Structure−Activity Relationships of *N*-Acyl Pyrroloquinolone PDE-5 Inhibitors

James C. Lanter,* Zhihua Sui, Mark J. Macielag, James J. Fiordeliso, Weiqin Jiang, Yuhong Qiu, Sheela Bhattacharjee, Patricia Kraft, T. Mathew John, Donna Haynes-Johnson, Elizabeth Craig, and Joanna Clancy

Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development LLC, 1000 Route 202 S, P.O. Box 300, Raritan, New Jersey 08869

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The discovery of the potent and selective PDE-5 inhibitory activity of a pyrroloquinolone scaffold prompted us to explore the SAR of its acyl derivatives. During the course of these studies, three structural series were found with K_i values for PDE-5 in the subnanomolar range. Systematic modification of one of these leads produced a compound with excellent selectivity for PDE-5 over other phosphodiesterases and oral bioavailability of 15% in male rats. This compound also displayed in vivo efficacy in an anesthetized canine model of erection when dosed intravenously.

Introduction

The nucleotides 3′,5′-cyclic adenosine monophosphate (cAMP) and 3′,5′-cyclic guanosine monophosphate (cGMP) are important secondary messengers that regulate many physiological processes. Extracellular stimulation causes a rapid change in cyclic nucleotide levels, producing the physiological responses elicited by the stimulus. The levels of these intracellular cyclic nucleotides are modulated by the cyclases that synthesize and phosphodiesterases (PDEs) that degrade them.

To date, 21 mammalian PDE genes have been cloned and classified into 11 families according to the sequence homology and biochemical properties.[1] These enzymes are expressed in multiple tissues, and, generally speaking, in any given tissue multiple PDEs coexist. Nevertheless, PDEs show differential expression patterns, making tissue-selective inhibition of PDE activity feasible. Sildenafil is a successful example of tissue-selective action. Its molecular target, PDE-5, is the major cGMP-hydrolyzing enzyme in human corpus cavernosal smooth muscle.[2] Normal erection is mediated through nitric oxide (NO)/cGMP pathway[3−5] wherein sexual stimulation causes the release of NO from noncholinergic, nonadrenergic neurons innervating the corpus cavernosum. Concurrently, soluble guanylyl cyclases in corpus cavernosal smooth muscle are activated and begin to synthesize cGMP from GTP, triggering a cascade of downstream events leading to smooth muscle relaxation and erection. By inhibiting the ability of PDE-5 to break down cGMP, sildenafil increases the half-life of this second messenger, indirectly extending the activity of NO in penile tissue.[6] This is particularly effective in male erectile dysfunction patients who are unable to generate sufficient amounts of NO during the initial phase of erection.

This effectiveness coupled with the fact that sildenafil is the first oral agent for the treatment of male erectile dysfunction has sparked tremendous interest in the search for new PDE-5 inhibitors.[7] Opportunities for next generation compounds reside mainly in their activity profile versus PDE-1 and PDE-6 over which sildenafil has demonstrated limited selectivity. For example, cross inhibition of PDE-6 causes the highly publicized visual disturbance side effect occurring in some patients taking sildenafil.[8]

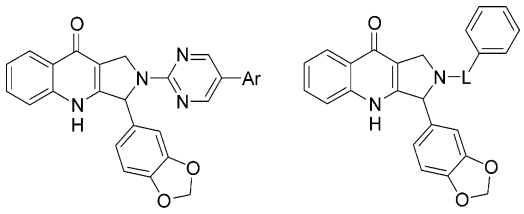

Figure 1. Lead structure and study prototype.

Results and Discussion

Recently[9] we reported the discovery of *N*-pyrimidinyl pyrroloquinolones **1** as potent PDE-5 inhibitors (Figure 1). Key features of the SAR that emerged from that study include the importance of substituents on C-3 and the necessity of N-2 substitution for PDE-5 inhibition. The pendant phenyl ring on pyrimidine **1** was found to tolerate both polar and nonpolar substituents. In an effort to further delineate the SAR for PDE-5 inhibition of the pyrroloquinolone (PQ) core, we undertook a study to examine the replacement of the heterocyclic moiety with an acyclic linker as noted in structure **2**.

Using known methodology,[10] we assembled the precursor tetrahydro β-carboline **3** in one step from tryptamine and piperonal (Scheme 1). Acylation of this intermediate with a variety of agents proceeded smoothly, but subsequent Winterfeldt oxidation[11] of the acylated products **4** failed to deliver the desired products in anything more than trace amounts. Direct oxidation of **3** to **7**, in addition to affording a poor yield of the desired product, resulted in the formation of significant quantities of dihydro and fully aromatized β-carboline. Isolation of pure **7** from this mixture proved difficult due to its poor solubility characteristics and relatively unstable nature. Ultimately, we chose to first benzylate **3** and

* To whom correspondence should be addressed. Phone: (908)-704-4688, Fax: (908)-526-6469, jlanter@prdus.jnj.com.

