 (s, 6H, Me); Compound **2**: mp 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (s, 5H, ArH), 5.41 (brs, 1H, NH), 5.10 (s, 2H, $ArCH_2$), 1.73 (s, 2H, NH_2), 1.55 (s, 6H, Me).

5.2. Benzyl 2-cyanopropan-2-ylcarbamate (*3*)

To a stirred solution of compound **2** (96 mmol) and Et_3N (0.21 mol) in CH_2Cl_2 (0.5 L) was added trifluoroacetic anhydride (0.11 mol) at 0 °C under N_2. The mixture was allowed to warm to room temperature for 10 h, then washed with saturated aq. $NaHCO_3$ solution (100 mL), H_2O (100 mL) and brine (100 mL), and dried with anhydrous $MgSO_4$ and concentrated in vacuo to give nitrile **3** (19.9 g, 95% yield). Mp 75–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (s, 5H, ArH), 5.14 (s, 2H, $ArCH_2$), 5.03 (brs, 1H, NH), 1.69 (s, 6H, Me).

5.3. Benzyl 1-amino-1-(hydroxyimino)-2-methylpropan-2-ylcarbamate (*4*)

Hydroxylamine hydrochloride (0.12 mol) was added to an equimolar stirred solution of potassium hydroxide in H_2O (7.5 mL) at 0 °C. The mixture was stirred for 15 min then added to the solution of nitrile **3** (0.1 mol) in isopropanol at 60 °C, and the solution was stirred for 4 h, then cooled to 0–5 °C and added pentane (60 mL). The mixture was stirred for 2 h, then filtered and washed with brine to afford the title product **4** (21.6 g, 86% yield). Mp 162–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (brs, 1H, NOH), 7.35 (s, 5H, ArH), 7.11 (brs, 1H, NH), 5.22 (brs, 2H, $ArCH_2$), 4.98 (s, 2H, NH_2), 1.38 (s, 6H, Me).

Table 1
Inhibition activity of synthesized compounds **20**–**42** toward HIV.

Compd.	Inhibition rate[a] (10 μM) (%)	IC_{50}[a] (nM)	Compd.	Inhibition rate[a] (10 μM) (%)	IC_{50}[a] (nM)
20	94.1	–	**33**	94.2	6.2×10^{-2}
21	92.9	2.0×10^{2}	**34**	93.7	6.4×10^{-1}
22	47.5	–	**35**	92.8	8.0×10^{-5}
23	95.1	1.0	**36**	77.7	0.3
24	92.7	2.4	**37**	19.8	–
25	100	4.0×10^{-5}	**38**	44.9	–
26	94.9	4.0×10^{-2}	**39**	59.3	–
27	94.2	7.3	**40**	28.6	–
28	88.3	32.8	**41**	52.1	–
29	93.4	14.7	**42**	6.0	–
30	79.7	1.2×10^{3}	**MK-0518**	95.1	8
31	−2.7	–	**AZT**	100	30
32	95.5	0.5			

"–": IC_{50} values were not tested.
[a] Values are measured with cell-based assay method with pseudotyped virus stocks.

5.4. Methyl 2-(2-(benzyloxycarbonylamino)propan-2-yl)-5-hydroxy-6-oxo-1,6-dihydropyrimidine-4-carboxylate (*5*)

To a solution of compound **4** (0.08 mol) in chloroform (500 mL) was added dimethyl acetylenedicarboxylate (0.09 mol) and the reaction was stirred at −10 °C for 3 h, room temperature for 2 h and then reflux for 5 h. After cooling to room temperature, the mixture was washed with H_2O (3 × 0.5 L), dried with anhydrous $MgSO_4$ and concentrated in vacuo. The resulting residue was taken into xylene (300 ml) and heated at 90 °C for 2 h, 125 °C for 2 h then refluxed for 16 h at N_2. After cooling to room temperature, the reaction mixture was concentrated in vacuo and added MeOH (30 mL) and diethyl ether (280 mL). The mixture was stayed at 0 °C for 36 h then filtered to give compound **5** (12.6 g, 42% yield for two steps). Mp 182–183 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.61 (brs, 1H, NH), 10.27 (brs, 1H, OH), 7.48 (brs, 1H, NH), 7.35 (s, 5H, ArH), 4.98 (s, 2H, $ArCH_2$), 3.82 (s, 3H, CO_2CH_3), 1.47 (s, 6H, Me).

5.5. Methyl 5-(benzoyloxy)-2-(2-(benzyloxycarbonylamino)propan-2-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxylate (*6*)

The solution of compound **5** (0.05 mol) and benzoic anhydride (0.06 mol) in pyridine (30 mL) was stirred at room temperature for 10 h and concentrated in vacuo. The resulting residue was taken into ethyl acetate (350 ml), washed with 5% HCl, dried with anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (PE/EA, 3:2, v/v) to afford compound **6** (23.23 g, 95.32% yield). Mp 56–58 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.32 (brs, 1H, NH), 8.14 (d, $J = 7.2$ Hz, 2H, ArH), 7.62–7.67 (m, 1H, ArH), 7.42–7.58 (m, 2H, ArH), 7.31 (s, 5H, ArH), 5.55 (brs, 1H, NH), 5.04 (s, 2H, $ArCH_2$), 3.82 (s, 3H, CO_2CH_3), 1.66 (s, 6H, Me).

