



Published in final edited form as:

*J Med Chem.* 2015 June 11; 58(11): 4610–4623. doi:10.1021/acs.jmedchem.5b00159.

## **N-Substituted Quinolinonyl Diketo Acid Derivatives as HIV Integrase Strand Transfer Inhibitors and Their Activity against RNase H Function of Reverse Transcriptase**

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### **Abstract**

Bifunctional quinolinonyl DKA derivatives were first described as nonselective inhibitors of 3'-processing (3'-P) and strand transfer (ST) functions of HIV-1 integrase (IN), while 7-aminosubstituted quinolinonyl derivatives were proven IN strand transfer inhibitors (INSTIs) that also displayed activity against ribonuclease H (RNase H). In this study, we describe the design, synthesis, and biological evaluation of new quinolinonyl diketo acid (DKA) derivatives characterized by variously substituted alkylating groups on the nitrogen atom of the quinolinone ring. Removal of the second DKA branch of bifunctional DKAs, and the amino group in position 7 of quinolinone ring combined with a fine-tuning of the substituents on the benzyl group in

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Author Contributions

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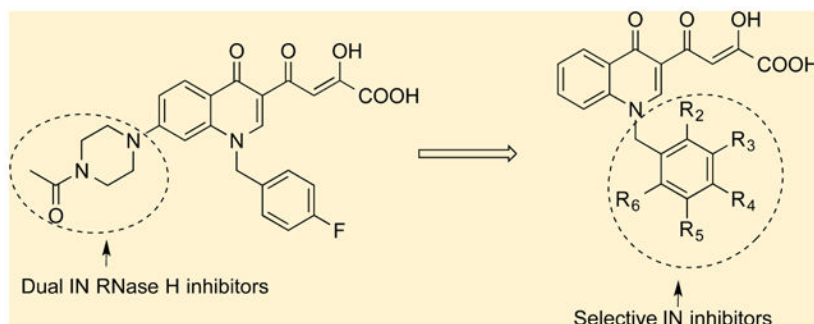
Supporting Information

Chemical, physical and spectroscopic data of compounds **12a–c**, **13a–aa**, analyses of compounds **10a–aa** **11a–aa**, and molecular modeling. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00159.

The authors declare no competing financial interest.

position 1 of the quinolinone, increased selectivity for IN ST activity. In vitro, the most potent compound was **11j** ( $IC_{50} = 10$  nM), while the most active compounds against HIV infected cells were ester derivatives **10j** and **10l**. In general, the activity against RNase H was negligible, with only a few compounds active at concentrations higher than  $10 \mu M$ . The binding mode of the most potent IN inhibitor **11j** within the IN catalytic core domain (CCD) is described as well as its binding mode within the RNase H catalytic site to rationalize its selectivity.

## Graphical Abstract



## INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) integrase (IN) and reverse transcriptase (RT) are two enzymes encoded by the *pol* gene and are essential for virus replication. IN mediates insertion of the double-stranded DNA provirus into the host genome, while RT synthesizes this DNA using viral RNA as a template and degrading RNA of the RNA–DNA replication intermediate in the process. As such, both events have been desirable therapeutic targets.<sup>1</sup>

The role of IN during HIV-1 replication begins in the cytoplasm after completion of reverse transcription. The resulting DNA double-stranded DNA is subjected to two reactions. The first of these involves removing of the 3'-terminal GT dinucleotides adjacent to the highly conserved CA at 3'-end creating reactive 3'-OH ends in both strands of the viral DNA and is termed 3'-processing (3'-P). During this step, IN, viral DNA, and both host (barrier to autointegration factor (BAF), heat-shock protein 60 (HSP60), high mobility group protein (HMG), lens epithelium-derived growth factor (LEDGF))<sup>2</sup> and viral (matrix, viral protein R (Vpr), p7/nucleocapsid and RT)<sup>3</sup> proteins form a preintegration complex (PIC), which is transported into the nucleus. This is followed by a strand transfer (ST) step, involving a concerted nucleophilic attack by the two reactive 3'-OH recessed ends of the viral DNA (DNA donor), formed during 3'-P, on the host DNA (DNA acceptor). The gap formed after the nucleophilic attack between the 5'-end of DNA donor and 3'-end of DNA acceptor is filled probably by the cellular DNA repair enzymes, finishing the integration process.

Integration is the final step before irreversible and productive HIV-1 infection of the target cell.<sup>4</sup> Although identified as an attractive target more than 12 years ago,<sup>5</sup> the first drug targeting IN (raltegravir, **1**) was approved by the Food and Drug Administration (FDA) (Figure 1) only in late 2007<sup>6</sup> as part of combination antiretroviral therapy.

RT is the most actively and longest studied among the enzymes encoded by the *pol* gene, displaying both DNA polymerase and ribonuclease H (RNase H) activities, the latter catalyzing degradation of RNA of the RNA–DNA hybrid intermediate to permit the synthesis of (+) strand DNA. Although RT is a multifunctional enzyme, all approved nucleoside and non-nucleoside RT inhibitors target only its DNA polymerase function. IN and the RNase H domain of RT belong to a broader class of polynucleotidyl transferases<sup>7</sup> and share structural similarities.<sup>8</sup> Both enzymes require Mg<sup>2+</sup> for catalysis, and the metal ion is chelated by a highly conserved motif consisting of three acidic amino acid residues, also referred to as DDE motif. Within the IN catalytic core, this motif is represented by the three conserved acidic residues D64, D116, and E152 involved in the coordination of the two catalytic Mg<sup>2+</sup> ions.<sup>9</sup> In the RNase H domain, this motif comprises D443, E478, D498, and D549 amino acids which also chelate two magnesium ions.<sup>10</sup>

Given the structural similarities between the catalytic domains of these HIV-1 enzymes, several compounds initially developed as IN inhibitors, including diketo acids (DKAs) **2** and **3** (Figure 1)<sup>11</sup> and DNA aptamers<sup>12</sup> have also been screened against RNase H. Recently, three structurally different compounds have been described as dual inhibitors: tropolone **4**,<sup>13</sup> 2-hydroxyisoquinolin-1,3(2*H*,4*H*)-dione **5**,<sup>14</sup> and madurahydroxylactone **6**<sup>15</sup> (Figure 1).

Each one of these classes of inhibitors characteristically presents three aligned heteroatoms capable of binding divalent metal ions.

Recently, quinolinonyl diketo acid-derived HIV-1 IN inhibitors were described.<sup>16</sup> First bifunctional DKA **7** (Figure 2) was reported as nonselective 3'-P and ST inhibitor, interacting with both the acceptor and donor DNA binding sites of the enzyme. Indeed, 3'-P was inhibited with an IC<sub>50</sub> of 0.20 μM, in comparison with ST reaction that was inhibited with IC<sub>50</sub> of 0.012 μM. This compound was modified to obtain a series of quinolinonyl diketo acid derivatives in which the 6-diketo acid chain responsible of 3'-P inhibition was removed, providing selective ST inhibitors. In particular, compound **8** showed good inhibitory activity with an IC<sub>50</sub> value of 33 nM with no activity against 3'-P step up to a 50 μM concentration (Figure 2). The proven capability of the DKA chain to chelate the catalytically critical metal ions made these potential inhibitors of both enzymes.<sup>17</sup>

In recent years, a number of IN inhibitors have been tested against RNase H function, and recently we reported derivatives of **9** as IN inhibitors endowed with a moderate activity against RNase H.<sup>18</sup> However, the amino function that was introduced in that series does not increase either the potency against the IN enzyme or the activity against RNase H. Thus, we decided to design new quinolinone derivatives to better understand the structure–activity relationships within this series of inhibitors. In particular, we describe herein the design, synthesis, and the biological evaluation of a series of quinolinonyl DKA derivatives **10a–aa** and **11a–aa** that are characterized by a variously substituted benzyl group or by an aryl or heteroaryl alkyl moiety on nitrogen atom of the quinolinone ring (Figure 3). In fact, the benzyl portion of IN strand transfer inhibitors (INSTIs) is known to be relevant for the binding of these inhibitors to the IN/DNA complex. Thus, a study of its influence on the activity of this series of quinolinonyl DKAs against IN and its role on the inhibition of

RNase H function is vital. Moreover, the role of the arylmethyl group linked on quinolonyl nitrogen was not previously studied within this series of inhibitors.

## RESULT AND DISCUSSION

### Chemistry.

Compounds **10a–aa** and **11a–aa** were synthesized according to the pathway reported in Scheme 1. 3-Acetyl-4(1*H*)-quinolinones **12a–c** were prepared by reaction of aniline with ethyl orthoformate and ethyl acetoacetate, which were thermally condensed in the presence of an inert heating medium (Dowtherm A) under argon atmosphere, according to the Yoshizawa procedure.<sup>19</sup> *N*-1 substituted quinolones **13a–aa** were obtained via alkylation of compounds **12a–aa** with the proper aryl alkyl bromide in alkaline medium (K<sub>2</sub>CO<sub>3</sub>). Compounds **12a–aa** were then subjected to a Claisen condensation reaction with diethyl oxalate and sodium ethoxide as the catalyst to provide ethyl esters **10a–aa**, which were in turn hydrolyzed with 1 N NaOH to afford the corresponding acids **11a–aa** (Scheme 1). Chemical, physical, and spectroscopic data of final products **10a–aa** and **11a–aa** are described in the Experimental Section, while data of intermediates **12a–c**, **13a–aa** and analyses of **10a–aa** and **11a–aa** are reported in the Supporting Information.

### Evaluation of Biological Activities: In Vitro Assays.

Newly synthesized compounds **10a–aa** and **11a–aa** were tested in vitro with respect to RT-associated RNase H function as well as IN-associated 3'-P and ST activities. ST and 3'-P were evaluated using gel-based assay carried out in the presence of Mg<sup>2+</sup>, and the corresponding IC<sub>50</sub> values were calculated using a dose–response curves. The results of these assays are summarized in Tables 1 and 2.

Several of the newly synthesized compounds were potent IN inhibitors showing high activity against the ST reaction and low activity against 3'-P and RNase H function. As expected from previous findings, acid derivatives were always more active than the corresponding esters when tested against IN. Moreover, the *N*-benzyl substituted derivatives **11a–w** were more active than the *N*-arylalkyl substituted counterparts **11x–aa** on both IN and RNase H (Tables 1 and 2).

### Anti-IN Activities.

Acid derivatives **11a–w** were very active ST inhibitors, showing IC<sub>50</sub> values in the nanomolar range (640–10 nM), with the exception of **11k,o,q**, which exhibited IC<sub>50</sub> values in the micromolar range (3.2–7.0 μM).

Mono- (**11a–i**) and disubstituted (**11j–w**) benzyl derivatives showed comparable activities against IN. In fact, in the first case the IC<sub>50</sub> values were in the range of 0.64–0.015 μM, while in the second one, values were in the range of 0.22–0.010 μM.

The most active compound of the series was the 2,4-F<sub>2</sub> acid derivative **11j**, with an IC<sub>50</sub> value of 10 nM. Four additional compounds, the 2-F, 3-F, 2,6-F<sub>2</sub> and 2,3-F,Cl derivatives (**11b,d,l,s**), demonstrated comparable potencies (IC<sub>50</sub> values of 16, 15, 19, and 18 nM,

respectively). Interestingly, the fluorine substituent was best tolerated, particularly when bound in position 2 of the benzyl ring, both in mono and disubstituted-benzyl derivatives. This trend was highlighted in the series of the disubstituted benzyl derivatives, comparing the most active compound **11j** with the other compounds **11l–u**. In fact, when the fluorine atom was bound in position 2 of the benzyl ring, inhibitory activity increased even if there was a second substituent on the phenyl ring. For example, compounds **11l** (2,6-F<sub>2</sub>) and **11s** (2,3-F,Cl) showed inhibitory activities comparable to **11j**. When the fluorine atom in position 2 was replaced by a chlorine atom (**11o–p**) or a methyl group (**11q**), activity decreased by 1–2 orders of magnitude (IC<sub>50</sub> values of 2.3, 0.27, and 7.0 μM, respectively). An exception was the derivative **11v**, 2-fluorobenzyl substituted, characterized by a chlorine atom in 8-position of the aromatic ring of the quinolinonic nucleus. This derivative was 10 times less active than its parent 2-fluoro benzyl substituted counterpart **11b** (IC<sub>50</sub> values of 0.13 and 0.016 μM, respectively).

Among monosubstituted benzyl derivatives **11a–i**, replacing the fluorine atom in the 2-position with a methoxy group led to a decrease in the activity (**11c**, IC<sub>50</sub> value of 38 nM), as well as its substitution with an hydrogen atom (**11a**, IC<sub>50</sub> value of 34 nM). The shift of the fluorine atom from position 2 to 3 gave a compound that had the same inhibitory activity (**11d**, IC<sub>50</sub> value of 15 nM), while its shift to position 4 slightly decreased activity (**8**, IC<sub>50</sub> value of 40 nM). When the fluorine atom in position 4 was replaced by other atoms (**11f**) or group (**11g–i**), activity decreased by an order of magnitude (IC<sub>50</sub> values of 0.45–0.64 μM).

#### Anti-RH Activities.

The quinolinonyl diketo acid derivatives **10a–aa** and **11a–aa** were tested for RNase H inhibition, calculated as a percentage of inhibition at a concentration of 10 μM. IC<sub>50</sub> values have been determined for derivatives showing % of inhibition >30%.

In general, the ester derivatives **10a–aa** poorly inhibited the RNase H function at 10 μM, and in all cases the measured IC<sub>50</sub>s were always >50 μM. Although the acid counterparts were more effective, their IC<sub>50</sub> were poor compared to their anti-IN potency. The most active compounds were the acid derivatives **11g**, **11l**, and **11p**, showing IC<sub>50</sub> values of ~10 μM. Among the monosubstituted benzyl derivatives (**10a–i** and **11a–i**), substitution in position 4 was best tolerated. In particular, in the ester series, a nitro group (**10i**) increased the inhibitory activity of the molecule (31.9% inhibition); among this series, inhibitory activity decreased in the following order: NO<sub>2</sub> > OH > Cl > OCH<sub>3</sub> > F. In the acid series, increased activity was highlighted by the presence of a chlorine atom (**11f**, 56.2% inhibition); among this series, inhibitory activity decreased in the order: Cl > F > OCH<sub>3</sub> ≈ OH ≈ NO<sub>2</sub>.

