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Design, synthesis, and biological evaluation of novel trifluoromethyl indoles as potent HIV-1 NNRTIs with an improved drug resistance profile†

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A novel series of trifluoromethyl indole derivatives have been designed, synthesized and evaluated for anti-HIV-1 activities in MT-2 cells. The hydrophobic constant, acute toxicity, carcinogenicity and mutagenicity were predicted. Trifluoromethyl indoles **10i** and **10k** showed extremely promising activities against WT HIV-1 with IC_{50} values at the low nanomolar level, similar to efavirenz, better than nevirapine, and also possessed higher potency towards the drug-resistant mutant strain Y181C than nevirapine. Preliminary SAR and docking studies of detailed binding mode provided some insights for discovery of more potent NNRTIs.

Introduction

Human immunodeficiency virus (HIV) reverse transcriptase (RT) is responsible for producing the DNA copy of the viral RNA genome that will be integrated into the human DNA. HIV RT, together with protease and integrase, is one of three key enzymes in the HIV life cycle and the primary target of many antiviral drugs.[1] Non-nucleoside reverse transcriptase inhibitors (NNRTIs) target an allosteric binding pocket on HIV-1 RT in a noncompetitive manner, causing distortion in the three-dimensional structure of the enzyme to inhibit RT catalytic function.[2] Currently, five NNRTIs drugs have been approved by the U.S. Food and Drug Administration (FDA) (Fig. 1). The first-generation NNRTIs drugs nevirapine (NVP), efavirenz (EFV) and delavirdine (DLV) are limited in their clinical use due to the rapid emergence of drug resistance. In particular, K103N and Y181C are the two mutations most frequently observed in patients exposed to various NNRTIs.

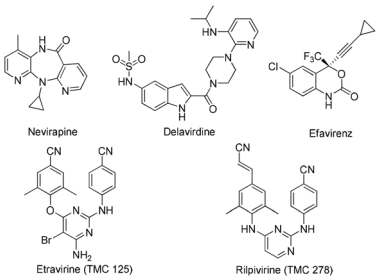

Fig. 1 Current marketed NNRTIs.

Etravirine (TMC125) and rilpivirine (TMC278), which belong to the diarylpyrimidine (DAPY) family, were approved as second generation NNRTIs by the FDA in 2008 and 2011 respectively, and demonstrated activities against HIV-1 strains that developed resistance to first generation NNRTIs. In patients failing etravirine-containing regimens, Y181C was shown to commonly occur.[3–5] Furthermore, the pharmacokinetic profiles of most DAPY derivatives are not satisfactory due to their low water solubility.[6–8] There are also many undesired side effects to TMC125, such as toxic epidermal necrolysis and erythema multiforme, hypersensitivity reactions characterized by rash, and sometimes even organ dysfunction, including hepatic failure. Efforts to develop the next generation of NNRTIs[9–11] based on novel scaffolds have focused

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†Electronic supplementary information (ESI) available: Copies of ^1H, ^{13}C NMR, MS, IR and HPLC spectra of target compounds **9a–9e** and **10a–10q**. See DOI: 10.1039/c3ob42186d

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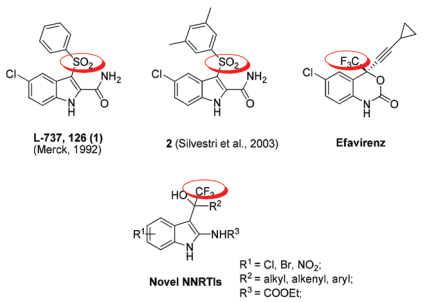


Fig. 2 Structure of reference L-737, 126 (**1**) and IAS **2**.

on the design of compounds with high potency, low toxicity, improved resistant profiles, and better pharmacokinetic profiles.

The development of indolylarylsulfones (IASs) NNRTIs was based on the Merck derivative L-737, 126 (**1**)[12] as a reference compound (Fig. 2). The potent activity of IAS **2** against the NNRTI resistant mutants was correlated to the presence of a 3-(3,5-dimethylphenyl) sulfonyl moiety.[13] The sulfone group[14] is similar to the trifluoromethyl group in efavirenz allowing the inhibitor to assume a butterfly-like conformation, which is commonly found in many other NNRTIs. Inspired by the good potency and bioavailability profiles[15–17] of the trifluoromethyl group, if the sulfonyl moiety of IASs could be replaced by a trifluoromethyl group to produce a new potential NNRTI, it would be possible to improve the activity against resistant mutants and reduce toxicity.

With a novel scaffold in mind, we simultaneously sought to streamline the discovery process. The main goal is to minimize the number of compounds that have to be synthesized and assayed to obtain possible drug candidates. The hydrophobic constant, acute toxicity, carcinogenic toxicity and mutagenic toxicity of several of the designed compounds with representative structures were first predicted to exclude the compounds that did not obey "Lipinski's rule of five"[18] or with potential toxicity. Further molecular docking studies were performed in order to validate whether the compounds can efficiently target the nonnucleoside binding site (NNBS) of HIV-1 RT. Herein, we report the design, synthesis, anti-HIV-1 evaluation and preliminary structure–activity relationships (SARs) of a new series of trifluoromethyl indoles.

Results and discussion

Prediction

The predictions of the hydrophobic constant (log *P*), acute toxicity, carcinogenicity and mutagenicity of some representative and structurally diverse compounds are listed in Table 1. The data listed in Table 1 show that the log *P* values (predicted by CISOC-log *P*[19]) of compounds **9d**, **10e**, **10l**, **10i**, **10k**, **10j** and **10m** were in line with "Lipinski's rule of five".[18] The acute toxicity (predicted by CISOC-PSAT[20]) of compounds **10i**, **10k** and **10j** was at a non-toxic level ($LD_{50} \geq 5000$ mg kg^{-1} (non tox.)), and that of **9d**, **10e**, **10l** and **10m** was at a low-toxic level ($500 \leq LD_{50} < 5000$ mg kg^{-1} (low tox.)). The carcinogenicity (predicted by CISOC-PSCT)[21] of compounds **10e**, **10l**, **10i**, **10k**, **10j** and **10m** was *N* (probability of non carcinogenic is high), except for TMC278 and compound **9d**, which were *P* (probability of non carcinogenic is low). AMES (predicted by CISOC-PSMT)[22] of all compounds, as listed in Table 1, was negative. For TMC278, the prediction results almost corresponded to experimental results.

Therefore, the preliminary prediction of some drug-like properties served as an encouragement for us to synthesize these novel trifluoromethyl indoles.

Chemistry

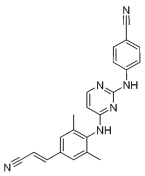
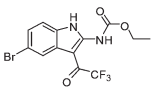
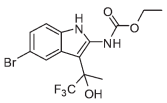
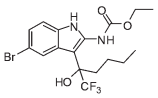
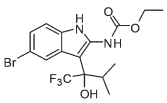
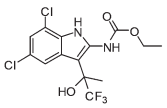
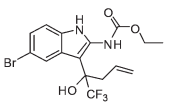
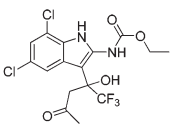
The synthesis of new trifluoromethyl indoles is depicted in Scheme 1.

Variously substituted indoles were prepared with a view to examining the SARs by typical Fischer indole synthesis. The phenylhydrazine **3** reacted with methylpyruvate in methanol to afford the phenylhydrazone **4**, followed by Fischer cyclization of phenylhydrazone **4** to give the aromatic indole **5** under acidic conditions. The detailed mechanism for the Fischer reaction[23] of phenylhydrazine **3** and methylpyruvate is as follows (Scheme 2): phenylhydrazone **4** is firstly formed from the condensation reaction of commercially available methylpyruvate and phenylhydrazine **3**, followed by the isomerization of phenylhydrazone **4** to result in the enamine **A**. After protonation, enamine **A** is transformed into imine **B** through a cyclic [3,3]-sigmatropic rearrangement. Then aminoacetal **C** is produced by cyclization of the resulting imine **B**. Subsequently imine **D** is obtained by aminoacetal **C** losing one molecule of NH_3 under acid catalysis, followed by protonation to deliver the energetically favorable aromatic indole **5**.

Aromatic indole **5** was converted into carbohydrazide **6** with $N_2H_4{\cdot}H_2O$ (85%) in ethanol, followed by diazotization reaction with $NaNO_2$ in acetic acid to produce azide **7**. Subsequently, the Curtius rearrangement of azide **7** yielded carbamate **8** in ethanol. The detailed mechanism for the rearrangement[24] is as follows (Scheme 3): nitrene **F** is formed from substituted azide **7** by loss of one molecule of nitrogen, followed by the rearrangement of acyl nitrene **F** by migration of the indole group to form the desired isocyanate **G**. The nucleophilic addition reaction of the resulting isocyanate **G** and EtOH delivers the desired ethyl carbamate **8**. Subsequently, carbamate **8** reacts with trifluoroacetic anhydride in dry DMF or dry diethyl ether at 0 °C to afford the desired compounds **9a–9e**. Finally, some of the most potent trifluoromethyl indole analogues, **10a–10l**, were prepared in dry THF by reaction with various Grignard reagents at 0 °C for 1–2 hours.

