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Discovery of 3,4-dihydropyrimidin-2(1*H*)-ones with inhibitory activity against HIV-1 replication

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ABSTRACT

3,4-Dihydropyrimidin-2(1*H*)-ones (DHPMs) were selected and derivatized through a HIV-1 replication assay based on GFP reporter cells. Compounds **14, 25, 31**, and **36** exhibited significant inhibition of HIV-1 replication with a good safety profile. Chiral separation of each enantiomer by fractional crystallization showed that only the *S* enantiomer retained anti-HIV activity. Compound (*S*)-**40**, a novel and potent DHPM analog, could serve as an advanced lead for further development and the determination of the mechanism of action.

In 2009 more than 33 million people were infected with the human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), which currently accounts for the highest number of deaths by any single infectious agent.[1] There are currently 25 drugs belonging to 6 different inhibitor classes approved 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 considerably improved the life quality of infected people by delaying the progression of the disease and reducing disabilities, making HIV/AIDS a chronic disease, not a death sentence. However, there are serious drawbacks of HAART due to the tendency of HIV-1 to rapidly mutate. Prolonged HAART treatment leads to the emergence of drug-resistant strains of the virus.[3] Also, the side effects of combination therapy have limited their clinical effectiveness.[4] Therefore, the continued development of novel anti-HIV drugs with acceptable toxicity and resistance profiles is clearly needed.

In a high-throughput screening campaign for the discovery of novel antiretrovirals employing a HIV full replication assay based on reporter cells harboring an EGFP expression cassette under the control of the HIV promoter, we identified a series of compounds containing a dihydropyrimidinone scaffold (Fig 1) that exhibited inhibitory activities against HIV replication at low micromolar concentrations. Dihydropyrimidinone derivatives have been reported to exhibit diverse biological activities, such as, anti-bacterial,[5] anti-fungal,[6] anti-cancer,[7] and anti-oxidant activities,[8] whereas an anti-HIV activity has not been previously documented.[9] Here, we report the preliminary SAR of dihydropyrimidinones as novel inhibitors of HIV-1 replication and a scale-up procedure for the preparation of enantiomerically pure forms using a fractional crystallization method.

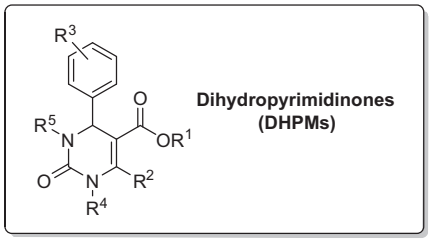

Figure 1. Hit compounds from cell-based HIV-1 replication assay.

