# Design of Non-nucleoside Inhibitors of HIV-1 Reverse Transcriptase with Improved Drug Resistance Properties. 2.

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HIV-1 nonnucleoside reverse transcriptase inhibitors (NNRTIs) are part of the combination therapy currently used to treat HIV infection. The features of a new NNRTI drug for HIV treatment must include selective potent activity against both wild-type virus as well as against mutant virus that have been selected by use of current antiretroviral treatment regimens. Based on analogy with known HIV-1 NNRTI inhibitors and modeling studies utilizing the X-ray crystal structure of inhibitors bound in the HIV-1 RT, a series of substituted 2-quinolones was synthesized and evaluated as HIV-1 inhibitors.

## Introduction

In the life cycle of HIV, the causative agent of AIDS, one of the enzymes required for replication is the virally encoded human immunodeficiency virus reverse transcriptase (HIV RT). HIV RT converts single-stranded RNA into double-stranded DNA that is ultimately incorporated into the human genome.[1] The utility of HIV RT as a therapeutic target has been amply established by the availability of drugs aimed at its inhibition. Six nucleoside inhibitors (AZT, D4T, 3TC, FTC, ddI, ddC and ABC), the recently FDA-approved nucleotide, tenofovir disoproxil fumarate (TDF), and three nonnucleoside reverse transcriptase inhibitors (NNRTIs) (nevirapine,[2] delavirdine[3] and efavirenz[4]) all FDA-approved drugs for the treatment of HIV infections, exert their therapeutic efficacy by inhibiting HIV RT.

NNRTIs bind in a pocket approximately 10 Å away from the catalytic site where nucleotides bind[5] and are generally specific for HIV-1 RT. Treatment of HIV infection with NNRTIs has trailed behind nucleosides because of rapid emergence of resistant strains of virus. It was only after the success of combination therapy with nevirapine in sustaining a viral load reduction that a firm footing for NNRTIs was finally established.[6] Efavirenz[4] has set the standard for NNRTI therapy, primarily because of its favorable antiviral profiles and effectiveness in both drug-naïve and drug-experienced patients. Yet despite favorable antiviral potency against a range of relevant NNRTI mutants, clinical resistance to efavirenz eventually develops due primarily to the K103N mutation. The presence of the K103N mutant has indeed become its Achilles' heel; it is the most observed mutant in AIDS patients who failed treatment with efavirenz.[7−9]

The ready availability of X-ray crystal structures of inhibitor−HIV RT complexes, coupled with the abundance of drug resistance data,[10] has enabled us to pursue a de novo approach to drug design. One aim of the design was to study NNRTI interactions with the inhibitor binding site and to identify a common pharmacophore from the array of known structures upon which a novel chemical structure (template) could be built. Analogue synthesis would then provide an avenue for the optimization of contacts with residues around the binding pocket potentially leading to maximal effectiveness against the wild-type virus and minimal loss of potency should mutations occur. Of course, our additional aim was to identify a template that was amenable to synthesis so that we could construct a structure−activity relationship (SAR) around the molecule. As mentioned in the preceding paper,[11] the common pharmacophore identified was that of structure **A** shown in Figure 1. After consideration of several possible templates, the quinolone as shown by structure **1** was chosen, which allowed for the incorporation of the pharmacophore. Such a structure had the additional benefit of feasibility of synthesis and amenability to substituent variations.

We envisaged that an SAR could be established from modifications of the R, R′ and R″ groups in **1**. Synthesis of compounds **1a**−**d** and their subsequent antiviral results suggested that the quinolone scaffold was potentially suitable to test our design hypothesis.[11] We now report the synthesis and SAR studies of additional

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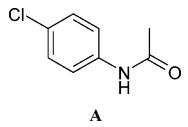


A

**Figure 1.** Common pharmacophore derived from the analysis of known NNRTIs used as a basis for template design.[11]

analogues, which culminated in the identification of a series of compounds that manifest a broad-spectrum antiviral activity against a panel of clinically relevant NNRTI resistant strains.

## Results and Discussion

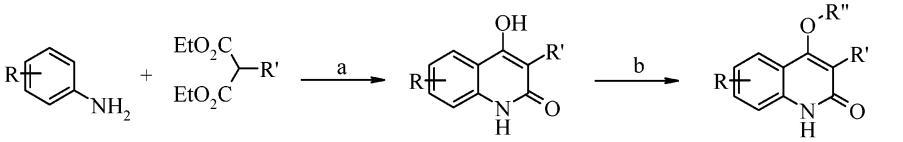
**Chemistry.** Quinolones **1a**−**h** and **1l**−**aa** were prepared according to the procedures shown in Scheme 1. Appropriately substituted anilines **2** were treated with an excess of diethyl malonate and heated in diphenyl ether to 250 °C for 24 h.[12a,b] Alkylation of the resulting quinolones **4** with alkyl halides using sodium hydride in DMF resulted in **1a**−**h, 1l**−**aa** listed in Table 1.

Compounds **1i** and **1j** were prepared according to procedures shown in Scheme 2. Methyl 5-Chloroanthranilate **5a** and methyl 5-fluoroanthranilate **5b** were first acylated with ethoxyacetyl chloride to give intermediates **6a** and **6b**.[13] Ring closure using potassium hexamethyldisilylamide in toluene gave quinolones **4q** and **4r**. This step was then followed by alkylation with cyclobutylmethyl bromide using sodium hydride as the base, resulting in **1i** and **1j**.

The synthesis of **1k** began with isatoic anhydride **7**, which was reacted with ethyl cyanoacetate **8** to give intermediate **9**. Ring closure of **9** gave quinolone **4s**, which was alkylated with cyclobutylmethyl bromide using sodium hydride as the base to give **1k**. The series of procedures are depicted in Scheme 3.

Compounds **1bb**, **1cc**, **1ee**−**hh** were prepared according to procedures shown in Scheme 4. Quinolones **4c**, **4d**, **4e**−**g** and **4t** were allowed to react with 1-bromo-2,2,2-trifluoroethane in the presence of sodium hydride

**Scheme 1**[a]



**2a** R = 4-Cl
**2b** R = 4-F
**2c** R = 4-Br
**2d** R = 7-$OCH_3$
**2e** R = 3-$NO_2$, 4-F
**2f** R = H
**2g** R = 4-$CH_3$
**2h** R = 4-$OCH_3$
**2i** R = 4-$OCF_3$
**2j** R = 4- CN

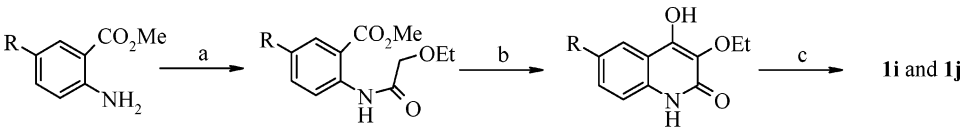
**3a** R' = $CH_2CH_2CH_3$
**3b** R' = $CH_3$
**3c** R' = $CH_2CH_3$
**3d** R' = $CH(CH_3)_2$
**3e** R' = $CH_2CH(CH_3)_2$
**3f** R' = $CH_2(CH_3)CH_2CH_3$

**4a** R = 6-Cl, R' = $CH_2CH_2CH_3$
**4b** R = 6-F, R' = $CH_3$
**4c** R = 6-F, R' = $CH_2CH_3$
**4d** R = 6-F, R' = $CH_2CH_2CH_3$
**4e** R = 6-F, R' = $CH(CH_3)_2$
**4f** R = 6-Cl, R' = $CH(CH_3)_2$
**4g** R = 7- $OCH_3$, R' = $CH_2CH_2CH_3$
**4h** R = 6-F,5-$NO_2$, R' = $CH_2CH_2CH_3$
**4i** R = H, R' = $CH_2CH_2CH_3$
**4j** R = Br, R' = $CH_2CH_2CH_3$
**4k** R = 6-Cl, R' = $CH_2CH_3$
**4l** R = 6-Cl, R' = $CH_2CH(CH_3)_2$
**4m** R = 6-Cl, R' = $CH_2(CH_3)CH_2CH_3$
**4n** R = 6-$CH_3$, R' = $CH_2CH_2CH_3$
**4o** R = 6-$OCH_3$, R' = $CH_2CH_2CH_3$
**4p** R = 6-$OCF_3$, R' = $CH_2CH_2CH_3$
**4q** R = 6-$CN_3$, R' = $CH_2CH_2CH_3$

**1a-h, 1l-1aa** (Table 1)

[a] Conditions: (a) $(Ph)_2O$, 250 °C; (b) RX, NaH, DMF, 100 °C.

**Scheme 2**[a]



**5a**, R = Cl
**5b**, R = F

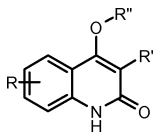
**6a**, R = Cl
**6b**, R = F

**4q**, R = Cl
**4r**, R = F

**1i** and **1j**

[a] Conditions: (a) $EtOCH_2COCl$, $Et_3N$, $CH_2Cl_2$; (b) KHMBS, toluene; (c) cyclobutyl$CH_2Br$, NaH, DMF.

**Table 1.** Antiviral Activity of Quinolinones **1a**−**hh** against Wild-Type HIV-1 and Nevirapine-Resistant Strain in the MT4 Assay



**1**

| | | | | $IC_{50}$ value (nM) vs | | |
|---|---|---|---|---|---|---|
| compd | R | R′ | R″ | HIV-1 | Nev-R | $CC_{50}/IC_{50}$ |
| **1a** | 6-Cl | *n*-propyl | cyclopropylCH$_2$ | 1100 | 8300 | >40000 |
| **1b** | 6-Cl | *n*-propyl | cyclobutyl | 470 | 2500 | >8000 |
| **1c** | 6-F | methyl | cyclobutylCH$_2$ | 990 | 3900 | >8000 |
| **1d** | 6-F | ethyl | cyclobutylCH$_2$ | 39 | 230 | >8000 |
| **1e** | 6-F | *n*-propyl | cyclobutylCH$_2$ | 69 | 190 | >8000 |
| **1f** | 6-Cl | *n*-propyl | cyclobutylCH$_2$ | 72 | 390 | >40000 |
| **1g** | 6-F | isopropyl | cyclobutylCH$_2$ | 26 | 230 | >500 |
| **1h** | 6-Cl | isopropyl | cyclobutylCH$_2$ | 29 | 230 | >500 |
| **1i** | 6-F | ethoxy | cyclobutylCH$_2$ | 470 | 1070 | >8000 |
| **1j** | 6-Cl | ethoxy | cyclobutylCH$_2$ | 290 | 2090 | >8000 |
| **1k** | H | cyano | cyclobutylCH$_2$ | >8000 | >8000 | >8000 |
| **1l** | 7-OCH$_3$ | *n*-propyl | cyclobutylCH$_2$ | >8000 | >8000 | >8000 |
| **1m** | 6-F,5-NO$_2$ | *n*-propyl | cyclobutylCH$_2$ | 1200 | 1800 | >8000 |
| **1n** | H | *n*-propyl | cyclopentyl | 380 | 3900 | >40000 |
| **1o** | 6-Br | *n*-propyl | cyclopentyl | 120 | 1700 | >40000 |
| **1p** | 6-F | *n*-propyl | cyclopentyl | 150 | 1300 | >40000 |
| **1q** | 6-Cl | *n*-propyl | cyclopentyl | 96 | 1700 | >40000 |
| **1r** | 6-Cl | ethyl | cyclopentyl | 78 | 1020 | >40000 |
| **1s** | 6-Cl | isobutyl | cyclopentyl | 52 | 1600 | >40000 |
| **1t** | 6-Cl | *sec*-butyl | cyclopentyl | 9 | 290 | >40000 |
| **1u** | 6-CH$_3$ | *n*-propyl | cyclopentyl | 970 | 6400 | >40000 |
| **1v** | 6-OCH$_3$ | *n* -propyl | cyclopentyl | 1010 | 24000 | >40000 |
| **1w** | 6-OCF$_3$ | *n* -propyl | cyclopentyl | 380 | 2500 | >40000 |
| **1x** | 6-CN | *n* -propyl | cyclopentyl | 120 | 2000 | >40000 |
| **1y** | 6-Cl | *n* -propyl | cyclohexyl | 58 | 760 | >5000 |
| **1z** | 6-Cl | isopropyl | cyclohexyl | 23 | 360 | >40000 |
| **1aa** | 6-Cl | *n* -propyl | cyclohexylCH$_2$ | 5100 | 1300 | >40000 |
| **1bb** | 6-F | ethyl | cyclopropylethynyl | 25 | 15 | >8000 |
| **1cc** | 6-F | *n*-propyl | cyclopropylethynyl | 72 | 81 | >8000 |
| **1dd** | 6-Cl | *n*-propyl | cyclopropylethynyl | 109 | 120 | >8000 |
| **1ee** | 6-F | isopropyl | cyclopropylethynyl | 29 | 25 | 16000 |
| **1ff** | 6-Cl | isopropyl | cyclopropylethynyl | 24 | 31 | >8000 |
| **1gg** | 6-F | isobutyl | cyclopropylethynyl | 150 | 120 | >8000 |
| **1hh** | 6-F | isopropyl | *sec*-butylethynyl | 17 | 32 | >6500 |

in DMF at 150 °C to give trifluoroethoxy intermediates **10a**−**e** that were then protected with *tert*-butyldimethylsilyl chloride in DMF using triethylamine to give intermediates **11**. Reaction with cyclopropylethynyllithium gave alkynes **12**.[15] Deprotection of **12** was accomplished with Dowex 50 acidic resin in a mixture of THF and methanol at room temperature providing the desired **1bb**, **1cc**, **1ee**−**gg**. Compound **1hh** was synthesized using the same procedure, except in this case, the lithium salt of 3-methylpentyne was used.