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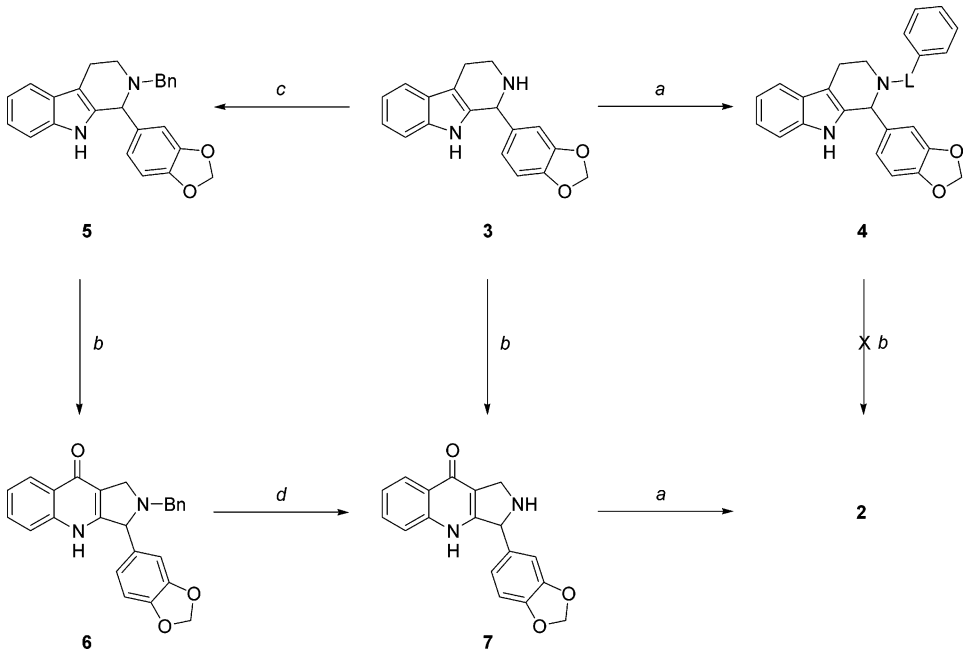
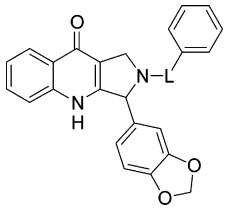
Scheme 1: Synthesis of Pyrroloquinolone Targets[a]



[a] Conditions: a. Cl-L-Ph, (4-$NO_2C_6H_4$)OC(O)OCH_2Ar or OCN-CH_2Ph, Et_3N, THF; b. KO^{t}Bu, O_2/DMF; c. BnBr, Et_3N/DMF; d. HCl, H_2, Pd−C(cat)/MeOH.

Table 1. Linker SAR Studies

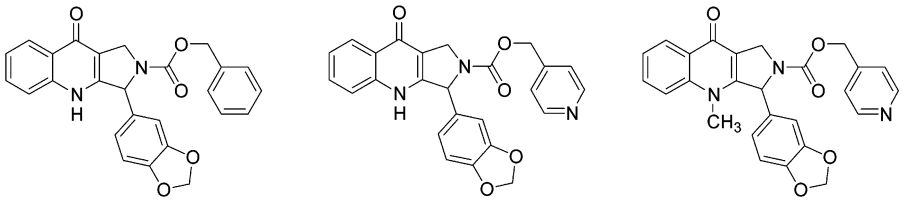


compound	L	K_i, nM (SD)
8	C(O)C≡C-	0.35 (± 0.04)
9	C(O)CH=CH	0.22 (± 0.16)
10	$C(O)CH_2CH_2$	4.53 (± 1.32)
11	$C(O)CH_2$	12.5 (± 2.89)
12	C(O)	2.49 (± 1.16)
13	CH_2CH=CH	23.3 (± 6.14)
14	SO_2CH=CH	118 (± 8.77)
15	SO_2CH_2	113 (±17.9)
16	$C(O)NHCH_2$	1.06 (± 0.23)
17	$C(O)NMeCH_2$	11.0 (±1.49)
18	$C(O)OCH_2$	0.58 (± 0.16)
sildenafil	−	1.91 (± 0.33)

then subject **5** to the Winterfeldt protocol. Benzylated pyrroloquinolone **6** could be isolated in excellent purity by precipitation from the reaction mixture. This material was amenable to long term storage and was easily converted to **7** as needed by hydrogenolysis in acidic methanol. Elaboration of **7** with a variety of acylation and alkylation reagents produced the initial series of compounds for the SAR study.

First we considered amide derivatives of **7**. The unsaturated amides, propynoyl (**8**) and propenoyl (**9**) showed excellent PDE-5 inhibitory activity in the subnanomolar range (Table 1). Saturation of the double bond in **9** resulted in a 1 order of magnitude loss in potency (**10**). This is probably due unfavorable entropic factors resulting from the increased flexibility of the alkane relative to the alkene offsetting any additional van der Waals interactions. Shortening the linker between the amide and the phenyl ring (**11** and **12**) failed to return the inhibitory activity to its previous levels. Although these two analogues do not have as many degrees of freedom as the propionamide, more favorable entropic factors are probably balanced by their lower potential for hydrophobic interaction. Removal of the carbonyl (**13**) resulted in a 100-fold decrease in potency relative to the cinnamoyl compound, implying that the carbonyl group plays an important role in binding to the enzyme active site. Surprisingly, changing from the amide to a sulfonamide functionality (**14**) caused a loss of 3 orders of magnitude inhibitory activity relative to **9**. Attempting to shorten the linker (**15**) to account for the greater size of the sulfur atom failed to have any effect, indicating that the carbonyl interaction is fairly sensitive and might not tolerate the longer sulfur−oxygen bond length. In addition, the presence of the second sulfur oxygen bond could project a polar group into a hydrophobic region of the enzyme. With these results in mind, we replaced the cinnamide with a benzyl urea (**16**) in a bid to access a carbonyl compound with rigidity similar to a cinnamide but lacking its electrophilic character. Although **16** was a reasonable PDE-5 inhibitor ($K_i \sim 1$ nM), attempts to improve its solubility characteristics by methylating the urea nitrogen (**17**) caused a 10-fold drop in potency. This is probably due a predisposition toward the "*S*-trans" conformation analogous to that of secondary amides,[12] a bias lacking in disubstituted analogues such as **17**. Since esters are known to prefer the same conformation as secondary amides, it seemed logical that replacement of the urea with a carbamate should serve as a reasonable cinnamide mimetic with better physical properties than **16**. We were pleased to note that when the pendant nitrogen of **16** was replaced with oxygen (**18**), the PDE-5 inhibitory activity returned to the level of our two initial leads.

