Design, Synthesis, and Biological Activity of Potent and Selective Inhibitors of Blood Coagulation Factor Xa

J. Adam Willardsen,[†,‡] Danette A. Dudley,[†] Wayne L. Cody,[†] Liguo Chi,[§] Thomas B. McClanahan,[§] Thomas E. Mertz,[§] Ronald E. Potoczak,[§] Lakshmi S. Narasimhan,[∥] Debra R. Holland,[∥] Stephen T. Rapundalo,[⊥] and Jeremy J. Edmunds*[,†]

Departments of Chemistry, Cardiovascular Therapeutics, Discovery Technologies, and Cardiovascular Molecular Sciences, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, Michigan 48105

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Factor Xa (FXa) has materialized as a key enzyme for the intervention of the blood coagulation cascade and for the development of new antithrombotic agents. FXa is the lone enzyme responsible for the production of thrombin and therefore is an attractive target for the control of thrombus formation. We have designed and synthesized a unique series of quinoxalinone FXa inhibitors. This series resulted in 3-{4-[5-((2*S*,6*R*)-2,6-dimethylpiperidin-1-yl)pentyl]-3-oxo-3,4-dihydroquinoxolin-2-yl}benzamidine (**35**) with 0.83 nM activity against FXa and excellent selectivity over similar serine proteases. An X-ray crystal structure of compound **35** bound to trypsin along with molecular modeling has led to a predicted binding conformation of compound **35** in FXa. Compound **35** has also been proven to be efficacious in vivo in both the rabbit veno-venous shunt and dog electrolytic injury models. In addition, it was shown that compound **35** did not significantly increase bleeding times in a rabbit model except at the highest doses and plasma concentrations were elevated in a dose dependent manner following a bolus dose and continuous intravenous infusion.

Introduction

Cardiovascular disease is the leading cause of death in the United States, and within the cardiovascular disease class, heart disease is the primary source of mortality.[1] In 1996, myocardial infarction, stroke, deep vein thrombosis, and pulmonary embolism accounted for approximately two million deaths in the United States alone.[2] Inappropriate thrombus formation is a common cause of the above clinical conditions, and therefore there has been a large effort devoted to the discovery of new antithrombotic therapies.[3] The coagulation cascade is composed of a complex series of plasma proteins, serine proteases, and cellular receptors. Each of these helps play a delicate balancing act, through positive and negative feedback controls, between thrombosis and hemostasis.

Recently, significant effort has been put forth to develop inhibitors of the key serine proteases that are part of the coagulation cascade. Thrombin was the initial target, and many small molecule inhibitors have been reported.[4] Being that Factor Xa (FXa) is at the convergence of the intrinsic and extrinsic pathways of the coagulation cascade, it represents an attractive target for the control of thrombus formation and inhibitors for this enzyme have also been pursued.[5] In the route to the physiological formation of a clot, FXa combines with Factor Va (FVa) and calcium ions on a phospholipid membrane to create the prothrombinase complex. This complex is then responsible for the conversion of prothrombin (FII) into thrombin (FIIa).[6] Thrombin proceeds to catalyze the formation of the fibrin clot. The inhibition of FXa provides for a small, but hemostatically important, amount of thrombin to be produced.[7] This provides evidence for favorable benefit-to-risk properties for FXa inhibitors and makes the development of a direct FXa inhibitor an appealing method for the control of thrombotic diseases.

We have previously discussed a new class of potent and selective inhibitors of FXa, which originated from a high-throughput screen (HTS) of our chemical library.[8] Herein, we will report on modifications to the benzoxazinone skeleton, which was identified from our HTS screen, along with the in vitro and in vivo data that led to the clinical candidate compound **35**, a quinoxalinone based FXa inhibitor, with a *p*-hydroxybenzamidine binding in the S1 pocket.[9]

Chemistry

The molecules of interest replaced the oxygen atom of the benzoxazinone ring of **1**[8] and **2**[10] with sulfur, nitrogen, and carbon (Figure 1). The desired sulfur analogues **22a** and **22b** of the previously reported compounds **1** and **2** were synthesized as outlined in Schemes 1, 2, and 6. 3-Cyanobenzaldehyde was treated with aluminum foil and lead(II) bromide in carbon tetrachloride to furnish the trichlorocarbinol **4** (Scheme 1).[11] In the presence of base and 2-aminothiophenol, **4** was readily cyclized to the benzothiazinone **5a**.[11,12] The desired target compound was then obtained via the standard route outlined in Scheme 6. The amide nitrogen was first alkylated with 1,5-dibromopentane to yield

* To whom correspondence should be addressed. Phone: (734) 622-4984, fax: (734) 622-3909, e-mail: jeremy.edmunds@pfizer.com.
[†] Department of Chemistry.
[‡] Present address: Myriad Genetic Pharmaceuticals Inc., 320 Wakara Way, Salt Lake City, UT 84108.
[§] Department of Cardiovascular Therapeutics.
[∥] Department of Discovery Technologies.
[⊥] Department of Cardiovascular Molecular Sciences.

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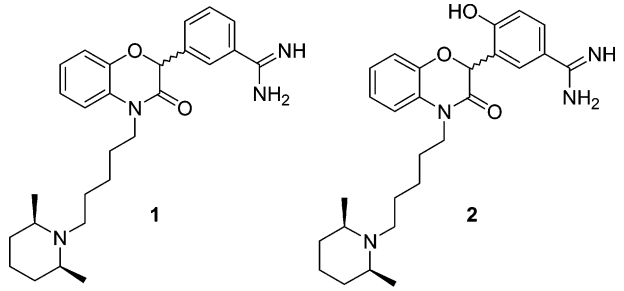
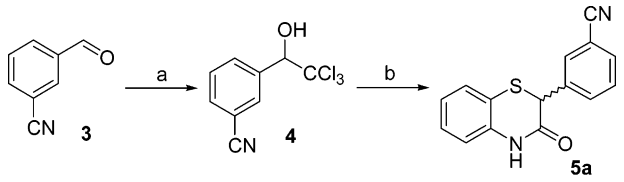


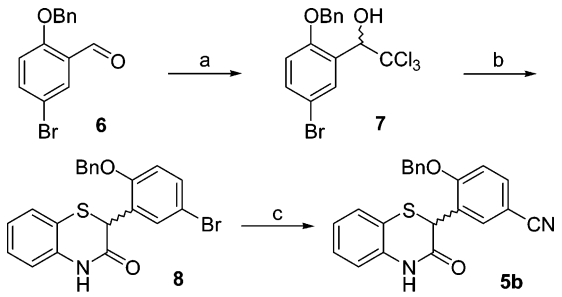
Figure 1.

Scheme 1[a]



[a] Reagents and conditions: (a) Al foil, $PbBr_2$, CCl_4, DMF; (b) 2-$(HS)C_6H_4NH_2$, NaH, DMF.

Scheme 2[a]

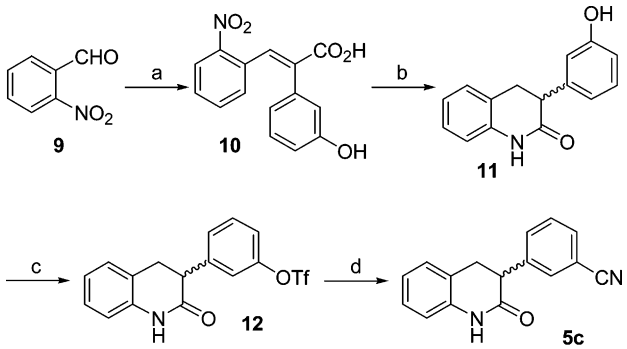


[a] Reagents and conditions: (a) Al foil, $PbBr_2$, CCl_4, DMF; (b) 2-$HSC_6H_4NH_2$, NaH, DMF; (c) CuCN, DMF, 160 °C.

20a. In the cases outlined in Scheme 6, only a small amount of *O*-alkylated product was observed, which was easily removed via column chromatography. Displacement of the alkyl bromide of **20a** with *cis*-2,6-dimethylpiperidine resulted in **21a** in excellent yields.

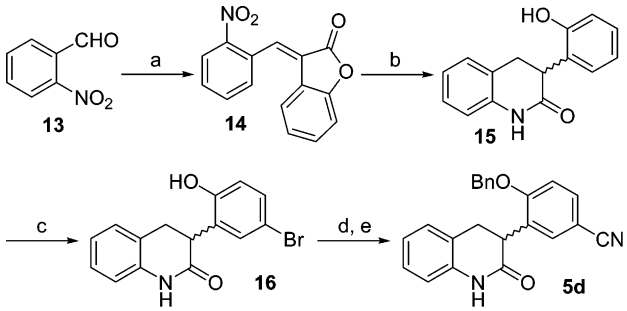
Although there are many reported methods available for the formation of amidines,[13,14] it was found that proceeding through the amidoxime was usually the most advantageous. The amidoxime was formed by treating the benzonitrile with hydroxylamine, followed by acylation with trifluoroacetic anhydride. Acylation resulted in cyclization to produce an oxadiazole, which when subjected to hydrogenation over palladium on carbon revealed the amidine **22a**.[15] The *p*-hydroxyamidine derivative of **22a** was obtained in a similar manner; trichlorocarbinol **7** was converted to the benzothiazinone **8** as discussed, previously (Scheme 2). By heating the arylbromide **8** in a mixture of cuprous cyanide and DMF, the bromide was displaced to give the nitrile **5b**. The intermediate **5b** was taken on using the same procedure as described before, with one note: as seen in Scheme 6, the benzyl ether was cleanly removed during the hydrogenation of the oxadiazole to give the second desired dihydrobenzothiazinone analogue **22b**.For the tetrahydroquinolinone series 22c and 22d, the carbon skeleton was assembled in a slightly different manner (Schemes 3 and 4). Treating 3-hydroxyphenylacetic acid with 2-nitrobenzaldehyde (**9**) and acetic anhydride gave compound **10** (Scheme 3).[16] Reduction

Scheme 3[a]



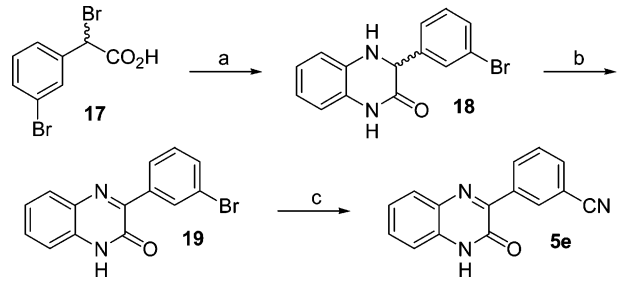
[a] Reagents and conditions: (a) 3-Hydroxyphenylacetic acid, Ac_2O, Et_3N, 150 °C; (b) H_2, Pd/C, MeOH; (c) *N*-phenyltrifluoromethanesulfonimide, NaH, THF; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 100 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) 2-Hydroxyphenylacetic acid, Ac_2O, Et_3N, 150 °C; (b) H_2, Pd/C, MeOH; (c) Br_2, CS_2, CH_2Cl_2; (d) BnBr, Cs_2CO_3, DMF; (e) CuCN, DMF, 150 °C.