5.6. Methyl 5-(benzoyloxy)-2-(2-(benzyloxycarbonylamino) propan-2-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (7) and methyl 5-(benzoyloxy)-2-(2-(benzyloxycarbonylamino) propan-2-yl)-6-methoxypyrimidine-4-carboxylate (*8*)

The solution of compound **6** (0.05 mol) and LiH (0.08 mol) in 1,4-dioxane (180 mL) was stirred at 40 °C for 1 h, then cooled to room temperature and added dropwise $(CH_3)_2SO_4$ (0.10 mol). The

mixture stirred at 60 °C for 3 h and concentrated in vacuo. The resulting residue was taken into ethyl acetate (200 ml), washed with saturated aq. $NaHCO_3$ solution (100 mL) and brine(100 mL), then dried with anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (PE/EA, 3:2, v/v) to afford compound **7** (15.55 g, 65% yield) and compound **8** (7.4 g, 31% yield). For **7**: mp 141–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, $J = 7.5$ Hz, 2H, ArH), 7.64 (t, $J = 6.9$ Hz, 1H, ArH), 7.51 (t, $J = 7.2$ Hz, 2H, ArH), 7.33 (s, 5H, ArH), 5.42 (brs, 1H, NH), 5.04 (s, 2H, $ArCH_2$), 3.80 (s, 3H, CO_2CH_3), 3.65 (brs, 3H, NCH_3), 1.74 (s, 6H, Me). For **8**: 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, $J = 7.2$ Hz, 2H, ArH), 7.68 (t, $J = 7.5$ Hz, 1H, ArH), 7.54 (t, $J = 7.9$ Hz, 2H, ArH), 7.29–7.42 (s, 5H, ArH), 6.62 (brs, 1H, NH), 5.11 (s, 2H, $ArCH_2$), 4.01 (brs, 3H, OMe), 3.83 (s, 3H, CO_2CH_3), 1.80 (s, 6H, Me).

5.7. 2-[5-(Benzoyloxy)-4-(methoxycarbonyl)-1-methyl-6-oxo-1, 6-dihydropyrimidin-2-yl]propan-2-amine hydrochloride (**9**)

A solution of compound **7** (6.26 mol) in MeOH (180 mL) and 1 equiv of 6 N HCl (1.04 mL) was stirred in the presence of 10% Pd/C (600 mg) under an H_2 atmosphere at atmospheric pressure for 3 h. The mixture was then filtered and the Pd residues washed with MeOH (100 mL). The combined organics were concentrated under reduced pressure to yield the title compound **9** (2.25 g, 94% yield). Mp 227–229 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.79 (brs, 3H, HCl, NH_2), 8.09 (d, $J = 7.7$ Hz, 2H, ArH), 7.81 (t, $J = 7.2$ Hz, 1H, ArH), 7.65 (t, $J = 7.7$ Hz, 2H, ArH), 3.79 (s, 3H, CO_2CH_3), 3.63 (s, 3H, NMe), 1.82 (s, 6H, Me).

5.8. Methyl 5-(benzoyloxy)-1-methyl-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxylate (**10**)

To a stirred suspension of potassium 5-methyl-1,3,4-oxadiazole-2-carboxylate (12.5 mmol) in oxalyl chloride (180 mL) at room temperature, a solution of oxalylchloride (11.9 mmol) in dichloromethane (15 mL) was added dropwise over 10 min. DMF (10 drops) was added, and vigorous gas evolution was observed. The resulting reaction mixture was stirred for 50 min, then cooled to 0 °C and stirred for a further 10 min. Then, a solution of compound **9** (6.3 mmol) and Et_3N (25.1 mmol) in dichloromethane (180 mL) was added dropwise over 15 min. The resulting solution was stirred for 2 h, and then the mixture was washed with saturated aq. $NaHCO_3$ solution (150 mL), H_2O (150 mL), and brine (150 mL). The mixture was dried (Na_2SO_4) and concentrated under reduced pressure. The material was purified by column chromatography on silica gel (PE/EA, 1:4, v/v) to afford compound **10** (2.55 g, 89% yield). Mp 200–202 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.03 (s, 1H, NH), 8.09 (d, $J = 7.7$ Hz, 2H, ArH), 7.79 (t, $J = 7.3$ Hz, 1H, ArH), 7.63 (t, $J = 7.7$ Hz, 2H, ArH), 3.78 (s, 3H, CO_2CH_3), 3.56 (s, 3H, NMe), 2.58 (s, 3H, OCMe), 1.75 (s, 6H, Me).