Interestingly, compound **8** was tested as a representative example of the nonbasic quinolonyl DKA series previously reported<sup>16b</sup> and was confirmed weak inhibitor, showing IC<sub>50</sub> = 42 μM against recombinant RNase H.

The disubstituted benzyl derivatives **10j–w** and **11j–w** showed a good inhibitory activity when two chlorine atoms were the substituents of the benzyl moiety (**10o–p**, 51 and 55% inhibition, respectively; **11o–p**, 68 and 86.3% inhibition, respectively). In general, the

fluorine atom led to decreased activity, even then in the F<sub>2</sub>-benzyl and Cl,F-benzyl disubstituted derivatives (**10j–n** and **10s–u**, 3–24% inhibition; **11j–n** and **11s–u**, 30–57.9% inhibition), with respect to the most active acid compound 2,6-Cl<sub>2</sub>-disubstituted derivative **11p** (86.3% inhibition).

### Cell-Based Assays.

Newly synthesized compounds were tested for antiviral activity and cytotoxicity using HeLa-CD4-LTR- $\beta$ -gal cells infected by HIV-1(IIIB). Elvitegravir (EVG) and **1** were used as reference compounds. EC<sub>50</sub> and CC<sub>50</sub> values for compounds **10a–aa** and **11a–aa** are reported in Tables 1 and 2. Among these, derivatives **10a–e,g,j,l–n,p,s,t,w** and **11a–d,f,j,l,m,s,w,y** show EC<sub>50</sub> < 50  $\mu$ M. It is noteworthy that although less active in biochemical assays, esters **10a–e,g,j,l–n,p,s,t,w** and acids **11a–d,f,j,l,m,s,w,y** are almost equipotent in cell-based assays. In particular, the most active compounds are the ester derivatives **10j** and **10l** and acid **11f** that are active in the submicromolar range (EC<sub>50</sub> = 0.58, <0.2, and 0.87  $\mu$ M, respectively), with selectivity indices (SI) higher than 50 (>86.2, >250, >57.5, respectively). Notably, compound **10l** showed an increased antiviral activity if compared with quinolinonyl DKA reported in our previous papers.<sup>16,18</sup> All active compounds were characterized by low cytotoxicity against the same HeLa-CD4-LTR- $\beta$ -gal cells, showing CC<sub>50</sub> values >50  $\mu$ M, with the sole exception of **10e** showing a CC<sub>50</sub> of 44  $\mu$ M.

### Molecular Modeling.

To better rationalize structure–activity relationships (SARs) for the newly identified quinolinonyl DKAs, molecular docking studies were performed on compound **11j**, which is the most potent INSTI (10 nM) within this series while displaying insignificant activity against RNase H. Computations were aimed at elucidating the structural requisites responsible for binding at the IN active sites but with residual activity against RNase H.

Our recently built homology model of the IN catalytic core domain (CCD)/viral DNA was used to carry out docking studies.<sup>18</sup> This model was constructed starting from the coordinates of the full-length structure of prototype foamy virus (PFV) IN in complex with **1** (PDB code 3OYA) and two active site Mg<sup>2+</sup> ions required for both catalytic activity and chelation by INSTIs.<sup>21</sup> According to docking results, compound **11j** lies at the IN/DNA interface with the *O, O, O* donor triad of the DKA branch chelating the Mg<sup>2+</sup> ions, and the central hydroxyl anionic oxygen atom bridging between the two cations (Figure 4a). This results in formation of 5- and 6-membered chelate rings with the two metal centers. Interestingly, the 5-,6-membered chelate ring binding motif appears to be one of the most critical features of highly effective INSTIs, as underscored by recent theoretical studies on the role of the metal binding groups within this class of anti HIV-1 drugs.<sup>22</sup> Additionally, chelation by the carboxylate group might explain the higher inhibitory potency of acidic compounds with respect to the corresponding esters (see **10a–aa** vs **11a–aa**). Besides coordination of the metal centers, additional key interactions are established by our reference compound within the active site. In particular, the quinolinonyl ring establishes hydrophobic contacts with the C $\beta$  and C $\gamma$  carbons of Q148 and is involved in a parallel-displaced  $\pi$ – $\pi$  interaction with the terminal adenosine at the 3' end of the nucleic acid substrate, which is known to be displaced through an induced-fit mechanism.<sup>21</sup> Furthermore,



the *p*-fluorobenzyl group fits into a narrow pocket created by rearrangement of the aforementioned terminal adenosine and the enzyme surface. Herein, the *p*-F-benzyl branch establishes a  $\pi$ -stacking interaction with the penultimate cytidine of the DNA reactive strand and favorable hydrophobic contacts with Q146 and P145. Interestingly, these two amino acids are directly involved in separation of the viral DNA strands upon the ST reaction.<sup>23</sup> Indeed, these interactions appear to influence the inhibitory potency of these INSTIs as demonstrated by the potencies of compounds bearing the fluorine atoms in different positions of the aromatic ring (compounds **11b,d,j-n,s,t,u,v**). This is in line with SARs data achieved for different INSTIs by us and other groups.<sup>24</sup> Nevertheless, it should be pointed out that the role of the fluorobenzyl group in the IN recognition is rather “enigmatic” as outlined by Hare and co-workers.<sup>23</sup> On the other hand, the importance of the fluorine substitution on the benzyl group is also proved by compounds bearing different halogens (**11f,o,p**) and different groups (**11e,g-i,o-q**), which are generally less active than the fluorinated analogues. Additionally, limited extension of the pocket at the IN/DNA interface would also explain why replacement of the benzyl substituent with bulkier groups (compounds **11x-aa**) results in a loss of the inhibitory activity against IN.

Overall, these results support the theory according to which INSTIs should inhibit the ST reaction by contacting the  $Mg^{2+}$  cofactors and displacing the terminal 3'-adenosine of the viral DNA, thus interfering with the recognition between IN/viral DNA binary complex and the host cell DNA (see Supporting Information Figure S1).<sup>23</sup> Indeed, the binding mode provided by our docking calculations is shared by the majority of INSTIs including other quinolinonyl DKAs<sup>25-28</sup> and also **1** (Figure 4b).<sup>21</sup>

A rationalization for residual activity against RNase H shown by some compounds of this series was also performed. The 2.09 Å resolution crystal structure of full-length HIV-1 RT in complex with a RNase H pyrimidinol carboxylic acid inhibitor and the nonnucleoside inhibitor nevirapine (PDB code 3QIP) was selected for docking calculations. This structure shows the RNase H domain in complex with an inhibitor which is more structurally related to our DKA compounds as compared to the other ligands cocrystallized with the full-length enzyme or the isolated RNase H domain.<sup>29-31</sup> Before running docking calculations,  $Mn^{2+}$  ions in complex with this structure were replaced with physiologically relevant  $Mg^{2+}$  cations, which were also present in the biochemical and cell-based assays. In the predicted binding conformation, **11j** chelates both catalytic  $Mg^{2+}$  cations with its DKA branch (Figure 5) in a 5-, 6-membered chelate ring binding arrangement, whereas the carboxylate terminal group H-bonds with the amide of N474. As already observed for IN, such a chelate geometry would explain the general higher inhibitory activity displayed by acidic ligands compared to the corresponding esters. In addition to chelating the metal cations, **11j** establishes favorable lipophilic interactions with the  $C\beta$  and  $C\gamma$  carbons of Q475 through its quinolinonyl nucleus, while the benzyl substituent, although being partially solvent exposed, is able to contact the lipophilic residues Q500, and W535. Indeed, the generally lower RNase H activity of this series with respect our previous study<sup>18</sup> can be ascribed to the lack of additional interactions established with residues such as Y501, which is highly conserved and is a critical component of the RNase H primer grip. Moreover, recent experimental data

demonstrate that mutating this residue is also negatively affecting the inhibitory potency of other chemo-types bearing the DKA moiety.<sup>32</sup>

In conclusion, and in support of previously reported series,<sup>18</sup> we suggest that the quinolinonyl DKAs presented herein could exert their RNase H inhibitory activity by sterically hindering fitting of the viral DNA:RNA hybrid at the catalytic site, thus hampering the degradation of the RNA strand (see Supporting Information Figure S2).<sup>18</sup>

A final consideration should be done on the higher activity of **10j** in cell-based assays compared to that of **11j**. Although ester **10j** showed a similar binding pose of **11j** at both the IN and RNase H active sites (see Supporting Information Figure S3 and compare with Figures 4a) and 5), its higher activity against HIV infected cells would be rationalized by hypothesizing that **10j** is likely to better penetrate the cell membrane than its corresponding acid and, once in the cell, it would be hydrolyzed to **11j**, which may represent the effective inhibiting form of both the IN and RNase H enzymes.

## CONCLUSION

In this paper, we describe the design, synthesis, and biological assays of a series of new quinolinonyl diketo acid derivatives that are characterized by variously substituted alkylating groups on the nitrogen atom of the quinolinone ring. These derivatives were tested in both enzyme- and cell-based assays as anti-HIV-1 agents to selectively target the ST step of integration, testing in parallel the RNase H function of RT. We generated new compounds which are able to selectively inhibit IN and only marginally the RNase H. Five acid compounds resulted in being active against IN in the nanomolar range and, among them, compound **11j** (IC<sub>50</sub> = 10 nM against IN) inhibited RNase H activity with an IC<sub>50</sub> = 35.9  $\mu$ M. These compounds were also active against HIV-1 replication in acutely infected cells as well. However, the potency in cell-based assays was lower if compared to the activity against ST, suggesting that the acid derivatives are less prone to cell membrane penetration. Thus, the most active compounds against HIV infected cells turned out to be ester derivatives **10j** and **10l**.

In conclusion, the design of these new quinolinonyl DKAs was based on (i) removal of the second DKA branch of bifunctional DKAs that directed the activity against 3'-P, (ii) removal of the amino group in position 7 of quinolinone ring that addressed the activity on both IN and RNase H, and (iii) fine-tuning of the substituents on the benzyl group in position 1 of the quinolinone that preferentially addressed activity against IN ST. Further studies will be necessary to improve the activity of these compounds against HIV infected cells.

## EXPERIMENTAL SECTION

### Chemistry: General.

Melting points were determined on a Bobby Stuart Scientific SMP1 melting point apparatus and are uncorrected. Compounds purity were always >95% determined by high pressure liquid chromatography (HPLC). HPLC analysis were carried out with a Shimadzu LC-10AD



VP CTO-10AC VP. Column used was generally Discovery Bio Wide Pore C18 (10 cm × 4.6 mm, 3 μm). IR spectra were recorded on a PerkinElmer Spectrum-One spectrophotometer. <sup>1</sup>H NMR spectra were recorded at 400 MHz on a Bruker AC 400 Ultrashield 10 spectrophotometer (400 MHz). Dimethyl sulfoxide-*d*<sub>6</sub> 99.9% (code 44,139-2) (Aldrich) was used. Column chromatographies were performed on silica gel (Merck; 70–230 mesh). All compounds were routinely checked on TLC by using aluminum-baked silica gel plates (Fluka DC-Alufohlen Kieselgel 60 F254). Developed plates were visualized by UV light. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of rotary evaporator (Büchi) operating at a reduced pressure (ca. 20 Torr). Organic solutions were dried over anhydrous sodium sulfate (Merck). All reactions were carried out under nitrogen; all solvents were freshly distilled under nitrogen and stored over molecular sieves for at least 3 h prior to use. Analytical results agreed to within ±0.40% of the theoretical values.

### 3-Acetyl-4(1*H*)-quinolinones **12a–c**.

Ethyl orthoformate (4.0 g, 27 mmol), ethyl acetoacetate (3.5 g, 27 mmol), the proper aniline (27 mmol), and Dowtherm A (5.6 mL) were charged in a round-bottom three-necked flask equipped with a water separator. This mixture was stirred under argon atmosphere, while the temperature was increased to 95 °C in 1 h, then gradually up to 162 °C for a further hour. Then the mixture was stirred at this temperature for 6 h. After this time, the resulting solution was added in portions during 3 h into 42 mL of Dowtherm A stirred in a round-bottom three-necked flask equipped with thermometer and water separator and heated at 253–254 °C. After the addition, the mixture was heated at the same temperature for 2 h. Then the mixture was cooled at 90 °C, treated with 2-propanol (10 mL), cooled at 30 °C, filtered, and washed with 2-propanol and light petroleum ether in turn to give pure derivatives **12a–c**. For chemical, physical, analytical, and spectroscopic data of compounds **12a–c**, see Supporting Information.

### General Procedure for the Synthesis of 3-Acetyl-1-(aryl)-methyl-4(1*H*)-quinolinones (**13a–aa**).

A mixture of **12a–c** (1.1 mmol), the proper arylmethyl bromide (3.3 mmol), and anhydrous K<sub>2</sub>CO<sub>3</sub> (210 mg, 1.5 mmol) in dry DMF (10 mL) was stirred at 100 °C for 1 h. After the mixture was cooled, water was added (40 mL), and the formed precipitate was filtered, washed with water and light petroleum ether in turn, and then dried under an IR lamp to provide pure derivatives **13a–aa**. For chemical, physical, analytical, and spectroscopic data of compounds **13a–aa**, see Supporting Information.

### General Procedure for the Synthesis of Diketo Esters **10a–aa**.

Sodium ethoxide (390 mg, 5.5 mmol) was added into a well stirred mixture of the appropriate acetyl derivative **13a–aa** (2.7 mmol) and diethyl oxalate (790 mg, 5.4 mmol) in anhydrous THF (2.7 mL) under nitrogen atmosphere. The mixture was stirred at room temperature for 2 h, then was poured into *n*-hexane (50 mL). The collected precipitate was vigorously stirred for 30 min in 1 N HCl (50 mL). The formed yellow solid was filtered,

washed with water, and dried under an IR lamp to afford the pure diketo esters **10a–aa**. Yield (%), melting point (°C), recrystallization solvent, IR, <sup>1</sup>H NMR, and analytical data for each of the following compounds are reported.

