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Design, synthesis, and biological evaluations of novel quinolones as HIV-1 non-nucleoside reverse transcriptase inhibitors

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Abstract—A novel series of quinolones was discovered as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) using a structure-based approach. The lead quinolones exhibited single digit nanomolar potency in the HIV-1 replication assays. The preliminary SAR of these quinolones was also established via systematic structural modifications. These novel and potent quinolones could serve as advanced leads for further optimization.

HIV-1 reverse transcriptase (RT) is a key enzyme in HIV replication and is a well-established target for anti-HIV drug discovery.[1–3] Three non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been approved by FDA. They are nevirapine (Viramune®), delavirdine (Rescriptor®) and efavirenz (Sustiva®), and are key components of the combination therapy.[4–6] Since significant resistance has been developed against these drugs, there is an urgent need to develop new NNRTIs that would overcome the current drug resistance.[7] Previously, we have reported the discovery of novel NNRTIs via HTS and SAR studies.[8–11] In this letter, we wish to report our discovery of a series of quinolones as NNRTIs based on rational design.

Among the reported NNRTIs, a number of them share common structural features. For example, efavirenz (**1**), HBY-097 (**2**),[12] and oxindole **3**[8,9] all share a fused aromatic core, which contains a hydrophobic phenyl moiety, and an amide or carbamide moiety. By carefully examining these structures, we designed a quinolone scaffold (**4**). Based on molecular modeling studies, quinolone **4** fits into the NNRTI binding site and aligns nicely with efavirenz in space (Fig. 1). The quinolone core of **4** and the dihydrobenzoxazinone core of efavirenz almost overlap in the center of the binding pocket.

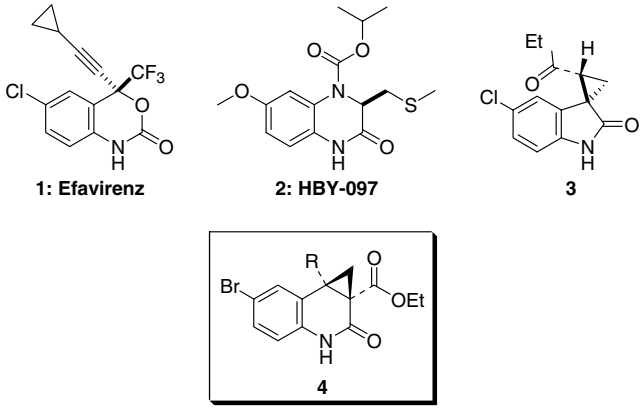

Figure 1.

The halophenyl moieties in both molecules occupy the same hydrophobic pocket. An alkyl R group in **4** and the cyclopropylethynyl group in efavirenz could occupy the same hydrophobic pocket. The cyclopropyl moiety in **4** and the trifluoromethyl group in efavirenz fill into another small hydrophobic space. Both molecules could form critical hydrogen bonds between the lactam moieties and K101 of the enzyme. The ethyl ester group in **4** extends out into the hydrophobic pocket and points to the solvent exposed regions.

Encouraged by these modeling studies, we carried out the synthesis of the designed quinolones (Scheme 1).[13] Starting from the commercially available aniline **5**, the

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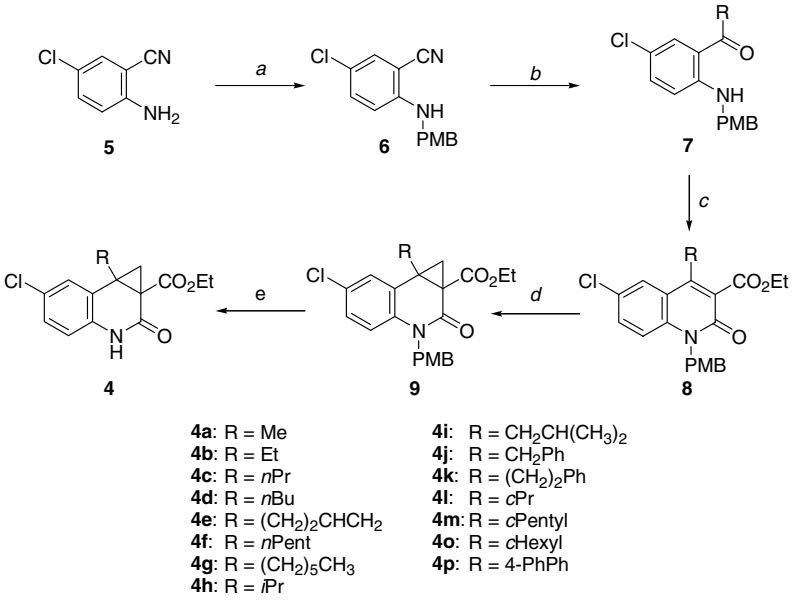


