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Structure–Activity Relationship of Pyrrolyl Diketo Acid Derivatives as Dual Inhibitors of HIV-1 Integrase and Reverse Transcriptase Ribonuclease H Domain

Giuliana Cuzzucoli Crucitti[†], **Mathieu Métifiot**[‡], **Luca Pescatori**[†], **Antonella Messore**[†], **Valentina Noemi Madia**[†], **Giovanni Pupo**[†], **Francesco Saccoliti**[†], **Luigi Scipione**[†], **Silvano Tortorella**[†], **Francesca Esposito**[§], **Angela Corona**[§], **Marta Cadeddu**[§], **Christophe Marchand**[‡], **Yves Pommier**[‡], **Enzo Tramontano**[§], **Roberta Costi**[*,†,‖], **Roberto Di Santo**[†,‖]

[†] Dipartimento di Chimica e Tecnologie del Farmaco, Istituto Pasteur-Fondazione Cenci Bolognetti, “Sapienza” Universitàdi Roma, Rome, I-00185, Italy

[‡] Developmental Therapeutics Branch and 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, Cagliari, 09124, Italy

Abstract

The development of HIV-1 dual inhibitors is a highly innovative approach aimed at reducing drug toxic side effects as well as therapeutic costs. HIV-1 integrase (IN) and reverse transcriptase-associated ribonuclease H (RNase H) are both selective targets for HIV-1 chemotherapy, and the identification of dual IN/RNase H inhibitors is an attractive strategy for new drug development. We newly synthesized pyrrolyl derivatives that exhibited good potency against IN and a moderate inhibition of the RNase H function of RT, confirming the possibility of developing dual HIV-1 IN/RNase H inhibitors and obtaining new information for the further development of more effective dual HIV-1 inhibitors.

Graphcal Abstract

[*]**Corresponding Author** Phone: +39-06-49693247. Fax: +39-06-49913133. roberta.costi@uniroma1.it.

[‖]Author Contributions

R.C. and R.D.S. contributed equally.

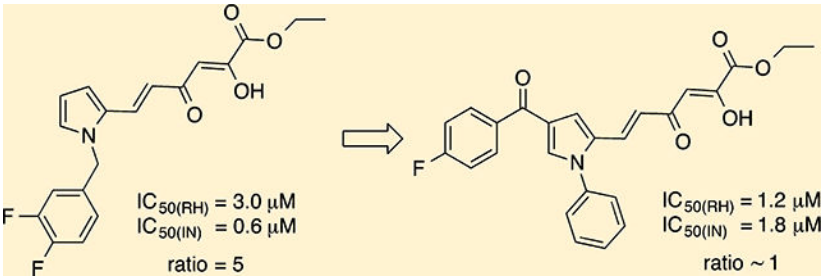
The manuscript was written through contribution of all authors. All authors have given approval to the final version of the manuscript.

ASSOCIATED CONTENT

Supporting Information

Elemental analysis results of compounds **6a–l** and **7a–m**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.



INTRODUCTION

The development of dual-action drugs is a promising approach to ameliorate drug–drug interactions, reduce toxic side effects, and suppress viral resistance selection.[1–4] Among dual-action drugs, dual inhibitors are single compounds that are able to inhibit two enzyme activities. Several reports have shown that dual inhibitors may have a role in the treatment of different diseases such as Alzheimer,[5] Parkinson,[6] inflammation,[7] and cancer.[1,8,9] This approach had been attempted also in the virological arena, aiming to inhibit rhinovirus replication.[10] Recently, tropolones,[11–13] madurahydroxylactone,[14] and 2-hydroxyisoquinolin-1,3(2*H*,4*H*)-diones[15,16] have been reported to act as dual inhibitors against HIV-1, targeting viral integrase (IN) and reverse transcriptase (RT) ribonuclease H (RNase H) activities.

HIV-1 IN is the viral enzyme responsible for the integration of the proviral dsDNA into the cell host chromosome through two coordinated enzyme functions, both accomplished by the same active site.[17] In the first reaction, termed 3′-end processing (3′-P), IN removes the two terminal nucleotides (GT) from each 3′-end of the dsDNA.[17] In the second reaction, termed strand-transfer (ST), IN catalyzes a nucleophilic attack by the free 3′-OH of the viral processed DNA to the target chromosomal DNA, resulting in covalent joining of the two molecules. Several classes of integrase inhibitors have been identified;[18] among these the diketoacids (DKAs) showed greatest promise, and the first DKA bioisoster, raltegravir (**1**), has been approved in 2007 for HIV-1 therapy.[19]

HIV-1 IN belongs to the functionally diverse superfamily of DDE(D) nucleotidyltransferases, whose other notable members include RNaseH and MuA, Tn5, and Mos1 transposases.[17] The active sites of these enzymes typically contain three essential carboxylates that coordinate a pair of divalent metal cations (usually Mg^{2+}). Thus, chelating inhibitors can be active across several classes of viral metal-dependent enzymes and chelation has been successfully used in drug design, also of dual inhibitors.[20] In particular, DKAs have been reported to chelate the divalent cations in the IN active site,[17,18] and notably, DKAs originally developed against HIV-1 IN have been also reported to inhibit the HIV-1 RNase H.[21,22] The HIV-1 RT-associated RNase H function hydrolyzes the RNA strand of the replicative intermediate RNA:DNA hybrid and, hence, is essential for viral replication.[23] Even though several compounds have been recently identified to inhibit this RT activity,[24–30] up to today no RNase H inhibitor has been approved for HIV therapy. Therefore, this viral function is a very attractive target for drug development.

The first DKA IN inhibitor later described also as RNase H inhibitor was the 4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid (BTDBA, **2**) discovered by Merck.[21] Recently we reported that 6-[1-(4-fluorophenyl)methyl-1*H*-pyrrol-2-yl)]-2,4-dioxo-5-hexenoic acid ethyl ester (RDS 1643, **3**) inhibited the HIV-1 RT-associated RNase H function in biochemical assays with an IC_{50} value of 8 μM, the HIV-1 IN ST (the IC_{50} value was 98 μM) and blocked the HIV-1 replication in cell based assays with an EC_{50} value of <0.2 μM (Chart 1).[22] Starting from these observations, more recently we designed a small library of **3** analogues with the aim of obtaining dual IN/RNase H HIV-1 inhibitors and found compounds active at micromolar concentration against RNase H and low nanomolar IC_{50} values against IN in recombinant assays.[31] The best dual inhibition was found for compounds showing a diketo ester group and fluorine atoms as substituents on the benzyl portion, as exemplified by compound **4** (Chart 1, $IC_{50,IN}$ = 0.6 μM, $IC_{50,RH}$ = 3.0 μM, EC_{50} = 2 μM). Furthermore, we extended these results to a quinolonyl diketo acid series, in which less marked dual activity was found, having in the best case $IC_{50,IN}$ = 26.2 μM, $IC_{50,RH}$ = 2.4 μM, and EC_{50} = 3.6 μM (compound **5**).[32]

Herein we present the design, synthesis, and biological evaluation of new compounds related to **3** to better define the structure–activity relationship (SAR) within the most interesting pyrrolyl diketo acid series. All the newly designed pyrrolyl DKAs, both ester **6** and acid **7** derivatives, are shown in Chart 2. Basically, starting from **3**, we fixed the pyrrole ring and the DKA chain while wider tranformations included one or more of the following modifications: (i) introduction of aromatic substituent in position 4 of the pyrrole ring; (ii) shift of the diketo hexenoic chain from 2 to 3 position of the pyrrole moiety; (iii) shortening of the diketo hexenoic branch into a diketo butanoic group; (iv) introduction of alkyl or aryl group replacing the fluorobenzyl moiety; (v) replacement of carboxylic function with a triazole ring; (vi) introduction of alkyl group within the DKA branch; (vii) replacement of keto group of DKA moiety with NH_2 function. Notably, among the compounds described in this paper, **7k** has been the first DKA derivative reported by Merck as selective ST IN inhibitor.[33] We decided to include this compound in this study to define its properties as dual inhibitor of IN and RNase H.

RESULTS AND DISCUSSION

Chemistry.

The synthesis of derivatives **6a**–**l** and **7a**–**m** is outlined in Schemes 1–4. Derivatives **6a**–**d** and **7a**–**d** were synthesized according to the pathway described in Scheme 1. The acetyl or propionyl pyrrole intermediates **9a**–**c** were obtained by two different procedures: (1) the alkylation with 4-fluorobenzyl bromide in alkaline medium (K_2CO_3) of 1-(4-phenyl-1*H*-pyrrol-3-yl)ethanone[34] or derivatives **8**, achieved via toluene-4-sulfonylmethyl isocyanide (TosMIC) reaction from (*E*)-1-phenylpent-1-en-3-one;[35] (2) termal transposition of the acetyl chain of 2-acetyl-1-[(4-fluorophenyl)methyl]pyrrole[36] from 2- to 3-position of the pyrrole ring in the presence of CF_3COOH. Derivatives **9a**–**c** were condensed in turn with diethyl oxalate in the presence of sodium ethoxide to provide the diketobutanoic ethyl esters **6a**–**c**. Compound **6a** was used as substrate to provide (i) **6d**, obtained by reacting the enol **6a** with ammonium acetate in acid medium (CH_3COOH) following a known procedure

reported for DKA derivarives,[37] and (ii) **7a**, achieved by hydrolysis of ester **6a** in the presence of 6 N NaOH. The last conditions have also been used to obtain **7b–c** starting from **6b–c**, respectively. A sligthly different condition has been used to obtain **7d**, as previously described.[37]

Derivatives **6e–h** and **7e–h** were obtained according to Scheme 2. The pyrroles **10** and **11** were used as starting materials to obtain the key intermediates **12e–h**. Compound **10** was obtained by alkylation of commercially available 4-iodo-2-formylpyrrole in alkaline medium (K_2CO_3) with 4-*F*-benzyl bromide.

Derivative **11** was obtained starting from pyrrole that underwent a two-step one-pot reaction comprising (i) formylation in the presence of oxalyl chloride and DMF and (ii) Friedel–Crafts reaction with 4-*F*-benzoyl chloride. Thus, compound **11** was arylated to **12f** or alkylated on nitrogen atom of pyrrole ring with the appropriate alkyl halide furnishing **12e** and **12g**, while intermediate **10** underwent a Suzuki coupling in position 4 to obtain **12h**. The formylpyrroles **12e–h** were condensed with acetone in alkaline medium (4 N NaOH) to obtain α,β-insaturated ketones **13e–h**. The last compounds were in turn condensed with diethyl oxalate in the presence of sodium ethoxide to provide the diketobutanoic ethyl esters **6e–h** that were finally hydrolyzed by reaction with 1 N NaOH to give the corresponding carboxylic acids **7e–h**.

The synthetic pathway to obtain derivatives **6i**,**j** and **7i**,**j** is outlined in Scheme 3. The enones **17i**,**j** were obtained starting from **15** and **16**, respectively. Pyrrole **15** has been achieved starting from pyrrole-2-carboxaldehyde that was alkylated in alkaline medium (K_2CO_3) to obtain **14**. The termal transposition of the formyl chain from position 2 to 3 of the pyrrole ring in the presence of trifluoroacetic acid led to derivative **15**. In a parallel pathway (3*E*,5*E*)-6-phenylhexa-3,5-dien-2-one[38] underwent ring closure by reacting with TosMIC, giving the pyrrole derivatives **16**. Interestingly, the attack of the TosMIC reagent was specific on the 5,6 double bond of the starting dienone. Afterward, derivative **15** was condensed with acetone, affording **17i**; conversely, compound **16** was converted into **17j** by reaction with 4-*F*-benzyl bromide in alkaline medium (K_2CO_3). Finally, intermediates **17i**,**j** were converted into esters **6i**,**j** by condensation with diethyl oxalate and then converted to acids **7i**,**j** by alkaline hydrolysis.

