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Synthesis and Evaluation of Anti-HIV-1 Activities of Novel 7-Hydroxy-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-4-carboxylate Derivatives

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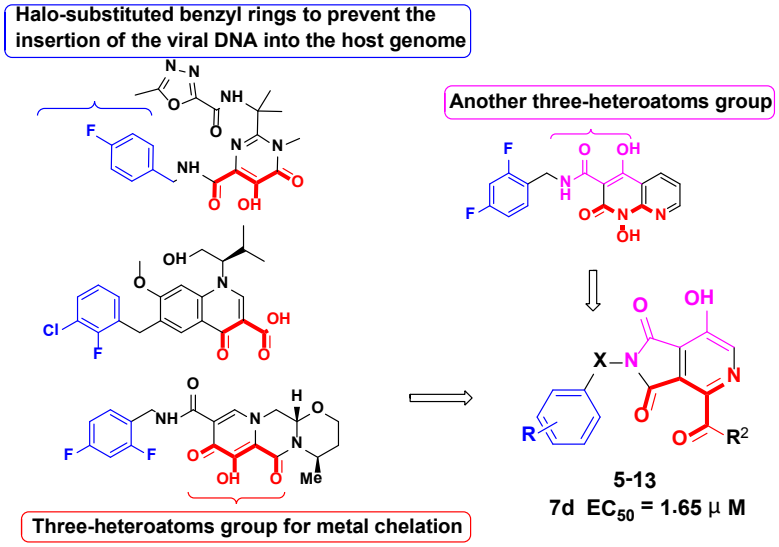
Abstract

Aim: To design and synthesize a series of novel 7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate derivatives and evaluate their anti-HIV-1 activities. Methods: Holding the same triad of metal-chelating heteroatoms for the catalytic site of IN and introducing a new hydroxyl group into the adjacent position of the amide to form another three-heteroatoms group for metal chelation, a series of novel 7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate derivatives were designed and synthesized through multi-step chemical reactions. All the synthesized compounds were evaluated for their inhibitory activities against HIV-1 replication. Results: Thirty-five new compounds (**5**–**13**) have been designed, synthesized and bioassayed. Their structural features were determined by ^{1}H-NMR spectra, and low- and high-resolution mass spectra. Most of the synthesized compounds showed moderate to potent activities against HIV-1.

Among the analogs, compounds **7d**, **7f**, **7i**-**7j**, **8d** and **9c** exhibited potent anti-HIV-1 activities (EC_{50}<10 μM). In particular, **7d** exhibited significant anti-HIV-1 activities with EC_{50} values of 1.65 μM. Conclusion: This study provides a new template for further development of potent anti-HIV-1 drugs, and the preliminary SAR among the newly synthesized analogs provided useful indications for guiding further rational design of potent anti-HIV-1 agents.



Keywords: AIDS; Anti-HIV-1; Design; Integrase; SAR; Synthesis

Introduction

Acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus (HIV) infection are global health hazards with huge social, economic, and ethical consequences [1,2]. HIV-1 integrase (IN) is a virally encoded enzyme essential for replication, which catalyzes the integration of viral DNA into the host chromatin. As a result of this unique retroviral step, and the absence of any known human homolog, IN has become an attractive target for drug discovery in the treatment of HIV-1 infection [3-6]. After years of sustained effort, Raltegravir (RAL, 1) (October 2007)[7,8] and Elvitegravir (EVG, 2) (August 2012) [9,10] were the first generation of IN inhibitors (INIs) to be approved by the United States of America's Food and Drug Administration (FDA), thus opening up a new class of antiretroviral agents (Figure 1). Unfortunately, treatment with RAL and EVG could lead to the development of resistance, and there is extensive shared cross-resistance [11-13]. The need to overcome these problems has driven the development of a second generation of INIs such as Dolutegravir (DTG, 3, Figure 1) approved on August 2013, which displays superior characteristics to RAL and EVG, but partially shares

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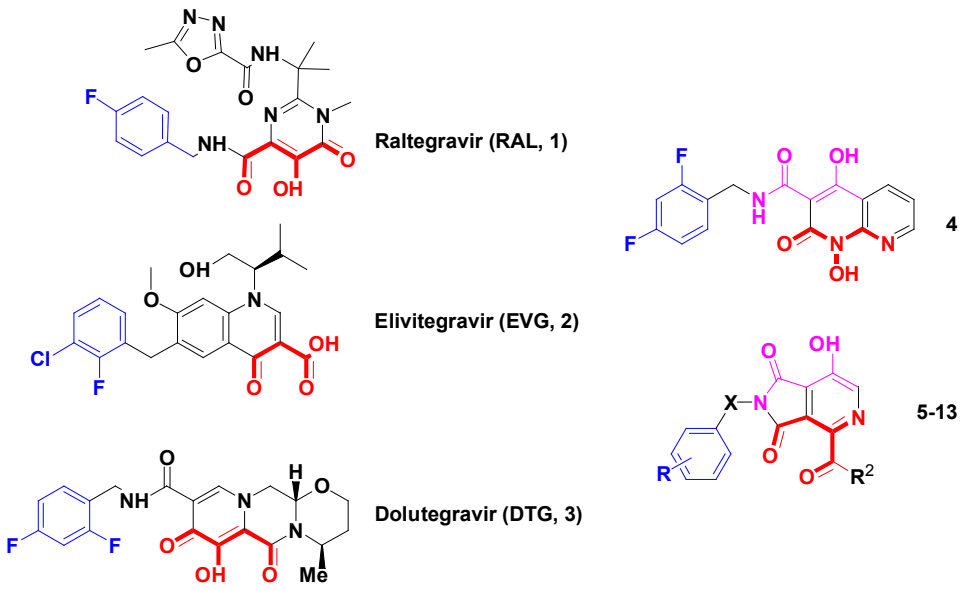


Figure 1: HIV-1 integrase inhibitors.

the resistance pathways [14-16]. Therefore, an intensified search for structurally novel INIs to combat resistance is urgently needed.

INIs share key structural features that trace their conceptual ancestry to diketoacid (DKA) progenitors containing a triad of heteroatoms, whose function is to chelate the two catalytically-essential divalent metal ions at the IN active site [17]. Additionally, halo-substituted benzyl rings are present, appended through amine or amide groups, which interact with the penultimate cytosine base near the 3'-end of the viral DNA to prevent the insertion of the viral DNA into the host genome [18]. Based on the hits discovered using virtual screening and bioassays, we identified a new anti-HIV-1 compound, 1-pyrrolidineacetamide, through a new IN-binding site [19], which has a modified scaffold of 5,6-dihydroxypyrimidines and shows potent anti-HIV-1 activity, with therapeutic index (TI) values of >1000 [20]. Recently, the introduction of a hydroxyl group into the adjacent position of the carboxamide substituents resulted in analogs **4** (Figure 1), which is effective in suppressing the spread of HIV-1 infection in cells [21]. As a continuation of our research in this field, we have designed some novel small molecules **5-13** (Figure 1), by holding the same triad of metal-chelating heteroatoms for the catalytic site of IN (red color), and introducing a new hydroxyl group into the adjacent position of the amide substituted by halobenzyl substituents (blue color), to form another three-heteroatoms group for metal chelation (pink color). Herein, we report the synthesis and bioassay of these compounds, in which further explorations with hydrophobic groups at position N-2 and an ester or amide at the 4-position were performed.

