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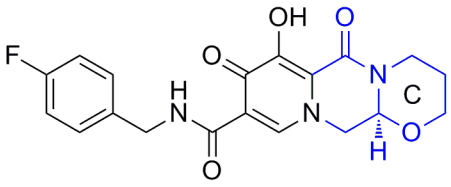
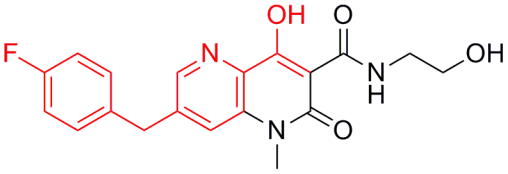


Carbamoylpyridone

$^{pHIV}IC_{50}$ = 2 nM

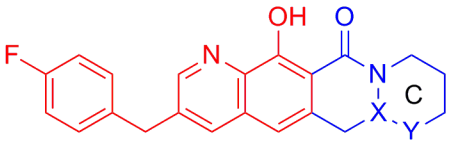
Q148K = 2.4 FC



Naphthyridinone

$^{pHIV}IC_{50}$ = 3 nM

Q148K = 40 FC



8-Hydroxyquinoline Tetracyclic

12 examples

$^{pHIV}IC_{50}$ = 0.6 - 18 nM

The Design of 8-Hydroxyquinoline Tetracyclic Lactams as HIV-1 Integrase Strand Transfer Inhibitors

Emile J. Velthuisen, Brian A. Johns, David P. Temelkoff, Kevin W. Brown, Susan C. Danehower*

GlaxoSmithKline Research & Development, Infectious Diseases Therapeutic Area Unit, Five Moore Drive, Research Triangle Park, NC 27709, United States

*Corresponding author. *E-mail address:* emile.j.velthuisen@gsk.com

Abstract— A novel series of HIV-1 integrase strand transfer inhibitors were designed using the venerable two-metal binding pharmacophore model and incorporating structural elements from two different literature scaffolds. This manuscript describes a number of 8-hydroxyquinoline tetracyclic lactams with exceptional antiviral activity against HIV-1 and little loss of potency against the IN signature resistance mutations Q148K and G140S/Q148H.

Keywords: HIV, AIDS, integrase, integrase inhibitor, strand transfer inhibitor, quinoline

Abbreviations: ADME, absorption, distribution, metabolism, excretion; AIDS, acquired immunodeficiency syndrome; Cl, clearance; CLND, chemi-luminescent nitrogen detection; CCD, catalytic core domain; CTD, C-terminal domain; DIPEA, diisopropyl ethyl amine; DMPK, drug metabolism and pharmacokinetics; DNAUC, dose-normalized area under the curve; dsDNA, double-stranded deoxyribonucleic acid; EVG, elvitegravir; F, oral bioavailability; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HQT, hydroxyquinoline tetracyclic; IN, Integrase; IN-ST, Integrase strand transfer; NTD, N-terminal domain or naphthyridinone; RAL, raltegravir; Teoc, 2-(trimethylsilyl)ethyl carbamate; TMS, trimethylsilyl.

1. Introduction

HIV-1 integrase (IN) is one of the three essential enzymes required for viral replication and has been the subject of intense research for two decades.[1,2,3,4,5] The enzyme is 32 kDa in size, and consists of a 288 amino acid sequence that can be divided into three domains: N-terminal (NTD), C-terminal (CTD), and the catalytic core (CCD). The catalytic core possesses a triad of carboxylate residues that coordinate a pair of Mg^{2+} ions and is ultimately responsible for catalyzing two biochemical steps required for incorporation of reverse transcribed viral DNA into host chromatin.[6,7] In the first reaction, IN removes the two terminal nucleotides from the respective 3'-ends of viral DNA. The enzyme then facilitates nicking of host chromosomal DNA by the recessed 3'-hydroxyl of the viral DNA ends, resulting in strand transfer and effective integration of the viral dsDNA.[8] Although there have been efforts to find inhibitors of both

biochemical process, strand transfer inhibitors have proven to be much more amenable to inhibition that is pharmacologically relevant.[9,10,11,12,13,14]

In general, strand transfer inhibitors contain three chelating heteroatoms in a rigidified co-planar orientation linked to a flexible hydrophobic tail.[15,16,17] These molecules act as a bioisostere of the substrate phosphodiester transition state by chelating two magnesium ions and preventing progress of the strand transfer of viral DNA into the host's chromatin. This generic pharmacophore model [18] has proven highly successful in designing strand transfer inhibitors and to date there have been three agents approved by the FDA for use in patients infected by HIV-1.[2]

In 2007, raltegravir (RAL) was approved as the first-in-class integrase strand transfer inhibitor (Figure 1).[19] Although it requires a relatively high dose of 400 mg twice daily, it was well tolerated, and provided a very rapid and exceptional viral load drop in patients.[20] However, a noteworthy deficiency of this therapeutic was formation of three primary IN signature mutation pathways: Y143, Q148, and N155.[21] These mutations can include additional mutations to recover viral fitness (e.g. Q148H/G140S) and lead to decreased susceptibility and eventual virological failure.[22] In 2012, a second strand transfer inhibitor was approved – elvitegravir (EVG).[23] This agent was differentiated from RAL with the option of once daily dosing with the co-administration of a CYP3A4 inhibitor such as ritonavir or cobicistat.[24] However, EVG was also susceptible to formation of IN mutations albeit through a slightly different resistance pathway and demonstrates significant cross-resistance with RAL.[25]

Figure 1

(insert figure 1)

With the advent of two marketed strand transfer inhibitors there was little justification for introducing a third agent unless it was clearly differentiated in terms of the dosing regimen (i.e. low dose, no PK booster) and/or resistance profile to existing IN resistance mutations. Fortunately, GSK had several such molecules under clinical investigation with the tricyclic carbamoylpyridone class of inhibitors.[18,26,27,28] These molecules represented a true second generation profile in that they were not cross-resistance to RAL or EVG and the preclinical pharmacokinetics were suggestive of unboosted once daily pharmacokinetics. The realization that optimization of integrase inhibitors designs was feasible as evidenced by the carbamoylpyridone scaffold coupled with early discovery uncertainties inherent to any drug

discovery program led to an interest in identifying additional scaffolds with such attributes to serve as backup to the clinical assets.[29]

2. Results and Discussion

2.1 HQT Scaffold Design

The carbamoylpyridone class of inhibitor is believed, in part, to have improved potency against wild-type and key resistance mutations due to the additional rings built into the metal chelating architecture.[28,30] Accordingly, we sought to combine this type of strategy with our earlier work on naphthyridinone (NTD) inhibitors.[31] In combining the additional ring system "C" of the carbamoylpyridone (CAB, **3**)[26,27,28] with the 3-benzylpyridine portion of the naphthyridinone **4**,[32,33,34] we would create a hydroxyquinoline tetracyclic scaffolding (HQT, **5**) that has the additional structural elements believed necessary for a superior resistance profile (Scheme 1).

Scheme 1

(insert Scheme 1)

2.2 HQT Chemistry

Our initial attempt to construct the hydroxyquinoline tetracyclic ring system **16** was through an anionic Michael-Dieckmann condensation of pyridine **9** and cyclic hydrazide **14** (Scheme 2). Synthesis of pyridine **9** started with Negishi coupling of (3-chloro-2-fluorobenzyl)zinc bromide and commercially available methyl 5-bromo-2-chloronicotinate (**6**). The methyl ester was reduced with sodium borohydride and the resulting 2-chloropyridine **8** carbonylated to afford lactone **9**. Synthesis of the coupling partner **14** commenced with alkylation of known *tert*-butoxycarbonyl pyrazolidine **10**[35] with allyl bromide. Following removal of the *tert*-butoxycarbonyl protecting group under acidic conditions the pyrazolidine **12** was acylated with acryloyl chloride and the olefins metathesized to provide cyclic hydrazide **14**. The key Michael-Dieckmann condensation was accomplished in a two step sequence. Lactone **9** was first deprotonated with LiHMDS and treated with Michael acceptor **14**. The intermediate alcohol **15** was then activated using methanesulfonyl chloride and subsequently eliminated to afford the desired hydroxyquinoline tetracyclic ring system **16** in 24% yield. Although the key step provided a low yield of desired product, optimization was delayed until the antiviral activity of HQT **16** was

confirmed and could validate the additional structural complexity translating into an improved profile.

Scheme 2

(insert Scheme 2)

Reagents and Conditions: (a) $Pd(PPh_3)_4$, (3-chloro-2-fluorobenzyl)zinc bromide, THF (72%); (b) $NaBH_4$, THF (69%); (c) $Pd(OAc)_2$, Et_3N, DPPP, DMF (22%); (d) Allyl bromide, K_2CO_3; (e) TFA; (f) acryloyl chloride (41% over 3-steps); (g) Grubbs 2 (85%); (h) LiHMDS, THF; (i) MsCl, Et_3N, CH_2Cl_2 (24% over 2-steps).

Fortunately, the initial HQT analog **16** was potent in a pseudotyped antiviral assay ($^{pHIV}IC_{50}$ = 16 nM) with an 9-fold shift in the presence of 40% human serum albumin ($^{pHIV}PAIC_{50}$ = 136 nM). In addition, there was no loss of potency against theQ148K virus and only a 2.3-fold loss in potency against G140S/Q148H. It is noteworthy that both raltegravir (**1**) and elvitegravir (**2**) demonstrated a much greater loss of potency against these two integrase resistance mutations (Figure 1). These results provided the necessary justification to reinvestigate the key Michael-Dieckmann condensation and to further explore the SAR of this series.

Several attempts were made to optimize the Michael-Dieckmann cyclization, varying the solvent, temperature, and base which had little impact on the outcome of the reaction. Instead, we sought to fundamentally redesign the lactone coupling partner in order to decrease the pKa of the benzylic position.[36,37] Synthesis of such a substrate commenced with activation of benzylic alcohol **8** with methanesulfonyl chloride and subsequent displacement with sodium cyanide (Scheme 3). The 2-chloropyridine **17** was then carbonylated in the presence of methanol to afford methyl ester **18**. In contrast to the previous Michael-Dieckmann cyclization of lactone **9**, deprotonation of pyridine **18** with LiHMDS and subsequent treatment with hydrazide **14** afforded the desired dihydroquinoline product **19** very efficiently. However, much to our disappointment, the oxidation of the dihydroquinoline **19** to the corresponding HQT did not occur under several conditions.

Scheme 3

(insert Scheme 3)

Reagents and Conditions: (a) NaCN (97%); (b) $Pd(OAc)_2$, Et_3N, DPPP, DMF (72%); (c) LiHMDS, THF (60%); (d) DDQ, DCM or Pd/C, air, MeOH.

