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Synthesis, Potency, and in Vivo Evaluation of 2-Piperazin-1-ylquinoline Analogues as Dual Serotonin Reuptake Inhibitors and Serotonin 5-HT_{1A} Receptor Antagonists

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On the basis of the previously reported clinical candidate, SSA-426 (**1**), a series of related 2-piperazin-1-ylquinoline derivatives **3**−**16** were synthesized and evaluated as dual-acting serotonin (5-HT) reuptake inhibitors and 5-HT_{1A} receptor antagonists. In particular, compound **7** exhibits potent functional activities at both the 5-HT transporter and 5-HT_{1A} receptor, good selectivity over the α_1-adrenergic and dopaminergic receptors, acceptable pharmacokinetic properties, and a favorable in vivo profile.

Introduction

Selective serotonin reuptake inhibitors (SSRIs[a]) have achieved great success in treating depression and related psychiatric illnesses. While SSRIs are efficacious in 60−80% of patients, they require weeks of treatment before efficacy is observed.[1] This delay in efficacy is believed to be due to stimulation of inhibitory 5-HT_{1A} autoreceptors, and the onset of antidepressant activity is consistent with a time-dependent desensitization of these autoreceptors.[2] The combination of an SSRI and a 5-HT_{1A} receptor antagonist within one molecule could inhibit the 5-HT transporter while simultaneously antagonizing 5-HT_{1A} autoreceptors to prevent feedback inhibition of neuronal firing. This dual mechanism of action would maximize serotonergic function and result in an immediate increase in synaptic levels of 5-HT in forebrain regions. Over the past decade, both preclinical and clinical data have positioned SSRI/5-HT_{1A} receptor antagonism as an important target for the development of new antidepressant therapeutics.[3,4] As a result, this approach has been pursued by many pharmaceutical companies[5,6] and has been the focus of a major effort in our laboratories.[7−9]

(*S*)-2-((4-(1*H*-Indol-3-yl)-5,6-dihydropyridin-1(2*H*)-yl)-methyl)-8-methyl-2,3-dihydro-[1,4]dioxino-[2,3-*f*]quinoline (SSA-426, **1**, Figure 1),[7,8] Wyeth's first advanced SSRI/5-HT_{1A} antagonist, was tested extensively in preclinical animal models, lending further support to the potential of this approach for the treatment of depression. In vivo, this compound produced an immediate and dose-dependent increase in cortical extracellular levels of 5-HT following oral administration. This compound also showed efficacy in several behavioral models that are predictive of antidepressant-like activity.[8] To further investigate the SAR and build chemically diverse series of analogues, we initiated a study to replace the 3-indolyl tetrahydropyridinyl core (A region, Figure 1) with other 5-HT transporter pharmacophores.[9] In one of our initial efforts, we incorporated 6-nitroquipazine[10] (**2**, Figure 1) as the 5-HT transporter moiety. The resulting compound **3** demonstrated good binding affinity for both the 5-HT transporter and the 5-HT_{1A} receptor. We then turned our attention to modification of the substitutent at the C6-position of the quipazine moiety, and of the "linker" (B region, Figure 1) between the 8-methyl-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinolin-2-yl and 2-quinolinyl core. Herein, we report the synthesis and in vitro biological evaluation of compounds **3**−**16**, as well as in vivo characterization of compound **7**.