Materials and Methods

Chemistry

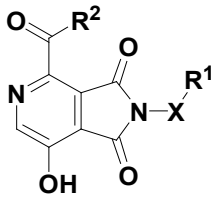
Compound design: The inhibitory activity of DKA analogs against HIV depends on the triad of heteroatoms groups and halo-substituted benzyl rings of the DKA core. 35 new analogues (5–13) were designed by keeping the key groups of DKA and introducing a new hydroxyl group into the adjacent position of the amide (Table 1). We changed the length and bulk of the linker X, used various aromatic, heterocyclic, and aliphatic groups to substitute R^1, and changed R^2 from ester groups to amide groups.

Synthetic procedures: As outlined in Scheme 1, target compounds **5-13** were synthesized successfully from commercially available reagents. In a similar reported synthetic method, the treatment of glycine ethylester with diethyl oxalate and Et_3N in ethanol followed by cyclization of the resulting ethyl 2-((2-ethoxy-2-oxoethyl)amino)-2-oxoacetate **M1** with phosphorous pentoxide (P_2O_5) in anhydrous acetonitrile without further purification, gave the intermediate ethyl 5-ethoxy-l,3-oxazole-2-carboxylate **M2** [22]. Another intermediate **M3** was obtained from maleic anhydride by treatment of the corresponding amines or hydrazines in acetic anhydride in the presence of sodium acetate [23]. Subsequently, reacting **M3** with **M2** in water under conditions of microwave irradiation produced target compounds **5-7** and **9-13** *via* a Diels-Alder reaction. Compound **8** was prepared from **7d**, **7f** and **7i** by reacting them with the corresponding amine in anhydrous acetonitrile. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and mass spectroscopy (MS) characterized all the target compounds.

The reagents (chemicals) were purchased from commercial sources (Alfa, Acros, Sigma-aldrich and Shanghai Chemical Reagent Company), and used without further purification. Analytical thin layer chromatography (TLC) was HSGF 254 (0.15-0.2 mm thickness, Yantai Huiyou Company, China). The microwave reactor, chempower 18920570, was manufactured by Shanghai Chubo Instrument Limited Company. Column chromatography was performed with CombiFlash® Companion system (Teledyne Isco, Inc.). The products were characterized by their NMR and MS spectra. ^{1}H NMR spectra were obtained on Varian Mercury-300 or Varian Mercury-400 spectrometers. Low- and high-resolution mass spectra (LRMS and HRMS) were measured on Finnigan MAT 95 and LCQ-DE-CA mass spectrometer.

General procedure for the synthesis of M1-M3

Ethyl 2-((2-ethoxy-2-oxoethyl)amino)-2-oxoacetate M1: To a solution of ethyl 2-aminoacetate (2 g, 19.4 mmol) and diethyl oxalate (5.29 mL, 38.8 mmol) in ethanol (25 mL), was added TEA (2.68 mL, 19.4 mmol). The reaction mixture was stirred at 50°C for 4 h. After removing of the solvent, the residue was dissolved in water (40 mL), extracted with CH_2Cl_2 (DCM) (20 mL*3), dried over Na_2SO_4, filtered and concentrated to give the title compound as white solid without further purification (2.72 g, 69%); LRMS (ESI) *m/z* 204 $[M+H]^+$.



Entry	5-13 Comp.	X	R^1	R^2	EC_{50}[b] (μM)	CC_{50}[c] (μM)	TI[d]
1	**5a**	CH_2	Ph	OEt	63.37	234.02	3.69
2	**5b**	CH_2	2-FPh	OEt	252.56	>581.40	>2.30
3	**5c**	CH_2	4-FPh	OEt	88.89	323.32	3.64
4	**5d**	CH_2	2-OMePh	OEt	57.58	395.39	6.87
5	**5e**	CH_2	3-OMePh	OEt	213.17	>561.80	>2.64
6	**5f**	CH_2	4-OMePh	OEt	69.18	243.69	3.52
7	**5g**	CH_2	3,4-OMePh	OEt	288.91	>518.13	>1.79
8	**5h**	CH_2	naphthalen-1-yl	OEt	48.32	>531.91	>11.01
9	**5i**	$CH(CH_3)$	Ph	OEt	68.79	320.09	4.65
10	**6a**	NH	4-FPh	OEt	32.61	213.01	6.53
11	**6b**	NH	4-ClPh	OEt	14.59	135.46	9.28
12	**7a**	CH_2CH_2	Ph	OEt	55.91	376.18	6.73
13	**7b**	CH_2CH_2	2-FPh	OEt	44.83	148.80	3.32
14	**7c**	CH_2CH_2	3-FPh	OEt	52.26	176.42	3.38
15	**7d**	CH_2CH_2	4-FPh	OEt	**1.65**	13.16	7.98
16	**7e**	CH_2CH_2	4-ClPh	OEt	15.16	145.43	9.59
17	**7f**	CH_2CH_2	4-MePh	OEt	**2.34**	15.45	6.60
18	**7g**	CH_2CH_2	4-SO_2NH_2	OEt	136.42	173.46	1.27
19	**7h**	CH_2CH_2	4-OMePh	OEt	163.92	232.97	1.42
20	**7i**	CH_2CH_2	3,4-OMePh	OEt	**9.15**	33.52	3.66
21	**7j**	CH_2CH_2	indol-3-yl	OEt	**9.37**	26.73	2.85
22	**8a**	CH_2CH_2	4-MePh	NH(4-FBn)	40.37	164.60	4.08
23	**8b**	CH_2CH_2	4-MePh	NH(2-OMeBn)	32.70	215.82	6.60
24	**8c**	CH_2CH_2	4-MePh	$NHCH_2CH(CH_2CH_2)$	36.41	306.17	8.41
25	**8d**	CH_2CH_2	4-FPh	NH(4-FBn)	**5.70**	30.76	5.40
26	**8e**	CH_2CH_2	4-FPh	NH(2-OMeBn)	63.01	224.25	3.56
27	**8f**	CH_2CH_2	3,4-OMePh	NH(2-OMeBn)	149.25	217.70	1.46
28	**9a**	CH_2CO	4-BrPh	OEt	54.27	>464.04	>8.55
29	**9b**	$CH_2CH(CH_3)$	Ph	OEt	25.14	146.10	5.81
30	**9c**	$CH_2CH(Ph)$	Ph	OEt	**3.10**	25.22	8.14
31	**9d**	$CH(COOMe)CH_2$	Ph	OEt	185.55	346.88	1.87
32	**10**	$CH_2CH_2CH_2$	Ph	OEt	59.15	206.41	3.49
33	**11**	1,2,3,4-tetrahydronaphthalen-1-yl		OEt	36.64	132.92	3.63
34	**12**	propyl		OEt	352.81	>719.42	>2.03
35	**13**	^{t}-butyl		OEt	119.38	>684.93	>5.74
36	**AZT**[e]	-	-	-	0.0085	3779	444588

