Bicyclic 1-Hydroxy-2-oxo-1,2-dihydropyridine-3-carboxamide-Containing HIV-1 Integrase Inhibitors Having High Antiviral Potency against Cells Harboring Raltegravir-Resistant Integrase Mutants

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ABSTRACT: Integrase (IN) inhibitors are the newest class of antiretroviral agents developed for the treatment of HIV-1 infections. Merck’s Raltegravir (RAL) (October 2007) and Gilead’s Elvitegravir (EVG) (August 2012), which act as IN strand transfer inhibitors (INSTIs), were the first anti-IN drugs to be approved by the FDA. However, the virus develops resistance to both RAL and EVG, and there is extensive cross-resistance to these two drugs. New “2nd-generation” INSTIs are needed that will have greater efficacy against RAL- and EVG-resistant strains of IN. The FDA has recently approved the second generation INSTI, GSK’s Dolutegravir (DTG) (August 2013). Our current article describes the design, synthesis, and evaluation of a series of 1,8-dihydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides, 1,4-dihydroxy-2-oxo-1,2-dihydro-1,8-naphthylidine-3-carboxamides, and 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthylidine-3-carboxamides. This resulted in the identification of noncytotoxic inhibitors that exhibited single digit nanomolar EC_{50} values against HIV-1 vectors harboring wild-type IN in cell-based assays. Importantly, some of these new inhibitors retain greater antiviral efficacy compared to that of RAL when tested against a panel of IN mutants that included Y143R, N155H, G140S/Q148H, G118R, and E138K/Q148K.

INTRODUCTION

After 30 years of intensive research, approximately 30 drugs have been approved for the treatment of acquired immunodeficiency syndrome (AIDS).1,2 Of these, integrase (IN) inhibitors are the newest drug class3 with Merck’s Raltegravir (RAL, 1) (October 2007) and Gilead’s Elvitegravir (EVG, 2) (August 2012)4 being the first IN inhibitors to be approved by the FDA (Figure 1). These agents selectively block the strand transfer step (ST) of the integration reaction as compared with the 3′-processing step (3′-P). For this reason, these drugs are called IN strand transfer inhibitors (INSTIs).5 INSTIs, including RAL and EVG, share key structural features. These include a coplanar arrangement of three heteroatoms, whose function is to chelate the two catalytically important Mg^{2+} ions at the IN active site, which are held in place by a conserved DDE motif (D64-D116, and E152 in HIV-1 IN).6 Additionally, a halobenzyl ring is present that interacts with the penultimate cytosine base near the 3′-end of the viral DNA. This displaces the dA at the very 3′-end of the viral DNA and prevents the insertion of the viral DNA into the host genome.7 Treatment with RAL and EVG can lead to the development of resistance, and there is extensive shared cross-resistance.8–10 Therefore, “2nd-generation” INSTIs are being developed that have greater efficacies against RAL and EVG-resistant strains of IN.11 GSK’s Dolutegravir (DTG, 3, Figure 1) is a second-generation INSTI that has recently received FDA approval for the treatment of AIDS.1,12,13 However, the finding that DTG, like all anti-HIV drugs, selects for resistant viruses,12 emphasizes the continuing need to develop INSTIs that can effectively inhibit HIV strains that carry the common/extant resistance mutations.

An attractive property of DTG is its ability to maintain high potencies against mutant strains of HIV that are resistant to RAL and EVG.14 Although DTG contains key structural features that are found in other INSTIs (outlined above and highlighted in Figure 1), it differs in having its halobenzyl group appended via an amide carbonyl that is proximal to but not part of the triad of metal chelating heteroatoms. This is in contrast to RAL and several other INSTIs, in which the halobenzyl amide carbonyl serves as one of the metal-chelating elements in the heteroatom triad. As a result, the halobenzyl linker moiety of DTG has greater flexibility than RAL structurally related INSTIs. This flexibility may contribute to DTG’s ability to bind tightly to both wild-type (WT) and mutant IN–DNA complexes.15–18 While differing in their halobenzyl linker arrangements, both RAL and DTG have a hydroxyl group as the central component of their metal-chelating triad of Mg^{2+} complexes.
heteroatoms. We noted that hydroxyl amides can function as high affinity metal-chelating groups. In fact, hydroxylamide-containing INSTIs (for example 4) have been reported, which combine a centrally located hydroxylamide metal chelating functionality with flexible halobenzylamide group like the one in DTG. Alternatively, inhibitors such as 5 employ a hydroxylamide group as the terminal member of the metal-chelating triad, and the halobenzyl group is appended through a 1H-pyrrole ring (Figure 1). Our current report describes new INSTIs (6–8, Figure 1) that have a hydroxylamide group as the central component of a triad of metal-chelating heteroatoms, which originate from within bicyclic frameworks. Importantly, the halobenzylamide moieties of many of these inhibitors are appended in a fashion that may not require participation of their amide carbonyls in metal chelation. These inhibitors exhibit high potencies against viral vectors that carry WT IN and the major RAL-resistant IN mutants.