Chemistry

The requisite 2-(piperazin-1-yl)quinolines (**2**, **22**−**26**, and **30**−**34**) and 2-(homopiperazin-1-yl)quinolines (**27**−**28** and **35**) were either commercially available (**2**, **32**−**35**) or were readily prepared (**22**−**28** and **30**−**31**) by the method outlined in Schemes 1−2. The 2-(piperazin-1-yl)- and 2-(homopiperazine-1-yl)quinoline intermediates **22**−**28** were prepared by nucleophilic aromatic substitution of the corresponding 2-chloroquinolines **17**−**20** with the piperazines **21a**−**c** or homopiperazine **21d** in *N*,*N*-dimethylformamide and in the presence of potassium carbonate at 110−140 °C. The construction of 2-(piperazin-1-yl)quinoline-6-carbonitrile (**30**) was achieved in two steps utilizing a nucleophilic aromatic substitution reaction with piperazine-1-carbaldehyde (**29**). Subsequent heating of 2-(4-formylpiperazin-1-yl)quinoline-6-carbonitrile in aqueous sulfuric acid (4.0 M) at 130 °C triggered decarbonylation to give the piperazine analogue **30**. 2-(Piperazin-1-yl)quinoline-6-carboxamide (**31**) was obtained from the corresponding cyano analogue **30** via hydrogen peroxide-catalyzed nucleophilic addition of sodium hydroxide (Scheme 2). Finally, the 2-(piperazin-1-yl)- and 2-(homopiperazine-1-yl)quinolines (**2**, **22**−**28**, and **30**−**35**) underwent *N*-alkylation under thermal, basic conditions with (*R*)-(8-methyl-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinolin-2-yl)methyl 4-bromo-benzenesulfonate (**36**)[11] to provide the target compounds **3**−**16** (Scheme 3).

Results and Discussion

Structure−Activity Relationships. Compounds were evaluated in vitro to determine their binding affinities for both

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[a]Abbreviations: SSRIs, selective serotonin reuptake inhibitors; 5-HT, serotonin; r-5-HT-T, rat serotonin transporter; h-5-HT_{1A}, human 5-HT_{1A} receptor; CYP450, cytochrome P450.

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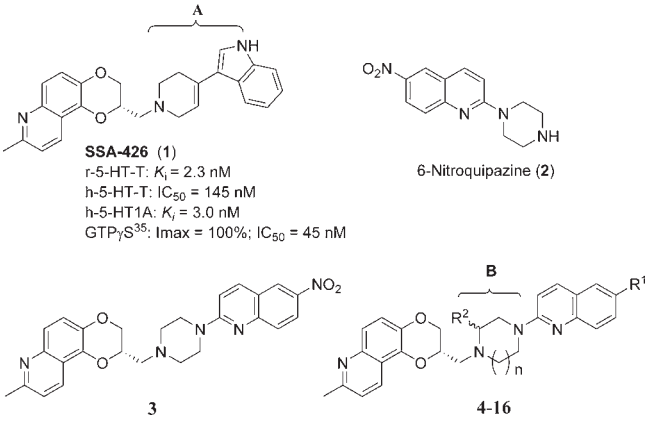
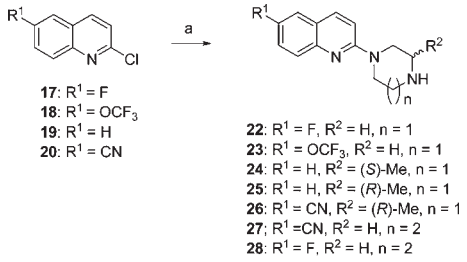


Figure 1. Compound Design.

Scheme 1[a]



[a] Reagents and conditions: (a) piperazine (**21a**), or (*S*)-2-methylpiperazine (**21b**) or (*R*)-2-methylpiperazine (**21c**) or 1,4-diazepane (**21d**), DMF, 110−140 °C.

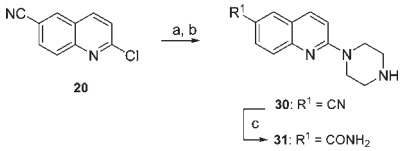
the serotonin transporter (r-5-HT-T) and the 5-HT_{1A} receptor (h-5-HT_{1A}). Compounds were also evaluated for their h-5-HT_{1A} receptor functional activity. Antagonism at the 5-HT_{1A} receptor was determined using a [^{35}S]-GTPγS binding assay. WAY-100635 (a 5-HT_{1A} receptor antagonist) and fluoxetine (a 5-HT transporter inhibitor) were used as reference standards (Table 1).