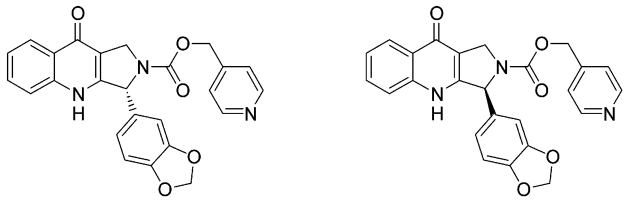
During studies to adjust the aqueous solubility of structures such as **9**, we noted its propensity to undergo



18, K_i = 0.58 nM **19**, K_i = 0.29 nM **20**, K_i = 601 nM

Figure 2. Carbamate modification.

Table 2. Selectivity Data for the Enantiomers of **19**



R-(-)-**19** *S*-(+)-**19**

compound	K_i (nM)	PDE-1/PDE-5	PDE-2/PDE-5	ratio PDE-3/PDE-5	PDE-4/PDE-5	PDE-6(cone)/PDE-5	PD-6(rod)/PDE-5
R-**19**	0.23	283 000	77 100	441 000	42 800	28.3	52.4
S-**19**	3.48	28 700	560	1320	2710	32.4	13.2
sildenafil	1.91	140	8910	12 900	2560	8.0	11.6

conjugate addition. As a result we focused our attention on modification of **18**. The 4-pyridyl derivative (**19**), in addition to being readily accessible from commercially available 4-pyridyl carbinol, was also known to increase the stability[13] of the carbamate moiety (Figure 2). We were pleased to see that **19** had the same potency as **9**. Methylation of **19** caused a 2000-fold drop in inhibitory activity, reinforcing our previous observations regarding the importance of the NH moiety. Separation of **19** into its enantiomers was accomplished through the use of chiral HPLC. These enantiomers were then examined for their selectivity for PDE-5 relative to other PDE isozymes (Table 2). The *R*-(−)-enantiomer is an order of magnitude stronger inhibitor of PDE-5 than either its antipode or sildenafil. Even more striking is its selectivity profile versus that of sildenafil. The (*R*)-enantiomer of **19** is more than 280000 times more selective for PDE-5 versus PDE-1, 28-fold more potent for PDE-5 than for PDE-6 (cone), and 52-fold more discriminating for PDE-5 than PDE-6 (rod). By comparison, sildenafil is only 140-, 8-, and 11-fold selective for PDE-5 over these same isozymes. Considering that PDE-1 and PDE-6 are the major cGMP hydrolyzing enzymes in the vasculature and retinal tissues, respectively, these results have important implications regarding potential side effects of our structures.

Due to its ease of synthesis, racemic **19** was studied both in vitro in a functional cell model and in vivo in a canine model for erection. The functional assay utilized rat fetal lung fibroblast (RFL-6) cells treated with sodium nitroprusside as a baseline to measure a compound's ability to elevate intracellular cGMP levels. By blocking the action of PDE-5, an inhibitor should be able to raise cGMP levels in a dose-dependent manner over this baseline. As noted in Figure 3, *rac*-**19** possessed efficacy equal to sildenafil in the cell-based assay, inducing a 3-fold increase at a lower concentration than sildenafil and a 5-fold increase at the same concentration. The in vivo model of erection utilizes electrically stimulated anesthetized dogs to measure the effects of

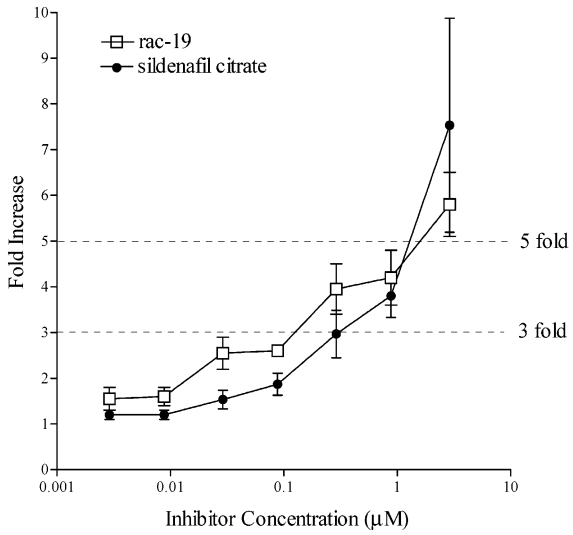


Figure 3. RFL-6 functional data.

administered compounds on the intracavernosal blood pressure (ICP). A PDE-5 inhibitor should cause a rise in ICP as cGMP levels are elevated and the penis becomes engorged. In our experiment, **19** was dosed to the animals by the intravenous route using sildenafil citrate as a positive control. In this model, **19** was shown to reach the same maximum efficacy as sildenafil, but was determined to be at least 1 order of magnitude less potent (Figure 4). Preliminary pharmacokinetics studies indicated that **19** had an oral bioavailability of 15% in male rats as compared with 11% for sildenafil in the same study, demonstrating the potential of the carbamate series as an oral therapy for male erectile dysfunction.