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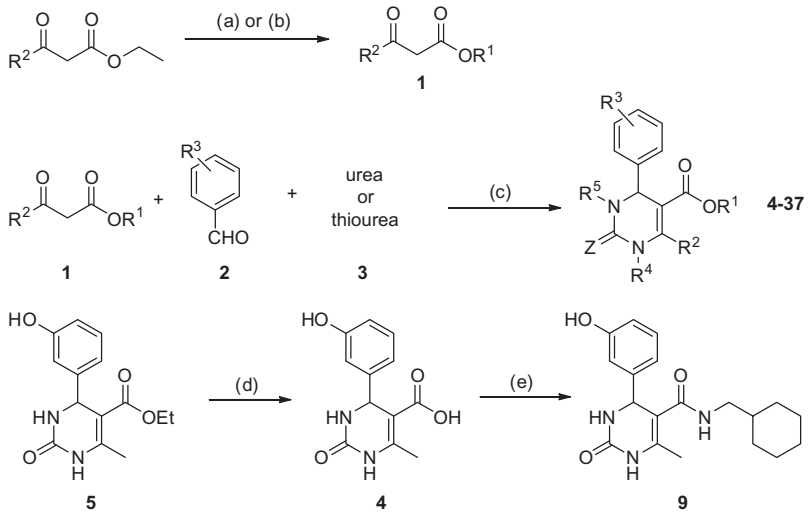
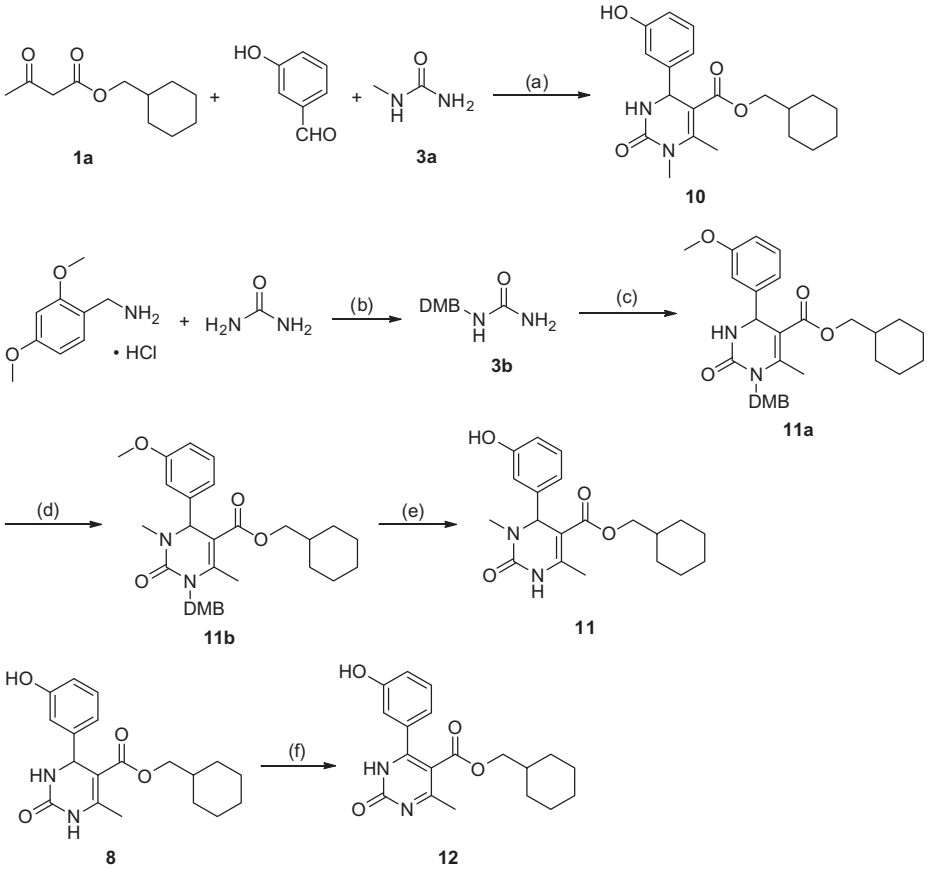
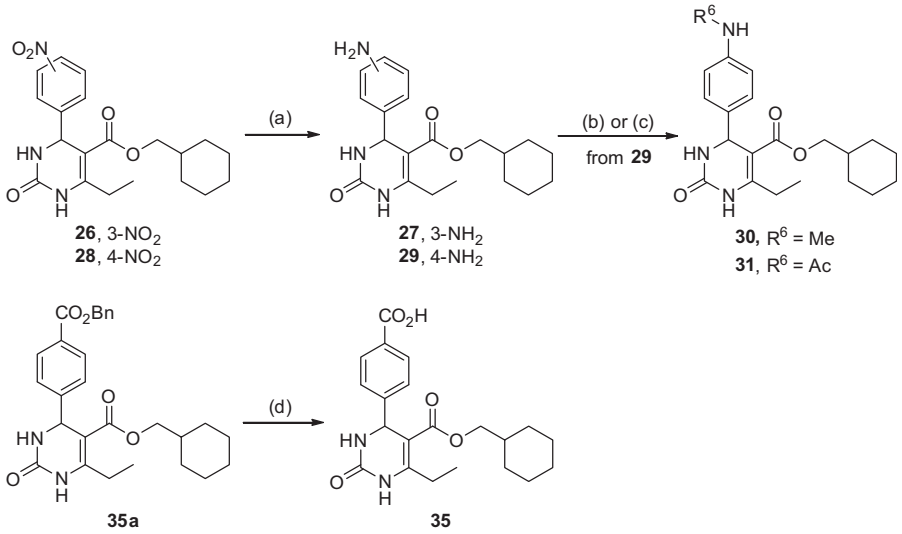


Scheme 1. Synthesis of dihydropyrimidinones **4**–**37**. Reagents and conditions: (a) cat. I_2, R^1OH, toluene, reflux 12 h; (b) NaOH, H_2O, 25 °C, 12 h; then EDC, DMAP, R^1OH, CH_2Cl_2, 25 °C, overnight; (c) cat. $Yb(OTf)_3$, THF, reflux, overnight; (d) NaOH, $MeOH/H_2O$, 25 °C, 48 h; (e) EDC, HOBt, cyclohexylmethylamine, DMF, 25 °C, 4 h.