Scheme 5[a]

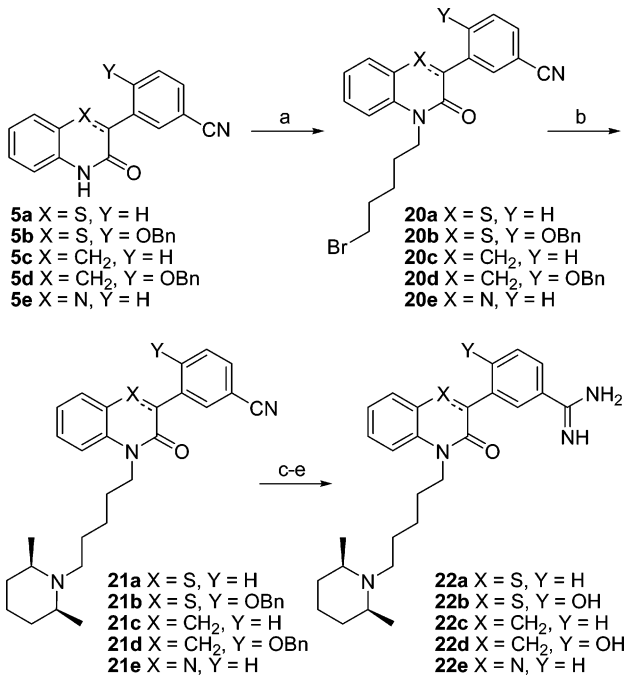


[a] Reagents and conditions: (a) 1,2-Phenylenediamine, KOH, H_2O, 60 °C; (b) DDQ, toluene, 120 0C; (c) CuCN, DMF, 150 °C.

of the aryl nitro group resulted in spontaneous cyclization to yield **11** as a mixture of enantiomers. Phenol **11** was converted to the corresponding triflate and coupled with zinc cyanide using $Pd(PPh_3)_4$ to give the core structure of **5c**.[17] For the 4-hydroxyamidine analogue, a mixture of 2-hydroxyphenylacetic acid and 2-nitrobenzaldehyde gave the lactone **14** (Scheme 4).[16] Again, reduction of the nitro group of **14** yielded the cyclized product **15**. Electrophilic bromination resulted in the bromide **16**, which was converted to the nitrile **5d** with cuprous cyanide as described previously for **5b**. Tetrahydroquinolinone analogues **5c,d** were transformed to the desired amidine analogues **22c**,**d** as shown in Scheme 6.

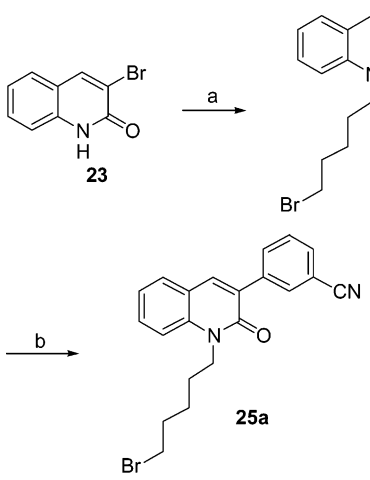
For the dihydroquinolinone series, **27a** and **27b**, conversion of 3-bromo-1*H*-quinolin-2-one (**23**)[18] to the nitrile **25a** via a palladium(0)-mediated coupling followed by alkylation of the amide nitrogen, as previously outlined for other analogues, led to complex reaction

Scheme 6[a]



[a] Reagents and conditions: (a) $Br(CH_2)_5Br$, NaH, DMF, 70 °C; (b) *cis*-2,6-dimethylpiperidine, DMF, 70 °C; (c) $H_2NOH{\cdot}HCl$, DIEA, MeOH; (d) TFAA; (e) H_2, Pd/C, TFA.

Scheme 7[a]

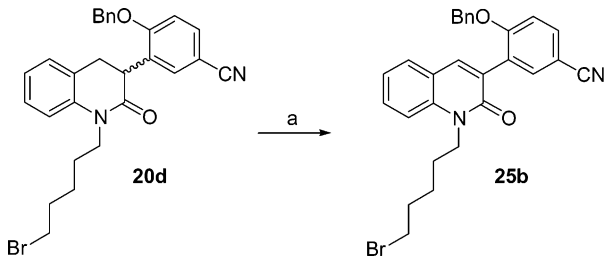


[a] Reagents and conditions: (a) $Br(CH_2)_5Br$, NaH, DMF, 70 °C; (b) (3-cyanophenyl)boronic acid, $(Ph_3P)_4Pd$, $NaHCO_3$, toluene H_2O, EtOH.

mixtures. When the amide nitrogen of **23** was first alkylated with dibromopentane to give **24**, the subsequent palladium(0) coupling proceeded cleanly to give **25a** (Scheme 7).[19] As shown in Scheme 8, an intermediate from the tetrahydroquinolinone series **20d** was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give **25b** for the dihydroquinolinone series of compounds. Again, **25a**,**b** were carried on to the desired products by addition of *cis*-2,6-dimethylpiperidine and formation of the amidine to give **27a**,**b** (Scheme 9).

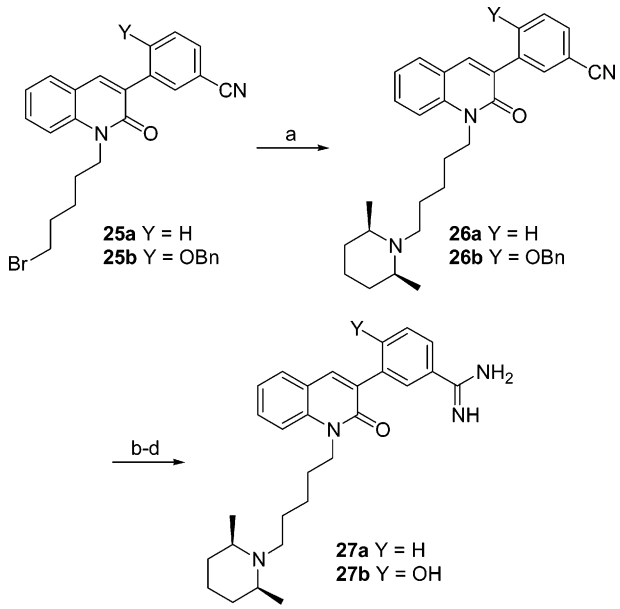
The quinoxalinone, nitrogen analogue, **5e** was obtained via the addition of 1,2-phenylenediamine to a solution of **17** (Scheme 5). Upon treatment of intermediate **18** with DDQ, the bromide **19** was obtained. Conversion of the bromide **19** tothe nitrile **5e** was accomplished with cuprous cyanide as shown previously (Scheme 2).

Scheme 8[a]



[a] Reagents and conditions: (a) DDQ, toluene 80 °C.

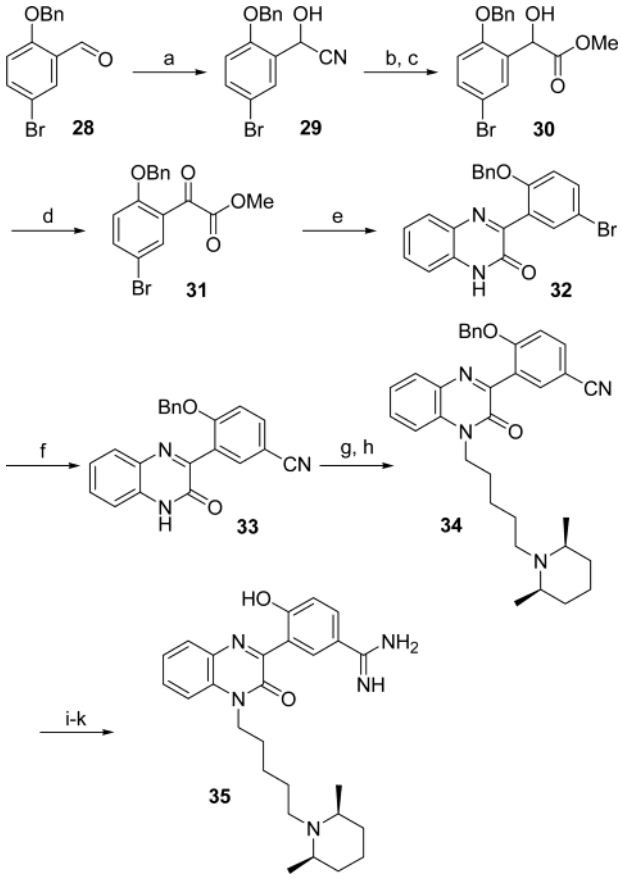
Scheme 9[a]



[a] Reagents and conditions: (a) *cis*-2,6-Dimethylpiperidine, DMF, 75 °C; (b) $H_2NOH{\cdot}HCl$, DIEA, MeOH; (c) TFAA; (d) H_2, Pd/C, TFA.