Scheme 1. Synthesis of quinolones **4a**–**4p**. Reagents and conditions: (a) PMBOH (1.5 equiv), *p*-TsOH (cat.), CH_3CN, 70 °C, 12 h, 99%; (b) RMgCl (2.0 equiv), THF, 0–25 °C, 1 h, 75–92%; (c) $ClCOCH_2CO_2Et$ (2.0 equiv), benzene, 80 °C, 4 h, 73–88%; (d) $(CH_3)_2SOCH_3I$ (2.0 equiv), NaH (3.0 equiv), DMSO, 0–90 °C, 51–78%; (e) CAN (2.0 equiv), CH_3CN/H_2O = 9:1, 0–25 °C, 12 h, 74–90%.

amino group was first protected with a PMB group, and the nitrile was then converted into a ketone by reacting with various Grignard regents. Treatment of the resulting ketone with ethyl chlorocarbonylacetate led to the formation of the quinolone core **8**. The cyclopropane moiety was installed by reacting with dimethyloxosulfonium methylide.[14] Removal of the PMB group gave rise to quinolones **4a**–**4p**. These quinolones were tested against the WT HIV-1 virus, and their antiviral activities are shown in Table 1. As suggested by molecular modeling studies, a number of these quinolones exhibited potent antiviral activity, and a clear SAR emerged. The R group prefers straight aliphatic chains, and the best R groups are *n*-butyl (**4d**), *n*-4-pentenyl (**4e**), and *n*-pentyl (**4f**), and all three compounds exhibited low single digit nanomolar potency. Either longer or shorter R groups resulted in reduced activity (**4a**–**4c**, **4g**). With the exception for the *i*-butyl analog **4i** and phenethyl analog **4k**, all other quinolones with branched alkyl, phenyl alkyl or phenyl groups (**4h**, **4j**, and **4l**–**4p**) showed poor antiviral activity. These data are consistent with the modeling studies, which suggest a narrow hydrophobic pocket in the enzyme which can interact with this region of the molecule (Fig. 2).

To explore the role of the ethyl ester moiety, a nitrile analog **13** was prepared (Scheme 2). Ketone **7d** was first converted into the corresponding cyanoquinolone **10** in excellent yield by reacting with cyanoacetyl chloride. Treatment of **10** with dimethyloxosulfonium methylide led to the isolation of the desired cyclopropane analog **11**. In addition to **11**, another product was isolated, which had a molecular weight of 14 mass units more than that of **11**. Spectroscopic studies established its structure as a cyclobutane analog **12**, which was likely due to the reaction of **11** with an additional equivalent of dimethyloxosulfonium methylide. Removal of the PMB groups in **11** and **12** led to the final products **13** and **14**, respectively. Antiviral testing indicated that both **13** and **14** lost activity significantly, representing more than a 60- and 30-fold reduction in potency, respectively (Table 1). These data demonstrate the important role of the ester moiety in the interaction with the enzyme, as well as the cyclopropane moiety in anchoring the molecule for optimal interactions.

To further explore the SAR in the ester region, a library of esters and amides (**16a**–**16y**) were prepared via the corresponding acid intermediate **15** (Scheme 3). Biological testing suggested that small esters are preferred with the methyl (**16a**) and allyl (**16b**) esters being among the most potent. The slightly larger esters, cyanoethyl (**16c**), isobutenyl (**16d**), and phenyl analogs (**16e**–**16f**), suffered a dramatic loss in antiviral activity. In the case of **16c**, the polar nitrile moiety could have resulted in unfavorable interactions with the enzyme. Replacement of the ethyl ester with a primary amide still gave a potent inhibitor (**16g**), although it suffered some loss of activity.