In conclusion, we have discovered a novel series of potent and selective PDE-5 inhibitors. The lead compound was shown to have in vivo efficacy in a canine erection model and oral bioavailability in preliminary pharmacokinetic studies.

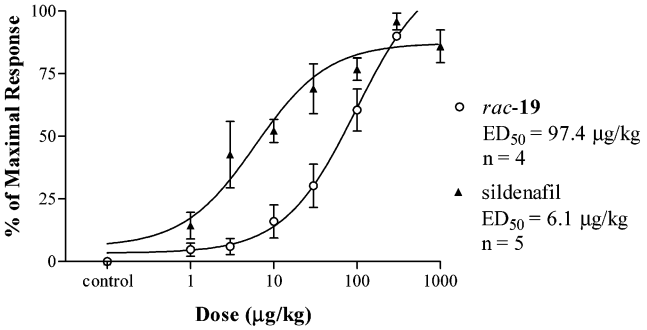


Figure 4. In vivo results.

Experimental Section

PDE Isolation. PDE-1, -2, -3, -4, -5, and -6 were isolated from human heart, corpus cavernosum, platelet, skeletal muscle, corpus cavernosum, and retina, respectively, according to the protocol described by Boolell[6] et al. with minor modifications. Briefly, the tissues were homogenized in an ice-cold buffer containing 20 mM HEPES (pH 7.2), 0.25 M sucrose, 1 mM EDTA, and 1 mM phenylmethyl sulfonyl fluoride (PMSF). The homogenates were centrifuged at 100 000g for 60 min at 4 °C. The supernatant was filtered through 0.2 μM filter and loaded on a Pharmacia Mono Q anion exchange column (1 mL bed volume) that was equilibrated with 20 mM HEPES, 1 mM EDTA, and 0.5 mM PMSF. After washing out unbound proteins, the enzymes were eluted with a linear gradient of 100-600 mM NaCl in the same buffer. The column was run at flow rate of 1 mL/min, and 1 mL fractions were collected. The PDE1-5 were identified using selective inhibitors as shown in Qiu[14] et al. The fractions comprising various PDE activities were pooled separately and used in later studies.

PDE Assay and K_i Determination. The PDE assay was carried out essentially as described by Thompson and Appleman[15] with minor modifications to adapt the assay to a 96-deep well format. PDE-1, -5, and -6 were assayed with cGMP as substrate and PDE-2, -3, and -4 were assayed with cAMP as substrate. Briefly, the enzyme was assayed in 5 mM $MgCl_2$, 15 mM Tris HCl (pH 7.4), 0.5 mg/mL bovine serum albumin, 30 nM [^{3}H]-cGMP, or [^{3}H]-cAMP. The total volume of the assay was 100 μL. The reaction mixture was incubated at 30 °C for 30 min. The reaction was stopped by boiling for 1 minute and then cooled on ice. The resulting [^{3}H]5′-mononucleotides were further converted to uncharged [^{3}H]-nucleosides by adding 25 μL of 1 mg/mL snake venom (*Ophiophagus hannah*) and incubating at 30 °C for 10 min. The reaction was stopped by the addition of 1 mL Bio-Rad AG1-X2 resin slurry (1:3). After centrifugation, an aliquot of 200 μL supernatant was counted by liquid scintillation. For compound inhibition study on the PDEs, stock solution of the compounds were prepared in 100% DMSO, diluted in 100% DMSO to the appropriate concentrations, and added to the assay buffer to give a final concentration of 2% DMSO. The amount of enzyme used in each reaction was such that the hydrolysis of substrates did not exceeded 15% so that the amount of product increased linearly with time. Duplicates were run in each assay. IC_{50} values were obtained from a nonlinear regression curve fitting program. At very low substrate concentrations ([S]$<<$Km), IC_{50} values approximate the K_i values.

RFL-6 Cell-Based Functional Assay. Rat fetal lung fibroblast (RFL-6) cells (passage number 4 from ATCC frozen stock) were plated at 5.0×10^5 cells per well in 48-well plates and grown to confluency for 48 h at 37 °C. The cells were treated with the PDE inhibitors (final DMSO concentration 0.3%) for 15 min and then with freshly prepared sodium nitroprusside (SNP) (final concentration 5 μM) for 10 min. After several washes, the cells were lysed and the lysates frozen overnight at 80 °C. Intracellular levels of cGMP in acetylated cell lysates were determined using IRA (Amersham Pharmacia Biotech code RPA540). The cGMP levels induced by 6 μM SNP was designated as baseline. Results were expressed as the fold increase in cGMP levels over baseline. For each compound concentration, an *n* of six treatments was obtained. The compound concentrations required to elicit threefold or fivefold increase were determined by nonlinear regression analysis.

