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Discovery of a Potent HIV Integrase Inhibitor that Leads to a Prodrug with Significant anti-HIV Activity

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Abstract

Worldwide research efforts in drug discovery involving HIV integrase have produced only one compound, raltegravir, that has been approved for clinical use in HIV/AIDS. As resistance, toxicity and drug-drug interactions are recurring issues with all classes of anti-HIV drugs, the discovery of novel integrase inhibitors remains a significant scientific challenge. We have designed a lead HIV-1 strand transfer (ST) inhibitor (IC_{50} 70 nM), strategically assembled on a pyridinone scaffold. A focused structure-activity investigation of this parent compound led to a significantly more potent ST inhibitor, **2** (IC_{50} 6 ± 3 nM). Compound **2** exhibits good stability in pooled human liver microsomes. It also displays a notably favorable profile with respect to key human cytochrome P450 (CYP) isozymes and human UDP glucuronosyl transferases (UGTs). The prodrug of inhibitor **2**, i.e., compound **10**, was found to possess remarkable anti-HIV-1 activity in cell culture (EC_{50} 9 ± 4 nM, CC_{50} 135 ± 7 μM, therapeutic index = 15,000).

Keywords

Integrase inhibitor; pyridinone; CYP/UGT profile; anti-HIV prodrug

The retroviral enzyme, HIV-1 integrase, which is encoded at the 3′-end of the *pol* gene of the human immunodeficiency virus (HIV), is essential for HIV replication and is a significant target for the discovery and development of anti-HIV therapeutic agents.[1–11] However, research efforts in the area of anti-HIV integrase inhibitors for the treatment of acquired immunodeficiency syndrome (AIDS) has resulted in only one compound, raltegravir (Isentress), that has been approved by the FDA for the clinical treatment for HIV-AIDS.[7,8] However, as resistance, toxicity and drug-drug interactions are recurring issues with all classes of anti-HIV drugs, the discovery of new, anti-HIV active integrase inhibitors remains a significant scientific challenge. HIV-1 integrase is a 32 kDa protein,[1,12,13] which catalyzes the incorporation of HIV DNA into host chromosomal DNA through a specifically defined sequence of reactions, which involves 3′-processing and a key strand transfer (ST) step.[1,3,12–15] Initiation of integration occurs in the cytoplasm, where a complex is formed between viral cDNA, previously produced by reverse transcription, and HIV integrase. Following this is site-specific endonucleatic cleavage of two nucleotides from each 3′-end of double-stranded viral DNA, which produces truncated viral DNA with terminal CAOH-3′ (3′-processing). The next step, ST, occurs in the nucleus and involves staggered nicking of

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SUPPORTING INFORMATION AVAILABLE

Antiviral assays, microsome stability assays, cytochrome P450 inhibition assays, UDP-glucuronosyltransferase substrate assays, synthesis of integrase inhibitor and its prodrug, analytical data for all compounds and HPLC purity trace of integrase inhibitor. This material is available free of charge *via* the internet at http://pubs.acs.org

chromosomal DNA and joining of each 3′-end of the recessed viral DNA to the 5′-ends of the host DNA, followed by repair/ligation. The ST step is carried out after transport of the processed, preintegration complex from the cytoplasm into the nucleus. Both 3′-processing and ST steps require divalent metal ion cofactors.

To explore whether a significantly anti-HIV active integrase inhibitor could be discovered that would also possess a favorable *in vitro* drug-drug interaction profile with respect to key cytochrome P450 (CYP) and UDP glucuronosyltransferase (UGT) isozymes, we carried out the design of such an inhibitor from a lead compound discovered in our laboratory. This lead compound was 4-(1,5-dibenzyl-1,2-dihydro-2-oxopyridin-3-yl)-2-hydroxy-4-oxobut-2-enoic acid (**1**, Figure 1), which was an inhibitor of the ST step of HIV-1 integrase (IC_{50} 70 nM). Using compound **1** as a starting point, we undertook lead optimization studies on **1**.[16]

