Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b01759 • Publication Date (Web): 23 Feb 2017

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

High-potency phenylquinoxalinone cystic fibrosis transmembrane conductance regulator (CFTR) activators

Jung-Ho Son[†], Jie S. Zhu[†], Puay-Wah Phuan[‡], Onur Cil[‡], Andrew P. Teuthorn[†], Colton K. Ku[†], Sujin Lee[‡], Alan S. Verkman[‡], Mark J. Kurth[†]*

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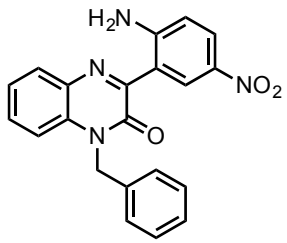
ABSTRACT: We previously identified phenylquinoxalinone **CFTR$_{act}$-J027** (**4**) as a cystic fibrosis transmembrane conductance regulator (CFTR) activator with an EC_{50} of ~200 nM, and demonstrated its therapeutic efficacy in mouse models of constipation. Here, structure-activity studies were done on 36 synthesized phenylquinoxalinone analogs to identify compounds with improved potency and altered metabolic stability. Synthesis of the phenylquinoxalinone core was generally accomplished by condensation of 1,2-phenylenediamines with substituted phenyloxoacetates. Structure-activity studies established, among other features, the privileged nature of a properly positioned nitro moiety on the 3-aryl group. Synthesized analogs showed improved CFTR activation potency compared to **4** with EC_{50} down to 21 nM and with greater metabolic stability. CFTR activators have potential therapeutic indications in constipation, dry eye, cholestatic liver diseases, and inflammatory lung disorders.

INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride channel expressed in mammalian epithelia in the respiratory, gastrointestinal, and reproductive systems, as well as in exocrine glands and other tissues.[1] Loss-of-function mutations in CFTR cause cystic fibrosis, and CFTR over activation causes certain secretory diarrheas including cholera and Travelers' diarrhea.[2] CFTR is considered an important drug target, with activators of CFTR of potential benefit for constipation,[3,4] dry eye,[5] inflammatory lung disorders,[6] and cholestatic liver disease; inhibitors of wildtype CFTR may be useful for treatment of certain secretory diarrheas and polycystic kidney disease;[7,8] and correctors and potentiators of mutant CFTRs for treatment of cystic fibrosis.[9]

We previously identified by high-throughput screening the phenylquinoxalinone **$CFTR_{act}$-J027** (**4**; Figure 1) as a CFTR activator and demonstrated its efficacy in normalizing stool output, hydration, and intestinal transit in a mouse model of opioid-induced constipation.[3] Phenylquinoxalinone **4** activated CFTR chloride conductance with an EC_{50} of ~200 nM and showed no apparent off-target actions or toxicity following chronic administration in mice. In a follow-up study,[4] **4** was shown by patch-clamp and biochemical studies to target CFTR directly, and was demonstrated to activate CFTR in human enterocytes and normalize stool parameters in mouse models of acute and chronic constipation. Side-by-side comparisons of intestinal fluid secretion and



$CFTR_{act}$-J027 (**4**)
EC_{50} = 200 nM

Figure 1. A phenylquinoxalinone CFTR activator identified by high-throughput screening.

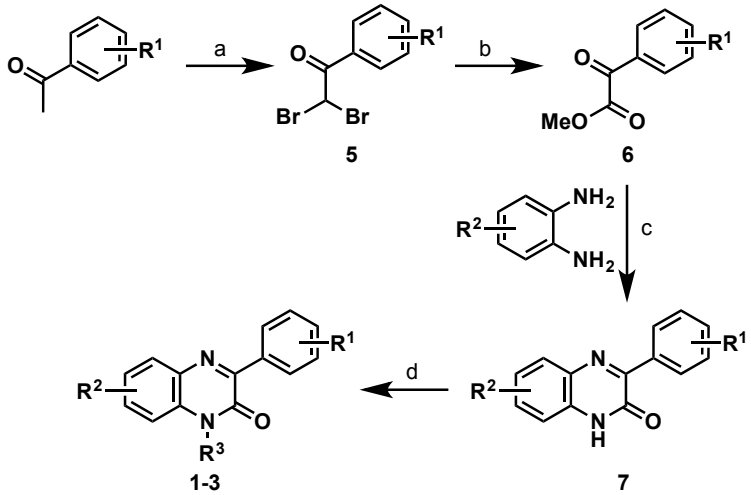
stool output in constipation models showed greater efficacy of **4** than supramaximal doses of the FDA-approved drugs lubiprostone and linaclotide.

Here, motivated by the potential therapeutic utility of phenylquinoxalinone-based CFTR activators in constipation and other diseases, we synthesized 36 analogs of phenylquinoxalinone **4** in order to establish structure-activity relationships and to identify compounds with greater potency. Also, while the rapid hepatic metabolism of **4** results in minimal systemic exposure following oral administration in mice, which is desirable for treatment of constipation, we also sought phenylquinoxalinone CFTR activators with greater metabolic stability for treatment of lung and liver disorders where systemic exposure is necessary.

RESULTLS AND DISCUSSION

Chemistry. General synthesis of phenylquinoxalinones. Most of the phenylquinoxalinone in this study were expediently synthesized in four steps starting from acetophenones (Scheme 1). We generated the phenylquinoxalinone core by condensing *o*-phenylenediamines with substituted phenyloxoacetates (**6**), which were synthesized following literature methods.[10]

Scheme 1. Synthesis of phenylquinoxalinones **1-3**.

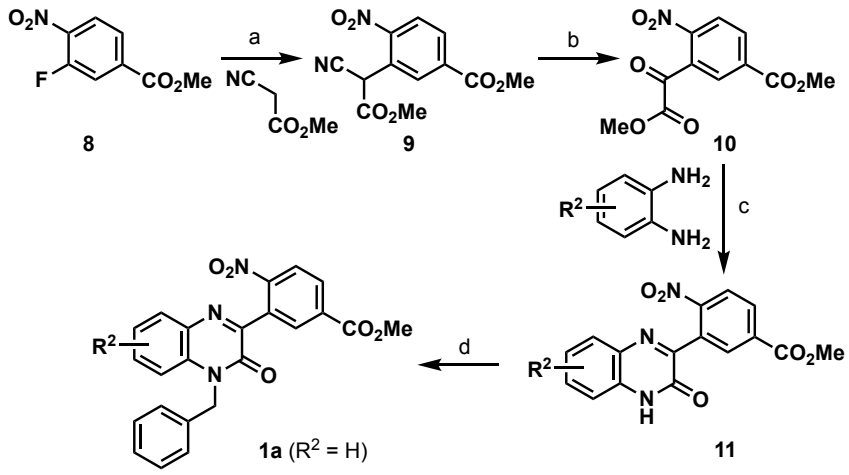


(a) Br_2, 1,4-dioxane; (b) DMSO, Δ; MeOH; (c) toluene, Δ; (d) R-Br, K_2CO_3, DMF.

Briefly, substituted acetophenone was doubly brominated with bromine in 1,4-dioxane to give **5**, then heated in DMSO followed by addition of methanol to give **6**. Phenylquinoxalinone **7** was N^1-alkylated using K_2CO_3 and R-X in DMF[11] and pure products (**1-3**) were obtained via column chromatography.

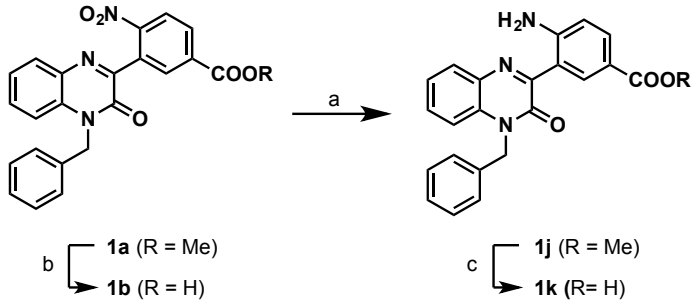
Phenylquinoxalinone **1a** was prepared as outlined in Scheme 2. Treatment of methyl 3-fluoro-4-nitrobenzoate (**8**) with methyl 2-cyanoacetate under basic conditions delivered intermediate **9**.[12] Subsequent copper(I) iodide-catalyzed aerobic oxidation[12] delivered methyl 2-oxo-2-phenylacetate **10** and, from here, target **1a** was prepared in parallel to the chemistry employed in Scheme 1. With **1a** in hand, saponification and nitro reduction were accomplished as outlined in Scheme 3 to deliver analogs **1b**, **1j**, and **1k**.

Scheme 2. Synthesis of phenylquinoxalinone **1a**.



(a) Cs_2CO_3, DMSO; (b) CuI, 1,10-phen, ACN, O_2; (c) toluene, Δ; (d) K_2CO_3, DMF, BnBr.