The final quinoxalinone derivative **35** was synthesized in a slightly different manner to the above examples as shown in Scheme 10. The benzaldehyde analogue **28** was hydrocyanated with potassium cyanide to yield compound **29**.[20] Under Pinner conditions (HCl, ethanol followed by NH_3), **29** was converted into the α-hydroxy ester **30**.[14] Swern conditions were used to oxidize the hydroxyl functionality to the α-ketoester **31**. Condensation of the ester **31** and 1,2-phenylenediamine resulted in the parent quinoxalinone **32**. Due to the previously mentioned cuprous cyanide methodology leading to many byproducts, a palladium(0)-mediated coupling between aryl bromide **32** and zinc cyanide was used to install the aryl nitrile functionality of **33**. Since the previously mentioned methods for alkylation of the quinoxalinone nucleus resulted in poor yields, it was found that using cesium carbonate and DMF resulted in a nearly quantitative yield of a 1:1 mixture on N- and O-alkylated products. The *N*-alkylated component was separated via column chromatography and carried on to the piperidine derivative **34**. Formation of the amidine via the hydroxylamine method vida infra resulted in a complex reaction mixture; therefore, Pinner conditions were used to introduce the amidine functionality. Finally, the phenolic benzyl ether was removed by hydrogenation to give the desired quinoxalinone derivative **35**.

Scheme 10[a]



[a] Reagents and conditions: (a) KCN, AcOH, MeOH; (b) HCl, Dioxane, MeOH, Et_2O, 0 °C; (c) H_2O, dioxane; (d) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C; (e) 1,2-phenylenediamine, MeOH, 65 °C; (f) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 100 °C; (g) $Br(CH_2)_5Br$, Cs_2CO_3, DMF, 0 °C−RT; (h) *cis*-2,6-dimethylpiperidine, DMF, 75 °C; (i) HCl, EtOH; (j) NH_3, MeOH; (k) H_2, Pd/C, TFA.

Results and Discussion

The aim of this program was to develop an anticoagulant therapy that utilized a selective inhibitor of coagulation FXa. This research was initiated by conducting an in vitro high throughput screen of our chemical library. The results of this high throughput screen and subsequent design of benzoxazinone **1** have been previously reported.[8] Although **1** was highly potent (K_i = 0.885 nM against FXa) and selective for FXa over other similar serine proteases, development would be hindered by the easily epimerized asymmetric center. Subsequent studies on the benzoxazinone template resulted in incorporation of the *p*-hydroxyphenylamidine moiety[9] to provide racemic **2**, which further improved potency (K_i = 0.160 nM against FXa). While an asymmetric synthesis of **2** resulted in the demonstration that the *S*-isomer had the greatest affinity for FXa (K_i = 0.08 nM); however, this enantiomer was easily racemized at pH > 8.[10]

Due to the difficulty of obtaining crystal structures of a small molecule bound to FXa, in these studies, trypsin was used as a surrogate to predict the binding conformations in FXa. Using this rationale, molecular modeling suggested that the high potency of this series of compounds could be retained if the oxygen atom of the benzoxazinone nucleus was replaced with sulfur, carbon, or nitrogen. Advantageously, in the cases of carbon and nitrogen, this would allow for the design of inhibitors lacking the troublesome asymmetric center.

Table 1. Selectivity Data for Specified Compounds as Evidenced by the Inhibition of Various Serine Proteases

compd	X	Y	FXa K_i (nM)	thrombin IC_{50} (μM)	trypsin IC_{50} (μM)
1	O	H	3.0 (IC_{50})	2.0	0.5
2	O	OH	0.16	1.0	0.4
22a	S	H	67	1.3	1.0
22b	S	OH	2.3	3.6	0.7
22c	CH_2	H	60.5	0.9	0.3
22d	CH_2	OH	12.9	4.8	0.7

Table 2. Selectivity Data for Specified Compounds as Evidenced by the Inhibition of Various Serine Proteases

compd	X	Y	FXa K_i (nM)	thrombin IC_{50} (μM)	trypsin IC_{50} (μM)
22e	N	H	18.9	2.9	2.0
27a	CH	H	177.0 (IC_{50})	10.7	0.3
27b	CH	OH	23.5	4.8	0.7
35	N	OH	0.8	3.5	2.5

For the benzothiazinone derivatives **22a** and **22b**, the affinity for FXa was somewhat diminished (Table 1). Interestingly, X-ray crystallography analysis in the trypsin active site revealed that the *R* enantiomer was the preferred configuration for this molecule. The increased radius of the sulfur resulted in van der Waals interactions with the Cys192−Cys220 disulfide bridge and caused repulsion of **22a** and **22b** from the active site. Inversion of the asymmetric center enables the phenyl amidine to maintain the two-on-two hydrogen bond with Asp189. In the case of the quinolinone derivatives **22c** and **22d**, the binding to FXa was reduced, as was the selectivity over other serine proteases. Crystallization of **22d** with trypsin reveled that it was bound in the active site in a fashion very similar to **2**. To our surprise, this crystallization of **22d** with trypsin and modeling studies in FXa did not reveal reasonable indications as to why there was a decrease in affinity or selectivity. It is assumed that entropic factors associated with these saturated species are responsible for the loss of affinity for FXa. Although, the SAR on the previous compounds was important, they still contained the problematic asymmetric center, and therefore this problem still needed to be addressed.

When the oxygen atom in the core structure of **1** and **2** is replaced by a methine, dihydroquinoxalinone **27a**,**b**, it was shown that there is at least a 150-fold loss of activity against FXa (Table 2). These compounds also show a large loss in selectivity over similar serine proteases. Exchanging the oxygen for nitrogen to give the quinoxalinones **22e** and **35**, a loss in activity of 20- and 5-fold respectively was seen. These results were promising; quinoxalinone **35**, although less potent than **2**, still maintained sub-nanomolar activity. More importantly, this molecule lacks the asymmetric center. Another important consideration is that **35** maintains a 390- and 275-fold selectivity over thrombin and trypsin, respectively.

Being that **35** still had some affinity for trypsin, X-ray crystallographic experiments were conducted to determine its binding mode in these trypsin-like serine proteases (Figure 2). As expected, the amidine was positioned in the S1 pocket of the protein and forms a strong two-on-two hydrogen bond with Asp189. The

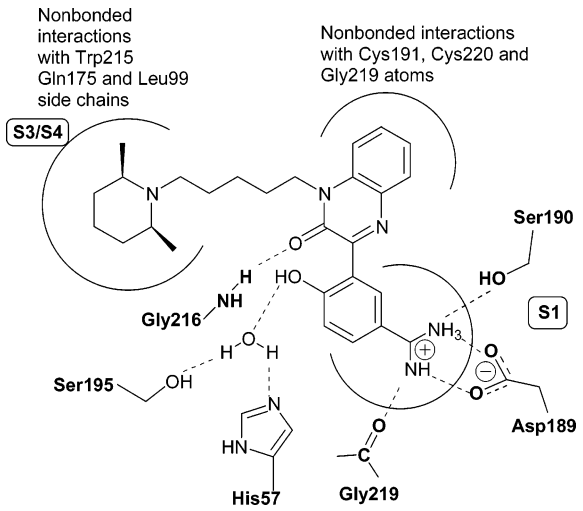


Figure 2. Interactions of compound **35** with trypsin.

Table 3. Concentration of Compound **35** (micromolar) Needed to Double the Desired Coagulation Parameter in the Specified Species

species	PT	aPTT
rat	1.3	5.2
dog	0.9	1.6
human	0.3	0.4

Ser190 hydroxyl and the Gly219 carbonyl also form hydrogen bonds with the amidine. The phenol, which lies para to the amidine, makes use of a water molecule to form hydrogen bonds with the Ser195 hydroxyl and the His57 imidazole residues. This interaction alone was responsible for the greater than 20-fold increase in affinity over **22e**. Another interaction, which plays a key role in the high affinity of compound **35** for FXa, was the hydrogen bond formed between the amide carbonyl and the Gly216 amine. Also, the phenyl ring of the quinoxalinone template makes significant nonbonding interactions with Cys191, Cys220, and Gly219. The X-ray structure revealed that the dimethylpiperidine fragment does not have hydrogen bonding interactions to trypsin, but modeling of this fragment into FXa shows that it made nonbonded (van der Waals) interactions with the side chains of Trp215, Gln175, and Leu99 (see Figure 2).[8]

With these encouraging in vitro results, **35** was tested ex vivo in rabbit, dog, and human plasma to determine the effect it had on the standard coagulation parameters (Table 3). As seen with compound **1**, quinoxalinone **35** was most effective in human plasma and showed species dependent inhibition of coagulation FXa. To determine the effects that **35** had on thrombosis and hemostasis, two in vivo studies were also conducted. First, anesthetized rabbits were subjected to a veno-venous shunt model, which has previously been described.[21] A bolus injection of **35** was followed by a constant infusion of **35** for 120 min as outlined in Table 4. In control animals, all vessels occluded in approximately 10 min. With the introduction of **35** at the highest doses (480 + 16), five out of the six vessels remained patent for the duration of the experiment (120 min). Table 4 also shows an expected dose dependent increase in the average time for the vessels to occlude in this experiment. Thus, providing more evidence that **35** may be useful in treating coagulation disorders.

Table 4. Results of Compound **35** in the Rabbit Veno-venous Shunt Model[21]

dose (bolus μg/kg + maintenance μg/kg/min)	incidence: occluded/total	time to occlusion, min
saline	7/7	10 ± 1
30 + 1	5/6	34 ± 18
60 + 2	3/6	71 ± 22
480 + 16	1/6	100 ± 19

A second in vivo test involved the electrolytic injury of the femoral artery (or vein) of a dog in the presence of restricted blood flow.[22] The electrolytic injury induced the formation of a thrombus and in control studies all vessels occluded in approximately 100 min. Upon constant infusion of a solution of **35**, a dose dependent response was seen in both the arterial and venous systems (Table 5) with most vessels in the two highest doses remaining patent for the duration of the study. At the highest dose, it actually appears that the time to occlusion decreased, but there is a great deal of variability in the formation of an occlusive clot in this model that could account for this. In addition to the extension in the time for a vessel to occlude any thrombi were also analyzed. In the arteries, the thrombus weight was decreased from 46 ± 10 mg to 15 ± 5 mg and in the vein from 136 ± 26 mg to 10 ± 2 mg at the highest doses. In addition, it was shown that compound **35** did not significantly increase bleeding times in a continuous infusion rabbit model except at the highest dose (Table 6) and plasma drug concentrations were elevated in a dose dependent manner after a bolus dose and followed by continuous intravenous infusion (Table 7).