**4-[1-Phenylmethyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10a).**—Yield 89%; 141–142 °C; benzene. IR  $\nu$  3400 (OH), 1740 (C=O ester), 1648, 1629, and 1609 (C=O ketone)  $\text{cm}^{-1}$ . <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.29 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.29 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.76 (s, 2H, CH<sub>2</sub>), 7.22–7.29 (m, 5H, benzene H), 7.48 (m, 1H, quinolinone C6-H), 7.70–7.71 (m, 2H, quinolinone C7-H and C8-H), 8.01 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.12 (s, 1H, quinolinone C2-H), 15.50 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>19</sub>NO<sub>5</sub>) C, H, N.

**4-[1-(2-Fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10b).**—Yield 92%; 152–153 °C; toluene. IR  $\nu$  3200 (OH), 1744 (C=O ester), 1651, and 1612 (C=O ketone)  $\text{cm}^{-1}$ . <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.28 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.29 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.81 (s, 2H, CH<sub>2</sub>), 7.12–7.28 (m, 4H, benzene H), 7.47 (m, 1H, quinolinone C6-H), 7.68–7.76 (m, 2H, quinolinone C7-H and C8-H), 7.99 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.09 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>18</sub>FNO<sub>5</sub>) C, H, F, N.

**4-[1-(2-Methoxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10c).**—Yield 98%; 154–156 °C; toluene. IR  $\nu$  3200 (OH), 1726 (C=O ester), 1631, and 1601 (C=O ketone)  $\text{cm}^{-1}$ . <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.29 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 3.82 (s, 3H, CH<sub>3</sub>), 4.29 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.63 (s, 2H, CH<sub>2</sub>), 6.89 (t, 1H, benzene H), 7.06 (d, 1H, benzene H), 7.13 (d, 1H, benzene H), 7.30 (t, 1H, benzene H), 7.49 (m, 1H, quinolinone C6-H), 7.71–7.76 (m, 2H, quinolinone C7-H and C8-H), 8.00 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.05 (s, 1H, quinolinone C2-H), 14.20 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>21</sub>NO<sub>6</sub>) C, H, N.

**4-[1-(3-Fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10d).**—Yield 94%; 174–175 °C; methanol. IR  $\nu$  3138 (OH), 1740 (C=O ester), 1650 and 1613 (C=O ketone)  $\text{cm}^{-1}$ . <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.27 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.30 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.78 (s, 2H, CH<sub>2</sub>), 7.01–7.20 (m, 3H, benzene H), 7.38 (m, 1H, benzene H), 7.49 (m, 1H, quinolinone C6-H), 7.65–7.74 (m, 2H, quinolinone C7-H and C8-H), 8.01 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.12 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>18</sub>FNO<sub>5</sub>) C, H, F, N.

**4-[1-(3-Methoxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10e).**—Yield 93%; 177–179 °C; toluene. IR  $\nu$  3146 (OH), 1729 (C=O ester), 1628 and 1603 (C=O ketone)  $\text{cm}^{-1}$ . <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.29 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 3.69 (s, 3H, CH<sub>3</sub>), 4.27 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.72 (s, 2H, CH<sub>2</sub>), 6.74 (d, 1H, benzene H), 6.84–6.89 (m, 2H, benzene H), 7.24 (t, 1H, benzene H), 7.49 (m, 1H, quinolinone C6-H), 7.69–7.75 (m, 2H, quinolinone C7-H and C8-H), 8.01 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.10 (s, 1H, quinolinone C2-H), 15.40 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>21</sub>NO<sub>6</sub>) C, H, N.

**4-[1-(4-Chlorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10f).**—Yield 91%; 174–176 °C; toluene. IR  $\nu$  3130 (OH), 1745 (C=O ester), 1643 and 1611 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.30 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.29 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.77 (s, 2H,  $\text{CH}_2$ ), 7.30–7.32 (m, 2H, benzene H), 7.40–7.42 (m, 2H, benzene H), 7.50 (m, 1H, quinolinone C6-H), 7.67 (m, 1H, quinolinone C8-H), 7.73 (m, 1H, quinolinone C7-H), 8.01 (s, 1H, butenoate C3-H), 8.30 (m, 1H, quinolinone C5-H), 9.14 (s, 1H, quinolinone C2-H), 15.60 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{18}\text{ClNO}_5$ ) C, H, Cl, N.

**4-[1-(4-Hydroxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10g).**—Yield 64%; 179–181 °C; methanol. IR  $\nu$  3141 (OH), 1733 (C=O ester), 1600 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.30 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.31 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.61 (s, 2H,  $\text{CH}_2$ ), 6.71–6.73 (m, 2H, benzene H), 7.13–7.15 (m, 2H, benzene H), 7.50 (m, 1H, quinolinone C6-H), 7.74 (m, 1H, quinolinone C7-H), 7.79 (m, 1H, quinolinone C8-H), 8.00 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.06 (s, 1H, quinolinone C2-H), 9.51 (bs, 1H, OH phenole), 14.00 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{19}\text{NO}_6$ ) C, H, N.

**4-[1-(4-Methoxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10h).**—Yield 90%; 154–156 °C; methanol. IR  $\nu$  1734 (C=O ester), 1638 and 1602 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.28 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 3.69 (s, 3H,  $\text{CH}_3$ ), 4.29 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.67 (s, 2H,  $\text{CH}_2$ ), 6.89–6.91 (m, 2H, benzene H), 7.24–7.26 (m, 2H, benzene H), 7.50 (m, 1H, quinolinone C6-H), 7.71–7.79 (m, 2H, quinolinone C7-H and C8-H), 8.00 (s, 1H, butenoate C3-H), 8.31 (m, 1H, quinolinone C5-H), 9.10 (s, 1H, quinolinone C2-H), 15.60 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{21}\text{NO}_6$ ) C, H, N.

**4-[1-(4-Nitrophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10i).**—Yield 88%; 175–176 °C; toluene. IR  $\nu$  1718 (C=O ester), 1638 and 1607 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.30 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.32 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.94 (s, 2H,  $\text{CH}_2$ ), 7.46–7.54 (m, 3H, benzene H and quinolinone C6-H), 7.60 (m, 1H, quinolinone C8-H), 7.68 (m, 1H, quinolinone C7-H), 8.02 (s, 1H, butenoate C3-H), 8.18–8.20 (m, 2H, benzene H), 8.33 (m, 1H, quinolinone C5-H), 9.18 (s, 1H, quinolinone C2-H), 15.60 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_7$ ) C, H, N.

**4-[1-(2,4-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10j).**—Yield 96%; 180 °C; methanol. IR  $\nu$  (C=O ester), and (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.35 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.36 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.84 (s, 2H,  $\text{CH}_2$ ), 7.10 (t, 1H, quinolinone C6-H), 7.41–7.57 (m, 3H, benzene H), 7.76–7.83 (m, 2H, quinolinone C8-H and quinolinone C7-H), 8.05 (s, 1H, butenoate C3-H), 8.38 (d, 1H, quinolinone C5-H), 9.14 (s, 1H, quinolinone C2-H), 14.60 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{17}\text{F}_2\text{NO}_5$ ) C, H, F, N.

**4-[1-(2,5-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10k).**—Yield 62%; 160–163 °C; toluene. IR  $\nu$  3432 (OH), 1736 (C=O ester), 1637 and 1602 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.35 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.35 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.86 (s, 2H,  $\text{CH}_2$ ), 7.18–7.41 (m, 3H, benzene H), 7.58 (t,

1H, quinolinone C6-H), 7.75 (m, 1H, quinolinone C8-H), 7.82 (m, 1H, quinolinone C7-H), 8.06 (s, 1H, butenoate C3-H), 8.38 (m, 1H, quinolinone C5-H), 9.16 (s, 1H, quinolinone C2-H), 14.60 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>17</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(2,6-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10l).**—Yield 85%; 172–173 °C; benzene/toluene. IR  $\nu$  3432 (OH), 1736 (C=O ester), 1637 and 1602 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.28 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.30 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.84 (s, 2H, CH<sub>2</sub>), 7.14–7.19 (t, 2H, benzene H), 7.43–7.53 (m, 2H, benzene H and quinolinone C6-H), 7.68 (t, 1H, quinolinone C7-H), 7.80 (d, 1H, quinolinone C8-H), 8.00 (s, 1H, butenoate C3-H), 8.30 (m, 1H, quinolinone C5-H), 9.11 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>17</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(3,4-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10m).**—Yield 100%; 175–176 °C; benzene. IR  $\nu$  3432 (OH), 1719 (C=O ester), 1648 and 1612 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.35 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.38 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.80 (s, 2H, CH<sub>2</sub>), 7.19 (t, 1H, quinolinone C6-H), 7.41–7.57 (m, 3H, benzene H), 7.72–7.80 (m, 2H, quinolinone C8-H and quinolinone C7-H), 8.06 (s, 1H, butenoate C3-H), 8.38 (d, 1H, quinolinone C5-H), 9.17 (s, 1H, quinolinone C2-H), 14.60 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>17</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(3,5-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10n).**—Yield 90%; 174–175 °C; methanol. IR  $\nu$  3432 (OH), 1736 (C=O ester), 1642 and 1626 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.34 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.36 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.84 (s, 2H, CH<sub>2</sub>), 7.05–7.23 (m, 3H, benzene H), 7.58 (t, 1H, quinolinone C6-H), 7.69 (d, 1H, quinolinone C8-H), 7.79 (t, 1H, quinolinone C7-H), 8.04 (s, 1H, butenoate C3-H), 8.38 (m, 1H, quinolinone C5-H), 9.16 (s, 1H, quinolinone C2-H), 14.60 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>17</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(2,4-Dichlorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10o).**—Yield 94%; 200–201 °C; toluene. IR  $\nu$  1735 (C=O ester), 1641 and 1632 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.34 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.35 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.84 (s, 2H, CH<sub>2</sub>), 6.99 (d, 1H, benzene H), 7.37 (dd, 1H, benzene H), 7.50–7.61 (m, 2H, quinolinone C6-H and C8-H), 7.78–7.83 (m, 2H, benzene H and quinolinone C7-H), 8.06 (s, 1H, butenoate C3-H), 8.41 (dd, 1H, quinolinone C5-H), 9.13 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>17</sub>Cl<sub>2</sub>NO<sub>5</sub>) C, H, Cl, N.

**4-[1-(2,6-Dichlorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10p).**—Yield 82%; 184–186 °C; toluene. IR  $\nu$  (C=O ester), and (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.32 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 4.35 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.90 (s, 2H, CH<sub>2</sub>), 7.61–7.67 (m, 2H, benzene H), 7.73–7.75 (m, 2H, quinolinone C6-H and C8-H), 7.94 (t, 1H, benzene H), 8.03–8.04 (m, 2H, quinolinone C7-H and butenoate C3-H), 8.42 (d, 1H, quinolinone C5-H), 8.46 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. (C<sub>22</sub>H<sub>17</sub>Cl<sub>2</sub>NO<sub>5</sub>) C, H, Cl, N.

**4-[1-(2,4-Dimethylphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid Ethyl Ester (10q).**—Yield 95%; 181 °C; toluene. IR  $\nu$  1727 (C=O ester),

1638 and 1600 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.32 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 2.24 (s, 3H,  $\text{CH}_3$ ), 2.36 (s, 3H,  $\text{CH}_3$ ), 4.32 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.69 (s, 2H,  $\text{CH}_2$ ), 6.58 (d, 1H, benzene H), 6.90 (d, 1H, benzene H), 7.12 (s, 1H, benzene H), 7.53–7.61 (m, 2H, quinolinone C6-H and C8-H), 7.76 (m, 1H, quinolinone C7-H), 8.05 (s, 1H, butenoate C3-H), 8.37 (d, 1H, quinolinone C5-H), 8.94 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{24}\text{H}_{23}\text{NO}_5$ ) C, H, N.

**4-[1-(3,5-Dimethylphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10r).**—Yield 89%; 181 °C; methanol. IR  $\nu$  1716 (C=O ester), 1625 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.35 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 2.24 (s, 6H,  $\text{CH}_3$ ), 4.35 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.72 (s, 2H,  $\text{CH}_2$ ), 6.89–6.96 (m, 3H, benzene H), 7.54 (t, 1H, quinolinone C6-H), 7.71–7.77 (m, 2H, quinolinone C7-H and C8-H), 8.05 (s, 1H, butenoate C3-H), 8.37 (d, 1H, quinolinone C5-H), 9.13 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{24}\text{H}_{23}\text{NO}_5$ ) C, H, N.

**4-[1-(3-Chloro-2-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10s).**—Yield 79%; 167–169 °C; benzene. IR  $\nu$  1746 (C=O ester), 1648 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.32 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.32 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.89 (s, 2H,  $\text{CH}_2$ ), 7.13–7.18 (m, 2H, benzene H and quinolinone C6-H), 7.55–7.77 (m, 4H, quinolinone C7-H, C8-H and benzene H), 8.03 (s, 1H, butenoate C3-H), 8.35 (d, 1H, quinolinone C5-H), 9.13 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{17}\text{ClFNO}_5$ ) C, H, Cl, F, N.

**4-[1-(3-Chloro-4-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10t).**—Yield 84%; 160–161 °C; methanol. IR  $\nu$  1746 (C=O ester), 1648 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.32 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.32 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.77 (s, 2H,  $\text{CH}_2$ ), 7.30–7.72 (m, 6H, quinolinone C6-H, C7-H, C8-H and benzene H), 8.03 (s, 1H, butenoate C3-H), 8.33 (d, 1H, quinolinone C5-H), 9.15 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{17}\text{ClFNO}_5$ ) C, H, Cl, F, N.

**8-Chloro-4-[1-(3-chloro-4-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10u).**—Yield 70%; 166–167 °C; ethanol. IR  $\nu$  1746 (C=O ester), 1648 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.32 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.33 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 6.10 (s, 2H,  $\text{CH}_2$ ), 7.11 (m, 1H, quinolinone C6-H), 7.34–7.50 (m, 3H, benzene H), 7.80–7.85 (m, 2H, quinolinone C7-H and C8-H) 7.95 (s, 1H, butenoate C3-H), 8.38 (d, 1H, quinolinone C5-H), 8.91 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{16}\text{Cl}_2\text{FNO}_5$ ) C, H, Cl, F, N.

