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6-(1-Benzyl-1*H*-pyrrol-2-yl)-2,4-dioxo-5-hexenoic Acids as Dual Inhibitors of Recombinant HIV-1 Integrase and Ribonuclease H, Synthesized by a Parallel Synthesis Approach

Roberta Costi[*,†], **Mathieu Métifiot**[‡], **Francesca Esposito**[∫], **Giuliana Cuzzucoli Crucitti**[†], **Luca Pescator**[†], **Antonella Messore**[†], **Luigi Scipione**[†], **Silvano Tortorella**[†], **Luca Zinzula**[∫], **Ettore Novellino**[§], **Yves Pommier**[‡], **Enzo Tramontano**[∫], **Christophe Marchand**[*,‡], **Roberto Di Santo**[†]

[†]Dipartimento di Chimica e Tecnologie del Farmaco, Istituto Pasteur-Fondazione Cenci Bolognetti, “Sapienza” Università di Roma, P. le Aldo Moro 5, Rome I-00185, Italy

[‡]Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 37, Room 5068, Bethesda, Maryland 20892-4255, United States

[∫]Department of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, Cagliari I-09124, Italy

[§]Dipartimento di Farmacia, Università di Napoli “Federico II”, Via D. Montesano 49, I-80131 Napoli, Italy

Abstract

The increasing efficiency of HAART has helped to transform HIV/AIDS into a chronic disease. Still, resistance and drug–drug interactions warrant the development of new anti-HIV agents. We previously discovered hit **6**, active against HIV-1 replication and targeting RNase H in vitro. Because of its diketo-acid moiety, we speculated that this chemotype could serve to develop dual inhibitors of both RNase H and integrase. Here, we describe a new series of 1-benzyl-pyrrolyl diketohexenoic derivatives, **7a–y** and **8a–y**, synthesized following a parallel solution-phase approach. Those 50 analogues have been tested on recombinant enzymes (RNase H and integrase) and in cell-based assays. Approximately half (22) exibited inhibition of HIV replication. Compounds **7b**, **7u**, and **8g** were the most active against the RNase H activity of reverse-transcriptase, with IC_{50} values of 3, 3, and 2.5 μM, respectively. Compound **8g** was also the most potent integrase inhibitor with an IC_{50} value of 26 nM.

[*]**Corresponding Authors** R.C.: phone, +39-06-49693247; fax, +39-06-49913133; roberta.costi@uniroma1.it.; C.M.: phone, +1-301-435-2463; fax, +1-301-402-0752; marchand@nih.gov.

Author Contributions

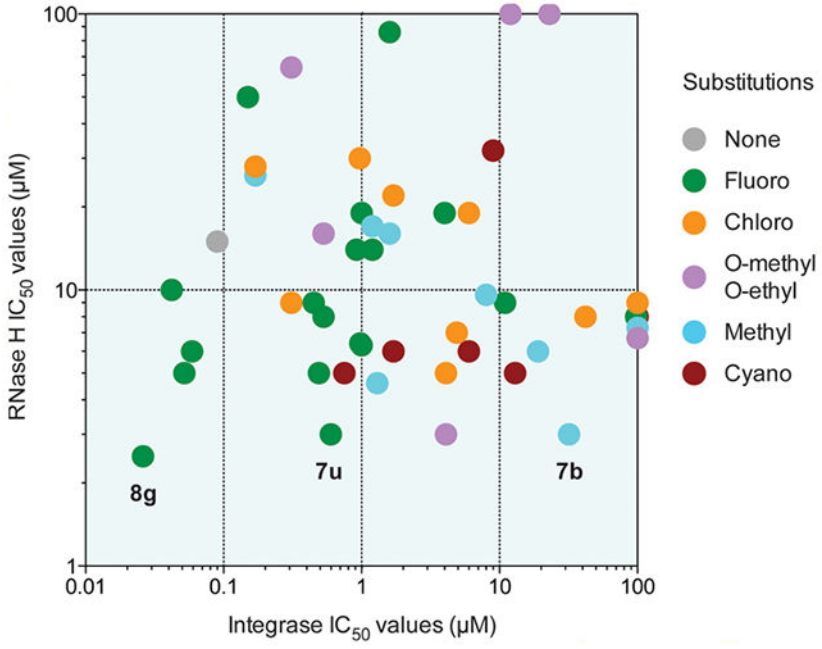
R.C. and M.M. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Supporting Information

Spectroscopic data and analyses of compounds **7a–y**, **8a–y**, **9a–y**, and **10a–y**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

Graphical Abstract



INTRODUCTION

After the introduction of the highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV) infection has been transformed into a chronic disease.[1] The main reason for this success is the use of combinatorial therapy targeting different viral targets, which drastically reduces the selection of drug-resistant virus strains. However, HAART exhibits several liabilities including long-term toxicity, emergence of drug resistant virus strains, drug–drug interactions, and a significant cost burden associated with the use of multiple single agent formulations.[2] Therefore, the development of new anti-HIV drugs is warranted, particularly if targeted against novel viral functions and/or if exploring new pharmacological approaches. One of such innovative approaches is the use of dual-action drugs that combine two different desired pharmacological actions at a similarly effective dose. This approach has already been validated in the oncology arena, where dual inhibitors of tyrosine and phosphoinositide kinases show very promising physiological activity[3] in the anti-infective field of malaria[4] and currently represents an active aspect of drug development.[5]

Among the drugs currently used in HAART regimens, raltegravir (**1**, Chart 1) received approval by the U.S. Food and Drug Administration (FDA) in 2007. **1** inhibits HIV-1 integrase (IN), the viral coded enzyme responsible for the insertion of the HIV retro-transcribed dsDNA into a cell host chromosome.[6] HIV-1 IN catalyzes two enzymatic reactions with the same active site. In the first reaction, called 3′-processing (3′-P), IN removes the two terminal nucleotides (GT) from each 3′-end of the dsDNA. In the second reaction, named “strand transfer” (ST), IN catalyzes a nucleophilic attack by the free 3′-OH of the viral processed DNA onto the target chromosomal DNA, resulting in covalent joining

of the two DNA molecules. IN belongs to the polynucleotidyl transferase family, and its active site shares structural similarities with the HIV-1 reverse transcriptase (RT)-associated ribonuclease H (RNase H) active site.[7] In particular, the arrangement of the active site residues and metal ion coordination in HIV IN and HIV RNase H are very similar.[6,8] It is also important to note that (i) the RT-associated RNase H function is essential for HIV-1 replication, (ii) it is a validated target for drug development, and (iii) no drug that targets RNase H has yet been approved.[8-10] Given the structural similarities between the domains of these two HIV-1 enzymes, several compounds initially developed as IN inhibitors have also been screened against RNase H and vice versa, such as diketo acid (DKA) derivatives like 4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid (BTDBA, **2**)[11] (Chart 1) and DNA aptamers.[12] Recently, three kind of structurally different compounds as dual inhibitors were described, including tropolones (i.e., **3**),[13-15] madurahydroxylactone derivatives like compound **4**,[16] and 2-hydroxyisoquinolin-1,3(2*H*,4*H*)-diones (i.e., **5**)[17,18] (Chart 1).

We have previously reported that DKAs initially synthesized as IN inhibitors were also able to inhibit the HIV-1 RNase H activity of RT.[19,20] In particular, the IN inhibitor 6-[1-(4-fluorophenyl)methyl-1*H*-pyrrol-2-yl)]-2,4-dioxo-5-hexenoic acid ethyl ester (RDS 1643, **6**, Chart 1),[19,21] has been shown to chelate Mg^{2+} in the RNase H catalytic site and to be inactive against the RT-associated DNA polymerase function.[19] Thus, pursuing our decennial studies on IN inhibitors[21-28] and considering the above observations, we decided to design a small library of analogues of compound **6**, as dual IN/RNase H inhibitors. Taking into account that the benzyl ring of DKAs plays a crucial role in binding to both the vDNA bases and the hydrophobic pocket within the catalytic core of IN enzyme,[29] we wanted to investigate the influence of the benzyl substituents on the anti-IN and anti-RNase H activities while keeping unmodified both the diketohexenoic moiety and the pyrrolyl nucleus. In this study, we describe a small library of pyrrolyl diketohexenoic derivatives (**7a–y** and **8a–y**), designed by replacement of the fluorine atom in the 4-fluorobenzyl moiety of **6** with several substituents endowed with various electronic and steric properties to obtain dual inhibitors of IN and RNase H. This small library of pyrrolyl derivatives was set up by a simple and efficient parallel solution-phase synthesis approach. The newly synthesized compounds **7a–y** and **8a–y** have been evaluated against both RNase H and HIV IN and for their cytotoxicity and activity against HIV-1 infected cells.

RESULT AND DISCUSSION

Chemistry.

The synthesis of derivatives **7a–y** and **8a–y** is reported in Scheme 1. The mono- and disubstituted-*N*-benzyl-pyrrolyl diketohexenoic acids were synthesized in a four-step parallel procedure (Scheme 1) using a Büchi SynCor reactor fitted with 12 or 13 tubes (50 mL). Pyrrole-2-carboxaldehyde was alkylated with the appropriate benzyl bromide in the presence of NaOH to afford the *N*-substituted-pyrrole-2-carboxaldehydes **9a–y**, which were converted to the corresponding 3-buten-2-ones **10a–y** by condensation with acetone using NaOH as a base. Compounds **10a–y** were then reacted with diethyl oxalate in the presence of NaOEt as a base to obtain hexenoic esters **7a–y** that were finally hydrolyzed in basic medium to give the corresponding acids **8a–y**.

