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A novel 3,4-dihydropyrimidin-2(1*H*)-one: HIV-1 replication inhibitors with improved metabolic stability

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ABSTRACT

Following the previous SAR of a novel dihydropyrimidinone scaffold as HIV-1 replication inhibitors a detailed study directed towards optimizing the metabolic stability of the ester functional group in the dihydropyrimidinone (DHPM) scaffold is described. Replacement of the ester moiety by thiazole ring significantly improved the metabolic stability while retaining antiviral activity against HIV-1 replication. These novel and potent DHPMs with bioisosteres could serve as advanced leads for further optimization.

Since the discovery of the human immunodeficiency virus (HIV) as the causative agent of the acquired immunodeficiency syndrome (AIDS) in 1983, the virus has rapidly spread around the world and HIV/AIDS is currently considered a pandemic.[1] According to UNAIDS 2009 report [Data.unaids.org], worldwide some 60 million people have been infected, with some 25 million deaths, and 14 million orphaned children in southern Africa alone since the epidemic began. This major global health threat triggered intensive drug discovery efforts and the first FDA-approved antiretroviral drug, AZT, was available in 1987. To date, 25 anti-HIV drugs belonging to six different inhibitor classes have been approved by FDA for the treatment of HIV infection, including nucleoside or non-nucleosides reverse transcriptase inhibitors (NRTIs/NNRTIs), protease inhibitors (PIs), integrase inhibitors, entry and fusion inhibitors.[2] The introduction of highly active antiretroviral therapy (HAART)—a regimen combining 3–4 antiretrovirals from different inhibitor classes—has significantly improved the life quality of patients by delaying the progression of the disease and reducing disabilities, transforming HIV/AIDS into a chronic manageable disease. However, the lack of an effective and safe vaccine, the emergence and spread of drug-resistant viral variants[3] and the inability of current regimens to eradicate the virus enforce the continuous development of novel anti-HIV drugs.

As a part of our HIV program aimed at the discovery of novel antiretrovirals via HTS using a cell-based assay, we successfully identified a novel class of 3,4-dihydropyrimidin-2(1*H*)-one (DHPM) HIV-1 replication inhibitors (Fig. 1).[4] Initial structure–activity relationship (SAR) studies focused on altering the substitution patterns of R groups attached to the DHPM core. These studies revealed that only the *S* enantiomer retained anti-HIV activity and R^2 with an ethyl group is optimal. Compound **1** was developed as an early lead with potent anti-HIV activity. These results have encouraged further structural optimization studies to search for more potent antiviral agents with the DHPM scaffold. Although compound **1** has a good antiviral activity (EC_{50} = 38 nM), it lacked metabolic stability due to the ester moiety in the DHPM scaffold. Analogs (**1, 2**) with ester group were metabolically unstable in liver microsomal stability test of human and rat and corresponding free acids were inactive against HIV-1. As the next step in this project, the main focus was

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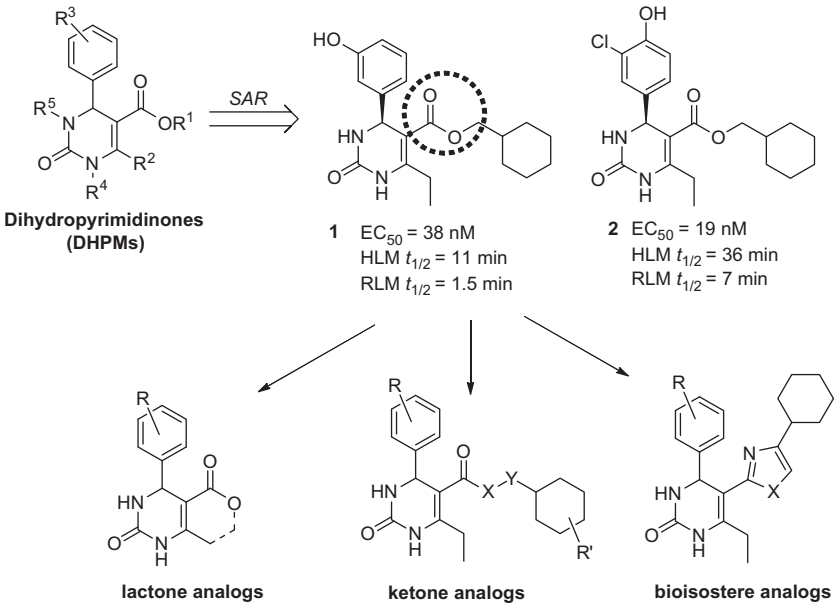


Figure 1. Structural modifications of ester moiety in DHPMs to improve metabolic stability.