**RESULTS AND DISCUSSION**

**Inhibitor Design.** In designing the current series of inhibitors, we examined two classes of bicyclic platforms that differed in their presentation of the “left” terminal member of the metal-chelating heteroatom triad. Both of these platforms utilized a 1-hydroxyxpyridin-2(1H)-one moiety as the central and “right” members of the heteroatom triad. However, one class of inhibitor utilized a phenolic hydroxyl as the “left” terminal metal-chelating heteroatom [1,8-dihydroxyquinolin-2(1H)-ones 6], while the second class employed a ring-embedded nitrogen to give 1-hydroxy-1,8-naphthyridine-2(1H)-ones (7). The latter class of inhibitors was also varied by the introduction of a 4-hydroxyl substituent [1,4-dihydroxy-1,8-naphthyridine-2(1H)-ones 8] (Figure 1). Three different halogen-substituted benzylcides were examined at the 3-position of the “right” 1-hydroxyxpyridin-2(1H)-one ring because the nature and pattern of halogen phenyl substitution is known to significantly affect the IN inhibitory potency of INSTIs.

![Figure 1. Structures of HIV-1 integrase inhibitors discussed in the text. Mg²⁺-chelating heteroatoms are shown in red with the halogen-substituted aromatic functionality shown in blue. The amide linkers are shown in magenta.](image)

**Synthesis.** Modification of a previously reported low-yield Knoevenagel condensation of 3-nitrobenzaldehyde 9a with diethyl malonate under similar conditions but without the inclusion of benzene gave the corresponding diethyl 2-(3-methoxy-2-nitrobenzylidene)malonate 10b in 90% yield. Initial hydrogenation of 10a (0.15 equiv of platinum(IV) oxide) using previously reported conditions that did not contain DMSO, provided a very low yield of the desired methyl 1-hydroxy-2-oxo,1,2-dihydroquinoline-3-carboxylate 11a, accompanied by significant reduction of the C3–C4 double bond and the N-hydroxyl group. However, when the reduction was performed on 10b with the inclusion of DMSO (1.6 equiv), the desired ethyl 1-hydroxy-8-methoxy-2-oxo,1,2-dihydroquinoline-3-carboxylate 11b was obtained in 82% yield. In choosing the composition and pattern of halogen substitution on the benzyl amide group, we selected 3′-chloro-4′-fluorobenzyl 13a and 3′,4′-difluorobenzyl 13b, respectively, based on our previous findings that these can provide improved IN inhibitory potencies. In addition, we prepared the 2′,4′-difluorobenzyl-substituted pattern 12c and 13c found in DTG. Demethylation of 13a-c with boron tribromide gave the desired final products 1,8-dihydroxy-2-oxo,1,2-dihydroquinoline-3-carboxamides, 6a-c (Scheme 1).
μ the case of the 3-in vitro tested, 3-chloro-4-(Scheme 1). These results are consistent with our previous compounds to inhibit the 3-Compounds were evaluated in an IN biochemical assay using dine-3-carboxamides (over-reduced 4-dihydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides (7), which lack a substituent at the corresponding position. Accordingly, in a fashion similar to reported methodology, treatment of commercially available methyl 2-fluoronicotinate 14 with benzoxylamine in DMSO followed by acylation of the resulting methyl 2-((benzoxyl)-amino)nicotinate with methyl 3-chloro-3-oxopropanoate gave the intermediate methyl 2-(N-(benzoxyl)-3-methoxy-3-oxopropanamido)nicotinate 15 (Scheme 2). This was treated with sodium methoxide in methanol to yield the cyclized product, methyl 1-((benzoxyl)-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate 16 (66% yield for three steps from 14) (Scheme 2). Removal of the 4-hydroxy group was achieved by conversion of 16 to its triflate 17 followed by reduction, and the resulting methyl 1-((benzoxyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate 18 was reacted individually with neat 3-chloro-4-fluorobenzylamine (a), 3,4-difluorobenzylamine (b) and 2,4-difluorobenzylamine (c) to provide the corresponding amides 19a–c (Scheme 2). Finally, hydrolytically deprotection of the N-hydroxyl group gave the desired 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides (7a–c) (Scheme 2). Alternatively, debenzylation of 16 followed by treatment with halobenzenamines (a, b, and c) in DMF afforded the desired 1,4-dihydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides 8a–c along with over-reduced 4-dihydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides (20a–c) (Scheme 2).

