European Journal of Medicinal Chemistry 104 (2015) 127–138

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis of dihydropyrimidine α,γ-diketobutanoic acid derivatives targeting HIV integrase

Ozkan Sari [a], Vincent Roy [a], Mathieu Métifiot [b], Christophe Marchand [b], Yves Pommier [b], Stéphane Bourg [a, c], Pascal Bonnet [a], Raymond F. Schinazi [d], Luigi A. Agrofoglio [a, *]

[a] Univ. Orléans et CNRS, ICOA, UMR 7311, F-45067, Orléans, France
[b] Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, 20892, USA
[c] CNRS, CBM, UPR 4301, Univ. Orléans, F-45071, Orléans, France
[d] Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine and Veterans Affairs Medical Center, Atlanta, GA, 30322, USA

ARTICLE INFO

Article history:
Received 21 June 2015
Received in revised form
9 September 2015
Accepted 10 September 2015
Available online 25 September 2015

Keywords:
Dihydropyrimidine α,γ-diketobutanoic acid
Synthesis
Docking analysis
Antiviral activity
Integrase-HIV

ABSTRACT

The synthesis and antiviral evaluation of a series of dihydropyrimidinone and thiopyrimidine derivatives bearing aryl α,γ-diketobutanoic acid moiety are described using the Biginelli multicomponent reaction as key step. The most active among 20 synthesized novel compounds were **4c, 4d** and **5b,** which possess nanomolar HIV-1 integrase (IN) stand transfer (ST) inhibition activities. In order to understand their mode of interactions within the IN active site, we docked all the compounds into the previously reported X-ray crystal structure of IN. We observed that compounds **4c**, **4d and 5b** occupied an area close to the two catalytic Mg^{2+} ions surrounded by their chelating triad (E221, D128 and D185), DC16, Y212 and the β-diketo acid moiety of **4c**, **4d** and **5b** chelating Mg^{2+}. As those compounds lack anti-HIV activities in cell, their prodrugs were synthetized. The prodrug **4c**′ exhibited an anti-HIV activity of 0.19 μM in primary human lymphocytes with some cytotoxicity. All together, these results indicate that the new analogs potentially interact within the catalytic site with highly conserved residues important for IN catalytic activity.

© 2015 Elsevier Masson SAS. All rights reserved.

1. Introduction

The genome of HIV in each viral capsid includes two RNA strands along with three enzymes required for the replication cycle: reverse transcriptase (RT), protease (PR) and integrase (IN). While some prior treatments like the *highly active antiretroviral therapy* (HAART) used to focus solely on RT and PR, IN became, in the past decade, a crucial target since it lacks a mammalian counterpart [1–5]. IN acts by inserting the viral DNA into host cell chromosomes. More specifically, in the cytoplasm, IN catalyzes the 3′-processing step (3′-P), which consists in the removal of a GT dinucleotide from the 3′-end of both extremities of the viral genome (U3 and U5 long terminal repeats (LTRs)) [6]. Subsequently, IN stay bound to the LTRs in the preintegration complex and moves to the nucleus where the strand transfer step (ST) takes place. Integration is essential for the generation of the proviral genes, which is required for production of future virions.

Since 2007, three compounds have been approved by the FDA as IN inhibitors: Raltegravir **1** (Isentress®, Merck), Elvitegravir **2** (Vitekta®, Gilead) and Dolutegravir **3** (Tivicay®, ViiV Healthcare), (Fig. 1). These drugs are unique by their ability to selectively inhibit ST during the integration process by complexing the two catalytic Mg^{2+} ions in the active site.

The design of the β-hydroxy-(amide) functions, responsible for the chelation, was inspired by the pioneering studies made with the α,γ-diketobutanoic acid (DKA). In fact, the discovery of DKAs was decisive in the validation of IN as a therapeutic target toward the inhibition of HIV replication [7]. Therefore, as part of our drug discovery program, we have developed a library of diversely substituted dihydropyrimidine α,γ-diketobutanoic acid derivatives targeting selectively ST. A metal chelating aryl α,γ-diketobutanoic acid moiety was attached to these structures *via* derivation at N-1 for dihydropyrimidinone (**4**) or C-2 in the case of thiopyrimidines (**5**) (Fig. 2).

* Corresponding author.
E-mail address: luigi.agrofoglio@univ-orleans.fr (L.A. Agrofoglio).

http://dx.doi.org/10.1016/j.ejmech.2015.09.015
0223-5234/© 2015 Elsevier Masson SAS. All rights reserved.

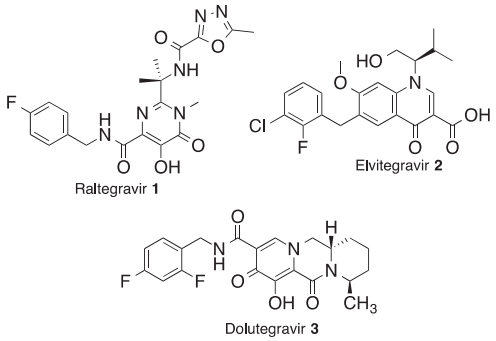


Fig. 1. FDA-approved integrase inhibitors: Raltegravir **1**, Elvitegravir **2**, Dolutegravir **3**.

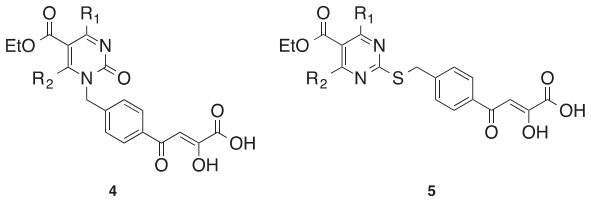
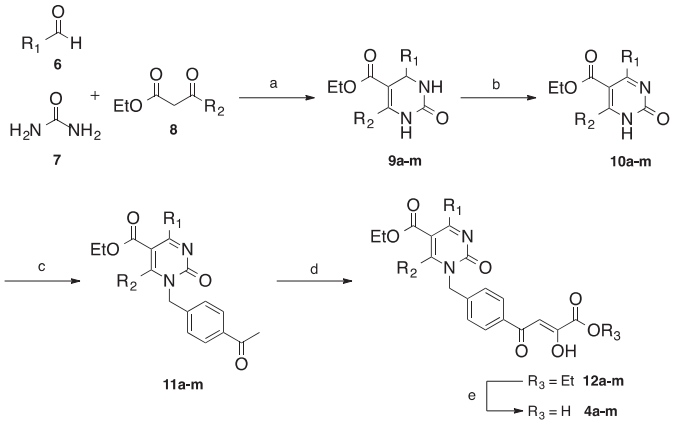


Fig. 2. Target dihydropirimidine derivatives bearing a α,γ-diketobutanoic acid moiety at N-1 (**4**) or C-2 (**5**).

2. Results and discussion

2.1. Chemical synthesis

The preparation of the N-1 derived compounds starts with the synthesis of the 3,4-dihydropyrimidin-2(1H)-ones **4a-m** (DHPMs) using the Biginelli multicomponent reaction as key step [8]. This reaction allowed us to easily introduce diversity at position C-4 and C-6 of the DHPMs by modulation of the aldehyde **6** and the β-ketoester **8**. To begin with, the DHPMs **9a-d** substituted by a methyl group at position 6 were synthesized in high yield under solvent-less conditions using 5 mol% of $Ce(NO_3)_3.6H_2O$ at 80 °C during 15 min [9].The synthesis of DHPMs **9e** substituted by a benzyl group at C-4 and **9f-m** substituted either by an ethyl, an isopropyl or a phenyl group at C-6 has proven to be difficult using the previously described conditions. The low reactivity of the aliphatic aldehyde or β-ketoesters involved in their preparation led us to apply a different protocol with a higher catalyst loading and a longer reaction time. Hence, preparation of these DHPMs was performed using 25 mol% of $CeCl_3.7H_2O$ in refluxing ethanol during 24 h to generate the desired derivatives in moderate to good yields (33–76%) [10]. These DHPMs were then involved in an oxidative aromatization step to isolate the N-1 for alkylation. Therefore, a treatment with 65% nitric acid following the methodology described by Kappe et al. [11] generated efficiently the desired pseudo-aromatized compounds **10a-m** in good to excellent yields (59–92%). The obtained compounds were then alkylated with 4-(bromomethyl)acetophenone, which was prepared by radical bromination of 4-methylacetophenone (*not described*) [12]. The alkylated derivatives **11a-m** were isolated in moderate yields (31–56%) after 24 h at room temperature. Finally, the methyl ketone intermediates were treated with LiHMDS and reacted with diethyl oxalate to produce the α,γ-diketoesters derivatives, which were subsequently submitted to base-promoted hydrolysis (saponification) and acidification to yield the desired α,γ-diketobutanoic acids **4a-m** in good yields, over two steps [13]. It is noteworthy that 6-methyl substituted derivatives led to lower yields due to side reactions with LiHMDS, which generated undesired diketoacids derivatives (Scheme 1).



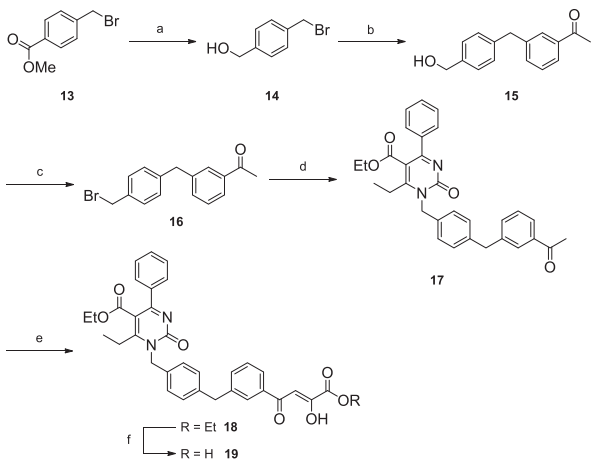
Scheme 1. Reagents and conditions: a) $Ce(NO_3)_3.6H2O$ (5 mol%), 80 °C, 15 min (65–92%) or $CeCl_3.7H2O$ (25 mol%), EtOH, 80 °C, 24 h (33–76%); b) HNO_3 (65%), 30 min, 0 °C to rt (59–92%); c) 4-(bromomethyl)acetophenone, Cs_2CO_3, DMF, rt, 24 h (31–56%); d) LiHMDS, THF, -78 °C, 30 min, then diethyl oxalate; e) LiOH (1 M), THF, rt, 1 h (51–98% over two steps).

In order to study the effect of the proximity of the α,γ-diketobutanoic acid moiety to the dihydropyrimidine building block, a compound bearing a diaryl spacer was synthesized. The spacer **16** was prepared in 3 steps from the methyl 4-(bromomethyl)benzoate **13** successively by reduction with DIBAL-H, Suzuki coupling with the 3-acethylphenylboronic acid and bromination (Scheme 2) [14]. Then, the diaryl spacer was attached to the dihydropyrimidine derivative **10f** to generate the methyl ketone intermediate **17** in 52% yield. It is noteworthy that the derivative **10f**, having an ethyl group at position 6, was chosen to avoid the side reaction discussed above for the 6-methyl derivatives. The α,γ-diketobutanoic acid moiety was elaborated following the previously described conditions to afford the compound **19** in 96% yield over two steps.

The analogs **5a-f**, bearing the α,γ-diketobutanoic acid moiety at C-2, were prepared following a slightly modified sequence, respectively. Thus, thiopyrimidines were synthesized using the previously described $CeCl_3.7H_2O$ catalyzed procedure to afford **21a-f** in good yields (Scheme 3, 70–94%). C-2 derivation was achieved by selective *S*-alkylation with 4-(bromomethyl)acetophenone to afford **22a-f** as a mixture of isomers which were subsequently treated with MnO_2 under microwave conditions to yield the aromatized derivatives **23a-f** (35–49% over two steps) [15]. These intermediates were then converted as described above to the corresponding β-diketoacids **5a-f**.

