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Design, Synthesis, and Biological Evaluation of Novel Trifluoromethyl Indole Derivatives as Potent HIV-1 Non-nucleoside Reverse Transcriptase Inhibitors 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 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 as well as 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 to cause distortion of the three-dimensional structure of the enzyme to inhibit RT catalytic function.[2] Currently, five NNRTIs drugs have been approved by U.S. Food and Drug Administration (FDA) (Fig. 1). The first-generation NNRTIs drugs, Nevirapine (NVP), Efavirenz (EFV) and Delavirdine (DLV) are limited in clinical use due to rapid emergence of drug resistance. Especially, K103N and Y181C are the two mutations frequently observed in patients exposed to various NNRTIs.

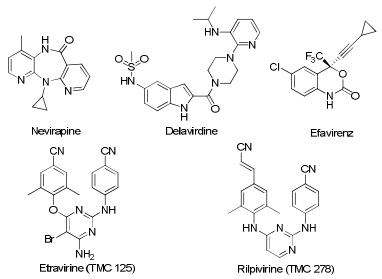


Fig.1 Current marketed NNRTIs.

Etravirine (TMC125) and Rilpivirine (TMC278) belonging to the diarylpyrimidine (DAPY) family, as the second generation NNRTIs, were approved by FDA in 2008 and 2011 respectively, and demonstrated activities against HIV-1 strains that developed resistance to the first generation NNRTIs drugs. Nevertheless, 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 the low water solubility.[6–8] And there are a lot of undesired side effects of TMC125, such as toxic epidermal necrolysis and erythema multiforme, as well as hypersensitivity reactions characterized by rash, even sometimes organ dysfunction, including hepatic failure. Efforts to develop the next-generation NNRTIs[9–11] possessing novel scaffolds have focused on the design of compounds with high potency, low toxicity, improved resistant profiles, and better pharmacokinetic profiles.

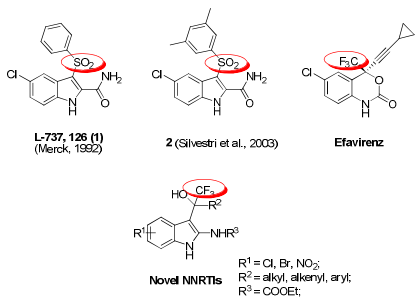


Fig. 2 Structure of reference L-737, 126 (**1**) and IAS **2**.

The development of indolylarylsulfones (IASs) NNRTIs was based on the Merck derivative L-737,126 (**1**)[12] as 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 trifluoromethyl group in Efavirenz allowing the inhibitor to assume a butterfly-like conformation, which is commonly found in many other NNRTIs. On the other hand, inspired by the good potency and bioavailability profiles[15–17] of trifluoromethyl group, if the sulfonyl moiety of IASs can be replaced by trifluoromethyl group to produce a new potential NNRTI, it is possible to improve the activity against resistant mutants and reduce toxicity.

With a novel scaffold in mind, we have 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 designed compounds with representative structures were first predicted to exclude the compounds out of "Lipinski's 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 relationship (SAR) of a new series of trifluoromethyl indoles.

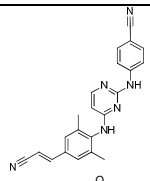
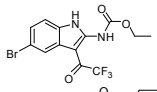
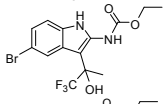
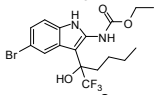
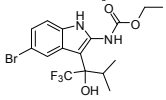
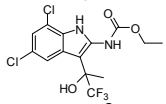
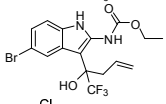
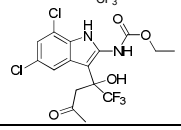
Results and discussion

Prediction

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

Therefore, preliminary prediction of some drug-like properties served as a modest stimulant to induce us to synthesize these novel trifluoromethyl indoles.