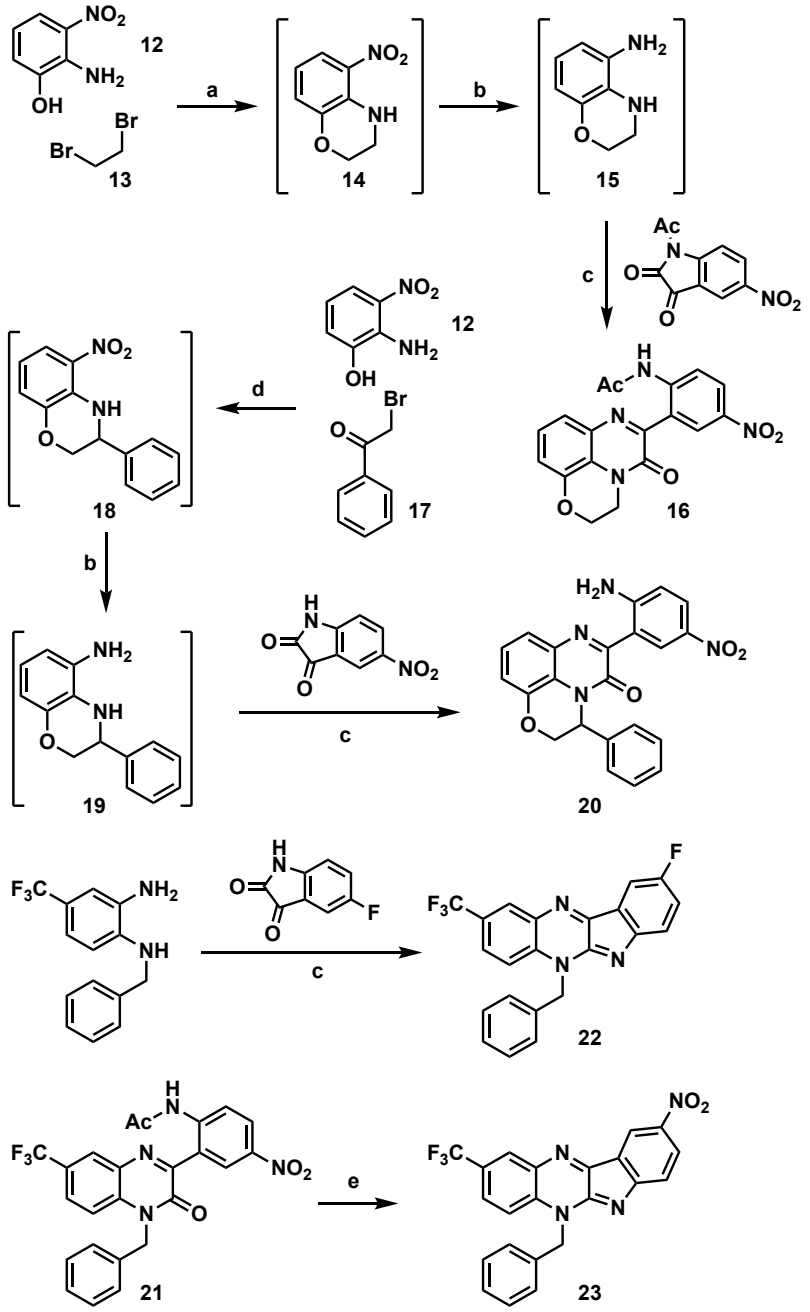
Scheme 3. Diversification from phenylquinoxalinone **1a**.



(a) H_2, Pd/C, MeOH; (b) NaOH, EtOH; (c) KOH, MeOH.

Constrained ring phenylquinoxalinone analogs **16** and **20** were prepared as outlined in Scheme 4. 2-Amino-3-nitrophenol was *N*- and *O*-alkylated with 1,2-dibromoethane to deliver intermediate **14** and subsequent nitro reduction and condensation of the resulting diamine with 1-acetyl-5-nitroindoline-2,3-dione led smoothly to analog **16**.[13] Employing 2-bromo-1-phenyle-than-1-one in place of 1,2-dibromoethane and 5-nitroindoline-2,3-dione in place of 1-acetyl-5-nitroindoline-2,3-dione delivered analog **20**.[14] Interestingly, the reaction of *N*-benzyl-1,2-dia-minobenzene with 5-fluoroisation (in analogy with the protocol employed to prepare compounds **16**, **20** and **4**) led to **22** and the attempted de-acylation of **21** (X = NO_2) led to **23**.

Scheme 4. Synthesis of constrained ring phenylquinoxalinone analogs.

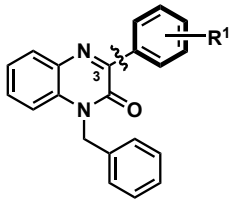


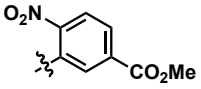
(a) KOH, DMF; (b) H_2, Pd/C, MeOH; (c) HOAc, toluene; (d) K_2CO_3, ACN (e) HCl, MeOH.

Modifications of the 3-aryl ring. Compound **4** contains a 2-amino-5-nitro phenyl ring at the 3-position of the quinoxalinone core (Figure 1) and our first effort was to modify this ring. Several compounds (Table 1) were rationally synthesized and their activities were determined using a plate reader assay. The most active compound was **CFTR$_{act}$-J125** (**1c**), which only lacks the 2-

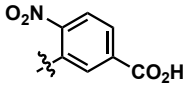
amino group at C_2 of the 3-aryl ring compared to **4**, and it showed an ~10-fold increased potency when compared to **4**. We then synthesized a series of analogs retaining this amino group deletion. In contrast to the high activity of **1c**, compounds without the 3-nitro group had significantly lower activity (**1h**), indicating the privileged nature of the 3-nitro group in **1c**.

Table 1. CFTR activation with variation in the 3-aryl ring (EC_{50} reported in μM ± S.E.M.).

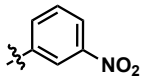




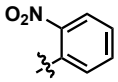
1a
EC_{50} = 9.9 ± 2



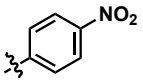
1b
EC_{50} = >10



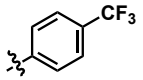
1c (J125)
EC_{50} = 0.009 ± 0.003



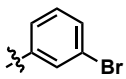
1d
EC_{50} = 1 ± 0.2



1e
EC_{50} = 1 ± 0.1

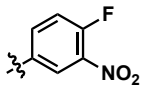


1f
EC_{50} = 1 ± 0.2



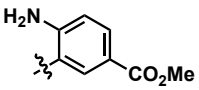
1g
EC_{50} = 0.33 ± 0.07

1h
EC_{50} = 5 ± 0.4

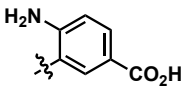


1i
EC_{50} = 0.69 ± 0.22

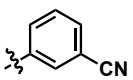
Nitro bioisosteres



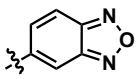
1j
EC_{50} = 0.55 ± 0.1



1k
EC_{50} = >10



1l
EC_{50} = 1 ± 0.4



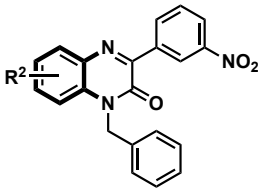
1m
EC_{50} = 0.018 ± 0.006

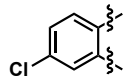
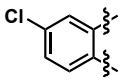
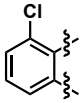
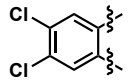
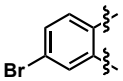
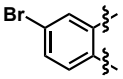
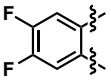
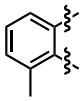
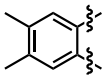
Bioisosteric compounds containing –COOR replacements of the $–NO_2$ moiety in the 3-aryl group were synthesized according to Schemes 2 and 3. It was found that carboxylic acid analog

1k was inactive, while methyl ester analog **1j** was moderately potent. Combining a 2-NO_2 with either a 5-COOMe or a 5-COOH (**1a** and **1b**, respectively) resulted in very low activity. Positional deviation of the nitro group also resulted in reduced activity, as with 2-nitro or 4-nitro analogs **1d** and **1e**, respectively. Introduction of other functional groups, such as 4-CF_3 or 3-Br, in place of the 3-NO_2 of **1c** also showed low activity (**1f** and **1g**, respectively). Introducing a fluorine atom into **1c** (i.e., **1i**) reduced activity. Replacements of the nitro group with a bioisosteric nitrile (**1l**) greatly reduced activity, but interestingly, the bioisosteric benzoxadiazole (**1m**) showed comparable potency to the nitro analog (**1c**).

Modifications of the quinoxalinone core. Moving forward with **1c** as the lead, quinoxalinone backbone modifications were undertaken (Table 2). Halogen substitution at the 6-position, such as 6-Cl (**2b**) or 6-Br (**2f**), showed good activity albeit less than that of **1c**. Substitution at the 5-, 7-, or 8-positions generally resulted in lower activity. Disubstitution at the 6- and 7-positions (dichloro; **2d** / difluoro; **2g** / dimethyl; **2i**) reduced activity.

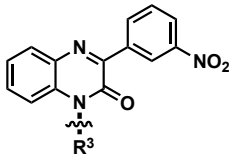
Table 2. CFTR activation with variation in the quinoxalinone core (EC_{50} reported in μM ± S.E.M.).

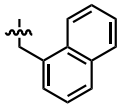
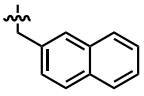
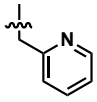


 **2a** EC_{50} = 0.76 ± 0.38	 **2b** EC_{50} = 0.07 ± 0.03	 **2c** EC_{50} = 0.11 ± 0.04
 **2d** EC_{50} = >10	 **2e** EC_{50} = Inactive	 **2f** EC_{50} = 0.063 ± 0.03
 **2g** EC_{50} = >10	 **2h** EC_{50} = >10	 **2i** EC_{50} = >10

Modifications of the N^1-substituent. We next modified the N^1-benzyl group (Table 3). We reported previously that placing substituents on the benzyl ring was not tolerated.[4] However, heterocycle analogs such as thiophenyl (**3j**), furanyl (**3i**), and pyridyl (**3h**) showed comparable activity. Among short chains, allyl and ethyl groups showed moderate activity, but methyl and propyl groups had poor activity (**3a-e**). Replacing the phenyl with a larger aromatic group, such as naphthyl, significantly reduced activity (**3f** and **3g**).