2.2. Biological evaluation

The twenty newly synthesized compounds **4a-m**, **19** and **5a-f** were evaluated for their ability to inhibit the enzymatic activity of HIV IN (3′-P and ST) in vitro. The results are summarized in Table 1. As expected, some of these compounds (**4c,d**, **5b** and **19**) displayed selective inhibition of ST with sub-micromolar activities. The obtained results demonstrated that steric hindrance at C-6 was not well tolerated, since compounds substituted by either a phenyl or an isopropyl group exhibited the lowest inhibitions. Nevertheless, compounds substituted at by a methyl or an ethyl group gave interesting results. Among them, compound **4c**, substituted by a benzyl group at C-4 position, displayed a promising inhibition with an IC_{50} of 0.19 μM. Concerning the substitution on the aryl group at C-4 of both compounds, the presence of halogen such as a fluorine, of the methoxy or 3,5-dimethyl group did not affect significantly the ST inhibition ($IC_{50} < 6.5$ μM). A phenyl group at C-4 and an ethyl group at C-6 generated compound **4d** with an IC_{50} of 0.64 μM.



Scheme 2. Reagents and conditions: a) DIBAL-H, CH_2Cl_2, 1.5 h, −78 °C, quant.; b) 3-acethylphenylboronic acid, $Pd(OAc)_2$, PPh_3, K_3PO_4, toluene, rt, 48 h, 77%; c) NBS, PPh_3, THF, 0 °C, 1 h, 98%; d) 10f, Cs_2CO_3, DMF, rt, 24 h, 52%; e) LiHMDS, THF, −78 °C, 1 h, then diethyl oxalate; e) LiOH (1 M), THF, rt, 1 h, 96% over two steps.

Attaching a diaryl spacer such as in compound **19**, or moving the α,γ-diketobutanoic acid moiety at C-2 position as in compounds **5a,f** did not affect remarkably the activity providing a slightly less potent compound with an IC_{50} of 0.85 μM and 2.23 μM for **19** and **5a,** respectively when compared to the parent structure **4d**.

In a first attempt, antiviral evaluation of the DKAs against HIV-1 in CEM cells (data not shown) did not exhibit any anti-HIV activity (EC_{50} > 65 μM), which is in total disconnection with enzymatic potencies. This lack of activity has been imputed to the low cell penetration of the compounds caused by the presence of a highly polar carboxylic acid group. Therefore, the best ST inhibitors **4c,d,l**, **5b** and **19** were converted back to their α,γ-diketoester form to overcome this issue. Recently, Nair et al. have reported the isopropyl ester as an efficient prodrug of DKAs [16]. They also showed that the α,γ-diketoester form was quickly hydrolyzed in human liver microsomes to produce the active acid form. Thus, the isopropyl ester prodrug version of our compounds were prepared *via* one-step acid-catalyzed esterification of DKA with 2-propanol (Scheme 4) and these prodrugs were screened to evaluate their anti-HIV-1 activity.

The cellular anti-HIV activity of α,γ-diketoester prodrugs **4′c,d,l**, **19′** and **5'b** is reported on Table 2. As expected, a slight improvement in cellular activities were observed especially for the best ST inhibitor **4′c** with an EC_{50} of 17.2 μM, which most likely implies that the prodrug forms are able to penetrate into cells. However, the compounds with antiviral activity are also showing some cytotoxicity.

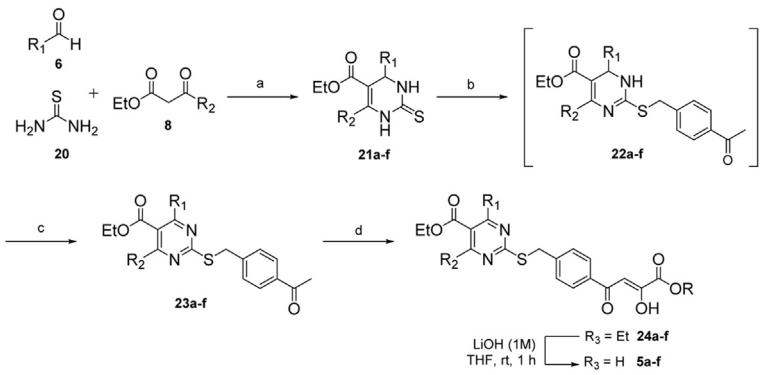
2.3. Molecular modeling

In order to investigate the binding mechanism of our compounds, we performed molecular docking experiments. Our studies relied on the crystal structure of the Prototype Foamy Virus (PFV) intasome complexed with raltegravir [17] which is an established model for the development of HIV-1 IN strand transfer inhibitors as reported by Billamboz et al. [18] and shown by Hare et al. [19,20] First, we validated our approach by extracting and redocking raltegravir in the binding site, and analyzed the result by superimposing the docked poses with the crystallographic conformation. The enolic tautomeric form was retained for the ligand, as it is the well-established form in solution for diketo acids [20]. A RMSD value of 0.3 Å for the best pose considering the heavy atoms was found. The pharmaphoric features of the crystallographic binding mode are well reproduced such as the strong chelation of the two Mg^{2+} cations by (Glu221), (Asp128) and (Asp185) residues, and two π − π stacking interactions, one between (Tyr212) and oxadiazol ring and the other between DC16 and p-fluorobenzyl ring. Next we docked compounds **4c**, **4d** and **5b** following the same protocol. The best poses are superimposed and compared to raltegravir on Fig. 3. Compounds **4c**, **4d** and **5b** chelate the two Mg^{2+} cations by their aryl α,γ-diketobutanoic acid moiety, as does raltegravir with (Glu221), (Asp128) and (Asp185) residues. The ester moiety of the three compounds **4c**, **4d** and **5b** are parallel to the DNA strand and pointing towards the solvent area. They form hydrogen bond interactions between the carbonyl of the ester and DNA donor nucleotide, **4c** and **4d** with DC16 and **5b** DA15. In contrast to raltegravir, the central aromatic six-membered ring of the compounds is not able to interact *via* π − π stacking interactions with DC16. Additionally, no π − π stacking interaction is observed with (Tyr212).

The Mg^{2+} chelation and the hydrogen bonds could explain the activity of the compounds against IN, but those hydrogen bond interactions are probably not sufficient to counterbalance the loss of the π − π stacking interactions with DC16 observed with raltegravir.

3. Conclusions

Twenty newly α,γ-diketobutanoic acid derivatives were successfully synthesized through the Biginelli reaction to achieve pyrimidine building blocks. As expected, compounds **4a-m**, **19** and **5a-f** which were evaluated for their ability to inhibiti HIV-1 IN



Scheme 3. Reagents and conditions: a) $CeCl_3.7H_2O$ (25 mol%), EtOH, reflux, 24 h (77–94%); b) 4-(bromomethyl)acetophenone, K_2CO_3, DMF, 60 °C, 1 h (35–49%); c) MnO_2 (10 equiv.), CH_2Cl_2, MW, 100 °C, 30 min (89–98%); d) LiHMDS, THF, −78 °C, 30 min, then diethyl oxalate; e) LiOH (1 M), THF, rt, 1 h 89–98% (over two steps).

Table 1
Inhibition of integrase activities (3′-Processing and Strand transfer).

Compounds	-R_2	-R_1	IC_{50} (μM) ± S.D.[a]	
			3′-Processing	Stand transfer
4a	-CH_3		>111	2.4 ± 0.5
4b	-CH_3		17.4 ± 2.1	1.3 ± 0.1
4c	-CH_3		16.9 ± 3.4	0.19 ± 0.12
4d	-CH_2-CH_3		19.1 ± 4.0	0.64 ± 0.16
4e	-CH_2-CH_3		35 ± 8	1.1 ± 0.4
4f	-CH_2-CH_3		72 ± 4.5	5.6 ± 4.6
4g	-$CH(CH_3)_2$		37.5 ± 5.1	2.3 ± 0.7
4h	-$CH(CH_3)_2$		40 ± 12	3.6 ± 0.8
4i	-$CH(CH_3)_2$		19.2 ± 5.1	3.3 ± 1.4
4j			18.4 ± 1.7	2.0 ± 0.3
4k			16.6 ± 2.4	2.4 ± 0.6
4l			17.2 ± 3.2	1.9 ± 0.8
4m			16.3 ± 3.5	1.3 ± 0.5
19	–		22.5 ± 2.0	0.85 ± 0.12
5a	-CH_2-CH_3		21.1 ± 1.2	2.23 ± 0.27
5b	-CH_2-CH_3		29.3 ± 3.5	0.92 ± 0.14
5c	-CH_2-CH_3		30.7 ± 2.7	2.27 ± 0.28
5d			30 ± 4	6.1 ± 1.4
5e			22 ± 4	2.8 ± 0.9
5f			23 ± 4	1.2 ± 0.3
Raltegravir	–		–	0.013 ± 0.001

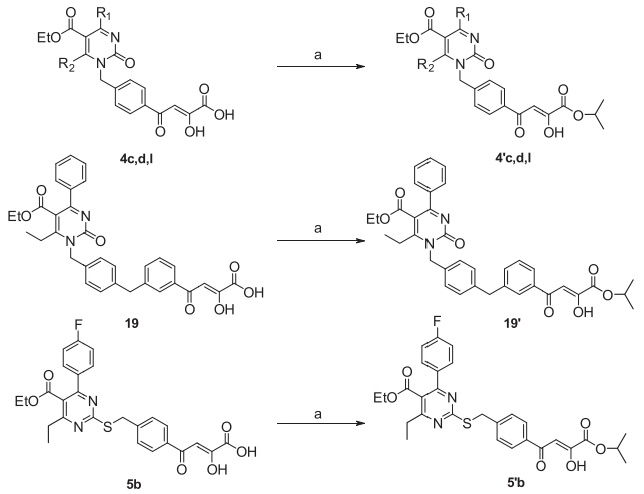
[a] Concentration required to induce 50% of inhibition.

in vitro and showed IC_{50} values in the low nanomolar range with some of them in nanomolar concentration (**4c**). Assuming that compounds suffered from low cell penetration, ester prodrug forms of selected compounds were prepared. Micromolar antiviral activities were observed for these prodrugs but unfortunately they were also associated with some cytotoxicity. The results of molecular docking indicate that the new analogs potentially interact with the highly conserved residues important for IN catalytic activities. The data reported in this work should be considered as a starting point for developing new HIV-IN inhibitors.

4. Experimental section

4.1. Chemistry

Commercially available chemicals were of reagent grade and used as received. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60F254, E. Merck). Column chromatography was performed on



Scheme 4. Synthesis of ester prodrug forms. a) *i*PrOH, H_2SO_4 (cat), 85 °C, 3 h (24–38%).

Table 2
Evaluation of the antiviral activity against human immunodeficiency virus (HIV) and cytotoxicity against PBM, CEM, and VERO cells in vitro, expressed in μM, of synthesized of α,γ-dicetoester analogs.

Prodrug	EC_{50}[a] (μM)	CC_{50}[b] (μM)		
		PBM	CEM	VERO
4′c	17.2	77.0	7.3	32.9
4′d	>100	>100	21.4	>100
4′l	80.6	>100	25.3	>100
19′	36.8	47.6	8.9	>100
5′b	25.6	43.2	5.2	39.5
AZT	0.0074	>100	56.1	39.5

[a] Concentration required to induce 50% of inhibition.
[b] Cytostatic Concentration required to reduce cell growth by 50%.