Table 1
Cell-based antiviral activity of DHPM analogs against HIV-1

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)
1	0.038	>10	**16f**	0.983	>10
2	0.019	>10	**16g**	0.480	>10
6	>10	>10	**16h**	0.087	>10
12	>10	>10	**16i**	>10	>10
16a	0.420	>10	**16j**	1.40	>10
16b	0.403	>10	**26a**	0.116	>10
16c	0.329	>10	**26b**	0.078	>10
16d	>10	>10	**26c**	>10	>10
16e	3.28	>10	NVP[c]	0.150	>10

[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compounds **1**–**26c**, $n = 2$ and the values are the geometric mean of two determinations; all individual values are within 25% of the mean.
[b] CC_{50} is the cytotoxic concentration of compound that reduces viability of uninfected cells by 50%.
[c] Nevirapine (NVP) was used as a positive control.

Table 2
Metabolic stability of DHPMs

Compound	EC_{50}[a] (μM)	Metabolic stability ($t_{1/2}$, min)[b]	
		Human	Rat
1	0.038	11	2
2	0.019	36	7
16b	0.403	22	5
16f	0.983	18	7
16h	0.087	24	16
26a	0.116	132	54
26b	0.078	682	30

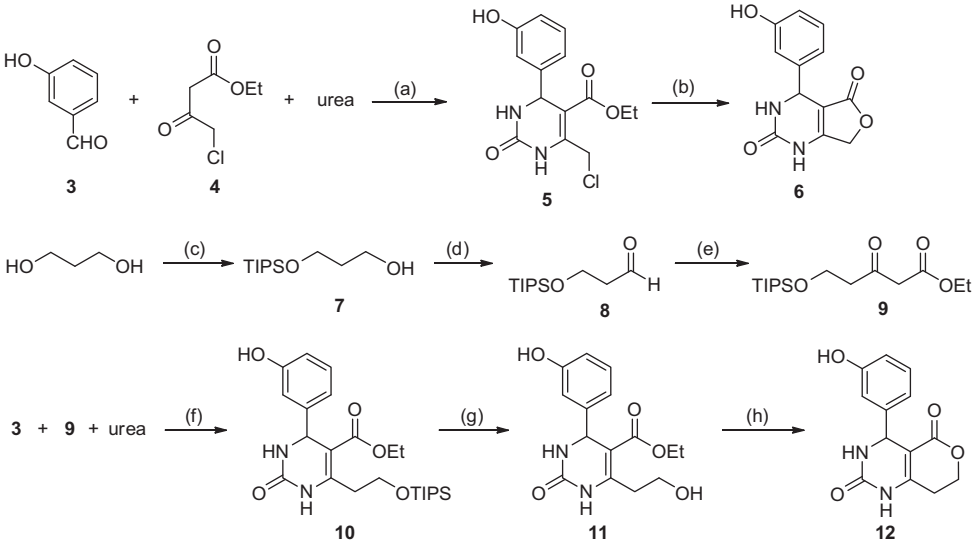
[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compounds **1**–**26b**, $n = 2$ and the values are the geometric mean of two determinations; all individual values are within 25% of the mean.
[b] Liver microsomal stability.

shifted to identify metabolically stable equivalents of the ester group that retained anti-HIV potency as well as continue to explore SAR. Three different structural modifications were designed to resolve this key issue; (i) lactone analogs, (ii) ketone analogs and (iii) bioisostere analogs (Fig. 1). We describe here our efforts for the identification of novel DHPM HIV-1 replication inhibitors with greatly improved metabolic stability.