Evaluating in vitro IN Biochemical Assays.
Table 2. Inhibitory Potencies of 1-Hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides 7(a−c) Using an in Vitro IN Assay

![Chemical structure](image)

| no. | Z          | IC50 values (μM) | 3′-processing | strand transfer |
|-----|------------|-----------------|---------------|----------------|
| 7a  | 3′-Cl-4′-F | 9.4 ± 1.5       | 0.014 ± 0.003 |
| 7b  | 3′, 4′-dif  | 13.6 ± 1.8      | 0.017 ± 0.003 |
| 7c  | 2′, 4′-dif  | 6.1 ± 0.8       | 0.041 ± 0.012 |

RAL- and EVG-resistant strains of mutant IN,15−17 we inserted a hydroxyl group at the 4-position of the 1-hydroxy-1,8-naphthyridine-2(1H)-one ring system to ask whether this could potentially influence the inhibitory profiles by hydrogen bonding with the 3-carboxamide carbonyl. However, this modification resulted in a slight loss of ST inhibitory potencies (8a−c, Table 3). We also showed that removing the hydroxylamide group resulted in a more than two-orders of magnitude loss of ST inhibitory potency (20a−c, Table 3). This is consistent with the important role this hydroxyl may play as the central component of the metal-chelating triad.

Table 3. Inhibitory Potencies of 1,4-Dihydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides 8(a−c) and 4-Dihydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides 20(a−c) Using an in Vitro IN Assay

![Chemical structure](image)

| no. | Y  | Z          | IC50 values (μM) | 3′-processing | strand transfer |
|-----|----|------------|-----------------|---------------|----------------|
| 8a  | OH | 3′-Cl-4′-F | 4.7 ± 0.7       | 0.027 ± 0.006 |
| 8b  | OH | 3′, 4′-dif | 3.8 ± 0.6       | 0.040 ± 0.010 |
| 8c  | OH | 2′, 4′-dif | 1.2 ± 0.2       | 0.055 ± 0.008 |
| 20a | H  | 3′-Cl-4′-F | 125 ± 15        | 7.8 ± 1.1     |
| 20b | H  | 3′, 4′-dif | 93 ± 8          | 8.1 ± 1.8     |
| 20c | H  | 2′, 4′-dif | >333            | 7.1 ± 1.8     |

Evaluation of the Compounds in Cell-Based Antiviral Assays. A primary objective in developing second-generation IN inhibitors is to overcome mutations that are associated with resistance to first-generation inhibitors, such as RAL. To determine the abilities of our current inhibitors to retain efficacy against resistant variants, we evaluated the antiviral potencies in cells infected with one-round HIV-1 vectors that carry mutations that cause resistance to RAL (Table 4).10,34,35 In these assays, RAL (1) exhibited an EC50 value (effective concentration resulting in 50% reduction of luciferase reporter signal) of 4 nM in cells infected with virus containing WT IN. Cells infected with viruses containing either the Y143R or the N155H mutant forms of IN, showed significantly elevated EC50 values (162 nM and 154 nM, respectively). An even greater loss of efficacy (EC50 = 1900 nM) was observed with viruses containing the G140S/Q148H double mutant form of IN (Table 4). In similar assays, treatment of WT-infected cells with inhibitors 7a−c and 8a−c gave EC50 values in the low nanomolar range, with analogues 7c and 8c having the 2′, 4′-dif substituted benzy group (EC50 values of 5 nM and 6 nM, respectively) showing greater efficacy by several fold relative to analogues having either 3′-Cl-4′-F (7a and 8a) or 3′, 4′-dif (7b and 8b) substituted benzy groups. This is in contrast to in vitro assays, where analogues bearing the 2′, 4′-dif-substituted benzy group were slightly less effective ST inhibition (Tables 2 and 3).

An important feature of the new inhibitors is their ability to maintain high efficacies against RAL-resistant strains. For example, compound 7c exhibits single digit nanomolar antiviral potencies against both WT and the Y143R mutant, while showing a lower potency against the G140S/Q148H mutant (EC50 = 438 nM). However, it is much improved when compared to RAL (1) (EC50 = 1900 nM). Compound 8c also retains good efficacy against the Y143R mutant (EC50 = 11 nM) as well as both the N155H mutant (EC50 = 31 nM; RAL has EC50 = 154 nM) and the G140S/Q148H mutant (EC50 = 308 nM) (Table 4).

While showing nanomolar EC50 values against cells infected with HIV-1 vectors carrying WT and efficacy against some RAL-resistant mutants, analogues 7a−c exhibited very low cytotoxicity, with CC50 values (cytotoxic concentrations were measured as the level of the compound that reduced cellular ATP levels by 50%) greater than 250 μM. In particular, for the 2′, 4′-difluorobenzyl amide 7c with an EC50 value of 5.1 nM against WT, this gives a selectivity index (SI = CC50/EC50) greater than 49,000 (Table 4). Analogues 8a−c showed CC50 values greater than 6,579 nM.