Silica Gel 60 M (0.040–0.063 mm, E. Merck). The ^1H and ^{13}C NMR spectra were recorded on a Varian InovaUnity 400 spectrometer (400 MHz) in (d4) methanol, $CDCl_3$, shift values in parts per million relative to $SiMe_4$ as internal reference. High Resolution Mass spectra were performed on a Bruker maXis mass spectrometer.

4.1.1. General procedure for the Biginelli reactions (*9a-e*)

Procedure A: A mixture of β-ketoester (1 mmol), aldehyde (1 mmol) and urea (1.5 mmol) was heated at 80 °C during 15 min in the presence of $Ce(NO_3)_3.6H_2O$ (5 mol%). The obtained solid was filtered and washed with ice-cold water and recrystallized from hot ethanol to afford the desired 1,3-dihydropyrimidinone derivative.

Procedure B: A solution of β-ketoester (1 mmol), aldehyde (1 mmol) and urea (1.5 mmol) in EtOH (20 mL) was heated at reflux (80 °C) during 24 h in the presence of $CeCl_3.7H_2O$ (25 mol%). The mixture was cooled down to room temperature, poured into crushed ice and stirred/triturated until precipitation was observed. The solid was filtered and washed with ice-cold water and recrystallized from hot ethanol to afford the desired 1,3-dihydropyrimidinone derivative.

4.1.2. General procedure for aromatization reactions (*10a-m*)

To a solution of 65% nitric acid (5 mL) in an open vessel was added portion-wise 1,3-dihydropyrimidinone derivative (1 mmol) at 0 °C. After stirring 5 min at 0 °C, the solution was left to reach room temperature and stirred for an additional 30 min. The mixture was poured into crushed ice, neutralized (pH 7–8) with K_2CO_3 and extracted with CH_2Cl_2 (3 × 20 mL). Combined organic

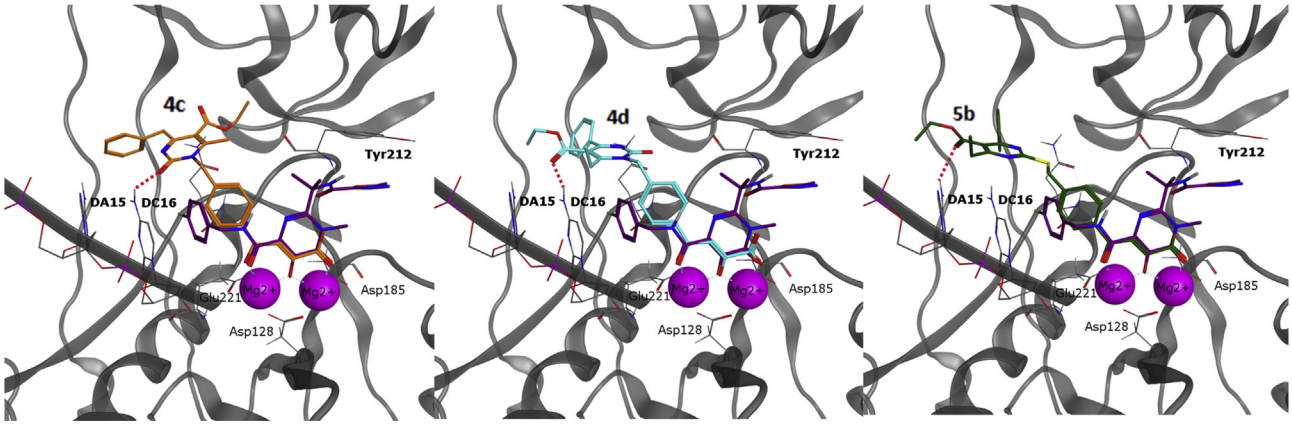


Fig. 3. Docking results of compounds **4c**, **4d** and **5b** regarding to raltegravir. Integrase and DNA are shown as gray ribbons, Mg^{2}+ metal ions as magenta spheres and chelating triad (Glu221, Asp128 and Asp185), (DC16) and (Tyr212) as element colored sticks. Ligands are C atom colored: raltegravir in purple, **4c** in orange, **4d** in cyan and **5b** in green. **4c** (left), **4d** (center) and **5b** (right) are superimposed to raltegravir. Hydrogen bonds are represented by red dashed line (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

layers were washed with water (20 mL), brine (20 mL), dried over $MgSO_4$ and concentrated in *vacuo*. The resulting solid was recrystallized from hot EtOH to afford the desired aromatized 1,3-dihydropyrimidinone derivative.

*4.1.3. General procedure for alkylation reactions (**11a-m**)*

To a solution of aromatized dihydropyrimidinone (1 mmol) in DMF (2 mL) was added 4-(bromomethyl)acetophenone (1 mmol) and Cs_2CO_3 (1.5 mmol). The mixture was stirred at room temperature during 24–36 h and evaporated under reduced pressure. The residue was purified over silica gel column chromatography with Petroleum ether/EtOAc to afford the desired alkylated derivative as a colorless oil.

*4.1.4. General procedure to β-diketoacids (**4a-m** and **5a-f**)*

A solution of methyl ketone derivative (1 equiv.) in dry THF (0.1 M) under positive pressure of nitrogen was treated with LiHDMS (1.1 equiv.) at −78 °C. After stirring 30 min at −78 °C, diethyloxalate (1.1 equiv.) was added dropwise and the mixture was left to reach room temperature. Stirring was continued for 2 h and the solution was concentrated under reduced pressure. The residue was taken with 1 M HCl and extracted with CH_2Cl_2. Combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated in vacuo. The remainder was dissolved in THF (0.1 M) and 1 M LiOH was added. The mixture was stirred 1 h at room temperature, acidified with 1 M HCl and extracted with DCM. Combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified over short reverse phase silica gel column with H_2O/ACN to afford the desired β-diketoacid derivative.

*4.1.5. Ethyl 6-methyl-2-oxo-4-phenyl-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9a**)*

Yield: 98%, white solid. CAS: 5395-36-8.

*4.1.6. Ethyl 4-(4-fluorophenyl)-6-methyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate **(9b)***

Yield: 92%, white solid. CAS: 5937-24-6.

*4.1.7. Ethyl 4-benzyl-6-methyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9c**)*

Yield: 40%, white solid. CAS: 378186-60-8.

*4.1.8. Ethyl 6-ethyl-2-oxo-4-phenyl-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9d**)*

Yield: 76%, white solid. CAS: 205999-91-3.

*4.1.9. Ethyl 6-ethyl-4-(4-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9e**)*

Yield: 75%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.38–7.17 (m, 2H), 6.99 (t, J = 8.6 Hz, 2H), 6.16 (s, 1H), 5.39 (d, J = 2.2 Hz, 1H), 4.20–3.96 (m, 2H), 2.73 (ddd, J = 30.7, 13.2, 7.2 Hz, 2H), 1.22 (t, J = 7.5 Hz, 3H), 1.17 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.2, 162.3 (d, J = 246.5 Hz), 153.9, 151.8, 139.7 (d, J = 3.2 Hz), 128.3 (d, J = 8.2 Hz), 115.5 (d, J = 21.5 Hz), 100.5, 60.1, 55.0, 25.3, 14.1, 12.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_3$: 293.1296, found: 293.1298.

*4.1.10. Ethyl 6-ethyl-4-(4-methoxyphenyl)-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9f**)*

Yield: 66%, white solid. CAS: 205999-93-5.

*4.1.11. Ethyl 6-isopropyl-2-oxo-4-phenyl-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9g**)*

Yield: 50%, white solid. CAS: 868755-03-7.

*4.1.12. Ethyl 4-(4-fluorophenyl)-6-isopropyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9h**)*

Yield: 38%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.34–7.20 (m, 2H), 6.99 (t, J = 8.7 Hz, 2H), 6.12 (s, 1H), 5.37 (d, J = 2.9 Hz, 1H), 4.28–4.13 (m, 1H), 4.06 (q, J = 7.1 Hz, 2H), 1.21 (d, J = 7.0 Hz, 2H), 1.18 (d, J = 7.0 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 163.5, 161.1, 154.4, 153.1, 139.7, 139.7, 128.3, 128.2, 115.7, 115.4, 60.1, 55.0, 27.5, 19.7, 19.5, 14.0. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}N_2O_3$: 307.1452, found: 307.1452.

*4.1.13. Ethyl 6-isopropyl-4-(4-methoxyphenyl)-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9i**)*

Yield: 33%, white solid. ^{1}H NMR (400 MHz, DMSO) δ 8.79 (s, 1H), 7.63 (s, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 5.08 (d, J = 3.2 Hz, 1H), 4.11 (dt, J = 14.0, 7.0 Hz, 1H), 4.03–3.89 (m, 2H), 3.71 (s, 3H), 1.13 (d, J = 7.1 Hz, 2H), 1.11 (d, J = 7.1 Hz, 2H), 1.07 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.8, 158.9, 156.5, 153.0, 137.3, 127.8, 114.2, 98.9, 59.7, 55.5, 53.7, 27.4, 19.6, 19.4, 14.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{23}N_2O_4$: 349.1547, found: 349.1548.

*4.1.14. Ethyl 2-oxo-4,6-diphenyl-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9j**)*

Yield: 48%, white solid. CAS: 34906-28-0.

*4.1.15. Ethyl 4-(4-fluorophenyl)-2-oxo-6-phenyl-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9k**)*

Yield: 76%, white solid. CAS: 397882-37-0.

*4.1.16. Ethyl 4-(4-methoxyphenyl)-2-oxo-6-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**9l**)*

Yield: 71%, white solid. CAS: 380655-10-7.

*4.1.17. Ethyl 4-(3,5-dimethylphenyl)-2-oxo-6-phenyl-3,4-dihydro-1H-pyrimidine-5-carboxylate (**9m**)*

Yield: 65%, white solid. ^{1}H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 7.75 (s, 1H), 7.40–7.30 (m, 5H), 6.97–6.91 (m, 3H), 5.16 (s, 1H), 3.70 (q, J = 6.6 Hz, 2H), 0.72 (t, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.5, 152.5, 149.1, 144.9, 137.8, 135.6, 129.3, 128.8, 128.2, 124.5, 100.9, 59.5, 54.6, 21.5, 13.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_3$: 351.1703, found: 351.1703.

*4.1.18. Ethyl 6-methyl-2-oxo-4-phenyl-1H-pyrimidine-5-carboxylate (**10a**)*

Yield: 84%, white solid. CAS: 69207-36-9.

*4.1.19. Ethyl 4-(4-fluorophenyl)-6-methyl-2-oxo-1H-pyrimidine-5-carboxylate (**10b**)*

Yield: 59%, white solid. CAS: 1091909-58-8.

*4.1.20. Ethyl 4-benzyl-6-methyl-2-oxo-1H-pyrimidine-5-carboxylate (**10c**)*

Yield: 92%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.54 (s, 1H), 7.31–7.15 (m, 5H), 4.22 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 2.49 (s, 3H), 1.25 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 158.2, 129.1, 128.6, 127.0, 111.7, 61.7, 14.0. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}N_2O_3$: 273.1234, found: 273.1235.

*4.1.21. Ethyl 6-ethyl-2-oxo-4-phenyl-1H-pyrimidine-5-carboxylate (**10d**)*

Yield: 86%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.68 (s, 1H, NH), 7.62–7.56 (m, 2H), 7.51–7.38 (m, 3H), 4.04 (q, J = 7.1 Hz, 2H), 2.88 (q, J = 7.6 Hz, 2H), 1.38 (t, J = 7.6 Hz, 3H), 0.93 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 158.6, 130.8, 128.39, 128.0, 111.0, 61.7, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}N_2O_3$: 273.1234, found: 273.1237.