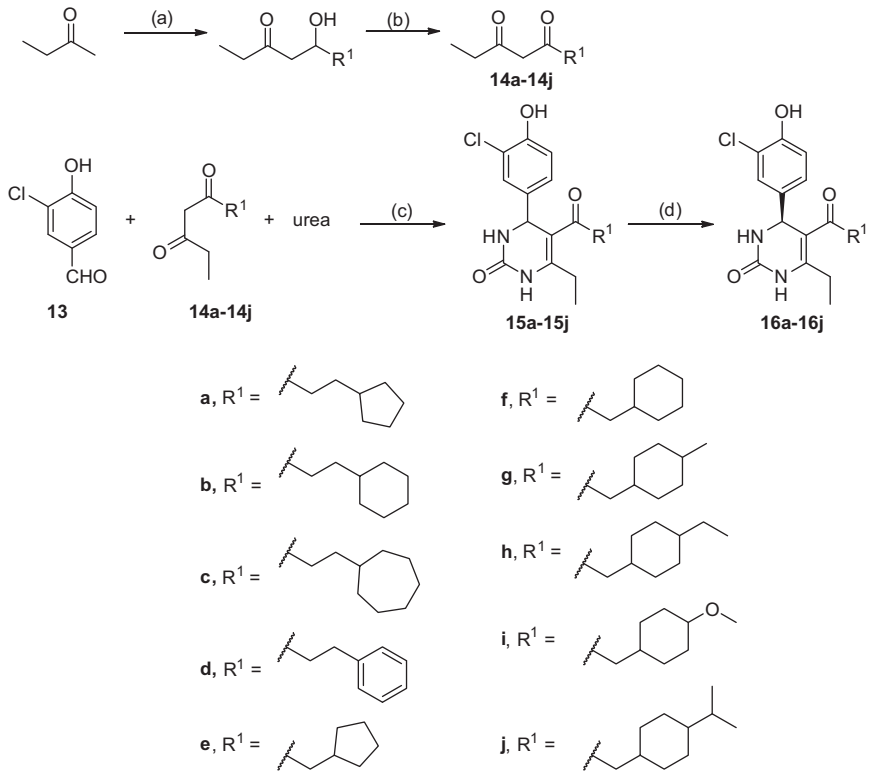
The target compounds (Tables 1 and 2) were synthesized as outlined in Schemes 1–3. The first series of ester modification in DHPMs focused on lactone analogs (**6**, **12**). For the synthesis of lactone analog **6**, Biginelli adduct **5** was obtained in a one-pot condensation reaction of an aldehyde **3**, β-keto ester **4**, and urea in the presence of 10 mol % VCl_3.[5] Subsequent thermolysis of ester **5** at high temperature gave the five-membered lactone **6** in good yield.[6] To synthesize the six-membered lactone analog **12**, the required β-keto ester **9** was prepared in three-steps from 1,3-propanediol. After the monoprotection of diol with TIPSCl, the resulting alcohol **7** was oxidized to aldehyde **8** and then subjected to $SnCl_2$-promoted Roskamp homologation[7] to give β-keto ester **9**. Condensation adduct **10** was synthesized via $Yb(OTf)_3$ catalyzed Biginelli reaction.[8] After deprotection of TIPS group, the resulting alcohol **11** underwent tin-mediated lactonization to afford lactone **12**.[9]

To explore the SAR of DHPMs with ketone moiety, isosteric ketone derivatives **15a–15j** were prepared in a similar procedure using Biginelli reaction with various 1,3-diketones **14a–14j**, which were synthesized by two-step protocol via aldol reaction[10] and subsequent oxidation.[11] The racemic ketone analogs (**15a–15j**) were separated into its enantiomer (**16a–16j**) via chiral separation.[12]

The next series of ester modifications to be explored was bioisostere replacement. The concept of bioisosterism has been widely utilized as one strategy for lead optimization in the drug discovery.[13] Three different bioisosteric replacements were designed and synthesized as shown in Scheme 3. To access an oxazole surrogate, α-bromo ketone **17** which was prepared from cyclohexyl methyl ketone by bromination underwent a cyclization with acetamide to give 2-methyl-1,3-oxazole **18**.[14] After the formation of 2-(lithiomethyl)oxazole via regioselective deprotonation with lithium diethylamide, the resulting anion was reacted with ethyl propionate to afford acylated product **20** in good yield.[15] Similarly, the synthesis of corresponding thiazole derivative **21** was achieved using thioacetamide. In order to obtain the substituted imidazole **24**, commercially available acetamidine hydrochloride salt was neutralized with sodium hydroxide and then subjected to cyclization with α-bromo ketone **17** to give 2-methyl-5-cyclohexyl imidazole **22**. After the extensive trials with various protecting groups,[16] we found trityl group is suitable for the following regioselective lithiation at 2-methyl position. Deprotonation/acylation