Table 4. Antiviral Potencies of Compounds 7(a−c) and 8(a−c) in Cells Infected with HIV-1 Vectors Containing Wild-Type (WT) or Mutant IN

![Chemical structure](image)

| no. | CC50 (μM)a | EC50 (nM) WTb | Y143R | N155H | G140S/Q148H | SI d | EC50 (nM, IN mutants) |
|-----|-------------|---------------|-------|-------|-------------|------|-----------------------|
| 1   | >100        | 4 ± 2         | 162 ± 16 | 154 ± 33 | 1900 ± 300 | >25,000 |
| 7a  | >250        | 38 ± 15       | 34 ± 6 | 90 ± 6 | N/A* | >6,579 |
| 7b  | >250        | 62 ± 14       | 40 ± 13 | 2200 ± 61 | N/A’ | >4,032 |
| 7c  | >250        | 5.1 ± 1.9     | 4.9 ± 0.8 | 134 ± 23 | 438 ± 121 | >49,020 |
| 8a  | 102 ± 18    | 35 ± 12       | 54 ± 9 | 148 ± 8 | 489 ± 62 | 2,914 |
| 8b  | 192 ± 19    | 20 ± 6        | 45 ± 12 | 189 ± 70 | 507 ± 125 | 9,600 |
| 8c  | 137 ± 20    | 6.2 ± 2.9     | 11 ± 2 | 31 ± 8 | 308 ± 125 | 22,097 |

aCytotoxic concentration resulting in 50% reduction in the level of ATP in human osteosarcoma (HOS) cells. bValues obtained from cells infected with the lentiviral vector harboring WT IN. cCells were infected with viral vectors carrying IN mutations and indicated values in EC50. dSelectivity index calculated as the ratio of CC50 to EC50. eNot available.
Table 5. Fold Change (FC) of Amides 7c and 8c Compared with That of RAL (1) in Cells Infected with HIV-1 Constructs Carrying WT or Mutant IN

| No. | EC50 (nM) WT  | Y143R | N155H | G140S/Q148H | G118R | E138K/Q148K |
|-----|-------------|-------|-------|-------------|-------|-------------|
| 1   | 4 ± 2       | 41x   | 38x   | 475x        | 9x    | 375x        |
| 7c  | 5.1 ± 1.9   | 1x    | 26x   | 86x         | N/A   | N/A         |
| 8c  | 6.2 ± 2.9   | 2x    | 5x    | 50x         | 6x    | 32x         |

Values obtained from cells infected with a lentiviral vector harboring WT IN. Cells were infected with viral constructs carrying IN mutations, and the indicated values correspond to the fold-change (FC) in EC50 relative to WT. *Not available.

values between approximately 100 μM (8a) and 200 μM (8b). In the case of 8c, which showed a WT EC50 value of 6.2 nM, a SI value greater than 20,000 was achieved.

Because compounds 7c and 8c show antiviral potencies similar to those of RAL (1) against HIV-1 vectors carrying WT IN, it is important to compare the relative effectiveness of our inhibitors against mutant strains of IN in terms of fold-loss relative to WT. Table 5 contains data showing the efficacy of compounds against the G118R and E138K/Q148K mutants, which have recently been identified through in vitro selection studies with second-generation inhibitors.76 Of particular note is the ability of 8c to maintain efficacy equivalent to that of RAL against the G118R mutant while showing approximately one order of magnitude greater efficacy than RAL against the remaining mutants in the table.

**CONCLUSIONS**

A series of bicyclic inhibitors were prepared that employed 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (7) and 1,8-dihydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (8) ring systems. Key features of these inhibitors include the use of a hydroxylamide group as a metal-chelating component and the inclusion of halobenzylamide functionality that is appended through a linker whose carboxamide carbonyl is not an obligatory component of the key metal-chelating heteroatom triad. Among these IN inhibitors, amides 7c and 8c have single digital nanomolar antiviral EC50 potencies against HIV-1 vectors carrying WT IN. Several compounds have selectivity indices of greater than 20,000, and certain of these inhibitors have greater antiviral efficacies than RAL against a panel of IN mutants that included Y143R, N155H, G118R, and the double mutants G140S/Q148H and E138K/Q148K. Compounds 7c and 8c represent potentially useful platforms for further structural variations intended to find compounds that are more broadly effective against additional resistant strains of the virus.