Evaluation of Biological Activities.

All the newly synthesized compounds **7a–y** and **8a–y** were tested in vitro in biochemical assays both against rRT to test their ability to reduce the activity of the RT-associated RNase H function, and against rIN, to evaluate inhibitory activities against the ST step of the integration reaction. Corresponding IC_{50} values were calculate using a dose–response curve and are reported in Table 1.

The IC_{50} values obtained for both recombinant RNase H and IN were plotted against each other in a correlation plot where single dots correspond to single compounds (Figure 1). As seen in Figure 1A, this graph does not reveal any particular correlation between RNase H and IN inhibition. The compounds organize themselves around two perpendicular axes crossing the IN IC_{50} axis (*X* axis) at 1 μM and the RNase H IC_{50} axis (*Y* axis) at 10 μM (bolded crosshair in the center of the graph, Figure 1). These two axes splice the graph into four quarters corresponding to RNase H/IN dual inhibitors (lower left quarter), RNase H-selective inhibitors (lower right quarter), IN selective inhibitors (upper left quarter), and inhibitors of lower potency (upper right quarter).

When the compounds are categorized according to their ester (blue, Figure 1A) or acidic function (red, Figure 1B), it appears that the acidic function is preferred for IN potency, with a majority of the red dots (acids) in the two left quarters and a majority of blue dots (esters) in the two right quarters of the plot, respectively (Figure 1A). This preference of the acidic over the ester function is not observed for RNase H inhibition because red dots (acidic) are equally distributed in the two upper and two lower quarters of the graph. RNase H inhibition may even be more prone to ester containing compounds with two-thirds of the blue dots distributed in the two lower quarters of the graph (Figure 1A).

We then categorized the compounds according to the type of substitutions and found that 8 out of 11 compounds in the lower left quarter of the graph are fluoro substituted derivatives (green dots, Figure 1B) and that out of these compounds, seven are difluoro substituted. Therefore, difluoro substitutions may be a hallmark of RNase H/IN dual inhibitors. Chloro substituents (orange dots), which distribute around the center of the graph, do not seem to select for inhibition of one enzyme over the other (Figure 1B). Methyl (cyan dots) and *O*-alkyl substitutions (lavender dots) do not seem to favor IN inhibition because they are in vast majority located in the two right quarters of the plot (Figure 1B). Moreover, these substituents do not seem to impact RNase H inhibition with an even distribution among the upper and lower quarters of the graph. In contrast, the cyano substituted compounds (dark-red dots) appear to be more selective for RNase H inhibition (lower right quarter, Figure 1B).

Finally, the compounds were categorized according to the position of their substitutions (Figure 1C). Compounds bearing di-*ortho* (2,6) substitutions (blue dots) do not inhibit RNase H efficiently because these compounds all distribute in the two upper quarters of the graph in contrast to compounds bearing at least one *para* substituent (magenta, position 4), which are more potent RNase H inhibitors with 90% of the compounds in the two lower quarters of the plot (Figure 1C). These two positions do not impact IN inhibition.

The most part of the newly synthesized pyrrolyl derivatives **7a–y** and **8a–y** exhibited good potency in inhibiting the ST step of the HIV-1 IN. All the newly synthesized acids compounds were selective inhibitors of the ST step of the integration process catalyzed by IN, confirming that the DKA derivatives were ST vs 3′-P selective inhibitors. In fact, the IC_{50} values on the 3′-P step were around 2–3 orders of magnitude higher if compared with the ones on ST (data not shown).

The acids were more potent than the corresponding esters. In fact, **8a–y** showed IC_{50} values in the range 0.026–6.0 μM for the ST step, while the corresponding esters **7a–y** were less active, showing IC_{50} values in the range of >0.45–111 μM for the ST step. Among the acid derivatives, 15 compounds (**8a,b,e–h,k–m,o,q–t,y**) resulted in having submicromolar activity values when tested against the rIN ST step while four compounds (**8i,j,q,w**) can be considered almost inactive (IC_{50} values in the range of 4–6 μM). The other six acid compounds (**8c,d,lp,u,v**) were moderate IN inhibitors because their IC_{50} values were in the range of 1.2–1.6 μM.

The inhibition of the rIN by the monofluoro substituted- and difluoro substituted-*N*-benzyl-pyrrolyl diketohexenoic acids was higher if compared to that of compounds bearing other substituents (Table 1). The most potent anti-IN compound within this series was the 4-fluorobenzyl-pyrrolyl diketohexenoic acid (**8g**), which showed an IC_{50} of 26 nM. Generally, the most active compounds were fluoro derivatives **8g**, **8q**, **8r**, and **8s** (4-F, 2,3-F_2, 2,4-F_2, and 2,5-F_2 substituted respectively) with IC_{50} values ranging from 26 to 59 nM. Among the ester series, the difluoro substituted derivatives (**7q**,**s**,**u**,**v**) resulted in being the most active compounds with IC_{50} values in the range of 0.49–0.60 μM.

The anti-IN activities of monosubstituted- and disubstituted-*N*-benzyl-pyrrolyl diketohexenoic acids were comparable. In fact, their IC_{50} values were in the range of 6–0.026 and 4.9–0.042 μM, respectively.

The substitution of fluorine atom in *para* position of the benzyl group with either electron-donor or -withdrawing groups leads to compounds with decreased activity (**8d**,**i**,**l**,**n**, IC_{50}s from 1.2 to >4.1 μM). On the contrary, a good activity persisted when the fluorine atom in the 4-position was replaced by a hydrogen atom, such as for compound **8a** (IC_{50} = 0.090 μM). When the fluorine atom of the benzyl ring of **8a** was shifted from *para* to *ortho* or *meta* position, the resulting compounds **8e** and **8f** were 10-fold less active than **8a** (IC_{50} values 0.98 and 0.92 μM, respectively).

Among the disubstituted derivatives, the difluoro compounds **8q–s** (2,3-F_2, 2,4-F_2, and 2,5-F_2, respectively), showed IC_{50} values (42–59 nM) comparable to that of the 4-fluoro counterpart (**8g**), while **8t–v** (2,6-F_2, 3,4-F_2, and 3,5-F_2, respectively) were 6–60 times less potent than **8g** (IC_{50} range 0.15–1.6 μM). Similarly to **8t–v**, the dimethylderivative **8p** and the dichloroderivatives **8w–y** showed reduced activities (IC_{50} range 0.17–4.9 μM).

In general, activities of acid **7a–y** and ester **8a–y** derivatives against HIV-1 RT-associated RNase H function were comparable. In fact, 27 compounds (**7b–g**,**i**,**k**,**l**,**n**,**p**,**q**,**s**,**u–w** and

8e,**g–l**,**n**,**q**,**s**,**w**) were characterized by IC_{50} values <9.6 μM. The most selective inhibitor of the RNase H function was compound **7n** that showed an IC_{50} RNase H/IC_{50} IN ratio of 16.

Compounds **7b**,**u** and **8g** (2-Me, 3,4-F_2, and 4-F substituted, respectively) were the most active derivatives as RNase H inhibitors. Although **7b** and **7u** were ester derivatives, while **8g** was an acid compound, it is worth noting that their IC_{50} values against RNase H were comparable (3, 3, and 2.5 μM, respectively). On the contrary, a direct comparison of their anti-IN activities showed that the acid derivative **8g** was more potent than the esters **7b** and **7u** (0.026, 32, and 0.6 μM, respectively). Compounds **7u** and **8g** were more selective in inhibiting the IN enzyme because their IC_{50} RNase H/IC_{50} IN ratios were ≈10 and 100, respectively. Conversely, **7b** was more active on RNase H (IC_{50} IN/IC_{50} RNase H ratio ≈10).

Within the *ortho*- and *meta*-benzylsubstituted pyrrolyl diketohexenoic derivative series, the inhibitory activities against RNase H were well correlated with the nature of the substituent. In fact, in both cases the activity decreases with the following order: Me > Cl, CN, F > OMe, OEt, where the derivative ester **7b** (2- Me) and the acid **8c** (3- Me) showed the best activity with an IC_{50} value of 3 and 4.6 μM, respectively.

Among the *ortho*-benzyl substituted pyrrolyl diketohexenoic acids series, fluoro and cyano groups (**8e**,**j**) are well-tolerated in comparison with methyl, methoxy, and ethoxy groups (**8b**,**m**,**o**). The same was observed when comparing the *para*-benzylsubstituted esters **7g**,**i**,**l** (fluoro, chloro, and cyano, respectively) with the esters **7d**,**n** (methyl and methoxy, respectively).

The well tolerated combination of substituents on the benzyl moiety among the disubstituted acids derivatives was observed when the fluorine atom is present in positions 4 (**8r**) or 3 (**8q**) or when the fluorine atoms were changed with the chlorine ones (**8w**). Among the disubstituted esters derivatives, the well tolerated combination of substituents on the benzyl mojety was the 3,4-F_2 (**7u**), with an IC_{50} value of 3 μM. A comparable activity was observed when the fluorine atom was shifted from 4 to 5 position (**7v**) or when the fluorine atoms were changed with the methyl group (**7p**).

Cell-Based Assays.