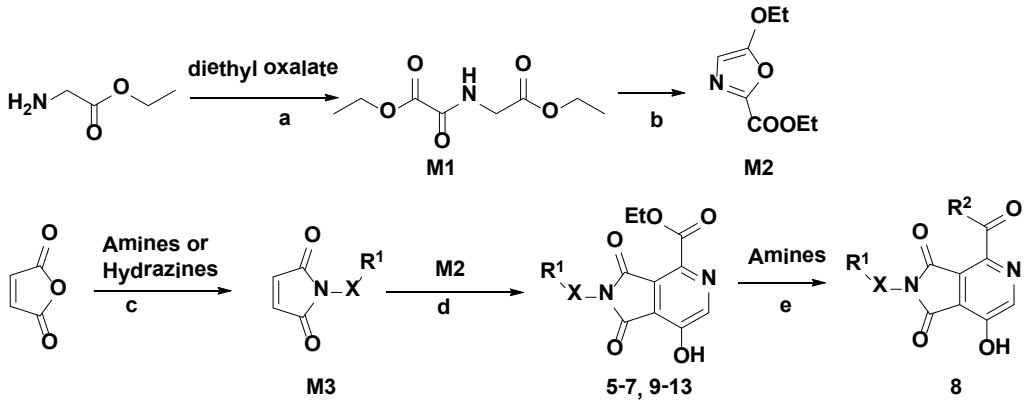
[a]Values are means of two separate experiments.
[b]CC_{50} (50% cytotoxic concentration), concentration of drug that causes 50%reduction in total C8166 cell number.
[c]EC_{50} (50% effective concentration), concentration of drug that reduces syncytiaformation by 50%.
[d]*In vitro* therapeutic index (CC_{50} value/EC_{50} value).
[e]AZT was used as a positive control.

Table 1: Anti-HIV activity of compounds **5-13** *in vitro*[a].

Ethyl 5-ethoxy-l,3-oxazole-2-carboxylate M2: To a solution of P_2O_5 (9.4 g, 67.0 mmol) in acetonitrile (50 mL) stirred under N_2 at 0°C, was added **M1** (2.72 g, 13.4 mmol) dropwise during 30 min. The reaction mixture was heated to 70°C for 4 h. Then the reaction was quenched with saturated brine, extracted with ethyl acetate (EtOAc) (20 mL*3), the combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (4:1, Petro Ether(PE)-EtOAc) to afford the title compound as a white solid (1.72 g, 47.8%); ^{1}H-NMR(300 MHz, $CDCl_3$) δ 1.41 (t, 3H, *J* = 5.1 Hz), 1.47 (t, *J* = 5.4 Hz, 3H), 4.25(m, 2H), 4.41(m,2H),6.34(s,1H);LC-MS(ESI)*m/z*186[M+H]$^+$.

General procedures for preparations of **M3** (**M3-1**–**35**) are described as those for 1-benzyl-1*H*-pyrrole-2,5-dione **M3-1.**

In a 100 mL three-necked flask provided with a stirrer, a reflux condenser, and a dropping funnel were placed 0.98 g (10 mmol) of maleic anhydride and 25 mL of diethyl ether. The maleic anhydride dissolved upon stirring, at which point a solution of 1 equiv (10 mmol) of benzylamine in 5 mL of diethyl ether was run through the dropping funnel. The resulting thick suspension was stirred at room temperature for 1 h and was then cooled in an ice bath, filtered and dried. Subsequently, the residue was added to a flask containing a solution of anhydrous sodium acetate (0.33 g, 4 mmol) in acetic anhydride (5 mL)



Scheme 1: Synthetic Procedures. (a) Et_3N/EtOH; (b) P_2O_5, CH_3CN, N_2; (c) NaOAc, $(Ac)_2O$, Et_2O, reflux; (d) H_2O, microwave, 100°C, 30min; (e) CH_3CN.

and stirred reflux for 30 min. The reaction mixture was then cooled to room temperature in a cold water bath and was then poured into 30 mL of an ice-water mixture. The precipitated product was recovered by filtration, washed three times with 10 mL portions of ice-cold water, and dried as white solid (1.50g, 80%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.30 (m, 5H), 6.72 (s, 2H), 4.69 (s, 2H); MS (EI, m/z) 187 $[M]^+$; HRMS (EI) m/z calcd $C_{11}H_9NO_2$ $[M]^+$ 187.0633, found 187.0638.

1-(2-fluorobenzyl)-1*H*-pyrrole-2,5-dione **M3-2.** Pale yellow solid (1.52 g, 74%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30-7.25 (m, 2H), 7.12-7.04 (m, 2H), 6.74 (s, 2H), 4.77 (s, 2H); MS (EI, m/z) 205 $[M]^+$; HRMS (EI) m/z calcd $C_{11}H_8FNO_2$ $[M]^+$ 205.0539, found 205.0542.

1-(4-fluorobenzyl)-1*H*-pyrrole-2,5-dione **M3-3.** Pale yellow solid (1.48 g, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.32 (m, 2H), 7.03-6.97 (t, *J* = 8.7 Hz, 2H), 6.72 (s, 2H), 4.65 (s, 2H); MS (EI, m/z) 205 $[M]^+$; HRMS (EI) m/z calcd $C_{11}H_8FNO_2$ $[M]^+$ 205.0539, found 205.0538.

1-(2-methoxybenzyl)-1*H*-pyrrole-2,5-dione **M3-4**. Pale yellow solid (1.65 g, 76%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25-7.22 (m, 1H), 7.12-7.09 (m, 1H), 6.92-6.85 (m, 2H), 6.73 (s, 2H), 4.74 (s, 2H), 3.84 (s, 3H); MS (EI, m/z) 217 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{11}NO_3$ $[M]^+$ 217.0739, found 217.0734.

1-(3-methoxybenzyl)-1*H*-pyrrole-2,5-dione **M3-5**. Pale yellow solid (1.52 g, 70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26-7.21 (t, *J* = 8.1 Hz, 1H), 6.94-6.80 (m, 3H), 6.72 (s, 2H), 4.66 (s, 2H), 3.79 (s, 3H); MS (EI, m/z) 217 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{11}NO_3$ $[M]^+$ 217.0739, found 217.0740.

1-(4-methoxybenzyl)-1*H*-pyrrole-2,5-dione **M3-6**. Pale yellow solid (1.65 g, 76%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.29 (d, *J* = 9.0 Hz, 2H), 6.86-6.83 (d, *J* = 8.7 Hz, 2H), 6.69 (s, 2H), 4.62 (s, 2H), 3.78 (s, 3H); MS (EI, m/z) 217 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{11}NO_3$ $[M]^+$ 217.0739, found 217.0733.

1-(3,4-dimethoxybenzyl)-1*H*-pyrrole-2,5-dione **M3-7**. Pale yellow solid (1.75 g, 71%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94-6.91 (d, *J* = 8.4 Hz, 2H), 6.81-6.78 (d, *J* = 8.1 Hz, 1H), 6.70 (s, 2H), 4.61 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H); MS (EI, m/z) 247 $[M]^+$; HRMS (EI) m/z calcd $C_{13}H_{13}NO_4$ $[M]^+$ 247.0845, found 247.0844.

1-(naphthalen-1-ylmethyl)-1*H*-pyrrole-2,5-dione **M3-8**. Yellow solid (1.61 g, 68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28-8.25 (d, *J* = 8.1 Hz, 1H), 7.89-7.79 (m, 2H), 7.61-7.41 (m, 4H), 6.71 (s, 2H), 5.16 (s, 2H); MS (EI, m/z) 237 $[M]^+$; HRMS (EI) m/z calcd $C_{15}H_{11}NO_2$ $[M]^+$ 237.0790, found 237.0787.

1-(1-phenylethyl)-1*H*-pyrrole-2,5-dione **M3-9**. Yellow solid (1.25 g, 62%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44-7.41 (m, 2H), 7.36-7.27 (m, 3H), 6.64 (s, 2H), 5.40-5.33 (q, 1H), 1.85-1.82 (d, *J* = 7.2 Hz, 3H); MS (EI, m/z) 201 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{11}NO_2$ $[M]^+$ 201.0790, found 201.0792.