Finally, compound **1dd** was prepared according to procedures similar to those shown in Scheme 4. As shown in Scheme 5, compound **10e**, which was prepared according to the procedure described above, was protected with *p*-methoxybenzyl chloride.[16] The resultant intermediate **11** was then reacted with cyclopropylethynyllithium to give compound **12**. Deprotection with ammonium cerium nitrate gave **1dd**.[17]

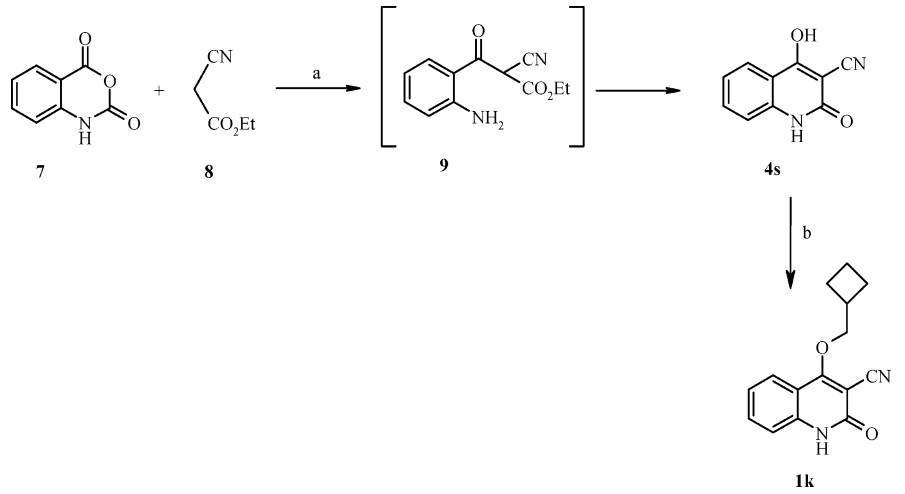
**Biology.** We decided, as an initial task, to categorize the synthesis of the quinolone analogues into four subgroups where the R″ moieties were made up of cyclopropylethynyl, cyclobutylmethyl and cyclopentyl groups (see Figure 3). As synthesis progressed, additional four substituents were investigated, although in a limited sense, which was designed to extend our understanding of the binding of these quinolone analogues. These were the cyclohexyl, *sec*-butylethynyl, cyclopropylmethyl, cyclobutyl and cyclohexylmethyl.

Within the subgroups consisting of the above substituents, variations were then made around the R and R′ groups in rings A and B, of **1**, respectively (Figure 2a). The R group included mainly the fluoro and chloro substituents. On the basis of the modeling work, the NH proton was expected to form a hydrogen bond with the backbone carbonyl group of the K103 residue, similar to that of efavirenz. Therefore, differences in the electron-withdrawing properties of the R substituents were expected to have an effect on the acidity of the NH proton and thus the strength of the hydrogen bond. As shown in Table 1, other R substituents, intended to answer certain SAR questions, were also included.

As for the R′ group, we focused mainly on the isopropyl and *n*-propyl substituents. These were the groups that were viewed to be the optimal from the design work. Other substituents were also included, primarily to confirm our understanding of the activities of the analogues having the *n*-propyl and isopropyl groups. The antiviral and enzyme inhibition data for all synthesized compounds are summarized in Tables 1 and 2.

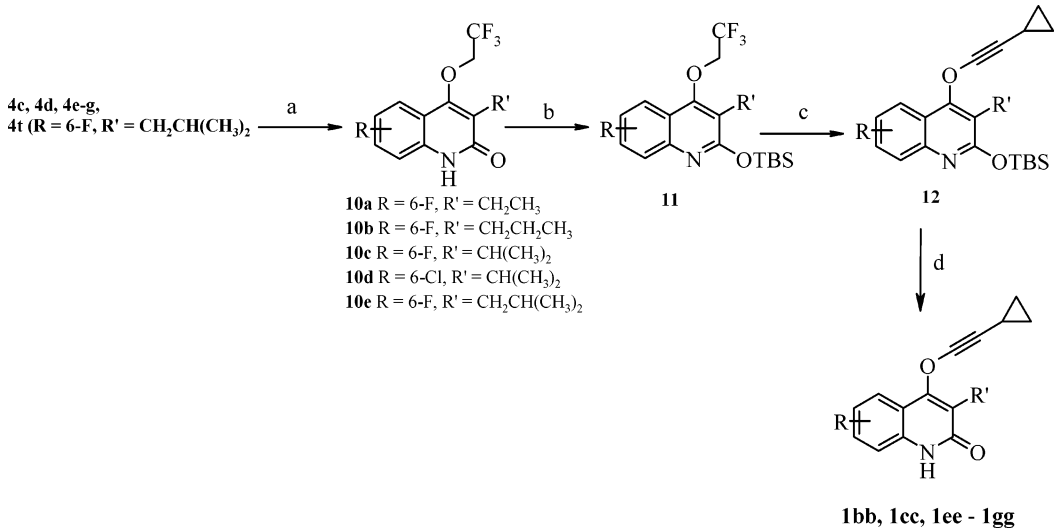
The compounds were initially assayed in a MT4 cell infection model with wild-type HIV-1 and mutant

**Scheme 3**[a]




[a] Conditions: (a) $Et_3N$, DMF, 150 °C; (b) cyclobutyl$CH_2Br$, NaH, DMF.

**Scheme 4**[a]

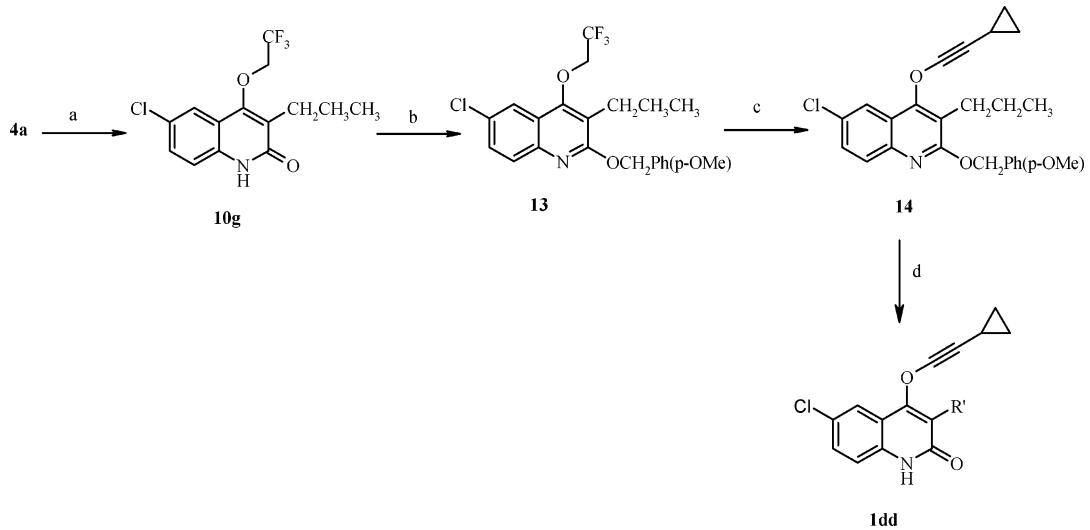



[a] Conditions: (a) $BrCH_2CF_3$, NaH, DMF, 150 °C; (b) TBDMSCl, $Et_3N$, DMF, RT; (c) cyclopropylethynyllithium, *sec*-BuLi, $Et_2O$, −78 °C to RT; (d) Dowex 50 $H^+$, THF, MeOH, RT.

strains of HIV-1.[18] Using an arbitrary $IC_{50}$ cutoff value of ≤300 nM, 24 compounds were found to fall at or below the cutoff against the wild-type virus and eleven against the nevirapine resistant strain (Nev-R), which contains the mutation Y181C in the HIV-1 RT gene. The data from the list of compounds in Table 1 suggested the following: for the R group in template **1**, fluoro and chloro groups appeared to be optimal for antiviral activity. Conversely, moieties such as methyl and methoxy led to compounds (**1u** and **1v**) that showed much reduced antiviral activity. The C6 position appeared to be optimal as evidenced from the lack of antiviral activity of compound **1l** (with 7-methoxy group) compared to compound **1v** (with 6-methoxy group). For the R′ group, variations between ethyl, *n*-propyl, isopropyl and isobutyl did not appear to have a striking difference in antiviral activity, provided that the optimal groups were used in R. Even an ethoxy group was well tolerated (compounds **1i** and **1j**), although both compounds were more active against the wild-type virus than against the Nev-R strain. This is in direct contrast to what was found in our modeling work, which suggested that isopropyl and *n*-propyl groups would impart optimal antiviral activities.

The cyclopropylethynyl subgroup had the largest number of active compounds (**1bb**−**gg**). All six compounds within this subgroup showed antiviral activity against both the wild type and the Nev-R strains that were much less than the cutoff $IC_{50}$ value of ≤300 nM. Indeed, compounds **1ee** and **1ff** were two of the most potent analogues against both strains. Undeniably, the cyclopropylethynyl moiety imparted the antiviral potency since the corresponding analogues in the other subgroups (see compounds **1a**, **1g**, **1h**) were less active. The cyclopropylethynyl motif was favorably exploited in the 1,4-dihydro-2*H*-3,1-benzoxazin-2-one work that culminated in the FDA-approved efavirenz.[19] In fact, our modeling suggested that when quinolone **1ee** and efavirenz were superimposed in the NNRTI binding pocket, the cyclopropylethynyl moiety for both compounds projected into a common subpocket defined by the Y181 and Y188 residues. Because the electronic properties of the fluoro group are markedly different from the chloro group ($\sigma$ values of 0.06 and 0.23,

**Scheme 5**[a]




[a] Conditions: (a) $BrCH_2CF_3$, NaH, DMF, 150 °C; (b) $ClCH_2Ph(p\text{-}OMe)$, $Ag_2O$, DMF; (c) cyclopropylethynyllithium, *sec*-BuLi, $Et_2O$, −78 °C to RT; (d) $(NH_4)_2Ce(NO_3)_6$, $CH_3CN$, $H_2O$.

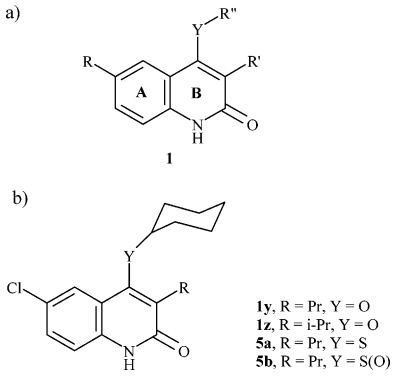



**Figure 2.** (a) Quinolone template incorporating NNRTI pharmacophore A. SAR determined from modification of substituents R, R′ and R″. (b) Initial quinolone leads.[11]

respectively,[20] we expected that the acidity of the NH proton would be stronger for compound **1ff**, and thus its hydrogen bond capability to the backbone carbonyl group of the K103 residue, than for compound **1ee**. Nonetheless, compounds **1ff** and **1ee** showed equipotency against the wild-type virus. In fact, this equipotent tendency was observed in the rest of the analogues with similar R′ and R″ substitutents (see Table 1), suggesting therefore that antiviral potency was not dependent on the strength of the hydrogen bond between NH of the quinolone analogues and the K103 backbone carbonyl group.

After the initial determination of antiviral activity, the compounds were further assayed against a panel of recombinant, isogenic viruses[21] containing the wild type HIV-1 and NNRTI resistant strains encoding the following single and double mutations: K103N, V106A, G190A, Y188C, V108I, Y181C, K103N/Y181C, K103N/G190A, V106A/Y181C, K103N/V108I, and V108I/Y181C. These mutations occur in the binding site where the NNRTIs come in close contact with and are generally associated with NNRTI-treatment failures.

It is now known that the most common mutations that manifest high cross-resistance to a vast array of NNRTIs are the K103N and the Y181C while other mutations are generally compound specific. In addition to virus strains encoding the K103N and the Y181C mutations, resistance to nevirapine is also due to strains encoding the G190A, Y188C and the V106A mutations, and the P236L mutation in the case of delavirdine.[22]

Again, using an arbitrary $IC_{50}$ value cutoff of ≤300 nM, almost the same number of compounds active against the wild-type and Y181C strains were identified as that in the MT4 assay. Twenty-eight compounds were active against the G190A strain and twenty-seven against the Y188C mutant. The compounds generally showed weak activity against the K103N strain with only two compounds (**1d** and **1z**) showing $IC_{50}$ values of <300 nM against the K103N resistant strain. Similarly, the activity against strains containing two mutations was lacking, except for compounds **1bb**, **1dd**, **1ee**, **1ff** and **1hh**. Five compounds (**1dd**, **1ee**, **1ff**, **1f**, and **1q**) were active against the V106A strain.