As an alternative to the late stage oxidation of dihydroquinoline **19** we sought to increase the oxidation state of the hydrazide coupling partner **14** by creating an ynoate. Synthesis of the new Michael acceptor began with alkylation of *tert*-butoxycarbonyl pyrazolidine **10** with propynyl methanesulfonate (Scheme 4). The alkyne **21** was deprotonated with *n*-butyl lithium and quenched with methyl chloroformate to afford ynoate **22**. Upon treatment of pyridine **18** with LiHMDS and subsequent addition of ynoate **22**, the desired Michael-Dieckmann cyclization occurred. Following removal of the *tert*-butoxycarbonyl protecting group under acidic conditions, the lactam formed spontaneously to provide HQT **20** in 89% yield.

Scheme 4

(insert Scheme 4)

Reagents and Conditions: (a) prop-2-yn-1-yl methanesulfonate, Cs_2CO_3, DMF (67%); (b) i) n-BuLi, ii) methyl chloroformate (66%); (c) i) LiHMDS, ii) TFA (89%).

In a pseudotyped antiviral assay HQT **20** was very potent ($^{pHIV}IC_{50}$ = 2.2 nM) but demonstrated a significant 188-fold shift in the presence of 40% human serum albumin ($^{pHIV}PAIC_{50}$ = 415 nM). There was a 1.3-fold loss in potency against the Q148K virus and only a 8-fold loss against the double mutant G140S/Q148H. With an improved HQT synthesis and an additional functional handle to exploit, a variety of analogs were pursued using this strategy.

2.3 HQT Structure Activity Relationship

To follow up on the initial hit we were interested in constructing either proline or pipecolinic acid derived hydroxyquinoline tetracyclic ring systems in order to investigate the effect of ring size and chirality on antiviral potency, protein shift, and *in vivo* DMPK. Accordingly, a variety of chiral ynoates were prepared from known aldehydes[38] as illustrated in Scheme 5. The sequence started with homologation of a chiral aldehyde with Ohira-Bestmann reagent[39] followed by formation of the propiolate from deprotonation of the terminal alkyne and quenching with methyl chloroformate. In addition to the *tert*-butoxy carbonyl protected derivatives, it was also possible to use a non-acid labile protecting group such as a trimethylsilylethyl carbamate (Teoc) by performing a late stage protecting group swap.

Scheme 5

(insert scheme 5)

Reagents and Conditions: (a) K_2CO_3, dimethyl (diazomethyl)phosphonate, K_2CO_3, MeOH; (b) i) *n*-BuLi, ii) methyl chloroformate; (c) TFA, DCM; (d) Teoc-Cl, pyridine

The chiral ynoates described in Scheme 5 were used in Michael-Dieckmann cyclizations with pyridine **18** to construct a variety of HQT analogs (Scheme 6). In general, the LiHMDS promoted cyclization proceeded with good conversion to the desired substituted quinoline. However, following deprotection of the *tert*-butoxy carbonyl protected proline or piperidine, the nitrogen could either cyclize with the C7-ester to afford the desired lactam **40** or react with the C5-nitrile to provide an undesired amidine ring system **41**. Fortunately when attempting to create a 6-membered ring, lactam **40** would form preferentially. However, when constructing a 5-membered ring, the acidic conditions required for removal of the *tert*-butoxy carbonyl protecting group promoted formation of amidine **41**. In order to circumvent this undesired reaction pathway a non-acid labile nitrogen protecting group such as trimethylsilylethyl carbamate (Teoc) was employed. Overall, the combined yields for the Michael-Dieckmann cyclization and subsequent deprotection/lactam formation ranged from 12-78% (Table 1).

Scheme 6

(insert Scheme 6)

Reagents and Conditions: (a) LiHMDS, THF; (b) TFA, DCM; (c) NaOH, MeOH; (d) TBAF, THF or CsF, THF/DMF.

Table 1

(insert table 1)

[1] Reported yield is over 3-steps for R = Boc (a, b, c) and 2-steps for R = Teoc (a, d).
[2] Assay run in the presence of 40% human serum albumin.

In general, all the HQT analogs were potent in the pseudotyped antiviral assay ($^{pHIV}IC_{50}$ = 5 – 18 nM) although many demonstrated a significant shift in potency when the assay was run in the presence of 40% human serum albumin (Table 1). Against the single mutant Q148K virus and the double mutant G140S/Q148H virus, 6-membered lactams (**42** and **43**) exhibited a smaller fold change in potency relative to the 5-membered lactams (**44** – **47**). Stereochemistry appeared to be less of a predictor of antiviral potency against both wild type and mutant viruses although

there was a significant difference in protein shift for the matched stereoisomers of both the 5- and 6-memererd lactams (**42**/**43**, **44**/**45**, and **46**/**47**).

In order to further define the HQT structure activity relationship a variety of conditions were explored to hydrolyze the C5-nitrile to the corresponding carboxylic acid. Unfortunately majority of the standard conditions afforded decomposition products with the exception of an acidic hydrolysis with acetic acid and sulfuric acid that provided a primary amide (Figure 2). Interestingly these analogs proved to be exceptionally potent antiviral agents.

Figure 2[1]

(insert figure 2 scheme)

(insert figure 2)

[1] Reagents and Conditions: (a) AcOH/H_2SO_4, H_2O.
[2] Assay run in the presence of 40% human serum albumin.

The HQT C5-primary amides were very potent in the pseudotyped antiviral assay ($^{pHIV}IC_{50}$ = 0.6 – 8.0 nM) with reasonably small serum shifts that ranged from 2x to 5x (Figure 2). Most encouraging was their potency against the IN resistance mutations. All four analogs were nearly equipotent to, or more potent against the Q148K mutant. Against the double mutant G140S/Q148H there was a slightly larger fold shift but this still translated into single digit nanomolar potency which is a requirement for a true second generation integrase strand transfer inhibitor.

2.4 HQT in vivo DMPK

In addition to an excellent virological profile it was also necessary to evaluate the *in vivo* metabolic stability of the series (Table 2). In order to do so, HQT **52** was dosed both intravenously and orally in Sprague-Dawley rats and Harlan beagle dogs. The compound had a good $t_{1/2}$, low i.v. clearance, but poor oral bioavailability in rats (7.2 h, 1.4 mL min^{-1} kg^{-1}, 2 %) and dogs (11.8 h, 2.8 mL min^{-1} kg^{-1}, 14 %). Despite moderate permeability (P_{APP} = 60 nm/s), poor intrinsic aqueous solubility (cLND = 25 μM) is likely limiting oral absorption for this compound.

Table 2

(insert table 2)

3.0 Conclusion

In conclusion, this manuscript describes the design and synthesis of novel IN strand transfer inhibitors using the venerable two-metal binding pharmacophore model and incorporating structural elements from two different literature scaffolds. Several examples of the 8-hydroxyquinoline tetracyclic lactams have a virological profile consistent with other second generation integrase stand transfer inhibitors and represent an excellent starting point for further optimization wherein improvements in the pharmacokinetic profiles are needed.

4.0 Experimental Section

4.1 Chemistry

All commercially available reagents were used without further purification. Column chromatography was carried out on silica gel (70-230 mesh). TLC was conducted on silica gel 250 micron, F254 plates. ^{1}H NMR spectra were recorded at 300 or 400 MHz. Melting points are uncorrected. Purities of test compounds were established by analytical HPLC (C-18 column, 5.0 micron, 0→100% CH_3CN (or MeOH)/water with 0.05-0.1% HCOOH (or TFA) and UV detection with or without mass spectrometer detection. All test compounds showed >95% purity (AUCs by UV detection).

*4.1.1 Methyl 2-chloro-5-(3-chloro-2-fluorobenzyl)nicotinate (**7**).* A suspension of zinc (5.0 g) in 4 N HCl (10 mL) was stirred at 30 ºC for 10 min and then filtered. The residue was crushed to yield fine particles and then washed with water (5x) and acetone (2x). The activated zinc was then dried *in vacuo* for 24 h.

A solution of 3-chloro-2-flurobenzyl bromide (1.12 g, 5 mmol) in THF (3 mL) was added dropwise to suspension of activated zinc (0.98 g, 15 mmol), 1,2-dibromoethane (0.034 mL, 0.4 mmol), trimethylchlorosilane (0.038 mL, 0.3 mmol) and THF (1.5 mL) at 23 ºC. The reaction was heated to 45 ºC and maintained at this temperature for 1.5 h. TLC indicated complete consumption of 3-chloro-2-flurobenzyl bromide (sm: R_f = 0.4; 30% EtOAc-hexanes).

A solution of (3-chloro-2-fluorobenzyl)zinc bromide in THF (7.98 ml, 7.98 mmol, 1.0 M) was added dropwise over a 10 min period to a stirring mixture of 5-bromo-2-chloronicotinate (2.0 g, 7.98 mmol), tetrakis(triphenylphosphine)palladium (0.923 g, 0.798 mmol), and THF (26.6 ml) at 65 ℃. The resulting mixture was stirred for 1 h and then poured into 10% aq. NH_4Cl and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organics layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (10-100% EtOAc-hexanes gradient) to yield the title compound as a yellow oil. (1.8 g, 71.8 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.41 (d, J=2.38 Hz, 1 H), 7.97 (d, J=2.38 Hz, 1 H), 7.37–7.29 (m, 1 H), 7.08–7.04 (m, 3 H), 4.04 (s, 2 H), 3.97–3.92 (m, 3 H); ES^+ MS: 315 (M + 1).

*4.1.2 {2-Chloro-5-[(3-chloro-2-fluorophenyl)methyl]-3-pyridinyl}methanol (**8**).* To a –78 ℃ solution of methyl 2-chloro-5-(3-chloro-2-fluorobenzyl)nicotinate (1.5 g, 4.77 mmol) in THF (24 mL) was added DiBAL-H (10.50 ml, 10.50 mmol, 1.0 M in CH_2Cl_2). After 30 min, the reaction mixture was warmed to –30 ℃ and maintained at this temperature for 2 h. The reaction mixture was poured into 1 N HCl and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organics layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (10-60% EtOAc-hexanes gradient) to afford the title compound as a white solid (1.1 g, 69% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.22 (d, *J*=2.20 Hz, 1 H), 7.70 (d, *J*=1.65 Hz, 1 H), 7.31 (m, 1 H), 7.10–7.03 (m, 2 H), 4.76 (d, *J*=5.86 Hz, 2 H), 4.02 (s, 2 H); ES^+ MS: 287 (M + 1).

*4.1.3 3-(3-chloro-2-fluorobenzyl)furo[3,4-b]pyridin-7(5H)-one (**9**)*. A solution of {2-Chloro-5-[(3-chloro-2-fluorophenyl)methyl]-3-pyridinyl}methanol (0.6 g, 2.1 mmol), and triethylamine (1.45 mL, 10.5 mmol) in DMF (15 mL) was degassed with N2 for 10 min. The mixture was then treated with palladium acetate (0.05 g, 0.21 mmol) and DPPP (0.09 g, 0.21 mmol) and subsequently purged/backfilled with N_2 3x and CO 3x. The reaction mixture was then placed under an atmosphere of CO (50 psi) and heated to 90 ℃. After 18 h, the reaction mixture was cooled to ambient temperature and filtered through a pad of celite. The filtrate was partitioned between EtOAc/water and the organics washed with water, brine, dried (MgSO4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (10-50% EtOAc/hexanes) to afford the title compound (0.13 g, 22% yield) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.82 (s, 1 H), 7.68 (s, 1 H), 7.36 (td, *J*=7.28, 2.11 Hz, 1 H), 7.16–7.04 (m, 2 H), 5.34 (s, 2 H), 4.18 (s, 2 H); ES^+ MS: 278 (M + 1).