Consistent with the observations in the ester series, all tertiary amides, phenyl and pyridyl amides (**16q**–**16y**), showed significantly less activity. Only the 3-cyanophenyl analog **16t** displayed good antiviral potency (160 nM). The activity difference between **16s** and **16t** could be the result of the steric interactions of the ortho nitrile group in **16s** with the quinolone core that led to less favorable interactions with the enzyme. As the compounds were tested in a cell-based assay, different cell permeability of these analogs might have played a role in their reduced antiviral activity.

Alcohol **19a** and ethers **19b**–**19e** were also prepared to further understand the SAR in this region (Scheme 4). Ester **9d** was first selectively reduced to alcohol **17**,

Table 1. Anti-HIV activities of quinolones.[15]

Compound	EC_{50} (μM)	CC_{50} (μM)
Efavirenz	0.001	~10
Nevirapine	0.050	>10
2	0.002	>10
4a	>10	>10
4b	5.039	>10
4c	0.045	>10
4d	0.003	>10
4e	0.001	>10
4f	0.001	>10
4g	0.025	>10
4h	>10	>10
4i	0.057	>10
4j	>10	>10
4k	0.221	>10
4l	>10	>10
4m	3.248	>10
4n	1.462	>10
4o	>10	>10
4p	>10	>10
13	0.197	>10
14	0.102	>10
16a	0.001	>10
16b	0.001	>10
16c	0.113	>10
16d	2.361	>10
16e	>10	>10
16f	1.095	>10
16g	0.012	>10
16h	0.747	>10
16i	0.489	>10
16j	6.300	>10
16k	2.775	>10
16l	5.691	>10
16m	2.821	8.822
16n	5.205	>10
16o	>10	>10
16p	>10	>10
16q	>10	>10
16r	>10	>10
16s	1.713	6.088
16t	0.160	>10
16u	1.327	>10
16v	4.551	>10
16w	2.695	>10
16x	8.259	>10
16y	>10	>10
19a	0.743	>10
19b	0.045	>10
19c	0.065	>10
19d	0.240	>10
19e	0.037	>10
23	0.265	>10
24	0.166	>10
25	1.398	>10

which was then treated with methyl, ethyl, and allyl iodide in the presence of sodium hydride, giving rise to the corresponding PMB-protected ethers **18b**–**18d**. Standard acylation of the hydroxy group in **17** led to acetate **18e**. Deprotection of the PMB groups in **17**, **18b**–**18e** with CAN furnished the alcohol analogs **19a**–**19e**. Interestingly, most of these analogs exhibited good antiviral activity. In particular, **19b**, **19c**, and **19e** showed EC_{50}s of 45, 65, and 37 nM, respectively, which are consistent with the SAR observed for the corresponding esters.