**EXPERIMENTAL SECTION**

**General Synthesis.** 1H and 13C NMR data were obtained on a Varian 400 MHz spectrometer or a Varian 500 MHz spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which the spectra were collected. The solvent was removed by rotary evaporation under reduced pressure, and anhydrous solvents were obtained commercially and used without further drying. Purification by silica gel chromatography was performed using CombiFlash Rf 200h with EtOAc–hexanes solvent systems. Preparative high pressure liquid chromatography (HPLC) was conducted using a Waters Prep LC4000 system having photodiode array detection and Phenomenex C18 columns (Cat. No. 00G-4436-P0-AX, 250 mm × 21.2 mm, 10 μm particle size, 110 Å pore) at a flow rate of 10 mL/min. Binary solvent systems consisting of A = 0.1% aqueous TFA and B = 0.1% TFA in acetonitrile were employed with gradients as indicated. Products were obtained as amorphous solids following lyophilization. Electrospray ionization-mass spectra (ESI-MS) were acquired with an Agilent LC/MSD system equipped with a multimode ion source. Purities of samples subjected to biological testing were assessed using this system and shown to be ≥95%. High-resolution mass spectra (HRMS) were acquired by LC/MS-ESI using an LTQ-Orbitrap-XL at 30K resolution.

**General Procedure A for the Synthesis of Diethyl 2-(2-Nitrobenzylidene)malonates (10a and 10b).** Commercially available 2-nitrobenzaldehydes (9a and 9b) (5 mmol) were added to a solution of dimethyl malonate or diethyl malonate (60 mmol), acetic acid (20 mmol), and piperidine (6 mmol). The mixture was irradiated with microwave radiation with stirring (80 °C, 15 h). The mixture was partitioned between EtOAc (60 mL) and aqueous NaHCO3 (30 mL), and the organic phase was dried (Na2SO4) and filtered, and the filtrate was concentrated. The resulting residue was purified by Combiflash silica gel chromatography (hexanes and EtOAc) to yield either dimethyl (10a) or diethyl 2-(2-nitrobenzylidene)malonate (10b).

**Diethyl 2-(2-Nitrobenzylidene)malonate (10a).** Reaction of commercially available 2-nitrobenzaldehyde (9a) and dimethyl malonate in benzene (8 mL) as described in general procedure A with the inclusion of benzene (8.0 mL) provided 10a as a colorless oil in 100% yield. 1H NMR (500 MHz, CDCl3) δ 8.19 (t, J = 4.1 Hz, 2H), 7.64 (t, J = 7.6 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.39 (d, J = 7.6 Hz, 1H), 3.85 (s, 3H), 3.59 (s, 3H). 13C NMR (125 MHz, CDCl3) δ 164.94, 162.88, 151.25, 140.28, 135.29, 131.38 (2C), 127.75, 120.06, 114.03, 62.00, 61.73, 56.55, 13.98, 13.72. ESI-MS m/z: 266.0 (M+H+). 1H NMR (500 MHz, CDCl3) δ 8.19 (s, 1H), 7.36 (t, J = 8.2 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 4.24 (q, J = 7.2 Hz, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). 13C NMR (100 MHz, CDCl3) δ 166.94, 162.88, 151.25, 140.28, 135.29, 131.38 (2C), 127.75, 120.06, 114.03, 62.00, 61.73, 56.55, 13.98, 13.72. ESI-MS m/z: 324 (M+H+).
General Procedure C for the Synthesis of N-(Benzyloxy)-1-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (12c and 13a–13c). A mixture of 11a or 11b (0.4 mmol) with benzylamine (3 mL) was heated with stirring (60 °C, 14 h), and the resulting product mixture was purified by reverse-phase HPLC to provide 12c or 13a–13c.

N-(2,4-Difluorobenzyl)-1-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (12c). Treatment of 11a with 2,4-difluorobenzylamine as outlined in general procedure C with purification by preparative reverse-phase HPLC (linear gradient of 30% B to 65% B over 30 min; retention time = 24.6 min) provided 6c as a white solid in 38% yield. 1H NMR (400 MHz, DMSO-d$_6$) δ 9.99 (t, J = 6.6 Hz, 1H), 8.67 (s, 1H), 7.41 (dd, J = 15.7, 8.0 Hz, 2H), 7.23–7.11 (m, 3H), 7.04 (dd, J = 19.0, 8.2 Hz, 2H), 4.54 (d, J = 5.9 Hz, 2H). ESI-MS m/z: 347.1 (M+H$^+$). HRMS calcd C$_{12}$H$_{12}$F$_2$N$_2$O$_3$ [M+H$^+$], 347.0838; found, 347.0847.