5.9. Raltegravir (**11**)

The solution of compound **10** (5.7 mmol) and 4-fluorobenzylamine (17.1 mmol) in MeOH (150 mL) was refluxed for 20 h under N_2, then concentrated under reduced pressure. The resulting residue was taken into 0.5 N NaOH solution (250 mL) and washed with diethyl ether (3 × 100 mL), then acidified to a pH of 2 with 6 N HCl at 0 °C and extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and concentrated in vacuo to give raltegravir (1.93 g, 73% yield). Mp 140–142 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.22 (s, 1H, NH), 9.87 (s, 1H, NH), 9.10 (brs, 1H, OH), 7.40 (dd, $J = 8.1, 5.9$ Hz, 2H, ArH), 7.18 (t, $J = 8.9$ Hz, 2H, ArH), 4.51 (d, $J = 6.1$ Hz, 2H, $ArCH_2$), 3.48 (s, 3H, NMe), 2.57 (s, 3H, OCMe), 1.74 (s, 6H, Me); HRMS (ESI) calcd for $C_{20}H_{21}FN_6NaO_5$ $(M + Na)^+$ 467.1450, found 467.1442.

5.10. Raltegravir potassium (**12**)

A standard 0.5 N solution of KOH in H_2O (4.5 mL) was added to a solution of **11** (2.3 mmol) in MeCN (40 mL), and the mixture was stirred at room temperature for 20 min then concentrated under reduced pressure. The resulting residue was taken into EtOH (50 mL), and refluxed for 10 min, then cooled to 0 °C, and left to stand for 4 h. The resulting crystals were filtered to give compound 12 (0.88 g, 81% yield). Mp 275–277 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 11.74 (brs, 1H, NH), 9.74 (s, 1H, NH), 7.28–7.37 (m, 2H, ArH), 7.12 (t, $J = 7.8$ Hz, 2H, ArH), 4.43 (d, $J = 3.4$ Hz, 2H, $ArCH_2$), 3.38 (s, 3H, NMe), 2.57 (s, 3H, OCMe), 1.69 (s, 6H, Me).

5.11. Potassium 5-methyl-1,3,4-oxadiazole-2-carboxylate (**16**)

The stirred solution of 5-methyl-1H-tertazole (0.24 mol) and Et_3N (0.25 mol) in toluene (300 mL) was added dropwise a solution of ethyl oxalylchloride (0.25 mol) in toluene (60 mL) at 0 °C. The mixture was stirred for a further hour, filtered, and the solid was washed with cold toluene (300 mL). The combined filtrates were kept at 0 °C and were slowly added to a hot solution of toluene (50 °C, 150 mL) over 90 min, then the solution was stirred at 65 °C for 1 h. After cooling to room temperature, the solution was washed with bine (200 mL) and dried with anhydrous $MgSO_4$ and concentrated in vacuo. The resulting residue was taken into EtOH (400 mL), cooled to 10 °C, then 20% KOH (70 mL) was added. The mixture was stirred at room temperature for 40 min, then cooled to 0 °C, and left to stand for 30 min, then filtered to afford compound **16** (33.71 g, 85.3% yield). Mp 255–257 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 2.71 (s, 3H, Me).

5.12. 2-[5-(Benzoyloxy)-4-(methoxycarbonyl)-6-methoxypyrimidin-2-yl]propan-2-amine hydrochloride (**18**)

Following the procedure described for **9**, treatment of **8** with 10% Pd/C under an H_2 atmosphere to afford the title compound **18** in 90% yield. Mp 206–208 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.70 (brs, 3H, HCl, NH_2), 8.12 (d, $J = 7.4$ Hz, 2H, ArH), 7.82 (t, $J = 7.5$ Hz, 1H, ArH), 7.65 (t, $J = 7.8$ Hz, 2H, ArH), 4.11 (s, 3H, OMe), 3.80 (s, 3H, CO_2CH_3), 1.69 (s, 6H, Me).

5.13. Methyl 5-(benzoyloxy)-6-methoxy-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)pyrimidine-4-carboxylate (**19**)

Following the procedure described for **10**, treatment of **18** with 5-methyl-1,3,4-oxadiazole-2-carboxylic chloride to afford the title compound **19** in 86% yield. Mp 98–100 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.17 (s, 1H, NH), 8.20 (d, $J = 7.4$ Hz, 2H, ArH), 7.68 (t, $J = 7.5$ Hz, 1H, ArH), 7.54 (t, $J = 7.8$ Hz, 2H, ArH), 4.11 (s, 3H, OMe), 3.88 (s, 3H, CO_2CH_3), 2.64 (s, 3H, OCMe), 1.94 (s, 6H, Me).