Table 3. CFTR activation with variation in the N^1-substituent (EC_{50} reported in μM ± S.E.M.).

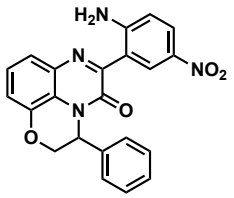


 **3a** EC_{50} = >10	 **3b** EC_{50} = >10	 **3c** EC_{50} = 0.065 ± 0.02	 **3d** EC_{50} = 0.177 ± 0.048
 **3e** EC_{50} = 0.055 ± 0.01	 **3f** EC_{50} = Inactive	 **3g** EC_{50} = 4.3 ± 0.6	
 **3h** EC_{50} = 0.052 ± 0.02	 **3i** EC_{50} = 0.02 ± 0.01	 **3j (J170)** EC_{50} = 0.01 ± 0.005	

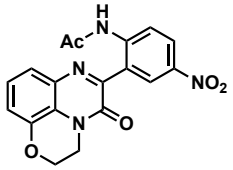
Compounds with constrained rings. Motivated by our prior findings that constrained rings can enhance the activity of a CFTR corrector,[15] we next examined two different types of constrained analogs (Scheme 4) with hindered rotation of the N^1-alkyl group (**16** and **20**) or phenyl ring (**22** and **23**). As previously shown, the *N*-acetylated version of **4** has very low activity;[4] we therefore attempted to synthesize **16** without an *N*-acyl group, but both deacylation of **16** and

condensation with 5-nitroisatin failed. The activities of neither **20** (EC_{50} = >10 μM) nor **16** (EC_{50} = 0.82 μM) were greater than that of **4** (see Table 4). Phenyl-ring constrained compounds **22** and **23** were unexpected by-products of the deacylation reaction (see Scheme 4 and Table 4). These compounds are purple and red, respectively – a consequence of their extended aromatic systems – and those colors are different from the bright yellow color of most derivatives of **4**. Intramolecular heterocycle formation in this system might have been facilitated by the presence of the electron withdrawing CF_3 group in the quinoxalinone backbone under these acidic deacylation conditions. With other substituents in the quinoxalinone backbone, only small amounts of uncharacterized reddish byproduct formed during the deacylation step, suggesting a minimal amount of by-product formation.

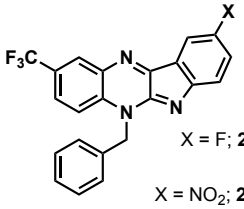
Table 4. CFTR activation with constrained ring analogs (EC_{50} reported in μM ± S.E.M.).



20 EC_{50} = >10



16 EC_{50} = 0.82 ± 0.25



X = F; **22** EC_{50} = >10

X = NO_2; **23** EC_{50} = >10

Biology. In vitro characterization of phenylquinoxalinones. Phenylquinoxalinone CFTR activators with the highest potency as determined by plate-reader assay, **1c** and **$CFTR_{act}$-J170** (**3j**; Table 3), were further characterized. Short-circuit current measurements were done using CFTR-expressing FRT cells in the presence of a transepithelial chloride gradient and with permeabilization of the cell basolateral membrane; consequently, current is a direct, linear measure

of CFTR chloride conductance. Compounds **1c** and **3j** were added to the apical side of the monolayer. Representative data in Figure 2 for **1c** and **3j** shows a small increase in current following addition of a low concentration of forskolin, followed by concentration-dependent increases in current following activator additions. EC_{50} values were determined to be 21 ± 4 and 70 ± 11 nM for **1c** and **3j**, respectively. The potency for CFTR inhibition by **1c** was similar in non-permeabilized and permeabilized CFTR-expressing FRT cells (data not shown).

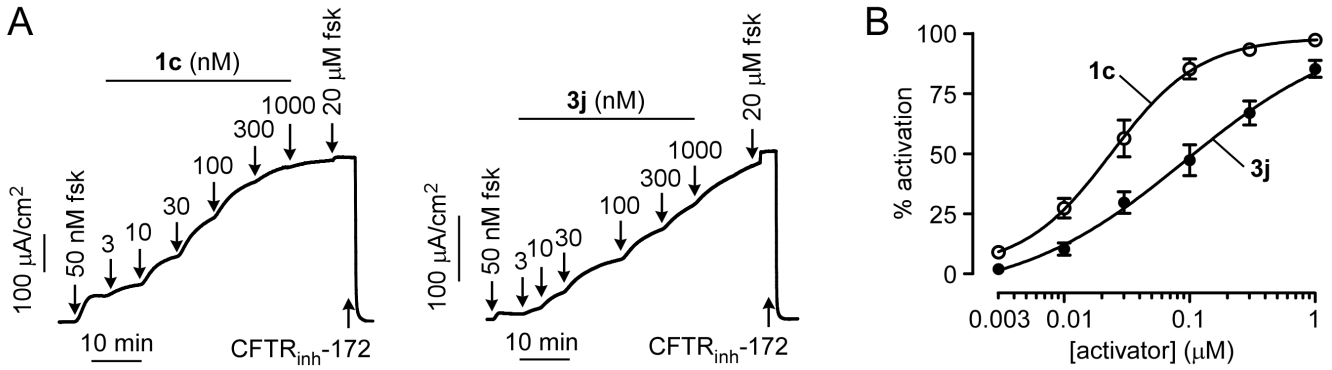


Figure 2. Short-circuit current measurement of CFTR activation by **1c** and **3j**. *A.* Measurements done in FRT cells expressing human wild-type CFTR showing responses to indicated concentrations of forskolin, **1c** or **3j**, and 10 μM CFTR inhibitor $CFTR_{inh}$-172. *B.* Concentration-dependent activation of CFTR (mean ± S.E.M., n=3).

The CFTR specificity of the most potent compound, **1c**, was further studied. At 10 μM, **1c** did not affect the cellular cAMP level (Figure 3A), nor did it elevate cytoplasmic calcium or inhibit the ATP-stimulated elevation in cytoplasmic calcium (Figure 3B). In addition, **1c** at 10 μM neither inhibited nor activated calcium-activated chloride channels in HT-29 cells (Figure 3C) or in FRT cells expressing TMEM16A (Figure 3D).

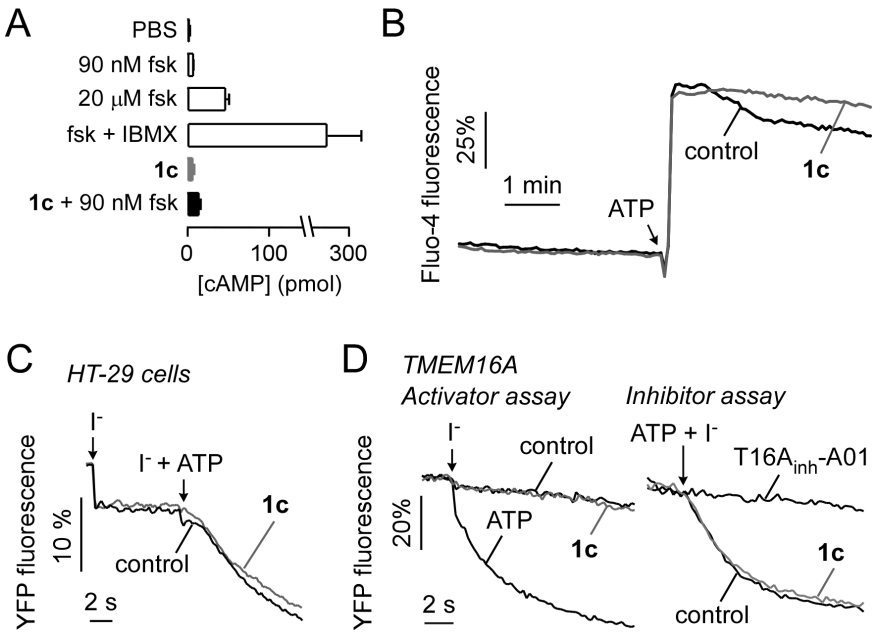


Figure 3. In vitro characterization of **1c**. *A.* Cellular cAMP in FRT cells in response to incubation for 10 min with 10 μM **1c** without or with 90 nM forskolin (fsk). Positive controls included fsk (100 nM and 20 μM) and fsk + IBMX (20 μM + 100 μM) (mean ± S.E.M., n = 4). *B.* Cytoplasmic calcium measured by Fluo-4 fluorescence. FRT cells were pretreated for 5 min with 10 μM **1c** (or control), with 100 μM ATP added as a calcium agonist as indicated. *C.* CaCC activity measured in HT-29 cells expressing YFP showing no activation (iodide addition) or inhibition (iodide + ATP addition) by 10 μM **1c**. *D.* TMEM16A activity measured in FRT cells expressing YFP showing no activation (iodide addition) or inhibition (iodide + ATP addition) by 10 μM **1c**.

Efficacy of 1c in a loperamide-induced mouse model of acute constipation. We previously demonstrated the efficacy of **4** in a loperamide-induced mouse model of constipation.[3,4] Here, the efficacy of **1c** was tested. Phenylquinoxalinone **1c** was administered orally to mice 1 h prior to loperamide and 3-h stool samples were collected after loperamide. Figure 4 shows that orally administered **1c** fully normalized stool weight, pellet number and hydration with half-maximal effective dose (ED_{50}) < 1 mg/kg.