Antiviral activity was initially assessed using HeLa-CD4-LTR-β-gal reporter cells (Table 1). In this assay, reference compound **1** exhibited low nanomolar activity with an EC_{50} value of 24 ± 5 nM (Table 1). Among **7a–y** and **8a–y**, 18 derivatives (**7a**,**e**,**g**,**h**,**m**,**o**,**q–t** and **8a**,**e**,**g**,**m**,**o**,**q**,**r**,**t**) showed submicromolar EC_{50} values when tested against HIV-1(IIIB) infected HeLa-CD4-LTR-β-gal cells. Selected compounds were also tested for their ability to inhibit the HIV-1-induced cytopathic effect (CPE) in MT-4 cells. From the 10 active compounds we selected (**7a**,**c**,**e**,**f**,**g** and **8a**,**b**,**c**,**f**,**g**), all were antiviral in the CPE assay. The inactive compound **8i** was also found inactive in this assay (Table 1). Among the acid derivatives, the most potent compounds were **8m**,**o**,**t**, with EC_{50} values below 0.2 μM, while the most potent ester derivatives were **7g**,**m**,**q**,**r**,**t**, with EC_{50} values of 0.2 μM or below.

In parallel, the newly synthesized compounds have been tested for their cytotoxcity using HeLa-CD4-LTR-β-gal cells. Compounds **7a–y** and **8a–y** exhibited limited cytotoxicity, with CC_{50} values ranging from 30 to >250 μM (Table 1). This resulted in 13 compounds with selctivity indexes (SI) above 100. The most selective compound were **7g**,**m**,**q**,**r**,**t** and **8o**,**t**, with SI values above 250.

In general, antiviral activity data correlated with inhibition data obtained on recombinant enzymes. Compounds harboring an ester function were usually less active on recombinant IN than their acid counterparts. This is may be explained by the fact that a free carboxylic function is required for the inhibition of IN by metal cofactor chelation. Still, some of those acid compounds with good potency against recombinant enzymes (e.g., **8d**,**h–k**,**u**,**y**) lacked activity against HIV-1 replication in a cellular context (Table 1). Neutralization of their acid function in the corresponding ester derivatives (**7d**,**h–k**,**u**,**y**) rescued their antiviral activity (Table 1). Similarly, our most potent inhibitor **8g** (2.5 μM and 26 nM against RNase H and IN, respectively) is not the most potent antiviral in this series (EC_{50} value of 630 nM), while the ester **7g**, inactive against recombinant enzymes, is one of the most potent antiviral (EC_{50} value below 200 nM). All together, our data indicate that acid derivatives may suffer from poor cellular uptake and that esterification of the carboxylic group may improve bioavailability. Furthermore, our ester derivatives seem to be metabolized to the more active acidic form in cells. Thus, in our series of derivatives, ester variants may represent potential pro-drug candidates.

CONCLUSION

Despite the major progress of HAART, limitations arising from resistance and drug–drug interaction warrant the development of original antiviral agents. In this study, we took advantage of the similarities between IN and RNase H domain of RT to design dual inhibitors. A series of pyrrolyl diketohexenoic derivatives has been synthesized using a simple and efficient parallel solution-phase approach. The best compounds achieved single-digit micromolar inhibition of RNase H and low nanomolar IC_{50} values against IN in recombinant assays. Correlation analysis of the in vitro potency data highlighted key structural requirements for the selective inhibition of one or the other enzyme. Further studies taken into account these results should allow the rational development of dual inhibitors with increased RNase H potency.

EXPERIMENTAL SECTION

Chemistry. General.

Melting points were determined with a Büchi 530 capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum-One spectrophotometer. ^1H NMR spectra were recorded on a bruker AC 400 spectrometer. Merck silica gel 60 F_{254} plates were used for analytical TLC. Developed plates were visualized by UV light. Column chromatography was performed on silica gel (Merck; 70–230 mesh) or aluminum oxide (Merck; 70–230 mesh). Compounds purity were always >95% determined by high-pressure liquid chromatography (HPLC). HPLC analysis was carried out using Shimadzu LC-10AD VP and CTO-10AC VP. Column used was generally Suplex pKb-100 (250 mm × 4.6 mm, 5

μm). Büchi SynCor was used for parallel synthesis using 50 mL test tubes. Solvents were reagent grade and, when necessary, purified and dried by standard methods. Organic solutions were dried over anhydrous sodium sulfate (Merck). Concentration of solution after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure of approximately 20 Torr. Analytical results agreed to within ±0.40% of the theoretical values. Dimethylsulfoxide-d_6 99.9% (code 44,139-2) and deuterochloroform 98.8% (code 41,675-4) of isotopic purity (Aldrich) were used.

General Procedure for the Synthesis of *N*-[(Phenyl)methyl]-pyrrole-2-carboxaldehyde 9a–y.—Twenty-five tubes (25 mL each, divided in two sessions of 12 and 13 tubes, respectively) were charged with a solution of pyrrole-2-carboxaldehyde (0.016 mol) in 30 mL of dry DMF treated with NaOH (0.018 mol) and placed in the Büchi SynCor reactor. Then the appropriate benzyl halide (0.011 mol) was added, and the reaction mixtures were stirred with bascular stirring in Büchi Syncor at room temperature at 250 rpm overnight. The solution was diluted with water and extracted with ethyl acetate. The collected organic extract was washed with brine (three times) and dried, and the solvent was evaporated under reduced pressure to obtain crude **9a–y**, which was purified by column chromatography. Chemical, physical, and analytical data of derivatives **9a–y** are reported in Table 1 of the Supporting Information, together with the spectral data of representative compounds.

General Procedure for the Synthesis of 4-(Pyrrol-3-yl)but-3-en-2-one 10a–y.—Twenty-five tubes (25 mL each, divided in two sessions of 12 and 13 tubes, respectively) were charged with a solution of the appropriate aldehyde **9a–y** (0.0068 mol) in 14 mL of acetone and were treated with 9.8 mL of NaOH 5 N and stirred at 50 °C with bascular stirring in Büchi Syncore at room temperature at 250 rpm overnight and then treated with water. The reaction mixture was extracted with ethyl acetate. The collected organic extract was washed with brine (three times) and dried, and the solvent was evaporated under reduced pressure to obtain crude derivatives **10a–y**, which was purified by column chromatography. Chemical, physical, and analytical data of derivatives **10a–y** are reported in Table 1 of the Supporting Information, together with the spectral data of representative compounds.

General Procedure for the Synthesis of Diketo Esters 7a–y.

Twenty-five tubes (25 mL each, divided in two sessions of 12 and 13 tubes, respectively) were charged with a solution of the appropriated acetyl derivative **10a–y** (0.077 mol) and diethyl oxalate (0.077 mol) dissolved in 8 mL of dry THF and placed in the Büchi SynCor reactor. The reaction mixtures were treated, under argon stream, with NaOEt obtained by the dissolution of Na (0.0155 mol) in 17 mL of absolute ethanol. The mixture was stirred with bascular stirring in Büchi SynCor at room temperature at 250 rpm for 1 h 30 min and then was poured into *n*-hexane (60 mL). The collected precipitate was vigorously stirred for 30 min in 1 N HCl (60 mL). The solid that formed was filtered, washed with water and light petroleum ether, and dried under IR lamp to afford the pure diketo ester **7a–y**. Yield (%), melting point (°C), recrystallization solvent, IR, ^{1}H NMR, and analytical data are reported for each of the following compounds.

6-[1-(Phenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7a).—Yield 68%; 79–81 °C; ligroin. IR ν 3400 (OH), 1732 (C═O ester), 1621 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (t, 3H, CH_2CH_3), 4.38 (q, 2H, CH_2CH_3), 5.27 (s, 2H, CH_2), 6.33–6.43 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.90–6.97 (m, 1H, pyrrole β), 7.11 (m, 1H, pyrrole α), 7.30–7.38 (m, 5H, benzene H), 7.69 (d, 1H, J_t = 15.0 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

6-[1-(2-Methylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7b).—Yield 89%; 104–105 °C; benzene/cyclohexane. IR ν 3400 (OH), 1720 (C═O ester), 1610 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.43 (t, 3H, CH_2CH_3), 2.36 (s, 1H, CH_3), 4.41 (q, 2H, CH_2CH_3), 5.22 (s, 2H, CH_2), 6.27–6.45 (m, 3H, J_t = 15.3 Hz, hexanoate C5–H, hexanoate C3–H and pyrrole β), 6.69–6.72 (m, 3H, benzene H, pyrrole α, β), 7.16–7.31 (m, 3H, benzene H), 7.65 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

6-[1-(3-Methylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7c).—Yield 71%; 76–78 °C; cyclohexane. IR ν 3400 (OH), 1700 (C═O ester), 1590 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (t, 3H, CH_2CH_3), 2.32 (s, 3H, CH_3), 4.33 (q, 2H, CH_2CH_3), 5.19 (s, 2H, CH_2), 6.32–6.40 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.38–7.26 (m, 6H, benzene H, pyrrole α and pyrrole β), 7.63 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

6-[1-(4-Methylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7d).—Yield 60%; 85–86 °C; *n*-hexane. IR ν 3400 (OH), 1721 (C═O ester), 1605 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (t, 3H, CH_2CH_3), 2.36 (s, 3H, CH_3), 4.39 (q, 2H, CH_2CH_3), 5.23 (s, 2H, CH_2), 6.32–6.43 (m, 3H, J_t = 15.4 Hz, pyrrole β, hexanoate C3–H, hexanoate C5–H), 6.90–6.96 (m, 2H, pyrrole α, β), 7.00 (d, 2H, benzene H), 7.18 (m, 2H, benzene H), 7.69 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

6-[1-(2-Fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7e).—Yield 87%; 103–105 °C; benzene. IR ν 3400 (OH), 1731 (C═O ester), 1680 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.44 (t, 3H, CH_2CH_3), 4.41 (q, 2H, CH_2CH_3), 5.34 (s, 2H, CH_2), 6.37 (t, 1H, pyrrole β), 6.41–6.46 (m, 2H, J_t = 15.2 Hz, hexanoate C5–H and hexanoate C3–H), 6.86–6.92 (m, 2H, benzene H, pyrrole β), 7.00 (s, 1H, pyrrole α), 7.12–7.18 (m, 2H, benzene H), 7.36 (m, 1H benzene H), 7.73 (d, 1H, J_t = 15.2 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{18}FNO_4$) C, H, N, F.

