Novel Quinolinonyl Diketo Acid Derivatives as HIV-1 Integrase Inhibitors: Design, Synthesis, and Biological Activities

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Novel quinolinonyl diketo acids were designed to obtain integrase (IN) inhibitors selectively active against the strand transfer (ST) step of the HIV integration process. Those new compounds are characterized by a single aryl diketo acid (DKA) chain in comparison to 4, a bifunctional diketo acid reported by our group as an anti-IN agent highly potent against both the 3′-processing and ST steps. Compound 6d was the most potent derivative in IN enzyme assays, while 6i showed the highest potency against HIV-1 in acutely infected cells. The selective inhibition of ST suggested the newly designed monofunctional DKA bind the IN—DNA acceptor site without affecting the DNA donor site.

Introduction

Three different classes of chemotherapeutic agents are generally combined to block the replication of human immunodeficiency virus type 1 (HIV-1) responsible for AIDS and to prevent the occurrence of resistance: reverse transcriptase inhibitors (RTI), protease inhibitors (PRI), and fusion inhibitors. This widespread triple combination therapy is referred to as HAART (highly active antiretroviral therapy).1 HAART effectively inhibits HIV replication to such an extent that the virus becomes undetectable in the blood. However, it fails to eradicate viruses that are integrated in the host genome or that persist in cellular and anatomical “reservoirs”. In addition, prolonged drug exposure led to HIV drug resistance, thus reducing patients’ therapeutically available options.2 The above considerations and the toxicity of a number of antiretroviral agents have fueled the discovery of drugs against additional targets. Among them, HIV integrase (IN), which has no cellular counterpart, has been intensely studied over the past 15 years.3,5 IN has recently been fully validated as a therapeutic target with the first FDA approved IN inhibitor raltegravir.6

IN catalyzes the insertion of the viral cDNA (generated by reverse transcription of the viral RNA) into the host cell genome. Integration occurs via a sequence of reactions, which start with the IN-mediated cleavage of terminal dinucleotide from the 3′-end of the viral cDNA (termed “3′-processing”, 3′-P) shortly after reverse transcription in the cytoplasm. Following transfer of the resulting processed viral cDNA into the nucleus, IN catalyzes the insertion of both ends into target cellular host DNA. That second reaction is referred as “strand transfer” (ST).4 In the past 15 years, a range of natural and synthetic compounds have been identified as inhibitors of recombinant IN enzyme in biochemical assays. Interestingly, polyhydroxylated aromatics and diketo compounds were among the first inhibitors identified.3,7–9 However, those early polyhydroxylated derivatives were later demonstrated to inhibit viral entry or to be too toxic to be pursued as therapeutic IN inhibitors.10 More recently, the Merck and Shionogi companies discovered aryl diketo acid (DKA) derivatives as selective anti-HIV agents that block the viral replication cycle via IN inhibition in vivo. Those compounds are typified by 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)propenone (5CITEP, 1) synthesized by Shionogi & Co. Ltd.11 and by the pyrrole derivative L-731,988 (2) developed by Merck Research Laboratories12 (Figure 1). They are characterized by their ability to preferentially inhibit ST versus 3′-P. Chemically, DKA is characterized by a diketo acid moiety (α and γ ketone and a carboxylic acid), which is believed to be essential for the inhibitory activity, although the carboxylic group can be effectively replaced by a bioisoster azole ring (triazole, tetrazole) (i.e., 1)11 and the 1,3-diketo acid moiety can be mimicked by a 8-hydroxy-[1,6]naphthyridine ring13 (i.e., compound 3, Figure 1).

Recent studies on quinolinonyl diketo acid derivatives led us to discover the bifunctional compound 4 as a potent IN inhibitor for both 3′-P and ST.14 Moreover, 4 inhibits HIV-1 replication in acutely infected cells.14 Docking studies on the binding mode of 4 to the IN catalytic site also suggested a
peculiar interaction of the drug involving both the acceptor and the donor DNA binding sites of the enzyme. This hypothesis was confirmed by our recent cross-linking experiment studies that pointed out a specific interaction of 4 with K156 and K159 amino acid residues of the IN catalytic core domain.15 This binding mode could account for the high potency of 4 against both 3′-P and ST.