1-((4-fluorophenyl) amino)-1*H*-pyrrole-2,5-dione **M3-10**. Yellow solid (1.07 g, 52%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98-6.93 (m, 2H), 6.82 (s, 2H), 6.77-6.73 (m, 2H); MS (EI, m/z) 206 $[M]^+$; HRMS (EI) m/z calcd $C_{10}H_7FN_2O_2$ $[M]^+$ 206.0492, found 206.0495.

1-((4-chlorophenyl)amino)-1*H*-pyrrole-2,5-dione **M3-11**. Yellow solid (1.22 g, 55%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22-7.19 (m, 2H), 6.83 (s, 2H), 6.40-6.31 (m, 2H); MS (EI, m/z) 222 $[M]^+$; HRMS (EI) m/z calcd $C_{10}H_7ClN_2O_2$ $[M]^+$ 222.0196, found 222.0201.

1-phenethyl-1*H*-pyrrole-2,5-dione **M3-12**. Pale yellow solid (1.69 g, 84%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.19 (m, 5H), 3.79-3.75 (t, *J* = 7.5 Hz, 2H), 2.93-2.88 (t, *J* = 7.5 Hz, 2H); MS (EI, m/z) 201 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{11}NO_2$ $[M]^+$ 201.0790, found 201.0781.

1-(2-fluorophenethyl)-1*H*-pyrrole-2,5-dione **M3-13**. Pale yellow solid (1.73 g, 79%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23-7.12 (m, 2H), 7.07-6.99 (m, 2H), 6.66 (s, 2H), 3.83-3.77 (t, *J* = 7.2 Hz, 2H), 2.98-2.93 (t, *J* = 6.9 Hz, 2H); MS (EI, m/z) 219 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{10}FNO_2$ $[M]^+$ 219.0696, found 219.0693.

1-(3-fluorophenethyl)-1*H*-pyrrole-2,5-dione **M3-14**. Pale yellow solid (1.80 g, 82%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.22 (m, 1H), 6.99-6.89 (m, 3H), 6.68 (s, 2H), 3.79-3.74 (t, *J* = 7.5 Hz, 2H), 2.93-2.88 (t, *J* = 7.5 Hz, 2H); MS (EI, m/z) 219 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{10}FNO_2$ $[M]^+$ 219.0696, found 219.0691.

1-(4-fluorophenethyl)-1*H*-pyrrole-2,5-dione **M3-15**. Pale yellow solid (1.80 g, 82%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18-7.13 (m, 2H), 7.00-6.94 (m, 2H), 6.66 (s, 2H), 3.77-3.72 (t, *J* = 7.2 Hz, 2H), 2.91-2.88 (t, *J* = 7.2 Hz, 2H); MS (EI, m/z) 219 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{10}FNO_2$ $[M]^+$ 219.0696, found 219.0690.

1-(4-chlorophenethyl)-1*H*-pyrrole-2,5-dione **M3-16**. Pale yellow solid (1.69 g, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27-7.24 (d, *J* = 8.4 Hz, 2H), 7.15-7.12 (d, *J* = 8.4 Hz, 2H), 6.67 (s, 2H), 3.77-3.72 (t, *J* = 7.5 Hz, 2H), 2.91-2.86 (t, *J* = 7.5 Hz, 2H); MS (EI, m/z) 235 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{10}ClNO_2$ $[M]^+$ 235.0400, found 235.0398.

1-(4-methylphenethyl)-1*H*-pyrrole-2,5-dione **M3-17**. Pale yellow solid (1.74 g, 81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (s, 4H), 6.66 (s, 2H), 3.77-3.72 (t, *J* = 7.8 Hz, 2H), 2.89-2.84 (t, *J* = 7.8 Hz, 2H), 2.32 (s, 3H); MS (EI, m/z) 215 $[M]^+$; HRMS (EI) m/z calcd $C_{13}H_{13}NO_2$ $[M]^+$ 215.0946, found 215.0942.

4-(2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl) benzenesulfonamide **M3-18**. Yellow solid (1.51 g, 54%); ^{1}H NMR (300 MHz, CD_3OD) δ 7.81-7.78 (d, *J* = 8.1 Hz, 2H), 7.37-7.34 (d, *J* = 8.1 Hz, 2H), 6.76 (s, 2H), 3.79-3.74 (t, *J* = 7.5 Hz, 2H), 3.00-2.95 (t, *J* = 7.8 Hz, 2H); MS (EI, m/z) 280 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_{12}N_2O_4S$ $[M]^+$ 280.0518, found 280.0519.

1-(4-methoxyphenethyl)-1*H*-pyrrole-2,5-dione **M3-19**. Pale yellow solid (1.69 g, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13-7.10 (d, *J* = 8.4 Hz, 2H), 6.84-6.81 (d, *J* = 9.0 Hz, 2H), 6.66 (s, 2H), 3.79 (s, 3H), 3.75-3.70 (t, *J* = 7.5 Hz, 2H), 2.89-2.82 (t, *J* = 7.5 Hz, 2H); MS (EI, m/z) 231 $[M]^+$; HRMS (EI) m/z calcd $C_{13}H_{13}NO_3$ $[M]^+$ 231.0895, found 231.0893.

1-(3,4-dimethoxyphenethyl)-1*H*-pyrrole-2,5-dione **M3-20**. Yellow solid (1.75 g, 67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78-6.71 (m, 3H), 6.66 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.77-3.72 (t, *J* = 7.5 Hz, 2H), 2.88-2.83 (t, *J* = 7.8 Hz, 2H); MS (EI, m/z) 261 $[M]^+$; HRMS (EI) m/z calcd $C_{14}H_{15}NO_4$ $[M]^+$ 261.1001, found 261.0992.

1-(2-(1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2,5-dione **M3-21**. Pale yellow solid (1.68 g, 70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69-7.66 (d, *J* = 7.5 Hz, 1H), 7.37-7.34 (d, *J* = 7.8 Hz, 2H), 7.21-7.14 (m, 2H), 7.06-7.05 (br, 1H), 6.66 (s, 2H), 3.87-3.82 (t, *J* = 7.5 Hz, 2H), 3.10-3.05 (t, *J* = 7.5 Hz, 2H); MS (EI, m/z) 240 $[M]^+$; HRMS (EI) m/z calcd $C_{14}H_{12}N_2O_2$ $[M]^+$ 240.0899, found 240.0903.

1-(2-(4-bromophenyl)-2-oxoethyl)-1*H*-pyrrole-2,5-dione **M3-22**. Yellow solid (1.23 g, 42%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85-7.82 (d, *J* = 8.4 Hz, 2H), 7.68-7.65 (d, *J* = 8.4 Hz, 2H), 6.84 (s, 2H), 4.92 (s, 2H); MS (EI, m/z) 293 $[M]^+$; HRMS (EI) m/z calcd $C_{12}H_8BrNO_3$ $[M]^+$ 292.9688, found 292.9691.

1-(2-phenylpropyl)-1*H*-pyrrole-2,5-dione **M3-23**. Yellow solid (1.08 g, 50%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31-7.08 (m, 5H), 6.61 (s, 2H), 3.74-3.58 (m, 2H), 3.27-3.22 (m, 1H), 1.28-1.26 (d, *J* = 6.9 Hz, 2H); MS (EI, m/z) 215 $[M]^+$; HRMS (EI) m/z calcd $C_{13}H_{13}NO_2$ $[M]^+$ 215.0946, found 215.0941.