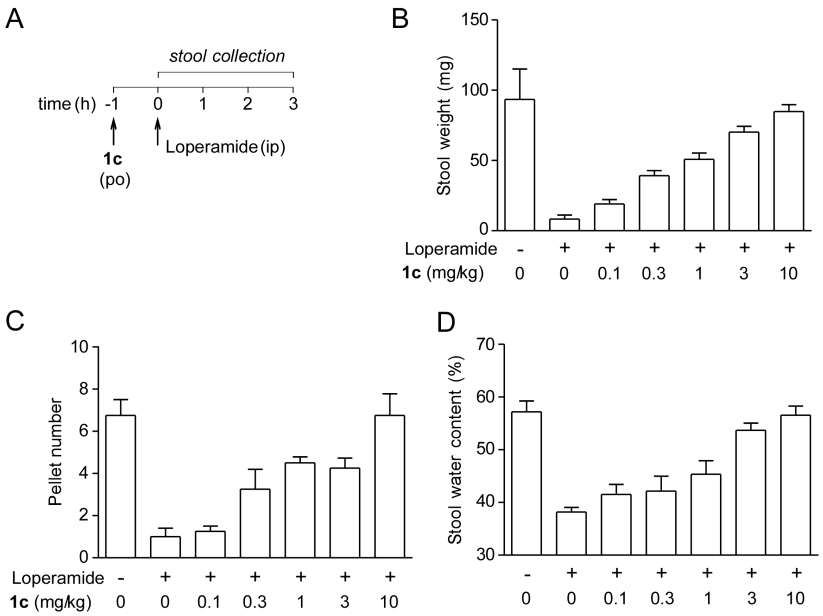


Figure 4. Efficacy of **1c** in a mouse model of acute constipation. *A*. Experimental protocol. Mice were treated with **1c** (orally) or vehicle 1 hour before loperamide (0.3 mg/kg, intraperitoneal). Weight, pellet number and water content were determined for stool collected at 3 h. *B-D.* Stool weight, pellet number and water content after treatment with 0, 0.1, 0.3, 1, 3, 10 mg/kg of **1c** (mean ± S.E.M., 4 mice per group). The control was without treatment with loperamide and **1c**.

Phenylquinoxalinone **4** was shown previously to have minimal oral bioavailability and rapid metabolism.[3,4] Though these properties are favorable for 'topical' applications in the treatment of constipation and dry eye in which systemic exposure is not needed, they are not favorable for treatment of liver and lung diseases where systemic exposure and organ accumulation are desired. Figure 5 shows substantially slower in vitro hepatic microsomal metabolism of **1c** compared with **4**, with nearly 80% of **4** metabolized at 60 min compared with <40% metabolism of **1c**.

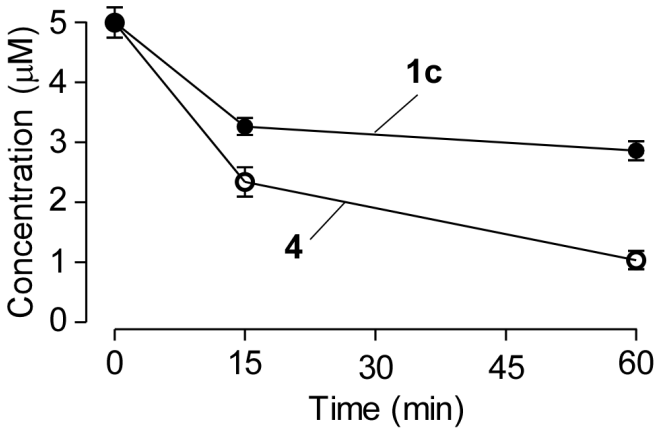


Figure 5. In vitro metabolic stability of **1c**. Remaining **1c** following incubation with human hepatic microsomes in the presence of NADPH after incubation for specified times (0, 15, 60 min), comparing with reference compound **4** (mean ± S.E.M., n=3).

Indeed, a number of important structure-activity relationships have been revealed in the present study. Previously, the amino group on the 3-aryl ring of phenylquinaxolinones – present in all of the 175 analogs reported in our preliminary study – was assumed to be a central structural feature (see **4** with an EC_{50} = 200 nM).[4] Through the series of compounds synthesized and assayed in this work, we determined that deletion of this amino group generally improves the potency of this class of CFTR activators (compare **1c** – the amino-deleted analog of **4** – with an EC_{50} = 21 nM). Additional amino-deleted analogs further established that an unsubstituted quinoxalinone core affords the best potency (compare **1c** with the quinoxalinone core analogs depicted in Table 2). Indeed, there appears to be a delicate balance between electronic and steric effects, especially considering that the highest performing analog with a substituted core (**2f**) underperforms by nearly an order of magnitude compared to unsubstituted **1c**. Quinoxalinone *N*-substituent effects were also thoroughly explored in this work (see Table 3) and, in general, *N*-benzyl or *N*-heteroaromatic groups provide optimal potency. Finally, structure-activity results reported here establish that in vitro hepatic microsomal metabolism is also dramatically

variable – see Figure 5 comparing the 60 min metabolic results for **1c** (<40% metabolized) with that for **4** (nearly 80% metabolized).

Small molecule CFTR activators have potential utility in various diseases involving epithelial fluid secretion. Compounds with minimal systemic absorption are of interest for treatment of dry eye disorders[5] with topical (eye drop) compound administration, and for constipation[3] with oral administration, as CFTR is expressed in surface-exposed epithelial cells in these tissues. However, compounds that are absorbed and accumulate in relevant tissues are needed to treat disorders of cholestasis[16] (by increasing bile flow) and inflammatory lung diseases (by increasing airway surface liquid volume).[17]

CONCLUSIONS

In conclusion, synthesis of 36 phenylquinoxalinones established structure-activity relationships and identified compounds with ~10-fold improved potency and greater metabolic stability than reference compound **4**. The most potent analog, **1c**, showed CFTR selectivity and efficacy in a mouse model of acute opioid-induced constipation. CFTR activation by phenylquinoxalinones may have utility in constipation and dry eye, as supported by prior experimental animal data,[3,4] as well as in inflammatory lung disorders and hepatic cholestasis.

EXPERIMENTAL SECTION

General Experimental: All compounds described in this manuscript have ≥95% purity. The analytical method used to determine purity was ^{1}H NMR (see the accompanying Supporting Information file which provides the ^{1}H- and ^{13}C-NMR for the thirty-six compounds assayed) and HPLC/HRMS. For HRMS analysis, samples were analyzed by flow-injection analysis into a Thermo Fisher Scientific LTQ Orbitrap (San Jose, CA) operated in the centroided mode. Samples were injected into a mixture of 50% MeOH/H_2O and 0.1% formic acid at a flow of 0.2

mL/min. Source parameters were 5.5kV spray voltage, capillary temperature of 275 ºC and sheath gas setting of 20. Spectral data were acquired at a resolution setting of 100,000 FWHM with the lockmass feature, which typically results in a mass accuracy <2ppm.

CFTR functional assays. Fischer Rat Thyroid (FRT) cells stably co-expressing human wildtype CFTR and the halide-sensitive yellow fluorescent protein (YFP)-H148Q were cultured as described.[18] Fluorescence platereader assays of CFTR function were done as described,[18] in which 96-well plates containing near-confluent cell cultures were washed with phosphate-buffered saline (PBS) and incubated for 10 min with PBS containing test compound and 125 nM forskolin. Assays of iodide influx into cells were done in single wells by continuous measurement of YFP fluorescence just for 2 s before (for baseline) and 12 s after addition of an iodide containing solution (final 140 mM iodide). TMEM16A activity assay was done similarly, as described,[19] using FRT cells co-expressing YFP and TMEM16A. Activity of non-TMEM16A CaCC activity was assayed as described[20] in HT-29 cells expressing YFP. In each assay, iodide influx rate and concentration-dependent curves were computed as described.[18-20] For short-circuit current measurement cells were cultured on porous filters and current was measured in the presence of a transepithelial chloride gradient and following permeabilization of the basolateral membrane, as described.[21] Cyclic AMP and cytoplasmic calcium measurement were done as described.[19,22]

Loperamide model of acute constipation in mice. All mouse experiments were approved by the UCSF Institutional Animal Care and Use Committee (approval number: AN108711-02A) and were conducted in accordance with the NIH guidelines for the care and use of animals. As described,[3] CD1 mice (age 8-10 weeks) were administered 0.3 mg/kg loperamide intraperitoneally (ip) and placed in metabolic cages with free access to food and water. Stool samples were

collected for 3 h for determination of total stool weight, number of fecal pellets, and stool water content (by wet and dry weight measurements). Compound **1c** (or vehicle control) was administered orally 1 h prior to loperamide. The vehicle consisted of saline containing 5% DMSO and 10% Kolliphor HS 15.

In vitro metabolic stability. Test compound (at 5 μM) was incubated for specified times at 37 °C with mouse liver microsomes (1 mg protein/ml; Sigma-Aldrich, St. Louis, MO) in potassium phosphate buffer containing 1 mM NADPH, as described.[3] Following ethyl acetate extraction, non-metabolized parent compound was assayed by LC/MS.

Statistical analysis. Data are presented as mean ± S.E.M. Comparisons between two groups were performed using the unpaired Student's *t*-test. $P < 0.05$ was considered as statistically significant.

General procedure for synthesis of dibromophenylpropanedione derivatives (**5**). A flask was charged with 1,4-dioxane (15 mL) was bubbled with N_2 for 10 min with stirring. Br_2 (2 mL, 39 mmol) was added and the solution was stirred for 30 min with slow N_2 bubbling. Substituted acetophenone (12 mmol) was dissolved in 1,4-dioxane (20 mL) and added. The mixed solution was stirred for 3 h, poured in water, and extracted with ethyl acetate. The organic layer was washed with water (3x) and brine, then dried over magnesium sulfate. Solvent was removed in vacuo to yield reddish oil of **5**, which was used for next step without further purification.