Meanwhile, compounds **10m** and **10n** were produced in dry DMSO by proline/AcK-catalyzed aldol reaction at 40 °C for 3–4 days. The detailed catalytic mechanism for the proline-catalyzed direct aldol reaction[25] is as follows (Scheme 4): enamine **I** is firstly formed from the condensation reaction of commercially available proline and acetone promoted by AcK. Subsequently, ketone **9c** or **9e** is added to the reaction mixture and

Table 1 Prediction results of log *P*, acute, carcinogenic and mutagenic toxicity

Compd	Structure	Log *P* (exp./pred.)	Acute tox.[a] (exp./pred.)	Car. tox.[b] (exp./pred.)	AMES[c] (exp./pred.)
TMC278		3.80–5.47[d]/3.70	Oral, rat: LD_{50} = 980 mg/kg[e]/3.46	P[f]/*P*[b]	N[g]/*N*[h]
9d		Null[h]/4.23	Null[h]/4.62	Null[h]/*P*[b]	Null[h]/*N*[h]
10i		Null[h]/3.35	Null[h]/5.02	Null[h]/*N*[b]	Null[h]/*N*[h]
10k		Null[h]/4.80	Null[h]/5.08	Null[h]/*N*[b]	Null[h]/*N*[h]
10j		Null[h]/4.20	Null[h]/5.02	Null[h]/*N*[b]	Null[h]/*N*[h]
10e		Null[h]/3.58	Null[h]/4.97	Null[h]/*N*[b]	Null[h]/*N*[h]
10l		Null[h]/3.93	Null[h]/4.88	Null[h]/*N*[b]	Null[h]/*N*[h]
10m		Null[h]/2.93	Null[h]/4.89	Null[h]/*N*[b]	Null[h]/*N*[h]

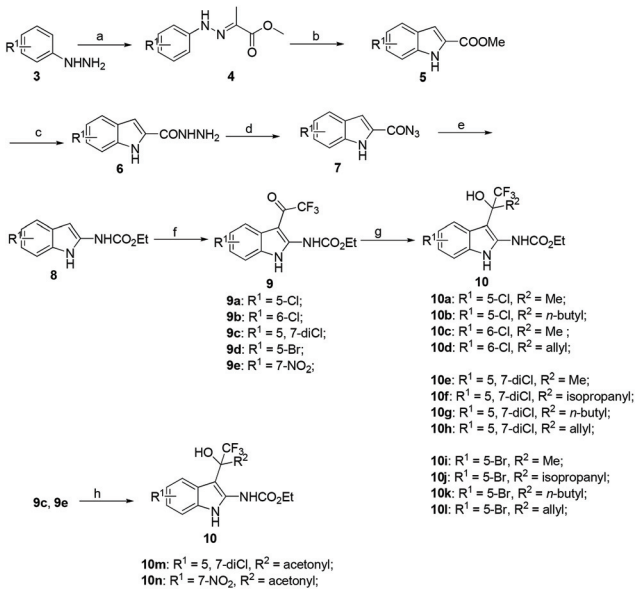
[a] Rat, oral, less than 2, LD_{50} < 1 mg kg^{-1}; equal or more than 2 and less than 3, 1 ≤ LD_{50} < 50 mg kg^{-1}; equal or more than 3 and less than 4, 50 ≤ LD_{50} < 500 mg kg^{-1}; equal or more than 4 and less than 5, 500 ≤ LD_{50} < 5000 mg kg^{-1}; equal or more than 5, LD_{50} ≥ 5000 mg kg^{-1}. [b] Rat, oral, *P*: probability of non carcinogenic is low; *N*: probability of non carcinogenic is high. [c] *Salmonella typhimurium*. [d] http://www.drugbank.ca/drugs/DB08864. [e] http://www.drugbank.ca/drugs/DB08864. [f] http://www.drugbank.ca/system/fda_labels/DB08864.pdf?1368312297. [g] http://www.drugbank.ca/system/fda_labels/DB08864.pdf?1368312297. [h] P: positive; N: negative; Null: no experimental data.

is attacked by the enamine **I** to deliver the iminium product **J**. Finally, iminium salt **J** is hydrolyzed to give aldol product **10m** or **10n** with the release of one molecule of proline, which allows the catalytic cycle to continue until the conversion is finished.

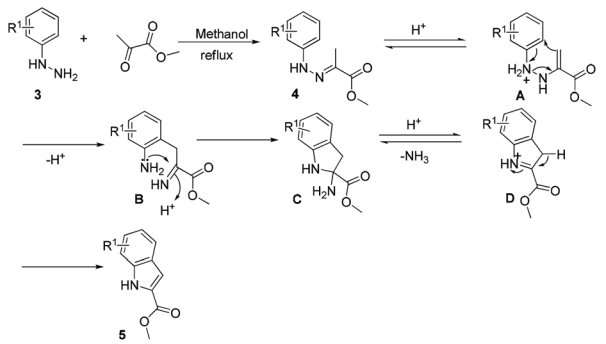
Following the same procedure as for compounds **10a–10l**, we also tried to synthesize compounds with a phenyl or 3,5-dimethylphenyl group as R^2 (**10o**, **10p** and **10q**) (Fig. 3). However, these compounds were very unstable and their purities as detected by HPLC decreased by about 10% in 24 hours. Since the stabilities of compounds **10o**, **10p** and **10q** were too poor to meet the requirements for biological assays, we decided not to continue with our efforts to synthesize compounds with an aryl moiety as R^2.

Biological activity

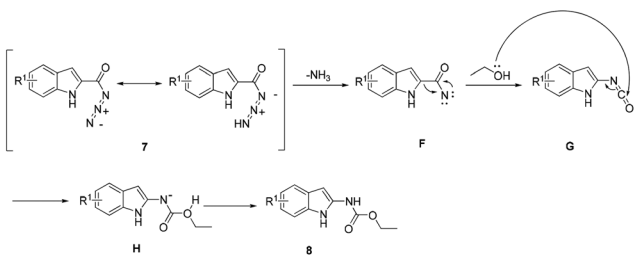
The *in vitro* Cell-Titer Glo assay method was used to evaluate the 19 new trifluoromethyl indoles (**9a–9e** and **10a–10n**) along with two FDA-approved drugs, NVP and EFV, as reference com-



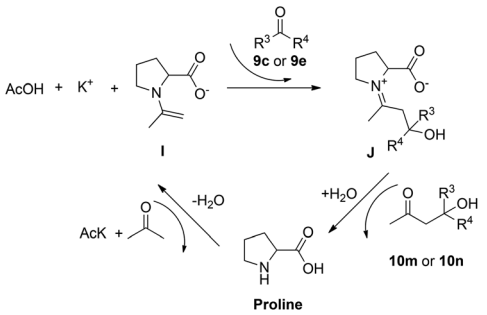
Scheme 1 Synthesis of compounds **9a–10n**. *Reagents and conditions*: (a) methylpyruvate, MeOH, reflux; (b) 4 N HCl–MeOH, reflux; (c) $N_2H_4{\cdot}H_2O$ (85%), EtOH, reflux; (d) $NaNO_2$, AcOH, 1,4-dioxane, 0 °C; (e) EtOH, reflux; (f) Trifluoroacetic anhydride, DMF or diethyl ether, 0 °C; (g) R^2MgCl/R^2MgBr, THF, 0 °C; (h) Acetone, Proline/AcK (1/1), DMSO, 40 °C.



Scheme 2 A plausible mechanism for the Fischer indole synthesis.



Scheme 3 A plausible mechanism for the Curtius rearrangement.



Scheme 4 A plausible mechanism for the proline-catalyzed direct aldol reaction.

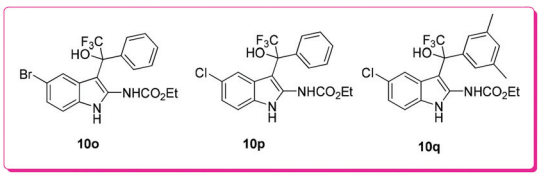


Fig. 3 Structure of **10o**, **10p** and **10q**.

Table 2 Anti-HIV activities and cytotoxicity of compounds **9a–9e** and **10a–10n** in MT-2 cells[a]

Compd		IC_{50}[b] (μM) WT IIIB	IC_{50}[b] (μM) Y181C	TC_{50}[c] (μM)	TI[d]
"A" type	**9a**	2.716	200	>200	>73.6
	9b	>200	142.758	>200	>1
	9c	57.531	200	67.5	>1
	9d	57.068	162.289	>200	>3.5
	9e	200	200	>200	>1
"B" type	**10a**	20.182	21.047	20.5	1.0
	10b	0.022	6.252	58.0	2672.6
	10c	5.839	ND[e]	>200	>34.3
	10d	12.831	15.760	54.9	>4.3
	10e	12.061	38.425	>200	>16.6
	10f	6.556	10.169	28.3	4.3
	10g	3.335	18.152	85.1	25.5
	10h	9.897	13.343	>200	>20.2
	10m	2.337	29.875	35.5	15.2
	10i	0.003	13.901	23.3	6849.5
	10j	5.048	8.010	33.2	6.6
	10k	0.003	1.932	24.0	7051.8
	10l	1.471	7.627	54.3	36.9
	10n	200	51.744	>200	>1
NVP		0.031	>30		
EFV		0.003	0.021		

[a] Data represent the mean of at least three separate experiments. [b] Compound concentration required to protect MT-2 cells against viral cytopathogenicity by 50%. [c] Compound concentration that decreases the uninfected MT-2 cell viability by 50%. [d] Selectivity index: TC_{50}/IC_{50} (WT) ratio. [e] ND: not determined.

pounds. These compounds were assayed for their cytotoxicities and anti-HIV-1 activities in MT-2 cells infected with wild-type (WT) HIV-1 (strain IIIB), and Y181C mutant HIV-1 strain, where Tyr181 was replaced by Cys. The results, expressed as TC_{50} (50% cytotoxic concentration), IC_{50} (50% HIV-1 cytoprotective concentration against HIV-1 induced cytopathic effect)

Table 3 Inhibitory activity of compounds **10i** and **10k** against WT HIV-1 RT[a]

Compd	EC_{50} [b] (μM) WT IIIB
10i[b]	133.33
10k[b]	18.59
EFV[c]	0.08
NVP[c]	0.4

[a] Data represent the mean of at least three separate experiments. [b] Effective concentration (EC_{50}, μM) required to inhibit the RT activity of the indicated strain by 50%. [c] Ref. 14*c*, IC_{50} (μM).

and TI (selectivity index represented by the TC_{50}/IC_{50} ratio) values, are listed in Table 2.