1-(2,2-diphenylethyl)-1*H*-pyrrole-2,5-dione **M3-24**. Pale yellow solid (1.80 g, 65%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.20 (m, 10H), 6.55 (s, 2H), 4.64-4.58 (t, *J* = 8.7 Hz, 1H), 4.16-4.13 (d, *J* = 7.6 Hz, 2H); MS (EI, m/z) 277 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{15}NO_2$ $[M]^+$ 277.1103, found 277.1108.

methyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-phenylpropanoate **M3-25**. Pale yellow solid (1.58 g, 61%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27-7.21 (m, 5H), 6.60 (s, 2H), 4.99-4.94 (m, 1H), 3.79 (s, 3H), 3.50-3.43 (m, 2H); MS (EI, m/z) 259 $[M]^+$; HRMS (EI) m/z calcd $C_{14}H_{13}NO_4$ $[M]^+$ 259.0845, found 259.0843.

1-(3-phenylpropyl)-1*H*-pyrrole-2,5-dione **M3-26**. White solid (1.59 g, 74%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28-7.26 (m, 2H), 7.20-7.17 (m, 3H), 6.66 (s, 2H), 3.61-3.56 (t, *J* = 7.5 Hz, 2H), 2.66-2.61 (t, *J* = 7.5 Hz, 2H), 2.00-1.90 (m, 2H); MS (EI, m/z) 215 $[M]^+$; HRMS (EI) m/z calcd $C_{13}H_{13}NO_2$ $[M]^+$ 215.0946, found 215.0945.

1-(1,2,3,4-tetrahydronaphthalen-1-yl)-1*H*-pyrrole-2,5-dione **M3-27**. Pale yellow solid (1.41 g, 62%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16-7.08 (m, 3H), 6.88-6.85 (d, *J* = 7.8 Hz, 1H), 6.71 (s, 2H), 5.38-5.33 (m, 1H), 3.04-2.93 (m, 1H), 2.84-2.76 (m, 1H), 2.35-2.23 (m, 1H), 2.11-2.00 (m, 2H), 1.84-1.79 (m, 1H); MS (EI, m/z) 227 $[M]^+$; HRMS (EI) m/z calcd $C_{14}H_{13}NO_2$ $[M]^+$ 227.0946, found 227.0941.

1-propyl-1*H*-pyrrole-2,5-dione **M3-28**. Colorless oil (1.03 g, 74%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 2H), 3.52-3.47 (t, *J* = 6.9 Hz, 2H), 1.66-1.58 (m, 2H), 0.93-0.88 (t, *J* = 7.8 Hz, 2H); MS (EI, m/z) 139 $[M]^+$; HRMS (EI) m/z calcd $C_7H_9NO_2$ $[M]^+$ 139.0633, found 139.0634.

1-*t*-butyl-1*H*-pyrrole-2,5-dione **M3-29**. Colorless oil (1.04 g, 68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (s, 2H), 1.58 (s, 9H); MS (EI, m/z) 153 $[M]^+$; HRMS (EI) m/z calcd $C_8H_{11}NO_2$ $[M]^+$ 153.0790, found 153.0793.

General procedures for preparations of **5-7**, **9-13** are described as those for ethyl 2-benzyl-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **5a.**

Compound **M2** (100 mg, 0.54 mmol) and compound **M3-1** (123 mg, 0.54 mmol) were dissolved in 4 mL water. The vial was sealed and the mixture was irradiated for 30 min at 100°C in the microwave reactor. The precipitated product was recovered by filtration, dried, and dissolved in DCM. The title compound was afford by chromatography (20:1, DCM-MeOH) as a yellow solid (130 mg, 74%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.13 (s, 1H), 7.33-7.18 (m, 5H), 4.70 (s, 2H), 4.43-4.36 (dd, *J* = 6.9 and 14.1 Hz, 2H), 1.43-1.38 (t, *J* = 6.9 Hz, 3H); MS (ESI, m/z) 325 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{17}H_{13}N_2O_5$ $[M-H]^+$ 325.0824, found 325.0842.

Ethyl 2-(2-fluorobenzyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine -4-carboxylate **5b.** Yellow solid (124 mg, 67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.43-7.37 (m, 1H), 7.34-7.27 (m, 1H), 7.14-7.07 (m, 2H), 4.93 (s, 2H), 4.56-4.49 (q, 2H), 1.48-1.43 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 344 $[M]^+$; HRMS (EI) m/z calcd $C_{17}H_{13}FN_2O_5$ $[M]^+$ 344.0808, found 344.0792.

Ethyl 2-(4-fluorobenzyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine -4-carboxylate **5c.** Yellow solid (132 mg, 71%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.12 (s, 1H), 7.37-7.33 (m, 2H), 7.02-6.96 (t, *J* = 7.8 Hz, 2H), 4.72 (s, 2H), 4.42-4.39 (dd, *J* = 7.5 and 14.4 Hz, 2H), 1.42-1.37 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 343 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{17}H_{12}FN_2O_5$ $[M-H]^+$ 343.0730, found 343.0733.

Ethyl 2-(2-methoxybenzyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyri- dine-4-carboxylate **5d.** Yellow solid (131 mg, 68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.31-7.25 (m, 2H), 6.94-6.86 (m, 2H), 4.90 (s, 2H), 4.56-4.48 (q, 2H), 3.84 (s, 3H), 1.48-1.43 (t, *J* = 6.9 Hz, 3H); MS (EI, m/z) 356 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{16}N_2O_6$ $[M]^+$ 356.1008, found 356.1000.

Ethyl 2-(3-methoxybenzyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyri- dine-4-carboxylate **5e.** Yellow solid (144 mg, 75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.26-7.23 (m, 1H), 7.02-6.96 (m, 2H), 6.86-6.83 (m, 1H), 4.81 (s, 2H), 4.56-4.49 (q, 2H), 3.80 (s, 3H), 1.48-1.44 (t, *J* = 6.9 Hz, 3H); MS (EI, m/z) 356 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{16}N_2O_6$ $[M]^+$ 356.1008, found 356.1006.

Ethyl 2-(4-methoxybenzyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyri- dine-4-carboxylate **5f.** Yellow solid (135 mg, 70%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.16 (s, 1H), 7.16-7.14 (d, *J* = 6.9 Hz, 2H), 6.74-6.71 (d, *J* = 7.5 Hz, 2H), 4.62 (s, 2H), 4.42-4.36 (dd, *J* = 7.5 and 14.4 Hz, 2H), 3.72 (s, 3H), 1.43-1.38 (t, *J* = 6.9 Hz, 3H); MS (ESI, m/z) 355 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{18}H_{15}N_2O_6$ $[M-H]^+$ 355.0930, found 355.0938.

Ethyl 2-(3,4-dimethoxybenzyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*] pyridine-4-carboxylate **5g.** Yellow solid (110 mg, 53%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.03-6.99 (m, 2H), 6.82-6.79 (m, 1H), 4.77 (s, 2H), 4.56-4.49 (q, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 1.48-1.44 (t, *J* = 6.9 Hz, 3H); MS (EI, m/z) 386 $[M]^+$; HRMS (EI) m/z calcd $C_{19}H_{18}N_2O_7$ $[M]^+$ 386.1114, found 386.1120.

