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Synthesis and evaluation of *N*-aryl pyrrolidinones as novel anti-HIV-1 agents. Part 1

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Abstract—The synthesis and preliminary structure–activity relationship of a series of pyrrolidinones are described. These pyrrolidinones have been characterized as novel non-nucleoside reverse transcriptase inhibitors (NNRTIs) which are highly potent against wild-type and drug-resistant human immunodeficiency viruses (HIV-1).
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Highly active antiretroviral therapy (HAART) combination regimens have dramatically decreased the morbidity and mortality among patients with HIV infections.[1] Four main classes of antiretroviral drugs are currently available for the treatment of HIV infection. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have become the key components in the combination regimens. NNRTIs bind to an allosteric site of reverse transcriptase (RT) in a noncompetitive manner which causes distortion of the three-dimensional structure of the enzyme and inhibits its catalytic function.[2] Currently, three NNRTIs (see Fig. 1) have been approved for the treatment of HIV infection, namely nevirapine (**1**),[3] delavirdine (**2**),[4] and efavirenz (**3**).[5] Due to the low genetic barrier to resistance for these marketed NNRTIs, the rapid emergence of resistance to NNRTIs, and cross resistance within this class, has become a major drawback in the clinic for NNRTIs. As the reverse transcriptase is essential to the life cycle of the virus, many research activities in this field have been focused on searching for novel NNRTIs with high potency against wild-type (WT) and drug-resistant viruses.

We have initiated a program to identify novel small molecule inhibitors of HIV infection by screening our in-house library using a cell-based HIV infection inhibition assay.[6] Approximately 230,000 compounds were screened and several lead scaffolds were discovered with anti-HIV activity. Among these lead scaffolds, a series of *N*-aryl pyrrolidinones represented by **4** (Fig. 2) were identified as novel NNRTIs. For the convenience of discussion, the rings of this compound are designated as ring A, B, C, and D as described in Figure 2. This compound showed an EC_{50} of 125 nM against WT HIV reporter virus in the 293T target cells.[6] It also has an IC_{50} of 2 μM in a RT inhibition enzymatic assay. Efavirenz showed an IC_{50} of 0.8 μM in the same ELISA. A systematic SAR study is then undertaken and the preliminary results are reported herein.

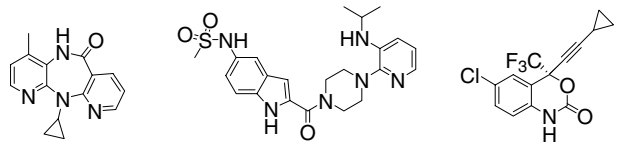

Figure 1. Currently approved NNRTIs.

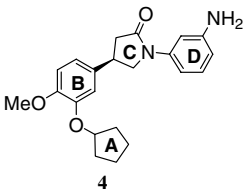

Figure 2. Structure of HTS hit **4**.

Keywords: Antiviral; HIV; NNRTI; Pyrrolidinone.
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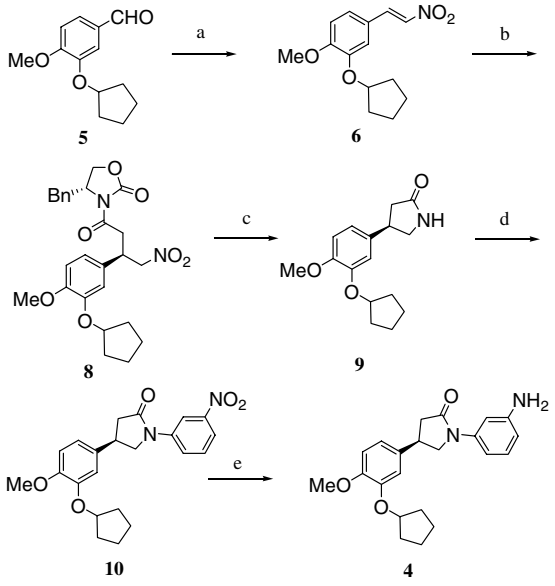
The synthesis of hit **4** is shown in Scheme 1. Following the literature protocol,[7] condensation of aldehyde **5** with nitromethane gave nitro olefin **6**. Michael addition of oxazolidone **7** to olefin **6** afforded **8** in 75% yield and 98% ee after a single re-crystallization. Reduction of the nitro group followed by intramolecular cyclization provided lactam **9** in 88% yield. Lactam **9** was coupled with 3-iodo-nitrobenzene catalyzed by CuI[8] in the presence of a diamine as ligand, and the resulting product **10** was subjected to hydrogenation to furnish the desired product **4**.