6-[1-(3-Fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7f).—Yield 76%; 84–86 °C; ligroin. IR ν 3400 (OH), 1700 (C═O ester), 1600 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (t, 3H, CH_2CH_3), 4.33 (q, 2H, CH_2CH_3), 5.25 (s, 2H, CH_2), 6.33–6.41 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.71 (s, 1H, pyrrole β), 6.82–7.06 (m, 4H, benzene H and pyrrole α), 7.32 (m, 1H, benzene H), 7.58 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{18}FNO_4$) C, H, N, F.

6-[1-(4-Fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7g).—Yield 78%; 96–97 °C; ligroin. IR ν 3400 (OH), 1698 (C═O ester), 1605 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.42 (t, 3H, CH_2CH_3), 4.39 (q, 2H, CH_2CH_3), 5.25 (s, 2H, CH_2), 6.36–6.44 (m, 3H, J_t = 15.5 Hz, pyrrole β, hexanoate C3–H, hexanoate C5–H), 6.80 (m, 1H, pyrrole β), 6.96 (m, 1H, pyrrole α), 7.05–7.09 (m, 4H, benzene H), 7.69 (d, 1H, J_t = 15.5 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{18}FNO_4$) C, H, N, F.

6-[1-(3-Chlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7h).—Yield 75%; 93–95 °C; ligroin. IR ν 3400 (OH), 1721 (C═O ester), 1574 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (t, 3H, CH_2CH_3), 4.33 (q, 2H, CH_2CH_3), 5.21 (s, 2H, CH_2), 6.32–6.40 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.86–7.01 (m, 4H, benzene H and pyrrole α, β), 7.26 (m, 2H, benzene H), 7.59 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{18}ClNO_4$) C, H, N, Cl.

6-[1-(4-Chlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7i).—Yield 60%; 134–136 °C; ligroin. IR ν 3400 (OH), 1700 (C═O ester), 1600 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.42 (t, 3H, CH_2CH_3), 4.37 (q, 2H, CH_2CH_3), 5.24 (s, 2H, CH_2), 6.35–6.43 (m, 3H, hexanoate C3–H, hexanoate C5–H and pyrrole β), 6.88–7.04 (m, 4H, benzene H and pyrrole α and pyrrole β), 7.34 (m, 2H, benzene H), 7.62 (m, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{18}ClNO_4$) C, H, N, Cl.

6-[1-(2-Cyanophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7j).—Yield 80%; 179–180 °C; benzene/cyclohexane. IR ν 3400 (OH), 2227 (CN), 1746 (C═O ester), 1578 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.43 (t, 3H, CH_2CH_3), 4.40 (q, 2H, CH_2CH_3), 5.52 (s, 2H, CH_2), 6.41–6.46 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.81 (d, 1H, benzene H), 6.94 (m, 1H, pyrrole β) 7.01 (m, 1H, pyrrole α), 7.46 (m, 1H, benzene H), 7.56–7.60 (m, 2H, benzene H and hexanoate C6–H), 7.78 (m, 1H, benzene H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

6-[1-(3-Cyanophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7k).—Yield 83%; 105–106 °C; acetone. IR ν 3400 (OH), 2227 (CN), 1746 (C═O ester), 1578 (c═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.82 (t, 3H, CH_2CH_3), 4.80 (q, 2H, CH_2CH_3), 6.11 (s, 2H, CH_2), 6.87 (m, 1H, pyrrole β), 6.92 (s, 1H, hexanoate C3–H), 7.15 (d, 1H, J_t = 15.5 Hz, hexanoate C5–H), 7.55 (m, 1H, pyrrole α), 7.18 (s, 1H, pyrrole β) 7.95–8.12 (m, 3H, benzene H), 8.17–8.21 (m, 2H, J_t = 15.4 Hz,benzene H and hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

6-[1-(4-Cyanophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7l).—Yield 82%; 128–130 °C; ligroin. IR ν 3400 (OH), 2227 (CN), 1746 (C═O ester), 1578 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.82 (t, 3H, CH_2CH_3), 4.79 (q, 2H, CH_2CH_3), 6.11 (s, 2H, CH_2), 6.87 (m, 1H, pyrrole β), 6.92 (s, 1H, hexanoate C3–H), 7.15 (d, 1H, J_t = 15.5 Hz, hexanoate C5–H), 7.55 (m, 1H, pyrrole α), 7.18 (s, 1H, pyrrole β), 8.02 (m, 2H, benzene H), 8.17–8.21 (m, 4H, benzene H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

6-[1-(2-Methoxyphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7m).—Yield 100%; 116–118 °C; benzene/cyclohexane. IR ν 3400 (OH), 1730 (C═O ester), 1620 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (t, 3H, CH_2CH_3), 3.90 (s, 3H, CH_3), 4.34 (q, 2H, CH_2CH_3), 5.21 (s, 2H, CH_2), 6.27 (t, 1H, pyrrole β), 6.35 (d, 1H, J_t = 15.3 Hz, hexanoate C5–H), 6.37 (s, 1H, hexanoate C3–H), 6.73 (m, 1H, pyrrole β), 6.83–6.94 (m, 5H, benzene H, pyrrole β and pyrrole α), 7.25 (m, 1H, benzene H), 7.74 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{21}NO_5$) C, H, N.

6-[1-(4-Methoxyphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7n).—Yield 64%; 84–86 °C; ethanol. IR ν 3400 (OH), 1701 (C═O ester), 1598 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (t, 3H, CH_2CH_3), 3.82 (s, 3H, CH_3), 4.37 (q, 2H, CH_2CH_3), 5.20 (s, 2H, CH_2), 6.32–6.43 (m, 3H, J_t = 15.4 Hz, pyrrole β, hexanoate C3–H, hexanoate C5–H), 6.86–7.08 (m, 6H, benzene 4H and pyrrole α and pyrrole β), 7.70 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{20}H_{21}NO_5$) C, H, N.

6-[1-(2-Ethoxyphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7o).—Yield 85%; 73–75 °C; benzene/cyclohexane. IR ν 3400 (OH), 1731 (C═O ester), 1680 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (t, 3H, CH_2CH_3), 1.45 (t, 3H, CH_2CH_3), 4.12 (q, 2H, CH_2CH_3), 4.35 (q, 2H, CH_2CH_3), 5.25 (s, 2H, CH_2), 6.27 (t, 1H, pyrrole β), 6.33 (s, 1H, hexanoate C3–H), 6.35 (d, 1H, J_t = 15.3 Hz, hexanoate C5–H), 6.83–6.89 (m, 3H, benzene H, pyrrole β), 6.97 (s, 1H, pyrrole α), 7.22 (d, 1H, benzene H), 7.73 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{21}H_{23}NO_5$) C, H, N.

6-[1-(3,5-Dimethylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7p).—Yield 46%; 99–101 °C; ligroin. IR ν 3400 (OH), 1729 (C═O ester), 1616 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.38 (t, 3H, CH_2CH_3), 2.27 (s, 6H, CH_3), 4.34 (q, 2H, CH_2CH_3), 5.14 (s, 2H, CH_2), 6.30 (m, 1H, pyrrole β), 6.33–6.37 (m, 2H, J_t = 15.1 Hz, hexanoate C5–H and hexanoate C3–H), 6.67 (s, 1H, benzene H), 6.84 (m, 1H, pyrrole β), 6.90–6.93 (m, 3H, pyrrole α and benzene H), 7.65 (d, 1H, J_t = 15.1 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{21}H_{23}NO_4$) C, H, N.

6-[1-(2,3-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7q).—Yield 79%; 67–69 °C; acetone. IR ν 3400 (OH), 1727 (C═O ester), 1611 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.80 (t, 3H, CH_2CH_3), 4.77 (q, 2H, CH_2CH_3), 6.05 (s, 2H, CH_2), 6.81–6.87 (m, 2H, pyrrole β and hexanoate C3–H), 7.11–7.26 (m, 2H, pyrrole α and J_t = 15.9 Hz, hexanoate C5–H), 7.48 (s, 1H, pyrrole β), 7.61–7.84 (m, 3H, benzene 3H), 8.22 (d, 1H, J_t = 15.9 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}F_2NO_4$) C, H, N, F.

6-[1-(2,4-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7r).—Yield 82%; 119–121 °C; ligroin. IR ν 3400 (OH), 1737 (C═O ester), 1601 (C═O ketone) cm^{-1}. ^1H NMR ($CDC1_3$) δ 1.46 (t, 3H, CH_2CH_3), 4.39 (q, 2H, CH_2CH_3), 5.29 (s, 2H, CH_2), 6.37 (m, 1H, pyrrole β), 6.41–6.46 (m, 2H, hexanoate C5–H and hexanoate C3–H), 6.85–6.99 (m, 5H, pyrrole α, pyrrole β and benzene H) 7.65 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}F_2NO_4$) C, H, N, F.