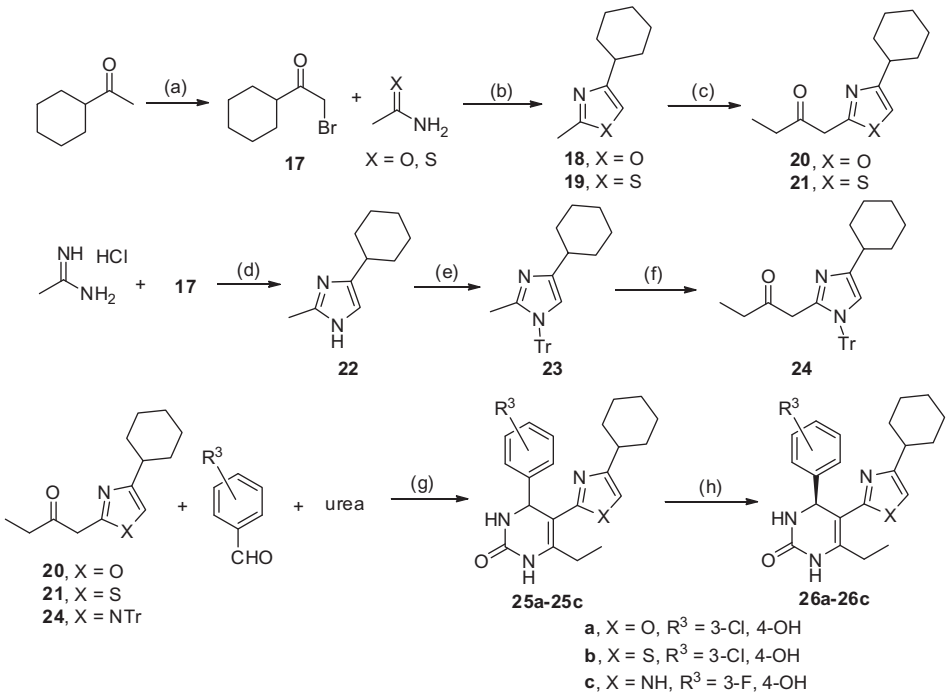
Scheme 1. Synthesis of lactone analogs **6** and **12**. Reagents and conditions: (a) 10 mol % VCl_3, CH_3CN, reflux, 8 h, 28%; (b) neat, 210 °C, 6 min, 91%; (c) NaH, THF, TIPSCl, 25 °C, 7 h, 92%; (d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 to 25 °C, 30 min, 93%; (e) 10 mol % $SnCl_2$, ethyl diazoacetate, CH_2Cl_2, 25 °C, 16 h, 86%; (f) 10 mol % $Yb(OTf)_3$, CH_3CN, 90 °C, 24 h, 56%; (g) TBAF, THF, 25 °C, overnight, 80%; (h) $(n\text{-}Bu_3Sn)_2O$, MeOH, 70 °C, 24 h, 95%.



Scheme 2. Synthesis of ketone analogs **16a–16j**. Reagents and conditions: (a) LDA, −78 °C, THF, 1 h, then R^1CHO, −78 °C, 1 h; (b) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 °C, 1 h; (c) 10 mol % Yb(OTf), THF, reflux, overnight, 44–63%; (d) Chiral HPLC separation.

was achieved successfully with 2 equiv of *n*-BuLi at low temperature, followed by trapping the resulting anion with ethyl propionate to generate β-keto imidazole **24** in moderate yield. After all the requisite β-keto heteroazoles were in hand, substituted oxazole **20**, thiazole **21**, and imidazole **24** were converted into Biginelli adducts **25a–25c** by heating in acetic acid with aldehyde and urea.[17] *N*-Trityl group in imidazole analog **25c** was deprotected simultaneously during acidic media condensation. The racemic bioisostere analogs (**25a–25c**) were also resolved into its enantiomer (**26a–26c**) using chiral separation.