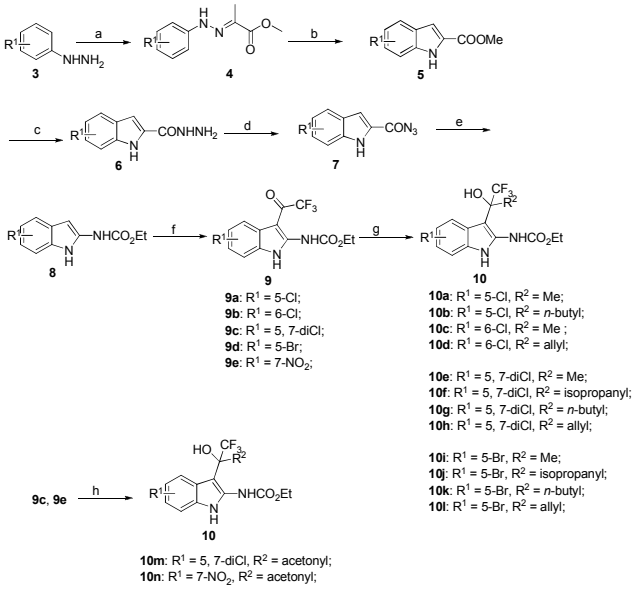
Table 1 Prediction results of logP, Acute, Carcinigenic and Mutagenic Toxicity

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} <1mg/kg; equal or more than 2 and less than 3, 1≤LD_{50}<50 mg/kg; equal or more than 3 and less than 4, 50≤LD_{50}<500 mg/kg; equal or more than 4 and less than 5, 500≤LD_{50}<5000 mg/kg; equal or more than 5, LD_{50}≥5000 mg/kg. [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.

Chemistry

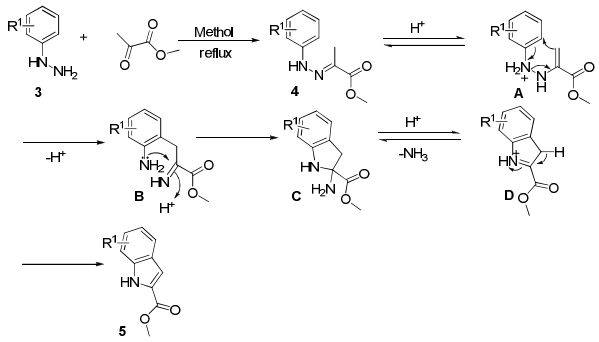
The synthesis of new trifluoromethyl indoles was depicted in Scheme 1.



Scheme 1 Synthesis of compounds **9a**–**10n**.[a]

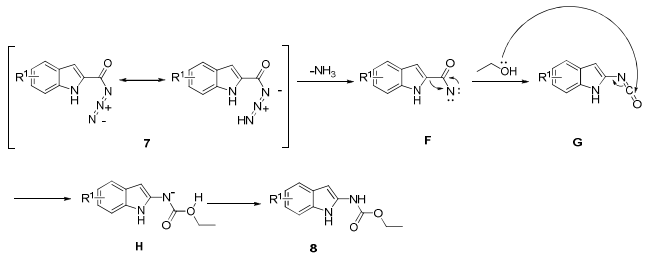
[a]*Reagents and conditions*: (a) methylpyruvate, MeOH, reflux; (b) 4N HCl-MeOH, reflux; (c) $N_2H_4 \cdot H_2O$ (85%), EtOH, reflux; (d) $NaNO_2$, AcOH, 1, 4-dioxane, 0 °C; (e) EtOH, reflux; (f) Trifluoroacetic anhydride, DMF or diethyl ether, 0 °C; (g) R^2MgCl/ R^2MgBr, THF, 0 °C; (h) Acetone, Proline/AcK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

Variously substituted indoles were prepared to examine SAR by typical Fischer indole reaction. 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 acid conditions. The detailed mechanism for Fischer indole reaction[23]of phenylhydrazine **3** and methylpyruvate was provided as follows (Scheme 2): Phenylhydrazone **4** is firstly formed from the condensation reaction of commercially available methylpyruvate and phenylhydrazine **3**, following the isomerization of Phenylhydrazone **4** to result in the enamine **A**. After the protonation, the enamine **A** is transformed to the imine **B** through a cyclic [3,3]-sigmatropic rearrangement reaction. Then the aminoacetal **C** is produced by cyclization of the resulting imine **B**. Subsequently the imine **D** is obtained by the aminoacetal **C** lossing of one molecule of NH_3 under acid catalysis, after protonation to deliver energetically favorable aromatic indole **5**.