As shown in Table 2, most trifluoromethyl indoles showed moderate to potent inhibitory activities against WT HIV-1 with IC_{50} values in the range of 57.068–0.003 μM. Among them, compounds **10i** and **10k** displayed good anti-HIV-1 activities against WT HIV-1 with an IC_{50} value of 0.003 μM, and compound **10k** showed the greatest selectivity (TI > 7079). Furthermore, compound **10k** also showed potential activity against the Y181C mutant virus with an IC_{50} value of 1.932 μM.

Based on the results of the antiviral assay (Table 2), some important SAR information can be summarized as follows: (1) "B" type trifluoromethyl indoles (**10a–10n**) were more potent against both the WT HIV-1 and the Y181C resistant mutant virus than "A" type (**9f–9e**); (2) substituents at the C5 position of the benzene ring influenced the activity: Br > Cl; (3) indoles with an NO_2 substituent at the C7 position of the benzene ring lost their activities against WT HIV-1 strain and their toxicity increased (**9e** and **10n**); (4) as far as the R^2 group of "B" type indoles was concerned, as for the R^1 group at the same position on the benzene ring, derivatives with R^2 = *n*-butyl were more potent than those with R^2 = methyl, isopropyl, allyl or acetonyl. Compounds **10b**, **10k** and **10g** were superior to other compounds against WT HIV-1.

HIV-1 RT inhibitory activities for compounds **10i** and **10k** with the better cellular antiviral activities are listed in Table 3.

Molecular modeling

In order to investigate the binding mode of our new compounds, interactions between the NNBS of HIV-1 RT and compounds **10e**, **10l**, **10i** and **10k** were calculated by FlexX.[26] Coordinates of the NNBS were taken from the crystal structure of the WT HIV-1 RT/TMC278 complex (PDB code: 2ZD1)[27] and Tyr181Cys mutant HIV-1 RT/EFV complex (PDB code: 1JKH).[28]

The values for the calculation of the interactions between the six compounds and target WT HIV-1 RT (PDB code: 2ZD1) are shown in Table 4 and Fig. 4. According to the calculation results, the location of TMC278 in the active site was basically consistent with that in the crystal structure. In general, the lower the total score of a compound, the higher its activity.

The docking results of ligands and WT HIV-1 RT showed that not only were there hydrogen bonds between EFV, **10i** or **10k**, and the key residue Lys101, but the total scores of the three compounds were almost identical. Their total scores were higher than for TMC278, which corresponded to the IC_{50} values in Table 2. On the other hand, although the total score of EFV was still higher than for compounds **10i** and **10k**, the length of the hydrogen bond between EFV and LYS101 (O96) was shorter than for LYS101 (O96) and **10i** or **10k**. Taken together, these results suggest that it is reasonable for the IC_{50} values of **10i** and **10k** to be similar to EFV.

Furthermore, although **10e** and **10l** were located in the active site, the IC_{50} value of **10l** is almost 6-fold lower than **10e**. This result is probably due to the fact that the allyl group of compound **10l** can bind into the region formed by Tyr 181, Tyr188, Phe227 and Trp229 while the methyl group of compound **10e** can't.[29] However, the extension of the allyl group of compound **10l** did not appear to fill the space between Tyr 181, Tyr188 and Trp229 any better than the *n*-butyl group of compound **10k**. In addition, the total scores of compounds **10e** and **10l** were higher than for compounds **10i** and **10k**, which all fits with the IC_{50} values in Table 2.

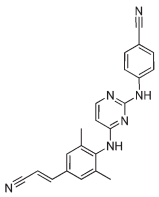
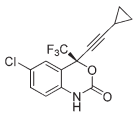
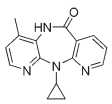
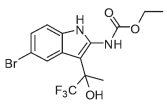
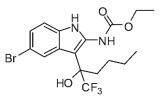
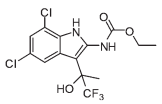
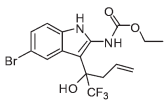
The superimpositions of EFV and **10i**, **10k**, **10e** and **10l** are shown in Fig. 5. In general, the superimpositions of the benzene moiety of EFV and the corresponding benzene moieties of compounds **10i**, **10k** and **10l** were better than for EFV and compound **10e**.

The values for the calculation of the interactions between the six compounds and their target, Y181C mutant HIV-1 RT (PDB code: 1JKH), are shown in Table 5 and Fig. 6. According to the calculation results, the location of EFV in the active site is also basically consistent with the crystal structure.

The calculation of the interactions between ligands and Y181C mutant HIV-1 RT showed that: (1) the total scores of compounds **10i** and **10k** corresponded well to their biological assays. (2) The length of the hydrogen bond between residue Lys101 and EFV was shorter than for Lys101 and **10i** or **10k**. Furthermore, in the active site, the superimposition of compound **10k** and EFV was better than for **10i** and EFV. These data were consistent with the biological assay results: the IC_{50} value of EFV was lower than those of **10k** and **10i**, and the IC_{50} value of **10k** was lower than that of **10i**. (3) Although the hydrogen bond lengths between Lys101 and **10i** or **10k** were identical, the IC_{50} value of **10k** was almost 10-fold lower than that of **10i**. This result is probably due to the fact that the *n*-butyl group of compound **10k** could bind into the region formed by Cys181, Tyr188, Phe227, and Trp229.[29] Its extension was seen to fill the space between Cys181, Tyr188, and Trp229. In addition, the IC_{50} value of **10i** for the Y181C mutant was 1000-fold higher than for the wild-type strain. The mutation of Tyr181 to Cys181 also prevented the methyl group from entering the region.

The binding modes of compounds NVP, **10e** and **10l** in the Y181C mutant HIV-1 RT NNBS showed that the three compounds were bound outside of the active site. Although there were hydrogen bonds between Lys101 and the three compounds NVP, **10e** and **10l**, these hydrogen bonds were

Table 4 Interactions between ligands and WT HIV-1 RT (2ZD1)

Compd	Structure	IC_{50} (μM)	Total score (kcal mol^{-1})	H-bond (Å)
TMC278[a] (Cry.)		0.0004	No calculation	Lys101 (O96)–TMC278 (H40): 1.78 Lys101 (H92)–TMC278 (N2): 2.22
TMC278 (Cal.)			−34.27	Lys101 (O96)–TMC278(H40): 1.62 Lys101 (H92)–TMC278(N2): 2.20
EFV		0.003	−13.58	LYS101 (O96)–EFV(H25): 1.38 LYS101 (H92)–EFV(O5): 1.92
NVP		0.031	−16.17	LYS103 (H154)–NVP(N17): 1.82 LYS103 (H156)–NVP(N3): 1.82
10i		0.003	−18.27	Lys101 (O96)–**10i** (H27): 1.91 Lys101 (H92)–**10i** (O12): 1.58 Lys101 (H92)–**10i** (O13): 2.65
10k		0.003	−18.27	Lys101 (O96)–**10k** (H30): 2.14 Lys101 (H92)–**10k** (O12): 1.67
10e		12.0614	−11.47	Lys101 (O96)–**10e** (H27): 1.81 Lys101 (H92)–**10e** (O21): 2.23
10l		1.4712	−16.36	Lys101 (O96)–**10l** (H29): 2.16 Lys101 (H92)–**10l** (O21): 1.65

[a] Ref. 30.

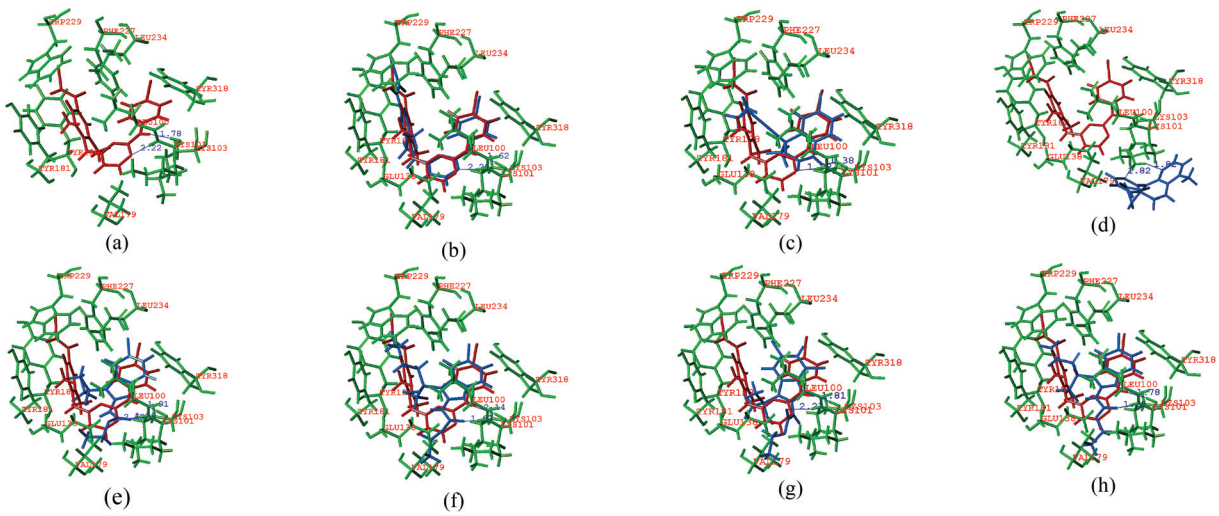


Fig. 4 Interactions between HIV-1 RT WT (PDB: 2ZD1) and TMC278 (red), other compounds. (a) TMC278 (cry.); (b) TMC278 (red, cry.), blue one docking result; (c) TMC278 (red, cry.), EFV (blue, docking result); (d) TMC278 (red, cry.), NVP (blue, docking result); (e) TMC278 (red, cry.), **10i** (blue, docking result); (f) TMC278 (red, cry.), **10k** (blue, docking result); (g) TMC278 (red, cry.), **10e** (blue, docking result); (h) TMC278 (red, cry.), **10l** (blue, docking result).