In the discovery of lead compound **1**, it was established that the specific nature of the modified nucleobase scaffold (i.e., the pyridinone ring) and the nature of the substituents on the scaffold (the functional components as well as the hydrophobic benzyl groups) were critical for integrase inhibitory activity. For this reason, we focused our optimization studies on substituents on the hydrophobic phenyl groups of the pyridinone scaffold. In the subsequent study, we examined the effects of various substituents, e.g., methoxy, chloro, alkyls and mixed halo/alkyl and others on the phenyl rings and their effect on the enzymology involving ST step inhibition. There was considerable variation in the ST inhibitory activity for these compounds (IC_{50} <10 nM to >1500 nM). Fluoro substitution IC_{50} data, however, were more compelling. Among this entire group of fluorinated compounds, the difluoro, trifluoro and tetrafluoro substituted compounds all had ST inhibitory IC_{50s} falling in the range of <10 nM, showing significant improvement over lead compound **1**. Within this group of fluorinated compounds, the trifluoroaryl (*o*- and *o,p*) and tetrafluoroaryl (*o,p* and *o,p*) substituted analogs (involving both phenyl rings) were the most active in terms of the integrase IC_{50} and IC_{90} data (≤ 6 nM and <100 nM, respectively). While the detailed reason for the increase in inhibitory potency with appropriate fluorine substitution is not fully understood, hydrophobic and/or electrostatic interactions may contribute.[17–19] In the next level of lead optimization, we investigated the antiviral cell culture data for these compounds. The results are summarized in Table 1 and show that the anti-HIV-1 EC_{50} values were largely in the 1–3 μM range. However, two compounds emerged from these studies that exhibited anti-HIV EC_{50} values of 500 nM or less. They were 4-(5-(2,4-difluorobenzyl)-1-(2-fluoro-benzyl)-2-oxo-1,2-dihydro-pyridin-3-yl)-2-hydroxy-4-oxobut-2-enoic acid (**2**, Entry 56 Table 1) and 4-(1,5-bis(2,4-difluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-4-hydroxy-2-oxobut-3-enoic acid (Entry 11, Table 1). Their ST inhibition IC_{50} data were 6 ±3 nM and 5.5 ±1.5 nM, respectively. The eventual selection of compound **2** over entry 11 as the key compound to move forward is discussed in the prodrug section below.

A highly-efficient synthesis of compound **2** (Scheme 1) was developed in our laboratory. Only 7 steps (aromatic nucleophilic addition, demethylation/deoxygenation, radical bromination, benzylation, palladium-catalyzed cross-coupling, Claisen condensation and acid-catalyzed hydrolysis), [20–26] were required for the total synthesis of **2** from commercially available 5-bromo-2-methoxypyridine (**3**). The overall yield from **3** was 37 %.

Stability studies of compound **2** in pooled human liver microsomes were carried out by pre-incubation, initiation with NADPH, incubation at 37 °C, quenching of samples at various time intervals with cold acetonitrile, centrifugation to remove precipitated proteins and finally HPLC analysis that utilized UV detection.[27–29] These studies revealed that integrase inhibitor **2** was relatively stable in human liver microsomes, exhibiting an *in vitro* half-life of > 3 h, as evidenced from HPLC data, which showed that 80% of compound **2** remained

after the 3 h incubation in microsomes (Figure 2). The key metabolite, which was slowly produced, was identified by HPLC and HRMS data to be the product of the retro-Claisen cleavage of the diketo group of **2** to produce the acetyl pyridinone **8**.

The *in vitro* drug interaction profile involving key cytochrome P450 (CYP) isozymes[28–30] and appropriate substrates in pooled human liver microsomes with varying concentrations of **2**, followed by kinetic analysis (Table 2), showed that compound **2** was not an inhibitor of CYP3A4 and CYP2D6 isozymes and was a very weak inhibitor of the CYP2C8 isozyme (Table 1). These key isozymes account for a total of over 80% of drugs that are metabolized by different CYP isoforms. In addition, compound **2** did not exhibit any activation of these CYP isozymes. Thus, our studies suggest that this integrase-based, anti-HIV compound is anticipated to have a favorable drug interaction profile with respect to key CYP isozymes.