General procedure for synthesis of oxophenylacetate derivatives (**6**). Anhydrous DMSO (15 mL) was added to the oily product of **5**, and heated at 75 °C overnight. The solution was cooled to RT, and methanol (10 mL) was added and stirred overnight. The solution was poured

in water, extracted with ethyl acetate. The organic layer was washed with water (3x) and brine, and dried over magnesium sulfate. The brown oily product was used in the next step without purification.

General procedure for synthesis of *N*-H phenylquinoxalinone derivatives (**7**). Substituted phenyloxoacetate (**6**, 1 mmol) was mixed with *o*-phenylenediamine (1 mmol) in toluene (20 mL), and heated at 70 °C overnight. The precipitate that formed was collected by filtration, triturated with toluene and hexane, and used in the next step without purification.

General procedure for *N*-alkylation of phenyquinaxolinone (**1-3**). Compound **7** (0.5 mmol) was dissolved in DMF (20 mL), benzyl bromide (0.6 mmol) and K_2CO_3 (1 mmol) were added, and the mixture was stirred overnight. The solution was diluted with water, and extracted with ethyl acetate. The organic layer was washed with water three times. The organic layer was washed with brine and dried over magnesium sulfate. The final product obtained after solvent evaporation was purified by flash column chromatography.

Methyl 3-(4-benzyl-3-oxo-3,4-dihydroquinoxalin-2-yl)-4-nitrobenzoate (**1a**). Methyl 3-fluoro-4-nitrobenzoate (**8**, 1.0 g, 5 mmol) was mixed with Cs_2CO_3 (3.3 g, 10 mmol) in DMSO (20 mL). Methyl cyanoacetate (1.0 g, 10 mmol) was added and the solution was heated at 130 °C for 4 h and then maintained at 90 °C overnight. Upon cooling, the reaction mixture was extracted with ethyl acetate and the organic layer was washed with 1N HCl, water (3x), and brine. After drying over magnesium sulfate and filtration, the solvent was removed in vacuo to yield **9** as a purple oil, which was used without purification in the next step.

Crude intermediate **9** (1.8 g, 6.5 mmol) was dissolved in acetonitrile (20 mL), and CuI (1 g, 5.3 mmol) and 1,10-phenanthroline (0.23 g, 1.3 mmol) were added. The mixture was reacted at

50 °C overnight with an O_2 balloon overnight. After cooling, the solution was filtered through Celite and concentrated in vacuo. The product was purified by flash column chromatography to yield colorless **10** after solvent evaporation. Yield = 0.66 g (38 %).

Intermediate **10** (163 mg, 0.61 mmol) was mixed with *o*-phenylenediamine (78 mg, 0.72 mmol) in toluene (30 mL) and heated at 70 °C overnight. The resulting tan precipitate of **11** was collected by filtration, triturated with toluene and hexane, and air dried; it was used in the next step without purification. Yield = 185 mg (93 %).

Intermediate **11** (185 mg, 0.57 mmol) was mixed with benzyl bromide (150 mg, 0.88 mmol) and K_2CO_3 (170 mg, 1.2 mmol) in DMF (10 mL) and stirred overnight at RT. After dilution with water, the solution was extracted with ethyl acetate, washed with water (3x) and brine, and dried over $MgSO_4$. Solvent removal and purification by column chromatography gave **1a**. Yield = 149 mg (63 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 1.8 Hz, 1H), 8.22 (dd, *J* = 8.5, 1.9 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 7.89 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.50 – 7.37 (m, 1H), 7.36 – 7.06 (m, 7H), 5.44 (s, 2H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.83, 154.65, 154.19, 151.57, 134.82, 134.73, 133.30, 133.17, 132.98, 131.80, 131.61, 131.28, 130.79, 129.05, 127.83, 126.89, 124.22, 124.20, 114.82, 52.91, 46.25. HRMS $[C_{23}H_{17}N_3O_5+H]^+$: calcd 416.1247 / found 416.1253.

3-(4-Benzyl-3-oxo-3,4-dihydroquinoxalin-2-yl)-4-nitrobenzoic acid (**1b**). Compound **1a** (10 mg, 0.024 mmol) was dissolved in hot ethanol (30 mL). Sodium hydroxide (0.1 g, 2.5 mmol) dissolved in water (5 mL) was added and the mixture stirred for 1 h. The cooled solution was acidified with 1 N HCl and extracted with ethyl acetate. The organic layer was washed with water and brine, then dried over $MgSO_4$. Solvent was removed in vacuo, and product was purified by flash column chromatography. Yield = 9 mg (93 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H), 8.30 (d, *J* = 1.4 Hz, 2H), 7.98 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.62 (ddd, *J* = 8.6, 7.2, 1.5 Hz, 1H),

7.56 – 7.39 (m, 2H), 7.39 – 7.09 (m, 5H), 5.53 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.89, 154.75, 154.16, 151.48, 135.90, 133.43, 133.14, 132.95, 132.23, 131.82, 131.49, 130.53, 129.19, 127.93, 127.19, 124.88, 124.65, 115.88, 45.52. HRMS $[C_{22}H_{15}N_3O_5+H]^+$: calcd 402.1090 / found 402.1085.

1-Benzyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**1c**). Yield = 150 mg (70 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (t, *J* = 2.0 Hz, 1H), 8.88 (ddd, *J* = 7.9, 1.7, 1.1 Hz, 1H), 8.37 (ddd, *J* = 8.3, 2.3, 1.1 Hz, 1H), 8.03 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.55 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.47 – 7.30 (m, 7H), 5.63 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.51, 151.07, 148.25, 137.44, 135.48, 135.02, 133.08, 132.97, 131.27, 130.92, 129.00, 128.93, 127.82, 126.92, 124.70, 124.13, 114.50, 109.99, 46.23. HRMS $[C_{21}H_{15}N_3O_3+H]^+$: calcd 358.1192 / found 358.1188.

1-Benzyl-3-(2-nitrophenyl)quinoxalin-2(1H)-one (**1d**). Yield = 108 mg (69 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.97 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.87 – 7.73 (m, 2H), 7.65 (ddd, *J* = 8.7, 7.0, 2.0 Hz, 1H), 7.48 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.42 – 7.24 (m, 7H), 5.52 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.76, 154.35, 149.16, 135.01, 133.82, 133.30, 132.94, 131.72, 131.66, 131.00, 130.58, 129.04, 127.78, 126.96, 126.92, 124.09, 124.08, 114.83, 46.19. HRMS $[C_{21}H_{15}N_3O_3+H]^+$: calcd 358.1192 / found 358.1187.

1-Benzyl-3-(4-nitrophenyl)quinoxalin-2(1H)-one (**1e**). Yield = 94 mg (49 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 – 8.56 (m, 2H), 8.42 – 8.23 (m, 2H), 8.01 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.56 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.47 – 7.29 (m, 7H), 5.62 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.55, 151.50, 148.67, 141.76, 134.97, 133.20, 133.02, 131.58, 131.08, 130.67, 129.05,

127.90, 126.93, 124.25, 123.14, 114.58, 46.29. HRMS $[C_{21}H_{15}N_3O_3+H]^+$: calcd 358.1192 / found 358.1187.

1-Benzyl-3-(4-(trifluoromethyl)phenyl)quinoxalin-2(1H)-one (**1f**). Yield = 120 mg (83 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (d, *J* = 8.2 Hz, 2H), 7.98 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.50 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H), 7.42 – 7.26 (m, 7H), 5.59 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.61, 152.55, 139.23, 135.16, 133.25, 132.96, 131.91 (q, J_{C-F}= 33 Hz), 130.90, 130.85, 129.97, 128.93, 127.74, 126.92, 124.88 (br), 124.23 (q, J_{C-F}=325 Hz), 123.92, 114.40, 46.18. HRMS $[C_{22}H_{15}F_3N_2O+H]^+$: calcd 381.1215 / found 381.1206.

1-Benzyl-3-(3-bromophenyl)quinoxalin-2(1H)-one (**1g**). Yield = 295 mg (75 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (t, *J* = 1.8 Hz, 1H), 8.42 (dt, J = 7.9, 1.3 Hz, 1H), 7.99 (dd, J = 8.0, 1.5 Hz, 1H), 7.65 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H), 7.50 (ddd, J = 8.6, 7.3, 1.6 Hz, 1H), 7.44 – 7.29 (m, 8H), 5.60 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.58, 152.33, 137.85, 135.18, 133.32, 133.20, 132.83, 132.49, 130.82, 130.76, 129.60, 129.00, 128.35, 127.78, 126.93, 123.99, 122.29, 114.42, 46.20. HRMS $[C_{21}H_{15}BrN_2O+H]^+$: calcd 391.0446 / found 391.0442.

1-Benzyl-3-phenylquinoxalin-2(1H)-one (**1h**). Yield = 62 mg (44 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (ddd, *J* = 6.3, 2.9, 1.5 Hz, 2H), 7.99 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.53 (tt, *J* = 3.9, 2.4 Hz, 3H), 7.48 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.40 – 7.18 (m, 7H), 5.61 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.80, 154.23, 136.01, 135.37, 133.38, 132.76, 130.60, 130.45, 130.32, 129.66, 128.95, 128.13, 127.71, 126.99, 123.81, 114.36, 46.15. HRMS $[C_{21}H_{16}N_2O+H]^+$: calcd 313.1341 / found 313.1341.