5.14. N-(2-(4-(4-fluorobenzylcarbamoyl)-5-hydroxy-6-methoxypyrimidin-2-yl)propan-2-yl)-5-methyl-1,3,4-oxadiazole-2-carboxamide (**20**)

Following the procedure described for **11**, treatment of **19** with 4-fluorobenzylamine, then purified by column chromatography on silica gel (PE/EA, 3:1, v/v) to afford compound **20** in 67% yield. Mp 161–162 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.08 (s, 1H, NH), 9.59 (s, 1H, NH), 9.25 (s, 1H, OH), 7.40 (dd, $J = 8.3, 5.8$ Hz, 2H,

ArH), 7.18 (t, $J = 8.8$ Hz, 2H, ArH), 4.51 (d, $J = 6.3$ Hz, 2H, $ArCH_2$), 3.94 (s, 3H, OMe), 2.56 (s, 3H, OCMe), 1.74 (s, 6H, Me); HRMS (ESI) calcd for $C_{20}H_{21}FN_6NaO_5$ $(M + Na)^+$ 467.1450, found 467.1442.

5.15. Potassium 4-(4-fluorobenzylcarbamoyl)-6-methoxy-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)pyrimidin-5-olate (**21**)

To a solution of KOH (0.5 mmol) in MeOH (10 mL) was added the solution of compound **20** (0.5 mmol) in MeOH. The mixture was stirred at room temperature for 1 h, and concentrated in vacuo, then taken into EtOH (5 mL). The solution was cooled to 0 °C, and left to stand for 10 h, then filtered to afford compound **21** (0.21 g, 85% yield). Mp 285–287 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.18 (s, 1H, NH), 9.80 (s, 1H, NH), 7.34 (t, $J = 7.9$ Hz, 2H, ArH), 7.13 (t, $J = 8.8$ Hz, 2H, ArH), 4.47 (d, $J = 5.6$ Hz, 2H, ArCH_2), 3.81 (s, 3H, OMe), 2.60 (s, 3H, OCCH_3), 1.70 (s, 6H, C$(CH_3)_2$).

5.16. N-(2-(4-(4-fluorobenzylamino)-6-(4-fluorobenzylcarbamoyl)-5-hydroxypyrimidin-2-yl)propan-2-yl)-5-methyl-1,3,4-oxadiazole-2-carboxamide (**22**)

The solution of compound **19** (3.3 mmol) and 4-fluorobenzylamine (33 mmol) in MeOH (30 mL) was refluxed for 30 h under N_2, and concentrated in vacuo, then taken into dichloromethane (150 mL). The mixture was washed with 1 N HCl, and dried with anhydrous Na_2SO_4, then concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to afford compound **22** in 54% yield. Mp 177–178 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.91 (s, 1H, NH), 9.34 (s, 1H, OH), 8.23 (br, 1H, NH), 7.37 (m, 4H, ArH), 7.04 (m, 4H, ArH), 6.08 (t, $J = 5.6$ Hz, 1H, NH), 4.72 (d, $J = 5.9$ Hz, 2H, $ArCH_2$), 4.62 (d, $J = 6.2$ Hz, 2H, $ArCH_2$), 2.62 (s, 3H, OCMe), 1.79 (s, 6H, Me); Anal. Calcd. for $C_{26}H_{25}F_2N_7O_4$: C, 58.10; H, 4.69; N, 18.24. Found: C, 58.40; H, 4.58; N, 18.50.

5.17. Methyl 5-(benzoyloxy)-2-(2-(2-(diethylamino)-2-oxoacetamido)propan-2-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (**31**)

Following the procedure described for **10**, treatment of **9** with 2-(diethylamino)-2-oxoacetyl chloride to afford the title compound **31** in 86% yield. Mp 158–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.92 (s, 1H, N–H), 7.61–7.68 (m, 1H, Ar–H), 7.47–7.54 (m, 2H, Ar–H), 3.81 (s, 3H, CO_2CH_3), 3.67–3.73 (m, 2H, NCH_2CH_3), 3.66 (s, 3H, NMe), 3.35–3.46 (m, 2H, NCH_2CH_3), 1.81 (s, 6H, C$(CH_3)_2$), 1.14–1.23 (m, 6H, NCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 163.3, 160.4, 160.1, 159.5, 158.5, 139.7, 136.9, 133.9, 130.6, 128.6, 57.7, 52.8, 43.4, 42.5, 42.3, 38.3, 33.3, 27.2, 14.8, 14.0, 12.6, 12.5; *Anal*. Calcd. for $C_{23}H_{28}N_4O_7$: C, 58.47; H, 5.97; N, 11.86. Found: C, 58.27; H, 6.04; N, 11.87.

5.18. N^1,N^1-Diethyl-N^2-(2-(4-(4-fluorobenzylcarbamoyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)propan-2-yl)oxalamide (**32**)

Following the procedure described for **11**, treatment of **31** with 4-fluorobenzylamine to afford the title compound **32** in 70% yield. Mp 108–110 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.21(s, 1H, OH), 9.37 (s, 1H, N–H), 9.09 (t, $J = 5.6$ Hz, 1H, N–H), 7.40 (t, $J = 7.3$ Hz, 2H, Ar–H), 7.17 (t, $J = 8.7$ Hz, 2H, Ar–H), 4.51 (d, $J = 5.9$ Hz, 2H, CH_2), 3.56 (s, 3H, NMe), 3.21–3.33 (m, 4H, NCH_2CH_3), 1.67 (s, 6H, C$(CH_3)_2$), 1.05–1.12 (m, 6H, NCH_2CH_3); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 168.4, 163.6, 163.4, 162.5, 160.1, 158.5, 152.0, 145.6, 134.9, 129.5, 129.5, 124.4, 115.2, 115.0, 56.6, 41.6, 38.2, 32.7, 26.9, 14.1, 12.5; HRMS (ESI) calcd for $C_{22}H_{29}FN_5O_5$ $(M + H)^+$ 462.2147, found 462.2140.