Because isozymes of UGT also play an important role in determining drug-drug interactions, we investigated the substrate activity of **2** towards key human UGTs.[27,31] Compound **2** was not a substrate for the following key UGT isozymes: 1A1, 1A4, 1A6, 1A9 and 2B7. In comparison, the major mechanism for rapid clearance of raltegravir in humans is through UGT 1A1-mediated glucuronidation.[27] The integrase inhibitor, S/GSK 1349572, is also a substrate for UGT 1A1 and its primary route of metabolism and subsequent clearance is glucuronidation.[10]

Antiviral data in cell culture of compound **2** revealed a significant disconnect of almost two or more orders of magnitude between the anti-HIV-1 activity (EC_{50} 500 nM, MAGI cells) and the ST inhibition data for **2** (IC_{50} 6 nM). For HIV-1 integrase inhibitors, there is normally a reasonably strong correlation between ST IC_{50} data and cell culture EC_{50} data.[3,5] Because the disconnect between the IC_{50} and EC_{50} data for compound **2** and also other compounds, including entry 11 (Table 1), appeared to be a problem associated with the cellular permeability of the inhibitors, we examined prodrugs of these compounds. The isopropyl ester prodrug, **10** (Figure 3), was easily synthesized from compound **2** through acid-catalyzed esterification with 2-propanol. The cLog P values for compounds **2** and the isopropyl ester **10** are 2.38 and 4.24, respectively, suggesting that compound **10** is significantly less polar than compound **2** and thus would be expected to be more cellularly permeable. The antiviral data confirmed this, as prodrug **10** exhibited remarkable anti-HIV-1 activity (EC_{50} = 9 ± 4 nM, MAGI cells). This was the best activity achieved of all of the prodrugs studied in this work (EC_{50s} ranged from 9 nM to 46 nM for the isopropyl ester prodrugs). The isopropyl ester prodrug of entry 11 (Table 1) was significantly less active than compound **10**, exhibiting an EC_{50} of 46 ± 18 nM (MAGI cells). The overall performance of the assay was validated by the MOI-sensitive positive control compound, raltegravir, which exhibited the expected level of antiviral activity (EC_{50} < 6 nM).[7] Cell viability data for **10** showed only low toxicity at higher test concentrations (CC_{50} = 135 ± 7 μM, CC_{90} >200 μM), although a CC_{90} was not reached at the highest test concentration (200 μM). It is of relevance to mention that the EC_{50} and EC_{90} data for **10** correlate exceptionally well with the ST inhibition IC_{50} and IC_{90} data for **2** (6 nM and 97 nM, respectively). The therapeutic (selectivity) index TI (CC_{50}/EC_{50}) for **10** was 15,000. Finally, isopropyl ester, **10**, was a poor inhibitor of HIV integrase in enzymatic studies (IC_{50} > 475 nM), suggesting that the anti-HIV activity of **10** was most likely the result of its hydrolysis in cell culture to produce the cellularly active anti-HIV compound **2**. Consistent with this conclusion, was our observation that CYP and UGT studies on compound **10** were precluded by its rapid hydrolysis in human liver microsomes to produce compound **2** (100 % conversion in 15 minutes).

While a number of structurally diverse compounds have been reported to be inhibitors of HIV integrase, the data of two of these compounds (Figure 4), that have received

considerable attention and that have a relationship, albeit peripheral, to the compounds described herein, are worthy of mention. Both compounds are ST inhibitors [IC_{50} 20 nM (S-1360) and 170 nM (L-731,988)]. The *in vitro* anti-HIV data for the β-diketo triazole, S-1360,[32] showed an EC_{50} of 140 nM and a CC_{50} of 110 μM (PBMC) resulting in a TI of 790. Compound, L-731,988, is somewhat less active (EC_{50} 1 μM in MT-4 cells).[33] The CC_{50} value was not given.