*4.1.22. Ethyl 6-ethyl-4-(4-fluorophenyl)-2-oxo-1H-pyrimidine-5-carboxylate (**10e**)*

Yield: 62%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.72 (s, 1H), 7.85–7.49 (m, 2H), 7.13 (t, J = 8.3 Hz, 2H), 4.10 (q, J = 7.0 Hz, 2H), 2.89 (q, J = 7.3 Hz, 2H), 1.40 (t, J = 7.4 Hz, 3H), 1.03 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 164.4 (d, J = 251.9 Hz), 158.5, 130.3 (d, J = 8.8 Hz), 115.6 (d, J = 22.2 Hz), 110.9, 61.8, 13.6. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{16}FN_2O_3$: 291.1139, found: 291.1144.

*4.1.23. Ethyl 6-ethyl-4-(4-methoxyphenyl)-2-oxo-1H-pyrimidine-5-carboxylate (**10f**)*

Yield: 81%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.58 (s, 1H), 7.62 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.86 (s, 3H), 2.86 (q, J = 7.5 Hz, 2H), 1.38 (t, J = 7.5 Hz, 3H), 1.06 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 162.0, 158.6, 130.1, 113.8, 61.7, 55.4, 13.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{19}N_2O_4$: 303.1339, found: 303.1341.

*4.1.24. Ethyl 6-isopropyl-2-oxo-4-phenyl-1H-pyrimidine-5-carboxylate (**10g**)*

Yield: 88%, white solid. CAS: 868755-10-6.

*4.1.25. Ethyl 4-(4-fluorophenyl)-6-isopropyl-2-oxo-1H-pyrimidine-5-carboxylate (**10h**)*

Yield: 74%, white solid. CAS: 1313372-06-3.

*4.1.26. Ethyl 6-isopropyl-4-(4-methoxyphenyl)-2-oxo-1H-pyrimidine-5-carboxylate (**10i**)*

Yield: 82%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.79 (s, 1H), 7.60 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.86 (s, 3H), 3.32–3.12 (m, 1H), 1.42 (s, 3H), 1.40 (s, J = 6.6 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 161.9, 158.3, 129.9, 114.0, 61.8, 55.4, 20.7, 13.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}N_2O_4$: 317.1496, found: 317.1501.

*4.1.27. Ethyl 2-oxo-4,6-diphenyl-1H-pyrimidine-5-carboxylate (**10j**)*

Yield: 59%, white solid. CAS: 34906-29-1.

*4.1.28. Ethyl 4-(4-fluorophenyl)-2-oxo-6-phenyl-1H-pyrimidine-5-carboxylate (**10k**)*

Yield: 71%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.17 (s, 1H), 7.71–7.58 (m, 4H), 7.57–7.43 (m, 3H), 7.15 (t, J = 8.6 Hz, 2H), 3.93 (q, J = 7.1 Hz, 2H), 0.87 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 164.5 (d, J = 252.3 Hz), 157.8, 131.3, 130.5 (d, J = 8.9 Hz), 128.8, 128.0, 115.9 (d, J = 22.0 Hz), 111.9, 61.9, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{16}FN_2O_3$: 339.1139, found: 339.1142.

*4.1.29. Ethyl 4-(4-methoxyphenyl)-2-oxo-6-phenyl-1H-pyrimidine-5-carboxylate (**10l**)*

Yield: 74%, white solid. CAS: 913696-98-7.

*4.1.30. Ethyl 4-(3,5-dimethylphenyl)-2-oxo-6-phenyl-1H-pyrimidine-5-carboxylate (**10m**)*

Yield: 85%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.91 (s, 1H), 7.66–7.58 (m, 2H), 7.56–7.42 (m, 3H), 7.22 (s, 2H), 7.13 (s, 1H), 3.94 (q, J = 7.1 Hz, 2H), 2.35 (s, 6H), 0.90 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 157.7, 138.3, 132.8, 131.0, 128.6, 128.0, 125.7, 111.9, 61.7, 21.3, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{21}N_2O_3$: 349.1547, found: 349.1548.

*4.1.31. Ethyl 1-[(4-acetylphenyl)methyl]-6-methyl-2-oxo-4-phenyl-pyrimidine-5-carboxylate (**11a**)*

Yield: 84%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.65–7.53 (m, 4H), 7.52–7.40 (m, 3H), 5.58 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 2.61 (s, 3H), 2.59 (s, 3H), 1.04 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.8, 168.9, 168.1, 166.5, 163.6, 141.8, 137.6, 136.7, 130.8, 128.5, 128.4, 128.2, 127.9, 120.4, 68.5, 61.7, 26.6, 22.8, 13.6. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{23}N_2O_4$: 391.1652, found: 391.1653.

*4.1.32. Ethyl 1-[(4-acetylphenyl)methyl]-4-(4-fluorophenyl)-6-methyl-2-oxo-pyrimidine-5-carboxylate (**11b**)*

Yield: 35%, yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 2H), 7.67–7.53 (m, 4H), 7.12 (t, J = 8.7 Hz, 2H), 5.56 (s, 2H), 4.17 (q, J = 7.1 Hz, 2H), 2.60 (s, 3H), 2.57 (s, 3H), 1.09 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 169.0, 168.0, 165.2, 164.02 (d, J = 250.9 Hz), 163.6, 141.7, 136.7, 133.7, 130.4 (d, J = 8.6 Hz), 128.5, 127.8, 120.2, 115.6 (d, J = 21.8 Hz), 68.5, 61.8, 26.6, 22.8, 13.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{22}FN_2O_4$: 409.1558, found: 409.1560.

*4.1.33. Ethyl 1-[(4-acetylphenyl)methyl]-4-benzyl-6-methyl-2-oxo-pyrimidine-5-carboxylate (**11c**)*

Yield: 33%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 8.2 Hz, 2H), 7.32–7.12 (m, 5H), 5.48 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 4.14 (s, 2H), 2.60 (s, 3H), 2.49 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 169.4, 168.7, 167.3, 163.5, 141.7, 137.3, 136.6, 129.2, 128.4, 128.4, 127.9, 126.7, 120.8, 68.3, 61.7, 41.8, 26.6, 23.2, 14.0. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}N_2O_4$: 405.1809, found: 405.1808.

*4.1.34. Ethyl 1-[(4-acetylphenyl)methyl]-6-ethyl-2-oxo-4-phenyl-pyrimidine-5-carboxylate (**11d**)*

Yield: 46%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.3 Hz, 2H), 7.62–7.56 (m, 4H), 7.49–7.39 (m, 3H), 5.58 (s, 2H), 4.14 (q, J = 7.1 Hz, 2H), 2.85 (q, J = 7.5 Hz, 2H), 2.60 (s, 3H), 1.31 (t, J = 7.5 Hz, 3H), 1.03 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 173.2, 168.2, 166.5, 163.9, 141.9, 137.7, 136.7, 130.1, 128.5, 128.4, 128.24, 127.9, 120.0, 68.5, 61.7, 29.0, 26.6, 13.6, 12.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}N_2O_4$: 405.1809, found: 405.1809.

*4.1.35. Ethyl 1-[(4-acetylphenyl)methyl]-6-ethyl-4-(4-fluorophenyl)-2-oxo-pyrimidine-5-carboxylate (**11e**)*

Yield: 43%, colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 2H), 7.72–7.55 (m, 4H), 7.14 (t, J = 8.7 Hz, 2H), 5.59 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 2.85 (q, J = 7.5 Hz, 2H), 2.61 (s, 3H), 1.33 (t, J = 7.5 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 173.3, 168.1, 164 (d, J = 250.1 Hz), 163.9 (s), 141.8, 136.7, 133.8 (d, J = 3.2 Hz), 130.4 (d, J = 8.7 Hz), 128.5, 127.9, 119.9, 115.5 (d, J = 21.9 Hz), 68.5, 61.8, 29.0, 26.6, 13.7, 12.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{24}FN_2O_4$: 423.1715, found: 423.1714.

*4.1.36. Ethyl 1-[(4-acetylphenyl)methyl]-6-ethyl-4-(4-methoxy phenyl)-2-oxo-pyrimidine-5-carboxylate (**11f**)*

Yield: 49%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 2H), 7.61 (t, J = 7.8 Hz, 4H), 6.95 (d, J = 8.8 Hz, 2H), 5.58 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 3.86 (s, 3H), 2.83 (q, J = 7.5 Hz, 2H), 2.61 (s, 3H), 1.31 (t, J = 7.5 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 172.9, 168.6, 165.6, 163.8, 161.4, 142.0, 136.6, 130.0, 129.9, 128.4, 127.9, 119.5, 113.9, 68.4, 61.7, 55.4, 29.0, 26.6, 13.8, 12.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{27}N_2O_5$: 435.1914, found: 435.1918.

*4.1.37. Ethyl 1-[(4-acetylphenyl)methyl]-6-isopropyl-2-oxo-4-phenyl-pyrimidine-5-carboxylate (**11g**)*

Yield: 53%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.70–7.56 (m, 4H), 7.54–7.38 (m, 3H), 5.59 (s, 2H), 4.15 (q, J = 7.1 Hz, 2H), 3.22 (hept, J = 6.7 Hz, 1H), 1.31 (s, 3H), 1.30 (s, 3H), 1.04 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 176.4, 168.3, 166.5, 164.1, 142.0, 137.7, 136.6, 130.1, 128.4, 128.4, 128.2, 128.0, 119.8, 68.4, 61.7, 33.1, 26.6, 21.6, 13.6. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{27}N_2O_4$: 419.1965, found: 419.1967.

*4.1.38. Ethyl 1-[(4-acetylphenyl)methyl]-4-(4-fluorophenyl)-6-isopropyl-2-oxo-pyrimidine-5-carboxylate (**11h**)*

Yield: 56%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.68–7.56 (m, 4H), 7.13 (t, J = 8.6 Hz, 2H), 5.58 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 3.20 (hept, J = 6.7 Hz, 1H), 2.61 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H), 1.10 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 176.5, 168.2, 165.3, 164.1, 164.0 (d, J = 250.8 Hz), 141.9, 136.7, 133.8 (d, J = 3.3 Hz), 130.4 (d, J = 8.6 Hz), 128.4, 128.0, 119.6, 115.5 (d, J = 21.8 Hz), 68.5, 61.8, 33.2, 26.6, 21.6, 13.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{26}FN_2O_4$: 437.1871, found: 437.1874.

*4.1.39. Ethyl 1-[(4-acetylphenyl)methyl]-6-isopropyl-4-(4-methoxyphenyl)-2-oxo-pyrimidine-5-carboxylate (**11i**)*

Yield: 54%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.3 Hz, 2H), 7.62 (t, J = 8.3 Hz, 4H), 6.95 (d, J = 8.8 Hz, 2H), 5.58 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 3.18 (hept, J = 6.7 Hz, 1H), 2.61 (s, 3H), 1.30 (s, 3H), 1.29 (d, J = 6.7 Hz, 6H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.8, 176.1, 168.7, 165.7, 164.0, 161.34, 142.2, 136.6, 130.0, 129.9, 128.4, 128.0, 119.2, 113.8, 68.4, 61.7, 55.4, 33.1, 26.6, 21.6, 13.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{29}N_2O_5$: 449.2071, found: 449.2075.

*4.1.40. Ethyl 1-[(4-acetylphenyl)methyl]-2-oxo-4,6-diphenyl-pyrimidine-5-carboxylate (**11j**)*

Yield: 39%, colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 2H), 7.81–7.55 (m, 6H), 7.53–7.37 (m, 6H), 5.62 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 2.61 (s, J = 4.3 Hz, 3H), 0.95 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 168.1, 167.4, 163.8, 141.8, 137.3, 136.7, 130.2, 128.5, 128.5, 128.3, 127.9, 120.3, 68.7, 61.8, 26.7, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{25}N_2O_4$: 453.1809, found: 453.1811.