6-[1-(2,5-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7s).—Yield 82%; 84–85 °C; acetone. IR ν 3400 (OH), 1724 (C═O ester), 1618 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.83 (t, 3H, CH_2CH_3), 4.80 (q, 2H, CH_2CH_3), 6.04 (s, 2H, CH_2), 6.86 (s, 1H, pyrrole β) 6.96 (s, 1H, hexanoate C3–H), 7.01–7.19 (m, 2H, J_t = 15.3 Hz, pyrrole α and hexanoate C5–H), 7.55 (s, 1H, pyrrole β), 7.64–7.78 (m, 3H, benzene H), 8.26 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}F_2NO_4$) C, H, N, F.

6-[1-(2,6-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7t).—Yield 50%; 125–127 °C; ligroin. IR ν 3400 (OH), 1710 (C═O ester), 1610 (C═O ketone) cm^{-1}. ^1H NMR ($CDC1_3$) δ 1.42 (t, 3H, CH_2CH_3), 4.38 (q, 2H, CH_2CH_3), 5.27 (s, 2H, CH_2), 6.27 (m, 1H, pyrrole β), 6.40 (d, 1H, J_t = 15.2 Hz, hexanoate C5–H), 6.48 (s, 1H, hexanoate C3–H), 6.80 (m, 1H, pyrrole β), 6.91–7.01 (m, 3H, benzene H and pyrrole α), 7.35 (m, 1H, benzene H), 7.95 (d, 1H, J_t = 15.2 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}F_2NO_4$) C, H, N, F.

6-[1-(3,4-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7u).—Yield 60%; 74–75 °C; acetone. IR ν 3400 (OH), 1737 (C═O ester), 1633 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.83 (t, 3H, CH_2CH_3), 4.79 (q, 2H, CH_2CH_3), 6.01 (s, 2H, CH_2), 6.84 (m, 1H, pyrrole β) 6.93 (s, 1H, hexanoate C3–H), 7.14 (d, 1H, J_t = 15.4 Hz, hexanoate C5–H), 7.51–7.63 (m, 3H, benzene H and pyrrole β), 7.78–7.86 (m, 2H, benzene H and pyrrole α) 8.20 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}F_2NO_4$) C, H, N, F.

6-[1-(3,5-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7v).—Yield 58%; 75–78 °C; ligroin. IR ν 3400 (OH), 1735 (C═O ester), 1630 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.46 (t, 3H, CH_2CH_3), 4.40 (q, 2H, CH_2CH_3), 5.28 (s, 2H, CH_2), 6.40–6.45 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.57–6.59 (m, 2H, pyrrole α and pyrrole β), 6.78 (m, 1H, benzene H), 6.92 (m, 1H, benzene H), 6.97 (m, 1H, benzene H) 7.57 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}F_2NO_4$) C, H, N, F.

6-[1-(2,4-Dichlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7w).—Yield 100%; 110–113 °C; benzene/cyclohexane. IR ν 3400 (OH), 1773 (C═O ester), 1610 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.30 (t, 3H, CH_2CH_3), 4.26 (q, 2H, CH_2CH_3), 5.48 (s, 2H, CH_2), 6.35–6.36 (m, 2H, pyrrole β and hexanoate C3–H), 6.52 (d, 1H, benzene H), 6.77 (m, 1H, hexanoate C5–H), 7.02 (s, 1H, pyrrole β), 7.26 (s, 1H, pyrrole α) 7.41–7.47 (m, 3H, benzene H and hexanoate C6–H), 7.74 (d, 1H, benzene H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}Cl_2NO_4$) C, H, N, Cl.

6-[1-(2,6-Dichlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7x).—Yield 69%; 146–148 °C; ligroin. IR ν 3400 (OH), 1730 (C═O ester), 1578 (C═O ketone) cm^{-1}. ^1H NMR ($CDC1_3$) δ 1.45 (t, 3H, CH_2CH_3), 4.43 (q, 2H, CH_2CH_3), 5.51 (s, 2H, CH_2), 6.27 (m, 1H, pyrrole β), 6.49–6.53 (m, 2H, J_t = 15.2 Hz, hexanoate C5–H and hexanoate C3–H), 6.64 (s, 1H, pyrrole β), 6.90 (m, 1H, pyrrole α),

7.36 (m, 1H, benzene H), 7.45–7.47 (d, 2H, benzene 2H), 7.98 (d, 1H, J_t = 15.2 Hz, hexanoate C6–H), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}Cl_2NO_4$) C, H, N, Cl.

6-[1-(3,5-Dichlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid Ethyl Ester (7y).—Yield 62%; 112–113 °C; ligroin. IR ν 3400 (OH), 1723 (C═O ester), 1646 (C═O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.38 (t, 3H, CH_2CH_3), 4.35 (q, 2H, CH_2CH_3), 5.20 (s, 2H, CH_2), 6.35–6.42 (m, 3H, pyrrole β, hexanoate C3–H and hexanoate C5–H), 6.88–6.91 (m, 4H, benzene H and pyrrole β), 7.28 (m, 1H, pyrrole α), 7.54 (d, 1H, J_t = 15.3 Hz, hexanoate C6–h), 12–16 (sb, 1H, OH). Anal. ($C_{19}H_{17}Cl_2NO_4$) C, H, N, Cl.

General Procedure for the Synthesis of Diketo Acids 8a–y.

Twenty-five tubes (25 mL each, divided in two sessions of 12 and 13 tubes, respectively) were charged with a mixture of 1 N NaOH (9.48 mL) and the appropriated ester **7a–y** (0.0028 mol) in 1:1 THF–methanol (12 mL) was stirred with bascular stirring in Büchi Syncor at room temperature at 250 rpm for 1 h 30 min and then poured into crushed ice. The aqueous mixture was treated with 1 N HCl until pH 3 was reached and extracted with ethyl acetate (three times). The collected organic extract was washed with brine (three times) and dried, and the solvent was evaporated under reduced pressure to give the pure diketo acids **8a–y**. Yield (%), melting point (°C), recrystallization solvent, IR, ^1H NMR, and analytical data are reported for each of the following compounds.

6-[1-(Phenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8a).—Yield 68%; 146–148 °C; toluene. IR ν 3400 (OH), 1710 (C═O acid), 1598 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.43 (s, 2H, CH_2), 6.32 (m, 1H, pyrrole β), 6.45 (s, 1H, hexanoate C3–H), 6.62 (d, 1H, J_t = 15.4 Hz, hexanoate C5–H), 7.00 (m, 1H, pyrrole β), 7.10 (m, 1H, pyrrole α), 7.27–7.40 (m, 5H, benzene H), 7.61–7.79 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{15}NO_4$) C, H, N.

6-[1-(2-Methylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8b).—Yield 95%; 146–147 °C; benzene/toluene. IR ν 2923 (OH), 1700 (C═O acid), 1580 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 5.39 (s, 2H, CH_2), 6.30–6.43 (m, 2H, pyrrole β and hexanoate C3–H), 6.65 (d, 1H, J_t = 15.2 Hz, hexanoate C5–H), 6.98 (s, 1H, pyrrole β), 7.06–7.25 (m, 5H, benzene H and pyrrole α), 7.48 (d, 1H, J_t = 15.2 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

6-[1-(3-Methylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8c).—Yield 95%; 121–123 °C; benzene/cyclohexane. IR ν 3400 (OH), 1700 (C═O acid), 1590 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 5.33 (s, 2H, CH_2), 6.28–6.33 (m, 2H, hexanoate C3–H and pyrrole β), 6.63 (d, 1H, J_t = 15.7 Hz, hexanoate C5–H), 6.79–7.34 (m, 6H, benzene H, pyrrole α, β), 7.58 (d, 1H, J_t = 15.7 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

6-[1-(4-Methylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8d).—Yield 72%; 149–151 °C; benzene/toluene. IR ν 3400 (OH), 1705 (C═O acid), 1575 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.27 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.27 (m, 1H,

pyrrole β) 6.37 (d, 1H, hexanoate C5–H), 6.55 (s, 1H, hexanoate C3–H), 6.90 (m, 1H, pyrrole β), 7.13–7.24 (m, 5H, benzene H and pyrrole α), 7.46 (d, 1H, J_t = 15.6 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

6-[1-(2-Fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8e).— Yield 85%; 132–133 °C; benzene/toluene. IR ν 3400 (OH), 1710 (C═O acid), 1590 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.46 (s, 2H, CH_2), 6.32 (m, 1H, pyrrole β), 6.68 (d, 1H, J_t = 16.0 Hz, hexanoate C5–H), 6.87–6.95 (m, 2H, hexanoate C3–H and pyrrole β), 7.14–7.38 (m, 5H, benzene H and pyrrole α), 7.64 (m, 1H, J_t = 16.0 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{14}FNO_4$) C, H, N, F.

6-[1-(3-Fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8f).— Yield 76%; 132–135 °C; toluene. IR ν 3400 (OH), 1700 (C═O acid), 1600 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.44 (s, 2H, CH_2), 6.31 (m, 1H, pyrrole β), 6.40 (s, 1H, hexanoate C3–H), 6.64 (d, 1H, J_t ≈ 15 Hz, hexanoate C5–H), 7.79–7.19 (m, 4H, benzene H and pyrrole β), 7.26–7.47 (m, 2H, benzene H), 7.62 (d, 1H, J_t ≈ 15 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{14}FNO_4$) C, H, N, F.

6-[1-(4-Fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8g).— Yield 54%; 98–99 °C; benzene/toluene. IR ν 3400 (OH), 1689 (C═O acid), 1598 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.38 (s, 2H, CH_2), 6.25–6.33 (m, 2H, pyrrole β and hexanoate C3–H), 6.62 (d, 1H, J_t = 15.6 Hz, hexanoate C5–H), 6.93–7.01 (m, 3H, benzene H and pyrrole β), 7.15 (d, 2H, benzene H), 7.26 (s, 1H, pyrrole α), 7.46 (d, 1H, J_t = 15.6 Hz, hexanoate C6– H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{14}FNO_4$) C, H, N, F.