Ethyl 2-(naphthalen-1-ylmethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*] pyridine-4-carboxylate **5h.** Yellow solid (128 mg, 63%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.34-8.32 (d, *J* = 8.4 Hz, 1H), 7.89-7.83 (m, 2H), 7.66-7.43 (m, 4H), 5.32 (s, 2H), 4.56-4.49 (q, 2H), 1.48-

1.43 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 376 $[M]^+$; HRMS (EI) m/z calcd $C_{21}H_{16}N_2O_5$ $[M]^+$ 376.1059, found 376.1062.

Ethyl 2-(1-phenylethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine- 4-carboxylate **5i.** Yellow solid (92 mg, 50%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.51-7.48 (m, 2H), 7.35-7.27 (m, 3H), 5.58-5.51 (q, 1H), 4.56-4.48 (q, 2H), 1.94-1.92 (d, *J* = 7.2 Hz, 3H), 1.48-1.44 (t, *J* = 6.9 Hz, 3H); MS (EI, m/z) 340 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{16}N_2O_5$ $[M]^+$ 340.1059, found 340.1052.

Ethyl 2-((4-fluorophenyl)amino)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*] pyridine-4-carboxylate **6a.** Yellow solid (129 mg, 69%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 1H), 6.97-6.88 (m, 4H), 4.55-4.48 (q, 2H), 1.47-1.42 (t, *J* = 6.9 Hz, 3H); MS (EI, m/z) 345 $[M]^+$; HRMS (EI) m/z calcd $C_{16}H_{12}FN_3O_5$ $[M]^+$ 345.0761, found 345.0758.

Ethyl 2-((4-chlorophenyl)amino)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*] pyridine-4-carboxylate **6b.** Yellow solid (125 mg, 64%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.25-7.22 (d, *J* = 9.0 Hz, 2H), 6.81-6.78 (d, *J* = 9.0 Hz, 2H), 4.93 (s, 2H), 4.55-4.48 (q, 2H), 1.47-1.42 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 361 $[M]^+$; HRMS (EI) m/z calcd $C_{16}H_{12}ClN_3O_5$ $[M]^+$ 361.0465, found 361.0460.

Ethyl 2-phenethyl-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo [3,4-*c*]pyridine-4-carboxylate **7a.** Yellow solid (138 mg, 75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.30-7.22 (m, 5H), 4.57-4.50 (q, 2H), 3.96-3.91 (t, *J* = 7.2 Hz, 2H), 3.02-2.97 (t, *J* = 7.2 Hz, 2H), 1.50-1.45 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 340 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{16}N_2O_5$ $[M]^+$ 340.1059, found 340.1069.

Ethyl 2-(2-fluorophenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7b.** Yellow solid (151 mg, 78%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H), 7.19-7.17 (m, 2H), 7.05-6.99 (m, 2H), 4.40-4.33 (dd, *J* = 7.2 and 13.8 Hz, 2H), 3.86-3.81 (t, *J* = 6.9 Hz, 2H), 3.00-2.95 (t, *J* = 6.9 Hz, 2H), 1.40-1.35 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 357 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{18}H_{14}FN_2O_5$ $[M-H]^+$ 357.0887, found 357.0887.

Ethyl 2-(3-fluorophenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7c.** Yellow solid (145 mg, 75%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.14 (s, 1H), 7.25-7.20 (m, 1H), 7.01-6.89 (m, 3H), 4.41-4.34 (dd, *J* = 7.8 and 14.1 Hz, 2H), 3.84-3.79 (t, *J* = 7.5 Hz, 2H), 2.96-2.91 (t, *J* = 7.5 Hz, 2H), 1.41-1.37 (t, *J* = 6.9 Hz, 3H); MS (ESI, m/z) 357 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{18}H_{14}FN_2O_5$ $[M-H]^+$ 357.0887, found 357.0897.

Ethyl 2-(4-fluorophenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7d.** Yellow solid (130 mg, 67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.22-7.17 (m, 2H), 7.02-6.96 (m, 2H), 4.57-4.50 (q, 2H), 3.93-3.88 (t, *J* = 7.8 Hz, 2H), 3.00-2.95 (t, *J* = 7.2 Hz, 2H), 1.49-1.44 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 358 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{15}FN_2O_5$ $[M]^+$ 358.0965, found 358.0957.

Ethyl 2-(4-chlorophenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7e.** Yellow solid (150 mg, 74%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H), 7.25-7.16 (m, 4H), 4.40-4.33 (dd, *J* = 7.2 and 14.1 Hz, 2H), 3.82-3.77 (t, *J* = 6.9 Hz, 2H), 2.93-2.88 (t, *J* = 7.5 Hz, 2H), 1.41-1.36 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 373 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{18}H_{14}ClN_2O_5$ $[M-H]^+$ 373.0591, found 373.0584.

Ethyl 2-(4-methyphenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7f.** Yellow solid (155 mg, 81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.06-7.02 (m, 4H), 4.46-4.39 (q, 2H), 3.85-3.80 (t, *J* = 7.5 Hz, 2H), 2.92-2.87 (t, *J* = 7.8 Hz, 2H), 2.26 (s, 3H), 1.42-1.37 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 354 $[M]^+$; HRMS (EI) m/z calcd $C_{19}H_{18}N_2O_5$ $[M]^+$ 354.1216, found 354.1220.

Ethyl 2-(4-sulfamoylphenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7g.** Yellow solid (174 mg, 77%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.07 (s, 1H), 7.80-7.77 (d, *J* = 8.1 Hz, 2H), 7.41-7.39 (d, *J* = 8.4 Hz, 2H), 4.40-4.33 (dd, *J* = 6.9 and 14.1 Hz, 2H), 3.88-3.83 (t, *J* = 8.1 Hz, 2H), 3.06-3.01 (t, *J* = 6.9 Hz, 2H), 1.41-1.36 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 418 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{18}H_{16}N_3O_7S$ $[M-H]^+$ 418.0709, found 418.0730.

Ethyl 2-(4-methoxyphenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7h.** Yellow solid (146 mg, 73%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.16 (s, 1H), 7.10-7.07 (d, *J* = 8.1 Hz, 2H), 6.80-6.77 (d, *J* = 8.1 Hz, 2H), 4.42-4.35 (dd, *J* = 6.9 and 14.1 Hz, 2H), 3.79-3.72 (m, 5H), 2.86-2.81 (t, *J* = 7.5 Hz, 2H), 1.41-1.37 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 369 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{19}H_{17}N_2O_6$ $[M-H]^+$ 369.1087, found 369.1100.

Ethyl 2-(3,4-dimethoxyphenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4- *c*]pyridine-4-carboxylate **7i.** Yellow solid (171 mg, 79%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 6.77-6.75 (m, 3H), 4.57-4.49 (q, 2H), 3.94-3.89 (t, *J* = 7.5 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 2.97-2.92 (t, *J* = 7.8 Hz, 2H), 1.49-1.44 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 400 $[M]^+$; HRMS (EI) m/z calcd $C_{20}H_{20}N_2O_7$ $[M]^+$ 400.1271, found 400.1281.

Ethyl 2-(2-(1*H*-indol-3-yl)ethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **7j.** Yellow solid (149 mg, 73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.71-7.68 (d, *J* = 8.4 Hz, 1H), 7.27 (s, 1H), 7.18-7.13 (m, 3H), 4.58-4.51 (q, 2H), 4.04-3.99 (t, *J* = 7.5 Hz, 2H), 3.19-3.15 (t, *J* = 7.5 Hz, 2H), 1.50-1.45 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 379 $[M]^+$; HRMS (EI) m/z calcd $C_{20}H_{17}N_3O_5$ $[M]^+$ 379.1168, found 379.1171.