*4.1.4 1-Acryloyl-2-(2-propen-1-yl)pyrazolidine (**13**).* A solution of 1,1-dimethylethyl 1-pyrazolidinecarboxylate[40] (17.7 g, 103 mmol) in toluene (206 mL) was treated with potassium carbonate (28.4 g, 206 mmol) and allyl bromide (13.34 ml, 154 mmol) and heated. After 4 h, the reaction mixture was poured into water and the layers separated. The aqueous phase was extracted with EtOAc and the combined organics layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. The crude residue was dissolved in dichloromethane and cooled to 0 ℃. Trifluoroacetic acid (103 ml, 1336 mmol) was added and the reaction mixture was then warmed to 23 ℃. After 16 h, the reaction mixture was concentrated *in vacuo* and azeotroped with toluene (2x). The crude residue was dissolved in THF (500 mL) and triethyl amine (43.0 mL, 308 mmol) was added. The reaction mixture was then cooled to –78 ℃ and treated dropwise with acryloyl chloride (9.2 mL, 113 mmol). The cooling bath was allowed to expire and the reaction mixture was stirred for an additional 16 h. The reaction mixture was poured into sat. aq. NH_4Cl and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organics layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by Kugelrohr distillation (0.2 torr, 175 ℃ oven temperature) to afford the title compound (7.0 g, 41%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.98 (dd, *J*=17.3, 10.4 Hz, 1 H), 6.36 (dd, *J*=17.3, 2.1 Hz, 1 H), 5.97–5.79 (m, *J*=17.0, 10.3, 6.5, 6.5 Hz, 1 H), 5.64 (dd, *J*=10.4, 2.1 Hz, 1 H), 5.26–5.14 (m, 2 H), 4.14–3.32 (m, 2 H), 3.25 (d, *J*=6.5 Hz, 2 H), 2.99 (dd, *J*=0.8, 0.4 Hz, 2 H), 2.27–2.06 (m, 2 H).

*4.1.5 2,3-dihydro-1H-pyrazolo[1,2-a]pyridazin-5(8H)-one (**14**).* A solution 1-Acryloyl-2-(2-propen-1-yl)pyrazolidine (2.5 g, 15.04 mmol) in dichloromethane (500 ml) was degassed with N_2 for 15 min and shielded from ambient light. Benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium (Grubbs Catalyst, 2nd Generation)(1.50 g, 0.11 mmol) was added and the reaction mixture was heated to reflux. After 15 h, ethyl vinyl ether (7.23 mL, 75 mmol) was added and stirring was continued for 15 min. The reaction mixture was cooled to 23 ℃ and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% ethyl acetate-dichloromethane) to afford the title compound (1.5 g, 85%) as a black oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.56 (dt, *J*=9.7, 4.2 Hz, 1 H), 6.06–6.00 (m, 1 H), 3.77–3.69 (m, 2 H), 3.51 (dd, *J*=3.9, 1.4 Hz, 2 H), 3.05 (t, *J*=6.5 Hz, 2 H), 2.22–2.12 (m, 2 H).

*4.1.6 3-[(3-chloro-2-fluorophenyl)methyl]-13-hydroxy-9,10-dihydro-8H-pyrazolo[1,2-b]pyrido[2,3-g]phthalazin-12(6H)-one (**16**).* A –78 ℃ solution of 3-[(3-chloro-2-fluorophenyl)methyl]furo[3,4-*b*]pyridin-7(5*H*)-one (0.2 g, 0.720 mmol) in dichloromethane (2.8 ml) was treated with lithium

bis(trimethylsilylamide) (0.72 mL, 0.72 mmol, 1.0 M THF) dropwise. After 12 min, a solution of 2,3-dihydro-1*H*-pyrazolo[1,2-*a*]pyridazin-5(8*H*)-one (0.104 g, 0.756 mmol) in THF (2.8 mL) was added dropwise over a 5 min period. After an additional 30 min, the reaction mixture was treated with lithium bis(trimethylsilylamide) (0.72 mL, 0.72 mmol, 1.0 M THF) and stirring continued for 20 min. The reaction mixture was poured into sat. aq. NH_4Cl and the layers separated. The aqueous phase was extracted with ethyl acetate and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude residue was dissolved in THF (3.0 mL), cooled to 0 ℃ and treated sequentially with triethyl amine (0.60 mL, 4.32 mmol) and methane sulfonyl chloride (0.23 mL, 2.88 mmol). After 30 min, the reaction mixture was poured into sat. aq. NH_4Cl and the layers separated. The aqueous phase was extracted with ethyl acetate, dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (0-10% methanol-dichloromethane) to afford the title compound (63 mg, 24%) as a brown solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.85 (d, *J*=1.83 Hz, 1 H), 7.78 (s, 1 H), 7.32 (td, *J*=7.32, 1.65 Hz, 1 H), 7.17–6.98 (m, 2 H), 6.94 (s, 1 H), 4.20 (s, 2 H), 4.13 (s, 3 H), 3.95–3.87 (m, 2 H), 3.22 (t, *J*=6.41 Hz, 2 H), 2.35–2.22 (m, 2 H); ES^+ MS: 398 (M + 1).

*4.1.7 {2-Chloro-5-[(3-chloro-2-fluorophenyl)methyl]-3-pyridinyl}acetonitrile (**17**).* A 0 ℃ solution of {2-chloro-5-[(3-chloro-2-fluorophenyl)methyl]-3-pyridinyl}methanol (1.6 g, 5.59 mmol) and Et_3N (3.12 ml, 22.37 mmol) in dichloromethane (50 mL) was treated with methanesulfonyl chloride (0.865 ml, 11.18 mmol). After 20 min the reaction mixture was poured into sat. sat. NH_4Cl and the layers were separated. The aqueous phase was extracted with dichloromethane and the combined organics layers were dried ($MgSO_4$), filtered and concentrated. The crude residue was dissolved in *N*,*N*–dimethylformamide (50 mL) and sodium cyanide (0.301 g, 6.15 mmol) was added in one portion. After stirring overnight, the reaction mixture was poured into a water/diethyl ether mixture and the layers were separated. The aqueous phase was extracted with diethyl ether and the combined organics layers were washed with water, brine, dried ($MgSO_4$), filtered and concentrated. The crude residue was used without further purification (1.6 g, 97%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.29 (d, *J*=1.83 Hz, 1 H), 7.70 (d, *J*=1.46 Hz, 1 H), 7.33 (m, 1 H), 7.11–7.03 (m, 2 H), 4.04 (s, 2 H), 3.83 (s, 3 H); ES^+ MS: 296 (M + 1).

*4.1.8 Methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (**18**).* A pressure vessel was charged with methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (1.60 g, 5.42 mmol), triethylamine (3.76 ml, 27.1 mmol), *N*,*N*–

dimethylformamide (27 ml), and methanol (27 ml). After degassing with N_2 for 10 min, $Pd(OAc)_2$ (0.122 g, 0.542 mmol) and DPPP (0.224 g, 0.542 mmol) were added. The reaction mixture was then purged/backfilled with N_2 (3x), followed by CO (3x) and finally pressurized with CO at 50 psi and heated to 90 ℃. After stirring 18 h, the r eaction mixture was cooled to 23 ℃ and filtered through a pad of celite and rinsed with diethyl ether. The filtrate was poured into water and the layers were separated. The aqueous phase was extracted with diethyl ether and the combined organics layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (10-100% EtOAc-hexanes gradient) to afford the title compound as an off-white solid (1.25 g, 72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.62 (s, 1 H), 7.79 (s, 1 H), 7.34 (td, *J*=6.96, 2.75 Hz, 1 H), 7.12–7.04 (m, 2 H), 4.27 (s, 2 H), 4.13 (s, 2 H), 4.01 (s, 3 H); ES^+ MS: 319 (M+1).

*4.1.9 3-(3-chloro-2-fluorobenzyl)-12,13-dioxo-5a,6,8,9,10,12,12a,13-octahydro-5H-pyrazolo[1,2-b]pyrido[2,3-g]phthalazine-5-carbonitrile (**19**).* A -78 ℃ solution of Methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (30 mg, 0.094 mmol) in THF (1 mL) was treated with LiHMDS (104 µL, 0.104 mmol). After 20 min, the reaction mixture was treated with a solution of 2,3-dihydro-1H-pyrazolo[1,2-a]pyridazin-5(8H)-one (15.6 mg, 0.113 mmol) in THF (0.2 mL). After 20 min the reaction mixture was warmed to ambient temperature. After an additional 30 min, the reaction mixture was quenched with 10% NH4Cl and diluted with EtOAc. The layers were partitioned and the organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (0-10% MeOH/DCM) to afford the title compound (24 mg, 60% yield).

*4.1.10 1,1-dimethylethyl 2-(2-propyn-1-yl)-1-pyrazolidinecarboxylate (**21**).* A 0 ℃ solution of propargyl alcohol (2.90 ml, 49.8 mmol) in dichloromethane (100 mL) was treated with triethylamine (13.89 ml, 100 mmol) and methanesulfonyl chloride (5.81 ml, 74.7 mmol). After 20 min, the reaction mixture was poured into sat. aq. NH_4Cl and the layers separated. The aqueous phase was extracted with dichloromethane and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*.

A solution of 1,1-dimethylethyl 1-pyrazolidinecarboxylate[40] (4.29 g, 24.91 mmol) and DMF (80 mL) was treated with cesium carbonate (12.2 g, 37.4 mmol) and a solution of 2-propyn-1-yl methanesulfonate (6.67 g, 49.8 mmol) and heated to 50 ℃. After 16 h, the reaction mixture was poured into water and diethyl ether and the layers separated. The aqueous phase was extracted with diethyl ether and the combined organic extracts were washed with water, brine, dried

($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (0-50% ethyl acetate-hexanes) to afford the title compound (3.5 g, 67%) as an orange solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.64–3.53 (m, 4 H), 3.23 (t, J=6.87 Hz, 2 H), 2.24–2.13 (m, 2 H), 2.05 (s, 1 H), 1.50 (s, 9 H).