In summary, our search for new integrase-based, anti-HIV compounds led to the discovery of a highly potent ST inhibitor of HIV-1 integrase, **2** (IC_{50} 6 nM). This compound was relatively stable in pooled human liver microsomes (80% of compound remained after incubation for 3 h). It displayed a favorable interaction profile with respect to key human CYP isozymes as it was not an inhibitor or activator of these isozymes. Also of significance was the observation that inhibitor **2** was not a substrate of important human UGTs. A prodrug of **2**, i.e., compound **10**, exhibited remarkable anti-HIV-1 activity in cell culture (EC_{50} = 9 nM, CC_{50} = 135 μM). The therapeutic or selectivity index of prodrug **10**, which was 15,000, was also a notable finding. Further biological studies are in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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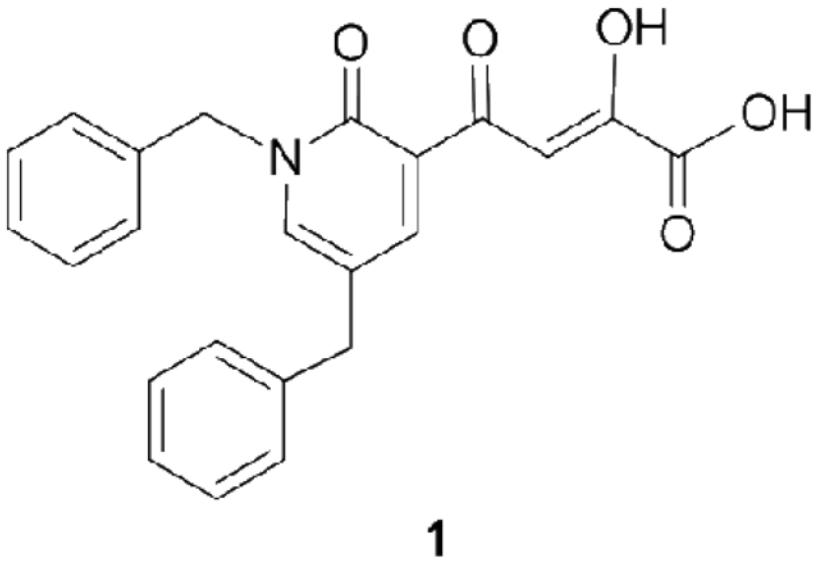


Figure 1.
Structure of lead compound **1**

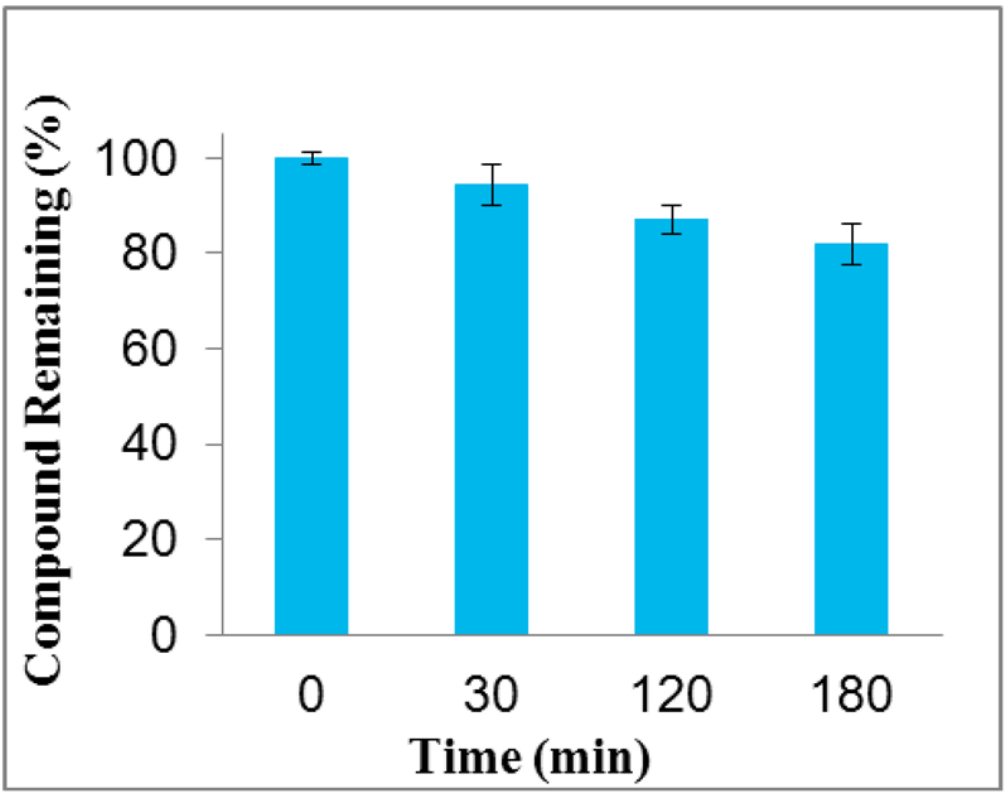
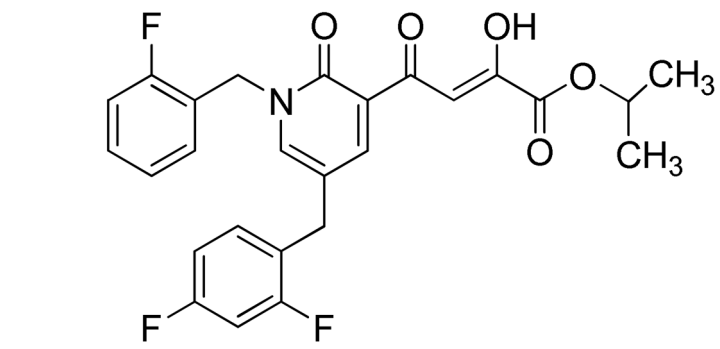
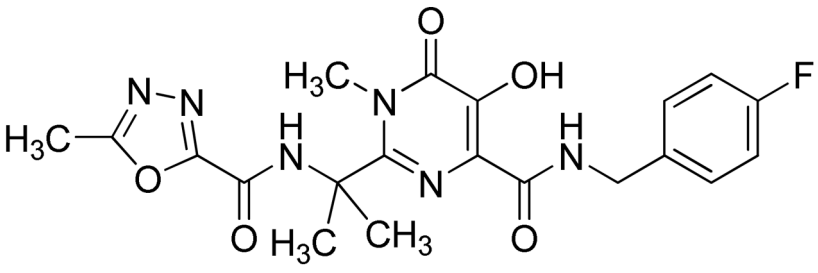