5.19. Methyl 5-(benzoyloxy)-2-(2-(2-(diethylamino)-2-oxoacetamido)propan-2-yl)-6-methoxypyrimidine-4-carboxylate (**37**)

Following the procedure described for **10**, treatment of **18** with 2-(diethylamino)-2-oxoacetyl chloride to afford the title compound **37** in 83% yield. Mp 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1H, N–H), 8.21 (dd, $J = 1.3$, 8.5 Hz, 2H, Ar–H), 7.69 (t, $J = 6.3$ Hz, 1H, Ar–H), 7.55 (t, $J = 7.9$ Hz, 2H, Ar–H), 4.09 (s, 3H, OMe), 3.86 (s, 3H, CO_2CH_3), 3.75 (q, $J = 7.0$, 14.1 Hz, 2H, NCH_2CH_3), 3.45 (q, $J = 7.1$, 14.3 Hz, 2H, NCH_2CH_3), 1.87 (s, 6H, C$(CH_3)_2$), 1.28 (t, $J = 7.0$ Hz, 3H, NCH_2CH_3), 1.22 (t, $J = 7.1$ Hz, 3H, NCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 163.9, 163.7, 163.1, 162.5, 161.1, 146.8, 134.1, 131.8, 130.5, 128.7, 128.2, 58.3, 55.1, 53.1, 43.3, 41.9, 26.4, 14.7, 12.5; *Anal*. Calcd. for $C_{23}H_{28}N_4O_7$: C, 58.47; H, 5.97; N, 11.86. Found: C, 58.53; H, 6.10; N, 11.79.

5.20. N^1,N^1-Diethyl-N^2-(2-(4-(4-fluorobenzylcarbamoyl)-5-hydroxy-6-methoxypyrimidin-2-yl)propan-2-yl)oxalamide (**38**)

Following the procedure described for **20**, treatment of **37** with 4-fluorobenzylamine to afford the title compound **38** in 59% yield. Mp 189–191 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.00 (s, 1H, OH), 9.66 (t, $J = 6.3$ Hz, 1H, N–H), 8.82 (s, 1H, N–H), 7.41 (dd, $J = 5.7$, 8.5 Hz, 2H, Ar–H), 7.17 (t, $J = 8.9$ Hz, 2H, Ar–H), 4.52 (d, $J = 6.4$ Hz, 2H, CH_2), 3.98 (s, 3H, OMe), 3.24–3.31 (m, 4H, NCH_2CH_3), 1.64 (s, 6H, C$(CH_3)_2$), 1.02–1.08 (m, 6H, NCH_2CH_3); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 167.9, 164.4, 163.8, 162.5, 160.2, 160.1, 160.0, 141.3, 134.6, 132.8, 129.7, 129.6, 115.2, 115.0, 79.2, 57.2, 54.0, 41.5, 37.9, 26.9, 13.9, 12.5; HRMS (ESI) calcd for $C_{22}H_{29}FN_5O_5$ $(M + H)^+$ 462.2147, found 462.2153.

5.21. General procedure for the preparation of **23**–**30**, **33**–**36** and **39**–**42**

To the solution of **11** or **20** or **32** or **38** (0.5 mmol) and Et_3N (1.4 mmol) in dichloromethane was added dropwise corresponding carbonyl or sulfonyl chloride (0.9 mmol), then stirred at room temperature or reflux for 3 h. After cooling to room temperature, the mixture was washed with saturated aq. $NaHCO_3$ (50 mL), brine (50 mL), and dried with anhydrous Na_2SO_4, then concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to afford title compounds.

5.21.1. 4-(4-Fluorobenzylcarbamoyl)-1-methyl-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)-6-oxo-1,6-dihydropyrimidin-5-yl acetate (**23**)

Treatment of **11** with acetylchloride at reflux to afford **23** in 87% yield, mp 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H, CO$NHC(CH_3)_2$), 7.97 (t, $J = 5.9$ Hz, 1H, CO$NHCH_2$), 7.33 (dd, $J = 5.4$, 8.3 Hz, 2H, Ar–H), 7.03 (t, $J = 8.6$ Hz, 2H, Ar–H), 4.57 (d, $J = 6.1$ Hz, 2H, Ar-CH_2), 3.66 (s, 3H, NMe), 2.63 (s, 3H, CH_3CNN), 2.42 (s, 3H, CH_3CO_2), 1.87 (s, 6H, NHC$(CH_3)_2$); HRMS (ESI) calcd for $C_{22}H_{24}FN_6O_6$ $(M + H)^+$ 487.1736, found 487.1744.