Our SAR study began with an evaluation of the 6-nitroquipazine analogue **3**. Compared to **1**, compound **3** showed a 9-fold increase in binding affinity for the 5-HT transporter (K_i = 0.25 nM) but had weaker affinity for the 5-HT_{1A} receptor (K_i = 20 nM). Encouraged by these results, we evaluated the effects of other electron-withdrawing substitutents at the C6-position of the 2-(piperazin-1-yl)quinoline ring.

As summarized in Table 1, replacement of the C6-nitro group on the quipazine moiety with a C6-halogen resulted in substantially decreased binding affinity for the 5-HT transporter but the binding affinity for the 5-HT_{1A} receptor was maintained. The exception was the chloro derivative **5**, which showed a 10-fold decrease in binding affinity for the 5-HT_{1A} receptor. Interestingly, the C6-cyano and C6-carboxamide analogues (**7** and **8**) demonstrated a 3−5 fold increase in 5-HT_{1A} receptor binding affinity compared to the corresponding C6-nitro analogue **3**. Replacement of the C6-nitro group with a trifluoromethoxy group was found to be less favorable; compound **9** exhibited reduced binding affinities for both the 5-HT transporter and the 5-HT_{1A} receptor. For comparison purposes, the corresponding C6-unsubstituted analogue **10** was prepared as well. As depicted in Table 1, compound **10** had only moderate binding affinities for both the 5-HT transporter and the 5-HT_{1A} receptor.

After optimization of the C6-substitution of compound **3**, we turned our attention toward modification of the linker B region with the goal of further increasing the 5-HT transporter binding affinity while retaining the 5-HT_{1A} receptor binding affinity. The first modification examined the effect of introducing a methyl group to the C3-position of the piperazine, using the C6-unsubstituted analogue **10** as a starting point. As summarized in Table 1, within the pair of diastereomers **11** and **12**, the (*R*)-methyl piperazinyl analogue **12** exhibited better binding affinity for the 5-HT_{1A} receptor than its corresponding (*S*)-2-methyl isomer **11** but less affinity for the 5-HT transporter. It was our hope that minor structural changes, such as introduction of the C6-cyano on the quipazine ring of compound **12**, would afford an increase in binding affinity for the 5-HT transporter, as suggested by the enhanced binding affinity of the 6-cyano analogue **7** when compared with C6-unsubstituted analogue **10**. Surprisingly, this goal was not realized: compound **13** demonstrated a 2.7-fold loss in 5-HT transporter binding affinity when compared to the compound **12**.

Scheme 2[a]



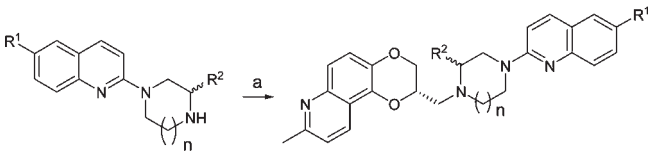
[a] Reagents and conditions: (a) 1-piperazinecarboxaldehyde (**29**), DMF, 130 °C; (b) H_2SO_4, 130 °C; (c) NaOH, 30% H_2O_2, DMSO.