Figure 2.
Stability of **2** in pooled human liver microsomes monitored by HPLC/UV.

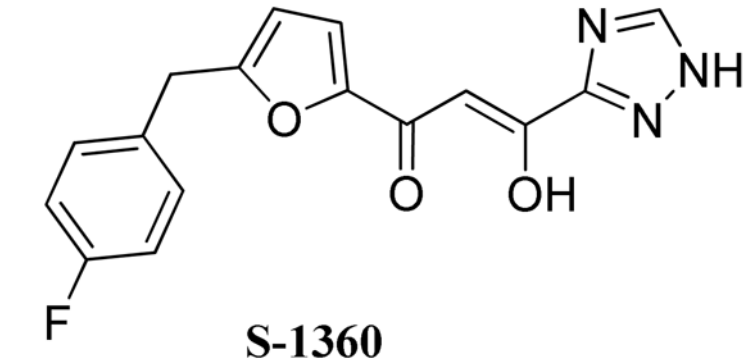


10

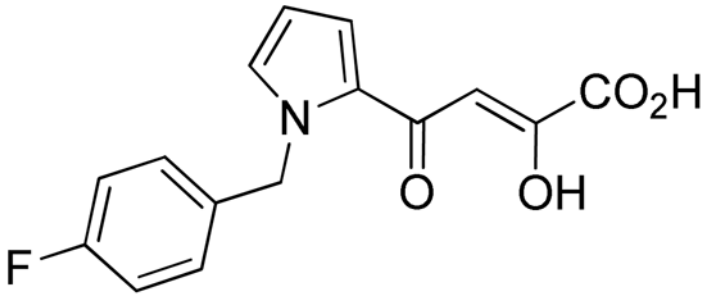


raltegravir

Figure 3.
Structures of prodrug **10** and raltegravir.