*4.1.11 3-[(3-chloro-2-fluorophenyl)methyl]-13-hydroxy-12-oxo-6,9,10,12-tetrahydro-8H-pyrazolo[1,2-b]pyrido[2,3-g]phthalazine-5-carbonitrile (**20**).* A –78 ℃ solution of methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (300 mg, 0.941 mmol) in THF (1.3 mL) was treated with lithium bis(trimethylsilyl amide) (1035 µl, 1.035 mmol) dropwise. After stirring for 20 min, a solution of 1-dimethylethyl 2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrazolidinecarboxylate (303 mg, 1.129 mmol) in THF (0.9 mL) was added and stirred at this temperature for 20 min. The reaction mixture was then warmed to 0 ℃ for 30 min and poured into 0.1 M HCl and the layers separated. The aqueous phase was extracted with ethyl acetate and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude residue was dissolved in 1,2-dichloroethane (1.3 mL) and treated with trifluoroacetic acid. After stirring at 23 ℃ for 1 h, the reaction mixture was heated to 90 ℃. After 20 min, the reaction mixture was cooled to ambient temperature and concentrated *in vacuo* to afford the title compound (355 mg, 89%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.93 (s, 1 H), 8.19 (s, 1 H), 7.34 (t, *J*=6.87 Hz, 1 H), 7.17–7.03 (m, 2 H), 4.39 (s, 2 H), 4.27 (s, 2 H), 3.91 (t, *J*=7.42 Hz, 2 H), 3.29 (t, *J*=6.32 Hz, 2 H), 2.43–2.25 (m, 2 H); ES^+ MS: 423 (M+1).

*4.1.12 1,1-dimethylethyl 2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrazolidinecarboxylate (**22**).* A –78 ℃ solution of 1,1-dimethylethyl 1-pyrazolidine carboxylate (1.61 g, 7.66 mmol) in THF (30.6 mL) was added n-BuLi (5.74 ml, 9.19 mmol, 1.6 M hexanes) dropwise. After stirring for 30 min, the reaction mixture was cannulated into a –78 ℃ s olution of methyl chloroformate (0.652 ml, 8.42 mmol) in THF (30 mL). The reaction mixture was warmed to 23 ℃ and after 16 h, poured into sat. aq. NH_4Cl and the layers were separated. The aqueous phase was extracted with ethyl acetate and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (0-30% ethyl acetate-hexanes) to afford the title compound (1.35 g, 66% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.78 (s, 3 H), 3.76 (s, 2 H), 3.60 (t, *J*=7.23 Hz, 2 H), 3.22 (t, *J*=6.96 Hz, 2 H), 2.20 (quin, *J*=7.10 Hz, 2 H), 1.50 (s, 9 H).

*4.1.13 1,1-dimethylethyl (2S)-2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrrolidinecarboxylate (**25**).* A suspension of potassium carbonate (3.20 g, 23.16 mmol) in methanol (20 mL) was cooled to 0 ℃ and diethyl 1-diazo-2-oxopropylphosphonate (2. 83 mL, 17.37 mmol) was added. 1,1-dimethylethyl (*S*)-tert-butyl 2-(2-oxoethyl)pyrrolidine-1-carboxylate[41] (2.34 g, 10.97 mmol) was added, the ice bath was removed and the mixture was stirred for 2 hours after which time the yellow solution took on a green hue. Saturated NH_4Cl was added and the mixture was extracted with ether. The ethereal extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated to give the crude, 1,1-dimethylethyl (2*S*)-2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrrolidinecarboxylate (2.61 g, quantitative), as pale yellow oil. TLC (ethyl acetate/hexanes 1:4, R_f = 0.53). To a -78 ℃ solution of 1,1-dimethylethyl (2S)-2-(2-propyn-1-yl)-1-pyrrolidinecarboxylate (2.611 g, 12.48 mmol) in THF (25 mL) was slowly added *n*-butyllithium (8.32 mL, 13.32 mmol, 1.6 M in hexanes) to give a dark mixture. After 30 minutes at -78 ℃ the dark solution was added to an awaiting -78 ℃ solution of methyl chloroformate (0.987 mL, 12.74 mmol) in THF (10 mL) via cannula. After the addition the dark mixture was stirred for 5 minutes at -78 ℃ and then the bath was removed. After 5 minutes TLC was taken and showed the reaction to be complete. Saturated NH_4Cl was added and the mixture was diluted with ethyl acetate. After extraction with ethyl acetate, the extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (0-15-30% ethyl acetate,hexanes) to give 1,1-dimethylethyl (2*S*)-2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrrolidinecarboxylate (2.30 g, 78% over 3 steps) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.03 – 3.88 (m, 1 H), 3.77 (s, 3 H), 3.48 – 3.32 (m, 2 H), 2.87 – 2.42 (m, 2 H), 2.16 – 1.77 (m, 4 H), 1.47 (s, 9 H); TLC (ethyl acetate/hexanes 15:85, R_f = 0.30).

4.1.14 *1,1-dimethylethyl (2R)-2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrrolidinecarboxylate (**26**).* A suspension of potassium carbonate (0.49 g, 3.56 mmol) in methanol (3 mL) was cooled to 0 ℃ and diethyl 1-diazo-2-oxopropylphosphonate (0.58 mL, 2.67 mmol) was added. (*R*)-tert-butyl 2-(2-oxoethyl)pyrrolidine-1-carboxylate[42] (0.38 g, 1.78 mmol) was added, the ice bath was removed and the mixture was stirred for 2 hours after which time the yellow solution took on a green hue. Saturated NH_4Cl was added and the mixture was extracted with ether. The ethereal extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated to give the crude, 1,1-dimethylethyl (2*R*)-2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrrolidinecarboxylate (0.25 g, quantitative), as pale yellow oil. To a -78 ℃ solution of 1,1-dimethylethyl (*2R*)-2-(2-propyn-1-yl)-1-pyrrolidinecarboxylate (0.25 g, 1.19 mmol) in THF (6 mL) was slowly added *n*-butyllithium

(0.72 mL, 1.79 mmol, 2.5M solution in hexanes) to give a dark mixture. After 30 minutes at -78 ℃ the dark solution was added to an awaiting -78 ° C solution of methyl chloroformate (0.138 mL, 12.74 mmol) in THF (3 mL) via cannula. After the addition the dark mixture was stirred for 5 minutes at -78 ℃ and then the bath was removed. A fter 5 minutes TLC was taken and showed the reaction to be complete. Saturated NH_4Cl was added and the mixture was diluted with ethyl acetate. After extraction with ethyl acetate, the extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (0-15-30% ethyl acetate,hexanes) to give 1,1-dimethylethyl (2*R*)-2-[4-(methyloxy)-4-oxo-2-butyn-1-yl]-1-pyrrolidinecarboxylate (0.1 g, 31% yield) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.03 – 3.88 (m, 1 H), 3.77 (s, 3 H), 3.48 – 3.32 (m, 2 H), 2.87 – 2.42 (m, 2 H), 2.16 – 1.77 (m, 4 H), 1.47 (s, 9 H).

*4.1.15 1,1-dimethylethyl (2S)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (**29**).* (*S*)-*tert*-butyl 2-formylpyrrolidine-1-carboxylate[43] (21 g, 0.11 mol) and diethyl 1-diazo-2-oxopropylphosphonate (30.4 g, 0.158 mol) were dissolved in methanol (200 mL), cooled to 0 ℃ and potassium carbonate (30.4 g, 0.158 mol) was added. The mixture was stirred at ice bath temp for 30 minutes. After 18h, the mixture was quenched with saturated NH_4Cl and extracted with ether. The extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated to a yellow oil (14 g) that was used in the next step without further purification. A solution of the crude alkyne in THF (280 mL) was cooled to -78 ℃ a nd treated slowly with a *n*-butyllithium (31.7 mL, 7.314 mmol, 2.5 M in hexanes). After 30 min, the reaction mixture was transferred via cannula into a -78 ℃ solution of methyl chloroform ate (5.92 mL, 76 mmol) in THF (60 mL). After 30 min, the reaction mixture was allowed to warm to ambient temperature and quenched with NH_4Cl and extracted with ethyl acetate. The extracts were washed with brine, dried, filtered and concentrated. The crude material was purified by silica gel chromatography (0-30% ethyl acetate/hexanes) to give 1,1-dimethylethyl (2*S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (7.7 g, 46% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.69 – 4.48 (m, 1 H), 3.78 (br. s., 3 H), 3.54 – 3.26 (m, 2 H), 2.24 – 1.86 (m, 4 H), 1.49 (s, 9 H).

*4.1.16 1,1-dimethylethyl (2R)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (**30**).* (*R*)-*tert*-butyl 2-formylpyrrolidine-1-carboxylate[44] (1.22 g, 6.14 mmol) and diethyl 1-diazo-2-oxopropylphosphonate (1.499 mL, 9.21 mmol) were dissolved in methanol (20 mL), cooled to 0 ℃ and potassium carbonate (1.697 g, 12 .28 mmol) was added. The mixture was

stirred at ice bath temp for 30 minutes. After 18h, the mixture was quenched with saturated NH_4Cl and extracted with ether. The extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated to a yellow oil (1.24 g) that was used in the next step without further purification. A solution of the crude alkyne in THF (12 mL) was cooled to -78 ℃ and treated slowly with a *n*-butyllithium (1.6 M in hexanes) (4.57 mL, 7.31 mmol). The resultant was stirred for 30 minutes at -78 ℃ and transferred by cannula to a -78 ℃ solution of methyl chloroformate (0.54 mL, 7.0 mmol). After 30 min, the cooling bath was removed and the mixture was allowed to warm to rt. The mixture was quenched with NH_4Cl and extracted with ethyl acetate. The extracts were washed with brine, dried, filtered and concentrated. The crude material was purified by silica gel chromatography (0-30% ethyl acetate/hexanes) to give 1,1-dimethylethyl (2*R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (0.780 g, 50% over two steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.69 – 4.48 (m, 1 H), 3.78 (br. s., 3 H), 3.54 – 3.26 (m, 2 H), 2.24 – 1.86 (m, 4 H), 1.49 (s, 9 H).

4.1.17 2-(trimethylsilyl)ethyl (2S)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (**31**). A solution of 1,1-dimethylethyl (*2S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (7.7 g, 30.4 mmol) in dichloromethane (30 mL) was treated with trifluoroacetic acid (9.75 mL) to give a tan colored solution. The mixture was stirred for 15 minutes and concentrated *in vacuo*, and azeotroped twice with toluene and then placed under vacuum overnight to give methyl 3-[(2*S*)-2-pyrrolidinyl]-2-propynoate trifluoroacetate as a pale yellow oil. The crude trifluoroacetate salt was dissolved in dichloromethane (162 mL) and then cooled to 0 ℃. Pyridine (12.3 mL, 152 mmol) was added followed by slow addition of 2-(trimethylsilyl)ethyl chloridocarbonate (6.05 g, 33.5 mmol). The mixture was stirred at 0 ℃ for 20 minutes then room temperature for 20 minutes. Water was added and the mixture was diluted with dichloromethane. The layers were separated and the organic layer was washed with saturated $NaHCO_3$ (x2) and brine (x2), dried over $MgSO_4$, filtered and concentrated. The crude material was purified by silica gel chromatography (0-15-50% EtOAc/hexanes gradient) to give 2-(trimethylsilyl)ethyl (2*S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (6.0 g, 67% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.73 – 4.55 (m, 1 H), 4.26 – 4.16 (m, 2 H), 3.76 (s, 3 H), 3.59 – 3.30 (m, 2 H), 2.22 – 1.90 (m, 4 H), 1.10 – 0.97 (m, 2 H), 0.05 (s, 9 H).