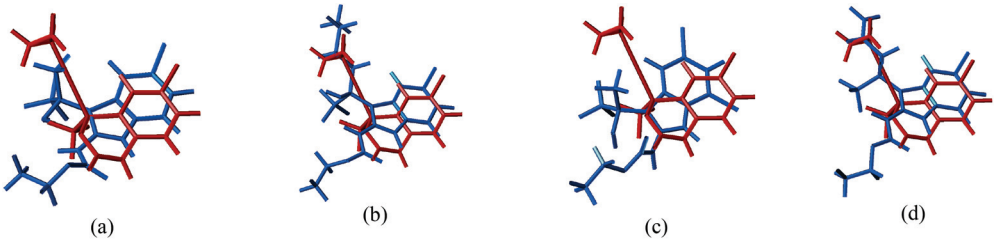


Fig. 5 Superimpositions of EFV and compounds **10i**, **10k**, **10e** and **10l**. (a) EFV (red), **10i** (blue); (b) EFV (red), **10k** (blue); (c) EFV (red), **10e** (blue); (d) EFV (red), **10l** (blue).

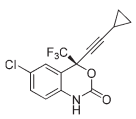
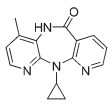
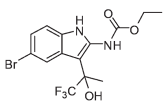
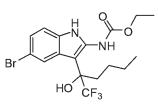
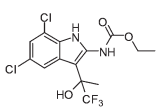
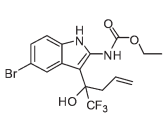
different from those between Lys101 and EFV, **10i** and **10k**. The main difference was in the particular atoms of Lys101 that were involved in hydrogen bonds to **10e** and **10l**. The total scores of **10e** and **10l** were also higher than for **10i** and **10k**, which was consistent with the IC_{50} values in Table 2.

Based on the molecular modelling investigations on the binding mode of ligands to the NNBS of Y181C mutant HIV-1 RT, together with the SAR studies as described above, we postulated that selecting a suitable substituent (R^2) to fit the pocket formed by Cys181, Tyr188, Phe227, and Trp229 would be beneficial towards enhancing the interaction between the inhibitors and Y181C mutant HIV-1 RT.

Moreover, a hydrogen bond was formed between **10k** (F25)–Cys181 (H214) in the mutant Y181C, which was unusual for most NNRTIs binding to the active site. Generally, the larger the difference in electronegativity between the H atom and the other atom (N, O, or F), the stronger the H-bond. This also inspired us to pay more attention to special H–F hydrogen bonds for further fluorine-containing drugs design, which will also contribute to strengthen interaction of the ligand and target and improve biological activity.

In summary, the calculation results of the interaction between the targets and our newly designed and synthesized compounds was consistent with the biological evaluation. For the Tyr181Cys mutant in the active site, two different molecular models provided insights for further molecular design and structural optimization.

Table 5 Interaction between ligands and Y181C mutant HIV-1 RT (1JKH)

Compd	Structure	IC_{50} (μM)	Total score	H-bond (Å)
EFV (cry.) EFV (cal.)		0.021	No calculation −22.99	Lys101 (H56)–EFV (O5): 2.72 Lys101 (H56)–EFV (O5): 1.99 Lys101 (O60)–EFV (H25): 1.77
NVP		>30	−13.81	Lys101 (H74)–NVP (N3): 1.91 Lys101 (H76)–NVP (N17): 1.90
10i		13.9	−11.35	Lys101 (H56)–**10i** (O12): 2.06 Lys101 (H56)–**10i** (O13): 2.71 Lys101 (O60)–**10i** (H27): 1.89
10k		1.93	−12.58	Lys101 (H56)–**10k** (O12): 2.06 Lys101 (H56)–**10k** (O13): 2.71 Lys101 (O60)–**10k** (H30): 1.89 Cys181 (H214)–**10k** (F25): 2.60
10e		38.4248	−10.95	Lys101 (H76)–**10e** (O22):1.83 Lys103 (H118)–**10e** (O21): 1.91
10l		7.6286	−8.80	Lys101 (H76)–**10l** (O22):1.78 Lys103 (H118)–**10l** (O21): 1.78 Lys103 (H118)–**10l** (O14): 2.47 Lys103 (H119)–**10l** (O14): 2.01

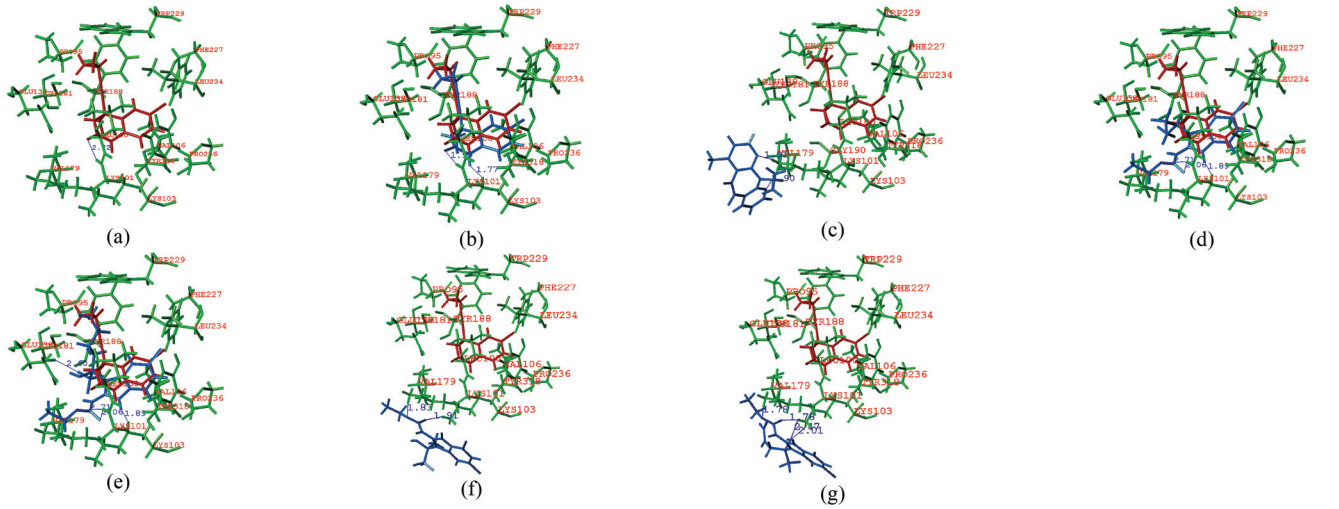


Fig. 6 Interaction between the Y181C mutant HIV-1 RT (PDB: 1JKH), EFV (red) and other compounds. (a) EFV (cry.); (b) EFV (red, cry.; blue, docking result); (c) EFV (red, cry.), NVP (blue, docking result); (d) EFV (red, cry.), **10i** (blue, docking result); (e) EFV (red, cry.), **10k** (blue, docking result); (f) EFV (red, cry.), **10e** (blue, docking result); (g) EFV (red, cry.), **10l** (blue, docking result).

Experimental

Chemistry

All commercially available reagents and solvents were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) and column chromatography was carried out on silica gel H (10 ± 40 mm). IR spectra were recorded on a FT-IR spectrometer and only major peaks are reported. NMR spectra were recorded on a Bruker DRX 300 or 400 MHz spectrometer. Mass spectra were obtained using an Agilent 592N spectrometer and a Shimadzu LCMS-2010EV. The purity of compounds was determined by analytical HPLC using a Kromasil 5u 100A C18 column (4.6 × 150 mm, Nacalai Tesque, Inc., Kyoto, Japan) at a flow rate of 1.0 mL min^{-1} on a Shimadzu SPD20A LC-20AT (Shimadzu Corp., Ltd, Kyoto, Japan). Gradient conditions: (1) solvent A (acetonitrile) and solvent B (water): 0–20.00 min, 10/90 (A/B)–100/0 (A/B) (linear gradient); 20.00–30.00 min, 100% A. Eluting products were detected by UV at 254 nm; (2) solvent A (acetonitrile) and solvent B (water): 0–20.00 min, 80/20 (A/B). Eluting products were detected by UV at 214 nm.

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). Data were processed using non-linear regression and a standard sigmoidal dose response model to obtain IC_{50} values. Dose–response data are presented as means standard errors from three independent experiments, with triple wells for each concentration.

General procedure for preparation of target compounds 9a–9e

General procedure for preparation of target compounds 4. To a solution of phenylhydrazine hydrochloride **3** (5 mmol) in MeOH (20 mL) was added methylpyruvate (1.4–1.5 mmol) and the reaction was stirred at reflux until the starting material was consumed completely (monitored by TLC). The solvents were removed under reduced pressure and the residue was purified with silica gel column to give the product methyl 2-(2-phenylhydrazono) propanoate **4** in a yield of 80–95%.

General procedure for preparation of target compounds 5. A solution of hydrazone **4** (1 mmol) in MeOH (20 mL) with cat. H_2SO_4 or 4 N HCl–MeOH was refluxed under Ar. After 2.5 hours, the resulting reaction solution was poured into crushed ice and extracted with DCM (2 × 100 mL). The organic layer was washed with sat. $NaHCO_3$ (2 × 10 mL), water and then brine, separated, dried with $MgSO_4$, filtered and evaporated under reduced pressure to give the product **5** in a yield of 65–85%.