The aim of the present project was the design of new quinolinonyl diketo acid derivatives endowed with a selective activity against ST. This project could provide new information regarding the interactions of quinolinonyl diketo acids with the IN active site and consequently increase our knowledge about the catalytic mechanisms of IN, which in the absence of structural information on IN−DNA and drug molecular structures are still far from being totally elucidated. In the present manuscript, we describe novel quinolinonyl diketo acid derivatives 5a−i, 6a−i, 7a,b, and 8a,b designed by replacement of the 6-diketo acid chain of 4, responsible for binding to the donor DNA binding site, with smaller substituents in the 6-, 7-, or 8-position of the quinolinone ring (Figure 2). These substituents should exhibit reduced binding to the donor viral cDNA site due to their small size/length or to their limited ability to form hydrogen bonds. Thus, these structural modifications should provide novel quinolinonyl diketo acid derivatives with increased ST selectivity.

Results and Discussion

Chemistry. Synthesis of derivatives 5a−i, 6a−i, 7a,b, and 8a,b is outlined in Schemes 1−3. 3-Acetyl-4(1H)-quinolinones 9a−h were prepared by reaction of the proper 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.16 Then, 9a−h were alkylated with 4-fluorobenzyl bromide in alkaline medium (K2CO3) to give the N-1 substituted quinolones 10a−h. These compounds were condensed with diethyl oxalate in the presence of sodium ethoxide to provide ethyl esters 5a−h, which were in turn hydrolyzed with 6 N NaOH to afford the corresponding acids 6a−h (Scheme 1).

Synthesis of 1-unsubstituted derivatives 7a,b and 8a,b was achieved in a similar fashion by coupling of 4-quinolinones 9e,f with diethyl oxalate in the presence of the sodium ethoxide, followed by alkaline hydrolysis of the resulting ethyl esters 7a,b to provide acids 8a,b (Scheme 2).
Table 1. Chemical, Physical, and Analytical Data of Derivatives 9a−h and 10a−i

| compd   | R₆   | R₇   | R₈   | mp (°C) | recryst solvent* | yield (%) | analysis |
|---------|------|------|------|---------|------------------|-----------|----------|
| 9a<sup>a</sup> | H    | H    | H    | 242−244 | a                | 56        | C. H. N (C₁₇H₂₉NO₂) |
| 9b<sup>a</sup> | F    | H    | H    | >300    | b                | 29        | C. H. N. F (C₁₉H₄FNO₂) |
| 9c<sup>a</sup> | F    | F    | H    | 270−271 | c                | 41        | C. H. N. F (C₁₉H₄FNO₂) |
| 9d<sup>a</sup> | H    | H    | F    | 268−270 | b                | 40        | C. H. N. F (C₁₉H₂FNO₂) |
| 9e<sup>a</sup> | Cl   | H    | Cl   | >300    | b                | 29        | C. H. N. Cl (C₁₉H₃ClNO₂) |
| 9f<sup>a</sup> | H    | Cl   | H    | >300    | b                | 96        | C. H. N. Cl (C₁₉H₃ClNO₂) |
| 9g<sup>a</sup> | H    | H    | Cl   | >300    | b                | 55        | C. H. N. Cl (C₁₉H₃ClNO₂) |
| 9h<sup>a</sup> | Cl   | Cl   | H    | 114−115 | d                | 19        | C. H. N. Cl (C₁₉H₃ClNO₂) |
| 10a<sup>a</sup> | H    | H    | H    | 213−214 | d                | 65        | C. H. N. F (C₁₅H₂FNO₂) |
| 10b<sup>a</sup> | F    | H    | H    | 234−235 | e                | 94        | C. H. N. F (C₁₉H₂FNO₂) |
| 10c<sup>a</sup> | F    | F    | H    | 186−187 | e                | 100       | C. H. F (C₁₉H₂ClNO₂) |
| 10d<sup>a</sup> | H    | H    | F    | 193−194 | f                | 78        | C. H. F (C₁₉H₂ClNO₂) |
| 10e<sup>a</sup> | Cl   | H    | H    | 214−216 | e                | 92        | C. H. N. Cl, F (C₁₉H₂ClFNO₂) |
| 10f<sup>a</sup> | H    | Cl   | H    | 185−187 | e                | 73        | C. H. N. Cl, F (C₁₉H₂ClFNO₂) |
| 10g<sup>a</sup> | H    | H    | Cl   | 215−217 | g                | 92        | C. H. N. Cl, F (C₁₉H₂ClFNO₂) |
| 10h<sup>a</sup> | Cl   | Cl   | H    | 235−237 | d                | 49        | C. H. N. Cl, F (C₁₉H₂ClFNO₂) |
| 10i<sup>a</sup> | H    | 1-pyrrolidinyl | H | 175−177 | e                | 56        | C. H. N. F (C₂₁H₂FNO₂) |