4.1.18 2-(trimethylsilyl)ethyl (2R)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (**32**). A solution of 1,1-dimethylethyl (*2R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (0.489 g, 1.931 mmol) in dichloromethane (2 mL) was treated with trifluoroacetic acid (0.4 mL) to give a tan colored solution. After stirring for 1 hour, additional trifluoroacetic acid (0.2 mL) was added. The mixture was stirred for 15 minutes and judged complete by TLC. The mixture was concentrated, azeotroped twice with toluene and then placed under vacuum overnight to give methyl 3-[(2*R*)-2-pyrrolidinyl]-2-propynoate trifluoroacetate as a pale yellow oil. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.40 – 9.30 (m, 1 H), 8.33 – 7.78 (m, 1 H), 4.58 (t, *J*=6.87 Hz, 1 H), 3.80 (s, 3 H), 3.60 – 3.43 (m, 2 H), 2.50 – 2.40 (m, 1 H), 2.32 – 2.09 (m, 3 H). The crude trifluoroacetate salt was dissolved in dichloromethane (10 mL) and then cooled to 0 ℃. P yridine (0.781 mL, 9.65 mmol) was added followed by slow addition of 2-(trimethylsilyl)ethyl chloridocarbonate (0.384 mL, 2.124 mmol). The mixture was stirred at 0 ℃ for 20 minutes then room temperature for 20 minutes. Water was added and the mixture was diluted with dichloromethane. The layers were separated and the organic layer was washed with saturated $NaHCO_3$ (x2) and brine (x2), dried over $MgSO_4$, filtered and concentrated. The crude material was purified by silica gel chromatography (0-15-50% EtOAc/hexanes gradient) to give 2-(trimethylsilyl)ethyl (2*R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-pyrrolidinecarboxylate (0.481, 84% over 2 steps) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.73 – 4.55 (m, 1 H), 4.26 – 4.16 (m, 2 H), 3.76 (s, 3 H), 3.59 – 3.30 (m, 2 H), 2.22 – 1.90 (m, 4 H), 1.10 – 0.97 (m, 2 H), 0.05 (s, 9 H); TLC (ethyl acetate/hexanes 15:85, R_f = 0.29).

*4.1.19 1,1-Dimethylethyl (2S)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (**35**).* To an ice cold suspension of potassium carbonate (38.6 g, 0.28 mol) and diethyl 1-diazo-2-oxopropylphosphonate (41 g, 0.21 mmol) was added (*S*)-*tert*-butyl 2-formylpiperidine-1-carboxylate[45] (30 g, 0.14 mol) via pipet. The mixture was stirred for 1 hour at which time the reaction was judged complete by TLC (15:85 EtOAc/hexanes). The mixture had turned a pale green in color. The reaction was quenched by the addition of saturated NH_4Cl and extracted with ether. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF (400 mL), cooled to -78 ℃ and treated dropwise with n-BuLi (47.2 mL, 118 mmol, 2.5M in THF). After 30 minutes at -78 ℃, the dark solution was cannulated into a -78 ℃ solution of methyl chlorof ormate (11.2 g, 118 mmol) in THF (50 mL). The mixture was stirred for 5 minutes at -78 ℃ the n warmed to ambient temperature. Saturated

NH_4Cl was added the mixture was diluted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified by silica gel chromatography (0-30% ethyl acetate/hexanes) to give 1,1-dimethylethyl (*2S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (13.5 g, 59%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.39 – 5.12 (m, 1 H), 3.91 – 4.07 (m, 1 H), 3.78 (s, 3 H), 3.08 – 2.91 (m, 1 H), 1.90 – 1.32 (m, 15 H).

*4.1.20 1,1-Dimethylethyl (2R)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (**36**).* To an ice cold suspension of potassium carbonate (4.51 g, 32.64 mmol) and diethyl 1-diazo-2-oxopropylphosphonate (4.20 g, 24.48 mmol) was added 1,1-dimethylethyl (*R*)-*tert*-butyl 2-formylpiperidine-1-carboxylate[46] (3.48 g, 16.32 mmol) via pipet. The mixture was stirred for 1 h at which time the reaction was judged complete by TLC (15:85 EtOAc/hexanes). The mixture had turned a pale lime green in color. The reaction was quenched by the addition of saturated NH_4Cl and extracted with ether. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF (25 mL), cooled to -78 ℃ and treated dropwise with n-BuLi (13.89 m L, 22.23 mmol, 1.6M in THF). After 30 minutes at -78 ℃, the dark solution was cannulated into a -78 ℃ solution of methyl chloroformate (1.579 mL, 20.38 mmol) in THF (10 mL). The mixture was stirred for 5 minutes at -78 ℃ then warmed to room temp. TLC right before dry ice bath was removed indicated the reaction was nearly complete by formation of new more polar spot. Saturated NH_4Cl was added the mixture was diluted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified by silica gel chromatography (0-15-30% ethyl acetate/hexanes) to give 1,1-dimethylethyl (*2R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (2.86 g, 58%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.39 – 5.12 (m, 1 H), 3.91 – 4.07 (m, 1 H), 3.78 (s, 3 H), 3.08 – 2.91 (m, 1 H), 1.90 – 1.32 (m, 15 H); TLC (ethyl acetate/hexanes 15:85, R_f = 0.49).

*4.1.21 2-(trimethylsilyl)ethyl (2S)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (**37**).* A solution of 1,1-dimethylethyl (*2S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (1.0 g, 3.74 mmol) in dichloromethane (4 mL) was treated with trifluoroacetic acid (1.2 mL). After 30 minutes, the reaction mixture was concentrated and then azeotroped with toluene. The crude amine was dissolved in dichloromethane (20 mL), cooled to

0 ℃ and then treated with pyridine (1.5 mL, 18.7 m mol) followed by slow addition of Teoc-Cl (0.74 g, 4.11 mmol). The mixture was stirred for 5 minutes at 0 ℃ and then warmed to room temperature for 30 minutes. The mixture was diluted with dichloromethane. The organic phase was washed with saturated $NaHCO_3$ (x2) and then brine, dried with $MgSO_4$, filtered and concentrated. The crude material was purified by silica gel chromatography (0-30% EtOAc/hexanes) to give 2-(trimethylsilyl)ethyl (*2S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (0.35 g, 30%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.38 – 5.17 (m, 1 H), 4.20 (t, J=8.42 Hz, 2 H), 4.10 – 3.95 (m, 1 H), 3.78 (s, 3 H), 3.11 – 2.98 (m, 1 H), 1.92 – 1.63 (m, 5 H), 1.48 – 1.35 (m, 1 H), 1.05 – 0.98 (m, 2 H), 0.05 (s, 9 H).

*4.1.22 2-(trimethylsilyl)ethyl (2R)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (**38**).* A solution of 1,1-dimethylethyl (*2R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (1.119 g, 4.19 mmol) in dichloromethane (12 mL) was treated with trifluoroacetic acid (4 mL). After 30 minutes, TLC indicated complete consumption of starting material and only baseline material (15:85 ethyl acetate/hexanes). The mixture was concentrated and then azeotroped with toluene. The crude amine was dissolved in dichloromethane (15 mL), cooled to 0 ℃ and then tr eated with pyridine (1.016 mL, 12.56 mmol) followed by slow addition of Teoc-Cl (0.840 mL, 4.60 mmol). The mixture was stirred for 5 minutes at 0 ℃ and then warmed to room temperature for 30 minutes. The mixture was diluted with dichloromethane. The organic phase was washed with saturated $NaHCO_3$ (x2) and then brine, dried with $MgSO_4$, filtered and concentrated. The crude material was purified by silica gel chromatography (0-15-30% EtOAc/hexanes) to give 2-(trimethylsilyl)ethyl (*2R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (1.092 g, 84%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.38 – 5.17 (m, 1 H), 4.20 (t, J=8.42 Hz, 2 H), 4.10 – 3.95 (m, 1 H), 3.78 (s, 3 H), 3.11 – 2.98 (m, 1 H), 1.92 – 1.63 (m, 5 H), 1.48 – 1.35 (m, 1 H), 1.05 – 0.98 (m, 2 H), 0.05 (s, 9 H).

*4.1.23 (R)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-6,6a,7,8,9,11-hexahydroindolizino[7,6-g]quinoline-5-carbonitrile (**42**).* A -78 ℃ solution of methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (0.200 g, 0.63 mmol) in THF (3.0 mL) was treated with lithium bis(trimethylsilyl)amide (0.75 mL, 0.75 mmol, 1.0 M in THF). After 1 h, the deep red solution was treated with a solution of (*R*)-*tert*-butyl 2-(4-methoxy-4-oxobut-2-yn-1-yl)pyrrolidine-1-carboxylate (0.168 g, 0.75 mmol) in THF (1.0 mL). After 20 min, the reaction

mixture was warmed to 0 ℃. After an additional 30 min, the reaction mixture was quenched with 0.1 N HCl and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was dissolved in DCM (8.0 mL) and treated with TFA (2.0 mL). After 3h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in MeOH (5 mL), and treated with 1N NaOH (2.5 mL). A precipitate formed and the reaction mixture was then acidified to pH 3 with 1N HCl and extracted with EtOAc. The organic later was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was suspended in MeOH and filtered to afford the title compound (50 mg, 20% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ = 14.95 (s, 1H), 8.92 (s, 1H), 8.16 (s, 1H), 7.56 - 7.38 (m, 2H), 7.23 (t, *J*=7.9 Hz, 1H), 4.38 (s, 2H), 3.75 - 3.65 (m, 1H), 3.58 - 3.46 (m, 2H), 3.16 - 3.04 (m, 1H), 2.39 - 2.29 (m, 1H), 2.13 - 2.01 (m, 1H). ES+ MS: 422 (M + 1).

*4.1.24 (S)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-6,6a,7,8,9,11-hexahydroindolizino[7,6-g]quinoline-5-carbonitrile (**43**).* A -78 ℃ solution of methyl 5-[(3-chloro-2-fluoroph enyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (0.300 g, 0.94 mmol) in THF (5.0 mL) was treated with lithium bis(trimethylsilyl)amide (1.13 mL, 1.13 mmol, 1.0 M in THF). After 1 h, the deep red solution was treated with a solution of (*S*)-*tert*-butyl 2-(4-methoxy-4-oxobut-2-yn-1-yl)pyrrolidine-1-carboxylate (0.168 g, 0.75 mmol) in THF (1.0 mL). After 20 min, the reaction mixture was warmed to 0 ℃. After an additional 30 min, the rea ction mixture was quenched with 0.1 N HCl and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was dissolved in DCM (4.0 mL) and treated with TFA (1.0 mL). After 2h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in MeOH (5 mL), and treated with 1N NaOH (2.5 mL). A precipitate formed and the reaction mixture was then acidified to pH 3 with 1N HCl and extracted with EtOAc. The organic later was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was suspended in MeOH and filtered to afford the title compound (189 mg, 48% yield) as a tan solid. ^{1}H NMR (400MHz, DMSO-d_6) δ = 14.95 (s, 1H), 8.92 (d, *J*=1.8 Hz, 1H), 8.16 (s, 1H), 7.55 - 7.38 (m, 2H), 7.27 - 7.19 (m, 1H), 4.38 (s, 2H), 4.00 - 3.89 (m, 1H), 3.74 - 3.64 (m, 1H), 3.58 - 3.45 (m, 2H), 3.09 (dd, *J*=13.3, 15.8 Hz, 1H), 2.40 - 2.01 (m, 2H), 1.93 - 1.69 (m, 2H). ES+ MS: 422 (M + 1).