General procedure for preparation of target compounds 6. To a solution of methyl 1*H*-indole-2-carboxylate **5** (5 mmol) in EtOH (10 mL) was added hydrazine hydrate (4 ml, 85%) and the reaction solution was stirred at reflux for 5 hours. After cooling to room temperature, the solid was collected by filtration, washed with cold EtOH and dried to give the product **6** in a yield of 85–95%.

General procedure for preparation of target compounds 7. To a solution of 1*H*-indole-2-carbohydrazide **6** (10 mmol) in dioxane (10 mL) was added AcOH (10 mL) at 0 °C with stirring. Then $NaNO_2$ (800 mg) in H_2O (2 mL) was added dropwise at 0 °C and the reaction mixture was stirred at the same temperature for 0.5–1 hour. The solids were collected by filtration, washed well with ice water and dried to give the azide product **7** in a yield of 80–95%.

General procedure for preparation of target compounds 8. A solution of azide **7** (1 mmol) in dry EtOH (20 mL) was refluxed for 1–5 hours. After cooling to room temperature, the solids were removed by filtration, and the filtrate was concentrated under reduced pressure to give the carbamate product **8** in a yield of 85–90%.

General procedure for preparation of target compounds 9a–9e. To a solution of carbamate **8** (1 mmol) in dry DMF (1 mL) or dry diethyl ether (10 ml) was added dropwise TFAA

(2.5 mmol) at 0 °C with stirring. After completion (monitored by TLC), water was added and the solution was extracted with EtOAc (2 × 100 mL). The organic layer was separated, dried with Na_2SO_4, filtered and concentrated to give the residue. The residue was purified with silica gel column to give the trifluoroacetyl carbamate products **9a–9e** in a yield of 75–90%.

*Ethyl 5-chloro-3-(2,2,2-trifluoroacetyl)-1*H*-indol-2-ylcarbamate (**9a**).* Yield 85%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 7.67–7.65 (m, 2H), 7.24 (dd, J = 8.6, 1.8 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H); ^{1}H NMR (300 MHz, in DMSO-d_6): δ 1.30–1.34 (m, 3H), 4.29–4.34 (m, 2H), 7.30 (d, J = 8.1 Hz, 1H), 7.53 (s, 1H), 7.65 (d, J = 8.7 Hz, 1H), 10.70 (s, 1 H), 12.80 (m, 1 H). ^{13}C NMR (101 MHz, in acetone-d_6): δ 173.31 (q, J = 36 Hz), 152.75, 150.12, 132.28, 128.48, 123.22, 118.57 (q, J = 6 Hz), 117.37 (q, J = 286 Hz), 114.14, 94.46, 63.09, 13.55; MS (EI) m/z: 334[M]$^+$, 288, 265, 256, 237, 226, 219, 203, 193, 178, 165, 158, 149, 137, 129, 125, 109, 97, 91, 77, 69, 57, 51, 43, 41; IR (cm^{-1}): 3373.7, 3276.2, 2992.4, 2914.3, 1736.4, 1639.1, 1568.2, 1531.4, 1476.7, 1454.1, 1435.2, 1377.8, 1330.5, 1276.6, 1256.1, 1215.7, 1194.8, 1183.2, 1145.2, 1125.5, 1083.7, 1062.8, 1014.9, 954.5, 863.8, 817.9, 775.6, 768.1, 750.2, 714.4, 690.9, 638.4, 537.3, 456.7, 435.7. HPLC purity: 99% (254 nm), t_R: 21.29 min; 99% (214 nm), t_R: 6.10 min.

*Ethyl 6-chloro-3-(2,2,2-trifluoroacetyl)-1*H*-indol-2-ylcarbamate (**9b**).* Yield 87%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 11.88 (br s, NH), 10.80 (br s, NH), 7.73 (s, 1H), 7.72 (d, J = 10.3 Hz, 1H), 7.30 (d, J = 7.4 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 173.46 (q, J = 36 Hz), 152.84, 150.19, 134.39, 128.35, 123.34, 120.71, 120.29 (q, J = 5 Hz), 117.42 (q, J = 285 Hz), 112.75, 94.62, 63.10, 13.55; MS (EI) m/z: 334[M]$^+$, 314, 288, 261, 233, 219, 201, 193, 165, 137, 129, 110, 102, 87, 75, 64, 58, 51, 43; IR (cm^{-1}): 3359.7, 3252.8, 3072.3, 2989.2, 2910.7, 1741.3, 1637.8, 1581.1, 1563.3, 1530.9, 1489.9, 1477.0, 1454.4, 1378.9, 1353.2, 1327.5, 1301.1, 1275.7, 1254.8, 1196.6, 1172.9, 1151.1, 1133.4, 1116.7, 1065.0, 1016.2, 972.6, 943.8, 906.5, 886.2, 811.9, 772.8, 743.6, 706.7, 692.1, 641.1, 596.3, 577.7, 527.1, 490.4, 407.7. HPLC purity: 100% (254 nm), t_R: 21.10 min; 100% (214 nm), t_R: 5.65 min.

*Ethyl 5,7-dichloro-3-(2,2,2-trifluoroacetyl)-1*H*-indol-2-ylcarbamate (**9c**).* Yield 82%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 7.66 (d, J = 1.2 Hz, 1H), 7.45 (d, J = 1.7 Hz, 1H), 4.45 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 173.82 (q, J = 33 Hz), 153.36, 150.36, 128.87, 128.74, 124.32, 122.58, 117.78 (q, J = 6 Hz), 117.63, 117.06 (q, J = 286 Hz), 95.08, 63.64, 13.51; MS (EI) m/z: 368 [M]$^+$, 322, 299, 271, 255, 227, 220, 199, 192, 171, 163, 136, 127, 109, 100, 95, 86, 76, 69, 62, 52, 43; IR (cm^{-1}): 3365.4, 3262.0, 3121.5, 3075.4, 2994.7, 2947.1, 1734.5, 1647.1, 1619.8, 1576.0, 1528.1, 1462.2, 1446.2, 1421.6, 1371.0, 1350.7, 1332.2, 1279.1, 1231.1, 1218.3, 1197.6, 1179.5, 1143.4, 1096.8, 1071.0, 1024.0, 995.9, 954.3, 893.3, 871.0, 860.0, 769.1, 754.5, 715.8, 698.5, 633.9, 586.2, 560.1, 459.2, 441.2. HPLC purity: 99% (254 nm), t_R: 23.63 min; 99% (214 nm), t_R: 12.83 min.

*Ethyl 5-bromo-3-(2,2,2-trifluoroacetyl)-1*H*-indol-2-ylcarbamate (**9d**).* Yield 80%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 11.87 (br s, NH), 10.75 (br s, NH), 7.82 (s, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.39 (dd, J = 8.6, 1.7 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 173.33 (q, J = 36 Hz), 152.78, 150.01, 132.60, 126.00, 123.75, 121.54 (q, J = 6 Hz), 117.4 (q, J = 286 Hz), 116.13, 114.54, 94.32, 63.10, 13.55; MS (EI) m/z: 380(99.36), 378(100) [M]$^+$, 354, 334, 309, 299, 283, 265, 237, 226, 201, 183, 158, 129, 102, 75, 64, 51, 43; IR (cm^{-1}): 3370.4, 3279.7, 2991.2, 1735.1, 1639.1, 1566.9, 1529.5, 1471.3, 1431.9, 1377.3, 1332.1, 1271.6, 1255.8, 1214.7, 1193.4, 1182.3, 1144.2, 1126.9, 1072.8, 1061.6, 1013.7, 950.5, 862.5, 814.9, 775.4, 767.7, 742.8, 706.2, 688.3, 641.8, 585.8, 532.9, 456.3, 419.7. HPLC purity: 99% (254 nm), t_R: 20.95 min; 98% (214 nm), t_R: 5.76 min.

*Ethyl 7-nitro-3-(2,2,2-trifluoroacetyl)-1*H*-indol-2-ylcarbamate (**9e**).* Yield 75%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.26 (br s, NH), 10.67 (br s, NH), 8.17 (br d, J = 8.5 Hz, 1H), 8.14 (br d, J = 7.9 Hz, 1H), 7.40 (t, J = 8.2 Hz, 1H), 4.43 (q, J = 7.1 Hz, 2H), 1.42 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 174.15 (q, J = 38 Hz), 153.33, 150.47, 133.38, 126.36 (q, J = 5 Hz), 125.15, 123.27, 119.10, 117.06 (q, J = 286 Hz), 94.56, 63.77, 13.52; MS (EI) m/z: 345 [M]$^+$, 329, 300, 284, 276, 256, 248, 232, 226, 214, 204, 184, 158, 129, 102, 90, 75, 69, 51, 43; IR (cm^{-1}): 3359.2, 3282.4, 3086.4, 3031.7, 3018.2, 2997.1, 1728.1, 1645.1, 1575.1, 1530.2, 1517.2, 1471.3, 1461.3, 1434.3, 1376.4, 1338.9, 1309.1, 1271.7, 1238.9, 1221.8, 1187.8, 1152.0, 997.3, 951.2, 891.5, 860.9, 806.2, 776.4, 738.9, 705.5, 683.8, 653.1, 562.1, 477.0, 418.3. HPLC purity: 98% (254 nm), t_R: 21.00 min; 97% (214 nm), t_R: 5.57 min.

General procedure for preparation of target compounds 10a–10l and 10o–10q

To a solution of trifluoroacetyl carbamate **9a–9d** (1 mmol) in dry THF (10 mL) Grignard reagents (2–4 mmol) were added dropwise at 0 °C under Ar. After stirring at 0 °C for 1–2 hours, the resulting reaction mixture was quenched with sat. NH_4Cl and extracted with EtOAc (2 × 100 mL). The organic layer was separated, washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the residue. The residue was purified with silica gel chromatography to give the products **10a–10l** in a yield of 52–85%.