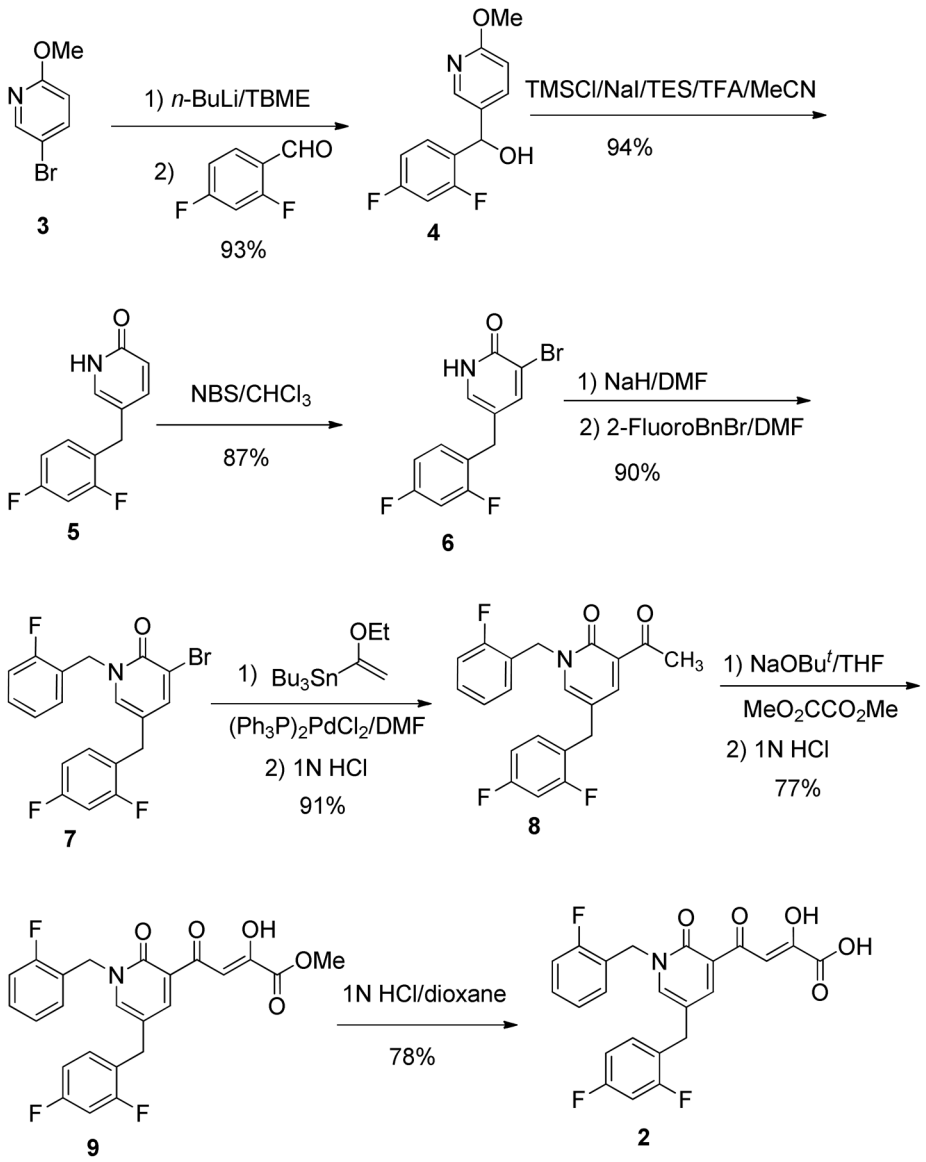


S-1360



L-731,988

Figure 4.
Two well-known β-diketo compounds.



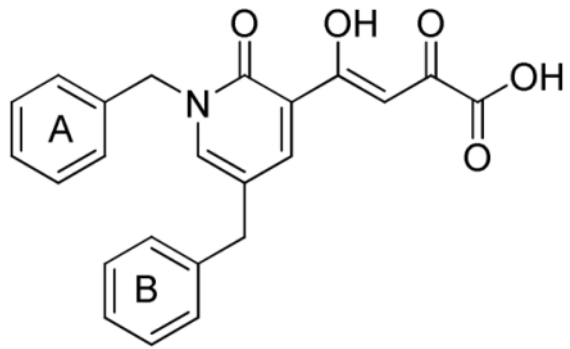
Scheme 1.

Methodology developed for the synthesis of integrase inhibitor **2**.

Abbreviations: (i) *tert*-butylmethyl ether (TBME); (ii) trimethylsilyl chloride (TMSCl), triethylsilane (TES), trifluoroacetic acid (TFA); (iii) N-bromosuccinimide (NBS); (iv-v) dimethylformamide (DMF); (vi) tetrahydrofuran (THF).

Table 1

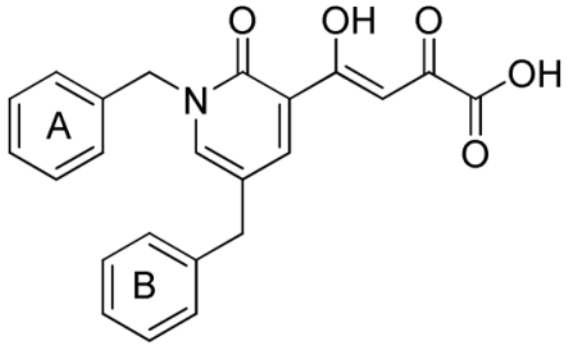
In Vitro Anti-HIV Data for Analogs of Compound **1**