*4.1.25 (R)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-5b,6,7,8,9,11-hexahydroindolizino[1,2-g]quinoline-5-carbonitrile (**44**).* A -78 ℃ solution of methyl 5-[(3-chloro-

2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (0.255 g, 0.800 mmol) in THF (4.0 mL) was treated with lithium bis(trimethylsilyl)amide (0.960 mL, 0.960 mmol, 1.0 M in THF). After 1 h, the deep red solution was treated with a solution of 2-(trimethylsilyl)ethyl (2*R*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (0.299 g, 0.960 mmol) in THF (1.0 mL). After 20 min, the reaction mixture was warmed to 0 ℃. After an additional 20 min, the reaction mixture was quenched with 0.1 N HCl and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was dissolved in THF (7.0 mL) and treated with TBAF (2.4 mL, 2.4 mmol, 1.0 M in THF) and heated to 70 ℃. After 3h, the reaction mixture was treated with 1.0N HCl and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. Methanol an diethyl ether were added and a precipitate formed. The solvents were removed and the solid was washed repeatedly with minimal amounts of methanol. The solid was dried under vacuum to give the title compound (0.264 g, 50%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.92 (d, J=1.81 Hz, 1 H), 8.31 (d, J=1.20 Hz, 1 H), 7.37 – 7.32 (m, 1 H), 7.17 – 7.12 (m, 1 H), 7.11 – 7.05 (m, 1 H), 4.67 (dd, J=11.84, 3.81 Hz, 1 H), 4.41 - 4.55 (m, 1 H), 4.29 (s, 2 H), 3.10 – 3.00 (m, 1 H), 2.90 – 2.82 (m, 1 H), 2.14 – 2.06 (m, 1 H), 1.94 – 1.86 (m, 1 H), 1.83 – 1.69 (m, 1 H), 1.54 – 1.41 (m, 1 H), 1.32 - 1.19 (m, 1 H); ES^+ MS: 422 (M + 1).

*4.1.26 (S)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-5b,6,7,8,9,11-hexahydroindolizino[1,2-g]quinoline-5-carbonitrile (**45**).* A -78 ℃ solution of methyl 5-[(3-chloro-2-fluoroph enyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (0.502 g, 1.45 mmol) in THF (9.0 mL) was treated with lithium bis(trimethylsilyl)amide (2.5 mL, 2.5 mmol, 1.0 M in THF). After 1 h, the deep red solution was treated with a solution of 2-(trimethylsilyl)ethyl (2*S*)-2-[3-(methyloxy)-3-oxo-1-propyn-1-yl]-1-piperidinecarboxylate (0.54 g, 1.73 mmol) in THF (3.0 mL). After 20 min, the reaction mixture was warmed to 0 ℃. After an additional 20 min, the reaction mixture was quenched with 0.1 N HCl and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was dissolved in THF (7.0 mL) and treated with TBAF (5.8 mL, 5.8 mmol, 1.0 M in THF) and heated to 70 ℃. After 3h, the reaction mixture was treated with 1.0N HCl and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. Methanol an diethyl ether were added and a precipitate formed. The solvents were removed and the solid was washed repeatedly with minimal amounts of methanol. The solid was dried under vacuum to give the title compound (0.07 g, 12%) as an orange solid. ^{1}H NMR (400MHz, DMSO-d_6) δ = 8.96 (s, 1H), 8.25 (s, 1H), 7.55 -

7.38 (m, 2H), 7.28 - 7.17 (m, 1H), 4.74 (dd, J=3.5, 11.5 Hz, 1H), 4.40 (s, 2H), 4.21 (dd, J=3.6, 13.0 Hz, 1H), 3.00 - 2.89 (m, 1H), 2.58 (d, J=10.2 Hz, 1H), 2.02 - 1.84 (m, 1H), 1.81 - 1.63 (m, 2H), 1.37 - 1.03 (m, 2H); ES+ MS: 422 (M + 1).

*4.1.27 (R)-3-(3-chloro-2-fluorobenzyl)-11-hydroxy-10-oxo-6,7,8,10-tetrahydro-5bH-pyrrolizino[1,2-g]quinoline-5-carbonitrile (**46**).* A -78 ºC solution of methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (0.237 g, 0.744 mmol) in THF (4.0 mL) was treated with lithium bis(trimethylsilyl)amide (0.892 mL, 0.892 mmol, 1.0M in THF). The red mixture was stirred at -78 ºC for 20 min before adding a solution of (*R*)-2-(trimethylsilyl)ethyl 2-(3-methoxy-3-oxoprop-1-yn-1-yl)pyrrolidine-1-carboxylate (0.265 g, 0.892 mmol) in THF (1 mL). The mixture was stirred at -78 ℃ for 20 minutes and then warmed to 0 ℃. After 15 minutes the reaction was judged compl ete by LCMS. The mixture was quenched with 0.1 N HCl and extracted with ethyl acetate. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF/DMF (1:1 - 5.0 mL) and treated with cesium fluoride (0.282 g, 1.859 mmol) and heated to 90 ℃. After 7 hours, the reaction mixture was cooled to ambient temperature and 1.0 N HCl was added. The mixture was extracted with ethyl acetate. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The material was by silica get chromatography (0-100% ethyl acetate /hexanes gradient) to afford the title compound (60 mg, 20%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.90 (m, 1 H), 8.29 (m, 1 H), 7.38 – 7.32 (m, 1 H), 7.18 – 7.06 (m, 2 H), 5.0 (dd, J=10.63, 6.02 Hz, 1 H), 4.30 (s, 2 H), 3.85 – 3.77 (m, 1 H), 3.52 – 3.44 (m, 1 H), 2.73 – 2.64 (m, 1 H), 2.50 – 2.34 (m, 2 H), 1.48 – 1.37 (m, 1 H); ES+ MS: 408 (M + 1).

*4.1.28 (S)-3-(3-chloro-2-fluorobenzyl)-11-hydroxy-10-oxo-6,7,8,10-tetrahydro-5bH-pyrrolizino[1,2-g]quinoline-5-carbonitrile (**47**).* A -78 ºC solution of methyl 5-[(3-chloro-2-fluorophenyl)methyl]-3-(cyanomethyl)-2-pyridinecarboxylate (0.153 g, 0.480 mmol) in THF (3.0 mL) was treated with lithium bis(trimethylsilyl)amide (0.576 mL, 0.576 mmol, 1.0M in THF). The red mixture was stirred at -78 ºC for 20 min before adding a solution of (*R*)-2-(trimethylsilyl)ethyl 2-(3-methoxy-3-oxoprop-1-yn-1-yl)pyrrolidine-1-carboxylate (0.171 g, 0.576 mmol) in THF (0.6 mL). The mixture was stirred at -78 ℃ for 20 minutes and then warmed to 0 ℃. After 25 minutes the reaction was judged compl ete by LCMS. The mixture was quenched with 0.1 N HCl and extracted with ethyl acetate. The extracts were washed with brine, dried

over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF (5.0 mL) and treated with TBAF (0.96 mL, 0.96 mmol) and heated to 70 ℃. After 8h, the reaction mixture was treated with 1.0N HCl and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (0-100% EtOAc/hexanes) to afford the title compound (16 mg, 22%) as an orange solid. ^{1}H NMR (400MHz, $CDCl_3$) δ = 8.87 (s, 1H), 8.27 (s, 1H), 7.33 (s, 1H), 7.17 - 7.02 (m, 2H), 4.97 (dd, *J*=6.0, 10.6 Hz, 1H), 4.27 (s, 2H), 3.79 (d, *J*=11.5 Hz, 1H), 3.51 - 3.40 (m, 1H), 2.71 - 2.60 (m, 1H), 2.49 - 2.31 (m, 2H), 1.40 (dd, *J*=8.5, 11.1 Hz, 1H). ES+ MS: 408 (M + 1).

*4.1.29 (R)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-6,6a,7,8,9,11-hexahydroindolizino[7,6-g]quinoline-5-carboxamide (**50**).* A suspension of (R)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-6,6a,7,8,9,11-hexahydroindolizino[7,6-g]quinoline-5-carbonitrile (0.034 g, 0.081 mmol) in acetic acid (0.6 mL) and water (0.6 mL) was treated with sulfuric acid (0.6 mL) to affect dissolution and heated to 120 ºC. After 8 h, the reaction mixture was concentrated *in vacuo* and the crude product recrystallized from MeOH to afford the title compound (24 mg, 67%) as a yellow solid. ^{1}H NMR (400MHz, DMSO-d_6) δ = 14.15 (s, 1H), 8.82 (d, *J*=1.8 Hz, 1H), 7.96 (s, 2H), 7.82 (br. s., 1H), 7.49 (t, *J*=6.9 Hz, 1H), 7.42 - 7.35 (m, 1H), 7.25 - 7.17 (m, 1H), 4.31 - 4.23 (m, 2H), 3.87 - 3.63 (m, 2H), 3.57 - 3.46 (m, 1H), 3.31 - 3.22 (m, 2H), 2.86 - 2.72 (m, 1H), 2.31 (dd, *J*=5.5, 11.3 Hz, 1H), 2.11 - 1.98 (m, 1H), 1.92 - 1.65 (m, 2H) ES^+ MS: 440 (M + 1).

*4.1.30 (S)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-6,6a,7,8,9,11-hexahydroindolizino[7,6-g]quinoline-5-carboxamide (**51**).* A suspension of (S)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-6,6a,7,8,9,11-hexahydroindolizino[7,6-g]quinoline-5-carbonitrile (0.082 g, 0.194 mmol) in acetic acid (0.7 mL) and water (0.7 mL) was treated with sulfuric acid (0.7 mL) to affect dissolution. The mixture was heated at 125 ºC for 8 hours after which time the reaction was judged complete by LCMS. The mixture was purified by reverse phase chromatography to give the title compound (74 mg, 87%) as a yellow solid. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 8.77 (s, 1H), 8.14 (s, 1H), 7.35 - 7.30 (m, 1H), 7.14 - 7.01 (m, 2H), 6.32 (br. s., 1H), 4.14 (s, 2H), 3.81 (br. s., 1H), 3.70 (t, J=10.5 Hz, 1H), 3.52 - 3.39 (m, 2H), 2.81 - 2.67 (m, 1H), 2.41 - 2.30 (m, 1H), 2.18 - 2.06 (m, 1H), 1.97 - 1.83 (m, 1H), 1.78 - 1.63 (m, 1H). ES+ MS: 440 (M + 1).