Synthesized target compounds (**6**, **12, 16a–16j, 26a–26c**) were evaluated for their inhibitory activity against HIV-1 replication in CEM cells and Nevirapine was used as a positive control.[18] The assay results of compounds are summarized in Table 1. It was clear that the anti-HIV activity of the DHPMs is sensitive to structural modifications. Both lactone analogs (**6, 12**) resulted in inactive compounds, which indicated that a large lipophilic substituent in the right hand side is essential for anti-HIV activity. We next investigated the effect of R^1 moiety in the DHPM ketone analogs (**16a–16j**). Ketone analog **16b** revealed moderate antiviral activity



Scheme 3. Synthesis of bioisostere analogs **26a**–**26c**. Reagents and conditions: (a) Br_2, MeOH, H_2O, 25 °C, overnight, 97%; (b) neat, 130 °C, 2.5 h, **18** 26%, **19** 89%; (c) $LiNEt_2$, THF, ethyl propionate, −78 to 25 °C, 2 h, **20** 81%, **21** 53%; (d) NaOH, MeOH, 0 °C, 3 h; then **17**, K_2CO_3, CH_3CN/H_2O, 25 °C, overnight, 52%; (e) TritylCl, Et_3N, DMF, 25 °C, overnight, 100%; (f) *n*-BuLi (2.0 equiv), THF, −78 to 25 °C, 1 h; then ethyl propionate, −78 to 25 °C, overnight, 48%; (g) AcOH, 100 °C, 16 h, **25a** 28%, **25b** 49%, **25c** 20%; (h) Chiral HPLC separation.

compared with corresponding ester analog **2**. Variations in the ring size of R^1 displayed moderately improved antiviral activities depending on the ring sizes from five-membered **16a** (EC_{50} = 420 nM), six-membered **16b** (EC_{50} = 403 nM), and seven-membered **16c** (EC_{50} = 329 nM), respectively. However, the phenyl analog **16d** completely lost its antiviral activity. This result exemplified that a hydrophobic alicyclic R^1 group is a critical moiety for maintaining anti-HIV activity. Reducing the linker size from ethylene to methylene markedly increased the sensitivity of molecules to steric nature of substituents. Six-membered analog **16f** was three-time more active than five-membered analog **16e**. Variations in steric bulkiness at the 4-position of the cyclohexyl ring exhibited clear SAR. Attachment of a methyl group showed twofold increased potency in analog **16g**. Increasing substituents from methyl (**16g**) to ethyl group (**16h**) led to fivefold increase in potency. Further increase in size from ethyl to *iso*-propyl group (**16j**) and replacing an ethyl group with a methoxy group (**16i**) resulted in the loss of activity. These results clearly showed the subtleness at this position. Based on the SAR, the optimal substituent in the ketone analogs is a methylene linker and a cyclohexyl ring with an ethyl group at the 4-position (**16h**, EC_{50} = 87 nM).

After examining lactone and ketone derivatives, we evaluated the effect of replacing ester with bioisosteres for compounds **26a–26c**. The ester group was replaced with bioisosteric fragments such as oxazole and thiazole, and those compounds were minimized using CHARMM force field[19] implemented in Discovery Studio (ver. 3.1).[20] Minimized conformation of compounds **2** (Fig. 2a), **26a** (data not shown) and **26b** (Fig. 2b) were well aligned to each other when aligned according to steric and electrostatic fields. The carboxylic group and thiazole overlapped well with each other retaining similar physicochemical properties (Fig. 2c). In respect to minimized energy, compound **26b** (13.21 kcal/mol) was more stable than compound **2** (14.74 kcal/mol). Indeed, oxazole and thiazole analogs (**26a**, **26b**) exhibited similar activities with EC_{50} of 116 and 78 nM, respectively. On the other hand, imidazole analog