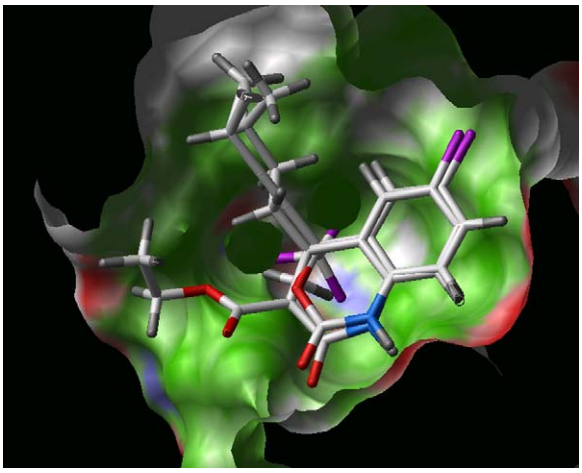


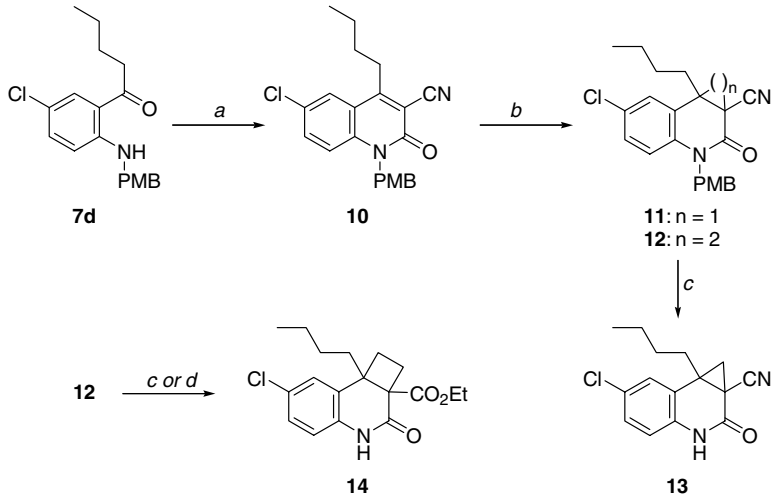
Figure 2. Docking of **4d** and efavirenz in the NNRTI binding site.[15]

These data also illustrate the importance of the ester carbonyl oxygen of **4d** in the interaction with the enzyme.

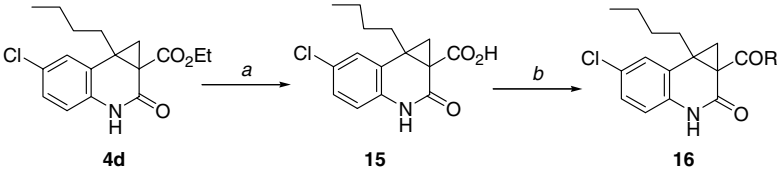
A ketone analog (**23**) of **4d** was synthesized according to Scheme 5. Hydrolysis of the ester in **9d** with lithium hydroxide gave the corresponding acid **20**, which was converted into Weinreb amide **21**. Reaction of amide **21** with propyl magnesium chloride furnished ketone **22**. Ketone **23** was obtained after the removal of the PMB group using CAN. Although **23** still exhibited good antiviral activity, its EC_{50} suffered more than 20-fold loss compared to that of **4d**, demonstrating the role of the ester oxygen in the interaction with the enzyme. Molecular modeling was carried out to understand the interaction of the ester region with the enzyme. The activity of ester (**4d**), ether (**19c**), amide (**16g**), keto (**23**), and ethylamide (**16h**) quinolones correlates quite well with their corresponding free energies of binding, which are −33.7, −28.0, −32.2, −29.8, and −24.6 kcal/mol, respectively. The q^2 value for this correlation is 0.54. The lower potency of keto-quinolone (**23**) could be due to the lack of a polar atom (oxygen in ester or nitrogen in amide) that occupies a polar sub-pocket in mostly hydrophobic binding site as identified with Sitemap, Schrodinger, LLC.[16]

Quinolones **24** and **25** were prepared to further understand the SAR in the cyclopropane region (Scheme 6). The olefinic analog **24** was conveniently synthesized by simply removing the PMB group in **8d**. Conjugate addition of lithium dimethylcuprate[17] to the double bond in **8d** followed by the removal of the PMB group furnished **25** as a mixture of two diastereoisomers in a ratio of 1:1. While **24** retained a 166 nM EC_{50} against the WT HIV virus, **25** had only micromolar potency. These data again support the important role of the cyclopropane moiety in rigidifying the molecules for optimal interactions.

A number of quinolones were assayed against a panel of NNRTI resistant mutants. These quinolones exhibited good antiviral activity against a number of these mutants, including E138K, F227L, I135V, L100I, and