4.1.31 *(R)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-5b,6,7,8,9,11-hexahydroindolizino[1,2-g]quinoline-5-carboxamide (**52**)*. A suspension of (*R*)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-5b,6,7,8,9,11-hexahydroindolizino[1,2-g]quinoline-5-carbonitrile (0.092 g, 0.218 mmol) in acetic acid (0.7 mL) and water (0.7 mL) was added sulfuric acid (0.5 ml) to affect dissolution. The solution was stirred at room temperature for 15 minutes and then heated to 90 ℃ for 1 hour. Heating was increased to 125 ℃ for 4 hours and then the mixture was cooled and allowed to stir overnight at room temperature. LCMS indicated conversion to the primary amide and decarboxylation products (1:1 ratio). The mixture was purified by reverse phase chromatography twice to give the title compound (25 mg, 26%) as a grey-tinged solid. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 8.87 (m, 1 H), 8.48 (m, 1 H), 7.43 – 7.29 (m, 2 H), 7.19 – 7.12 (m, 1 H), 4.81 – 4.74 (m, 1 H), 4.43 – 4.32 (m, 3 H), 3.10 – 3.00 (m, 1 H), 2.58 – 2.51 (m, 1 H), 2.05 – 1.95 (m, 1 H), 1.89 – 1.67 (m, 2 H), 1.48 – 1.32 (m, 1 H), 1.23 – 1.09 (m, 1 H); ES+ MS: 440 (M + 1).

4.1.32 *(S)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-5b,6,7,8,9,11-hexahydroindolizino[1,2-g]quinoline-5-carboxamide (**53**).* A suspension of (*S*)-3-(3-chloro-2-fluorobenzyl)-12-hydroxy-11-oxo-5b,6,7,8,9,11-hexahydroindolizino[1,2-g]quinoline-5-carbonitrile (0.050 g, 0.12 mmol) in acetic acid (0.5 mL) and water (0.5 mL) was added sulfuric acid (0.5 ml) to affect dissolution and then heated to 95 ℃. After 6h, the reaction mixture was cooled to ambient temperature and poured into water and DCM/MeOH, The layers were partitioned and the organic phase washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by reverse phase chromatography twice to give the title compound (10 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.87 (m, 1 H), 8.48 (m, 1 H), 7.43 – 7.29 (m, 2 H), 7.19 – 7.12 (m, 1 H), 4.81 – 4.74 (m, 1 H), 4.43 – 4.32 (m, 3 H), 3.10 – 3.00 (m, 1 H), 2.58 – 2.51 (m, 1 H), 2.05 – 1.95 (m, 1 H), 1.89 – 1.67 (m, 2 H), 1.48 – 1.32 (m, 1 H), 1.23 – 1.09 (m, 1 H); ES+ MS: 440 (M + 1).

4.2 Virology

Reagents. Reverse transcriptase and integrase were prepared as previously described.[47] Deoxynucleotide triphosphates (dNTPs) and protease-free Bovine Serum Albumin (BSA;

Fraction V) were acquired from Roche Applied Science (Indianapolis, IN). [8-^{3}H(N)]-deoxyguanosine 5'-triphosphate (dGTP) and streptavidin-coated polystyrene SPA imaging beads were acquired from PerkinElmer (Waltham, MA). 1,4-dithiothreitol (DTT), CHAPS and dimethly sulfoxide (DMSO) were acquired from Sigma-Aldrich (St. Louis, MO). NP-40 was acquired from Thermo Scientific (Rockford, IL). Nuclease-free dH_2O, Tris pH 8.0, NaCl, KCl, MgCl, and EDTA were acquired from Life Technologies (Grand Island, NY). All compounds were prepared as 10 mM stocks in DMSO and stored at -20 ℃.

The following oligonucleotides were used and were acquired from Midland Certified Reagent Company (Midland, TX) and Integrated DNA Technologies (Coralville, IA).

Oligo 1, 5'-AGGUG AGUGA GAUGA UAACA AAUUU GCGAG CCCCA GAUGC-3'

Oligo 2, 5'-Biotin-GCATC TGGGG CTCGC AAATT TG-3'

Oligo 3, 5'-CCCCC CCCCC CCCCC AGGTG AGTGA GATGA TAAC-cordycepin-3'

All lyophilized oligonucleotides were reconstituted to 200 µM in 10 mM Tris pH 8.0, 10 mM NaCl and stored at -20℃ in small aliquots prior to use.

Substrate A was prepared by equilibrating 20 µM of Oligo 1 and 20 µM of Oligo 2 in 10 mM Tris pH 8.0, 10 mM NaCl at 95℃, followed by a gradual d ecrease to 80℃ (0.1℃ decrease /6 seconds) and then to 4℃ (0.1C/20 seconds) with a D yad DNA Engine thermal cycler (BioRad; Hercules, CA).

Pseudo-HIV assay. The antiviral activities of compounds were measured in a single-round assay using a self-inactivating PHIV lentiviral vector. CIP4 cells (2 _ 104/well) infected with an amount of PHIV sufficient to produce approximately 50,000 relative light units were added to 96-well black, clear-bottom plates and were incubated for 2 days with S/GSK1349572 at varying concentrations.Infected cells were measured as a function of luciferase activity in a luminometer using the Steady-Glo reagent (Promega Corporation).[29]

4.3 *In vivo DMPK*

Male non-fasted Sprague Dawley (CD) rats (n=2 per study arm) received test article at doses of 1 mg/kg i.v. (1 mL/kg) or 5 mg/kg p.o. (5 mL/kg), formulated in a 10% DMSO/10% Solutol/80% 0.05 M N-methylglucamine dosing vehicle. For all animals, water was provided *ad libitum*. Blood samples were withdrawn from a surgically-implanted venous cannula at timed intervals

for 24 h after dose administration, treated with EDTA, and centrifuged to harvest plasma for LC/MS/MS analysis. Plasma concentration-time data for individual rats were analyzed using non compartmental analysis (WinNonlin v. 4.1a; Pharsight, Mountain View CA) to generate pharmacokinetic parameter estimates.

All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

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Figure 1. Representative integrase strand transfer inhibitors

Scheme 1. Design of the 8-hydroxyquinoline tetracyclic lactam scaffold

Scheme 2. Initial synthesis of the HQT scaffold

Scheme 3. Unsuccessful strategy to synthesizing the HQT scaffold

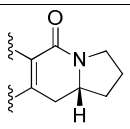
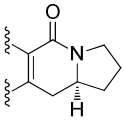
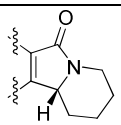
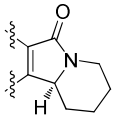
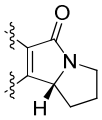
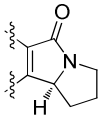
Scheme 4. Successful Michael-Dieckmann cyclization with an ynoate coupling partner

Scheme 5. Synthesis of ynoate coupling partners

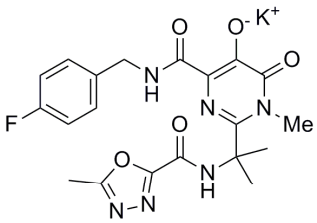
Scheme 6. Synthesis of HQT analogs using a Michael-Dieckmann cyclization

Figure 2. Hydrolysis of the C5-nitrile to afford a primary amide

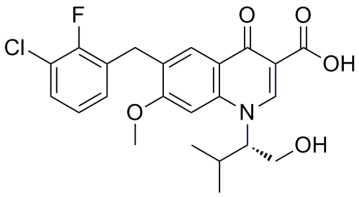
Table 2. *In vivo* DMPK profile of HQT analog **52**

Cmpd	Structure	Ynoate R=	Cyclization Yield[1]	pHIV IC_{50} (μM)	pHIV $PAIC_{50}$ (μM)[2]	pHIV Q148K (FC)	pHIV G140S/Q148H (FC)	Toxicity CC_{50} (μM)
42		Boc	20%	0.007	4.54	0.8x	1.5x	12.8
43		Boc	48%	0.014	0.066	1.4x	2.8x	50
44		Teoc	78%	0.015	0.457	8.8x	17x	7.05
45		Teoc	12%	0.018	1.28	17x	93x	3.36
46		Teoc	20%	0.005	0.294	216x	456x	7.27
47		Teoc	22%	0.010	3.32	31x	131x	1.83

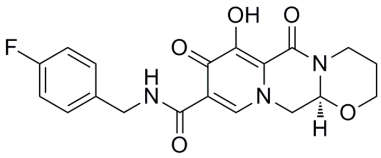
Species	*Clt (mL min^{-1} kg^{-1})*	% HBF	$t_{1/2}$ (h)	V_{dss}	F (%)	Permeability (nm/s)	CLND (µM solubility)
Rat	1.4	2	7.2	0.4	2	60	25
Dog	2.8	5	11.8	1.2	14		



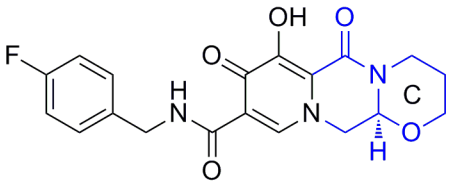
Raltegravir (1)
$^{pHIV}IC_{50}$ = 2 nM
Q148K = 83 FC
Q148H/G140S = 480 FC



Elvitegravir (2)
$^{pHIV}IC_{50}$ = 0.7 nM
Q148K = 97 FC
Q148H/G140S = >1700 FC



Carbamoylpyridone (3)
$^{pHIV}IC_{50}$ = 2 nM
Q148K = 2.4 FC

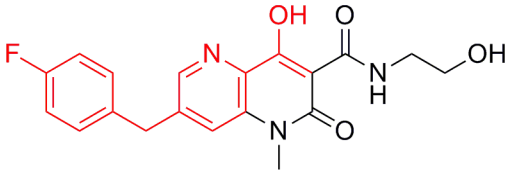


3

Carbamoylpyridone

$^{pHIV}IC_{50}$ = 2 nM

Q148K = 2.4 FC

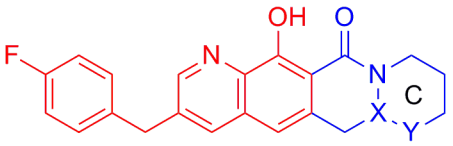


4

Naphthyridinone

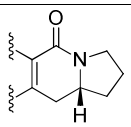
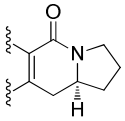
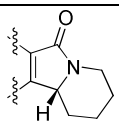
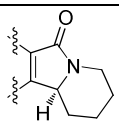
$^{pHIV}IC_{50}$ = 3 nM