6-[1-(3-Chlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8h).— Yield 79%; 144–145 °C; toluene/cycloexane. IR ν 3400 (OH), 1750 (C═O acid), 1610 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.43 (s, 2H, CH_2), 6.31 (m, 1H, pyrrole β), 6.38 (s, 1H, hexanoate C3–H), 6.66 (d, 1H, J_t = 15.2 Hz, hexanoate C5–H), 6.97–7.40 (m, 6H, benzene H and pyrrole β, α), 7.58 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{14}ClNO_4$) C, H, N, Cl.

6-[1-(4-Chlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8i).— Yield 76%; 170–172 °C; benzene/toluene. IR ν 3400 (OH), 1685 (C═O acid), 1548 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.44 (s, 2H, CH_2), 6.37 (m, 1H, pyrrole β), 6.48 (s, 1H, hexanoate C3–H), 6.71 (d, 1H, J_t = 15.5 Hz, hexanoate C5–H), 7.05 (s, 1H, pyrrole β), 7.10 (m, 2H, benzene H), 7.32 (m, 1H, pyrrole α), 7.42 (m, 2H, benzene H), 7.60 (d, 1H, J_t = 15.5 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{14}ClNO_4$) C, H, N, Cl.

6-[1-(2-Cyanophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8j).— Yield 58%; 179–180 °C; 2-propanol. IR ν 3400 (OH), 2227 (CN), 1746 (C═O acid), 1578 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.71 (s, 2H, CH_2), 6.42 (m, 1H, pyrrole β), 6.53 (s, 1H, hexanoate C3–H), 6.72–6.76 (m, 2H, hexanoate C5–H and benzene H), 7.09 (s, 1H, pyrrole β), 7.31 (s, 1H, pyrrole α), 7.53 (m, 1H, benzene H), 7.63–7.71 (m, 2H, benzene

H and hexanoate C6–H), 7.95 (m, 1H, benzene H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

6-[1-(3-Cyanophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8k).— Yield 44%; 126–128 °C; 2-propanol/isopropyl ether. IR ν 3400 (OH), 2231 (CN), 1717 (C═O acid), 1582 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.52 (s, 2H, CH_2), 6.34 (m, 1H, pyrrole β), 6.46 (s, 1H, hexanoate C3–H), 6.68 (d, 1H, J_t = 15.3 Hz, hexanoate C5–H), 7.01 (s, 1H, pyrrole β), 7.34–7.37 (m, 2H, benzene H), 7.52–7.66 (m, 3H, J_t = 15.3 Hz, benzene H, hexanoate C6–H and pyrrole α), 7.79 (d, 1H, benzene H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

6-[1-(4-Cyanophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8l).— Yield 93%; >250 °C; washed with isopropyl ether. IR ν 3400 (OH), 2227 (CN), 1746 (C═O acid), 1578 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.59 (s, 2H, CH_2), 6.37 (m, 1H, pyrrole β), 6.48 (s, 1H, hexanoate C3–H), 6.71 (d, 1H, J_t = 15.4 Hz, hexanoate C5–H), 7.05 (s, 1H, pyrrole β), 7.23 (m, 2H, benzene H), 7.36 (m, 1H, pyrrole α), 7.60 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 7.86 (m, 2H, benzene H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

6-[1-(2-Methoxyphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8m).— Yield 100%; 142–143 °C; toluene. IR ν 3400 (OH), 1732 (C═O acid), 1601 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 6.25 (m, 1H, pyrrole β), 6.41 (s, 1H, hexanoate C3–H), 6.62 (d, 1H, J_t = 15.3 Hz, hexanoate C5–H), 6.69–7.03 (m, 4H, benzene H and pyrrole β), 7.20–7.23 (m, 2H, benzene H, pyrrole α), 7.66 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{17}NO_5$) C, H, N.

6-[1-(4-Methoxyphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8n).— Yield 64%; >300 °C; washed with isopropyl ether. IR ν 3400 (OH), 1698 (C═O acid), 1600 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.73 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.27–6.40 (m, 2H, pyrrole β and hexanoate C3–H) 6.66 (d, 1H, hexanoate C5–H), 6.89–6.93 (m, 3H, benzene H and pyrrole β), 7.07 (d, 2H, benzene H), 7.28 (s, 1H, pyrrole α), 7.66 (d, 1H, J_t = 15.6 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{18}H_{17}NO_5$) C, H, N.

6-[1-(2-Ethoxyphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8o).— Yield 80%; 159–160 °C; toluene. IR ν 2923 (OH), 1731 (C═O acid), 1601 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.32–1.38 (t, 3H, CH_2CH_3), 4.05–4.10 (q, 2H, CH_2CH_3), 5.33 (s, 2H, CH_2), 6.24 (t, 1H, pyrrole β), 6.42 (s, 1H, hexanoate C3–H), 6.62 (d, 1H, J_t = 15.4 Hz, hexanoate C5–H), 6.74 (d, 1H, benzene H), 6.83 (t, 1H, bezene H), 6.95 (s, 1H, pyrrole β), 6.99 (d, 1H, benzene H), 7.20–7.23 (m, 2H, benzene H, pyrrole α), 7.66 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{19}H_{19}NO_5$) C, H, N.

6-[1-(3,5-Dimethylphenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8p).— Yield 78%; 145–147 °C; benzene. IR ν 3400 (OH), 1713 (C═O acid), 1608 (C═O

ketone) cm^{-1}. ^1H NMR (DMF-d_7) δ 2.18 (s, 6H, CH_3), 5.29 (s, 2H, CH_2), 6.25 (m, 1H, pyrrole β), 6.44 (s, 1H, hexanoate C3–H), 6.61 (d, 1H, J_t = 15.4 Hz, hexanoate C5–H), 6.68 (s, 1H, benzene H), 6.86 (s, 1H, pyrrole β), 6.95–6.96 (d, 1H, benzene H), 7.26 (s, 1H, pyrrole α), 7.61 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

6-[1-(2,3-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8q). —Yield 47%; 147–148 °C; 2-propanol/*n*-hexane. IR ν 3400 (OH), 1758 (C═O acid), 1630 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.54 (s, 2H, CH_2), 6.35 (m, 1H, pyrrole β), 6.50 (s, 1H, hexanoate C3–H), 6.69–6.75 (m, 2H, pyrrole β and hexanoate C5–H), 7.03 (s, 1H, pyrrole α), 7.19–7.45 (m, 3H, benzene H), 7.72 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}F_2NO_4$) C, H, N, F.

6-[1-(2,4-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8r). —Yield 67%; 130–132 °C; ethyl acetate. IR ν 3400 (OH), 1758 (C═O acid), 1630 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.46 (s, 2H, CH_2), 6.31 (m, 1H, pyrrole β), 6.50 (s, 1H, hexanoate C3–H), 6.72 (d, 1H, J_t = 15.7 Hz, and hexanoate C5–H), 6.97–7.03 (m, 2H, pyrrole β and benzene H), 7.10 (m, 1H, benzene H) 7.28–7.37 (m, 2H, pyrrole α and benzene H), 7.69 (d, 1H, J_t = 15.7 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}F_2NO_4$) C, H, N, F.

6-[1-(2,5-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8s). —Yield 51%; 141–143 °C; toluene/*n*-hexane. IR ν 3400 (OH), 1747 (C═O acid), 1711 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.50 (s, 2H, CH_2), 6.35 (m, 1H, pyrrole β), 6.51 (s, 1H, hexanoate C3–H), 6.68–6.77 (m, 2H, pyrrole β and hexanoate C5–H), 7.04 (s, 1H, pyrrole α), 7.21–7.39 (m, 3H, benzene H), 7.72 (d, 1H, J_t = 15.2 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}F_2NO_4$) C, H, N, F.

6-[1-(2,6-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8t). —Yield 64%; 182–183 °C; toluene. IR ν 3400 (OH), 1720 (C═O acid), 1610 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.40 (s, 2H, CH_2), 6.22 (m, 1H, pyrrole β), 6.50 (s, 1H, hexanoate C3–H), 6.70 (d, 1H, J_t = 15.4 Hz, hexanoate C5–H), 6.91 (m, 1H, pyrrole β), 7.10–7.20 (m, 2H, pyrrole α and benzene H), 7.45–7.50 (m, 2H, benzene H), 7.87 (d, 1H, J_t = 15.4 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}F_2NO_4$) C, H, N, F.

6-[1-(3,4-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8u). —Yield 100%; >270 °C; DMF/H_2O. IR ν 3400 (OH), 1758 (C═O acid), 1630 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.38 (s, 2H, CH_2), 5.80 (s, 1H, pyrrole β), 6.30 (s, 1H, hexanoate C3–H), 6.53 (m, 1H, hexanoate C5–H), 6.80–6.90 (m, 2H, pyrrole β and benzene H), 7.14–7.60 (m, 4H, benzene H, hexanoate C6–H and pyrrole α), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}F_2NO_4$) C, H, N, F.

6-[1-(3,5-Difluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8v). —Yield 60%; 146–149 °C; washed with ethanol. IR ν 3400 (OH), 1712 (C═O acid), 1595 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.29 (s, 2H, CH_2), 6.36 (s, 1H, pyrrole β),

6.47 (s, 1H, hexanoate C3–H), 6.70–6.78 (m, 3H, benzene H and hexanoate C5–H), 7.03 (s, 1H, pyrrole β), 7.20 (m, 1H, pyrrole α), 7.35 (s, 1H, benzene 1H), 7.60 (d, 1H, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}F_2NO_4$) C, H, N, F.