A simple and direct method for the synthesis of dihydropyrimidinones (DHPMs), first reported by Biginelli in 1893,[10] involves a reaction in which three reactants come together in a one-pot to form a new product that contains all the components. The classical Biginelli reaction is a one-pot condensation reaction of an aryl aldehyde, β-keto ester, and urea or thiourea with catalytic acid in a protic solvent, which often suffer from low yields especially in the case of substituted aromatic and aliphatic aldehydes.[11] Since then numerous improved synthetic methods have been reported.[12] To explore the SAR of DHPMs, we prepared a series of dihydropyrimidone derivatives using $Yb(OTf)_3$ as a catalyst under refluxing condition in THF (Scheme 1).[13] Various β-keto esters **1** were



Scheme 2. Synthesis of dihydropyrimidinones **10**–**12**. Reagents and conditions: (a) cat. $BF_3{\cdot}OEt_2$, CuCl, AcOH, THF, reflux, 12 h; (b) HCl, H_2O, reflux, overnight; (c) **1a**, 3-methoxybenzaldehyde, cat. $BF_3{\cdot}OEt_2$, CuCl, AcOH, THF, 65 °C, 12 h; (d), NaHMDS (1.0 M in THF), THF, −78 °C, then $(CH_3O)_2SO_2$, −78 °C to 25 °C, 2 h; (e) BBr_3 (1.0 M in CH_2Cl_2), CH_2Cl_2, −78 °C, 1 h to 25 °C, 10 min; (f) CAN, $NaHCO_3$, acetone/H_2O, 0–25 °C, overnight.



Scheme 3. Synthesis of dihydropyrimidinones **26**–**35**. Reagents and conditions: (a) 10% Pd/C, H_2 (1 atm), MeOH, 25 °C, 2 h; (b) MeI, DIPEA, CH_2Cl_2/DMF (1:1), 25 °C, overnight; (c) EDC·HCl, DMAP, Ac_2O, THF, 25 °C, 12 h (d) 10% Pd/C, H_2 (1 atm), MeOH, 25 °C, 2 h.

synthesized by the methods known in the literatures.[14] Hydrolysis of the ester **5** in sodium hydroxide gave the corresponding carboxylic acid **4**, which was converted into amide analog **9** using EDC coupling reaction. In order to synthesize *N*-methyl substituted analogs (**10, 11**), substituted ureas (**3a, 3b**) were used in $BF_3{\cdot}OEt_2$ catalyzed Biginelli reaction (Scheme 2). For compound **11**,