In Vivo Efficacy Study. The anesthetized dog model was established with modifications from the models described in the literature.[16,17] Male beagles, with body weight of 8 to 15 kg, were fasted overnight. The animals were anesthetized with sodium pentobarbital. Anesthesia was maintained throughout the course of the experiment by continuous intravenous infusion. A catheter was introduced into the femoral artery for recording systemic arterial blood pressure. Catheters were also inserted into the right and left femoral veins for delivery of test compounds and pentobarbital infusion, respectively. A mid-line abdominal incision was made and bladder was completely emptied. The pelvic nerve plexus was identified, dissected free from surrounding tissues, and placed into a subminiature electrode. The penis was denuded of skin down to the base. A 19-gauge needle attached by a flexible catheter to a pressure transducer was inserted into the corpus cavernosum to record intracavernosal pressure (ICP) changes in response to various frequencies of pelvic nerve stimulation. Parameters were recorded on Crystal Biotech CBI-8000 (Gould Instrument System, Valley View, OH), and the data acquisition was carried out through a PONEMAH P3 (Gould Instrument System, Valley View, OH) series. Pelvic nerve was stimulated with a Grass S88K square pulse stimulator and a CCU1 constant current unit (Grass, West Warwick, RI 02893). A current output set at 15−30 mA, 3-millisecond pulse duration, 1−10 Hz frequency range, and 1−2 min stimulation.

After period of a stabilization, the control ICP response curves were generated at an appropriate current output setting (ICP increase to 20−30% of systolic pressure). When similar ICP increases were obtained from at least two control stimulations applied in 15-min interval, the baseline was established. Area under the curve was computed and designated as baseline. Ascending doses of compound were administrated via bolus injection to the right femoral vein at 40 min intervals. The effects of compound on ICP increase were evaluated 15 min after each dosing by electrical stimulation with the frequency setting the baseline. To determine the effect of the compound, the area under the curve (AUC) for ICP at each stimulation was computed and subtracted from the baseline AUC. At the end of each experiment, 300 mg/kg sildenafil was given intravenously. This dose was showed to induce a

maximal response in our hands. For each animal, the highest ICP increase was designated as 100%.

Synthesis of Compounds Reported. General. NMR spectra were obtained at 400 MHz and 300 MHz on a Brucker AVANCE300 and AVANCE400 spectrometer. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Magnesium sulfate was employed to dry organic extracts prior to concentration by rotary evaporation. Flash chromatography was done using EM science silical gel 60 (230−400 mesh). Standard solvents from J. T. Baker were used as received. Anhydrous solvents from J. T. Baker or Aldrich and all other commercially available reagents were used without further purification. Mass spectra were obtained on a Hewlett-Packard 5989A quadrupole mass spectrometer. Thin-layer chromatography was performed on Analtech silica gel GF pre-scored plates (250 μm). HPLC analysis was carried on Agilent 1100 Series LC/MSD equipment. The two methods used for purity analysis employed a Phenomenex Luna Su C18(2) 100 × 4.60 mm reverse phase column utilizing either methanol (isocratic) or 20−80% acetonitrile/1% aqueous TFA (gradient) as the eluant.

1-(3,4-Methylenedioxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (**3**) was prepared according to the process described in reference 10.

1-Benzo[1,3]dioxol-5-yl-2-benzyl-2,3,4,9-tetrahydro-1*H*-*β*-carboline (**5**). To a solution of **3** (3.00 g, 10.3 mmol) in dry DMF (12 mL) were added triethylamine (1.43 mL, 10.3 mmol) and benzyl bromide (1.22 mL, 10.3 mmol). The reaction mixture was stirred at room temperature overnight before being added dropwise to a flask containing rapidly stirred water (100 mL). The resulting tan precipitate was removed by vacuum filtration, washed with water, and dissolved in a 1:1 mixture of chloroform and ether (100 mL). After being dried ($MgSO_4$), the solvent was removed in vacuo to afford the product (3.76 g, 96%) which was used without further purification. ^{1}H NMR 300 MHz ($CDCl_3$) δ 2.57−2.89 (series of m, 3 H), 3.18−3.23 (m, 1 H), 3.33 (d, J = 13.7 Hz, 1 H), 3.93 (d, J = 13.7 Hz, 1 H), 4.55 (s, 1 H), 5.94 (d, J = 2.2 Hz, 2 H), 6.77−7.52 (series of m, 13 H); MS (*m*/*z*) 383 (MH^+); HRMS calcd MH^+ for $C_{25}H_{22}N_2O_2$ is 383.1759; found 383.1763.

3-Benzo[1,3]dioxol-5-yl-2-benzyl-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**6**). 1-(3,4-Methylenedioxyphenyl)-2-benzyl-2,3,4,9-tetrahydro-1*H*-*β*-carboline (0.79 g, 2.0 mmol) (**5**) was dissolved in dry DMF (15 mL). Potassium *tert*-butoxide (0.56 g, 5.0 mmol) was added, followed by oxygen, bubbled in via syringe needle. The mixture was maintained at room temperature for 1 h and then poured onto a mixture of 1 N HCl (5 mL), water (35 mL) and ethyl acetate (35 mL). A fluffy yellow precipitate was collected, the organic layer removed, and the aqueous solution extracted with ethyl acetate (15 mL). The extracted layer was agitated for overnight, and an additional quantity of product (as a precipitate) was collected. Drying the combined solids provided the product as a yellow powder (0.38g, 31%). MS (*m*/*z*): 397 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.65 (m, 2 H), 4.01 (d, J = 13.2 Hz, 1 H), 3.93 (dd, J = 11.9, 3 Hz, 1 H), 4.95 (m, 1 H), 6.05 (s, 2 H), 6.82 (d, J = 7.6 Hz, 1 H), 6.96 (d, J = 8.1 Hz, 1 H), 7.07 (s, 1 H), 7.23−7.38 (m, 6 H), 7.51 (t, J = 8.4 Hz, 1 H), 8.08 (s, 1 H), 8.34 (d, J = 12.2 Hz, 1 H). HRMS calcd MH^+ for $C_{25}H_{20}N_2O_3$ is 397.1552; found 397.1543.