Entry	Aryl Ring A			Aryl Ring B			EC_{50} (μM)[a,b]
	o	*m*	*p*	*o*	*m*	*p*	
1	H	H	H	H	H	H	2.1
2	F	H	H	H	H	H	0.9
3	H	H	F	H	H	H	1.0
4	F	H	F	H	H	H	0.6
5	F	H	H	F	H	H	0.7
6	H	H	OMe	H	H	H	1.8
7	H	H	F	H	H	F	0.8
8	H	H	Me	H	H	F	1.6
9	H	Cl	F	H	H	H	0.8
10	H	H	OMe	H	H	OMe	>5.0
11	F	H	F	F	H	F	0.3
12	H	F	H	H	H	H	2.0
13	H	Cl	H	H	Cl	H	1.1
14	H	Cl	F	H	Cl	H	1.2
15	F	H	H	H	H	F	0.7
16	H	Cl	F	H	Cl	F	2.4
17	H	H	Me	H	Cl	F	>5.0

Entry	Aryl Ring A			Aryl Ring B			EC_{50} (μM)[a,b]
	o	*m*	*p*	*o*	*m*	*p*	
18	H	H	Me	H	Cl	H	2.5
19	H	H	Me	F	H	H	0.7
20	H	H	F	H	Cl	F	>5.0
21	F	Cl	F	H	H	H	0.6
22	H	H	F	H	Cl	H	1.4
23	2,6-diF	H	H	H	H	F	1.4
24	H	H	F	F	H	H	0.8
25	F	Cl	H	F	Cl	H	2.4
26	H	H	Cl	H	Cl	H	2.5
27	H	Cl	H	H	Cl	H	1.4
28	F	Cl	H	H	Me	H	1.4
29	F	H	F	F	H	H	0.6
30	H	Me	H	H	Cl	F	2.2
31	F	Cl	H	H	H	H	0.7
32	F	5-Cl	H	H	H	H	0.9
33	F	Cl	H	H	Cl	H	1.4
34	F	H	F	H	Cl	F	1.4
35	H	Cl	H	H	H	F	1.2
36	H	Cl	H	F	H	H	0.7

Entry	Aryl Ring A			Aryl Ring B			EC_{50} (μM)[a,b]
	o	*m*	*p*	*o*	*m*	*p*	
37	2,6-di-F	H	H	H	Cl	F	5.1
38	2,6-di-F	H	H	H	H	H	1.5
39	H	H	H	F	H	F	0.8
40	F	H	H	H	Cl	F	2.2
41	H	F	H	F	H	H	1.9
42	F	H	H	H	Cl	H	1.9
43	F	F	H	H	H	H	0.9
44	H	Me	H	F	H	H	0.9
45	F	F	H	H	H	F	0.7
46	H	H	CN	F	H	H	1.1
47	F	F	H	F	H	H	0.8
48	H	F	H	H	H	F	1.0
49	F	H	F	Cl	H	H	0.8
50	H	Cl	H	F	Cl	H	1.5
51	H	H	F	F	H	F	0.6
52	H	Me	H	F	H	F	0.7
53	H	H	H	H	COOH	H	>5.0
54	H	H	H	Me	H	F	1.3
55	H	H	F	Me	H	F	1.0



Entry	Aryl Ring A			Aryl Ring B			EC_{50} (μM)[a,b]
	o	*m*	*p*	*o*	*m*	*p*	
56	F	H	H	F	H	F	0.5
57	F	H	H	Me	H	F	1.3

[a] EC_{50} values are the average of three determinations. Standard deviations for the EC_{50} are within 31% of the average.

[b] EC_{50} = concentration for 50% inhibition of the replication of HIV-1.

Table 2

IC_{50} Data for Inhibition of Key Cytochrome P450 Isozymes.

CYP450 Isozyme	Substrate/Stock Solution	Conc. (μM)	Protein (mg/mL)	Incubation (min)	IC_{50} Data (μM)
CYP3A4	Testosterone (50 mM)	100	0.3	30	>200 μM
CYP3A4	Triazolam (50 mM)	200	0.4	30	>200 μM
CYP2D6	Dextromethorphan (50 mM)	200	2.0	60	>200 μM
CYP2C8	Amodiaquine (5 mM)	200	0.4	30	>65 μM