As was found in the MT4 assay, the active compounds came mainly from the cyclopropylethynyl subgroup. In fact, broad-spectrum activity against the panel of mutants was seen among analogues within this subgroup. Among them, compound **1bb** was the most broad-spectrum in its antiviral activity, and was then followed by compounds **1dd**, **1ee** and **1ff**. However, in general, compounds **1ee** and **1ff** had higher potency.

As alluded to above, for an NNRTI to be considered truly effective, particularly among drug-experienced patients, the compound needs to be able to overcome the cross-resistance barrier imposed by the K103N and the Y181C mutations and to be active against mutants insensitive to currently available drugs. As shown in the bar diagram of Figure 4, efavirenz was generally potent against key NNRTI resistant strains listed in Table 2, which thus makes it currently the most effective NNRTI in the market. On the other hand, nevirapine and delavirdine did not generally fare well against the same set of resistant strains. When compared with nevirapine, delavirdine and efavirenz, a few of the quinolone analogues such as **1ee** and **1ff** (see Figure 4) were more broad-spectrum in their antiviral activities than nevirapine and delavirdine and were quite similar to that of efavirenz. Additionally, both

**Table 2.** Antiviral Activity of Quinolones **1a**−**1hh** and Standards against Wild Type HIV-1 and Those Containing NNRTI-Resistance Mutations in HeLa−CD4 MAGI Assay

| | R | R′ | R″ | WTRVA | K103N | V106A | G190A | Y188C | V108I | Y181C | K103N/ Y181C | K103N/ G190A | V106A/ Y181C | K103N/ V108I | V108I/ Y181C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | 6-Cl | *n*-propyl | cyclopropyl-methyl | 120 | >2000 | 630 | 49 | 89 | 200 | 210 | >2000 | >2000 | >2000 | >2000 | 1700 |
| **1b** | 6-Cl | *n*-propyl | cyclobutyl | 300 | >2000 | >2000 | 220 | 290 | 690 | 1700 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1c** | 6-F | methyl | cyclobutyl-methyl | 610 | >2000 | >2000 | 250 | 840 | 1700 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1d** | 6-F | ethyl | cyclobutyl-methyl | 89 | 270 | 1700 | 110 | 130 | 250 | 390 | >2000 | 1100 | >2000 | >2000 | 870 |
| **1e** | 6-F | *n*-propyl | cyclobutyl-methyl | 63 | 1800 | 1700 | 46 | 60 | 290 | 240 | >2000 | >2000 | 1700 | >2000 | 560 |
| **1f** | 6-Cl | *n*-propyl | cyclobutyl-methyl | 36 | 980 | 124 | 15 | 16 | 77 | >2000 | >2000 | 1300 | 630 | >2000 | 600 |
| **1g** | 6-F | isopropyl | cyclobutyl-methyl | 49 | 1700 | 1900 | 16 | 30 | 440 | 280 | >2000 | 530 | >2000 | >2000 | 700 |
| **1h** | 6-Cl | isopropyl | cyclobutyl-methyl | 15 | 560 | 380 | 9 | 32 | 79 | 210 | >2000 | 560 | 1900 | >2000 | 440 |
| **1I** | 6-F | ethoxy | cyclobutyl-methyl | 580 | >2000 | >2000 | 86 | 360 | 1500 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1j** | 6-Cl | ethoxy | cyclobutyl-methyl | 340 | >2000 | >2000 | 170 | 390 | 890 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1k** | H | cyano | cyclobutyl-methyl | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1l** | 7-$CH_3O$ | *n*-propyl | cyclobutyl-methyl | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1m** | 6-F, 5-$NO_2$ | *n*-propyl | cyclobutyl-methyl | 1100 | >2000 | >2000 | 540 | 250 | ~1000 | >2000 | >2000 | >2000 | 1000 | >2000 | >2000 |
| **1n** | H | *n*-propyl | cyclopentyl | 350 | >2000 | >2000 | 270 | 180 | 1220 | 630 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1o** | 6-Br | *n*-propyl | cyclopentyl | 49 | >2000 | 430 | 32 | 9 | 120 | >2000 | >2000 | >2000 | >2000 | >2000 | 520 |
| **1p** | 6-F | *n*-propyl | cyclopentyl | 150 | >2000 | >2000 | 370 | 42 | 350 | 1800 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1q** | 6-Cl | *n*-propyl | cyclopentyl | 60 | >2000 | 270 | 25 | 31 | 200 | >2000 | >2000 | >2000 | >2000 | >2000 | 670 |
| **1r** | 6-Cl | ethyl | cyclopentyl | 72 | >2000 | >2000 | 12 | 54 | 260 | 6 | >2000 | 640 | ~1000 | >2000 | 780 |
| **1s** | 6-Cl | isobutyl | cyclopentyl | 150 | >2000 | 1500 | 42 | 59 | 280 | 37 | >2000 | >2000 | >2000 | >2000 | 1100 |
| **1t** | 6-Cl | *sec*-butyl | cyclopentyl | 9.3 (900) | 970 | >2000 | ~2000 | 670 | 1100 | 320 | >2000 | >2000 | ~2000 | >2000 | >2000 |
| **1u** | $CH_3$ | *n*-propyl | cyclopentyl | 400 | >2000 | >2000 | 150 | 240 | 710 | 410 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1v** | 6-$CH_3O$ | *n*-propyl | cyclopentyl | 420 | >2000 | >2000 | 710 | 160 | 540 | 710 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1w** | 6-$CF_3O$ | *n*-propyl | cyclopentyl | 270 | >2000 | >2000 | 47 | 360 | 300 | 990 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1x** | 6-CN | *n*-propyl | cyclopentyl | 65 | >2000 | 1300 | 1 | 52 | 240 | 1700 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **1y** | 6-Cl | *n*-propyl | cyclohexyl | 32 | >2000 | 980 | 54 | 12 | 180 | 470 | >2000 | 1000 | 1100 | ~2000 | 900 |
| **1z** | 6-Cl | isopropyl | cyclohexyl | 38 | 170 | 1100 | 27 | 12 | 110 | 1400 | >2000 | 190 | 430 | >2000 | ~800 |
| **1aa** | 6-Cl | *n*-propyl | cyclohexyl-methyl | 1200 | >2000 | >2000 | 260 | 23 | >2000 | 690 | >2000 | >2000 | >2000 | >2000 | 1000 |
| **1bb** | 6-F | ethyl | cyclopropyl-ethynyl | 25 | 420 | 340 | 6 | 100 | 60 | 24 | 300 | 68 | 230 | 240 | 130 |
| **1cc** | 6-F | *n*-propyl | cyclopropyl-ethynyl | 66 | >2000 | 440 | 21 | 290 | 160 | 1 | 1400 | 490 | 380 | >2000 | 370 |
| **1dd** | 6-Cl | *n*-propyl | cyclopropyl-ethynyl | 38 | >2000 | 220 | 15 | 70 | 60 | 92 | 1800 | 300 | 120 | 1400 | 230 |
| **1ee** | 6-F | isopropyl | cyclopropyl-ethynyl | 10 | 400 | 150 | 5 | 23 | 26 | 15 | 620 | 100 | 220 | 720 | 11 |
| **1ff** | 6-Cl | isopropyl | cyclopropyl-ethynyl | 16 | 320 | 57 | 3 | 18 | 36 | 39 | 620 | 52 | 120 | 1200 | 120 |
| **1gg** | 6-F | isobutyl | cyclopropyl-ethynyl | 130 | >2000 | >2000 | 16 | 220 | 230 | 150 | >2000 | 1800 | 660 | 1900 | 240 |
| **1hh** | 6-F | isopropyl | *sec*-butyl-ethynyl | 28 | 540 | 420 | 21 | 21 | 110 | 84 | 640 | 230 | 130 | >2000 | 140 |
| | | | efavirenz | 1 | 23 | 1 | 7 | 1 | 1.4 | 2 | 43 | 552 | 3 | 74 | 3 |
| | | | delavirdine | 159 | 2651 | 870 | 35 | 124 | 97 | | 1250 | 8000 | 10000 | 3400 | 10000 |
| | | | neviripine | 89 | 7100 | ~10000 | >10000 | 2200 | 340 | >10000 | >50000 | – | >10000 | 21000 | >10000 |

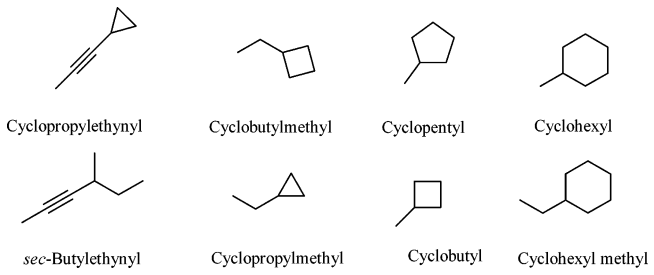



**Figure 3.** R″ substituents used in the synthesis of analogues of **1**.

analogues were generally more potent than nevirapine and delavirdine, but fell short of the potency of efavirenz.

**Crystallography. Comparison of RT-GW490-745X (1ee) with other RT−Quinolone Complexes.** GW490745X bound at the NNRTI site is shown in Figure 4a. We have reported the structures of four RT-quinolone complexes (RT-**2a**, RT-**2b**, RT-**5**, and RT-**6**; Hopkins et al., preceding paper). Comparing the protein core of 110 residues around the NNRTI site of RT-GW490745X with those of RT and quinolone analogue complexes more than 87% of the CA atoms can be overlapped with rms deviations of 0.56 Å, 0.57 Å, 0.55 Å and 0.56 Å, respectively. The chemical differences between the inhibitors result in slight alterations in the binding modes at the NNRTI site, which are accommodated by conformational changes in the protein, mainly in side-chains but also to a lesser extent in the main-chain (Figure 4b). GW490745X has a cyclopropylethynyl group instead of the cyclohexyloxy or cyclohexylthio of other quinolone analogues (Hopkins et al., preceding paper). A result of this differing substituent is that GW490745X binds in a significantly different

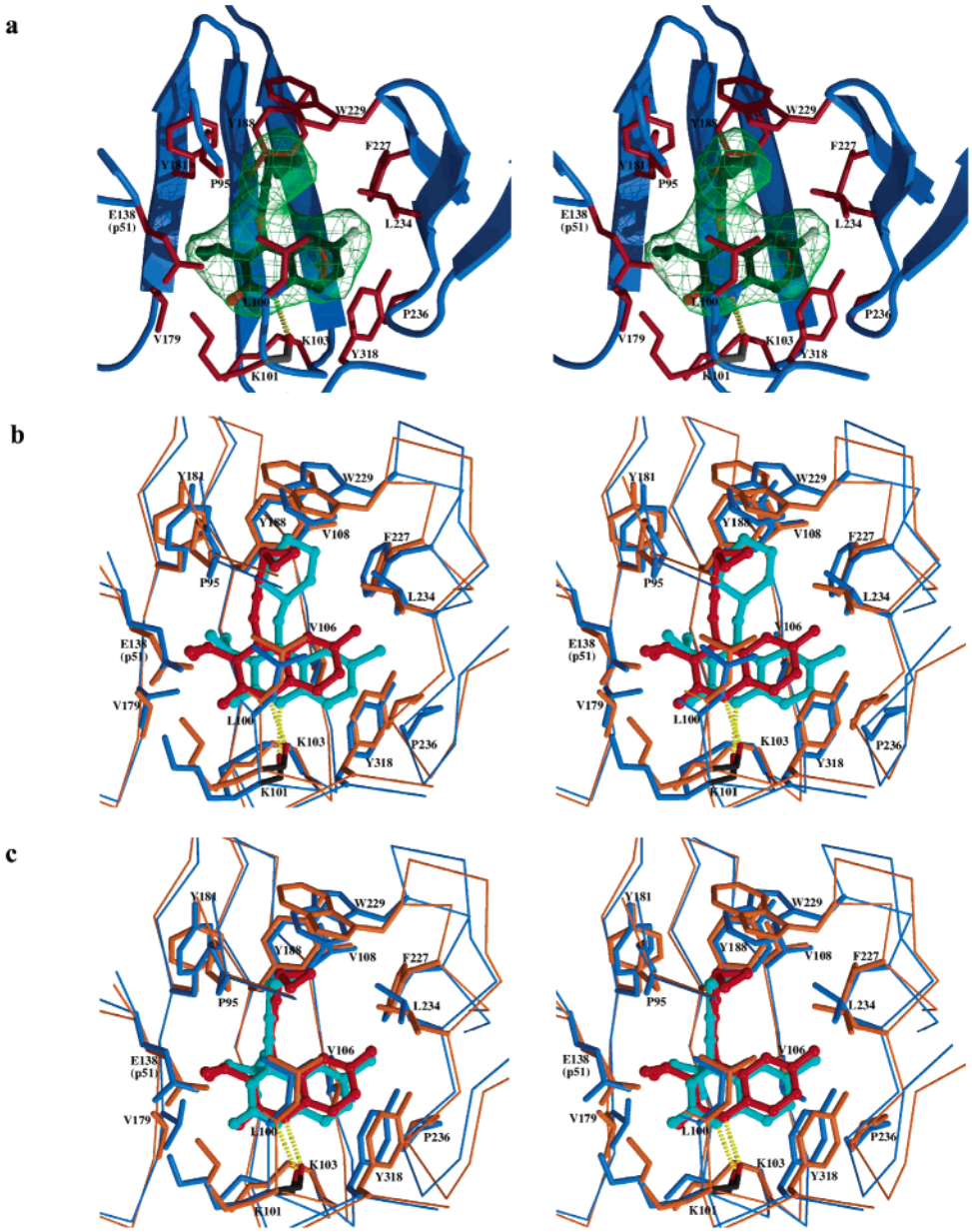



**Figure 4.** Stereodiagrams showing the NNRTI binding site. (a) **1ee** at the NNRTI site. The CA backbone of the protein is draw as ribbons and coils and colored in blue, and protein side-chains are shown as red sticks. The inhibitor is shown as ball-and-stick representation and colored by atoms. The simulated annealing omit electron density map for the inhibitor contoured at 3.5$\sigma$ is shown as semi-transparent green surface. The yellow broken stick represent the hydrogen bond between the carbonyl oxygen of residue K101 and the inhibitor. (b) comparison of the NNRTI sites between RT−**1ee** and RT−**2b** complexes. The protein CA backbones and side-chains are shown as thinner and thicker sticks with RT−**1ee** and RT−**2b** colored in orange and blue, respectively. The inhibitors are shown as ball-and-sticks with **1ee** colored in red and **2b** in cyan. (c) Comparison of the NNRTI sites in RT−**1ee** and RT−efavirenz complexes. The drawing scheme is the same as b) with RT−**1ee** colored in orange and red, and RT−efavirenz in blue and cyan.