Derivatives **6k**,**l** and **7k**,**l** were obtained according to the pathway described in Scheme 4. The iodination of 2-acetyl-1-[(4-fluorophenyl)methyl]pyrrole in the presence of *N*-iodosuccinimide (NIS) afforded derivative **18**, which underwent a Suzuki coupling reaction to give **19**. Intermediates **19** and 2-acetyl-1-[(4-fluorophenyl)methyl]pyrrole were converted into the diketobutanoic acid derivatives **7k**,**l** through the ethyl esters **6k**,**l**, according to the condensation/hydrolysis procedure described above.

Finally, 2-acetyl-1-[(4-fluorophenyl)methyl]pyrrole was subjected to a condensation reaction in the presence of 1-trityl-1*H*-[1,2,4]triazole-3-carboxylic acid ethyl ester[39] to afford the triazole-protected derivative **20**, which was deprotected by hydrolysis in the presence of 3 M HCl to obtain the triazole derivative **7m**.

Evaluation of Biological Activities.

All newly synthesized compounds **6a**–**l** and **7a**–**m** were tested in vitro in enzyme assays against both recombinant RNase H and IN. The IC_{50} values obtained for each compound in the inhibition of both the IN ST reaction and HIV-1 RT-associated RNase H function were plotted against each other in correlation plots (Figure 1). In these plots, single dots correspond to single compounds. The compounds are distributed 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 each 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). As seen in Figure 1, these graphs do not show any particular correlation between RNase H and IN inhibition.

The newly synthesized pyrrolyl derivatives **6a**–**l** and **7a**–**m** exhibited good potency of inhibition when tested on the HIV-1 IN ST (Table 1). In general, as reported previously,[31,32] the acid derivatives **7a**–**m** were endowed with the highest potency showing IC_{50} values in the range of 26–0.019 μM. In our assay Merck compound **7k** was confirmed as potent IN ST inhibitor showing IC_{50} = 0.057 μM (literature data IC_{50} = 0.08 μM).[33] As seen in Figure 1A, 80% of the acid compounds (red dots) are distributed in the left half of the graph, suggesting the acid function is critical for IN inhibition but not critical for RNase H inhibition. Among them, seven compounds (**7a**,**c**,**d**,**e**,**h**,**k**,**l**) were proven to have submicromolar activity (IC_{50} value were in the range of 66–19 nM), while two derivatives (**7b**,**i**) can be considered less active to almost inactive (IC_{50} values were 111 and 26 μM, respectively). The most active compound was the 4-phenylpyrrole derivative **7l** with an IC_{50} value of 19 nM, 3 times less active than **1** in inhibiting of the ST reaction of IN. Interestingly, even though not all the synthesized analogues were tested on the HIV-1 3′-P activity, results showed that the newly synthesized acids were selective inhibitors of the ST step, with the IC_{50} values on the 3′-P step 2–3 orders of magnitude higher with respect to the ones obtained on ST, thus confirming that the DKAs are selective ST inhibitors (data not shown).

The newly synthesized pyrrolyl DKA derivatives **6a**–**l** and **7a**–**m** can be divided into two classes: the diketobutanoic (**6a**–**d**,**k**,**l** and **7a**–**d**,**k**,**l**) and the diketohexenoic (**6e**–**j** and **7e**–**j**) derivatives. In the diketobutanoic structures of derivatives **7a**–**d**, **7k**,**l**, two main differences involving the substitution of the pyrrole ring emerged: the diketobutanoic chain can be linked on the pyrrole ring into two different positions (2- or 3-position), along with a phenyl ring, which can be substituted in position 4 of the pyrrole ring. The two variables did not influence the IN inhibitory activity. Only the phenyl substitution at position 4 of the pyrrole ring (R substituent) seems to favor slightly IN vs RNase H inhibition with a majority of compounds bearing this substitution distributed in the two left quarters of the correlation graphs (green dots, Figure 1B). In fact, from a comparison of the inhibition data of diketobutanoic/diketohexenoic **7a**/**7l** and **7c**/**7k**, which are characterized respectively by the presence of the phenyl ring and by its absence, a correspondence in the orders of magnitude of their IC_{50} values was observed. Moreover, **7a** and **7c** linked the diketobutanoic chain in

position 3 of the pyrrole ring, while **7k** and **7l** linked it in 2-position of the same ring. All these compounds were characterized by similar potencies (IC_{50} values of 22, 24, 57, and 19 nM, respectively). The 4-fluorobenzoyl substitution at position 4 of the pyrrole ring (R substituent) does not seem to favor the inhibition of either enzymes (orange dots, Figure 1B).

The other three modifications involved exclusively the diketobutanoic chain: (1) the substitution of the enolic OH with a NH_2 (**7d**), (2) the introduction of a methyl group in 3-position (**7b**), and (3) the substitution of the carboxylic acid function with its bioisoster triazole ring (**7m**). The NH_2 (**7d)** and triazole (**7m**) derivatives were 2-fold less active with respect to their OH (**7a**) and COOH (**7h**) counterparts, respectively (IC_{50} values were 43, 110, 22, and 57 nM, respectively). On the contrary, the 3-methylbutenoate derivative (**7b**) completely lost its ability to inhibit IN enzyme (IC_{50} values of >111 μM). It appears that IN seems to tolerate a wide variety of substitutions at the R_1 position, including the absence of substituent, with a majority of compounds with such substitutions distributed in the two left quarters of the correlation graphs (Figure 1C). No preferential inhibition pattern can be observed for compounds with substitutions at the R_2 position on the correlation plot presented in Figure 1 D.

When the two series of diketobutanoic and the diketohexenoic esters and acids were tested on the HIV-1 RT-associated RNase H function in biochemical assays, the most active derivatives were the diketohexenoic ester **6f** and the diketobutanoic acid **7d**, with IC_{50} value of 1.8, and 2 μM, respectively. Interestingly, both compounds were more active then the references 3 and 4. Noteworthy, the structure of **6f** is related to the reference 3, since both are pyrrolyl diketohexenoic derivatives, but **6f** bring a phenyl ring on nitrogen replacing the 4-F-benzyl group of **3** and a 4-F-benzoyl moiety linked in 4 position of the pyrrole ring. In the matter of compound **7d,** it is a 3-pyrrolyl diketobutanoic acid derivative characterized by the presence of an amine function that replaces the enol OH in position 3 of the diketobutanoic chain. Since **7d** is a carboxylic acid, its stronger inhibition toward IN than RNase H function was expected; conversely, the ester **6f** is the best dual inhibitor IN/RNase H of this series. From a first analysis of the results we can state that (i) as known, the acid function confers a better inhibitory activity on IN, (ii) the ester function is amenable for inhibition of RNase H function of RT, confirming the results recently reported by the means of docking studies and mutagenesis experiments,[40] and (iii) contemporaneously, the ester function is necessary for a dual inhibition IN/RNase H.

Among the ester derivatives (**6a**–**l**), the diketohexenoic compounds (**6e**–**j**, IC_{50} values in the range of 1.8–55 μM) were able to inhibit the HIV-1 RNase H function with a slightly higher potency then the diketobutanoic counterpart (**6a**–**d**,**k**,**l**, IC_{50} values in the range of 6–72 μM). Differently, when the acid derivatives (**7a**–**m**) were tested, this difference was not observed.

In both the diketohexenoic and the diketobutanoic ester and acid derivatives, the presence of a phenyl substituent in position 4 of the pyrrole ring influenced the inhibitory activity. In fact, the 4-phenyl substituted diketobutanoic ester and acid **6a**/**7a** (IC_{50} values of 10 μM, 7.5 μM, respectively) and diketohexenoic **6j**/**7j** (IC_{50} values of 3.0 μM, 7.0 μM, respectively)

were consistently more active than the unsubstituted counterparts **6c**/**7c** and **6i**/**7i** (IC_{50} values of >100, 41, 55, and 69 μM, respectively).

In general, when the DKA chain was shifted from 2- to 3-position, a 2- to 4-fold increase in potency of inhibition was observed (compare **6h**, **7h**, and **7l** with **6j**, **7j**, and **7a**: IC_{50} values of 13.4, 23, 14, 3, 7, and 7.5 μM, respectively), with the sole exception of **6a** and **6l**, which showed comparable potency (IC_{50} values of 10 and 6 μM, respectively).

The substitution of the enolic OH on the diketobutanoic chain (**6a**/**7a**) with a NH_2 group (**6d**/**7d**) in the ester series led to a 7-fold decrease of the potency of RNase H inhibition (**6d**, IC_{50} values of 72 μM) with respect to the unmodified counterpart (**6a**, IC_{50} value of 10 μM), while within the acid series, the NH_2 derivative improved its potency of inhibition (**7d**, IC_{50} value of 2 μM; **7a**, IC_{50} value of 7.5 μM). The introduction of a methyl group in position 3 of the diketobutanoic chain (**7b**) reduced the inhibition (**7b** and **7a** IC_{50} values of 64 and 7.5 μM, respectively) and likewise the substitution of the carboxylic acid function with its bioisoster triazole (**7a** and **7m**, IC_{50} values of 7.5 μM and 26 μM, respectively).

Among all the newly synthesized derivatives, the 2-pyrrolyl diketohexenoic ester **6f**, athough not very potent, emerged as dual inhibitor showing similar IC_{50} values against both IN enzyme and RNase H function of RT (1.2 and 1.8 μM, respectively). This compound retains the ester function that is demonstrated to be necessary for the dual inhibiton[31] and is characterized by a phenyl ring on nitrogen replacing the 4-*F*-benzyl group of **3** and by a 4-*F*-benzoyl moiety linked in 4 position of the pyrrole ring.

Cell-Based Assays.

Among the newly synthesized pyrrolyl DKA derivatives **6a**–**l** and **7a**–**m**, seven derivatives (**6a**,**c**,**l** and **7a**,**c**,**d**,**l**) were characterized by a good anti-HIV activity showing a EC_{50} values in the submicromolar concentration (EC_{50} in the range of 0.56–0.9 μM) and a good selectivity index (SI). Three compounds, the **6k**,**j** and **7k** derivatives, showed an anti-HIV activity in the range of the micromolar concentration (EC_{50} in the range of 1–4.3 μM), while 10 compounds were less active or inactive (**6d**–**i** and **7b**,**e**,**f**,**i**,**m**, EC_{50} in the range of 17.2 to >50 μM). In general, all the compounds had a low citotoxicity index (CC_{50} > 50 μM). Compound **7c**, characterized by the diketobutanoic chain in 3-position of the pyrrole ring, showed the best antiviral efficacy on HIV-1 infected cell (EC_{50} = 0.58 μM) and a low cytotoxicity (CC_{50} > 50 μM, SI > 86). The best dual inhibitor derivative **6f** that showed activity at micromolar concentration in biochemical assays was 20 times less potent in cell-based assays.

CONCLUSION

The development of HIV-1 dual inhibitors is a highly innovative approach aimed at reducing drug toxic side effects and therapeutic costs. Since HIV-1 IN and RNase H are both selective targets for HIV-1 chemotherapy, the identification of dual IN/RNase H inhibitors is an attractive strategy for new drug development. The newly synthesized pyrrolyl derivatives **6a**–**l** and **7a**–**m** exhibit good potency against IN and a moderate inhibition of the RNase H function of RT. In general, by comparison of the inhibition data among the ester and the acid

derivatives, a different behavior was observed. As expected, the acid derivatives showed a higher potency of IN inhibition with respect to the corresponding esters, while the latter compounds have been often found more potent than the corresponding acids in inhibiting the RNase H function of RT enzyme. Notably, compound **6f**, although not very potent on HIV-infected cell, showed a good correlation between HIV-1 IN and RNase H inhibition. It is characterized by a diketoester function, a phenyl ring on nitrogen, and a 4-*F*-benzoyl moiety linked in 4 position of the pyrrole ring. We can state that although the acid function confers a better inhibitory activity on IN, an ester function is amenable for inhibition of RNase H function of RT, and as a consequence, the ester function is necessary for a dual inhibition IN/RNase H. These basic chemical features should be considered for development of more potent dual inhibitors. Overally, the data reported in this work confirm the possibility of developing dual HIV-1 IN/RNase H inhibitors and give new information for the further development of effective dual HIV-1 inhibitors.

EXPERIMENTAL SECTION

Chemistry. General.