N-(2,4-Difluorobenzyl)-1,8-dihydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (6c). Treatment of 13c as outlined in general procedure D with purification by preparative reverse-phase HPLC (linear gradient of 30% B to 65% B over 30 min; retention time = 24.6 min) provided 6c as a white solid in 38% yield. 1H NMR (400 MHz, DMSO-d$_6$) δ 9.99 (t, J = 6.6 Hz, 1H), 8.67 (s, 1H), 7.41 (dd, J = 15.7, 8.0 Hz, 2H), 7.23–7.11 (m, 3H), 7.04 (dd, J = 19.0, 8.2 Hz, 2H), 4.54 (d, J = 5.9 Hz, 2H). ESI-MS m/z: 347.1 (M+H$^+$). HRMS calcd C$_{12}$H$_{12}$F$_2$N$_2$O$_3$ [M+H$^+$], 347.0838; found, 347.0847.
General Procedure F for the Synthesis of 1-(Benzyloxy)-N-(halobenzyl)-2-oxo-1,2,4-dihydro-1,8-naphthyridine-3-carboxamides (19a–19c). A solution of 18 (0.6 mmol) and halobenzylamine (1 mL) was heated with stirring (60 °C, 14 h). The mixture was then extracted (CHCl₃) and washed sequentially with aqueous 1 N HCl and brine and dried (Na₂SO₄). The organic phase was filtered and concentrated, and the crude residue was purified by CombiFlash silica gel chromatography (hexanes and EtOAc) to provide amides 19a–c.

1-(Benzyloxy)-N-(3-chloro-4-fluorobenzyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (19a). Treatment of 18 with 3-chloro-4-fluorobenzylamine as outlined in general procedure F provided 19a as a white solid in 31% yield. HRFMS (900 MHz, CDCl₃) δ 9.90 (t, J = 5.7 Hz, 1H), 8.81 (s, 1H), 8.75 (dd, J = 4.6, 1.8 Hz, 1H), 8.06 (dd, J = 7.8, 1.8 Hz, 1H), 7.61–7.60 (m, 1H), 7.59 (t, J = 1.7 Hz, 1H), 7.36–7.32 (m, 2H), 7.31–7.27 (m, 3H), 7.19–7.15 (m, 1H), 7.03 (t, J = 8.7 Hz, 1H), 5.27 (s, 2H), 4.56 (d, J = 6.0 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 162.45, 159.38, 157.35 (d, J = 248.3 Hz), 153.33, 148.97, 141.41, 138.72, 135.31 (d, J = 3.9 Hz), 133.56, 129.94 (2C), 129.87, 129.24, 128.49 (2C), 128.36 (d, J = 15.3 Hz), 127.43 (d, J = 7.3 Hz), 123.36, 120.00, 116.61 (d, J = 21.2 Hz), 114.12, 78.57, 42.58. ESI-MS m/z: 438.1 (M+H⁺).

1-(Benzyloxy)-N-(3,4-difluorobenzyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (19b). Treatment of 18 with 3,4-difluorobenzylamine as outlined in general procedure F provided 19b as a white solid in 51% yield. HRFMS (900 MHz, CDCl₃) δ 9.95 (t, J = 5.8 Hz, 1H), 8.85 (s, 1H), 8.78 (dd, J = 4.7, 1.8 Hz, 1H), 8.09 (dd, J = 7.8, 1.7 Hz, 1H), 7.65–7.64 (m, 1H), 7.62 (d, J = 1.7 Hz, 1H), 7.37–7.30 (m, 4H), 7.20–7.13 (m, 1H), 7.09–7.03 (m, 1H), 6.98–6.94 (m, 1H), 5.29 (s, 2H), 4.59 (d, J = 6.0 Hz, 2H). ESI-MS m/z: 422.1 (M+H⁺).

1-(Benzyloxy)-N-(2,4-difluorobenzyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (19c). Treatment of 18 with 2,4-difluorobenzylamine as outlined in general procedure F provided 19c as a white solid in 22% yield. HRFMS (900 MHz, CDCl₃) δ 9.92 (d, J = 5.4 Hz, 1H), 8.87 (s, 1H), 8.82 (dd, J = 4.7, 1.7, 0.6 Hz, 1H), 8.13 (d, J = 7.8, 1.7 Hz, 1H), 7.69 (d, J = 2.4 Hz, 1H), 7.68 (dd, J = 3.3, 1.3 Hz, 1H), 7.42–7.34 (m, 5H), 6.88–6.76 (m, 2H), 5.34 (s, 2H), 4.69 (d, J = 5.9 Hz, 2H). ESI-MS m/z: 422.1 (M+H⁺).

General Procedure G for the Synthesis of 1-(Benzyloxy)-N-(halobenzyl)-2-oxo-1,2,4-dihydro-1,8-naphthyridine-3-carboxamides (7a–c). A solution of amide (19a–19c) (0.2 mmol) in a solution of MeOH (10 mL) and EtOAc (3 mL) was added Pd–C (10%, 20 mg), and the mixture was degassed and stirred at room temperature under H₂ (1 h). The mixture was then filtered through a small pad of silica gel, the filtrate was concentrated, and the residue was purified by preparative reverse-phase HPLC to provide the target amides 7a–c.