**4-[7-Chloro-1-(2-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (10w).**—Yield 85%; 175 °C; methanol. IR  $\nu$  1721 (C=O ester), 1627 and 1585 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.35 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.36 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.88 (s, 2H,  $\text{CH}_2$ ), 7.22–7.48 (m, 4H, benzene H), 7.60 (t, 1H, quinolinone C6-H), 7.90 (m, 2H, quinolinone C7-H and C8-H), 8.00 (s, 1H, butenoate C3-H), 8.35 (d, 1H, quinolinone C5-H), 9.11 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{22}\text{H}_{17}\text{FCINO}_5$ ) C, H, N, F, Cl.

**2-Hydroxy-4-oxo-4-(4-oxo-1-(4-phenylbutyl)-1,4-dihydroquinolin-3-yl)but-2-enoic Acid Ethyl Ester (10x).**—Yield 95%; 88–90 °C; cyclohexane. IR  $\nu$  1725 (C=O ester), 1630 and 1604 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.24 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 1.60 (m, 2H, butyl H), 1.75 (m, 2H, butyl H), 2.58 (m, 2H, butyl H), 4.25 (m, 2H, butyl H), 4.45 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 7.11–7.25 (m, 5H, benzene H and quinolinone C6-H), 7.49 (m, 1H, quinolinone C8-H), 7.74–7.78 (m, 2H, butenoate C3-H and quinolinone C7-H), 8.30 (d, 1H, quinolinone C5-H), 8.76 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{25}\text{H}_{25}\text{NO}_5$ ) C, H, N.

**2-Hydroxy-4-(1-(naphthalen-2-ylmethyl)-4-oxo-1,4-dihydroquinolin-3-yl)-4-oxobut-2-enoic Acid Ethyl Ester (10y).**—Yield 87%; 153–154 °C; DMF/ $\text{H}_2\text{O}$ . IR  $\nu$  1734 (C=O ester), 1629 and 1603 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.28 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.32 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.94 (s, 2H,  $\text{CH}_2$ ), 7.49–7.56 (m, 4H, naphthyl H), 7.66–7.75 (m, 2H, naphthyl H), 7.79 (s, 1H, naphthyl H), 7.84–7.92 (m, 3H, quinolinone C6-H, C7-H and C8-H), 8.04 (s, 1H, butenoate C3-H), 8.32 (d, 1H, quinolinone C5-H), 9.20 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{26}\text{H}_{21}\text{NO}_5$ ) C, H, N.

**2-Hydroxy-4-oxo-4-(4-oxo-1-(pyridin-4-ylmethyl)-1,4-dihydroquinolin-3-yl)but-2-enoic Acid Ethyl Ester (10z).**—Yield 66%; 168–169 °C; DMF/ $\text{H}_2\text{O}$ . IR  $\nu$  1717 (C=O ester), 1645 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.28 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.28 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.82 (s, 2H,  $\text{CH}_2$ ), 7.08 (s, 2H, picoline H), 7.49 (t, 1H, quinolinone C6-H), 7.55 (d, 1H, quinolinone C8-H), 7.79 (t, 1H, quinolinone C7-H), 7.95 (s, 1H, butenoate C3-H), 8.32 (m, 1H, quinolinone C5-H), 8.50 (s, 2H, picoline H), 9.10 (s, 1H, quinolinone C2-H), 15.50 (bs, 1H, OH). Anal. ( $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_5$ ) C, H, N.

**2-Hydroxy-4-oxo-4-(4-oxo-1-(quinolin-2-ylmethyl)-1,4-dihydroquinolin-3-yl)but-2-enoic Acid Ethyl Ester (10aa).**—Yield 100%; 237–238 °C; DMF/ $\text{H}_2\text{O}$ . IR  $\nu$  1712 (C=O ester), 1634 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.16 (t, 3H,  $\text{CH}_2\text{CH}_3$ ), 4.30 (q, 2H,  $\text{CH}_2\text{CH}_3$ ), 5.98 (s, 2H,  $\text{CH}_2$ ), 7.11–7.94 (m, 9H, quinolinone C6-H, C7-H, C8-H, quinoline H), 8.25–8.37 (m, 2H, quinolinone C5-H and quinoline H), 9.04 (s, 1H, quinolinone C2-H), 15.00 (bs, 1H, OH). Anal. ( $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}_5$ ) C, H, N.

#### General Procedure for the Synthesis of Diketo Acids 11a–aa.

A mixture of 1 N NaOH (6.5 mL) and the appropriate ester **10a–aa** (1.3 mmol) in 1:1 THF/methanol (12 mL) was stirred at room temperature for 40 min and then poured onto crushed ice. The aqueous layer was separated and treated with 1 N HCl until pH 3 was reached, and the formed yellow solid was collected by filtration, then washed with water, hot dry ethanol, and light petroleum ether to afford pure acids **11a–aa**. Yield (%), melting point (°C), recrystallization solvent, IR,  $^1\text{H}$  NMR, and analytical data for each of the following compounds are reported.

**4-[1-Phenylmethyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid (11a).**—Yield 92%; 141–142 °C; DMF/ $\text{H}_2\text{O}$ . IR  $\nu$  3424 (OH), 1726 (C=O acid), 1625 and 1603 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  5.75 (s, 2H,  $\text{CH}_2$ ), 7.22–7.35 (m, 5H, benzene H), 7.44 (m, 1H, quinolinone C6-H), 7.65–7.69 (m, 2H, quinolinone C7-H and C8-



H), 7.90 (s, 1H, butenoate C3-H), 8.30 (m, 1H, quinolinone C5-H), 9.08 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>19</sub>H<sub>15</sub>NO<sub>5</sub>) C, H, N.

**4-[1-(2-Fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11b).**—Yield 83%; 172–174 °C; toluene. IR  $\nu$  3200 (OH), 1748 (C=O acid), 1621 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.81 (s, 2H, CH<sub>2</sub>), 7.14–7.27 (m, 3H, benzene H), 7.35 (m, 1H, benzene H), 7.50 (m, 1H, quinolinone C6-H), 7.70–7.73 (m, 2H, quinolinone C7-H and C8-H), 7.95 (s, 1H, butenoate C3-H), 8.31 (d, 1H, quinolinone C5-H), 9.08 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>14</sub>FNO<sub>5</sub>) C, H, F, N.

**4-[1-(2-Methoxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11c).**—Yield 94%; 172–175 °C; washed with dry ethanol. IR  $\nu$  3149 (OH), 1742 (C=O acid), 1620 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.82 (s, 3H, CH<sub>3</sub>), 5.61 (s, 2H, CH<sub>2</sub>), 6.89 (t, 1H, benzene H), 7.01–7.12 (m, 2H, benzene H), 7.0 (t, 1H, benzene H), 7.42–7.49 (m, 1H, quinolinone C6-H), 7.71–7.74 (m, 2H, quinolinone C7-H and C8-H), 7.94 (s, 1H, butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 9.02 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>21</sub>H<sub>17</sub>NO<sub>6</sub>) C, H, N.

**4-[1-(3-Fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11d).**—Yield 60%; 158–159 °C; washed with hot dry ethanol. IR  $\nu$  3392 (OH), 1719 (C=O acid), 1667 and 1620 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.76 (s, 2H, CH<sub>2</sub>), 7.05–7.18 (m, 3H, benzene H), 7.36 (m, 1H, benzene H), 7.47 (m, 1H, quinolinone C6-H), 7.66–7.68 (m, 2H, quinolinone C7-H and C8-H), 7.88 (s, 1H, butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 9.07 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>14</sub>FNO<sub>5</sub>) C, H, F, N.

**4-[1-(3-Methoxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11e).**—Yield 80%; 179–181 °C; washed with dry ethanol. IR  $\nu$  3002 (OH), 1748 (C=O acid), 1602 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.70 (s, 3H, CH<sub>3</sub>), 5.72 (s, 2H, CH<sub>2</sub>), 6.75 (d, 1H, benzene H), 6.84–6.89 (m, 2H, benzene H), 7.24 (t, 1H, benzene H), 7.49 (m, 1H, quinolinone C6-H), 7.69–7.74 (m, 2H, quinolinone C7-H and C8-H), 7.98 (s, 1H, butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 9.08 (s, 1H, quinolinone C2-H), 13.90 (bs, 1H, OH), 15.60 (bs, 1H, OH). Anal. (C<sub>21</sub>H<sub>17</sub>NO<sub>6</sub>) C, H, N.

**4-[1-(4-Chlorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11f).**—Yield 96%; 177–179 °C; washed with hot dry ethanol. IR  $\nu$  3250 (OH), 1741 (C=O acid), 1643 and 1611 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.76 (s, 2H, CH<sub>2</sub>), 7.25–7.31 (m, 2H, benzene H), 7.39–7.41 (m, 2H, benzene H), 7.49 (m, 1H, quinolinone C6-H), 7.65–7.73 (m, 2H, quinolinone C8-H and C7-H), 7.96 (s, 1H, butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 9.11 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>14</sub>ClNO<sub>5</sub>) C, H, Cl, N.

**4-[1-(4-Hydroxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11g).**—Yield 77%; 207–208 °C; washed with dry ethanol. IR  $\nu$  3135 (OH), 1718 (C=O acid), 1600 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.59 (s, 2H,

CH<sub>2</sub>), 6.71–6.73 (m, 2H, benzene H), 7.11–7.13 (m, 2H, benzene H), 7.47 (m, 1H, quinolinone C6-H), 7.67–7.78 (m, 2H, quinolinone C8-H and C7-H), 7.92 (s, 1H, butenoate C3-H), 8.29 (d, 1H, quinolinone C5-H), 9.00 (s, 1H, quinolinone C2-H), 9.50 (bs, 1H, OH phenole), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>15</sub>NO<sub>5</sub>) C, H, N.

**4-[1-(4-Methoxyphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11h).**—Yield 100%; 152 °C; washed with dry ethanol. IR  $\nu$  3400 (OH), 1726 (C=O acid), 1620 and 1611 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.69 (s, 3H, CH<sub>3</sub>), 5.66 (s, 2H, CH<sub>2</sub>), 6.88–6.90 (m, 2H, benzene H), 7.24–7.26 (m, 2H, benzene H), 7.50 (m, 1H, quinolinone C6-H), 7.70–7.78 (m, 2H, quinolinone C7-H and C8-H), 7.93 (s, 1H, butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 9.07 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>21</sub>H<sub>17</sub>NO<sub>6</sub>) C, H, N.

**4-[1-(4-Nitrophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11i).**—Yield 90%; 230–232 °C; washed with dry ethanol. IR  $\nu$  3100 (OH), 1731 (C=O acid), 1634 and 1602 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.87 (s, 2H, CH<sub>2</sub>), 7.45–7.52 (m, 3H, benzene H and quinolinone C6-H), 7.58 (m, 1H, quinolinone C8-H), 7.68 (m, 2H, quinolinone C7-H), 7.92 (s, 1H, butenoate C3-H), 8.18–8.20 (m, 2H, benzene H), 8.32 (d, 1H, quinolinone C5-H), 9.12 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>14</sub>N<sub>2</sub>O<sub>7</sub>) C, H, N.

**4-[1-(2,4-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11j).**—Yield 84%; 182 °C; ethanol. IR  $\nu$  3400 (OH), 1729 (C=O acid), and 1620 and 1608 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.84 (s, 2H, CH<sub>2</sub>), 7.11 (t, 1H, quinolinone C6-H), 7.37–7.40 (m, 2H, benzene H), 7.57 (d, 1H, quinolinone C8-H), 7.77–7.81 (m, 2H, benzene H and quinolinone C7-H), 8.01 (s, 1H, butenoate C3-H), 8.37 (d, 1H, quinolinone C5-H), 9.13 (s, 1H, quinolinone C2-H), 14.60 (bs, 1H, OH), 15.90 (bs, 1H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(2,5-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11k).**—Yield 46%; 176–178 °C; washed with dry ethanol. IR  $\nu$  3402 (OH), 1746 (C=O acid), 1622 and 1602 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.85 (s, 2H, CH<sub>2</sub>), 7.21–7.38 (m, 3H, benzene H), 7.56–7.80 (m, 3H, quinolinone C6-H, C7-H and C8-H), 7.99 (s, 1H, butenoate C3-H), 8.38 (d, 1H, quinolinone C5-H), 9.11 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(2,6-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11l).**—Yield 89%; 171–172 °C; washed with dry ethanol. IR  $\nu$  3450 (OH), 1747 (C=O acid), 1621 and 1603 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.82 (s, 2H, CH<sub>2</sub>), 7.13–7.18 (t, 2H, benzene H), 7.45–7.49 (m, 2H, benzene H and quinolinone C6-H), 7.67 (d, 1H, quinolinone C8-H), 7.77 (t, 1H, quinolinone C7-H), 7.86 (s, 1H, butenoate C3-H), 8.29 (d, 1H, quinolinone C5-H), 9.06 (s, 1H, quinolinone C2-H), 13.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(3,4-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11m).**—Yield 86%; 188–190 °C; washed with dry ethanol. IR  $\nu$  3000

(OH), 1730 (C=O acid), 1638 and 1610 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.79 (s, 2H, CH<sub>2</sub>), 7.17 (t, 1H, quinolinone C6-H), 7.45–7.54 (m, 3H, benzene H), 7.74–7.76 (m, 2H, quinolinone C8-H and quinolinone C7-H), 8.00 (s, 1H, butenoate C3-H), 8.37 (d, 1H, quinolinone C5-H), 9.15 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(3,5-Difluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11n).**—Yield 85%; 188 °C; ethanol. IR  $\nu$  3200 (OH), 1721 (C=O acid), 1626 and 1602 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.83 (s, 2H, CH<sub>2</sub>), 7.05–7.22 (m, 3H, benzene H), 7.55 (t, 1H, quinolinone C6-H), 7.68 (d, 1H, quinolinone C8-H), 7.78 (t, 1H, quinolinone C7-H), 8.02 (s, 1H, butenoate C3-H), 8.36 (d, 1H, quinolinone C5-H), 9.14 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, F, N.