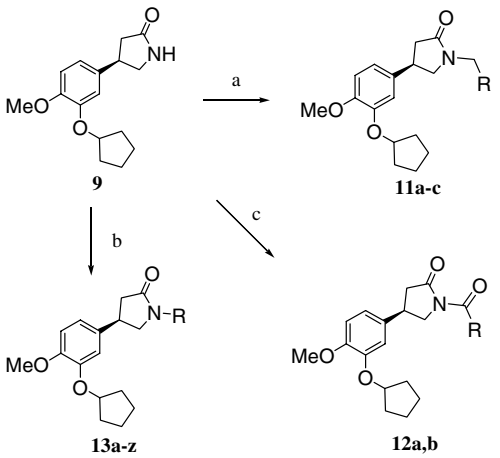
In order to explore the SAR of D ring, different groups were attached to lactam **9** (Scheme 2). Direct alkylation of **9** with substituted benzyl bromides gave *N*-alkylated analogs **11a**–**c**. Simple *N*-acyl analogs **12a** and **12b** were made by treating lactam **9** with acyl chlorides in pyridine. Following the N-arylation protocol for the preparation of **10**, a series of *N*-aryl analogs **13a**–**z** were synthesized.



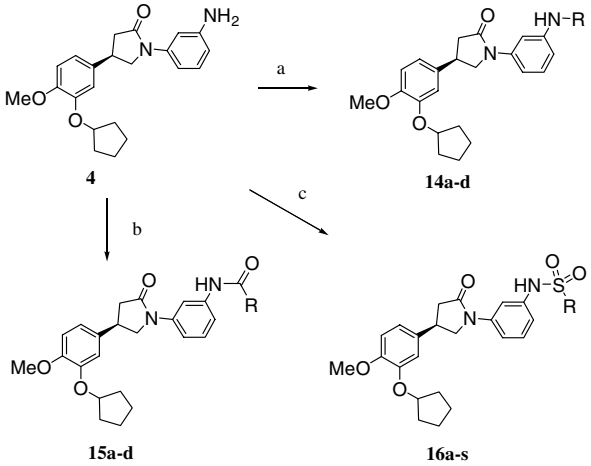
Scheme 1. Reagents and conditions: (a) AcOH, CH_3NO_2, NH_4OAc, reflux 2 h, 45%; (b) (*R*)-3-acetyl-4-benzyl-oxazolidin-2-one (**7**), LDA, THF, −78 °C, then **6**, 75%; (c) EtOH, AcOEt, Raney Nickel, H_2, 88%; (d) 3-iodo-nitrobenzene, CuI, K_3PO_4, 1,2-cyclohexanediamine, DMF, 110 °C, 12 h, 92%; (e) 10% Pd/C, EtOH, H_2, 86%.

To further define the SAR around the aniline group as well as substitutions on aromatic D ring, additional amine derivatives of **4** were made for biological evaluation (Scheme 3). Thus, reductive amination of **4** with different aldehydes provided amines **14a**–**d**. Direct coupling of **4** with different acyl chlorides in pyridine afforded amides **15a**–**d**. Finally, treatment of **4** with substituted sulfonyl chlorides in pyridine gave a series of sulfonamides **16a**–**s**. All final products were purified either by normal phase silica gel column chromatography or reverse-phase preparative LC–MS.[9] All analogs that passed QC by analytical LC–MS were tested in the biological assays.

The antiviral activities of these analogs were evaluated by a cell-based single cycle replication assay.[6] In general, this series of analogs did not show cytotoxicity up to 10 μM.[10] Table 1 summarizes the antiviral activities of analogs **4**, **11a**–**c**, **12a**,**b**, and **13a**–**z**. The antiviral activity of **4** was confirmed to possess an EC_{50} of 125 nM, and nevirapine (NVP) was included for comparison. As shown in Table 1, replacement of the aromatic D ring with simple alkyl groups gave analogs **11a**–**c** which exhibited no antiviral activity up to a 10 μM concentration. Likewise, simple acyl analogs **12a**,**b** and the *N*-des-aryl analog **13a** are not active. Therefore, further efforts were then focused on the modification of the aromatic D ring. To assess the contribution of the amino group to the antiviral activity, analogs **13b**–**j** were evaluated. It is clear that this amino group plays a critical role in the antiviral activity of the pyrrolidinones. This was supported by the fact that the simple removal of the amino group resulted in analog **13b** with 20-fold reduction in the antiviral activity. At the same time, the *N*-methyl analog **13d** showed an approximately 40-fold loss in activity. This indicated that the amino