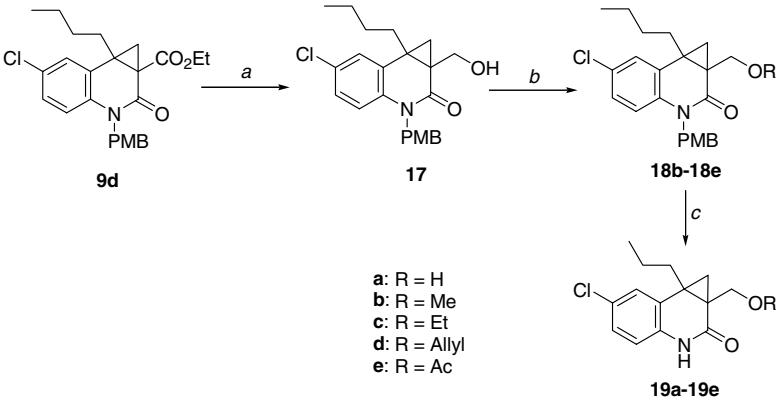


Scheme 2. Synthesis of quinolones **13** and **14**. Reagents and conditions: (a) $NCCH_2COCl$ (2.0 equiv), benzene, 80 °C, 2 h, 89%; (b) $(CH_3)_2SOCH_3I$ (3.0 equiv), NaH (4.2 equiv), DMSO, 60 °C, 12 h, 77%, **11**:**12** = 1:1; (c) **11**, CAN (2.0 equiv), CH_3CN/H_2O = 9:1, 0–25 °C, 12 h, 76% for **13**, 82% for **14**; (d) Concd H_2SO_4 (cat.), EtOH, reflux, 24 h, 47%.



16a: R = OMe
16b: R = OAllyl
16c: R = $O(CH_2)_2CN$
16d: R = OCH_2CHCMe_2
16e: R = OPh
16f: R = OPh-4-CN
16g: R = NH_2
16h: R = NHEt
16i: R = NHAllyl
16j: R = *n*Bu
16k: R = NH*i*Pr
16l: R = CH_2*c*Pr
16m: R = NHBn
16o: R = $NH(CH_2)_2CO_2H$
16p: R = $N(CH_2)_3$
16q: R = $N(CH_2)_4$
16r: R = N(Me)Et
16s: R = NHPh-2-CN
16t: R = NHPh-3-CN
16u: R = NHPh-2-$CONH_2$
16v: R = NHPh-4-$CONH_2$
16w: R = NH-2-Py
16x: R = NH-4-Py
16y: R = NHPh-4-CO_2H

Scheme 3. Synthesis of quinolones **16a**–**16y**. Reagents and conditions: (a) LiOH (3.0 N), THF/MeOH/H_2O = 3:2:1, 70 °C, 5 h, 90%; (b) EDCI (2.0 equiv), *i*Pr_2NEt (3.0 equiv), CH_2Cl_2, 25 °C, 12 h, 77–94%.

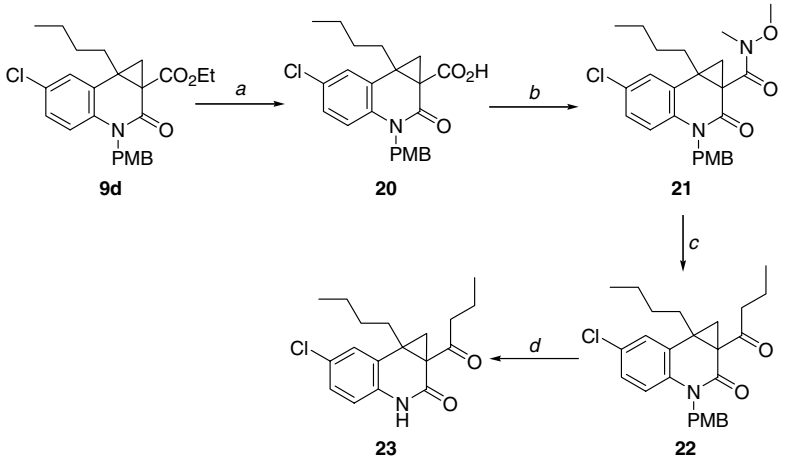


a: R = H
b: R = Me
c: R = Et
d: R = Allyl
e: R = Ac

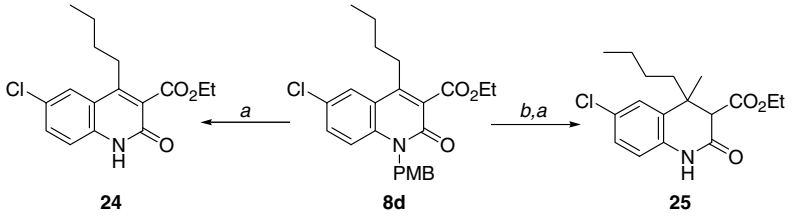
Scheme 4. Synthesis of quinolones **19a**–**19e**. Reagents and conditions: (a) LAH (1.1 equiv), THF, 0 °C, 1 h, 96%; (b) for **18b**–**18d**: RI (1.3 equiv), NaH (2.0 equiv), DMSO, 77–86%; for **18e**: Ac_2O (2.0 equiv), Et_3N (3.0 equiv), CH_2Cl_2, 0–25 °C, 1 h, 82%; (c) CAN (2.0 equiv), CH_3CN/H_2O = 9:1, 0–25 °C, 12 h, 82–91%.