*4.1.41. Ethyl 1-[(4-acetylphenyl)methyl]-4-(4-fluorophenyl)-2-oxo-6-phenyl-pyrimidine-5-carboxylate (**11k**)*

Yield: 43%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 2H), 7.74–7.58 (m, 6H), 7.55–7.42 (m, 3H), 7.16 (t, J = 8.7 Hz, 2H), 5.63 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 2.62 (s, 3H), 0.97 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 168.1, 167.5, 166.2, 164.0 (d, J = 251.0 Hz), 163.8, 141.7, 137.2, 136.7, 133.4 (d, J = 3.3 Hz), 130.5 (d, J = 8.7 Hz), 130.3, 128.5, 128.5, 128.3, 127.9, 120.1, 115.6 (d, J = 21.9 Hz), 68.7, 61.9, 26.7, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{24}FN_2O_4$: 471.1715, found: 471.1715.

*4.1.42. Ethyl 1-[(4-acetylphenyl)methyl]-4-(4-methoxyphenyl)-2-oxo-6-phenyl-pyrimidine-5-carboxylate (**11l**)*

Yield: 44%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99–7.94 (m, 2H), 7.75–7.56 (m, 6H), 7.54–7.38 (m, 3H), 6.99–6.93 (m, 2H), 5.62 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 3.86 (s, 3H), 2.61 (s, 3H), 0.98 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.8, 168.5, 167.3, 166.6, 163.7, 161.5, 141.9, 137.5, 136.7, 130.1, 130.8, 129.6, 128.5, 128.4, 128.2, 127.9, 119.8, 113.9, 68.6, 61.8, 55.4, 26.67, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{27}N_2O_5$: 483.1914, found: 483.1916.

*4.1.43. Ethyl 1-[(4-acetylphenyl)methyl]-4-(3,5-dimethylphenyl)-2-oxo-6-phenyl-pyrimidine-5-carboxylate (**11m**)*

Yield: 36%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.2 Hz, 2H), 7.70–7.58 (m, 4H), 7.54–7.41 (m, 3H), 7.26 (s, J = 4.5 Hz, 2H), 7.12 (s, 1H), 5.63 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 2.62 (s, 3H), 2.36 (s, 6H), 1.00 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 168.2, 167.7, 167.1, 163.8, 141.9, 138.0, 137.4, 137.2, 136.7, 131.9, 130.1, 128.5, 128.4, 128.3, 127.9, 126.1, 120.3, 68.6, 61.7, 26.6, 21.3, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{29}N_2O_4$: 481.2122, found: 481.2123.

*4.1.44. 4-[4-[(5-ethoxycarbonyl-6-methyl-2-oxo-4-phenyl-pyrimidin-1-yl)methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4a**)*

Yield: 51%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.4 Hz, 1H), 7.67–7.60 (m, 2H), 7.52–7.39 (m, 1H), 7.15 (s, J = 9.8 Hz, 1H), 5.61 (s, 1H), 4.17 (q, J = 7.1 Hz, 1H), 2.62 (d, J = 4.3 Hz, 1H), 1.05 (t, J = 7.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.8, 169.0, 168.0, 166.6, 163.5, 161.9, 143.0, 137.5, 132.8, 130.3, 128.5, 128.2, 128.2, 128.0, 120.5, 95.5, 68.4, 61.8, 22.7, 13.6. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{23}N_2O_7$: 463.1499, found: 463.1492.

*4.1.45. 4-[4-[[5-ethoxycarbonyl-4-(4-fluorophenyl)-6-methyl-2-oxo-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4b**)*

Yield: 57%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.3 Hz, 2H), 7.74–7.56 (m, 4H), 7.15 (t, J = 8.6 Hz, 1H), 5.60 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 2.60 (s, 2H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.8, 174.4, 169.1, 168.3, 167.9, 165.3, 163.4, 161.9, 161.6, 143.0, 132.8, 130.41 (d, J = 8.7 Hz), 128.1, 128.05, 120.4, 115.64 (d, J = 21.8 Hz), 95.5, 68.4, 61.9, 22.7, 13.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{22}FN_2O_7$: 481.1405, found: 481.1406.

*4.1.46. 4-[4-[(4-benzyl-5-ethoxycarbonyl-6-methyl-2-oxo-pyrimidin-1-yl)methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4c**)*

Yield: 66%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.3 Hz, 5H), 7.55 (d, J = 8.2 Hz, 5H), 7.31–7.16 (m, 16H), 7.13 (s, 2H), 5.50 (s, 5H), 4.31 (q, J = 7.1 Hz, 5H), 4.16 (s, 5H), 2.52 (s, 7H), 1.31 (t, J = 7.1 Hz, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.5, 169.6, 168.8, 167.2, 163.3, 162.5, 142.8, 137.1, 133.1, 129.2, 128.4, 128.2, 128.0, 126.7, 121.0, 99.9, 96.0, 68.3, 61.8, 41.8, 23.0, 14.0. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_2O_7$: 477.1656, found: 477.1657.

*4.1.47. 4-[4-[(5-ethoxycarbonyl-6-ethyl-2-oxo-4-phenyl-pyrimidin-1-yl)methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4d**)*

Yield: 98%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.3 Hz, 2H), 7.71–7.57 (m, 4H), 7.12 (s, 1H), 6.97 (d, J = 8.9 Hz, 2H), 5.60 (s, 2H), 4.22 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 2.85 (q, J = 7.5 Hz, 2H), 1.33 (t, J = 7.5 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.4, 173.7, 173.0, 168.4, 165.8, 163.6, 162.3, 161.5, 143.1, 133.1, 130.0, 129.7, 128.2, 128.0, 119.6, 113.9, 95.9, 68.3, 61.8, 55.4, 53.4, 28.9, 13.8, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_2O_7$: 477.1656, found: 477.1656.

*4.1.48. 4-[4-[[5-ethoxycarbonyl-6-ethyl-4-(4-fluorophenyl)-2-oxo-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4e**)*

Yield: 91%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.3 Hz, 2H), 7.73–7.57 (m, 4H), 7.23–7.07 (m, 3H), 5.61 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 2.88 (q, J = 7.5 Hz, 2H), 1.35 (t, J = 7.5 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 187.5, 173.4, 168.0, 165.4, 164.1 (d, J = 251.3 Hz), 163.7, 162.4, 142.9, 133.5 (d, J = 3.2 Hz), 133.2, 130.4 (d, J = 8.7 Hz), 128.2, 128.1, 120.1, 115.6 (d, J = 21.9 Hz), 96.0, 68.5, 61.9, 29.0, 13.7, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{24}FN_2O_7$: 495.1562, found: 495.1562.

*4.1.49. 4-[4-[[5-ethoxycarbonyl-6-ethyl-4-(4-methoxyphenyl)-2-oxo-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4f**)*

Yield: 93%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.3 Hz, 2H), 7.72–7.56 (m, 4H), 7.12 (s, 1H), 6.97 (d, J = 8.9 Hz, 2H), 5.60 (s, 2H), 4.22 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 2.85 (q, J = 7.5 Hz, 2H), 1.33 (t, J = 7.5 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.4, 173.7, 173.0, 168.4, 165.8, 163.6, 162.3, 161.5, 143.1, 133.1, 130.0, 129.7, 128.2, 128.0, 119.6, 113.9, 95.9, 68.3, 61.8, 55.4, 53.4, 28.9, 13.8, 12.9. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_2O_8$: 507.1762, found: 507.1762.

*4.1.50. 4-[4-[(5-ethoxycarbonyl-6-isopropyl-2-oxo-4-phenyl-pyrimidin-1-yl)methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4g**)*

Yield: 93%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4 Hz, 2H), 7.71–7.59 (m, 4H), 7.52–7.41 (m, 3H), 7.13 (s, 1H), 5.60 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.30–3.17 (m, 1H), 1.32 (s, 1H), 1.30 (s, J = 6.7 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.1, 176.6, 168.2, 166.6, 164.0, 162.0, 143.3, 137.6, 132.9, 130.2, 128.5, 128.4, 128.2, 128.0, 119.9, 95.7, 68.4, 61.8, 33.2, 21.6, 13.6. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_2O_7$: 491.1813, found: 491.1814.

*4.1.51. 4-[4-[[5-ethoxycarbonyl-4-(4-fluorophenyl)-6-isopropyl-2-oxo-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4h**)*

Yield: 92%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.2 Hz, 2H), 7.71–7.59 (m, 4H), 7.15 (t, J = 8.6 Hz, 2H), 7.10 (s, 1H), 5.60 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 3.22 (hept, J = 6.7 Hz, 1H), 1.33 (s, 3H), 1.31 (s, 3H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.1, 176.7, 172.4, 168.1, 165.4, 164.0 (d, J = 251.1 Hz), 163.9, 163.0, 142.9, 133.5 (d, J = 3.3 Hz), 133.4, 130.4 (d, J = 8.7 Hz), 128.3, 128.1, 119.8, 115.6 (d, J = 21.8 Hz), 96.6, 68.5, 62.0, 33.3, 21.6, 13.7. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{26}FN_2O_4$: 437.1871, found: 437.1870.

*4.1.52. 4-[4-[[5-ethoxycarbonyl-6-isopropyl-4-(4-methoxyphenyl)-2-oxo-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4i**)*

Yield: 96%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.3 Hz, 2H), 7.72–7.57 (m, 4H), 7.11 (s, 1H), 6.96 (d, J = 8.8 Hz, 2H), 5.59 (s, 2H), 4.22 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 3.19 (hept, J = 6.7 Hz, 1H), 1.31 (s, 3H), 1.30 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.1, 176.2, 174.0, 168.6, 165.8, 163.9, 162.1, 161.4, 143.3, 132.9, 130.0, 129.8, 128.4, 128.0, 119.4, 113.9, 95.6, 68.3, 61.8, 55.4, 33.2, 21.6, 13.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{29}N_2O_8$: 521.1918, found: 521.1916.

*4.1.53. 4-[4-[(5-ethoxycarbonyl-2-oxo-4,6-diphenyl-pyrimidin-1-yl)methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4j**)*

Yield: 92%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.3 Hz, 2H), 7.67 (dd, J = 7.7, 1.1 Hz, 6H), 7.60–7.40 (m, 6H), 7.15 (s, 1H), 5.66 (s, 2H), 4.05 (q, J = 7.1 Hz, 2H), 0.95 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.7, 168.0, 167.4, 163.7, 161.8, 143.1, 137.2, 132.8, 130.3, 128.5, 128.3, 128.1, 120.4, 95.4, 68.5, 61.9, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{25}N_2O_7$: 525.1656, found: 525.1657.

*4.1.54. 4-[4-[[5-ethoxycarbonyl-4-(4-fluorophenyl)-2-oxo-6-phenyl-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4k**)*

Yield: 94%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.2 Hz, 2H), 7.76–7.60 (m, 6H), 7.58–7.40 (m, 3H), 7.16 (t, J = 8.6 Hz, 3H), 5.65 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 0.97 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0, 167.6, 165.0 (d, J = 259.0 Hz), 143.0, 137.1, 130.6, 130.5, 130.4, 128.5, 128.3, 128.1, 120.3, 115.6 (d, J = 21.9 Hz), 95.5, 68.6, 62.0, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{24}FN_2O_7$: 543.1562, found: 543.1560.