Scheme 2. Reagents and conditions: (a) RX, NaH, DMF, 50–76%; (b) RX, K_3PO_4, CuI, DMF, 1,2-cyclohexanediamine, 110 °C, 75–92%; (c) RCOCl, DMAP, Py, 66–74%.



Scheme 3. Reagents and conditions: (a) RCHO, DMF, $NaB(O_2CCH_3)_3H$, 62–90%; (b) RCOCl, Py, 82–91%; (c) RSO_2Cl, Py, 86–92%.

Table 1. Antiviral activity of **6**, **11**, **12** and **13** against WT HIV-1 virus[6]

Compound	R	WT-EC_{50} (μM)	CC_{50} (μM)
4	3-Amino-phenyl	0.125	>10
11a	3-Cyano-phenyl	>10	>10
11b	3-Amino-phenyl	>10	>10
11c	3-Nitro-phenyl	>10	>10
12a	Methyl	>10	>10
12b	Phenyl	>10	>10
13a	H	>10	>10
13b	Phenyl	2.280	>10
13c	3-Hydroxy-phenyl	1.780	>10
13d	3-Dimethylamino-phenyl	4.710	>10
13e	3,5-Diamino-phenyl	>10	>10
13f	3-Amino-4-methyl-phenyl	0.196	>10
13g	3-Amino-2-methyl-phenyl	>10	>10
13h	2-Amino-phenyl	>10	>10
13i	4-Amino-phenyl	1.860	>10
13j	3-Amino-5-fluoro-phenyl	0.080	>10
13k	2-Cyano-phenyl	>10	>10
13l	3-Cyano-phenyl	0.520	>10
13m	4-Cyano-phenyl	0.660	>10
13n	2-Methyl-phenyl	>10	>10
13o	3-Methyl-phenyl	0.419	>10
13p	4-Methyl-phenyl	3.260	>10
13q	2-Trifluoromethyl-phenyl	>10	>10
13r	3-Trifluoromethyl-phenyl	2.950	>10
13s	4-Trifluoromethyl-phenyl	3.620	>10
13t	2-Pyridyl	2.630	>10
13u	3-Pyridyl	2.370	>10
13v	4-Pyridyl	0.11	>10
13w	3-Methanesulfinyl-phenyl	>10	>10
13x	4-Methanesulfinyl-phenyl	0.040	7.51
13y	3-Methanesulfonyl-phenyl	>10	>10
13z	4-Methanesulfonyl-phenyl	0.180	>10
NVP[a]		0.050	>10

[a] NVP, nevirapine.

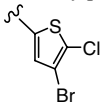
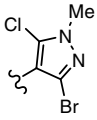
group likely makes an H-bond interaction with amino acid residues inside the binding pocket and functions as an H-bond donor. For comparison, the corresponding phenol analog **13c** showed much lower antiviral activity. The diamino analog (**13e**) showed diminished antiviral activity, probably due to the interference of the other amino group on binding to the enzyme. Based on this result, substituted aromatic D ring analogs with an amino group were studied in detail. Surprisingly, analogs **13f** and **13g** showed a dramatic difference in their antiviral activity. The 2-Me analog **13g** lost its antiviral activity completely, while its positional isomer **13f** possessed similar antiviral activity compared to **4**. To further explore the SAR in this region, analogs **13h–s** were tested. It was found that a substitution at the position 2 abolished the antiviral activity as observed for **13h**, **13k**, **13n**, and **13q**. All these analogs were inactive against HIV replication up to 10 μM concentration. Analogs possessing substituents at the 3 or 4 position showed low micromolar to sub-micromolar antiviral activities (**13i**, **13j**, **13l**, **13m**, **13o**, **13p**, **13r**, and **13s**). It is most likely that a substituent at the 2 position changes the conformation between the C and D rings which led to the diminished antiviral activity.