3-Benzo[1,3]dioxol-5-yl-1,2,3,4-tetrahydro-pyrrolo-[3,4-*b*]quinolin-9-one (**7**). To a suspension of 1,2,3,4-tetrahydro-2-benzyl-3-(3,4-methylenedioxyphenyl)-9*H*-pyrrolo-[3,4-*b*]quinolin-9-one (**6**) (1.12 g, 2.82 mmol) in methanol (50 mL) and 10% Pd/C (500 mg) was added HCl (1.41 mL, 2 N in ether, 2.82 mmol). The reaction mixture was agitated under a hydrogen atmosphere (45 psi) in a Parr apparatus for 6 h. The resulting solution was filtered through a plug of celite and was concentrated in vacuo to yield the product as a pink powder. ^{1}H NMR ($CDOD_3$) δ 4.18 (d, J = 13.6 Hz, 1H), (dd, 4.34 J = 13.6 Hz, 0.8 Hz, 1 H), 5.42 (s, 1 H), 5.97 (d, J = 0.8 Hz, 2 H), 6.78 (s, 1 H), 6.88 (s, 2 H), 7.41 (t, J = 7.6 Hz, 1 H), 7.55 (d, J = 6.1 Hz, 1 H), 7.68 (t, J = 6.3 Hz, 1 H), 8.34 (d, J = 7.6 Hz, 1 H); MS (*m*/*z*) 307 (MH^+); HRMS calcd MH^+ for $C_{18}H_{14}N_2O_3$ is 307.1082; found 307.1081.

General Procedure for the Reaction of 7 with Electrophiles . To a stirred mixture of **7** (1 equiv) in THF (10 mL/mmol) containing triethylamine (2 equiv) was added a solution of the electrophile (1 equiv) in THF (5 mL/mmol). After stirring at room temperature for 2 h, water (1 mL/mL of THF) was added and the mixture extracted twice with ethyl acetate (2 mL/mL of THF). The organic layers were combined, washed with brine (1 mL/mL of THF), dried over $MgSO_4$, and concentrated. Purification was achieved by using silica gel flash chromatography with 0−10% methanol−dichloromethane as the eluant.

3-Benzo[1,3]dioxol-5-yl-2-(3-phenyl-propynoyl)-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**8**). Prepared using phenyl propynoyl chloride[18] as the electrophile. Yield: 16% MS (*m*/*z*): 435 (MH^+); ^{1}H NMR (400 MHz, DMSO) (*mixture of rotamers) δ 4.71/4.98* (d, J = 14.9 Hz, 1 H), 4.81/5.10* (dd, J = 11.9, 2.2 Hz, 1 H), 6.01 (m, 2 H), 6.19/6.43* (nd, J = 2.2 Hz, 1 H), 6.91−7.00 (m, 3 H), 7.32-8.17 (series of m, 9 H), 11.78/11.93* (br s, 1 H). HRMS calcd MH^+ for $C_{27}H_{18}N_2O_4$ is 435.1377; found 435.1364.

3-Benzo[1,3]dioxol-5-yl-2-(3-phenyl-acryloyl)-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**9**). Prepared using phenyl cinnamoyl chloride as the electrophile. Yield: 41% MS (*m*/*z*): 435 (M − H); ^{1}H NMR (400 MHz, $CDCl_3$) (*mixture of rotamers) δ 4.79/4.95* (d, J = 12.5 Hz, 1 H), 4.89/5.09* (dd, J = 12.5, 2.4 Hz, 1 H), 5.68/5.69* (s, 2 H), 5.73/6.05* (nd, J = 2.4 Hz, 1 H), 6.42−6.77 (series of m, 4H), 7.29−7.59 (series of m, 9 H), 8.25/8.38* (d, J = 8.1 Hz, 1 H), 10.61/10.82* (br s, 1 H). HRMS calcd MH^+ for $C_{27}H_{20}N_2O_4$ is 437.1502; found 437.1501.

3-Benzo[1,3]dioxol-5-yl-2-(3-phenyl-propionyl)-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**10**). Prepared using 3-phenylpropionyl chloride as the electrophile. Yield: 58% MS (*m*/*z*): 437 (M − H); ^{1}H NMR (400 MHz, DMSO) (*mixture of rotamers) δ 2.58-2.82 (m, 4 H) 4.62−4.86 (m, 2 H) 5.99−6.21 (series of s, 3 H), 6.82−7.63 (series of m, 12 H), 8.12 (d, J = 8.1 Hz, 1 H), 11.73/11.80* (br s, 1 H). HRMS calcd MH^+ for $C_{27}H_{22}N_2O_4$ is 439.1658; found 439.1654.

3-Benzo[1,3]dioxol-5-yl-2-phenylacetyl-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**11**). Prepared using 2-phenyl-acetyl chloride as the electrophile. Yield: 39% MS (*m*/*z*): 423 (M − H); ^{1}H NMR (400 MHz,

DMSO) δ (*mixture of rotamers) 3.68−3.85 (m, 2 H) 4.64−4.92 (m, 2 H) 5.98−6.31 (series of s, 3 H), 6.81−7.64 (series of m, 11 H), 8.13 (d, J = 7.9 Hz, 1 H), 11.73/11.81* (br s, 1 H)). HRMS calcd MH^+ for $C_{26}H_{20}N_2O_4$ is 425.1502; found 425.1494.