Table 1
Cell-based antiviral activity of **4**–**37** against HIV-1

compound	Z	R^1	R^2	R^3	R^4	R^5	EC_{50}[a] (μM)	CC_{50}[b] (μM)
4	o	H	Me	3-OH	H	H	>10	>10
5	o	Et	Me	3-OH	H	H	>10	ND[d]
6	o	Bn	Me	3-OH	H	H	>10	>10
7	0	*c*-Hex	Me	3-OH	H	H	>10	>10
8	o	CH_2-*c*-Hex	Me	3-OH	H	H	0.529	>10
9	o	$NHCH_2$-*c*-Hex	Me	3-OH	H	H	>10	ND
10	o	CH_2-*c*-Hex	Me	3-OH	Me	H	>10	>10
11	o	CH_2-*c*-Hex	Me	3-OH	H	Me	>10	>10
12	0	CH_2-*c*-Hex	Me	3-OH	H	H	>10	>10
13	s	CH_2-*c*-Hex	Me	3-OH	H	H	>10	>10
14	o	CH_2-*c*-Hex	Et	3-OH	H	H	0.087	>10
15	o	CH_2-*c*-Hex	Pr	3-OH	H	H	0.286	>10
16	0	CH_2-*c*-Hex	*i*-Pr	3-OH	H	H	>10	ND
17	o	CH_2-*c*-Hex	Cyclopropyl	3-OH	H	H	>10	>10
18	o	CH_2-*c*-Hex	$-CH_2Cl$	3-OH	H	H	0.359	>3
19	o	CH_2-*c*-Hex	$-C{\equiv}CH$	3-OH	H	H	2.20	>1
20	o	CH_2-*c*-Hex	Ph	3-OH	H	H	>10	>10
21	s	CH_2-*c*-Hex	Et	3-OH	H	H	>10	>10
22	o	CH_2-*c*-Hex	Me	2-OH	H	H	>10	>10
23	o	CH_2-*c*-Hex	Me	3-OMe	H	H	1.27	>10
24	o	CH_2-*c*-Hex	Me	4-OH	H	H	0.431	>10
25	0	CH_2-*c*-Hex	Et	4-OH	H	H	0.031	>10
26	o	CH_2-*c*-Hex	Et	3-N02	H	H	0.335	>10
27	o	CH_2-*c*-Hex	Et	3-NH2	H	H	0.159	>10
28	o	CH_2-*c*-Hex	Et	4-NO2	H	H	0.141	>10
29	0	CH_2-*c*-Hex	Et	4-NH2	H	H	0.151	>10
30	o	CH_2-*c*-Hex	Et	4-NHMe	H	H	0.108	>10
31	o	CH_2-*c*-Hex	Et	4-NHAc	H	H	0.063	>10
32	o	CH_2-*c*-Hex	Et	4-NMe2	H	H	0.177	>10
33	o	CH_2-*c*-Hex	Et	4-CN	H	H	0.061	>10
34	0	CH_2-*c*-Hex	Et	4-C02Me	H	H	0.122	>10
35	o	CH_2-*c*-Hex	Et	4-C02H	H	H	>10	>10
36	o	CH_2-*c*-Hex	Et	4-F	H	H	0.024	>10
37	o	CH_2-*c*-Hex	Et	4-Cl	H	H	0.088	>10
NVP[c]							0.150	>10

[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compound **4–37**, $n = 2$ 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.
[d] Not determined.

Table 2
Cell-based antiviral activity of separated enantiomers against HIV-1

Compound	R^2	Stereochemistry	EC_{50}[a] (μM)	CC_{50}[b] (μM)
8	Me	Racemic	0.529	>10
(*S*)-38		(*S*)-	0.233	>10
(*R*)-39		(*R*)-	>10	>10
14	Et	Racemic	0.087	>10
(*S*)-40		(*S*)-	0.038	>10
(*R*)-41		(*R*)-	>10	>10

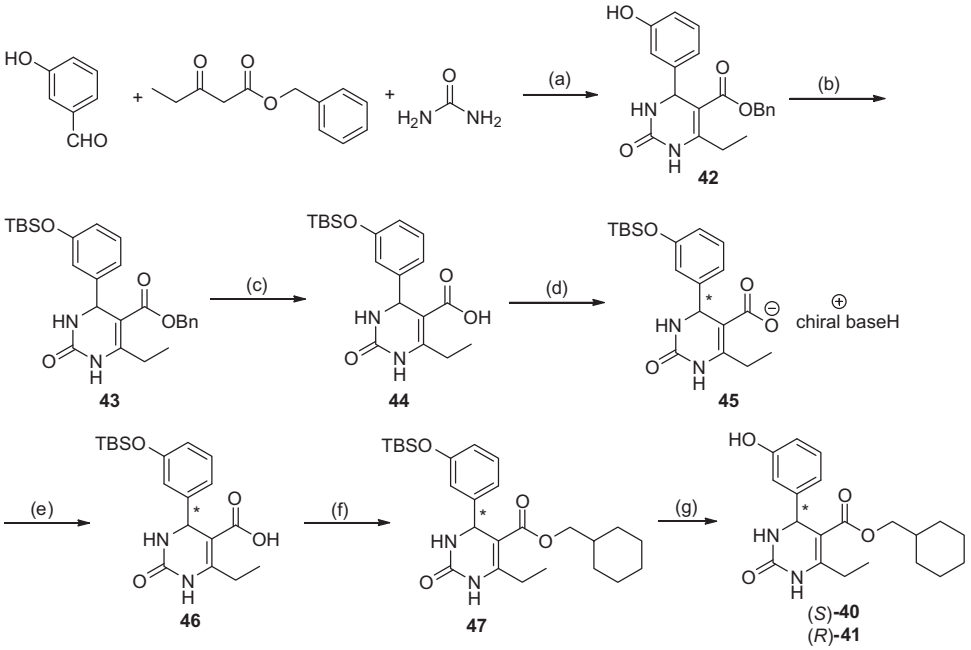
[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compound, $n = 2$ and the values are the geometric mean of two determinations; all individual values are within 25% of the mean.