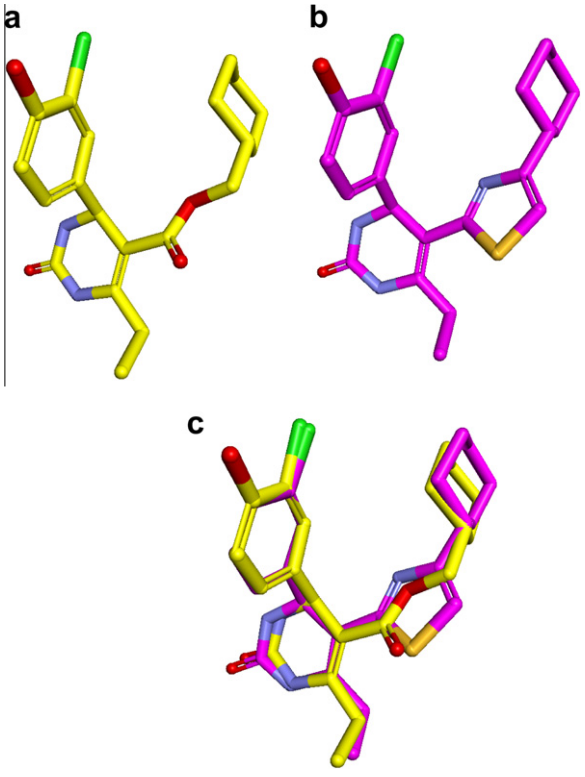


Figure 2. Lowest energy conformers of **2** (a) and **26b** (b) are well superimposed (c). All compounds are shown in atom-colored stick form with different colored carbons: yellow for compound **2**, and pink for compound **26b**. Hydrogens were removed for clarity purpose.

26c did not show any antiviral activity, which is consistent with the results from previously reported amide analogs.[4]

Compounds which exhibited significant potency against HIV-1 replication in a cell-based assay were further evaluated for their metabolic stability in vitro.[21] Test results were summarized in Table 2. Ketone analogs (**16b, 16f, 16h**) displayed poor metabolic stability similar to the corresponding ester analog **2**. Gratifyingly, however, oxazole analog **26a** and thiazole analog **26b** exhibited markedly improved metabolic stability in vitro while retaining significant antiviral activity against HIV-1 replication.

In summary, novel dihydropyrimidinone analogs (**6**, **12**, **16a**–**16j**, **26a**–**26c**) were designed and synthesized to optimize metabolic stability. In particular, replacement of the ester moiety by bioisosteres dramatically improved the metabolic liability while retaining antiviral activity against HIV-1. These novel and potent DHPMs containing ester bioisosteres could serve as advanced lead compounds for further development. Mode of action and further optimization of this lead compound will be reported in due course.