position and orientation compared with **2b**. GW490745X shifts about 1 Å toward V179, with the quinolone ring tilted by some 10° and the isopropyl group rotated about 180°. To avoid a clash with the isopropyl group of the inhibitor the side-chain of V179 is rotated by 120°. The cyclopropylethynyl group is positioned more distantly from Y188 and hence has fewer contacts with this residue. There is a more than 0.5 Å main-chain movement at residues 98−101 such that a similar H-bond distance from the inhibitor to the carbonyl oxygen is maintained (2.64 Å, **2a**:2.55 Å, **5**:2.67 Å, **6**:2.81 Å, **2b**: 2.78 Å). The position and smaller size of the fluorine atom of GW490745X (chlorine in the other quinolones) also introduces changes at the $\beta$10-$\beta$11 loop whose flexible nature allows for the NNRTI site to accommodate inhibitors very different in size.[26,29] The conformation difference of the W229 side-chain can be attributed to the difference in unit cell for the crystals.[30]

**Comparison of RT-GW490745X and RT−Efavirenz Complexes.** 92 out of 110 CA atoms of the protein core around the NNRTI site of RT-GW490745X can be superimposed on that of RT−efavirenz[31] with an rms deviation of 0.44 Å. The quinolone ring of GW490745X is tilted by about 10° from the benzoxazin-2-one ring of efavirenz such that the fluorine end of the quinolone ring is positioned about 0.5 Å higher and also drags

V106 with it (Figure 4c). The cyclopropyl group of GW490745X is positioned higher up in the site due to its extra oxygen atom, thereby making more interactions with W229. The isopropyl group is positioned closer to V179, about 1.5 Å offset from the structurally related trifluoromethyl group of efavirenz which occupies the so-called "Tyrosine 181 trigger" position.[26] The H-bond from the ring NH to the carbonyl oxygen of K101 is shorter (2.64 Å) than that of RT−efavirenz (2.72 Å). There are also differences in protein side-chain conformation when comparing the two complexes, for example at residues V106, V179, Y181 and L234 (47° difference in the $\chi_2$ angle of Y181; 1.6 Å distance between the CG1 atoms of V179).

**Structure and Antiviral Activity Relationship.** The differences in position and orientation between RT−GW490745X (**1ee**) and the four other quinolone compounds in the NNRTI pocket can be attributed to the substitution of the cyclohexyloxy or cyclohexylthio by a cyclopropylethynyl group in **1ee**. Indeed the cyclopropylethynyl group is positioned much closer to cyclopropylpropynyl group of efavirenz. Crystal structures of complexes of RT with potent NNRTIs invariably have a substituent that occupies the same volume as filled by the trifluoromethyl group of efavirenz (Figure 4c).[26,32] Interestingly, although the trifluoromethyl is at the 4-position on the benzoxazin-2-one ring of efavirenz and is perpendicular to the ring plane, for **1ee** the isopropyl group fulfills this role but is in the 3-position of the quinolone and in the plane of the ring. Thus it is probably the tendency of the isopropyl group to occupy the "Tyrosine 181 trigger" volume that tilts the quinolone ring compared to the benzoxazin-2-one ring of efavirenz. Despite this, **1ee** has higher spatial overlap with efavirenz in the NNRTI pocket compared to the other four quinolone analogues, which explains its better antiviral activity. There are less hydrophobic interactions from the smaller cyclopropylethynyl group of **1ee** to Y181 and Y188 compared to the cyclohexyloxy or cyclohexylthio groups of the four other quinolone analogues, explaining the smaller reduction of the antiviral activity due to Y181C or Y188C mutation.

The details of NNRTI binding derived from the crystal structures of the quinolone analogues correlate well with the SAR data (Table 1). The isopropyl group of **1ee** forms strong van de Waals interactions with V179. Replacement of the group with ethyl (**1bb**) or *n*-propyl (**1cc**) would either reduce the hydrophobic interactions or introduce structural clashes, therefore weakening the antiviral activity (Table 2). A larger group such as isobutyl (**1gg**) would reposition or reorient the inhibitor in the NNRTI pocket, resulting in significant loss of activity. The 6-fluoro group has a close contact (3.3 Å) with the CB atom of residue L234, a larger Cl substitute (**1ff**) would position the quinolone ring closer to the benzoxazin-2-one ring of efavirenz as well as to V106. That **1ff** has higher activity against the V106A mutant virus is probably because A106 would be able to move closer to the inhibitor, partially compensating the loss of protein−inhibitor interactions in the mutant structure. **1ee** and efavirenz have a very similar spectrum of activity against V108I, Y181C and Y188C mutant viruses, which is reflected in the crystal structures where the two inhibitors have similar interactions with these residues. The G190A mutant virus is 6-fold less sensitive to efavirenz, but 2-fold more sensitive to **1ee** compared to wild-type virus. The trifluoromethyl group of efavirenz makes several hydrophobic contacts with G190, while **1ee** has no interaction with this residue, the closest approach to the CA atom of G190 is 5.1 Å. There is enough room for an alanine side-chain between the quinolone ring and G190, and the G190A mutation would be predicted to give greater protein−inhibitor interaction and increase the tightness of inhibitor binding.

## Conclusions

We selected compound **1ee** for further evaluation of its druglike properties. The pharmacokinetic properties of compound **1ee** showed acceptable parameters including clearance, half-life and oral bioavailability, 44 mL/min/kg, 3.7 h, and 34% respectively. The therapeutic index based on the ratio of the $IC_{50}$ values of the MT4 cell lines versus the $IC_{50}$ value against the wild-type virus and Nev-R was >4000. In the MT4 assay, compound **1ee** showed an approximately 8-fold decrease in wild-type $IC_{50}$ value in the presence of 45% human serum and a 2-fold decrease in the presence of 15% fetal calf serum.

In summary, we have shown that, based on known crystal structures and resistance data, a quinolone template was designed, and synthesis led to a group of analogues with a broad spectrum of activity against both wild-type HIV-1 and key NNRTI-relevant mutant viruses. Analogues **1ee** and **1ff** demonstrated the most broad-spectrum activity among these analogues and were more potent than nevirapine and delavirdine against mutant viruses that generally are insensitive to currently known NNRTIs. The quinolone class of NNRTIs, as predicted by their design, have proven to be effective and potent against a wide range of diverse drug resistant mutants, far more so than the first generation NNRTIs. However, in the range of analogues reported here, we did not improve upon the potency of efavirenz, particularly in comparison with their activity against the K103N strain. In conclusion, we believe the data presented here justifies our initial assumption that the structural analysis of the binding modes and drug resistant mechanisms of NNRTIs can lead to valuable insights to aid the design of the next generation of nonnucleoside inhibitors of HIV-1 reverse transcriptase. The principles established in our previous paper[11] and demonstrated here for the quinolone series may also be applicable to other potential NNRTI templates to guide the search for inhibitors with improved drug resistance properties.

## Experimental Section

**Compound Synthesis. 6-Chloro-4-hydroxy-3-propyl-2(1*H*)-quinolinone (4a).** 4-Chloroaniline (**2b**) (10 g, 80 mmol) and **3a** (16 mL, 80 mmol) were combined in phenyl ether (100 mL) and heated to 250° overnight. The reaction was cooled to 50 °C. A precipitate formed and was collected by filtration. After washing with hexane, 15 g (84%) of **4a** was obtained as a solid, which was used without further purification. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.3 (bs, 1H, OH), 10.0 (bs, 1H, NH), 7.82 (s, 1H, ArH), 7.44 (d, 1H, ArH), 7.21 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.40 (m, 2H, $CH_2$), 0.86 (t, 3H, $CH_3$). MS (ES+): *m/z* 238.0 (M + 1).

**6-Chloro-4-cyclopropylmethoxy-3-propyl-2(1*H*)-quinolinone (1a).** Intermediate **4a** (0.50 g, 2.1 mmol) was dissolved in DMF (Aldrich, Sure Seal, 6 mL). Sodium hydride (0.126 g, 3.2 mmol, 1.5 equiv) was added and the solution stirred for 30 min. The reaction was placed in a 100 °C oil bath and stirred for 5 min. Bromomethylcyclopropane (1 mL, 10.5 mmol, 5 equiv) was added and the reaction heated for 2 h. The reaction was poured into ice−water (100 mL). The product was extracted with ethyl acetate (1 vol), dried with magnesium sulfate and filtered. The crude product was purified by flash column chromatography on silica gel eluted with hexane/ethyl acetate (3:1, v/v) followed by recrystallization from ethyl acetate giving **1a** (0.185 g, 30%). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.70 (s, 1H, ArH), 7.53(d, 1H, ArH), 7.33 (d, 1H, ArH), 3.88 (m, 2H, $CH_2$), 2.53 (overlap DMSO, 2H), 1.5 (m, 2H, $CH_2$), 1.40 (m, 1H, CH), 0.95 (t, 3H, $CH_3$), 0.62 (m, 2H, $CH_2$), 0.36 (m, 2H, $CH_2$). MS (ES+): *m/z* 292.0 (M + 1).). Anal. ($C_{16}H_{18}ClNO_2$) C, H, N, Cl.

**6-Chloro-4-(cyclohexyloxy)-3-propyl-2(1*H*)-quinolinone (1y).** Intermediate **4a** (2.50 g, 10.5 mmol), cyclohexyl bromide (2.6 mL, 21 mmol), potassium carbonate (1.6 g, 46 mmol) and triethylamine (1.05 mL, 14 mmol) were combined in DMF (Aldrich, Sure Seal, 50 mL) and heated in a 165 °C oil bath for 36 h. The reaction was poured into ice−water (200 mL). The product was extracted with ethyl acetate (1 vol.), dried with magnesium sulfate and filtered. The crude product was purified by chromatography on silica gel eluted with hexane/ethyl acetate (3:1, v/v) giving **1y** (0.03 g, 1%). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.62 (s, 1H, ArH), 7.49 (d, 1H, ArH), 7.28 (d, 1H, ArH), 3.95 (m, 1H, CH), 1.95 (m, 2H, $CH_2$), 1.70 (m, 2H, $CH_2$), 1.50 (m, 2H, $CH_2$), 1.28−1.20 (m, 10H, alkyls). MS (ES+): *m/z* 320.0 (M + 1).

**6-Chloro-4-(cyclobutyloxy)-3-propyl-2(1*H*)-quinolinone (1b).** The title compound was prepared from **4a** by the method used in the synthesis of **1y**, except the reaction was heated in a 70 °C oil bath for 7 h. Sodium hydride (0.04 g) was then added and the reaction heated in a 120 °C oil bath for 24 h. **1b** (27% yield) was obtained following recrystallization from ethyl acetate. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.56 (s, 1H, ArH), 7.53 (d, 1H, ArH), 7.32 (d, 1H, ArH), 4.46 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 2.3 (m, 3H, alkyls), 1.7 (m, 1H, alkyl), 1.50 (m, 4H, $CH_2$), 0.95 (t, 3H, $CH_3$). MS (ES+): *m/z* 292.0 (M + 1). Anal. ($C_{16}H_{18}ClNO_2$) C, H, N, Cl.