1-Benzyl-3-(4-fluoro-3-nitrophenyl)quinoxalin-2(1H)-one (**1i**). Yield = 127 mg (71 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.31 (dd, *J* = 7.5, 2.3 Hz, 1H), 8.87 (ddd, *J* = 8.8, 4.3, 2.3 Hz, 1H), 7.98 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.52 (ddd, *J* = 8.6, 7.2, 1.5 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.36 – 7.31 (m, 3H), 7.28 (dt, *J* = 9.7, 3.1 Hz, 3H), 5.59 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.19, 154.54 (d, JC-F=12 Hz), 149.65, 136.75, 136.63, 134.89, 132.92 (br), 132.79 (br), 131.41, 130.86, 129.03, 127.88, 127.67 (br), 126.87, 124.28, 118.06, 117.79, 114.56. 46.25. HRMS $[C_{21}H_{14}FN_3O_3+H]^+$: calcd 376.1098 / found 376.1092.

Methyl 4-amino-3-(4-benzyl-3-oxo-3,4-dihydroquinoxalin-2-yl)benzoate (**1j**). Compound **1a** (120 mg, 0.29 mmol) was dissolved in hot ethanol (50 mL). After cooling, a saturated NH_4Cl solution (30 mL) and Zn dust (1 g) were added and the mixture stirred for 3 h. The solution was filtered through Celite, concentrated, and purified by flash column chromatography. Yield = 100 mg (90 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.26 – 8.97 (m, 1H), 7.91 (ddd, *J* = 8.6, 2.1, 0.9 Hz, 1H), 7.83 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.45 (ddt, *J* = 8.5, 7.2, 1.2 Hz, 1H), 7.38 – 7.11 (m, 7H), 6.77 (dd, *J* = 8.6, 0.8 Hz, 1H), 6.18 (s, 2H), 5.59 (s, 2H), 3.87 (d, *J* = 0.9 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.68, 154.23, 151.69, 135.24, 134.67, 132.68, 132.44, 132.17, 130.20, 129.51, 128.90, 127.67, 126.94, 123.79, 118.49, 117.26, 116.47, 114.44, 51.61, 46.44. HRMS $[C_{23}H_{19}N_3O_3+H]^+$: calcd 386.1505 / found 386.1509.

4-Amino-3-(4-benzyl-3-oxo-3,4-dihydroquinoxalin-2-yl)benzoic acid (**1k**). Compound **1j** (20 mg, 0.052 mmol) was dissolved in methanol (100 mL) at 85 °C. KOH (0.2 g, 3.6 mmol) and water (30 mL) were added and the solution refluxed overnight. Solvent was removed in vacuo, and acidified with 1 N HCl. The product was extracted with dichloromethane. Yield = 13 mg (67 %). ^{1}H NMR (600 MHz, acetone-d_6) δ 9.20 (d, *J* = 2.1 Hz, 1H), 7.89 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.84 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.51 (ddd, *J* = 8.5, 7.1, 1.5 Hz, 1H), 7.47 (dd, *J* = 8.5, 1.4 Hz, 1H),

7.43 – 7.30 (m, 5H), 7.30 – 7.19 (m, 1H), 7.08 (s, 2H), 6.93 (d, *J* = 8.7 Hz, 1H), 5.67 (s, 2H). ^{13}C NMR (151 MHz, acetone-d_6) δ 166.89, 154.55, 154.34, 152.99, 136.23, 135.27, 132.57, 132.28, 132.11, 130.02, 129.31, 128.70, 127.32, 126.98, 123.52, 116.81, 116.56, 115.85, 114.84, 45.73. HRMS $[C_{22}H_{17}N_3O_3+H]^+$: calcd 372.1348 / found 372.1351.

3-(4-Benzyl-3-oxo-3,4-dihydroquinoxalin-2-yl)benzonitrile (**1l**). Yield = 27 mg (62 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (t, *J* = 1.7 Hz, 1H), 8.72 (dt, *J* = 8.1, 1.5 Hz, 1H), 7.97 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.76 (dq, *J* = 7.7, 1.4 Hz, 1H), 7.60 (td, *J* = 7.9, 1.8 Hz, 1H), 7.51 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.41 – 7.26 (m, 7H), 5.58 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.54, 151.35, 137.00, 135.02, 133.85, 133.45, 133.13, 132.93, 131.27, 130.90, 129.04, 128.92, 127.88, 126.94, 124.20, 118.73, 114.53, 112.46, 46.26. HRMS $[C_{22}H_{15}N_3O+H]^+$: calcd 338.1293 / found 338.1290.

3-(Benzo[c][1,2,5]oxadiazol-5-yl)-1-benzylquinoxalin-2(1H)-one (**1m**). Yield = 30 mg (75 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 – 9.25 (m, 1H), 8.83 (ddd, *J* = 7.8, 1.7, 1.1 Hz, 1H), 8.34 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 8.00 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.71 – 7.55 (m, 3H), 7.42 (ddd, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.25 (dd, *J* = 5.2, 1.3 Hz, 2H), 7.20 (dt, *J* = 3.5, 1.0 Hz, 1H), 6.97 (dd, *J* = 5.1, 3.5 Hz, 1H), 5.70 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.04, 151.18, 148.25, 137.33, 136.88, 135.52, 133.15, 132.49, 131.35, 131.18, 129.04, 127.58, 126.85, 126.01, 124.84, 124.70, 124.31, 113.96, 41.24. HRMS $[C_{21}H_{14}N_4O_2+H]^+$: calcd 355.1195 / found 355.1207.

1-Benzyl-7-chloro-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2a**). Yield = 31 mg (15 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (t, *J* = 2.0 Hz, 1H), 8.86 (dt, *J* = 7.9, 1.3 Hz, 1H), 8.36 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.99 – 7.85 (m, 1H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.44 – 7.30 (m, 7H), 5.56 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.27, 151.03, 148.24, 137.44, 137.07, 135.52, 134.44,

133.77, 131.97, 131.58, 129.19, 129.09, 128.12, 126.95, 125.02, 124.79, 124.71, 114.47, 46.42. HRMS $[C_{21}H_{14}ClN_3O_3+H]^+$: calcd 392.0802 / found 392.0810.

1-Benzyl-6-chloro-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2b**). Yield = 56 mg (31 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (t, *J* = 2.0 Hz, 1H), 8.87 (dt, *J* = 7.9, 1.3 Hz, 1H), 8.39 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 8.02 (d, *J* = 2.4 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.49 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.42 – 7.20 (m, 6H), 5.60 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.22, 152.30, 148.24, 137.00, 135.59, 134.61, 133.57, 131.59, 131.31, 130.09, 129.60, 129.15, 129.11, 128.06, 126.85, 125.19, 124.86, 115.71, 46.44. HRMS $[C_{21}H_{14}ClN_3O_3+H]^+$: calcd 392.0802 / found 392.0819.

1-Benzyl-5-chloro-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2c**). Yield = 8 mg (8 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.51 (t, *J* = 2.0 Hz, 1H), 8.96 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.39 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.71 (t, *J* = 8.1 Hz, 1H), 7.57 – 7.40 (m, 2H), 7.40 – 7.18 (m, 6H), 5.63 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.25, 150.92, 148.29, 137.14, 135.84, 135.73, 134.66, 134.45, 131.23, 129.73, 129.16, 129.12, 128.01, 126.80, 125.26, 125.10, 125.03, 113.41, 46.67. HRMS $[C_{21}H_{14}ClN_3O_3+H]^+$: calcd 392.0802 / found 392.0813.

1-Benzyl-6,7-dichloro-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2d**). Yield = 13 mg (31 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (t, *J* = 2.0 Hz, 1H), 8.86 (ddt, *J* = 7.9, 2.8, 1.3 Hz, 1H), 8.46 – 8.28 (m, 1H), 8.11 (s, 1H), 7.70 (td, *J* = 8.1, 3.8 Hz, 1H), 7.46 (s, 1H), 7.43 – 7.28 (m, 5H), 5.56 (d, *J* = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.00, 152.23, 148.25, 136.73, 135.57, 134.16, 132.21, 132.08, 131.98, 131.53, 129.28, 129.18, 128.27, 126.90, 125.35, 124.85, 124.73, 115.94, 46.56. HRMS $[C_{21}H_{13}Cl_2N_3O_3+H]^+$: calcd 426.0412 / found 426.0405.

1-Benzyl-7-bromo-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2e**). Yield = 47 mg (15 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (t, *J* = 2.0 Hz, 1H), 8.86 (dt, *J* = 7.9, 1.3 Hz, 1H), 8.37 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.69 (t, *J* = 8.1 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.46 – 7.29 (m, 5H), 5.56 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.22, 151.28, 148.25, 137.08, 135.52, 134.44, 133.89, 132.07, 131.89, 129.20, 129.11, 128.13, 127.65, 126.97, 125.69, 125.05, 124.73, 117.46, 46.40. HRMS $[C_{21}H_{14}BrN_3O_3+H]^+$: calcd 436.0297 / found 436.0291.

1-Benzyl-6,7-difluoro-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2g**). Yield = 13 mg (10 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (t, *J* = 2.0 Hz, 1H), 8.85 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.38 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.82 (dd, *J* = 10.0, 8.2 Hz, 1H), 7.70 (t, *J* = 8.1 Hz, 1H), 7.47 – 7.22 (m, 5H), 7.15 (dd, *J* = 11.3, 7.0 Hz, 1H), 5.55 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.16, 152.20 (dd, $J_{C\text{-}F}$=257, 15 Hz), 151.47, 148.25, 147.15 (dd, $J_{C\text{-}F}$= 250, 15 Hz), 136.87, 135.49, 134.17, 130.27 (br), 129.41 (br), 129.28, 129.15, 128.26, 126.85, 125.17, 124.73, 118.26 (dd, $J_{C\text{-}F}$ = 18, 2 Hz), 103.22 (d, $J_{C\text{-}F}$ = 23 Hz), 46.87. HRMS $[C_{21}H_{13}F_2N_3O_3+H]^+$: calcd 394.1003 / found 394.0996.