5.21.2. 4-(4-Fluorobenzylcarbamoyl)-1-methyl-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)-6-oxo-1,6-dihydropyrimidin-5-yl methanesulfonate (**24**)

Treatment of **11** with methanesulfonyl chloride at reflux to afford **24** in 94% yield, mp 101–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H, CO$NHC(CH_3)_2$), 7.88 (t, $J = 5.8$ Hz, 1H, CO$NHCH_2$), 7.31–7.35 (m, 2H, Ar–H), 7.03 (t, $J = 8.7$ Hz, 2H, Ar–H), 4.60 (d, $J = 6.0$ Hz, 2H, CH_2), 3.67 (s, 3H, NMe), 3.54 (s, 3H, CH_3SO_3), 2.61 (s, 3H, CH_3CNN), 1.86 (s, 6H, NHC$(CH_3)_2$); HRMS (ESI) calcd for $C_{21}H_{23}FN_6NaO_7S$ $(M + Na)^+$ 545.1225, found 545.1222.

*5.21.3. 4-(4-Fluorobenzylcarbamoyl)-1-methyl-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)-6-oxo-1,6-dihydropyrimidin-5-yl benzoate (**25**)*

Treatment of **11** with benzoyl chloride at room temperature to afford **25** in 89% yield, mp 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (d, J = 7.1 Hz, 2H, Ar–H), 8.06 (s, 1H, NH), 7.98 (s, 1H, NH), 7.65 (t, J = 5.9 Hz, 1H, Ar–H), 7.48–7.56 (m, 2H, Ar–H), 7.30–7.34 (m, 2H, Ar–H), 7.03 (t, J = 7.2 Hz, 2H, Ar–H), 4.57 (d, J = 4.8 Hz, 2H, CH_2), 3.71 (s, 3H, NMe), 2.66 (s, 3H, CH_3CNN), 1.91 (s, 6H, NHC(CH_3)$_2$); HRMS (ESI) calcd for $C_{27}H_{25}FN_6NaO_6$ $(M + Na)^+$ 571.1712, found 571.1715.

*5.21.4. 4-(4-Fluorobenzylcarbamoyl)-1-methyl-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)-6-oxo-1,6-dihydropyrimidin-5-yl 2,4,6-trimethylbenzenesulfonate (**26**)*

Treatment of **11** with mesitylene sulfonyl chloride at reflux to afford **26** in 92% yield, mp 224–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H, CONHC(CH_3)$_2$), 7.64 (t, J = 6.0 Hz, 1H, CONHCH$_2$), 7.33–7.36 (m, 2H, Ar–H), 6.98–7.04 (m, 4H, Ar–H), 4.57 (d, J = 6.0 Hz, 2H, Ar-CH_2), 3.57 (s, 3H, NMe), 2.68 (s, 6H, Ar-CH_3), 2.62 (s, 3H, CH_3CNN), 2.31 (s, 3H, Ar-CH_3), 1.85 (s, 6H, NHC(CH_3)$_2$); HRMS (ESI) calcd for $C_{29}H_{30}FN_6O_7S$ $(M–H)^-$ 625.1886, found 625.1880.

*5.21.5. 4-(4-Fluorobenzylcarbamoyl)-6-methoxy-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)pyrimidin-5-yl acetate (**27**)*

Treatment of **20** with acetylchloride at room temperature to afford **27** in 91% yield, mp 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H, NH), 8.07 (s, 1H, NH), 7.29 (s, 2H, Ar–H), 6.97 (s, 2H, Ar–H), 4.50 (d, J = 3.3 Hz, 2H, CH_2), 4.02 (s, 3H, OMe), 2.55 (s, 3H, CH_3CNN), 2.35 (s, 3H, CH_3CO_2), 1.80 (s, 6H, NHC(CH_3)$_2$); HRMS (ESI) calcd for $C_{22}H_{23}FN_6NaO_6$ $(M + Na)^+$ 509.1555, found 509.1550.

*5.21.6. 4-(4-Fluorobenzylcarbamoyl)-6-methoxy-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)pyrimidin-5-yl methanesulfonate (**28**)*

Treatment of **20** with methanesulfonyl chloride at room temperature to afford **28** in 81% yield, mp 51–53 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H, NH), 8.10 (t, J = 2.2 Hz, 1H, NH), 7.38 (s, 2H, Ar–H), 7.06 (t, J = 1.9 Hz, 2H, Ar–H), 4.62 (s, 2H, CH_2), 4.18 (s, 3H, OMe), 3.54 (s, 3H, CH_3SO_3), 2.64 (s, 3H, CH_3CNN), 1.88 (s, 6H, NHC(CH_3)$_2$); HRMS (ESI) calcd for $C_{21}H_{23}FN_6NaO_7S$ $(M + Na)^+$ 545.1225, found 545.1230.