Melting points were determined on a Bobby Stuart Scientific SMP1 melting point apparatus and are uncorrected. Compound purities were always >95% determined by high pressure liquid chromatography (HPLC). HPLC analyses were carried out with a Shimadzu LC-10AD VP CTO-10AC VP. Column used was generally Discovery Bio Wide Pore C18 (10 cm × 4.6 mm, 3 μm). Infrared (IR) spectra were recorded on a PerkinElmer Spectrum-One spectrophotometer. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AC 400 Ultrashield 10 spectrophotometer (400 MHz). Dimethyl sulfoxide-d_6 99.9% (code 44,139–2) and deuterochloroform 98.8% (code 41,675–4) of isotopic purity (Aldrich) were used. Column chromatographies were performed on silica gel (Merck; 70–230 mesh) column or aluminum oxide (Sigma-Aldrich; 150 mesh) column. All compounds were routinely checked on TLC by using aluminum-baked silica gel plates (Fluka DC-Alufolien Kieselgel 60 F254) or aluminum oxide (Fluka DC-Alufolien). Developed plates were visualized by UV light. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of rotary evaporator (Büchi) operating at a reduced pressure (~20 Torr). Organic solutions were dried over anhydrous sodium sulfate (Merck). All reactions were carried out under nitrogen. All solvents were freshly distilled under nitrogen and stored over molecular sieves for at least 3 h prior to use.

Microwave Irradiation Experiments.

Microwave reactions were conduced using a CEM Discover system 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 A (GP-A): Synthesis of Pyrrole Nucleus.

A solution of α,β-unsaturated ketone (5.42 mmol) and toluene-4-sulfonylmethyl isocyanide (1.16 g, 5.96 mmol, 1.1 equiv) dissolved in a mixture of anhydrous dimethyl sulfoxide/ethyl ether (14:30 mL) was added dropwise into a well-stirred suspension of sodium hydride (60% in paraffine oil; 0.48 g, 11.93 mmol, 2.2 equiv) in dry ethyl ether (30 mL) under argon atmosphere. After the addition the mixture was stirred at room temperature for 1 h. The reaction was treated with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude product was purified by chromatography on aluminum oxide (chloroform as eluent) to afford the pure product. Yield (%), melting point (°C), recrystallization solvent, IR, and ^{1}H NMR are reported for each compound.

General Procedure B (GP-B): Alkylation of the Pyrrole Nitrogen.

A mixture of the appropriate pyrrole (1.1 mmol), alkylating agent (3.3 mmol), and anhydrous K_2CO_3 (210 mg, 1.5 mmol) in dry DMF (10 mL) was stirred at 100 °C for 2 h. Then the mixture was cooled, treated with water (40 mL), and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude product was purified by chromatography on silica gel to afford the pure product. Chromatography eluent, yield (%), melting point (°C), recrystallization solvent, IR, and ^{1}H NMR are reported for each compound.

General Procedure C (GP-C): Condensation of Pyrrole Carboxaldehyde with Acetone.

The proper pyrrole carboxaldehyde (0.075 mol) was dissolved in 250 mL of acetone. To this mixture was added 4 N NaOH (110 mL), and the mixture was stirred at room temperature for 24 h. After this period water (300 mL) and ethyl acetate (250 mL) were added. The organic layer was separated, washed with water (2 × 100 mL), dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel to obtain pure products. Chromatography eluent, yield (%), melting point (°C), recrystallization solvent, IR, and ^{1}H NMR are reported for each compound.

General Procedure (GP-D): Acetyl Transposition.

A mixture of opportune α acetyl-substituted pyrrole (1.23 mmol) in trifluoroacetic acid (5 mL) was heated at 80 °C for 20 h. After this period the reaction was quenched with water (30 mL) and extracted with ethyl acetate (2 × 50 mL). The organic layers were collected, dried over sodium sulfate, filtered, and evaporated under vacuum. The crude product was purified by chromatography on silica gel (chloroform as eluent) to afford pure product as a brown oil. Yield (%), melting point (°C), recrystallization solvent, IR, and ^{1}H NMR are reported for each compound.

General Procedure E (GP-E): Suzuki Reaction.

$Pd_2(dba)_3$ (0.1 g, 1.7 mmol) was added into a well stirred mixture of appropriate 4-iodopyrrole (1.7 mmol), phenylboronic acid (0.85 g, 7.0 mmol), Cs_2CO_3 (0.665 g, 2.0 mmol), and P(t-But)$_3$ in dioxane (20 mL). The mixture was stirred at 80 °C for 24 h under

argon atmosphere. Then the mixture was cooled to room temperature, filtered, and washed with dioxane. The organic layer was evaporated under vacuum. The raw material was extracted with water (50 mL) and ethyl acetate (50 mL). The organic phase was separated, dried over sodium sulfate, filtered, and evaporated under vacuum. The raw material was purified by silica gel chromatography. Chromatography eluent, yield (%), melting point (°C), recrystallization solvent, IR, and ^{1}H NMR are reported for each compound.

General Procedure F (GP-F): Synthesis of Diketo Esters.

Freshly prepared sodium ethoxide (390 mg, 5.5 mmol) was added into a well-stirred mixture of the appropriate acetyl derivative (2.7 mmol) and diethyl oxalate (790 mg, 5.4 mmol) in anhydrous THF (2.7 mL) under nitrogen atmosphere. The mixture was stirred at room temperature for 2 h and then was poured into *n*-hexane (50 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. Yield (%), melting point (°C), IR, and ^{1}H NMR are reported for each compound.

General Procedure G (GP-G): Synthesis of Diketo Acids.

A mixture of 1 N NaOH (6.5 mL) and the appropriate ester (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 treated with 1 N HCl until pH 3 (pH 7 for **1d**) was obtained, and the solid that formed was collected by filtration, then washed with water and dried under warming lamp to afford pure acids. Yield (%), melting point (°C), IR, and ^{1}H NMR are reported for each compound.

1-(4-Phenyl-1*H*-pyrrol-3-yl)propan-1-one (8).[41]

Compound **8** was prepared from (*E*)-1-phenylpent-1-en-3-one[35] by means of GP-A. 79% as a yellow solid; 169–170 °C; toluene. Anal. ($C_{13}H_{13}NO$) C, H, N.

1-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)ethanone (9a).

Compound **9a** was prepared from 1-(4-phenyl-1*H*-pyrrol-3-yl)ethanone[32] by means of GP-B, using 4-fluorobenzyl bromide as alkylatig agent. Chloroform; 100% as brown oil; IR ν 1705 (C=O ketone) cm^{-1}; ^{1}H NMR (DMSO d_6) δ 2.26 (s, 3H, CH_3), 5.04 (s, 2H, CH_2), 6.63 (s, 1H, pyrrole α-proton), 7.06 (m, 2H, benzyl H), 7.19 (m, 2H, benzyl H), 7.25–7.41 (m, 6H, benzene H and pyrrole α-proton). Anal. ($C_{19}H_{16}FNO$) C, H, N, F.

1-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)propan-1-one (9b).

Compound **9b** was prepared from **8** by means of GP-B, using 4-fluorobenzyl bromide as alkylating agent. Chloroform/acetate 50:1; 66% as brown oil; IR ν 1656 (C=O ketone) cm^{-1}. ^{1}H NMR (DMSO d_6) δ 1.04 (t, 3H, J = 8 Hz, CH_2CH_3), 2.75 (q, 2H, J = 8 Hz, CH_2CH_3), 5.18 (s, 2H, CH_2), 7.07 (d, 1H, J = 2.2 Hz, pyrrole C5-H), 7.2–7.3 (m, 3H, benzene H), 7.32 (t, 2H, benzyl H), 7.4 (m, 2H, benzene H), 7.47 (m, 2H, benzyl H), 7.87 (d, 1H, J = 2 Hz, pyrrole C2-H). Anal. ($C_{20}H_{18}FNO$) C, H, N, F.

1-(1-(4-Fluorobenzyl)-1*H*-pyrrol-3-yl)ethanone (9c).

Compound **9c** was prepared from 1-(1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl)ethanone[36] by means of GP-D. 50% as brown oil; IR ν 1655 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 5.03 (s, 2H, CH_2), 6.60–6.63 (m, 2H, pyrrole C4-H and C5-H), 7.03 (t, 2H,benzene H), 7.12 (m, 2H, benzene H), 7.28 (t, 1H, J = 2.0 Hz, pyrrole C2-H,). Anal. ($C_{13}H_{14}FNO$) C, H, N, F.

1-(4-Fluorobenzyl)-4-iodo-1*H*-pyrrole-2-carboxaldehyde (10).

Compound **10** was prepared from commercially available 4-iodopyrrole-2-carboxaldehyde by means of GP-B, using 4-fluorobenzyl bromide as alkylating agent. Chloroform/*n*-hexane 4:1; 39% as brown oil; IR ν 1651 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.47 (s, 2H), 6.9–7.0 (m, 4H, pyrrole α-proton, pyrrole β-proton, and benzene H), 7.02 (m, 2H, benzene H), 9.48 (s, 1H, CHO). Anal. ($C_{12}H_9FINO$) C, H, N, F, I.

4-(4-Fluorobenzoyl)-1*H*-pyrrole-2-carboxaldehyde (11).

To a well stirred solution of DMF (3.9 mL, 50 mmol) in 1,2-dichloroethane (100 mL) refrigerated in an ice bath was added dropwise a solution of oxalyl chloride (6.35 g, 50 mmol) in 1,2 dichloroethane (100 mL) in a period of 15 min. After addition, the suspension was stirred at room temperature for 15 min. After this time the reaction mixture was refrigerated in ice bath and treated with a solution of pyrrole (49.9 mmol) in 1,2-dichloroethane (100 mL). The mixture was stirred for 15 min at room temperature and then treated with $AlCl_3$ (14.6 g, 109 mmol) and 4-fluorobenzoyl chloride (50 mmol). The reaction was maintained at room temperature for 4 h. After this period the reaction was quenched with ice and water and extracted with ethyl acetate, dried over sodium sulfate, filtered, and evaporated under vacuum. The crude product was purified with chromatography on aluminum oxide (1:1 ethyl acetate–chloroform as eluent) to afford **5** as yellow solid. 85%; 110–111 °C; benzene/cyclohexane; IR ν 2900 (enol), 1660 (C=O ketone) 1640 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.1–7.4 (m, 3H, benzene H and pyrrole β-proton), 7.45 (s, 1H, pyrrole α-proton), 7.8–7.9 (m, 2H, benzene H), 9.60 (s, 1H, CHO), 12 (sb, 1H, NH). Anal. ($C_{12}H_8FNO_2$) C, H, N, F.

1-Benzyl-4-(4-fluorobenzoyl)-1*H*-pyrrole-2-carbaldehyde (12e).

Compound **12e** was prepared from **11** by means of GP-B, using benzyl bromide as alkylatig agent. Acetate/*n*-hexane 1:2; 34% as brown oil; IR ν 1672 (C=O aldehyde), 1638 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.62 (s, 2H, CH_2), 7.1–7.2 (m, 4H, benzyl H and pyrrole β-proton), 7.3–7.4 (m, 4H, benzyl H and benzoyl H), 7.58 (s, 1H, pyrrole α-proton), 7.8–7.9 (m, 2H, benzoyl H), 9.62 (s, 1H, CHO). Anal. ($C_{19}H_{14}FNO_2$) C, H, N, F.

4-(4-Fluorobenzoyl)-1-phenyl-1*H*-pyrrole-2-carbaldehyde (12f).

Compound **11**, phenylboronic acid, pyridine, and copper(II) acetate anhydrous were dissolved in NMP (2.4 mL) in a microwave reactor tube and left to react at 60 W, 120 °C for 6 min. After this period the reaction was quenched with water and extracted with ethyl acetate (5 × 20 mL), washed with water (5 × 20 mL), dried over sodium sulfate, filtered, and evaporated under vacuum. The crude product was purified with chromatography on silica gel

(1:1 ethyl acetate–hexane as eluent) to afford **12f** as brown solid (63% yield). 130–131 °C. Benzene/cyclohexane; IR ν 1680 (C=O aldehyde), 1632 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.21 (t, 2H, benzoyl H), 7.4–7.5 (m, 2H, benzene H), 7.5–7.6 (m, 4H, benzene H and pyrrole β-proton), 7.67 (d, 1H, J = 2 Hz, pyrrole α-proton), 7.9–8.0 (m, 2H, benzoyl H), 9.68 (s, 1H, CHO). Anal. ($C_{18}H_{12}FNO_2$) C, H, N, F.