Conclusions

In summary, we have designed and synthesized a novel and selective quinoxalinone inhibitor of coagulation FXa. As previously discussed, compound **35** originated from a high-throughput screen of our chemical library. Through the use of molecular modeling and a Topliss tree analysis approach,[23] and structure−activity relationships (SAR) from other FXa inhibitors,[10] the 6 μM high throughput-screening lead ultimately led to the design of the sub-nanomolar compound **35**. Compound **35** has been shown to be efficacious in two antithrombotic animal models and shows a dose dependent response in each. Quinoxalinone **35** shows promise for the parental treatment of thrombotic diseases and is being investigated for these uses.

Experimental Section

General Chemistry. All starting materials were obtained from commercial sources and were used without further purification unless otherwise specified in the experimental. Proton NMR spectra were obtained on a Varian Unity 400 or 300 MHz spectrometer. Elemental analyses were determined by Robertson Microlit, Inc. (Madison, NJ), and the results were within 0.4% of the theoretical values for the elements indicated. Mass spectral data were obtained on a VG Analytical 7070 E/HF mass spectrometer. Flash column chromatography was performed on Merck silica gel 60, 230−400 mesh, purchased from Mallinckrodt. Reactions were monitored by thin-layer chromatography (TLC) on Merck glass plates precoated with 0.25 mm of silica gel. Standard analytical high performance liquid chromatography (HPLC) conditions unless stated otherwise are Beckman 235328 C18 column, 5 μm, 4.6 mm × 25 cm, eluted with a gradient of (80:20 to 10:90 H_2O: CH_3CN)

Table 5. Results of Compound **35** in the Canine Electrolytic Injury Model[22]

mg/kg/min	time to occlusion (vein, min)	occluded/total	time to occlusion (artery, min)	occluded/total
saline	86 ± 15	7/7	106 ± 21	14/14
1	91 ± 21	5/5	101 ± 23	10/10
3	175 ± 25	5/5	130 ± 18	9/10
5	240 ± 0	0/5	240 ± 0	0/5
10	205 ± 22	1/5	225 ± 15	3/10

Table 6. Effects of Compound **35** on the Bleeding Time in Seconds in Rabbits[a]

	baseline	15 min	60 min	120 min
control	122 ± 14	117 ± 17	112 ± 15	120 ± 27
30 μg/kg + 1 μg/kg/min	113 ± 19	169 ± 67	93 ± 12	78 ± 3
60 μg/kg + 2 μg/kg/min	99 ± 6	84 ± 27	96 ± 25	68 ± 8
480 μg/kg + 16 μg/kg/min	116 ± 14	170 ± 36	170 ± 7	206 ± 42

[a] Values expressed as mean ± SEM; $n = 6$ all groups except 480+16 group, $n = 5$. The ear bleeding time was determined at baseline, 15, 60, and 120 min of compound infusion.

+ 0.1% TFA over 23 min with a flow rate of 1.5 mL/min and detected at 214 and 280 nm.

Biochemistry. Compounds were evaluated for their ability to inhibit the serine proteases, FXa, thrombin, trypsin, and plasmin. In vitro kinetic assays were conducted at 37 °C using a kinetic spectrophotometric plate reader. An optimized concentration of human enzyme in buffer (0.5 nM final concentration for thrombin and trypsin, and 1.0 nM for FXa and plasmin) was combined with 2 μL of inhibitor dilutions (creating a final concentration range of 1.0 nM to 100 μM) and preincubated for 1 h at room temperature. The assay was initiated by the addition of an appropriate synthetic substrate (S-2765 (Pharmacia Hepar) for FXa, CHROMOZYM TH (Boehringer Mannheim) for thrombin, S-2222 (Pharmacia Hepar) for trypsin, and S-2403 (Pharmacia Hepar) for plasmin, at predetermined $2 \times K_m$. Absorbance at 405 nM was determined over 10 min, and percent inhibition was calculated from the slope of the progress curves during the linear part of the time course at each concentration. The IC_{50} was defined as that concentration of test substance that inhibited 50% of the respective protease activity. Typically an $n = 2$ was performed on each compound such that data reported is an average of the two measurements.

Crystallization. Bovine pancreatic trypsin and benzamidine were obtained from Sigma Chemical Company. Crystals of the trypsin−benzamidine complex were obtained by the hanging drop vapor diffusion method. 8 μL drops of protein solution containing trypsin (20 mg/mL), benzamidine (0.5 M), calcium chloride (0.01 M), Tris-HCl (0.05 M), and ammonium sulfate (1.25 M) at pH 7.0 were allowed to equilibrate against a reservoir containing ammonium sulfate (600 μL, 2.5 M) and Tris-HCl (0.1 M) at pH 6.5. Rectangular rod shaped crystals of the trypsin−benzamidine complex appeared after 48 h and continued to grow for several weeks. Crystals of the trypsin−benzamidine complex are orthorhombic, space group P212121 with unit cell dimensions of $a = 63.70$, $b = 63.40$, and $c = 69.10$. Crystals of the complex of trypsin with **35** were obtained by soaking pregrown trypsin−benzamidine crystals in solution containing: **35** (10 mM), calcium chloride (0.01 M), Tris-HCl (0.10 M), and ammonium sulfate (3.0 M) at pH 7.0 and 24 °C for 72 h.

Crystal Data Collection. For X-ray data collection, crystals of the trypsin−**35** complex were sealed in thin walled glass capillaries in equilibrium with soaking solution. X-ray diffraction data to 1.7 Å resolution were collected at 24 °C using a MarResearch Image Plate X-ray detector and a Rigaku Ru-200B rotating anode X-ray generator operating at 50 kV and 120 mA. The crystals of the complex are of the orthorhombic, space group P212121, with unit cell dimensions of $a = 63.70$, $b = 63.40$, $c = 69.40$Å. A total of 28352 reflections (out of a possible 28523) with $I/(I) \geq 2.0$ were measured to 1.7 Å resolution.

Crystal Structure Determination and Refinement. The structure was solved by difference electron density methods and the published coordinates for the bovine pancreatic trypsin structure.[24] Difference electron density maps using data to 1.7Å resolution revealed the positions and conformations of a single bound **35** ligand located in the active site of the trypsin molecule (Figure 1). Compound **35** was fit to the difference electron density using QUANTA and the complex structure including 80 water molecules was refined to 1.7 Å resolution using XPLOR.[25] The final crystallographic *R*-factor was 0.200 using diffraction data from 8.0 to 1.7 Å.

3-(2,2,2-Trichloro-1-hydroxyethyl)benzonitrile (4). To a mixture of lead(II) bromide (1.69 g, 4.60 mmol) and finely cut aluminum foil (1.25 g, 45.8 mmol) in DMF (226 mL) were added 3-cyanobenzaldehyde (6.01 g, 45.8 mmol) and carbon tetrachloride (8.84 mL, 91.7 mmol.). The mixture was stirred at ambient temperature for 3 h before being quenched with HCl (1 N, 100 mL). The aqueous mixture was extracted with ethyl acetate (3×), and the combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to give the trichloromethyl carbinol (**4**) as a brown oil in quantitative yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.94 (m, 1H), 7.86 (m, 1H), 7.66 (m, 1H), 7.48 (m, 1H), 5.24 (s, H), 4.98 (bs, 1H).

2-(3-Cyanophenyl)-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (5a). To 2-aminothiophenol (2.3 mL, 22.3 mmol) in DMF (60 mL) was added sodium hydride (3.61 g, 90.3 mmol). After bubbling and the evolution of heat ceased, a solution of **4** (5.67 g, 22.6 mmol) in DMSO (40 mL) was added dropwise over 60 s. After the evolution of heat ceased, the mixture was stirred at 50 °C for 6 h. and at room temperature for 16 h. The reaction mixture was cooled, diluted with water and extracted with ethyl acetate (3×). The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo. The residue was absorbed onto silica gel and purified by gradient chromatography (1% to 4% methanol in dichloromethane). The residue was crystallized from ethyl acetate and hexanes to provide 1.71 g (29%) of the title compound **5a** as a tan solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.97 (s, 1H), 7.75 (m, 1H), 7.57 (m, 2H), 7.32 (m, 1H), 7.20 (m, 1H), 7.00 (m, 3H), 5.10 (s, 1H).

1-(2-Benzyloxy-5-bromophenyl)-2,2,2-trichloroethanol (7). Prepared using the same procedure as to synthesize **4.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 1H), 7.30−7.40 (m, 6H), 6.85 (d, 1H), 5.71 (d, 1H), 5.15 (d, 2H).

2-(2-Benzyloxy-5-bromophenyl)-4*H*-benzo[1,4]thiazin-3-one (8). Prepared via the same route to Compound **4**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18−7.43 (m, 9H), 7.02 (t, 1H), 6.98 (d, 1H), 6.80 (d, 1H), 5.16 (s, 1H), 5.11 (s, 2H). MS (CI) *m*/*z* 426/428 [M + H]. Anal. ($C_{21}H_{16}BrNO_2S$) C, H, N.

4-Benzyloxy-3-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazin-2-yl}benzonitrile (5b). To a solution of **8** (1.11 g, 2.6 mmol) in DMF (10 mL) was added CuCN (460 mg, 5.2 mmol), and the mixture was warmed to 160 °C for 12 h or until HPLC showed disappearance of starting **8**. The mixture was diluted with water and extracted with ethyl acetate (3×). The combined organics were washed with brine and dried over $MgSO_4$. After concentration, the title compound was obtained in quantitative yield and used as is without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (dd, 1H), 7.20−7.75 (m, 9H), 6.96−7.40 (m, 1H), 6.89 (d,1H), 5.19 (s, 1H), 5.16 (s, 1H). MS (CI) *m*/*z* 373 [M + H].