In the next aspect of the SAR of the linker B region, we explored the effect of expansion of the piperazine ring to the homopiperazine. We prepared the C6-unsubstituted analogue **14** first. It is interesting to note that homopiperazine analogue **14** exhibited nearly equal potencies for both 5-HT transporter (K_i = 7.40 nM) and the 5-HT_{1A} receptor (K_i = 6.79 nM). However, compound **14** showed an increase in 5-HT_{1A} receptor intrinsic activity (I_{max} = 58%). Introduction of an electron withdrawing group (fluoro or cyano) at the C6-position of quipazine ring, exemplified by analogues **15** and **16**, provided further improvement in binding affinities at both 5-HT transporter and 5-HT_{1A} receptor when compared to compound **14**. However, 5-HT_{1A} receptor partial agonist functions were retained in compounds **15** and **16.**

At this stage, we further examined a few compounds selected from Table 1 for their functional activities at the human 5-HT transporter site by incubating test compounds with a human carcinoma cell line (JAR cells) previously treated with staurosporine to enhance the endogenous expression of the 5-HT transporter.[16] Because SSRIs can show different affinities at the rat versus human transporter,[17] determination of functional activity was done to confirm activity at human 5-HT transporters. Compound **7** proved to be one of the most potent 5-HT transporter inhibitors (IC_{50} = 57.7 ± 14.6 nM vs IC_{50} = 145 nM for compound **1** and IC_{50} = 39.4 nM for fluoxetine) in this assay. Considering that the potency for blocking 5-HT_{1A} receptor is similar (IC_{50} = 102.1 ± 19.1 nM), this dual mechanism compound was tested further in microdialysis and the behavioral model.

Further selectivity profiling of compound **7** across the α_1 adrenergic receptor and the dopamine receptors is depicted in Table 2. Selectivity of compound **7** for the α_1 adrenergic receptor was determined by incubating rat cortical membranes with [^{3}H]-prazosin.[18] Affinity for the dopamine D_2, D_3, and D_4 receptors was determined using [^{3}H]-spiperone in

Scheme 3[a]

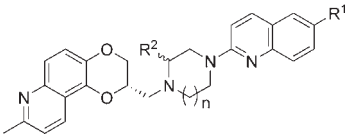


2: $R^1 = NO_2$, R^2 = H, n = 1
22: R^1 = F, R^2 = H, n = 1
32: R^1 = Cl, R^2 = H, n = 1
33: R^1 = Br, R^2 = H, n = 1
30: R^1 = CN, R^2 = H, n = 1
31: $R^1 = CONH_2$, R^2 = H, n = 1
23: $R^1 = OCF_3$, R^2 = H, n = 1
34: R^1 = H, R^2 = H, n = 1
24: R^1 = H, R^2 = (*S*)-Me, n = 1
25: R^1 = H, R^2 = (*R*)-Me, n = 1
26: R^1 = CN, R^2 = (*R*)-Me, n = 1
35: R^1 = H, R^2 = H, n = 2
27: R^1 = CN, R^2 = H, n = 2
28: R^1 = F, R^2 = H, n = 2

3: $R^1 = NO_2$, R^2 = H, n = 1
4: R^1 = F, R^2 = H, n = 1
5: R^1 = Cl, R^2 = H, n = 1
6: R^1 = Br, R^2 = H, n = 1
7: R^1 = CN, R^2 = H, n = 1
8: $R^1 = CONH_2$, R^2 = H, n = 1
9: $R^1 = OCF_3$, R^2 = H, n = 1
10: R^1 = H, R^2 = H, n = 1
11: R^1 = H, R^2 = (*S*)-Me, n = 1
12: R^1 = H, R^2 = (*R*)-Me, n = 1
13: R^1 = CN, R^2 = (*R*)-Me, n = 1
14: R^1 = H, R^2 = H, n = 2
15: R^1 = CN, R^2 = H, n = 2
16: R^1 = F, R^2 = H, n = 2

[a] Reagents and conditions: (a) (*R*)-(8-methyl-2,3-dihydro-[1,4]dioxino[2,3-f]quinolin-2-yl)methyl 4-bromobenzenesulfonate (**36**), Et_3N or $^iPr_2EtN$, DMSO, 90 °C.