1-Methyl-4-(4-fluorobenzoyl)-1*H*-pyrrole-2-carboxaldehyde (12g).

Compound **12g** was prepared from **11** by means of GP-B, using iodomethane as alkylatig agent. Chloroform; 90% as gray solid; 115–116 °C; benzene/cyclohexane; IR ν 1660 (C=O aldehyde), 1640 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.03 (s, 3H, N–CH_3), 7.2 (m, 2H, benzoyl H), 7.4 (d, 1H, pyrrole β-proton), 7.5 (s, 1H, pyrrole α-proton), 7.8–7.9 (m, 2H, benzoyl H), 9.60 (s, 1H, CHO). Anal. ($C_{13}H_{10}FNO_2$) C, H, N, F.

1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrole-2-carbaldehyde (12h).

Compound **12h** was prepared from **10** by means of GP-E. 1:1 ethyl acetate–hexane as eluent; 95% as brown oil; IR ν 1642 (C=O aldehyde) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.54 (s, 2H, CH_2), 6.82 (d, 2H, J = 7.0 Hz, benzene H), 6.91 (t, 1H, J = 7.0 Hz, benzene H), 6.99 (t, 2H, benzyl H), 7.16–7.24 (m, 4H, pyrrole β-proton, pyrrole α-proton, benzyl H), 7.48 (d, 2H, J = 7 Hz, benzene H), 9.58 (s, 1H, CHO). Anal. ($C_{18}H_{14}FNO$) C, H, N, F.

4-(1-Benzyl-4-(4-fluorobenzoyl)-1*H*-pyrrol-2-yl)but-3-en-2-one (13e).

Compound **13e** was prepared from **12e** by means of GP-C. 71% as yellow solid; 94–95 °C; benzene/cyclohexane; IR ν 1675 (C=O ketone), 1637 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 5.29 (s, 2H, CH_2), 6.60 (d, 1H, J = 16 Hz, butenone C3-H), 7.1–7.2 (m, 5H, butenone C4-H, benzyl H and pyrrole β-proton), 7.3–7.4 (m, 4H, benzyl H and benzoyl H), 7.46 (d, 1H, J = 2 Hz, pyrrole α-proton), 7.90 (m, 2H, benzoyl H). Anal. ($C_{22}H_{18}FNO_2$) C, H, N, F.

4-(1-Phenyl-4-(4-fluorobenzoyl)-1*H*-pyrrol-2-yl)but-3-en-2-one (13f).

Compound **13f** was prepared from **12f** by means of GP-C. 30% as yellow solid; 145–146 °C; benzene/cyclohexane; IR ν 1680 (C=O ketone), 1634 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.24 (s, 3H, CH_3), 6.56 (d, 1H, J = 16 Hz, butenone C3-H), 7.19 (t, 2H, benzoyl H), 7.24 (d, 1H, J = 16 Hz, butenone C4-H), 7.35 (d, 1H, J = 2 Hz, pyrrole β-proton), 7.38 (d, 1H, J = 2 Hz, pyrrole α-proton), 7.5–7.6 (m, 5H, benzene H), 7.94 (m, 2H, benzoyl H). Anal. ($C_{21}H_{16}FNO_2$) C, H, N, F.

4-(1-Methyl-4-(4-fluorobenzoyl)-1*H*-pyrrol-2-yl)but-3-en-2-one (13g).

Compound **13g** was prepared from **12g** by means of GP-C. 70% as yellow solid; 117–118 °C; benzene/cyclohexane; IR ν 1660 (C=O ketone), 1640 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.78 (s, 3H, N-CH_3), 6.62 (d, 1H, J = 16 Hz, butenone C3-H), 7.1–7.2 (m, 3H, benzene H and pyrrole β-proton), 7.35 (d, 1H, J = 3.7 Hz, pyrrole α-proton), 7.43 (d, 1H, J = 16 Hz, butenone C4-H), 7.8–7.9 (m, 2H, benzene H). Anal. ($C_{16}H_{14}FNO_2$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-2-yl)but-3-en-2-one (13h).

Compound **13h** was prepared from **12h** by means of GP-C. 15% as yellow solid; 160–161 °C; benzene/cyclohexane; IR ν 1604 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, CH_3), 5.24 (s, 2H, CH_2), 6.57 (d, 1H, J = 16 Hz, butenone C3-H), 7.0–7.1 (m, 5H, pyrrole β-proton and benzyl H), 7.18 (d, 1H, J = 2 Hz, pyrrole α-proton,), 7.24 (t, 1H, J = 7 Hz, benzene H), 7.3–7.4 (m, 3H, butenone C4-H and benzene H), 7.5–7.6 (m, 2H, benzene H). Anal. ($C_{21}H_{18}FNO$) C, H, N, F.

1-(4-Fluorobenzyl)-1*H*-pyrrole-2-carboxaldehyde (14).

Compound **14** was prepared from commercially available pyrrole-2-carboxaldehyde by means of GP-B, using 4-fluorobenzyl bromide as alkylating agent. Chloroform; 80% as brown oil; IR ν 1640 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.54 (s, 2H, CH_2), 6.30 (t, 1H, J = 4 Hz, pyrrole C4-H), 6.9–7.0 (m, 4H, benzene H), 7.15 (d, 1H, J = 4 Hz, pyrrole C3-H), 7.17 (d, 1H, J = 4 Hz, pyrrole C5-H), 9.57 (s, 1H, CHO). Anal. ($C_{12}H_{10}FNO$) C, H, N, F.

1-(4-Fluorobenzyl)-1*H*-pyrrole-3-carboxaldehyde (10).

Compound **10** was prepared from **9** by means of GP-D. 55% as brown oil; IR ν 1640 (C=O aldehyde) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.08 (s, 2H, CH_2), 6.6 (s, 1H, pyrrole C4-H), 6.7 (s, 1H, pyrrole C-2H), 7.0–7.2 (m, 4H, benzene H), 7.31 (s, 1H, pyrrole C2-H), 9.75 (s, 1H, CHO). Anal. ($C_{12}H_{10}FNO$) C, H, N, F.

4-(4-Phenyl-1*H*-pyrrol-3-yl)but-3-en-2-one (16).[42]

Compound **16** was prepared from (3*E*,5*E*)-6-phenylhexa-3,5-dien-2-one[38] by means of GP-A. 56% as brown solid; toluene; IR ν 1640 (C=O ketone) cm^{-1}. Anal. ($C_{14}H_{13}NO$) C, H, N.

4-(1-(4-Fluorobenzyl)-1*H*-pyrrol-3-yl)but-3-en-2-one (17i).

Compound **17i** was prepared from **15** by means of GP-C. 70% as brown oil; IR ν 1655 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.43–6.48 (m, 2H, pyrrole C4-H and butenone C3-H), 6.71 (s, 1H, pyrrole C2-H), 7.0 (s, 1H, pyrrole C5-H), 7.06–7.12 (m, 2H, benzene H), 7.15–7.18 (m, 2H, benzene H), 7.49 (d, 1H, butenone C4-H, J = 16 Hz). Anal. ($C_{15}H_{14}FNO$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)but-3-en-2-one (17j).

Compound **17j** was prepared from **16** by means of GP-B, using 4-fluorobenzyl bromide as alkylating agent. Chloroform; 63% as brown oil; IR ν 1640 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.82 (s, 1H, J = 16.3 Hz, butenone C3-H), 6.90 (s, 1H,), 6.99–7.54 (m, 11H, pyrrole C2-H, pyrrole C5-H, benzene H and benzyl H), 7.80 (s, 1H, J = 16.3 Hz, butenone C4-H). Anal. ($C_{21}H_{18}FNO$) C, H, N, F.

1-(1-(4-Fluorobenzyl)-4-iodo-1*H*-pyrrol-2-yl)ethanone (18).

A mixture of 1-(1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl)ethanone[36] (4 g, 18.4 mmol) in dry acetone (100 mL) was cooled at −78 °C. NIS (4.98 g, 22.1 mmol) was added. The mixture was stirred, and the temperature was slowly increased to 25 °C in a period of 96 h. After this period the mixture was evaporated, and ethyl acetate (50 mL) and $NaHCO_{3(aq)}$ (50 mL) were

added. The organic phase was separated, dried over sodium sulfate, filtered, and evaporated under vacuum. The raw material was purified with a column chromatography on silica gel (1:10 ethyl acetate–hexane as eluent) to afford **18** as white solid with a yield of 40%. 81–82 °C; *n*-hexane; IR ν 1640 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 5.50 (s, 2H, CH_2), 6.92 (d, 1H, J = 2.0 Hz, pyrrole C3-H), 7.00 (t, 2H, benzene H), 7.07 (d, 1H, J = 2.0 Hz, pyrrole C5-H), 7.1–7.2 (m, 2H, benzene H). Anal. ($C_{13}H_{11}FINO$) C, H, N, F, I.

1-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-2-yl)ethanone (19).

Compound **19** was prepared from **18** by means of GP-E. 1:7 ethyl acetate–hexane as eluent. 43% as colorless oil; IR ν 1650 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3), 5.58 (s, 2H, CH_2), 6.97–7.02 (t, 2H, benzyl H), 7.14–7.27 (m, 5H, benzene H, pyrrole C3-H and pyrrole C5-H), 7.35–7.39 (t, 2H, benzyl H), 7.51 (d, 2H,benzene H). Anal. ($C_{18}H_{21}FNO$) C, H, N, F.

1-(1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl)-3-hydroxy-3-(1-trityl-1*H*-1,2,4-triazol-3-yl)prop-2-en-1-one (20).

A solution of 1-[1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl]ethanone[36] (1 g, 4.6 mmol) in anhydrous THF (5 mL) was thermostated at −32 °C. LHMDS (9.2 mL) was added, and the mixture was stirred at the some temperature for 2 h. A solution of 1-trityl-1*H*-[1,2,4]triazole-3-carboxylic acid ethyl ester[39] (2 g, 5.3 mmol) in anhydrous THF (18 mL) was added dropwise to the solution thermostated at −32 °C. After the addition, the reaction mixture was stirred for 1.5 h at room temperature. The reaction was poured into 1 N HCl (100 mL) and extracted with ethyl acetate. The organic phase was separated, washed with water, dried over sodium sulfate, filtered, and evaporated under vacuum obtaining 2.6 g of crude product as light yellow solid. The raw material was purified by recrystallization from benzene/cyclohexane, obtaining 1.84 g of pure **20**. 54%; 110–112 °C; benzene/cyclohexane; IR ν 2954 (OH enol), 1626 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.62 (s, 2H, CH_2), 6.3 (t, 1H, pyrrole C4-H), 6.9–7.0 (m, 4H, butenoate C3-H, pyrrole C3-H, benzyl H), 7.1–7.2 (m, 9H, benzyl H, pyrrole C5–H and benzene H), 7.3–7.4 (m, 9H, benzene H), 8.01 (s, 1H, triazole H), 15 (br s, 1H, OH enol). Anal. ($C_{35}H_{27}FN_4O_2$) C, H, N, F.

Ethyl 4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxobut-2-enoate (6a).

Compound **6a** was prepared from **9a** by means of GP-F. 88%; 111–112 °C; benzene; IR ν 2900 (OH enol), 1720 (C=O ester), 1620 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.29 (t, 3H, CH_3CH_2), 4.25 (q, 2H, CH_2CH_3), 5.06 (s, 2H, CH_2 benzyl), 6.48 (s, 1H, butenoate C3-H), 6.67 (d, 1H, J = 1.5 Hz, pyrrole C5-H), 7.02–7.46 (m, 10H, pyrrole C2-H, benzene H and benzyl H), 15 (br s, 1H, OH enol). Anal. ($C_{23}H_{20}FNO_4$) C, H, N, F.