(*E*)-2-(3-Hydroxyphenyl)-3-(2-nitrophenyl)-2-propenoic Acid (10). To a mixture of 2-nitrobenzaldehyde (19.22 g, 127 mmol) and 3-hydroxyphenylacetic acid (19.30 g, 127 mmol)

Table 7. Plasma Concentrations of Compound **35** (ng/mL) Following Intravenous Administration to Rabbits

	time postdose (min)					
dose	0	5	15	60	120	140
30 μg/kg + 1 μg/kg/min.	BLQ[a]	80.0 ± 17.8	80. 3± 15.5	70.8 ± 12.1	79.7 ± 10.5	102 ± 17.1
60 μg/kg + 2 μg/kg/min.	BLQ	135 ± 14.4	119 ± 16.7	188 ± 43.7	187 ± 37.1	176 ± 18.4
480 μg/kg + 16 μg/kg/min.	BLQ	830 ± 116.5	784 ± 60.7	907 ± 75.9	1009 ± 55.8	1012 ± 90.6

[a] BLQ = Below the limit of quantitation.

in acetic anhydride (87 mL) was added triethylamine (17.5 mL, 126 mmol), and the reaction mixture was stirred and heated at reflux (150 °C) for 45 min. The mixture was diluted with water (200 mL), cooled, diluted with NaOH (2 N, 200 mL), and washed with ether. The aqueous solution was then acidified with HCl (6 N) to pH 3 and stirred for 3 h. The solid was collected and dried under high vacuum at 45 °C to give 23.1 g (64%) of the desired compound **10**. ^{1}H NMR (300 MHz, DMSO): δ 8.09 (m, 1H), 7.93 (s, 1H), 7.48 (m, 2H), 7.00 (m, 2H), 6.59 (m, 1H), 6.48 (m, 2H). MS (CI) *m*/*z* 285 [M + H].

3-(3-Hydroxyphenyl)-3,4-dihydro-2(1*H*)-quinolinone (11). To (*E*)-2-(3-hydroxyphenyl)-3-(2-nitrophenyl)-2-propenoic acid (**10**) (26.29 g, 92.0 mmol) in methanol (600 mL) was added 20% Pd/C (1.5 g), and the mixture was hydrogenated at 45 psi of hydrogen and 30 °C for 3.5 h. The mixture was filtered through Celite and the filter pad washed with methanol. The combined filtrate and washings were concentrated in vacuo, and the product was crystallized from methanol in water to give 17.9 g (82%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO): δ 10.30 (s, 1H), 9.31 (s, 1H), 7.14 (m, 2H), 7.05 (m, 1H), 6.90 (m, 2H), 6.61 (m, 3H), 3.70 (m, 1H), 3.20−3.06 (m, 2H).

3-(2-Oxo-1, 2,3,4-tetrahydro-3-quinolinyl) phenyl trifluoromethanesulfonate (12). To 3-(3-hydroxyphenyl)-3,4-dihydro-2(1*H*)-quinolinone (**11**) (3.01 g, 12.6 mmol) in THF (40 mL) was added sodium hydride (0.55 g, 13.8 mmol), and the mixture was stirred at room temperature for 5 min. To this mixture was added *N*-phenyltrifluoromethanesulfonimide (4.92 g, 13.8 mmol), and the reaction mixture was stirred at room temperature for 1 h. The mixture was cooled, diluted with H_2O, and extracted with EtOAc (3×). The combined organic extracts were washed with brine (2×), dried with $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified via silica gel chromatography (20% to 40% ethyl acetate in hexanes). The product **12** (4.29 g, 92%) was isolated as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.40−7.15 (m, 6H), 7.00 (m, 1H), 6.76 (m, 1H), 3.87 (m, 1H), 3.22 (m, 2H). MS (CI) *m*/*z* 372 [M + H].

3-(2-Oxo-1,2,3,4-tetrahydro-3-quinolinyl)benzenecarbonitrile (5c). To 3-(2-oxo-1,2,3,4-tetrahydro-3-quinolinyl)-phenyl trifluoromethanesulfonate (**12**) (0.410 g, 1.11 mmol) in DMF (5 mL) were added zinc cyanide (0.078 g, 0.690 mmol) and tetrakis(triphenylphosphine)palladium(0) ($Pd(Ph_3P)_4$ (0.128 g, 0.11 mmol). The reaction mixture was stirred and heated at 100 °C for 1 h, followed by cooling, dilution with water (200 mL) and 2 M sulfuric acid (20 mL), and extraction with EtOAc (3×). The combined organic extracts were washed with brine (2×), dried over magnesium sulfate, filtered, and evaporated in vacuo. The product **5c** (0.222 g, 81%) was crystallized from ethyl acetate and hexanes to provide a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (s, 1H), 7.70−7.18 (m, 6H), 7.04 (m, 1H), 6.81 (m, 1H), 3.89 (m, 1H), 3.24 (m, 2H). MS (CI) *m*/*z* 249 [M + H].

3-[(*E*)-(2-Nitrophenyl)methylidene]-1-benzofuran-2-one (14). 2-Hydroxyphenylacetic acid (1.83 g, 10.0 mmol) was added to a mixture of 2-nitrobenzaldehyde (1.83 g, 10.0 mmol), acetic anhydride (10 mL), and triethylamine (1.7 mL, 12.0 mmol). The reaction mixture was heated at 140 °C for 15 min. The mixture was allowed to cool to 80 °C at which point water (10 mL) was carefully added. The precipitated material was filtered, washed with methanol, and dried in vacuo to afford the required product **14** (0.905 g, 28%). A further amount of product (1.523 g) was recovered from the filtrate and recrystallized from methanol/water. ^{1}H NMR (400 MHz, DMSO): δ 8.36 (d, 1H), 8.15 (1H, s), 8.00−7.80 (m, 3H), 7.42 (t, 1H), 7.31 (d, 1H), 7.04 (m, 2H). MS (CI) *m*/*z* 267 [M + H].

3-(2-Hydroxyphenyl)-3,4-dihydro-2(1*H*)-quinolinone (15). To 3-[(*E*)-(2-nitrophenyl)methylidene]-1-benzofuran-2-one (**14**) (2.18 g, 7.65 mmol) in methanol (70 mL) and THF (30 mL) was added Pd/C 20% (50 mg), and the mixture was hydrogenated for 5 h. Filtration and flash chromatography (30−50% ethyl acetate in hexanes) afforded the desired product **15** (0.913 g, 49%). ^{1}H NMR (300 MHz, DMSO): δ 9.52 (s, 1H), 7.15 (m, 2H), 7.05 (m, 1H), 6.97 (m, 1H), 6.89 (d, 2H), 6.83 (d, 1H), 6.67 (t, 1H), 3.96 (dd, 1H), 3.19 (dd, 1H), 2.99 (dd, 1H). MS (CI) *m*/*z* 240 [M + H]. Anal. ($C_{15}H_{13}N_1O_2$) C, H, N.

3-(5-Bromo-2-hydroxyphenyl)-3,4-dihydro-2(1*H*)-quinolinone (16). To 3-(2-hydroxyphenyl)-3,4-dihydro-2(1*H*)-quinolinone (**15**) (0.650 g, 2.72 mmol) in carbon disulfide (10 mL) and dichloromethane (10 mL) was slowly added (10 min) a solution of bromine (0.17 mL, 3.30 mmol) in carbon disulfide (5 mL). After 2 h, a precipitate had formed which was collected and washed with ethyl ether. This afforded the title compound **16** (0.738 g, 85%). ^{1}H NMR (400 MHz, DMSO): δ 10.29 (s, 1H), 7.21 (m, 2H), 7.16 (m, 2H), 6.91 (m, 2H), 6.78 (d, 1H), 3.93 (dd, 1H), 3.19 (dd, 1H), 2.97 (dd, 1H). MS (CI) *m*/*z* 318 [M − H], 320 [M + H].

3-[2-(Benzyloxy)-5-bromophenyl]-3,4-dihydro-2(1*H*)-quinolinone. To 3-(5-bromo-2-hydroxyphenyl)-3,4-dihydro-2-(1*H*)-quinolinone (**16**) (3.16 g, 9.94 mmol) in DMF (15 mL) was added cesium carbonate (5.2 g, 31.0 mmol) followed by benzyl bromide (1.18 mL, 9.92 mmol). The reaction mixture was stirred at room temperature for 16 h, diluted with ethyl acetate (200 mL), washed with brine, dried over magnesium sulfate, combined, and concentrated under reduced pressure. Flash chromatography (20% ethyl acetate in hexanes) afforded the title compound (2.77 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (brs, 1H), 7.30 (m, 7H), 7.17 (d1, H), 7.11 (d, 1H), 6.98 (t, 1H), 6.84 (d, 1H), 6.78 (d, 1H), 5.07 (s, 2H), 4.09 (dd, 1H), 3.38 (dd, 1H), 2.99 (dd, 1H). MS (CI) *m*/*z* 408 [M − H], 410 [M + H].

4-(Benzyloxy)-3-(2-oxo-1,2,3,4-tetrahydro-3-quinolinyl)-benzenecarbonitrile (5d). The title compound was synthesized as per compound **5b** and isolated as a colorless oil (1.077 g, 45%). ^{1}H NMR (300 MHz, DMSO): δ 10.40 (s, 1H), 7.77 (dd, 1H), 7.69 (d, 1H), 7.40−7.10 (m, 8H), 6.89 (m, 2H), 5.22 (m, 2H), 4.04 (dd, 1H), 3.31(dd, 1H), 2.93 (dd, 1H). MS (CI) *m*/*z* 355 [M + H].

3-(3-Bromophenyl)-3,4-dihydro-1*H*-quinoxalin-2-one (18). To 2-bromo-(3-bromophenyl)acetic acid (4.95 g, 16.9 mmol) as a suspension in water (40 mL) was added powdered NaOH (0.65 g, 16.7 mmol) to yield a red solution. 2-Phenylenediamine (1.83 g, 16.9 mmol) was added, and the mixture was stirred at 60 °C for 30 min. The formed solid was filtered, washed with water, and dried in vacuo. The remaining residue was recrystallized from ethanol and water to afford 1.88 g (37%) of the title compound. ^{1}H NMR (400 MHz, DMSO) δ 7.40−7.50 (m, 2H), 7.20−7.40 (m, 2H), 6.65−6.80 (m, 2H), 6.58−6.63 (m, 2H). MS (CI) *m*/*z* 303/305 [M + H] and 300/303 [M − H].