*5.21.7. 4-(4-Fluorobenzylcarbamoyl)-6-methoxy-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)pyrimidin-5-yl benzoate (**29**)*

Treatment of **20** with benzoyl chloride at reflux to afford **29** in 85% yield, mp 55–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 1H, N–H), 8.15 (dd, J = 7.4, 14.5 Hz, 2H, Ar–H), 7.74 (t, J = 7.7 Hz, 1H, NH), 7.61 (t, J = 7.3 Hz, 2H, Ar–H), 7.51 (t, J = 7.6 Hz, 1H, Ar–H), 7.44 (t, J = 7.5 Hz, 2H, Ar–H), 7.34 (t, J = 7.6 Hz, 1H, Ar–H), 6.93 (t, J = 8.5 Hz, 1H, Ar–H), 5.13 (s, 2H, Ar-CH_2), 3.98 (s, 3H, OMe), 2.68 (s, 3H, CH_3CNN), 1.80 (s, 6H, NHC(CH_3)$_2$); HRMS (ESI) calcd for $C_{27}H_{24}FN_6O_6$ $(M–H)^-$ 547.1747, found 547.1750.

*5.21.8. 4-(4-Fluorobenzylcarbamoyl)-6-methoxy-2-(2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl)pyrimidin-5-yl 2,4,6-trimethylbenzenesulfonate (**30**)*

Treatment of **20** with mesitylene sulfonyl chloride at reflux to afford **30** in 74% yield, mp 183–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84 (s, 1H, NH), 7.72 (t, J = 1.4 Hz, 1H, NH), 7.39 (dd, J = 3.0, 6.1 Hz, 2H, Ar–H), 7.03–7.09 (m, 4H, Ar–H), 4.58 (d, J = 5.6 Hz, 2H, CH_2), 3.84 (s, 3H, OMe), 2.65 (s, 9H, Ar–CH_3), 2.37 (s, 3H, CH_3CNN), 1.87 (s, 6H, NHC(CH_3)$_2$); HRMS (ESI) calcd for $C_{29}H_{31}FN_6NaO_7S$ $(M + Na)^+$ 649.1851, found 649.1850.

*5.21.9. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-5-yl acetate (**33**)*

Treatment of **32** with acetylchloride at room temperature to afford **33** in 87% yield, mp 76–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (s, 1H, NH), 7.96 (s, 1H, NH), 7.30 (s, 2H, Ar–H), 7.02 (s, 2H, Ar–H), 4.54 (s, 2H, CH_2), 3.63 (s, 3H, NMe), 3.33–3.72 (m, 4H, CH_3CH_2), 2.41 (s, 3H, CH_3CO_2), 1.75 (s, 6H, NHC(CH_3)$_2$), 1.13–1.23 (m, 6H, CH_3CH_2); HRMS (ESI) calcd for $C_{24}H_{30}FN_5NaO_6$ $(M + Na)^+$ 526.2072, found 526.2077.

*5.21.10. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-5-yl methanesulfonate (**34**)*

Treatment of **32** with methanesulfonyl chloride at room temperature to afford **34** in 90% yield, mp 203–205 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 1H, NH), 7.86 (s, 1H, NH), 7.33 (dd, J = 8.0, 13.2 Hz, 2H, Ar–H), 7.05 (dd, J = 8.6, 15.1 Hz, 2H, Ar–H), 4.60 (d, J = 5.9 Hz, 2H, CH_2), 3.66 (s, 3H, NMe), 3.64–3.72 (m, 2H, CH_3CH_2), 3.58 (s, 3H, CH_3SO_3), 3.36–3.44 (m, 2H, CH_3CH_2), 1.76 (s, 6H, NHC(CH_3)$_2$), 1.14–1.24 (m, 6H, CH_3CH_2); HRMS (ESI) calcd for $C_{23}H_{30}FN_5NaO_7S$ $(M + Na)^+$ 562.1742, found 562.1734.

*5.21.11. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-5-yl benzoate (**35**)*

Treatment of **32** with benzoyl chloride at room temperature to afford **35** in 82% yield, mp 101–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, J = 3.4 Hz, 2H, Ar–H), 8.04 (s, 1H, NH), 7.99 (s, 1H, NH), 7.57–7.63 (m, 1H, Ar–H), 7.44–7.52 (m, 2H, Ar–H), 7.24–7.31 (m, 2H, Ar–H), 6.94–7.03 (m, 2H, Ar–H), 4.51 (s, 2H, CH_2), 3.65 (s, 3H, NMe), 3.31–3.72 (m, 4H, CH_3CH_2), 1.75 (s, 6H, NHC(CH_3)$_2$), 1.11–1.28 (m, 6H, CH_3CH_2); HRMS (ESI) calcd for $C_{29}H_{32}FN_5NaO_6$ $(M + Na)^+$ 588.2229, found 588.2228.