6-[1-(2,4-Dichlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8w).—Yield 68%; 145–146 °C; toluene. IR ν 3400 (OH), 1758 (C═O acid), 1630 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.51 (s, 2H, CH_2), 6.37 (m, 1H, pyrrole β), 6.47 (s, 1H, hexanoate C3–H), 6.56 (m, 1H, benzene H), 6.63 (d, 1H, J_t = 15.3 Hz, hexanoate C5–H), 7.07 (s, 1H, pyrrole β), 7.29 (s, 1H, pyrrole α), 7.42 (m, 1H, benzene H), 7.7 (d, 1H, J_t = 15.3 Hz, hexanoate C6–H), 7.73 (d, 1H, benzene 1H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}Cl_2NO_4$) C, H, N, Cl.

6-[1-(2,6-Dichlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8x).—Yield 84%; 132–134 °C; toluene. IR ν 3400 (OH), 1758 (C═O acid), 1630 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.55 (s, 2H, CH_2), 6.23 (m, 1H, pyrrole β), 6.61 (s, 1H, hexanoate C3–H), 6.67 (s, 1H, pyrrole β), 6.81 (d, 1H, J_t = 15.7 Hz, hexanoate C5–H), 7.01 (s, 1H, pyrrole α), 7.52 (m, 1H, benzene H), 7.63–7.65 (m, 2H, benzene H), 7.94 (d, 1H, J_t = 15.7 Hz, hexanoate C6–H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}Cl_2NO_4$) C, H, N, Cl.

6-[1-(3,5-Dichlorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic Acid (8y).—Yield 95%; >300 °C; toluene/cyclohexane. IR ν 3400 (OH), 1743 (C═O acid), 1521 (C═O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.38 (s, 2H, CH_2), 6.24 (s, 1H, pyrrole β), 6.54 (m, 1H, hexanoate C3–H), 6.33 (s, 1H, hexanoate C5–H), 7.02–7.35 (m, 5H, pyrrole α, pyrrole β and hexanoate C6–H), 7.50 (s, 1H, benzene H), 12–18 (sb, 2H, OH acid and OH enole). Anal. ($C_{17}H_{13}Cl_2NO_4$) C, H, N, Cl.

Biological Methods.

HIV-1 RNase H Inhibition.—The RT-associated RNase H activity was measured in a polymerase-independent cleavage assay in which the Poly(dC)-[^{3}H]Poly(rG) hybrid was used as reaction substrate as previously described.[30]

HIV-1 IN Inhibition.—ST activity was monitored using an electrochemiluminescent plate-based assay as previously described.[27] Briefly, donor DNA obtained from BioVeris (Gaithersburg, MD) was incubated for 30 min at 37 °C in the presence of 250 nM of recombinant HIV-1 IN. After addition of the compounds, the integration reaction was initiated by addition of target DNA and carried out for 60 min at 37 °C. Finally, plates were read on the BioVeris M-SERIES analyzer.

HIV-1 Replication Inhibition.—Compounds antiviral activity was determined in a cell-based assay according to the procedure described previously[31] and modified as follows. HeLa-CD4-LTR-β-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 μL of DMEM medium complemented with 10% serum. On day one, each drug is serial diluted directly on cells following a 3-fold dilution over 6 points and each well

is then filled to 200 μL with either fresh medium or concentrated viral supernatant (HIV-1(IIIB), Advanced Biotechnologies Inc.). The highest compound concentration tested was 50 μM. On day two, cells are washed three times with PBS before adding 200 μL of a solution containing 50 mM Tris-HCl pH 7.5, 100 mM β-mercaptoethanol, 0.05% Triton X100, and 5 mM 4-methyl-umbelliferyl-β-D-galactopyranoside (4-MUG, Sigma). On day three, sealed plates are read in a SpectraMax GEMINI-XS (Molecular Devices) with $\lambda_{ex/em}$ = 360/460 nm.

Selected compounds (see Table 1) were tested for anti-HIV activity against the replication of HIV-1 (IIIB) in MT-4 cells. MT-4 cells were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 0.1% sodium bicarbonate, and 20 μg/mL gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) was used in all experiments. Inhibition of virus-induced cytopathic effect by the compounds was monitored by a classical survival assay. Briefly, 50 μL of HIV-1 (100–300 $CCID_{50}$ (50% cell culture infective dose)) were added to a flat-bottomed microtiter tray with 50 μL of medium containing various concentrations of the test compounds. MT-4 cells were added at a final concentration of 6×10^5 cells/mL. After 5 days of incubation at 37 °C, cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT).

Cellular Toxicity.—Similarly to the antiviral assays, plates were prepared with 10000 HeLa-CD4-LTR-β-gal cells per well and a serial dilution of compounds in 100 μL. After 24 h of culture, 100 μL of ATPlite reagent (Perkin-Elmer) was added to each well. After 5 min at room temperature, plates' luminescence was quantified using an EnVision multilabel reader (Perkin-Elmer) according to manufacturer's instructions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
RNase H	ribonuclease H
RT	reverse transcriptase

IN	integrase
rRT	recombinant reverse transcriptase
rIN	recombinant integrase
FDA	Food and Drug Administration
3′-P	3′-processing
ST	strand transfer
GT	guanine thymine
DKA	diketo acids
IR	infrared
HPLC	high-performance liquid chromatography
NMR	nuclear magnetic resonance

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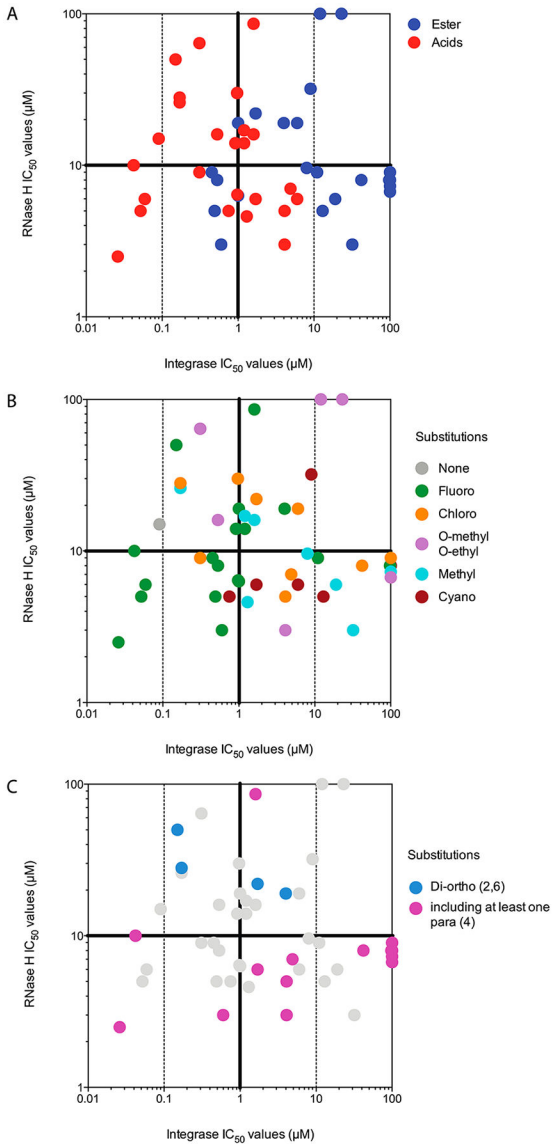


Figure 1.

Scatter plot for inhibition of RNase H and IN enzymes: (A) Compounds are categorized according to their acidic or ester function. (B) Compounds are categorized according to the nature of their substitutions. (C) Compounds are categorized according to the position of their substitutions. Compounds with one IC_{50} value missing such as **7a** have been left out of the plot and compounds with IC_{50} values above 111 μM have been arbitrary positioned at the 100 μM value.