* Recrystallization solvents: (a) aceton, (b) ethanol, (c) isopropanol, (d) toluene, (e) toluene/cyclohexane, (f) benzene/cyclohexane, (g) benzene. Reference 17.

Derivatives 5i and 6i were synthesized as described in Scheme 3. Pyrrolidinyl derivative 10i was obtained in few minutes with good yields by substitution of fluorine atom of 10c with pyrrolidine in the presence of N,N₃ under microwave irradiation. 10i was then condensed with ethyl oxalate, and the ester 5i that formed was hydrolyzed to afford the required acid 6i. Chemical, physical, and analytical data of intermediates 9a−h and 10a−i are reported in Table 1, while spectroscopic data are shown in Supporting Information. Data for final products 5a−i, 6a−i, 7a,b, and 8a,b are listed in Experimental Section.

Evaluation of Biological Activities. In Vitro Assays. Derivatives 5a−i, 6a−i, 7a,b, and 8a,b were tested in vitro for ST inhibition in the presence of magnesium (Mg²⁺) using a recently described high throughput electrochemiluminescent (HTECL) assay.14 IC₅₀ values were generated from duplicate experiments (Table 2). Compounds 5a−i, 6a−i, 7a,b, and 8a,b were also tested for ST and 3’-P using gel-based assays carried out in the presence of Mg²⁺ (Figure 3A). IC₅₀ values were calculated using dose response curves (Figure 3B) and are summarized in Table 2. The newly synthesized DKAs exhibited high potency against IN with high selectivity against ST, thus confirming the hypothesis that the removal of the diketo acid branch in the 6-position of the quinolinone ring of 4 can lead to ST-selective inhibitors (6a−i showed 3’-P(ST)/ST ratios ranging from 45 to 667). The acid derivatives 6a−i were more potent than the corresponding esters 5a−i (Figure 3A and Figure 3B, compare compounds 6h and 5h), and the 1-p-F-benzyl substituted quinolones were markedly more active than their unsubstituted counterparts (7a,b and 8a,b). This trend is consistent with the results obtained previously on the bifunctional DKA (BDKA) series.15 In particular, the relevant role played by the p-F-benzyl moiety at the 1-position of the quinolinone ring was previously noted for the DKA derivatives.12,13 The newly synthesized acid derivatives 6a−i were potent inhibitors, showing IC₅₀ values in the range of 18−40 nM for the ST step (Table 2, HTECL assay). The most active compound of this series is 6d with a ST IC₅₀ value of 18 nM, which is comparable to reference drugs 2 and 3. Removal of the diketo acid chain from the 6-position of our BDKA lead molecule (compound 4)14 led to compound 6a that retains the potent anti-IN activity of the parent derivative with an IC₅₀ value of 40 nM for ST (Table 2, HTECL assay). However, 6a is a more ST selective inhibitor than 4.14 Introduction of a halogen (F or Cl) in the 6-, 7-, or 8-position of 6a led to compounds 6b−g, which show similar anti-IN activities (IC₅₀ = 18−30 nM, Table 2, HTECL assay) as their unsubstituted counterpart 6a. Interestingly, the anti-IN activities appear to be influenced by the position of the halogen. Indeed, inhibitory activities decrease in the following order: 8-F(Cl) > 7-F(Cl) > 6-F(Cl) and 7-Cl > 8-Cl > 6-Cl. The introduction of a second chlorine atom in the structure of the potent inhibitor 6f led to 6h, which is approximately 1.5 times less potent than the parent compound. Similarly, replacement of the chlorine atom of 6f with a pyrrolidine ring led to 6i, which is approximately 1.5 times less potent than the parent compound. In conclusion, the activities of compounds with a substituent in the 7-position decrease in the following order: Cl > F > 1-pyrrolidinyl > H (Table 2, HTECL assay).