Table 1. 2-Piperazin-1-ylquinoline Derivatives **3**−**16**[a]



	R^1	n	R^2	r-5-HT-T K_i (nM)[b]	h-5-HT_{1A} K_i (nM)[c]	GTPS I_{max} %	GTPS IC_{50} (nM)[d]
fluoxetine				2.7			
WAY-100635					0.9		
1				2.34 ± 0.59	3.02 ± 0.1	98.3 ± 1.4	56.7 ± 11.7
3	NO_2	1	H	0.25 ± 0.00	20.0 ± 5.2	79.0 ± 1.4	294 ± 153
4	F	1	H	66.0 ± 3.5	32.9 ± 0.5	100 ± 0	2165 ± 898
5	Cl	1	H	0.88 ± 0.54	214.1 ± 15.7	62.0 ± 1.4	781 ± 255
6	Br	1	H	12.7 ± 1.7	17.2 ± 0.5	96.0 ± 0.7	156 ± 8.9
7	CN	1	H	13.8 ± 1.0	3.7 ± 0.5	88.6 ± 6.11	102.1 ± 19.7
8	$CONH_2$	1	H	17.0 ± 0.1	7.2 ± 0.9	100 ± 0	164.0 ± 10
9	OCF_3	1	H	148.5 ± 47.4	172.0 ± 30.4	85.0 ± 1.4	588.0 ± 24.0
10	H	1	H	75.0 ± 6.2	47.3 ± 7.8	100 ± 0	4870 ± 2641
11	H	1	(*S*)-CH_3	10.3 ± 4.9	167.0 ± 15.6	76.5 ± 19.1	1899 ± 1556
12	H	1	(*R*)-CH_3	32.8 ± 13.9	5.5 ± 1.7	85.0 ± 1.4	117 ± 14.0
13	CN	1	(*R*)-CH_3	89.6 ± 54.3	2.8 ± 1.2	92.0 ± 0.2	180 ± 23.4
14	H	2	H	7.4 ± 2.3	6.8 ± 0.3	58.0 ± 2.8	70.0 ± 18.0
15	CN	2	H	1.1 ± 0.4	2.0 ± 0.2	53.0 ± 1.4	58.0 ± 6.4
16	F	2	H	3.9 ± 0.1	1.5 ± 0.5	74.0 ± 0.7	70.0 ± 19.0

[a] K_I values are the mean of at least two experiments performed in triplicate, determined from nine concentrations and all K_i values were calculated from IC_{50} values using the method of Cheng and Prusoff.[12] [b] Binding affinity at rat cortical 5-HT reuptake sites labeled with [^{3}H]-paroxetine.[13] [c] Binding affinity at human 5-HT_{1A} receptors in CHO cells labeled with [^{3}H]-8-OH-DPAT.[14] [d] Stimulation of GTPγS^{35} binding in CHO cells expressing the 5-HT_{1A} receptor.[15] I_{max} is the maximum percent inhibition.

CHO cells transfected with human D_2, D_3, and D_4 receptors.[19] Gratifyingly, compound **7** displayed high selectivities against α_1 adrenergic receptor and dopamine D_2, D_3, and D_4 receptors. At this point, we have identified a molecule that met our primary in vitro criteria. We then proceeded to evaluate this compound in vivo.

Pharmacokinetic Profile. The pharmacokinetic profile of compound **7** was determined in rats. As summarized in Table 3, the iv bolus pharmacokinetics of compound **7** exhibited moderate clearance, volume of distribution and elimination half-life. Upon oral administration at 3 mg/kg, compound **7** was quickly absorbed, had a moderate terminal half-life, and good oral bioavailability. However, upon oral dosing at 10 mg/kg, both the maximum plasma concentration (C_{max}) and systemic exposure ($AUC_{0-\infty}$) increased in a less than dose-proportional manner, suggesting saturation of absorption.