Ethyl 4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-2-hydroxy-3-methyl-4-oxobut-2-enoate (6b).

Compound **6b** was prepared from **9b** by means of GP-F. **6b** was extracted with ethyl acetate. The organic phase was separated, washed with brine, dried over sodium sulfate, filtered, and evaporated under vacuum obtaining a crude product that was purified with column chromatography on silica gel (ethyl acetate/*n*-hexane 1:2). 30% as yellow oil; IR ν 1730

(C=O ester), 1650 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 1.14 (d, 3H, J = 7 Hz, CH_3), 1.34 (t, 3H, J = 7.5 Hz, CH_3CH_2), 4.33 (q, 2H, J = 7.5 Hz, CH_2CH_3), 5.25 (s, 2H, CH_2), 7.14 (d, 1H, J = 1.9 Hz, pyrrole C2-H), 7.22–7.51 (m, 9H, benzene H and benzyl H), 8.08 (s, 1H, J = 1.9 Hz, pyrrole C5-H), 14 (br s, 1H, enol). Anal. ($C_{24}H_{22}FNO_4$) C, H, N, F.

Ethyl 4-(1-(4-Fluorobenzyl)-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxobut-2-enoate (6c).

Compound **6c** was prepared from **9c** by means of GP-F. 93% as yellow solid; 63–65 °C; ligroin; IR ν 3500–2500 (OH enol), 1726 (C=O ester), 1633 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 1.28 (t, 3H, J = 7 Hz, CH_3CH_2), 4.27 (q, 2H, J = 7 Hz, CH_2CH_3), 5.17 (s, 2H, CH_2), 6.62 (t, 1H, pyrrole C4-H), 6.73 (s, 1H, butenoate C3-H), 7.02 (t, 1H, pyrrole C5-H), 7.18 (t, 2H, benzene H), 7.36 (dd, 2H, benzene H), 8.07 (s, 1H, pyrrole C2-H), 15 (br s, 1H, enol). Anal. ($C_{17}H_{18}FNO_4$) C, H, N, F.

Ethyl 2-Amino-4-(1-(4-fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-4-oxobut-2-enoate (6d).

To a stirred mixture of **6a** (1 g, 2.5 mmol) and ammonium acetate (0.22 g, 2.9 mmol) in benzene (30 mL) was added acetic acid glacial (0.2 mL, 3.9 mmol). The mixture was stirred at reflux for 20 h with a Dean–Stark apparatus. After this period the mixture was cooled to room temp and washed with a saturated solution of $NaHCO_3$ (50 mL). The organic layer was separated, dried over sodium sulfate, filtered, and evaporated under vacuum. The raw material was purified with chromatography on aluminum oxide (chloroform as eluent) to afford **6d** as yellow solid with a yield of 50%. 135–136 °C. benzene; IR ν 3500 (NH_2), 1720 (C=O ester), 1620 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H, CH_3CH_2), 4.25 (q, 2H, CH_3CH_2), 5.08 (s, 2H, CH_2), 6.13 (s, 1H, butenoate C3-H), 6.67 (d, 1H, J = 1.5 Hz, pyrrole C5-H), 7.0–7.6 (m, 10H, benzene H, benzyl H, and pyrrole C2-H), 9 (br s, 2H, NH_2). Anal. ($C_{23}H_{21}FN_2O_2$) C, H, N, F.

Ethyl 6-(1-Benzyl-4-(4-fluorobenzoyl)-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoate (6e).

Compound **6e** was prepared from **13e** by means of GP-F. 62% as yellow solid; 154–155 °C; benzene/cyclohexane; IR ν 1730 (C=O ester), 1636 (C=O ketone) cm^{-1}. ^1H NMR (acetone-d_6) δ 1.34 (t, 3H, CH_3CH_2), 4.32 (q, 2H, CH_2CH_3), 5.60 (s, 2H, CH_2), 6.86 (d, 1H, J = 15.6 Hz, hexanoate C5-H), 7.2–7.5 (m, 9H, J = 1.6 Hz, benzene H, pyrrole β-proton, benzoyl H, and hexanoate C3-H), 7.72 (d, 1H, J = 15.6 Hz, hexanoate C6-H), 7.90 (d, 1H, J = 1.6 Hz, pyrrole α-proton), 7.9–8.0 (m, 2H, benzoyl H), 14 (bs, 1H, enol). Anal. ($C_{26}H_{22}FNO_5$) C, H, N, F.

Ethyl 6-(4-(4-Fluorobenzoyl)-1-phenyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoate (6f).

Compound **6f** was prepared from **13f** by means of GP-F. 80% as yellow solid; 129–130 °C; benzene/cyclohexane; IR ν 3500 (OH enol), 1720 (C=O ester), 159 (C=O ketone) cm^{-1}. ^1H NMR (CD_3OD) δ 1.36 (t, 3H, J = 7.5 Hz, CH_3CH_2), 4.31 (q, 2H, J = 7.5 Hz, CH_2CH_3), 7.26–7.48 (m, 3H, benzoyl H and hexanoate C5-H), 7.39 (d, 1H, J = 15.6 Hz, hexanoate C6-H), 7.43–7.48 (m, 3H, benzene H and hexanoate C3-H), 7.58–7.62 (m, 4H, benzene H and

pyrrole β-proton), 7.72 (s, 1H, pyrrole α-proton), 7.97–8.01 (m, 2H, benzoyl H), 14 (bs, 1H, OH enol). Anal. ($C_{25}H_{20}FNO_5$) C, H, N, F.

Ethyl 6-(4-(4-Fluorobenzoyl)-1-methyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoate (6g).

Compound **6g** was prepared from **13g** by means of GP-F. 83% as yellow solid; 166–167 °C; benzene/cyclohexane; IR ν [1] 2900 (OH enol), 1720 (C=O ester), 1650 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.39 (t, 3H, J = 7.5 Hz, CH_3CH_2), 3.81 (s, 3H, N-CH_3), 4.39 (q, 2H, J = 7.5 Hz, CH_2CH_3), 6.45 (s, 1H, hexanoate C3-H), 6.50 (d, 1H, J = 15.4 Hz, hexanoate C5-H), 7.12–7.20 (m, 3H, benzene H and pyrrole β-proton), 7.36–39 (m, 1H, pyrrole α-proton), 7.63 (d, 1H, J = 15.4 Hz, hexanoate C6-H), 7.83–7.91 (m, 2H, benzene H), 15 (bs, 1H, enol). Anal. ($C_{20}H_{18}FNO_5$) C, H, N, F.

Ethyl 6-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoate (6h).

Compound **6h** was prepared from **13h** by means of GP-F. 92% as red solid; 169–170 °C; benzene; IR ν 2900 (OH enol), 1723 (C=O ester), 1602 (C=O ketone) cm^{-1}. ^1H NMR (acetone-d_6) δ 1.35 (t, 3H, J = 7.5 Hz, CH_3CH_2), 4.32 (q, 2H, J = 7.5 Hz, CH_2CH_3), 5.53 (s, 2H, CH_2), 6.46 (s, 1H, hexanoate C3-H), 6.74 (d, 1H, J = 16 Hz, hexanoate C5-H), 7.13–7.24 (m, 3H, benzyl H and benzene H), 7.30–39 (m, 4H, benzyl H, hexanoate C6-H, and pyrrole β-proton), 7.43 (s, 1H, pyrrole α-proton), 7.65 (d, 2H, benzene H), 7.7–7.8 (m, 2H, benzene H), 15 (bs, 1H, enol). Anal. ($C_{25}H_{22}FNO_4$) C, H, N, F.

Ester 6-(1-(4-Fluorobenzyl)-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxohexa-2,5-dienoate (6i).

Compound **6i** was prepared from **17i** by means of GP-F. 41% as yellow solid; 88–90 °C; ligroin; IR ν 3400 (OH enol), 1725 (C=O ester), 1625 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.44 (t, 3H, J = 7 Hz, CH_3CH_2), 4.41 (q, 2H, J = 7 Hz, CH_2CH_3), 5.08 (s, 2H, CH_2), 6.39 (s, 1H, J = 16 Hz, hexanoate C5-H), 6.5–6.6 (m, 2H, pyrrole C4-H and hexanoate C3-H), 6.7 (t, 1H, pyrrole C5-H), 7.0 (t, 1H, pyrrole C2-H), 7.08–7.20 (m, 4H, benzene H), 7.75 (s, 1H, J = 16 Hz, hexanoate C6-H), 14 (br s, 1H, enol). Anal. ($C_{19}H_{18}FNO_4$) C, H, N, F.

Ethyl 6-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxohexa-2,5-dienoate (6j).

Compound **6j** was prepared from **17j** by means of GP-F. 56% as yellow solid; 121–122 °C; cyclohexane; IR ν 2900 (OH enol), 1720 (C=O ester), 1620 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.43 (t, 3H, CH_3CH_2), 4.40 (q, 2H, CH_2CH_3), 5.09 (s, 2H, CH_2), 6.73 (s, 1H, hexanoate C3-H), 6.87 (d, 1H, J = 16.6 Hz, hexanoate C5-H), 7.01 (s, 1H, pyrrole C5-H), 7.08–7.54 (m, 10H, pyrrole C2-H, benzene H and benzyl H), 7.74 (d, 1H, hexanoate C6-H), 15 (bs, 1H, enol). Anal. ($C_{25}H_{21}FNO_4$) C, H, N, F.

Ethyl 4-(1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxobut-2-enoate (6k).

Compound **6k** was prepared from 1-(1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl)ethanone[36] by means of GP-F. 41% as yellow solid; 88–89 °C; ligroin; IR ν 2900 (OH enol), 1720 (C=O ester), 1620 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 1.26 (t, 3H, J = 7.5 Hz, CH_3CH_2), 4.25 (q, 2H, J = 7.5 Hz, CH_2CH_3), 5.59 (s, 2H, CH_2), 6.32 (dd, 1H, J = 2.5 Hz, J = 3.5 Hz,

pyrrole C4-H), 6.84 (s, 1H, butenoate C3-H), 7.0–7.2 (m, 4H, benzene H), 7.43 (d, 1H, J = 3.5 Hz, pyrrole C3-H), 7.53 (s, 1H, pyrrole C5-H), 14 (br s, 1H, enol). Anal. ($C_{17}H_{16}FNO_4$) C, H, N, F.

Ethyl 4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxobut-2-enoate (6l).

Compound **6l** was prepared from **19** by means of GP-F. 95% as yellow solid; 102–103 °C; benzene/cyclohexane; IR ν 3400 (OH enol), 1720 (C=O ester), 1620 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 1.34 (t, 3H, J = 7.5 Hz, CH_3CH_2), 4.33 (q, 2H, J = 7.5 Hz, CH_2CH_3), 5.68 (s, 2H, CH_2), 7.06 (s, 1H, butenoate C3-H), 7.17–7.31 (m, 5H, benzene H and benzyl H), 7.40–7.44 (m, 2H, benzene H), 7.75 (d, 2H, benzene H), 8.01 (s, 1H, pyrrole C5-H), 8.13 (s, 1H, pyrrole C3-H), 14 (bs, 1H, enol). Anal. ($C_{22}H_{20}FNO_4$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxobut-2-enoic Acid (7a).

Compound **7a** was prepared from **6a** by means of GP-G. 98% as brown solid; 109–110 °C; toluene; IR ν 3500–2000 (OH acid and enol), 1740 (C=O acid), 1620 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.06 (s, 2H, CH_2), 6.48 (s, 1H, butenoate C3-H), 6.67 (d, 1H, J = 1.5 Hz, pyrrole C5-H), 7.06–7.10 (t, 2H, benzyl H), 7.18–7.25 (m, 2H, benzyl H), 7.30–7.39 (m, 5H, benzene H), 7.49 (s, 1H, pyrrole C2-H), 14 (br s, 2H, OH acid and enol). Anal. ($C_{21}H_{16}FNO_4$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-2-hydroxy-3-methyl-4-oxobut-2-enoic Acid (7b).