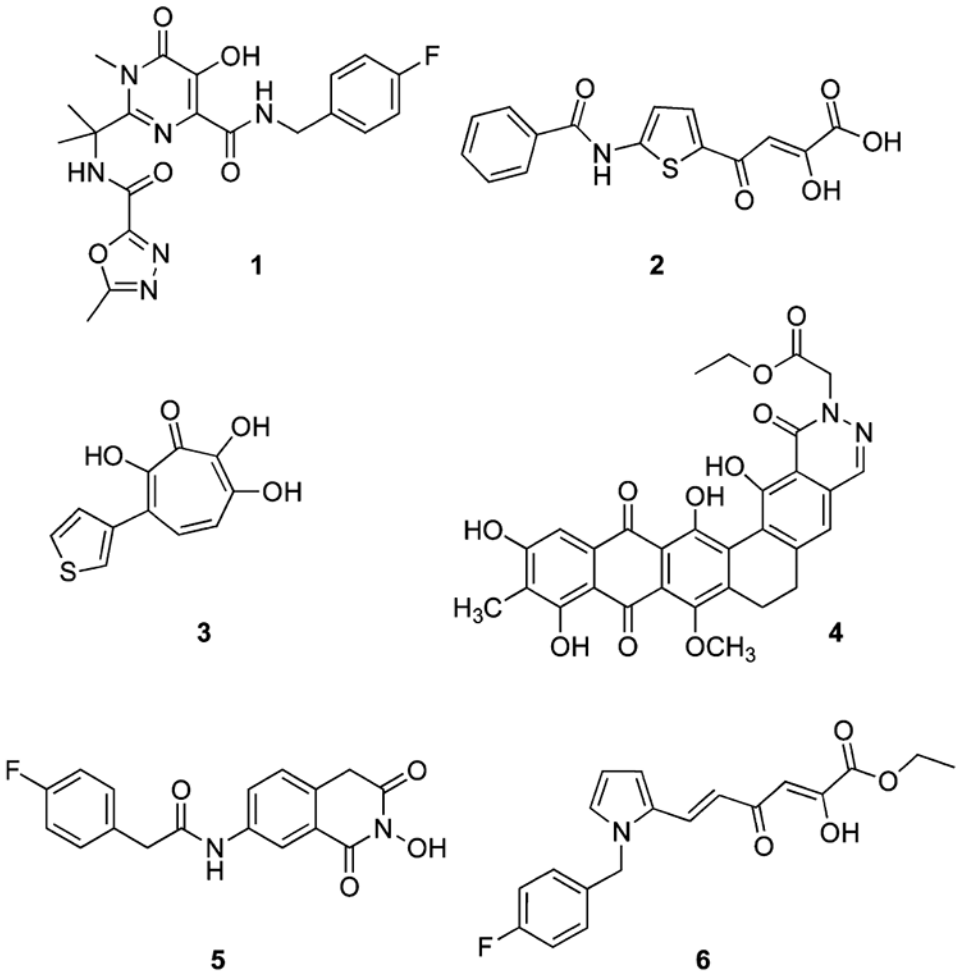
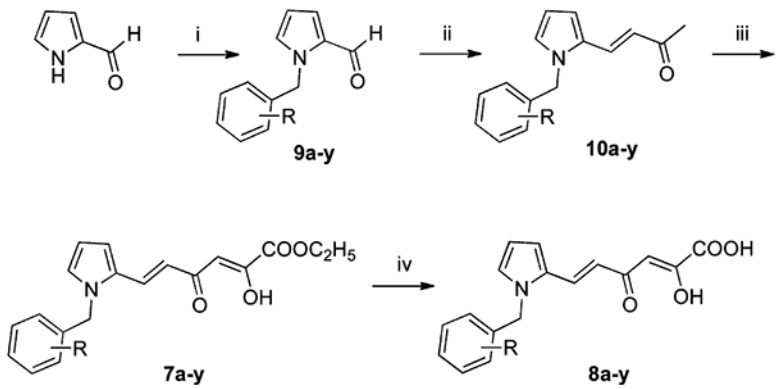


Chart 1.
Selected Inhibitors of HIV-1 RNase H Function of RT and/or IN Enzyme

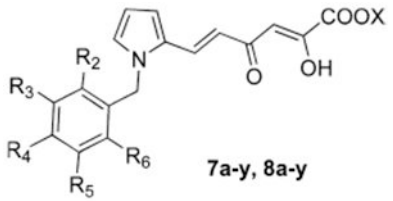


Scheme 1. Synthesis of Derivatives 7a–y and 8a–y[a]

[a]Reagents and conditions: (i) substituted benzyl bromide, NaH, room temp; (ii) acetone, 5 N NaOH, 50 °C; (iii) diethyl oxalate, sodium ethoxide, THF_a, room temp; (iv) 1 N NaOH, 1:1 THF–methanol, room temp.

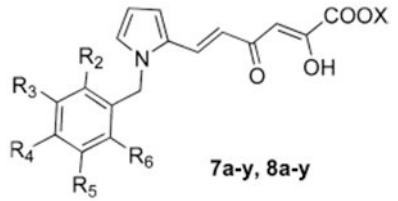
Table 1.

Cytotoxicity, Enzymatic, and Antiviral Activities of Compounds 7a–y and 8a–y



7a-y, 8a-y

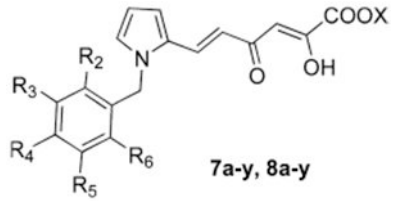
							activity in enzyme assay, IC_{50} [a]		antiviral activity			
compd	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**X**	**RH** [b]	**ST** [c]	**EC_{50}** [d] **HeLa**	**EC_{50}** [e] **MT-4**	**CC_{50}** [f]	**SI** [g]
7a	H	H	H	H	H	Et		15	0.25	1.5	41	164
7b	Me	H	H	H	H	Et	3.0	32	1.4		>50	>35
7c	H	Me	H	H	H	Et	9.6	8.0	2.4	2.2	37	15
7d	H	H	Me	H	H	Et	7.3	>111	17.4		>50	>2
7e	F	H	H	H	H	Et	6.3	1.0	0.31	1.2	>50	>161
7f	H	F	H	H	H	Et	9.0	11	2.2	5.4	42	19
7g	H	H	F	H	H	Et	8	98	<0.2	1.7	>50	>250
7h	H	Cl	H	H	H	Et	19	6	0.79		>50	>63
7i	H	H	Cl	H	H	Et	8.0	42	26.3		>50	>1
7j	CN	H	H	H	H	Et	32	9.0	17		>50	>2
7k	H	CN	H	H	H	Et	5	13	7.1		48	7
7l	H	H	CN	H	H	Et	8	>111	>50		>50	
7m	OMe	H	H	H	H	Et	>100	23	0.2		>50	>250
7n	H	H	OMe	H	H	Et	6.7	110	>50		>50	
7o	OEt	H	H	H	H	Et	>100	12	0.6		>50	>83
7p	H	Me	H	Me	H	Et	6	19	13.8		>50	>3
7q	F	F	H	H	H	Et	8	0.53	<0.2		>50	>250
7r	F	H	F	H	H	Et	19	1.00	<0.2		>50	>250
7s	F	H	H	F	H	Et	9	0.45	0.81		>50	>16
7t	F	H	H	H	F	Et	19	4.00	0.2		>50	>250



7a-y, 8a-y

							activity in enzyme assay, IC_{50} [a]		antiviral activity			
compd	R_2	R_3	R_4	R_5	R_6	X	RH[b]	ST[c]	EC_{50} [d] HeLa	EC_{50} [e] MT-4	CC_{50} [f]	SI[g]
7u	H	F	F	H	H	Et	3	0.60	2		>50	>25
7v	H	F	H	F	H	Et	5	0.49	3.9		>50	>12
7w	Cl	H	Cl	H	H	Et	9	>111	>50		>50	
7x	Cl	H	H	H	Cl	Et	22	1.70	2.5		>50	>20
7y	H	Cl	H	Cl	H	Et		8.0	3		30	10
8a	H	H	H	H	H	H	15	0.09	0.35	0.83	>50	>142
8b	Me	H	H	H	H	H	26	0.17	3.7	1.6	>50	>13
8c	H	Me	H	H	H	H	4.6	1.3	1.9	4.7	>50	>26
8d	H	H	Me	H	H	H	17	1.2	>50		>50	
8e	F	H	H	H	H	H	6.4	0.98	0.35		>50	>142
8f	H	F	H	H	H	H	14	0.92	5.1	2.6	>50	>9
8g	H	H	F	H	H	H	2.5	0.026	0.63	1.7	>50	>79
8h	H	Cl	H	H	H	H	9	0.31	>50		>50	
8i	H	H	Cl	H	H	H	5.0	4.1	>50	>50	>50	
8j	CN	H	H	H	H	H	6	6	>50		>50	
8k	H	CN	H	H	H	H	5	0.75	>50		>50	
8l	H	H	CN	H	H	H	6	1.7	>50		>50	
8m	OMe	H	H	H	H	H	16	0.53	<0.2		42	>210
8n	H	H	OMe	H	H	H	3.0	4.1	17.4		>50	>2
8o	OEt	H	H	H	H	H	64	0.31	<0.2		>50	>250
8p	H	Me	H	Me	H	H	16	1.6	9.8		30	3
8q	F	F	H	H	H	H	6	0.059	0.52		>50	>128
8r	F	H	F	H	H	H	10	0.042	0.5		44	88

J Med Chem. Author manuscript; available in PMC 2020 October 22.



7a-y, 8a-y

							activity in enzyme assay, IC_{50} [a]		antiviral activity			
compd	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**X**	**RH** [b]	**ST** [c]	**EC_{50}** [d] **HeLa**	**EC_{50}** [e] **MT-4**	**CC_{50}** [f]	**SI** [g]
8s	F	H	H	F	H	H	5	0.052	2.5		>50	>20
8t	F	H	H	H	F	H	50	0.15	<0.2		>50	>250
8u	H	F	F	H	H	H	86	1.60	>50		>50	
8v	H	F	H	F	H	H	14	1.2	1.7		>50	>29
8w	Cl	H	Cl	H	H	H	7	4.9	16.2		>50	>3
8x	Cl	H	H	H	Cl	H	28	0.17	1.4		>50	>35
8y	H	Cl	H	Cl	H	H	30	0.97	>50		>50	
1								0.007	0.024	0.016	>250	>15000
2							3.2	1.9	ND	>50		

[a] Inhibitory concentration 50% (μM) determined from dose–response curves.

[b] Experiments performed against HIV-1 RT-associated RNase H activity.

[c] Experiments performed against HIV-1 IN ST activity.

[d] Effective concentration 50% (μM) in HIV-1 infected HeLa cells.

[e] Effective concentration 50% (μM) in HIV-1 infected MT-4 cells.

[f] Cytotoxic concentration 50% (μM).

[g] Selectivity index = CC_{50}/EC_{50} in HeLa cells.