Ethyl 5-chloro-3-(1,1,1-trifluoro-2-hydroxypropan-2-yl)-1*H*-indol-2-ylcarbamate (10a). Yield 83%; ^{1}H NMR (300 MHz, in acetone-d_6): δ 10.86 (br s, 1H), 9.15 (br s, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.35 (s, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.40 (br s, 1H), 4.11 (q, J = 7.1 Hz, 2H), 1.91 (s, 3H), 1.16 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.04, 135.85, 131.26, 126.71 (q, J = 286.0 Hz), 126.20, 125.21, 119.79, 117.68, 112.69, 92.54, 74.81 (q, J = 30.7 Hz), 61.63, 22.42, 13.77; LR-ESI: [M − H]$^+$ 349.0; IR (cm^{-1}): 3425.6, 3364.3, 2999.0, 2981.4, 1697.6, 1635.9, 1586.7, 1489.6, 1473.6, 1440.9, 1381.5, 1361.1, 1298.6, 1257.1, 1235.6, 1204.2, 1178.5, 1161.5, 1134.2, 1095.2, 1074.7, 1038.5, 924.0, 909.1, 862.6, 835.9, 796.2, 763.1, 740.0, 705.1, 687.4, 649.7, 606.5, 585.6, 566.3, 529.6, 438.6, 412.1; HPLC purity: 97% (254 nm), t_R: 18.76 min; 96% (214 nm), t_R: 3.42 min.

Ethyl 5-chloro-3-(1,1,1-trifluoro-2-hydroxyhexan-2-yl)-1*H*-indol-2-ylcarbamate (10b). Yield 81%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.97 (br s, 1H), 9.37 (br s, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.45 (s, 1H), 7.04 (dd, J = 8.6, 1.7 Hz, 1H), 6.30 (br s, 1H), 4.24 (q, J = 7.1 Hz, 2H), 2.65 (td, J = 13.9, 4.5 Hz, 1H), 2.05 (m, 1H), 1.54 (m, 1H), 1.36 (dd, J = 14.6, 7.2 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.13 (m, 1H), 0.84 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.04, 137.06, 131.23, 126.79 (q, J = 286.8 Hz), 126.07, 125.32, 119.83, 117.27 (d, J = 2.0 Hz), 112.68, 89.92, 78.24 (q, J = 29.4 Hz), 61.61, 32.92, 24.57, 22.32, 13.78, 13.27; LR-ESI: $[M - H]^-$ 391.1; IR (cm^{-1}): 3422.0, 3352.6, 2961.3, 2935.8, 2872.1, 1694.1, 1636.3, 1587.4, 1488.8, 1440.5, 1385.0, 1363.1, 1281.8, 1256.2, 1234.6, 1212.3, 1183.1, 1152.4, 1080.5, 1059.8, 1014.0, 983.3, 959.1, 933.4, 863.1, 796.8, 788.6, 766.2, 737.5, 716.6, 688.4, 652.2, 594.4, 532.1; HPLC purity: 96% (254 nm), t_R: 20.69 min; 99% (214 nm), t_R: 4.97 min.

Ethyl 6-chloro-3-(1,1,1-trifluoro-2-hydroxypropan-2-yl)-1*H*-indol-2-ylcarbamate (10c). Yield 82%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.94 (br s, 1H), 7.63 (d, J = 1.6 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.05 (dd, J = 8.7, 2.0 Hz, 1H), 4.26 (q, J = 7.1 Hz, 2H), 2.05 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.08, 135.20, 133.30, 126.73 (q, J = 284 Hz), 125.06, 123.80, 120.13, 119.59, 111.23, 92.75, 74.88 (q, J = 30 Hz), 61.61, 22.47, 13.78; LR-ESI: $[M + H]^+$ 351.0; IR (cm^{-1}): 3442.5, 3370.4, 2993.1, 2960.3, 2926.9, 2871.6, 2854.3, 1692.6, 1636.1, 1599.2, 1565.7, 1488.4, 1475.6, 1437.8, 1381.1, 1363.1, 1291.6, 1262.3, 1233.9, 1204.3, 1179.3, 1162.9, 1131.4, 1162.9, 1090.7, 1069.7, 1041.0, 943.1, 927.9, 887.2, 836.6, 798.8, 762.6, 732.4, 710.6, 643.2, 609.0, 587.7, 525.7, 457.5, 428.8; HPLC purity: 95% (254 nm), t_R: 18.55 min; 96% (214 nm), t_R: 3.23 min.

Ethyl 6-chloro-3-(1,1,1-trifluoro-2-hydroxypent-4-en-2-yl)-1*H*-indol-2-ylcarbamate (10d). Yield 85%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.97 (br s, 1H), 9.30 (br s, 1H), 7.64 (d, J = 1.5 Hz, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.07 (dd, J = 8.7, 1.9 Hz, 1H), 6.35 (br s, 1H), 5.67 (ddt, J = 17.1, 10.2, 6.9 Hz, 1H), 5.20 (d, J = 17.2 Hz, 1H), 5.01 (d, J = 10.2 Hz, 1H), 4.26 (q, J = 7.1 Hz, 2H), 3.51 (dd, J = 15.0, 6.5 Hz, 1H), 2.88 (dd, J = 15.1, 7.2 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.08, 136.40, 133.31, 130.80, 124.31 (q, J = 284 Hz), 120.23, 119.61, 119.16, 111.25, 90.04, 77.70 (q, J = 29 Hz), 61.64, 37.78, 13.80; LR-ESI: $[M - H]^-$ 375.2; IR (cm^{-1}): 3418.8, 3364.9, 2981.2, 2933.3, 1704.2, 1635.7, 1592.7, 1567.3, 1487.7, 1441.5, 1383.9, 1300.6, 1276.6, 1259.8, 1233.9, 1206.0, 1186.0, 1172.6, 1150.2, 1132.1, 1067.2, 1020.5, 996.4, 956.6, 931.9, 899.4, 884.7, 855.8, 802.3, 765.9, 727.6, 670.6, 589.0, 523.3; HPLC purity: 97% (254 nm), t_R: 19.51 min; 96% (214 nm), t_R: 3.74 min.

Ethyl 5,7-dichloro-3-(1,1,1-trifluoro-2-hydroxypropan-2-yl)-1*H*-indol-2-ylcarbamate (10e). Yield 83%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.71 (br s, 1H), 9.33 (br s, 1H), 7.52 (s, 1H), 7.18 (d, J = 1.4 Hz, 1H), 6.59 (br s, 1H), 4.31 (q, J = 7.1 Hz, 2H), 2.07 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.65, 136.76, 127.82, 127.20, 126.54 (q, J = 284 Hz), 125.63, 119.24, 117.21, 116.22, 94.40, 74.70 (q, J = 31 Hz), 62.16, 22.25, 13.73; LR-ESI: $[M - H]^-$ 383.2; IR (cm^{-1}): 3473.4, 3406.0, 3358.7, 3004.0, 2983.8, 1693.2, 1636.5, 1587.6, 1503.4, 1485.7, 1473.6, 1441.2, 1386.5, 1362.9, 1293.7, 1250.2, 1209.3, 1185.9, 1163.5, 1095.7, 1044.9, 1020.8, 929.3, 908.7, 873.2, 842.8, 809.1, 764.1, 743.0, 706.2, 692.0, 650.7, 623.1, 608.0, 584.5, 557.7, 443.0; HPLC purity: 96% (254 nm), t_R: 21.61 min; 97% (214 nm), t_R: 6.81 min.

Ethyl 5,7-dichloro-3-(1,1,1-trifluoro-2-hydroxy-3-methylbutan-2-yl)-1*H*-indol-2-ylcarbamate (10f). Yield 75%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.75 (br s, 1H), 9.47 (br s, 1H), 7.53 (s, 1H), 7.19 (s, 1H), 6.02 (br s, 1H), 4.31 (q, J = 7.1 Hz, 2H), 3.01 (dt, J = 13.7, 6.8 Hz, 1H), 1.35 (t, J = 7.1 Hz, 3H), 1.27 (dd, J = 6.8, 1.4 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.65, 137.51, 127.86, 126.62, 126.58 (q, J = 287 Hz), 125.60, 119.29, 117.27, 116.17, 93.63, 81.15 (q, J = 28 Hz), 62.13, 31.72, 16.91, 15.13, 13.71; LR-ESI: $[M - H]^-$ 411.2; IR (cm^{-1}): 3455.1, 3414.7, 3352.9, 2981.5, 2937.5, 1704.6, 1690.5, 1638.4, 1587.2, 1559.7, 1491.2, 1473.2, 1447.7, 1388.1, 1374.9, 1308.0, 1283.6, 1253.9, 1209.8, 1186.3, 1151.7, 1113.5, 1091.2, 1070.6, 1017.6, 982.7, 889.5, 873.6, 834.9, 808.2, 766.2, 740.2, 717.0, 652.1, 591.1, 543.8, 444.0; HPLC purity: 95% (254 nm), t_R: 23.23 min; 95% (214 nm), t_R: 10.66 min.