**6-Fluoro-4-hydroxy-3-methyl-2(1*H*)-quinolinone (4b).** The title compound was prepared according to the procedure used in the synthesis of **4a** starting from **2b** (71.6 mmol, 7.95 g) and **3b** (164 mmol, 28.54 g) to give **4b** (9.8 g, 71%) as a white solid. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.38 (s, 1H), 10.19 (s, 1H), 7.55 (dd, 1H, $J$ = 2.9, 9.9 Hz), 7.39−7.22 (m, 2H), 2.04 (d, 0.5H, $J$ = 1.8 Hz), 1.96 (s, 2.5H). MS (ES+): *m/z* 194 (M +1),

**4-Cyclobutylmethoxy-6-fluoro-3-methyl-2(1*H*)-quinolinone (1c).** The title compound was prepared from **4b** by the method used in the synthesis of **1y** except the oil bath temperature was 70 °C and the reaction time was 24 h. **1c** (9% yield) was isolated by chromatography on silica gel eluted with $CHCl_3$/MeOH (95/5). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 12.3 (bs,1H, NH), 7.44−7.41 (m, 2H, ArH), 7.24−7.19 (m,1H, ArH), 4.01 (d, 2H, $OCH_2$), 2.9 (m, 1H, CH), 2.2 (m, 5H, alkyls), 2.0 (m, 4H, alkyls). MS (ES+): *m/z* 262.0 (M + 1). Anal. ($C_{15}H_{16}FNO_2$) C, H, N, F.

**3-Ethyl-6-fluoro-4-hydroxy-2(1*H*)-quinolinone (4c).** To a round-bottom flask equipped with a stir bar, an addition funnel and nitrogen on demand was added **3c** (5.0 mL, 5.0 g, 27 mmol). A solution of KOH (1.8 g, 32 mmol) in absolute ethanol (50 mL) was added dropwise via an addition funnel, and the reaction was allowed to stir at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and the resultant crude solid washed with $Et_2O$ (30 mL) and concentrated again under reduced pressure to afford potassium 2-(ethoxycarbonyl)butanoate (1.0 g, 95%) as a white solid. This butanoate was dissolved in $CH_2Cl_2$ in a round-bottom flask equipped with a stir bar and nitrogen on demand. Oxalyl chloride (0.44 mL, 0.64 g, 5.0 mmol) was added dropwise via a syringe, followed by the addition of a catalytic amount of DMF (1 drop). The reaction mixture was allowed to stir at room temperature for 18 h. When judged complete, the mixture was concentrated under reduced pressure to provide ethyl 2-(chlorocarbonyl)butanoate (0.95 g, >100%) as an orange oil, which was dissolved in $CH_2Cl_2$ and added dropwise via an addition funnel to a solution of **2b** (1.9 mL, 2.2 g, 20 mmol) and $Et_3N$ (10 mL, 7.3 g, 73 mmol) in $CH_2Cl_2$ (100 mL). The resultant mixture was allowed to stir at RT. When judged complete, the reaction mixture was partitioned between EtOAc and water. The organic layer was separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford ethyl 2-[(4-fluoroanilino)carbonyl]butanoate (4.0 g, 80%) as a crystalline solid. This solid was dissolved in phosphorus pentoxide methanesulfonic acid (PPMA) (70 mL, (Cho, H.; Matsuki, S. *Heterocycles* **1996**, *43* (1), 127−131) and heated to 170 °C for 1 h. The mixture was allowed to cool to room temperature and then poured over ice and filtered. The filtrate was extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to a yellow solid. The crude product was dissolved in 0.5 N NaOH, extracted with toluene and acidified to pH∼3 using concentrated HCl. The resulting white precipitate was filtered and dried to provide **4c** (1.3 g, 43%) as a white solid. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.37 (s, 1H), 7.62 (d, 1H), 7.32 (m, 2H), 2.58 (q, 2H), 1.02 (t, 3H).

**4-(Cyclobutylmethoxy)-3-ethyl-6-fluoro-2(1*H*)-quinolinone (1d).** To NaH (35 mg of 60% dispersion in oil, 0.87 mmol) in a round-bottom flask equipped with a stir bar and nitrogen on demand was added anhydrous DMF (5 mL). A solution of **4c** (0.15 g, 0.72 mmol) in anhydrous DMF (1 mL) was added dropwise, followed by the dropwise addition of (bromomethyl)-cyclobutane (0.10 g, 0.07 mL, 0.65 mmol), and the reaction was allowed to stir at room temperature for 48 h. The reaction mixture was quenched by the dropwise addition of water and extracted with ethyl acetate. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford a yellow oil. The crude product was filtered through a pad of silica gel, eluding with $CH_2Cl_2$, and the filtrate was concentrated in vacuo. The solid was rinsed with $Et_2O$, and filtered to provide **1d** (20 mg, 10%) as a white solid. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.77 (s, 1H), 7.37 (m, 3H), 4.00 (d, 2H), 2.88 (m, 1H), 2.58 (q, 2H), 2.15 (m, 2H), 1.95 (m, 4H), 1.02 (t, 3H). MS (ES) 274 $(M - H)^-$.

**6-Fluoro-4-hydroxy-3-propyl-2(1*H*)-quinolinone (4d).** The title compound was prepared from **2b** and **3a** by the method used in the synthesis of **4a**. A 56% yield of **4d** was obtained. $^1$H NMR (MesSO-$d_6$) $\delta$ 11.3 (bs, 1H, OH), 10.0 (bs, 1H, NH), 7.87 (d, 1H, ArH), 7.42 (dd, 1H, ArH), 7.22 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.40 (m, 2H, CH2), 0.86 (t, 3H, CH3), MS (ES+): *m/z* 222.0 (M + 1).

**4-Cyclobutylmethoxy-6-fluoro-3-propyl-2(1*H*)-quinolinone (1e).** The title compound was prepared from **4d** and cyclobutylmethyl bromide by the method used in the synthesis of **1y** except the oil bath temperature was 60 °C. A 40% yield of **1e** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.32 (m, 3H, ArH), 4.00 (d, 2H, $OCH_2$), 2.86 (m, 1H, CH), 2.14 (m, 2H, alkyls), 1.95 (m, 4H, alkyls), 1.65 (m, 2H, $CH_2$), 0.95 (t, 3H, $CH_3$). MS (ES+): *m/z* 290.0 (M + H). Anal. ($C_{17}H_{20}FNO_2$) C, H, N, F.

**6-Chloro-4-(cyclobutylmethoxy)-3-propyl-2(1*H*)-quinolinethione (1f).** The title compound was prepared from **4a** and cyclobutylmethyl bromide by the method outlined in the synthesis of **1y**. An 8% yield of **1f** was obtained after chromatography on silica gel (4 by 7 cm column) eluted with $CHCl_3$/MeOH (97:3, v/v, 2 times). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 13.6 (bs, 1H, NH), 7.67 (s, 1H, ArH), 7.62 (s, 2H, ArH), 4.00 (d, 2H, $OCH_2$), 2.83 (t, 3H, alkyls), 2.1 (m, 2H, alkyls), 1.9 (m, 4H, alkyl), 1.55 (m, 2H, alkyls), 0.90 (t, 3H, $CH_3$). MS (ES+): *m/z* 322.0 (M + 1). Anal. ($C_{17}H_{20}ClNO_2$) C, H, N, Cl.

**6-Fluoro-4-hydroxyl-3-isopropyl-2(1*H*)-quinolinone (4e).** The title compound was prepared from **2b** (10 g, 80 mmol) and **3d** by the method used in the synthesis of **4a** except that the reaction was run for 5 d. A 40% yield of **4e** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 10.9 (bs, 1H, OH), 10.1 (bs, 1H, NH),

7.7 (d, 1H, ArH), 7.4−7.20 (m, 2H, ArH), 3.3 (m, overlapping with DOH), 1.29 (d, 6H, $CH_3$). MS (ES+): *m/z* 222.0 (M + H).

**4-(Cyclobutylmethoxy)-6-fluoro-3-isopropyl-2(1H)-quinolinone (1g).** The title compound was prepared from **4e** by the method used in the synthesis of **1y**. A 2% yield of **1g** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.7 (bs, 1H, NH), 7.4−7.3 (m, 3H, ArH), 3.94 (d, 2H, $OCH_2$), 2.9 (m, 1H, CH), 2.1 (m, 2H, alkyls), 1.93 (m, 4H, alkyls), 1.93 (d, 6H, $CH_3$). MS (EI+): *m/z* 305.0 ($M^+$). Anal. ($C_{17}H_{20}FNO_2$) C, H, N, F.

**6-Chloro-4-hydroxy-3-isopropyl-2(1*H*)-quinolinone (4f).** The title compound was prepared from **2a** and **3d** by the method used in the synthesis of **4a**. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.3 (bs, 1H, OH), 10.0 (bs, 1H, NH), 7.87 (s, 1H, ArH), 7.44 (d, 1H, ArH), 7.22 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.22 (m, 6H, $CH_3$). MS (ES+): *m/z* 238.0 (M + H).

**6-Chloro-4-cyclobutylmethoxy-3-isopropyl-2(1*H*)-quinolinone (1h).** The title compound was prepared from **4f** and cyclobutylmethyl bromide using the method employed for the synthesis of **1y**. A 22% yield of **1h** was obtained. $^1$H NMR ($CDCl_3$) $\delta$ 12.5 (bs, 1H, NH), 7.70 (s, 1H, ArH), 7.40 (d, 1H, ArH), 7.30 (d, 1H, ArH), 3.94 (d, 2H, $OCH_2$), 3.45 (m, 1H, CH), 2.91 (m, 1H, CH), 2.22 (m, 2H, alkyls), 1.99 (m, 4H, alkyls), 1.45 (d, 6H, $CH_3$). MS (EI+): *m/z* 305.0 ($M^+$). Anal. ($C_{17}H_{20}ClNO_2$) C, H, N, Cl.

**Methyl 5-chloro-2-[(2-ethoxyacetyl)amino]benzoate (6a).** Methyl 2-amino-5-chlorobenzoate, **5a** (1.0 g, 5.4 mmol, Sigma-Aldrich), was dissolved in $CH_2Cl_2$ (10 mL). Ethoxyacetyl chloride (1.0 g, 8.1 mmol) (see footnote for preparation) was added followed by $Et_3N$ (2.2 mL, 16 mmol). The reaction was stirred at room temperature for 5 min. Water was added, and the mixture was transferred to a separatory funnel. The organic layer was washed with saturated ammonium chloride (2×). The solvent was removed in vacuo, and the product was isolated by chromatography on silica gel (4 × 15 cm column) eluted with chloroform/methanol (98:2, v/v-500 mL followed by 95:5, 300 mL) to give a 55% yield of **6a**. $^1$H NMR ($CDCl_3$) $\delta$ 11.1 (bs, 1H, NH), 8.78 (d, 1H, ArH), 8.01 (s, 1H, ArH), 7.49 (d, 1H, ArH), 4.1 (s, 2H,$CH_2$), 3.95 (s, 3H, $OCH_3$), 3.65 (m, 2H, $CH_2$), 1.38 (m, 3H, $CH_3$). MS (ES+): *m/z* 272.0 (M + 1).

**6-Chloro-3-ethoxy-4-hydroxy-2(1*H*)-quinolinone (4q).** **6a** (0.20 g, 0.74 mmol) was boiled in toluene to remove excess $H_2O$. The excess toluene was removed in vacuo. The concentrate was dissolved in THF (8 mL) and chilled to −78 °C. Potassium bis(trimethylsilyl)amide (4.4 mL of a 0.5 M solution in toluene, 2.2 mmol) was added over 5 min. The reaction was stirred for 5 min and transferred to a 0 °C bath. The reaction was stirred for 30 min and then allowed to warm to room temperature and stir overnight. The reaction was poured onto ice−water (15 mL) and extracted with ethyl acetate (20 mL). The solvent was removed in vacuo, and **4q** (22% yield) was isolated by filtration through silica gel (10 g) eluted with ethyl acetate/hexane (1:4, v/v). $^1$H NMR ($CDCl_3$) $\delta$ 11.6 (bs, 1H, NH), 10.6 (bs, 1H, OH), 7.68 (s, 1H, ArH), 7.42 (d, 1H, ArH), 7.22 (d, 1H, ArH), 4.04 (q, 2H, $OCH_2$), 1.22 (t, 2H, $CH_3$). MS (ES+): *m/z* 240.0 (M + 1).

**6-Chloro-4-(cyclobutylmethoxy)-3-ethoxy-2(1*H*)-quinolinone (1i).** The title compound was prepared from **4q** and cyclobutylmethyl bromide by the method used for the synthesis of **1y** except a 60 °C oil bath was used. The reaction time was 48 h. **1i** (11% yield) was isolated by chromatography on silica gel (4 × 7 cm column) eluted with ethyl acetate/hexane (1:1). $^1$H NMR ($CDCl_3$) $\delta$ 10.4 (bs, 1H, NH), 7.8(s, 1H, ArH), 7.36 (d, 1H, ArH), 7.14 (d, 1H, ArH), 4.42 (d, 2H, $OCH_2$), 4.2 (q, 2H, $OCH_2$), 2.81 (m, 1H, CH), 2.2−1.2 (m, alkyls), 0.87 (m, 2H). MS (ES+): *m/z* 308.0 (M + 1).