[b] CC_{50} is the cytotoxic concentration of compound that reduces viability of uninfected cells by 50%.

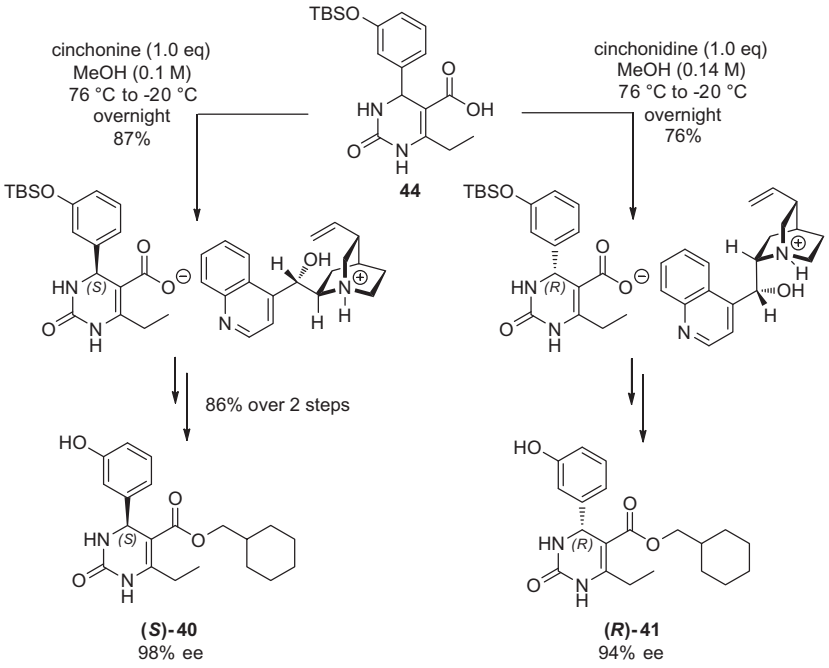
2,4-dimethoxybenzyl protected urea was prepared in acidic condition and subjected into following cyclization reaction. Treatment of Biginelli adduct **11a** with NaHMDS and trapping with dimethyl sulfate afforded *N*-methylated compound **11b.** Subsequent deprotection of methoxy and DMB group with BBr_3 gave the desired analog **11**. To evaluate the effect of chiral center in DHPM analogs, compound **12** was synthesized via CAN-mediated oxidation from compound **8**. For the synthesis of amine analogs (**27–31**), nitro group was reduced to the corresponding amine, which was subjected to alkylation and acylation to give compound **30** and **31**, respectively. Carboxylic acid analog **35** was synthesized from ester analog **35a** by reductive removal of the benzyl group in the presence of 10% Pd/C under H_2 (Scheme 3).