Scheme 2 A plausible mechanism for Fischer indole reaction

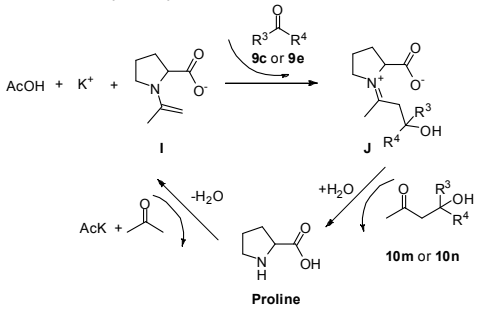
Then the aromatic indole **5** was converted with $N_2H_4 \cdot H_2O$ (85%) in ethanol into carbohydrazide **6**, followed by diazotization reaction with $NaNO_2$ in acetic acid to produce azide **7**. Subsequently, the Curtius rearrangement of the azide **7** yielded the carbamate **8** in ethanol. The detailed mechanism for the rearrangement[24] reaction was provided as follows (Scheme 3): The nitrene **F** is formed from a substituted azide **7** by loss of one molecule of nitrogen gas, following the rearrangement of the acyl nitrene **F** by migration of indole group to form the desired the isocyanate **G**, after the nucleophilic addition reaction of the resulting the isocyanate **G** and EtOH to deliver the desired ethyl carbamate **8**. Subsequently, resulting carbamate **8** reacted 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.



Scheme 3 A plausible mechanism for Curtius rearrangement reaction

Meanwhile, compounds **10m** and **10n** were also produced in dry DMSO by proline/AcK-catalyzed aldol reaction at 40 °C for 3–4 days. The detailed catalytic mechanism for proline-catalyzed direct aldol reaction[25]was provided as follows (Scheme 4): Enamine **I** is firstly formed from the condensation reaction of commercially available proline and acetone promoting with AcK. Subsequently substrate ketone **9c** or **9e** is added to the reaction mixture and is attacked by the enamine **I** to deliver the iminium product **J**. Finally, the iminium **J** is hydrolyzed to give aldol product **10m** or **10n** with releasing of one molecule of proline,

which allows the catalytic cycle to continue until the conversion is finished.



Scheme 4 A plausible proline-catalyzed direct aldol reaction pathways

Following the same procedure as compounds **10a**–**10l**, we also tried to synthesize compounds with the phenyl or 3, 5-dimethylphenyl group as the R^2, such as compounds **10o**, **10p** and **10q** (Fig. 3). However, these compounds were very unstable and their purities detected by HPLC decreased by about 10% in 24 hours. Since stabilities of compounds **10o**, **10p** and **10q** were too poor to meet the requirements of biological assays, we had to give up synthesizing these compounds with the aryl moieties as the R^2.

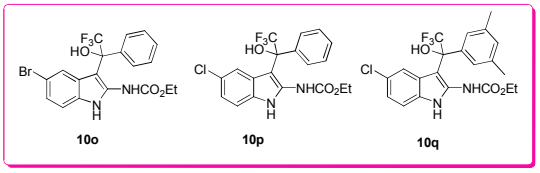


Fig. 3 Structure of **10o, 10p** and **10q.**

Biological activity

In vitro Cell-Titer Glo assay method was used to evaluate 19 new trifluoromethyl indoles (**9a**–**9e** and **10a**–**10n**) along with two FDA-approved drugs: NVP and EFV as reference compounds. 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, with Tyr181 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) and TI (selectivity index represented by the TC_{50}/IC_{50} ratio) values, were 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, the compounds **10i** and **10k** displayed good anti-HIV-1 activities against WT HIV-1 with an IC_{50} values of 0.003 μM respectively, and compound **10k** showed the greatest selectivity (TI >7079). Furthermore, compound **10k** was also proved potential activity against Y181C mutant virus with an IC_{50} value of 1.932 μM.

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

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

Compd		IC_{50} (μM)[b]		TC_{50} (μM)[c]	TI[d]
		WT IIIB	Y181C		
"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.

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

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 by 50% the RT activity of the indicated strain. [c] Reference 14c, IC_{50} (μM).

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] respectively.

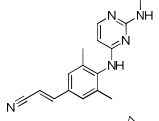
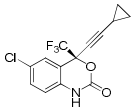
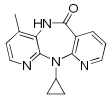
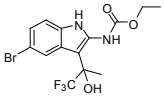
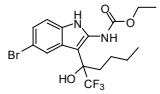
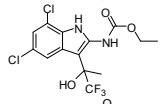
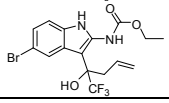
The calculation of interactions between the six compounds and target WT HIV-1 RT (PDB code: 2ZD1) was listed in Table 4 and Figure 4. According to the calculation results, the location of TMC278 in the active site was basically consistent with that in crystal structure. In general, the lower total score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

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

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

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

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

Compd	Structure	IC_{50} (μM)	Total Score (Kcal/mol)	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] Literature 30.