3-(3-Bromophenyl)-1*H*-quinoxalin-2-one (19). To a solution of 3-(3-bromophenyl)-3,4-dihydro-1*H*-quinoxalin-2-one (1.73 g, 5.71 mmol) in toluene (50 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.30 g, 5.73 mmol) and the mixture was warmed to reflux for 3 h. The formed solid was filtered to afford the title compound (2.49 g, 66%), which was used without further purification. ^{1}H NMR (400 MHz, DMSO) d 8.45 (s, 1H), 8.25 (d, 1H), 7.80 (d, 1H), 7.66 (d, 1H), 7.41 (t, 1H),

7.30 (t, 1H), 7.28 (m, 2H). MS CI *m/z* 300/302 [M + H] and 298/300 [M − H]. Anal.($C_{14}H_9N_2OBr$) C, H, N.

3-(3-Oxo-3,4-dihydroquinoxalin-2-yl)benzonitrile (5e). Following the preparation for **5b** gave the title compound in 92% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.76 (t, 1H), 8.62 (dt, 1H), 7.91 (m, 1H), 7.80 (d, 1H), 7.71 (t, 1H), 7.55 (t, 1H), 7.20−7.35 (m, 2H). MS CI *m/z* 248 [M + H] and 247 [M$^-$]. Anal. ($C_{15}H_9N_3O$) C, H, N.

4-(5-Bromopentyl)-2-(3-cyanophenyl)-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (20a). To the benzothiazinone **5a** (0.75 g, 2.82 mmol) in DMF (5 mL) was added sodium hydride (0.124 g, 3.09 mmol), and the solution was stirred at 70 °C for 15 min, at which point, the bubbling had stopped. To this solution was added 1,5-dibromopentane (1.54 mL, 11.2 mmol), and the reaction mixture was stirred at 70 °C for an additional 16 h. The reaction mixture was cooled, diluted with water, and extracted with ethyl acetate (3×). The combined organic extracts were washed with brine (2×), dried over magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified via gradient chromatography (20% to 30% ethyl acetate in hexanes). The title compound (**20a**) was isolated (0.66 g, 52%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (m, 3H), 7.38 (m, 2H), 7.25 (m, 1H), 7.11−7.01 (m, 2H), 4.64 (s, 1H), 4.09 (m, 2H), 3.40 (m, 2H), 1.91 (m, 2H), 1.70 (m, 2H), 1.53 (m, 2H).

4-Benzyloxy-3-[4-(5-bromopentyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazin-2-yl}benzonitrile (20b). Prepared via the same route as compound **20a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, 1H), 7.20−7.74 (m, 9H), 6.95−7.05 (m, 2H), 5.18 (s, 2H), 5.04 (s, 1H), 4.03−4.19 (m, 2H), 3.40 (t, 2H), 1.83−1.98 (m, 2H), 1.66−1.80 (m, 2H), 1.47−1.58 (m, 4H). MS (CI) *m/z* 521/523 [M + H].

3-[1-(5-Bromopentyl)-2-oxo-1,2,3,4-tetrahydro-3-quinolinyl]benzenecarbonitrile (20c). Prepared via the same route as **20a** and the product **20c** was isolated (1.59 g, 36%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58−7.28 (m, 5H), 7.20 (m, 1H), 7.05 (m, 2H), 3.99 (m, 2H), 3.85 (m, 1H), 3.41 (m, 2H), 3.19 (m, 2H), 1.91 (m, 2H), 1.70 (m, 2H), 1.54 (m, 2H). MS (CI) *m/z* 397/399 [M + H], 395/396 [M − H].

4-(Benzyloxy)-3-[1-(5-bromopentyl)-2-oxo-1,2,3,4-tetrahydro-3-quinolinyl]benzenecarbonitrile (20d). Prepared via the same route as compound **20a** and isolated as an oil (0.230 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (dd, 1H), 7.42 (d, 1H), 7.40−7.20 (m, 6H), 7.13 (d, 1H), 7.02 (m, 3H), 5.15 (m, 2H), 4.04 (dd, 1H), 3.98 (m, 2H), 3.39 (t, 2H), 3.30 (dd, 1H), 2.96 (dd, 1H), 1.88 (m, 2H), 1.75−1.45 (m, 4H). MS (CI) *m/z* 505 [M + H], 503 [M − H].

3-[4-(5-Bromopentyl)-3-oxo-3,4-dihydroquinoxalin-2-yl]benzonitrile (20e). Prepared via the same route as compound **20a** to yield 46% along with 29% of the O-alkylated analogue. ^{1}H NMR (400 MHz, DMSO) δ 8.65 (t, 1H), 8,42 (dt, 1H), 8.18 (d, 1H), 7.85 (d, 1H), 7.60−7.80 (m, 4H), 4.61 (t, 2H), 3.46 (t, 2H), 1.88−2.05 (m, 4H), 2.65−2.78 (m, 2H). MS (CI) *m/z* 396/398 [M + H] and 395/397 [M − H].

5-[(2*R*,6*S*)-2,6-Dimethylpiperidin-1-yl]pentyl-2-(3-cyanophenyl)-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (21a). To **20a** (0.66 g, 1.59 mmol) was added *cis*-2,6-dimethylpiperidine (6.0 mL, 44.0 mmol), and the reaction mixture was stirred at 70 °C for 48 h. A small amount of DMF was added to obtain a homogeneous mixture before heating. The solution was cooled, diluted with water, and extracted with ethyl acetate (3×). The combined organic extracts were washed with saturated sodium bicarbonate (2×), brine (2×), dried with magnesium sulfate, filtered, and evaporated in vacuo. The residue was coevaporated with toluene and dried under high vacuum to give 0.69 g (97%) of **21a** as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55 (m, 3H), 7.37 (m, 2H), 7.24 (m, 1H), 7.10−7.00 (m, 2H), 4.63 (s, 1H), 4.07 (m, 2H), 2.74 (m, 2H), 2.47 (m, 2H), 1.76−1.22 (m, 12H), 1.08 (m, 6H). HPLC 14.2 min.

4-Benzyloxy-3-{1-[5-((2*S*,6*R*)-2,6-dimethylpiperidin-1-yl)pentyl]-3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazin-2-yl}benzonitrile (21b). Prepared via the same route as compound **21a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (dd, 1H), 7.05−7.40 (m, 9H), 6.90−7.00 (m, 2H), 5.12 (s, 2H), 4.97 (s, 1H), 4.04 (m, 2H), 2.35−2.72 (m, 4H), 0.75−1.65 (m, 20H). MS (CI) *m/z* 554 [M + H]. HPLC 17.4 min.

3-{1-[5-[(2*R*,6*S*)-2,6-Dimethylpiperidin-1-yl]pentyl-2-oxo-1,2,3,4-tetrahydro-3-quinolin-2-yl)benzonitrile (21c). Prepared via the same route as compound **21a**, and the title compound was isolated in quantitative yield as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50−6.96 (m, 8H), 3.91 (m, 2H), 3.80 (m, 1H), 3.13 (m, 2H), 2.67 (s, 2H), 2.36 (s, 2H), 1.66−1.15 (m, 12H), 1.01 (m, 6H). HPLC 14.2 min.

4-Benzyloxy-3-{1-[5-[(2*R*,6*S*)-2,6-dimethylpiperidin-1-yl]pentyl-2-oxo-1,2,3,4-tetrahydro-3-quinolinyl}benzonitrile (21d). Prepared via the same route as compound **21a**, but the title compound was not isolated, but rather taken directly on to **22d**.

3-{4-[5-((2*S*,6*R*)-2,6-Dimethylpiperidin-1-yl)pentyl]-3-oxo-3,4-dihydroquinoxalin-2-yl}benzonitrile (21e). Prepared via the same route as compound **21a** and wa s used without further purification. MS CI *m/z* 429 [M + H] and 428 [M$^-$]. HPLC 14.6 min.

3-(4-[5-[(2*R*,6*S*)-2,6-Dimethylpiperidin-1-yl]pentyl]-3-oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)-1-benzenecarboximidamide (22a). To **21a** (0.30 g, 0.67 mmol) in methanol (10 mL) were added *N*-hydroxylamine hydrochloride (0.116 g, 1.67 mmol) and diisopropylethylamine (DIEA, 0.12 mL, 0.67 mmol). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated in vacuo, and the remaining residue was dried under high vacuum. To the residue was added trifluoroacetic anhydride (2 mL), and the resulting solution was stirred at room temperature for 2 h. The reaction mixture was diluted with acetic acid, and the mixture was concentrated in vacuo to give a yellow oil. To the yellow oil in acetic acid (2 mL) was added 20% Pd/C (45 mg), and the mixture was hydrogenated at 23 °C for 2 h. The mixture was filtered through Celite and the filter pad washed with acetic acid. The combined filtrate and washings were evaporated in vacuo and purified by preparative HPLC (Vydac 218TP1022 C-18, 95:5 (H_2O:CH_3CN + 0.1% TFA) to 60:40 (H_2O:CH_3CN + 0.1% TFA) over 90 min), and appropriate fractions were combined and lyophilized to provide 50 mg (16%) of the title compound **22a** as a fluffy off-white solid. ^{1}H NMR (400 MHz, DMSO): δ 9.33 (s, 1H), 9.18 (s, 1H), 7.69 (m, 3H), 7.55 (m, 1H), 7.41 (m, 2H), 7.33 (m, 1H), 7.07 (m, 1H), 5.11 (s, 1H), 4.09 (m, 2H), 3.38 (m, 2H), 3.24 (m, 2H), 3.10 (m, 2H), 2.91 (m, 2H), 1.84 (m, 2H), 1.66−1.33 (m, 6H), 1.24 (m, 6H). MS (CI) *m/z* 465 [M + H] 464 [M − H]. HPLC 8.9 min.