Synthesized compounds **4**–**37** were evaluated for their inhibitory activity against HIV-1 replication in CEM cells and Nevirapine was used as a positive control.[15] Assay results of compound **4**–**37** are summarized in Table 1. It was apparent that the anti-HIV activities of the DHPMs are sensitive to structural perturbations. Compounds **4**–**8** have the common DHPM core but with varying R^1 groups. Results showed that a hydrophobic alicyclic R^1 is preferred and **8** exhibited EC_{50} of 0.529 μM. Typical alkyl ester (**5**), benzyl ester (**6**) or polar carboxylic acid (**4**) itself resulted in the loss of cellular activities. Also the distance of alicyclic group R^1 from the carbonyl group of carboxyl ester is a critical factor and analog 7 without methylene group showed reduced potency. Replacement of ester moiety with amide **9** did not show any inhibitory activity. Methyl substitutions on nitrogen (**10, 11**) resulted in complete loss of activity. Oxygen atom at position 2 in dihydropyrimidinones was a mandatory requirement for activity as was shown in compounds (**8, 14**) compared with sulfur analogs (**13, 21**), respectively. The spatial arrangement of phenyl group at C-4 of DHPMs plays a pivotal role and an oxidized, achiral analog **12** was inactive up to 10 μM. We next investigated the effect of R^2 moiety in DHPM analogs **14**–**20** and their results displayed clear SAR. Increasing the size of R^2 from methyl, ethyl, and propyl showed cellular activities from 529, 87, and 286 nM, respectively. Other analogs similar to two carbon unit (*iso*-Pr **16**, cyclopropyl **17**, chloromethyl **18** and ethynyl **19**) and phenyl analog **20** exhibited reduced or complete loss of activities. From these results, it is clear that the R^2 position is sensitive to steric and electronic nature of substituents. The optimal substitution at this position is an ethyl group (**14**, EC_{50} = 87 nM). The same effect was also shown again by 13-fold activity difference between **24** and **25**. After examining the R^1 and R^2 regions, we evaluated the substituent effect on phenyl ring with compounds **22**–**37**. Both *para*-OH analog **8** and *meta*-OH analog **24** showed comparable activities of 529 and 431 nM, respectively. However, *ortho*-OH analog **22** suffered the loss of antiviral activity. Methoxy analog **23** was twofold less active than hydroxyl analog **8**. Generally, amino analogs (**27, 29**) displayed slightly reduced potency compared to corresponding hydroxyl analogs (**14**, **25**). Mono-substituted amine analogs (NH-Me **30**, NH-acetyl **31**) exhibited slightly improved activity than corresponding di-substituted amine analog **32**. Analogs with electron-withdrawing group at *para*-position on phenyl ring (4-NO_2 **28**, 4-CN **33**, 4-CO_2Me **34**, 4-F **36**, 4-Cl **37**) maintained significant inhibitory activities except carboxylic acid analog **35**, which was presumably due to its low cell membrane permeability in our cell-based assay system.

The resolution of **8** and **14** into their enantiomers was performed to evaluate the impact of stereochemistry at C-4.[16] The



Scheme 4. Synthetic route of enantiomer (*S*)-**40** using fractional crystallization method. Reagents and conditions: (a) cat. $Yb(OTf)_3$, THF, reflux, 24 h, 77%; (b) TBSCl, imidazole, DMF, 25 °C, overnight, 100%; (c) Pd/C, H_2 (3 bars), MeOH/Et_3N, 25 °C, 3 h; then 1 N HCl, H_2O, 91%; (d) fractional crystallization with chiral amines; (e) 1 N HCl, H_2O; (f) EDC, DMAP, cyclohexylmethanol, 50 °C, overnight; (g) TBAF, CH_2Cl_2, 0 °C, 30 min, 86% over 2 steps.



Scheme 5. Conditions for fractional crystallization of a racemic mixture (**44**).

absolute stereochemistry of separated enantiomers was confirmed by circular dichroism (CD) spectroscopy.[17] Interestingly, test results against HIV-1 revealed that only (*S*)-enantiomers (**38, 40**) exhibited antiviral activity, whereas (*R*)-enantiomers (**39, 41**) were completely inactive up to 10 μM (Table 2).

Encouraged by these results, we developed a new synthetic route to access enantiomers for further evaluation and optimization. The most potent enantiomer (*S*)-**40** could be scaled up using a fractional crystallization method as outlined in Scheme 4. Compound **42** is readily accessible via Biginelli reaction and the resulting hydroxyl group was protected with TBSCl. Subsequent conversion of **43** to carboxylic acid **44** was achieved by reductive removal of the benzyl group in the presence of 10% Pd/C under H_2 (3 bars). Racemic carboxylic acid **44** was then co-crystallized with various chiral amines. Enantiomeric excess was determined after the conversion of resolved carboxylic acid into esters via EDC coupling and subsequent deprotection of TBS group. After examining the various chiral amines, we found that cinchonine/cinchonidine series could separate each enantiomer selectively with 98% ee for (*S*)-**40** and 94% ee for (*R*)-**41** (Scheme 5).[18] This combination could be applied to other dihydropyrimidinone compounds.