Compounds 5a−i and 6a−i also inhibited integrase with similar potency in the presence of Mg²⁺ or Mn²⁺ (data not shown). The activity in the presence of Mg²⁺ in addition to the selectivity for ST represents a trademark of the most potent DKAs active against HIV replication in cells.19

Cell-Based Assays. Cytotoxicity and antiviral activities of compounds 5a−i, 6a−i, 7a,b, and 8a,b are presented in Table 2. Among the tested compounds, derivatives 5a,i, and 6a,e,h,i show EC₅₀ < 50 μM when tested against HIV-1-infected H9/HTLVIIIB cells. In particular, 5i and 6a,h,i are active in the micromolar range, with SI ranging from 48.8 to >1176. The most potent derivative of this series is 6i (EC₅₀ = 0.17 μM and SI > 1176). Compound 6i is in fact more potent than reference derivatives 2−4. In general, all the active compounds were characterized by low cytotoxicity against human histiocytic lymphoma (U937) cell line, showing CC₅₀ values in the range of 99 to >200 μM.

Conclusions

We designed, synthesized, and tested a series of novel quinolinonyl diketo acid derivatives in both enzyme- and cell-based assays as anti-HIV-1 agents to selectively target the ST step of integration. We were able to generate such new compounds, which are potent ST-selective IN inhibitors and active against HIV-1 replication in acutely infected cells. These compounds could be useful tools to study the mechanism of action of HIV-1 IN.

Experimental Section

Chemistry. General. Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum-one spectro-
Figure 3.

d electrochemiluminescent (HTECL) assay (ST assay in the presence of Mg$^{2+}$) on silica gel Merck 70–230 mesh, visualized by UV light. Column chromatographies were performed on silica gel plates for analytical TLC. Developed plates were visualized under longwave ultraviolet light with a UV lamp. NMR spectra were recorded at 400 MHz on a Bruker AC 400 Ultrashield spectrometer. Merck silica gel 60 F254 plates were used for analytical TLC. Developed plates were visualized under longwave ultraviolet light with a UV lamp.

Table 2. Cytotoxicity, Antiviral, and Anti-Integrase Activities of Derivatives 5a–i, 6a–i, 7a,b, and 8a,b