**Methyl 5-fluoro-2-[(2-ethoxyacetyl)amino]benzoate (6b).** The title compound was prepared from **5b** (made from the acid, Sigma-Aldrich, by esterification in ethanol/toluene with one drop of concd $H_2SO_4$) by the method used for **6a** and was used without chromatography in the synthesis of **1j**. $^1$H NMR ($CDCl_3$) $\delta$ 11.7 (bs, 1H, NH), 8.78 (dd, 1H, ArH), 7.72 (dd, 1H, ArH), 7.24 (m, 1H, ArH), 4.4 (m, 2H,$OCH_2$), 4.1 (m, 2H, $OCH_2$), 3.7 (s, 2H, $OCH_2$), 1.2 (m, 6H, $CH_3$).

**6-Fluoro-4-(cyclobutylmethoxy)-3-ethoxy-2(1*H*)-quinolinone (1j).** The title compound was prepared from **4r** and cyclobutylmethyl bromide by the method used for the synthesis of **1y** except a 60 °C oil bath was used. The reaction time was 48 h. **1j** (8% yield) was isolated by chromatography on silica gel (4 × 7 cm column) eluted with ethyl acetate/hexane (1:1). $^1$H NMR ($CDCl_3$) $\delta$ 11.1 (bs,1H, NH), 7.52 (d, 1H, ArH), 7.24 (m, 1H, ArH), 7.15 (m, 1H, ArH), 4.42 (d, 2H, $OCH_2$), 4.2 (q, 2H, $OCH_2$), 2.81 (m, 1H, CH), 2.2−1.2 (m, alkyls), 0.87 (m, 2H). MS (ES+): *m/z* 292.0 (M + 1).

**4-Hydroxy-7-methoxy-3-propyl-2(1*H*)-quinolinone (4g).** The title compound was prepared from **2d** and **3a** by the method used in the synthesis of **4a**. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.1 (bs, 1H, OH), 9.9 (bs, 1H, NH), 7.8 (d, 1H, ArH), 6.7−6.8 (m, 2H, ArH), 3.8 (s, 3H, MeO), 2.5 (m, overlapping with DMSO, 2H, $CH_2$), 1.3−1.5 (m, 2H, $CH_2$), 0.9 (t, 3H, $CH_3$).

**7-Methoxy-4-(cyclobutylmethoxy)-3-propyl-2(1*H*)-quinolinone (1l).** The title compound was prepared from **4g** and cyclobutylmethyl bromide by the method used for the synthesis of **1y** except a 60 °C oil bath was used. The reaction time was 48 h. **1l** (22% yield) was isolated by chromatography on silica gel (4 × 7 cm column) eluted with MeOH/$CH_2Cl_2$ (3:97). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.5 (bs,1H, NH), 7.5 (d, 1H, ArH), 6.7−6.8 (m, 2H, ArH), 3.9 (d, 2H, $OCH_2$), 3.8 (s, 3H, $OCH_3$), 2.7−2.8 (m, 1H, CH), 2.3−2.4 (m,), 2−2.1 (m, 2H, $CH_2$), 1.9−2 (m, 6H, cyclobut), 1.4−1.5 (m, 2H, $CH_2$), 0.9 (t, 3H, $CH_3$).

**6-Fluoro 4-hydroxy-5-nitro-3-propyl-2(1*H*)-quinolinone (4h).** A mixture of **2e** (1.5 g, 4.8 mmol), **3a** and $Na_2CO_3$ (1.0 g, 9.6 mmol) in $H_2O$ (20 mL) was heated to reflux for 1 h. The reaction mixture was cooled to room temperature and acidified with 2 N HCl. A precipitate formed and was collected by filtration and washed repeatedly with water. After drying, 1.24 g of a yellow solid was obtained. The solid was mixed with polyphosphoric acid (20 mL) and was heated to 120 °C for 4 h. The reaction mixture was cooled to room temperature, 2 N HCl was added (20 mL). A precipitate formed and was collected and dried. **4h** (0.94 g, 74% yield) was obtained as a light yellow solid. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (s, 1H), 11.0 (br s, 1H), 7.72 (t, 1H), 7.48 (dd, 1H), 2.5 (m, overlapping with DMSO), 1.4−1.5 (m, 2H), 0.92 (t, 3H).

**4-(Cyclobutylmethoxy)-6-fluoro-5-nitro-3-propyl-2(1*H*)-quinolinone (1m).** To a stirred suspension of NaH (0.25 g of a 50% dispersion in oil, 5.3 mmol) in DMF (10 mL) was added portionwise 0.94 g of **4h**. After stirring for 10 min, cyclobutylmethyl bromide (0.47 mL, 4.2 mmol) was added. The reaction mixture was stirred for 24 h. The mixture was then concentrated and dry-packed on silica gel. Flash column chromatography on silica with 5% MeOH in $CH_2Cl_2$ gave **1m** (0.16 g, 14% yield) as a peach-colored solid. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 12.23 (br s, 1H), 7.75 (t, 1H), 7.54 (dd, 1H), 3.8 (d, 2H), 2.6−2.8 (m, 1H), 2.5 (m, overlapping with DMSO), 1.8−2.2 (m, 6H), 1.5−1.7 (m, 2H), 0.95 (t, 3H).

**4-Hydroxy-3-propyl-2(1*H*)-quinolinone (4i).** The title compound was prepared from **2f** and **3a** by the method outlined for the synthesis of **4a** in 73% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.2 (bs, 1H, OH), 9.94 (bs, 1H, NH), 7.82 (d, 1H, ArH), 7.39 (t, 1H, ArH), 7.19 (d, 1H, ArH), 7.09 (t, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.40 (m, 2H, $CH_2$), 0.86 (t, 3H, $CH_3$). MS (ES+): *m/z* 204.0 (M + 1).

**4-Cyclopentyloxy-3-propyl-2(1*H*)-quinolinone (1n).** The title compound was prepared from **4i** using the method employed for the synthesis of **1y**. A 9% yield of **1n** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.6 (bs, 1H, NH), 7.64 (d, 1H, ArH), 7.42 (t, 1H, ArH), 7.25 (d, 1H, ArH), 7.15 (t, 1H, ArH), 4.70 (s, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, alkyls), 1.60 (m, 2H, $CH_2$), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 272.0 (M + 1). Anal. ($C_{17}H_{21}NO_2$) C, H, N.

**6-Bromo-4-hydroxy-3-propyl-2(1*H*)-quinolinone (4j).** The title compound was prepared from **2c** and **3a** by the method outlined for the synthesis of **4a** in 58% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.4 (bs, 1H, OH), 10.2 (bs, 1H, NH), 7.95 (s, 1H, ArH), 7.54 (d, 1H, ArH), 7.15 (d, 1H, ArH), 2.5 (m,

overlapping with DMSO), 1.40 (m, 2H, $CH_2$), 0.86 (t, 3H, $CH_3$). MS (ES+): *m/z* 284.0 (M + 1).

**6-Bromo-4-(cyclopentyloxy)-3-propyl-2(1*H*)-quinolinone (1o).** The title compound was prepared from **4j** using the method employed in the synthesis of **1y**. A 7% yield of **1o** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.70 (s, 1H, ArH), 7.60 (d, 1H, ArH), 7.21 (d, 1H, ArH), 4.69 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, alkyls), 1.60 (m, 2H, $CH_2$), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 352.0 (M + 1). Anal. ($C_{17}H_{20}BrNO_2 \cdot 0.25C_6H_{14}$) C, H, N.

**4-Cyclopentyloxy-6-fluoro-3-propyl-2(1*H*)-quinolinone (1p).** The title compound was prepared from **4d** and cyclopentyl bromide by the method used in the synthesis of **1y** except the oil bath temperature was 50 °C. A 22% yield of **1p** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.7 (bs, 1H, NH), 7.32 (m, 3H, ArH), 4.70 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, alkyls), 1.60 (m, 2H, $CH_2$s), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 290.0 (M + 1).

**6-Chloro-4-(cyclopentyloxy)-3-propyl-2(1*H*)-quinolinone (1q).** Starting from **4a** and using the procedure described for the synthesis of **1y**, compound **1q** (20% yield) was obtained after chromatography on silica gel (4 by 7 cm column) eluted with hexane/EtOAc (2:1, v/v). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.56 (s, 1H, ArH), 7.49 (d, 1H, ArH), 7.28 (d, 1H, ArH), 4.70 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, alkyls), 1.60 (m, 2H, $CH_2$s), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 306.0 (M + 1). Anal. ($C_{17}H_{20}ClNO_2$) C, H, N, Cl.

**6-Chloro-3-ethyl-4-hydroxyl-2(1*H*)-quinolinone (4k).** The title compound was prepared from **2a** and **3c** by the method outlined for the synthesis of **4a** in 69% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.4 (bs, 1H, OH), 10.2 (bs, 1H, NH), 7.82 (s, 1H, ArH), 7.44 (d, 1H, ArH), 7.21 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 0.97 (t, 3H, $CH_3$). MS (ES+): *m/z* 224.0 (M + 1).

**6-Chloro-4-(cyclopentyloxy)-3-ethyl-2(1*H*)-quinolinone (1r).** The title compound was prepared from **4k** and cyclopentyl bromide using the method employed for the synthesis of **1y**. A 12% yield of **1r** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.62 (s, 1H, ArH), 7.5 (d, 1H, ArH), 7.33 (d, 1H, ArH), 4.8 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.9 (m, 6H, alkyls), 1.60 (m, 2H, $CH_2$s), 1.12 (t, 3H, $CH_3$). MS (ES+): *m/z* 292.0 (M + 1).

**6-Chloro-4-hydroxyl-3-isobutyl-2(1*H*)-quinolinone (4l).** The title compound was prepared from **2a** and **3e** by the method outlined for the synthesis of **4a** except the reaction was run for 72 h. A 51% yield of **4l** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.4 (bs, 1H, OH), 10.1 (bs, 1H, NH), 7.83 (s, 1H, ArH), 7.44 (d, 1H, ArH), 7.22 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.86 (m, 1H, CH), 0.82 (m, 6H, $CH_3$). MS (ES+): *m/z* 252.0 (M + H).

**6-Chloro-4-(cyclopentyloxy)-3-isobutyl-2(1*H*)-quinolinone (1s).** The title compound was prepared from **4l** using the method employed for the synthesis of **1y**. A 13% yield of **1s** was obtained. $^1$H NMR ($CDCl_3$) $\delta$ 10.4 (bs, 1H, NH), 7.70 (s, 1H, ArH), 7.35 (d, 1H, ArH), 7.15 (d, 1H, ArH), 4.73 (m, 1H, OCH), 2.60 (d, 2H, $CH_2$), 2.08−1.54 (m, 9H, alkyls), 0.90 (t, 6H, $CH_3$). MS (ES+): *m/z* 320.0 (M + 1). Anal. ($C_{18}H_{22}ClNO_2 \cdot 0.05CHCl_3$) C, H, N.

**3-(*sec*-Butyl)-6-chloro-4-hydroxyl-2(1*H*)-quinolinone (4m).** The title compound was prepared from **2a** and **3f** by the method outlined for the synthesis of **4a** in 35% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.3 (bs, 1H, OH), 10.0 (bs, 1H, NH), 7.87 (s, 1H, ArH), 7.44 (d, 1H, ArH), 7.22 (d, 1H, ArH), 3.12 (m, 1H, CH), 1.87 (m, 1H, CH), 1.6 (m, 1H, CH), 1.2 (d, 3H, $CH_3$), 0.7 (m, 3H, $CH_3$). MS (ES+): *m/z* 252.0 (M + H).

**3-(*sec*-Butyl)-6-chloro-4-(cyclopentyloxy)-2(1*H*)-quinolinone (1t).** The title compound was prepared from **4m** and cyclopentyl bromide using the method employed for the synthesis of **1y**. A 2% yield of **1t** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.6 (bs, 1H, NH), 7.56 (s, 1H, ArH), 7.49 (d, 1H, ArH), 7.28 (d, 1H, ArH), 4.60 (m, 1H, OCH), 3.0 (m, 1H, CH), 1.80 (m, 8H, $CH_2$), 1.60 (m, 2H, $CH_2$), 1.25 (d, 3H, $CH_3$), 0.73 (t, 3H, $CH_3$). MS (ES+): *m/z* 320.0 (M + 1).

**4-Hydroxy-6-methyl-3-propyl-2(1*H*)-quinolinone (4n).** The title compound was prepared from **2g** and **3a** by the method outlined for the synthesis of **4a** in 75% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.3 (bs, 1H, OH), 9.8 (bs, 1H, NH), 7.62 (s, 1H, ArH), 7.21 (d, 1H, ArH), 7.09 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 2.31 (s, 3H, $CH_3$), 1.40 (m, 2H, $CH_2$), 0.86 (t, 3H, $CH_3$). MS (ES+): *m/z* 218.0 (M + 1).