Ethyl 5,7-dichloro-3-(1,1,1-trifluoro-2-hydroxyhexan-2-yl)-1*H*-indol-2-ylcarbamate (10g). Yield 83%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.75 (br s, 1H), 9.43 (br s, 1H), 7.49 (s, 1H), 7.18 (d, J = 1.6 Hz, 1H), 6.35 (br s, 1H), 4.31 (q, J = 7.1 Hz, 2H), 2.66 (td, J = 14.4, 4.5 Hz, 1H), 2.16–2.03 (m, 1H), 1.75–1.50 (m, 1H), 1.47–1.26 (m, 5H), 1.22–1.08 (m, 1H), 0.86 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.65, 138.00, 127.82, 127.07, 126.60 (q, J = 285 Hz), 125.77, 119.30, 116.81, 116.22, 91.72, 78.18 (q, J = 30 Hz), 62.15, 32.84, 24.54, 22.30, 13.73, 13.25; LR-ESI: $[M - H]^-$ 425.2; IR (cm^{-1}): 3403.2, 3370.5, 2959.0, 2932.3, 2871.3, 1690.1, 1638.9, 1590.0, 1560.3, 1490.0, 1473.2, 1445.2, 1387.4, 1362.0, 1283.5, 1254.2, 1220.1, 1205.0, 1182.3, 1155.2, 1087.8, 1068.5, 975.4, 936.8, 873.6, 844.7, 809.5, 767.7, 717.7, 691.2, 652.1, 635.4, 588.9, 525.4, 442.4; HPLC purity: 98% (254 nm), t_R: 23.92 min; 99% (214 nm), t_R: 13.84 min.

Ethyl 5,7-dichloro-3-(1,1,1-trifluoro-2-hydroxypent-4-en-2-yl)-1*H*-indol-2-ylcarbamate (10h). Yield 82%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.62 (br s, 1H), 8.91 (br s, 1H), 7.32 (s, 1H), 7.12 (d, J = 1.3 Hz, 1H), 5.64 (dt, J = 16.3, 8.3 Hz, 1H), 5.33 (d, J = 11.2 Hz, 1H), 5.29 (d, J = 4.5 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 3.31 (dd, J = 14.6, 6.2 Hz, 1H), 2.86 (dd, J = 14.6, 8.4 Hz, 2H), 1.35 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.65, 137.94, 130.46, 127.82, 126.92, 126.33 (q, J = 285 Hz), 125.76, 119.44, 119.33, 117.19, 116.17, 91.69, 77.53 (q, J = 30 Hz), 62.18, 37.60, 13.71; LR-ESI: $[M - H]^-$ 409.2; IR (cm^{-1}): 3414.6, 3351.0, 3095.3, 2996.1, 2930.0, 1863.5, 1686.1, 1639.0, 1589.3, 1559.1, 1489.8, 1472.7, 1445.9, 1420.1, 1389.8, 1340.9, 1260.6, 1210.0, 1184.5, 1167.4, 1152.6, 1091.3, 1075.6, 1201.4, 991.2, 940.2, 940.6, 928.3, 872.0, 838.2, 771.1, 743.5, 723.5, 654.0, 620.4, 590.0, 538.8, 440.1; HPLC purity: 96% (254 nm), t_R: 23.39 min; 98% (214 nm), t_R: 8.25 min.

Ethyl 5-bromo-3-(1,1,1-trifluoro-2-hydroxypropan-2-yl)-1*H*-indol-2-ylcarbamate (10i). Yield 85%; ^{1}H NMR (400 MHz, in

acetone-d_6): δ 10.84 (br s, 1H), 9.15 (br s, 1H), 7.50 (s, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.02 (d, J = 7.9 Hz, 1H), 6.37 (br s, 1H), 4.10 (q, J = 7.1 Hz, 2H), 1.90 (s, 3H), 1.15 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.08, 135.73, 131.56, 126.87, 126.72 (q, J = 287 Hz), 122.50, 120.66, 113.18, 112.89, 92.47, 74.82 (q, J = 30 Hz), 61.66, 22.45, 13.78; LR-ESI: $[M - H]^-$ 393.0; IR (cm^{-1}): 3424.4, 3364.7, 2978.8, 1697.5, 1635.9, 1591.6, 1488.2, 1472.6, 1439.9, 1383.0, 1359.5, 1297.0, 1257.3, 1235.4, 1204.3, 1177.9, 1159.3, 1135.4, 1095.1, 1064.3, 1039.5, 861.8, 834.9, 793.9, 763.2, 733.1, 704.0, 674.5. 647.8. 605.6, 584.4, 533.6, 437.0; HPLC purity: 95% (254 nm), t_R: 21.01 min; 95% (214 nm), t_R: 3.30 min.

Ethyl 5-bromo-3-(1,1,1-trifluoro-2-hydroxy-3-methylbutan-2-yl)-1*H*-indol-2-ylcarbamate (10j). Yield 78%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 11.00 (br s, 1H), 9.45 (br s, 1H), 7.65 (s, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.18 (dd, J = 8.6, 1.8 Hz, 1H), 5.98 (br s, 1H), 4.25 (q, J = 7.1 Hz, 2H), 3.02 (dt, J = 13.7, 6.9 Hz, 1H), 1.31 (dd, J = 9.5, 4.7 Hz, 3H), 1.26 (dd, J = 6.8, 1.5 Hz, 3H), 0.89 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.06, 136.43, 131.58, 126.76 (q, J = 287 Hz), 126.26, 122.53, 120.73, 113.15, 112.85, 91.72, 81.23 (q, J = 28 Hz), 61.61, 31.67, 16.98, 15.17, 13.77; LR-ESI: $[M - H]^-$ 421.3; IR (cm^{-1}): 3409.4, 2980.0, 1709.8, 1632.7, 1585.6, 1485.4, 1433.6, 1384.9, 1358.9, 1255.1, 1230.5, 1207.7, 1185.5, 1148.5, 1111.6, 1071.6, 1033.0, 982.2, 887.0, 864.3, 796.2, 766.5, 708.5, 681.5, 648.8, 589.6, 541.2; HPLC purity: 96% (254 nm), t_R: 20.49 min; 95% (214 nm), t_R: 4.80 min.

Ethyl 5-bromo-3-(1,1,1-trifluoro-2-hydroxyhexan-2-yl)-1*H*-indol-2-ylcarbamate (10k). Yield 75%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.85 (br s, 1H), 9.22 (br s, 1H), 7.46 (s, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.03 (dd, J = 8.5, 1.8 Hz, 1H), 6.13 (br s, 1H), 4.10 (q, J = 7.1 Hz, 2H), 2.57–2.44 (m, 1H), 1.97–1.85 (m, 1H), 1.49–1.33 (m, 1H), 1.29–1.19 (m, 2H), 1.16 (t, J = 7.1 Hz, 3H), 1.00 (ddd, J = 17.1, 10.1, 5.4 Hz, 1H), 0.68 (t, J = 8 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.08, 136.95, 131.53, 126.79 (q, J = 288 Hz), 126.73, 122.53, 120.29, 113.17, 112.97, 89.83, 78.27 (q, J = 30 Hz), 61.64, 32.95, 24.55, 22.31, 13.78, 13.24; LR-ESI: $[M - H]^-$ 435.1; IR (cm^{-1}): 3421.4, 3357.1, 2961.2, 2935.3, 2867.0, 1692.6, 1634.8, 1585.2, 1487.1, 1444.6, 1385.9, 1362.0, 1281.8, 1257.0, 1235.7, 1213.4, 1183.3, 1157.3, 1099.7, 1072.5, 1007.4, 983.1, 952.1, 932.4, 862.1, 794.6, 766.5, 714.0, 678.9, 649.4, 591.2, 560.7, 528.9, 421.7; HPLC purity: 96% (254 nm), t_R: 21.04 min; 97% (214 nm), t_R: 5.50 min.

Ethyl 5-bromo-3-(1,1,1-trifluoro-2-hydroxypent-4-en-2-yl)-1*H*-indol-2-ylcarbamate (10l). Yield 75%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 11.02 (br s, 1H), 9.34 (br s, 1H), 7.67 (s, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.19 (dd, J = 8.6, 1.7 Hz, 1H), 6.42 (br s, 1H), 5.75–5.60 (m, 1H), 5.22 (dd, J = 17.1, 1.2 Hz, 1H), 5.04 (d, J = 10.2 Hz, 1H), 4.26 (q, J = 7.1 Hz, 2H), 3.51 (dd, J = 15.1, 6.4 Hz, 1H), 2.89 (dd, J = 15.2, 7.3 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.08, 136.92, 131.57, 130.77, 126.57, 126.52 (q, J = 286 Hz), 122.59, 120.61, 119.24, 113.17, 113.00, 89.72, 77.63 (q, J = 30 Hz), 61.68, 37.73, 13.79; LR-ESI: $[M - H]^-$ 419.2; IR (cm^{-1}): 3407.1, 3367.6, 2983.2, 2933.3, 1703.0, 1633.6, 1582.8, 1491.2, 1443.5, 1361.0, 1296.9, 1275.5, 1258.2, 1235.3, 1211.7, 1182.4, 1145.1, 1094.5, 1072.4, 1019.3, 995.8, 962.3, 931.9, 912.7, 882.1, 856.8, 800.8, 193.3, 766.3, 728.6, 702.1, 671.1, 590.1, 522.2, 475.1; HPLC purity: 95% (254 nm), t_R: 19.55 min; 97% (214 nm), t_R: 3.77 min.

Ethyl 5-bromo-3-(2,2,2-trifluoro-1-hydroxy-1-phenylethyl)-1*H*-indol-2-ylcarbamate (10o). Yield 63%; ^{1}H NMR (300 MHz, in acetone-d_6): δ 11.07 (br s, 1H), 9.27 (br s, 1H), 7.64 (m, 2H), 7.45 (m, 4H), 7.04 (dd, J = 8.6, 1.9 Hz, 1H), 6.45 (br s, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.05, 138.85, 136.41, 131.29, 128.99, 128.45 (2C), 127.66 (q, J = 2.0 Hz) (2C), 127.39, 126.49 (q, J = 287.0 Hz), 122.36, 120.41, 112.92, 112.58, 92.64, 78.90 (q, J = 30.0 Hz), 61.75, 13.78; LR-ESI: $[M - H]^-$ 455.0; IR (cm^{-1}): 3386.2, 3063.8, 2983.0, 2934.7, 1718.1, 1633.9, 1586.2, 1485.5, 1449.7, 1388.1, 1358.0, 1230.4, 1254.1, 1203.6, 1164.5, 1078.1, 1002.2, 959.0, 935.0, 882.9, 859.2, 795.2, 765.2, 732.3, 709.0, 698.7, 681.3, 668.7, 653.5, 626.1, 590.7, 549.4; HPLC purity: 91% (254 nm), t_R: 19.99 min.