In summary, a series of dihydropyrimidinone analogs (**4–37**) was synthesized and evaluated as HIV-1 replication inhibitors in vitro. Among the derivatives, compounds **14**, **25**, **31**, and **36** exhibited significant inhibitory activity against HIV-1 in a cell-based assay. Chiral separation of the enantiomers showed that the *S* configuration on the C-4 in dihydropyrimidinone ring is a crucial factor for antiviral activity. Each enantiomer could be scaled up and separated selectively via fractional crystallization. Mode of action and further optimization of this lead compound will be reported in due course.

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13. A mixture of $Yb(OTf)_3$ (0.05 mmol, 0.1 equiv), β-ketoester (0.5 mmol, 1.0 equiv), aryl aldehyde (0.5 mmol, 1.0 equiv) and urea (0.5 mmol, 1.0 equiv) in THF (2 mL) was refluxed 20–30 h under Ar. After adding H_2O (5 mL), the mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (SiO_2, *n*-Hexanes/EtOAc/AcOH, 50:50:0.5) to afford the desired dihydropyrimidinone compound.
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15. HIV full replication assay. CEMx174-LTR-GFP cells (clone CG8) were seeded with a microplate dispenser (WellMate; Thermo Scientific Matrix; U.S.A.) 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_{LAI} 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.; U.S.A.). 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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18. The β-keto ester (1.0 g, 4.84 mmol), 3-hydroxybenzaldehyde (590 mg, 4.84 mmol), urea (290 mg, 4.84 mmol), and $Yb(OTf)_3$ (30 mg, 0.484 mmol) were dissolved in THF (9.6 mL) and stirred under Argon for 24 h at 90. 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 via flash column chromatography (SiO_2, *n*-Hexanes/EtOAc/AcOH = 5:1:0.5) to give Biginelli adduct **42** (1.31 g, 77%) as a pale yellow solid. To a solution of Biginelli adduct **42** (1.30 g, 3.69 mmol) in DMF (19 mL) was added TBSCl (834 mg, 5.53 mmol) and imidazole (377 mg, 5.53 mmol) at 25 °C. After stirring for overnight at 25 °C, the reaction mixture was quenched by the addition of H_2O (30 mL), extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (30 mL) and dried over Na_2SO_4. After filtration and concentration in vacuo, the residue was purified via flash column chromatography (SiO_2, *n*-Hexanes/Et_2O = 5:1 → *n*-Hexanes/EtOAc = 2:1 to 1:1 → CH_2Cl_2/MeOH = 20:1) to give a TBS protected product **43** with a quantitative yield as a white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.14–7.12 (m, 3H), 7.01–6.98 (m, 3H), 6.70 (d, *J* = 7.2 Hz, 1H), 6.61 (bs, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 5.48 (bs, 1H), 5.21 (s, 1H), 4.91 (s, 2H), 2.67–2.57 (m, 2H), 1.09–1.05 (m, 3H), 0.81 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 156.1, 153.2, 152.5, 144.9, 136.0, 129.8, 128.4, 128.0, 127.9, 119.7, 119., 118.1, 99.9, 65.9, 55.4, 25.7, 25.3, 18.2, 12.5, −4.4; TLC R_f (CH_2Cl_2–MeOH 10:1) = 0.51. To a solution of TBS protected product **43** (721 mg, 1.545 mmol) in MeOH (16 mL) was added 10% Pd on carbon (72 mg) and Et_3N (215 μL, 1.545 mmol) at room temperature. The reaction mixture was hydrogenated with H_2 gas (3 bars) for 3 h at 25 °C. The mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was resuspended in water (20 mL) and acidified with 1 N HCl (~3 mL, pH <2). The resulting suspension was sonicated for 10 min and then filtered and washed with H_2O. After freeze drying in vacuo, the resulting acid **44** (white solid, 529 mg, 91%) was used in the following step without further purification. To a suspension of