**4-Cyclopentyloxy-6-methyl-3-propyl-2(1*H*)-quinolinone (1u).** The title compound was prepared from **4n** using the method employed for the synthesis of **1y** except the reaction was heated for 2 h and a second portion of NaH (0.025 g, 0.50 eq) was added. The reaction was heated overnight. NaH (0.025 g, 0.50 equiv) was added again and heating at 60 °C was continued another 4 h. Purification was accomplished by chromatography on silica gel eluted with $CHCl_3$/MeOH (98:2, v/v) resulting in **1u** (2% yield). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.5 (bs, 1H, NH), 7.41 (s, 1H, ArH), 7.24 (d, 1H, ArH), 7.15 (d, 1H, ArH), 4.70 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 2.32 (s, 3H, $CH_3$), 1.9−1.2 (m, 12H, $CH_2$s), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 286.0 (M + 1).

**4-Hydroxy-6-methoxy-3-propyl-2(1*H*)-quinolinone (4o).** The title compound was prepared from **2h** and **3a** by the method outlined for the synthesis of **4a** in 72% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.2 (bs, 1H, OH), 9.9 (bs, 1H, NH), 7.38(s, 1H, ArH), 7.19 (d, 1H, ArH), 7.1 (d, 1H, ArH), 3,80 (s, 3H, $OCH_3$), 2.5 (m, overlapping with DMSO), 1.40 (m, 2H, $CH_2$), 0.92 (t, 3H, $CH_3$). MS (ES+): *m/z* 234.0 (M + 1).

**4-Cyclopentyloxy-6-methoxy-3-propyl-2(1*H*)-quinolinone (1v).** The title compound was prepared from **4o** using the method employed for the synthesis of **1y** except the reaction was heated in a 100 °C oil bath for 24 h. A 16% yield of **1v** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.5 (bs, 1H, NH), 7.20 (s, 1H, ArH), 7.10 (d, 1H, ArH), 7.06 (d, 1H, ArH), 4.70 (m, 1H, OCH), 3.75 (s, 3H, $OCH_3$), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, $CH_2$s), 1.60 (m, 2H, $CH_2$s), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 302.0 (M + 1).). Anal. ($C_{18}H_{23}NO_3$) C, H, N.

**4-Hydroxy-3-propyl-6-trifluoromethoxy-2(1*H*)-quinolinone (4p).** The title compound was prepared from **2i** and **3a** by the method outlined for the synthesis of **4a** in 40% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.5 (bs, 1H, OH), 10.3 (bs, 1H, NH), 7.8 (s, 1H, ArH), 7.5 (d, 1H, ArH), 7.3 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.40 (m, 2H, $CH_2$), 0.92 (t, 3H, $CH_3$). MS (ES+): *m/z* 288.0 (M + 1).

**4-(Cyclopentyloxy)-3-propyl-6-trifluoromethoxy-2(1*H*)-quinolinone (1w).** The title compound was prepared from **4p** using the method employed in the synthesis of **1y**. **1w** was obtained in 9% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.50 (s, 1H, ArH), 7.46 (d, 1H, ArH), 7.34 (d, 1H, ArH), 4.68 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, $CH_2$s), 1.60 (m, 2H, $CH_2$s), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (ES+): *m/z* 356.0 (M + 1). Anal. ($C_{18}H_{20}F_3NO_3 \cdot 0.05CHCl_3$) C, H, N, F.

**4-Hydroxy-2-oxo-3-propyl-1,2-dihydro-6-quinolinecarbonitrile (4q).** The title compound was prepared from **2j** and **3a** by the method outlined for the synthesis of **4a** in 77% yield. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.7 (bs, 1H, OH), 10.5 (bs, 1H, NH), 8.23 (s, 1H, ArH), 7.77 (d, 1H, ArH), 7.33 (d, 1H, ArH), 2.5 (m, overlapping with DMSO), 1.4 (m, 2H, $CH_2$), 0.87 (t, 3H, $CH_3$). MS (ES+): *m/z* 229.0 (M + 1).

**4-Cyclopentyloxy-2-oxo-3-propyl-1,2-dihydro-6-quinolinecarbonitrile (1x).** The title compound was prepared from **4q** using the method employed in the synthesis of **1y**. A 15% yield of **1x** was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 12.0 (bs,1H, NH), 8.00 (s, 1H, ArH), 7.82 (d, 1H, ArH), 7.37 (d, 1H, ArH), 4.74 (m, 1H, OCH), 2.5 (m, overlapping with DMSO), 1.80 (m, 6H, $CH_2$s), 1.60 (m, 2H, $CH_2$s), 1.50 (m, 2H, $CH_2$), 0.88 (t, 3H, $CH_3$). MS (APCH-): *m/z* 295.0 (M-1). Anal. ($C_{18}H_{20}N_2O_2$) C, H, N.

**6-Chloro-4-(cyclohexyloxy)-3-isopropyl-2(1*H*)-quinolinone (1z).** The title compound was prepared from **4f** by the method outlined for the synthesis of **1y**. $^1$H NMR ($Me_2SO$-$d_6$)

$\delta$ 11.6 (bs, 1H, NH), 7.59 (s, 1H, ArH), 7.46 (d, 1H, ArH), 7.25 (d, 1H, ArH), 3.95 (m, 1H, CH), 1.95 (m, 1H, CH), 1.60 (d, 6H, $CH_3$), 1.28−1.20 (m, 10H, alkyls). MS (ES+): *m/z* 320.0 (M + 1).

**6-Chloro-4-(cyclohexylmethoxy)-3-propyl-2(1*H*)-quinolinone (1aa).** The title compound was prepared from **4a** by the same method used in the synthesis of **1y** except the reaction was heated in a 50 °C oil bath for 9 h followed by heating in a 75 °C oil bath for 4 h. Following chromatography on silica gel (4 by 7 cm column) eluted with $CHCl_3$/MeOH (97:3, v/v), **1aa** (10% yield) was obtained. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.8 (bs, 1H, NH), 7.61 (s, 1H, ArH), 7.53 (d, 1H, ArH), 7.34 (d, 1H, ArH), 3.80 (m, 2H, $OCH_2$), 2.5 (m, overlapping with DMSO), 2.0−0.9 (m, 17H, alkyls). MS (ES+): *m/z* 334.0 (M + 1).

**6-Chloro-3-propyl-4-(2,2,2-trifluoroethoxy)-2(1*H*)-quinolinone (10g). 4a** (1.5 g, 6.3 mmol) was dissolved in DMF (12 mL) and treated with NaH (0.55 g of a 60% oil dispersion, 13 mmol, 2.1 equiv) at room temperature for 5 min. 2-Bromo-1,1,1-trifluoroethane (1.8 mL, 19.8 mmol) was added and the reaction heated in a 150 °C oil bath for 48 h. The solution was cooled to room temperature and poured onto ice−water (70 mL). The resulting precipitate was collected by filtration. The product was isolated by filtration through silica gel (50 g) eluted with EtOAc/hexane (3:7, v/v). The solvents were removed in vacuo and the residue slurried in $Et_2O$ to give **10g** (0.5 g, 25%). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.4 (bs, 1H, NH), 7.70 (s, 1H, ArH), 7.45 (d, 1H, ArH), 7.26 (d, 1H, ArH), 4.35 (q, 2H, $CH_2$), 2.66 (m, 2H, $CH_2$), 1.66 (m, 2H, $CH_2$), 1.01 (t, 3H, $CH_3$). MS (ES-): *m/z* 318.0 (M −1).

**6-Chloro-2-[(4-methoxybenzyl)oxy]-3-propyl-4-quinolinyl 2,2,2-trifluoroethyl Ether (13). 10g** (0.26 g, 0.80 mmol) was dissolved in DMF (3 mL). Silver(I) oxide (0.20 g, 0.86 mmol) was added followed by 4-methoxybenzyl chloride (0.135 mL,1.0 mmol) The reaction was heated in a 100 °C oil bath overnight. The oil bath temperature was raised to 145 °C for 1 h. Silver(I) oxide (0.1 g, 0.4 mmol) and 4-methoxybenzyl chloride (0.050 mL, 0.37 mmol) were added, the oil bath temperature was adjusted to 110 °C and the reaction was heated for 4 h. Silver(I) oxide (0.1 g, 0.4 mmol) and 4-methoxybenzyl chloride (0.050 mL, 0.37 mmol) were again added and the reaction was heated in the 145 °C oil bath for an additional 1.5 h. The reaction was cooled to room temperature and filtered. The filtrate was poured into water (25 mL), and the resulting precipitate was collected by filtration. Following filtration through silica gel (40 g) eluted with neat $CHCl_3$, **13** was obtained as white solid in 47% yield. GCMS (EI): *m/z* 439 ($M^+$), one peak.

**6-Chloro-4-[(2-cyclopropylethynyl)oxy]-2-[(4-methoxybenzyl)oxy]-3-propylquinoline (14). 13** (0.14 g, 0.33 mmol) was dissolved in ether (5 mL) and chilled to −78 °C. A solution of cyclopropyllithium was prepared by the reaction of cyclopropyl bromide (0.93 mL, 12 mmol) with lithium wire (0.16 g, 23 mmol) in ether (10 mL) in a 0 °C bath for 1.5 h. Cyclopropyllithium solution (4.0 mL, 2.3 mmol) was added at −78 °C, and the reaction was allowed to warm to room temperature overnight. The reaction was washed with sat. $NH_4Cl$. The organic phase was dried with $MgSO_4$ and filtered and the solvent removed in vacuo. Following purification by chromatography on silica gel (4 × 7 cm column) eluted with $CHCl_3$/c-hexane (1:4, v/v), **14** was obtained in 32% yield. $^1$H NMR ($CDCl_3$) $\delta$ 8.06 (s, 1H, ArH), 7.78 (d, 1H, ArH), 7.55 (d, 1H, ArH), 7.43 (d, 2H, ArH), 6.91 (d, 2H, ArH), 5.47 (s, 2H, CH2), 3.82 (s, 3H, $OCH_3$), 2.84 (m, 2H, $CH_2$), 1.60 (m, 2H, $CH_2$), 1.15 (m, 1H, CH), 0.97 (t, 3H, $CH_3$), 0.65 (m, 2H, $CH_2$), 0.55 (m, 2H, $CH_2$). GCMS (CI+): *m/z* 439.0 (M + $NH_4$).

**6-Chloro-4-[(2-cyclopropylethynyl)oxy]-3-propyl-2(1*H*)-quinolinone (1dd). 12** (0.04 g, 0.1 mmol) was dissolved in $CH_3CN$ (20 mL). A 10-mL aqueous solution of ammonium cerium(IV) nitrate (0.055 g, 0.10 mmol) was then added to the mixture. An immediate precipitate formed. Acetonitrile (20 mL) was added, along with ammonium cerium(IV) nitrate (0.005 g, 0.01 mmol). **1dd** (0.006 g, 20%) precipitated out and was collected by filtration. $^1$H NMR ($CDCl_3$) $\delta$ 11.9 (bs, 1H, NH), 7.90 (s, 1H, ArH), 7.69 (d, 1H, ArH), 7.45 (d, 1H, ArH), 2.81 (m, 2H, $CH_2$), 1.67 (m, 2H, $CH_2$), 1.2 (m, 1H, CH), 1.0 (m, 3H, $CH_3$), 0.65 (m, 2H, $CH_2$), 0.55 (m, 2H, $CH_2$). MS (ES+): *m/z* 302.0 (M + 1).

**6-Fluoro-3-isopropyl-4-(2,2,2-trifluoroethoxy)-2(1*H*)-quinolinone (10c).** The title compound was prepared from **4e** using the method described for the synthesis of **10g**. $^1$H NMR ($CDCl_3$) $\delta$ 12.6 (bs, 1H, NH), 7.42−7.35 (m, 2H, ArH), 7.28−7.23 (m, 1H, ArH), 4.31 (q, 2H, $CH_2$), 3.4 (m, 1H, CH), 1.4 (d, 6H, $CH_3$). MS (ES+): *m/z* 304.0 (M + 1).

**2-[*tert*-Butyl(dimethyl)silyl]oxy-6-fluoro-3-isopropyl-4-(2,2,2-trifluoroethoxy)quinoline (11c). 10c** (0.92 g, 3.0 mmol) was dissolved in DMF (30 mL). *tert*-butyldimethylsilyl chloride (0.95 g, 6.0 mmol) was added followed by $Et_3N$ (1.6 mL, 10.5 mmol). The reaction was stirred at room temperature for 2.5 h. The mixture was poured into ice−water (250 mL). The product was extracted with ether. The solution was dried with $MgSO_4$, filtered, and the solvent removed in vacuo to give **11c** (1 g, 80%). $^1$H NMR ($CDCl_3$) $\delta$ 7.70 (dd, 1H, ArH), 7.48 (dd, 1H, ArH), 7.32 (dd, 1H, ArH), 4.33 (q, 2H, $CH_2$), 3.55 (m, 1H, CH), 1.4 (d, 6H, $CH_3$), 1.06 (s, 9H, Si-t-Bu), 0.43 (s, 6H, Si−$CH_3$). MS (CI+): *m/z* 418.0 (M + 1).