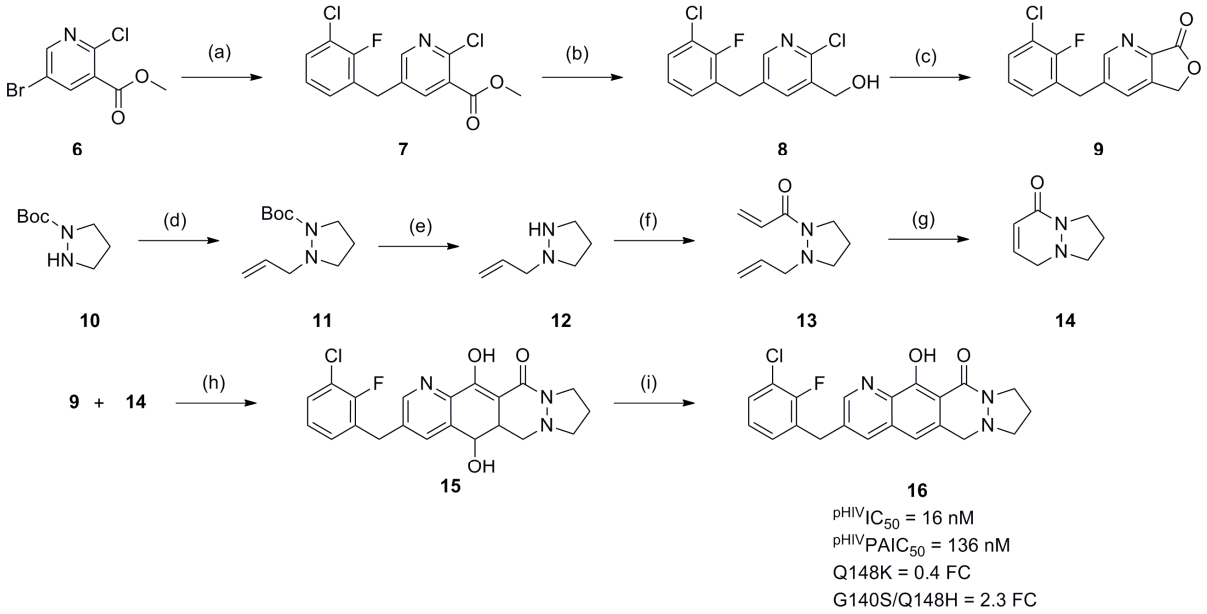
Q148K = 40 FC



5

8-Hydroxyquinoline Tetracyclic Lactam

Cmpd	Structure	Hydrolysis Yield	pHIV IC_{50} (μM)	pHIV $PAIC_{50}$ $(\mu M)^2$	pHIV Q148K IC_{50} (FC)	pHIV G140S/Q148H IC_{50} (FC)	Toxicity CC_{50} (μM)
50		67%	0.008	0.030	0.6x	0.4x	21.1
51		90%	0.003	0.015	0.6x	2.6x	36.8
52		26%	0.0007	0.001	1.4x	2.8x	24.4
53		100%	0.0006	0.002	0.1x	3.7x	5.18

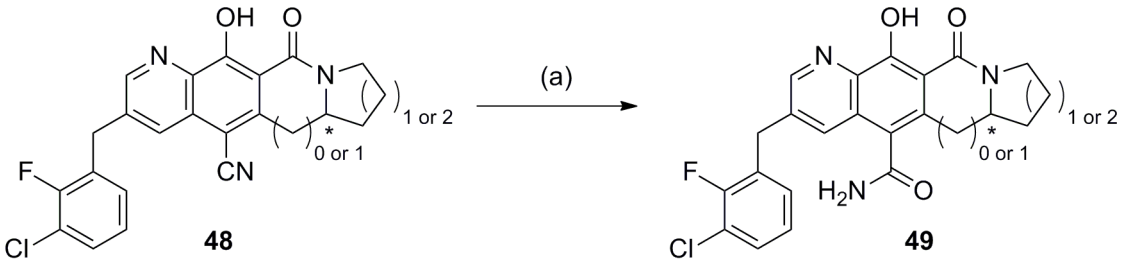


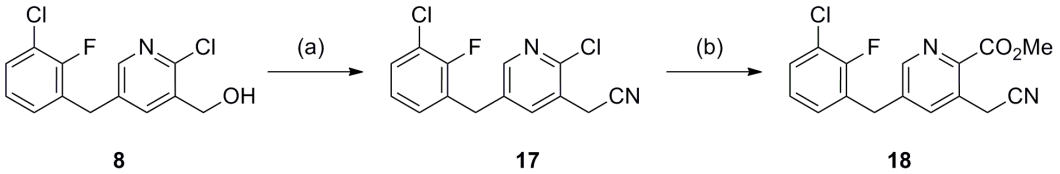
$^{pHIV}IC_{50}$ = 16 nM

$^{pHIV}PAIC_{50}$ = 136 nM

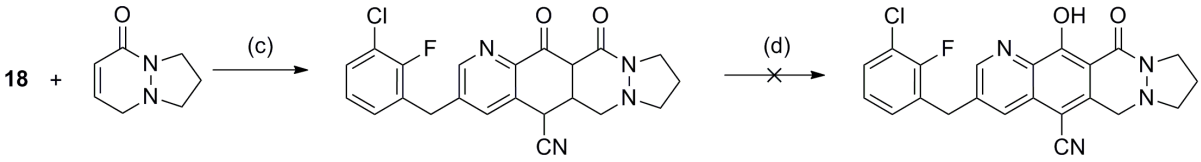
Q148K = 0.4 FC

G140S/Q148H = 2.3 FC

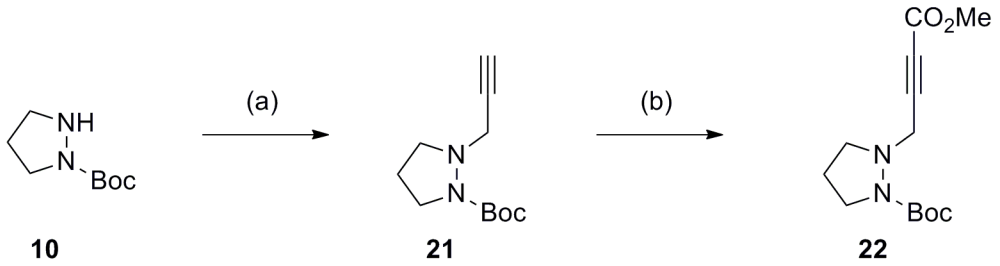




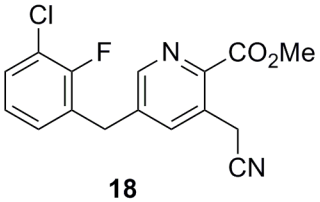
8 **17** **18**



14 **19** **20**



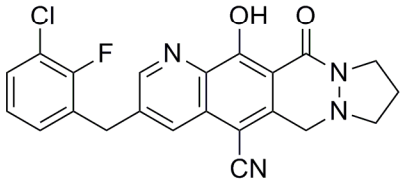
10 **21** **22**



18

\+ **22**

(c)



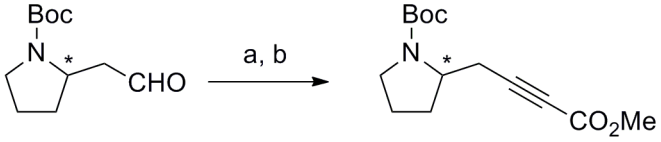
20

$^{pHIV}IC_{50}$ = 2.2 nM

$^{pHIV}PAIC_{50}$ = 415 nM

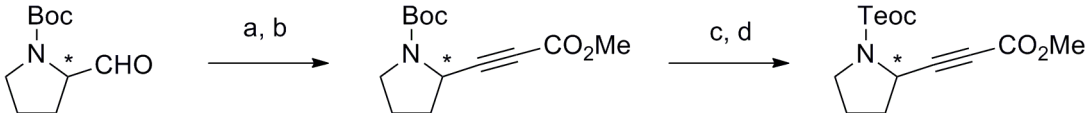
Q148K = 1.3 FC

G140S/Q148H = 8 FC



(*S*) **23**
(*R*) **24**

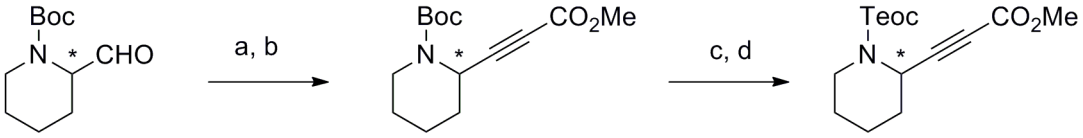
(*S*) **25**
(*R*) **26**



(*S*) **27**
(*R*) **28**

(*S*) **29**
(*R*) **30**

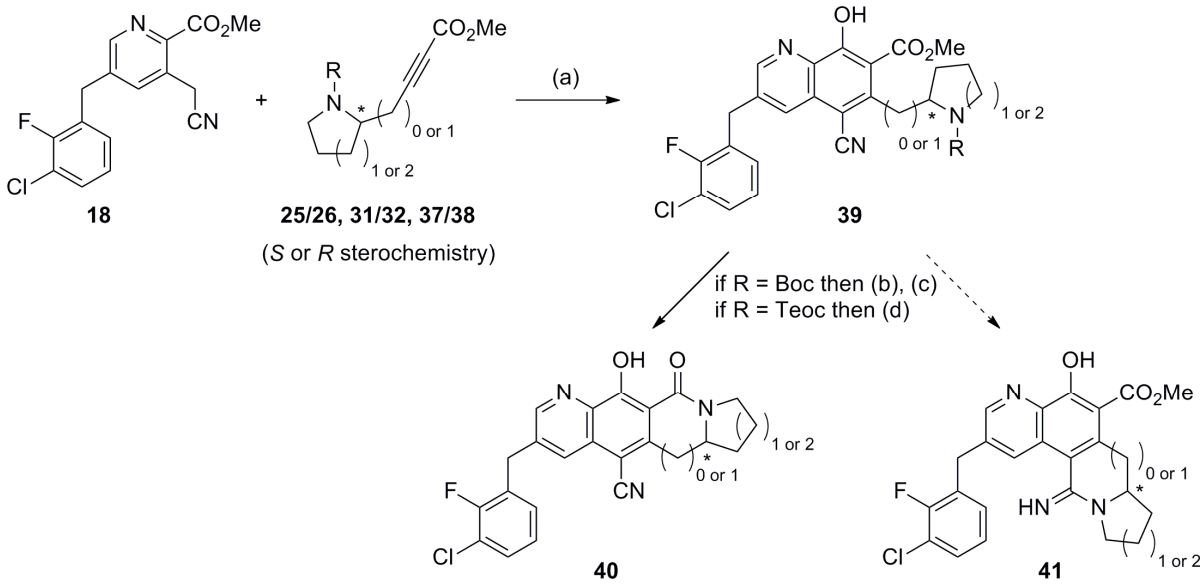
(*S*) **31**
(*R*) **32**



(*S*) **33**
(*R*) **34**

(*S*) **35**
(*R*) **36**

(*S*) **37**
(*R*) **38**



Highlights

► A structurally unique HIV-1 integrase strand transfer inhibitor was designed by incorporating features from two different literature scaffolds. ► A number of examples demonstrated exquisite antiviral potency with a little loss of activity against integrase signature resistance mutations Q148K and G140S/Q148H ► Rat and dog *in vivo* studies demonstrated poor oral bioavailability likely attributed to solubility limited absorption.