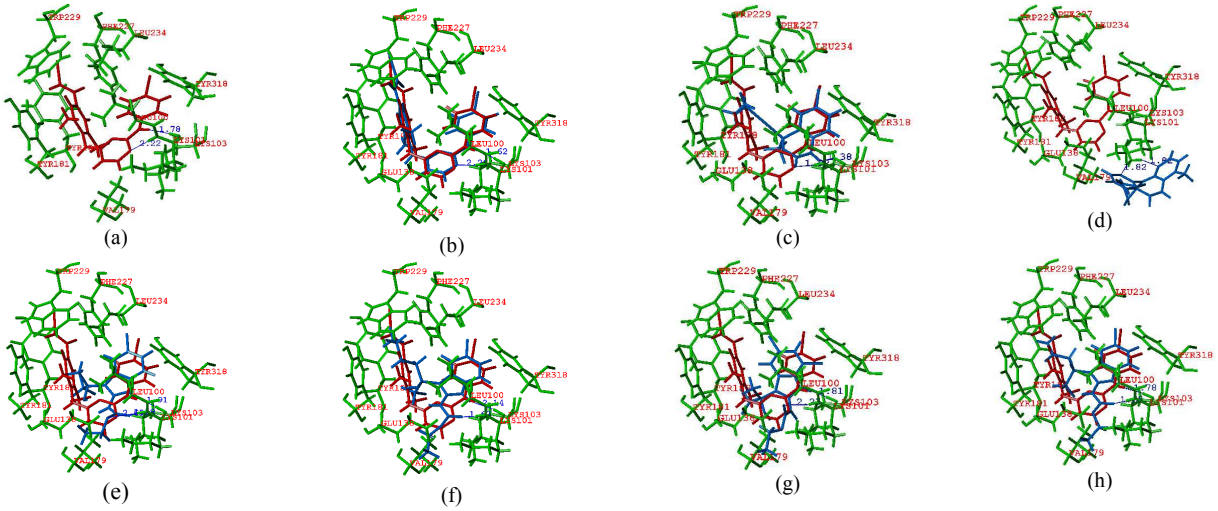
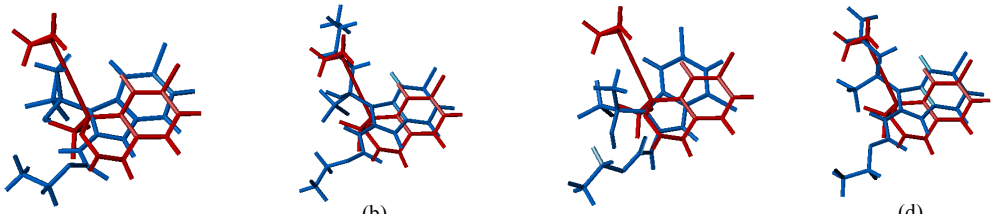


Fig. 4 Interactions between HIV-1 RT WT (PDB: 2ZD1) and TMC278 (red), other compounds. (a) TMC278 (Cry.); (b) TMC278 (red, cry.), blue one docking result; (c) TMC278 (red, cry.), EFV (blue, docking result); (d) TMC278 (red, cry.), NVP (blue, docking result); (e) TMC278 (red, cry.), **10i** (blue, docking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

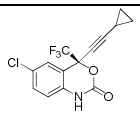
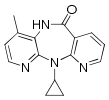
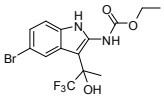
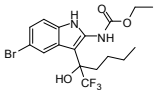
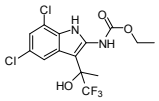
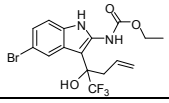