Compound **7b** was prepared from **6b** by means of GP-G. 38% as white solid; 233–234 °C; benzene; IR ν 3500–2500 (OH acid and enol), 1700 (C=O acid), 1640 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 1.14 (d, 3H, CH_3, J = 7 Hz), 5.13 (s, 2H, CH_2), 7.1–7.2 (m, 3H, benzyl H and pyrrole C5-H), 7.34 (t, 2H, benzyl H), 7.47 (s, 1H, pyrrole C2-H), 7.51 (m, 3H, benzene H), 7.9–8.0 (m, 2H, benzene H), 14 (br s, 2H, enol and acid). Anal. ($C_{22}H_{18}FNO_4$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxobut-2-enoic Acid (7c).

Compound **7c** was prepared from **6c** by means of GP-G. 57% as yellow solid; 146–147 °C; toluene; IR ν 3500–2500 (OH acid and enol), 1727 (C=O acid), 1621 (C=O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.16 (s, 2H, CH_2), 6.60 (dd, 1H, J_1 = 1.5 Hz, J_2 = 3.0 Hz, pyrrole C4-H), 6.72 (s, 1H, butenoate C3-H), 7.00 (dd, 1H, J_1 = 1.5 Hz, J_2 = 3 Hz, pyrrole C5-H), 7.18 (t, 2H, benzene H), 7.36 (dd, 2H, benzene H), 8.04 (s, 1H, pyrrole C2-H), 14 (br s, 1H, enol), 15 (br s, 1H, COOH). Anal. ($C_{15}H_{12}FNO_4$) C, H, N, F.

6-(1-Benzyl-4-(4-fluorobenzoyl)-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoic Acid (7e).

Compound **7e** was prepared from **6e** by means of GP-G. 87% as orange solid; 165–166 °C; benzene; IR ν 3500–2500 (OH acid and enol), 1727 (C=O acid), 1630 (C=O ketone) cm^{-1}. ^1H NMR (CD_3OD) δ 5.22 (s, 2H, CH_2), 6.69 (s, 1H, J = 15.2 Hz, hexanoate C5-H), 7.15–7.44 (m, 9H, hexanoate C3-H, benzyl H, benzoyl H, and pyrrole β-proton), 7.65 (s, 1H, J = 15.2 Hz, hexanoate C6-H), 7.79 (s, 1H, pyrrole α-proton), 7.92–7.97 (m, 2H, benzoyl H). Anal. ($C_{24}H_{18}FNO_5$) C, H, N, F.

6-(4-(4-Fluorobenzoyl)-1-phenyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoic Acid (7f).

Compound **7f** was prepared from **6f** by means of GP-G. 85% as yellow solid; 183–184 °C; benzene; IR ν 3500–2500 (OH acid and enol), 1724 (C=O acid), 1596 (C=O ketone) cm^{-1}. ^1H NMR (CD_3OD) δ 6.69 (s, 1H, J = 16 Hz, hexanoate C5-H), 7.26 (t, 2H, benzoyl H), 7.41 (d, 1H, hexanoate C6-H), 7.47–7.49 (m, 2H, pyrrole β-proton and hexanoate C3-H), 7.73 (s, 1H, pyrrole α-proton), 7.98–8.01 (m, 2H, benzoyl H). Anal. ($C_{23}H_{16}FNO_5$) C, H, N, F.

6-(4-(4-Fluorobenzoyl)-1-methyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoic Acid (7g).

Compound **7g** was prepared from **6g** by means of GP-G. 95% as orange solid; 162–163 °C; ethanol; IR ν 3500–2500 (OH acid and enol), 1700 (C=O acid), 1600 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 3.32 (s, 3H, N-CH_3), 6.52 (s, 1H, hexanoate C3-H), 6.93 (d, 1H, hexanoate C5-H), 7.32–7.86 (m, 7H, benzene H, pyrrole β-proton, pyrrole α-proton, and hexanoate C6-H), 14 (br s, 2H, OH enol and acid). Anal. ($C_{18}H_{14}FNO_5$) C, H, N, F.

6-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxohexa-2,5-dienoic Acid (7h).

Compound **7h** was prepared from **6h** by means of GP-G. 73% as red solid; decompose; benzene; IR ν 3500–2500 (OH acid and enol), 1707 (C=O acid), 1571 (C=O ketone) cm^{-1}. ^1H NMR (acetone-d_6) δ 5.51 (s, 2H, CH_2), 6.49 (s, 1H, hexanoate C3-H), 6.7, (d, 1H, hexanoate C5-H), 7.07–7.42 (m, 7H, benzyl H, pyrrole β-proton, pyrrole α-proton, and hexanoate C6-H), 7.6–7.8 (m, 5H, benzene H), 14 (br s, 2H, enol and acid). Anal. ($C_{23}H_{18}FNO_4$) C, H, N, F.

6-(1-(4-Fluorobenzyl)-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxohexa-2,5-dienoic Acid (7i).

Compound **7i** was prepared from **6i** by means of GP-G. 92% as yellow solid; >300 °C; DMF/H_2O; IR ν 3500–2500 (OH acid and enol), 1720 (C=O acid), 1630 (C=O ketone) cm^{-1}. ^1H NMR (DMF-d_7) δ 5.34 (s, 2H, benzyl), 6.39 (s, 1H, hexanoate C3-H), 6.54 (d, 1H, hexanoate C5-H), 6.7 (bs, 1H, pyrrole C4-H), 7.38–7.67 (m, 7H, pyrrole C2-H, pyrrole C5-H, benzene H, and hexanoate C6-H), 14 (br s, 2H, OH enol and acid). Anal. ($C_{17}H_{16}FNO_4$) C, H, N, F.

6-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-2-hydroxy-4-oxohexa-2,5-dienoic Acid (7j).

Compound **7j** was prepared from **6j** by means of GP-G. 75% as yellow solid; 128–129 °C; benzene; IR ν 3500–2500 (OH acid and enol), 1700 (C=O acid), 1600 (C=O ketone) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.20 (s, 2H, CH_2), 6.75 (bs, 1H, hexanoate C3-H), 6.80 (d, 1H, hexanoate C5-H), 7.1–7.6 (m, 12H, pyrrole C2-H, pyrrole C5-H, benzene H, benzyl H, and hexanoate C6-H), 14 (br s, 2H, enol and acid). Anal. ($C_{23}H_{17}FNO_4$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxobut-2-enoic Acid (7k).

Compound **7k** was prepared from **6k** by means of GP-G 80% as yellow solid; 156–157 °C; benzene; IR ν 3500–2500 (OH acid and enol), 1700 (C=O acid), 1620 (C=O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.59 (s, 2H, CH_2), 6.30 (dd, 1H, J = 2.5 Hz, J = 3.5 Hz, pyrrole C4-H), 6.81 (s, 1H, butenoate C3-H), 7.0–7.09–7.16 (m, 4H, benzene H), 7.39 (d, 1H, J = 3.5 Hz, pyrrole C3-H), 7.51 (s, 1H, pyrrole C5-H) 14 (br s, 2H, OH enol and acid). Anal. ($C_{15}H_{12}FNO_4$) C, H, N, F.

4-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-pyrrol-2-yl)-2-hydroxy-4-oxobut-2-enoic Acid (7l).

Compound **7l** was prepared from **6l** by means of GP-G. 82% as yellow solid; 195–196 °C; toluene; IR ν 3500–2000 (OH acid and enol), 1740 (C=O acid), 1620 (C=O ketone) cm^{-1}. ^1H NMR (DMSO d_6) δ 5.68 (s, 2H, CH_2 benzyl), 7.05 (s, 1H, butenoate C3-H), 7.1–7.3 (m, 5H, benzyl H and benzene H), 7.41 (t, 2H, benzene H), 7.76 (d, 2H, benzene H), 8.01 (s, 1H, pyrrole C3-H), 8.13 (s, 1H, pyrrole C5-H) 14 (br s, 1H, OH enol), 15 (bs, 2H, OH acid). Anal. ($C_{20}H_{16}FNO_4$) C, H, N, F.

(*E*/*Z*)-2-Amino-4-(1-(4-fluorobenzyl)-4-phenyl-1*H*-pyrrol-3-yl)-4-oxobut-2-enoic Acid (7d).

Compound **6d** (340 mg, 0.9 mmol) was dissolved in anhydrous THF (4.5 mL) under argon atmosphere and cooled to 0 °C. To this was added dropwise a solution of 0.5 N KOH (2 mL, 1.0 mmol), and the mixture was allowed to stir at room temperature overnight. The reaction mixture was concentrated in vacuo and the residue partitioned between water and ethyl acetate. The aqueous layer was cooled on ice and acidified with 1 N HCl. After chilling at 4 °C for 2 h, the resulting precipitate was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum to yield the acid **7d** (250 mg). 76% yellow solid; 86–88 °C; toluene; IR ν 3000–2500 (OH acid and enol), 1700 (C=O acid) cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.95 (m, 2H, CH_2 E/Z form), 6.70 (s, 1H, butenoate C3-H), 6.8–7.43 (m, 11H, pyrrole C2-H, pyrrole C5-H, benzene H, and benzyl H), 14 (br s, 2H, acid and enol). Anal. ($C_{21}H_{17}FN_2O_3$) C, H, N, F.

1-(1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl)-3-hydroxy-3-(1*H*-1,2,4-triazol-3-yl)prop-2-en-1-one (7m).

20 (1.84 g, 1.8 mmol) was suspended in 12 mL of dioxane and treated with 4.4 mL of 1 N HCl. The reaction mixture was stirred at 70 °C for 4 h. After cooling, the mixture was poured into 4.4 mL of 1.5 N NaOH. The formed precipitate was filtered and portioned between ethyl acetate and 1 N NaOH. The water phase was separated, and acidification until pH 4 was obtained was done with concentrated HCl. The formed solid was filtered, washed with water, and recrystallized from absolute ethanol, obtaining 430 mg of pure **7m.** 64%; 188–189 °C; ethanol; IR ν 3200–2400 (NH, OH enol, and acid), 1712 (C=O ketone) cm^{-1}. ^1H NMR (DMSO-d_6) δ 4.50 (s, 1H, butanoate C3-H), 5.47 and 5.62 (s, 2H, CH_2 keto and enol form), 6.21 and 6.29 (t, 1H, pyrrole C4-H), 6.91 (s, 1H, butenoate C3-H), 7.06–7.16 (m, 6H, benzene H keto and enol form), 7.22 and 7.29 (m, 1H, pyrrole C3-H), 7.36 and 7.45 (s, 1H, pyrrole C2-H keto and enol form), 8.59 (bs, 1H, NH), 14 (br s, 1H, OH enol and acid). Anal. ($C_{16}H_{13}FN_4O_2$) C, H, N, F.

Biological Methods.

RT Expression and Purification.—The recombinant HIV-1 RT protein, whose coding gene was subcloned in the p6HRT_prot plasmid, was expressed in *E. coli* strain M15.[43,44] The bacteria cells were grown up to an OD_{600} of 0.8 and induced with 1.7 mM IPTG for 5 h. HIV-1 RT purification was carried out as described. Briefly, cell pellets were resuspended in lyses buffer (20 mM Hepes, pH 7.5, 0.5 M NaCl, 5 mM β-mercaptoethanol, 5 mM imidazole, 0.4 mg/mL lysozime), incubated on ice for 20 min, sonicated, and centrifuged at 30 000*g* for 1 h. The supernatant was applied to a His-binding resin column and washed

thoroughly with wash buffer (20 mM Hepes, pH 7.5, 0.3 M NaCl, 5 mM β-mercaptoethanol, 60 mM imidazole, 10% glycerol). The RT protein was eluted with elute buffer. The enzyme-containing fractions were pooled, dialyzed, and aliquots were stored at −80 °C.

HIV-1 RT RNase H Inhibition.—The RT-associated RNase H function 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.[43]

HIV-1 IN Inhibition.—HIV-1 IN gel-based assays were carried out as previously published.[45]

HIV-1 Replication Inhibition.—The antiviral activity of compounds was determined in a cell-based assay according to the procedure described previously[46] 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 to experimentation, 96-well plates were prepared to contain 10 000 cells per well in 100 μL of Dulbecco's modified Eagle medium (DMEM) complemented with 10% serum. On day 1, each drug was serial diluted directly on cells following a 3-fold dilution over 6 points, and each well was 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 2, cells were 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-methylumbelliferyl-β-D-galactopyranoside (4-MUG, Sigma). On day 3, sealed plates were read in a SpectraMax GEMINI-XS (Molecular Devices) with λ_{ex} = 360 nm and λ_{em} = 460 nm.