1-Benzyl-8-methyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2h**). Yield = 36 mg (28 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.91 (dt, *J* = 7.9, 1.3 Hz, 1H), 8.43 – 8.10 (m, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.50 – 7.20 (m, 8H), 5.60 (s, 2H), 2.79 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.44, 148.96, 148.33, 139.84, 137.88, 135.41, 135.20, 133.19, 131.65, 131.10, 128.92, 128.87, 127.70, 126.84, 125.30, 124.69, 124.54, 112.42, 46.31, 17.59. HRMS $[C_{22}H_{17}N_3O_3+H]^+$: calcd 372.1348 found 372.1343

1-Benzyl-6,7-dimethyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2i**). Yield = 13 mg (31 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (t, *J* = 2.0 Hz, 1H), 8.86 (dt, *J* = 7.8, 1.4 Hz, 1H), 8.33 (ddd, *J*

= 8.2, 2.4, 1.1 Hz, 1H), 7.77 (s, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.43 – 7.22 (m, 5H), 7.13 (s, 1H), 5.59 (s, 2H), 2.38 (d, *J* = 1.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.59, 149.83, 148.21, 141.67, 137.78, 135.44, 135.23, 133.39, 131.66, 131.12, 130.88, 129.00, 128.93, 127.76, 126.91, 124.59, 124.47, 114.91, 46.11, 20.84, 19.21. HRMS $[C_{23}H_{19}N_3O_3+H]^+$: calcd 386.1505 / found 386.1499.

1-Benzyl-6-bromo-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**2j**). Yield = 106 mg (34 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 – 9.22 (m, 1H), 8.87 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.38 (ddd, *J* = 8.2, 2.4, 1.2 Hz, 1H), 8.18 (d, *J* = 2.3 Hz, 1H), 7.70 (t, *J* = 8.1 Hz, 1H), 7.61 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.44 – 7.26 (m, 5H), 7.23 (d, *J* = 9.0 Hz, 1H), 5.59 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.22, 152.22, 148.24, 136.98, 135.59, 134.58, 134.02, 133.86, 133.18, 132.02, 129.14, 128.07, 126.97, 126.85, 125.20, 124.86, 116.82, 115.98, 46.41. HRMS $[C_{21}H_{14}BrN_3O_3+H]^+$: calcd 436.0297 / found 436.0290.

3-(3-Nitrophenyl)quinoxalin-2(1H)-one (**3a**). Yield = 220 mg (75 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 9.31 – 9.12 (m, 1H), 8.78 (ddd, J = 7.9, 1.7, 1.1 Hz, 1H), 8.38 (ddd, J = 8.2, 2.4, 1.1 Hz, 1H), 7.96 – 7.88 (m, 1H), 7.82 (t, J = 8.0 Hz, 1H), 7.71 – 7.48 (m, 1H), 7.47 – 7.25 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.02, 152.12, 147.99, 137.43, 135.81, 132.85, 132.34, 131.58, 130.08, 129.52, 125.19, 124.22, 124.15, 115.77. HRMS $[C_{14}H_9N_3O_3+H]^+$: calcd 268.0722 / found 268.0713. HRMS $[C_{14}H_9N_3O_3+H]^+$: calcd 268.0722 / found 268.0713.

1-Methyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3b**). Yield = 39 mg (74 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.79 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.33 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 7.99 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.74 – 7.54 (m, 2H), 7.54 – 7.32 (m, 2H), 3.81 (s, 3H). ^{13}C NMR

(151 MHz, $CDCl_3$) δ 154.43, 151.10, 148.27, 137.51, 135.36, 133.59, 132.86, 131.25, 130.83, 128.89, 124.65, 124.64, 124.05, 113.68, 29.34. HRMS $[C_{15}H_{11}N_3O_3+H]^+$: calcd 282.0879 / found 282.0870.

1-Ethyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3c**). Yield = 54 mg (70 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 – 9.14 (m, 1H), 8.83 (ddd, *J* = 7.9, 1.7, 1.1 Hz, 1H), 8.34 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 8.09 – 7.88 (m, 1H), 7.77 – 7.58 (m, 2H), 7.53 – 7.36 (m, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 1.47 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.96, 151.05, 148.19, 137.50, 135.49, 133.16, 132.53, 131.32, 131.10, 128.97, 124.71, 123.96, 113.61, 37.80, 12.44. HRMS $[C_{16}H_{13}N_3O_3+H]^+$: calcd 296.1035 / found 296.1037.

1-Propyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3d**). Yield = 13 mg (23 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.30 (t, *J* = 1.9 Hz, 1H), 8.80 (dt, *J* = 7.9, 1.3 Hz, 1H), 8.32 (ddd, *J* = 8.1, 2.3, 1.1 Hz, 1H), 7.99 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.73 – 7.55 (m, 2H), 7.52 – 7.32 (m, 2H), 4.38 – 4.17 (m, 2H), 1.87 (hept, *J* = 7.5 Hz, 2H), 1.10 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.18, 151.04, 148.25, 137.55, 135.40, 133.11, 132.82, 131.14, 131.04, 128.86, 124.67, 124.61, 123.85, 113.72, 44.19, 20.67, 11.34. HRMS $[C_{17}H_{15}N_3O_3+H]^+$: calcd 310.1192 / found 310.1182.

1-Allyl-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3e**). Yield = 91 mg (79 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (t, *J* = 2.0 Hz, 1H), 8.92 – 8.73 (m, 1H), 8.35 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 8.02 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.77 – 7.54 (m, 2H), 7.51 – 7.32 (m, 2H), 6.02 (ddt, *J* = 17.3, 10.4, 5.2 Hz, 1H), 5.42 – 5.15 (m, 2H), 5.03 (dt, *J* = 5.2, 1.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.02, 151.05, 148.20, 137.41, 135.51, 133.01, 132.81, 131.28, 130.92, 130.36, 128.99, 124.77, 124.71, 124.15, 118.44, 114.30, 44.88. HRMS $[C_{17}H_{13}N_3O_3+H]^+$: calcd 308.1035 / found 308.1036.

1-(Naphthalen-1-ylmethyl)-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3f**). Yield = 54 mg (35 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.39 (t, *J* = 2.0 Hz, 1H), 8.89 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.32 (ddd, *J* = 8.3, 2.4, 1.1 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.08 – 8.01 (m, 1H), 7.94 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 7.71 – 7.62 (m, 2H), 7.59 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.46 – 7.33 (m, 2H), 7.32 – 7.22 (m, 1H), 7.08 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.83 (dd, *J* = 7.3, 1.2 Hz, 1H), 6.04 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.50, 150.92, 148.33, 137.43, 135.48, 133.92, 133.15, 133.12, 131.33, 130.85, 130.52, 129.24, 129.17, 128.91, 128.19, 128.17, 126.71, 126.12, 125.38, 124.73, 124.20, 122.30, 122.07, 114.77, 44.04. HRMS $[C_{25}H_{17}N_3O_3+H]^+$: calcd 408.1348 / found 408.1342.

1-(Naphthalen-2-ylmethyl)-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3g**). Yield = 80 mg (75 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (t, *J* = 2.0 Hz, 1H), 8.91 (ddd, *J* = 7.9, 1.7, 1.1 Hz, 1H), 8.38 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 8.09 – 7.98 (m, 1H), 7.91 – 7.80 (m, 2H), 7.78 (dd, *J* = 6.2, 3.4 Hz, 1H), 7.74 – 7.65 (m, 2H), 7.57 – 7.44 (m, 4H), 7.41 (ddd, *J* = 8.3, 7.6, 1.2 Hz, 2H), 5.79 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.64, 151.22, 148.26, 137.47, 135.59, 133.33, 133.16, 132.99, 132.85, 132.47, 131.39, 130.98, 129.06, 127.75, 126.53, 126.24, 125.72, 124.86, 124.79, 124.72, 124.27, 114.63, 46.52. HRMS $[C_{25}H_{17}N_3O_3+H]^+$: calcd 408.1348 found 408.1339.

3-(3-Nitrophenyl)-1-(pyridin-2-ylmethyl)quinoxalin-2(1H)-one (**3h**). Yield = 82 mg (90 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (t, *J* = 2.0 Hz, 1H), 8.86 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.61 (dt, *J* = 4.8, 1.4 Hz, 1H), 8.36 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.01 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.75 – 7.62 (m, 2H), 7.62 – 7.51 (m, 2H), 7.41 (ddd, *J* = 8.2, 6.5, 2.0 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.24 (ddd, *J* = 7.5, 4.9, 1.1 Hz, 1H), 5.73 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.11, 154.53,

151.10, 149.49, 148.23, 137.43, 137.27, 135.52, 133.10, 131.43, 130.79, 129.03, 124.82, 124.74, 124.31, 122.98, 122.23, 115.06, 48.25. HRMS $[C_{20}H_{14}N_4O_3+H]^+$: calcd 359.1144 / found 359.1138.

1-(Furan-2-ylmethyl)-3-(3-nitrophenyl)quinoxalin-2(1H)-one (**3i**). Yield = 238 mg (67 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (t, *J* = 2.0 Hz, 1H), 8.83 (dt, *J* = 7.8, 1.4 Hz, 1H), 8.34 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.00 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.78 – 7.51 (m, 3H), 7.44 (ddd, *J* = 8.3, 7.0, 1.5 Hz, 1H), 7.39 (s, 1H), 6.49 (d, *J* = 3.2 Hz, 1H), 6.36 (dd, *J* = 3.3, 1.9 Hz, 1H), 5.56 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.03, 151.07, 148.57, 148.19, 142.62, 137.34, 135.51, 133.01, 132.82, 131.33, 130.92, 129.00, 124.79, 124.69, 124.26, 114.38, 110.74, 109.85, 39.18. HRMS $[C_{19}H_{13}N_3O_4+H]^+$: calcd 348.0985 / found 348.0984.