**4-[(2-Cyclopropylethynyl)oxy]-6-fluoro-3-(isopropyl)-2(1*H*)-quinolinone (1ee). 11c** (0.30 g, 0.75 mmol) was dissolved in EtOAc/$CH_3CN$ (10 mL, 1:1, v/v). *tert*-butylammonium fluoride hydrate (0.20 g, 1.1 mmol) was added. The reaction was stirred at room temperature for 5 min. Sat. NaCl solution was added and the product extracted with EtOAc. The product was isolated by chromatography on silica gel (4 × 7 cm column) eluted with EtOAc/hexane (1:2, v/v). Final purification was accomplished by chromatography on C18 (Water's Symmetry C18 column, 19 × 150 mm) eluted with MeOH/water (3:2, v/v) to give **1ee** (0.043 g, 20%). $^1$H NMR ($CDCl_3$) $\delta$ 11.6 (bs, 1H, NH), 7.61 (d, 1H, ArH), 7.58−7.22 (m, 2H, ArH), 3.60 (m, 1H, CH), 1.4 (d, 6H, $CH_3$), 1.15 (m, 1H, CH), 0.68 (m, 2H, $CH_2$), 0.56 (m, 2H, $CH_2$). MS (ES+): *m/z* 286.0 (M + 1). Anal. ($C_{17}H_{16}FNO_2$·0.25 $H_2O$) C, H, N, F.

**4-[(2-Cyclopropylethynyl)oxy]-6-fluoro-3-propyl-2(1*H*)-quinolinone (1cc).** The title compound was prepared from **4d** according to the method described for compound **1ee**. $^1$H NMR ($CDCl_3$) $\delta$ 10.9 (bs, 1H, NH), 7.59 (d, 1H, ArH), 7.25 (m, 2H, ArH), 2,78 (m, 2H, $CH_2$), 1.66 (m, 2H, $CH_2$), 1.19 (m, 1H, CH), 1.04 (t, 3H, $CH_3$), 0.68 (m, 2H, $CH_2$), 0.56 (m, 2H, $CH_2$). MS (ES+): *m/z* 286.0 (M + 1).). Anal. ($C_{17}H_{16}FNO_2$) C, H, N.

**4-[(2-Cyclopropylethynyl)oxy]-3-ethyl-6-fluoro-2(1*H*)-quinolinone (1bb).** In a round-bottom flask equipped with a stir bar and nitrogen on demand, **12a** (0.2 g, 0.5 mmol) was dissolved in anhydrous $Et_2O$ (6 mL) and cooled to −78 °C by means of a dry ice/acetone bath. Cyclopropyllithium (3.5 mL of a 0.50M solution in $Et_2O$, 1.76 mmol) was added dropwise, and the mixture was allowed to stir at −78 °C for 1h and was then allowed to warm to room temperature. When judged to be complete, the reaction mixture was quenched with sat. $NH_4Cl$, and the layers were separated. The organic layer was dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The remaining residue was dissolved in THF and acidic resin (BioRad, AG 50W−X4, 200−400 mesh, hydrogen form) was added to the mixture. When the deprotection was judged to be complete, the reaction was filtered and concentrated in vacuo. The crude material was purified by column chromatography, eluding with 3:1 hexanes/EtOAc to afford **1bb** (18 mg, 10%) as a white solid. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 12.11 (s, 1H), 7.41 (m, 3H), 2.68 (q, 2H), 1.25 (m, 1H), 1.15 (t, 3H), 0.76 (m, 2H), 0.55 (m, 2H). MS (ES) 270 (M−H). Anal. ($C_{16}H_{14}FNO_2$) C, H, N.

**6-Chloro-4-[(2-Cyclopropylethynyl)oxy]-3-isopropyl-2(1*H*)-quinolinone (1ff). 10d** (108 mg, 0.25 mmol), prepared from **4f** by the method used to make **10g**, was treated as previously described in examples **1bb** to give the crude product. Purification was accomplished by flash chromatography on silica gel (20% EtOAc/hexanes) to give **1ff** (10 mg, 9%) as a white solid. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 12.47 (s, 1H), 7.92 (d, 1H), 7.47 (m, 1H), 7.33 (d, 1H), 3.64 (m, 1H), 1.45

(d, 6H), 1.18 (m, 1H), 0.71 (m, 2H), 0.59 (m, 2H). MS (ES-) 300 $(M - H)^-$.

**6-Fluoro-4-hydroxy-3-isobutyl-2(1*H*)-quinolone (4r).** The title compound was prepared according to the method of Cai et al. (Cai, S. X.; Zhou, Z.; Haung, J.; Whittemore, E. R.; Egbuwoku, Z. O.; Lu, Y.; Hawkinson, J. E.; Woodward, R. M.; Weber, E.; Keana, J. F. W. *J. Med. Chem.* **1996**, *39*, 3248−3255): **2b** (15.87 g, 143 mmol) and **3e** (71.06 g, 329 mmol) were heated to 180 °C. The EtOH produced was collected in a Dean−Stark trap (∼ 5 mL). After 6 h, the solution was cooled to room temperature and a precipitate formed. The mixture was combined with MeOH (80 mL), water (400 mL), and $Na_2CO_3$ (52 g) and heated to reflux for 1 h. Incomplete hydrolysis was observed. The mix was neutralized with 2 N HCl and filtered. The resulting solid was treated with 2 N LiOH (200 mL) and THF (200 mL) with stirring for 48 h. The THF was removed in vacuo, and a precipitate was observed. The solid was filtered, the filtrate was acidified to pH 1 with concentrated HCl and the resulting solid filtered. The solid was treated with polyphosphoric acid (300 mL) at 140 °C for 3 h and then cooled to room temperature. Aqueous HCl (1 N, 400 mL) was added and stirred for 4 h. The pH was adjusted to 4 with 20% NaOH. The resulting solid was filtered and dried in vacuo overnight to give **4r** (21 g, 62%). $^1$H NMR ($Me_2SO$-$d_6$, 400 MHz) $\delta$ 11.30 (s, 1H), 7.58 (q, 1H), 7.30 (m, 1H), 7.22 (m, 1H), 2.43 (d, 2H), 1.87 (m, 1H), 0.83 (d, 6H). MS (ES-) 234 $(M-H)^-$.

**6-Fluoro-3-isobutyl-4-(2,2,2-trifluoroethyl)-2(1*H*)-quinolone (10e).** 6-Fluoro-4-hydroxy-3-isobutyl-2(1*H*)-quinolone (**4r**) (5 g, 21 mmol) was alkylated according to the procedure described for the synthesis of **10g** to give **10e** (634 mg, 9.5%) as a white powder. $^1$H NMR ($CDCl_3$) $\delta$ 7.30 (m, 3H), 4.36 (q, 2H), 2.61 (d, 2H), 2.13 (m, 1H), 0.97 (d, 6H). MS (ES+) 318 $(M + H)^+$.

**4-[(2-Cyclopropylethynyl)oxy]-6-fluoro-3-isobutyl-2(1*H*)-quinolinone (1gg).** Compound **10e** was protected according to the procedure described for **11a** to give **11e** as a partially pure oil after flash chromatography (2% EtOAc/hexane, neutral alumina). Without further purification, **10e** was converted to **1gg** following the procedure described for the synthesis of **1ee**. Flash chromatography (20% EtOAc/hexane, neutral alumina), afforded compound **1gg** (17 mg, 10%) as a white solid. $^1$H NMR ($CDCl_3$) $\delta$12.45 (s, 1H), 7.65 (dd, 1H), 7.40 (dd, 1H), 7.29 (m, 1H), 2.76 (d, 2H), 2.13 (quint, 1H), 1.18 (m, 1H), 1.00 (d, 6H), 0.71 (m, 2H), 0.59 (m, 2H). MS (ES+) 300 $(M + H)^+$. ($C_{18}H_{18}FNO_2 \cdot 0.3H_2O$) C, H, N, F.

**6-Fluoro-3-isopropyl-4-[(3-methylpent-1-ynyl)oxy]-2(1*H*)-quinolinone (1hh).** Starting with **4e** and following the procedure described for the synthesis of **1ee**, compound **1hh** was obtained. $^1$H NMR ($CDCl_3$) $\delta$ 12.1 (bs, 1H, NH), 7.68 (d, 1H, ArH), 7.38−7.26 (m, 2H, ArH), 3.68 (m, 1H, CH), 2.33 (m, 1H), 1.4 (m, 8H), 1.12 (m, 3H, $CH_3$), 0.92 (m, 3H, $CH_3$). MS (ES+): *m/z* 302.0 (M + 1).

**3-Cyano-4-hydroxy-2(1*H*)-quinolone (4s).** Isatoic anhydride (**7**) (1.63 g, 10 mmol), ethyl cyanoacetate (**8**) (1 mL, 10 mmol) and $Et_3N$ (3 mL, 20 mmol) were mixed and heated in 20 mL of DMF at 150 °C for 18 h. The reaction mixture was concentrated in vacuo followed by the addition of 1 N HCl. Precipitate was collected by filtration, washed with $H_2O$ and dried. This resulted in **4s** (1.52 g, 82%). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 11.7 (s, 1H, OH), 8 (d, 1H, ArH), 7.6 (t, 1H, ArH), 7.25 (d, 1H, ArH), 7.2 (t, 1H). MS (ES) 185 (M − H).

**3-Cyano-4-(cyclobutylmethoxy)-2(1*H*)-quinolinone (1k).** The title compound was prepared from **4s** by the method used in the synthesis of **1y. 1k** (5% yield) was isolated by chromatography on silica gel eluted with MeOH/EtOAc (1:9). $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 7.9 (d, 1H, ArH), 7.4 (t, 1H, ArH), 7.2 (d, 1H, ArH), 6.95(t, 1H), 4.1 (d, 2H, $CH_2$), 2.5−2.7 (m, 1H, CH), 1.6−1.9 (m, 6H, cyclobutyl). MS (ES) 185 (M−H).

**Preparation of Ethoxyacetyl Chloride.** Ethoxyacetic acid was reacted neat with thionyl chloride, 1.7 equiv, at room temperature for 1 h followed by heating in a 70 °C oil bath for 30 min. The product was purified by distillation at 120 °C at room pressure from a 180 °C oil bath.

**Table 3.** Statistics for Crystallographic Structure Determination of the RT−GW490745X Complex

| | |
|---|---|
| data collection site | PF BL-6A |
| detector | Fuji BAS III |
| wavelength (Å) | 1.000 |
| unit cell dimensions (*a, b, c* in Å) | 138.2, 109.7, 72.8 |
| resolution range (Å) | 30.0−2.85 |
| observations | 112938 |
| unique reflections | 26233 |
| completeness (%) | 99.1 |
| average $I/\sigma(I)$ | 6.4 |
| $R_{merge}$[a] | 0.163 |
| Outer Resolution Shell | |
| resolution range (Å) | 2.95−2.85 |
| unique reflections | 2527 |
| completeness (%) | 98.0 |
| average $I/\sigma(I)$ | 0.9 |
| Refinement Statistics | |
| Resolution range (Å) | 30.0−2.85 |
| No. of reflections(working/test) | 24824/1260 |
| *R*-factor[b] ($R_{work}/R_{free}$) | 0.220/0.285 |
| *R*-factor[b] (all data) | 0.213 |
| no. atoms (protein/inhibitor) | 7725/21 |
| rms bond length deviation (Å) | 0.0077 |
| rms bond angle deviation (deg) | 1.42 |
| mean *B*-factor (Å$^2$)[c] | 66/71/46 |
| rms backbone *B*-factor deviation (Å$^2$) | 3.8 |

[a] $R_{merge} = \sum|I - \langle I\rangle|/\sum\langle I\rangle$; [b] *R*-factor $= \sum|F_o - F_c|/\sum F_o$; [c] Mean *B* factor for main-chain, side-chain and inhibitor.

**Crystallization, Data Collection and Structure Determination.** Crystals of the RT−GW490745 complex were grown from solutions containing RT at 26 mg/mL with a 1:1.1 molar ratio of compound, 6% PEG 3400, buffered with citrate/phosphate, pH 5.0, using the sitting-drop method as described previously.[23] The crystals were partially dehydrated by increasing the PEG concentration to 50% over 3 days prior to data collection. X-ray data were collected at the Photon Factory, KEK, Japan, at 100 K using a Weissenberg camera.[24] Data frames of 3.5° with a coupling constant 1.5°/mm were collected with an exposure time of 210 s. Indexing and integration of data images were carried out with DENZO, and data were merged with SCALEPACK.[25]

The orientation and position of the RT molecule in the cell were determined using rigid-body refinement with an initial model of RT−MKC442 (PDB code 1rt1,).[26] Rounds of positional, simulated annealing and individual *B*-factor refinement with bulk solvent correction and model rebuilding resulted in the current structure with *R*-factor of 0.213 for all the data and rms deviations of 0.008 Å and 1.4° from ideality for bond lengths and bond angles, respectively. Both the bound inhibitor as well as the protein residues around it, have well-defined electron density (Figure 4a). Due to the limited reflections-to-parameters ratio, atoms distal to the NNRTI site (defined as >20 Å from the Cα of Y188) were tightly restrained to their positions after domain-wise rigid-body refinement. Refinement and model rebuilding were carried out with programs CNS[27] and O.[28] Table 3 shows the X-ray data and crystallographic refinement statistics.

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