*4.1.55. 4-[4-[[5-ethoxycarbonyl-4-(4-methoxyphenyl)-2-oxo-6-phenyl-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4l**)*

Yield: 95%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4 Hz, 2H), 7.76–7.61 (m, 6H), 7.57–7.37 (m, 3H), 7.14 (s, 1H), 7.06–6.90 (m, 2H), 5.65 (s, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.88 (s, 3H), 0.99 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.3, 187.0, 168.4, 167.4, 166.6, 163.6, 162.1, 161.5, 143.2, 137.3, 132.9, 130.2, 130.1, 129.4, 128.4, 128.2, 128.1, 119.9, 114.0, 95.6, 68.5, 61.9, 55.4, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{31}H_{27}N_2O_8$: 555.1762, found: 555.1760.

*4.1.56. 4-[4-[[4-(3,5-dimethylphenyl)-5-ethoxycarbonyl-2-oxo-6-phenyl-pyrimidin-1-yl]methyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**4m**)*

Yield: 89%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.1 Hz, 2H), 7.77–7.57 (m, 4H), 7.56–7.39 (m, 3H), 7.26 (s, 2H), 7.14 (d, J = 10.5 Hz, 2H), 5.65 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 2.37 (s, 6H), 1.00 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 167.8, 167.2, 163.6, 143.2, 138.10, 137.3, 137.0, 132.0, 130.2, 128.5, 128.3, 128.1, 126.1, 120.4, 95.5, 77.2, 68.5, 61.79, 21.3, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{32}H_{29}N_2O_7$: 553.1969, found: 553.1969.

*4.1.57. 4-Bromomethylbenzyl alcohol (**14**)*

To a solution of methyl 4-bromomethylbenzoate **13** (3.09 g, 13 mmol) in dry CH_2Cl_2 (80 mL) cooled to −78 °C with stirring under nitrogen was added dropwise a solution of DIBAL-H (47 mL, 1.0 M solution in THF). Stirring was continued for 1.5 h at −78 °C, and the reaction mixture was then allowed to warm to 0 °C and quenched with H_2O. The organic layer was separated and the aqueous was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated to yield quantitatively the desired alcohol as a white solid. CAS: 71831-21-5.

*4.1.58. 1-(3-(4-(Hydroxymethyl)benzyl)phenyl)ethanone (**15**)*

To a solution of alcohol **14** (2.01 g, 10 mmol) in toluene (30 mL) were added 3-acethylphenylboronic acid (2.46 g, 15 mmol), K_3PO_4 (20 mmol), PPh_3 (104 mg, 0.4 mmol) and $Pd(OAc)_2$ (45 mg, 0.2 mmol). The resulting yellow suspension was stirred at room temperature for 2 days under Ar. The reaction mixture was then poured into 100 mL of ether and washed with 1 N NaOH and brine. The organic was dried over $MgSO_4$ and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, petroleum ether/EtOAc, 7:3) to afford the title compound (1.8 g, 77%) as a light yellow solid. CAS: 1237521-77-5.

*4.1.59. 1-(3-(4-(Bromomethyl)benzyl)phenyl)ethanone (**16**)*

To a solution of alcohol **15** (450 mg, 1.87 mmol) and PPh_3 (786 mg, 1.87 mmol) in THF (12 mL) was added NBS (0.534 g, 1.87 mmol) in one portion at 0 °C. The resulting yellow solution was stirred at 0 °C for 1 h. The reaction was then quenched by adding 20 mL of H_2O and was extracted with CH_2Cl_2 (20 mL × 3). The combined organic extracts were dried over MgSO4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (Petroleum ether/EtOAc, 95:5 to 90:10) to afford the title compound (559 mg, 98%) as a pale yellow oil. CAS: 1237521-89-9.

*4.1.60. (Z)-4-[3-[[4-[(5-ethoxycarbonyl-6-ethyl-2-oxo-4-phenyl-pyrimidin-1-yl)methyl]phenyl]methyl] phenyl] -2-hydroxy-4-oxo-but-2-enoic acid (**19**)*

Yield: 96%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 7.1 Hz, 1H), 7.70–7.61 (m, 3H), 7.53–7.40 (m, 7H), 7.23 (d, J = 7.9 Hz, 2H), 7.02 (s, 1H), 5.50 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 4.09 (s, 2H), 2.92 (q, J = 7.5 Hz, 2H), 1.35 (t, J = 7.5 Hz, 3H), 1.07 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.2, 173.3, 171.5, 168.2, 166.7, 163.9, 162.7, 142.2, 139.9, 137.6, 134.5, 134.3, 130.2, 129.3, 129.3, 129.1, 128.5, 128.3, 125.7, 119.9, 97.0, 69.5, 61.8, 41.1, 28.9, 13.6, 13.1. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{33}H_{31}N_2O_7$: 567.2126, found: 567.2126.

*4.1.61. Ethyl 6-ethyl-4-phenyl-2-thioxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**21a**)*

Yield: 77%, white solid. CAS:134074-29-6.

*4.1.62. Ethyl 6-ethyl-4-(4-fluorophenyl)-2-thioxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**21b**)*

Yield: 94%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.87 (s, 1H), 7.27 (dd, J = 8.5, 5.0 Hz, 2H), 7.01 (t, J = 8.7 Hz, 2H), 5.39 (d, J = 3.0 Hz, 1H), 4.18–4.05 (m, 2H), 2.77 (q, J = 7.5 Hz, 2H), 1.24 (t, J = 7.5 Hz, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 164.8, 162.5 (d, J = 247.4 Hz), 148.3, 138.4 (d, J = 3.2 Hz), 128.6 (d, J = 8.3 Hz), 115.8 (d, J = 21.6 Hz), 102.0, 60.5, 55.3, 24.9, 14.0, 12.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_2S$: 309.1067, found: 309.1071.

*4.1.63. Ethyl 6-ethyl-4-(4-methoxyphenyl)-2-thioxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**21c**)*

Yield: 77%, white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.29 (s, 1H), 9.58 (s, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 5.11 (d, J = 3.6 Hz, 1H), 4.02 (q, J = 7.1 Hz, 2H), 3.73 (s, 3H), 2.82–2.61 (m, 2H), 1.11 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d6) δ 174.8, 165.2, 159.2, 150.7, 136.14, 128.0, 114.4, 100.6, 60.0, 55.5, 53.8, 23.9, 14.4, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{21}N_2O_3S$: 321.1267, found: 321.1268.

*4.1.64. Ethyl 4,6-diphenyl-2-thioxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**21d**)*

Yield: 90%, white solid. CAS: 154866-93-0.

*4.1.65. Ethyl 4-(4-fluorophenyl)-6-phenyl-2-thioxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**21e**)*

Yield: 89%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.53–7.31 (m, 8H), 7.09 (t, J = 8.6 Hz, 2H), 5.55 (d, J = 3.1 Hz, 1H), 3.97–3.77 (m, 2H), 0.84 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.8, 164.7, 162.7 (d, J = 247.7 Hz), 143.7, 138.1 (d, J = 3.2 Hz), 133.9, 130.0, 128.6 (d, J = 8.4 Hz), 128.5, 128.0, 116.0 (d, J = 21.7 Hz), 103.7, 60.5, 55.9, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{18}FN_2O_2S$: 357.1067, found: 357.1068.

*4.1.66. Ethyl 4-(4-methoxyphenyl)-6-phenyl-2-thioxo-3,4-dihydro-1H-pyrimidine-5-carboxylate (**21f**)*

Yield: 77%, white solid. CAS: 134074-40-1.

*4.1.67. General procedure for alkylation and aromatization of thiopyrimidines (**23a-f**)*

A 2–5 mL microwave vial was charged with a DMF solution (0.5 M) of thiopyrimidine (1 mmol), 4-(bromomethyl)acetophenone (1.2 mmol) and K_2CO_3 (2 mmol). The mixture was irradiated at 60 °C during 1 h. Volatiles were evaporated and the residue was purified by silica gel column chromatography (EtOAc/Petroleum ether, 2:8) to afford the alkylated derivative as a mixture of isomers. Subsequently, in a 10–20 mL microwave vial, the crude alkylated product was dissolved in CH_2Cl_2 (0.05 M) and MnO_2 (10 equiv.) was added. The mixture was irradiated at 100 °C during 30 min, filtrated through a pad of Celite© and concentrated under reduced pressure. The remainder was purified by a short silica gel column (EtOAc/petroleum ether, 1:9) to afford the desired aromatized derivative.

*4.1.68. Ethyl 2-[(4-acetylphenyl)methylsulfanyl]-4-ethyl-6-phenyl-pyrimidine-5-carboxylate (**23a**)*

Yield: 48% in two steps, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 2H), 7.64–7.53 (m, 4H), 7.52–7.40 (m, 3H), 4.51 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 2.85 (q, J = 7.5 Hz, 2H), 2.60 (s, 3H), 1.33 (t, J = 7.5 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 171.1, 170.0, 167.9, 163.9, 143.7, 137.6, 136.0, 130.1, 129.2, 128.49, 128.46, 128.3, 121.2, 61.8, 34.9, 29.0, 26.6, 13.6, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}N_2O_3S$: 421.1580, found: 421.1582.

*4.1.69. Ethyl 2-[(4-acetylphenyl)methylsulfanyl]-4-ethyl-6-(4-fluorophenyl)pyrimidine-5-carboxylate (**23b**)*

Yield: 43% in two steps, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 2H), 7.63–7.59 (m, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.20–7.08 (m, 2H), 4.50 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 2.84 (q, J = 7.5 Hz, 2H), 1.32 (t, J = 7.5 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.6, 171.2, 170.1, 167.9, 164.0 (d, J = 251.0 Hz), 162.6, 143.6, 136.0, 133.6 (d, J = 3.3 Hz), 130.4 (d, J = 8.6 Hz), 129.2, 128.5, 121.0, 115.6 (d, J = 21.8 Hz), 61.9, 34.9, 29.0, 26.6, 13.7, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{24}FN_2O_3S$: 439.1486, found: 439.1486.

*4.1.70. Ethyl 2-[(4-acetylphenyl)methylsulfanyl]-4-ethyl-6-(4-methoxyphenyl)pyrimidine-5-carboxylate (**23c**)*

Yield: 49% in two steps, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 7.57 (d, J = 8.3 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 4.51 (s, 2H), 4.22 (q, J = 7.1 Hz, 2H), 3.88 (s, 3H), 2.82 (q, J = 7.5 Hz, 2H), 2.60 (s, 3H), 1.31 (t, J = 7.5 Hz, 3H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 170.8, 169.8, 168.4, 163.0, 161.4, 143.8, 136.0, 130.0, 129.8, 129.2, 128.5, 120.7, 113.9, 61.8, 55.4, 34.9, 29.0, 26.6, 13.8, 12.9. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{27}N_2O_4S$: 451.1686, found: 451.1687.

*4.1.71. Ethyl 2-[(4-acetylphenyl)methylsulfanyl]-4,6-diphenyl-pyrimidine-5-carboxylate (**23d**)*

Yield: 35% in two steps, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.3 Hz, 2H), 7.72–7.61 (m, 4H), 7.56 (d, J = 8.2 Hz, 2H), 7.52–7.39 (m, 6H), 4.52 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 2.58 (s, 3H), 0.95 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.6, 171.3, 167.9, 164.6, 143.5, 137.2, 136.0, 130.2, 129.3, 128.5, 128.4, 121.4, 61.9, 35.1, 26.6, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for C28H25N2O3S: 469.1580, found: 469.1581.