| compd | R₁ | R₂ | R₃ | X       | Mg$^{2+}$ | Mg$^{2+}$ | Mg$^{2+}$ | CC₅₀<sup>50</sup> | EC₅₀<sup>50</sup> | SI<sup>50</sup> |
|-------|----|----|----|---------|-----------|-----------|-----------|-----------------|-----------------|-----------|
| 5a    | H  | H  | H  | C₂H₅   | 0.67      | 0.79 0.44 | 135       | 99              | 34.8            | 2.8       |
| 5b    | F  | H  | H  | C₂H₅   | 0.58      | 0.23 0.44 | >333       | >200            | >50             |           |
| 5c    | H  | F  | H  | C₂H₅   | 1.3       | 1.2 2.9   | >333       | >200            | >50             |           |
| 5d    | H  | H  | F  | C₂H₅   | 26        | 5.2      | >333       | >200            | >50             |           |
| 5e    | Cl | H  | H  | C₂H₅   | 21        | 3.0      | >333       | nd<sup>50</sup> | nd<sup>50</sup>   |           |
| 5f    | H  | Cl | H  | C₂H₅   | 14        | 3.6      | >333       | nd<sup>50</sup> | nd<sup>50</sup>   |           |
| 5g    | H  | H  | Cl | C₂H₅   | 32        | 5.2      | >333       | >200            | >50             |           |
| 5h    | Cl | Cl | H  | C₂H₅   | 3.9       | 1.8      | 135        | 72              | >50             |           |
| 5i    | H  | Py<sup>b</sup> | H  | C₂H₅   | 2.3       | 2.7      | 110        | >200            | 4.1             | 48.8      |
| 6a    | H  | H  | H  | H      | 0.040     | 0.06 0.16 | 14         | >200            | 1.17            | >171      |
| 6b    | F  | H  | H  | H      | 0.030     | 0.14     | 2.3        | >200            | >50             |           |
| 6c    | H  | F  | H  | H      | 0.020     | 0.050    | 4.4        | >200            | >50             |           |
| 6d    | H  | H  | F  | H      | 0.018     | 0.16     | 16         | >200            | >50             |           |
| 6e    | Cl | H  | H  | H      | 0.028     | 0.40     | 6.4        | >200            | 46.1            | 4.3       |
| 6f    | H  | Cl | H  | H      | 0.019     | 0.11     | 4.0        | >200            | >50             |           |
| 6g    | H  | H  | Cl | H      | 0.023     | 0.51     | 44         | >200            | >50             |           |
| 6h    | Cl | Cl | H  | H      | 0.033     | 0.075    | 11.3       | 156             | 3.2             | 62.5      |
| 6i    | H  | Py<sup>b</sup> | H  | H      | 0.028     | 0.18     | 14.9        | >200            | 0.17            | >1176     |
| 7a    | Cl | H  | H  | C₂H₅   | 3.3       | 2.1      | 120        | nd<sup>50</sup> | nd<sup>50</sup>   |           |
| 7b    | Cl | Cl | H  | C₂H₅   | >111      | 224      | >333       | nd<sup>50</sup> | nd<sup>50</sup>   |           |
| 8a    | Cl | H  | H  | H      | >111      | 56       | 166        | nd<sup>50</sup> | nd<sup>50</sup>   |           |
| 8b    | H  | Cl | H  | H      | 37        | 17       | >333       | nd<sup>50</sup> | nd<sup>50</sup>   |           |
| 5a<sup>c</sup> | 2.1 <sup>d</sup> | 0.05 | 1.0 <sup>b</sup> | 0.01 | 0.016 | 0.017 | 0.44 | >200 | 4.29 | >47 |

<sup>a</sup> Inhibitory concentration 50% (µM) determined from dose response curves. <sup>b</sup> Experiments performed in duplicate using the high throughput electrochemiluminescent (HTECL) assay (ST assay in the presence of Mg$^{2+}$). <sup>c</sup> Experiments performed in a gel-based assay in the presence of Mg$^{2+}$ (see Figure 3). <sup>d</sup> Cytotoxic concentration 50% (µM). <sup>e</sup> Effective concentration 50% (µM). <sup>f</sup> Selectivity index = CC₅₀/EC₅₀. <sup>g</sup> nt: not tested. <sup>h</sup> Py = 1-pyrrolidinyl.

1H NMR spectra were recorded at 400 MHz on a Bruker AC 400 Ultrashield spectrometer. Merck silica gel 60 F254 plates were used for analytical TLC. Developed plates were visualized by UV light. Column chromatographies were performed on silica gel Merck 70–230 mesh. 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 a rotatory evaporator (Büchi) operating at a reduced pressure of approximately 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results agreed to within ±0.40% of the theoretical values.

**Microwave Irradiation Experiments.** Microwave reactions were conducted using a CEM Discover synthesis unit (CEM Corp., Matthews, NC). The machine consists of a continuous focused microwave-power delivery system with operator-selectable power output from 0 to 300 W. The temperature of the contents of the vessel was monitored using a calibrated infrared temperature control mounted under the reaction vessel. All experiments were performed using a stirring option whereby the contents of the vessel are stirred by means of a rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar in the vessel.

**General Procedure for the Synthesis of 3-Acetyl-1-(4-fluorophenyl)methyl-7-(pyrrolidin-1-yl)-4(1H)-quinolines 9a–h.** Ethyl orthoformate (4.0 g, 27 mmol), ethyl acetoacetate (3.5 g, 27 mmol), the appropriate substituted aniline (27 mmol), and Dowtherm A (5.6 mL) were charged in a 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 in 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 three-necked flask equipped with thermometer and water separator and heated at 253–254 °C. After addition the mixture was heated at the same temperature for 2 h. Then the mixture was cooled at 90 °C, treated with isopropanol (10 mL), cooled at 30 °C, filtered, and washed with isopropanol and light petroleum ether in turn to give pure derivatives 9a–h. Chemical, physical, and analytical data of derivatives 9a–h are reported in Table 1. For spectroscopic data see Supporting Information.