1-(Benzyloxy)-N-(3-chloro-4-fluorobenzyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (17c). To a solution of 16 (268 mg, 0.82 mmol) and triethylamine (0.27 mL, 1.97 mmol) in CH₂Cl₂ (15 mL) was added trifluoromethanesulfonic anhydride (0.17 mmol, 0.98 mL) at 0 °C, and the solution was stirred at 0 °C (0.5 h). The mixture was concentrated and purified by CombiFlash silica gel chromatography to obtain 17 as a white solid (301 mg, 80% yield). HRFMS (900 MHz, CDCl₃) δ 8.86 (dd, J = 4.7, 1.7 Hz, 1H), 8.21 (dd, J = 8.1, 1.7 Hz, 1H), 7.70–7.67 (m, 2H), 7.43–7.38 (m, 4H), 5.34 (s, 2H), 4.01 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 160.87, 155.20, 154.02, 153.96, 148.76, 148.09, 133.32, 133.23, 130.12, 129.33, 128.04, 128.81, 128.11, 119.86, 116.67, 109.73, 78.72, 53.50. ESI-MS m/z: 459.0 (M+H⁺).

1-Methyl-(Benzyloxy)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (18). To a mixture of 17 (34 mg, 0.074 mmol) and PdCl₂(PPh₃)₂ (5 mg, 0.07 mmol) in DMF (1 mL), triethylamine (0.030 mL, 0.22 mmol) and trisopropylsilane (17 mg, 0.15 mmol) were added. The mixture was heated to 85 °C (24 h). The resulting crude mixture was purified by CombiFlash silica gel chromatography (hexanes and EtOAc) to provide 18 as a white solid (12 mg, 52% yield). HRFMS (400 MHz, CDCl₃) δ 8.84 (d, J = 4.7, 1.8 Hz, 1H), 8.41 (s, 1H), 7.99 (dd, J = 7.8, 1.7 Hz, 1H), 7.69 (dd, J = 7.6, 1.8 Hz, 1H), 7.38–7.30 (m, 3H), 7.26–7.23 (m, 1H), 5.29 (s, 2H), 3.96 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 164.47, 154.31, 141.78, 138.27, 133.86, 132.08, 131.99, 130.10 (2C), 129.05, 128.50, 128.37 (2C), 119.32, 113.30, 78.21, 52.90. ESI-MS m/z: 311.1 (M+H⁺).
Regression analysis on the data. CC50 values were determined from the normalized to cytotoxicity in the absence of target compounds. The human osteosarcoma cell line, HOS, was obtained from Dr. Richard Schwartz (Michigan State University, East Lansing, MI) and grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) supplemented with 5% (v/v) fetal bovine serum, 5% newborn calf serum, and penicillin (50 units/mL) plus streptomycin (50 µg/mL; Quality Biological, Gaithersburg, MD). On the day prior to the screen, HOS cells were seeded in a 96-well luminescence cell culture plate at a density of 4000 cells in 100 µL per well. On the day of the screen, the cells were treated with compounds in the appropriate concentration range chosen and incubated at 37 °C for 2 h and then incubated by the addition of an equal volume of loading buffer (formamide containing 1% sodium dodecyl sulfate, 0.25% bromophenol blue, and xylene cyanol). Reaction products were separated in 16% polyacrylamide-denaturing sequencing gels. Dried gels were visualized using Typhoon 8600 (GE Healthcare, Piscataway, NJ). Densitometric analyses were performed using ImageQuant 5.1 software from GE Healthcare. The data analyses (linear regression, 50% inhibitory concentration [IC50] determination, and standard deviation [SD]) were performed from at least 3 independent determinations using Prism 5.0c software from GraphPad.

Cellular Cytotoxicity Assays. The human osteosarcoma cell line, HOS, was obtained from Dr. Richard Schwartz (Michigan State University, East Lansing, MI) and grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) supplemented with 5% (v/v) fetal bovine serum, 5% newborn calf serum, and penicillin (50 units/mL) plus streptomycin (50 µg/mL; Quality Biological, Gaithersburg, MD). On the day prior to the screen, HOS cells were seeded in a 96-well luminescence cell culture plate at a density of 4000 cells in 100 µL per well. On the day of the screen, the cells were treated with compounds in the appropriate concentration range chosen and incubated at 37 °C for 48 h. Cytotoxicity was measured by monitoring ATP levels via a luciferase reporter assay. Cells were lysed in 50 µL of cell lysis buffer (PerkinElmer, Waltham, MA) and shaken at 700 rpm at room temperature for 5 min. After the addition of 50 µL of ATPlute buffer (PerkinElmer) directly onto the lysed cells and shaking at 700 rpm at room temperature (5 min), ATP levels were monitored by measuring luciferase activity using a microplate reader. Activity was normalized to cytotoxicity in the absence of target compounds. KaleidaGraph (Synergy Software, Reading, PA) was used to perform regression analysis on the data. EC50 values were determined from the fit model.