In Vivo Microdialysis. An in vivo microdialysis assay was used to investigate the effect compound **7** on 5-HT levels in rat dorsal lateral frontal cortex, a brain region intimately linked to impairments in cognition and motor function in depressed patients.[20] Initially, six dialysate samples were taken prior to drug injection to demonstrate a steady baseline. At the end of the sixth baseline sample, animals received an oral administration of compound **7** (3−30 mg/kg, po) or vehicle (2% Tween 80, po), and dialysis samples were collected for the following 3 h. As illustrated in Figure 2, acute administration of compound **7**

Table 2. Binding Affinities at α_1 Adrenergic and Dopaminergic Receptors

	r-α_1	dopaminergic receptors		
compd	K_i (nM)	h-D_2 K_i (nM)	h-D_3 K_i (nM)	h-D_4 K_i (nM)
7	1110	1306	1316	1418

Table 3. Pharmacokinetic Profile of **7** in Rats[a]

	rat PK		
	1 mg/kg (iv)[b]	3 mg/kg (oral)[c]	10 mg/kg (oral)[c]
C_0 (ng/mL)	903		
C_{max} (ng/mL)		456 ± 227	815 ± 189
T_{max} (h)		1.3 ± 0.6	1 ± 0.0
$t_{1/2}$ (h)	4	3.8 ± 0.3	3.8 ± 0.6
AUC (h·ng/mL)	1268	2320 ± 759	4620 ± 1450
CL (mL/h/kg)	13.2		
V_{ss} (mL/kg)	2.5		
F (%)		61	36

[a] Results are expressed as mean ± SD of $n = 3$. [b] Compound dosed intravenously in 20/80, DMSO/PEG200; $n = 2$. [c] Compound dosed orally in 2% Tween/0.5% methyl cellulose.

(10−30 mg/kg, po) rapidly and dose-dependently elevated extracellular levels of 5-HT in the rat frontal cortex, an effect shown to be similar to acute coadministration of fluoxetine and WAY-100635.[21] This effect was not observed with acute fluoxetine treatment alone.[21] At 30 mg/kg, maximal elevations in 5-HT were 71% above baseline. These results indicated that combining a serotonin reuptake inhibitor and a 5-HT_{1A} receptor antagonist component within the compound **7** successfully limited the negative feedback through blockade of the 5-HT_{1A} autoreceptor and allowed an immediate increase in synaptic levels of 5-HT, suggesting rapid onset antidepressant effects.

Resident-Intruder Model. The rat resident-intruder model described by Mitchell and Redfern[22] is sensitive to the effects of antidepressant mechanisms. The acute treatment with a wide range of antidepressant drugs commonly reduces the aggressive behavior[22] of resident rats when confronted with an unknown conspecific intruder. In the test, resident animals were separated 3 days prior to each test day and housed individually with food and water available ad libitum. Thirty min prior to the social encounter, the resident rats were treated with either vehicle or compound **7** (3.3, 10.0, and 30 mg/kg, sc). A drug-free unfamiliar intruder was then introduced into the resident rats' home cage and the ensuing social behavior recorded on to videotape for 10 min.[22] As indicated in Figure 3, compound **7** demonstrated a dose-dependent reduction in the level of aggressive behavior ($ID_{50} = 12.5$ mg/kg, sc; $F(3,21) = 10.642$, $p = 0.0004$) at doses that had no effect on total behavior score ($ID_{50} \gg 30.0$ mg/kg, sc; $F(3,21) = 0.832$, $p = 0.4487$), consistent with antidepressant-like effects.

Cytochrome P450 (CYP450) Inhibition. The CYP450 activities of compound **7** in human liver microsomes were also evaluated. As a result, compound **7** showed virtually no inhibition of CYP2A6 or CYP2D6 (tested up to 100 μM concentration) and only weakly inhibition of CYP2C9 ($IC_{50} = 358$ μM). Compound **7** moderately inhibited CYP2C8 and CYP2C19 with $IC_{50} = 29$ and 98 μM, respectively. However, compound **7** exhibited potent inhibition of CYP3A4 ($IC_{50} = 10$ μM).