**4-[1-(2,4-Dichlorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11o).**—Yield 83%; 220 °C (dec); washed with 2-propanole/isopropilic eter. IR  $\nu$  3200 (OH), 1722 (C=O acid), 1621 and 1608 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.83 (s, 2H, CH<sub>2</sub>), 6.97 (d, 1H, benzene H), 7.35 (d, 1H, benzene H), 7.49–7.59 (m, 2H, quinolinone C6-H and C8-H), 7.75–7.81 (m, 2H, benzene H quinolinone C7-H), 8.02 (s, 1H, butenoate C3-H), 8.39 (d, 1H, quinolinone C5-H), 9.10 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>Cl<sub>2</sub>NO<sub>5</sub>) C, H, Cl, N.

**4-[1-(2,6-Dichlorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11p).**—Yield 98%; 193–195 °C; washed with dry ethanol. IR  $\nu$  3041 (OH), 1743 (C=O acid), 1630 and 1605 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.89 (s, 2H, CH<sub>2</sub>), 7.60–7.66 (m, 2H, benzene H), 7.72–7.74 (m, 2H, quinolinone C6-H and C8-H), 7.91–8.04 (m, 3H, benzene H, quinolinone C7-H and butenoate C3-H), 8.41 (d, 1H, quinolinone C5-H), 8.44 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>20</sub>H<sub>13</sub>Cl<sub>2</sub>NO<sub>5</sub>) C, H, Cl, N.

**4-[1-(2,4-Dimethylphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11q).**—Yield 86%; 192 °C; washed with dry ethanol. IR  $\nu$  3500 (OH), 1731 (C=O acid), 1625 and 1602 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  2.27 (s, 3H, CH<sub>3</sub>), 2.38 (s, 3H, CH<sub>3</sub>), 5.72 (s, 2H, CH<sub>2</sub>), 6.61 (d, 1H, benzene H), 6.94 (d, 1H, benzene H), 7.15 (s, 1H, benzene H), 7.53–7.63 (m, 2H, quinolinone C6-H and C8-H), 7.78 (m, 1H, quinolinone C7-H), 8.05 (s, 1H, butenoate C3-H), 8.40 (d, 1H, quinolinone C5-H), 8.96 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>22</sub>H<sub>19</sub>NO<sub>5</sub>) C, H, N.

**4-[1-(3,5-Dimethylphenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenic Acid (11r).**—Yield 82%; 197–198 °C; washed with dry ethanol. IR  $\nu$  3200 (OH), 1736 (C=O acid), 1620 and 1602 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  2.23 (s, 6H, CH<sub>3</sub>), 5.72 (s, 2H, CH<sub>2</sub>), 6.85–7.16 (m, 3H, benzene H), 7.50–7.95 (m, 4H, butenoate C3-H, quinolinone C6-H, C7-H and C8-H), 8.49 (d, 1H, quinolinone C5-H), 9.26 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>22</sub>H<sub>19</sub>NO<sub>5</sub>) C, H, N.

**4-[1-(3-Chloro-2-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid (11s).**—Yield 38%; 164–165 °C; washed with dry ethanol. IR  $\nu$  3067 (OH), 1731 (C=O acid), 1622 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.87 (s, 2H,  $\text{CH}_2$ ), 7.01–7.19 (m, 2H, benzene H and quinolinone C6-H), 7.51–7.75 (m, 4H, quinolinone C7-H, C8-H and benzene H), 7.85 (s, 1H, butenoate C3-H), 8.34 (d, 1H, quinolinone C5-H), 9.08 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. ( $\text{C}_{20}\text{H}_{13}\text{ClFNO}_5$ ) C, H, Cl, F, N.

**4-[1-(3-Chloro-4-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid (11t).**—Yield 85%; 162–163 °C; washed with dry ethanol. IR  $\nu$  3060 (OH), 1732 (C=O acid), 1620 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.75 (s, 2H,  $\text{CH}_2$ ), 7.25 (m, 1H, quinolinone C6-H), 7.39 (t, 1H, quinolinone C7-H), 7.47–7.51 (m, 2H, quinolinone C8-H and benzene H), 7.60–7.72 (m, 3H, benzene H), 7.80 (s, 1H, butenoate C3-H), 8.33 (d, 1H, quinolinone C5-H), 9.08 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. ( $\text{C}_{20}\text{H}_{13}\text{ClFNO}_5$ ) C, H, Cl, F, N.

**8-Chloro-4-[1-(3-chloro-4-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid (11u).**—Yield 100%; 158–159 °C; washed with dry ethanol. IR  $\nu$  3040 (OH), 1730 (C=O acid), 1625 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  6.08 (s, 2H,  $\text{CH}_2$ ), 7.08 (m, 1H, quinolinone C6-H), 7.37–7.48 (m, 4H, quinolinone C7-H and benzene H), 7.83 (s, 1H, butenoate C3-H), 8.40 (d, 1H, quinolinone C5-H), 8.86 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. ( $\text{C}_{20}\text{H}_{12}\text{Cl}_2\text{FNO}_5$ ) C, H, Cl, F, N.

**8-Chloro-4-[1-(2-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid (11v).**—Yield 70%; 163–164 °C; washed with dry ethanol. IR  $\nu$  3400 (OH), 1728 (C=O acid), 1625 and 1601 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  6.14 (s, 2H,  $\text{CH}_2$ ), 6.90–6.96 (m, 2H, benzene H), 7.08–7.15 (m, 2H, benzene H), 7.22–7.37 (m, 2H, quinolinone C6-H and benzene H), 7.48 (m, 1H, quinolinone C7-H), 7.82 (s, 1H, butenoate C3-H), 8.38 (d, 1H, quinolinone C5-H), 8.88 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. ( $\text{C}_{20}\text{H}_{13}\text{ClFNO}_5$ ) C, H, Cl, F, N.

**4-[7-Chloro-1-(2-fluorophenyl)methyl-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid (11w).**—Yield 83%; 186–187 °C; washed with dry ethanol; IR  $\nu$  3631 (OH), 1732 (C=O acid), 1600 and 1583 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  5.87 (s, 2H,  $\text{CH}_2$ ), 7.22–7.46 (m, 4H, benzene H), 7.60 (d, 1H, quinolinone C6-H), 7.90 (s, 1H, quinolinone C8-H), 7.93 (s, 1H, butenoate C3-H), 8.35 (d, 1H, quinolinone C5-H), 9.10 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. ( $\text{C}_{20}\text{H}_{13}\text{FCINO}_5$ ) C, H, N, F, Cl.

**2-Hydroxy-4-oxo-4-(4-oxo-1-(4-phenylbutyl)-1,4-dihydroquinolin-3-yl)but-2-enoic Acid (11x).**—Yield 90%; 136–138 °C; DMF/ $\text{H}_2\text{O}$ . IR  $\nu$  3400 (OH), 1747 (C=O acid), 1623 and 1606 (C=O ketone)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$  1.63 (m, 2H, butyl H), 1.77 (m, 2H, butyl H), 2.59 (m, 2H, butyl H), 4.46 (m, 2H, butyl H), 7.11–7.25 (m, 5H, benzene H and quinolinone C6-H), 7.49 (m, 1H, quinolinone C8-H), 7.77–7.83 (m, 2H,

quinolinone C7-H and butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 8.85 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>23</sub>H<sub>21</sub>NO<sub>5</sub>) C, H, N.

**2-Hydroxy-4-(1-(naphthalen-2-ylmethyl)-4-oxo-1,4-dihydroquinolin-3-yl)-4-oxobut-2-enoic Acid (11y).**—Yield 85%; 212–213 °C; washed with dry ethanol. IR  $\nu$  (C=O acid), and (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.89 (s, 2H, CH<sub>2</sub>), 7.38–47 (m, 4H, naphthyl H), 7.64–7.71 (m, 2H, naphthyl H), 7.75 (s, 1H, naphthyl H), 7.85–7.91 (m, 3H, quinolinone C6-H, C7-H and C8-H), 8.12 (s, 1H, butenoate C3-H), 8.30 (d, 1H, quinolinone C5-H), 9.13 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>24</sub>H<sub>17</sub>NO<sub>5</sub>) C, H, N.

**2-Hydroxy-4-oxo-4-(4-oxo-1-(pyridin-4-ylmethyl)-1,4-dihydroquinolin-3-yl)but-2-enoic Acid (11z).**—Yield 65%; 225 °C; washed with dry ethanol. IR  $\nu$  3240 (OH), 1700 (C=O acid), 1620 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.79 (s, 2H, CH<sub>2</sub>), 7.18 (m, 2H, picoline H), 7.45–7.66 (m, 3H, quinolinone C6-H, C7-H and C8-H), 7.98 (s, 1H, butenoate C3-H), 8.33 (m, 1H, quinolinone C5-H), 8.51 (m, 2H, picoline H), 9.12 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>19</sub>H<sub>14</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

**2-Hydroxy-4-oxo-4-(4-oxo-1-(quinolin-2-ylmethyl)-1,4-dihydroquinolin-3-yl)but-2-enoic Acid (11aa).**—Yield 62%; >300 °C; washed with dry ethanol. IR  $\nu$  3429 (OH), 1700 (C=O acid), 1633 (C=O ketone) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.98 (s, 2H, CH<sub>2</sub>), 7.40–7.82 (m, 9H, quinolinone C6-H, C8-H, quinoline H), 7.93–7.95 (m, 2H, quinolinone C7-H and butenoate C3-H), 8.29–8.40 (m, 2H, quinolinone C5-H and quinoline H), 8.94 (s, 1H, quinolinone C2-H), 14.00–16.00 (bs, 2H, OH). Anal. (C<sub>23</sub>H<sub>16</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

## Biological Methods.

**HIV-1 IN Inhibition.**—HIV-1 IN gel-based assays were carried out as previously published.<sup>33</sup> Briefly, drugs or an equivalent volume of 100% DMSO (dimethyl sulfoxide, used as the drug solvent) was added to a reaction mixture containing 20 nM <sup>32</sup>P-labeled DNA substrate and 400 nM IN in 50 mM MOPS, pH 7.2, 7.5 mM MgCl<sub>2</sub>, and 14 mM 2-mercaptoethanol. Reactions were performed at 37 °C for 1 h and stopped by the addition of an equal volume of loading buffer [formamide containing 1% SDS (sodium dodecyl sulfate), 0.25% bromophenol blue, and xylene cyanol]. Products were separated in 16% polyacrylamide denaturing sequencing gels. Dried gels were visualized using a Typhoon 8600 (GE Healthcare). Densitometric analyses were performed using the ImageQuant 5.1 software from GE Healthcare. Data analyses (linear regression, IC<sub>50</sub> determination, and standard deviation) were performed using Prism 5.0c software from GraphPad.

**HIV-1 RT RNase H Inhibition.**—IC<sub>50</sub> values were determined as previously reported<sup>34</sup> using an 18-nt 3'-fluorescein-labeled RNA annealed to a complementary 18-nt 5'-dabsyl-labeled DNA. To a 96-well plate was added 1  $\mu$ L of each inhibitor (in DMSO), followed by 10  $\mu$ L of the appropriate RT (15–80 ng/mL) in reaction buffer. Hydrolysis was initiated by adding 10  $\mu$ L of RNA/DNA hybrid (2.5  $\mu$ M). Final assay conditions were 50 mM Tris-HCl, pH 8.0, 60 mM KCl, 10 mM MgCl<sub>2</sub>, 1% DMSO, 150–800 ng of RT, 250 nM substrate, and

increasing concentrations of inhibitor. Wells containing only DMSO were used as negative control. Plates were incubated at 37 °C in a Spectramax Gemini EM fluorescence spectrometer for 10 min, and fluorescence ( $\lambda_{\text{ex}} = 485 \text{ nm}$ ;  $\lambda_{\text{em}} = 520 \text{ nm}$ ) was measured at 1 min intervals such that linear initial rates could be measured in the presence ( $v_i$ ) and absence ( $v_0$ ) of inhibitor. Percent inhibition was calculated as  $100(v_0 - v_i)/v_0$ , and plotted against  $\log[I]$ .  $IC_{50}$  values were determined using Prism5 (GraphPad Software). All assays were performed in triplicate.

**HIV-1 Replication Inhibition.**—The compounds' antiviral activity was determined in a cell-based assay according to the procedure described previously<sup>35</sup> and modified as follows. HeLa-CD4-LTR- $\beta$ -gal cells were maintained in DMEM with 10% serum and 0.5 mg/mL G418. The day prior experimentation, 96-well plates were prepared to contain 10000 cells per well in 100  $\mu\text{L}$  of DMEM medium complemented with 10% serum. On day one, each drug was serially diluted directly on cells following a 3-fold dilution over six points, and each well was then filled to 200  $\mu\text{L}$  with either fresh medium or concentrated viral supernatant (HIV-1(HIB), Advanced Biotechnologies Inc.). The highest compound concentration tested was 50  $\mu\text{M}$ . On day two, cells were washed three times with PBS before adding 200  $\mu\text{L}$  of a solution containing 50 mM Tris-HCl pH 7.5, 100 mM  $\beta$ -mercaptoethanol, 0.05% Triton X100, and 5 mM 4-methyl-umbelliferyl- $\beta$ -D-galactopyranoside (4-MUG, Sigma). On day three, sealed plates were read in a SpectraMax GEMINI-XS (Molecular Devices) with  $\lambda_{\text{ex/em}} = 360/460 \text{ nm}$ .

**Cellular Toxicity.**—Similar to the antiviral assays, plates were prepared with 10000 HeLa-CD4-LTR- $\beta$ -gal cells per well and a serial dilution of compounds in 100  $\mu\text{L}$ . After 24 h of culture, 100  $\mu\text{L}$  of ATPlite reagent (PerkinElmer) was added to each well. After 5 min at room temperature, luminescence was quantified using an EnVision multilabel reader (PerkinElmer) according to manufacturer's instructions.