3-Benzo[1,3]dioxol-5-yl-2-benzoyl-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**12**). Prepared using benzoyl chloride as the electrophile. Yield: 61% MS (*m*/*z*): 409 (M − H); ^{1}H NMR (400 MHz, DMSO) (*mixture of rotamers) δ 4.71/4.98* (d, J = 12.8 Hz, 1 H), 4.92/5.09* (d, J = 11.0 Hz, 1 H), 5.91−6.40 (series of s, 3 H), 6.65−7.66 (series of m, 11 H), 8.10 (d, J = 9 Hz, 1 H), 11.70/11.90* (br s, 1 H). HRMS calcd MH^+ for $C_{25}H_{18}N_2O_4$ is 411.1345; found 411.1347.

3-Benzo[1,3]dioxol-5-yl-2-(3-phenylallyl)-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**13**). Prepared using cinnamyl bromide as the electrophile. Yield 45 % MS (*m*/*z*): 422 (M − H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 (dd, J = 13.7, 7.7 Hz, 1 H), 3.58 (dd, J = 13.6, 4.6 Hz, 1 H), 3.83 (dd, J = 12.3, 3.7 Hz, 1 H), 4.49 (dd, J = 12.3, 2.8 Hz, 1 H), 4.92 (br s, 1 H), 5.97 (s, 1 H), 5.98 (s, 1 H), 6.17−6.25 (m, 1 H), 6.55 (d, J = 15.9 Hz, 1 H), 6.82 (d, J = 7.8 Hz, 1 H), 6.91 (dd, J = 7.8, 1.2 Hz, 1 H), 6.97 (nd, J = 1.2 Hz, 1 H), 7.20−7.33 (m, 5 H), 7.50 (t, J = 8.5 Hz, 1 H), 7.92 (br s, 1 H), 8.38 (d, J = 8.0, 1 H) HRMS calcd MH^+ for $C_{27}H_{22}N_2O_3$ is 423.1714; found 423.1714.

3-Benzo[1,3]dioxol-5-yl-2-(2-phenylethenesulfonyl)-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**14**). Prepared using 2-phenylethenesulfonyl chloride as the electrophile. Yield 38 % MS (*m*/*z*): 471 (M − H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (dd, J = 12.6, 3.4 Hz, 1 H), 4.76 (d, J = 11.5 Hz, 1 H), 5.68 (s, 1 H), 5.82 (s, 1 H), 5.90 (br s, 1 H), 6.36 (d, J = 15.4 Hz, 1 H), 6.67 (d, J = 5.3 Hz, 1 H), 6.69 (s, 1 H), 6.83 (dd, J = 8.1, 1.7 Hz, 1 H), 7.21−7.60 (m, 9H), 8.29 (d, J = 7.1, 1 H), 9.45 (br s, 1 H). HRMS calcd MH^+ for $C_{26}H_{20}N_2O_5S$ is 473.1171; found 473.1195.

3-Benzo[1,3]dioxol-5-yl-2-phenylmethanesulfonyl-1,2,3,4-tetrahydro-pyrrolo[3,4-*b*]quinolin-9-one (**15**). Prepared using phenylmethanesulfonyl chloride as the electrophile. Yield 55% MS (*m*/*z*): 459 (M − H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (d, J = 13.9 Hz, 1 H), 4.02 (d, J = 13.9 Hz, 1 H), 4.23 (dd, J = 12.6, 3.0 Hz, 1 H), 4.71 (d, J = 12.7 Hz, 1 H), 5.80 (s, 1 H), 5.94 (s, 2 H), 6.61 (s, 1 H), 6.70−6.75 (m, 2 H), 7.15−7.37 (m, 8 H), 7.58 (t, J = 8.0 Hz, 1 H), 8.28 (d, J = 8.0 Hz, 1 H), 9.48 (br s, 1 H). HRMS calcd MH^+ for $C_{25}H_{20}N_2O_5S$ is 461.1171; found 461.1179.

3-Benzo[1,3]dioxol-5-yl-9-oxo-1,3,4,9-tetrahydro-pyrrolo[3,4-*b*]quinoline-2-carboxylic Acid Benzylamide (**16**). Prepared using benzyl isocyanate as the electrophile. Yield 61% MS (*m*/*z*): 438 (M − H); ^{1}H NMR (400 MHz, DMSO) δ 4.12 (dd, J = 15.9, 5.6 Hz, 1 H), 4.32 (dd, J = 15.9, 5.6 Hz, 1 H), 4.57 (d, J = 12.9 Hz, 1 H), 4.71 (dd, J = 12.9, 3.6 Hz, 1 H), 5.99 (nd, J = 3.8 Hz, 2 H), 6.04 (nd, J = 3.2 Hz, 1 H), 6.78−7.35 (series of m, 10 H), 7.54−7.64 (m, 2 H), 8.13 (d, J = 7.9 Hz, 1 H), 11.78 (s, 1H). HRMS calcd MH^+ for $C_{26}H_{21}N_3O_4$ is 440.1610; found 440.1612.