*4.1.72. Ethyl 2-[(4-acetylphenyl)methylsulfanyl]-4-(4-fluorophenyl)-6-phenyl-pyrimidine-5-carboxylate (**23e**)*

Yield: 49% in two steps, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.1 Hz, 2H), 7.70–7.61 (m, 4H), 7.56 (d, J = 8.2 Hz, 2H), 7.53–7.44 (m, 3H), 7.16 (t, J = 8.5 Hz, 2H), 4.52 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 2.60 (s, 3H), 0.97 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.6, 171.3, 167.8, 164.8, 164.03 (d, J = 251.1 Hz), 163.4, 143.4, 137.1, 136.1, 133.26 (d, J = 3.3 Hz), 130.58 (d, J = 8.7 Hz), 130.3, 129.3, 128.5, 128.5, 128.3, 121.2, 115.65 (d, J = 21.9 Hz), 62.0, 35.1, 26.6, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for C28H24FN3O3S: 487.1486, found: 487.1486.

*4.1.73. Ethyl 2-[(4-acetylphenyl)methylsulfanyl]-4-(4-methoxyphenyl)-6-phenyl-pyrimidine-5-carboxylate (**23f**)*

Yield: 39% in two steps, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 2H), 7.69–7.60 (m, 4H), 7.56 (d, J = 8.2 Hz, 2H), 7.53–7.43 (m, 3H), 6.98 (d, J = 8.9 Hz, 2H), 4.53 (s, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.89 (s, 3H), 2.61 (s, 3H), 0.99 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 171.0, 168.2, 164.6, 163.8, 161.4, 143.6, 137.4, 136.0, 130.1, 130.0, 129.4, 129.3, 128.5, 128.4, 128.3, 120.8, 114.0, 61.9, 55.4, 35.1, 26.6, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{27}N_2O_4S$: 499.1686, found: 499.1687.

*4.1.74. (Z)-4-[4-[(5-ethoxycarbonyl-4-ethyl-6-phenyl-pyrimidin-2-yl)sulfanylmethyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**5a**)*

Yield: 96%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.88 (m, 2H), 7.67–7.55 (m, 4H), 7.55–7.39 (m, 3H), 7.14 (s, 1H), 4.52 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 2.86 (q, J = 7.5 Hz, 2H), 1.32 (t, J = 7.5 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.9, 172.9, 170.9, 170.2, 167.9, 164.01, 163.1, 145.1, 137.5, 132.5, 130.2, 129.7, 128.5, 128.3, 128.1, 121.3, 96.3, 61.9, 35.0, 29.0, 13.6, 12.9. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_2O_6S$: 493.1428, found: 493.1428.

*4.1.75. (Z)-4-[4-[[5-ethoxycarbonyl-4-ethyl-6-(4-fluorophenyl) pyrimidin-2-yl]sulfanylmethyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**5b**)*

Yield: 94%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.1 Hz, 2H), 7.66–7.56 (m, 4H), 7.17–7.12 (m, 3H), 4.51 (s, J = 14.7 Hz, 2H), 4.20 (q, J = 7.1 Hz, 2H), 2.85 (q, J = 7.5 Hz, 2H), 1.32 (t, J = 7.5 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.8, 171.0, 170.2, 167.8, 164.1 (d, J = 229.8 Hz), 162.8, 162.7, 145.0, 133.5 (d, J = 3.2 Hz), 132.5, 130.4 (d, J = 8.7 Hz), 129.6, 128.1, 121.1, 115.6 (d, J = 21.9 Hz), 96.2, 62.0, 35.0, 29.0, 13.7, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{24}FN_2O_6S$: 511.1334, found: 511.1335.

*4.1.76. (Z)-4-[4-[[5-ethoxycarbonyl-4-ethyl-6-(4-methoxyphenyl) pyrimidin-2-yl]sulfanylmethyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**5c**)*

Yield: 89%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 5.2 Hz, 2H), 7.60–758 (m, 4H), 7.13 (s, 1H), 6.95 (d, J = 8.5 Hz, 2H), 4.50 (s, 2H), 4.22 (q, J = 7.0 Hz, 2H), 3.86 (s, 3H), 2.81 (q, J = 7.4 Hz, 2H), 1.30 (t, J = 7.5 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.8, 168.3, 163.1, 161.42, 130.0, 129.7, 129.6, 128.0, 120.7, 113.9, 61.8, 55.4, 34.9, 28.9, 13.8, 12.9. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_2O_7S$: 523.1533, found: 523.1533.

*4.1.77. (Z)-4-[4-[(5-ethoxycarbonyl-4,6-diphenyl-pyrimidin-2-yl) sulfanylmethyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**5d**)*

Yield: 98%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.3 Hz, 2H), 7.75–7.57 (m, 6H), 7.57–7.40 (m, 6H), 7.15 (s, 1H), 4.54 (s, 2H), 4.05 (q, J = 7.1 Hz, 2H), 0.94 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.2, 171.0, 167.8, 164.7, 162.3, 145.1, 137.1, 132.2, 130.3, 129.8, 128.5, 128.3, 128.0, 121.4, 95.6, 61.9, 35.1, 13.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{25}N_2O_6S$: 541.1428, found: 541.1425.

*4.1.78. (Z)-4-[4-[[5-ethoxycarbonyl-4-(4-fluorophenyl)-6-phenyl-pyrimidin-2-yl]sulfanylmethyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**5e**)*

Yield: 92%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.3 Hz, 2H), 7.71–7.57 (m, 6H), 7.55–7.43 (m, 3H), 7.17 (t, J = 8.7 Hz, 2H), 7.14 (s, 1H), 4.53 (s, 2H), 4.06 (q, J = 7.1 Hz, 2H), 0.97 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.8, 172.8, 171.1, 167.8, 164.84, 164.06 (d, J = 251.2 Hz), 163.5, 163.0, 144.9, 137.0, 133.14 (d, J = 3.3 Hz), 132.5, 130.6 (d, J = 8.7 Hz), 130.35, 129.7, 128.5, 128.3, 128.1, 121.2, 115.7 (d, J = 21.9 Hz), 96.3, 62.1, 35.1, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{24}FN_2O_6S$: found: 559.1328.

*4.1.79. (Z)-4-[4-[[5-ethoxycarbonyl-4-(4-methoxyphenyl)-6-phenyl-pyrimidin-2-yl]sulfanylmethyl]phenyl]-2-hydroxy-4-oxo-but-2-enoic acid (**5f**)*

Yield: 90%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.2 Hz, 2H), 7.70–7.54 (m, 6H), 7.53–7.42 (m, 3H), 7.12 (s, 1H), 6.99 (d, J = 8.9 Hz, 2H), 4.52 (s, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 0.98 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.2, 172.4, 170.8, 168.2, 164.7, 163.9, 163.3, 161.5, 145.0, 137.2, 132.6, 130.2, 129.7, 129.3, 128.5, 128.3, 128.1, 120.9, 114.0, 96.6, 62.0, 55.4, 35.1, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{31}H_{27}N_2O_7S$: 571.1533, found: 571.1530.

4.1.80. General procedure for the preparation of prodrug forms

To a stirred solution of β-diketocid derivative (50 mg) in 2-propanol (3 mL) was added conc. H_2SO_4 (1 drop) and the mixture was heated for 3 h at 85 °C. The reaction was concentrated under reduced pressure and the remainder (unstable on silica gel) was purified by preparative reversed phase HPLC.

*4.1.81. Ethyl (Z)-4-benzyl-1-(4-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl)benzyl)-6-methyl-2-oxo-1,2-dihydropyrimidine-5-carboxylate (**25c**)*

Yield: 32%. White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.1 Hz, 2H), 7.38–7.15 (m, 5H), 7.06 (s, 1H), 5.51 (s, 2H), 5.26 (p, J = 6.3 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 4.15 (s, 2H), 2.51 (s, 3H), 1.43 (s, 3H), 1.41 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.1, 170.4, 169.5, 168.7, 167.3, 163.5, 161.7, 142.5, 137.3, 134.4, 129.2, 128.4, 128.2, 128.0, 126.7, 120.9, 97.8, 70.7, 68.3, 61.7, 41.8, 23.2, 21.7, 14.0. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{31}N_2O_7$: 519.2126, found: 519.2130.

*4.1.82. Ethyl (Z)-6-ethyl-1-(4-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl)benzyl)-2-oxo-4-phenyl-1,2-dihydropyrimidine-5-carboxylate (**25d**)*

Yield: 29%. White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.2 Hz, 2H), 7.78–7.57 (m, 4H), 7.53–7.41 (m, 3H), 7.08 (s, 1H), 5.62 (s, 2H), 5.25 (p, J = 6.3 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 2.88 (q, J = 7.5 Hz, 2H), 1.42 (s, 3H), 1.41 (s, 3H), 1.34 (t, J = 7.5 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.1, 173.3, 170.4, 168.1, 166.6, 163.8, 161.7, 142.7, 137.6, 134.5, 130.2, 128.5, 128.2, 128.2, 128.0, 120.1, 97.8, 70.7, 68.4, 61.8, 29.0, 21.7, 13.6, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{31}N_2O_7$: 519.2126, found: 519.2134.

*4.1.83. Ethyl (Z)-1-(4-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl) benzyl)-4-(4-methoxyphenyl)-2-oxo-6-phenyl-1,2-dihydropyrimidine-5-carboxylate (**25l**)*

Yield: 24%. White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.3 Hz, 2H), 7.78–7.61 (m, 6H), 7.55–7.41 (m, 3H), 7.08 (s, 1H), 6.99 (d, J = 8.8 Hz, 2H), 5.66 (s, 2H), 5.26 (p, J = 6.3 Hz, 1H), 4.09 (q, J = 7.1 Hz, 2H), 3.89 (s, 3H), 1.42 (s, 3H), 1.41 (s, 3H), 1.00 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.1, 170.4, 168.5, 167.3, 166.6, 163.7, 161.7, 161.5, 142.8, 137.5, 134.5, 130.1, 129.5, 128.4, 128.3, 128.2, 128.0, 119.9, 113.9, 97.8, 70.7, 68.5, 61.9, 55.4, 29.7, 21.7, 13.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{33}N_2O_8$: 597.2231, found: 597.2227.

*4.1.84. Ethyl (Z)-6-ethyl-1-(4-(3-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl)benzyl)benzyl)-2-oxo-4-phenyl-1,2-dihydropyrimidine-5-carboxylate (**26**)*

Yield: 30%. White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91–7.82 (m, 2H), 7.64 (dd, J = 7.9, 1.7 Hz, 2H), 7.51–7.41 (m, 7H), 7.21 (d, J = 8.0 Hz, 2H), 7.05 (s, 1H), 5.51 (s, 2H), 5.25 (p, J = 6.3 Hz, 1H), 4.16 (q, J = 7.1 Hz, 2H), 4.08 (s, 2H), 2.86 (q, J = 7.5 Hz, 2H), 1.42 (s, 3H), 1.40 (s, 3H), 1.34 (t, J = 7.5 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.7, 173.1, 170.2, 168.3, 166.4, 164.1, 161.8, 142.0, 140.1, 137.8, 135.2, 134.6, 134.4, 130.0, 129.1, 128.9, 128.9, 128.4, 128.3, 128.2, 125.9, 119.7, 97.9, 70.7, 69.1, 61.7, 41.5, 29.7, 29.0, 21.7, 13.6, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{36}H_{37}N_2O_7$: 609.2595, found: 609.2590.