**General Procedure for the Synthesis of 3-Acetyl-1-(4-fluoroephynyl)methyl-4(1H)-quinolones 10a–h.** A mixture of the proper 9a–h derivative (1.1 mmol), 4-fluorophenethyl bromide (610 mg, 3.3 mmol), and anhydrous K₂CO₃ (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 precipitate that formed was filtered, washed with water and light petroleum ether in turn, and then dried under IR lamp to provide pure derivatives 10a–h.

**3-Acetyl-1-(4-fluoroephynyl)methyl-7-(pyrrolidin-1-yl)-4(1H)-quinoline (10i).** A mixture of 10e (2.7 g, 8.5 mmol), pyrrolidine (1.8 g, 25.6 mmol), and NEt₃ (0.7 g, 7.7 mmol) in dry DMF (40 mL) was irradiated with microwave at 153 °C for 10 min (applied power 100 W), in an open vessel equipped with a condenser. After cooling, the reaction mixture was diluted with water (100 mL) and treated with 1 N HCl until pH 7. The solid that formed was collected by filtration. Purification of crude product was performed by chromatography on silica gel column (chloroform/ethyl acetate 1:1
as eluent) to give 1.7 g (56%) of pure 10i. Chemical, physical, and analytical data of derivatives 10i are reported in Table 1. For spectroscopic data see Supporting Information.

**General Procedure for the Synthesis of Diketo Esters 5a–i and 7a,b.** Sodium ethoxide (390 mg, 5.5 mmol) was added into a well stirred mixture of the appropriate acetyl derivative 9a–h or 10a–i (2.7 mmol) and diethyl oxalate (790 mg, 5.4 mmol) in anhydrous THF (2.7 mL). The collected precipitate was vigorously stirred for 30 min in 1 N HCl (50 mL). The yellow solid that formed was filtered, washed with water, and dried under IR lamp to afford the pure diketo esters 5a–i and 7a,b. Yield (%), melting point (°C), recrystallization solvent, IR, $^1$H NMR, and analytical data are reported for each of the following compounds.

4-[(4-Fluorophenyl)methyl]-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5a). 82%; 175–177 °C; ethanol; IR ν 3400 (OH), 1721 (C=O ester), 1661, 1635, and 1607 (C=O ketone) cm$^{-1}$. $^1$H NMR (CDCl$_3$) δ 1.44 (t, 3H, CH$_3$), 4.42 (q, 2H, CH$_2$CH$_3$), 5.48 (s, 2H, CH$_2$), 7.06–7.26 (m, 4H, benzene H), 7.37–7.65 (m, 3H, quinolinone C6–H, C7–H, and C8–H), 8.16 (s, 1H, butenoate C3–H), 8.56 (m, 1H, quinolinone C5–H), 8.77 (s, 1H, quinolinone C2–H), 15.50 (bs, 1H, OH). Anal. (C$_{22}$H$_{18}$FNO$_5$)C, H, N, F.

4-[6-Fluoro-1-(4-fluorophenyl)methyl]-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5b). 92%; 152–153 °C; toluene/cyclohexane; IR ν 1740 (C=O ester), 1650 and 1624 (C=O ketone) cm$^{-1}$. $^1$H NMR (CDCl$_3$) δ 1.29 (t, 3H, CH$_3$), 4.30 (q, 2H, CH$_2$CH$_3$), 5.78 (s, 2H, CH$_2$), 7.16–7.20 (m, 2H, benzene H), 7.29–7.37 (m, 2H, benzene H), 6.66 (m, 1H, quinolinone C7–H), 7.81 (m, 1H, quinolinone C8–H), 7.97 (m, 1H, quinolinone C5–H), 8.00 (s, 1H, butenoate C3–H), 9.13 (s, 1H, quinolinone C2–H), 15.33 (bs, 1H, OH). Anal. (C$_{22}$H$_{17}$FNO$_5$) C, H, N, F.