### Molecular Modeling.

**Ligands Setup.**—The 3D structures of all of the compounds were generated with the Maestro Build Panel<sup>36</sup> and were then submitted to Polak–Ribiere conjugate gradient minimization (0.0005 kJ/( $\text{\AA}$  mol) convergence) using MacroModel (version 9.9).<sup>37</sup> DKAs were modeled in their enolic tautomeric form because it has been clearly established that such compounds mainly exist in this form in solution and also given the influence of the two metal cations in the binding site.<sup>38</sup>

**Proteins Setup.**—The HIV-1 CCD/DNA homology model and the RNase H crystal structure (PDB code 3QIP) were prepared for docking calculations using the “Protein Preparation Wizard” panel of Schrödinger 2010 molecular modeling package.<sup>36</sup> In particular, using the “preprocess and analyze structure” tool, the bond orders and disulfide bonds were assigned, all the hydrogen atoms were added, and all the water molecules in a distance greater than 5  $\text{\AA}$  from any heterogroup were deleted; in addition, both  $\text{Mn}^{2+}$  ions in the RNase H crystal structure were replaced with  $\text{Mg}^{2+}$  cations. Using Epik 2.0, a prediction of the side chains heterogroups ionization and tautomeric states was performed.<sup>39</sup> An optimization of the hydrogen-bonding network was performed using the “H-bond



assignment” tool. Finally, using the “impref utility”, the positions of the hydrogen atoms were optimized by keeping all the heavy atoms in place.

**Docking Calculations.**—Docking studies at both the HIV-1 IN and RNase H active sites were carried out with Glide v. 5.7 (Schrödinger).<sup>40</sup> Glide is a grid-based ligand docking with energetics approach and searches for favorable interactions between ligands and receptors. The shape and properties of the receptor are represented on a grid by different sets of fields that provide progressively more accurate scoring of the ligand pose. These fields are generated as preprocessing steps in the calculation and hence need to be computed only once for each receptor. For the grid generation of both the receptors, a box centered on the Mg<sup>2+</sup> cations was created. This box gives a more precise measure of the effective size of the search space. However, ligands can move outside this box during grid minimization. The Cartesian coordinates of the outer box, *X*, *Y*, and *Z* length, were set to 25 Å. The conformational space of the ligand is defined by Glide by several lowest-energy poses that are subjected to a Monte Carlo procedure that examines nearby torsional minima. This procedure is needed in some cases to properly orient peripheral groups and occasionally alters internal torsion angles. The default value (1.00) for the van der Waals radii scaling factor was chosen, which means no scaling for the nonpolar atoms was performed (no flexibility was simulated for the receptor). In the present study, the standard precision (SP) mode of GlideScore function was used to score the obtained binding poses. The force field used for the docking was the OPLS-2005.<sup>41</sup> All of the pictures were rendered with the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.<sup>42</sup>

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

The authors thank the Italian MIUR for financial support, PRIN 2010-2011 (2010W2KM5L\_002) and “Sapienza” Ateneo C26A133H7B. R. Di Santo and R. Costi thank the FP7 CHAARM project for support. This work was also supported by the NIH Intramural Research Program, Center for Cancer Research, National Cancer Institute (Z01 BC 007333).

## ABBREVIATIONS USED

<b>IN</b>	integrase
<b>RT</b>	reverse transcriptase
<b>3'-P</b>	3'-processing
<b>BAF</b>	barrier to autointegration factor
<b>HSP60</b>	heat-shock protein 60
<b>HMG</b>	high mobility group protein
<b>LEDGF</b>	lens epithelium-derived growth factor

<b>Vpr</b>	viral protein R
<b>RNase H</b>	ribonuclease H
<b>PIC</b>	preintegration complex
<b>ST</b>	strand transfer
<b>DKA</b>	diketo acid
<b>rRT</b>	recombinant reverse transcriptase
<b>rIN</b>	recombinant integrase
<b>EVG</b>	elvitegravir
<b>SI</b>	selectivity index
<b>SAR</b>	structure–activity relationship
<b>CCD</b>	catalytic core domain
<b>PFV</b>	prototype foamy virus
<b>INSTI</b>	strand transfer inhibitor

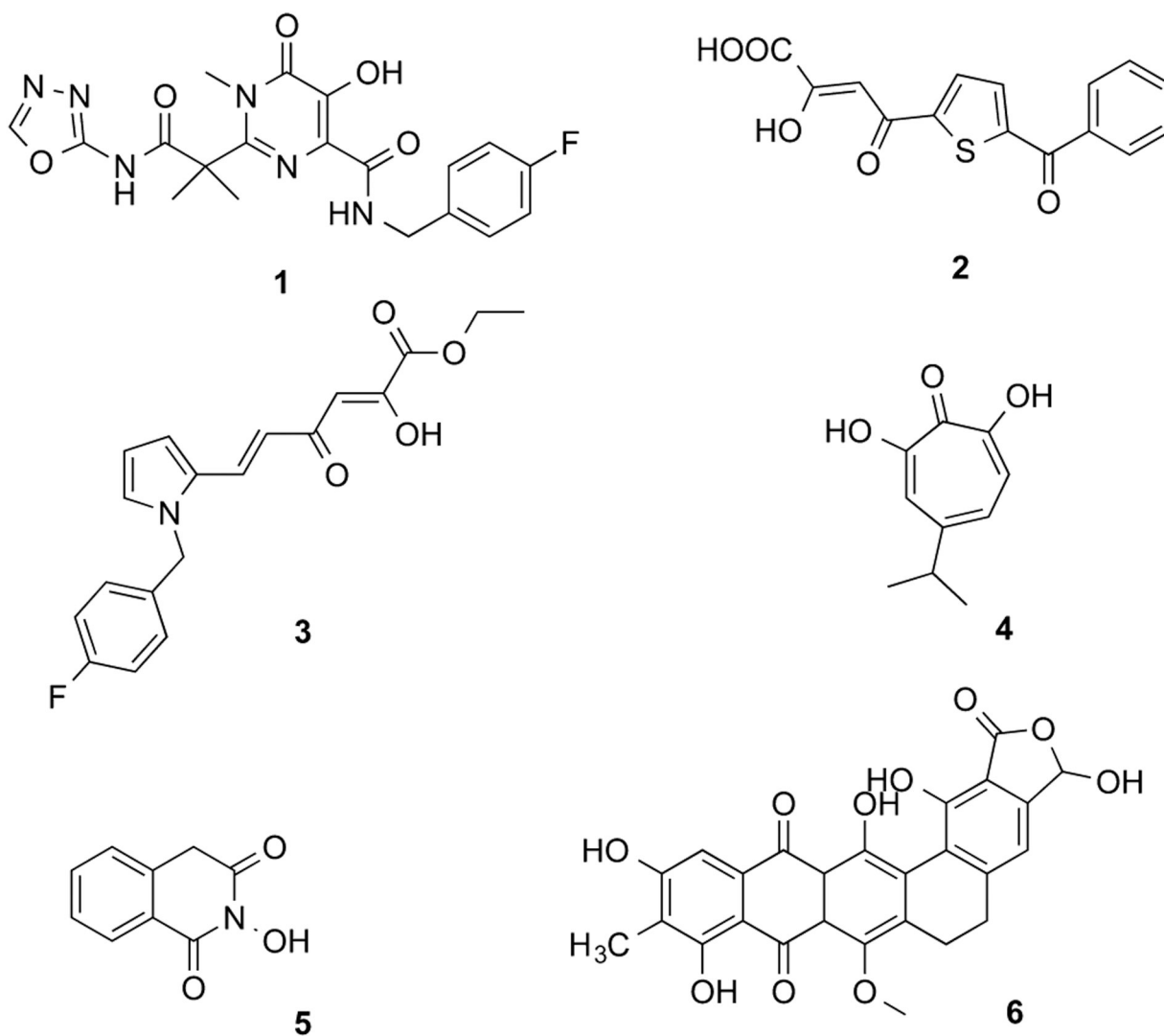
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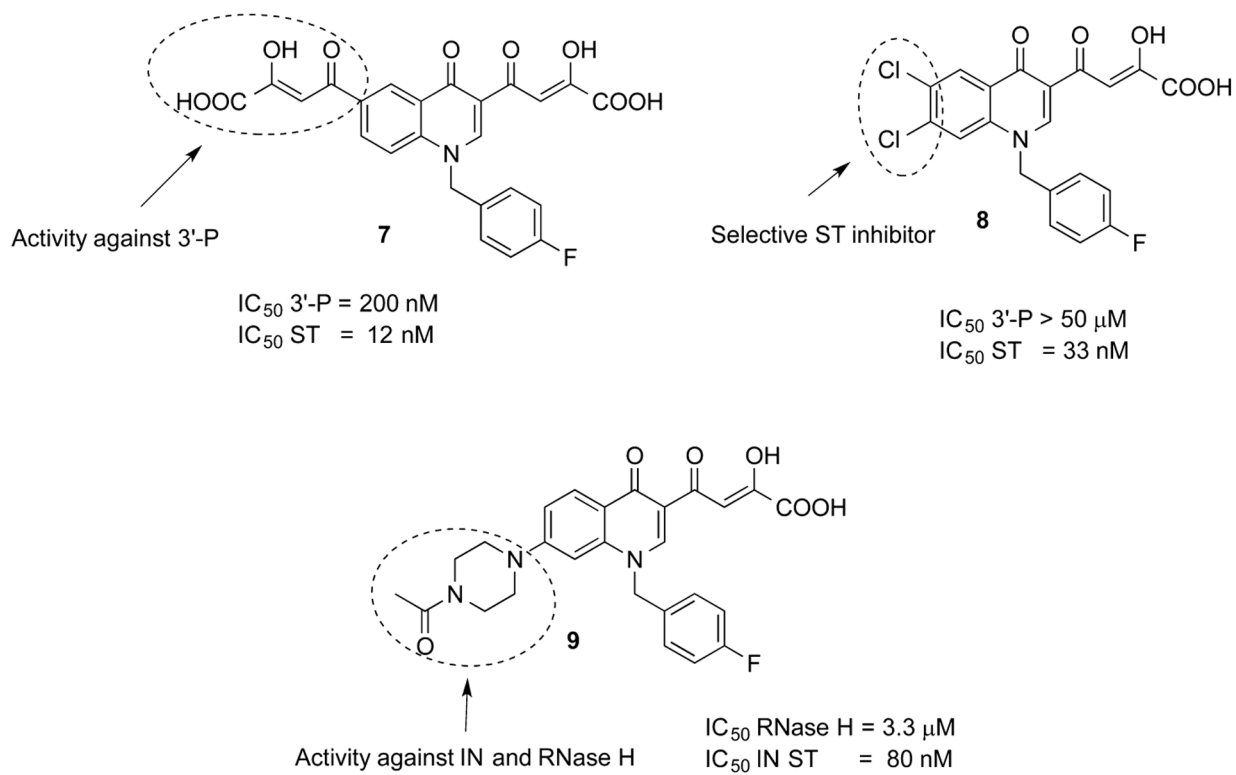
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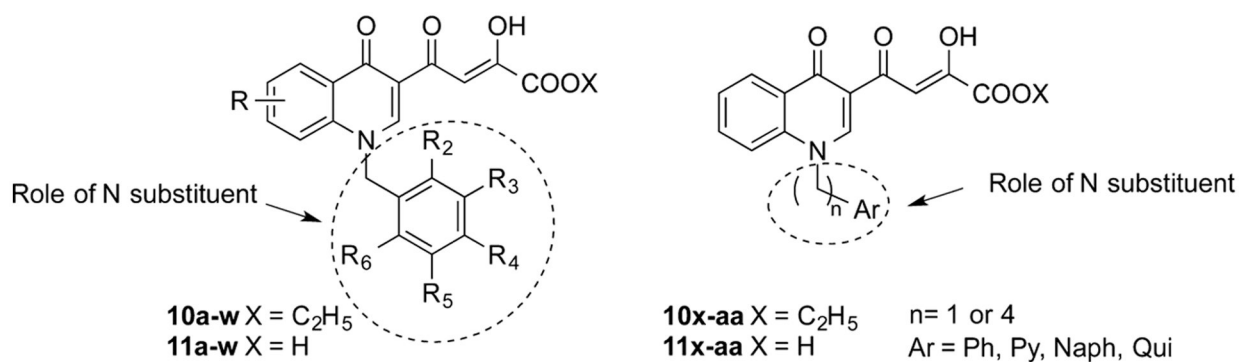