*4.1.85. Ethyl(Z)-4-ethyl-6-(4-fluorophenyl)-2-((4-(3-hydroxy-4-isopropoxy-4-oxobut-2-enoyl)benzyl)thio)pyrimidine-5-carboxylate (**27**)*

Yield: 38%. Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 2H), 7.77–7.53 (m, 4H), 7.14 (t, J = 8.7 Hz, 2H), 7.05 (s, 1H), 5.25 (p, J = 6.3 Hz, 1H), 4.52 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 2.84 (q, J = 7.5 Hz, 2H), 1.42 (s, 3H), 1.40 (s, 3H), 1.32 (t, J = 7.5 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.1, 171.0, 170.3, 170.2, 167.8, 164.0 (d, J = 251.0 Hz), 162.6, 161.7, 144.5, 133.8, 133.6 (d, J = 3.2 Hz), 130.4 (d, J = 8.6 Hz), 129.5, 128.0, 121.1, 115.6 (d, J = 21.8 Hz), 97.8, 70.7, 61.9, 35.0, 29.0, 21.7, 13.7, 12.8. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{30}FN_2O_6S$: 553.1803, found: 553.1798.

4.2. Enzymatic activity evaluation

HIV-1 IN recombinant protein was expressed in bacteria and purified as previously described [21]. 3′-P and ST activity were monitored using a gel-based assay in the following condition. Compounds or an equivalent volume of the drug solvent (100% DMSO) was mixed to 20 nM ^{32}P-labeled DNA substrate and 400 μM IN in a buffer containing 50 mM MOPS pH 7.2, 7.5 mM $MgCl_2$, and 14 mM 2-mercaptoethanol. After 1 h incubation at 37 °C, the reaction was quenched by addition of an equal volume of loading buffer (formamide supplemented with 1% sodium dodecyl sulfate, 0.25% bromophenol blue, and xylene cyanol). Products were separated in 16% polyacrylamide denaturing sequencing gels. Dried gels were visualized using a Typhoon 8600 (GE Healthcare). Densitometric analyses were performed using the ImageQuant 5.1 software from GE Healthcare. Data analyses (linear regression, IC_{50} determination, and standard deviation) were performed using Prism 5.0c software from GraphPad.

4.3. Antiviral activity evaluation

The antiviral activity of compounds described in Table 2 was evaluated against HIV-1 in activated primary human PBM cells [22]. Cytotoxicity was evaluated in normal PBM cells, along with CEM and Vero cells [23].

4.4. Molecular modeling

Software: molecular modeling studies were performed with the Schrodinger Molecular Modeling Suite [24] within Maestro, the interface piloting different modules. Glide was used to dock ligands. Analysis and visualization tasks were performed within MOE software [25].

Structure preparation: crystal structure of IN in complex with raltegravir (PDB code 3OYA) was retrieved from the Protein Data Bank. Next this structure was prepared using the Protein Preparation Wizard workflow of the Schrodinger Molecular Modeling Suite. Receptor was pre-processed (hydrogen atoms added, incomplete residues filled), bond orders and connections of ligand were manually corrected and non structural water removed. An exhaustive sampling was conducted regarding hydrogen bond assignment and the complex was finally refined by a minimization stage with a constraint to converge to a structure with an RMSD of 0.3 Å (OPLS2005 force field), essentially in order to remove steric clashes. Ligands, other than the one co-crystallized, were built with Marvin Sketch 5.8.0 [26] and were submitted to Corina [27] [MM09], a 3D structure generator. Finally, 3D structures were submitted to the LigPrep module of the Schrodinger Molecular Modeling Suite in order to take into account tautomerization and ionization *via* the Epik module. The resulting structures became the starting point for docking simulations. Docking parameters: docking calculations were performed with extra precision. Ligand flexibility was taken into account and the option of sampling of ring conformation was activated.

Acknowledgments

This work was supported in part by the NIH Intramural Research Program, Center for Cancer Research, National Cancer Institute (Z01 BC 007333-09LMP). O.S. is grateful to the French Ministère de l'Enseignement Supérieur MNERST for a Ph.D. scholarship. We thank the LABEX SynOrg (ANR-11-LABX-0029) for financial support. RFS is supported in part by NIH Grant 5P30-AI-50409 (CFAR) and by the Department of Veterans Affairs.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.09.015.

References

[1] E. De Clercq, New approaches towards anti-HIV chemotherapy, J. Med. Chem. 48 (2005) 1297–1313.
[2] T.E. Park, A. Mohamed, J. Kalabalik, R. Sharma, Review of integrase strand transfer inhibitors for the treatment of human immunodeficiency virus infection, Expert Rev. Anti Infect. Ther. 9 (2015) 1–18.
[3] J. Agapkina, D. Yanvarev, A. Anisenko, S. Korolev, J. Vepsäläinen, S. Kochetkov, M. Gottikh, Specific features of HIV-1 integrase inhibition by bisphosphonate derivatives, Eur. J. Med. Chem. 73 (2014) 73–82.
[4] M. Carcelli, D. Rogolino, M. Sechi, G. Rispoli, E. Fisicaro, C. Compari, N. Grandi, A. Corona, E. Tramontano, C. Pannecouque, L. Naesens, Antiretroviral activity of metal-chelating HIV-1 integrase inhibitors, Eur. J. Med. Chem. 83 (2014) 594–600.
[5] L. De Luca, F. Morreale, F. Christ, Z. Debyser, S. Ferro, R. Gitto, New scaffolds of natural origin as integrase-LEDGF/p75 interaction inhibitors/Virtual screening assays, Eur. J. Med. Chem. 68 (2013) 405–411.
[6] Y. Pommier, A.A. Johnson, C. Marchand, Integrase inhibitors to treat HIV/AIDS, Nat. Rev. Drug Discov. 4 (2005) 236–248.
[7] D.J. Hazuda, S.D. Young, J.P. Guare, N.J. Anthony, R.P. Gomez, J.S. Wai, J.P. Vacca, L. Handt, S.L. Motzel, H.J. Klein, G. Dornadula, R.M. Danovich, M.V. Witmer, K.A. Wilson, L. Tussey, W.A. Schleif, L.S. Gabryelski, L. Jin, M.D. Miller, D.R. Casimiro, E.A. Emini, J.W. Shiver, Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques, Science 305 (2004) 528–532.
[8] C. Limberakis, Biginelli Pyrimidone Synthesis, Wiley and Sons, Hoboken, NJ, 2005, pp. 509–520.
[9] M. Adib, K. Ghanbary, M. Mostofi, M. Ganjali, Efficient $Ce(NO_3)_3 \cdot 6H_2O$-catalyzed solvent-free synthesis of 3,4-dihydropyrimidin-2(1H)-ones, Molecules 11 (2006) 649–654.
[10] D.S. Bose, L. Fatima, H.B. Mereyala, Green chemistry approaches to the synthesis of 5-alkoxycarbonyl-4-aryl-3,4-dihydropyrimidin-2(1H)-ones by a three-component coupling of one-pot condensation reaction: comparison of ethanol, water, and solvent-free conditions, J. Org. Chem. 68 (2002) 587–590.
[11] A. Puchala, F. Belaj, C.O. Kappe, J. Bergman, On the reaction of 3,4-dihydropyrimidones with nitric acid. Preparation and x-ray structure analysis of a stable nitrolic acid, J. Heterocycl. Chem. 38 (2001) 1345–1352.
[12] J.C. Pieck, D. Kuch, F. Grolle, U. Linne, C. Haas, T. Carell, PNA-based reagents for the direct and site-specific synthesis of thymine dimer lesions in genomic DNA, J. Am. Chem. Soc. 128 (2006) 1404–1405.
[13] (a) X.Z. Zhao, E.A. Semenova, C. Liao, M. Nicklaus, Y. Pommier, T.R. Burke Jr., Biotinylated biphenyl ketone-containing 2,4-dioxobutanoic acids designed as HIV-1 integrase photoaffinity ligands, Bioorg. Med. Chem. 14 (2006) 7816–7825;
(b) S.H. Hwang, K.M. Wagner, C. Morisseau, J.-Y. Liu, H. Dong, A.T. Wecksler, B.D. Hammock, Synthesis and structure–activity relationship studies of urea-containing pyrazoles as dual inhibitors of cyclooxygenase-2 and soluble epoxide hydrolase, J. Med. Chem. 54 (2011) 3037–3050;
(c) S.Hare, A. M.Vos, R. F.Clayton, J. W.Thuring, M. D.Cummings, Cherepanov P. Molecular mechanisms of retrieval integrase inhibition and the evolution of viral resistance. Proc. Natl. Acad. Sci. U. S. A., 107(2010) 20057–20062
[14] Z. Wang, E.M. Bennett, D.J. Wilson, C. Salomon, R. Vince, Rationally designed dual inhibitors of HIV reverse transcriptase and integrase, J. Med. Chem. 50 (2007) 3416–3419.
[15] M. Matloobi, C.O. Kappe, Microwave-assisted solution- and solid-phase synthesis of 2-amino-4-arylpyrimidine derivatives, J. Comb. Chem. 9 (2007) 275–284.
[16] B.I. Seo, V.R. Uchil, M. Okello, S. Mishra, X.-H. Ma, M. Nishonov, Q. Shu, G. Chi, V. Nair, Discovery of a potent HIV integrase inhibitor that leads to a prodrug with significant anti-HIV activity, ACS Med. Chem. Lett. 2 (2011) 877–881.
[17] M. Billamboz, F. Bailly, C. Lion, C. Calmels, M.-L. Andréola, M. Witvrouw, F. Christ, Z. Debyser, L. De Luca, A. Chimirri, P. Cotelle, 2-Hydroxyisoquinoline-1,3(2H,4H)-diones as inhibitors of HIV-1 integrase and reverse transcriptase RNase H domain: influence of the alkylation of position 4, Eur. J. Med. Chem. 46 (2010) 535–546.
[18] S. Hare, S.S. Guptaz, E. Volkov, A. Engelman, P. Cherepanov, Retroviral intasome assembly and inhibition of DNA strand transfer, Nature 464 (2010) 232–236.
[19] E. Valkov, S.S. Gupta, S. Hare, A. Helander, P. Roversi, M. McClure, P. Cherepanov, Functional and structural characterization of the integrase from the prototype foamy virus, Nucleic Acids Res. 37 (2009) 243–255.
[20] C. Maurin, F. Bailly, E. Buisine, H. Vezin, G. Mbemba, J.F. Mouscadet, P. Cotelle, Spectroscopic studies of diketoacids-Metal interactions. A probing tool for the pharmacophoric intermetallic distance in the HIV-1 integrase active site, J. Med. Chem. 47 (2004) 5583–5586.
[21] M. Métifiot, K. Maddali, A. Naumova, X. Zhang, C. Marchand, Y. Pommier, Biochemical and pharmacological analyses of HIV-1 integrase flexible loop mutants resistant to raltegravir, Biochemistry 49 (2010) 3715–3722.
[22] R.F. Schinazi, J.P. Sommadossi, V. Saalman, D.L. Cannon, M.W. Xie, G.C. Hart, E.F. Hahn, Activities of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1, Antimicrob. Agents Chemother. 34 (1990) 1061–1067.
[23] L.J. Stuyver, S. Lostia, M. Adams, J. Mathew, B.S. Pai, J. Grier, P. Tharnish, Y. Choi, Y. Chong, H. Choo, C.K. Chu, M.J. Otto, R.F. Schinazi, Antiviral activities and cellular toxicities of modified 2',3'-dideoxy-2',3'-didehydrocytidine analogues, Antimicrob. Agents Chemother. 46 (2002) 3854–3860.
[24] Schrödinger Release 2014-3: Maestro, Version 9.9, Schrödinger, LLC, New York, NY, 2014.
[25] Molecular Operating Environment, Version 2013.08, Chemical Computing Group Inc., Montreal, Quebec, Canada, 2013.
[26] http://www.chemaxon.com.
[27] J. Sadowski, J. Gasteiger, From atoms and bonds to three-dimensional atomic coordinates: automatic model builders, Chem. Rev. 93 (1993) 2567–2581. http://www.molecular-networks.com/products/corina.