4-[7-Fluoro-1-(4-fluorophenyl)methyl]-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5c). 84%; 151–152 °C; benzene/cyclohexane; IR ν 1726 (C=O ester), 1644 (C=O ketone) cm$^{-1}$. $^1$H NMR (DMSO-d$_6$) δ 1.30 (t, 3H, CH$_3$), 4.30 (q, 2H, CH$_2$CH$_3$), 5.72 (s, 2H, CH$_2$), 7.17–7.21 (m, 2H, benzene H), 7.31–7.40 (m, 3H, benzene H and quinolinone C6–H), 7.62 (m, 1H, quinolinone C8–H), 7.97 (s, 1H, butenoate C3–H), 8.36 (m, 1H, quinolinone C5–H), 9.10 (s, 1H, quinolinone C2–H), 14.00 (bs, 1H, OH). Anal. (C$_{22}$H$_{17}$F$_2$NO$_5$) C, H, N, F.

4-[8-Fluoro-1-(4-fluorophenyl)methyl]-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5d). 83%; 179–180 °C; benzene/cyclohexane; IR ν 3300 (OH), 1744 (C=O ester), 1648 and 1604 (C=O ketone) cm$^{-1}$. $^1$H NMR (DMSO-d$_6$) δ 1.27 (t, 3H, CH$_3$), 4.30 (q, 2H, CH$_2$CH$_3$), 5.78 (s, 2H, CH$_2$), 7.14–7.24 (m, 4H, benzene H), 7.48–7.53 (m, 1H, quinolinone C6–H), 7.59–7.69 (m, 1H, quinolinone C7–H), 7.97 (s, 1H, butenoate C3–H), 8.17–8.19 (m, 1H, quinolinone C5–H), 9.01 (s, 1H, quinolinone C2–H), 15.50 (bs, 1H, OH). Anal. (C$_{22}$H$_{17}$F$_2$NO$_5$) C, H, N, F.

4-[6-Chloro-1-(4-fluorophenyl)methyl]-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5e). 34%; 131–133 °C; washed with cyclohexane; IR ν 3400 (OH), 1722 (C=O ester), 1700 and 1628 (C=O ketone) cm$^{-1}$. $^1$H NMR (DMSO-d$_6$) δ 1.33 (t, 3H, CH$_3$), 4.33 (q, 2H, CH$_2$CH$_3$), 5.71 (s, 2H, CH$_2$), 7.14–7.34 (m, 4H, benzene H), 7.71–7.77 (m, 2H, quinolinone C7–H and C8–H), 7.91 (s, 1H, butenoate C3–H), 8.25 (m, 1H, quinolinone C5–H), 9.09 (s, 1H, quinolinone C2–H), 12.70 (bs, 1H, OH). Anal. (C$_{22}$H$_{17}$ClFNO$_5$) C, H, N, Cl, F.

4-[7-Chloro-1-(4-fluorophenyl)methyl]-4(1H)-quinolinon-3-yl]-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5f). 76%; 173–175 °C; washed with isopropanol; IR ν 3300 (OH), 1727 (C=O ester), 1647 (C=O ketone) cm$^{-1}$. $^1$H NMR (DMSO-d$_6$) δ 1.30 (t, 3H, CH$_3$), 4.32 (q, 2H, CH$_2$CH$_3$), 5.76 (s, 2H, CH$_2$), 7.16–7.25 (m, 2H, benzene H), 7.36–7.38 (m, 2H, benzene H), 7.54 (m, 1H, quinolinone C6–H), 7.88 (m, 1H, quinolinone C8–H), 7.96 (s,
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4-[7-Chloro-1(4-fluorophenyl)methyl(4(1H)-quinolinon-3-yl)-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5f), 95%; 164–165 °C; toluene; IR ν 3400 (OH), 1749 (C=O ester), 1644 (C=O ketone) cm⁻¹. H NMR (DMSO-d₆) δ 7.91 (s, 1H, quinolinone C₂–H), 7.91 (1H, butenoate C₃–H), 8.34 (m, 1H, quinolinone C₅–H), 8.81 (s, 1H, quinolinone C₂–H), 15.25 (bs, 1H, OH). Anal. (C₂₉H₂₁F₂NO₅) C, H, N, F.