Ethyl 2-(2-(4-bromophenyl)-2-oxoethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **9a.** Yellow solid (138 mg, 59%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.88-7.85 (d, *J* = 8.7 Hz, 2H), 7.71-7.68 (d, *J* = 8.7 Hz, 2H), 5.08 (s, 2H), 4.56-4.48 (q, 2H), 1.47-1.43 (t, *J* = 6.9 Hz, 3H); MS (EI, m/z) 432 $[M]^+$; HRMS (EI) m/z calcd $C_{18}H_{13}BrN_2O_6$ $[M]^+$ 431.9957, found 431.9964.

Ethyl 2-(2-phenylpropyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **9b.** Yellow solid (140 mg, 73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.31-7.21 (m, 5H), 4.56-4.48 (q, 2H), 3.90-3.74 (m, 2H), 3.37-3.29 (m, 1H), 1.48-1.43 (t, *J* = 6.9 Hz, 3H), 1.33-1.31 (d, *J* = 8.1 Hz, 3H); MS (EI, m/z) 354 $[M]^+$; HRMS (EI) m/z calcd $C_{19}H_{18}N_2O_5$ $[M]^+$ 354.1216, found 354.1210.

Ethyl 2-(2,2-diphenylethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **9c.** Yellow solid (175 mg, 78%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.30-7.19 (m, 10H), 4.74-4.68 (t, *J* = 8.1 Hz, 1H), 4.55-4.48 (q, 2H), 4.32-4.29 (d, *J* = 8.4 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 2.97-2.92 (t, *J* = 7.8 Hz, 2H), 1.49-1.44 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 416 $[M]^+$; HRMS (EI) m/z calcd $C_{24}H_{20}N_2O_5$ $[M]^+$ 416.1372, found 416.1368.

Ethyl 2-(1-methoxy-1-oxo-3-phenylpropan-2-yl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **9d.** Yellow solid (148 mg, 69%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.26-7.14 (m, 5H), 5.17-5.12 (m, 1H), 4.53-4.46 (q, 2H), 3.81 (s, 3H), 3.63-3.47 (m, 2H), 1.47-1.42 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 398 $[M]^+$; HRMS (EI) m/z calcd $C_{20}H_{18}N_2O_7$ $[M]^+$ 398.1114, found 398.1115.

Ethyl 2-(3-phenylpropyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **10.** Yellow solid (145 mg, 76%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.24 (s, 1H), 7.20-7.02 (m, 5H), 4.45-4.38 (dd, *J* = 6.9 and 14.1 Hz, 2H), 3.66-3.61 (m, 2H), 2.64-2.57 (m,

2H), 1.98-1.90 (m, 2H), 1.43-1.39 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 353 $[M+H]^+$; HRMS (ESI) m/z calcd $C_{19}H_{17}N_2O_5$ $[M-H]^+$ 353.1137, found 353.1146.

Ethyl 2-(1,2,3,4-tetrahydronaphthalen-1-yl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **11.** Yellow solid (134 mg, 68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.19-7.07 (m, 3H), 6.95-6.92 (d, *J* = 4.5 Hz, 1H), 5.57-5.52 (m, 1H), 4.56-4.49 (q, 2H), 3.03-2.79 (m, 2H), 2.40-2.29 (m, 1H), 2.17-2.05 (m, 2H), 1.91-1.82 (m, 1H), 1.48-1.44 (t, *J* = 7.2 Hz, 3H); MS (EI, m/z) 366 $[M]^+$; HRMS (EI) m/z calcd $C_{20}H_{18}N_2O_5$ $[M]^+$ 366.1216, found 366.1218.

Ethyl 2-propyl-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **12.** Yellow solid (99 mg, 66%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H), 4.42-4.35 (dd, *J* = 7.5 and 14.4 Hz, 2H), 3.57-3.52 (t, *J* = 7.5 Hz, 2H), 1.67-1.60 (m, 2H), 1.42-1.37 (t, *J* = 7.2 Hz, 3H), 0.93-0.88 (t, *J* = 7.5 Hz, 3H); MS (ESI, m/z) 277 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{13}H_{13}N_2O_5$ $[M-H]^+$ 277.0824, found 277.0815.

Ethyl 2-^{t}butyl-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate **13.** Yellow solid (100 mg, 63%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.05 (s, 1H), 4.41-4.34 (dd, *J* = 7.2 and 14.1 Hz, 2H), 1.64 (s, 9H), 1.41-1.36 (t, *J* = 7.2 Hz, 3H); MS (ESI, m/z) 291 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{14}H_{16}N_2O_5$ $[M-H]^+$ 292.1059, found 292.1063.

General procedures for preparations of **8** are described as those for *N*-(4-fluorobenzyl)-7-hydroxy-2-(4-methylphenethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxamide **8a.**

Compound **7f** (50 mg, 0.14 mmol) and (4-fluorophenyl) methanamine (21 mg, 0.17 mmol) were dissolved in 10 mL EtOH. The reaction mixture was refluxed for 5 h. After removing of the solvent, the residue was dissolved in DCM (20 mL), washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The title compound was afford by chromatography (20:1, DCM-MeOH) as a yellow solid (44 mg, 73%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.52 (s, 1H), 7.14-7.00 (m, 8H), 4.57 (s, 2H), 3.71-3.68 (m, 2H), 3.05-3.08 (m, 2H), 2.28 (s, 3H); MS (EI, m/z) 433 $[M]^+$; HRMS (EI) m/z calcd $C_{24}H_{20}FN_3O_4$ $[M]^+$ 433.1438, found 433.1431.

N-(2-methoxybenzyl)-7-hydroxy-2-(4-methylphenethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxamide **8b**. Yellow solid (31 mg, 50%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.56 (s, 1H), 7.14-7.03 (m, 8H), 4.58 (s, 2H), 3.83 (s, 3H), 3.69-3.65 (t, *J* = 6.9 Hz, 2H), 3.94-2.89 (t, *J* = 7.2 Hz, 2H), 2.28 (s, 3H); MS (EI, m/z) 445 $[M]^+$; HRMS (EI) m/z calcd $C_{25}H_{23}N_3O_5$ $[M]^+$ 445.1638, found 445.1632.

N-(cyclopropylmethyl)-7-hydroxy-2-(4-methylphenethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxamide **8c**. Yellow solid (32 mg, 61%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.56 (s, 1H), 7.18-7.08 (m, 4H), 3.73-3.68 (t, *J* = 6.9 Hz, 2H), 3.56-3.53 (d, *J* = 6.9 Hz, 2H), 2.97-2.92 (t, *J* = 6.9 Hz, 2H), 2.25 (s, 3H), 1.31-1.28 (m, 1H), 0.55-0.50 (m, 2H), 0.40-0.35 (m, 2H); MS (EI, m/z) 379 $[M]^+$; HRMS (EI) m/z calcd $C_{21}H_{21}N_3O_4$ $[M]^+$ 379.1532, found 379.1528.