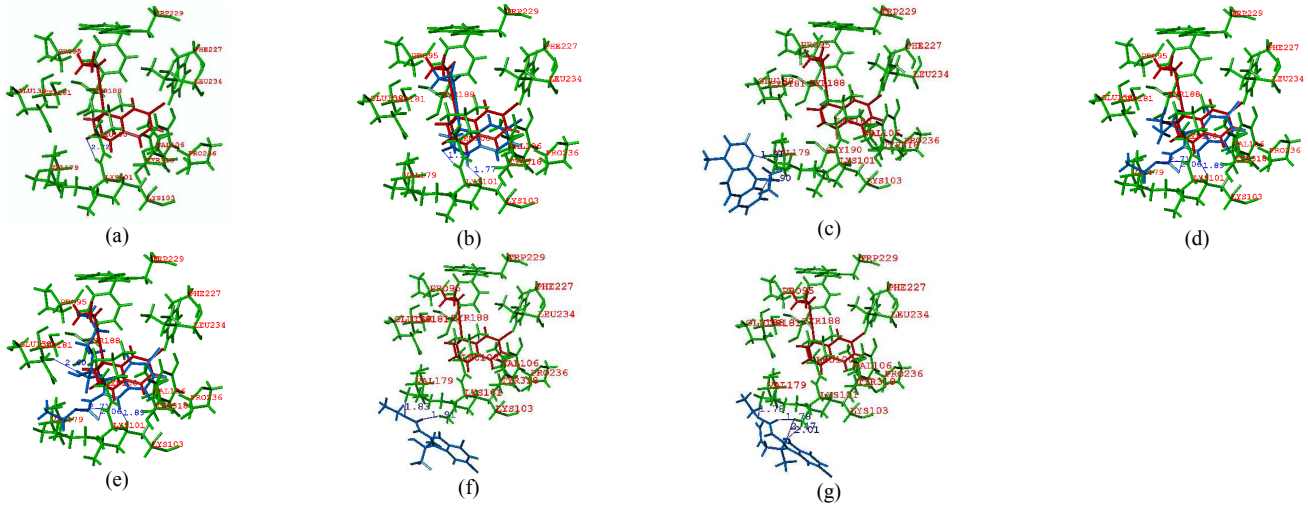
(a) (b) (c) (d)

Fig. 5 Superimpositions of EFV and compound **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).

The calculation of interactions between the six compounds and target, Y181C mutant HIV-1 RT (PDB code: 1JKH), was listed in Table 5 and Figure 6. According to the calculation results, the location of EFV in the active site was also basically consistent with that in crystal structure.

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

Compd	Structure	IC_{50} (μM)	Total Score	H-Bond (Å)
EFV (Cry.)		0.021	No calculation	Lys101 (H56) – EFV (O5): 2.72
EFV (Cal.)			-22.99	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



(a) (b) (c) (d) (e) (f) (g)

Fig. 6 Interaction between Y181C mutant HIV-1 RT (PDB: 1JKH) and EFV (red), other compounds. (a) EFV (Cry.); (b) EFV (red, cry.), blue one 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)

The calculation of interactions between ligands and Y181C mutant HIV-1 RT showed that 1) Total scores of compounds **10i** and **10k** corresponded well to their biological assays; 2) Length of hydrogen bond between residue Lys101 and EFV was shorter than that of Lys101 and **10i** or **10k**, respectively. Furthermore, in the active site, superimposition of compound **10k** and EFV was higher than that of **10i** and EFV. These data accorded 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 hydrogen bond length of residue Lys101 to **10i** and **10k** were identical, the IC_{50} value of **10k** was almost 10-fold lower than that of **10i**. This result was also probably due to the fact that the *n*-butyl group of compound **10k** could insert into the region formed by Cys181, Tyr188, Phe227, and Trp229.[29] Further its extension was seen to fill the space well between Cys181, Tyr188, and Trp229. In addition, the IC_{50} value of **10i** for Y181C mutant was 1000-fold higher than the wild-type strain. It was also because that the mutation of Tyr181 to Cys181 prevented the methyl group from entering the region.

The binding modes of compounds NVP, **10e** and **10l** in Y181C mutant HIV-1 RT NNBS showed that the three compounds were out of the active site. Although there were hydrogen bonds between residue Lys101 and the three compounds NVP, **10e** and **10l**, these hydrogen bonds were different from those between residue Lys101 and EFV, **10i** and **10k**. The main differences were the hydrogen bonds were formed between small molecule compounds and different oxygen or hydrogen atoms of residue Lys101. In addition, the total scores of the two compounds **10e** and **10l** were higher than those of compounds **10i** and **10k**, which also corresponded to the IC_{50} values in Table 2.