Summary

In this study, we synthesized a class of 2-(piperazin-1-yl) quinoline analogues **3**−**16**. The goal of creating a single molecular entity with dual activities as both a 5-HT transporter inhibitor and a 5-HT_{1A} receptor antagonist has been achieved. The identity of the substituent at the C6-position of quipazine and the piperazine linker in the B region are clearly important. Compound **7** from this series was chosen for further profiling in vitro and in vivo. This compound dose dependently increased synaptic 5-HT levels after acute oral administration in a way consistent with a more rapid antidepressant-like effect. It was also active in the model predictive of antidepressant activity. Thus, compound **7** represents an important advancement in this area.

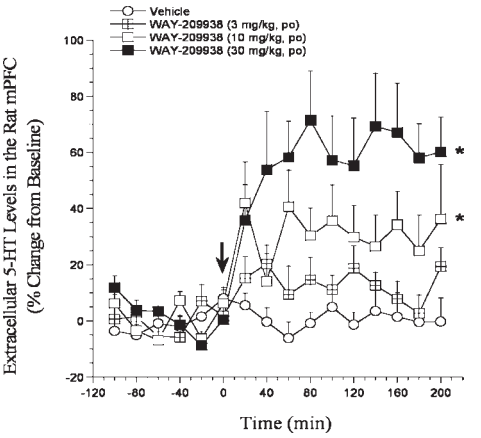


Figure 2. Effects of **7** on extracellular 5-HT in frontal cortex. *Represents overall significant ($p < 0.05$) treatment effect compared to vehicle group.

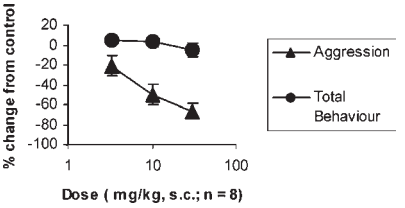


Figure 3. Effects of **7** in rat resident-intruder.

Experimental Section

General Procedure: Preparation of Compounds 3−16. A solution of [(2*R*)-8-methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinolin-2-yl]methyl 4-bromobenzenesulfonate[11] (**36**), 2-(1-piperazinyl) quinoline, and triethylamine (or diisopropylethyl amine) in dimethyl sulfoxide was heated under nitrogen at 90 °C for 12 h. The reaction was diluted with saturated aqueous sodium bicarbonate and extracted three times with methylene chloride. The combined organic layers were washed three times with water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (1/44/55 methanol/ethyl acetate/hexanes) to afford the desired product. The material was dissolved in ethyl acetate and made into its hydrochloride salt using excess ethereal hydrochloric acid.

(2*S*)-8-Methyl-2-{[4-(6-nitroquinolin-2-yl)piperazin-1-yl]-methyl}-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline (3). Yield: 48% of an orange foam. The hydrochloride salt was prepared and collected as a yellow solid; mp 200 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{26}H_{25}N_5O_4 \cdot 2.75HCl$) C, H, N.

(2*S*)-2-{[4-(6-Fluoroquinolin-2-yl)piperazin-1-yl]methyl}-8-methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline (4). Yield: 54% of a yellow solid; mp 140−141 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{26}H_{25}FN_4O_2$) C, H, N.

(2*S*)-2-{[4-(6-Chloroquinolin-2-yl)piperazin-1-yl]methyl}-8-methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline (5). Yield: 69% of

a light-yellow oil. The hydrochloride salt was prepared and collected as orange crystals; mp 209−218 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{26}H_{25}ClN_4O_2 \cdot 2.5H_2O \cdot 4HCl$) C, H, N.

(2*S*)-2-{[4-(6-Bromoquinolin-2-yl)piperazin-1-yl]methyl}-8-methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline (6). Yield: 39% of an off-white solid; mp 153−154 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{26}H_{25}BrN_4O_2$) C, H, N.