4-[6-Chloro-1(4-fluorophenyl)methyl(4(1H)-quinolinon-3-yl)-2-hydroxy-4-oxo-2-butenoic Acid Ethyl Ester (5h), 78%; 180–181 °C; with hot anhydrous ethanol; IR ν 3400 (OH), 1732 (C=O acid), 1634 and 1602 (C=O ketone) cm⁻¹. H NMR (DMSO-d₆) δ 5.86 (s, 2H, CH₂), 7.12–7.23 (m, 4H, benzene H), 7.45–7.50 (m, 1H, quinolinone C₅–H), 7.70 (s, 1H, butenoate C₃–H), 8.16–8.18 (m, 1H, quinolinone C₅–H), 8.96 (s, 1H, quinolinone C₂–H), 14.30 (bs, 2H, OH). Anal. (C₂₉H₁₈F₂NO₅) C, H, N, F.

4-[7-Chloro-1(4-fluorophenyl)methyl(4(1H)-quinolinon-3-yl)-2-hydroxy-4-oxo-2-butenoic Acid (6d), 64%; 188–190 °C; with isopropanol; IR ν 3400 (OH), 1734 (C=O acid), 1646 (C=O ketone) cm⁻¹. H NMR (DMSO-d₆) δ 5.75 (s, 2H, CH₂), 7.18–7.32 (m, 4H, benzene H), 7.75–7.85 (m, 2H, quinolinone C₇–H and C₈–H), 8.22 (m, 1H, C₅–H quinolinone), 7.90 (s, 1H, butenoate C₃–H), 9.08 (s, 1H, quinolinone C₂–H), 13.00 (bs, 2H, OH). Anal. (C₂₉H₂₁F₂NO₅) C, H, N, F.

**General Procedure for the Synthesis of Diketo Acids 6a–ı and 8a–b. A mixture of 1 N NaOH (6.5 mL) and the appropriate ester 5a–ı or 7a, b (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 yellow solid that formed was collected by filtration, then washed with water, hot dry ethanol, and light petroleum ether to afford pure acids 6a–ı and 8a–b. Yield (%), melting point (°C), recrystallization solvent, IR, 1H NMR, and analytical data are reported for each of the following compounds.

4-[1(4-Fluorophenyl)methyl(4(1H)-quinolinon-3-yl)-2-hydroxy-4-oxo-2-butenoic Acid (6a), 50%; 207–209 °C; with washed with anhydrous ethanol; IR ν 3400 (OH), 1732 (C=O acid), 1619 (C=O ketone) cm⁻¹. H NMR (CDCl₃) δ 5.79 (s, 2H, CH₂), 7.22–7.38 (m, 4H, benzene H), 7.53–8.34 (m, 5H, quinolinone C₆–H, C₇–H, C₈–H and butenoate C₃–H), 8.16 (s, 1H, quinolinone C₂–H), 15.50 (bs, 2H, OH). Anal. (C₂₀H₁₄F₂NO₅) C, H, N, F.

4-[6-Chloro-1(4-fluorophenyl)methyl(4(1H)-quinolinon-3-yl)-2-hydroxy-4-oxo-2-butenoic Acid (6b), 95%; 183–184 °C; washed with anhydrous ethanol; IR ν 3100 (OH), 1730 (C=O acid), 1613 (C=O ketone) cm⁻¹. H NMR (DMSO-d₆) δ 5.76 (s, 2H, CH₂), 7.16–7.20 (m, 2H, benzene H), 7.33–7.36 (m, 2H, benzene H), 7.65 (m, 1H, quinolinone C₇–H), 7.80 (1H, quinolinone C₈–H), 7.94–7.97 (m, 2H, quinolinone C₅–H and butenoate C₃–H), 9.10 (s, 1H, quinolinone C₂–H), 14.67 (bs, 2H, OH). Anal. (C₂₉H₂₃F₂NO₅) C, H, N, F.
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