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8. *General procedure for Biginelli reaction with β-keto esters:* The β-keto ester **9** (200 mg, 0.632 mmol), 3-hydroxybenzaldehyde (77 mg, 0.632 mmol), urea (57 mg, 0.948 mmol), and $Yb(OTf)_3$ (39 mg, 0.063 mmol) were dissolved in acetonitrile (1.6 mL) and stirred under Argon for 24 h at 90 °C. After cooling to room temperature, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layers were dried over Na_2SO_4. After filtration and concentration in vacuo, the residue was purified by flash column chromatography (SiO_2, *n*-hexanes/EtOAc = 5:1→1:1 then CH_2Cl_2/MeOH = 20:1) to give Biginelli adduct **10** (164 mg, 56%) as a yellow solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.88 (s, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.76–6.73 (m, 2H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.39 (s, 1H), 5.22 (s, 1H), 4.04–3.94 (m, 4H), 3.07–2.97 (m, 2H), 1.12–1.02 (m, 24H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.7, 156.5, 153.1, 148.8, 144.9, 129.7, 117.8, 114.9, 113.7, 101.5, 62.0, 60.1, 55.1, 33.6, 17.9, 14.0; TLC R_f (CH_2Cl_2:MeOH 10:1) = 0.42.
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16. Other protecting groups tried in this reaction were THP, 2,4-dimethoxybenzyl, 4-methoxybenzyl, MOM, Boc groups.
17. *General procedure for Biginelli reaction with β-keto heteroazoles:* To a mixture of aryl aldehyde (1.0 equiv), urea (1.5 equiv), and β-keto oxazole (thiazole, or imidazole) (1.0 equiv) was added acetic acid (0.2 M) and stirred at 110 °C for 16 h. The reaction was slowly quenched with saturated aqueous K_2CO_3 (caution: *gas evolution!*) and extracted with EtOAc (3×). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was redissolved with CH_2Cl_2 and crystallized by the slow addition of hexanes. The resulting precipitate was filtered and washed with cold CH_2Cl_2/*n*-hexanes (1:1) solution. Alternatively, the concentrated residue was directly subjected to flash column chromatography (SiO_2, CH_2Cl_2/MeOH = 19:1) to give Biginelli adducts. Compound **26a** ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (s, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 7.07 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 5.44 (s, 1H), 2.70 (m, 2H), 2.40 (m, 1H), 1.92 (m, 2H), 1.72 (m, 2H), 1.65 (m, 1H), 1.35–1.24 (m, 5H), 1.94 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD3OD) δ 159.6, 152.4, 146.4, 143.3, 136.1, 131.5, 128.0, 125.8, 120.1, 116.2, 98.0, 54.6, 35.6, 31.9, 31.4, 25.8, 25.7, 23.8, 20.2, 11.7; Enantiomerically pure forms were obtained by chiral HPLC (OD-H preparative column, 20% *i*-PrOH in *n*-hexanes, 6.5 mL/min, t_R = 20.5 min): **26b** mp 186 °C; $[\alpha]_D^{26}$ +8.1 (*c* 0.001, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.22 (d, *J* = 2.0 Hz, 1H), 7.06 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.86 (s, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 5.39 (s, 1H), 2.63 (m, 3H), 1.96 (m, 2H), 1.79 (m, 2H), 1.71 (d, *J* = 12.4 Hz, 1H), 1.46–1.25 (m, 5H), 1.22 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.7, 163.2, 155.7, 153.9, 142.1, 137.1, 129.8, 127.5, 122.5, 117.6, 111.5, 105.4, 58.6, 41.7, 34.1, 33.7, 27.4, 25.0, 12.7; TLC R_f (CH_2Cl_2/MeOH 19:1) = 0.42; HRMS (EI) *m/z* calcd for $C_{21}H_{24}ClN_3O_2S$ (M)$^+$ 417.1277, found 417.1282; Enantiomerically pure forms were obtained by chiral HPLC (OD-H preparative column, 20% *i*-PrOH in *n*-hexanes, 6.5 mL/min, t_R = 18.1 min).
18. HIV full replication assay. CEMx174-LTR-GFP cells (clone CG8) were seeded with a microplate dispenser (WellMate; Thermo Scientific Matrix; USA) at a density of 4000 cells/well into 384-well glass plates (Evotec. Hamburg, Germany) pre-dispensed with 10 μL of compound diluted in DMSO and incubated for 1 h at 37 °C, 5% CO_2. Then cells were infected with HIV-1_{IAI} at a multiplicity of infection (MOI) of 3 and incubated for 5 days at 37 °C, 5% CO_2. Fluorescence intensities were the determined using a multilabel plate reader (Victor3; PerkinElmer, Inc.; USA). And see: Sommer, P.; Vartanian, J. P.; Wachsmuth, M.; Henry, M.; Guetard, D.; Wain-Hobson, S. *J. Mol. Biol.* **2004**, *344*, 11.
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21. (a) *Materials:* Human, Rat Microsomes and NADPH regenerating system were purchased from BD Gentest (Woburn, MA). Liquid chromatographic analysis was performed on the instrument of Agilent 1200 series equipped with a diode-array detector and autosampler (Agilent Technology, Piscataway, NJ). An analytical column was applied after the trapping cartridges (Phenomenex, Gemini C18 50 mm × 2.0 mm, 3 μm, Torrance, CA). A quadrupole LC mass spectrometer (Agilent Technology, Piscataway, NJ), with electrospray ionization (ESI) was employed for sample analysis. Instruments were controlled by ChemStation software (Version 2.0, Agilent Technology, Piscataway, NJ).; (b) *Methods:* Compounds (2 μM final concentration) are incubated with liver microsomes (rat and human) in potassium phosphate buffer. The microsomal protein concentration in the assay is 0.5 mg/mL and the final percent DMSO is 0.2%. Reaction is started by the addition of NADPH and stopped either immediately or at 5, 10, 30, 60 and 120 min for a precise estimate of clearance. The corresponding loss of parent compound is determined by LC/MS. The mobile phases were (A) water with 0.1% of formic acid and (B) acetonitrile with 0.1% of formic acid at a flow rate of 0.3 mL/min. The LC conditions were 5% B at 0 min, a linear gradient from 5% to 50% B over 1 min, held at 50% for 0.5 min, then ramped from 50% to 95% over 0.5 min, followed by 95% B for 1.5 min and back to 5% B over 0.5 min, then held at 5% B for the remaining 3.5 min.; (c) *Data analysis:* % remaining of compound is calculated compared to the initial quantity at time zero. Half-life is then calculated based on first-order reaction kinetics.