Cellular Toxicity.—Similar to the antiviral assays, plates were prepared with 10 000 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 (PerkinElmer) was added to each well. After 5 min at room temperature, the plates' luminescence was quantified using an EnVision multilabel reader (PerkinElmer) according to the manufacturer's instructions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

IN	integrase
RT	reverse transcriptase
RNase H	ribonuclease H

3′-P	3′-processing
ST	strand transfer
DKA	diketo acid
BTDBA	4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid
SI	selectivity index
TosMIC	toluene-4-sulfonylmethyl isocyanide
NIS	*N*-iodosuccinimide
GP	general procedure
DMEM	Dulbecco's modified Eagle medium

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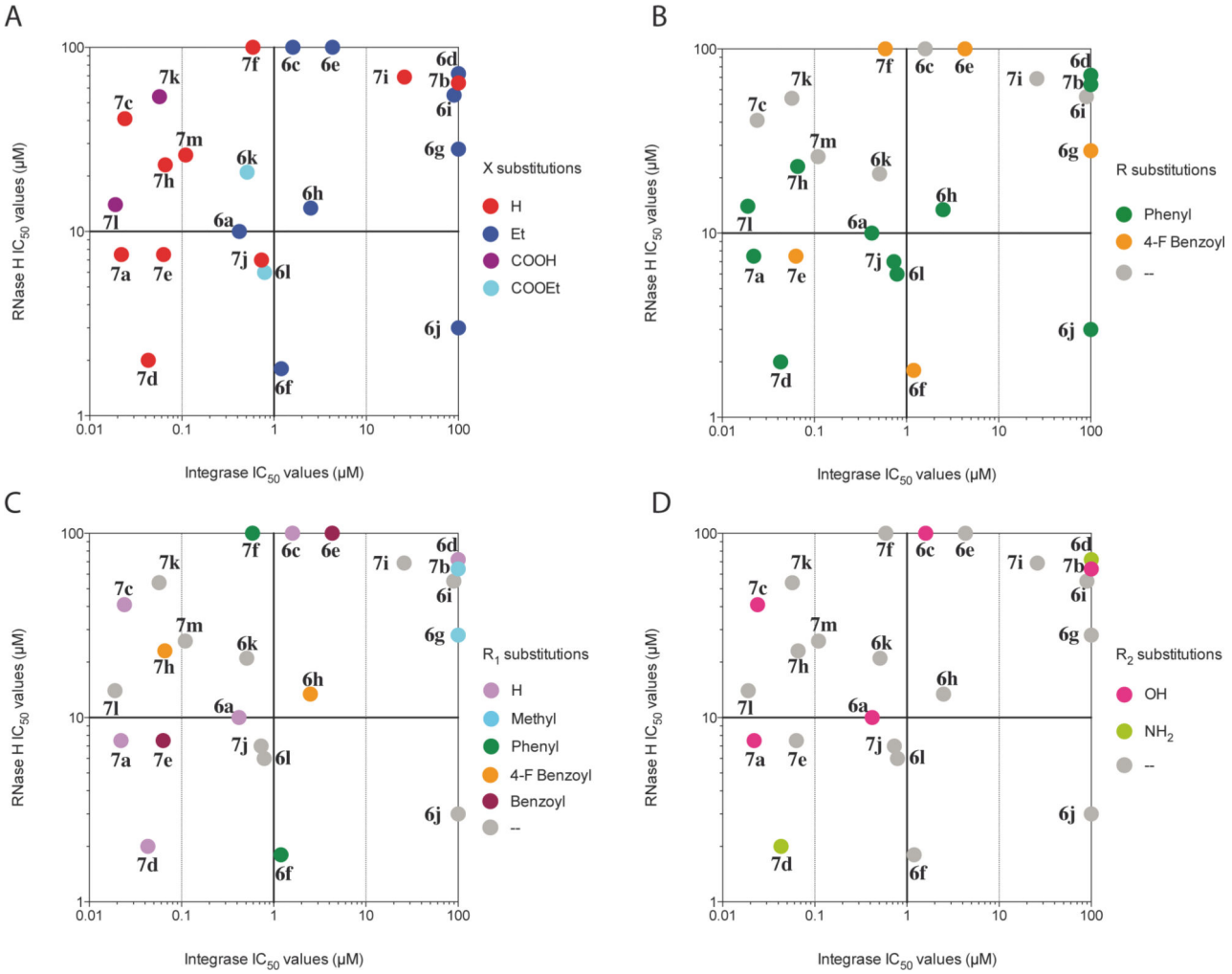
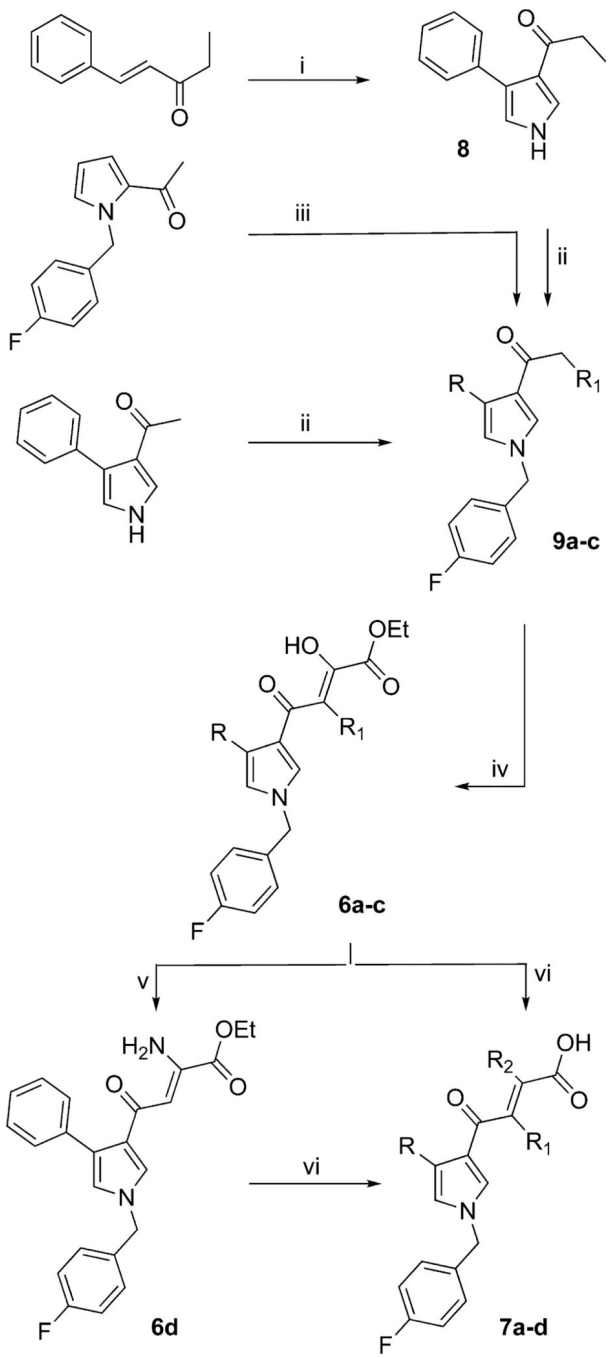


Figure 1.

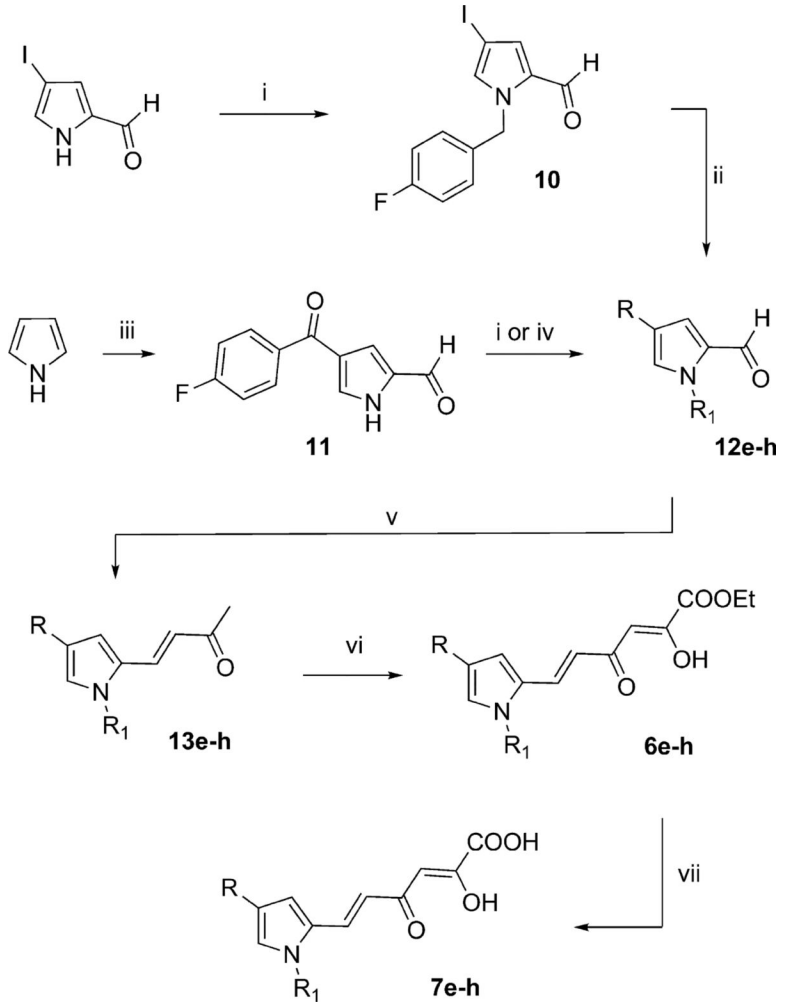
Scatter plot for the inhibition of RNase H and IN enzymes. (A) Compounds are categorized according to their acidic or ester function. (B) Compounds are categorized according to the nature of their R substitution. (C) Compounds are categorized according to the nature of their R_1 substitution. (D) Compounds are categorized according to the nature of their R_2 substitution. Compounds with one IC_{50} value missing such as **6d** 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.



Scheme 1.