3-(3-Nitrophenyl)-1-(thiophen-2-ylmethyl)quinoxalin-2(1H)-one (**3j**). Yield = 48 mg (35 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 – 9.28 (m, 1H), 8.83 (ddd, *J* = 7.8, 1.7, 1.1 Hz, 1H), 8.34 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 8.06 – 7.95 (m, 1H), 7.72 – 7.55 (m, 3H), 7.42 (ddd, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.25 – 7.23 (m, 1H), 7.20 (dq, *J* = 3.5, 0.9 Hz, 1H), 6.97 (dd, *J* = 5.1, 3.5 Hz, 1H), 5.70 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.05, 151.18, 148.25, 137.33, 136.88, 135.53, 133.15, 132.49, 131.35, 131.18, 129.05, 127.58, 126.86, 126.02, 124.85, 124.70, 124.32, 113.96, 41.24. HRMS $[C_{19}H_{13}N_3O_3S+H]^+$: calcd 364.0756 / found 364.0749.

***N*-(4-nitro-2-(5-oxo-2,3-dihydro-5H-[1,4]oxazino[4,3,2-de]quinoxalin-6-yl)phenyl)acetamide** (**16**). 2-Amino-3-nitrophenol (1 g, 6.5 mmol) was dissolved in DMF (15 mL). 1,2-Dibromoethane (0.7 mL, 8.1 mmol) and KOH (0.3 g, 5.3 mmol) were added, and the mixture was refluxed at 160 °C for 3 d. After cooling, the solution was poured into water and extracted with

ethyl acetate. The ethyl acetate solution was washed with water and brine, then dried with magnesium sulfate. After filtration and concentration in vacuo, the product was purified by column chromatography with a 30:70 mixture of ethyl acetate/hexane to yield intermediate **14** as a red crystalline product. This red product was dissolved in methanol (20 mL) and Pd/C (0.1 g) was added. H_2 was bubbled for 2 h until the solution turned nearly colorless. The solution was filtered through Celite, and solvent was removed in vacuo to yield 3,4-dihydro-2H-benzo[b][1,4]oxazin-5-amine (intermediate **15**) as a light brown oil (0.102 g, 10 %), which was used directly in the next step.

This 3,4-dihydro-2H-benzo[b][1,4]oxazin-5-amine (**15**: 0.102 g, 0.68 mmol) was dissolved in a mixture of 20 mL of acetic acid and 20 mL of toluene. *N*-Acyl-5-nitroisatin (0.22 g, 0.94 mmol) was added and the mixture refluxed at 90 °C overnight. Upon cooling, solvent was removed in vacuo, and the residue was washed with ethanol to yield **16** as a dark tan oil. Yield = 0.246 g (99 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.55 (s, 1H), 8.34 (s, 2H), 7.52 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 4.46 (t, J = 4.9 Hz, 2H), 4.19 (t, J = 4.8 Hz, 2H), 2.04 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.41, 154.38, 152.13, 143.50, 143.22, 142.31, 132.85, 127.02, 126.93, 125.65, 123.98, 122.47, 122.07, 121.23, 117.02, 63.92, 24.63. HRMS $[C_{22}H_{16}N_4O_4+H]^+$: calcd 401.1250 / found 401.1237.

6-(2-Amino-5-nitrophenyl)-3-phenyl-2,3-dihydro-5H-[1,4]oxazino[4,3,2-de]quinoxalin-5-one (**20**). 2-Amino-3-nitrophenol 0.83 g (5.4 mmol) was mixed with K_2CO_3 (1.13 g, 8.2 mmol) in acetonitrile (100 mL). 2-Bromoacetophenone (1.3 g, 6.5 mmol) was added portion-wise and stirred overnight. Ethyl acetate (100 mL) was added and the solution was filtered, washed with water, 1 N HCl, and brine. The solution was dried over magnesium sulfate, filtered, and solvent removed in vacuo. The resulting crude product (**18**) was partly dissolved in hot methanol (100 mL). After cooling, Pd/C (0.2 g) was added and H_2 was bubbled until the starting material was

consumed as monitored by TLC. The solution was filtered through Celite, and the solvent was removed in vacuo. The resulting diamine was purified by column chromatography with a 30:70 mixture of ethyl acetate/hexane. An orange-brown oil of intermediate **19** was obtained. Yield = 0.6 g (50 %).

This 3-phenyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-5-amine (**19**: 0.2 g, 0.9 mmol) was dissolved in a mixture of acetic acid (20 mL) and toluene (40 mL). 5-Nitroisatin (0.17 g, 0.88 mmol) was added, and the mixture refluxed at 100 °C for 2 h. Upon cooling, solvent was removed in vacuo, and the product was purified by column chromatography with a 30:70 mixture of ethyl acetate/hexane to yield **20**. Yield = 16 mg (5 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.21 (d, J = 2.8 Hz, 1H), 8.08 – 7.87 (m, 3H), 7.64 (dd, J = 8.1, 1.2 Hz, 1H), 7.39 – 7.21 (m, 4H), 7.21 – 7.09 (m, 3H), 6.90 (d, J = 9.2 Hz, 1H), 5.98 (t, J = 1.6 Hz, 1H), 4.74 (dd, J = 11.8, 1.3 Hz, 1H), 4.45 (dd, J = 11.7, 2.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.09, 153.39, 151.79, 142.99, 138.01, 135.39, 131.91, 129.46, 129.04, 128.08, 126.93, 126.72, 124.09, 122.12, 120.45, 116.57, 116.25, 115.20, 109.99, 69.48, 53.31. HRMS $[C_{18}H_{14}N_4O_5+H]^+$: calcd 367.1043 / found 367.1038.

5-Benzyl-9-fluoro-2-(trifluoromethyl)-5a,10a-dihydro-5H-indolo[2,3-b]quinoxaline (**22**). Yield = 98 mg (15 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.96 (dd, J = 7.6, 2.6 Hz, 1H), 7.83 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H), 7.63 (dd, J = 8.6, 4.2 Hz, 1H), 7.41 (td, J = 9.0, 2.7 Hz, 1H), 7.35 – 7.26 (m, 5H), 6.06 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.71 (d, $J_{C\text{-}F}$ = 240 Hz), 154.95, 147.04, 134.09, 133.93, 131.33, 129.19, 128.76 (br), 128.27, 126.80, 126.32 (br), 125.80 (d, $J_{C\text{-}F}$ = 33 Hz), 123.77, 123.73 (q, $J_{C\text{-}F}$ = 272 Hz), 120.58 (q, $J_{C\text{-}F}$ = 24 Hz), 119.80, 119.75, 115.35, 109.43 (d, $J_{C\text{-}F}$ = 24 Hz), 49.35. HRMS $[C_{22}H_{13}F_4N_3+H]^+$: calcd 396.1124 / found 396.1105.

5-Benzyl-9-nitro-2-(trifluoromethyl)-5a,10a-dihydro-5H-indolo[2,3-b]quinoxaline (**23**). Yield = 17 mg (22 %). ^{1}H NMR (800 MHz, DMSO-d_6) δ 9.01 (d, J = 2.4 Hz, 1H), 8.71 (d, J = 2.0 Hz, 1H), 8.59 (dd, J = 8.7, 2.4 Hz, 1H), 8.22 (d, J = 8.9 Hz, 1H), 8.17 (dd, J = 9.0, 2.2 Hz, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.45 – 7.37 (m, 2H), 7.35 – 7.31 (m, 2H), 7.31 – 7.23 (m, 1H), 6.25 (s, 2H). ^{13}C NMR (201 MHz, DMSO-d_6) δ 163.95, 154.04, 150.17, 141.98, 135.07, 134.96, 132.05, 129.30, 128.91, 128.50 (br), 128.33, 127.48, 127.44 (br), 125.48 (q, J_{C-F} = 26 Hz), 124.29 (q, J_{C-F} = 204 Hz), 123.34, 119.35, 118.91, 118.21, 49.67. HRMS $[C_{22}H_{13}F_3N_4O_2+H]^+$: calcd 423.1069 / found 423.1062.

ASSOCIATED CONTENT

Supporting Information: ^{1}H and ^{13}C NMR data (PDF) for all assayed compounds and molecular formula strings (CSV). The Supporting Information is available free of charge on the ACS Publications website at DOI: ####.

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Author Contributions

The manuscript was written by J.-H.S., J.S.Z., P.-W.P., A.S.V., and M.J.K. Synthesis of substrates was performed by J.-H.S., J.S.Z., A.P.T, and C.K.K. Assays and biological data collection were performed by O.C., P.-W.P., and S.L. All authors have given approval to the final version of the manuscript.

Notes

O. Cil and A. S. Verkman are named inventors on provisional patent filings, with rights owned by the University of California, San Francisco. The other authors have nothing to declare.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (DK072517, DK099803, DK075302, EY13574, DK101373, EB00415) and the UC Davis Tara K. Telford CF Fund (fellowship to J.S.Z.).

ABBREVIATIONS USED

CFTR, cystic fibrosis transmembrane conductance regulator; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; FRT, Fischer Rat Thyroid; YFP, yellow fluorescent protein; PBS, phosphate-buffered saline; RT, room temperature; TLC, thin layer chromatography.

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