3-{4-[5-((2*S*,6*R*)-2,6-Dimethylpiperidin-1-yl)-pentyl]-3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazin-2-yl}-4-hydroxybenzamidine (22b). Prepared via the same synthetic route as compound **22a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (dd, 1H), 7.40 (M, 4h), 7.05 (m, 2H), 4.88 (s, 1H), 4.00−4.22 (m, 4H), 2.80−3.20 (m, 4H), 1.20−1.80 (m, 16H). MS (CI) *m/z* 481 [M + H]. HPLC 8.77 min.

3-(1-[5-[(2*R*,6*S*)-2,6-Dimethyltetrahydro-1(2*H*)-pyridinyl]pentyl]-2-oxo-1,2,3,4-tetrahydro-3-quinolinyl)benzenecarboximidamide (22c). Prepared via the same synthetic route as compound **22a** and converted to an HCl salt by filtration through Amberlite 400(Cl) resin to give 619 mg (52%) of **22c** as an off-white solid. ^{1}H NMR (400 MHz, DMSO): δ 9.35 (s, 1H), 9.07 (s, 2H), 7.66 (m, 2H), 7.49 (m, 2H), 7.21 (m, 3H), 6.97 (m, 1H), 3.95 (m, 3H), 3.42 (s, 4H), 3.35−2.86 (m, 6H), 1.75−1.26 (m, 8H), 1.20 (m, 6H). MS (CI) *m/z* 447 [M + H]. HPLC 9.6 min.

3-(1-[5-[(2*R*,6*S*)-2,6-Dimethyltetrahydro-1(2*H*)-pyridinyl]pentyl]-2-oxo-1,2,3,4-tetrahydro-3-quinolinyl)-4-hydroxybenzenecarboximidamide (22d). Prepared via the same synthetic route as compound **22a** and isolated as a colorless powder (0.123 g, 50%). ^{1}H NMR (400 MHz, DMSO): δ 10.89 (s, 1H), 10.20 (brs, 1H), 9.09 (s, 2H), 8.80 (s, 1H), 7.62 (m, 2H), 7.20 (m, 3H), 7.00 (m, 2H), 3.90 (m, 3H), 3.40 (m, 1H), 3.20 (m, 2H), 3.00 (m, 1H), 2.85 (m, 2H), 1.80−1.20 (m, 18H). MS (CI) *m/z* 463 [M + H]. TOF HRMS calcd 463.3073, found 463.3069. HPLC 10.1 min.

3-{4-[5-((2*S*,6*R*)-2,6-Dimethylpiperidin-1-yl)pentyl]-3-oxo-3,4-dihydroquinoxalin-2-yl}benzamidine (22e). Pre-

pared via the same synthetic route as compound **22a**. ^{1}H NMR (400 MHz, DMSO) δ 8.70 (m, 1H), 8.68(d, 1H), 7.95 (t, 2H), 7.78 (m, 3H), 7.48 (m, 1H), 4.44 (m, 2H), 3.00−3.40 (m, 2H), 1.40−1.80 (m, 12H), 1.28 (d, 6H). MS CI *m/z* 446 [M + H]. TOF HRMS calcd 466.2920, found 466.2911. HPLC 8.5 min.

3-Bromo-1-(5-bromopentyl)quinolin-2-one (24). To a solution of **23** (2.0 g, 8.92 mmol) in DMF (6 mL) were added NaH (390 mg of a 60% oil dispersion, 9.75 mmol) and 1,5-dibromopentane (2.6 mL, 18 mmol). The reaction mixture was stirred for 3 h at ambient temperature before being diluted with water and extracted with ethyl acetate (3×). The combined organics were washed with brine and dried over magnesium sulfate. After evaporation, the residue was purified by column chromatography (20% ethyl acetate in hexanes) to yield 1.76 g (33%) of the desired product. ^{1}H NMR (300 MHz, DMSO) δ 8.13 (s, 1H), 7.48−7.63 (m, 2H), 7.36 (d, 1H), 7.25 (t, 1H), 4.35 (t, 2H), 3.43 (t, 2H), 1.92 (m, 2H), 1.80 (m 2H), 1.63 (m, 2H). MS (CI) *m/z* 371/373/375 [M + H].

3-[1-(5-Bromopentyl)-2-oxo-1,2-dihydroquinolin-3-yl]-benzonitrile (25a). To a mixture of **24** (720 mg, 1.93 mmol) in toluene (6 mL) and ethanol (6 mL) was added saturated aqueous sodium bicarbonate (6 mL). $Pd(PPh_3)_4$ was added followed by 3-cyanophenylboronic acid, and the mixture was stirred at 100 °C for 4 h. The mixture was diluted with and washed with water and brine. After the organics were dried over magnesium sulfate, the residue was purified by column chromatography (20% ethyl acetate in hexanes) to provide 560 mg of **25a** (74%). ^{1}H NMR (400 MHz, DMSO) δ 8.08 (s, 1H), 8.00 (d, 1H), 7.85 (s, 1H), 7.62 (m, 3H), 6.48 (t, 1H), 6.40 (d, 1H), 7.25 (d, 1H), 4.39 (t, 2H), 3.42 (t, 2H), 1.98 (m, 2H), 1.82 (m, 2H), 1.64 (m, 2H). MS (CI) *m/z* 397/399 [M + H].

4-Benzyloxy-3-[1-(5-bromopentyl)-2-oxo-1,2-dihydroquinolin-3-yl}benzonitrile (25b). To a solution of **20d** (600 mg, 1.19 mmol) in toluene (5 mL) was added DDQ (540 mg, 2.38 mmol). The mixture was warmed to 80 °C in a sealed tube. After 18 h, compound **20d** still remained. Additional DDQ (540 mg, 2.38 mmol) was added, and the mixture was stirred at 80 °C for 72 h. The reaction mixture was diluted with water and extracted with ethyl acetate (3×). The combined organics were washed with brine and dried over magnesium sulfate. The mixture was filtered, evaporated, and purified by flash chromatography (20% ethyl acetate in hexanes) to give 68% of the slightly impure desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.00−7.80 (m, 12H), 5.16 (s, 2H), 4.45 (t, 2H), 4.34 (t, 2H), 1.50−2.00 (m, 6H). MS (CI) *m/z* 501/503 [M + H].

3-{1-[5-((2*S*,6*R*)-2,6-Dimethylpiperidin-1-yl)pentyl]-2-oxo-1,2-dihydroquinolin-3-yl}benzonitrile (26a). Prepared via the same synthetic route as compound **21a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.92 (d, 1H), 7.78 (s, 1H), 7.40−7.62 (m, 3H), 7.33 (d, 1H), 7.05−7.25 (m, 2H), 4.29 (t, 2H), 2.60−2.80 (m, 2H), 2.30−2.50 (m, 2H). 1.00−1.80 (m, 18H). MS (CI) *m/z* 428 [M + H]. HPLC 14.2 min.

4-Benzyloxy-3-{1-[5-((2*S*,6*R*)-2,6-dimethylpiperidin-1-yl)-pentyl]-2-oxo-1,2-dihydroquinolin-3-yl}benzonitrile (26b). Prepared via the same synthetic route as compound **21a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.00−7.80 (m, 12H), 5.09 (s, 2H), 4.41 (t, 2H), 4.26 (t, 2H), 2.70 (m, 2H), 2.40 (m, 2H), 1.00−1.90 (m, 16H). MS (CI) *m/z* 534 [M + H]. HPLC 16.7 min.

3-{1-[5-((2*S*,6*R*)-2,6-Dimethylpiperidin-1-yl)pentyl]-2-oxo-1,2-dihydroquinolin-3-yl}benzamidine (27a). Prepared via the same synthetic route as compound **21a** The HCl salt was formed by treating an aqueous solution of the TFA salt with Amberlite IRA 400(Cl) resin followed by filtration and lyopholization. ^{1}H NMR (400 MHz, DMSO) δ 8.38 (s, 1H), 8.18 (s, 1H), 8.12 (d, 1H), 7.81 (t, 2H), 7.64 (m,3H), 7.32 (t, 1H), 4.23 (bs, 2H), 2.80−3.40 (m, 4H), 1.40−1.80 (m, 12H), 1.26 (d, 6H). MS (CI) *m/z* 445 (M + H). HPLC 9.2 min.

3-{1-[5-((2*S*,6*R*)-2,6-Dimethyl-piperidin-1-yl)-pentyl]-2-oxo-1,2-dihydroquinolin-3-yl}-4-hydroxybenzamidine (27b). Amidine was formed as previously mentioned for **22a**, and the benzyl group was removed via hydrogenation. The HCl salt was prepared as described for **27a** ^{1}H NMR (300 MHz, DMSO) δ 8.35 (s,1H), 7.61−7.83 m, 5H), 7.36 (t, 1H), 7.16 (d, 1H), 4.25−4.41 (m, 2H), 3.00−3.25 (m, 2H), 1.22−1.82 (m, 14H), 0.85 (m, 6H). MS (CI) *m/z* 461 [M + H]. HPLC 9.6 min.

5-Benzyloxy-2-bromobenzaldehyde (28). 5-Bromosalicylaldehyde (100 g, 496 mmol) was dissolved in ethanol (300 mL), and a solution of potassium hydroxide (27.8 g, 496 mmol) in water (80 mL) was added. After the mixture was stirred at ambient temperature for 30 min, benzyl bromide (71.2 mL, 596 mmol), which was previously passed through a plug of neutral alumina, was added. The resulting mixture was warmed to reflux for 18 h, at which time a colorless precipitate formed. After the mixture was cooled to room temperature, water (50 mL) was added and the title compound was filtered. After the mixture was washed with water, **28** was recrystallized from ethanol to provide 122 g (85%) of a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (d, 1H); 7.90 (d, 1H); 7.56 (dd, 1H); 7.30 (m, 5H); 6.91 (d, 1H); 5.14 (s, 2H). MS (CI) *m/z* 292 [M^+], 261 [M − CHO]. HPLC 20.4 min.