**Figure 1.**  
Structures of dual IN and RNase H inhibitors.

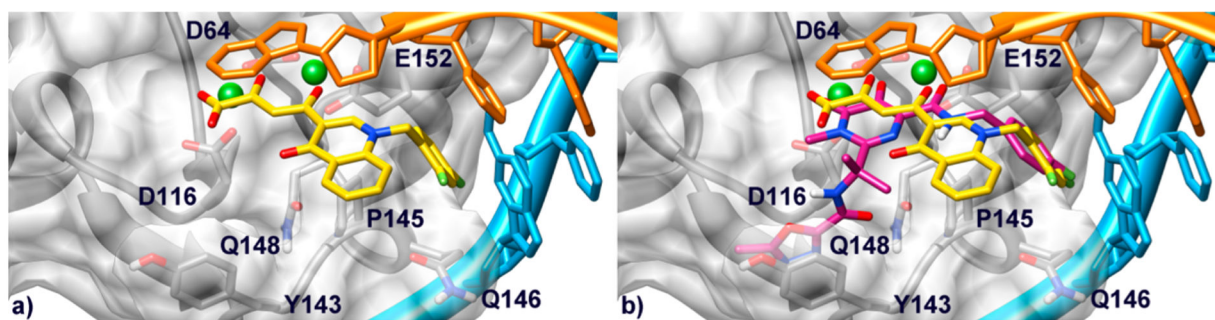




**Figure 2.**  
 HIV-1 IN inhibitors of the mono- and bifunctional quinolinonyl DKA class.

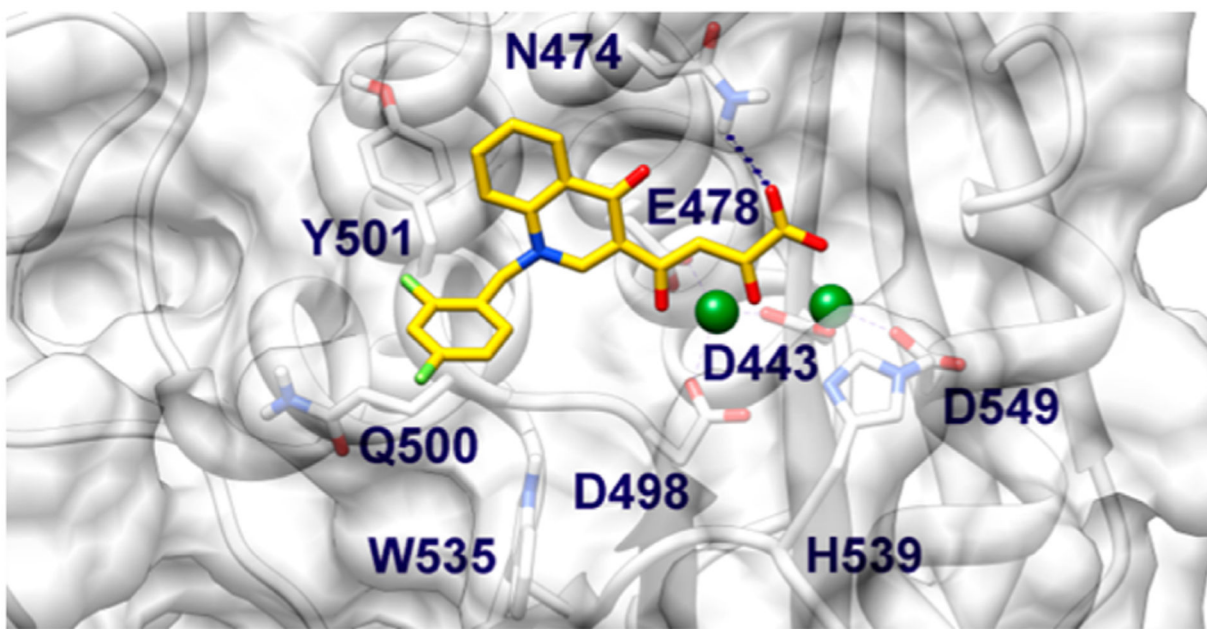
**Figure 3.**

Structures of the newly designed quinolinonyl diketo acid derivatives **10a–aa** and **11a–aa** (Ph = phenyl, Py = 2-pyridinyl, Naph = 2-naphthyl, Qui = 2-quinolinyl). For specific structures and molecules, see Tables 1 and 2.

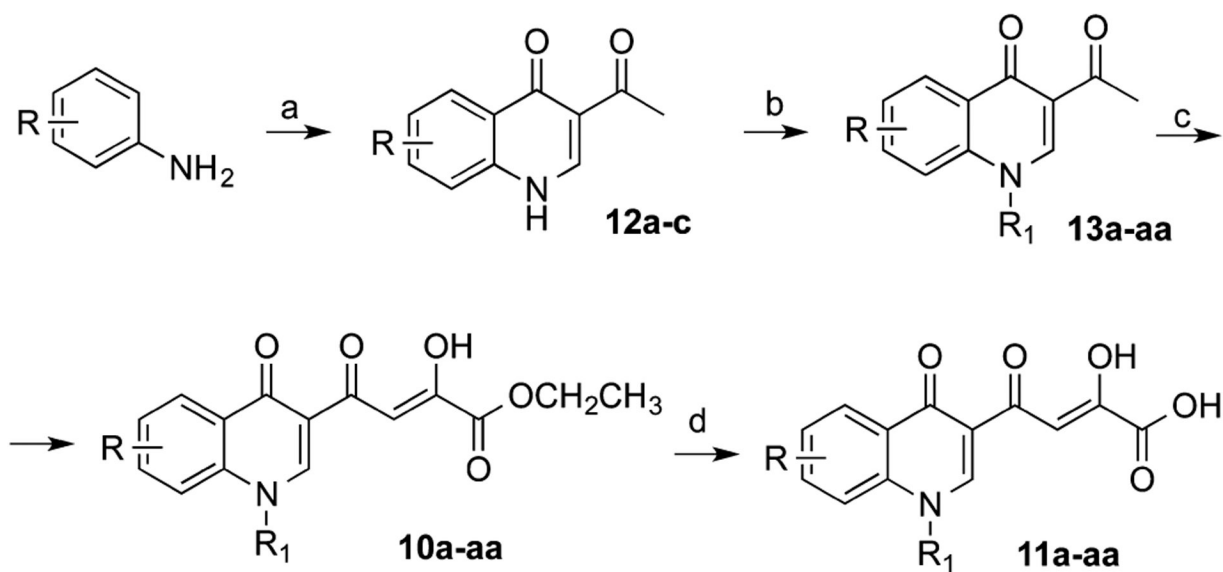


**Figure 4.**

(a) Binding mode of compound **11j** (yellow) within the HIV-1 IN/DNA model. Catalytic core domain is depicted as transparent light-gray surface and ribbons. Amino acid side chains important for the ligand binding are represented as sticks. The nontransferred (cyan) and reactive (orange) viral DNA strands are shown as ribbon and sticks.  $Mg^{2+}$  metal ions are represented as green spheres. (b) Overlay of compound **11j** and **1** (magenta) in the IN binding pocket.



**Figure 5.** Binding mode of compound **11j** (yellow) within the HIV-1 RNase H active site. CCD is shown as transparent white surface and ribbons. Amino acid side chains important for the ligand binding are represented as sticks.  $Mg^{2+}$  metal ions are depicted as green spheres.

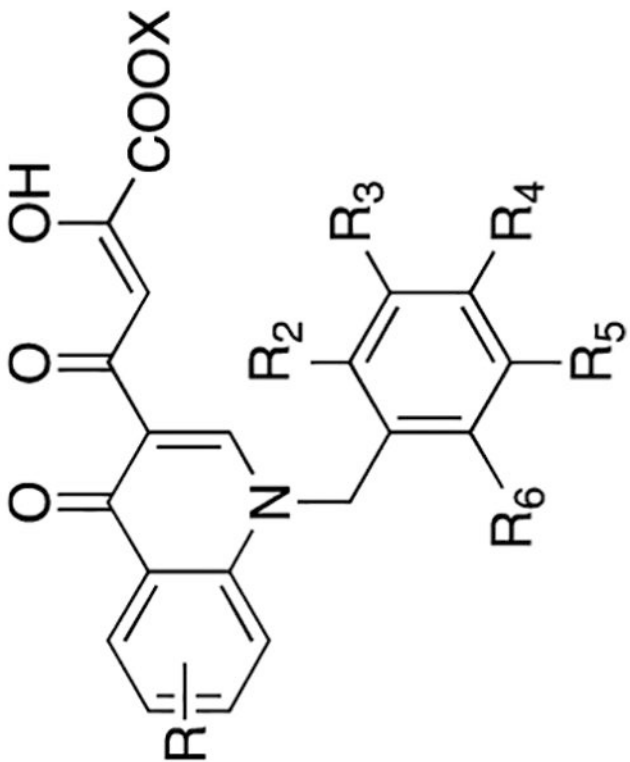


**Scheme 1. Synthetic Route to Quinolinonyl DKAs 10a-aa and 11a-aa<sup>a</sup>**

<sup>a</sup>Reagents and conditions: (a) ethyl orthoformate, ethyl acetoacetate, Dowtherm A, 95–254 °C, 8 h, 52–58% yield; (b) arylalkyl bromide, K<sub>2</sub>CO<sub>3</sub>, DMF, 100 °C, 50–75% yield; (c) diethyl oxalate, C<sub>2</sub>H<sub>5</sub>ONa, THF, room temp, 10 min, 62–100% yield; (d) 1 N NaOH, THF/CH<sub>3</sub>OH, room temp, 40 min, 38–100% yield.

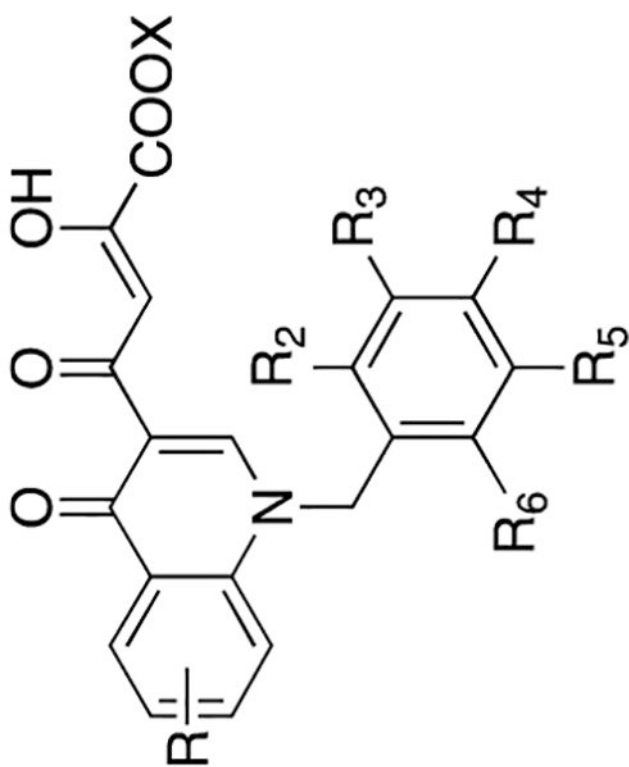
Table 1.

Cytotoxicity, Antiviral, Anti-IN, and Anti-RNase H Activities of Compounds 10a–w and 11a–w



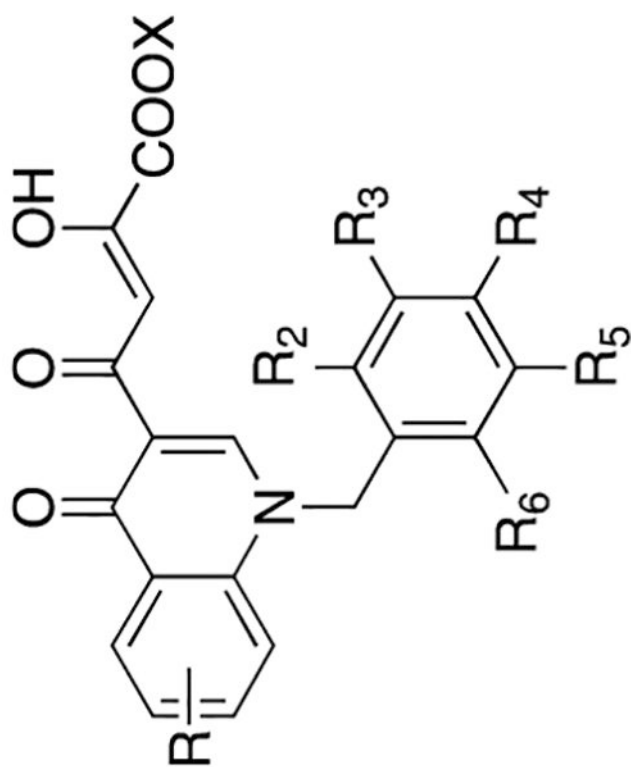
compd	activity in enzyme assays						antiviral activity and cytotoxicity							
	R	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	IN IC <sub>50</sub> (μM) <sup>a</sup>	ST	% in. at 10 μM <sup>b</sup>	RNase H IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
10a	H	H	H	H	H	H	H	>1000	0.70	-3.3	nd <sup>f</sup>	1	>50	>50
10b	F	F	H	H	H	H	H	125	0.28	2.8	nd	1.5	>50	>33.4
10c	H	OCH <sub>3</sub>	H	H	H	H	H	>333	1.4	11.4	nd	2.6	>50	>19.2
10d	H	H	F	H	H	H	H	>12.3	4.9	-9.4	nd	1.7	>50	>29.4
10e	H	H	OCH <sub>3</sub>	H	H	H	H	>333	16	4.9	nd	8.5	44	5.2
10f	H	H	H	Cl	H	H	H	>37	>37	16.1	nd	>50	nd	nd





activity in enzyme assays

compd	IN IC <sub>50</sub> (μM) <sup>a</sup>					RNase H			antiviral activity and cytotoxicity					
	R	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	X	3'-P	ST	% in. at 10 μM <sup>b</sup>	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
10g	H	H	H	OH	H	H	Et	>333	1.4	26.0	nd	30.9	>50	>1.6
10h	H	H	H	OCH <sub>3</sub>	H	H	Et	>37	>37	10.0	nd	>50	nd	
10i	H	H	H	NO <sub>2</sub>	H	H	Et	>37	>37	31.9	nd	>50	nd	
10j	F	F	H	F	H	H	Et		2.2	3	nd	0.58	>50	>86.2
10k	F	F	H	H	F	H	Et		3.7	30	nd	>50	nd	
10l	F	F	H	H	H	F	Et	>12.3	0.25	4.1	nd	<0.2	>50	>250
10m	H	H	F	F	H	H	Et		5.5	24	nd	9.8	>50	>5.1
10n	H	H	F	H	F	H	Et		11	24	nd	32.4	>50	>1.5
10o	Cl	Cl	H	Cl	H	H	Et		>111	51	>50	>50	nd	
10p	Cl	Cl	H	H	H	Cl	Et		32	55.0	>50	30.2	>50	>1.7



activity in enzyme assays

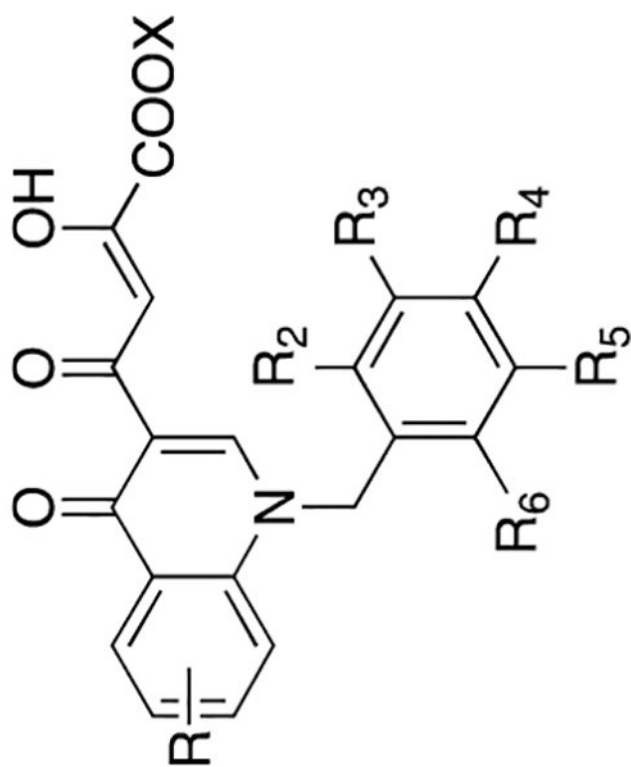
compd	activity in enzyme assays										antiviral activity and cytotoxicity				
	R	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	X	3'-P	IN IC <sub>50</sub> (μM) <sup>a</sup>	ST	% in. at 10 μM <sup>b</sup>	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
10q		CH <sub>3</sub>	H	CH <sub>3</sub>	H	H	Et		>111	33	>50	nd	>50	nd	
10r		H	CH <sub>3</sub>	H	CH <sub>3</sub>	H	Et		1.3	21	nd	>50	>50	nd	
10s		F	Cl	H	H	H	Et	1.08	1.08	21	nd	10	>50	>5	
10t		H	Cl	F	H	H	Et	2.66	3.1	18	nd	17.4	>50	>2.9	
10u	8-Cl	H	Cl	F	H	H	Et	>333	25.08	24	nd	>50	nd		
10w	7-Cl	F	H	H	H	H	Et			2.8	nd	11	>50	>4.5	
11a		H	H	H	H	H	H	4.0	0.034	9.6	nd	15.5	>50		
11b		F	H	H	H	H	H	1.2	0.016	45.1	>50	2.6	>50	>19.2	