2-(4-{[(2*S*)-8-Methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinolin-2-yl]methyl}piperazin-1-yl)-quinoline-6-carbonitrile (7). Yield: 72% of a colorless oil. The hydrochloride salt was prepared and collected as a yellow powder; mp > 275 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{25}N_5O_2 \cdot 1H_2O \cdot 2HCl$) C, H, N.

2-(4-{[(2*S*)-8-Methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinolin-2-yl]methyl}piperazin-1-yl)quino-line-6-carboxamide (8). Yield 44% of a colorless oil. The hydrochloride salt was prepared and collected as a yellow solid; mp 290 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{27}N_5O_3 \cdot 1H_2O \cdot 2HCl$) C, H, N.

(2*S*)-8-Methyl-2-({4-[6-(trifluoromethoxy)quinolin-2-yl]piperazin-1-yl}methyl)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinoline (9). Yield: 77% of a yellow solid; mp 174−176 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{25}F_3N_4O_3$) C, H, N.

(2*S*)-8-Methyl-2-[(4-quinolin-2-ylpiperazin-1-yl)methyl]-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline (10). Yield: 21% of a light-brown oil. The hydrochloride salt was prepared and collected as an orange−brown solid; mp 237−243 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{26}H_{26}FN_4O_2 \cdot 1.25H_2O \cdot 3HCl$) C, H, N.

(2*S*)-8-Methyl-2-{[(2*S*)-2-methyl-4-quinolin-2-ylpiperazin-1-yl]methyl}-2,3-dihydro[1,4]dioxino-[2,3-*f*]quinoline (11). Yield: 62% of an off-white solid; mp 141−144 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{28}N_4O_2 \cdot 0.2H_2O$) C, H, N.

(2*S*)-8-Methyl-2-{[(2*R*)-2-methyl-4-quinolin-2-ylpiperazin-1-yl]methyl}-2,3-dihydro[1,4]dioxino-[2,3-*f*]quinoline (12). Yield: 47% of an off-white solid; mp 78−82 °C. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{28}N_4O_2 \cdot 0.3H_2O$) C, H, N.

2-((3*R*)-3-Methyl-4-{[(2*S*)-8-methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinolin-2-yl]methyl}pipera-zin-1-yl)quinoline-6-carbonitrile (13). Yield: 67% of a light-yellow foam. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{28}H_{27}N_5O_2 \cdot 0.4H_2O$) C, H, N.

(2*S*)-8-Methyl-2-[(4-quinolin-2-yl-1,4-diazepan-1-yl)methyl]-2,3-dihydro[1,4]dioxino[2,3-*f*]-quinoline (14). Yield: 37% of a yellow foam. ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{28}N_4O_2 \cdot 0.5H_2O$) C, H, N.

2-(4-{[(2*S*)-8-Methyl-2,3-dihydro[1,4]dioxino[2,3-*f*]quinolin-2-yl]methyl}-1,4-diazepan-1-yl)-quinoline-6-carbonitrile(15). Yield: 2% of an off-white solid; mp 80−85 °C. ^{1}H NMR (400 MHz, DMSO-d_6); HRMS (ES) m/z 466.2237 $[M + H]^+$ calcd; m/z 466.2232 $[M + H]^+$ obsd.

(2*S*)-2-{[4-(6-Fluoroquinolin-2-yl)-1,4-diazepan-1-yl]methyl}-8-methyl-2,3-dihydro[1,4]dioxino-[2,3-*f*]quinoline (16). Yield: 50% of a yellow foam; ^{1}H NMR (400 MHz, DMSO-d_6). Anal. ($C_{27}H_{27}FN_4O_2 \cdot 1.5H_2O \cdot 2.5HCl$) C, H, N.

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Supporting Information Available: Experimental details for the synthesis of compounds **3**−**16** and **22**−**28** and elemental analysis results for compounds **3**−**14** and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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