acid **44** (1.00 g, 2.655 mmol) in MeOH (20 mL) was added cinchonine (782 mg, 2.655 mmol) at 76 °C. The resulting suspension was treated with the slow addition of MeOH (10 mL) at 76 °C. The resulting clear solution was slowly cooled to room temperature followed by overnight storage at −20 °C. The next day, the salt was filtered and rinsed with EtOH to give an acid/cinchonine salt (614 mg, 69%) as a white solid. The filtrate was concentrated in vacuo and the residue was re-subjected to fractional crystallization in MeOH (10 mL) to give an additional acid/cinchonine salt (125 mg, 14%). The salt above (80 mg) was resuspended in water (4 mL) and acidified with 1 N HCl (500 μL, pH <2). The resulting suspension was sonicated for 10 min and then centrifuged to remove the upper layer. After repeating the same procedure one more time, the resulting solid was washed with H_2O (3 × 4 mL) and freeze dried to give a salt-free acid **46** (43 mg, 97%) as a white solid: ^1H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 7.47 (s, 1H), 7.03 (t, *J* = 8.0 Hz, 1H), 6.70 (d, *J* = 7.6 Hz, 1H), 6.58 (s, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 4.90 (d, *J* = 3.6 Hz, 1H), 2.50–2.47 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H), 0.77 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.5, 155.8, 153.4, 147.1, 130.1, 120.1, 119.2, 118.1, 54.2, 26.2, 24.6, 18.6, 13.8, −3.8; TLC R_f (CH_2Cl_2–MeOH 10:1) = 0.31. To a solution of acid **46** (20 mg, 0.053 mmol) and alcohol (18 mg, 0.159 mmol) in DMF (5 mL) was added EDC (31 mg, 0.159 mmol) and DMAP (32 mg, 0.266 mmol) at room temperature. The resulting mixture was heated at 50 °C overnight under Argon. The reaction was quenched by the addition of saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (2 × 5 mL) and brine (5 mL) successively, and then dried over Na_2SO_4. After filtration and concentration in vacuo, the residue was purified via preparative TLC (SiO_2, 0.5 mm, CH_2Cl_2–MeOH = 10:1) to give an ester **47** with a quantitative yield as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 7.6 Hz, 1H), 6.75–6.72 (m, 1H), 6.71 (dd, *J* = 6.4, 1.6 Hz, 1H), 5.59 (s, 1H), 5.31 (d, *J* = 2.8 Hz, 1H), 3.85–3.76 (m, 2H), 2.81–2.68 (m, 2H), 1.65–0.78 (m, 11H), 1.22 (t, *J* = 7.2 Hz, 3H), 0.94 (s, 9H), 0.14 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.9, 148.7, 145.8, 144.5, 137.6, 122.4, 112.3, 112.0, 110.7, 92.9, 62.0, 48.1, 29.8, 22.3, 18.9, 18.5, 18.3, 17.9, 10.8, 5.1; TLC R_f (CH_2Cl_2–MeOH 10:1) = 0.47. To a 0 °C solution of the above ester **47** (0.053 mmol) in CH_2Cl_2 (2 mL) was added dropwise TBAF (1 M in THF, 64 μL, 0.064 mmol). After 10 min at 0 °C, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (2 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL) and dried over Na_2SO_4. After filtration and concentration in vacuo, the residue was purified via preparative TLC (SiO_2, 0.5 mm, CH_2Cl_2–MeOH = 10:1) to give a desilylated product (*S*)-**40** (17 mg, 86% over 2 steps) as a white solid. The enantiomeric excess was determined to be 98% ee by chiral HPLC (Daicel Chiralcel AD column, 0.85 mL/min, *n*-Hexanes/*i*-PrOH = 75:25): ^1H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.15 (s, 1H), 7.63 (s, 1H), 7.09 (t, *J* = 8.0 Hz, 1H), 6.66–6.61 (m, 3H), 5.04 (d, *J* = 3.2 Hz, 1H), 3.83 (dd, *J* = 10.8, 6.0 Hz, 1H), 3.72 (dd, *J* = 10.8, 6.0 Hz, 1H), 2.76–2.68 (m, 1H), 2.66–2.57 (m, 1H), 1.61–1.41 (m, 6H), 1.40–1.00 (m, 6H), 0.87–0.75 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.7, 158.1, 154.7, 152.9, 146.7, 129.9, 117.6, 114.8, 113.8, 98.8, 68.8, 54.6, 37.4, 29.72, 29.65, 26.4, 24.7, 13.7.