Y181H. However, they showed only moderate or weak activity for the rest of the mutant viruses (Table 2).

In summary, using a structure-based approach, a series of novel quinazoline NNRTIs was designed and synthesized. SAR studies revealed the critical role of the cyclopropane moiety in positioning the substituents on the quinolone nucleus for optimal interactions with the enzyme. The ester moiety also plays an important role for the antiviral activity. Several of these quinolones exhibited potent inhibitory activity against the WT virus and showed promising activity against several NNRTI



Scheme 5. Synthesis of quinolone **23**. Reagents and conditions: (a) LiOH (3.0 N), THF/MeOH/H_2O = 3:2:1, 70 °C, 5 h, 88%; (b) $MeSO_2Cl$ (1.1 equiv), Et_3N (3.0 equiv), HN(Me)OMe, CH_2Cl_2, 0–25 °C, 1 h, 88%; (c) *n*PrMgCl (1.0 equiv), THF, 0–25 °C, 12 h, 24%; (d) CAN (2.0 equiv), CH_3CN/H_2O = 9:1, 0 °C−25 °C, 12 h, 40%.



Scheme 6. Synthesis of quinolones **24** and **25**. Reagents and conditions: (a) CAN (2.0 equiv), CH_3CN/H_2O = 9:1, 0–25 °C, 12 h, 62–71%; (b) MeLi (5.5 equiv), CuI (5.0 equiv), Et_2O, 0 °C, 5 min, then **8d**, 0–25 °C, 1 h, 53%.

Table 2. Antiviral activities (EC_{50}, μM) of selected quinolones against HIV-1 resistant mutants[15]

Compound	WT	E138K	F227L	I135V	K103N	L100I	V106A	Y181C	Y188L	V179E	Y181H	Y181S
Efavirenz	0.001	0.002	0.001	0.001	0.032	0.010	0.004	0.001	0.280	0.006	0.002	0.001
Nevirapine	0.050	0.031	0.148	0.079	5.053	0.164	8.458	>10	>10	0.116	0.112	3.001
4c	0.045	0.132	0.026	0.097	>10	0.041	1.013	>10	>10	1.889	0.210	1.213
4d	0.003	0.047	0.007	0.028	0.884	0.014	0.279	1.653	>10	0.914	0.009	0.684
4e	0.001	0.057	0.009	0.033	1.247	0.035	0.260	>10	>10	0.304	0.089	1.681
4f	0.001	0.018	0.005	0.017	0.669	0.012	0.238	0.926	>10	0.234	0.016	0.369
4g	0.025	0.098	0.041	0.066	3.075	0.053	0.878	2.249	>10	1.597	0.061	0.929
4i	0.057	0.091	0.022	0.085	>10	0.063	2.941	>10	>10	3.953	0.210	0.901
16a	0.001	0.008	>10	0.012	0.682	0.004	0.057	0.727	7.339	0.244	0.008	0.581
16b	0.001	0.032	0.001	0.023	1.081	0.001	1.220	2.945	>10	0.233	0.020	0.932
16g	0.012	0.046	0.001	0.015	3.827	0.013	2.178	7.957	>10	0.439	0.063	2.525
19b	0.045	0.042	0.020	0.046	2.916	0.033	0.726	5.029	>10	0.721	0.121	2.704
19c	0.065	0.094	0.251	0.074	3.955	0.039	1.124	6.237	>10	1.034	0.216	4.180
19e	0.037	0.054	0.001	0.064	2.618	0.045	4.725	7.463	>10	0.735	0.059	2.354

resistant mutants. These novel quinolones could serve as advanced leads for further optimizations, the goal of which will be focused on overcoming the NNRTI resistance.

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