*5.21.12. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-5-yl 2,4,6-trimethylbenzenesulfonate (**36**)*

Treatment of **32** with mesitylene sulfonyl chloride at reflux to afford **36** in 22% yield, mp 243–245 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 1H, NH), 7.72 (t, J = 1.4 Hz, 1H, NH), 7.34 (s, 2H, Ar–H), 6.95–7.06 (m, 4H, Ar–H), 4.58 (s, 2H, CH_2), 3.54 (s, 3H, NMe), 3.33–3.71 (m, 4H, CH_3CH_2), 2.70 (s, 6H, Ar–CH_3), 2.33 (s, 3H, Ar–CH_3), 1.74 (s, 6H, NHC(CH_3)$_2$), 1.11–1.22 (m, 6H, CH_3CH_2); HRMS (ESI) calcd for $C_{31}H_{38}FN_5NaO_7S$ $(M + Na)^+$ 666.2368, found 666.2372.

*5.21.13. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-6-methoxypyrimidin-5-yl acetate (**39**)*

Treatment of **38** with acetylchloride at room temperature to afford **39** in 78% yield, mp 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (s, 1H, NH), 8.41 (t, J = 5.1 Hz, 1H, NH), 7.36 (dd, J = 5.6, 7.8 Hz, 2H, Ar–H), 7.05 (t, J = 8.6 Hz, 2H, Ar–H), 4.58 (d, J = 6.0 Hz, 2H, CH_2), 4.09 (s, 3H, OMe), 3.71 (q, J = 7.0, 14.0 Hz, 2H, NCH_2CH$_3$), 3.39 (q, J = 7.0, 14.1 Hz, 2H, NCH_2CH$_3$), 2.44 (s, 3H, CH_3CO_2), 1.80 (s, 6H, NHC(CH_3)$_2$), 1.25 (t, J = 6.9 Hz, 3H, NCH$_2CH_3$), 1.18 (t, J = 7.1 Hz, 3H, NCH$_2CH_3$); HRMS (ESI) calcd for $C_{24}H_{31}FN_5O_6$ $(M + H)^+$ 504.2253, found 504.2255.

*5.21.14. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-6-methoxypyrimidin-5-yl methanesulfonate (**40**)*

Treatment of **38** with methanesulfonyl chloride at room temperature to afford **40** in 98% yield, mp 45–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H, NH), 8.21 (s, 1H, NH), 7.35 (d,

J = 4.8 Hz, 2H, Ar–H), 7.02 (d, J = 7.6 Hz, 2H, Ar–H), 4.58 (s, 2H, CH_2), 4.15 (s, 3H, OMe), 3.66 (t, J = 6.5 Hz, 2H, CH_3CH_2), 3.52 (s, 3H, CH_3SO_3), 3.37 (t, J = 6.7 Hz, 2H, CH_3CH_2), 1.78 (s, 6H, NHC(CH_3)$_2$), 1.07–1.23 (m, 6H, CH_3CH_2); HRMS (ESI) calcd for $C_{23}H_{30}FN_5NaO_7S$ $(M + Na)^+$ 562.1742, found 562.1740.

*5.21.15. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-6-methoxypyrimidin-5-yl benzoate (**41**)*

Treatment of **38** with benzoyl chloride at room temperature to afford **41** in 82% yield, mp 47–49 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H, NH), 8.32 (s, 1H, NH), 8.14 (s, 2H, Ar–H), 7.14–7.56 (m, 5H, Ar–H), 6.83–6.94 (m, 2H, Ar–H), 4.44 (s, 2H, CH_2), 3.96 (s, 3H, OMe), 3.23–3.68 (m, 4H, CH_3CH_2), 1.71 (s, 6H, NHC(CH_3)$_2$), 1.02–1.23 (m, 6H, CH_3CH_2); HRMS (ESI) calcd for $C_{29}H_{32}FN_5NaO_6$ $(M + Na)^+$ 588.2229, found 588.2228.

*5.21.16. 2-(2-(2-(Diethylamino)-2-oxoacetamido)propan-2-yl)-4-(4-fluorobenzylcarbamoyl)-6-methoxypyrimidin-5-yl benzoate (**42**)*

Treatment of **38** with mesitylene sulfonyl chloride at reflux to afford **42** in 50% yield, mp 136–138 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H, NH), 7.94 (t, J = 5.8 Hz, 1H, NH), 7.34 (dd, J = 5.6, 8.0 Hz, 2H, Ar–H), 6.99–7.04 (m, 4H, Ar–H), 4.54 (d, J = 6.0 Hz, 2H, CH_2), 3.76 (s, 3H, OMe), 3.65 (q, J = 6.9, 13.9 Hz, 2H, CH_3CH_2N), 3.36 (q, J = 7.2, 14.3 Hz, 2H, CH_3CH_2N), 2.62 (s, 6H, Ar-CH_3), 2.33 (s, 3H, Ar-CH_3), 1.74 (s, 6H, NHC(CH_3)$_2$), 1.21 (t, J = 7.0 Hz, 3H, CH_3CH_2N), 1.15 (t, J = 7.1 Hz, 3H, CH_3CH_2N); HRMS (ESI) calcd for $C_{31}H_{38}FN_5NaO_7S$ $(M + Na)^+$ 666.2368, found 666.2368.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.02.015.

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