Further exploration of the SAR in this region led to interesting results. Analogs possessing an H-bond acceptor at the 4 position of D ring, including analogs **13v**, **13x**, and **13z**, demonstrated good antiviral activity. Shifting the H-bond acceptor to a different position resulted in a loss of activity by more than 20-fold (**13t**, **13u**, **13w**, and **13y**), supporting the notion that specific H-bond interactions in this region play a critical role.

Aniline derivatives were also evaluated (Table 2). As indicated in Table 2, simple *N*-alkyl analogs of aniline **14a–d** showed decreased activity, while N-acylation was somewhat tolerated. Fortuitously, the sulfonamide analogs displayed a range of antiviral activity (**16a–s**). In this series, many aryl sulfonamides showed improved antiviral activity. In general, substitutions at the 2 or 3 position were well tolerated and many analogs exhibited improved antiviral activity as indicated by analogs **16a**, **16e–j**, and **16l–q** which displayed EC_{50}'s in the low nanomolar range. Some heterocyclic sulfonamides (**16r** and **16s**) also showed good antiviral activity.

Analogs with low nanomolar EC_{50}'s were tested against a panel of mutant viruses. The results are presented in Table 3. NVP and efavirenz (EFV) were included for comparison. NNRTI-specific mutant viruses K103N, Y181C, L100I, and K103N/L100I were selected for testing based on their prevalence in patients failing the

Table 2. Antiviral activity of **14**, **15** and **16** against WT HIV-1 virus[6]

Compound	R	WT-EC_{50} (μM)	CC_{50} (μM)
14a	Tetrahydrofuran-3-yl-methyl	1.024	>10
14b	Furan-3-yl-methyl	1.963	>10
14c	3-Phenyl propyl	7.902	>10
14d	Butyl	6.960	>10
15a	3-Methoxy benzoyl	0.502	>10
15b	3-Dimethylamino-benzoyl	0.124	>10
15c	Thiophene-2-carboxyl	0.502	>10
15d	Acetyl	5.786	>10
16a	3-Methoxyphenyl	0.025	>10
16b	4-Methoxyphenyl	0.256	>10
16c	3-Trifluoromethoxyphenyl	0.800	>10
16d	2,5-Dimethoxyphenyl	0.104	7.587
16e	2-Methoxy-5-methyl-phenyl	0.033	>10
16f	2-Methyl-3-chloro-phenyl	0.020	>10
16g	2-Chlorophenyl	0.042	>10
16h	3-Chlorophenyl	0.025	>10
16i	2-Fluorophenyl	0.064	>10
16j	3-Fluorophenyl	0.043	>10
16k	4-Fluorophenyl	0.102	>10
16l	2,6-Difluorophenyl	0.048	>10
16m	2-Bromophenyl	0.042	>10
16n	3-Bromophenyl	0.071	>10
16o	3-Trifluorophenyl	0.067	>10
16p	2-Methylphenyl	0.024	>10
16q	3-Methylphenyl	0.046	>10
16r		0.046	>10
16s		0.053	>10
NVP[a]		0.050	>10

[a] NVP, nevirapine.

Table 3. Antiviral activity of selected pyrrolidinones against mutant HIV-1 viruses

Compound	WT (μM)	K103N (μM)	Y181C (μM)	L100I (μM)	K103N/L100I (μM)
16a	0.025	0.656 (26)[a]	0.990 (40)	0.033 (1.3)	0.076 (3)
16e	0.033	0.172 (5.2)	0.507 (15)	0.023 (0.7)	0.033 (1)
16f	0.020	0.162 (8.1)	0.389 (19)	0.008 (0.4)	0.010 (0.5)
16g	0.042	0.170 (4)	0.557 (13)	0.019 (0.45)	0.028 (0.67)
16h	0.025	0.689 (27)	2.320 (93)	0.040 (1.6)	0.056 (2.2)
16i	0.064	0.269 (4.2)	1.496 (21)	0.034 (0.53)	0.041 (0.64)
16j	0.043	0.261 (6)	0.684 (16)	0.020 (0.46)	0.026 (0.6)
16l	0.048	0.451 (9.4)	1.305 (27)	0.068 (1.4)	0.052 (1)
16m	0.042	0.292 (6.9)	0.794 (19)	0.016 (0.38)	0.022 (0.52)
16n	0.071	0.923 (13)	2.263 (32)	0.053 (0.74)	0.083 (1.1)
16o	0.067	1.157 (17)	2.163 (32)	0.091 (1.3)	0.148 (2.2)
16p	0.024	0.117 (4.9)	0.246 (10)	0.010 (0.42)	0.017 (0.7)
16q	0.046	0.158 (3.4)	0.284 (6.2)	0.014 (0.3)	0.019 (0.41)
16r	0.046	0.542 (12)	0.753 (16)	0.039 (0.84)	0.095 (2)
NVP[b]	0.050	5.053 (101)	>10 (>200)	0.164 (3.2)	4.386 (88)
EFV[c]	0.0005	0.032 (64)	0.001 (2)	0.010 (20)	2.526 (5000)