According to 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 the strategy of selection of suitable substituent (R^2) fitting the pocket well formed by Cys 181, Tyr188, Phe227, and Trp229 would be beneficial to enhance the interaction between the inhibitors and Y181C mutant HIV-1 RT.

Moreover, there was also a hydrogen bond of **10k** (F25) – Cys181 (H214) for the mutant Y181C, which was unusal for most NNRTIs binding to the active site. Generally, the larger the difference in electronegativity of the H atom and the other atom (N, O, and F) is, the stronger the H-bond is. It will also inspire us to pay more attention to special H-F hydrogen bond 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 corresponded to the biological evaluations. For Tyr181–Cys mutant in the active site, two different molecular models provided insights for the further molecular design and structural optimization in due course.

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 a 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 the 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 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 the yield of 80–95%.

*General procedure for preparation of target compounds **5***

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

*General procedure for preparation of target compounds **6***

To the solution of methyl 1H-indole-2-carboxylate **5** (5 mmol) in EtOH (10 mL) was added hydrazine hydrate (4ml, 85%) and the reaction solution was stirred at reflux for 5 hours. After cooled to room temperature, the solid was collected by filtration, washed

with cold EtOH, dried to give the product **6** in the yield of 85–95%.

General procedure for preparation of target compounds 7

To the solution of 1H-indole-2-carbohydrazide **6** (10 mmol) in dioxane (10 mL) was added AcOH (10 mL) at 0 ^{o}C with stirring. Then $NaNO_2$ (800 mg) in H_2O (2 mL) was added dropwise at 0 ^{o}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, dried to give the azide product **7** in the yield of 80–95%.

*General procedure for preparation of target compounds **8***

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

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

To the solution of carbamate **8** (1 mmol) in dry DMF (1 mL) or dry diethyl ether (10ml) was added dropwise of TFAA (2.5 mmol) at 0 ^{o}C with stirring. After completion (Monitored by TLC), water was added and 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 the yield of 75–90%.

Ethyl 5-chloro-3-(2, 2, 2-trifluoroacetyl)-1H-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 the solution of trifluoroacetyl carbamate **9a**–**9d** (1 mmol) in dry THF (10 mL) was added dropwise of Grignard Reagents (2–4 mmol) at 0 °C under Ar protection. After stirred at 0 °C for 1–2 hours, the resulting reaction was quenched with sat. NH_4Cl solution, 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 column to give the products **10a**–**10l** in the 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 = 287Hz), 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)-1H-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)-1H-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)-1H-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)-1H-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 the 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 ^{o}C under Ar protection. After stirred at 40 ^{o}C for 3–4 days, the solvent was evaporated under reduced pressure and the residue was purified with silica gel column to give the desired products **10m** and **10n** in the 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* = 30Hz), 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 the compounds **9a**–**9e** and **10a**–**10n** were evaluated against wild-type HIV-1 strain IIIB, resistant mutant strain Y181C HIV-1 in MT-2 cell cultures.

In Vitro Cytotoxicity Assay in MT-2 Cells

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/well) in wells of a 384-well plate. After incubation at 37℃ for 3 days, 20 μL of assay reagents was 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.

In general, 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 quantitated using TZM-bl as a reporter cell line. Briefly, MT-2 cells (1.5×10^4/well) were infected with an HIV-1 IIIB in 100μL of RPMI 1640 medium containing 10% FBS in the presence or absence of a test compound at graded concentrations for 3 days. Then 10μL culture supernatants were transfer to the same positions of a new 384-well plate containing TZM-bl cells (1.5×10^4/well). 24 hrs after infection, 20 μL of Bright-Glo Luciferase Assay reagents (Promega, Madison, WI) was 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 has a resistant mutation at 181which replaced by Cys was used to infect TZM-bl cells in the presence of various concentrations of compounds. 48hrs after infection, 20 μL of Bright-Glo Luciferase Assay regeants (Promega, Madison, WI) was added to the wells for measurement of luminescence with a Victor 2 luminometer. The 50% inhibitory concentration (IC_{50}) 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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§ Electronic Supplementary Information (ESI) available: [Copies of ^{1}H, ^{13}C NMR, MS, IR and HPLC spectra of target compounds **9a–9e** and **10a-10q**. See DOI: 10.1039/b000000x/

‖ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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