Synthetic Route to Pyrrolyl DKAs 6a–d and 7a–d[a]

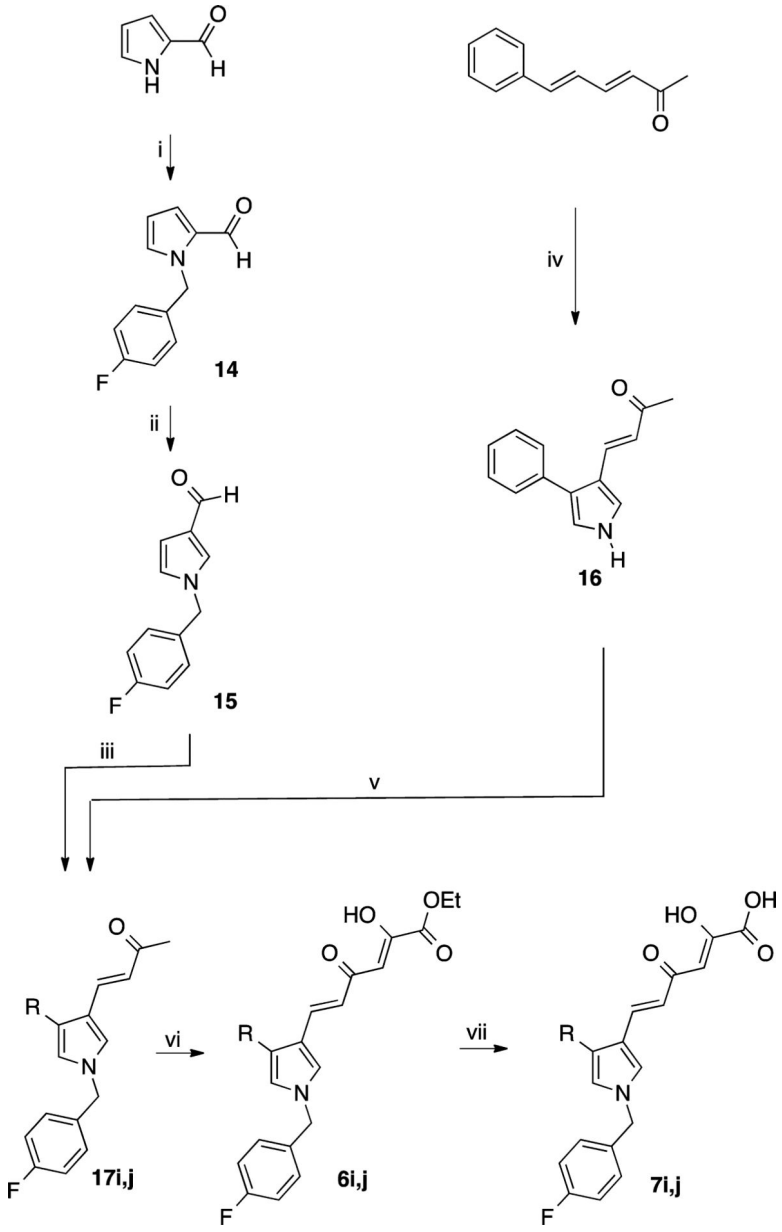
[a]Reagents and conditions: (i) TosMIC, NaH, Et_2O/DMSO, room temp, 1 h; (ii) trifluoroacetic acid, 80 °C, 24 h; (iii) 4-*F*-benzyl bromide, K_2CO_3, DMF, 100 °C, 24 h; (iv) diethyl oxalate, C_2H_5ONa, THF, room temp, 2 h; (v) CH_3COONH_4, benzene, glacial acetic acid, reflux, 20 h; (vi) 1 N NaOH, THF/CH_3OH, room temp, 1 h.



Scheme 2.

Synthetic Route to Pyrrolyl DKAs 6e–h and 7e–h[a]

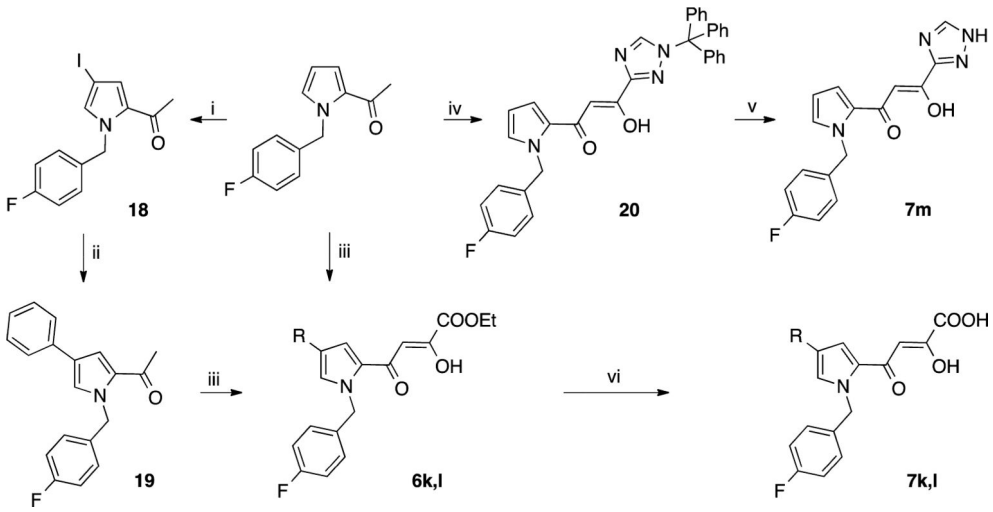
[a]Reagents and conditions: (i) alkylating agent, K_2CO_3, DMF, 100 °C, 24 h; (ii) phenylboronic acid, Cs_2CO_3, P(*t*-But)$_3$, $Pd_2(dba)_3$, dioxane, 80 °C, 24 h; (iii) (1) DMF, 1,2-dichloroethane dry, oxalyl chloride, 0 °C, 15 min, room temp, 15 min; (2) 4-*F*-benzoyl chloride, $AlCl_3$, room temp, 4 h; (iv) phenylboronic acid, copper(II) acetate anhydrous, pyridine/NMP 1:1, microwave at 60 W, 120 °C, 6 min; (v) acetone, 4 N NaOH, room temp, 24 h; (vi) diethyl oxalate, C_2H_5ONa, THF, room temp, 2 h; (vii) 1 N NaOH, THF/CH_3OH, room temp, 1 h.



Scheme 3.

Synthetic Route to Pyrrolyl DKAs 6i,j and 7i,j[a]

[a]Reagents and conditions: (i) 4-*F*-benzyl bromide, K_2CO_3, DMF, 100 °C, 24 h; (ii) trifluoroacetic acid, 80 °C, 24 h; (iii) acetone, 4 N NaOH, room temp, 24 h; (iv) Et_2O/DMSO, NaH, TosMIC, room temp, 1 h; (v) 4-*F*-benzyl bromide, K_2CO_3, DMF, 100 °C, 24 h; (vi) diethyl oxalate, C_2H_5ONa, THF, room temp, 2 h; (vii) 1 N NaOH, THF/CH_3OH, room temp, 1 h.



Scheme 4.

Synthetic Route to Pyrrolyl DKAs 6k,l and 7k,l[a]

[a]Reagents and conditions: (i) NIS, acetone, −78 °C, 96 h; (ii) phenylboronic acid, Cs_2CO_3, P(*t*-But)$_3$, $Pd_2(dba)_3$, dioxane, 80 °C, 24 h; (iii) diethyl oxalate, C_2H_5ONa, THF, room temp, 2 h; (iv) 1-trityl-1*H*-[1,2,4]triazole-3-carboxylic acid ethyl ester,[36] *n*-BuLi, THF, from −78 to 0 °C, 3.5 h; (v) 3 M HCl sol, 1,4-dioxane, 60 °C, 30 min; (vi) 1 N NaOH, THF/CH_3OH, room temp, 1 h.

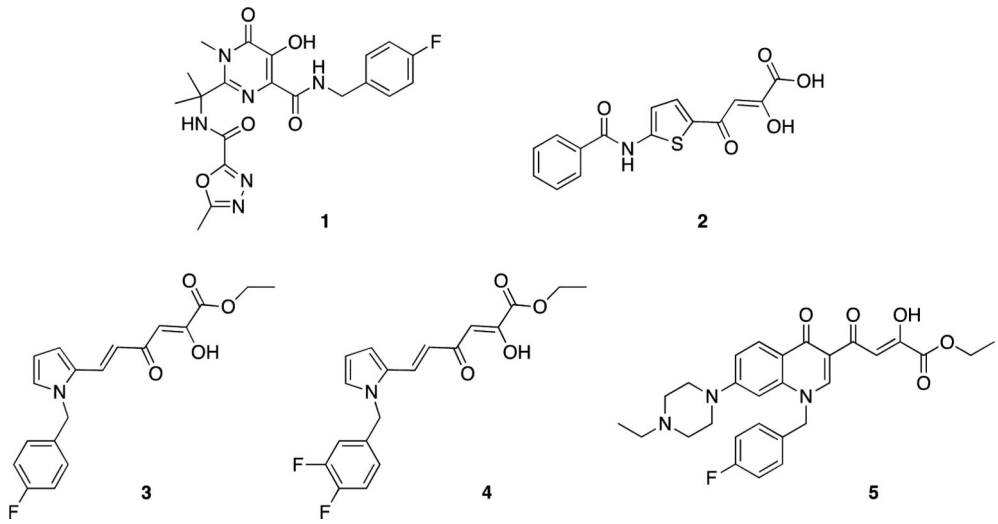


Chart 1.

Selective Inhibitor of IN Enzyme (1), First Described Dual IN/RNase H Inhibitor (2), and Recently Discovered Dual IN/RNase H Inhibitors 3–5

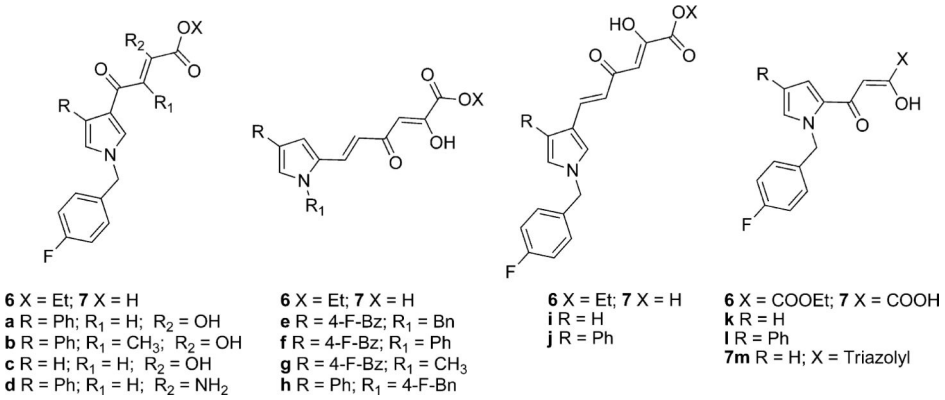


Chart 2.

Newly Designed Pyrrolyl DKA Derivatives 6a–l and 7a–m

Table 1.

Cytotoxicity, Enzymatic, and Antiviral Activities of Compounds 6a–l and 7a–m

					activity in enzyme assay, IC_{50} [a]		antiviral activity		
compd	**R**	**R_1**	**R_2**	**X**	**RH**[b]	**ST**[c]	**EC_{50}**[d]	**CC_{50}**[e]	**SI**[f]
6a	Ph	H	OH	Et	10	0.42	0.56	11	19.6
6b	Ph	Me	OH	Et					
6c	H	H	OH	Et	>100	1.6	0.90	>50	>55
6d	Ph	H	NH_2	Et	72	NT	19	>50	
6e	4-FBz[g]	Bn[h]		Et	>100	4.3	19	>50	
6f	4-FBz[g]	Ph		Et	1.8	1.2	20	>50	
6g	4-FBz[g]	Me		Et	28	>333	48	>50	
6h	Ph	4-FBn[h]		Et	13.4	2.5			
6i	H			Et	55	90	50	>50	
6j	Ph			Et	3.0	>21	4.3	26.9	6.3
6k	H			COOEt	21	0.51	1.2	33	27
6l	Ph			COOEt	6.0	0.79	0.70	3.9	6
7a	Ph	H	OH	H	7.5	0.022	0.66	>50	>75
7b	Ph	CH_3	OH	H	64	>111	>50	>50	
7c	H	H	OH	H	41	0.024	0.58	>50	>86
7d	Ph	H	NH_2	H	2.0	0.043	0.63	>50	>79
7e	4-FBz[g]	Bn[h]		H	7.5	0.063	>50		
7f	4-FBz[g]	Ph		H	100	0.59	>50		
7g	4-FBz[g]	Me		H	20				
7h	Ph	4-FBn[h]		H	23	0.066			
7i	H			H	69	26	>50	>50	
7j	Ph			H	7.0	0.73	17.2	>50	>2.9
7k[i]	H			COOH	54	0.057	1.0	28	28
7l	Ph			COOH	14	0.019	0.7	>50	>72

					activity in enzyme assay, IC_{50} [a]			antiviral activity		
compd	R	R_1	R_2	X	RH [b]	ST [c]	EC_{50} [d]	CC_{50} [e]	SI [f]
7m	H			Tr [j]	26	0.11	20.4	>50	
1						0.007	0.016	>250	>15000
2					3.2	1.9	>50		
3					8	98	<0.2	>50	>250
4					3	0.60	2	>50	>25
5					26.2	2.4	3.6	>50	>13.8

[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).

[e] Cytotoxic concentration 50% (μM).

[f] $SI = CC_{50}/EC_{50}$.

[g] Bz, benzoyl.

[h] Bn, benzyl.

[i] See also ref 33.

[j] Tr, triazolyl.