N-(4-fluorobenzyl)-2-(4-fluorophenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxamide **8d**. Yellow solid (41 mg, 67%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.56 (s, 1H), 7.36-7.33 (m, 2H), 7.22-7.17 (m, 2H), 7.07-6.95 (m, 4H), 4.84 (s, 2H), 3.69-3.65 (t, *J* = 6.9 Hz, 2H), 2.86-2.81 (t, *J* = 7.5 Hz, 2H); MS (ESI, m/z) 436 $[M-H]^+$; HRMS (ESI) m/z calcd $C_{23}H_{16}F_2N_3O_4$ $[M-H]^+$ 436.1109, found 436.1118.

N-(2-methoxybenzyl)-2-(4-fluorophenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxamide **8e**. Yellow solid (33 mg, 53%); ^{1}H NMR (300 MHz, CD_3OD) δ 7.91 (s, 1H), 7.24-7.15 (m, 3H), 7.02-6.87 (m, 4H), 6.73-6.68 (m, 1H), 4.57 (s, 2H), 3.91-3.82 (m, 5H), 2.94-2.92 (t, *J* = 7.2 Hz, 2H); MS (EI, m/z) 449 $[M]^+$; HRMS (EI) m/z calcd $C_{24}H_{20}FN_3O_5$ $[M]^+$ 449.1387, found 449.1395.

N-(2-methoxybenzyl)-2-(3,4-dimethoxyphenethyl)-7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxamide **8f**. Yellow solid (33 mg, 48%); ^{1}H NMR (300 MHz, d_6-DMSO) δ 8.55 (s, 1H), 7.29-7.24 (m, 1H), 7.07-7.01 (m, 2H), 6.90-6.74 (m, 4H), 4.72 (s, 2H), 3.82 (s, 3H), 3.69 (s, 6H), 3.49-3.43 (m, 2H), 2.79-2.74 (t, *J* = 7.5 Hz, 2H); MS (EI, m/z) 491 $[M]^+$; HRMS (EI) m/z calcd $C_{26}H_{25}N_3O_7$ $[M]^+$ 491.1693, found 491.1694.

Anti-HIV-1 evaluation

Target compounds were evaluated for their inhibitory activities against HIV-1 replication in acutely infected C8166 cells *in vitro* according to the previously described method [24,25]; AZT was used as a positive control.

Cytotoxicty assay: The cytotoxicty assay was performed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) method. Briefly, 100μl 4×10^4 C8166 cells were seeded each well with 100 μl different concentrations compounds in 96-well plate. The plate was incubated 72 hours at 37℃, 5% CO_2 incubator. 20 μl MTT (5 mg/ml) was added each well and the plate was incubated at 37℃ for 4 h. 100 μl supernatant was discarded and 100 μL 20% SDS-50% DMF was added. The plates were read at 570/630 nm by Bio-Tek Elx 800 reader. The 50% cytotoxic concentration (CC_{50}) was calculated.

Syncytia inhibition assay: The syncytia inhibition assay was applied by counting syncytia which were formed by HIV-1_{IIIB} infected C8166 cells. Briefly, HIV-1_{IIIB} supernatant and C8166 cells were seeded in 96-well plate with different concentration of compounds. The plate was incubated in a 37℃, 5% CO_2 incubator for 72 hours. Syncytia were counting under an inverted microscope and 50% effective concentration (EC_{50}) was calculated. Therapeutic index (TI) was calculated by the ratio of CC_{50}/EC_{50}.

Results

In total, 35 new compounds (**5**–**13**) were designed and synthesized whose chemical structures are shown in Table 1. These compounds were synthesized through the routes outlined in Scheme 1, and all the compounds were purified by silica gel thin-layer chromatography (>95%), and their structures were determined by ^{1}H-NMR and low- and high-resolution mass spectra.

The assay results of the target compounds are presented in Table 1, expressed as EC_{50}, CC_{50} and TI values. Among all the target compounds, **7d**, **7f**, **7i**-**7j**, **8d** and **9c** exhibited potent anti-HIV-1 activities (EC_{50} < 10 μM). Compound **7d** showed the most potent anti-HIV activity, with EC_{50} values of 1.65 μM.

Discussion

The structure–activity relationships (SARs) in these novel compounds were investigated by increasing the length of the linker X between N-2 and the side chain phenyl ring R^1, introducing substituents on the terminal aryl ring R^1, and changing R^2 from an ester group to an amide group. We initiated our work by extending the chain length between N-2 and substituting group R^1. As shown in Table 1, the length of the linker appears to be is important for the compounds' biological activities against HIV-1, appending an ethylidene and hydrazine linker could offer a better anti-HIV-1 activity (**6a** *vs.* **5c**, **7b** *vs.* **5b**, **7d** *vs.* **5c**, **7i** *vs.* **5g**), compared with that produced by introducing a methylene linker. The substitution style of the R^1 groups is also has a key impact on

anti-HIV-1 activities. Compared with the unsubstituted compound **7a**, introduction of a sulfamoyl (**7g**) and methoxyl (**7h**) in the 4-position of phenyl group decreased the anti-HIV-1 activities, whereas fluorine (**7d**), chlorine (**7e**) and methyl (**7f**) groups enhanced the antiviral potency against HIV-1. In particular, the 4-F-substituted compound **7d** displayed the most potent biological activity among the nine analogs (EC_{50} = 1.65 μM). However, introducing a fluorine atom into the 2- or 3-position of the phenyl group (**7b** and **7c**) had a negative impact on HIV-1 inhibition. Although the 4-methoxyl substituent showed poor anti-HIV-1 potency (**7h**), compound **7i** with a 3,4-dimethoxyl group showed an improved inhibitory profile, with an EC_{50} value of 9.15 μM. Interestingly, when we replaced the terminal benzene group with an indole ring (**7j**), good anti-HIV-1 activities were obtained (EC_{50} = 9.37 μM).

Encouraged by the improved potency of compounds **7d, 7f** and **7i-7j**, we continued our structural optimization effort by introducing some amino groups into the R^2 position (**8**). Although most of the tested compounds exhibited poor inhibitory potency against HIV-1, good biological activity was observed when 4-F-benzylamine was introduced into the molecule (**8d**, EC_{50} = 5.7 μM).

Subsequently, we have screened a diversity of linkers, and found that introducing bulky groups into the linker enhances the biological activities. In particular, a bulky phenyl substituent in the linker caused a large improvement in anti-HIV-1 activity (**9c** *vs* **7a**), with an IC_{50} value of 3.10 μM. We further lengthened the linker and introduced less flexible groups to explore the biological activities toward HIV-1. However, these strategies resulted in a slight decrease in the biological activity against HIV-1 (**10** *vs.* **7a** and **11** *vs.* **7a**, respectively). Finally, as exemplified by compounds **12** and **13**, replacement of the benzene ring with adipic groups led to loss of potency.

Conclusion

In summary, we designed and synthesized a series of 7-hydroxy-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-4-carboxylate derivatives (**5-13**) that exhibited anti-HIV-1 activities *in vitro*. Among the analogs, compounds **7d**, **7f**, **7i-7j**, **8d** and **9c** exhibited potent anti-HIV-1 activities (EC_{50}<10 μM). In particular, **7d** exhibited significant anti-HIV-1 activities with EC_{50} values of 1.65 μM. On the basis of this work, it would be possible to keep the ethylidene linker and 4-F-substituted phenyl group and systematically replace the other fragments with functional isosteres, potentially leading to more potent and less toxic compounds. The preliminary SAR among the newly synthesized analogs provided useful indications for guiding further rational design of potent anti-HIV-1 agents.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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