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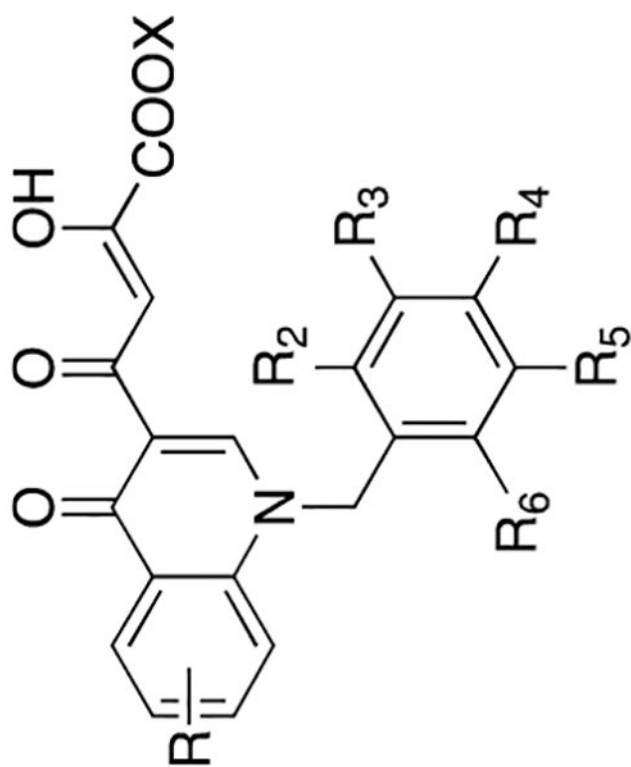
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activity in enzyme assays

compd	IN IC <sub>50</sub> (μM) <sup>a</sup>						RNase H			antiviral activity and cytotoxicity				
	R	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	X	3'-P	ST	% in. at 10 μM <sup>b</sup>	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
11c		OCH <sub>3</sub>	H	H	H	H	H	>4.1	0.038	45.2	16.3 ± 0.5	9.3	>50	>5.4
11d		H	F	H	H	H	H	4.6	0.015	2.4	nd	28.2	>50	>1.8
11e		H	OCH <sub>3</sub>	H	H	H	H	>4.1	0.14	1.9	nd	>50	nd	>57.5
11f		H	H	Cl	H	H	H	22	0.54	56.2	>50	0.87	>50	>50
11g		H	H	OH	H	H	H	>37	0.45	44.4	9.5 ± 0.4	>50	nd	>50
11h		H	H	OCH <sub>3</sub>	H	H	H	28	0.51	45.8	>50	>50	nd	>50
11i		H	H	NO <sub>2</sub>	H	H	H	27	0.64	42.4	47.0 ± 1.5	>50	nd	>50
11j		F	H	F	H	H	H	46	0.010	46	35.9 ± 0.8	13.8	>50	>3.6
11k		F	H	H	F	H	H	37	3.2	37	>50	>50	nd	>50



activity in enzyme assays

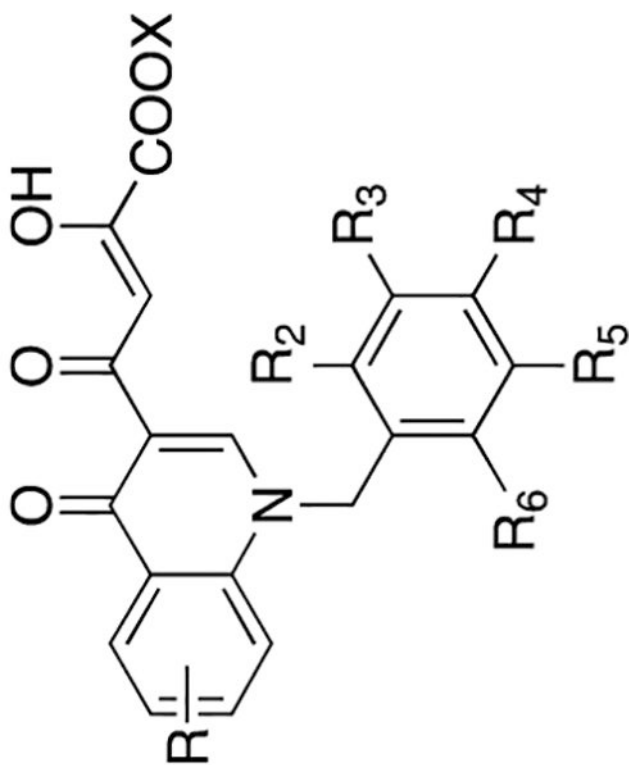
compd	IN IC <sub>50</sub> (μM) <sup>a</sup>					RNase H			antiviral activity and cytotoxicity					
	R	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	X	3'-P	ST	% in. at 10 μM <sup>b</sup>	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
11l		F	H	H	H	F	H	0.7	0.019	57.9	10.8 ± 0.4	8.1	>50	>6.2
11m		H	F	F	H	H	H		0.11	44	>50	16.2	>50	>3.1
11n		H	F	H	F	H	H		0.22	30	>50	>50	nd	
11o		Cl	H	Cl	H	H	H		2.3	68	44.7 ± 2.0	>50	nd	
11p		Cl	H	H	H	Cl	H		0.27	86.3	10.0 ± 0.3	>50	nd	
11q		CH <sub>3</sub>	H	CH <sub>3</sub>	H	H	H		7.0	52	19.6 ± 0.6	>50	nd	
11r		H	CH <sub>3</sub>	H	CH <sub>3</sub>	H	H		0.05	47	37.8 ± 0.9	>50	nd	
11s		F	Cl	H	H	H	H	1.75	0.018	12	nd	23.4	>50	>2.1
11t		H	Cl	F	H	H	H	21	0.19	25	nd	>50	nd	

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activity in enzyme assays

compd	activity in enzyme assays										antiviral activity and cytotoxicity				
	R	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	X	3'-P	IN IC <sub>50</sub> (μM) <sup>a</sup>	ST	% in. at 10 μM <sup>b</sup>	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
11u	8-Cl	H	Cl	F	H	H	H	9.8	0.096	16	nd	nd	50	nd	nd
11v	8-Cl	F	H	H	H	H	H	20.5	0.13	45	>50	>50	>50	nd	nd
11w	7-Cl	F	H	H	H	H	H			36.5	22.6 ± 0.5	17	>50	>2.9	>2.9
<b>1</b>								12.8 ± 6 <sup>f</sup>	0.087 ± 0.008 <sup>h</sup>	nd	>100	0.0236 ± 0.0046	>50	>2118	>2118
EVG								8.1 ± 4.2 <sup>g</sup>	0.028 ± 0.006 <sup>g</sup>	nd	91 ± 8	0.0142 ± 0.0052	>50	>3521	>3521

<sup>a</sup>Inhibitory concentration 50% (μM) determined from dose response curves.

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$b$  Percentage of inhibition determined at compound concentration of 10  $\mu$ M.

$c$  Effective concentration 50% ( $\mu$ M).

$d$  Cytotoxic concentration 50% ( $\mu$ M).

$e$  SI = CC50/EC50.

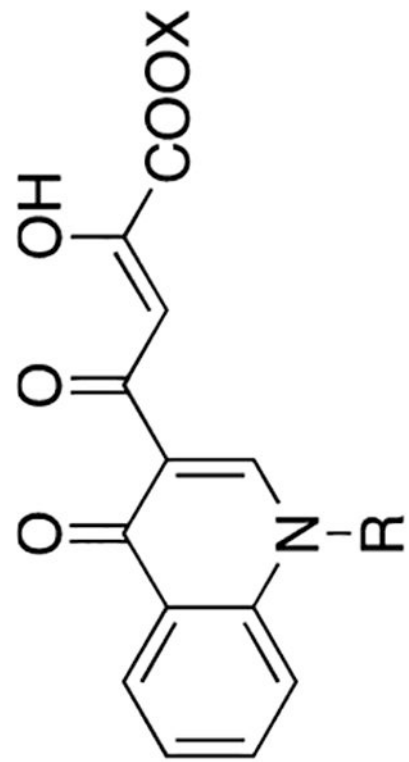
$f$  nd: not determined.


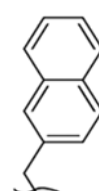
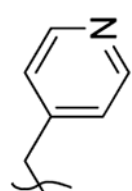
$g$  Reference 20.

$h$  Reference 18.



**Table 2.** Cytotoxicity, Antiviral, Anti-IN, and Anti-RNase H Activities of Compounds 10x–aa and 11x–aa



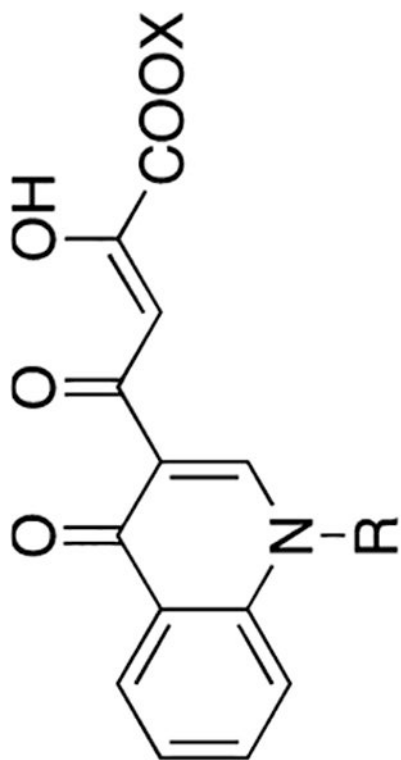
Activity in enzyme assays							Antiviral activity and cytotoxicity		
	R	X	3 <sup>1</sup> -P	IN IC <sub>50</sub> (μM) <sup>d</sup>	RNaseH	IC <sub>50</sub> (μM) <sup>e</sup>	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
10x		Et	>333	120	19.4	nd	>50	nd <sup>f</sup>	
10y		Et	>333	32	29.7	nd	>50	nd	
10z		Et	>333	14	-5.6	nd	>50	nd	

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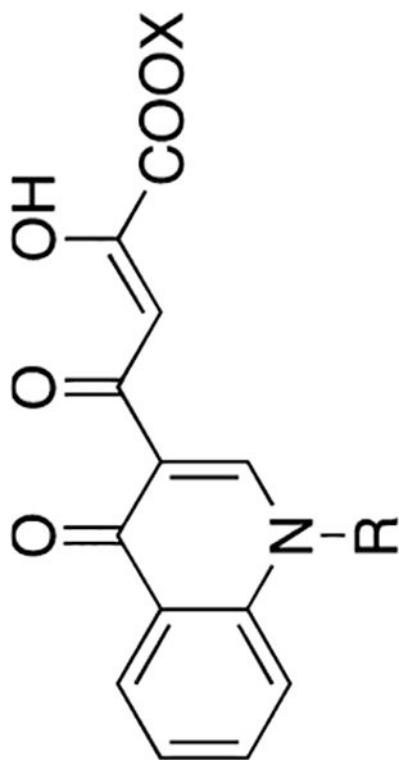
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## Activity in enzyme assays

	Activity in enzyme assays				Antiviral activity and cytotoxicity					
	R	X	3'-P	IN IC <sub>50</sub> (μM) <sup>a</sup>	ST	% in. at 10 μM <sup>b</sup>	RNaseH IC <sub>50</sub> (μM) <sup>x</sup>	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
<b>10aa</b>		Et	>1000	3.5	3.5	4.4	nd	>50	nd	
<b>11x</b>		H	>333	<0.45	<0.45	33.0	nd	>50	nd	
<b>11y</b>		H	18	<0.45	<0.45	40.7	nd	4.8	>50	>10.4
<b>11z</b>		H	>1000	16	16	-2.6	nd	>50	nd	



## Activity in enzyme assays

R	X	3'-P	IN IC <sub>50</sub> (μM) <sup>d</sup>		RNaseH		Antiviral activity and cytotoxicity		
			ST		% in. at 10 μM <sup>b</sup>	IC <sub>50</sub> (μM) <sup>x</sup>	EC <sub>50</sub> (μM) <sup>c</sup>	CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>
	H	70	0.40	16.4	nd	>50	nd	nd	

**11aa**<sup>a</sup>Inhibitory concentration 50% (μM) determined from dose response curves.<sup>b</sup>Percentage of inhibition determined at compound concentration of 10 μM.<sup>c</sup>Effective concentration 50% (μM).<sup>d</sup>Cytotoxic concentration 50% (μM).<sup>e</sup>SI = CC50/EC50.<sup>f</sup>nd: not determined.