(2-Benzyloxy-5-bromophenyl)-2-hydroxyacetonitrile (29). Aldehyde **28** (20 g, 69 mmol) was dissolved in methanol (150 mL) followed by the addition of potassium cyanide (20.1 g, 308 mmol). Acetic acid (6.3 mL, 109 mmol) was added dropwise over 20 min at room temperature. The resulting mixture was stirred at ambient temperature for 3 h. Acetic acid (2 mL) was added, and the mixture was stirred for an additional 60 min. Additional acetic acid (30 mL) was added, and the mixture was diluted with water (60 mL). After concentration at reduced pressure, the aqueous residue was extracted with ethyl acetate (3×). The combined organics were washed with brine and dried over magnesium sulfate. Filtration and concentration left a pale yellow oil, which was used without further purification. The title compound could be isolated in pure form by gradient column chromatography (10 to 20% ethyl acetate in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, 1H); 7.24 (m, 6H); 6.86 (d, 1H); 5.52 (d, 1H); 5.13 (s, 2H); 3.38 (d, 1H). MS (CI) *m/z* 199, 201 [M − Bn − CN]. HPLC 20.4 min.

(2-Benzyloxy-5-bromophenyl)-2-hydroxyacetic Acid Methyl Ester (30). Crude **29**, from above, was dissolved in anhydrous *p*-dioxane (25 mL), ethyl ether (25 mL), and methanol (6 mL). After the mixture was cooled to 0 °C, it was saturated with HCl gas and warmed to room temperature. A precipitate formed, the mixture was stirred for 90 min, and anhydrous ethyl ether was added as necessary to maintain viscousity. The reaction mixture was diluted with ethyl ether (200 mL) and filtered. The colorless solid was washed with ethyl ether and was used without further purification. The solid was suspended in water (60 mL) and *p*-dioxane (60 mL) and stirred vigorously for approximately 4 h. The reaction was complete when all the solids had disappeared and an oily residue remained. The mixture was poured into water (150 mL) and extracted with ethyl acetate (3×). The combined organics were washed with brine and dried over magnesium sulfate. Filtration and concentration yielded the crude title compound **30**, which was used without purification. The title compound could be isolated in pure form by column chromatography (15% ethyl acetate in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4 (m, 7H), 6.82 (m, 1H), 5.32 (d, 1H), 5.90 (ABq, 2H), 3.70 (s, 3H), 3.63 (d, 2H). MS (CI) *m/z* 352 [M^+]. HPLC 17.5 min.

(2-Benzyloxy-5-bromophenyl)-2-oxoacetic Acid Methyl Ester (31). Dimethyl sulfoxide (DMSO, 12.4 mL, 164 mmol) was added slowly to a solution of oxalyl chloride (7.2 mL, 82.4 mmol) in dichloromethane (100 mL) at −78 °C. After addition, the mixture was stirred at −78 °C for 15 min. A solution of the alcohol **30** (68.6 mmol) in dichloromethane (60 mL) was added via a cannula. After the mixture was stirred for 30 min at −78 °C, triethylamine (47.8 mL, 343 mmol) was added, and the mixture was allowed to warm to room temperature. After the mixture was stirred for 1 h at room temperature, it was poured into water (200 mL). The layers were separated, and the aqueous layer was washed with ethyl acetate (3×). The combined organics were washed with brine and dried over magnesium sulfate and filtered, and after concentration, the title compound **31** was crystallized from ethyl acetate/hexanes

to yield 13.5 g (60% from aldehyde **28**) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, 1H); 7.65 (dd, 1H); 7.40 (m, 5H); 6.95 (d, 1H); 5.06 (s, 2H); 3.34 (s, 3H). MS (CI) *m/z* 256, 258[M^+]. HPLC 22.6 min.

3-(2-Benzyloxy-5-bromophenyl)-1-quinoxalin-2-one (32). Ester **31** (7.0 g, 20.0 mmol) was dissolved in methanol (60 mL), and 1,2-phenylenediamine was added (2.2 g, 20.0 mmol). The mixture was warmed to reflux overnight, at which time a precipitate was formed. The mixture was cooled to room temperature, and water (10.mL) was added. The product was filtered and washed with a small amount of cold methanol to yield 7.7 g (94%) of the title compound as a colorless solid. ^{1}H NMR (400 MHz, DMSO) δ 12.54 (s, 1H); 7.73 (d, 1H); 7.65 (m, 2H); 7.23 (m, 8H); 7.10 (d, 1H); 5.08 (s, 2H). MS (CI) *m/z* 407, 409[M^+]. HPLC 24.1 min.

4-Benzyloxy-3-(3-oxo-3,4-dihydroquinoxalin-2-yl)-benzonitrile (33). Bromide **32** (20.4 g, 50.0 mmol) was dissolved in anhydrous DMF (200 mL) followed by the addition of zinc cyanide (3.52 g, 30.0 mmol) and $Pd(Ph_3P)_4$ (5.78 g, 5.0 mmol). The resulting mixture was warmed to 100 °C for 5 h. The dark green solution was cooled to room temperature and stirred overnight. The title compound formed as a precipitate, which was separated by filtration and washed with ethyl ether to yield 12.3 g (69%). ^{1}H NMR (400 MHz, DMSO) δ 12.63 (s, 1H); 7.92 (dd, 1H); 7.87 (d, 1H); 7.79 (d, 1H); 7.58 (m, 1H); 7.2−7.4 (m, 8H); 5.24 (s, 2H). MS (CI) *m/z* 354 [M^+]. HPLC 16.4 min.

4-Benzyloxy-3-[4[(5-bromopentyl)-3-oxo-3,4-dihydroquinoxalin-2-yl]benzonitrile To a solution of **33** (2.0 g, 5.7 mmol) in DMF (50 mL) was added cesium carbonate (2.21 g, 6.8 mmol) at 0 °C. The mixture was stirred for 30 min before the addition of 1,5-dibromopentane (4.62 mL, 40.0 mmol). The reaction mixture was stirred overnight, while slowly warming to room temperature, before being poured into water (100 mL). The aqueous solution was extracted into ethyl acetate (3×). The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated under pressure. The title compound (1.59 g, 53%, (R_f = 0.15; 15% ethyl acetate in hexanes)) was isolated by gradient column chromatography (10 to 25% ethyl acetate in hexanes) along with the O-alkylated analogue (1.17 g, 41%, (R_f = 0.31; 15% ethyl acetate in hexanes)). N−Alkylated: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, 2H); 7.88 (d, 1H); 7.70 (dd, 1H); 7.57 (t, 1H); 7.2−7.4 (m, 6H); 7.03 (d, 1H); 5.14 (s, 2H); 4.24 (t, 2H); 3.36 (t, 2H); 1.8−1.9 (m, 2H); 1.7−1.8 (m, 2H); 1.5−1.6 (m, 2H). MS (CI) *m/z* 502, 504 [M^+]. HPLC 23.5 min.

4-Benzyloxy-3-{4-[5-((2*S*,6*R*)-2,6-dimethylpiperidin-1-yl)pentyl]-3-oxo-3,4-dihydroquinoxolin-2-yl}benzonitrile (34). 4-Benzyloxy-3-[4[(5-bromopentyl)-3-oxo-3,4-dihydroquinoxalin-2-yl]benzonitrile (1.59 g, 3.17 mmol) was dissolved in anhydrous DMF (5 mL), and *cis*-2,6-dimethylpiperidine (15 mL) was added. The reaction mixture was warmed to 75 °C overnight. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (2×). The combined organics were washed with brine, dried over magnesium sulfate, dried, and concentrated to yield a light yellow oil, which was used without further purification. ^{1}H NMR (400 MHz, DMSO) δ 7.95 (dd, 1H); 7.93 (d, 1H); 7.86 (d, 1H) 7.6−7.7 (m, 2H); 7.2−7.4 (m, 7H); 5.22 (s, 2H); 4.23 (t, 2H), 0.9−2.5 (m 22H). MS (CI) 535 [M + H]. HPLC 18.8 min.

4-Benzyloxy-3-{4-[5-((2*S*,6*R*)-2,6-dimethylpiperidin-1-yl)pentyl]-3-oxo-3,4-dihydroquinoxolin-2-yl}benzamidine (35). A solution of crude **34** (assume 3.17 mmol), in anhydrous ethanol (20 mL), at 0 °C was saturated with HCl (gas). The gaseous HCl was bubbled through concentrated sulfuric acid prior to the introduction to the reaction mixture. The reaction mixture was sealed and warmed to room-temperature overnight. The mixture was concentrated in vacuo and used without further purification. The crude residue from above was dissolved in ammonia (2 M in ethanol, 20 mL), and the flask was sealed. After stirring for 18 h at room temperature, HPLC showed complete consumption of starting materials. The mixture was concentrated and the residue was subjected to hydrogenation (Pd/C, hydrogen, TFA, 24 h). The mixture was concentrated and purified via preparative HPLC (100% water to 20% water 80% acetonitrile with 0.1%TFA over 120 min) to yield **35**. The lyophilized solid from above was dissolved in water (100 mL), and Amberlite 400(Cl) resin was added. After being stirred for 3 h, the resin was filtered and the aqueous solution was lyophilized. The lyophilized solid was dissolved in methanol (5 mL), and the product was crystallized by the slow addition of a vast excess of ethyl acetate. Filtration yielded compound **35** as a yellow solid (630 mg, 30%). ^{1}H NMR (400 MHz, DMSO) δ 9.14 (s, 1H); 9.00 (s, 1H); 8.34 (d, 1H); 7.91 (d, 1H); 7.81 (dd, 1H); 7.71 (m, 2H); 7.47 (m, 1H); 7.11 (d, 1H); 4.31 (m, 2H); 3.0−3.8 (m, 2H); 1.2−1.8 (m, 20H). MS (CI) *m/z* 462 [M + H]. HPLC 14.3 min. HPLC method: Vydac 218TP54 C18 column, 5 μm, 4.6 mm × 25 cm, eluted with a gradient of (95:5 to 10:90 water:acetonitrile with 0.1% TFA).

Supporting Information Available: Combustion analysis of the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. In the version of the paper posted June 26, 2004, "acetic anhydride" in the synthetic procedure for **22a** has been changed to "trifluoroacetic anhydride". The revised version of the paper was posted July 7, 2004.

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