Single-Round HIV-1 Infectivity Assays. Human embryonic kidney cell line 293T was transfected with the pNLNgoMIVR-ΔLUC vector, which was made from pNLNgoMIVR-ΔEnv-LUC vector by removing the HSA reporter gene and replacing it with a luciferase reporter gene between the NotI and XhoI restriction sites.57 VSV-g-pseudotyped HIV was produced by transfections of 293T cells as described previously.58 On the day prior to transfection, 293T cells were plated on 100-mm-diameter dishes at a density of 1.5 x 10⁶ cells per plate. 293T cells were transfected with 16 µg of pNLNgoMIVR-ΔLUC and 4 µg of pCHMV-g (obtained from Dr. Jane Burns, University of California, San Diego) using the calcium phosphate method. At approximately 6 h after the calcium phosphate precipitate was added, the 293T cells were washed twice with phosphate-buffered saline (PBS) and incubated with fresh media (48 h). The virus-containing supernatants were then harvested, clarified by low-speed centrifugation, filtered, and diluted for preparation in infection assays. On the day prior to the screen, HOS cells were seeded in a 96-well luminescence cell culture plate at a density of 4000 cells in 100 µL per well. On the day of the screen, the cells were treated with the compounds from a concentration range of 10 µM to 0.0005 µM using 11 serial dilutions and then incubated at 37 °C (3 h). After this incubation, 100 µL of virus-stock diluted to achieve a maximum luciferase signal between 0.2 and 1.5 RLUs was added to each well, and the plates were incubated at 37 °C (48 h). Infectivity was measured by using the Steady-lite plus luminescence reporter gene assay system (PerkinElmer, Waltham, MA). Luciferase activity was measured by adding 100 µL of Steady-lite plus buffer (PerkinElmer) to the cells, incubating at room temperature (20 min), and measuring luminescence using a microplate reader. Activity was normalized to infectivity in the absence of target compounds. KaleidaGraph (Synergy Software, Reading, PA) was used to perform regression analysis on the data. EC50 values were determined from the fit model.

Vector Constructs. pNLNgoMIVR-ΔEnv-LUC has been described previously.57 The IN coding region was removed from pNLNgoMIVR-ΔEnv-LUC (between KpnI and SalI sites) and inserted between the KpnI and SalI sites of pbLuscript II K5+. Using that construct as the wild-type template, we prepared the following IN-resistant mutants via the QuikChange II XL (Stratagene, La Jolla, CA) site-directed mutagenesis protocol: G118R, Y143R, G140S + Q148H, G140A + Q148K, and E138K + Q148K. The following sense with cognate antisense (not shown) oligonucleotides (Integrated DNA Technologies, Coralville, IA) was used in the mutagenesis: G118R, 5′-GTATACATAGACAATTCGCAAGATTTTCACCAGTAC-3′; E138K, 5′-GGCCGGGATCAACGAGAAATTTGGCATTCCCCGC-3′; G140A, 5′-GGGGATCAACGAGAATTTCGCCATTTCCCTAATC-3′; G140S, 5′-GGGGATCAACGAGAATTTCGCCATTTCCCTAATC-3′; and N155H, 5′-GACGAATTTCGCCATTTCCCTAGGACTCCAAAGTCAAGGAGTAATAGAAT-3′. The double mutant G140S + Q148H was constructed by using the previously generated Q148H mutant and the appropriate oligonucleotide to introduce the second mutation, G140S. The double mutant G140A + Q148K was made by using the Q148K mutant and the appropriate oligonucleotide to introduce the second mutation, G140A. The double mutant E138K + Q148K was made by using the Q148K mutant and the appropriate oligonucleotide to introduce the second mutation, E138K.

The DNA sequence of each construct was verified independently by DNA sequencing. The mutant IN coding sequences from pbLuscript II K5+ were then subcloned into pNLNgoMIVR-ΔEnv-LUC (between the KpnI and SalI sites) to produce the full-length mutant HIV-1 IN constructs. These DNA sequences were additionally checked independently by DNA sequencing.

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Notes
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**ABBREVIATIONS USED**

HIV-1, human immunodeficiency virus type 1; AIDS, acquired immune deficiency syndrome; FDA, Food and Drug Administration; IN, integrase; RAL, Raltegravir; EVG, Eltirivatib; DTG, Dolutegravir; 3′-P, 3′-processing; ST, strand transfer; INSTIs, integrase strand transfer inhibitors; DNA, deoxyribonucleic acid; IC_{50}, half-maximum inhibitory concentration; EC_{50}, half-maximal effective concentration; WT, wild type; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; HPLC, high-pressure liquid chromatography; HRMS, high-resolution mass spectrometry

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