[a] The numbers in parentheses are the fold changes compared to wild type.
[b] NVP, nevirapine.
[c] EFV, efavirenz.

NNRTI-containing regimen. In general, with the exception of **16a**, **16h** and **16o**, these compounds only suffered moderate loss of activity against K103N, L100I and K103N/L100I mutant viruses compared to NVP and EFV. Most of the compounds lost significant activity (10- to 90-fold) against the Y181C mutant virus. However, **16q** was only 6-fold less potent against the Y181C virus while remaining much of its activity against the other mutant viruses. For comparison, NVP suffered a greater loss in potency against 4 of 5 mutant viruses while EFV exhibited a greater than 5000-fold loss in potency against the K103N/L100I double mutant virus (Table 3).

In summary, a novel class of NNRTI was discovered with potent antiviral activity. The SAR of the D ring region was established. This class of pyrrolidinones demonstrated distinct antiviral activity profiles against mutant viruses. In particular, it is worthy of note that analogs in this class showed potent antiviral activity against the K103N and K103N/L100I mutant viruses, which are prevalent in patients failing NNRTI regimens. Further investigations shall be focused on improving the antiviral activities of this class of compounds against WT virus as well as additional drug-resistant viruses.

References and notes

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6. HEK 293T cells are routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 1× Pen/Strep/Glutamine. The protocol is as follows: 293T cells are seeded in the 1536-well format at 700 cells/well (5 μL volume) using an Aquamax (Molecular Devices) liquid dispenser. Cells are cultured at 37 °C under 5% CO_2 for 24 h. 50 nL of each compound (serially diluted in DMSO) are transferred using the PinTool (GNF). After a 1 h at 37 °C incubation, HIV reporter virus is transferred to the cells using the Aquamax in a volume of 2 μL corresponding to a multiplicity of infection (MOI) of approximately 1.0. The treated and infected cells are cultured for an additional 48 h at 37 °C. Luciferase activity is monitored by addition of Bright-Glo (Promega, Cat. # E263B and E264B) luciferase reagent (5 μL/well, Aquamax) followed by plate reading on the CLIPR apparatus (Molecular Devices) using a 20 s shuttle speed.
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9. Analytic data of **16a**: ^{1}H NMR ($CDCl_3$): δ 7.70 (1H, br s), 7.37 (1H, d, J = 7.8 Hz), 7.19–7.29 (4H, m), 7.00 (2H, t, J = 7.6 Hz), 6.83 (1H, d, J = 8 Hz), 6.79 (1H, s), 6.79 (1H, d, J = 8 Hz), 4.76 (1H, br s), 4.11 (1H, t, J = 8.4 Hz), 3.83 (3H, s), 3.78 (1H, t, J = 8.4 Hz), 3.60 (1H, m), 3.02 (1H, q, J = 8.1 Hz), 2.85 (1H, q, J = 8.1 Hz), 1.61–1.88 (8H, m) ppm; LCMS *m/z*: 537.20 $[M+H]^+$.
10. 293T cells are seeded in the 1536-well format at 700 cells/well (5 μL volume) using an Aquamax (Molecular Devices) liquid dispenser. Cells are cultured at 37 °C under 5% CO_2 for 24 h. 50 nL of each compound (serially diluted in DMSO) is transferred using the PinTool (GNF). The treated and uninfected cells are cultured for an additional 48 h at 37°C. Cell viability is assessed by addition of 1 μL of Alamar Blue (Promega, Cat.# 00-100) diluted 1:1 in DMEM. Cells are further incubated for 4 h at room temperature and subsequent fluorescence intensity is read using an Acquest (TREK systems) with a 50/50 beam splitter.