Ethyl 5-chloro-3-(2,2,2-trifluoro-1-hydroxy-1-phenylethyl)-1*H*-indol-2-ylcarbamate (10p). Yield 58%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 11.03 (br s, 1H), 9.29 (br s, 1H), 7.68 (m, 2H), 7.50 (d, J = 8.6 Hz, 1H), 7.47–7.41 (m, 3H), 6.97 (s, 1H), 6.93 (dt, J = 8.6, 1.7 Hz, 1H), 6.36 (br s, 1H), 4.28 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.12, 138.91, 136.61, 131.03, 129.00, 128.47 (2C), 127.69 (q, J = 2.0 Hz) (2C), 126.82, 126.55 (q, J = 287.0 Hz), 124.95, 119.76, 117.43, 112.47, 92.82, 78.97 (q, J = 30.0 Hz), 61.78, 13.83; LR-ESI: $[M - H]^-$ 411.0; IR (cm^{-1}): 3391.6, 2933.4, 1699.5, 1635.3, 1588.7, 1487.9, 1450.4, 1385.8, 1361.0, 1230.0, 1254.6, 1204.2, 1165.3, 1082.4, 1002.6, 964.2, 935.2, 883.0, 860.5, 796.6, 765.4, 737.4, 714.4, 698.6, 668.6, 653.8, 626.9, 594.6, 549.8; HPLC purity: 92% (254 nm), t_R: 19.69 min.

Ethyl 5-chloro-3-(1-(3,5-dimethylphenyl)-2,2,2-trifluoro-1-hydroxyethyl)-1*H*-indol-2-ylcarbamate (10q). Yield 52%; ^{1}H NMR (300 MHz, in acetone-d_6): δ 10.89 (br s, 1H), 9.12 (br s, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.14 (s, 2H), 6.96 (s, 1H), 6.79 (d, J = 8.5 Hz, 1H), 6.67 (br s, 1H), 6.31 (s, 1H), 4.15 (q, J = 7.5 Hz, 2H), 2.17 (s, 6H), 1.19 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 153.09, 138.87, 137.82 (2C), 136.44, 131.01, 130.36, 126.86, 126.55 (q, J = 287.2 Hz), 125.28 (q, J = 2.0 Hz) (2C), 124.86, 119.73, 117.60, 112.42, 92.92, 78.94 (d, J = 29.7 Hz), 61.73, 20.58 (2C), 13.81; LR-ESI: $[M - H]^-$ 439.2; IR (cm^{-1}): 3515.2, 3422.5, 3399.3, 3381.4, 2982.1, 2918.6, 1725.6, 1639.0, 1595.3, 1486.4, 1474.4, 1456.9, 1379.6, 1361.4, 1288.1, 1251.3, 1207.2, 1229.4, 1182.6, 1146.7, 1087.3, 1011.4, 974.4, 945.5, 919.6, 887.7, 855.0, 790.7, 760.3, 747.9, 726.4, 717.9, 685.6, 632.4, 594.2, 553.7, 525.4, 434.9; HPLC purity: 91% (254 nm), t_R: 20.78 min.

General procedure for preparation of target compounds 10m and 10n

To a solution of trifluoroacetyl carbamate **9c** and **9e** (1 mmol) and Proline/AcK (1/1, 0.3 mmol/0.3 mmol) in dry DMSO (4 mL) was added acetone (1 ml) at 40 °C under Ar. After stirring at 40 °C for 3–4 days, the solvent was evaporated under reduced pressure and the residue was purified with silica gel

chromatography to give the desired products **10 m** and **10n** in a yield of 50–60%.

Ethyl 5,7-dichloro-3-(1,1,1-trifluoro-2-hydroxy-4-oxopentan-2-yl)-1*H*-indol-2-ylcarbamate (10m). Yield 60%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 10.78 (br s, 1H), 9.29 (br s, 1H), 7.60 (s, 1H), 7.19 (d, J = 1.5 Hz, 1H), 7.00 (br s, 1H), 4.31 (q, J = 7.1 Hz, 2H), 3.89 (d, J = 17.4 Hz, 1H), 3.54 (d, J = 17.4 Hz, 1H), 2.22 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 206.61, 153.70, 137.34, 127.78, 126.78, 125.77, 125.74 (q, J = 285 Hz), 119.43(2C), 117.11, 116.25, 92.59, 76.13 (q, J = 30 Hz), 62.20, 44.24, 30.54, 13.71; LR-ESI: $[M - H]^-$ 425.2; IR (cm^{-1}): 3380.7, 3349.8, 3246.0, 3072.2, 2997.5, 2910.9, 1725.6, 1701.6, 1636.4, 1584.0, 1556.5, 1498.9, 1484.1, 1469.6, 1433.1, 1388.6, 1361.4, 1330.4, 1268.1, 1236.0, 1171.7, 1151.1, 1090.5, 1079.3, 1040.1, 981.1, 922.6, 897.2, 873.3, 848.7, 767.2, 739.1, 726.0, 689.8, 611.3, 570.6, 530.6, 442.8, 409.5; HPLC purity: 98% (254 nm), t_R: 20.87 min; 99% (214 nm), t_R: 5.36 min.

Ethyl 7-nitro-3-(1,1,1-trifluoro-2-hydroxy-4-oxopentan-2-yl)-1*H*-indol-2-ylcarbamate (10n). Yield 60%; ^{1}H NMR (400 MHz, in acetone-d_6): δ 11.94 (br s, 1H), 9.37 (br s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.2 Hz, 1H), 7.29 (t, J = 8.1 Hz, 1H), 7.03 (br s, 1H), 4.35 (q, J = 7.1 Hz, 2H), 3.94 (d, J = 17.3 Hz, 1H), 3.56 (d, J = 17.3 Hz, 1H), 2.21 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, in acetone-d_6): δ 206.35, 153.56, 137.52, 132.54, 128.36, 126.17, 125.74 (q, J = 285 Hz), 125.70, 120.02, 116.67, 91.96, 76.23 (q, J = 31 Hz), 62.28, 44.55, 30.55, 13.74; LR-ESI: $[M - H]^-$ 402.2, $[M + Na]^+$ 426.3, $[M + H]^+$ 404.3; IR (cm^{-1}): 3403.9, 3387.8, 2995.5, 2910.2, 1724.8, 1643.3, 1593.8, 1520.4, 1568.8, 1499.4, 1459.6, 1429.0, 1415.6, 1364.2, 1312.5, 1343.0, 1234.4, 1201.7, 1168.1, 1150.4, 1114.7, 1084.5, 1039.2, 1021.2, 977.1, 917.9, 891.1, 871.3, 846.6, 800.2, 767.2, 734.6, 700.3, 634.9, 621.1, 565.7, 529.1, 487.5, 468.2; HPLC purity: 98% (254 nm), t_R: 18.01 min; 97% (214 nm), t_R: 2.90 min.

Anti-HIV activity assay

The anti-HIV activity and cytotoxicity of compounds **9a–9e** and **10a–10n** were evaluated against wild-type HIV-1 strain IIIB and resistant mutant strain Y181C HIV-1 in MT-2 cell cultures.

In vitro cytotoxicity assay in MT-2 cells

The *in vitro* cytotoxicity of compounds on MT-2 cells was measured using Cell-Titer Glo assay. Briefly, 10 μL of the test compound at graded concentrations were added to 90 μL of cells (1.5×10^4 per well) in wells of a 384-well plate. After incubation at 37 °C for 3 days, 20 μL of assay reagent were added for measurement of luminescence with a Victor 2 luminometer (Perkin Elmer). The TC_{50} (concentration for 50% cytotoxicity) values were calculated using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

Assay for measuring the inhibitory activity on HIV-1 IIIB replication in MT-2 cells

MT-2 cells were infected with HIV-1 IIIB at a multiplicity of infection (MOI) of 0.005 50% tissue culture infective doses (TCID50)/cell followed by incubation in the presence of serially diluted inhibitors for 3 days. Virus yields were quantified using TZM-bl as a reporter cell line. Briefly, MT-2 cells (1.5×10^4 per well) were infected with an HIV-1 IIIB in 100 μL of RPMI 1640 medium containing 10% FBS in the presence or absence of a test compound at graded concentrations for 3 days. Then 10 μL culture supernatants were transfer to the same positions of a new 384-well plate containing TZM-bl cells (1.5×10^4 per well). 24 hours after infection, 20 μL of Bright-Glo Luciferase Assay reagent (Promega, Madison, WI) were added to the wells for measurement of luminescence with a Victor 2 luminometer. The effective concentrations for 50% inhibition (EC_{50}) were calculated using GraphPad Prism 5.0.

HIV-1 infection assay using TZM-bl as a reporter cell line. Inhibition of HIV-1 infection was measured as reduction in luciferase gene expression after a single round of virus infection of TZM-bl cells as described previously. Briefly, 200 TCID50 of virus with a resistant mutation at 181 (where Tyr was replaced by Cys) was used to infect TZM-bl cells in the presence of various concentrations of compounds. 48 hours after infection, 20 μL of Bright-Glo Luciferase Assay reagent (Promega, Madison, WI) were added to the wells for measurement of luminescence with a Victor 2 luminometer. The 50% inhibitory concentration (IC50) was defined as the concentration that caused a 50% reduction of luciferase activity (Relative Light Units) compared to virus control wells.

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