3-Benzo[1,3]dioxol-5-yl-9-oxo-1,3,4,9-tetrahydro-pyrrolo[3,4-*b*]quinoline-2-carboxylic Acid Benzylmethylamide (**17**). Prepared using benzyl methyl carbamoyl chloride as the electrophile. Yield 31% MS (*m*/*z*): 452 (M − H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 (s, 3 H), 4.13 (d, J = 15.3 Hz, 1 H), 4.54 (d, J = 12.4 Hz, 1H), 4.70 (d, J = 15.3 Hz, 1 H), 4.92 (dd, J = 12.5, 3.4 Hz, 1 H), 5.86 (d, J = 5.3 Hz, 2 H), 6.43 (nd, J = 2.9 Hz, 1 H), 6.63 (d, J = 7.9 Hz, 1 H), 6.69 (s, 1 H), 6.78 (d, J = 7.9 Hz, 1 H), 7.15 (d, J = 6.8 Hz, 2 H), 7.20−7.35 (m, 6 H), 7.52 (t, J = 8.0 Hz, 1 H), 8.34 (t, J = 8.0 Hz, 1 H), 9.62 (br s, 1 H); HRMS calcd MH^+ for $C_{27}H_{23}N_3O_4$ is 454.1772; found 454.1775.

3-Benzo[1,3]dioxol-5-yl-9-oxo-1,3,4,9-tetrahydro-pyrrolo[3,4-*b*]quinoline-2-carboxylic Acid Benzyl Ester (**18**). Prepared using benzyl chloroformate as the electrophile. Yield 31% MS (*m*/*z*): 439 (M − H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.62−4.75 (m, 2 H), 4.92−5.25 (series of m, 2 H), 5.76−5.82 (m, 3 H), 6.50−6.67 (m, 3 H), 6.98−7.57 (series of m, 9 H), 8.25−8.30 (m, 1 H), 10.40 and 10.68* (br s, 1 H); HRMS calcd MH^+ for $C_{26}H_{20}N_2O_5$ is 441.1469; found 441.1482.

3-Benzo[1,3]dioxol-5-yl-9-oxo-1,3,4,9-tetrahydro-pyrrolo[3,4-*b*]quinoline-2-carboxylic Acid Pyridin-4-ylmethyl Ester (**19**). Prepared using the 4-nitrophenyl carbonate of 4-pyridyl carbinol as the electrophile. Yield 25% MS (*m*/*z*): 440 (M − H); ^{1}H NMR (reported for the (*S*)-enantiomer) (400 MHz, $CDCl_3$) (*mixture of rotamers) δ 4.72-5.25* (series of d, 4H), 5.85-5.91 (m, 3 H), 6.61−6.85* (series of m, 3 H), 7.19 (nd, J = 5.0 Hz, 1 H), 7.31−7.61 (series of m, 5 H), 8.38 (q, J = 10.9 Hz, 2 H), 8.53 (s, 1 H), 9.82 and 9.91* (br s, 1 H); HRMS calcd MH^+ for $C_{25}H_{19}N_3O_5$ is 442.1403; found 442.1383.

The enantiomers were separated using a Varian PrepStar HPLC fitted with Chiral Technologies Chiral-Pak AD 5 × 50, 20 μm column. Fractions were checked for purity on the Agilent LC/MSD previously mentioned but employing a Chiral Technologies ChiralPak AD 0.46 × 25 cm column. 2-Propanol was used as the mobile phase on the preparative column while methanol was utilized on the analytical column.

***R*-(−)-19**. The first peak to elute from the preparative column had a retention time of 3.581 min on the analytical system. NMR and MS were the same as reported above. $[\alpha]^{23}_D$ = -85, c = 0.135 in methanol. HRMS calcd MH^+ for $C_{25}H_{19}N_3O_5$ is 442.1403; found 442.1379.

***S*-(+)-19**. The second peak to elute from the preparative column had a retention time of 3.622 min on the analytical system. NMR and MS were the same as reported above. $[\alpha]^{23}_D$ = +75, c = 0.205 in methanol. HRMS calcd MH^+ for $C_{25}H_{19}N_3O_5$ is 442.1403; found 442.1382.

3-Benzo[1,3]dioxol-5-yl-4-methyl-9-oxo-1,3,4,9-tetrahydro-pyrrolo[3,4-*b*]quinoline-2-carboxylic Acid Pyridin-4-ylmethyl Ester (**20**) A suspension of sodium hydride (26 mg, 0.33 mmol, 60% dispersion in mineral oil) in dry DMF (4 mL) was treated with **19** (140 mg, 0.29 mmol). Dimethyl sulfate (30 μL, 0.33 mmol) was introduced via syringe and the resulting brown solution stirred at ambient temperature for 1 h. Water (10 mL) was added to the reaction mixture, and the emulsion was washed with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with 10% LiCl solution and brine, dried ($MgSO_4$), and concentrated in vacuo. Purification of the residue by flash chromatography (SiO_2, 5% MeOH/CH_2Cl_2) afforded 23 mg of the product as a tan solid (15%). MS (*m*/*z*): 456 (MH^+); ^{1}H

NMR (400 MHz, $CDCl_3$) (*mixture of rotamers) δ 3.49 (s, 3 H), 4.77−5.27* (series of m, 4 H), 5.91−6.14* (series of nd, 3 H), 6.73−7.03* (series of s and d, 3 H), 7.25−7.37 (m, 2 H), 7.39−7.46 (m, 2 H), 7.63−7.70 (m, 1 H), 8.50−8.59 (m, 3 H); HRMS calcd MH^+ for $C_{26}H_{21}N_3O_5$ is 456.1559; found 456.1565.

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Supporting Information Available: HPLC chromatograms and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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